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RESEARCH ARTICLE

Traditional Land and Food Systems: A Case of Uttarakhand State in North-western Indian Himalayas

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Like most of India, agriculture is one of the significant sectors of the economy of Uttarakhand state. About 86% of the state consists of hills, traditionally growing diverse crops as polycultures and sustaining about 50% of its population. As the contribution of agricultural biodiversity for food and nutritional security of native farming communities is often undervalued, the present communication succinctly documents the important features of traditional small-holder farming of Uttarakhand hills. About 70-80% of the native people living in rural areas depend on farming for their livelihoods, with the majority relying on small scale crop-livestock systems, including those that are integrated with long haul pastoral systems. The integration of crop and livestock production systems increases the diversity, along with environmental sustainability, of both sectors. At the same time it provides opportunities for increasing overall production and economics of farming. Being rich in agricultural biodiversity and food culture, use of locally available traditional food resources including wild harvested food resources in agricultural systems should be part of frontline strategies for nutrition interventions in Uttarakhand hills. The revitalization of local food systems is believed to be an imperative starting point in the local, regional and national framework of agrobiodiversity conservation, dietary diversification and food sovereignty in the Himalayan highlands. The present communication also suggests the need of a proactive alliance between local communities and their key allies across agriculture, food, and public health sectors in collaboratively creating a research and advocacy agenda in support of agrobiodiversity and the revival of local food systems and landscapes within the broader framework of indigenous food sovereignty.

Key Words: Community nutrition and health, Indigenous food sovereignty, Native food culture, Traditional hill farming

Introduction

Work on land tenure and traditional food systems are important for native farming communities and indigenous people world over. Indigenous food systems help people to realize the importance of maintaining their connections with nature and their own cultures, and between heart and mind, to reaffirm identity (Woodley *et al*., 2009). Where people identify themselves with their culture and natural environment, knowledge and use of traditional food systems to improve health builds community support and engagement for holistic health and well-being. Kuhnlein *et al.* (2009) asserts that "the dimensions of nature and culture that define a food system of an indigenous culture contribute to the whole health picture of the individual and the community-not only physical health but also the emotional, mental and spiritual aspects of health, healing and protection from disease". Those living in their rural homelands depend on traditional food systems rooted in historical continuity in their regions, where food is harvested with traditional knowledge from the natural environment, and prepared and served in local cultural settings. This is also an important aspect of cultural collectives of individuals and community – in recognizing continuity from the past, into the present and towards the future (Woodley *et al*., 2009).

Security of tenure, which is crucial to native communities' cultural identity and well-being, can be enhanced through recognition of customary tenure rules and practices. Common property regimes provide a basis for shared identity and livelihoods, and have been found to contribute to the health status of communities (Woodley *et al*., 2009). Indigenous Peoples' organizations are, however, concerned that various types of development activities have had negative impacts on native indigenous communities' traditional food and agro-ecosystems. Such impacts can only be avoided if development programmes are carried out with the free, prior and informed consent (FPIC) of the native communities with traditional rights to the lands, territories or resources concerned. Indigenous

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†Deceased on 14 December 2016

Peoples are often insufficiently aware of their rights, particularly those related to Intellectual Property Rights (IPR) and Access and Benefit-Sharing (ABS) as laid down in various UN treaties and conventions.

It is a well-accepted fact that crop diversity and wild-harvested plants and animals have made and continue to make appreciable contributions to human diets (Heywood, 2013). However, detailed evidence of their importance in terms of energy intake, micronutrient intake and dietary diversification is scarce and correlating agricultural biodiversity with human nutrition is generally difficult for a number of reasons including human diversity (DeClerck *et al*., 2011).

Further, the exploitation of agricultural biodiversity has provided enormous nutrition and health benefits but overexploitation of some resources and extensive habitat loss has negatively impacted dietary diversity, nutrition and health of some groups of society (Nakhauka, 2009). We are now faced with attempting to assess these impacts, learn lessons and seek a sustainable way forward (IAASTD, 2009). New approaches have been explored which aim to integrate environmental and human health, focusing especially on the many interactions between agriculture, ecology and human nutrition (Blasbalg *et al*., 2011; Heywood, 2013).

It is a well-known fact that despite the success of the agricultural revolution in providing enough food to feed the world, today we are faced with issues of over- and undernutrition – both forms of malnutrition. More than a billion people today are underfed and suffering from acute malnutrition thus making them more disease-prone, while much of the developed world is at the same time facing a crisis of obesity caused by overnutrition, a condition that is increasingly aggravated by an unhealthy lifestyle, leading to diet-related diseases, such as cardiovascular disease, hypertension, cancer, diabetes, etc. This tendency is not confined to the developed world but is also spreading to countries undergoing rapid societal transition – so-called development-driven obesity (Heywood, 2013). Worldwide 30 per cent more people are now obese than those who are underfed. The causes of these nutritional challenges are many and complex as are possible solutions.

Salient findings of a few exploratory case studies on native crop landrace diversity, experiential knowledge of farmer households in identification and use of wild harvested foods in agricultural systems, and potential of native biodiversity in addressing the food-based approach to community health and nutrition are highlighted in the present communication with regard to hilly areas of Uttarakhand state in north-western India where biodiverse traditional subsistence farming is still predominantly practiced.

The salient findings of the case study on wild harvested foods in agricultural systems are discussed in great detail in this communication as it forms an important but undervalued component of local food culture in Uttarakhand hills.

The aim of these exploratory case studies has been to build scientific credibility of local food systems, to use this information to improve the health of the people directly involved, and to share the findings to influence policies at the local and national levels. We also discuss how policies can be influenced to benefit native communities use their food systems, particularly by increasing access to the range of biodiversity available in overall framework of indigenous food sovereignty.

The studies on the potential of local food systems in addressing community health and nutrition will showcase how best the problem of malnutrition and food-related diseases can be addressed by reviving the native food culture. The need of a proactive alliance among diverse stakeholders collaboratively creating a research and advocacy agenda in support of agrobiodiversity and the revival of local food systems and landscapes within the broader framework of indigenous food sovereignty will be explored and advocated. Food sovereignty has yet not been formally adopted in the state policy of India in general and Uttarakhand hills in particular.

Representative Farming Situations in Uttarakhand Himalaya

Like most of India, agriculture is one of the significant sectors of the economy of Uttarakhand state. About 86% of the state consists of hills but most of the crop lands are in plains, the *Terai* and *Bhabar* region, where improved agriculture is practiced (Figure 1). On the other hand, the hilly areas of Uttarakhand state (parts of north-western Himalayas) traditionally grow diverse crops as polycultures. The crops include coarse cereals (minor millets-finger millet, barnyard millet, foxtail millet, little millet, prosomillet), rice, wheat, barley, pulses (horse gram, black gram, black-seeded local soybean, lentil, cowpea, rice bean), oilseeds (mustard, sesame), several

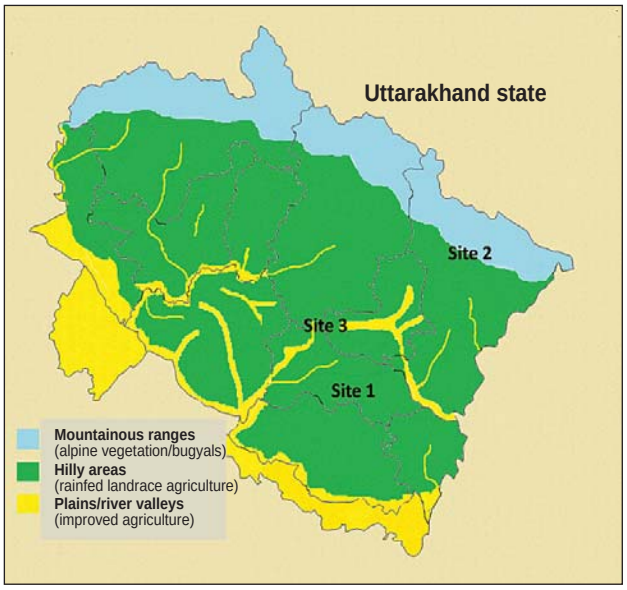

Fig. 1. Major agro-ecological regions in Uttarakhand (Site 1-3 are niche sites for representative farming situations)

underutilized crops (amaranths, buckwheat, chenopods, tubers, yams, cucurbits, minor leafy vegetables, perilla and others), numerous medicinal herbs, etc. Most of these native crops and their traditional landraces form the "functional foods" to hill farming communities with nutraceutical properties and high nutritional value.

The subsistence harvests and uses of *wild* resources by native communities have been another important feature of traditional hill farming. Fruits such as apples, oranges, pears, peaches, plums, and citrus are also grown and important to the food processing industry in certain areas. Livestock (buffalo, cattle, goats, sheep, and poultry) are integral to hill farming and contribute substantially to rural economy.

In Uttarakhand hills there exists a self-contained food system, specific to different farming situations and communities which are deeply rooted in local traditions and culture. However, the forces of globalization and the vulnerabilities arising from poverty, discrimination and marginalization in some sections of farmer households in the hill communities are increasingly affecting the native food culture. Limited access to diverse food resources is affecting the nutrition and health of native communities causing malnutrition among children and women.

The traditional hill agriculture is highly knowledge-based and farmer-led innovations are predominantly practiced. All farmer led innovations are socio-economic and are not of a technical nature. The driving forces for emergence of the farmer led innovation paradigm are constraints to crop and livestock production.

Non-availability of quality seed of native crop varieties, lack of human resource (farm labourers), poor market infrastructure for native crops, high land fragmentation, and the changing climate, especially greater frequency and severity of droughts during the past two decades or so, have been the major constraints to traditional rainfed agriculture of Uttarakhand hills. Further, poor management of common property resources (CPRs) leads to declining fodder resources for livestock in crop-livestock small-scale mixed farming and declining sources of wild harvested foods. The dynamic relationship among crops, livestock and CPRs is increasingly breaking down now.

During the exploratory surveys in parts of Uttarakhand hills we encountered the following three predominant representative farming situations (Fig. 1; Photo Plates 1-3):

1. Small-scale crop-livestock mixed-farming systems representing about 70% cropped area under rainfed farming (Plate 1). The farming situations could be characterized by high household food production and dietary diversity. Only farmer-led traditional innovations were predominantly practiced. In the mixed crop-livestock farming system of the hills, there still exists a dynamic relationship among CPRs, native crops and livestock. The livestock substantially contributes to household cash income whereas the surplus crop produce, if any, is sold locally and contributes very little to the household cash income.
2. High elevation mountainous areas adjoining Tibet with a mix of nomadic pastoralism, some arable land and wild harvesting including foraging and trading of medicinal herbs (Plate 2). Sheep and goat are the herded livestock. Level of household food production and dietary diversity is moderate to high. This system is representative of about 10% of the farming areas in hills of Uttarakhand. *Bhotia* and *Shoka* tribes are the main inhabitants of the region. The agricultural economy of the mountainous valleys consists of subsistence and export (market) farming, as well as tending of livestock and harvesting of herbs for export (market) sale. In addition to cultivated herbs, many valley residents engage in medicinal herb

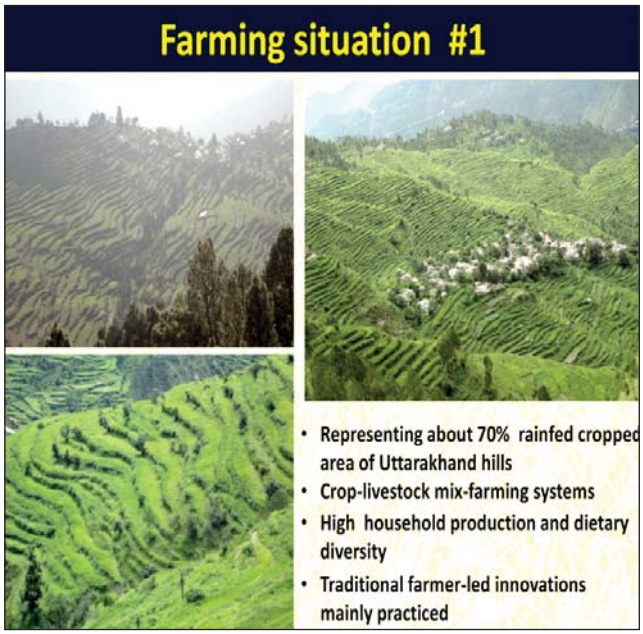

Plate-1

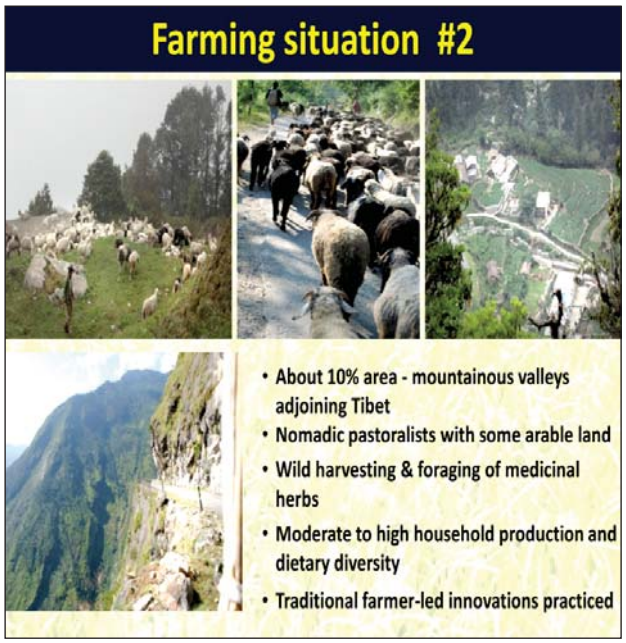

Plate-2

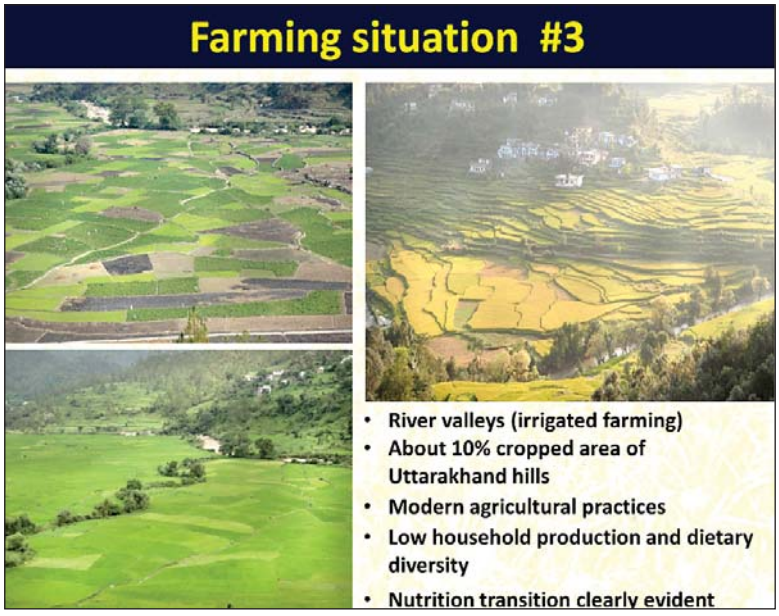

Plate-3

Plate-1 to 3: Representative farming situations in Uttarakhand hills (Sites 1-3)

foraging for income. Livestock grazing is practiced throughout the mountain valleys, although at rates significantly lower since the Indo-China conflict of 1962. Largely because the loss of trade with Tibet, demand for livestock and agriculture products as well as other professions linked to trade and agriculture including wool crafting and freight shepherding has dropped considerably.

Important crops include naked (hull-less) barley, tatary buckwheat (*Fagopyrum tataricum*), potato and many minor tubers, yams, leafy vegetables, and others. Consumption of meat (sheep and goat) is high in nomadic pastoralist societies. Cultivation and foraging of medicinal and other herbs, for export purposes in past and now for local and distant national markets viz. 'Jambu' or 'Faran'

(*Allium stracheyi*); 'Gandhrain' (*Angelica glauca*); 'Kala jeera' (*Carum carvi*); 'Chuk', Sea-buck-thorn (*Hippophae rhamnoides* ssp. *salicifolia*), etc. are common and a major economy for local communities.

Allium spp., *Dendrobenthamia capitata, Elaegnus parvifolia, Epilobium royleanum, Fagopyrum dibotrys, Fragaria nubicola, Gautheria trichophylla, Holboellia latifolia, Lonicera angustifolia, Malus baccata var. himalaica, Oxyria digyna, Paeonia emodi, Podophyllum haxandrum, Prunus* spp., *Rheum spp., Ribes* spp., *Schisandra grandiflora, Sorbus aucuparia, Taraxacum officinale, Viburnum* spp., etc. are other wild plant food resources of nomadic pastoralists commonly occurring in alpine and mountainous meadows/*bugyals*.

3. A few interspersed river valleys where improved agriculture under assured irrigation is practiced and rice-wheat or rice-potato is the predominant cropping pattern (Plate 3). Household food production and dietary diversity is very low. The livestock herding particularly rearing of goats is minimal with limited contribution of livestock to overall cash income of the households. The river valleys represent about 10% of the cropped area in Uttarakhand hills. The famous Someshwar and Garur valleys, for example, consisting of many villages have huge paddy fields as long as one can see; the valleys are pretty famous with the local for the ever-changing colours of the fields. Crossing through the valley would give an idea of how the daily life of a farmer is like in the hills; one would find more women working on the fields than men.

A nutrition transition is clearly evident in farming situations of river valleys with the emergence of cash crop economies and impact of globalization in recent years. Enhanced use of improved crop varieties from Formal Seed System (FSS), synthetic fertilizers and pesticides is commonly seen in this farming system. About 70-80% cropped area was planted by a few improved cultivars of rice, wheat and potato bred mainly by public sector institutions. Some farmers, however, still cultivate native landraces for their own household consumption. The produce of improved cultivars is generally sold in markets for cash income. Relatively reduced access to indigenous food resources in farmer households has resulted in the replacement of diets of the hitherto diversified food resources by energy-dense and nutrient-poor foods. With the nutrition transition resulting from increasing socio-economic change, the problems of overweight and underweight frequently co-exist. Socio-economic disparities and increased access to energy-dense foods are creating an "obesogenic" environment in river valley areas.

There existed a dynamic relationship among CPRs, crops and livestock of the mixed farming system in past in the hills. The same is now under increasing pressure from different sources. The livestock production systems are becoming quite dynamic in certain areas and households with accessibility to road networks and a market for milk. Farmers are being provided with a strong incentive to keep livestock; not just to fulfil the traditional role of providing draught power, milk, meat, and manure for households; but also to generate cash income through the sale of milk and meat. In certain areas, there has been a shift in management practices, with linkages to CPRs beginning to break down.

The small-scale crop-livestock traditional farming practices of the Uttarakhand hills, therefore, holds a promise for food and nutritional security of farmer households. The general ignorance of the nature and use of nutrient-rich indigenous and traditional food resources over the years has resulted in these foods being left out of most nutritional strategies put in place to address food security and nutrition problems of the local population. Awareness about enhanced use of minor millets, native pulses/legumes, leafy and underutilized vegetables, native tubers and yams, minor fruits, wild harvested foods, animal food products, etc. in daily diets, as "functional foods", can help address community health and nutrition in Uttarakhand hills.

Materials and Methods

Case Study on Farmer Household Production and Dietary Diversification

An exploratory case study, comparing the food production and dietary diversity of three representative farming situations of Uttarakhand hills, was conducted during 2015-16. The main representative target site was an agrobiodiversity rich niche village cluster in Tarikhet block of Almora district in Uttarakhand state (Fig. 1, Site 1). The study site comprised about 14 villages, 1000 farmer households and 1000 ha cropped area. The target site represented the crop-livestock small-scale mixed-farming, it being the predominant farming system of the Utarakhand hills. The two other representative

niche habitats for documenting information on household production and dietary diversity included, i) high elevation mountainous areas where long-haul pastoral/ nomadic livestock herding is practiced together with crop husbandry and cultivation, foraging and trading of medicinal herbs (Fig. 1, Site 2), and ii) river valleys where improved agriculture and crop monoculture is practiced (Fig. 1, Site 3). Information was documented from about 100 households in each of these two niche target sites representing a cluster of about 8-10 villages spread over 50-60 km^2 area.

A participatory approach was adopted for documenting information on household production and dietary diversity. Information on crop diversity, livestock diversity, food production and consumption diversity, and associated indigenous knowledge (IK) was documented. Consequent upon complete documentation of the community seed and livestock breed management systems; household production and dietary diversity; food and nutrition transition, etc., the farmer households expressed their opinion, in location-specific FGDs, on advantages/disadvantages of the existing farming systems; the pattern of change in production and dietary diversity over the years; the status of the existing indigenous knowledge in the community regarding biodiversity management and role of native diversity, including wild harvested foods in agricultural systems, in food and nutritional security of farmer households; and the way forward showcasing the potential of local food in eradicating malnutrition and addressing the nutritional security of farmer households.

The household production and dietary diversity scores were measured as per FAO (2011) and Sibhatu *et al*. (2015) with minor modifications.

An exploratory study was also conducted to relate the household production and dietary diversity with the nutrition and health of the above three *niche* target sites on the following health indicators, 1) Infant mortality rate, 2) Maternal mortality rate, 3) Level of malnutrition among children under five years, 4) Malnutrition among women of reproductive age, 5) Level of obesity among adults, 6) Level of coexistence of obesity in the adults and malnutrition in the children, 7) Formal education about malnutrition among women, 8) Incidence of communicable diseases (*e.g*. tuberculosis), and 9) Incidence of food-related non-communicable diseases (*e.g*. hypertension and diabetes).

Case Study on Wild Harvested Foods in Agricultural Systems

Data were collected through semi-structured interviews using a checklist of open ended questions. Since the data required will be mainly indigenous knowledge pertaining to wild edible plant identity and use, the free listing technique was used. In this technique, the respondents were asked to mention any plant that comes to their mind until they could not mention any more species. It is generally agreed that normally people remember plants which are important to them. Field guided walks to the farm lands, grazing lands, other open habitats and nearby forestry areas were led by the respondents to identify/collect the plants listed during the interview. One Focus Group Discussion (FGD) meeting each was held in all agrobiodiversity rich niche target sites to authenticate the data in questionnaires and capture additional responses.

The participatory interviews were done separately for men and women farmers of different age groups for local ecological knowledge (LEK) on wild harvested foods. Children (<15 yrs) were also interviewed for LEK. Data were documented from about 20 representative niche sites of three farming situations, i) traditional rain-fed farming areas (crop-livestock mix-farming, 10 niche sites), ii) mountainous regions-alpine meadows/ *bugyals* (nomadic pastoralists, 5 sites), and 3) river valleys (improved farming, 5 sites) of Uttarakhand hills (Plate 1-3). Average 10-15 farmer households were interviewed per niche site.

Results

Farmer Household Production and Dietary Diversity

A comparative study on production and dietary diversity as well as certain health indicators (Table 1 and Table 2) was performed under three different representative farming situations in Uttarakhand hills. It is evident from Table 1 that farmer household production and dietary diversity is more where traditional small scale crop-livestock mixed-farming is practiced followed by mountainous areas with a mix of nomadic pastoralism, crop husbandry, and wild harvesting and foraging of medicinal herbs, and river valleys where improved agriculture is practiced. With relatively greater per capita household income, the farmer households of river valleys has enhanced access to energy-dense foods low in nutrition leading to both malnutrition and obesity.

It is also evident from Table 2 that the problem of under-nutrition and obesity co-exist even in Himalayan highlands. With the nutrition transition resulting from increasing socio-economic change, the problems of overweight and underweight frequently co-exist even within the same village and even within the same household. Socio-economic disparities and increased access to energy-dense foods are creating an "obesogenic" environment particularly in river valleys as recorded in the present exploratory case study. In the present study we noticed that malnutrition is not always the result of food scarcity, it can also be caused by foods that are poor in essential nutrients. Lack of access to nutrient-rich food has in turn led to nutritional deformities (like stunting), which increases the likelihood of obesity later in life.

Wild Harvested Plant Food Resources in Agricultural Systems

A total of about 335 plant species have been recorded in different plant families of Angiosperms, and also Gymnosperms and Pteridophytes. A total of 97 families of dicotyledons and 12 families of monocotyledons, in Angiosperms; three families of Gymnosperms, and one family of Pteridophytes (Ferns) were represented (Table 3). Rosaceae (25), Polygonaceae (15), Fabaceae, (12), Asteraceae (11), Lamiaceae (10), Liliaceae (10), Urticaceae (10), Amaryllidaceae (Alliaceae, 8), Amaranthaceae (8), Caesalpinaceae (7), Rutaceae (7), etc. were the families with greater species representation among angiosperms.

Domestication programmes are being initiated by Uttarakhand state to bring many wild species, mainly the local herbs into farming systems. Examples of important plant species for domestication interventions include, *Allium humile, A. stracheyi, A. wallichi, Asendra butyracea, Malus baccata* var. *himalaica, Sorbus lanata, S. cuspidata*, *Fragaria nubicola*, *Rubus macilentus*, *R. ellipticus*/ *R. biflorus, R. nutans, Hippophae rhamnoides* spp. *salicifolia, Rosa sericea*/*R. macrophylla*, *Cleome viscosa* and others. These species can help provide livelihood support, health and nutritional benefits to local communities.

The Nutritional Value of Wild Foods

Malnutrition is a major health burden in many Uttarakhand hill communities/households and the recognition that nutritional security and biodiversity are linked is fundamental for enlisting policy support to secure wild food use and preserve habitats for wild

Table 1. Production and consumption pattern of major food groups in households (HH) of three different niche target sites of Uttarakhand state

Household characteristics	Agrobiodiversity rich niche site where traditional small scale crop-livestock mix-farming is practiced * (*Niche* site 1)	Niche site with nomadic pastoralism and some arable land** (*Niche* site 2)	Niche site where improved agriculture is practiced** * (*Niche* site 3)
Average per capita cash income/ annum (Rs.)/ HH (excluding home consumption of local agricultural produce)	33,259	36,425	52,124
Per cent contribution of livestock to HH cash income (without taking home consumption products into account)	29.60	34.60	5.50
Production diversity (no. of crop/livestock species produced/reared)	32.06	28.70	14.20
Food crop production diversity (no. of food crop species produced)	27.78	19.70	13.30
Food variety score (no. of food items consumed)	22.13	18.50	9.30
Food variety score only with respect to purchased foods (no. of food items consumed)	14.07	9.30	6.50
Dietary diversity score (no. of food groups consumed)	6.43	5.60	4.30
Dietary diversity score of healthy foods (no. of healthy food groups consumed)	5.49	4.50	3.40
Dietary diversity score only with respect to purchased foods (no. of healthy food groups consumed)	2.24	1.80	2.20
Food purchased from market, % of total food	29.37	21.32	18.30
Dietary diversity of wild harvested food	31.24	25.50	7.50

* The main target study site in Tarikhet block of Almora district; ** Higher Himalayan ranges (Johar valley in Pithoragarh district where nomadi cpastoralism is practiced, the herded livestock includes sheep and goats); *** Someshwar valley in Almora and adjoining Garur valley in Bageshwar district

Table 2. Comparison of some health indicators of households of three different niche target sites of Uttarakhand state (*last two decades*)

Health indicator	*Niche* site 1	*Niche* site 2	*Niche* site 3	Uttarakhand state
1. Infant mortality rate	Nil	Nil	Nil	38 /1000 (SRS 2011), 32/1000 (SRS 2013) 43/1000 (AHS 2011)
2. Maternal mortality rate	Nil	Nil	Nil	292/100000 (SRS 2010-12) 188/100000 (AHS 2011)
3. Level of malnutrition among children under five years*	Low (2%)	Low (2%)	Moderate (10%)	High (~40-50%)
4. Malnutrition among women of reproductive age**	Low (3%)	Low (4%)	Moderate (12%)	High (~40-50%)
5. Level of obesity among adults	Low (3%)	Low (5%)	Moderate (14%)	Data not available
6. Level of coexistence of obesity in the adults and malnutrition in the children	Low (2%)	Low (4%)	Moderate (8%)	Data not available
7. Formal education about malnutrition among women	Low	Low	Low	Low
8. Incidence of communicable diseases (e.g. tuberculosis)	Nil	Nil	Nil	170/100000 (State level health reports)
9. Incidence of non-communicable diseases				
• Hypertension	Low (2%)	Low (2%)	Moderate (7%)	8%
• Diabetes	Low (1%)	Low (2%)	Moderate (5%)	5.7% (MHFW-NPCDCS Survey 2010)

***Niche site1*:** Agro-biodiversity rich niche site where traditional small scale crop-livestock mix-farming is practiced
Niche site 2: Mountainous regions with nomadic pastoralism, some arable land and medicinal herb foraging
Niche site 3: River valleys where improved agriculture is practiced
*Based on three child malnutrition indicators, stunting, wasting and underweight
** Based on the concept of nutritional anaemia among women of reproductive age

edible species. Comprehensive food composition data is a critical first step. This is especially important for communities most vulnerable to malnutrition. However, understanding of wild foods' micro- and macro-nutritional properties currently lags behind that of cultivated species.

Though several studies have found that wild foods are important sources of micronutrients, their energy-density is generally low with a few exceptions. Several wild edible plants are sources of important micronutrients, Fe, Ca, P, Na, K, Zn etc. Many wild plants have edible parts that are commonly consumed and are critical suppliers of vitamins A, B_2, C, antioxidants, especially during seasonal lean periods. Many backyard plants and plants in agroforestry systems (CPRs) are important for ensuring year-round nutritional security in the face of possible food shortages, often used as famine foods. They are often superior in energy and micronutrient content compared with those from many cultivated species.

The Economic Value of Wild Foods

There is no comprehensive estimate of the economic value of wild foods. Quantitative analyses face methodological difficulties. First, case studies using different valuation methods and diverse scales are rarely comparable. Second, sale of wild products is very limited, often illegal, and therefore under-reported. Trade is often informal or occurs at local markets and is, therefore, missed by conventional accounting mechanisms.

While exact estimates of the economic value or volumes involved is difficult, what is not in dispute is that trade in and use of wild foods provide an important supplement to general incomes and are especially critical during economic hardship. In Table 4, we summarize findings from economic valuations of direct use values for wild foods in selected agro-ecologies/farming situations of Uttarakhand hills. From the limited data available, during exploratory surveys for some important species, it is clear that wild plants can provide Rs. 5,000–5,00,000 per households in specific agro-ecologies/niche areas.

An important aspect of wild food use is the relative importance of wild foods to poorer households. The conventional understanding holds that poorer households depend more on wild foods. However, detailed analyses on limited exploratory data do not show simple correlations between wealth and resource use. A range of context-specific social and economic factors (e.g. price, individual or cultural preference, and wealth) are

Table 3. Number of plant species used as wild harvested foods in Uttarakhand hills

Family	No. of species	Family	No. of species	Family	No. of species
Dicotyledons					
Acanthaceae	3	Fagaceae	2	Saurauiaceae	1
Amaranthaceae	8	Flacourteaceae	2	Saxifragaceae	2
Anacardiaceae	4	Gentianaceae	1	Schisandraceae	2
Annonaceae	1	Geraniaceae	1	Scrophulariaceae	2
Apiaceae	3	Grossulariaceae	3	Simaroubaceae	1
Apocynaceae	2	Hippocastanaceae	1	Solanaceae	3
Asclepiadaceae	5	Hydrocotylaceae	1	Sterculiaceae	1
Asteraceae	11	Juglandaceae	1	Taxaceae	1
Balsaminaceae	4	Lamiaceae	10	Tiliaceae	2
Begoniaceae	1	Lardizablaceae	1	Ulmaceae	4
Berberidaceae	1	Lauraceae	3	Urticaceae	10
Bignoniaceae	1	Loranthaceae	3	Verbenaceae	5
Bombacaceae	1	Lythraceae	1	Violaceae	4
Boraginaceae	1	Malvaceae	4	Vitaceae	3
Brassicaceae	6	Menispermaceae	2	Zygophyllaceae	1
Buxaceae	1	Molluginaceae	1	Total	**286**
Cactaceae	1	Moraceae	5		
Caesalpinaceae	7	Moringaceae	1	Monocotyledons	
Campanulaceae	1	Myricaceae	1	Amaryllidaceae (Alliaceae)	8
Cannabinaceae	1	Myrsinaceae	3	Araceae	5
Capparideceae	1	Myrtaceae	1	Comellinaceae	2
Capparifoliaceae	1	Nyctageneceae	1	Cyperaceae	3
Caryophyllaceae	3	Olacaceae	2	Dioscoraceae	5
Celastraceae	2	Oleaceae	1	Hypoxidaceae	1
Chenopodiaceae	3	Onagraceae	2	Liliaceae	10
Combretaceae	3	Oxalidaceae	1	Orchidaceae	2
Convolvulaceae	2	Paeoniaceae	1	Palmae	3
Coriariaceae	1	Phytolaccaceae	2	Poaceae	2
Cornaceae	2	Podophylaceae	1	Smilacaceae	1
Corylaceae	1	Polygonaceae	15	Zingiberaceae	2
Crassulaceae	1	Portulaceae	1	Total	**44**
Cucurbitaceae	5	Punicaceae	1		
Dillaneaceae	1	Ranunculaceae	1	Gymnosperms	
Dipsacaceae	1	Rhamnaceae	4	Ephedraceae	1
Dipterocarpaceae	1	Rosaceae	25	Pinaceae	2
Ebenaceae	1	Rubiaceae	5	Taxaceae	1
Ehretiaceae	4	Rutaceae	7	Total	4
Eleagnaceae	3	Sambucaceae	3		
Ericaceae	4	Sapindaceae	1	**Pteridophytes (**Ferns**)**	
Euphorbiaceae	5	Sapotaceae	1	Dryopteridaceae	**1**
Fabaceae	12	Sauraceae	1		
			G. Total: 335		

also relevant. In some households, consumption of wild foods increases with wealth and depends less on natural abundance than on socio-economic factors.

Drivers of Change in Wild Food Availability and Use

There are a number of important drivers for wild food availability and use. While some clearly increase or decrease the use of wild foods, the impact of others is ambiguous and context-dependent. The importance of understanding current trends for wild foods is underscored by the recognition that food insecurity is a particular problem among local communities. For instance, food insecurity was relatively more in households of crop-livestock small-scale mix farming situations followed

by nomadic pastoralists and least in households of river valleys. The interaction between drivers also deserves attention. In assessing links between local knowledge and socio-cultural continuity, we found that cultural identity and agrobiodiversity are strongly associated: 'culture and ecosystems co-evolve'. Thus, a biophysical driver (e.g. climate change) has knock-on effects on a cultural parameter (e.g. local knowledge), and the effect of the two combined could lead to either an increase or decrease in wild food use.

(a) Wild foods in a changing climate

Forecasting the precise impacts of the changing climate on the availability of wild foods is difficult. Studying resilience and vulnerability in communities of three distinct farming situations of Uttarakhand hills (Figure 1; Plate 1-3) revealed that there was insufficient evidence to predict the impacts that climate change would have on both human foraging and the interlinked processes of local ecological knowledge (LEK) transmission, cultural continuity and land-based subsistence livelihood.

Wild food species offer a potentially critical role for buffering against food stress caused by a changing climate. Nevertheless, the innate resilience of wild species to rapid climate change, which is often lacking in exotic species, means that they could play an increasingly important role during periods of low agricultural productivity associated with climate events.

(b) Land Use change and degradation

Decline in management of CPRs including expansion of intensive agriculture in river valleys and enhanced cash crop economies in some areas, limit the capacity of ecosystems to sustain food production and maintain the habitats of wild food species. Changes in land use and agriculture expansion/urbanization have significant implications for the availability of wild foods. The commercialization of agriculture-an important driver of land use change-potentially implies decreased reliance on wild foods. Agricultural and land use policy, infrastructure development and widened access to markets all drive land use change, and are implicated in declines of wild species in Uttarakhand hills.

Biodiversity in intensely managed CPRs and agroforestry systems has traditionally provided hill communities with the means to increase incomes, improve diets and increase labour productivity. Most of the wild food species used by local communities come from well managed CPRs and agroforestry systems rather than mature forests. Wild foods that accompanied CPRs are being lost, leading to decreased diversity and with it downgraded nutritional status, health and income, and the removal of a vital 'safety net' for the rural poor. In about 20 niche habitats (Table 4) surveyed in the present exploratory study, poor management of CPRs and deforestation had led to a decline in wild food species. Efforts by the local communities to stem this loss by domesticating important species were often unsuccessful, as many species do not survive outside their natural forested habitat.

Lack of sustainable intensification, will further threaten naturally biodiverse landscapes. This calls for a biodiversity-focused strategy in food, public health and poverty-alleviation policies.

(c) Unsustainable harvesting and changing dynamics

The Indian Himalayas including the Uttarakhand hills although one of the global hot-spots of biodiversity, have areas of greater malnutrition and hunger, which

Table 4. Direct use values of wild foods either as contributions to household consumption or income from sale in niche target areas of three representative farming situations (*in percentage terms*)

Farm household respondents from niche sites of different farming situations	Wild harvested foods (plant species used of the total reported in parentheses)	Other foods	Contribution of wild harvested food resources to household cash income	Major wild plant resources for household cash income from market sale
Crop-livestock small scale mix-farming systems (10 niche sites; 150 households)	8 (70)	92	14	*Myrica esculenta* (***Kaphal***); *Diplazium esculentum* (***Lingura,*** a vegetable fern); *Bauhinia variegata* (***Kuiral***, Mountain Ebony)
Mountain valleys, alpine meadows/ bugyals (5 niche sites; 70 households)	16 (40)	84	34	*Allium* spp. (***Jambu*** or ***Faran***); *Angelica glauca* (***Gandhrain***); *Carum carvi* (***Kala Jeera***)
River valleys (5 niche sites; 50 households)	2 (20)	98	1.5	*Diplazium esculentum* (***Lingura,*** a vegetable fern)

also places pressure on biodiversity for food provision. In certain niche habitats, unsustainable harvests have led to declines in wild food species.

In the long term, over-harvesting of all species especially from alpine medows/*bugyals* and higher mountain valleys will have a negative impact on wild food availability and thus on nutritional and livelihood security for those communities that rely on them. Unsustainable harvesting is regarded as a threat to wild flora in these habitats. Where species have traditionally been harvested sustainably, the entry of the market and the commercialization of species hitherto used exclusively for local subsistence can also result in over-harvesting. Unsustainable harvesting is a concern in the case of wild food resources of high elevation areas in Uttarakhand hills, more particularly "Jambu or Faran" (*Allium stracheyi* syn. *consanguineum* and *A. wallichii)* and "Gandhrain" (*Angelica glauca*), two important wild harvested commodities from alpine meadows/*bugyals* of Uttarakhand hills.

(d) Loss of local ecological knowledge

Local ecological knowledge is required for the identification and collection of species for preparation of foods with wild species. The distribution of LEK between individuals in a community is usually differentiated by gender, age or social role. In present exploratory case study, women scored higher on food-related knowledge. Data from about 20 niche sites, revealed that women above 40 years of age were able to describe the uses of 65 per cent of all edible species, while young men could only describe 15 per cent. Men and women also held specialized LEK. While men had more knowledge of hunting and fishing, women had more knowledge of wild food plants. LEK was also differentiated by age: children gathered fruit for consumption by the whole community, and unsurprisingly those under 30 had the most knowledge of wild fruits.

A significant decline was recorded in LEK where communities relied increasingly on market-bought foods having moved away from land-based livelihoods. It is thus possible that as young adults leave land-based livelihoods, knowledge transmission to younger generations will be diminished. In other cases, individuals' preferences change as they grow and thus, their stock of LEK changes, even if they remain within their community. It was observed that the grown-ups usually succumb to the culture of the society which regards the consumption of wild fruits as an inferior act. As climate change alters habitats, so knock-on effects are expected on LEK is feared.

LEK decline (in terms of species names and uses) is associated with increasing disconnection and livelihood independence from agricultural and wild systems as a consequence of modern economic growth. Our study reveals that LEK, a primary factor responsible for successful resource management, declines in association with economic growth. Therefore, in addition to the depletion of goods and services, the capacity of local communities to manage what environmental assets remain will decline in the future in association with economic growth. Hence time and money could be saved if the knowledge, experiences, and capacities of local peoples were protected and used in resource management efforts today.

(e) Socio-economic change and the expansion of markets

The nutrition transition associated with modernization of diets poses challenges to public health in hill communities. The replacement of wild foods by market-bought products is linked to reduced dietary diversity, rising rates of chronic lifestyle-related conditions such as obesity and type II diabetes, poor intake of micronutrients and malnutrition. Traditional species become undervalued and underused as exotic ones become available. Yet, the importance of wild foods to nutritional security means that they are not necessarily replaced by market-bought foods providing the same amount of calories. When more people depend solely on market-bought (cultivated foods), consuming wild foods will be marginalized.

As Uttarakhand hills have a strong food culture, traditional food systems can persist and wild foods are still prevalent enough to be considered an important part of local diets particularly in crop-livestock mix farming systems and pastoralist communities of higher Himalayan ranges.

The nutrition transition is driven by a changing climate as well as large-scale cultural changes and is expected to produce significant negative effects to physical and mental health at community level. Niche habitats where children use wild edible fruits and vegetables, level of malnutrition was low and the benefits of consuming traditional wild foods were clearly evident. Though wild foods have traditionally played a critical role in native communities, public health policy generally

operate within a model of food security that discounts the traditional food practices of the hill communities.

State of Food Sovereignty Movement in Uttarakhand

There is limited awareness about indigenous food sovereignty among farming communities of Uttarakhand hills. Indigenous food sovereignty is poorly understood conceptually by farmer households. The self-contained indigenous food systems are, however, largely dependent on landrace cultivation of native/naturalized crops, rearing of native livestock breeds and harvesting wild foods for subsistence from nearby agro-forestry/ forestry areas. Ecological food provisions maximises the contribution of ecosystems and improves resilience and adaptation of production and harvesting systems, especially important in the face of climate change.

Discussion

There has been a resurgence of interest in agricultural biodiversity within traditional food systems and the possible role these resources could play in ongoing efforts to steer populations away from carbohydrate and energy rich foods that are typical of simplified diets to more diversified diets that engender household food and nutrition security. In spite of nutrition transition trend in many other parts of Uttarakhand, it is widely acknowledged that in the representative target region rich in crop and livestock diversity and use of wild harvested foods, food traditions are still prevailing in the life of rural households to a greater extent. It is indeed heartening that the traditional food habits are still playing a significant role in contemporary food habits of the target communities, and the possibility of reversing the trends in favour of dietary diversification from dietary simplification look promising. It was found that the root cause of both malnutrition and over-nutrition/obesity is inadequate or improper nutrients. Consumption of an appropriate portion of food rich in essential nutrients can eliminate both pandemics.

The exploratory study of the household production and dietary diversity of different representative farming situations in Uttarakhand state clearly indicated that high production and dietary diversity is linked with better community health and nutrition. Growing a range of local crops supplemented by wild-harvested foods help provide much diversity in the diet in traditional farming areas. It may also be emphasized that better and balanced nutrition in the human diet depends not just on growing a diversity of crops but on the diversity within the crops (Mouille *et al*., 2010). The micronutrient superiority of landrace cultivars complemented with wild harvested food resources in traditional hill farming has been revealed by the present exploratory research findings. The past researches have shown substantial inter-varietal differences (Kennedy and Burlingame, 2003; Burlingame *et al*., 2009; Lutaladio *et al*., 2010). Intake of one variety rather than another can be the difference between micronutrient deficiency and micronutrient adequacy in traditional farming. Unfortunately, we lack detailed information about such diversity within most crops at the cultivar level and the role it plays in nutrition because of the general neglect by researchers/ professionals (Burlingame *et al*., 2009) and much of the evidence is anecdotal.

Coates *et al*. (2007) suggest dietary diversity, typically measured in the form of a count of food groups or food group frequency, as a proxy indicator for nutrient adequacy. Adequate human nutrition thus involves regular intake of a wide range of nutrients, some of which must be consumed on a frequent basis, even if in small quantities. The micronutrient superiority of some lesser-known cultivars and wild varieties over other has been confirmed by certain recent researches (Heywood, 2013).

We believe that the trend in nutrition transition can be slowed down and certain approaches are needed to move the nutrition transition in a more positive direction. Among the suggested public health promotion strategies, policies and intervention approaches (Smith, 2013) are:

- Holistic integrated food and nutrition interventions.
- Addressing under- and over-nutrition simultaneously.
- Involving communities in planning interventions using a bottom-up rather than top-down approach.
- Focusing on diversification of diets rather than a reliance on fortified foods and supplementation where possible.

Since the time of colonization, indigenous communities have witnessed a drastic decline in the health and integrity of indigenous cultures, ecosystems, social structures and knowledge systems which are integral to our ability to respond to our own needs

for adequate amounts of healthy indigenous foods. Within the larger society in which they live, despite the wealth of knowledge rural indigenous people have of their local environment and food system, they often face vulnerabilities derived from extreme poverty, discrimination and marginalization. It has been rightly argued that the emerging concept of food sovereignty emphasizes farmers' access to land, seeds, and water while focusing on local autonomy, local markets, local production-consumption cycles, energy and technological sovereignty, and community level farmer-to-farmer networks (Altieri, 2009).

The case study on household production and dietary diversity will set the stage for the future research to demonstrate how these local foods contribute to food security, nutrition and health. Our long-term objectives will be to address scientific issues, public health, and policy, with the goal of influencing local, national and international policies for environmental protection of indigenous peoples' land and food resources. In this way, communities can be encouraged to strengthen their use of local food and sustain knowledge of their local food systems for essential contributions to cultural protection, well-being and health. Local biodiversity should be recognized as a significant contribution to a sustainable agriculture–food–nutrition strategy alongside improvements in agricultural productivity and agronomic practice, nutritional enhancement of crops, industrial fortification, vitamin supplementation and other nutrition–agriculture interventions (Heywood, 2013).

Uttarakhand hills are primarily an agriculture-based society with a rich native food culture and traditions. The native food culture affects the holistic health of native communities individually and in community and cultural collectives - in recognizing continuity from the past, into the present and towards the future (Woodley *et al*., 2009; Kuhnlein *et al*., 2009). Indigenous people never separated food from medicine, depending upon which part of the plant is used, the season of the year and physiological condition of the person using the crop, the same plant can be consumed as food or medicine (Kuhnlein *et al*., 2009). Further sustainability of the environment happened to be one of the critical issues in the food acquisition and consumption of indigenous communities' world over (Demi, 2016). Industrial agriculture on the other hand is premised on a business model through neoliberal policies. The current WTO policy has worsened the plight of small-holder farmers, creating backlogs of unemployment in the Global South and widening the poverty gap between the rich and the poor in most countries.

One of the commonest criticisms of advocating a greater use of local agricultural biodiversity in the form of traditional crops, underutilized species and wild-harvested species to address under- or malnutrition is precisely that it is local and it is assumed therefore will have little impact on the global picture. Yet, at least 20 per cent of the world food supply comes from traditional multiple cropping systems, most of them small farm units often of 2 ha or less (Altieri, 2009). There is ample evidence on the ground that local biodiversity and ecosystem services play an essential role in the lives of communities throughout the developing world, by providing a social safety net for food, medicine, fibre, fuel wood etc. that can act as route out of poverty and a source of income generation, prevent people falling further into poverty or in extreme cases as an emergency lifeline through the provision of 'famine food' (Roe *et al*., 2010) [Not cited in References). It can also play a major part in addressing issues of malnutrition (Heywood, 2013).

With rich native food culture and more than 80% area under traditional farming, except a few river valleys, Uttarakhand state offers hospitable conditions for indigenous food sovereignty movement. Unlike food security, the food sovereignty has to be a process coming from the bottom up – from the peasant, from the communities (Schiavoni, 2015, 2017).

The native food culture of Uttarakhand hills can be discussed here in greater detail based on local IK in context of food viz. spirituality, food security, harvesting regulations/restrictions and reliance on locally available material as outlined by Demi (2016).

The predominantly crop-livestock small-scale mix farming systems of Uttarakhand hills encourages farmer households more on consumption of traditional crops instead of animal flesh except the nomadic pastoralists of high mountainous regions who depend relatively more on animal products. In indigenous animal husbandry of Uttarakhand hills, the livestock are mainly fed with crop by-products while substantive food is mainly reserved for human consumption. Such system saves humans from competing with livestock for food and ensuring food sufficiency.

Dependence of local communities of Uttarakhand hills on diverse plant resources including wild harvested

foods in local food culture ensures that the plant species are protected and this way an effective mechanism of sustainability that indigenous communities can employ to maintain a cosmic balance in the ecosystem could be duly showcased by the present case study research.

An important tradition of harvesting regulations/ restrictions commonly practiced in local farming communities/ food culture of Uttarakhand hills also ensures sustainability and helps control human desires which is considered an important learning in environmental education (Orr, 2004).

Use of local readily available materials, for example use of forest litter, animal waste, farm-yard manure, etc. and avoidance of synthetic fertilizers, except in river valleys where modern agricultural practices are followed, ensure that safe organic foods are produced for human consumption in Uttarakhand hills. These practices intend to improve human health and preserve the environment for future generations.

The exploratory case studies therefore suggest that the traditional food consumed by rural communities in Uttarakhand hills contain nutrients and calls to revert to the diets of our ancestors to regain lost nutrients. It is believed that such a shift would help to improve society's relationship with the Earth and restore human and environmental health. Many traditional and non-processed foods consumed by rural communities, such as millets, leafy greens, legumes, wild harvested food resources are nutrient-dense and offer healthy fatty acids, micronutrients and cleansing properties widely lacking in diets popular in high- and middle-income societies/ countries.

Indigenous diets worldwide are varied, suited to local environments, and can counter malnutrition and disease. For many indigenous communities, their food systems are complex, self-sufficient and deliver a very broad-based, nutritionally diverse diet. But the disruption of traditional lifestyles due to environmental degradation, and the introduction of processed foods, refined fats and oils, and simple carbohydrates, contributes to worsening health in native communities, and a decline in the production of nutrient-rich foodstuffs that could benefit all communities. Kuhnlein *et al*. (2009) says that "Indigenous peoples' food systems contain treasures of knowledge from long-evolved cultures and patterns of living in local ecosystems". Therefore, the traditional food systems need to be documented so that policy makers know what is at stake by ruining an ecosystem, not only for the native peoples living there, but for everyone.

Not only does food diversity have relevance in a public health and food policy sense, but also in individual counselling in clinical practice. Assessment of a patient's food variety can be rapid and semi-quantitative, encouraging small and consequential changes in diet. When ethnicity is taken into account, in the clinical setting, this process can be even more rewarding for the practitioner and patient (Wahlqvist, 2005).

The exploratory research outcome clearly indicates that malnutrition is not result of food scarcity but foods poor in essential nutrients. Although the problem of diminished food sovereignty and food insecurity is one that affects all people, not just indigenous communities, indigenous peoples are uniquely situated to offer solutions. Armed with ancient traditional knowledge and a deep connection to their lands, native communities, and particularly native women, are developing projects and building networks to revitalize local food capacity and strengthen food sovereignty.

The present study, therefore, shows that with a strong native food culture and agriculture, Uttarakhnd state provides us with a greater opportunity for food sovereignty research and activism. A recent article by Henderson (2017) argues that the articulation between the state and peasant organizations' internal structures – the class characteristics of their mass bases, their leaderships and the modes of interaction between the two – is critical for determining the nature of contemporary struggles guided by the discourse of food sovereignty. Schiavoni (2017) emphasizes on a historical, relational and interactive (HRI) framework, in context of Venezuela, that can help us to understand the crisis facing the food system, and implications for food sovereignty research and activism.

Wild harvested foods form a significant portion of the total food basket for farmer households of Uttarakhand hills. However, the focus on the contribution of wild harvested foods to total food and nutritional security is undervalued. The continued contribution of wild species to food and nutritional security is threatened by some of the processes that seek to increase agricultural production and enhance economic development. Further, the sustainable harvesting of wild economic species requires a strong policy support by ensuring its continued availability for livelihood security of local communities.

Wild harvested food species provide more than just food and income to hill communities. The hill communities with a tradition of wild food use; it is part of a living link with the land, a keystone of culture. The decline of traditional ways of life and decreased wild food use are interlinked. Research needs are twofold: (i) standardized, accessible and comparable studies on the nutritional and toxicological properties of currently used/underused wild species on a broad scale; (ii) the identification of priority areas for conservation of wild food species and the recording of food-relevant LEK. Polices on conservation, food-security and agriculture need to be integrated to recognize and preserve the importance of wild foods.

Traditional food revitalization projects aimed at increasing the consumption of wild foods, in order to provide health and cultural benefits to traditional communities otherwise subject to the nutrition transition is a necessity for hill communities. It is a well-recognized fact now that wild species and intra-species diversity have key roles in global nutrition security. The evidence shows that wild foods provide substantial health and economic benefits to those who depend on them. It is now clear that efforts to conserve biodiversity and preserve traditional food systems and farming practices need to be combined and enhanced.

It has been argued that healthy human nutrition is best achieved by an approach to agriculture that is biodiverse, providing a varied and ecologically sustainable food supply. But such an eco-nutrition model is sound in theory but very complex to achieve, as many variables are involved, in reality (Blasbalg *et al*., 2011). Such a biodiverse food-based approach should be seen as an element in an overall strategy that also includes continuing improvement of agricultural production, breeding cultivars that are more resistant to disease and stress, nutritional enhancement of crops, industrial fortification, vitamin supplementation and other nutrition- agriculture linkages (Chung *et al*., 2010).

There is therefore a necessity to broaden our approach even further and explore the linkages between agriculture, food production, nutrition, ethnobiology and ethnopharmacology and the resource base of wild and agricultural biodiversity in the context of accelerating global change. At an institutional level, both globally and nationally, these issues are very loosely (or not at all) coordinated (Heywood, 2011).

Conclusion

Nutrition improvements and health promotion interventions with native communities can be successful when they give full attention to the social context, social support, social capital and local food resources and provisioning. The exploratory research outcome offers the perspective that understanding how to use local foods to improve native communities' health benefits them directly, and also gives new insights for nutritional health promotion initiatives in general.

The authors hope that the evidence supplied from this study will stimulate others to promote traditional food systems for native communities' in their regions, and to contribute to mainstreaming food-based approaches with local resources. This issues a clear call for nutritionists and their colleagues in leadership roles in indigenous communities to experience the wide variety of unique foods and the social settings in which they are used, and to promote these important elements of local culture and ecosystems for their health benefits and their promise to provide sustainable solutions to food and nutrition security

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RESEARCH ARTICLE

Morphological Characterization for Identification of Drought Adapted Genotypes in Wheat (*Triticum aestivum* L.) under Drought Stress

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Drought is one of the major limitations to wheat production worldwide. This study was designed to identify the drought adapted genotypes among 160 wheat genotypes. Five morphological traits namely leaf morphology, leaf angle, leaf rolling, waxiness on leaf, spike fertility and nine yield traits namely days to heading, days to anthesis, days to maturity, grain filling duration, spikelets per spike, grains per spike, grain weight per spike (g), 1000-grain weight (g), and grain yield per plant (g). Grain yield per plant exhibited highly significant and positive correlation with 1000-grain weight, grain weight per spike, waxiness score, indicating dependency of yield on these traits. Based on path analysis, maximum positive direct effect on grain yield per plant was contributed mostly by days to maturity, followed by 1000 grain weight, waxiness score, number of grains per spike, grain weight per spike and leaf rolling score. From our results, waxiness score and leaf rolling score showed strong association with grain yield per plant. We have selected 18 genotypes based on high morphological score as-well-as high grain yield per plant namely; DBW50, HD2985, HD3043, HD2687, HD3059, HD3076, HD3093, HI1531, HW1105, PBN142, PBW502, WH1080, WH730, AUS30354, AUS30518, DRYSDALE, SB187, SSRT17. These genotypes can be utilized in drought breeding programme for development of mapping population, as well as drought resilience varieties for rainfed areas.

Key Words: Drought stress, Morphological traits, Wheat (*Triticum aestivum* L), Yield traits

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops across the globe. It is grown under broad range of environmental conditions in terms of water regimes, climates and soil types. At present, changes in global climate and increased variability in precipitation giving insistence to drought stress (Trenberth, 2011). Wheat production in semiarid and arid regions is increasingly constrained due to drought stresses (Gregersen *et al.*, 2013). Therefore improvement in drought tolerance as-well-as grain yield is very important in the selection of wheat cultivars for rainfed condition. The International Maize and Wheat Improvement Centre (CIMMYT) contributed to the worldwide adoption of modern wheat varieties through multi-environmental testing and collaboration with national breeding programmes (Manes *et al.*, 2012).

In wheat greater genetic variability can be explore with germplasm from its centres of origin and diversity (Dvorak *et al.*, 2011). In addition to cultivated wheat varieties and breeding lines, extensive variability for drought tolerance remains within wild relatives and landraces (Nevo and Chen, 2010; Dodig *et al.*, 2012). Manipulation of this diversity to improve drought tolerance among genotypes may be achieved through genetic modification and selection for adaptive mechanisms including drought escape, dehydration avoidance, and dehydration tolerance (Blum, 2010).

There are several approaches to investigate morphological traits for the purpose of increasing yield under moisture deficit conditions. Leaf rolling induced by loss of turgor and poor osmotic adjustment represents as an important drought avoidance mechanism (Richards, 1996). The erectophile leaf canopy has been proposed

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as a trait that could increase crop yield potential by improving radiation use efficiency in high radiation environments (Reynolds *et al.,* 1999).

Therefore, the grain yield and its contributing traits are two important selection criteria in moisture deficit conditions (Plaut *et al.,* 2004). Drought stress reduces the grain yield and an average yield loss of 17-70% has been estimated due to drought stress (Nouri-Ganbalani *et al.,* 2009). An understanding of yield components of a wheat crop in a particular environment is the key for a successful breeding program. Yield component traits, such as days to heading, days to anthesis, days to maturity, grain filling duration, spikelets per spike, grains per spike, grain weight per spike (g), 1000-grain weight (g), grain yield per plant (g) influence the tolerance to drought in wheat (Passioura, 1977).

Material and Methods

Experimental Site

The experiment was conducted in the experimental area of NE Borlaug Crop Research Centre (NEBCRC), G.B. Pant University of Agriculture and Technology. Pantnagar, Distt. US Nagar, Uttarakhand during 2014-15 and 2015-16 *Rabi* seasons. The Crop Research Centre is situated at 29ºN latitude, 79º29' E longitude and at an altitude of 243.84 m above the mean sea level.

Experimental Design

The experiment was conducted in Alpha lattice design (Patterson and Williams, 1976). The randomization of 160 cultivars was done with Crop Stat v7.2 software. The design constitutes of 8 × 20 i.e. eight blocks each of 20 genotypes, planted in rainfed (RF) condition with two replications. The each entry was planted two meter long rows, with three rows per plot. The plants were spaced 10 cm from each other and rows were 20 cm apart.

Water Deficit Environment

The experimental materials were evaluated in rainfed (RF) environment with two replications for 2 years 2014-15 and 2015-16. No irrigation was done to create the water deficit environment during the crop season.

Morphological Parameters

The data were recorded on five morphological traits namely morphology of leaves, leaf angle, leaf rolling, waxiness on leaves and spike fertility. The morphology of leaves was observed visually using 1 to 3 scale (1 = broad, 2 = medium and 3 = narrow). Leaf angle was observed visually using 1 to 3 scale (1 = droopy, 2 = semi-erect and 3 = erect). Leaf rolling of leaves was observed visually using 1 to 4 scale (1 = no rolling, 2 = weak rolling, 3 = semi-rolling and 4 = full rolling). Waxiness on leaves was observed visually using 1 to 4 scale (1 = absence, 2 = weak 3 = medium and 4 = strong). Spike fertility was also observed through hand detection using 1 to 3 scale (1 = sterile, 2 = semi-sterile, 3 = sterile) (Kundu, 2007).

Yield and Yield Components Traits

The data were recorded on nine yield and yield contributing traits namely days to heading, days to anthesis, days to maturity, grain filling duration, number of spikelets per spike, number of grains per spike, grains weight per spike (g), 1000 grain weight (g) and grain yield per plant (g).

Statistical Data Analysis

The data were subjected to analysis of variance using SAS GLM procedure release 9.3. Pearson correlation analyses were used to determine the association between morphological and yields traits. Path coefficient analyses were used for the causal relationship between these traits, with the degree of such relationship.

Results and Discussion

Leaf morphology is also associated with drought stress, plants having narrow leaves are more adapted for drought stress, because less loss of water through transpiration (Kumar *et al.*, 2016). Leaf angle is one of the most important parameters used to describe the structure of horizontal vegetation canopies of field crops, and affects how incident photosynthetically active radiation is distributed on plant leaves, thus directly affecting plant productivity (Zou *et al.,* 2014). Leaf rolling may be associated with improved grain yield under drought stress, rolled leaf loss less water through transpiration as compared to unrolled leaf (Rebetzke *et al.,* 2001). Waxiness on leaf may be well suited for drought condition because waxiness restricts transpiration in plant (Borrell *et al*., 2006). Some spike may be sterile or semi-fertile under drought stress due to metabolic imbalance (Shitsukawa *et al.,* 2009). Five morphological characters were recorded for characterization with respect to drought adaptation of wheat genotypes, the details of these traits are given below (Fig. 1a-e).

Leaf Morphology (Narrow/Medium/Broad): Leaves are the main organ for photosynthesis, providing major

assimilate source required for plant growth and panicle development. Under favourable conditions, wheat flag leaf contributes 45-58 % of photosynthate and 41-43 % of assimilates used in grain filling. Broad leaved plants had greener biomass, more chlorophyll content indicating suitable for green forage. On the other hand plants having narrow leaves become tolerant to abiotic stresses like drought, salinity, alkalinity and rainfed situation.

Leaf Angle (Erect/Semi-erect/Droopy): Leaf angle of leaves affect the extent to which a crop canopy intercepts solar radiation and light penetration through the canopy. Developmentally leaf angle is affected by temperature, light intensity, wavelength and growth regulators. The leaf erectness has been used to optimize plant architecture since erect leaves can enhance photosynthesis and dry matter production by greater sunlight capture. Brassinosteroid is a recent class of phytohormones that is related to erect leaf angle.

Leaf Rolling (Full/Semi/Weak/No-rolling): Leaf rolling may be associated with improved grain yield in some drought situations. The flag leaf of wheat plant rolls up into a cylinder in response to drought conditions. This is a desirable trait improving grain size particularly under drought. It may lead to a delay in the onset of leaf senescence and thus lead to greater water-use efficiency. The trait is mainly expressed in the flag leaf, which is one of the main organs contributing to grain yield.

Waxiness on Leaf (Strong/Medium/Weak/Absence): Epicuticular wax which imparts a bluish green cast, referred to as glaucousness has been associated with drought tolerance in several crop species. Genetic variation in epicuticular wax has an importance in breeding for drought tolerance. Genetic variation in epicuticular wax in wheat has been previously reported, but only in a limited range of genotypes. The present paper explores epicuticular waxiness in the large number genotypes and its relationship with yield and yield component traits of spring wheat cultivars.

Spike Fertility (Fertile/Semi-fertile/Sterile): Grain number per spike, which is greatly affected by floret fertility, is an important trait of wheat grain yield. Maximum floret primordia, fertile floret, and grain number per spikelet are three important factors of floret fertility. Floral degradation plays an important role in determining these three floret fertility-related traits. Spike is produced on the top of the main stem of each of the tiller that are fertile in a normal wheat cultivar, and those tillers that do not produce spike are referred to sterile tillers.

Adjusted Mean and Drought Adaptation Score: Adjusted mean of all the yield traits and adaptation score of all morphological traits given in the Table (1). For adjusted mean, we have taken from polled data of both the year 2014-15 and 2015-16, and for drought adaptation score, we have prepared based on sum of score value of all morphological traits for each genotype. We have selected 18 genotypes based on highest drought adaptation score and high grain yield per plant mentioned by bold figures (Table 1; Fig. 2).

Analysis of Variance

The data of analysis of variance (ANOVA) alpha lattice design both studied years and pooled are presented in Tables (2, 3, and 4). Results of these tables revealed that mean square of the replications had highly significantly differences for days to heading, days to anthesis, days to maturity, no. of spikelets per spike, 1000-grain weight (g) in both years and pooled data, remaining traits in the first year, second year and pooled data were non-significant. Mean square of the blocks had highly significantly differences for days to heading, days to anthesis, days to maturity, grain filling duration, grain weight per spike (g), 1000-grain weight (g), grain yield per plant (g) in the first year, days to heading, days to anthesis, days to maturity, grain filling duration, 1000-grain weight (g) in second year, days to heading, days to anthesis, days to maturity, grain filling duration, no. of spikelets per spike, no. of grains per spike, grain weight per spike (g), 1000-grains weight (g), grain yield per plant (g) in the pooled data. No. of spikelets per spike in the first year was significant only. Grain weight per spike (g) and grain yield per plant (g) in the second year were significant only. No. of grains per spike in the first year was non-significant. No. of spikelets per spike and no. of grains per spike were non-significant only in the second year. Mean square of the treatments had highly significantly differences for all the studied traits in both the years and pooled data. Mean square of replication x block were highly significant differences for days to anthesis, no. of spikelets per spike, no. of grains per spike in the first year, days to anthesis, no. of spikelets per spike, no. of grains per spike in second year, days to anthesis, grain filling duration, no. of spikelets per spike, no. of grains per spike, grain weight per spike (g) in the pooled data. Grain filling duration in the second year

Table 1. Adjusted mean of yield data and drought adaptation score of 160 wheat genotypes studied under rainfed condition

Sl. No.	Genotypes	Pedigree	DH	DA	DM	GFD	SLS	GS	GW (g)	TGW (g)	GY (g)	DTS
1	C 306	RGN/CSK3//2*C591/3/C217/N14//C281	86	92	127	34	16	43	2.41	41.17	11.74	4
2	Chirya 7	*	88	93	127	35	17	51	2.99	43.31	9.25	5
3	DBW 14	RAJ3765/PBW343	86	93	127	34	18	54	2.33	36.43	9.35	10
4	DBW 28	MILAN/PBW343	87	92	125	33	16	48	2.42	39.98	9.63	13
5	DBW 39	ATTILA/HUI	86	91	126	35	19	60	3.35	42.32	10.52	9
6	**DBW 50**	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	**87**	**92**	**126**	**34**	**19**	**57**	**2.83**	**43.07**	**12.26**	**12**
7	DBW 58	ATTILA/3*BCN//BAV92/3/TILHI	86	91	125	34	18	50	2.61	40.75	7.12	13
8	DBW 77	*	87	90	125	34	20	58	3.21	39.76	10.95	11
9	DBW 88	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	87	92	127	35	19	56	2.83	43.50	9.11	12
10	FLW 12	UP2338/Mega	85	90	125	34	18	52	1.78	40.64	7.27	7
11	FLW 13	WH542*/Yr15(CH25087)	90	94	127	33	18	52	2.21	42.04	8.02	7
12	FLW 3	UP2338/CHINA84	89	92	126	34	17	51	2.28	35.36	10.31	7
13	FLW 7	*	88	93	126	32	17	46	1.73	37.16	6.12	6
14	HALNA	HD1982/K816	84	88	124	35	19	53	2.18	41.89	12.90	8
15	HD 2643	VEE"S"/HD2407//HD2329	84	88	123	35	17	51	2.77	46.53	10.14	8
16	HD 2733	ATTILA/3/TUI/CARC//CHEN/CHTO/4/ATTILA	87	93	125	32	19	52	1.38	28.63	6.68	11
17	HD 2824	PTO-1/CNO79/PRL/GAA/3/HD1951	88	93	126	33	17	52	2.60	41.04	10.92	6
18	HD 2833	PBW226/HW1042//HD2285	82	88	123	35	18	55	2.52	35.90	8.12	6
19	HD 2864	DL509-2/DL377-8	85	91	127	36	17	47	1.84	39.79	10.45	5
20	HD 2877	CDWR9549/HD2347//HD2402	86	90	124	34	18	46	2.16	37.89	10.33	6
21	HD 2932	KAUZ/STAR//HD2643	86	93	124	32	18	53	2.38	39.19	11.39	9
22	HD 2967	ALD/COC//URES/HD2160M/HD2278	88	94	126	32	20	62	1.61	28.68	6.70	13
23	**HD 2985**	PBW343/PASTOR	86	92	125	33	19	57	2.57	41.12	12.88	11
24	HD 2987	HI1011/HD2348//MENDOS//IWP72/DL153-2	84	89	127	38	17	47	2.48	43.46	12.87	9
25	**HD 3043**	PJN/BOW//OPATA*2/3/CROC_1/Ae. squarrosa (224)//OPA	86	91	125	34	17	43	1.97	39.14	11.04	11
26	HD 2687	CPAN2009/HD2329	89	94	125	31	17	51	2.27	37.72	10.40	13
27	HD 3059	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	**88**	**93**	**127**	**34**	**18**	**55**	**2.72**	**40.81**	**11.59**	**14**
28	HD 3070	TAM 200/TUI/3/URES/JUN//KAUZ	88	91	126	35	17	49	2.10	35.38	5.15	12
29	**HD 3076**	BL2064//SW89.5124*2/FASAN/3/TILHI	**91**	**94**	**127**	**33**	**19**	**52**	**2.18**	**39.44**	**12.66**	**12**
30	HD 3086	DBW14/HD2733//HUW468	84	89	123	34	17	50	2.47	39.95	12.20	8
31	HD 3090	SFW/VAISHALI//UP2425	86	91	126	35	18	52	2.54	39.71	7.74	6
32	HD 3091	PICUS/3/KAUZ*2/BOW//KAUZ/4/TILHI	84	91	125	35	15	38	1.71	34.96	9.25	7
33	**HD 3093**	NW1012/HUW453	**88**	**91**	**127**	**36**	**16**	**44**	**2.35**	**41.68**	**11.07**	**11**
34	HD 3118	ATTILA*2/PBW65//WBLL1*2/TUKURU	86	90	125	35	16	51	2.91	38.74	11.31	10
35	HD 3121	MILAN//PRL/2*PASTOR/4/CROC_1/Ae. Squarrosa(213)//PGO/3/BAV92	85	90	125	35	18	54	2.78	40.27	10.72	10
36	HD 3122	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1	86	90	124	34	18	47	3.56	45.17	12.84	11
37	HD 3123	PASTOR//HXL7573/2*BAU/3/CMH82.575/CMH82.801	88	92	125	33	17	47	1.91	37.95	9.46	13
38	HI 1500	HW2002*2//STREMPALLI/PNC 5	86	91	128	37	18	53	3.10	41.11	12.20	6
39	**HI 1531**	HI1182/CPAN1990	88	93	**125**	**32**	**18**	**56**	**2.61**	**35.06**	**12.38**	**12**
40	HI 1544	HINDI 62/BOBWHITE/CPAN 2099	86	92	128	36	16	42	2.11	44.54	10.07	9
41	HI 1563	MACS 2496*2/MC10	84	89	127	38	18	53	2.69	41.08	7.62	4
42	HI 617	SELECTION FROM C 306	85	91	127	36	16	46	2.81	43.49	8.11	6
43	HUW 510	HD 2278/HUW234//DL230-16	85	89	126	37	17	52	2.12	35.89	8.84	7
44	HW 1105	C 306 *7//TR 380-14 #7/3AG14	**87**	**92**	**127**	**34**	**18**	**45**	**1.90**	**39.04**	**11.25**	**11**
45	HW 2004	*	87	91	126	35	18	52	2.73	46.25	10.46	6
46	HW 2005	*	86	91	126	35	16	48	1.67	35.13	9.49	6
47	HW 2009	*	80	86	123	37	17	50	2.24	31.13	6.57	8
48	HW 2036	*	86	91	126	35	18	55	2.83	41.66	10.09	10
49	HW 2039	*	83	87	125	38	16	49	2.28	41.02	11.09	6

Sl. No.	Genotypes	Pedigree	DH	DA	DM	GFD	SLS	GS	GW (g)	TGW (g)	GY (g)	DTS
50	HW 2066	MACS 2496*2//COOK*6/C80-1	86	91	125	34	18	56	2.62	28.84	6.92	11
51	HW 3620	*	88	91	125	34	17	49	1.89	30.42	6.00	5
52	HW 4002	*	82	88	127	40	17	49	2.77	43.24	10.58	5
53	HW 4008	*	82	87	123	37	16	49	2.04	40.86	8.96	6
54	HW 4009	HD2285*5/THATCHER*8/VPM	87	92	126	34	17	47	1.98	33.31	6.58	6
55	HW 4022	*	85	90	124	34	16	46	2.37	35.62	9.20	4
56	HW 4029	*	80	86	123	38	15	48	2.10	38.13	5.61	6
57	HW 4202	*	87	92	125	33	17	49	1.61	25.41	6.94	8
58	HW 4209	*	87	92	125	33	15	48	1.95	34.98	9.57	9
59	HW 4213	*	86	90	127	37	17	47	1.86	34.62	10.51	5
60	HW 4215	*	87	92	126	34	19	52	2.72	43.86	12.14	8
61	HW 4218	*	88	93	127	34	18	53	2.53	41.45	11.39	5
62	HW 4219	*	89	93	126	33	18	46	1.94	31.83	6.14	10
63	HW 5209	C 306 *7//TR 380-14 #7/3AG14	85	91	124	33	17	48	2.49	42.39	10.00	5
64	K 1016	PBW373/UP2338	81	92	121	29	17	53	2.49	40.17	8.27	12
65	Lok 1	S308/S331	85	88	126	38	15	44	2.08	47.97	12.75	6
66	Lok 45	CPAN 3066/K.SONA "S"/LOK 1/CNO 79/CPAN2081/J 24/SS-1063/CPAN 1907/CC 493//HD 2385	82	88	121	34	19	56	2.98	39.26	4.87	4
67	Lok 65	Lok1/J.24/SONALIKA"S"//HW2006/HD2358/HW2002	87	92	128	35	18	52	2.95	46.86	11.13	6
68	MACS 2496	SERI"S"	89	93	128	35	17	50	3.25	43.64	11.55	5
69	NI 5439	REMP80/3*NP710	87	93	125	32	18	54	2.46	33.43	6.35	9
70	NP 846	NP760/RIONEGRO	90	95	128	33	18	47	1.83	35.34	6.69	5
71	NW 1014	HAHN "S"	85	91	125	34	19	60	2.76	41.05	12.28	10
72	NW 2036	BOW/CROW/BUC/PVN	84	90	123	33	17	50	3.00	41.89	11.99	5
73	**PBN 142**	HD2189/NI917//AGANTHA	**87**	**92**	**125**	**33**	**18**	**52**	**2.39**	**39.20**	**10.55**	**14**
74	PBN 51	BUC'S'/FLK'S'	87	91	126	34	16	46	2.08	29.22	4.12	6
75	PBW 175	HD2160/WG1025	82	88	121	34	18	51	2.75	37.34	7.41	4
76	PBW 343	ND/VG9144//KAL/BB/3/YCO"S"/4/VEE#S "S"	90	95	126	31	18	53	2.37	35.84	10.06	10
77	PBW 373	ND/VG9144//KAL/BB/3/YCO"S"/4/VEE#5 "S"	87	91	125	34	16	51	2.25	35.90	9.50	9
78	**PBW 502**	W 485/PBW343//RAJ1482	**83**	**89**	**123**	**34**	**16**	**50**	**2.60**	**38.60**	**10.51**	**12**
79	PBW 550	WH 594/RAJ 3858//W485	85	89	126	37	17	53	2.52	37.04	10.55	8
80	RAJ 3765	HD2402/VL639	85	90	126	36	17	47	1.94	38.41	12.60	7
81	RAJ 4037	DL788-2/RAJ3717	87	91	124	33	15	42	1.94	35.43	6.37	6
82	RAJ 4083	PBW343/UP2442//WR258/UP2425	88	93	127	34	18	50	2.12	36.75	9.78	7
83	RAJ 4120	PBW343/V1	85	91	126	34	17	52	2.63	37.67	8.49	13
84	TEPOKO	*	87	91	125	34	16	48	2.57	34.65	7.66	7
85	UP 2691	UP2377/HW1085	90	95	127	32	18	49	2.54	44.80	10.21	9
86	UP 2828	CH01 X M95/ HUW562	87	91	124	33	19	53	2.33	40.45	9.22	11
87	WH 1021	NYOT95/SONAK	86	92	126	34	19	54	2.87	39.86	6.14	9
88	**WH 1080**	21STSAWSN151	87	92	127	35	17	**48**	**2.78**	**39.63**	**11.20**	**12**
89	WH 147	E 4870/C286/C273/4/S339/PV18	87	90	123	33	17	52	2.53	32.18	5.52	4
90	WH 157	NP876/S308//CNO/8156	88	93	127	34	19	47	2.05	42.07	7.20	8
91	WH 542	JUP /BJY"S"//URES	88	93	124	31	20	57	2.20	30.66	6.73	13
92	WH 711	ALD 'S' HUAC//HD2285/3/HFW-17	85	89	124	35	17	47	1.75	36.87	9.45	8
93	**WH 730**	*	**82**	**87**	**126**	**39**	**18**	**52**	**2.87**	**41.60**	**12.81**	**11**
94	ATTILA	CM85836-50Y-0M-0Y-3M-0Y	93	96	134	39	18	48	2.01	38.17	5.13	6
95	**AUS30354**	CMSS96Y02555S-040Y-020M-050SY-020SY-21M-0Y-0AUS	**90**	**93**	**127**	**34**	**19**	**51**	**2.16**	**45.01**	**10.44**	**13**
96	AUS30355	CMSS96Y02555S-040Y-020M-050SY-020SY-34M-0Y-0AUS	87	92	125	33	17	47	2.21	46.68	9.61	12
97	**AUS30518**	CMSA00M00114S-3M-2Y-0AUS	87	92	127	34	18	55	3.10	39.73	**10.43**	**12**
98	AUS30523	CMSA00M00114S-39M-3Y-0AUS	88	92	125	33	18	48	2.08	31.18	8.10	6
99	BABAX	CM92066	87	91	123	32	19	55	2.41	34.88	5.21	13
100	BACANORA 88	*	87	92	126	34	19	57	2.65	33.16	7.20	14

Sl. No.	Genotypes	Pedigree	DH	DA	DM	GFD	SLS	GS	GW (g)	TGW (g)	GY (g)	DTS
101	BARKARE	*	87	92	126	34	17	50	2.80	38.44	7.65	8
102	BAVIACORA M 92	CM92066-J-0Y-0M-0Y-4M-0Y-0MEX-48BBB-0Y	83	88	124	36	17	50	1.78	32.10	7.58	6
103	BAW898	CM92354-33M-0Y-0M-6Y-0B-0BGD-0Y	82	88	122	35	19	51	2.35	38.32	6.32	12
104	BERKUT	CMSS96M05638T	85	90	125	35	17	50	2.20	36.47	9.84	10
105	BWL 0814	*	87	92	126	34	15	47	2.12	36.53	10.81	8
106	BWL 0924	*	87	91	125	34	17	41	1.74	36.97	7.28	9
107	BWL 1771	*	86	88	125	37	19	54	2.17	39.65	10.32	6
108	BWL 1793	*	84	91	124	33	16	42	1.73	36.22	9.61	6
109	BWL 9022	*	88	94	126	31	19	44	1.30	35.64	7.69	12
110	CETTIA	CM92313-19Y-0H-0SY-5M-0RES-0HUA-0Y	87	92	126	34	17	50	2.23	34.40	10.05	6
111	DHARWAR DRY	*	87	93	127	34	21	59	2.66	40.01	12.89	7
112	**DRYSDALE**	-0AUS	**86**	**91**	**126**	**35**	**18**	**53**	**2.41**	**41.11**	**12.93**	**11**
113	EXCALIBUR	-DH_E165-0Y	89	94	127	33	19	50	2.00	33.49	7.21	6
114	GLADIUS	-0AUS	88	93	127	34	16	45	1.51	37.86	8.73	10
115	GRANERO INTA	CM49641-9Y-1M-2Y-3Y-0M-1P-0P-0ARG-0Y	87	90	126	36	18	52	2.24	35.76	8.01	12
116	HARTOG	CM8399-D-4M-4Y-2M-2Y-0M	86	89	125	36	16	38	1.79	30.00	9.29	6
117	IC 252803 CK9	*	87	92	125	32	18	52	2.38	40.89	8.82	5
118	IC 532653	*	95	98	131	33	19	53	2.40	32.69	7.60	7
119	IEPACA RABBE	*	89	92	126	34	19	57	3.07	38.88	11.57	8
120	JANZ	QT3685	88	93	126	34	18	48	1.75	30.44	7.57	5
121	KRICHAUFF	-0AUS	87	91	124	33	15	45	2.49	36.20	11.25	7
122	KUKRI	-0Y-0Y	89	93	127	34	18	49	2.30	35.04	8.49	6
123	LOVE-HH-129	-0Y	87	91	125	35	16	48	1.95	39.09	9.87	12
124	MACS 6272	*	88	93	127	34	18	52	2.23	33.68	9.45	14
125	MACS 6273	*	86	90	125	35	16	45	2.15	38.64	10.42	5
126	NACOZARI F 76	CM5287-J-1Y-2M-2Y-3M-0Y-0MEX-0Y	89	94	126	32	19	58	2.26	33.41	9.95	11
127	OTHERY EGYPT	*	88	93	128	35	16	48	1.85	42.06	8.63	5
128	PASTOR	CM85295	85	89	125	35	19	57	2.87	29.77	9.01	8
129	RAC875	-0Y-0Y	87	92	125	33	18	53	2.62	39.03	9.92	13
130	SB003	CMSS96Y04084S-0Y-1B-4TLA-0B-0Y-3B-0Y-0AUS	88	94	125	31	16	46	1.65	32.89	8.71	6
131	SB010	CMSS96Y04084S-0Y-1B-11TLA-0B-0Y-10B-0Y-0AUS	90	94	130	35	17	41	1.27	35.99	8.97	5
132	SB025	CMSS96Y04084S-0Y-1B-31TLA-0B-0Y-25B-0Y-0AUS	88	91	125	33	18	46	1.91	39.35	9.75	6
133	SB044	CMSS96Y04084S-0Y-1B-52TLA-0B-0Y-44B-0Y-0AUS	86	91	126	35	18	48	1.34	27.63	9.66	6
134	SB053	CMSS96Y04084S-0Y-1B-52TLA-0B-0Y-53B-0Y-0AUS	87	93	124	31	19	52	1.84	37.84	8.29	8
135	SB057	CMSS96Y04084S-0Y-1B-67TLA-0B-0Y-57B-0Y-0AUS	85	90	125	34	19	52	2.45	37.97	8.04	11
136	SB062	CMSS96Y04084S-0Y-1B-72TLA-0B-0Y-62B-0Y-0AUS	87	90	124	34	18	55	2.49	33.79	7.67	8
137	SB069	CMSS96Y04084S-0Y-1B-80TLA-0B-0Y-69B-0AUS	86	90	128	38	17	42	1.70	37.34	5.50	4
138	SB109	CMSS96Y04084S-0Y-1B-93TLA-0B-0Y-109B-0Y-0AUS	89	94	125	31	18	52	2.64	41.53	7.71	10
139	SB165	CMSS96Y04051S-0Y-1B-16TLA-0B-0Y-5B-0Y-0AUS	88	92	124	33	18	50	2.71	36.98	5.58	11
140	SB169	CMSS96Y04051S-0Y-1B-22TLA-0B-0Y-9B-0Y-0AUS	88	93	126	34	19	53	2.49	33.62	8.73	8
141	**SB187**	CMSS96Y04051S-0Y-1B-52TLA-0B-0Y-27B-0Y-0AUS	**85**	**90**	**125**	**34**	**19**	**49**	**2.75**	**36.74**	**10.54**	**13**
142	SERI M 82	CM33027-F-15M-500Y-0M-87B-0Y-0MEX	85	92	126	34	17	50	2.12	31.94	9.73	12
143	SILVERSTAR	*	86	90	125	35	18	52	2.10	32.04	10.00	9
144	SITTELLA	*	87	92	126	33	18	47	1.56	32.73	4.61	9
145	SOKOLL	CMSS97M00316S-0P20M-0P20Y-60M-010Y	92	95	129	35	20	50	1.78	38.07	4.20	5
146	SSRT02	*	85	90	125	35	20	56	2.33	34.76	7.35	10
147	SSRT09	*	83	90	123	33	18	48	1.49	29.68	4.25	6
148	SSRT14	*	81	87	124	37	16	47	1.67	35.09	5.41	6
149	SSRT16	*	89	92	126	34	18	53	2.77	38.75	6.62	9
150	**SSRT17**	*	**87**	**90**	**126**	**36**	**19**	**55**	**3.02**	**36.25**	**10.17**	**12**
151	SSRT65	*	87	93	126	34	18	54	2.79	37.21	8.01	11

Sl. No.	Genotypes	Pedigree	DH	DA	DM	GFD	SLS	GS	GW (g)	TGW (g)	GY (g)	DTS
152	SSRW35	*	88	91	125	34	17	63	3.31	41.96	9.70	9
153	SSRW47	*	83	88	120	32	17	48	1.99	31.14	6.51	5
154	TACUPETO F2001	CGSS95B00016F-099Y-099B-099Y-099B-15Y-0B-0MEX	89	93	124	31	19	55	2.30	35.59	5.56	9
155	VJ01	*	84	89	123	34	18	47	2.04	37.73	8.35	12
156	VJ10	*	88	92	126	34	19	54	3.25	42.34	11.42	10
157	VJ30	*	82	87	120	33	17	42	1.83	33.44	5.87	6
158	VJ99	*	85	90	129	39	18	40	2.09	39.32	6.83	4
159	VOROBEY	CMSS96Y02555S-040Y-020M-050SY-020SY-34M-0Y	90	95	127	33	19	54	2.06	30.62	7.71	11
160	WYALKATCHEM	-0AUS	88	92	128	36	17	46	1.74	30.04	4.11	8

*= Pedigrees not available

DH=Days to heading, DA=Days to anthesis, DM=Days to maturity, GFD=Grain filling duration, SLS=no. of spikelets per main spike, GS=no. of grains per spike, GW=Grains weight per spike, TGW=Thousand grain weight, GY=Grain yield per plant, DTS=Drought tolerance score

Table 2. Analysis of variance of different yield traits studied in wheat genotypes during 2014-15 under rainfed condition

Sources of variation	d.f.	DH	DA	DM	GFD	SLS	GS	GW (g)	TGW (g)	GY (g)
Replication	1	87.15^{**}	52.81^{**}	28.80^{**}	3.61^{ns}	80.00^{**}	2.62^{ns}	0.01^{ns}	206.08^{**}	0.26^{ns}
Blocks	7	49.46^{**}	22.22^{**}	10.41^{**}	11.67^{**}	3.47^{*}	54.22^{ns}	0.58^{**}	52.71^{**}	3.79^{**}
Treatments	159	10.36^{**}	7.51^{**}	5.60^{**}	5.44^{**}	2.47^{**}	36.14^{*}	0.40^{**}	36.35^{**}	9.63^{**}
Rep. x Blocks	7	3.28^{ns}	3.88^{ns}	1.55^{ns}	3.94^{ns}	4.10^{**}	83.88^{**}	0.22^{ns}	2.93^{ns}	0.70^{ns}
Error	145	2.74	2.23	2.33	2.83	1.45	26.27	0.14	10.12	1.10

** = Significant at 1 % level, * =Significant at 5 % probability level, ns = Non-Significant

Table 3. Analysis of variance of different yield traits studied in wheat genotypes during 2015-16 under rainfed condition

Sources of variation	d.f.	DH	DA	DM	GFD	SLS	GS	GW (g)	TGW (g)	GY (g)
Replication	1	29.40^{**}	69.37^{**}	75.07^{**}	0.11^{ns}	72.20^{**}	3.20^{ns}	0.12^{ns}	223.22^{**}	2.02^{ns}
Blocks	7	48.96^{**}	26.94^{**}	16.16^{**}	13.28^{**}	2.30^{ns}	56.30^{ns}	0.41^{*}	72.11^{**}	2.39^{*}
Treatments	159	10.99^{**}	8.57^{**}	7.25^{**}	6.04^{**}	2.77^{**}	38.21^{*}	0.39^{**}	41.99^{**}	10.35^{**}
Rep. x Blocks	7	3.15^{ns}	6.25^{**}	3.46^{ns}	6.51^{*}	4.18^{**}		0.26^{ns}	2.37^{ns}	0.39^{ns}
Error	145	2.51	2.21	2.48	2.53	1.43	27.94	0.17	9.40	1.02

** = Significant at 1 % level, * =Significant at 5 % probability level, ns = Non-Significant

Table 4: Pooled analysis of variance of different yield traits studied in wheat genotypes during 2014-15 and 2015-16 under rainfed condition

Sources of variation	d.f.	DH	DA	DM	GFD	SLS	GS	GW (g)	TGW (g)	GY (g)
Year	1	697.22^{**}	741.75^{**}	1758.93^{**}	216.22^{**}	72.90^{**}	690.97^{**}	10.52^{**}	179.73^{**}	86.17^{**}
Replication	1	108.90^{**}	121.62^{**}	98.43^{**}	1.22^{ns}	152.10^{**}	5.81^{ns}	0.02^{ns}	429.14^{**}	0.40^{ns}
Blocks	7	98.02^{**}	48.78^{**}	26.06^{**}	24.01^{**}	4.24^{**}	109.15^{**}	0.96^{**}	122.76^{**}	5.93^{**}
Treatments	159	20.92^{**}	15.88^{**}	12.49^{**}	10.87^{**}	5.10^{**}	74.01^{**}	0.78^{**}	75.78^{**}	19.84^{**}
Rep. x Blocks	7	6.10^{*}	9.83^{**}	4.54^{ns}	10.15^{**}	8.06^{**}	188.13^{**}	0.46^{**}	3.16^{ns}	1.02^{ns}
Year x Treat.	159	0.43^{ns}	0.21^{ns}	0.35^{ns}	0.61^{ns}	0.17^{ns}	0.36^{ns}	0.01^{ns}	2.56^{ns}	0.14^{ns}
Error	305	2.54	2.13	2.32	2.58	1.39	25.80	0.15	9.37	1.02

** = Significant at 1 % level, * =Significant at 5 % probability level, ns = Non-Significant

and days to heading in the pooled data were significant only, remaining traits in the first year, second year and pooled data were non-significant. Mean square of the year had highly significantly differences for all the traits in pooled data. Mean square of the year × treatment had non-significantly differences for all the traits in pooled data. These results indicated that considerable amount of genetic variation present in this material. These results are in agreement with those obtained by (Zarea-Fizabady and Ghodsi, 2004; Sajjad *et al.,* 2011; Abd EI-Mohsen and Abo-Hegazy, 2013).

Simple Correlation Coefficient Analysis: Correlation analysis is widely used in statistical evaluations and it shows efficiency of relationship between two variables. Correlation coefficient in the present study was estimated by taking the pooled mean of quantitative traits and correlated with the scores of morphological traits. According to the data presented in Table 5, grain yield per plant exhibited highly significant and positive correlation with 1000 grain weight (0.536), grain weight per spike (0.391), waxiness score (0.247), indicating dependency of yield on these traits. Other traits including, No. of grains per spike (0.131), grain filling duration (0.127), leaf rolling score (0.115), days to maturity (0.102), leaf angle score (0.086), spike fertility score (0.017), showed non-significant positive correlation with grain yield per plant; and days to anthesis (-0.051), No. of spikelets per spike (-0.043), days to heading (-0.039), leaf morphology score (-0.017) showed non-significant negative correlation with grain yield per plant, indicating that Non-dependency of yield on these traits. The results of present investigation show that waxiness score and leaf rolling score have strong association with grain yield per plant. The findings are in agreement with the results of (Rebetzke *et al.,* 2001; Sirault, 2007)

Path Coefficient Analysis: The knowledge of correlation alone is often misleading as the correlation observed may not be always true. Two characters might be show correlation just because they are correlated with a common third one. In such cases, it is necessary to use a method by which we can show the causal relationship between the variables, with the degree of such relationship. Path coefficient analysis measure the direct influence of one variable upon the other, and separate the correlation coefficients into components of direct and indirect effects. Portion of total correlation into direct and indirect effects provide actual information on contribution of characters and thus form the basis for selection to improve the grain yield. The results of correlation coefficient was partitioned into direct and indirect effects through various yield contributing characters. The estimates of direct and indirect effects of the thirteen attributes on grain yield are presented in Table (6).

Based on path analysis, maximum positive direct effect on grain yield per plant was contributed mostly by days to maturity (0.603), followed by 1000 grain weight (0.439), waxiness score (0.179), No. of grains per spike (0.147), grain weight per spike (0.094) and leaf rolling score (0.045). This means that a slight increase in one of these traits may directly contribute to grain yield. Similar results were reported by (Dhonde, 2000; Satya, 2002; Khan and Dar, 2010). The positive direct effects of spikes number, grains number and thousand-kernel weight were previously reported in wheat (Fellahi, 2013; Pirdashti, 2012). On the other hand, the maximum negative direct effect was exhibited by days to anthesis (-0.577), followed by grain filling duration (-0.483), No. of spikelet per spike (-0.201), days to heading (-0.050), leaf angle score (-0.049), spike fertility score (-0.035) and leaf morphology score (-0.007). Residual effects (0.078) indicated that thirteen characters included in this study explained high percentage of variation in grain yield.

The effect of leaf angle on the canopy-reflected spectrum have an importance, cannot be ignored in the inversion of leaf area index. Leaf angle affects light interception (Utsugi, 1999) which influences canopy reflectance (Roberts). Leaf rolling may be associated with improved grain yield in some drought conditions. Evidence of varietal differences for leaf rolling in wheat (*Triticum aestivum* L.) has been reported (Rebetzke *et al.,* 2001) but studies investigating the amount and nature of genotypic variation in leaf rolling of wheat are rare (Sirault, 2007). Leaf waxes are composed of cutins that form the cuticular matrix and waxes (Samuels *et al.,* 2008). The waxes embedded in the matrix are known as intracuticular wax, while waxes deposited on the leaf surface are known as epicuticular waxes, also referred to as glaucousness. Ishag (2003) suggested that the leaf waxes may reduce heat input, thereby lowering leaf temperatures. Leaf waxiness or glaucousness has been associated with cooler canopies under water limiting conditions (Bennett *et al.,* 2012). Grain number per spike, which is greatly influenced by floret fertility, is an important trait of wheat (*Triticum aestivum* L.) yield. Maximum floret primordia, fertile floret, and final grain number per spikelet are three crucial factors of floret fertility. Floral degradation plays a critical role in determining these three floret fertility-related traits (Guo and Schnurbusch, 2015). The results of present investigation showed that waxiness score and leaf rolling score have strong association with grain yield per plant. We have selected 18 genotypes based on high morphological score as-well-as high grain yield per plant namely; DBW50, HD2985, HD3043, HD2687, HD3059, HD3076, HD3093, HI1531, HW1105, PBN142,

Table 5. Simple correlation coefficients analysis between different morphological and yield traits studied under rainfed condition

Characters	LM	LA	LR	WX	SF	DH	DA	DM	GFD	SLS	GS	GW (g)	TGW (g)	GY (g)
Leaf morphology score	**1.000**	0.300**	0.418**	0.205*	-0.090	-0.001	0.094	-0.136	-0.246**	0.145	0.145	-0.081	-0.052	-0.017
Leaf angle score		**1.000**	0.484**	0.475**	-0.091	0.152	0.176*	0.041	-0.190*	0.200*	0.246**	0.111	0.028	0.086
Leaf rolling score			**1.000**	0.608**	0.000	0.057	0.113	-0.131	-0.263**	0.235**	0.290**	0.200*	-0.016	0.115
Waxiness score				**1.000**	0.013	0.187*	0.198*	0.034	-0.225**	0.306**	0.356**	0.259**	0.100	0.247**
Spike fertility score					**1.000**	-0.015	-0.069	-0.077	-0.001	0.165*	0.236**	0.186*	0.070	0.017
Days to heading						**1.000**	0.874**	0.642**	-0.359**	0.271**	0.094	-0.061	-0.064	-0.039
Days to anthesis							**1.000**	0.588**	-0.544**	0.282**	0.116	-0.096	-0.075	-0.051
Days to maturity								**1.000**	0.325**	0.111	-0.069	-0.047	0.143	0.102
Grain filling duration									**1.000**	-0.228*	-0.194*	0.073	0.227*	0.127
No. of spikelets per spike										**1.000**	0.642**	0.238**	-0.021	-0.043
No. of grains per spike											**1.000**	0.563**	0.054	0.131
Grain weight per spike (g)												**1.000**	0.506**	0.391**
1000-Grains weight (g)													**1.000**	0.536**
Grain yield per plant (g)														1.000

** = Significant at 1 % probability level, r=0.230, * = Significant at 5 % probability level, r=0.164

Table 6. Direct and indirect effects of different morphological and yield contributing traits on grain yield under rainfed condition

Characters	LM	LA	LR	WX	SF	DH	DA	DM	GFD	SLS	GS	GW (g)	TGW (g)	GY (g)
Leaf morphology score	**-0.007**	-0.015	0.019	0.037	0.003	0.000	-0.054	-0.082	0.119	-0.029	0.021	-0.008	-0.023	-0.017
Leaf angle score	-0.002	**-0.049**	0.022	0.085	0.003	-0.008	-0.102	0.025	0.092	-0.040	0.036	0.010	0.012	0.086
Leaf rolling score	-0.003	-0.024	**0.045**	0.109	0.000	-0.003	-0.065	-0.079	0.127	-0.047	0.043	0.019	-0.007	0.115
Waxiness score	-0.001	-0.023	0.028	**0.179**	0.000	-0.009	-0.114	0.020	0.109	-0.061	0.052	0.024	0.044	0.247**
Spike fertility score	0.001	0.004	0.000	0.002	**-0.035**	0.001	0.040	-0.047	0.000	-0.033	0.035	0.018	0.031	0.017
Days to heading	0.000	-0.007	0.003	0.033	0.001	**-0.050**	-0.504	0.387	0.173	-0.054	0.014	-0.006	-0.028	-0.039
Days to anthesis	-0.001	-0.009	0.005	0.036	0.002	-0.044	**-0.577**	0.355	0.263	-0.057	0.017	-0.009	-0.033	-0.051
Days to maturity	0.001	-0.002	-0.006	0.006	0.003	-0.032	-0.339	**0.603**	-0.157	-0.022	-0.010	-0.004	0.063	0.102
Grain filling duration	0.002	0.009	-0.012	-0.040	0.000	0.018	0.314	0.196	**-0.483**	0.046	-0.029	0.007	0.100	0.127
No. of spikelets per spike	-0.001	-0.010	0.011	0.055	-0.006	-0.014	-0.163	0.067	0.110	**-0.201**	0.094	0.022	-0.009	-0.043
No. of grains per spike	-0.001	-0.012	0.013	0.064	-0.008	-0.005	-0.067	-0.041	0.094	-0.129	**0.147**	0.053	0.024	0.131
Grain weight per spike (g)	0.001	-0.005	0.009	0.046	-0.006	0.003	0.056	-0.028	-0.035	-0.048	0.083	**0.094**	0.222	0.391**
1000-Grains weight (g)	0.000	-0.001	-0.001	0.018	-0.002	0.003	0.043	0.086	-0.110	0.004	0.008	0.048	**0.439**	0.536**

LM=Leaf morphology score, LA=Leaf angle score, LA=Leaf rolling score, WX=Waxiness score, SF=Spike fertility score, DH=Days to heading, DA=Days to anthesis, DM=Days to maturity, GFD=Grain filling duration, SLS=no. of spikelets per main spike, GS=no. of grains per spike, GW=Grains weight per spike (g), TGW=Thousand grain weight (g), GY=Grain yield per plant (g)

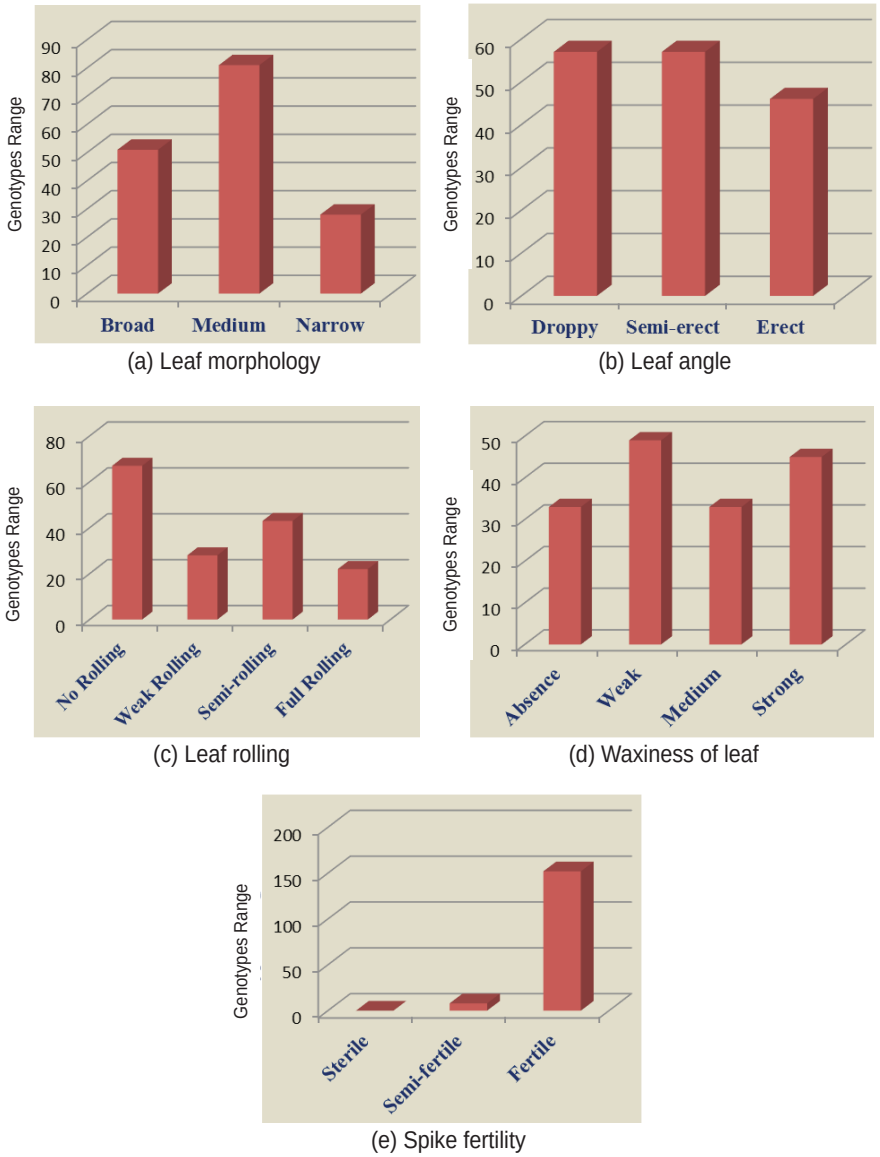

Fig. 1a-e. Frequency histograms of morphological characters recorded in 160 wheat genotypes studied under rainfed conditions.

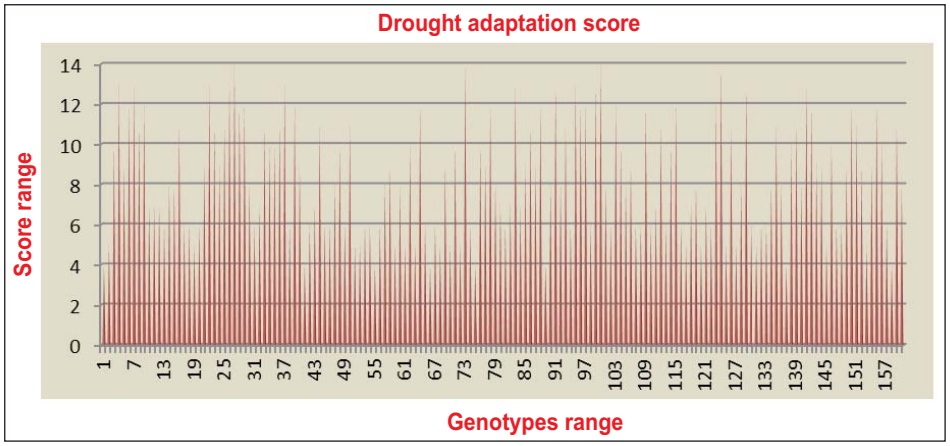

Fig. 2. Drought adaptation score studied in 160 wheat genotypes evaluated under rainfed condition.

PBW502, WH1080, WH730, AUS30354, AUS30518, DRYSDALE, SB187, SSRT17. These genotypes can be utilized in drought breeding programme for development of mapping population, as well as drought resilience varieties for rainfed areas.

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RESEARCH ARTICLE

Evaluation of Hot Pepper Germplasm for Multiple Disease Resistance against Root Knot Nematode and Viruses

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One hundred forty genotypes of hot pepper were screened for resistance against root knot nematode *Meloidogyne incognita* and two viruses *viz*, *Tomato leaf curl Joydebpur virus* (ToLCJV) and *Pepper mottle virus* (PepMov) prevalent under Punjab conditions. Artificial screening was done separately against each pathogen under controlled conditions. In case of *Tomato leaf curl Joydebpur virus* the resistant lines were further confirmed for presence of the virus by subjecting their DNA to PCR amplification using universal begomovirus specific AV/AC primers. For *Pepper mottle virus,* resistant lines were subjected to double antibody sandwich ELISA (DAS-ELISA) against *Pepper mottle virus* (PepMoV) specific antibodies. The results showed that for root knot nematode, *M. incognita* single genotype, PP 9950-5197-0107-7058 exhibited resistant reaction and four genotypes S-343, SL 472, SAS-39 and CH-27 exhibited moderately resistant reaction. For *Tomato leaf curl Joydebpur virus*, four genotypes *viz*., NSS-2, S-343, SL 473 and CH-27 were regarded as moderately resistant whereas, for *Pepper mottle virus,* eight genotypes, *viz*., VNR-314-2-1-4, IS 264, VR 527, EC-532386, PP0237-7508, IS-266, NSS-2, SL 472 gave negative ELISA reaction after sap inoculation with *Pepper mottle virus* and were thus regarded resistant to the virus. Further by comparing the screening results for individual pathogen it was observed that four genotypes *viz.,* SL 472, NSS-2, S-343 and PP 9950-5197-0107-7058 were found to have potential for multiple disease resistance to these three pathogens.

Key Words: Screening, Resistance, Root knot nematode, Viruses.

Introduction

Among the five recognized cultivated species of genus *Capsicum, Capsicum annuum* L. is the dominant species all over the world over for its pungent (chilli or hot pepper) and non-pungent (sweet pepper) fruits (Bosland and Votava, 2000). In India, hot-pepper is cultivated as an important commercial crop for vegetable, spice and industrial (capsaicin and oleoresin) purpose (Kumar and Rai, 2005).Root knot nematodes and viruses are among the major factors limiting the pepper cultivation in the country. Root knot nematodes belonging to the genus *Meloidogyne* are the important pest infecting pepper, worldwide (Khan and Haider, 1991). Several species of root knot nematode (*Meloidogyne incognita, M. javanica, M. hapla* and *M. arenaria*) are infecting peppers (Theis and Fery, 2002) among these, *M. incognita* and *M. javanica* have worldwide distribution and occur in varied warm-temperate & tropical climates and agro-ecosystems. Jain *et al*., 2007 reported economic losses amounting to approximately Rs. 210 million due to damage caused by root knot nematode in hot peppers. In spite of direct damage these nematodes also predispose the plants to other soil borne pathogens like bacteria and fungi by leaching plant nutrients into the soil.

Among viruses, whitefly transmitted begomoviruses causing leaf curl disease (syn. Pepper leaf curl disease) are the most destructive in terms of incidence and yield losses (Muniyappa and Veeresh, 1984). Leaf curl disease results in curling and cupping of leaves resulting in stunting of plants with either no or few undersized fruits leading to huge economic losses to the farmers. Early infection of the virus, on young plants results in flower bud abscission before attaining full size. The occurrence of *Tomato leaf curl Joydebpur virus, Tomato leaf curl New Delhi virus* and *Chilli leaf curl India virus* causing leaf curl disease in hot pepper has been reported in India (Senanayake *et al.,* 2006; Shih *et al.,* 2007). Apart from begomoviruses, some potyviruses are also causing significant damage to pepper crop globally. *Pepper mottle virus* (PepMoV) an aphid-transmitted virus belonging to potyvirus group is reported to infect *Capsicum* species (Shukla *et al.,* 1994). The virus cause mottling and

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puckering of leaves and along with misshapen leaves and fruits (Kim *et al.,* 2009). It was first reported in *Capsicum annuum* from Florida, USA (Zitter, 1972) and later on from different parts of the world. Earlier, in 1979 Sandhu and Chohan also reported mottle disease of chilli from India. Recently, the association of *Pepper mottle virus* (PepMoV) causing mottling disease has been confirmed from India (Kaur *et al.,* 2014).

Both nematodes and viruses are difficult to manage once they attack the crop. Most of the nematicides are banned due to their hazardous nature. Cultural control like crop rotation is effective but it also has limitation for root knot nematode due to polyphagous nature. Similarly for viruses, one has to manage vectors by applying insecticides, which fails to provide complete protection under field conditions. Host plant resistance is only the safer and economical way for management of these pathogens. Therefore, in the present study, hot pepper germplasm available with the Department of Vegetable Science, PAU, Ludhiana was screened in a systematic manner for resistance against root-knot nematode, *Meloidogne incognita*; begomovirus, *Tomato leaf curl Joydebpur virus* and a Potyvirus, *Pepper mottle virus* prevalent under Punjab conditions so as to identify the resistant source(s) which can be further utilized for resistance breeding purpose.

Materials and Methods

A total one hundred forty genotypes of pepper including improved varieties, inbread lines and hybrids representing two cultivated species *viz.*, *C. annuum* (138) and *C. frutescence* (2) were screened for resistance against root knot nematode (*Meloidogyne incognita*) a begomovirus, (*Tomato leaf curl Joydebpur virus*) and a Potyvirus (*Pepper mottle virus*). Three sets of genotypes were maintained separately for screening against each pathogen mentioned above.

Screening of Germplasm against Root Knot Nematode (Meloidogyne incognita)

Nursery of one hundred forty genotypes along with susceptible checks (Punjab Surkh and Kashi Anmol) was sown in plastic plug trays filled with steam sterilized coco-peat, perlite and vermiculite mixture in 3:1:1 ratio. At 3-4 leaf stage, plants were transplanted in 8 inch diameter pots filled with steam sterilized soil. After one week of transplanting when plants got established properly in the pots, inoculations were done with freshly hatched 2nd stage juveniles of *M. incognita* maintained in pure culture @ 500 juveniles per plant by making holes with the help of a glass rod near the roots of the plant. Four replications were maintained for each line along with susceptible check. Plants were watered regularly and properly maintained. Observations were recorded on root knot index and number of egg masses/ root system after 45 days of inoculations as per (0-5) scale given by Taylor and Sasser,1978.

Screening of Germplasm against Viruses

Same set of 140 genotypes along with local checks (Punjab Surkh and Kashi Anmol) were raised at Vegetable Research Farm, Department of Vegetable Sciences PAU, Ludhiana by following all the recommended cultural practices. No insecticide was sprayed so as to protect the vector population and initial screening for both the viruses was done under natural field conditions. The visual observations on appearance of symptoms were recorded at fortnight interval after transplanting the crop in end February till the month of August. For *Pepper mottle virus* (PepMoV) observations were recorded from the month of March to May and for leaf curl disease observations were recorded during July-August when the virus and vector load was at peak.

Screening of Germplasm against Leaf Curl Disease

Disease symptom severity recorded for each genotype was rated according to 0-5 scale given by Banerjee and Kalloo, 1987. According to the scale, Coefficient of Infection (CI) was calculated by multiplying disease incidence (DI) to response value, all the genotypes were assigned specific disease reaction (Table 1). The coefficient combined the amount of infection and its severity. The reaction grades assigned to each entry were added together for all observations and the mean values were calculated.

Non-viruliferous whitefly culture was maintained on cotton (*Gossipium hirsutum* L.) grown in 12 × 8 cm size plastic pots in insect-proof glass house. The day/ night temperature in the glass house was maintained at 28/20°C and the relative humidity at 70%. Inoculum of *Tomato leaf curl Joydebpur virus* (ToLCJV) was maintained on susceptible hot pepper cv. Kashi Anmol grown in the glass house by frequently inoculating 10-15 day old seedlings using viruliferous whitefly.

Promising entries along with susceptible checks were sown in plug trays filled with cocopeat, vermiculite

Table 1. Severity scale (0-5) for Leaf curl disease (Banerjee and Kalloo, 1987)

Symptom	Symptom severity grade	Response value	Coefficient of infection (CI)	Disease reaction
No visual symptom	0	0	0	Symptomless
0–5% curling of upper leaves	1	0.05	0.1–5	Highly Resistant (HR)
6-25% curling of leaves and swelling of veins	2	0.25	5.1–10	Resistant (R)
26–50% curling puckering and yellowing of leaves and swelling of veins	3	0.50	10.1–20	Moderately Resistant (MR)
51–75% leaf curling and stunted plant growth and blistering of internodes	4	0.75	20.1–40	Moderately Susceptible (MS)
More than 75% curling and deformed small leaves, stunted plant growth with small flowers and no or small fruit set	5	1.00	40.1–70	Susceptible (S)
			>70	Highly susceptible (HS)

and perlite mixture in 2:1:1 proportion. The trays were kept in the insect-proof glasshouse. Adults of non-viruleferous whitefly collected from stock culture were released into the plastic bottle cage containing ToLCJV infected branch and the flies were allowed to feed for 24 h acquisition access period. Ten seedlings from each genotype were inoculated at two-three leaf stage at twice weekly interval using 20 viruliferous whiteflies per plant. Inoculated plants were examined for ToLCJV symptoms expression daily till six weeks. The seedlings were rated according to (0-5) scale given by Banerjee and Kalloo,1987 as mentioned above. Total DNA was extracted from the genotypes showing resistant reaction on visual symptom bases and subjected to PCR amplification using begomovirus specific AV494/ AC1048 primers (Wyatt and Brown, 1996).

Screening of Germplasm against Pepper Mottle Virus

Preliminary screening for the virus was done under natural field conditions from the month of March to May as mentioned above. *Pepper mottle virus* symptom and severity was recorded following the 0-5 severity scale given by Mughal and Khan, 2001 with minor modification (Table 2).

The genotypes found resistant under field conditions were further confirmed by artificial screening. The *Pepper mottle virus* inoculum was maintained on susceptible line SL465 by sap inoculations to young healthy seedlings after every 15 days and maintained under insect proof conditions. The inoculum was prepared by grinding infected leaves in a grinder with phosphate buffer (0.01M).Ten plants for each genotype sown under insect proof conditions were sap inoculated with leaf rub method at 2-3 true leaf stage. After inoculation, leaves were washed with double distilled water to avoid any injury by carborandum powder and to remove excess inoculum. The inoculated plants were kept in an insect proof cage and observed regularly for symptom appearance up to five weeks. The disease incidence and severity was recorded on visual basis. The severity of plants was categorized as highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible.

The genotypes showing resistant reaction were further subjected to double antibody sandwich ELISA (DAS-ELISA) against *Pepper mottle virus* (PepMoV) specific antibodies (procured from Agdia, Elkhart, USA) as per manufacturers procedure. The colour changes were read visually and photo metrically with ELISA Reader (Tecan, Austria) at 405 nm. Readings of ELISA plates were taken by ELISA reader at OD value of 405nm. ELISA reaction was rated as negative, mild positive, positive and strong positive according to scale given by Moury *et al.,* 2005.

Table 2. Modified 0-5 severity scale for *Pepper mottle virus* (Mughal and Khan, 2001)

Symptoms	Severity grade	Disease reaction
No visible symptoms	0	HR
Mild mottling on the upper leaves	1	R
Banding of vein on few leaves, Mosaic initiation on all leaves	2	MR
Distinct mosaic and vein-banding symptoms on all leaves	3	MS
Severe mosaic and vein-banding along with narrowing of leaves, and misshapen leaf lamina	4	S
Severe mosaic and vein-banding, misshapen leaves and fruits, defoliation, small number of fruits	5	HS

Results and Discussion

Screening of Pepper Germplasm against Root Knot Nematode (Meloidogyne incognita)

Out of total 140 genotypes evaluated, one genotype (PP 9950-5197-0107-7058) was found resistant to root-knot nematode (*M. incognita*) with root gall/egg mass index 1.0 (Table 3). The roots of this genotype were free from egg masses. Four genotypes *viz.,* S-343, SL 472, SAS-39 and CH-27 exhibited moderately resistant reaction with 3-10 galls per root system and showing root gall index ranging from 1.25 to 2.0. These genotypes also showed lesser number of egg masses per root system as compared to the susceptible checks, Punjab Surkh and Kashi Anmol. Among others, 67 genotypes showed moderately susceptible and 67 showed susceptible reaction to root knot nematode. Genotype VR 526 was found highly susceptible to root-knot nematode with root gall index 4.5 (Table 3).

Resistance to *M. incognita, M. javanica* and *M. arenaria* had been identified in several *Capsicum* germplsm sources, including *C. annuum* L., *C. chacoense* L., *C. chinense* Jacq., and *C. frutescens* L. (Di Vito *et al.,* 1992; Theis and Fery, 2002). The resistance to root-knot nematode species that are considered important from economic stand point such as *M. incognita*, *M. javanica* and *M. arenaria* have been associated with at least nine dominant genes (*N, Me1, Me2, Me3, Me4, Me5, Me6, Me7, Mech1 e Mech2*) in pepper that are considered independently acting on gene-for-gene basis (Djian-Caporalino *et al.,* 2001; Wang and Bosland, 2006; Fazari *et al.,* 2012). Some of these genes (*Me1, Me3, Me7*) are considered thermostable and effective against a wide range of *Meloidogyne* spp., including *M. incognita, M. arenaria* and *M. javanica* (Djian-Caporalino *et al.,* 1999). The genotypes showing resistant and moderately resistant reaction to RKN (*M. incognita*) could be further investigated using molecular markers for the presence of resistance genes for exploitation in resistance breeding programmes.

Screening of Germplasm against Viruses

Screening of Germplasm against Leaf Curl Disease

Out of total 140 genotypes screened, fifteen genotypes (EC-532386, PP0237-7508, PC-410, CH-21312, LR 325, S-343, NSS-2, 09/CHIVAR-4, VS-524, SD 463, SL 473, PC-6, PP 0537-7541, CC-148 and CH-27) were found symptomless with coefficient of infection (CI) = 0.0. One genotype (SL 466) was found highly resistant with CI=5.0 and five genotypes (JL 282, PLS-1, AC-101, SL-8E-1and IS-268) were found resistant with CI ranging from 6.25-1.0 under field conditions. Besides, twenty four genotypes were found moderately resistant (with CI ranging from 10.70-20.0), fifty genotypes moderately susceptible (with CI from 21.40-40.0), thirty nine susceptible (with CI from 41.63-66.0) and six highly susceptible (CI = 75.0) to leaf curl disease.

Table 3. Screening of pepper germplasm to root-knot nematode, *M. incognita*

Reaction / No. of genotypes	Root gall/Egg mass index (0-5) scale	Genotypes/lines
Resistant (1)	0.1-1.0	PP 9950-5197-0107-7058.
Moderately Resistant (4)	1.1-2.0	S-343, SL 472, SAS -39, CH-27.
Moderately Susceptible (67)	2.1-3.0	MSC-31-4-1, VR521, IS 264, IS 265, ML-345, Pepsi-8-1,VR-36, EC-532390, Selection 44, TM 481, AC-103, IS-266, VS-528, SS-470, MSFL, PLS-1, SHHP-4884, MS-12-2, IS-267, MS-341, LS 324, PC-410, ATG, CH-21312, DL161, 2011/CHIVAR-7, 10/CHIVAR-4 (Yellow), IS-263, S-2530, PC-408, line 1-6-4, CH-21412(Orange), LR 325, PG-1-1, 2011/CHIVAR-1, NSS-2, 2011/CHIVAR-9, PLS-14, PLS-5, PLS-3-1, FZ-202, PC-1, VS-524, PLS-2, Perennial, PLS-12, JCA-283, UY 502, G-4, LLS, 10/CHIVAR-6, PLS 5-2,SR 467, PLS-15, IS 269, CH-21311, ACC-06-01, JCA-288, MSFL-4-2, PP 0537-7541, SL-474, VS-530, IS-261, GS-221,Ujwala KAU, Vellayani Atutlya, CARI-1.
Susceptible (67)	3.1-4.0	MSFL-4-3-1, MSFL-1-2, SL465, PP409, PP 402, PP 91-7195-1, SL 469, MSFL-5-2-1, MSC-31-6-1B, VR 527, EC-532386, PP 0437-7510, M-344, AC-102, CH-21511(orange), Saurian-2010, BCC-1, SHHP-404, JL 282, US 501, VS 522, PLS-3, AC-101, IS-262, CH 21411, SL 471, CH-2221, PLS-13, Surajmukhi, Dev Long, CH-1, C-142, MSC-31-10-1, YL 581,IS-263, S-2539, PL-411, SL-8E-1, ML 342, PLS-10, IS-268, 09/CHIVAR-4, SD-463, L-326,VS-529, Punjab Tej, SL 473, CS 147, PC-6, SL461, 09/CHIVAR-6, SL 466, IS-270, Annugraha, BL-121, CC-148, CAG-43, CAG-29, CAG-24, CART-2, CARI-5, CARI-4, CAG-3,CARI-3, S-217621, Punjab Surkh (Susceptible Check), Kashi Anmol (Susceptible Check).
Highly Susceptible (1)	4.1-5.0	VR 526.

The resistance exhibited by some genotypes under field conditions cannot be inferred as true resistance but they might have escaped the infection. In order to establish their nature of resistance, further confirmation was done by artificial inoculation.

Screening of Genotypes against Tomato Leaf Curl Joydebpur Virus under Artificial Conditions

The genotypes showing symptomless (EC-532386, PP0237-7508, PC-410, CH-21312, LR 325, S-343, NSS-2, 09/CHIVAR-4, VS-524, SD 463, SL 473, PC-6, PP 0537-7541, CC-148,CH-27), resistant (JL 282, PLS-1, AC-101, SL-8E-1, IS-268) and highly resistant (SL 466) reaction during field screening were subjected to artificial screening against most prevalent begomovirus (*Tomato leaf curl Joydebpur virus*) causing leaf curl disease in pepper under Punjab conditions.

It was observed that in susceptible check Kashi Anmol and genotypes, PLS-1, SL-8E-1, VS-524, Chilli colletion-4 and Surajmukhi symptoms started appearing on 18th day after inoculation and up to the 26thday after inoculation, all the genotypes were showing symptoms (Table 4). However, there was difference in symptom severity among the

Table 4. Screening of pepper genotypes against *Tomato leaf curl Joydebpur virus* under artificial conditions

S.No.	Genotype	Symptom appearance (Days after inoculations)	Reaction after Artificial Inoculation					
			Type of Symptoms	Severity Grade 0-5 scale	Disease Incidence (%)	Response Value	Coefficient of Infection	Disease Reaction
1	NSS-2	26	Mild curling of leaves	2.0	75.0	0.25	18.7	MR
2	S-343	22	Mild puckering no curling of leaves	2.0	100	0.25	25.0	MR
3	SL 473	23	Puckering without curling	2.0	70.0	0.25	17.5	MR
4	CH- 27	26	Mild curling of leaves	2.0	100	0.25	25.0	MR
5	LR 325	24	Mild curling of leaves and vein thickening	2.0	100	0.25	25.0	MR
6	EC-532386	22	Mild puckering, yellowing and curling of leaf margins	3.0	50.0	0.5	25.0	MS
7	PP0237-7508	23	Puckering, yellowing and curling	3.0	75.0	0.5	37.5	MS
8	PLS-1	18	Mild curling, twisting of leaf with swelling of veins	2.0	100	0.25	25.0	MS
9	AC-101	20	Curling of leaf margins with vein thickening	2.0	100	0.25	25.0	MS
10	PP 0537-7541	22	Puckering and downward curling of leaf margins	2.0	100	0.25	25.0	MS
11	Surajmukhi	18	Mild puckering and curling of leaf margins	3.0	50.0	0.5	25.0	MS
12	09/CHIVAR-4	23	Mild puckering and curling of leaves	2.0	100	0.25	25.0	MS
13	SD 463	20	Puckering and downward curling of leaf margins	2.0	100	0.25	25.0	MS
14	JL 282	20	Curling of leaves and vein swellings	2.0	100	0.25	25.0	MS
15	SL-8E-1	18	Pronounced puckering, curling and crinkling of leaves	3.0	100	0.5	50.0	S
16	IS-268	24	Mild puckering and curling of leaves	3.0	100	0.5	50.0	S
17	VS-524	18	Pronounced puckering and curling and twisting of leaf	3.0	88.0	0.5	44.0	S
18	SL 466	20	Puckering and downward curling of leaf margins	3.0	100	0.50	50.0	S
19	Chilli Collection-4	18	Pronounced puckering, curling and crinkling of leaves	3.0	100	0.50	50.0	S
20	PC-410	20	Puckering and downward curling of leaf margins	3.0	100	0.5	50.0	S
21	CH-21312	20	Downward curling of leaf margins	3.0	100	0.5	50.0	S
22	PC-6	20	Puckering and downward curling of leaf margins	3.0	100	0.5	50.0	S
23	KashiAnmol (Susceptible check)	18	Pronounced puckering, curling and crinkling of leaves, stunting of plants	5.0	100	1.0	100	HS
24	Punjab Surkh (Susceptible check)	20	Puckering, curling of leaves with thickening of veins	4.0	100	0.7	75.0	HS

genotypes. Five genotypes *viz.,* NSS-2, S-343, SL 473, CH-27 and LR 325 showed only mild puckering of leaves even 22 days after inoculation with CI ranging from 17.5 to 20.0. These genotypes were therefore, regarded as moderately resistant. The other genotypes which were symptomless (EC-532386, PP0237-7508, 09/CHIVAR-4, VS-524, SD463, CC-148, PP0537-7541, PC-410, CH-21312, PC-6, LR 325) highly resistant (SL 466) and resistant (JL 282, IS-268, Sel8-E, PLS-1, AC-101) under field conditions showed pronounced puckering, curling and blistering of leaves after artificial inoculations and were therefore rated as moderately susceptible and susceptible, respectively. The susceptible checks, Kashi Anmol and Punjab Surkh showed typical leaf curl type symptoms with pronounced puckering, curling and crinkling of leaves along with vein thickenings. The DNA of artificially inoculated lines giving resistant reaction when subjected to PCR amplification, showed presence of virus by amplifying ~570 bp band with begomovirus specific AV/AC primers. But the apparent symptoms vary with different genotypes which show that there is some resistant mechanism working in the genotypes NSS-2, S-343, SL 473, CH-27 and LR 325.

In India, a number of hot pepper lines resistant to leaf curl virus has been reported by different workers. Pepper cultivars like Perennial, BG-1, Lorai, and Punjab Lal are reported as important multiple disease resistant lines (Thakur *et al.,* 1987; Singh and Singh, 1989). The symptomless reaction of genotypes under field conditions can either be attributed to slow multiplication of virus which may be one of the probable reasons of resistance to leaf curl disease or may be simply due to escape (Banerjee and Kalloo, 1987). The resistance by escape could be because of a non-preference of whitefly under field conditions to a particular genotype planted along with several other genotypes or such lines may not be suitable for whitefly multiplication but suitable for virus multiplication and *vice-versa*. Hence, the field reaction of genotypes to particular organism may not be considered for having true resistance. However, if some genotype(s) show consistently low disease incidence under field conditions followed by artificial inoculation these genotypes might have some economic value. Even if viral DNA is detected using PCR assays these genotypes appear to be a good source of resistance for use in breeding programmes.

Screening of Genotypes against Pepper Mottle Virus

For *pepper mottle virus*, out of total 140 genotypes, 19 were found highly resistant, 11 resistant, 42 moderately resistant, 51 moderately susceptible, 16 susceptible and one genotype was found to be highly susceptible to the virus under natural field conditions. For further confirmation of resistance, a total of 49 lines comprising 19 genotypes showing highly resistant reaction, 11 resistant, 15 moderately resistant, 3 moderately susceptible and one highly susceptible reaction during natural field screening were tested under artificial conditions by sap inoculation.

It was observed that most of the genotypes exhibiting highly resistant and resistant reaction during field screening were found susceptible or moderately susceptible under artificial conditions (Table 5). Ten genotypes (VNR-314-2-1-4, IS 264, VR 527, EC-532386, PP0237-7508, IS-266, NSS-2, SL 472,Surajmukhi and MSFL-1-2) showed resistant reaction, nine genotypes (IS 269, VR521, BCC-1, EC-532390, Punjab Tej, IS-267, LR 325, S-343 and CH 27) showed moderately resistant reaction, seventeen genotypes (MSFL 4-3-1, DL161, ML-345, PC-408,CH-21411, MSC 31-10-1, PLS-14, SL-8E-1, AC 103, CH-2221, Line 1-6-4, PLS-13, Dev long, YL 581, PLS-3-1, Kashi Anmol and Punjab Surkh) showed moderately susceptible reaction, and ten genotypes (PP 402, MSC 31-4-1, SS-470, SHHP-4884, M-344, PG 1-1, CC-148, CART-2, CARI-1 and JCA-283) showed susceptible reaction. Three genotypes *viz*., CH-1, CARI-4 and SL465 showed highly susceptible reaction under artificial screening.

On confirmation of lines showing resistance during artificial screening by ELISA, it was observed that out of ten genotypes showing resistant reaction to PepMoV, eight genotypes (VNR-314-2-1-4, IS 264, VR 527, EC-532386, PP0237-7508, IS-266, NSS-2 and SL 472) gave negative ELISA reaction. While two genotypes *viz*., Surajmukhi and MSFL-1-2, showed mild positive (+) and positive (++) reaction with ELISA, respectively (Table 5). Among the genotypes showing moderately resistant reaction five genotypes *viz*., BCC-1, IS 269, VR521, S-343, CH-27 showed mild positive (+) reaction with ELISA whereas four genotypes *viz*., EC-532390, Punjab Tej, IS-267, LR 325 gave positive (++) reaction with ELISA. Similarly, lines showing moderately susceptible and susceptible reaction during artificial screening showed mild positive to strong positive reaction with ELISA. The genotypes CARI-4, SL465 and CH-1 showing highly susceptible reaction during artificial reaction gave strong positive (+++) reaction with ELISA.

Table 5. Screening of pepper germplasm against *Pepper mottle virus* under artificial conditions

S No.	Disease Reaction/ No. of genotypes	Severity Grade (0-5) Scale	Line /Genotype	ELISA Reaction*
1	Resistant (8)	1	VNR-314-2-1-4, IS 264, VR 527, EC-532386, PP0237-7508, IS-266SS-470, NSS-2, SL 472.	-
2	Resistant (1)	1	Surajmukhi	+
3	Resistant (1)	1	MSFL-1-2.	++
4	Moderately Resistant (5)	2	BCC-1, IS 269, VR521,S-343, CH-27.	+
5	Moderately Resistant (4)	2	EC-532390, Punjab Tej, IS-267, LR 325, PP 9950-5197-0107-7058	++
6	Moderately Susceptible (11)	3	DL-161, ML-345, PC-408, MSC-31-10-1, PLS-14,SL-8E-1,AC-103, CH-221 (Orange), Line 1-6-4, Dev Long, PLS-3-1.	+
7	Moderately Susceptible (5)	3	MSFL-4-3-1,PLS-13, YL 581, KashiAnmol (Susceptible Check), Punjab Surkh (Susceptible Check).	++
8	Moderately Susceptible (1)	4	CH-21411	+++
9	Susceptible (4)	4	SHHP-4884, CC-148, CART-2, JCA-283.	+
10	Susceptible (2)	4	MSC-31-4-1,M-344.	++
11	Susceptible (4)	4	PG-1-1, PP 402, SS-470, CARI-1.	+++
12	Highly Susceptible (3)	5	CARI-4, SL465, CH-1.	+++

*Scale for depicting ELISA results (Moury *et al.*,2005)

OD at 405 nm	Response	Symbol
Same as negative control	Negative	-
2-5 times higher than negative control	Mild positive	+
5-10 times higher than negative control	Positive	++
10-15 times higher than negative control	Strong positive	+++

Earlier, *Capsicum annuum* L. cv. Avelar was shown to have a monogenic, recessively inherited factor for resistance to *Pepper mottle potyvirus* (PepMoV) (Zitter and Cook, 1973; Guerini and Murphy, 1999). In India, not much information is available regarding hot pepper resistance to PepMoV. However, resistance to potyvirus viz., *Chilli veinal mottle virus* and *Pepper veinal mottle virus* was identified at AVRDC in two accessions of *C. annuum*, 'Perennial HDV' and 'PSP-11', which were originally of Indian origin (Anonymous, 1990; 1991).

Discussion

Hot pepper is one of the important spice and vegetable crop of India with high export potential. Problem of root-knot nematodes and viruses limits the pepper cultivation in many tracts of India particularly hilly and northern plains of the country. Due to the lack of effective management strategies, growing of resistant sources is the most economical strategy for management of these diseases. Identification of resistant source(s) and their utilization in resistant breeding programme are thus imperative to manage these pathogens in the long run. Thus, the present study was carried out keeping this objective in mind. The results showed that out of total 140 pepper genotypes evaluated against root knot nematode (*M. incognita*), *Tomato leaf curl Joydebpur virus* and *Pepper mottle virus*, one genotype (PP 9950-5197-0107-7058) was found resistant and four genotypes (S-343, SL 472, SAS-39 and CH-27) moderately resistant to *M. incognita*. For viruses, four genotypes *viz.*, NSS-2, S-343, SL 473 and CH-27 were found promising against *Tomato leaf curl Joydebpur virus* and eight genotypes (VNR-314-2-1-4, IS 264, VR 527, EC-532386, PP0237-7508, IS-266, NSS-2, SL 472) were found resistant against *Pepper mottle virus.* In addition to resistance against individual pathogens, four genotypes *viz.*, SL 472, NSS-2, S-343 and PP 9950-5197-0107-7058 were found promising for multiple disease resistance against these pathogens. On comparing together the individual screening results against each pathogen it was found that genotype 'S-343' showed moderately resistant reaction to both the viruses and root-knot nematode, SL 472 was found resistant to PepMoV and moderately resistant to RKN, NSS-2 was observed resistant to PepMoV and moderately resistant to *Tomato leaf curl Joydebpur virus* and genotype PP 9950-5197-0107-7058 was resistant to *M. incognita* and moderately resistant to PepMoV. Earlier, Pegard *et al.*, 2005 also reported disease resistance in *Capsicum annuum* against more than one pathogen.They found that the line CM334, being used by breeders as a source of resistance to

Phytophthora species and potyviruses, entirely suppresses reproduction of the root-knot nematode (*Meloidogyne* species). Thus, the promising genotypes identified in the present study can be exploited as source of resistance in breeding programme against these pathogens.

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RESEARCH ARTICLE

Grouping of Farmers' Varieties of Rice (*Oryza sativa* L.) Collected from West Bengal and Adjoining States

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A total of 132 Farmers' Varieties (FVs) of rice have been grouped into different classes based on the colour of basal leaf, days to 50% flowering, length of the stem (excluding panicle), length of decorticated grain, shape of decorticated grain (in lateral view), colour of decorticated grain, amylose content of endosperm and aroma of decorticated grain. Basal leaf colour of the most of the FVs was green. Based on the days to 50% flowering most of the FVs were classified under late to very late category. Again majority of the FVs were categorized into moderate and long in respect of length of the stem (excluding panicle). The rice genotypes with long slender decorticated grain have importance in international market. The FVs Uttar Banga Local-18, Birali-selection, Uttar Banga Local-17, Tulaipanji, Uttar Banga Local-14, Dudheswar-AD, Uttar Banga Local-16, Malbati, Rampha, Biroi, Uttar Banga Local-19, Kalturey, Tulaipanji-AD were classified as long slender. Decorticated coloured rice is reported to be potent sources of antioxidants and their consumption is encouraged. Decorticated grain colour of the FVs Chakhao Poireiton, Chakhao Angangbi, Chakhao Amubi, Chakhao-Selection-2, Sadabhatkalo were dark purple. Most of the FVs were non-aromatic, only 25 cultivars were found to have strong aroma on cooking and 23 were found to have mild aroma. The FVs which have been classified under desirable groups may be used for further improvement of rice based on specific character of donor.

Key Words: Grouping, Farmers' varieties, PPV&FR, Rice, *Oryza sativa*

Introduction

India rich in genetic diversity for scented and non-scented Farmers' Varieties (FVs) of rice in general and Northeastern India in particular (Roy *et al*., 1985; Talukdar *et al*., 2012; Chakravorty and Ghosh, 2013) and they are currently under production whose identity and distinctiveness needs to be established by various approaches. Landraces and wild species possess immense potential of most valuable genes which can be efficiently utilized in the breeding programmes to develop high yielding rice varieties with quality and resistance to biotic and abiotic stresses (Tirkey *et al*., 2013).

There is strong need that the local germplasm of rice to be collected, preserved and characterized in detail (Chakrabarty *et al*., 2012), subsequent development of suitable cultivar. Rice varieties have been developed traditionally by selection, hybridization and backcross with locally adapted high yielding genotypes. The numbers of parental lines are very limited which lead to narrow genetic base. Genetic uniformity in crop can be undesirable in terms of vulnerability of the crop to epidemics. A new important source for the introduction of new trait or development of new plant type is the existence of genetically diverse gene pool of scented and non-scented rice in our country. Until a collection is properly evaluated it is little practical use (Chang, 1976). Considering the importance of FVs of rice, the present investigation was planned to characterize the collected FVs of rice (Table 1) available in Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal and to identify the important traits which can be used in improvement of rice by the breeders. Based on DUS testing, the rice varieties usually are divided into different groups to facilitate the assessment of Distinctiveness. Following the guideline of Protection of Plant Varieties and Farmers' Rights (2007), the available FVs of rice at Uttar Banga Krishi Viswavidyalaya, Pundibari were grouped into different classes based on eight characters. This study will also be useful in generation of distinctiveness and subsequently registration of FVs under PPV & FRA.

Materials and Methods

One hundred thirty two Farmers' Varieties (FVs) of rice were used in this study, of which 48 were aromatic and

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Table 1. Name of the Farmers' Varieties of rice used in this study and their place of collection

Sl. No.	Name of farmers' variety	Place of collection/ source of the seed
1.	Ayangleima Phou	Central Agriculture University, Imphal, Manipur
2.	Badshabhog	Maldah district, West Bengal
3.	Baigonmacchua	Tarai Research Society, Alipurduar, West Bengal
4.	Baigonbuchi	Uttar Dinajpur district, West Bengal
5.	Betho	Alipurduar district, West Bengal
6.	Beto	Tarai Research Society, Alipurduar, West Bengal
7.	Binni	Tarai Research Society, Alipur Duar, West Bengal
8.	Birai	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
9.	Birali-Selection	UBKV, Pundibari, Cooch Behar, West Bengal
10.	Biroi	Tarai Research Society, Alipurduar West Bengal
11.	Bitti	PSBSG, Sitalkuchi, Cooch Behar district, West Bengal
12.	Boichi	Alipurduar district, West Bengal
13.	Bonnidhan	Tarai Research Society, Alipurduar, West Bengal
14.	Bora	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
15.	Chakhao Amubi	Central Agriculture University, Imphal, Manipur
16.	Chakhao Angangbi	Central Agriculture University, Imphal, Manipur
17.	Chakhao Poireiton	Central Agriculture University, Imphal, Manipur
18.	Chakhao-Selection-1	UBKV, Pundibari, Cooch Behar, West Bengal
19.	Chakhao-Selection-2	UBKV, Pundibari, Cooch Behar, West Bengal
20.	Chakhao-Selection-3	UBKV, Pundibari, Cooch Behar, West Bengal
21.	Chakhao Sempak	Central Agriculture University, Imphal, Manipur
22.	Chapka Chakhao	Central Agriculture University, Imphal, Manipur
23.	Chinakamani	BCKV, Mohanpur, West Bengal
24.	Dharam Phou	Central Agriculture University, Imphal, Manipur
25.	Dhyapa	Alipurduar district, West Bengal
26.	Dubari Komal	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
27.	Dudhekalam Motajosawa	Tarai Research Society, Alipurduar, West Bengal
28.	Dudhekalam-1	Tarai Research Society, Alipurduar, West Bengal
29.	Dudhekalam-9	Tarai Research Society, Alipurduar, West Bengal
30.	Dudheswar	Tarai Research Society, Alipurduar, West Bengal
31.	Dudheswar -AD	Tarai Research Society, Alipurduar, West Bengal
32.	Fudugey	Kalimpong, Darjeeling district, West Bengal
33.	Garu Chakhua	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
34.	Ghee Bora	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
35.	Gobindobhog	BCKV, Mohanpur, West Bengal
36.	Hatidat Komal	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
37.	Jailang Lal Komal	ICAR-CPRI- Kahikuchi, Kamrup, Assam
38.	Jaldhyapa-2	Sitalkuchi, Cooch Behar district, West Bengal
39.	Jaldhyapa-3	Cooch Behar district, West Bengal
40.	Jaldhyapa-AD	Tarai Research Society, Alipurduar, West Bengal
41.	Jasawa-AD	Alipurduar district, West Bengal
42.	Jashoya	Tarai Research Society, Alipurduar, West Bengal
43.	Jhagarikartik	Tarai Research Society, Alipur Duar, West Bengal
44.	Jhapaka	Kalimpong, Darjeeling district, West Bengal
45.	Jonroi Buna	Uttar Dinajpur district, West Bengal
46.	Kabra	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
47.	Kagey	Kalimpong, Darjeeling district, West Bengal
48.	Kaike	Tarai Research Society, Alipurduar, West Bengal
49.	Kalakali	Tarai Research Society, Alipur Duar, West Bengal
50.	Kalo Khasa	Uttar Dinajpur district, West Bengal
51.	Kalo Nunia	Cooch Behar district, West Bengal
52.	Kalobhog-Selection	UBKV, Pundibari, Cooch Behar, West Bengal
53.	Kaloboichi	Tarai Research Society, Alipur Duar, West Bengal
54.	Kalodhyapa	Tarai Research Society, Alipurduar, West Bengal
55.	Kalojeera	BCKV, Mohanpur, West Bengal
56.	Kalshepa	Tarai Research Society, Alipurduar, West Bengal

Sl. No.	Name of farmers' variety	Place of collection/ source of the seed
57.	Kalturey	Kalimpong, Darjeeling district, West Bengal
58.	Kashiya Binni	Tarai Research Society, Alipurduar, West Bengal
59.	Kataribhog	Alipurduar, West Bengal
60.	Kauka	Alipurduar district, West Bengal
61.	Kauka-Selection	UBKV, Pundibari, Cooch Behar, West Bengal
62.	Khaiyamdhan	Tarai Research Society, Alipurduar, West Bengal
63.	Khama	Alipurduar district, West Bengal
64.	Kharadhan	PSBSG, Sitalkuchi, Cooch Behar district, West Bengal
65.	Khasa	Malda district, West Bengal
66.	Kola Joha-Big	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
67.	Kola Joha-Small	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
68.	Konkoni Joha	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
69.	Ladu	PSBSG, Sitalkuchi, Cooch Behar district, West Bengal
70.	Lagidhan	Tarai Research Society, Alipurduar, West Bengal
71.	Laldhyapa	Tarai Research Society, Alipurduar, West Bengal
72.	Maitee	Kalimpong, Darjeeling, West Bengal
73.	Malbati	Tarai Research Society, Alipurduar, West Bengal
74.	Malshira	Tarai Research Society, Alipurduar, West Bengal
75.	Mangamuthi	Tarai Research Society, Alipurduar, West Bengal
76.	Marichsal	Tarai Research Society, Alipur Duar, West Bengal
77.	Mohanbhog	BCKV, Mohanpur, West Bengal
78.	Munimahuari	Kalimpong, Darjeeling district, West Bengal
79.	Pahariboichi	Jalpaiguri district, West Bengal
80.	Pahariboichi-Selection	UBKV, Pundibari, Cooch Behar, West Bengal
81.	Panikuthi Shyamlal	Uttar Dinajpur district, West Bengal
82.	Phoolpakari-1	Tarai Research Society, Alipurduar, West Bengal
83.	Phoolpakari-2	Tarai Research Society, Alipurduar, West Bengal
84.	Phoren Mubi	Central Agriculture University, Imphal, Manipur
85.	Radhatilak	PSBSG, Sitalkuchi, Cooch Behar district, West Bengal
86.	Radhatilak-AD	Tarai Research Society, Alipurduar, West Bengal
87.	Radhunipagal	BCKV, Mohanpur, West Bengal
88.	Rampha	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
89.	Ronga Komal	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
90.	Sada Nunia	Cooch Behar district, West Bengal
91.	Sadabhatkalo	PSBSG, Sitalkuchi, Cooch Behar district, West Bengal
92.	Sadamala	Tarai Research Society, Alipurduar, West Bengal
93.	Satia	Tarai Research Society, Alipurduar, West Bengal
94.	Seshphal	Alipurduar district, West Bengal
95.	Sial Bhomra	Tarai Research Society, Alipurduar, West Bengal
96.	Silathia Bora	ICAR-CPCRI- Kahikuchi, Kamrup, Assam
97.	Singara	Alipurduar district, West Bengal
98.	Sitalkuchi-1	UBKV, Pundibari, Coch Behar, West Bengal
99.	Sitalkuchi-2	UBKV, Pundibari, Coch Behar, West Bengal
100.	Sitalkuchi-3	UBKV, Pundibari, Coch Behar, West Bengal
101.	Sitalkuchi-4	UBKV, Pundibari, Coch Behar, West Bengal
102.	Sitalkuchi-5	UBKV, Pundibari, Coch Behar, West Bengal
103.	Sitalkuchi-6	UBKV, Pundibari, Coch Behar, West Bengal
104.	Tarapakari	Tarai Research Society, Alipurduar, West Bengal
105.	Tarapakari-Selection	UBKV, Pundibari, Cooch Behar, West Bengal
106.	Thuri	PSBSG, Sitalkuchi, Cooch Behar district, West Bengal
107.	Tarai Research Society-1	Tarai Research Society, Alipurduar, West Bengal
108.	Tarai Research Society -2	Tarai Research Society, Alipurduar, West Bengal
109.	Tarai Research Society -3	Tarai Research Society, Alipurduar, West Bengal
110.	Tarai Research Society -4	Tarai Research Society, Alipurduar, West Bengal
111.	Tulaipanji	Uttar Dinajpur, West Bengal
112.	Tulaipanji-AD	Alipurduar, West Bengal
113.	Tulsibhog	Alipurduar, West Bengal
114.	Tulsimukul	Tarai Research Society, Alipurduar, West Bengal
115.	Uttar Banga Local -2	Tarai Research Society, Alipurduar, West Bengal
116.	Uttar Banga Local -3	Tarai Research Society, Alipurduar, West Bengal

Sl. No.	Name of farmers' variety	Place of collection/ source of the seed
117.	Uttar Banga Local -3-1	UBKV, Pundibari, Cooch Behar, West Bengal
118.	Uttar Banga Local -5	Tarai Research Society, Alipurduar, West Bengal
119.	Uttar Banga Local -6	Tarai Research Society, Alipurduar, West Bengal
120.	Uttar Banga Local -7	Tarai Research Society, Alipurduar, West Bengal
121.	Uttar Banga Local -8	Tarai Research Society, Alipurduar, West Bengal
122.	Uttar Banga Local -9	Tarai Research Society, Alipurduar, West Bengal
123.	Uttar Banga Local-10	Tarai Research Society, Alipurduar, West Bengal
124.	Uttar Banga Local -11	Tarai Research Society, Alipurduar, West Bengal
125.	Uttar Banga Local -12	Tarai Research Society, Alipurduar, West Bengal
126.	Uttar Banga Local -13	Tarai Research Society, Alipurduar, West Bengal
127.	Uttar Banga Local -14	Tarai Research Society, Alipurduar, West Bengal
128.	Uttar Banga Local -15	Tarai Research Society, Alipurduar West Bengal
129.	Uttar Banga Local -16	Tarai Research Society, Alipurduar, West Bengal
130.	Uttar Banga Local -17	Tarai Research Society, Alipurduar, West Bengal
131.	Uttar Banga Local -18	Tarai Research Society, Alipurduar, West Bengal
132.	Uttar Banga Local -19	Tarai Research Society, Alipurduar, West Bengal

Table 2. Grain types based on decorticated grain length and grain length: breadth ratio

Classes	Parameters
Short-slender	Length less than 6 mm, L:B ration 3 and above
Short-bold	Length less than 6 mm, L:B ratio less than 2.5 mm
Medium-slender	Length less than 6 mm, L:B ratio 2.5 to 3.0 mm
Long-slender	Length 6 mm and above, L:B ratio 3 and above
Long-bold	Length 6 mm and above, L:B ratio less than 3.0 mm
Extr-long slender	Length 7.5 mm and above, L:B ratio 3.0 mm and above

84 were non-aromatic. Details of the genotypes studied are being given in Table 1. The field experiment was carried out at the Instructional Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, during *Kharif* season of 2013 and 2014. The farm is situated at 26°19′86″ N latitude and 89°23′53″ E longitude, at an elevation of 43 meter above mean sea level.

The FVs were grouped based on colour of basal leaf, days to 50% flowering, length of stem (excluding panicle), length of decorticated grain, shape of decorticated grain, colour of decorticated grain, amylose content of endosperm and aroma (Allidawati and Kustianto, 1989; Sood and Siddiq, 1978) to facilitate the assessment of Distinctiveness as per the Guidelines for the Conduct of Test for Distinctiveness, Uniformity and Stability on Rice (*Oryza sativa* L.) published by Protection of Plant Varieties and Farmers' Rights Authority (2007), Government of India. Characteristics, which are known from experience not to vary, or to vary only slightly within a variety and which in their various states are fairly evenly distributed across all varieties in the collection are suitable for grouping purpose. The experimental design used was randomized blocks design with 132 treatments (genotypes) and two replications.

Results and Discussion

The rice varieties for DUS testing usually are divided into groups to facilitate the assessment of Distinctiveness. Based on guideline of PPV&FRA (2007) FVs of rice were grouped and details are given below.

Basal Leaf Sheath Colour

The leaf sheath colour varied as green, light purple, purple lines and uniform purple. Out of 132 FVs, 110 FVs showed green coloured basal leaf sheath (Fig. 1a). This result also corroborates with the findings of many researchers that most of the FVs possess green coloured basal leaf sheath (Parikh *et al*., 2012; Chakrabarty *et al*., 2012; Chakravorty and Ghosh 2011, 2012, 2013; Tirkey *et al*., 2013; Subba Rao *et al*., 2013; Singh *et al*., 2015).

Seventeen FVs showed light purple coloured leaf sheath, four showed purple lines and one FV showed uniform purple coloured basal leaf sheath. Uniform purple colour of basal leaf sheath was also noted by Parikh *et al*. (2012). However, Chakravorty and Ghosh (2011) found no FV with uniform purple coloured basal leaf sheath among the 51 FVs.

Time of Heading (50% of plants with panicles)

Out of 132 FVs, only one FV (Fig. 1b) was found early time of heading, four were found medium, 39 were found late and 88 were found very late for 50%

heading. Findings of other researchers also showed that the most of the FVs fall under early and medium categories (Subba Rao *et al*., 2013). The early (Bitti) and medium early (Kalturey, Sada Nunia, Bora and Seshphal) may be tested for cultivation in other seasons, like *Boro* and *Aus*.

Stem Length (excluding panicle)

Stem length was categorized into five groups- very short (<91 cm), short (91-110 cm), medium (111-130 cm), long (131-150 cm) and very long (>150 cm). Five FVs were found very short stem length, eight were found to have short stem length (Fig. 1c), 64 were found to have medium stem length, 46 had long stem length, and nine were found to have very long stem length. Stem length is an important character which decides the susceptibility towards the lodging. In general, short to medium plant height is preferred as this makes easy all the intercultural activities. In addition, short height rice varieties are usually considered as lodging resistant. As per the findings of many rice workers the stem length of most of the FVs were medium and long (Chakravorty and Ghosh, 2012, 2013). However, Subba Rao *et al*. (2013) found that nearly all of the FVs they studied fallen under very short, short and medium categories. Only one variety (Kusum Kuntala) belongs to long category. Some other varieties with very long stem length are Muktasal, Jhulur, Manikachan (Chakravorty and Ghosh, 2012, 2013).

Decorticated Grain Length

In the present study, 63 FVs were found to possess short type of decorticated grain (Fig. 1d), 43 FVs were medium, 22 FVs were long type of decorticated grain length and only four FVs were had extra-long type of decorticated grain length. The decorticated grain length varied across the FVs of rice. This result also corroborates the findings of Chakrabarty *et al*. (2012), Subba Rao *et al*. (2013). Decorticated grain length is an important character for deciding grain shape with grain width. In general, longer grains are being preferred by most of the consumers.

Decorticated Grain Shape (in lateral view)

Shape is generally expressed as a ratio, between length and width. Shape of the decorticated grain was classified as short slender, short bold, medium slender, long bold, long slender, long slender (for Basmati type) and extra-long slender. Out of 132 FVs, none of them showed short slender and extra-long slender shape of decorticated grain. 46 FVs showed short bold shape of decorticated grain (Fig. 1e), 18 were medium slender, 54 were long-bold, and 13 showed long slender and one was found to be extra-long slender shape of decorticated grain.

Long grain rice has a low glycemic index than shorter grain rice. It also tends to be fluffier and less sticky than short grain. Medium slender rice is shorter and plumper than long grain and work better for a plant-based risotto or paella. Short grain rice is almost round in shape. The grains become glutinous and sticky when cooked, which is why it is also called 'sticky rice'. This is the best choice for rice puddings, sushi and rice balls. However, short grain rice is higher in glycemic index (https://www.huffingtonpost.com/ornish-living/make-the-switch-why-brown_b_8304558.html). As per the findings of Chakrabarty *et al*. (2012) majority of the FVs have been categorized under long bold and extra-long slender.

Decorticated Grain Colour

In present study out of 132, FVs 47 were found to have white decorticated grain colouration (Fig. 1f; Fig. 2), 34 were found light brown, seven were variegated brown, 13 were dark brown, 17 were light red, eight were red, one was purple and five FVs were found dark purple decorticated grain colouration. One FV was found to have variegated purple coloured decorticated grain.

The most common rice consumed by humans is white rice, followed by brown rice. However, rice genotypes with either red/purple or black bran layer have been cultivated for a long time in Asia (Ahuja *et al*., 2007). Coloured rice is reported to be potent sources of antioxidants and their consumption is encouraged (Yawadio *et al*., 2007, Anggraini *et al*., 2015). Black rice contains relatively high anthocyanin (primarily *cyaniding-3-O-glucoside and peonidin 3-O-glucoside*) in the pericarp layer which gives the dark purple color (Ryu *et al*., 1998; Takashi *et al*., 2001, Kristamtini *et al*., 2012). Anthocyanin is known for their bioactive properties and recognized as health-enhancing substances due to their antioxidant activities, anti-inflammatory, anticancer, anti-atherogenic, and anti-hypoglycemic effects (Wang and Stoner, 2008). Black rice is low in sugar but packed with healthy fibre and plant compounds that combat heart disease and cancer, according to scientists (Sutharut and Sudarat, 2012). Research suggests that the dark plant antioxidants, which mop up harmful molecules, can help

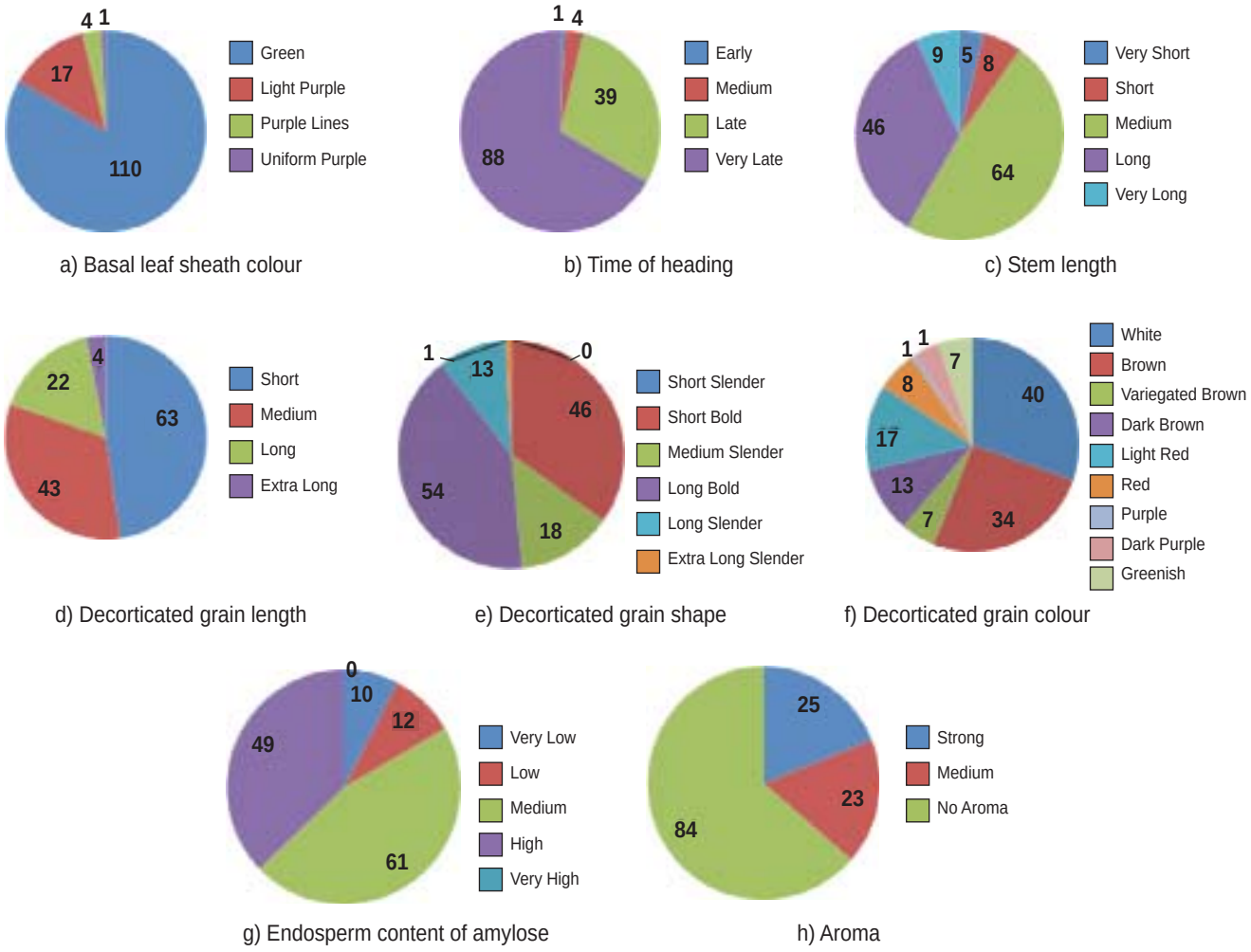

Fig. 1. Classification of the genotypes based on different characters as per PPV & FRA (2007). The numbers depicted in the pie-charts are the number of FVs in repective class.

protect arteries and prevent the DNA damage that leads to cancer. Colored rice have important roles in reducing risk of cancer and other chronic diseases because of their free radicals scavenging capacities (Wang and Stoner, 2008; Shih *et al*., 2007; Elisia *et al*., 2007; Elisia and Kitts, 2008). Black rice also contains higher levels of proteins, vitamins and minerals than common white rice (Suzuki *et al*., 2004).

Endosperm Content of Amylose

It is composed more than 80% starch and at molecular level starch contains amylose (linear chains glucose of α (1-4) linkages) and amylopectin (branched chain glucose with α(1-6) linkage (Henry and Kettlewell, 1996). The extent of amylose content in endosperm was classified into five categories, like very low (<10%), low (10-19%), medium (20-25%), high (26-30%) and very high (>30%). Ten FVs showed very low content of amylose in endosperm (Fig. 1g), 12 showed low amylose content, 61 showed medium and 49 showed high content of amylose in endosperm. None of them was showed very high content of amylose in endosperm. Amylose content is an important rice grain quality parameter in respect of consumer preference. In India, consumers prefer medium (20-25%) amylose content in the endosperm and this is an important parameter for promotion of rice entries during All India Rice Improvement Project (AICRIP) under Indian Council of Agricultural Research, India (Anonymous, 2017). Starch content (amylose) of rice is very important factors in grain yield, processing and palatability.

Decorticated Grain Aroma

Out of 132 FVs, 25 were found strong aromatic (Fig. 1h), 23 were found medium aromatic and remaining 84 were found non-aromatic. The Indian subcontinent has the *Natural Gift* of basmati rice that has been accepted as the best scented, long and slender grain in the national and international markets and gets high price. In addition to Basmati, many local landraces are grown traditionally which excel in aroma, grain quality and cooking quality.

A large number of aromatic FVs are available in our country, particularly in eastern and north-eastern states. Many researchers reported aromatic FVs of rice in their research articles (Chakrabary *et al*., 2012; Das *et al*., 2012; Tirkey *et al*., 2013; Semwal *et al*., 2014). Many FVs have strong aroma, but their other plant characters and/or grain characters may not be *at par* with the modern high yielding genotypes. Most of those aromatic FVs are poor yielders, for examples, Kalonunia, Tulaipanji, Kalojeera, Mohanbhog etc. On the other hand, few aromatic FVs possess high yielding ability, such as Gobindobhog, Badshabhog etc. Those FVs may be brought under cultivation through standardization of modern agronomic practices. In West Bengal, those strongly scented rice are generally used in preparation of *Khir* during special occasions and also offered during different pooja.

With growing demand for aromatic rice in the international market, high emphasis had been given for improvement of Basmati rice alone. The indigenous landraces with small and medium grained aromatic rice, which possesses outstanding aroma, grain quality, cooking quality and taste had been neglected. With introduction of high yielding genotypes of rice, the landraces that include aromatic quality are moving out of cultivation. A large number of aromatic rice has been already been lost and many are at the verge of extinction. Therefore, these local genotypes have to be collected and evaluated for their exploitation of genetic variability for development of suitable non-Basmati type aromatic cultivar. Further, management of the indigenous aromatic rice genetic resources by way of characterization and documentation helps in protection of these unique bio-resources. Improvement of aromatic rice employing the existing bio-resources would not only help farmers produce better-quality grain but would also generate more export income. Rice varieties have been

Fig. 2. Classification based on decorticated grain colour. A) White coloured decorticated grains (e.g., Seshphal, Baigonbuchi, Panikuthi Shyamlal etc.); B) Light brown coloured decorticated grains (e.g., Uttar Banga Local-9, Radhatlak-2, Uttar Banga Local-18 etc.); C) Dark brown coloured decorticated grains (e.g., Chakhao Sempak, Tarai Research Society-2, Uttar Banga Local-3 etc.); D) Red coloured decorticated grains (e.g., Sitalkuchi-2, Bitti, Kalshepa, Kaike etc.); E) Dark purple coloured decorticated grains (e.g., Chakhao Poireiton, Chakhao Angangbi, Sadabhatkalo etc.); F) Greenish coloured* decorticated grains (e.g., Uttar Banga Local-13, Uttar Banga Local-12, Kola Joha-Small etc.).
***This class has not been mentioned in 'Guidelines for the Conduct of test for DUS on rice (Oryza sativa L.). Protection of Plant Varieties and Famrer's Right Authority (PPV & FRA), Government of India, New Delhi. However some varieties showed greenish coloured decorticated grains (Surje Dinesh Tulsiram, 2016).**

developed traditionally by selection, hybridization and backcross selection with locally adopted high yielding genotypes. The numbers of parental lines are very limited which lead narrow genetic base. Genetic uniformity in crop can be undesirable in terms of vulnerability of the crop to epidemics. A new important source for the introduction of new trait or development of new plant type is the existence of genetically diverse gene pool of scented rice in our country.

In nutshell, desirable FVs could be selected for direct cultivation after standardization of modern agronomic practices based on grouping of the FVs in the present study the FVs from desirable groups may be used as donor in rice improvement programmes for different desirable traits.

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RESEARCH ARTICLE

Novel Rice (*Oryza sativa* L.) Genotypes Tolerant to Combined Effect of Submergence and Salt Stress

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The implications of both complete and partial submergence with saline water on growth and survival of different rice genotypes were investigated. The study showed that among the 199 genotypes, only five genotypes namely IC-459744, IC-469712, IC-464746, IC-459649 and IC-17024 were tolerant to both complete (plants under water) and partial submergence (water depth 45±5 cm) with saline water (12 dS m^{-1}). The association of Chlorophyll a, Chlorophyll b, total Chlorophyll, normalized difference vegetative index (NDVI) and photochemical reflectance index (PRI) with score under partial submergence with salt stress were highly negatively significant ($p<0.001$***). Biplot derived from principal component analysis showed that accessions such as IC-459744 and IC-459649, tolerant to both complete and partial submergence with salty water were in the same quarter where PRI was located with long vertex. The genotypes identified in this investigation have great potential as unique genetic resources for utilization in crop improvement programmes.

Key Words: Coastal area, Genetic resource, *Oryza sativa*, Submergence-salt stress combination, Tolerant genotypes

Introduction

Rice is adapted to various agro-climatic zones starting from favourable irrigated condition to highly unfavourable harsh environments. It is grown at altitudes ranging from below sea level (Kuttanad, Kerala, India) to 2761 m above sea level (Jumulla valley, Nepal), thus making its presence across environments (Chang, 2000). This adaptation to extremely variable conditions in rice offers a hope to mitigate the current challenges posed by variable abiotic stresses, as-well-as means to cope with the adverse effects of climate change, to secure food and livelihood (Das *et al*., 2004; Wassman *et al*., 2009; Mackill *et al*., 2010; Waziri *et al*., 2016; Ray *et al*., 2017). Rice genetic resources are vast, yet a fraction of it has been utilized for crop improvement programme, it is mainly due to the lack of knowledge on beneficial attributes of landraces (Glaszmann *et al*., 2010; Sarkar and Bhattacharjee, 2011; Singh and Sarkar, 2014; Kuanar *et al*., 2017). By taping the genetic resources, development of climate resilient rice varieties is possible. This is because genes associated with tolerance of various abiotic stresses are available within the cultivated rice gene pool (Ismail *et al*., 2007; Sarkar *et al*., 2009; Reddy *et al*., 2009; Thomson *et al*., 2010).

Farmers of coastal areas of eastern India still grow landraces that are photoperiod-sensitive, less-responsive to fertilisers and prone to lodging with limited yield potential and poor grain quality (Sarkar and Bhattacharjee, 2011). These landraces are frequently encountered by complete and partial submergence with or without saline water (Mahata *et al*., 2010; Yan *et al*., 2013). Therefore, the main aim of this investigation was to identify rice germplasm tolerant to complete and partial submergence along with salt stress. The germplasm lines showing tolerance to submergence under saline environment were tested further under partial submergence with saline water. The rice germplasm accessions thus identified through this study will be useful in developing high yielding rice varieties, which can tolerate both complete and partial submergence under saline environment. Due to complexity in evaluation of partial submergence with salt stress, additional physiological parameters were taken in categorizing the tolerance level. The association studies of some physiological parameters with plant performance under partial submergence with saline water were useful in identifying physiological indicator.

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Materials and Methods

Evaluation of Rice Germplasm for Submergence Tolerance under Saline Environment

One hundred and ninety nine cultivars were tested for submergence tolerance under saline water. Two cultivars namely Swarna (susceptible to submergence) and FR13A (tolerant to submergence both under saline and non-saline water) were used as checks (Sarkar and Ray, 2016). Seeds were sown directly in earthenware pots containing 2 Kg of farm soil and farmyard manure in a 3:1 ratio. Each pot was supplied with 192 mg single super phosphate (P_2O_5) and 80 mg murate of potash (K_2O). Ten days after germination, seedlings were thinned and ten plants per pot were maintained. Urea @ 100 mg was applied in each pot after thinning. The pots were placed inside the cemented tanks (2 m × 1 m × 1.2 m) after 20 days of growth. Complete submergence was provided with salty water (12 dS m^{-1}) for 15 days. The depth of water was 100 cm. Plant height was taken before and after submergence. Survival count was taken after 10 days of de-submergence. The following formulas were used to calculate elongation and survival percent.

Elongation (%) = {(Plant height after submergence – Plant height before submergence) / (Plant height before submergence)}*100.

Survival (%) = {(Plant number before submergence – Plant number after 10 days of de-submergence) / (Plant number before submergence)}*100.

Evaluation of Submergence Tolerant Rice Germplasm for Medium Depth (~50 cm) Partial Submergence under Saline Environment

The experiment was conducted with sixty six rice cultivars. Three cultivars such as FR13A (susceptible to salinity but tolerant to both complete and partial submergence), Varshadhan (susceptible to salinity but tolerant to partial submergence) and Rashpanjor (tolerant to both salinity and partial submergence) were used as checks (Sarkar *et al*., 2013; Sarkar and Ray, 2016). Complete submergence is more detrimental than partial submergence (personal observation). Therefore, greater emphasis was given in selection of accessions for further testing under partial submergence with salt stress. All the accessions which showed ≥ 60% survival were selected for the study leaving behind all the accessions which showed 100% mortality under complete submergence with salt stress. A set of 63 accessions was prepared with mostly tolerant to highly submergence tolerant type materials to test for partial submergence with salt stress. Plants were grown as described above; however, after 30 days of growth another dose of urea @ 100 mg pot^{-1} was applied. Partial submergence (depth of water 45 cm) with salinity (12.0 ± 0.2 dS m^{-1}) was imposed on forty five days old seedlings (Sarkar and Ray, 2016). Concentration of salt was checked in alternate days. The depth of water and salt concentration were maintained also in alternate days. Scoring was done after 10 and 15 days of treatment following 1-9 scale as follows:

Score 1: No apparent damage – highly tolerant, Score 3: Little damage of leaf, healthy stem – tolerant, Score 5: Greater damage of leaf, less damage of stem – medium tolerant, Score 7: Greater damage of both leaf and stem – susceptible, and Score 9: Almost dead – highly susceptible.

Measurement of Normalized Difference Vegetation Index (NDVI) and Photochemical Reflectance Index (PRI)

The NDVI and PRI readings were taken from 2nd leaf from top using Plant Pen NDVI 300 and PRI 200 (Photon System Instruments, Czech Republic), respectively according to the following formula after 10 days of partial submergence with saline water.

NDVI (Normalized Difference Vegetative Index) = (NIR760 – VIS635) / (NIR760 + VIS635)

PRI (Photochemical Reflectance Index) = (R525 – R592)/ (R525 + R592)

Chlorophyll Estimation

After taking *PRI and NDVI* reading, same leaf was used for the measurement of total chlorophyll (Chl) content, which comprised both Chl *a* and Chl *b*. Three leaves were pooled together, finely chopped and mixed thoroughly. One hundred mg chopped leaf was placed in a capped measuring tube containing 10 mL of pre-chilled 80% acetone, and placed inside a refrigerator (4°C) for 48 h. The chlorophyll was measured spectrophotometrically at 646.6 and 663.6 nm following Sarkar and Ray (2016).

Chl *a* and Chl *b* are calculated using the following equations:

Chl *a* (mg g^{-1} fresh weight) = $\{12.25 \times (A_{663.6}) - 2.25 \times (A_{646.6})\} \times 10$ / Sample weight (g) × 1/1000

Chl *b* (mg g^{-1} fresh weight) = {20.31 × ($A_{646.6}$) – 4.91 × ($A_{663.6}$)} × 10 / Sample weight (g) × 1/1000

Statistical Analysis

Statistical analysis for different parameters was done using the *CropStat* software (International Rice Research Institute, Philippines). Mean values were compared by the least significant difference (LSDp*<0.05), wherever the *F*-test was significant. Associations among different parameters were examined by simple correlation analysis using the same package.

Principal component analysis (PCA) was performed to understand the pattern of data matrix, identifying tolerant germplasm in response to important contributing factors under partial submergence with salt stress. Eigen vectors and principal components were calculated and a 2-D biplot was generated between PC-I and PC-II by SAS enterprise guide 4.3 (www. support.sas.com/publishing).

Results

Genotypes Tolerant to Combined Effect of Submergence and Salt Stress

Among the one hundred ninety nine accessions, only three genotypes such as IC-449848, IC-580261 and IC-43351 showed more than 90% survival under submergence with saline water (Table 1, Fig. 1). Elongation % varied greatly even among highly submergence tolerant accessions (Table 1). The genotypes were divided into five groups based on survival % under submergence with saline water (Fig. 2). Group 1 consisted of 91 highly susceptible genotypes where survival percentage was below 20%. Twenty three genotypes were in the sensitive Group 2 where survival % was more than 20%

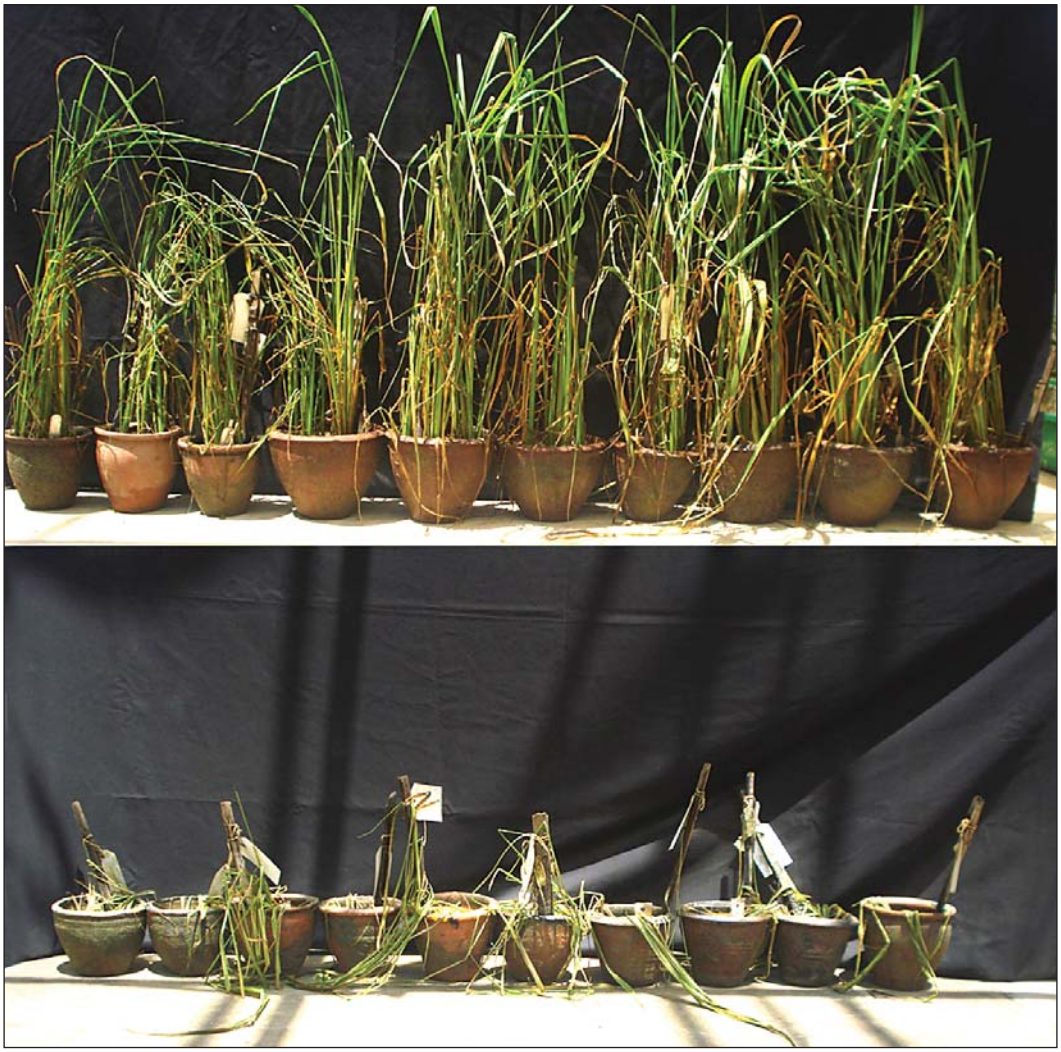

Fig. 1. Phenotypic characteristics of different rice genotypes under the impacts of submergence with salty water (12 dS m^{-1}).

Table 1. Elongation and survival percentage under submergence with saline water (12 dS m^{-1}) of highly tolerant accessions

Name	Height (BS) in cm	Height (AS) in cm	Elongation (cm)	Elongation (%)	Survival (%)
IC - 450262	31.6	36.7	5.1	16.2	80
IC-574839	34.4	57.6	23.2	67.4	80
IC-463054	36.9	49.3	12.3	33.4	80
IC-461222	36.4	56.6	20.2	55.7	80
IC-450092	43.2	54.5	11.3	26.3	82
IC-451031	35.8	53.8	18.0	50.3	82
AC-43409	38.9	43.7	4.8	12.2	84
IC-450639	34.6	46.3	11.8	34.1	84
AC-43365	39.7	48.3	8.7	21.9	85
IC - 459649	29.6	37.0	7.4	24.9	85
IC-419465	32.3	45.2	12.9	39.9	85
IC - 17024	37.3	47.7	10.3	27.7	86
IC - 449745	29.8	41.0	11.2	37.6	86
AC-43391	30.5	36.7	6.2	20.4	86
IC-449750	34.3	49.7	15.5	45.2	86
IC-145357	35.5	46.3	10.9	30.7	87
IC - 459362	37.7	43.7	6.0	15.8	88
IC-451469	37.4	44.3	6.9	18.4	88
IC-450292	33.3	44.4	11.2	33.6	90
FR 13A	31.4	34.7	3.3	10.4	90
IC-449848	30.2	45.5	15.3	50.7	92
IC-580261	26.8	33.8	7.0	26.0	92
AC-43351	31.7	35.7	4.0	12.7	95
$LSD_{*<0.05}$	3.6	4.9	2.4	9.8	6

BS-plant height before submergence; AS- plant height after 15 days of submergence.

but below 40%. Highly tolerant genotypes (Group 5), which showed more than 80% survival consisted of twenty three genotypes. Group 4 designated as tolerant group consisted of 17 genotypes (survival % > 60% but below 80%), whereas, intermediate tolerant Group 3 where survival % was above 40% but below 60% was comprised of 45 genotypes. Average elongation % was greater in Group 2 and Group 1, followed by Group 3, Group 4 and Group 5 (Fig. 3). It was 54.6%, 58.3%, 49.8%, 40.9% and 30.9%, respectively.

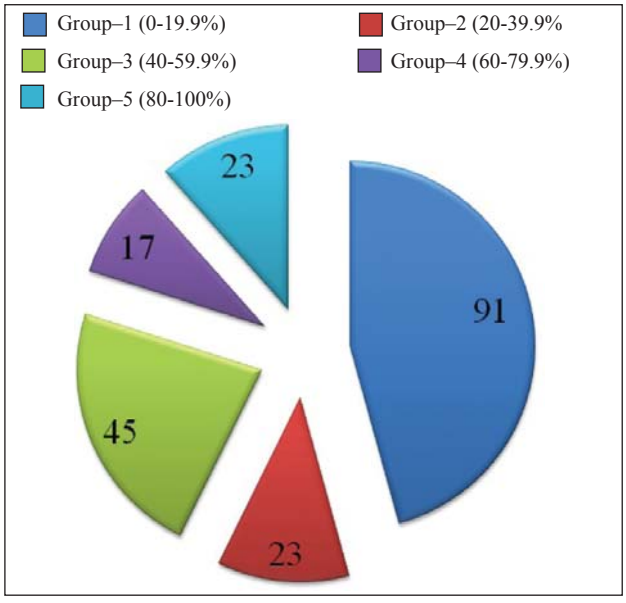

Fig. 2. Plant mortality differs greatly under the impacts of submergence with salty water (12 dS m^{-1}). Group was made base on survival %. Range of survival % is given in parenthesis.

Genotypes Tolerant to Combined Effect of Partial Submergence and Salt Stress

FR13A, a highly submergence tolerant cultivar showed score of 9 even after 7 days of partial submergence treatment with saline water (data not shown). After 10 days of treatment the cultivar FR13A was completely died out. Among the rest sixty five accessions two exhibited score of 9; twenty five showed score of 7, twenty five showed score of 5 and thirteen showed score of 3 (Table 2). None of the tested accessions showed score of 1. After 15 days of the treatment, the scoring increased greatly and none of the accessions even showed a score of 3. Among the thirteen accessions, which showed score of 3 after 10 days of partial submergence with salty water; ten showed score of 5 and three showed score of 7 after 15 days of the imposition of the treatment. The rest of

Table 2. Combined effect of stagnant flooding and salinity on plant pigment and survival score

Sl. No.	Genotypes	Chlorophyll content (mg g^{-1} fresh weight)			NDVI	PRI	Score	
		Chl *a*	Chl *b*	Chl T			10d	15d
1	IC-574839	0.74	0.25	0.99	0.324	0.008	9	9
2	IC-451031	0.66	0.20	0.82	0.355	0.009	9	9
3	Varshadhan	0.82	0.28	1.10	0.397	0.014	7	9
4	IC-545261	1.02	0.35	1.37	0.392	0.015	7	9
5	IC-459958	0.82	0.30	1.11	0.320	0.007	7	7
6	IC-450369	0.90	0.34	1.23	0.378	0.007	7	7
7	EC-496903	0.92	0.35	1.27	0.395	0.015	7	9
8	AC-41620	1.17	0.38	1.55	0.399	0.017	7	7
9	IC-381330	0.92	0.32	1.24	0.420	0.016	7	9
10	AC-1764	1.76	0.57	2.33	0.322	0.006	7	9
11	AC-43314	1.17	0.37	1.55	0.429	0.017	7	9
12	IC-461253	1.70	0.56	2.25	0.503	0.032	7	7
13	IC-450067	0.58	0.20	0.79	0.354	0.011	7	9
14	IC-578006	0.63	0.21	0.85	0.394	0.012	7	9
15	IC-450135	1.05	0.34	1.40	0.485	0.023	7	7
16	IC-459931	1.70	0.55	2.26	0.537	0.026	7	9
17	IC-463954	1.24	0.38	1.62	0.448	0.019	7	9
18	IC-463054	1.66	0.52	2.19	0.478	0.030	7	9
19	IC-461222	0.72	0.26	0.98	0.339	0.018	7	7
20	IC-450092	0.65	0.25	0.90	0.409	0.018	7	9
21	AC-43409	1.48	0.48	1.95	0.488	0.021	7	7
22	AC-43391	1.08	0.33	1.41	0.502	0.018	7	9
23	IC-145357	1.00	0.29	1.28	0.531	0.020	7	9
24	IC-459362	1.13	0.35	1.47	0.487	0.017	7	9
25	IC-451469	1.25	0.36	1.62	0.470	0.025	7	9
26	IC-450292	1.13	0.36	1.49	0.493	0.022	7	9
27	IC-580261	0.77	0.26	1.03	0.401	0.012	7	9
28	AC-43358	1.66	0.51	2.17	0.339	0.052	5	7
29	IC-98834	1.53	0.48	2.01	0.561	0.032	5	7
30	IC-459884	1.20	0.40	1.59	0.501	0.026	5	7
31	IC-459889	1.63	0.55	2.18	0.498	0.029	5	7
32	IC-449831	1.34	0.42	1.76	0.533	0.028	5	7
33	IC-381834	2.00	0.66	2.66	0.658	0.048	5	7
34	IC-461224	1.32	0.47	1.78	0.612	0.048	5	7
35	IC-261125	1.54	0.53	2.07	0.654	0.047	5	7
36	AC-43359	1.37	0.48	1.85	0.559	0.042	5	7
37	EC-516602	1.45	0.53	1.98	0.476	0.039	5	7
38	IC-330793	1.67	0.55	2.22	0.63	0.042	5	7
39	IC-451378	1.28	0.39	1.67	0.587	0.039	5	7
40	IC-399488	1.51	0.57	2.08	0.554	0.041	5	7
41	IC-450443	1.17	0.40	1.57	0.515	0.030	5	7
42	IC-459794	0.94	0.31	1.26	0.537	0.029	5	9
43	IC-450262	0.76	0.26	1.02	0.55	0.023	5	7
44	IC-450639	0.94	0.32	1.26	0.521	0.029	5	7
45	AC-43365	1.68	0.60	2.28	0.501	0.032	5	7
46	IC-419465	1.69	0.52	2.21	0.617	0.035	5	9
47	IC-449745	1.56	0.51	2.07	0.584	0.026	5	7
48	IC-449750	1.67	0.50	2.17	0.589	0.037	5	9
49	IC-449848	1.38	0.44	1.82	0.549	0.022	5	9
50	AC-43351	1.91	0.58	2.49	0.480	0.019	5	9

Contd.

Sl. No.	Genotypes	Chlorophyll content (mg g^{-1} fresh weight)			NDVI	PRI	Score	
		Chl *a*	Chl *b*	Chl T			10d	15d
51	IC-491212	2.08	0.79	2.87	0.552	0.031	5	9
52	AC-43353	1.57	0.54	2.11	0.543	0.034	5	9
53	Rashpanjor	2.10	0.61	2.72	0.560	0.046	3	5
54	IC-545518	1.23	0.43	1.65	0.517	0.036	3	5
55	IC-459733	1.87	0.58	2.45	0.659	0.057	3	5
56	IC-499780	2.30	0.75	3.05	0.631	0.054	3	5
57	IC-450250	2.60	0.80	3.41	0.596	0.047	3	7
58	IC-115617	2.11	0.72	2.83	0.557	0.049	3	5
59	AC-39460	1.34	0.54	1.88	0.547	0.033	3	7
60	AC-39293	2.59	0.82	3.41	0.660	0.053	3	5
61	IC-459744	1.21	0.42	1.63	0.518	0,032	3	5
62	IC-469712	2.48	0.85	3.33	0.672	0.057	3	5
63	IC-464746	1.00	0.41	1.42	0.590	0.043	3	7
64	IC-459649	1.84	0.61	2.45	0.646	0.058	3	5
65	IC-17024	1.37	0.46	1.83	0.614	0.041	3	7
	$LSD_{*p<0.05}$	0.23	0.18	0.35	0.061	0.006	–	–

NDVI, Normalized Difference Vegetative Index); PRI, Photochemical Reflectance Index; Score: No apparent damage (Score 1), Little damage of leaf, healthy stems (Score 3), Greater damage of leaf, less damage of stem (Score 5), Greater damage of both leaf and stem (Score 7), and Almost dead (Score 9)

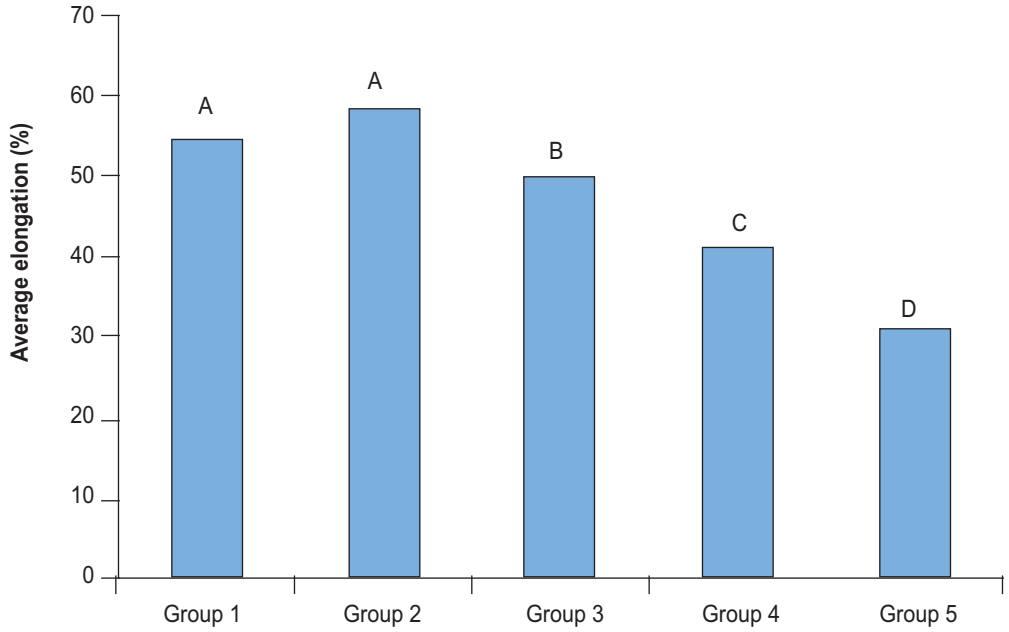

Fig. 3. Average elongation (%) in different survival groups under submergence with 12 dS m^{-1} saline water. Different letters across the groups indicate the significant differences whereas the same letter shows the non-significant difference.

the accessions either showed score of 7 or 9 after 15 days of the partial submergence with salty water.

Variation in Pigment Level, NDVI and PRI under Partial Submergence with Salinity

Levels of pigment content among the different accessions were significantly different (Table 2). Four accessions such as IC-491212, IC-499780, AC-39293 and IC-469712 maintained greater chlorophyll level compared to other. Total chlorophyll as well as both Chl *a* and Chl *b* contents were more than 3 mg g^{-1} fresh weight of leaf in these genotypes. NDVI reading ranged from 0.320 to 0.672 among the different accessions. NDVI reading

Table 3. Average value of different pigment content, light radiance (NDVI) and light reflectance (PRI) under different scoring group. Data represent as mean ± standard error

Score Group	Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	Chl T (mg g^{-1} FW)	NDVI	PRI
Score 9	0.70 ± 0.04	0.22 ± 0.03	0.92 ± 0.06	0.339 ± 0.015	0.009 ± 0.0001
Score 7	1.08 ± 0.07	0.36 ± 0.02	1.44 ± 0.09	0.439 ± 0.015	0.019 ± 0.001
Score 5	1.44 ± 0.06	0.48 ± 0.02	1.92 ± 0.08	0.548 ± 0.014	0.035 ± 0.002
Score 3	1.92 ± 0.15	0.63 ± 0.04	2.55 ± 0.19	0.601 ± 0.014	0.048 ± 0.002

Chl, chlorophyll; T, total; NDVI, Normalized Difference Vegetative Index; PRI, Photochemical Reflectance Index

more than 0.600 was observed in twelve accessions such as IC-381834, IC-461224, IC-261125, IC-330793, IC-419465, IC-491212, IC-459733, IC499780, AC-39293, IC-469712, IC-459649 and IC-17024. Similarly, the reading of PRI also varied among the different accessions. It ranged from 0.006 to 0.061. The PRI value was greater in IC-491212 (0.061), followed by IC-459649 (0.058), IC-469712 and IC-459733 (0.057), IC-499780 (0.054) and AC-39293 (0.053).

Table 3 depicts the average value of different pigment content, light radiance (NDVI) and light reflectance (PRI) under different scoring groups. The average values of Chl *a*, Chl *b*, Chl T, NDVI and PRI was greater in the group which showed score of 3 compared to score of Group 5, 7 and 9. The value was in the order of Score 3 > Score 5 > Score 7 > Score 9. The values of Chl *a*, Chl *b*, Chl T, NDVI and PRI under score 3 were 174%, 186%, 177%, 77% and 433% greater respectively, compared to score of 9 whereas in comparison to score 7 the values were 54%, 64%, 56%, 29% and 111% greater.

Correlation and Regression Analysis

Correlation coefficients were determined among different physiological parameters taken after 10 days of partial submergence with salinity and scoring (Table 4). Pigment content, light radiance and reflectance parameters showed highly significant (*p<0.001****) association. The 'r' values of Chl *a*, Chl *b*, Chl T, NDVI and PRI with the score taken after 10 days of treatment were -0.666***, -0.695***, -0.675***, -0.720*** and -0.811***, respectively. These parameters also showed highly significant negative association with the score taken after 15 days of treatment. The association between scoring after 10 days of treatment and after 15 days of treatment was highly positively significant (r = 0.662***).

Principal Component Analysis and Biplot Construction

PCA was done taking data of NDVI, PRI, Chl *a*, Chl *b*, Chl T and scoring at 10 days after partial submergence with saline water of 65 rice accessions. The first two principal components explained around 93% of the total variability among the germplasm accessions. Eigen vectors data (data not given) revealed that the positive effect of Chl *a* (0.42), Chl *b* (0.43), Chl T (0.43), NDVI (0.37), and PRI (0.41) and negative effect of score (–0.38) at 10 days after treatment contributed significantly to the first principal component (PC-I), and the negative effect of NDVI and PRI and the positive effect of Chl *a*, Chl *b*, Chl T and score contributed to PC-II. The Eigen vector size also depicted that Chl a, b, total chlorophyll and PRI contributed equally followed by NDVI and susceptibility score to the positional distribution of genotypes in the biplot based on PCA (Fig. 4). Rashpanjor, a popular rice genotype in coastal saline area tolerant to partial submergence and salinity belonged to a quarter of biplot with both positive effect of PC-I and PC-II. But due to

Table 4. Correlation among different pigment associated parameters with scoring under combined effect of stagnant flooding and salt stress

Parameters	Chl *a*	Chl *b*	Chl T	NDVI	PRI	Score 10d
Chl *b*	0.982***	---				
Chl T	0.999***	0.990***	---			
NDVI	0.619***	0.634***	0.624***	---		
PRI	0.731***	0.768***	0.745***	0.822***	---	
Score 10d	-0.666***	-0.695***	-0.675***	-0.720***	-0.811***	---
Score 15d	-0.423***	-0.484***	-0.439***	-0.501***	-0.643***	0.662***

***, significance at 0.001 level; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; Chl T, total chlorophyll; NDVI, Normalized Difference Vegetative Index; PRI, Photochemical Reflectance Index; Score 10d, 10 days after combined effect of stagnant flooding and salt stress.

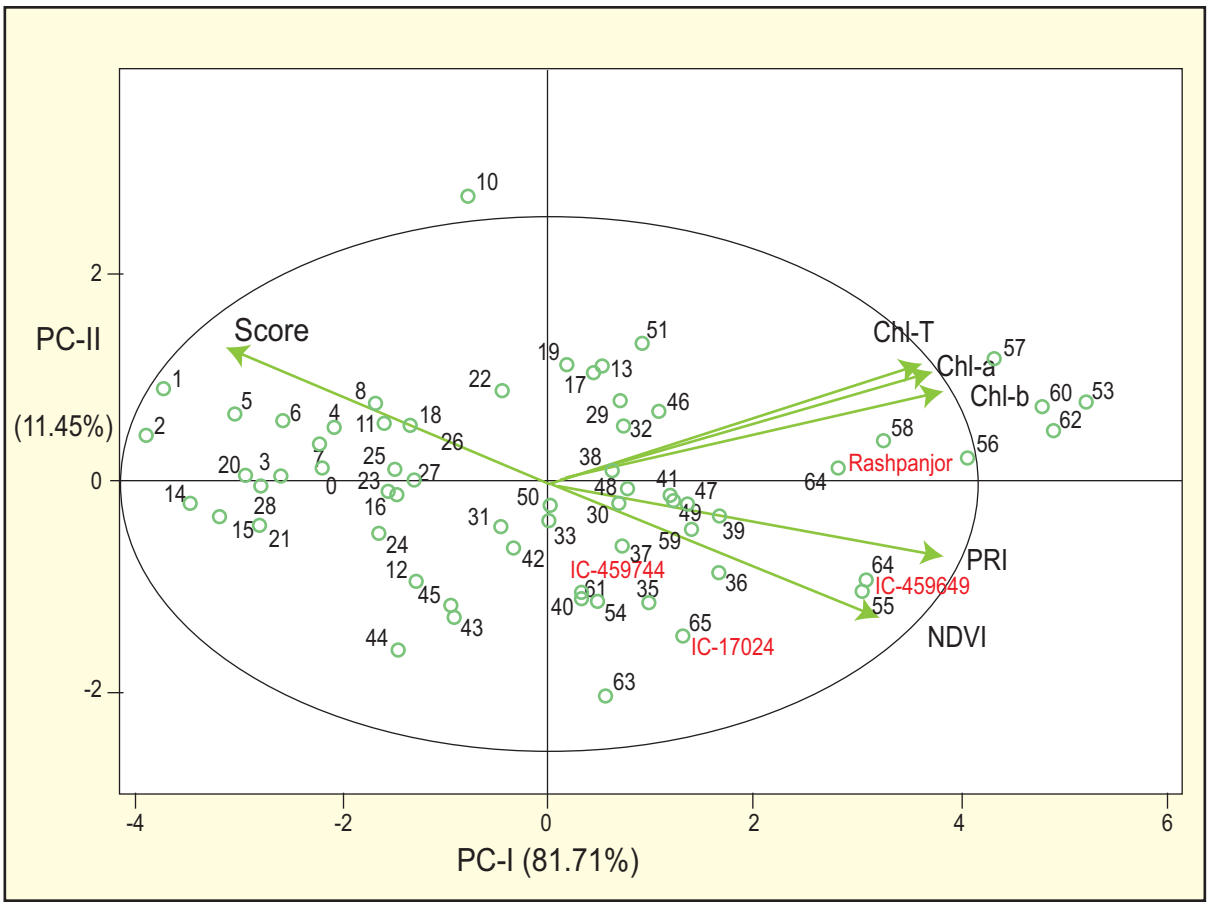

Fig. 4. Diversity analysis among sixty-four rice genotypes based on various phenotypic traits such as chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl T), normalized difference vegetative index (NDVI), photochemical reflectance index (PRI) and susceptibility score by principal component analysis under partial submergence with salt stress.

its position near to equatorial line in biplot, Chl *a*, Chl *b*, total chlorophyll, PRI and NDVI all are supposed to be with nearly equal contribution to its low scoring. In contrary tolerant rice genotypes for both complete and partial submergence with salty water, IC-459744, IC-459649 and IC-17024 belonged to a quarter of the biplot with positive effect of PC-I and negative effect of PC-II.

Discussion

Identification of specific genotype(s) within the cultivated gene pool and their utilization in crop improvement is still the most trusted and successful approach (Reddy *et al*., 2009; Sarkar *et al*., 2009; Chattopadhyay *et al*., 2014; Singh *et al.*, 2016). Coastal areas are more vulnerable than inlands. Multiple abiotic stresses are common in the same growing season depending on the rainfall pattern, high wind and higher tidal wave. Rice is the only crop that could be grown in this hostile environment when all other crops get lost (Mahata *et al*., 2010). Genotypes tolerant to complete submergence under saline environment are identified (Figs. 1-2, Table 1). FR13A, a genotype tolerant to submergence but susceptible to salinity (Sarkar *et al*., 2013) showed 90% survival under submergence with salt stress where as Rashpanjor, a genotype tolerant to salinity but susceptible to submergence totally succumbed under such conditions. Highly submergence tolerant genotypes showed less elongation compared to susceptible genotypes (Fig. 3). This is in conformity with earlier findings (Sarkar and Bhattacharjee, 2011; Ray *et al*., 2017). Floodwater quality largely determines the survival under submergence (Das *et al*., 2009). In this situation though floodwater was saline, the effect was as usual like normal water (Xu *et al*., 2006; Sarkar and Panda, 2009). Due to the lack of active transpiration under submergence, Na^+ built up inside the plant body was inhibited and thus effect of salinity was not noticed under complete submergence

with salt stress. In the same hypothesis, FR13A though susceptible to salinity could survive submergence stress under saline environment (Sarkar and Ray, 2016).

Genotypes showed tolerant reaction under submergence with salinity were not necessarily tolerant to partial submergence with salinity (Table 2). FR13A succumbed after 7 days of the imposition of the partial submergence with salt stress. Like FR13A, two highly submergence tolerant genotypes such as IC-574839 and IC-451031 showed susceptible reaction and after 10 days of the treatment were almost dead (Table 2). Rashpanjor which showed susceptible reaction under submergence with salinity exhibited tolerant reaction under partial submergence with salinity. This shows that salinity tolerant genotypes have an edge to withstand the partial submergence with salt stress over the salt-susceptible genotypes. Damage to plant under partial submergence with salinity increased with time. Thirteen genotypes showed tolerant reaction (score 3) after 10 days of partial submergence with salt stress. However, none of the genotypes showed sign of tolerant reaction after 15 days of the said stress (Table 2). Greater damage due to salinity with run of time was reported earlier (Singh and Sarkar, 2014).

The different physiological parameters such as chlorophyll levels, values of NDVI and PRI differed significantly under the combined stress of partial submergence and salt stress (Table 2). Few genotypes maintained greater values of these parameters compared to other genotypes. Restricting degradation of vital organ under stress is important for survival and growth as observed in any stress tolerance studies including this investigation. The genotypes were grouped into four categories with the score of 9, 7, 5 and 3 after 10 days of partial submergence with salty water. Average values of different pigment content, light radiance (NDVI) and light reflectance (PRI) under different scoring groups differed greatly (Table 3). The values of these parameters were maximum in the group with score 3, followed by 5, 7 and 9. Degradation of pigment level due to salinity as well submergence with salinity stresses were reported earlier (Yan *et al*., 2013; Singh and Sarkar, 2014; Sarkar and Ray, 2016). Different parameters showed distinct relationship among themselves (Table 4). Positive significant association of chlorophyll content and NDVI values with PRI was reported earlier (Hmimina *et al*., 2014; Soudani *et al*., 2014). Highly significant negative association of these parameters with scoring after 10 and 15 days of treatment revealed that maintenance of greater values of these parameters were the sign of plant fitness under stress.

Two dimensional biplot derived through PCA allowed to differentiate between genotypes with different tolerance levels (Fig.4). Some moderately tolerant genotypes co-localized with the vector for chlorophyll content traits in a cluster of the biplot (Fig. 4). But comparatively more tolerant genotypes with low susceptibility score localized in the just opposite cluster. The same cluster was dominated by PRI with long vector size. This indicated that PRI was the main determinant of tolerance for these genotypes.

Conclusion

Rice genetic resources tolerant to partial and complete submergence with salt stress are available among the tested genotypes which were mainly collected from coastal areas of India. The genotypes tolerant to submergence with salt stress were not always tolerant to partial submergence with salt stress. Genotypes tolerant to both partial and complete submergence with salt stress identifies in this investigation are of great value in developing the high yielding varieties tolerant to multiple abiotic stresses. The results show that the Photochemical Reflectance Index (PRI) provides a non-invasive and rapid method for investigating stress effects on photosynthetic machinery better compared to chlorophyll content and Normalized Difference Vegetative Index (NDVI).

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RESEARCH ARTICLE

Variability and Yield Contributing Principal Components in Nutmeg (*Myristica fragrans* Houtt.)

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In nutmeg, fruit weight, mace volume and pericarp thickness have direct and kernel volume, mace dry weight and fruit set have indirect effects on yield. Selection for higher yield should be based on most contributing principal component fruits/tree, followed by plant girth, canopy spread, flowers/10cm^2, fruit set and fruit weight.

Key Words: Core collections, Genetic variability, *Myristica fragrans*, Path analysis, Principal components

The nutmeg tree (*Myristica fragrans* Houtt.) is source of two distinct spices; namely nutmeg and mace, valued highly for flavouring and medicinal properties. The crop native to Moluccas, Indonesia, is presently cultivated in India and many other tropical countries. High amount of variability has been reported throughout the nutmeg growing tracts of India with respect to the growth rate and flushing pattern (Nazeem, 1979), productivity, size and shape of the leaf, flower size and shape of the fruit and nut (Senthilkumar *et al.*, 2010; Miniraj *et al*., 2015b). The crop improvement studies carried out in the nutmeg growing regions in India have given special emphasis on yield maximization. A tree producing 3000 fruits per year along with other economic characters is considered as high yielder (Miniraj *et al.,* 2015a). Inheritance of most of the economic traits is complex in nutmeg and mainly involves fruit and tree characteristics. The path coefficient method is an important tool for revealing the true nature of effect-cause interrelations between yield and its primary components. PCA is an exploratory tool designed by Karl (1901) to identify unknown trends in a multi-dimensional data set. In the past, there has been no attempt to use path-coefficient and principal component analysis to study the yield traits in nutmeg. The present study was undertaken to determine a classificatory analysis on the yield components of nutmeg core collections from diverse locations of Kerala, India by means of analysing the variability and partitioning it into direct and indirect effects by path and principal component analyses. This would enable us to classify the available morphotypes into distinct groups on the basis of their genetic diversity.

The study was carried out at Kerala Agricultural University, Thrissur during 2012-15. Core collections represented all the nutmeg growing regions of Kerala and planted in Chalakudy river basin central parts of Kerala (latitude 10.33ºN, longitude 76.33ºE), formed the material for study. Among the forty six selected accessions, forty two were females and four monoecious with same age group of fifteen years as per farmers registers. In each accession, observations were recorded from two trees per accession during two consecutive bearing seasons.

Following Completely Randomised Design (CRD), analysis of variance was done primarily for each of the 38 quantitative traits. Among them, 26 traits were selected based on their contribution to yield, statistical significance and economic importance. The analyses on variability, path coefficient and principal components were performed based on these 26 traits. The computer package SPSS v.16 (SPSS, 2007) was used for the analyses.

The accessions in the present study showed a wide range of variation for yield attributes (Table 1) *viz*.,

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Table 1. Variability in nutmeg morphotypes for yield contributing traits

Characters	Mean	Minimum	Maximum	CV (%)	Skewness	Kurtosis
Plant height (m)	7.79	3.20	12.35	4.53	0.24	0.73
Plant girth (cm)	44.77	20.33	63.51	5.54	-0.41	-0.09
Canopy spread (E-W)-(m)	5.80	3.11	9.03	8.51	0.50	0.12
Canopy spread (N-S)-(m)	5.77	3.13	8.85	6.54	0.30	0.02
Leaf area (cm^2)	33.94	20.29	54.63	10.45	0.53	0.88
No. of flowers/$10cm^2$	5.11	2.50	10.25	15.24	1.36	3.96
Fruit set percentage	22.96	6.15	44.15	5.29	0.05	-1.09
No. of fruits/m^2	14.66	2.75	31.50	18.59	0.44	-0.25
Fruit weight (g)	64.35	39.33	99.57	3.73	0.46	-0.28
Fruit length (mm)	57.55	42.15	66.25	2.18	-0.69	0.23
Fruit breadth (mm)	48.85	35.19	57.44	2.18	-0.68	0.39
Thickness of pericarp (mm)	11.94	8.26	15.70	6.21	0.16	-0.23
Fresh mace weight (g)	2.25	0.91	5.27	13.83	1.44	4.80
Dry mace weight (g)	1.12	0.46	2.62	13.47	1.32	2.72
Fresh nut weight (g)	10.01	4.42	13.67	8.74	-0.14	0.05
Dry nut weight (g)	7.07	3.56	11.01	6.93	0.27	0.14
Shell thickness (mm)	1.04	0.80	1.42	5.77	0.57	1.71
Kernel weight (g)	5.20	2.65	8.05	10.49	0.23	-0.49
Fruit volume (cm^3)	57.46	21.95	89.37	9.96	-0.32	1.74
Nut volume (cm^3)	9.49	4.00	13.21	6.18	-0.51	0.15
Mace volume (cm^3)	2.54	1.00	4.73	17.32	0.23	-0.71
Kernel volume (cm^3)	5.72	2.00	8.23	11.35	-0.64	0.80
Nut length (mm)	31.22	22.17	36.64	3.22	-0.54	-0.10
Nut breadth (mm)	23.99	16.88	34.72	2.45	0.23	3.33
Ratio of nut to mace	4.94	2.06	9.69	9.12	0.83	089
No. of fruits/tree	1398.09	27	4420	22.16	0.90	0.02

number of fruits/tree (22.16%), mace volume (17.32%), fresh mace weight (13.83%), dry mace weight (13.47%), kernel volume (11.35%) and kernel weight (10.49%), number of fruits/m^2 (18.59%), number of flowers/$10cm^2$ (15.24%) as well as leaf area (10.45%). The variation ranged from 27 to 4420 for number of fruits/tree, 1.0 to 4.73 cm^3 for mace volume, 0.91 to 5.27 g for fresh mace weight, 0.46 to 2.62 for dry mace weight, 2.0 to 8.23 cm^3 for kernel volume, 2.65 to 8.05 g for kernel weight, 2.75 to 31.50 for number of fruits/m^2, 2.50 to 10.25 for number of flowers/$10cm^2$ and 20.29 to 54.63 cm^2 for leaf area. The variability noticed in the fruit characters was ultimately reflected in the nutmeg yield. Senthilkumar *et al.* (2010) also reported wide range of variability in fruit characters of nutmeg under high altitude areas of Karnataka.

Significant skewness and kurtosis clearly exhibits normality of the distribution. It could be noted that majority of characters possessed positively skewed distribution and were controlled by additive gene action with complementary epistasis. The traits *viz*., plant girth, fruit length, fruit breadth, fresh nut weight, fruit volume, nut volume, kernel volume and nut length showed additive gene action with duplicate epistasis. Hence, if we go in selection programme based on these characters, we can bring improvement over base population. Similarly, majority of the characters possessed leptokurtic (positive) graphic distribution and were controlled by little genes (genes showing antagonistic affect on each other). Characters such as plant girth, fruit set percentage, number of fruits/m^2, fruit weight, thickness of pericarp, kernel weight, mace volume and nut length have shown platykurtic (negative) graphic distribution and were under the influence of many genes (genes showing synergetic affect each other). For all the genetic parameters, prediction of gene action was made as per suggestion given by Roy (2000). Similar results were reported by Shobha *et al.* (2013) in cashew.

Among the traits subjected to path analysis, fourteen traits showed positive direct effects on number of fruits per tree (Table 2). The pertinent data revealed that fruit weight (2.04), mace volume (1.98) and thickness of pericarp (1.29) exerted very high genotypic positive direct effect on number of fruits per tree and very high

Table 2. Path co-efficient analysis of nutmeg accessions for yield contributing characters

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	**22**	**23**	**24**	**25**	**rG**
1	**-0.23**	-0.01	-0.42	0.22	-0.10	-0.03	0.1	0.01	0.12	0.10	-0.21	0.45	0.40	-0.03	0.003	0.10	-0.01	-0.10	-0.09	0.48	-0.13	-0.23	-0.05	-0.003	0.0005	0.26
2	-0.13	**-0.02**	-0.43	0.23	-0.10	-0.043	0.023	0.004	0.46	-0.04	-0.25	0.58	0.10	0.09	0.002	-0.005	0.01	0.07	-0.35	0.12	-0.11	-0.03	-0.04	0.008	-0.001	0.16
3	-0.12	-0.01	**-0.81**	0.40	-0.09	0.098	0.20	0.013	0.53	0.02	-0.34	0.64	-0.53	0.25	-0.0003	-0.10	0.11	0.12	-0.35	0.016	0.23	0.04	-0.05	0.12	-0.005	0.38
4	-0.12	-0.01	-0.75	**0.42**	-0.09	0.10	0.15	0.005	0.43	0.02	-0.31	0.63	-0.61	0.29	-0.0003	-0.10	0.11	0.13	-0.31	-0.025	0.27	0.03	-0.02	0.10	-0.0060	0.34
5	-0.09	-0.01	-0.29	0.15	**-0.25**	0.01	-0.001	0.004	0.23	0.13	-0.12	0.39	0.29	-0.03	0.003	0.17	0.01	-0.17	-0.23	0.55	-0.26	-0.24	-0.10	-0.07	0.0002	0.07
6	0.01	0.002	-0.17	0.09	-0.01	**0.45**	-0.17	-0.01	0.18	-0.03	-0.09	0.15	-0.47	0.02	0.001	0.08	0.06	-0.09	-0.15	0.008	0.09	-0.07	-0.02	0.02	-0.005	-0.11
7	-0.02	-0.001	-0.19	0.08	0.0004	-0.10	**0.82**	0.054	0.53	-0.24	-0.24	0.31	-0.57	0.21	-0.01	-0.26	-0.01	0.37	-0.21	-0.90	0.23	0.37	0.18	0.19	0.005	0.62
8	-0.03	-0.001	-0.16	0.03	-0.014	-0.09	0.69	**0.064**	0.47	-0.16	-0.23	0.34	-0.005	-0.013	-0.004	-0.14	-0.01	0.23	-0.16	-0.70	0.10	0.23	0.09	0.18	0.008	0.72
9	-0.01	-0.004	-0.21	0.09	-0.03	0.04	0.21	0.01	**2.04**	-0.47	-0.72	1.16	-1.28	0.32	-0.01	-0.29	0.12	0.35	-1.69	-1.52	1.04	0.59	0.19	0.31	-0.003	0.27
10	0.02	-0.001	0.03	-0.013	0.05	0.02	0.28	0.01	1.36	**-0.70**	-0.46	0.72	-0.81	0.19	-0.01	-0.31	0.11	0.38	-1.24	-1.62	1.01	0.54	0.32	0.29	0.004	0.17
11	-0.06	-0.01	-0.36	0.17	-0.04	0.06	0.25	0.02	1.88	-0.41	**-0.77**	1.18	-1.32	0.33	-0.01	-0.26	0.14	0.33	-1.53	-1.31	1.04	0.56	0.16	0.33	-0.003	0.35
12	-0.08	-0.01	-0.40	0.21	-0.08	0.05	0.19	0.02	1.84	-0.39	-0.71	**1.29**	-1.10	0.32	-0.01	-0.25	0.11	0.39	-1.45	-1.01	0.73	0.36	0.10	0.30	-0.004	0.38
13	0.03	0.001	-0.15	0.09	0.03	0.08	0.16	0.0001	0.93	-0.20	-0.36	0.50	**-2.82**	0.76	-0.01	-0.20	0.13	0.24	-0.61	-0.87	1.65	0.39	0.12	0.20	-0.025	0.08
14	0.01	-0.002	-0.23	0.14	0.008	0.01	0.19	-0.001	0.75	-0.15	-0.29	0.47	-2.43	**0.88**	-0.005	-0.35	0.14	0.43	-0.41	-0.76	1.14	0.38	0.09	0.21	-0.022	0.19
15	0.04	0.0022	-0.02	0.01	0.06	-0.03	0.33	0.018	1.43	-0.47	-0.51	0.66	-1.25	0.35	**-0.01**	-0.55	0.11	0.68	-1.15	-2.23	1.18	0.83	0.31	0.40	0.003	0.21
16	0.04	-0.0001	-0.12	0.06	0.07	-0.05	0.32	0.013	0.89	-0.33	-0.31	0.49	-0.87	0.47	-0.012	**-0.66**	0.08	0.85	-0.68	-1.70	0.49	0.67	0.21	0.36	0.003	0.28
17	0.01	-0.001	-0.28	0.15	-0.008	0.09	-0.02	-0.003	0.79	-0.25	-0.34	0.48	-1.19	0.34	-0.005	-0.18	**0.30**	0.16	-0.56	-0.54	0.65	0.15	0.03	0.32	-0.007	0.10
18	0.03	-0.001	-0.12	0.07	0.05	-0.05	0.37	0.018	0.87	-0.32	-0.30	0.52	-0.82	0.45	-0.011	-0.66	0.06	**0.83**	-0.67	-1.67	0.51	0.71	0.21	0.33	0.002	0.41
19	-0.01	-0.003	-0.16	0.10	-0.032	0.04	0.095	0.006	1.94	-0.49	-0.67	1.05	-0.97	0.20	-0.01	-0.25	0.10	0.32	**-1.78**	-1.42	0.97	0.56	0.21	0.28	0.001	0.06
20	0.10	0.001	0.01	0.005	0.062	-0.002	0.33	0.02	1.38	-0.50	-0.45	0.58	-1.08	0.29	-0.014	-0.49	0.073	0.62	-1.12	**-2.25**	1.09	0.88	0.32	0.38	0.006	0.17
21	0.02	0.001	-0.09	0.06	0.034	0.02	0.10	0.003	1.08	-0.36	-0.41	0.47	-2.36	0.51	-0.008	-0.16	0.10	0.22	-0.87	-1.24	**1.98**	0.48	0.22	0.27	-0.015	-0.004
22	0.06	0.0004	-0.03	0.01	0.061	-0.03	0.30	0.015	1.22	-0.38	-0.44	0.47	-1.13	0.34	-0.011	-0.44	0.05	0.59	-1.01	-2.01	0.97	**0.99**	0.28	0.33	0.003	0.22
23	0.03	0.002	0.10	-0.02	0.062	-0.02	0.35	0.015	0.95	-0.54	-0.32	0.31	-0.79	0.19	-0.01	-0.33	0.024	0.42	-0.92	-1.75	1.05	0.68	**0.41**	0.20	0.007	0.09
24	0.002	-0.0003	-0.21	0.09	0.037	0.022	0.34	0.024	1.32	-0.42	-0.53	0.81	-1.19	0.38	-0.01	-0.48	0.20	0.57	-1.03	-1.76	0.88	0.68	0.17	**0.48**	-0.0002	0.38
25	-0.003	0.001	0.13	-0.08	-0.002	-0.075	0.14	0.015	-0.16	-0.09	0.08	-0.18	2.16	-0.59	-0.001	-0.06	-0.07	0.06	-0.08	-0.39	-0.93	0.09	0.09	-0.003	**0.03**	0.10

Residual value = -0.012 rG= Genotypic correlation coefficient of number of fruits per tree

1. Plant height
2. Plant girth
3. Canopy spread (E-W)
4. Canopy spread (N-S)
5. Leaf area
6. No. of flowers/10cm^2
7. Fruit set percentage
8. No. of fruits/m^2
9. Fruit weight
10. Fruit length
11. Fruit breadth
12. Thickness of pericarp
13. Fresh mace weight
14. Dry mace weight
15. Fresh nut weight
16. Dry nut weight
17. Shell thickness
18. Kernel weight
19. Fruit volume
20. Nut volume
21. Mace volume
22. Kernel volume
23. Nut length
24. Nut breadth
25. No. of fruits/tree
26. Ratio of nut to mace

negative direct effect was exerted by fresh mace weight (-2.82), nut volume (-2.25) and fruit volume (-1.78). Thondaiman and Rajamani (2014) in cocoa confirmed that pod characters should be considered with great emphasis as main yield components because these traits showed direct effects on yield. Findings of the present study again strongly confirms the reliability of the characters viz., fruit weight, mace volume, thickness of pericarp, kernel volume, dry weight of mace, fruit set percentage and number of fruits per tree towards selection of superior and high yielding genotypes.

The first ten principal component axes explained 86.90 per cent of the yield variability among the morphotypes under study (Table 3). The remaining fifteen axes contributed 13.01 per cent of the variability. The first principal component, plant height accounted 34.04 per cent contribution to the yield. While the subsequent nine principal components contributed 14.89, 10.75, 8.08, 5.20, 4.50, 3.55, 3.0 and 2.86 per cent, respectively. These characters were plant girth, canopy spread in E-W and N-S, leaf area, no. of flowers/10cm^2, fruit set percentage, no. of fruits/m^2 and fruit weight, respectively. The character contributing the maximum to the yield should be given greater emphasis in breeding programme (Majumder *et al*., 2013). The first principal yield component has additive gene action, which suggests that nutmeg breeding programme aimed for higher fruit yield should be through selection method of breeding strategy.

Yield levels in forty six morphotypes were differentiated by the Eigen vector (≥ 0.7) into different principal components (Table 4). The fruit weight, fruit length, fruit breadth, thickness of pericarp, fresh as well as dry nut weight, volume of fruit, nut and kernel and nut breadth were distinguished by the Eigen vector in the first principal component. The second principal component had plant height and canopy spread in both E-W & N-S directions which discriminated the forty six accessions. Number of fruits per tree significantly distinguished the yield levels in nutmeg in the third principal component. These traits played major role in all the four components for determining the yield of nutmeg.

Table 3. Eigen values and per cent variation of yield contributing traits of nutmeg

Sl. No.	Principal component axis	Eigen values	Variation (%)	Cumulative variation (%)
1	Plant height (m)	8.853	34.04	34.04
2	Plant girth (cm)	3.874	14.89	48.94
3	Canopy spread (E-W)-(m)	2.796	10.75	59.70
4	Canopy spread (N-S)-(m)	2.103	8.08	67.79
5	Leaf area (cm^2)	1.352	5.20	72.99
6	No. of flowers/10cm^2	1.171	4.50	77.49
7	Fruit set percentage	0.923	3.55	81.04
8	No. of fruits/m^2	0.781	3.00	84.04
9	Fruit weight (g)	0.744	2.86	86.90
10	Fruit length (mm)	0.520	2.00	88.90
11	Fruit breadth (mm)	0.462	1.77	90.68
12	Thickness of pericarp (mm)	0.396	1.52	92.20
13	Fresh mace weight (g)	0.361	1.38	93.59
14	Dry mace weight (g)	0.335	1.28	94.88
15	Fresh nut weight (g)	0.215	0.82	95.71
16	Dry nut weight (g)	0.190	0.73	96.44
17	Shell thickness (mm)	0.169	0.65	97.09
18	Kernel weight(g)	0.152	0.58	97.67
19	Fruit volume (cm^3)	0.134	0.51	98.19
20	Nut volume (cm^3)	0.117	0.44	98.64
21	Mace volume (cm^3)	0.104	0.39	99.04
22	Kernel volume (cm^3)	0.075	0.29	99.33
23	Nut length (mm)	0.059	0.22	99.56
24	Nut breadth (mm)	0.046	0.17	99.73
25	No. of fruits/tree	0.039	0.15	99.88
26	Ratio of nut to mace	0.030	0.11	100.00

Table 4. Principal component analysis on nutmeg yield traits (non-rotated values)

Sl. No.	Traits	Principal components (Eigen vector)			
		1	2	3	4
1	Plant height (m)	0.014	0.724	0.241	0.077
2	Plant girth (cm)	0.142	0.691	0.152	0.125
3	Canopy spread (E-W)-(m)	0.292	0.807	0.056	-0.117
4	Canopy spread (N-S)-(m)	0.289	0.798	-0.005	-0.121
5	Leaf area (cm^2)	-0.062	0.573	0.076	0.179
6	No. of flowers/10cm^2	0.030	0.111	-0.272	0.262
7	Fruit set percentage	0.496	0.021	0.520	-0.442
8	No. of fruits/m^2	0.361	0.116	0.614	-0.360
9	Fruit weight (g)	0.832	0.136	-0.058	0.352
10	Fruit length (mm)	0.729	-0.229	0.068	0.295
11	Fruit breadth (mm)	0.827	0.307	-0.032	0.295
12	Thickness of pericarp (mm)	0.711	0.432	-0.003	0.294
13	Fresh mace weight (g)	0.560	0.063	-0.656	-0.370
14	Dry mace weight (g)	0.549	0.134	-0.517	-0.515
15	Fresh nut weight (g)	0.847	-0.342	0.067	0.051
16	Dry nut weight (g)	0.723	-0.229	0.127	-0.244
17	Shell thickness (mm)	0.411	0.202	-0.311	0.093
18	Kernel weight(g)	0.688	-0.199	0.167	-0.335
19	Fruit volume (cm^3)	0.706	0.071	-0.068	0.563
20	Nut volume (cm^3)	0.834	-0.383	0.084	0.073
21	Mace volume (cm^3)	0.586	-0.058	-0.492	-0.043
22	Kernel volume (cm^3)	0.731	-0.316	0.063	-0.027
23	Nut length (mm)	0.659	-0.406	0.120	0.116
24	Nut breadth (mm)	0.844	-0.056	0.050	-0.053
25	No. of fruits/tree	-0.077	-0.270	0.778	0.384
26	Ratio of nut to mace	0.386	0.331	0.503	-0.411

The separation of the accessions under study into five clusters proves the high level of variability for yield traits (Fig.1). Cluster I housed only one nutmeg accession (Acc. 9) which was noticed distinct form other accessions with respect to the number of fruits per tree along with good nutmeg and mace weight.

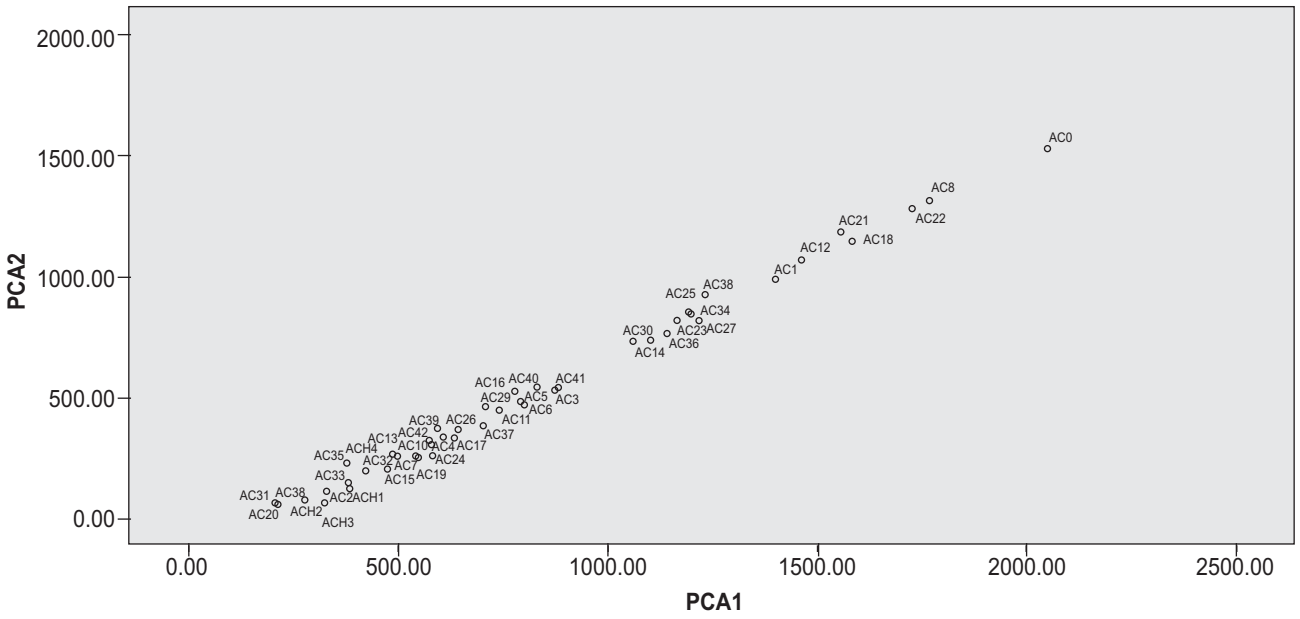

Fig. 1. Grouping of nutmeg accessions based on principal component analysis studied for yield contributing traits

This accession bears the highest number of fruits (4420) against the mean of 1398; mace weight 2.52 g and 1.44 g against the mean of 2.25 g and 1.12 g fresh as well as dry, respectively; nut weight 11.44 g and 8.37 g against the mean of 10.01 g and 7.07 g fresh as well as dry, respectively. Cluster II had two accessions viz., Acc. 8 and Acc. 22. Though these accessions are categorised into high yielding types along with Acc. 9 of cluster I, these cluster members possessed medium nut and mace weight. Further, cluster III included four cluster members viz., Acc. 1, Acc. 12, Acc. 18 and Acc. 21. All the trees in this group are medium yielders with respect to number of fruits per tree as well as other yield donating traits. In cluster IV, there were eight accessions which were medium yielding with average nut and mace weight, and other yield related traits. All the 31 accessions in cluster IV were very much similar in yield attributing traits. In this group both female as well as monoecious accessions clustered together because of average values in number of fruits per tree and nut and mace characters.

This study revealed that fruit weight, mace volume and thickness of pericarp had a very high direct effect on yield and kernel volume, dry weight of mace, and fruit set percentage had positive effect through number of fruits/tree. The PCA on yield parameters has shown that number of fruits/tree is most contributing towards the yield, followed by plant girth, canopy spread in E-W and N-S, leaf area, no. of flowers/10cm^2, fruit set percentage, and fruit weight. The skewness and kurtosis for the leading traits were number of fruits/tree as well as ratio of nut to mace and thus we suggest the selection method for breeding nutmeg for higher yield.

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RESEARCH ARTICLE

Morpho-molecular Variation in Indian Finger Millet (*Eleusine coracana* (L.) Gaertn.) Varieties and Landraces

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A total of 38 finger millet genotypes including 18 released varieties and 20 landraces from India were evaluated and compared based on RAPD, ISSR markers and morphological data collectively. RAPD markers showed higher polymorphism (61.62 %) as compared to ISSR (57.00 %) markers. The mean number of bands per primer and Jaccard's similarity coefficients generated for the markers RAPD, ISSR was 12.9; 0.810 and 14.3; 0.782 respectively. UPGMA clustering, and Structure analysis placed the landraces and varieties in separate groups with some exceptions. The data for 13 qualitative traits revealed diversity mainly for ear shape and ear size. ANOVA analysis of the 14 quantitative traits showed most of the characters to have significant difference, representing the presence of genotypic differences among the 38 genotypes studied. The morpho-variability as well as variation based on molecular markers was higher in the landraces compared to the varieties. The usage of more diverse landraces as genitors to augment the genetic base and identify genes for adaptive and nutritional variations as well as conservation of more landraces to maximize the available genetic diversity of finger millet was emphasized.

Key Words: ISSR, Landraces, Morphological, RAPD, Varieties

Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.), commonly known as ragi is an annual tetraploid and crop of immense importance due to its inherent resilience and nutritional qualities like high fibre, quality protein, rich mineral composition and neutraceuticals. It belongs to family Poaceae (Gramineae) and harbors enough genetic variability to be utilized for its further improvement as a nutritionally superior and subsistence crop for posterity. Finger millet is an important crop in India, particularly in the states viz. Karnataka, Tamil Nadu and Andhra Pradesh. Varieties used in the present study have been developed by pure line selection or hybridization (Indian × African; Indian × Indian). Indo-African crosses resulted in the development of many popular varieties. More and more finger millet genetic resources need to be characterized for their utilization as parents for development of improved varieties or generation of genomic resources. Landraces can serve as one such important genetically diverse plant genetic resource for crop improvement. Plant landraces were defined as "a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems" (Villa *et al.*, 2005). Landraces were also defined as "balanced populations – variable, in equilibrium with both environment and pathogens and genetically dynamic" (Harlan, 1971). Landraces are the repertoire of diverse and rare alleles and are unique with respect to different traits of importance viz. tolerance to various biotic and abiotic stresses. They are sources of novel variation which must be characterized for their conservation, management and utilization.

Morpho-molecular characterization of the available genetic resources will help us in providing a holistic assessment of the level of existing genetic variation in finger millet. There are limited reports in which RAPD and ISSR markers were used for molecular characterization of finger millet (Fakrudin *et al*., 2004, Babu *et al*., 2007, Panwar *et al*., 2010; Ramakrishnan *et al*., 2015; Arya *et al*., 2016). There is always a scope for characterization of different set of germplasm using different markers in combination with its phenotypic

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characterization for effective utilization of crop genetic resources. In this study, released varieties and landraces (Karnataka, India) of finger millet were evaluated and compared using RAPD, ISSR markers and quantitative and qualitative traits collectively. The information generated through the morphological and molecular markers will complement each other in germplasm management including their utilization.

Materials and Methods

Plant Materials

For the current study, the seeds of 18 varieties were obtained from Regional Research Station, Mandya, Karnataka and 20 landraces were taken from the active collection at University of Agricultural Sciences (UAS), Bangalore (Table 1).

Table 1. Details of varieties and landraces of finger millet used for this study

S. No.	Variety/Landraces	Pedigreee / Source/place of collection	Year of release/collection
Varieties			
1	Poorna(IC075476)	CO 1 × Aruna	1959
2	Hamsa*(IC475470)	Selection from germplasm at Hebbal	1968
3	PR 202(IC402988)	Pure Line selection from dry ragi (Mettachodi) of Arakuvelly of Andhra Pradesh	1976 and 1982
4	INDAF 1(IC402565)	Pure line selection from African germplasm	1978
5	INDAF 3(IC402566)	Cauvery × IE 927	1978
6	Shakthi(IC75479)	Roo 13 × H 22	1978
7	INDAF 5(IC403042)	Cauvery × IE 927	1978
8	INDAF 7(IC402911)	Annapurna × IE 927	1984
9	INDAF 8(IC474181)	Hullubele × IE 929	1986
10	INDAF 9(IC403043)	K 1 × IE 980 R	1988
11	HR 911(IC403096)	UAS 1 × IE 927	1986
12	INDAF 15(IC403101)	IE 927 × IE 67	1991
13	MR 2(IC403094)	PR 202 × IE 927	1996
14	GPU 26(IC312307)	INDAF 5 × (INDAF 9 × IE 1012)	1998
15	MR 1(IC403093)	Hamsa × IE 927	1999
16	L5(IC312321)	Malavi × INDAF 9	1999
17	GPU 28(425957)	INDAF 5 × (INDAF 9 × IE 1012)	2000
18	GPU 45	GPU 26 × L 5	2001
Landraces			
19	GE 1296(IC475994)	Kari Ragi II, Karnataka	1977
20	GE 3353(IC478155)	Karimuddagaragi, Panchavalli, Karnataka	1981
21	GE 656(IC476023)	Kotekariaragi, Telaginakuppepura, PeriyapatnaTq. Karnataka	1977
22	GE3314(IC477427)	Devanagiriragi, Arabethittu, Herugur Taluka, Karnataka	1981
23	GE 741(IC476358)	Madaiahanagiriragi, Kolegala Taluk, Karnataka	1977
24	GE 3322(IC477483)	Doddaragi, Kolipalya, CR Nagar, Karnataka	1981
25	GE 3321(IC477614)	Karikaddiragi, Kolipalya, CR Nagar, Karnataka	1981
26	GE 1447(IC475267)	Beligiddaragi, Karnataka	1977
27	GE 1412(IC475467)	Hasiruragi, Karnataka	1977
28	GE 3371(IC477213)	Pullapudhugaragi, Gangadahosahalli, HD kote	1981
29	GE 1364(IC474936)	Shivpura local, Karnataka	1977
30	GE 736(IC475369)	Bettapura local, Periyapatna Taluka, Karnataka	1977
31	GE 658(IC476081)	Yadiayalla local, Gundlupet, Karnataka	1977
32	GE 1308(IC475800)	Hullupareragi, Karnataka	1977
33	GE 632(IC475694)	Handaragi, Hassan District	1977
34	GE 1343(IC475465)	Challekere local, Karnataka	1977
35	GE 702(IC475594)	Kari ragi I, Yaachathi, Gundlupet Taluka	1977
36	GE 776(IC475802)	Gangoor local III, Hassan Dist.	1977
37	GE 328(IC476251)	Mannurragi, Hassan Dist.	1977
38	GE 904(IC476104)	Hosakote local, Hosakoite, Karnataka	1977

*White seeded finger millet

Evaluation of Morphological Diversity

The selected finger millet genotypes were evaluated in Randomized Block Design (RBD) with number of replications (3), number of rows/plot (2), row length (2 m) and spacing (inter and intra row/22.5 × 15 cm) at UAS (University of Agricultural Sciences), Bangalore and ICRISAT, Hyderabad. Thirteen qualitative (plant pigmentation, ear shape, ear size, finger branching, discontinuity of spikelets on finger, synchrony of ear maturity, spikelet shattering, grain covering, grain colour, grain shape, grain surface, pericarp persistence, susceptibility to lodging) and 14 quantitative traits (plant height, days to flowering, days to maturity, culm thickness, productive tillers, leaf number, flag leaf length and width, peduncle length, finger number, finger length, finger width, test weight and grain yield), were recorded.

DNA Extraction and PCR Amplification

Extraction of total genomic DNA was carried out following cetyl-trimethyl-ammonium bromide (CTAB) method (Saghai-Maroof *et al*., 1984) with minor modifications using bulk leaves samples (30 individual plants/sample) of 6-week-old plant. DyNA Quant 200 fluorometer (Hoefer Instruments, USA) was used for quantification of DNA and a working concentration of 5 ng/µl was prepared for PCR.

RAPD Analysis

PCR amplification was carried out with 25 ng of genomic DNA, 3 mM $MgCl_2$ (Fermentas Life Sciences), 1U Taq DNA polymerase (Fermentas Life Sciences), 1x PCR buffer without $MgCl_2$ (Fermentas Life Sciences), 0.2 µM decamer primers (Operon) and 0.2 mM of dNTP mix (Fermentas Life Sciences). PCR reactions were carried out in a Perkin Elmer GeneAmp PCR system 9600 thermocycler: initial denaturation at 94°C for 5 min, followed by forty cycles of denaturation at 94°C for 1 min, primer annealing at 37°C for 1 min and primer extension at 72°C for 2 min and final extension step at 72°C for 10 min.

ISSR Analysis

PCR amplification was carried out with 25 ng of genomic DNA, 2.5 mM $MgCl_2$, 1U *Taq* DNA polymerase, 1x PCR buffer without $MgCl_2$, 1.0 µM ISSR primer and 0.2 mM dNTP mix. Thermocycling conditions were as follows: denaturation at 94°C for 7 min.; thirty cycles of denaturation at 94°C for 30 sec., primer annealing at temperature specific to each primer for 45 sec. and primer extension at 72°C for 2 min. and final extension step at 72°C for 5 min.

Following PCR, RAPD and ISSR amplification products were loaded onto a 1.4% agarose gel in 1x TBE buffer and stained with EtBr. Electrophoresis was carried out at 90 V for 1.5 hours, followed by 70 V for 2 hours. The resolved RAPD products were visualized by UV and recorded using a Bio Imaging System (SynGene).

Statistical Analyses

Statistical analysis of morpho data was carried out using INDOSTAT package. Univariate analysis (ANOVA) and multivariate (cluster and ordination) analysis were performed for statistical analysis of quantitative traits and percentage frequency distribution was calculated for qualitative traits. RAPD and ISSR fragments were scored visually, as absent (0) or present (1). Jaccard's similarity coefficient was calculated for UPGMA clustering using NTSYS-pc. ver. 2.1 (Rohlf, 2000). GenAlEx software was used to calculate AMOVA (Peakall and Smouse, 2012). In addition, the software STRUCTURE (Pritchard *et al*., 2000) was used to investigate number of sub-groups using a burn-in of 100,000, a run length of 10,00,000 (admixture model). The number of sub-groups (K) in the population was determined by running the program at different K values (1 to 10) with five independent runs for each K value. Peak value of delta K was calculated using Structure Harvester (Earl and vonHoldt, 2012) to confer the number of distinct sub-groups.

Results and Discussion

Morphological Characterization

The data for 13 qualitative traits was recorded at Bangalore location and percentage frequency distribution was computed and cluster analysis was done using weighted average linkage dendrogram. Diversity was revealed mainly for ear shape and ear size. Semi-compact ear shape was found to be pre-dominant particularly among the varieties, while the landraces possessed both semi-compact and compact ear shapes. The varieties had a higher frequency of large ear size, but intermediate ear size was more pronounced in the material studied in general, and the landraces in particular. Semi-compact ear type and large ear of varieties may be due to the use of African germplasm as one of the parents in most of the varieties (Naik *et al*., 1993).

Analysis of variance (ANOVA) for 14 quantitative traits revealed significant differences for all the traits namely days to flowering, days to maturity, culm thickness, productive tillers, leaf number, flag leaf length and width, peduncle length, finger number, finger length, finger width, test weight and grain yield at both the locations. Combined ANOVA analysis showed most of the characters to have significant difference, indicating the presence of genotypic differences among the materials evaluated (Table 2). For location treatment all the characters showed significant difference. Number of productive tillers, flag leaf blade length and peduncle length were found to be non-significant for location and grain yield was observed to be non-significant for treatment. At both the locations, number of productive tillers showed a positively skewed distribution and high Kurtosis value (Table 3 and 4), indicating that the frequency distribution for this character is not strictly continuous. Differences were observed in the mean performance of the landraces and varieties between the two locations. The phenotypic coefficient of variation was high for characters such as, number of productive tillers, leaf number and finger length at both the locations. The variability was noticed to be higher in the landraces compared to varieties. In cluster analysis, the association between the clusters based on qualitative and quantitative characters was not distinct. But, some of the varieties, such as MR 1, MR 2, Indaf 15 and Indaf 8 grouped in one cluster; the varieties GPU 45, Indaf 5 and GPU 26 in a separate cluster and Hamsa and PR 202 grouped in a third cluster along with the landraces for both qualitative and quantitative characters. The inter-cluster distances for both locations were observed to be maximum between the cluster consisting predominantly of landraces and the cluster having mostly varieties. The landraces GE 632, GE 776, GE 328 collected from Hassan district and those collected from Kolipalaya namely GE 3321 and GE 3322 did not cluster together, indicating that the diversity pattern did not have strong association with geographical distribution.

Principal component analysis performed on standardized quantitative traits showed that first three most informative components accounted for 66.79% and 64.15% variation (Table 5), at Bangalore and Hyderabad location respectively. At Bangalore location important characters with greater weightage in principal component axis I were grain yield, days to maturity and days to flowering. Important characters in principal axis II

Table 2. Combined analysis of data for quantitative traits of locations, Bangalore and Hyderabad

Source	Df	DFL	PH	CT	PT	LN	FLBL	FLBW	PL	FN	FL	FW	DM	TW	GY
Location (L)	1	4522.98	21643.84**	9.05**	1.72^{ns}	346.84**	129.59**	0.02^{ns}	5.92^{ns}	4.43**	8.02*	0.86**	3533.71**	31.67**	510.63**
Replication (R)	4	5.93	609.28	0.026	1.45	6.82	50.55	0.06	17.19	0.16	0.86	0.04	39.57	0.19	11.83
Treatment (T)	37	234.28**	780.43**	0.05**	2.35**	29.49**	28.58*	0.07**	34.21**	3.46**	8.66**	0.02*	324.02**	0.46**	57.58^{ns}
L x T	37	14.73**	196.44**	0.006**	0.74**	8.84**	13.64**	0.14**	9.39**	0.58**	1.50**	0.01**	31.12**	0.20**	37.10**
Error	148	4.26	37.29	0.002	0.19	1.13	7.22	0.006	2.4	0.18	0.16	0.003	5.68	0.07	2.325

*P≤0.05, ** p ≤0.01, ^{ns}Not significant

Df= degree of freedom; DFL= Days to flowering; PH= Plant height; CT= Culm thickness; PT= Number of productive tillers; LN= leaf number on main tiller; FLBL= Flag leaf blade length; FLBW= Flag leaf blade width; PL= Peduncle length; FN= finger number; FL= Finger length; FW= Finger width; DM= Days to maturity; TW= Test weight; GY= Grain yield per plant

Table 3. Range of variation for quantitative traits at Bangalore location

Characters	Lowest	Highest	Mean	Kurtosis	Skewness	Standard Deviation	C.V
DFL	53.00	85.00	65.54	0.46	0.87	7.81	11.92
PH	46.85	119.40	81.06	-0.30	0.20	14.72	18.15
CT	0.73	1.19	0.93	-0.19	0.28	0.10	10.46
PT	2.33	7.30	3.80	3.41	1.58	0.92	24.32
LN	9.90	21.80	14.70	-0.43	0.27	2.46	16.73
FLBL	20.34	40.98	29.94	0.72	0.04	3.62	12.11
FLBW	0.76	1.32	1.05	-0.54	-0.17	0.12	11.76
PL	15.80	29.50	23.04	-0.48	-0.14	2.92	12.70
FN	4.80	10.10	7.14	0.83	0.32	0.92	12.83
FL	4.14	10.00	6.76	-0.34	0.06	1.20	17.83
FW	0.80	1.30	1.06	0.63	-0.30	0.09	8.16
DM	95.00	126.90	108.27	-0.32	0.51	8.93	8.25
TW	1.25	3.19	2.35	0.56	-0.26	0.36	15.32
GY	6.35	22.40	14.11	-0.73	-0.04	3.93	27.84

Df= degree of freedom; DFL= Days to flowering; PH= Plant height; CT= Culm thickness; PT= Number of productive tillers; LN= leaf number on main tiller; FLBL= Flag leaf blade length; FLBW= Flag leaf blade width; PL= Peduncle length; FN= finger number; FL= Finger length; FW= Finger width; DM= Days to maturity; TW= Test weight; GY= Grain yield per plant

Table 4. Range of variation for quantitative traits at Hyderabad location

Characters	Lowest	Highest	Mean	Kurtosis	Skewness	Standard Deviation	C.V
DFL	60.00	91.00	74.28	0.55	0.41	6.63	8.92
PH	68.40	127.10	100.37	-0.06	-0.30	11.76	11.72
CT	1.12	1.60	1.33	-0.24	0.22	0.10	7.90
PT	2.50	5.80	3.62	1.52	0.90	0.65	18.10
LN	10.60	24.70	17.04	-0.08	0.59	2.83	16.62
FLBL	20.82	35.30	25.52	0.56	0.06	3.78	9.98
FLBW	0.69	1.41	1.07	0.16	-0.44	0.13	12.56
PL	15.17	29.92	22.80	-0.21	-0.16	2.91	12.76
FN	5.30	9.50	7.42	0.22	-0.19	0.84	11.39
FL	4.37	9.77	7.14	-0.96	-0.06	1.45	20.28
FW	1.00	1.35	1.18	-0.46	0.35	0.08	6.65
DM	102.00	135.00	117.04	0.01	0.33	7.65	6.53
TW	2.10	4.80	3.10	2.12	-0.61	0.42	13.69
GY	9.42	29.70	17.12	-0.31	-0.03	4.29	25.06

Df= degree of freedom; DFL= Days to flowering; PH= Plant height; CT= Culm thickness; PT= Number of productive tillers; LN= leaf number on main tiller; FLBL= Flag leaf blade length; FLBW= Flag leaf blade width; PL= Peduncle length; FN= finger number; FL= Finger length; FW= Finger width; DM= Days to maturity; TW= Test weight; GY= Grain yield per plant

included flag leaf blade width and number of productive tillers. Finger number and peduncle length were the characters with greater weightage in principal component axis III. At Hyderabad location, days to flowering, leaf number and finger length had more weightage in principal component axis I. Finger number and flag leaf blade width were important in principal axis II, and peduncle length and flag leaf blade length in axis III. In general principal component analysis confirmed the groupings of the accessions obtained through cluster analysis.

Molecular Characterization

A total of 271 RAPD bands were generated using 21 random primers in the size range (250 to 3000 bp) with 61.62% polymorphism (Table 6). The number of bands generated per primer ranged from 5 (OPA07) to 23 (OPB11) with a mean of 12.9 bands per primer, and is higher than the earlier reports (Fakrudin *et al*., 2004, Babu *et al*., 2007, Panwar *et al*., 2010), which may be due to the primers selected for this study. For RAPD primers landraces showed 53.61% polymorphism as compared to 47.26% in finger millet varieties (Fig. 1).

Seven ISSR primers generated 100 PCR products in the size range 150 to 2200 bp and showed 57% polymorphism (Table 6). Eleven [(GA)9T] to 18 [(GA)9AAA(GA)5] ISSR bands were generated with a mean of 14.3 bands per primer. Landraces showed

Table 5. Principal components analysis for quantitative traits at both locations

PC Axes	Total variation explained		Character Weightage
	Percent	Cumulative	
Bangalore			
I	35.27	35.27	Grain yield (0.80), days to maturity (0.74), days to flowering (0.71)
II	17.72	52.98	Flag leaf blade width (0.75), number of productive tillers (0.69)
III	13.80	66.79	Finger number (0.65), peduncle length (-0.51)
Hyderabad			
I	39.04	39.04	Days to flowering (0.81), leaf number (0.80), finger length (0.76)
II	14.60	53.64	Finger number (0.70), flag leaf blade width, (-0.70), culm thickness (-0.49)
III	10.50	64.15	Peduncle length (-0.65), flag leaf blade length (-0.40)

53% and varieties 46.47% polymorphism with 100 ISSR markers (Fig. 1). For both the markers combined 53.44% and 47.04% of bands were polymorphic for landraces and varieties respectively.

The genetic similarity was determined on the basis of Jaccard's similarity coefficients. The mean values of the Jaccard's similarity coefficients for the markers RAPD, ISSR and both combined were 0.810, 0.782 and 0.802 respectively. The mean values for the markers combined were 0.824 for the varieties and 0.807 for the landraces. Both the %polymorphism and Jaccard's similarity coefficients values indicated that landraces were more diverse than varieties of finger millet.

For cluster analysis (Fig. 2) combined data of both the marker systems was used and two distinct clusters were obtained. Sixteen varieties along with one landrace GE 1343 formed cluster II and eighteen landraces along with only white seeded variety Hamsa formed cluster I, while GE 328 and variety Indaf 3 were present as outliers. The results indicated that all the varieties bred using African germplasm were closely related to each other and grouped in sub-cluster IIB and the varieties which were developed as pure line selections from India or Indian x Indian crosses were placed in sub-cluster IIA or as outlier of cluster II. Further in sub-cluster IIB varieties having common parents or showing pedigree relationship were closely grouped viz. MR 1 and MR 2; L 5 and Indaf 9; GPU 26, GPU 28 and GPU 45. Regarding landraces which were grouped in cluster I, the landraces GE 632, GE 776, GE 328 collected from Hassan district were placed separately like morphological markers but GE 3321 and GE 3322 collected from Kolipalaya clustered together, indicating that the molecular marker based diversity pattern have some association with geographical distribution.

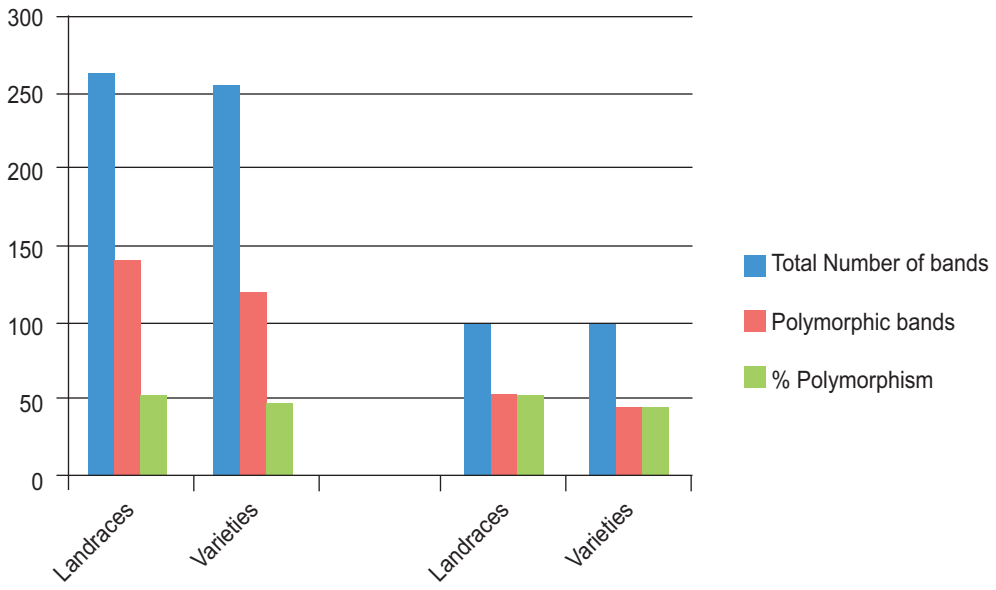

Fig. 1. Polymorphism summary statistics of landraces and varieties of finger millet based on RAPD and ISSR markers.

Table 6. Characteristics of bands generated using RAPD and ISSR primers

Primer	Total bands (no.)	Polymorphic bands (no.)	Polymorphism (%)	Size range of bands (bp)
RAPD Primers				
OPA 1	9	6	66.67	700-3000
OPA 7	5	4	80.00	500-2000
OPA 8	12	6	50.00	350-2500
OPA 13	17	15	88.24	300-2500
OPA 18	13	8	61.54	800-2000
OPB 4	14	8	57.14	500-3000
OPB 7	13	9	69.23	400-2000
OPB 8	13	7	53.84	550-3000
OPB 11	23	19	82.62	300-2400
OPB 12	7	5	71.43	450-900
OPB 18	14	11	78.57	300-2000
OPC 2	12	6	50.00	550-1800
OPC 6	10	4	40.00	600-2000
OPC14	19	11	57.89	450-3100
OPD 5	11	6	54.54	250-2000
OPD 8	16	7	43.75	750-2000
OPD 13	11	8	72.72	550-2000
OPK 1	11	6	54.54	400-2500
OPK 4	13	7	53.85	500-1900
OPF 14	14	6	42.86	400-2500
OPF 20	14	8	57.14	525-2300
Total	271	167	61.62	
ISSR Primers/Annealing temperature				
(GA)9T, 52°C	11	3	27.27	350-1400
(GA)9AC, 52°C	14	4	28.57	250-1500
(GA)9AY, 52°C	13	8	61.54	300-1400
(ACC)6T, 60°C	13	5	38.46	250-2000
(GACA)4, 52°C	14	10	71.43	300-2200
(AT)3(GT)15, 64°C	17	13	76.47	200-1800
(GA)9AAA(GA)5, 64°C	18	14	77.78	150-1500
Total	100	57	57.00	
Combined total	371	224	60.38	

Population structure analysis (Fig. 3) using RAPD and ISSR combined data resulted in to two groups, Group I (Varieties) and Group II (Landraces) based on peak value of delta K. All the landraces except GE 1447 and GE 1343 were in Group II and all the varieties except Hamsa, PR 202 and Indaf 3 were falling in Group I. All the varieties except Hamsa, PR 202, Indaf 3 and Poorna and all the landraces except GE 1447, GE 1412 and GE 1343 showed >86% membership coefficient in their respective groups (Table 7).

AMOVA was also conducted to analyze the separation between finger millet varieties and landraces. Between-variance component accounted for 14% variation compared to 86% within-variance component (Table 8). Results revealed that the level of genetic differentiation between the landraces and the varieties was low compared to variations within these groups. The probable reason may be that landraces might have been used in the development of these varieties as one of the parent along with African/Indian germplasm as the other parent in the lineage. Another reason for low component variance between the two groups could be that most of the landraces and varieties used in this study have originated/developed in Karnataka state of India. The landraces studied showed more diversity than varieties. The reason for this higher diversity may be due to the reason that landraces have been shaped over time due to natural selection by dynamic environmental conditions and human mediated selection. Forces like gene flow, selection, mutations and genetic drift

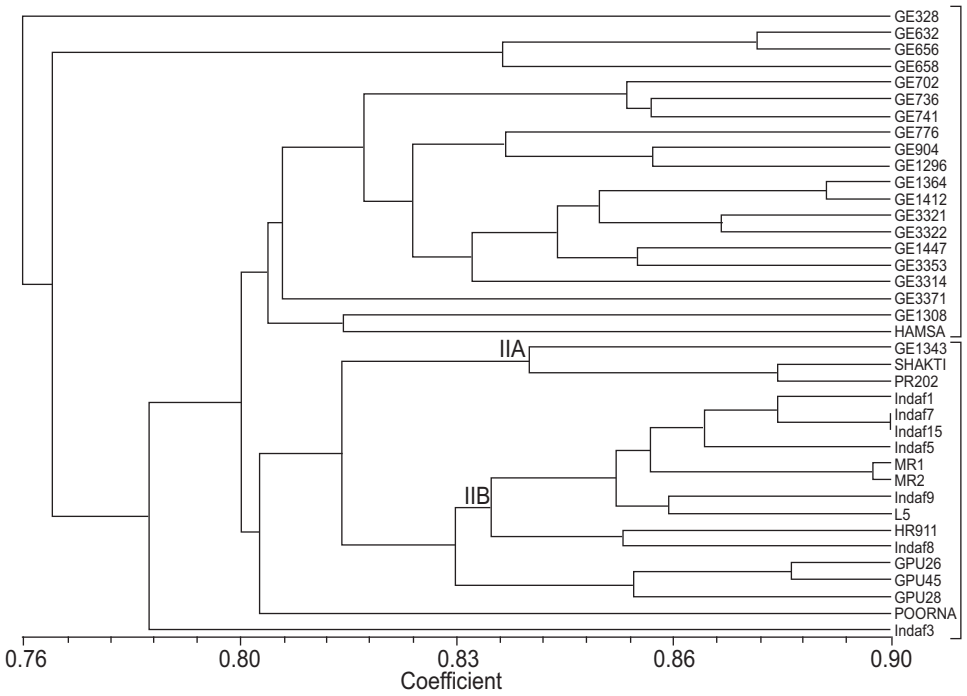

Fig. 2. UPGMA cluster analysis of finger millet varieties and landraces based on cumulative marker data (RAPD and ISSR).

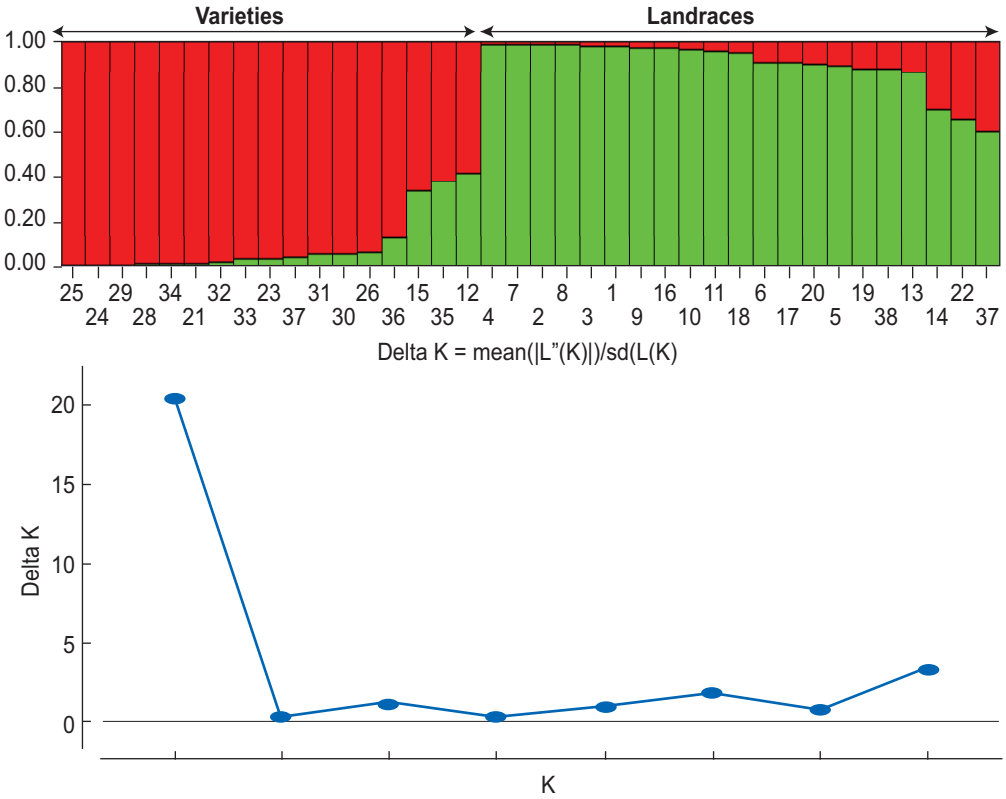

Fig. 3. Population structure analysis of 38 finger millet varieties and landraces and ΔK to predict the subgroups (Varieties and Landraces); 1: GE328, 2: GE632, 3: GE656, 4: GE658, 5: GE702, 6: GE736, 7: GE741, 8: GE776, 9: GE904, 10: GE1296, 11: GE1308, 12: GE1343, 13: GE1364, 14: GE1412, 15: GE1447, 16: GE3314, 17: GE3321, 18: GE3322, 19: GE3353, 20: GE3371, 21: Indaf1, 22: Indaf3, 23: Indaf5, 24: Indaf7, 25: Indaf15, 26: HR911, 27: Indaf8, 28: Indaf9, 29: L5, 30: GPU26, 31: GPU28, 32: GPU45, 33: MR1, 34: MR2, 35: POORNA, 36: SHAKTI, 37: PR202, 38: HAMSA.

Table 7. Membership coefficient of finger millet landraces and varieties in respective groups

Label	GI	GII
GE328	0.017	0.983
GE632	0.013	0.987
GE656	0.016	0.984
GE658	0.01	0.99
GE702	0.101	0.899
GE736	0.089	0.911
GE741	0.011	0.989
GE776	0.013	0.987
GE904	0.024	0.976
GE1296	0.031	0.969
GE1308	0.041	0.959
GE1343	0.582	0.418
GE1364	0.134	0.866
GE1412	0.297	0.703
GE1447	0.663	0.337
GE3314	0.024	0.976
GE3321	0.089	0.911
GE3322	0.049	0.951
GE3353	0.115	0.885
GE3371	0.096	0.904
Indaf1	0.981	0.019
Indaf3	0.337	0.663
Indaf5	0.962	0.038
Indaf7	0.99	0.01
Indaf15	0.991	0.009
HR911	0.93	0.07
Indaf8	0.951	0.049
Indaf9	0.984	0.016
L5	0.986	0.014
GPU26	0.936	0.064
GPU28	0.941	0.059
GPU45	0.973	0.027
MR1	0.964	0.036
MR2	0.983	0.017
POORNA	0.621	0.379
SHAKTI	0.865	0.135
PR202	0.397	0.603
HAMSA	0.12	0.88

make them more diverse. So there is a need to study landraces from different geographical areas in India at molecular and phenotypic level in order to explore their inherent genetic variability for utilization in crop improvement.

Comparison of molecular and morphological data revealed that the UPGMA clusters for RAPD and ISSR markers to some extent were similar to the clustering pattern based on quantitative characters. The landrace GE 1343 was found clustered with the varieties in quantitative trait analysis at Bangalore and dendrograms generated by RAPD and both the markers combined. The varieties Hamsa and PR 202, which were pureline selections from Indian germplasm were found to cluster with the landraces in Struture analysis based molecular and morphological characterization. Similarly, the varieties, such as MR 1, MR 2 and Indaf 15 grouped in one cluster and the varieties GPU 45 and GPU 26 in a separate cluster for qualitative, quantitative characters and molecular data.

Table 8. AMOVA of finger millet landraces and varieties based on ISSR and RAPD markers

Level of variation	df	SS	MS	Est. Var.	%
Among pops	1	114.388	114.388	4.514	14
Within pops	36	1039.033	28.862	28.862	86

degree of freedom (df); sum of squares (SS); mean of squares (MS)

In the present study, the materials evaluated showed significant genotypic variation. The morphological characterization accounted for higher variation than molecular characterization, which may be due to the reason that morphological traits are generally believed to be subject to natural selection and their expression is partly under the influence of environmental factors. Further, in contrast to morphological traits, molecular variation is based on DNA sequence variation. Molecular markers used are neutral and are not linked to any specific morphological adaptation, so the differences in diversity pattern were obvious.

The separate clustering of varieties in one group and the landraces in a separate group was more pronounced in the clustering patterns obtained through molecular markers. The forces causing high molecular differentiation could be due to genetic drift and no selection, particularly for landraces.

Conclusion

The landraces were found to be more diverse than the varieties and it is inevitable to introgress genes from landraces in to varieties to enhance the genetic base of finger millet and conserve more landrace accessions to maximize the diversity. Landraces can also serve as crucial genetic resources for association studies of genes responsible for adaptive variations, suggesting the *in situ* conservation of these landraces in the perspective of future climate change.

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RESEARCH ARTICLE

Phenotypic Diversity Analysis and Screening for Northern Corn Leaf Blight Resistance in Maize (*Zea mays* L.) Landraces Grown in North Eastern Hill Region of India

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A total of 139 diverse maize landraces from seven hill states of North East India were evaluated for genetic diversity and resistance to Northern Corn Leaf Blight (NCLB) in an augmented design. ANOVA for cob and flowering characteristics were highly significant. PCA revealed cob traits contributed to PC 1 while flowering traits loaded on PC 2. Agglomerative Hierarchical Clustering (Ward's method) grouped the landraces into four distinct classes on cob weight variation. For NCLB resistance, qualitative disease assessment under natural field conditions followed by controlled quantitative assessment based on AUDPC scores were consistent for the landraces studied implying genetic basis of inheritance. SSR markers which map close to resistant *Ht* genes when run on a subset of the landraces showed distinct polymorphism specifically between the most susceptible T(9)8) and resistant landrace M9(4) collected from Tripura and Meghalaya, respectively for markers close to resistant *Ht2* gene. Diversity and disease screening studies hereby establish presence of genetic variability for initiating or fortifying resistance breeding programmes.

Key Words: Cluster analysis, Maize landraces, Morphological diversity, NCLB, *Turcicum* blight resistance

Introduction

Maize possesses highest levels of diversity because of selective breeding over time in different regions of the world with most of the variation present in landraces (Mangelsdorf, 1986). Very high genetic variation for maize landraces also exists in the North Eastern Hill (NEH) Region of India (Sharma *et al.,* 2010; Singode and Prasanna, 2010). One of the secondary centers of diversity, thirteen highly prolific primitive maize landraces are distributed across NEH region. These landraces christened "Sikkim Primitives" were believed to have originated from a single popcorn variety and subsequently domesticated by different ethnic groups of the region (Prasanna and Sharma, 2005).

Although modern farming techniques have enhanced yield, greater crop uniformity has led to extensive loss of germplasm diversity. However, landraces till date represent one category of locally adapted germplasm which lack formal crop improvement, are genetically diverse, associated with traditional farming systems (Villa *et al*., 2005) and provide a ready source of alleles for improving disease and pest resistance, nutritional quality and other traits of interest (Yao *et al.,* 2007). Germplasm characterization in conserving genetic variation is critical to crop improvement and diversity studies to generate basic information about variability (Lucchin *et al.,* 2001) leading to better understanding of traits that have shaped quantitative variation in maize (Carvalho *et al.,* 2004).

Plant breeders are known to use variation found in landraces especially with respect to resistance to counter continuously evolving pests and diseases (Smale *et al.,* 2001). Prominent among foliar disease of maize is *Turcicum* blight also known as Northern Corn Leaf Blight (NCLB) caused by *Setosphaeria turcica* (Leonard & Suggs) with its conidial state *Exserohilum turcicum.* Damaging in both the tropics and the Himalayan Region with yield losses exceeding 70% during a severe attack, annual yield losses in maize growing nations from NCLB range from 15 to 30 % (Welz and Geiger, 2000; Wisser *et al.,* 2011; Haasbroek *et al.,* 2014). Genetic control of NCLB can be achieved qualitatively with dominant

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or partially dominant *Ht* genes or quantitatively, used separately or together (Ogliari *et al.,* 2005; Wisser *et al.,* 2006; Zwoniter *et al.,* 2009).

The present study was taken up for characterization of 139 landraces collected from NEH region for nine quantitative DUS descriptors and screening for NCLB disease resistance at the field as well as in controlled greenhouse environmental conditions based on AUDPC (Area Under Disease Progress Curve) values. Additionally, a subset of the landraces was also screened with SSR markers known to map close to *Ht* genes.

Materials and Methods

The study site was College of Post Graduate Studies, Umiam, Meghalaya India located 25º34'32"N latitude and 91º52'23"E longitude, set at an elevation of 950 m above mean sea level. Temperatures during the maize planting season range from 28º to 32ºC with a relative humidity of 85-95% which makes it a hot spot for occurrence of NCLB (Biswar *et al.,* 2007).

The experimental material comprised a collection of landraces from the seven hill states of North East India *viz*. Arunachal Pradesh (A), Manipur (Ma), Meghalaya (M), Mizoram (Mi), Nagaland (N), Sikkim(S) and Tripura (T). Local composities *viz.* RCM-1-1 and RCM-1-2 obtained from ICAR, NEH Region, Umiam Meghalaya and inbred lines *viz.* CM-145 (resistant) and V 334 (susceptible) from ICAR-VPKAS, Almora, Uttrakhand were used as standard checks for NCLB evaluation studies. These checks were assigned codes C1, C2, C5 and C6, respectively. While a total of 152 landraces were collected from various maize growing districts, 139 survived and were used for genetic diversity studies of which forty three landraces were collected from Meghalaya, twenty seven from Nagaland, twenty one from Manipur, eleven from Arunachal Pradesh, sixteen from Sikkim and five from Mizoram. Care was taken to collect only such landraces which have been traditionally conserved by farmers at their homes. The experiment was conducted over a span of two years from 2012-13 and 2013-14. The landraces were assigned alphanumerical codes according to their respective states of origin followed by the collection number assigned to a particular landrace.

An augmented design of field trial was laid out for the 139 maize landraces. Replicated checks were also planted in four blocks. Sowing was done in single rows of ten plants per landrace. Plant to plant spacing was maintained at 30 cm while row to row distance was fixed at 60 cm. Routine intercultural operations were carried out. Individual cobs from each plant within and between the landraces were always harvested separately. While data on 28 morphological descriptors both qualitative and quantitative were studied, data pertaining to nine economically important agronomic traits defined by DUS (Distinct, Uniform and Stable) requirements were used for further analysis. Tasselling (TA) and Silking (SI) data were calculated when 50 % of the plants of a particular landrace came into flowering based on which the Anthesis Silking Interval (ASI) was calculated. Data on cob weight with husk (WwH) in grams, cob weight without husk (WwoH) in grams, cob length without husk (CL) in centimeters, number of row grains (NRG), 100 grain weight (100GW) in grams and plant height (PH) in meters were the other observations used for analysis.

Qualitative scoring of the landraces for NCLB was carried out based on the method and disease ratings of 1 to 5 as per Payak and Sharma (1983). A score of 1 was used to characterize the resistant and a score of 5 to characterize the susceptible plant. Cobs of individual plants within and between landrace were harvested separately. Three plants which were treated as three replications from individual cobs with highest and lowest field disease scores were subsequently grown in the greenhouse to verify results of field screening.

Seedlings grown in the greenhouse, when at knee height stage were inoculated with *E. turcicum* spores under controlled environmental conditions of high humidity and temperature. All plants were inoculated with 0.5 ml spore suspension (3×10^4 conidia spore/ml, with 0.02% Tween 20) in leaf whorls and relative humidity levels were maintained at 80%. To measure disease progress, AUDPC was calculated based on area of disease spread on the leaf surface and, the progress recorded every three days from the onset of the disease as per Madden *et al.* (2000) till flowering. The disease progress of three lesions per plant were recorded and subsequently averaged over the three replications for final calculations of AUDPC.

Plant genomic DNA was extracted from leaves of individual plants at the three leaf emergence stage using SDS method of extraction. Based on high and low AUDPC scores recorded, ten landraces from either group were selected for bulk DNA studies as per Shen *et al*. (2003). DNA extraction was first done on individual basis. The individual genomic DNA was then bulked in

equal proportion in five separate bulks with each bulk containing DNA from landraces with similar AUDPC scores (Table 1). For each bulk, the same DNA samples were pooled twice to produce replicated samples. Twenty reported SSR primers that are known to tag close to resistant *Ht* genes from bin 8.06 and 2.08 were used to screen for polymorphism. The SSR sequences were obtained from Maize Genetics and Genomics Database (http://www.maizegdb.org/data_center/ssr#). PCR for bulked as well as individual DNA was carried out in a reaction volume of 10 µl. The amplification was carried out in thermal cycler using the PCR profile of 5 minutes initial denaturation at 95 ºC, 30 seconds at 55 ºC for annealing and 45 seconds at 72 ºC for extension and a final extension at 72 ºC for 5 minutes. Scoring was done using 3% agarose gel (Sigma, Ultrapure Agarose 1000) and the bands were scored based on length of the amplified products using the molecular ladder as reference. The number of polymorphic and monomorphic fragments was determined by observing the amplified fragments for each pair of primer.

Analysis of variance (ANOVA) for augmented design based on the morphological traits was done using adjusted treatment means. Data for the nine traits studied were further subjected to Principal Component Analysis using XLSTAT. Genetic diversity and cluster analysis for the quantitative traits were studied using Agglomerative Hierarchical Clustering (AHC) with Euclidean Distances and Ward's Method as measures of dissimilarity among populations using XLSTAT. The cluster diagram was generated using DARwin Version 6. For the molecular data analysis, data of polymorphic and monomorphic amplification was extracted to Excel table for further interpretation.

Result and Discussion

The maximum and minimum values for the nine different quantitative data studied under field conditions exhibited a range of variation around the mean data for the 139 landraces under study. ANOVA for the morphological descriptors based on quantitative data similarly confirmed presence of highly significant variation for the traits under study (Table 2). Anthesis Silking Interval was highly variable with differences between days to Tasseling and Silking being as low as 1 day in M25 to as high as 32 days in landrace T7. Similarly, cob weight was highly variable and was highest for landraces with higher Anthesis Silking Interval values. Landrace M31 with the highest average cob weight of 270 g without husk recorded an average ASI of 10 days. Cobs of landraces from Nagaland recorded the highest plant height, longer Anthesis Silking Interval, days to maturity and cob length and landrace N9 recorded the highest average 100 grain weight at 74.25g among all the landraces studied.

Landraces which do not serve as sources for improved maize germplasm contain untapped allelic variation in the form of resistance to biotic and abiotic stresses which can be effectively utilized in modern maize breeding (Warburton *et al.,* 2008).The goal of plant breeders has long been to produce broadly adapted, improved varieties by exploiting the available germplasm for variation (Mercer *et al.,* 2008). A substantial fraction of maize germplasm is maize landraces conserved in various pockets of NEHR by famers with small landholdings (Prasanna, 2010) but limited efforts have been made to characterize and explore the vast maize genetic variability available in the NEHR with only 2% of the available maize germplasm utilized in breeding programmes (Devi *et al.,* 2013). In the present study too, considerable variation for different yield contributing traits was present in the 139 landraces studied which can be effectively exploited in future breeding programmes.

Association studies (Table 3) using Pearson's correlation revealed highly significant correlation for days to 50% Silking with days to 50% Tasseling and Anthesis Silking Interval. Cob weight with husk was highly and significantly correlated with other cob traits *viz.* cob weight without husk, 100 grain weight and cob length. Number of row grains was also highly significantly correlated with cob length, 100 grain weight and plant height. Plant height was highly significantly correlated with cob weight with husk, cob weight without husk, cob length and number of row grains. The first four components of Principal Component Analysis could explain 83.80 % of the variation in the data. While PC1 accounted for 34.22 % of variability in the data, PC 2 could explain 22.48 % of the variability. PC 3 and PC 4 explained 14.76 % and 12.32 % variation in the data respectively. Cob traits cumulatively were found to have the highest loadings on PC1 accounting for 88.12 % of the total variation while plant height which also loaded on PC1contributed to around 10% to the total variation. Flowering traits days to 50% Silking and Anthesis Silking Interval had the highest loadings on PC2 contributing to 86.02 % of the total variation in PC2 of which, 44.30% was contributed by days to 50%

Silking alone. Traits, number of row grains and100 grain weight had high loadings on both PC1 and PC3 while days to 50% Tasseling had almost equal loadings on PC3 and PC4. Squared cosines of the variables studied to further confirm the contribution of the individual traits showed that cob length, cob weight with husk, cob weight without husk with values higher than 0.5 contributed to maximum variation in PC1, while Days to 50% Silking and Anthesis Silking Interval with cosine values higher than 0.5 in PC2 were the main contributors to variation.

Agglomerative Hierarchical Clustering (AHC) with Euclidean Distances using Wards Distance for the 139 landraces and the four reference checks studied generated four major classes (Figure 1). Cob weight with husk and cob weight without husk were the most variable parameters and appeared to influence grouping of the landraces in the different clusters. Cluster 1 comprised of 45 landraces, Cluster 2 of 29 landraces, Cluster 3 of 63 landraces and Cluster 4 of 6 landraces. Class 3 was markedly dissimilar from Classes 1, 2 and 4 and recorded the lowest cob weight and length. Classes 1 and 2 had very low levels of dissimilarity. In Class 4 clustered landraces were recorded with the highest cob weight and also the highest 100 grain weight. For all the nine quantitative traits studied, the maximum distance for the

Landraces:

A1	M18	M4	Ma3	N20	S3
A10	M19	M40	Ma4	N21	S4
A11	M2	M41	Ma5	N22	S5
A2	M20	M42	Ma6	N23	S6
A3	M21	M43	Ma7	N24	S7
A4	M22	M5	Ma8	N25	S8
A5	M23	M6	Ma9	N26	S9
A6	M24	M7	Mi1	N27	T1
A7	M25	M8	Mi2	N3	T10
A8	M26	M9	Mi3	N4	T11
A9	M27	Ma1	Mi4	N5	T12
C1	M28	Ma10	Mi5	N6	T13
C2	M29	Ma11	N1	N7	T14
C5	M3	Ma12	N10	N8	T15
C6	M30	Ma13	N11	N9	T16
M1	M31	Ma14	N12	S1	T2
M10	M32	Ma15	N13	S10	T3
M11	M33	Ma16	N14	S11	T4
M12	M34	Ma17	N15	S12	T5
M13	M35	Ma18	N16	S13	T6
M14	M36	Ma19	N17	S14	T7
M15	M37	Ma2	N18	S15	T8
M16	M38	Ma20	N19	S16	T9
M17	M39	Ma21	N2	S2	

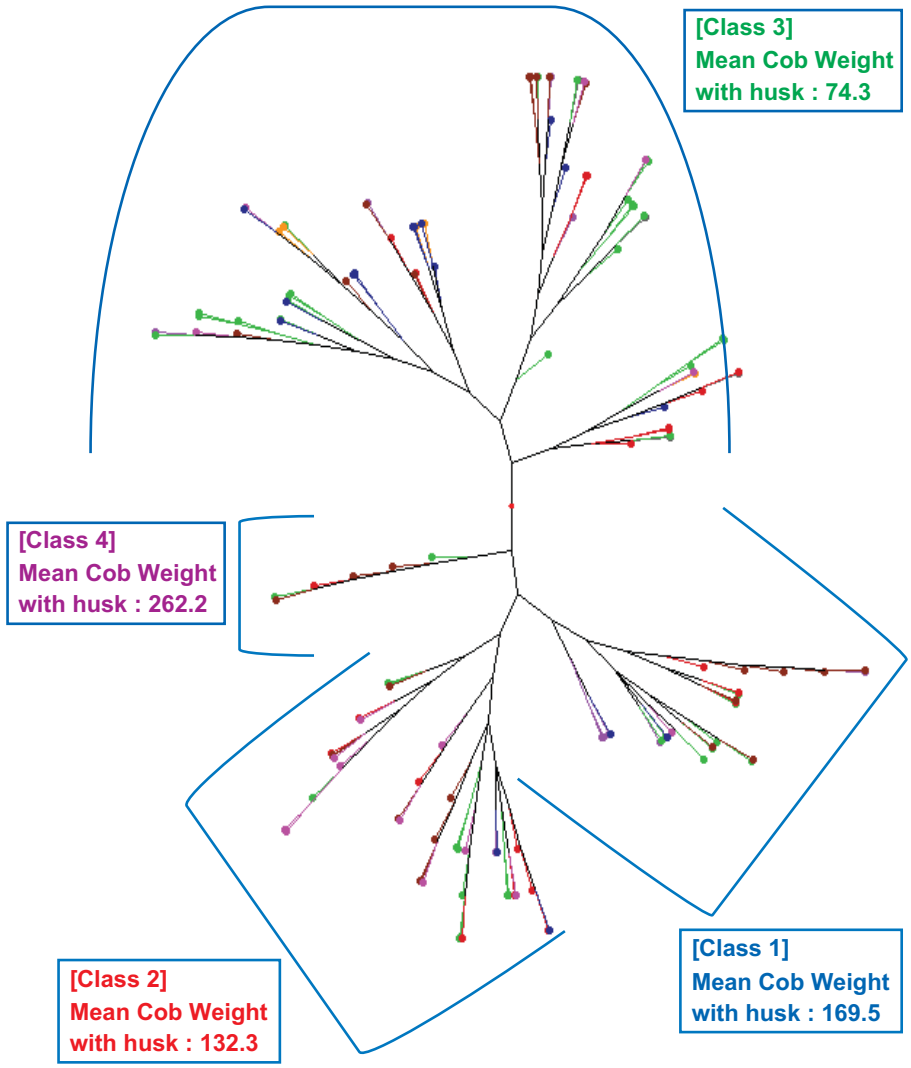

Fig. 1. A radial phylogenetic tree illustrating clustering pattern of the landraces under study using Agglomerative Hierarchical Clustering with Euclidean Distances and Ward's method. Clustering of the landraces appeared to be on the basis of cob weight with landraces from similar regions grouping into different classes without any geographical bias. Class 3 with the highest number of landraces clustering together had the lowest average cob weight without husk while in Class 4 were clustered landraces with highest mean cob weight.

class centroids was recorded between clusters 3 and 4. Landraces N24, T2, M30 and Ma7 were identified as central objects in clusters 1, 2, 3 and 4 respectively. The greatest distance between central objects was recorded between landraces of Classes 3 and 4.

Spread in the foothills of the Eastern Himalayan region at altitudes varying from around 280 m to more than 7000 m above the mean sea level (Mao *et al.*, 2009), North East India is an established secondary centre of diversity for maize. Locally grown maize which can be grouped into primitive, advanced or derived, recent introductions and hybrid races as a result of crosses between the Sikkim primitives and advanced races within the region (Medhi *et al.*, 2010) are found at varying altitudinal and climatic variations of the NEH region, where farmers grow maize to suit their diverse food and cultural habits. In the present study despite being representatives of diverse altitudes and climatic adaptations, the clustering of these 139 landraces was not based on geographical origin. A possible explanation is that because like elsewhere, landraces in the NEH region are also subjected to open pollination resulting in highly heterozygous cobs which as a result are distinct from each other even in neighboring fields of the same area. Also, selection for cobs in landraces at the individual farmer's level is always phenotypic, favouring the most vigorous plants/cobs leading to high heterogeneity within the collections of landraces. Heterogenity is also advantageous for plant breeders from evolution point of view as it promotes genetic variability and helps to stem genetic drift while promoting new genetic recombinations in the farmers' fields for selection. Qi-Lun *et al.* (2008) while studying the diversity of 124 landraces of maize had reported presence of considerable genetic variation within landraces because farmers select from the most heterozygous plants contributing to the high genetic variation.

For the 139 landraces studied, ANOVA, PCA and AHC studies established the significant contribution of cob weight as the highest contributor to variability in the dataset. Contribution of individual landraces to the PCs also revealed that the six landraces in Cluster 4 (N9, M31, N11, Ma7, M32 and N20) with highest mean cob weight had very high loadings on PC1. Since traits with higher loadings on PC1 are the highest contributors to variation in a dataset and serve as valuable indicators for selection in a crop improvement programme, selection based on high cob weight for the 139 landraces under study is expected to be effective.

Under natural field conditions, NCLB development was recorded in all 143 genotypes approximately 60 DAS (days after sowing), varying from highly resistant to moderately resistant to highly susceptible indicating presence of variability for NCLB resistance in the collection (Table 1). The standard inbred susceptible check C6 was also susceptible to NCLB in our field conditions. Under controlled green house conditions disease lesions appeared 47 days after planting and significant differences in the area of necrotized leaf tissue for the different landraces were observed. The susceptible landraces began by producing small lesions which enlarged and coalesced to form large necrotic patches expanding to cover the entire leaf blade whereas, in case of resistant plants, the necrotic lesions remained localized. The rate of sporulation as perceived by blackening of the necrotized surface was also lower in case of landraces with low AUDPC scores. With the exception of M9 (4) (where 4 indicates the 4^{th} of ten M9 plants grown in the field), every other plant inoculated artificially under green house conditions developed the disease in varying degrees and AUDPC scores ranged from a minimum of 0 in M9 (4) to a maximum of 273.4 in T9 (8) (where 8 indicates the 8^{th} of ten T9 plants grown in the field). The field scores based on qualitative scoring for M9 had also ranged for individual plants from 1(highly resistant) to 3 (moderately resistant) while field scores of T9 ranged from 3 (moderately resistant) to 5 (highly susceptible). Certain landraces like A7 in which were recorded plants with moderate resistance scores in field conditions were exceptions which recorded very high AUDPC values in green house conditions (Table 1). Given that AUDPC scores are more reliable in assessing disease resistance, the moderately resistant field scores for A7 can be attributed to environmental reasons. In general, the susceptible landraces recorded higher AUDPC values as a result of larger lesion size, faster onset of disease and higher number of lesions post artificial inoculation. Resistance to NCLB was not specific to landraces from a particular geographical region but was found in different landraces representing all the seven hill states.

The AUDPC studies were followed by bulked DNA analysis in a subset of individuals within landraces with the highest/lowest AUDPC scores using twenty SSR markers which map close to *Ht* genes in bins 8.06 and 2.08. Seven SSR markers screened *viz. umc2005*, *umc1947*, *umc1864*, *umc1149*, *umc1997*, *umc2361* and

Table 1. Panel of twenty selected plants used for molecular studies based on their AUDPC scores under controlled greenhouse conditions

DNA Bulk No.	Selected Individual Plants	Greenhouse Audpc Score	Qualitative Disease Scores
	M9(4)	0	1
1	M24(8)	11.2	1
	N25(4)	12.2	2
	M23(2)	12.8	2
2	S2(10)	15.2	3
	M23(9)	15.6	2
	N21(5)	15.6	2
3	S9(1)	18.2	3
	Mi4(1)	18.4	3
	N25(5)	18.6	2
4	T9(8)	273.4	5
	M25(1)	202.6	5
	Ma7(7)	200.4	5
	Ma6(7)	198.7	5
	T16(3)	193	5
5	T13(1)	185.2	5
	M3(3)	181.4	5
	A11(4)	180.8	5
	A7(1)	179	3
	M11(3)	178.6	5
	C1	25.8	5
	C2	5.6	5
	C5 (Resistant Inbred)	85.9	1
	C6 (Susceptible Inbred)	105.8	5

*Figures in parenthesis for selected individual plants, indicate the plant tag number for a particular landrace.

umc2119 were found to be polymorphic. For bulk DNA studies, M9(4) which did not show any disease incidence under greenhouse conditions was excluded from the bulks and used as reference along with the standard resistant (C5) and susceptible (C6) checks (Table 1). Of the seven polymorphic SSR markers, when run on individual plant DNA, markers *umc1149*, *umc2199* and *umc2361* exhibited distinct polymorphism (Figure 2) between the resistant M9 (4) and the highly susceptible T9 (8). A7 (1) behaved similarly as the susceptible T9 (8) for all the three SSR markers. Landraces M23 (9), S9 (1), Mi4 (1) were also polymorphic like M9 (4) for *umc1149* while, M23 (2) and S9 (1) showed similar polymorphism as M9 (4) for *umc2361*. In landraces M23, S9 and Mi4 were present individual plants with high to moderate resistance disease scores that were consistent with the low AUDPC values under greenhouse conditions.

Our studies of the 139 landraces based on field screening under natural disease conditions revealed variability for disease ratings not only between but also within the landraces. This variability also did not have a geographical basis and can be similarly explained by the fact that maize landraces are subjected to high degree of cross pollination at the farmer's level as a result of which landraces collected from individual farmers tend to be heterogenous seed mixtures. Consistency between the field scores of the individual plants within and between landraces and AUDPC scores recorded under controlled conditions for majority of the landraces establish a genetic basis for nature of inheritance of the disease. Distinct polymorphism between the resistant M9 (4) and susceptible T9 (8) for markers, *umc1149*, *umc2199* and *umc2361* known to tag close to *Ht2* gene was observed. Various workers (Yin *et al.,* 2003; Chung *et al.,* 2010) had reported the association of SSR markers *umc1149*, *umc2199* and *umc2361* and the *Ht2* major gene with genetic distance between SSR marker *umc1149* and the *Ht2* gene on chromosome 8 being as little as 7.2 centimorgan. Going by these reports, polymorphism in landraces M9 (4) and T9 (8) for *umc1149 umc2199* and *umc2361* indicate presence of resistance governed by the monogenic dominant resistant *Ht2* gene. Also, since quantitative disease resistance in plants is known to be frequently associated with basal resistance (Poland, 2011) these landraces can further be explored for presence of durable resistance.

Screening for NCLB resistant germplasm is critical to check destruction by an NCLB epidemic because the pathogen is fast evolving with several reported races. Dong *et al.* (2008) while studying the race distribution in northern China had reported variation in physiological races of *S. turcica* between neighbouring provinces while Human *et al.* (2016) have reported high diversity among *E. turcicum* isolates as a result of mixed modes of reproduction adopted by the pathogen for survival in South Africa. Successful gene pyramiding through seven different backcross populations and four resistant donors into elite lines for major and minor resistant genes to NCLB have been reported in India (Prasanna *et al.,* 2010) which makes disease resistant germplasm invaluable for crop improvement (Kraja *et al*., 2000).

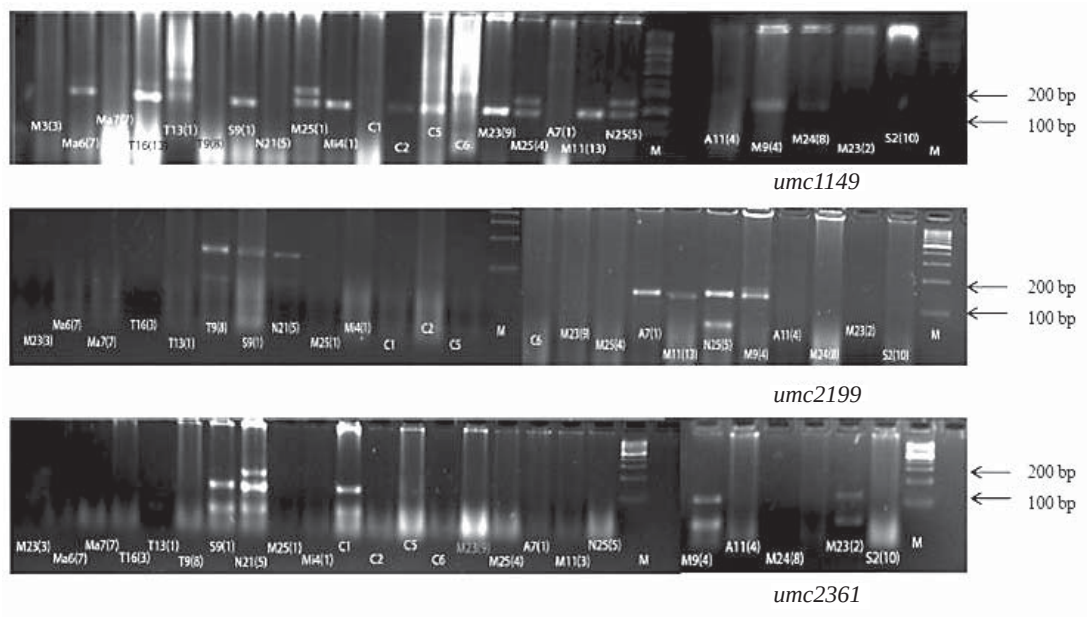

Fig. 2. Amplification patterns of SSR markers *umc1149*, *umc2199* and *umc2361* reported to map close to the *Ht2* gene for *Turcicum* blight resistance when run on a subset of individuals from the different landraces identified as resistant/susceptible based on their field and AUDPC scores. The individual lanes for the landraces are labeled accordingly where figures in parenthesis indicate individual plant tag number and M = molecular size ladder (100 base pair). C1, C2, C5 and C6 were the standard checks used in the study. The resistant M9 (4) and susceptible T9 (8) are polymorphic for all the three SSR markers.

To summarize, the current study was aimed at genetic diversity studies and characterization for NCLB resistance qualitatively and quantitatively in a collection of locally adapted and unexploited maize germplasm. ANOVA and genetic diversity studies established presence of variation for cob traits while field disease scores and AUDPC values established presence of variation for NCLB in the material studied. Both NCLB resistance and cob characteristics being critically important agronomic traits, the identified landraces can serve as raw material for exploitation in crop improvement. Also, important is that neither crop diversity nor resistance is localized to a specific geographical area within NEH region but is present across the representative germplasm studied. These landraces can therefore be further tapped to introduce variability

Table 2. Range, mean and MS (Mean Square) values of adjusted treatments and error estimated from ANOVA for the different quantitative parameters studied

Variables	Minimum value	Maximum value	Mean	Standard deviation	MS treatments (Adjusted)	MS error
SI	62.10	95.80	75.47	7.58	57.4**	10.6
TA	47.00	74.00	64.72	4.99	56.1**	3.0
ASI	1.04	32.00	10.75	6.03	36.4**	1.1
CL	3.00	7.00	4.18	1.00	1.0**	0.2
WwH	19.50	300.00	115.95	52.61	2747.4**	1409.8
WwoH	14.50	270.00	96.84	44.27	1786.6**	1264.4
NRG	3.00	22.62	11.04	4.19	1786.6**	0.4
100GW	3.50	74.25	21.50	10.02	55.5**	24.5
PH	3.20	9.00	6.43	1.48	1.7**	0.3

Days to 50% Tasseling (TA); Days to 50% Silking (SI); Anthesis Silking Interval (ASI);Cob length (CL); Cob weight with husk (WwH); Cob weight without husk (WwoH); 100 Grain weight (100GW); Number of row grains (NRG); Plant height (PH)
*- Significant at 5% level of significance; **-Significant at 1% level of significance

Table 3. Association studies based on Pearson's Correlation coefficients and squared cosines of Principal Component Analysis, for the nine quantitative parameters under study

	Pearson's Correlation coefficients:									Squared cosines of the variables			
Variables	SI	TA	ASI	CL	WwH	WwoH	NGR	100GW	PH	PC1	PC2	PC3	PC4
SI	1									0.014	**0.897**	0.085	0.004
TA	0.608**	1								0.046	0.291	0.012	0.57
ASI	0.753**	-0.063	1							0.001	**0.553**	0.076	0.301
CL	0.031	0.062	-0.012	1						**0.649**	0.003	0.002	0.008
WwH	-0.012	0.06	-0.065	0.558**	1					**0.642**	0.066	0.247	0.006
WwoH	-0.056	0.03	-0.095	0.516**	0.979**	1				**0.595**	0.091	0.256	0.006
NRG	0.127	0.153	0.032	0.491**	0.214*	0.182*	1			**0.449**	0.057	0.348	0.006
100GW	0.129	0.102	0.078	0.415**	0.232**	0.208*	0.719**	1		0.38	0.061	0.283	0.06
PH	-0.002	0.155	-0.131	0.414**	0.295**	0.274**	0.355**	0.154	1	0.306	0.005	0.019	0.148

Days to 50% Tasseling (TA); Days to 50% Silking (SI); Anthesis Silking Interval (ASI);Cob length (CL); Cob weight with husk (WwH); Cob weight without husk (WwoH); 100 Grain weight (100GW); Number of row grains (NRG); Plant height (PH)

*- Significant at 5% level of significance; **-Significant at 1% level of significance

For squared cosines of variables, figures in bold indicate high contribution of the trait in PC 1 and 2 respectively.

in maize germplasm for developing new hybrids and fortifying existing programmes via introgression.

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RESEARCH ARTICLE

Genetic Diversity through Morphological Characterisation in Betel Vine (*Piper betle* L.) of Malappuram District, Kerala, India

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The present investigation was carried out to study the diversity of betel vine in Tirur and nearby areas of Malappuram district of Kerala and to characterise the landraces based on morphological features. *Puthukodi, Chelan, Karinadan* and *Nadan* were the betel vine cultivars recorded from Malappuram District. *Puthukodi* and *Nadan* were the most common cultivars whereas *Chelan* and *Karinadan* were the cultivars conserved by few farmers. Morphological characterisation revealed distinctness of *Karinadan* and *Chelan* from other cultivars. *Karinadan* had dark green leaves with even leaf margin, short petiole, generally ovate lanceolate leaf lamina and high brittleness. The plant growth parameters like plant height and total number of leaves were significantly low in this cultivar. On the other hand, *Chelan* had light green leaves with wavy leaf margin, long petiole, ovate leaf lamina and round leaf base. The plant growth parameters such as plant height and total number of leaves were significantly high in this cultivar, resulting in higher number of leaves per plant. *Nadan, Puthukodi* and *Muvattupuzha Local* cultivars had green leaves with even margin and medium brittleness. *Puthukodi* recorded maximum leaf weight per unit area and optimum leaf parameters, making it as the most preferred land race in Malappuram district.

Key Words: Chelan, Karinadan, Muvattupuzha local, Nadan, Puthukodi

Introduction

Betel vine (*Piper betle* L.) is a dioecious, evergreen creeper belonging to the family Piperaceae. It grows in moist tropical and subtropical regions of different countries such as China, Thailand, Philippines, India, Bangladesh, Sri Lanka, etc. It is native to Central and Eastern Malaysia (Chattopadhyay and Maity, 1967). In India, it is cultivated in an approximate area of 45,000 ha as cash crop. Everyday about 15-20 million people in the country consume betel leaves. Sixty six per cent of the total production of betel leaf in India is contributed by West Bengal (Guha, 2006).

Betel vine is an indigenous medicinal plant with glabrous, deep green and heart shaped leaves as economically important part. The betel vine leaves are popularly known as *Paan* and is also known in other names like *Tamalapaku, Tambul* and *Vettilai* in different parts of India. *Bangla* had large thin leaves with nine main nerves and ovate lamina with cordate base. Leaf apex was pointed, short and not curved. Petiolar sinus of *Bangla* was more prominent than other varieties. *Desawari* had large thin leaves and cordate lamina with seven to nine nerves.

In Kerala, Tirur and nearby areas of Malappuram district are famous for betel vine cultivation with an area of 183 ha (FIB, 2014). In earlier days *Paan Bazar* in Tirur was an exclusive market for betel leaves. Best quality *Tirur betel vine* leaves are exported to Pakistan *via* North India and second grade leaves are sold in local markets (Nair, 2010). Presently *Tirur betel* leaves are also exported to Pakistan via Arab countries. Majority of people from Tirur and nearby areas depend on betel vine cultivation and allied sectors for their livelihood. Betel vine landraces from Tirur area possess some special morphological and biochemical characters like unique flavour and aroma because of geographical features, traditional cultural practices, specific genotypes, special soil characters and peculiar climatic features of area of production.

Even though *Tirur betel vine* leaf from Malappuram is a unique agricultural product of the country, studies on the variability of betel vine cultivars in Malappuram district are very scanty. Characterisation of popular betel vine types is a prerequisite to reveal the existing variability within the crop in Malappuram area. Studies on morphological characters are to be undertaken to identify

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the best cultivars for commercial cultivation and to use in both medicine and cosmetic production. Moreover, documentation of unique characters is necessary for the registration of *Tirur betel vine* as a Geographical Indication from Kerala. Presently an approved crop descriptor for betel vine is lacking. In this background, the present study was undertaken to document and characterise the betel vine cultivars of Malappuram district based on morphological characters.

Materials and Methods

The present study was undertaken in the Department of Plant Breeding and Genetics, College of Horticulture, Thrissur during 2012-2014. Experimental material was raised in farmer's field at Tirur, Malappuram which lies on the geographical coordinates of 10°54'0" N and 75° 55'0" E with an altitude of two meter above Mean Sea Level (MSL).

Information regarding betel vine landraces grown in Malappuram district and their special characteristic traits were collected through a preliminary survey conducted in seven Block Panchyaths located in the area of production of *Tirur vettila*. *Puthukodi, Chelan, Karinadan* and *Nadan* were the betel vine landraces grown in Malappuram district. Among these except *Chelan* all others were female landraces. Planting material of these four types were collected during survey. Planting material of *Muvattupuzha Local* was collected from Ashamanoor, Ernakulum district.

Puthukodi, Chelan and *Karinadan* along with *Nadan* (Local check variety) and *Muvattupuzha Local* type from Asamannoor as check variety were raised in farmer's field in Malappuram district during 2013-14. The five betel vine landraces were grown in a Randomized Complete Block Design with four replications. During the experimentation flowering was observed in all cultivars. The five betel vine types were characterized based on morphological characters.

Morphological Characterization

The betel vine types were characterised and evaluated based on morphological characters. Morphological characters were recorded from whole plant, lateral branch and leaf. Growth parameters were recorded from 15 Days After Lowering (DAL) upto 90 DAL at fifteen days interval. Leaf observations were recorded from fourth leaf from the tip of the lateral branch. Observation on days to lateral branching was recorded from the date of planting. Currently there is no approved descriptor for betel vine and hence the "Descriptor for Black Pepper" (IPGRI, 1995) and guidelines for the conduct of test for distinctiveness, uniformity and stability on black pepper (PPV & FR Authority, 2009) were followed (with suitable modifications) for characterisation of cultivars.

Qualitative Characters

The qualitative characters observed were internodal colour, lateral branch pattern, shoot tip colour, leaf margin, leaf brittleness, leaf colour, leaf lamina shape, leaf base shape and leaf apex shape. All other characters except leaf brittleness were visually observed. Leaf brittleness analyzed by slightly pressing the leaf by hand and the tendency to break was recorded and classified leaf brittleness as low, medium and high.

Quantitative Characters

The quantitative characters observed were plant height, total number of leaves, angle between orthotropic shoot and leaf petiole, days to lateral branching, days between lateral branch emergence, number of lateral branches, number of nodes per lateral branch, number of leaves per lateral branch, leaf length, leaf width, leaf area, total leaf weight, leaf weight per unit area, leaf petiole length and leaf tip angle.

Plant height was recorded in centimeters from the base to tip of the orthotropic shoot. The number of fully opened leaves were counted and expressed in number. Angle between orthotropic shoot and leaf petiole was measured and expressed in degree. Number of days from planting to lateral branching was counted and expressed in days. The days between the emergences of two consecutive lateral branches, number of lateral branches, number of nodes of the selected lateral branches and number of leaves of the selected lateral branches were counted and expressed in number. Leaf length was measured in centimeters from the base of midrib to tip of the leaf**.** Leaf width was measured in centimeters at the widest portion of leaf. Leaf area was measured by using leaf area meter model-LI -3000 model and expressed in centimeter squares. Leaf weight was measured using analytical laboratory digital weighing balance and expressed in grams. Leaf weight per unit area was calculated using the following formula and expressed in gram per centimeter square. Leaf petiole length was measured in centimeters from the petiole base to the insertion with the leaf lamina. Leaf tip angle was measured and expressed in degree.

$$\text{Leaf weight per unit area} = \frac{\text{Weight of the leaf (g)}}{\text{Area of the leaf (cm}^2\text{)}}$$

Results and Discussion

Status of Cultivation of Betel Vine

The area of cultivation of *Tirur betel vine* in Malappuram district during 2012-13 was 183 ha (FIB, 2014). It was cultivated in seven Block Panchayaths *viz.*, Ponmundam, Parappanangadi, Valanchery, Malappuram, Vengara, Tirur and Kondotty. In these Block Panchayaths most of the farmers were depending on betel vine cultivation for their livelihood. Variation was seen on different morphological characters of *Tirur betel vine* cultivars. Cultivars recorded from Tirur Block Panchayath were *Puthukodi, Karinadan, Nadan* and *Chelan*. *Puthukodi* was cultivated in larger area compared to other cultivars and exported to Pakistan. Leaves of *Nadan* were mostly preferred in local markets of Kerala. *Karinadan* and *Chelan* cultivars were conserved by few farmers in Malappuram district. Farmers mainly adopted organic method of cultivation.

Morphological Characterisation

Qualitative Characters

The qualitative characters of each landrace are presented in Table 1.

Internodal Colour

Orthotropic shoot and lateral branch expressed variability for internodal colour. Green internode with purple colour at nodal region was observed in *Nadan, Puthukodi*, and *Muvattupuzha Local*. *Chelan* showed internodal colour of light green with purple tinge whereas *Karinadan* showed uniform purple-green colour for internode. Internodal colour of lateral branch varied before and after spike formation. Purple pigmentation was present in all cultivars before spike formation. Purple colour with light green broken stripes was observed in lateral branches (without spike) of *Karinadan, Puthukodi, Nadan* and *Muvattupuzha Local* whereas *Chelan* showed light purple with light green broken stripes at internodal region. Purple pigmentation was absent in lateral branches with spikes in all landraces. Pinkish colouration in the stem of the betel vine was reported by Chaveerach *et al*. (2006).

Table 1. Qualitative morphological characters of betel vine cultivars of Malappuram district during 2013-14

Sl. No.	Morphological characters			Betel vine cultivars				
				Puthukodi	*Chelan*	*Karinadan*	*Nadan*	*Muvattupuzha Local*
1	Internodal colour	Orthotropic shoot		Green with purple colour at nodal region	Light green with purple tinge	Uniform purple green colour	Green with purple colour at nodal region	Green with purple colour at nodal region
		Lateral branch	With spike	Green	Light green	Green	Green	Green
			Without spike	Purple colour with light green broken stripes	Light purple with light green broken stripes	Purple colour with light green broken stripes	Purple colour with light green broken stripes	Purple colour with light green broken stripes
2	Lateral branch pattern			Hanging	Semi-erect	Hanging	Mostly hanging, rarely horizontal	Mostly hanging, rarely horizontal
3	Shoot tip colour			Dark purple colour with broken green stripes	Light purple with light green broken stripes	Dark purple colour with broken green stripes	Dark purple colour with broken green stripes	Dark purple colour with broken green stripes
4	Leaf margin			Even	Wavy	Even	Even	Even
5	Leaf brittleness			Medium	Low	High	Medium	Medium
6	Leaf colour			Green	Light green	Dark green	Green	Green
7	Leaf lamina shape			Mostly ovate elliptic, rarely ovate lanceolate	Ovate	Mostly ovate lanceolate, rarely ovate eliptic	Mostly ovate lanceolate, rarely cordate	Mosty ovate elliptic, rarely cordate
8	Leaf base shape			Cordate	Mostly round, rarely acute	Cordate	Mostly cordate, rarely round	Mostly cordate, rarely round
9	Leaf apex shape			Aristulate	Aristulate	Accuminate	Accuminate	Apiculate

Variations in the internodal colour could be used as a morphological marker in cultivar identification.

Lateral Branch Pattern

Three types of lateral branching pattern namely hanging, horizontal and semierect were shown by the betel vine cultivars. Hanging lateral branches were mainly seen in betel vine cultivars whereas, semi-erect lateral branches were the unique character of *Chelan*. *Nadan* and *Muvattupuzha Local* rarely produced horizontal lateral branches.

Shoot Tip Colour

All cultivars showed purple pigmentation at shoot tip. *Chelan* had light-purple with light-green broken stripes for shoot tip whereas all other cultivars showed dark purple colour with green broken stripes (Figure 1). Colour of shoot tips of *Tirur betel vine* cultivars was same as that in *Muvattupuzha Local*. An experiment conducted by Sreedevi *et al.* (2005) on black pepper recorded light purple for young orthotropic shoot tip.

Leaf Characters

Two types of leaf margins (even/entire and wavy) were found in betel vine cultivars. All cultivars, including check cultivar, *Muvattupuzha Local* showed even leaf margin except *Chelan* which produced wavy leaf margin (Figure 2). *Tirur betel vine* cultivars and *Muvattupuzha Local* showed same pattern in leaf margin. Similar findings were reported by Pariari and Imam (2012a).

Leaf brittleness varied among cultivars. Highly brittle leaves were produced by *Karinadan* and low brittle leaves by *Chelan*. Medium brittle leaves, which have more market preference were produced by *Puthukodi*, *Nadan* and *Muvattupuzha Local*. High brittleness of leaves in *Karinadan* and low brittleness of leaves in *Chelan* would have reduced their marketability.

Light-green leaves were the unique character of *Chelan* whereas dark-green leaves were the character of *Karinadan*. The remaining three cultivars *viz.*, *Puthukodi*, *Nadan* and *Muvattupuzha Local* produced green leaves. Light-green leaves and very dark-green leaves generally have less consumer preference. The dorsal surface of leaf lamina of all cultivars showed slight anthocyanin pigmentation along main veins and at the point of insertion of petiole with leaf lamina.

Leaf lamina shape varied with different cultivars in plagiotropic shoots. Four types of leaf lamina namely ovate, cordate, ovate-elliptic and ovate-lanceolate were seen in leaves from the lateral branches of the cultivars. Mostly ovate elliptic leaves were observed in *Puthukodi* and *Muvattupuzha Local*. *Karinadan* and *Nadan* mostly

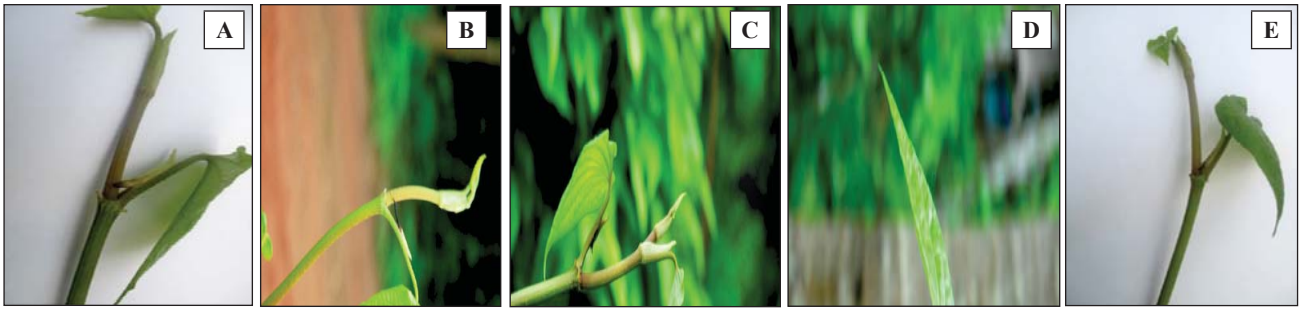


Fig. 1. Shoot tip colour in betel vine: (A) *Puthukodi*, (B) *Chelan*, (C) *Karinadan*, (D) *Nadan*, and (E) *Muvattupuzha Local*

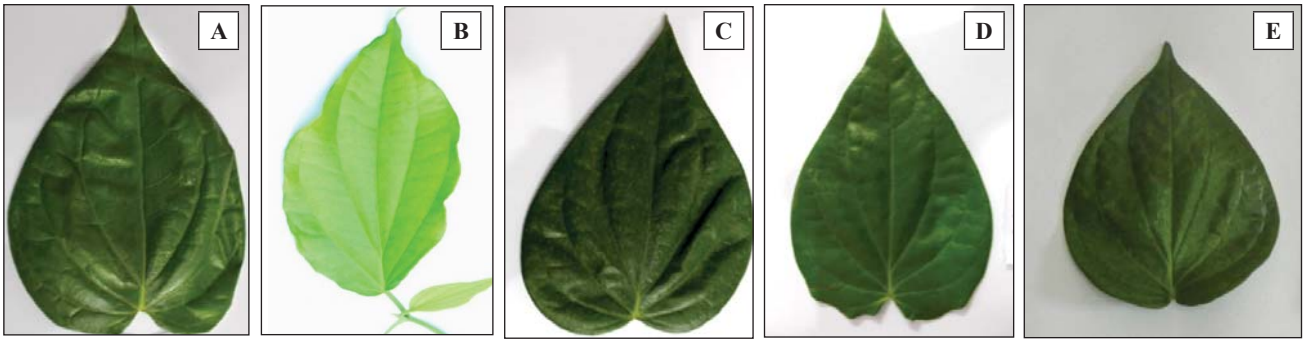


Fig. 2. Leaf margin types in betel vine: (A) *Puthukodi*, (B) *Chelan*, (C) *Karinadan*, (D) *Nadan*, and (E) *Muvattupuzha Local*

produced ovate-lanceolate leaf. *Chelan* was unique in the group to produce ovate leaf lamina. Chaveerach *et al.* (2006) observed ovate lamina for betel vine leaves whereas Pariari and Imam (2012a) observed cordate leaf lamina in plagiotropic shoots.

Generally three types leaf base were seen in betel vine cultivars. Cordate base was the most common type except in *Chelan.* In other qualitative characters, *Chelan* was found different from other cultivars with respect to leaf base also. Generally, *Chelan* produced distinguishable round leaf base and only very rarely, it produced acute leaf base. Other cultivars like *Puthukodi, Karinadan*, *Nadan* and *Muvattupuzha Local,* mostly produced cordate leaf base. However, *Nadan* and *Muvattupuzha Local* sometime also exhibit round leaf base. Chaveerach *et al.* (2006) found cordate base in *P. betle*. In black pepper, Sanchu (2000) reported cordate and round leaf bases.

Leaf apex shape varied slightly among cultivars. Three types of leaf apex namely accuminate, apiculate and aristulate were seen in leaves from the lateral branches of the cultivars. Leaf apex of *Nadan* and *Karinadan* was accuminate while aristulate leaf apex was observed in *Chelan* and *Puthukodi. Muvattupuzha Local* had apiculate leaf apex. However, slight variations from these cited shapes of leaf apex were also observed. Repeated studies are required to confirm leaf apex shape in each cultivar.

Number of lateral veins varied in cultivars. *Chelan* showed four lateral veins all others recorded six lateral veins in leaf. According to Singh (1994), *Bangla* cultivar had large thin leaves with nine main nerves. Colour of stipule or prophyll is light green in *Chelan* whereas all others showed violet tinge in stipule.

Quantitative Characters

The quantitative characters of each landrace are presented in Table 2.

Plant Height

Chelan showed significantly high value for plant height at all stages of crop growth; might be due to higher growth rate. *Karinadan* recorded lower plant height at all stages. At 90 DAL, all cultivars recorded a plant height of more than 260 cms. A study conducted by Pariari and Imam (2012a) revealed a vine length of 145.37 cm.

Total Number of Leaves

Faster growth rate in *Chelan* as evidenced by highest plant height, led to highest (280.35 per vine) total number of leaves at 90 DAL. On the other hand *Karinadan*, produced lowest leaf number (175.68 per vine). The work conducted by Pariari and Imam (2012a) showed higher annual leaf yield of 58.56 leaves per vine in *Simurali Deshi*. The number of leaves per vine at 90 DAL in *Tirur betel vine* cultivars ranged from 175.68 – 280.35. In the present study, method of trailing adopted in *Koottakodi* system would have resulted in higher leaf number per vine than the annual leaf yield (58.56 leaves per vine) in *Simurali Sanchi* recorded in '*bareja*' system by Pariari and Imam (2012a).

Lateral Branch Characters

As in the case of plant height and total number of leaves, number of lateral branches in *Chelan* recorded significantly high values. This would be due to faster

Table 2. Quantitative characters of betel vine cultivars

Sl. No.	Morphological characters	*Puthukodi*	*Chelan*	*Karinadan*	*Nadan*	*Muvattupuzha Local*
1	Days to lateral branching	134.00^{ab}	80.75^{c}	151.25^{a}	85.00^{c}	127.50^{b}
2	Days between lateral branch emergence	18.23^{c}	9.35^{d}	26.35^{a}	18.03^{c}	21.78^{b}
3	Angle between orthotropic shoot and leaf petiole (degree)	49.85^{c} (narrow)	77.95^{a} (wide)	49.12^{c} (narrow)	59.92^{b} (narrow)	57.55^{b} (narrow)
4	Leaf length (cm)	17.70^{c} (long)	14.78^{d} (medium)	19.48^{a} (long)	19.23^{b} (long)	15.25^{d} (medium)
5	Leaf width (cm)	12.63^{c} (medium)	11.50^{d} (medium)	14.40^{a} (broad)	13.80^{b} (broad)	11.75^{d} (medium)
6	Leaf area (cm^2)	156.13^{d} (medium)	134.25^{e} (low)	196.23^{a} (high)	184.08^{b} (high)	164.25^{c} (medium)
8	Leaf weight (g)	4.78^{a}	2.82^{c}	4.85^{a}	4.53^{a}	3.95^{b}
7	Leaf weight per unit area (g/cm^2)	0.028^{a}	0.021^{d}	0.025^{b}	0.022^{c}	0.022^{c}
8	Leaf petiole length (cm)	2.93^{c} (short)	4.85^{a} (long)	2.75^{c} (short)	3.30^{b} (medium)	3.35^{b} (medium)
9	Leaf tip angle (degree)	35.20^{c} (narrow)	38.90^{b} (narrow)	51.62^{a} (wide)	42.62^{b} (medium)	40.95^{b} (medium)

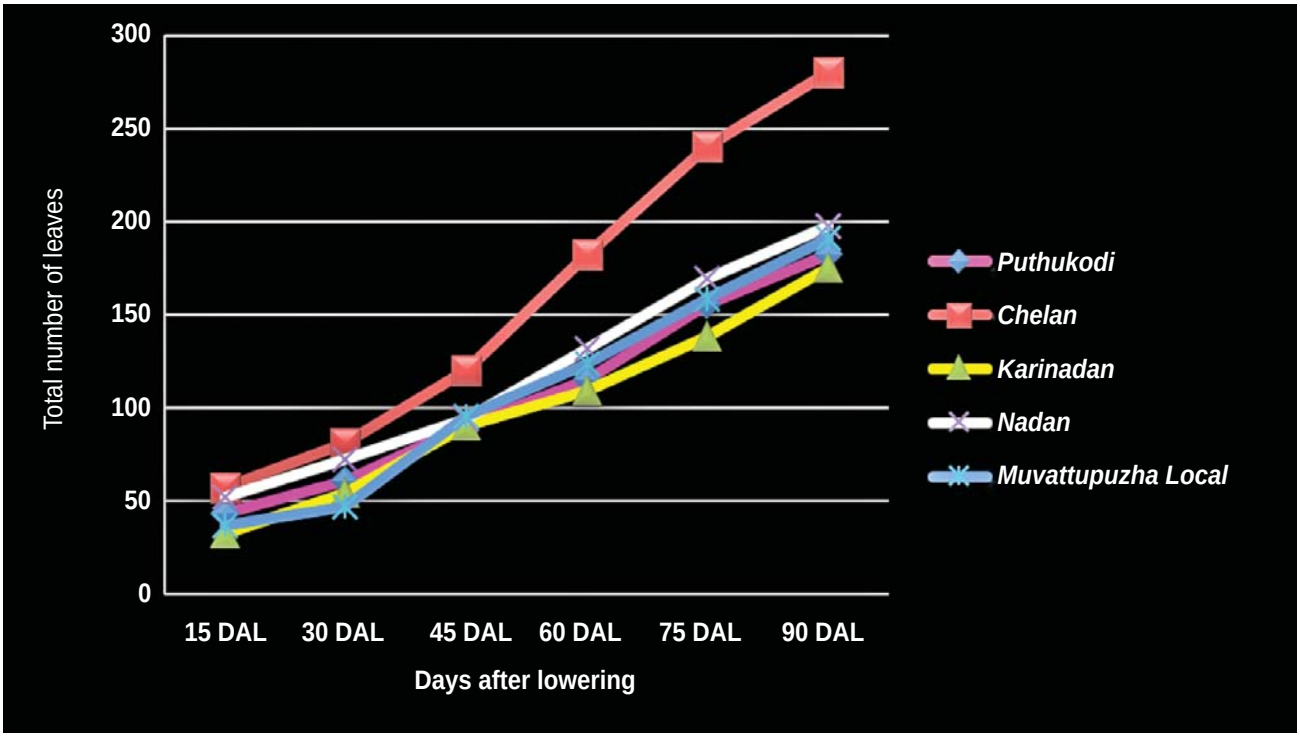


Fig. 3.Total number of leaves at different growth stages in betel vine cultivars of Malappuram district during 2013-2014

growth rate in this cultivar. *Karinadan* recorded less number of lateral branches as in other characters. *Chelan* recorded 16.88 lateral branches while *Karinadan* recorded 10.88 lateral branches at 90 DAL. *Muvattupuzha Local* recorded lower number of lateral branches than *Nadan* and more number than *Puthukodi*.

As in the case of other growth parameters, *Chelan* produced highest number of nodes per lateral branch (43.95) and *Karinadan* produced lowest number of nodes per lateral branch (26.28) at 90 DAL (Fig. 3). *Nadan* and *Muvattupuzha Local* produced almost same number of nodes per lateral branch during the same period of crop growth.

The data on number of leaves per lateral branch and number of nodes per lateral branch were same because from each node only one leaf was produced. Number of leaves per lateral branch increased in tune to increase in days after vine lowering. At all growth stages *Chelan* produced more number of leaves while *Karinadan* produced less number of leaves.

Leaves from lateral branches have more market value and are locally called as '*Kanni vettila*'. So, days to lateral branching are important with regard to production of marketable leaves. More days for the emergence of lateral branch would result in less leaf yield. Among the cultivars, *Karinadan* with slow rate of vine growth, took more number of days (151.25) for the commencement of lateral branch. This cultivar had expressed less number of total leaves also. *Chelan* with more plant height at all stages, showed early emergence of first lateral branch, and higher number of leaves at all growth stages.

Emergence of two consecutive lateral branches occurred very fast in *Chelan* as compared with other cultivars. *Chelan* with more plant height and less number of days in lateral branching, showed lesser days (9.35) between lateral branch emergence, indicating a faster growth rate. *Karinadan* with less plant height and more number of days in lateral branching took more days (26.35) between emergence of two lateral branches.

The angle between orthotropic shoot and leaf petiole is an important character in distinguishing different subtypes of *Piper* species. Except *Chelan,* which produced wide angle, all other cultivars showed narrow angle between orthotropic shoot and leaf petiole. The wide angle in *Chelan* resulted in spreading nature of leaves. Krishnamurthy *et al*. (2010) suggested that in black pepper, the leaf angle should be more at the bottom compared to top to help in filtering of more light to the bottom canopy.

Leaf Characters

Leaf length varied significantly among cultivars with highest leaf length (19.48 cm) in *Karinadan*. On the other hand lowest leaf length (14.78 cm) was observed in *Chelan*. In general, *Karinadan*, *Nadan* and *Puthukodi* showed long leaves. Pariari and Imam (2012a) also observed variation in leaf length between 14.75–16.73 cm among six cultivars namely *Ghanagette*, *Simurali Sanchi*, *Simurali Jhal*, CARI-2, CARI-6 and *Sanchi*.

The trend exhibited for leaf length was seen in leaf width also. Leaf width was more in *Karinadan* (14.40 cm) and less (11.50 cm) in *Chelan*. *Puthukodi* showed a leaf width of 12.63 cm. Optimum leaf length and leaf width are always preferred by traders to reduce handling difficulties.

Among the cultivars, maximum leaf area was recorded in *Karinadan* (196.23 cm^2) and minimum in *Chelan* (134.25 cm^2). The bigger leaves as well as small leaves of these cultivars would have added to their low acceptance in the market. *Muvattupuzha Local* (164.25 cm^2) and *Puthukodi* (156.13) recorded medium leaf area. Pariari and Imam (2012b) reported leaf area from 114.17 to 129.00 cm^2 for betel vine cultivars grown with different sources of organic manures.

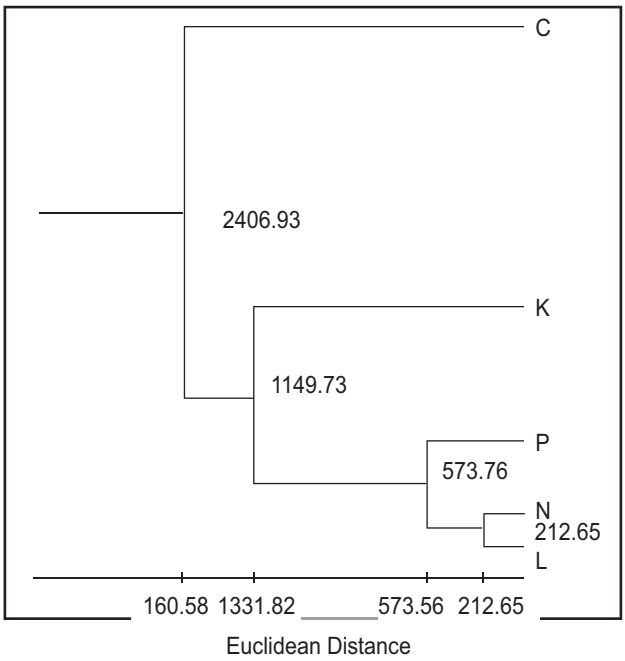
Leaf weight is an important criteria, deciding the market potential of betel leaf. *Karinadan* and *Puthukodi* produced leaves having maximum fresh weight while *Chelan* produced thin leaves with low weight. Pariari and Imam (2012b) reported a leaf weight of 3.64 gm for *Simurali Sanchi* which was close to the fresh leaf weight of *Muvattupuzha Local* (3.95 gm). The high fresh weight of leaves recorded in *Puthukodi,* would have been a reason for its high consumer acceptance. Leaf fresh weight per unit area ranged from 0.022–0.028 g/cm^2 with maximum in *Puthukodi* and minimum in *Chelan*. Pariari and Imam (2012b), in their field experiment, observed maximum fresh weight of 328.83 g for 100 leaves. Leaf weight is considered as an important parameter in the betel vine markets (Sumanasena *et al*., 2005). High fresh leaf weight might be one of the reasons for the high export potential of *Puthukodi* and on the other hand low fresh leaf weight and small leaf size are some of the probable reasons for less preference for leaves of *Chelan*.

Petiole length was significantly less in *Karinadan* and *Puthukodi*, significantly high in *Chelan*. Reddy (1996) reported 5.2-6.6 cm lengthy leaf petioles among the cultivars.

Narrow leaf tip angle (35.20^0) was seen in *Puthukodi* and *Chelan*. *Nadan* and *Muvattupuzha Local* showed medium leaf tip angle. Leaf tip angle in *Karinadan* was wide. Leaf tip angle determines the shape of leaf apex, and may act as morphological character for cultivar identification.

Diversity Analysis

The results of diversity analysis are presented in a dendrogram (Fig. 4) which illustrates the relationship between five landraces under study. The distance between two clusters or two cultivars is the measure of degree of diversification, greater the distance between two clusters, greater the divergence and *vice versa*. The dendrogram constructed, with Euclidean distance, based on morphological characters revealed that *Chelan* cultivar was distinct from other cultivars. *Chelan* showed 2406.93 Euclidean distance from cluster containing *Karinadan*, *Puthukodi*, *Nadan* and *Muvattupuzha Local*. *Karinadan* formed distinct cluster with a distance of 1149.73 from the cluster containing *Puthukodi*, *Nadan* and *Muvattupuzha Local*. It was interesting to note that *Muvattupuzha Local* and *Nadan* collected from different geographical locations, having almost similar morphological characters, proved their relatedness by



C – *Chelan,* K – *Karinadan,* P – *Puthukodi*, N – *Nadan,* L – *Muvattupuha Local*

Fig. 4. Dendrogram of taxonomic relationship of betel vine cultivars of Malappuram district during 2013-14

being in a single cluster with lowest Euclidean distance of 212.65. The cultivars from different clusters are generally opted for hybridization purpose and hence *Karinadan* and *Chelan* can be used for hybridization with the objective of combining their superior characters.

Conclusions

The study revealed that the *Chelan* is a very distinct, easily distinguishable among all the cultivar in Malappuram district in its leaf character with ovate, wavy, light-green leaves having round base. The internodes of orthotropic shoots are light-green in colour with purple tinge. The lateral branches are semi-erect. However, *Puthukodi*, *Nadan* and *Muvattupuzha Local* are morphologically quite similar to some extent showing green internode with purple colour at nodal region in orthotropic shoots, purple shoot tip, even leaf margin, medium leaf brittleness and green leaves. The shoot tip showed dark purple colour with green broken stripes in tender stem.

Chelan showed more vegetative growth with lesser days to lateral branch initiation and lesser days between two consecutive lateral branch emergence. However, leaves in this cultivar were small with less leaf length, less leaf width, less leaf area, less leaf weight per unit area and more petiole length reducing the market demand of the leaves. *Karinadan* showed less vegetative growth with more days to lateral branch emergence. The leaves are bigger in size with more leaf length, more leaf width, larger leaf area and short petiole. *Puthukodi* is identified as the most accepted landrace among farmers due to its optimum morphological characters suitable for cultivation, consumer preference and export.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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RESEARCH ARTICLE

Genetic Diversity of Sorghum (*Sorghum bicolor* (L) Moench) for Agronomic Traits and Shoot Fly Tolerance and Suitability for Winter Season

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Genetic diversity analysis of sorghum germplasm could provide useful information on selection of parental lines and diversifying the genetic base in winter sorghum wherein yield improvement has been marginal. An experiment was conducted on 4000 germplasm lines belonging to 28 countries of origin in augmented design in two winter sorghum growing regions of Maharashtra. Observations were recorded on yield parameters and traits governing shoot fly tolerance. Promising germplasm lines were identified for individual agronomic traits and shoot fly tolerance. The lines from Rwanda, Burundi and Kenya had more grain yield and the lines from India were tolerant to shoot fly. Though grain yield per plant was more variable among all the traits, the PCA analysis revealed that the panicle length, panicle width and shoot fly dead heart % contributed maximum towards divergence. A hierarchical cluster resulted in 11 clusters of originating countries with the accessions from Yemen, Gambia, Somalia, Mozambique, China and India clustering individually. Grain yield was positively associated with shoot fly resistance indicating possibility of co-evolution.

Key Words: Sorghum, Genetic Diversity, Grain yield, Shoot fly

Introduction

Sorghum is an important staple cereal for people residing in the semi-arid tropics of the world. It occupies fifth place after wheat, rice, maize and barley as one of the widely grown cereal (Sinha and Kumaravadivel, 2016). The sorghums grown in winter season (*rabi* or post-rainy season) assume importance in lieu of food, fodder and nutritional security especially in the Indian subcontinent. Across India, around 3 million tonnes of sorghum grain is produced from 5.7 million ha during the *rabi* season. The *rabi* cropping season follows the hot, wet rainy season (*kharif*) and is characterized by limited rainfall, cooler average temperature and shorter days, resulting in lower potential crop evapo-transpiration (Kholova *et al.,* 2013). The grain productivity of post-rainy season sorghum in India is very low as much of the cultivated area is under landraces that are poor in grain yield. For the past 30 years, several programs in India attempted to improve varieties and/or hybrid parents through pedigree breeding approach without much success (Sanjana Reddy *et al.,* 2009). M 35-1, a landrace selection developed in 1937 still dominates the post-rainy season sorghum areas in India (Sanjana Reddy *et al.,* 2012). Of 150 insect pests that attack sorghum, sorghum shoot fly, *Atherigona soccata* (Diptera: Muscidae) is a serious pest that causes a loss of 80–90% of grain, and 68% of fodder yield in India (Kahate *et al.,* 2014). Improvement of *rabi* sorghum productivity has a great impact on socio-economic status of the sorghum growing regions in India. Hence, there is an urgent need to identify and introgress new genetic diversity for developing improved lines with higher grain and fodder yields and tolerance to shoot fly.

Knowledge of genetic diversity of a crop usually helps the breeder in choosing desirable parents for the breeding program. The more diverse genotypes or accessions can be crossed to produce superior recombinants with high yield and resistance to abiotic and biotic stresses. Quantitative traits were reported as useful tool for preliminary evaluation of genetic diversity (Sinha and Kumaravadivel, 2016). In the present study, an attempt has been made to determine the extent of diversity among the 4000 sorghum accessions for yield traits and tolerance to shoot fly.

Materials and Methods

Plant Material

The plant material consisted 4000 accessions obtained from Indian Institute of Millets Research (IIMR)

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genebank, India. Among these, India is the major source of germplasm with 1494 accessions followed by Zimbabwe with 405 accessions and Uganda with 270 accessions (Table 1) in the current set of germplasm lines studied.

Experiment

The plant material was multiplied during 2012 *rabi* season at IIMR, Hyderabad, Telangana and evaluated at Mahatma Phule Krishi Vidyapeeth, Rahuri and Marathwada Agricultural University, Parbhani, Maharashtra during *rabi* 2013. The 4000 germplasm lines were evaluated in augmented design along with five checks viz., IS 18551 (shoot fly tolerant line), DJ 6514 (shoot fly susceptible line), CSV 8R, CSV 22, CSV 29R (high yielding varieties with *rabi* adaptation released at

Table 1. Variability parameters of sorghum germplasm lines evaluated at Parbhani and Rahuri during *rabi* 2013

Sl. No.	Region of origin	No. Acc.	Days to 50% flowering				Plant height (cm)				Panicle length (cm)				Panicle width (cm)			
			Mean	Range	SD	CV (%)	Mean	Range	SD	CV (%)	Mean	Range	SD	CV (%)	Mean	Range	SD	CV (%)
1	Benin	64	84	64-105	9.5	11.3	180	74-269	39.7	22.12	**25.7**	7.5-45.1	8.7	33.9	3.7	1.7-8.5	1.2	31.0
2	Burkina Faso	118	83	56-102	10.2	12.4	169	79-268	40.1	23.82	23.6	9.9-37.6	6.2	26.3	3.5	1.0-5.6	0.9	26.1
3	Burundi	44	88	68-108	9.0	10.2	164	78-230	29.1	17.74	18.5	12.7-31.4	3.8	20.5	3.7	1.7-6.4	1.1	29.5
4	Cameroon	80	**77**	56-103	10.5	13.6	**146**	62-229	35	23.92	18.1	6.8-34.7	6.5	36.1	3.6	2.0-5.6	0.8	22.9
5	China	12	80	67-93	8.4	10.6	147	90-181	26	17.64	17.8	10.1-28.7	5.5	31.2	**4.0**	1.8-6.2	1.3	31.5
6	Ethiopia	107	85	62-105	10.5	12.4	171	88-260	33.1	19.31	22.2	7.5-38.3	6.1	27.7	**4.0**	1.4-6.7	1.0	24.9
7	Gambia	15	88	70-102	8.1	9.2	166	104-241	34.7	20.87	**25.8**	15.9-35.9	6.7	26.1	3.7	1.4-5.1	1.1	28.9
8	Ghana	23	82	69-93	7.6	9.3	178	122-240	29.2	16.38	24.7	17.1-45.0	6.9	28.1	3.5	2.0-6.2	1.0	27.4
9	Hungary	20	**77**	57-91	7.9	10.2	147	90-192	29.8	20.24	20.7	13.8-39.4	6.6	31.8	3.7	1.7-5.5	1.0	26.7
10	India	1494	**77**	53-105	9.0	11.6	169	80-269	29.4	17.44	17.7	5.8-49.4	5.7	32.2	**4.1**	1.5-10.5	1.0	24.4
11	Kenya	59	82	63-103	9.3	11.4	156	82-223	32.5	20.87	20.2	10.1-37.4	5.4	26.6	3.8	2.0-5.9	0.9	24.0
12	Lesotho	123	81	57-103	9.1	11.2	**146**	82-225	27.3	18.73	19.9	9.3-37.6	5.1	25.5	3.8	1.5-7.3	1.0	25.8
13	Malawi	19	85	63-103	10.0	11.7	**190**	139-243	27.9	14.63	**27.7**	11.5-38.5	7.7	27.9	3.8	2.0-6.3	0.9	22.6
14	Mali	81	82	59-104	9.6	11.7	180	82-255	36.6	20.4	24.8	8.0-38.8	7.4	29.6	3.7	1.2-6.2	1.1	28.9
15	Mozam-bique	13	93	74-105	8.4	9.0	175	110-236	37.7	21.52	25.9	16.3-32.8	5.4	20.8	3.7	1.2-6.6	1.3	36.0
16	Nigeria	82	80	58-102	9.9	12.4	166	82-277	40.8	24.64	21.4	7.7-37.8	6.9	32.3	3.7	2.3-7.1	0.8	22.6
17	Rwanda	32	88	73-102	8.4	9.6	171	100-236	37.1	21.73	18.9	13.0-27.2	3.8	20.1	3.8	1.8-5.8	1.0	26.4
18	Somalia	12	**75**	64-88	8.1	10.8	157	117-216	27.2	17.34	15.4	6.6-34.6	8.7	56.3	3.2	2.0-5.4	1.0	31.9
19	South Africa	147	83	57-106	9.1	11.0	151	74-240	33.0	21.89	21.9	8.1-36.9	5.1	23.1	3.5	1.0-5.8	0.8	23.5
20	Sudan	142	82	56-104	9.6	11.6	162	82-270	31.6	19.54	21.2	7.0-40.1	6.3	29.6	3.5	1.0-5.7	0.9	26.5
21	Swaziland	80	86	62-105	8.9	10.3	153	87-236	30.0	19.57	22.2	8.8-33.4	4.7	21.3	3.4	1.2-6.5	0.9	26.6
22	Tanzania	132	86	65-106	9.5	11.0	176	105-273	34.4	19.57	21.9	9.0-46.1	6.6	29.9	3.9	1.0-6.7	1.2	29.8
23	Togo	78	80	60-101	9.7	12.2	175	88-259	40.5	23.15	23.2	8.1-37.6	7.3	31.7	3.6	1.4-6.7	0.9	24.3
24	Uganda	270	82	60-114	9.6	11.8	165	89-254	32.0	19.35	19.2	7.2-37.2	6.4	33.6	3.8	1.6-6.9	1.0	25.6
25	USA	129	79	59-96	7.9	9.9	154	79-242	35.3	22.88	20.1	6.3-35.7	5.5	27.5	3.7	1.0-6.7	0.9	25.3
26	Yemen, Republic of	102	84	58-104	10.8	12.9	166	69-262	32.0	19.26	17.7	7.0-35.1	5.2	29.3	3.7	1.0-6.5	1.2	31.3
27	Zambia	117	83	61-110	10.8	13.1	156	82-246	32.7	20.97	21.1	8.5-38.1	5.7	27.1	3.7	1.5-6.7	1.0	27.6
28	Zimbabwe	405	84	56-108	10.6	12.7	161	74-277	36.3	22.56	21.5	8.5-45.1	6.1	28.5	3.6	1.0-6.5	0.9	26.0
	Total	4000	81	53-114	10.0	12.4	165	62-277	33.43	20.28	19.9	5.8-49.4	6.4	32.1	3.9	1.0-10.5	1.0	26.4

1Leaf glossiness evaluated visually on a 1–5 scale (1 = highly glossy, and 5 = non-glossy) at fifth leaf stage

national level). The material was sown in 20 blocks with 200 entries in each block. The checks were randomised in each block. The experimental site at Rahuri is located at an altitude of 657m above mean sea level with latitude of 19.24°N and longitude of 74.39°E. The experimental site at Parbhani is located at an altitude of 357m above mean sea level with latitude of 19.25°N and longitude of 74.80°E. Both the locations receive a rainfall of 260-280 mm during crop growth period. Each accession at each location was raised in a single row of 2 meters length by adopting a spacing of 60cm × 15cm. All the recommended crop production packages were followed to raise a healthy crop. Observations were recorded on days to 50% flowering, plant height (m), panicle length (cm), panicle width (cm), grain yield per plant (g), glossy score (on a 1 to 5 scale where 1= more glossy and 5= less glossy) and the shoot fly infestation as per cent dead hearts at 28 days after emergence (Sharma and Nwanze, 1997). The mean values were utilized for statistical analysis to assess the genetic diversity among the accessions.

Statistical Analysis

The data was analyzed using augmented design for both the locations. The adjusted values were pooled and the mean, range and variances were calculated for the seven traits. Instead of standardisation of variables for cluster analysis, correlation matrix was used since correlations are themselves standardized. The mean values were used to perform principal component analysis (PCA) on Genstat 12 release (VSN International, 2010). The genotypes were grouped along their country of origin. Euclidean measure of genetic distance was used for estimating genetic distance among accessions (Mohammadi and Prasanna, 2003) and the hierarchial cluster analysis was performed based on the origin to group the country of origin. Phenotypic correlations were estimated among all quantitative characters and their significance was tested (Snedecor and Cochran, 1980).

Results and Discussion

Mean Performance

Agronomic traits: In sorghum, flowering is considered as a crucial event as it plays a key role in adaptation and geographical distribution of this crop (Mauro-Herrera *et al.,* 2013). The days to 50% flowering in the 4000 germplasm lines evaluated in the study ranged from 53 to 114 days (Mean: 81 days, SD: 10) (Table 1). The early (<55 days) and the late (>108 days) flowering lines are given in Table 2. The high yielding checks flowered in 76 to 84 days. Sorghum responds to the flowering time by two mechansims; firstly the sensitivity or insensitivity of genotypes to photoperiod and secondly, the inheritance of early or late flowering characteristic (Shehzad *et al*., 2014). On an average, the germplasm lines from Somalia, India, Hungary and Cameroon were early (75-77 days) while the lines from Mozambique were late (93 days). The lines from Cameroon were most variable (CV: 13.6) followed by Zambia (CV: 13.1) and Yemen (CV: 12.9) for flowering time. With a shorter daylength in the post-rainy season, all the accessions, including those from tropical African countries, flowered in a relatively shorter time as has been earlier reported by Appa Rao *et al.* (1996). As sorghum has been postulated to have originated in the north east quadrant of Africa (Doggett, 1988) and was transported to India only about 3000 years ago, it is a short day plant (Appa Rao *et al.*, 1996). The plant height ranged from 62 to 277 cm (Mean: 165 cm, SD: 33.4). The dwarf (<0.75 m) and the tall (>270 cm) lines are given in Table 2. The high yielding checks had a plant height of 169 to 208 cm. From the country means, it can be seen that lines from Cameroon, Hungary, China and Lesotho are dwarf (<147 cm) while those from Malawi were tall (190 cm). The lines from Nigeria were most variable (CV: 24.6) followed by Cameroon (CV: 23.9) and Burkina Faso (CV: 23.8). Panicle length and width are important components which contribute to yield. Panicle length is a stable character which is a characteristic feature of a particular race (Doggett 1988, Harlan 1992). The panicle length ranged from 5.8 to 49.4 cm (Mean: 19.9 cm, SD: 6.4). The 13 lines had a panicle length above 40 cm while the high yielding checks had a panicle length of 18.3 to 20.6 cm (Table 2). The lines from Malawi had a greater mean panicle length of 27.7 cm followed by Gambia (25.8 cm) and Benin (25.7 cm). The lines from Somalia were most variable (CV: 56.3) followed by Cameroon (CV: 36.1) and Benin (CV: 33.9) for panicle length. The panicle width ranged from 1 to 10.5cm (Mean: 3.9 cm, SD: 1.0). The 13 lines with stouter panicles (>7 cm) are given in Table 2. The high yielding checks had a panicle width of 4.4 to 5.2 cm. The lines from India, China and Ethiopia had greater panicle width (4.0 to 4.1 cm). The lines from Mozambique were more variable (CV: 36)

Table 2. Promising germplasm lines identified for yield parameters and shoot fly tolerance in sorghum germplasm lines evaluated at Parbhani and Rahuri during *rabi* 2013

Trait	Description	Value	Promising Germplasm Lines	Best Check
Days to 50% flowering	Early	<55 days	IS 480, EC 524565, IS 1385	C3: 76 days
	Late	>108 days	EC 483654, EC 483741, EC 524852, EC 524853	
Plant height (cm)	Tall	>270 cm	IC 290960, IS 1648, IS 1651, IS 1936, IS 2070, EC 524626	C4: 208 cm
	Dwarf	<75 cm	IC 253668, IS 1879, IS 2648, EC 533462, EC 524675	
Panicle length (cm)	Greater	>40 cm	EC 483888, IS 2154, EC 483502, EC 524644, EC 483887, IS 3735, EC 483656, IS 1961, IS 1976, IS 2137, IS 2434, EC 483857, EC 483862	C4: 20.6 cm, C5: 20.6 cm
Panicle width (cm)	Wider	>7 cm	EC 483117, EC 483213, IS 249, IS 323, EC 483255, IC - 253562, IS 1071, IS 1175, IS 1742, IS 1789, IS 1791, IS 1802, EC 524654	C4: 5.2 cm
Grain yield per plant (g)	Higher	>100 g	EC 483105, EC 483116, EC 483271, EC 483279, IS 585, IS 1119, IS 2168, IS 2343, IS 2597, EC 484181, IS 3366, EC 524799, EC 489763	C4: 57 g
Glossy score	More glossy	Score 1	IS 94, IS 222, IC 484370, IC 289482	C1: 1.9
Shoot fly dead hearts	Less	<15%	EC 483116, IS 182, EC 483203, EC 524490, EC 483226, IS 291, IC 291008, IS 1441	C1: 24%

1 Leaf glossiness evaluated visually on a 1–5 scale (1 = highly glossy, and 5 = non-glossy) at fifth leaf stage
C1: IS 18551, C2: DJ 6514, C3: CSV 8R, C4: CSV 22, C5: CSV 29R

followed by Somalia, Yemen and Benin (31.0 to 31.9). The grain yield per plant ranged from 4 to 144 g. The lines, EC 483105, EC 483116, EC 483271, EC 483279, IS 585, IS 1119, IS 2168, IS 2343, IS 2597, EC 484181, IS 3366, EC 524799, EC 489763 had more than 100 g/plant while the high yielding checks had 39 to 57 g/plant (Table 2). The lines from Rwanda, Burundi and Kenya had more average grain yield per plant (40.3 to 42.4 g). The lines from Gambia, Yemen and Ethiopia were more variable (51.4 to 55.4) (Table 1). Earlier workers identified greater diversity in the germplasm collections made from eastern parts of Ethiopia (Geleta and Labuschagne, 2005), Eritrea (Ghebru *et al.,* 2002), in the varieties collected from Burkina Faso (Barro-Kondombo *et al.,* 2010, Kondombo *et al.,* 2016) and in local landraces in Cameroon (Barnaud *et al.,* 2007).

Shoot fly tolerance traits: Host plant resistance (HPR) is one of the most economic and practical means for controlling shoot fly damage because it does not involve any extra cost to the farmers or require application skills in pest control techniques (Sharma, 1985). Although several improved varieties and hybrids have been developed and released, the yield gains at farmers' level are minimal (Sharma *et al.,* 2003). Therefore, there is a need to combine shoot fly resistance with high grain yield to increase the productivity of this crop. In the current study, the glossy score among the 4000 germplasm lines ranged from 1 to 5 (Mean: 3.1, SD: 0.7). Leaf glossiness has been reported to be associated with resistance to sorghum shoot fly (Agrawal and Abraham, 1985). There is negative correlation between glossiness with oviposition and deadhearts (Kamatar and Salimath, 2003). The intensity of leaf glossiness at the seedling stage is positively associated with the level of resistance to shoot fly (Sharma *et al.,* 1997). The lines IS 94, IS 222, IC 484370 and IC 289482 had a glossy score of 1 while the resistant check, IS 18551 had a score of 1.9 and the susceptible check, DJ 6514 had a score of 3.2 (Table 2). The lines from India and China has less mean glossy score of 2.8. The lines from China, Gambia, Rwanda and India were more variable (CV: 23.8 to 24.8) (Table 1). The shootfly infestation in terms of dead heart ranged from 8 to 100%. The lines, EC 483116, IS 182, EC 483203, EC 524490, EC 483226, IS 291, IC 291008, IS 1441 had less than 15% dead hearts while IS 18551 had 24%, DJ 6514 had 79% and the high yielding checks recorded 31-34% dead hearts (Table 2). The lines belonging to India had less mean dead heart % of 53 and more variable as well (CV: 29.5). The reason for less dead heart % in Indian lines might be due to local adaptation of the lines favouring resistance. Also, the majority of the resistance sources for shoot fly, stem borer and striga were found to originate from India (Appa Rao *et al.,* 1996). Among all the traits, grain yield per plant was more variable followed by panicle length.

Diversity Analysis

Principal Component Analysis (PCA) carried out using data of seven traits captured 81.5% of total variation from the first two principal components (PCs) (Table 3). The first principal component (PC) alone explained 68.5% of the total variation, mainly due to variation

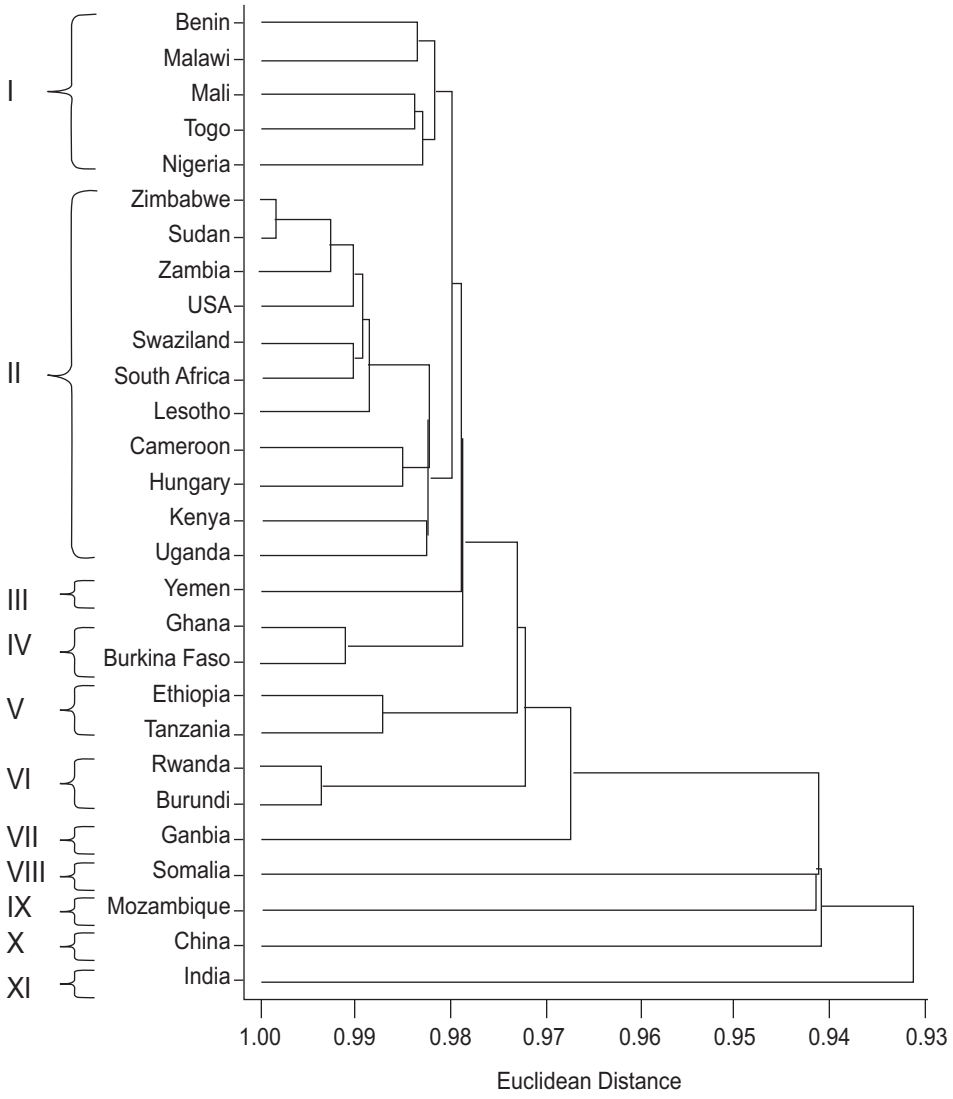

Fig. 1. Dendrogram generated by cluster analysis showing the relationships among the 28 regions of origin of 4000 sorghum accessions

Table 4. Characteristic means of 11 similarity cluster groups of origins of sorghum accessions

Cluster No.	Region of origin	No. of Acc	Days to 50% flowering	Plant height (cm)	Panicle length (cm)	Panicle width (cm)	Grain yield per plant (g)	Glossy score	Shoot fly dead hearts (%)
1	Benin, Malawi, Mali, Nigeria, Togo	324	82	178	25	4	34	3.2	63
2	Cameroon, Hungary, Kenya, Lesotho, South Africa, Sudan, Swaziland, Uganda, USA, Zambia, Zimbabwe	1572	81	154	21	4	36	3.2	64
3	Yemen, Republic of	102	84	166	18	3.7	36	3.3	68
4	Burkina Faso, Ghana	141	82	173	24	4	31	3.4	69
5	Ethiopia, Tanzania	239	86	173	22	4	39	3.3	64
6	Burundi, Rwanda	76	88	167	19	4	42	3.2	64
7	Gambia	15	88	166	26	3.7	30	3.5	65
8	Somalia	12	75	157	15	3.2	37	3.1	63
9	Mozambique	13	93	175	26	3.7	31	3.6	75
10	China	12	80	147	18	4.0	40	2.8	64
11	India	1494	77	169	18	4.1	39	2.8	53

Table 3. Principal component analysis for yield parameters and shoot fly tolerance in sorghum germplasm lines evaluated at Parbhani and Rahuri, 2013 *rabi* season

	PC I	PCII
Eigen value	0.35	0.67
% total variance	68.5	13.0
Cumulative variance %	68.5	81.49
Factor loading by various traits		
Days to 50% flowering	0.00	-0.01
Plant height	-0.01	0.03
Panicle length	**0.11**	0.02
Panicle width	-0.34	0.11
Grain yield per plant	0.02	-0.02
Glossy score	0.03	0.04
Shoot fly dead heart (%)	**0.27**	-0.04

in panicle length, shoot fly infestation as percent dead heart and the panicle width with negative loading. Panicle width contributed more to the second PC and accounted for 12.99 % of the total variation. The PCA analysis revealed that the panicle length, panicle width and shoot fly dead heart % contributed maximum towards divergence.

Depending on the divergence between clusters, emphasis is given for deciding the parents for hybridization (Rohman *et al.*, 2004). A hierarchical cluster resulted in 11 clusters of originating countries (Fig. 1 and Table 4). Cluster 1 consisted of accessions from five countries, cluster 2 from 11, cluster 4, 5, 6 from two each while the accessions from Yemen, Gambia, Somalia, Mozambique, China and India clustered individually. Cluster 1 had accessions with greater plant height while the second cluster had those with dwarf height. Cluster 6 grouped the accessions with greater grain yield per plant while cluster 7 for those having greater panicle length. Cluster 8 grouped the accessions with early flowering while cluster 9 for those having greater panicle length. Cluster 10 had the accessions with more panicle width, grain yield per plant and lesser glossy score. Cluster 10 had accessions from India which were the source for early flowering, more panicle width, grain yield per plant and shoot fly tolerance traits (less glossy score and less dead hearts%). Ramu *et al.* (2013) found that guineas from India and Western Africa formed two distinct clusters.

Correlations

The lines with more dead hearts (shoot fly susceptibility) was associated with delay in flowering, shorter plant height, greater panicle length, less panicle width and less grain yield. Grain yield was positively associated with shoot fly resistance (less glossy score and less dead hearts%), late flowering, greater plant height and more panicle width (Table 5). High positive and significant correlation of panicle weight with grain yield was reported by earlier researchers (Bakheit, 1989; Senthil and Palanisamy, 1995)

Conclusions

This study supports that quantitative traits are useful for preliminary evaluation of genetic diversity. The principle component analysis and hierarchical cluster analysis grouped the sorghum accessions under 11 clusters with six countries including India clustering individually. The Indian accessions were distinct from others and hence can be combined with the lines from other countries for generating diversity for yield traits. However, the lines from India were better tolerant to shoot fly than the countries from other clusters. Hence incorporation of stable resistance into elite genetic background will minimize the use of toxic chemicals leading to environmentally friendly and sustainable sorghum production in the semi-arid tropics. The germplasm lines identified superior for individual traits can be utilised in breeding program for generating diversity. Correlation studies clearly showed that the grain yield and shoot

Table 5. Correlation among yield parameters and shoot fly tolerant traits in sorghum germplasm lines evaluated at Parbhani and Rahuri during *rabi* 2013

Trait	Glossy score	Shoot fly dead hearts %	Days to 50% flowering	Plant height (cm)	Panicle length (cm)	Panicle width (cm)
Shoot fly dead hearts (%)	0.43**	1.00				
Days to 50% flowering	0.25**	0.27**	1.00			
Plant height	-0.13**	-0.12**	0.02	1.00		
Panicle length (cm)	0.15**	0.18**	0.19**	0.35**	1.00	
Panicle width (cm)	-0.21**	-0.19**	-0.10*	0.19**	0.04	1.00
Grain yield/ panicle (g)	-0.13**	-0.20**	0.11**	0.12**	-0.06	0.18**

* and **, Significant at 5% and 1% probability levels, respectively

fly resistance are positively associated paving way for simultaneous selection.

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RESEARCH ARTICLE

Molecular Characterization of Rice Accessions Using Microsatellite Markers

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Genetic improvement mainly depends on the extent of genetic variability present in the population. The molecular marker is a useful tool for assessing genetic variations and resolving cultivar identities. The objective of this study was to evaluate the genetic divergence of 24 rice accessions using SSR markers. A total of 48 alleles were detected in 24 accessions and the number of alleles per locus ranged from 1 to 5 with an average of 2 alleles per locus. The results revealed that out of twenty four, fourteen primers showed distinct polymorphism. The PIC value ranged from 0.07 to 0.79 with an average of 0.23. Markers having polymorphic reaction along with high PIC value *i.e.* RM 287 (0.79) and RM 22565 (0.69) should be potentially used for molecular characterization of accessions from various sources. The dendrogram based on SSR marker analysis grouped the rice accession into three clusters, where cluster II was the largest with 15 accessions. The cluster analysis showed higher level of genetic variation among the genotypes. Similarity coefficients ranged from 0.53 to 0.92. Coefficient of similarity revealed that the rice accessions of cluster I *viz.,* Kala jira and CGR: 7144; Jhingo of cluster II and Cross 116 of cluster III were genetically distant from rest of the accessions as shown by low genetical similarity coefficient. With the aid of microsatellite makers and clustering data, different distantly related rice genotypes may be combined by intercrossing genotypes from different clusters to get hybrid varieties to get better heterosis. The information obtained from the DNA fingerprinting studies helps to distinctly identify and characterize the various genotypes.

Keywords: Dendrogram, Genetic divergence, SSR markers

Introduction

Rice is the staple food for more than half of the world's population and it is a model plant for genomic research. Rice belongs to the grass family Poaceae, the genus having 21 wild and 2 cultivated species. It has rich genetic diversity in the form of thousands of land races and progenitor species. From the commercial point of view, DNA fingerprinting is a useful tool for varietal protection to prove ownership of plant lines. Moreover, the analysis of genetic diversity and relationship between or within different species, populations and individuals is a prerequisite for effective utilization and protection of plant genetic resources (Weising *et al.,* 1995). As DNA being the basis of genetic differences between distinct organisms, DNA fingerprinting is an effective method of biological differentiation. In principle, genetic uniqueness is brought about by two factors inheritance and new mutations. Genetic differences between individuals are laid down in the primary sequence of their genomic DNA; the most straight forward method is identifying an individual sequence for genomes under comparison (Krawczak and Schmidtke, 1994). Molecular marker technology is the powerful tool for determining genetic variation in rice varieties (Xu *et al.,* 1974). Among various PCR based markers, SSR markers are more popular in rice because they are highly informative, mostly mono locus, codominant, easily analyzed and cost effective (Gracia *et al.,* 2004). Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique accessions that compliments existing cultivars. Therefore, in the present context an attempt was made to evaluate the genetic divergence of 24 rice accessions with 24 SSR markers.

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Materials and Methods

Plant Material

A field experiment was conducted during *Kharif* 2015 at Research cum Instructional Farm, IGKV, Raipur. A total of twenty four rice accessions were used for the molecular analysis (Table 1).

Genomic DNA Isolation

DNA was isolated from fresh leaves by using CTAB method of DNA extraction (Zheng *et al*., 1995). The DNA samples were quantified using Nano Drop Spectrophotoscopy (NANODROP 2000). After

Table 1. List of the 24 rice accessions, their popular name and location of collection

S.No.	Collector's No.	IC No.	Accessions name	Village	Block	District
1	CGR:6323	IC-125558	Kanak chudi	Kurandi	Jagdalpur	Bastar
2	CGR:6366	IC-125601	Cross 116	Ludenga	Patthalgaon	Raigarh
3	CGR:6416	IC-125648	Deshi safri	Peeparkhar	ChhuiKhadan	Rajnandgaon
4	CGR:6644	IC-125878	Gada khuta	Muli	Bakawand	Bastar
5	CGR:6711	IC-125945	Gedrei	Buranga	Bagicha	Raigarh
6	CGR:7133	IC-114277	Jhaler genda	Kopa	Bagicha	Raigarh
7	CGR:7139	IC-114281	Jhili	Sarnadi	Balrampur	Sarguja
8	CGR:7142	NA	Jhilli	Siwani	Balod	Durg
9	CGR:7144	NA	Jhilli	Bancharoda	Abhanpur	Raipur
10	CGR:7159	IC-114289	Son jhilli	Marga	Jashpur Nagar	Raigarh
11	CGR:7168	IC-114295	Jhilli paragi	Femse	Duldula	Raigarh
12	CGR:7176	IC-114303	Jhingo	Sarjhoka	Baikundpur	Sarguja
13	CGR:7187	IC-114309	Jhumaki	Durgukondal	Durgukondal	Bastar
14	CGR:7189	IC-114311	Jhuna	Kewra	Lakhanpur	Sarguja
15	CGR:7209	IC-114325	Kala jira	Kalwa	Bagicha	Raigarh
16	CGR:7210	NA	Kanak jira	Dharampura	Dhamdha	Durg
17	CGR:7211	IC-114326	Maiphal jira III	Chotpan	Geedam	Bastar
18	CGR:7306	IC-114362	Kakadiha	Boda	Saraipali	Raipur
19	CGR:7367	IC-114403	Kali muchh	Chakserai	Kailaras	Muraina
20	CGR:7472	IC-114470	Kanthdudgi	Dharam Jai Garh	Dharam Jai Garh	Raigarh
21	CGR:7565	IC-114505	Kera ghul	Hukuram	GharGhoda	Raigarh
22	CGR:7593	IC-114518	Ketaki 11	Mallaur	Manendragarh	Sarguja
23	CGR:7629	IC-114539	Khira sar	Kewri	Lakhanpur	Sarguja
24	CGR:7634	IC-114543	Khira sar	Bilsiya	Surajpur	Sarguja

quantification, the DNA was diluted with TE such that the final concentration of DNA was approximately 40-50 ηg/µl for PCR amplification.

PCR Amplification and Electrophoresis

A set of 24 microsatellite markers distributed over 12 chromosomes of rice were used. 2 µl of diluted template DNA of each genotype was dispensed at the bottom of PCR plate. Separately cocktail was prepared in an Eppendorf tube as described in Table 2. Cocktail of 8 µl was added to each sample and the PCR was set up as the profile depicted in Table 3. Five per cent polyacrylamide gels (vertical) were used for better separation and visualization of PCR amplified products, since polyacrylamide gel (PAGE) have better resolution for amplified products.

Data Analysis

Clearly resolved unambiguous bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of matrix with'1'and'0', which indicate the presence and absence of bands in each accessions, respectively. Polymorphic information content (PIC) values were calculated for each of the SSR loci using the formula developed by Nei (1973).

$$PIC = 1-\Sigma Pi^2$$

Where, Pi is the allele frequency for the i^{th} allele.

Results and Discussion

Assessment of genetic diversity is an essential component in germplasm characterization and conservation. Selection increases the frequency of alleles or allelic combinations with favorable effects at the expense of others, eventually eliminating many of them (Cao *et al*., 1998).

In the present investigation 24 SSR markers (Simple Sequence Repeats) were used to characterize and assess genetic diversity among 24 rice accessions, out of which 14 markers showed polymorphism (Table 4). After analysis the data generated from 24 markers, a total of 48 alleles were detected in 24 rice accessions. The number of alleles per locus generated by each marker ranged from one to five alleles with an average number

Table 2. PCR mix for one reaction

Reagent	Stock concentration	Volume (µl)
Sterile and nanopure H_2O	-	5.2
PCR buffer A	10 X	1.0
dNTPs (Mix)	2.5 mM	0.5
Primer (forward+ reverse)	5 ρmol	1.0
Taq polymerase	3 U/µl	0.3
DNA template	50 ηg/µl	2.0
Total		10

Table 3.Temperature profile used for PCR amplification using micro-satellite markers

Steps	Temperature (°C)	Duration (min.)	Cycles	Activity
1	94	5	1	Denaturation
2	94	0.5		Denaturation
3	55	0.5	35	Annealing
4	72	1		Extension
5	72	7	1	Final Extension
6	4	∞		Storage

Table 4. List of 24 microsatellite markers with their number of alleles, allele size and PIC value found among 24 rice accessions

S. No.	Marker	Number of alleles	Allele size (bp)	PIC Value
1	RM 1	3	85, 105, 110	0.45
2	RM 11	1	135	0
3	RM 19	2	225, 250	0.46
4	RM 25	2	150, 155	0.07
5	RM 152	2	95, 105	0.15
6	RM 154	1	170	0
7	RM 161	1	170	0
8	RM 171	1	340	0
9	RM 215	2	150, 155	0.48
10	RM 242	1	250	0
11	RM 287	3	100, 118, 123	0.79
12	RM 316	2	150, 158	0.07
13	RM 408	2	125, 135	0.49
14	RM 431	1	310	0
15	RM 433	2	250, 275	0.48
16	RM 447	3	60, 70, 85	0.49
17	RM 454	1	225	0
18	RM 527	3	90, 95, 120	0.46
19	RM 22565	5	140, 175, 225, 235, 250	0.69
20	RM 22710	1	140	0
21	OSR 13	2	86, 90	0.27
22	Xa 13	1	240	0
23	Xa 21	2	766, 916	0.07
24	Xa 5s	1	250	0
	Total	48		
	Average	2		0.23

of two alleles per locus. The value is lower than that of 5.89, 5.66 and 2.6 alleles per locus as reported by Lapiton *et al.* (2007), Hoque *et al.* (2014) and Joshi *et al.* (2017), respectively. The highest number of alleles (five) was detected in the locus RM 22565. The PIC value ranged from 0.07 (RM 25, RM 316 and Xa 21) to 0.79 (RM 287) with an average of 0.23. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at specific locus (DeWoody *et al.,* 1995). The early studies on PIC values ranged from 0.24 to 0.94 with an average of 0.61 (Jain *et al.,* 2004), 0.19 to 0.90 with an average of 0.75 (Borba *et al.,* 2009), 0.356 to 0.798 with an average of 0.543 (Hoque *et al.,* 2014), which is relatively higher than that of present study. Markers having polymorphic reaction along with high PIC value *i.e.* RM 287 and RM 22565 should be potentially used for molecular characterization of accessions from various sources.

Microsatellite markers (SSR) are also used to detect the genetic similarity of rice accessions under study. The genetic similarity coefficient ranged from 0.53-0.92 as revealed by UPGMA cluster analysis using the 24 SSR markers. Jaccard coefficient showed a cut-off similarity coefficient level of 0.60, below which the similarity values narrowed conspicuously (Fig. 1). This dendrogram revealed that the accessions those are derivatives of genetically similar type clustered more together. The dendrogram revealed 3 clusters among the accessions. Cluster I had eight accessions and consisted of Kanak chudi, Deshi safri, Gada khuta, Jhaler genda, IC-114281, Jhumaki, Kala jira and CGR: 7144 whereas, cluster III had only one accession namely Cross 116. On the other hand, cluster II had maximum accessions (15) consisting of Gedrei, CGR: 7142, Kanak jira, Maiphal jira III, Kanthdudgi, Jhilli paragi, Jhuna, Kakadiha, IC-114539, Kali muchh, IC- 114543, Ketaki II, Son jhilli, Kera ghul and Jhingo.

Jaccard's coefficient of similarity revealed that high degree of similarity (92%) was observed between CGR: 7142 and Kanak jira, thus, are genetically similar. Whereas, Cross 116 showed low degree of similarity (59%), thus, is distant from members of other clusters. Similarity coefficient ranged between 0.62 and 0.88 in cluster I with other clusters of dendrogram. Cluster II with the similarity coefficient ranged between 0.61 and

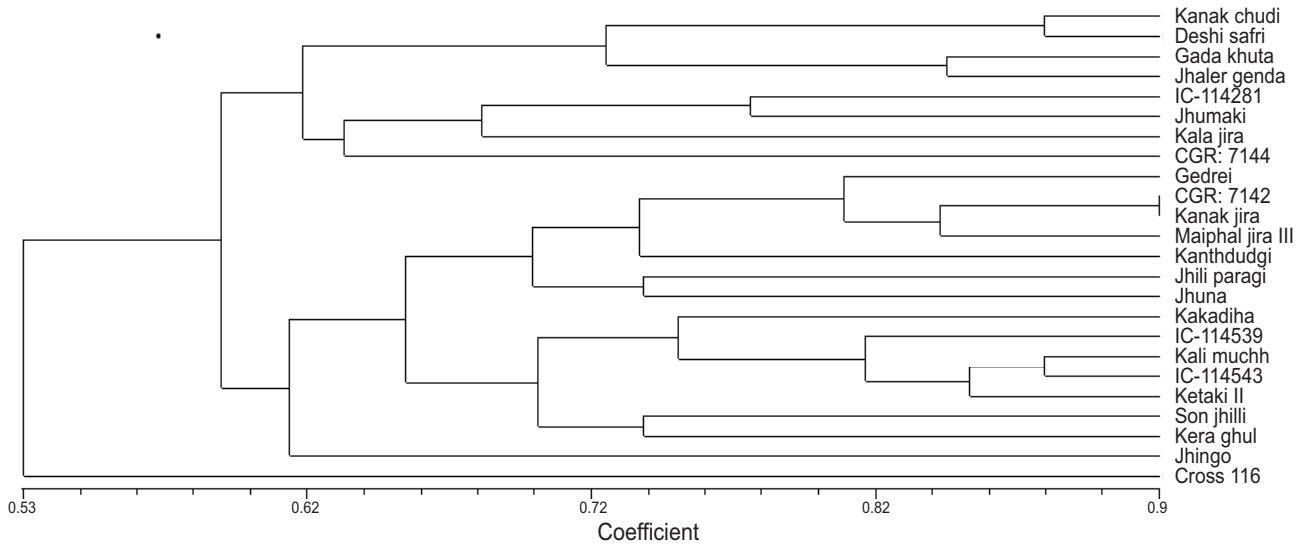

Fig. 1. UPGMA dendrogram showing the genetic relationships among 24 accessions of rice based on 24 SSR markers

0.92. While cluster III with the similarity coefficient ranged between 0.62 and 0.94.

In cluster I, coefficient of similarity revealed that high degree of similarity (>70%) was observed between Kanak chudi and Deshi safri; Gada khuta and Jhaler genda; IC-114281 and Jhumaki, thus, are genetically similar. On the other hand, low degree of similarity was observed between Kala jira and CGR: 7144, thus, are distant from each other.

In cluster II, high degree of similarity was observed between CGR: 7142 and CGR: 7210; CGR: 7211 with CGR: 7142 and CGR: 7210; CGR: 6711 with CGR: 7142, CGR: 7210 and CGR: 7211; CGR: 7472 with CGR: 6711, CGR: 7142, CGR: 7210 and CGR: 7211; CGR: 7168 and CGR: 7189; CGR: 7367 and CGR: 7634; CGR: 7593 with CGR: 7367 and CGR: 7634; CGR: 7634with CGR: 7367, CGR: 7629 and CGR: 7593; CGR: 7306 with CGR: 7367, CGR: 7634, CGR: 7593 and CGR: 7629; CGR: 7159 and CGR: 7565. On the other hand, CGR: 7176 showed low degree of similarity with rest of the accessions of cluster II, thus, is distant from rest of the accessions.

Cross 116 of cluster III showed low degree of similarity with rest of the accessions of cluster I and II, thus, is distant from rest of the accessions.

Thus, SSR markers provide adequate power of resolution to discriminate between rice accessions and it could serve as a potential tool in the identification and characterization of genetically distant cultivars from various sources.

The present investigation addresses the utilization of microsatellite markers to reveal genetic polymorphism and ensures unambiguous identification of 24 accessions of rice. The mean allele (2 alleles) across 48 loci obtained in our study was comparable with the result reported by Meti *et al.,* (2013) who found an average of 2.08 alleles per locus among 48 traditional indigenous aromatic rice germplasm using SSR markers with the range of one to five alleles per locus. They also found 25 bands as polymorphic and three as monomorphic bands.

Hossain *et al.* (2007) reported three to nine alleles per locus with an average of 4.53 alleles per locus for 30 microsatellite markers. Similarly, Rahman *et al.* (2012) found an average of 4.18 alleles per locus.

In the present study, the larger range of similarity values for cultivars revealed by microsatellite markers provides greater confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programme. With the aid of microsatellite markers and clustering data, different distantly related rice genotypes may be combined by intercrossing genotypes from different clusters to get hybrid varieties with the highest heterosis.

The present study revealed a wide variation among the accessions. Coefficient of similarity revealed that the rice accessions of cluster I *viz.,* Kala jira and CGR:

7144; Jhingo of cluster II and Cross 116 of cluster III were genetically distant from rest of the accessions as shown by low genetical similarity coefficient. The information about the genetic diversity will be very useful for proper identification and selection of appropriate parents for breeding programs, including gene mapping, and ultimately for emphasizing the importance of marker-assisted selection (MAS) in rice improvement worldwide.

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REVIEW ARTICLE

Plant Genomic DNA Isolation – the Past and the Present, a Review

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Successful isolation of a good quality of plant genomic DNA in sufficient quantity is a necessary prerequisite for its downstream applications,that are aimed to examine and analyze the molecular intricacies of the plant kingdom. The quantity and quality of isolated genomic DNA often determines the accuracy and robustness of results derived from many DNA based applications.The choice of the method adopted for DNA isolation depends on a large number of factors like the amount, nature and age of the starting plant tissue, the infrastructure available in terms of laboratory facilities, ready availability of required laboratory chemicals, time at hand and the downstream applications of the obtained DNA. A large number of modifications have been suggested to optimize DNA isolation from a particular plant species or tissue. There cannot be a universal protocol for DNA isolation across all plant species or tissues owing to the highly heterogeneous nature of plant cells. Hence, efforts need to be made to carry out suitable variations in the extraction method, so as to develop a protocol which is best suited to a particular plant species or tissue or application. So far, a large number of DNA isolation protocols from plants have been published, but there is no comprehensive report on suitability of these methods over each other and the different modifications which have been attempted to optimize the yield and quality of DNA. Many review papers have described various DNA isolation protocols and their underlying principles, that have been adopted in order to optimize DNA isolation from different plant species and tissues (Varma *et al*., 2007; Tan and Yiap 2009; Kumari *et al*., 2012). However, there is no comprehensive study which gives details of modifications at different steps of the plant genomic DNA isolation protocol, that have been attempted by researchers to overcome the specie and tissue specific limitations. This review gives a detailed account of the steps involved in genomic DNA isolation from various plant species and tissues and how each of these steps have been modified to overcome and eliminate the problem of contaminants in the extracted DNA so as to obtain a good quality of DNA amenable to downstream molecular biology applications.

Key Words: CTAB, DNA, Extraction buffer, High throughput methods, Kits

Introduction

The isolation of genomic DNA from plants is a routine procedure widely carried out in a plant molecular biology laboratory. This procedure is aimed to isolate the total genomic DNA from the plant for subsequent molecular analysis. The downstream application of the isolated DNA is crucial in determining the choice of method for DNA isolation. For applications like carrying out PCR for SSR analysis, small amounts of crude DNA can be used. However, PCR for RAPD, AFLP and ISSR analysis requires very small amount of good quality DNA. A highly pure and large quantity of DNA is required for applications like RFLP, southern blotting, cloning, genomic library construction, high throughput genotyping and sequencing (Tefler *et al*., 2013). Most commonly the isolated DNA is found to be contaminated by the presence of residual polyphenols, polysaccharides and other secondary metabolites, which result in inhibition of restriction endonuclease activity, amplification by *Taq* DNA polymerase and ligation by ligase (Moyo *et al*., 2008). Plant tissues, unlike animal tissues, pose a far greater challenge for DNA isolation because of the extensive variation and heterogeneity in the internal structures and composition (polysaccharides, storage proteins and secondary metabolites) of plant cells. As a result it is near impossible to have a single DNA isolation protocol which is universally applicable across different plant species or even different tissues from the same plant (Weising *et al.,* 2005). The presence of components like polysaccharides, cellulose, phenols and tannins, necessitates the standardization of the protocol which is able to remove these contaminants from the genomic DNA preparation. There are following five fundamental steps involved in all the DNA isolation methods:

a) **Cell disruption:** This means degradation of cell envelope (cell wall and cell membranes). The cell wall (cellulose) is disrupted by mechanical force and the cell

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membrane is broken by the addition of a detergent in the extraction buffer .The detergent disrupts the membranes due to the amphipathic (both hydrophilic and hydrophobic regions) nature of both cellular membranes and detergent molecules. When detergent comes close to the cell, it captures the lipids and proteins. The end result of cell disruption or lysis is that the contents of the plant cells are distributed in solution (cell extract).

b) **Deproteinization or organic extraction:** This step involves the use of phenol/chloroform/isoamyl alcohol to remove the proteins from the DNA. Phenol denatures proteins and dissolves denatured proteins. Chloroform is also a protein denaturant and also removes excess phenol from the preparation.

c) **Precipitation:** The DNA is recovered from the solution by insolubilization or precipitation on addition of isopropanol or ethanol. Isopropanol induces a structural change in DNA molecules that causes them to aggregate and precipitate out of solution. Ethanol in the presence of salt prevents DNA from dissolving in water causing it to precipitate out. The precipitated DNA is thereafter pelleted by centrifugation.

d) **Wash:** The precipitated DNA is washed with 70% ethanol to remove salts and other water soluble impurities.

e) **Resuspension:** The DNA thus obtained is suspended in a buffer (1X TE10 mM Tris:1mM EDTA) or in sterile distilled water.

In this review, we have attempted to compile various published protocols of DNA isolation, the specie -specific and tissue-specific modifications that have been carried out at different stages in the DNA isolation protocol and how these have led to troubleshooting in DNA isolation.

Discussion

Variables involved in DNA isolation

The steps involved in the genomic DNA isolation protocol are subjected to a large number of modifications in order to optimize the quantity and quality of the DNA. Efforts have been undertaken to streamline these modifications to develop the most suitable protocol for a particular plant species. The variables in the DNA isolation methodology, are described hereunder-:

a) **Plant tissue – age and type**: The source of the plant tissue to be used for DNA isolation greatly affects the quality and quantity of DNA obtained. Preferentially young leaf tissues are used because these have less concentration of starch and secondary metabolites (Varma *et al.*, 2007). However, it has been shown that the leaves of *in vitro* grown Himalayan herb, *Aconitum balfourii*, contain the highest amount of polyphenols compared to other tissues like shoots and roots (Sharma and Gaur, 2014). In some latex containing plants, etiolated leaves are also used as a tissue of choice for DNA isolation (Michiels *et al*., 2003). It has been reasoned that the photosynthetically active tissues like leaves contain more phenolic compounds as compared to etiolated leaves. These phenolics get oxidized during extraction and form irreversible complexes with proteins. Therefore, the use of etiolated leaves for DNA isolation is preferred in some plants. Mature leaves have also been used for DNA isolation but have been shown to yield lesser quantity of DNA compared to fresh leaves (Ribeiro and Lovato, 2007). However in plants like citrus and sweet potato, hardened and mature leaves have been used for DNA isolation (Varadarajan and Prakash, 1991).

Other tissues like seeds, embryos, endosperm, cotyledons, roots, floral parts, fruit rind, callus, rhizomes, cambial cells, wood tissues etc., have also been used for DNA isolation with limited success (Asish *et al*., 2010). It was found that flowers yielded the maximum amount of DNA in *Daucus carota*, as compared to seeds, leaves, calli and tap roots (Boiteux *et al*., 1999). Newly harvested seeds yielded a greater quantity and quality of DNA in maize compared to DNA isolated from leaves. In commercial timber yielding trees, bark tissue, heatwood, sapwood, cambium etc., have been used for DNA isolation (Asif and Cannon, 2005; Tnah *et al*., 2012). Other than differences in the biochemical composition of the tissues, the tissue specific variation in DNA yield and quality can also be attributed to differences in the number and size of cells in the tissue, the ratio of mitotic to interphasic nuclei and the amount of extranuclear DNA. Floral meristems of medicinal plants and grasses have been used for DNA isolation because of an expected high concentration of genomic DNA due to the presence of rapidly dividing cells and extensive DNA synthetic activity in the cells (Ibrahim, 2011). It has also been observed that not all tissues are amenable to DNA isolation with the same protocol. DNA could be isolated from seedlings and leaves, but not from seeds, of jute plant (*Corchorus*) from a protocol which did not employ phenol in organic extraction whereas DNA could be isolated from all the

tissues, using a protocol that used phenol for organic extraction (Haque *et al*., 2004).

b) **Collection and storage of tissue:** The tissue once harvested can be used directly for DNA isolation or may be stored conveniently at -80°C till the time of use. Freezing at this temperature inhibits the activity of nuclease enzymes and hence allows successful preservation of plant tissues. Another way of preservation of tissues for DNA isolation is immersing the tissue in a NaCl/CTAB preservation solution (Storchova *et al*., 2000). This tissue is later rinsed with tap water before grinding it for DNA isolation. The tissues can also be sun dried or heat treated at 65°C for 30 min before grinding (Elias *et al*., 2004; Sharma *et al*., 2013). Silica gel has also been used to achieve desiccation of tissue. Leaves are cut into small pieces and placed in bags containing silica gel (Moeller *et al*., 2014). This method is based on the principle that the rate of tissue desiccation is rapid enough to prevent degradation of DNA. Leaf tissues of many medicinal plants can also be dipped in a fixing solution (absolute alcohol) at room temperature which arrests the activities of enzymes (Sharma *et al*., 2010; Sharma and Gaur, 2014).

c) Homogenization: Tissue disruption in a ceramic mortar with the use of physical forces is the most common method used for tissue homogenization, more precisely cell wall disruption. Liquid nitrogen is widely used to aid the grinding process. It acts by making the tissue brittle and dry, suitable for grinding and also arrests the biochemical reactions taking place in a cell. Alternatively, prechilled mortar and pestle (at -80°C) can also be used for grinding the tissue (Biswas and Biswas, 2011). This averts the use of liquid nitrogen. Sterile sand has been used for grinding leaves of date palm (Arif *et al*., 2010). Grinding of tissues is now frequently carried out directly in eppendorf tubes or 96 well plates using mechanical tissue grinders and other high throughput systems. This prevents the chances of cross contamination which can otherwise occur while grinding in a mortar and pestle. The use of mechanical tissue lyser for high throughput lysis in a 96 well plate has drastically reduced the time for cell lysis (Hill Ambroz *et al*., 2002). Cell wall removal has also been achieved by ways other than application of physical forces. Alternate cold and heat treatment of the ground tissue, is one of the proposed ways to achieve it (Biswas and Biswas, 2011; Sahu *et al*., 2012). Chemical disruption of cell walls has also been achieved by using hydrolytic enzymes, such as cellulases, pectinases or cell wall macerases (Rogsatd *et al*., 2001). The leaf disks from different plant species were immersed in an enzymatic cocktail derived from *Trichoderma longibrachiatum*, which digests the cell walls (Manen *et al*., 2005).

d) **Extraction buffer:** The extraction buffer is essentially composed of individual chemicals which are prepared separately as stock solutions and mixed later. These individual constituents facilitate cell membrane dissolution (detergents), inactivation of nucleases (metal chelators, reducing agents) and removal of contaminants (salts, PVP etc.). Other than the common ingredients of the extraction buffer such as a suitable detergent (CTAB, SDS etc.), a buffering system to maintain the pH (Tris HCl), a salt to prevent DNA denaturation (NaCl), a protein denaturant (β-mercaptoethanol), a metal chelator or nuclease inactivator (EDTA), the extraction buffer is supplemented with various other ingredients to remove the contaminating residues . The latter includes RNA, proteins, polyphenolics (flavonoids, terpenoids and tannins), polysaccharides and non-nuclear DNA which can bind to the DNA and get co-precipitated with it. LiCl is incorporated in the extraction buffer to remove RNA (Arif *et al*., 2010). Proteinase K, a proteolytic enzyme is sometimes incorporated in the extraction buffer to inactivate nuclease enzymes and remove protein contaminants when protein rich seed tissue is used for DNA extraction (Thangjam *et al*., 2003; Pervaiz *et al*., 2011). When large amounts of phenolic compounds are present in the plants, a reducing agent or a phenol compound absorbent is frequently incorporated into the extraction buffer. The most commonly used phenol absorbents are polyvinyl pyrrolidon (PVP) or polyvinyl polypyrrolidon (PVPP).These prevent oxidation of polyphenols into quinines which bind to nucleic acids and hinder isolation of high quality DNA. Without the addition of PVP or PVPP (the two only differ in water solubility), the precipitated DNA is brown due to the presence of oxidized phenols. Various antioxidants like β-mercaptoethanol, BSA, sodium sulfite, sodium azide etc. are used with PVP for an effective removal of polyphenolics. A high concentration of PVP and β-mercaptoethanol has been used to remove tannins and polyphenolics from different tissue extracts of medicinal plant, *Aconitum heterophyllum* (Srivastava, 2010). Similarly, Mathew *et al*., 2014, used 1-3 % PVP, 0.1-1% sodium metabisulphite, 0.04-2% β-mercaptoethanol while grinding cardamom leaves to

remove polyphenolics from the tissue extract. However, in some cases like forest tree *Corymbia citriodora*, the use of 1% and 4% PVP compromised the quality of DNA and did not result in isolation of DNA which is amenable to NGS platforms (Healey *et al*., 2014). They instead used β-mercaptoethanol and carried out other minor modifications in the protocol, to rid the DNA of polyphenolics. Diethylthiocarbamic acid (DIECA) is a phenoloxidase inhibitor which helps to reduce brown colouring due to oxidation of polyphenols to quinines (Anuradha *et al*., 2013). L-ascorbic acid also prevents oxidation of phenolic compounds. The presence of gelling polysaccharides makes the DNA viscous, causing it to stick to the wells during gel electrophoresis (Diadema *et al*., 2003; Varma *et al*., 2007). To counter the problem of polysaccharides, a high salt (NaCl) concentration in the extraction buffer is most frequently favored. The NaCl concentration was increased to as high as 5M for removal of polysaccharides from *Urginea indica* extracts (Harini *et al*., 2008). A high CTAB concentration has also been used to remove polysaccharides from tissue extracts of *Chenopodium* (Akhtar *et al*., 2013). A 10% (w/v) CTAB solution containing 3M NaCl was used to remove polysaccharides from DNA isolated from leaves of cardamom (*Amomum subulatum*) (Mathew *et al*., 2014). The principle behind this CTAB based removal of polysaccharides is the formation of CTAB-polysaccharide complexes at high ionic strengths. Hence, a mere tweaking of salt concentrations can result in different functions of CTAB i.e., cell membrane disruption and nucleic acid precipitation or polysaccharide removal. The homogenate can also be diluted two or three times with the extraction buffer to reduce polysaccharide contamination (Sharma *et al*., 2000). The composition of extraction buffer used also differs with the age and nature of the tissue . A higher concentration of CTAB and NaCl in the extraction buffer was used for isolating DNA from dry leaves of rice while a lower concentration of CTAB and NaCl was present in the extraction buffer when the source of DNA was fresh leaves (Ahmadikhah, 2009). Similarly, different concentrations of PVP and β-mercaptoethanol were used for tissue grinding when DNA was isolated from fresh and dry leaves of cardamom (*Amomum subulatum*) (Mathew *et al*., 2014). N-phenylacylthiazolium B (PTB) has been extensively used to extract DNA from hard and woody tissues of timber yielding trees and mycorrhizal root tips (Asif and Cannon, 2005; Tnah *et al*., 2012). DNA obtained from bark tissue of such timber yielding trees by the modified CTAB method and kits failed to amplify, due to poor quality of DNA due to the presence of Maillard products. These are the condensation products of reducing sugars and primary amines. PTB cleaves these glucose derived protein cross linkages and helps to free the DNA entrapped in these crosslinks (Asif and Cannon, 2005). Different protocols have been suggested, varying in extraction buffer compositions and various other steps, for different classes of plant species differing in the amounts of secondary metabolites and DNases (Lutz *et al*., 2011). Hence, it can be concluded that depending upon the nature of the starting plant material, the composition of the extraction buffer in terms of presence or absence of individual chemicals or their concentrations is varied, to optimize the DNA yield. Thus, it can be concluded that the composition of extraction buffer has been subjected to most of the variations in order to optimize the quantity and quality of the extracted DNA . The composition of extraction buffer has been modified mostly by changing the concentration of salt or NaCl in tissues with high polysaccharide content (eg. potato tubers, citrus leaves, chickpea seeds); or by addition of PVP to tackle the problem posed by the presence of polyphenolics in plant tissues (eg. leaf tissues from medicinal and aromatic plants).Other changes that have been widely attempted and standardized for troubleshooting include the addition of chemicals like LiCl, ascorbic acid, sarcosyl, diethyldithiocarbamic acid to the extraction buffer or separately after homogenization of the tissue.

A detergent is added to the extraction buffer to effect the disruption of cell membranes. Based on the detergent used for lysis, most DNA isolation protocols can be broadly categorized into two different classes:

- CTAB based methods
- SDS based methods

CTAB based methods: These methods employ a cationic detergent, hexadecyltrimethylammonium bromide (CTAB) in the extraction buffer for cell membrane disruption. When CTAB is used in extraction buffer, a low ionic strength of the buffer is maintained. Under these conditions, CTAB precipitates nucleic acids and the protein contaminants lie in the supernatant.

Chronology of CTAB based DNA Isolation protocols:

A large number of CTAB based methods are used by researchers, which have been suitably modified to suit a particular plant genus or tissue type. Most of the current

methods of DNA isolation are modifications of these classical methods.

- 1980–Murray and Thompson Protocol: first used CTAB and employed cesium chloride (CsCl) density gradient technique to eliminate the polysaccharides.
- 1982–Taylor and Powell miniprep method
- 1984–Rogers and Benedich protocol
- 1984–Maroof and Saghai protocol- used lyophilized tissue
- 1987–Doyle and Doyle protocol

SDS based methods: These methods use the anionic detergent, sodium dodecyl sulfate (SDS) for cell disruption.

Chronology of SDS based DNA Isolation protocols:

- 1983–Dellaporta *et al*., Method
- 1997–Aljanabi and Martinez Method

There are some protocols which have shown the use of both CTAB and SDS or each of these with a different combination of detergents, in the extraction buffer e.g., the extraction buffer used for isolation of DNA from rice leaves, was a combination of two different solutions; solution A (150 mM sorbitol, 125 mM Tris, 25 mM EDTA, 500 mM NaCl, 20 mM Na_2SO_3, 0.8% CTAB and 2% sarkosyl, pH 7.5) and solution B (100 mM Tris, 500 mM KCl, 18 mM $MgCl_2$ and 1% Triton X-100, pH 9.0) in a defined proportion with supplementation of β-mercaptoethanol (Chuan *et al*., 2010). In cotton, two types of buffers termed as homogenization and lysis buffers were used in combination or successively to facilitate DNA extraction (Haiwen *et al*., 2001). Another cationic detergent, dodecytrimethylammonium bromide (DTAB), has been used for DNA extraction from many microalgal species (Fawley and Fawley, 2004). This method commonly referred to as the CTAB-DTAB method increases the DNA recovery rate and quality of the DNA obtained. Interestingly, household detergents and shampoos have also been used by some researchers (Inuwa *et al*., 2011).

Tables 1 and 2 enlist the published modifications (last 15 years) in the extraction buffer composition, for isolation of DNA from various plant species and tissues.

The volume of extraction buffer used in proportion to the amount of plant tissue taken, also affects the quality and yield of DNA (Krizman *et al*., 2006; Chen *et al*., 2009). The incubation time of the homogenate at a temperature of ~60°C or less (at 37°C) has also been shown to affect the quality of DNA (Ginwal and Maurya 2010; Ansari and Khan, 2012).

Comparative Studies on various DNA Isolation Protocols

Table 3 gives a succinct comparison of the classical DNA isolation methodologies. Most CTAB based DNA isolation methods reported in literature are variations of the original Doyle and Doyle method and most SDS based methods are variations of the original Dellaporta method. About 70 % of the studies reported from the year 2000 onwards, have used CTAB methods for DNA isolation. These methods have been adopted for a wide range of plant species, both monocots and dicots. Biophysically, it has been established that the self aggregation behaviour (micelle formation) of CTAB is stronger than that of SDS as determined by ESR (Bahri *et al*., 2006).

e) **Deproteinization or organic extraction:** This is a two step procedure which aims to remove protein and lipid contaminants present in the cell lysate. The first step involves treatment of the cell lysate with organic solvents like phenol or mixture of phenol:chloroform:isoamyl (25:24:1) alcohol or mixture of chloroform:isoamyl alcohol (24:1) or mixture of chloroform: octanol (24:1). In cases where phenol is used, the DNA is reextracted with chloroform or chloroform:isoamyl alcohol to ensure the removal of any trace phenol from the nucleic acid preparation (Adhikari *et al*., 2011; Akhtar *et al*., 2013). This is followed by centrifugation to enable separation of DNA containing aqueous phase from the organic phase containing protein and lipid contaminants. Most researchers have used chloroform:isoamyl alcohol for organic extraction as compared to using phenol because of a better quality of DNA obtained while using the former. More than one extractions can be carried out with chloroform:isoamyl alcohol to remove the cloudiness caused by PVP. Sharma *et al*. (2013) isolated DNA from different tissues of plants (cowpea, soybean, maize, *Dioscorea* sp., cassava, mango, banana and okra) for PCR amplification without phenol-chloroform extraction. Rehman *et al*. (2007) proposed a high throughput method of DNA extraction from wheat and canola by protocols with and without the use of any of

Table 1. Details of composition of CTAB based extraction buffer used for DNA isolation from different plant species and tissues

Plant/Family	Tissue used	Extraction Buffer Composition					Additional components/ Features	References
		Tris HCl	EDTA	NaCl	CTAB	β-mercap		
Allium sps.	Leaf,stem, flowers.	100mM	25mM	1.5M	2.5%	0.2%	1% PVP	Khanuja *et al*., 1999
Gossypium sps.	Leaf	0.1mM	.005mM	-	-	-	Sodium bisulfite (0.4g), 0.35 M sorbitol.	Haiwen *et al*., 2001
		200mM	0.05mM	2M	.055mM	-		
Agavaceae	Foliar tissue	100mM	20mM	1.4M	2%	10mM	4% PVP, 0.1% ascorbic acid	Keb Llanes *et al*., 2002
		100mM	50mM	0.1M	-	10mM	-	
Asteraceae (*Cichorium intybus, Taraxacum officinale, Lactuca sativa*)	Etiolated leaves	100mM	20mM	1.4M	2%	0.2%	2% PVP	Michiels *et al*., 2002
Manihot esculenta	Tuber	100mM	30mM	1.2M	3%	3%	-	Elias *et al*., 2004
Litchi chinensis	Leaf	100mM	20mM	2M	2%	5%	2%PVP,10mM ammonium acetate	Puchooa 2004
Foeniculum vulgare, Origanum vulgare, Cannabis sativa, Humulus lupulus, Coffea arabica	Leaf, seed, dried cones, beans	100mM	20mM	2M	2%	-	1% PVP, 0.5% activated charcoal	Krizman *et al*., 2006
Terminalia arjuna	Leaf	100mM	20mM	1.4M	2%	N.M	Combination of CTAB and column based purification system	Sarwat *et al*., 2006
Medicinal plants (*Sclerocarya birrea, Barleria greenii, Huernia hystrix, Aloe polyphylla*)	Leaf	100mM	20mM	1.4M	2%	N.M	3% PVPP added separately	Moyo *et al*., 2008
Ipomea batatas	Leaf	100mM	20mM	1.4M	2%	0.4 -1%	-	Borges *et al*., 2009
Arid trees (*Acacia, Prosopis, Calligonum*)	Leaf	100mM	20mM	4M	3%	0.2%	6% PVP	Sablok *et al*., 2009
Garcinia sps.	Fruit rind and leaf	100mM	30mM	1.4M	2-4%	0.3%	1.5%PVP	Ashish *et al*., 2010
Phoenix dactylifera	Leaf	1.21g	0.4g	8.12g	2%	-	PVP (2g), LiCl (0.2g)	Arif *et al*., 2010
Dalbergia sissoo	Leaf	100mM	20mM	1.42M	2%	3 ul	4% PVP-40, 5mM ascorbic acid	Ginwal and Maurya 2010
Allium stracheyi	Seed	100mM	25mM	2.5M	2.5%	3%	3% PVP, 0.15% sodium sulfite	Ranjan *et al*., 2010
Aconitum heterophyllum	Leaves from field grown and tissue culture raised plants, seeds	100mM	20mM	1.4M	2%	3%	15mM ascorbic acid, 3% PVP.	Srivastava *et al*., 2010
Cereals (rice, wheat, maize)	Seed and leaf	100mM	20mM	1.4M	2%	1%	1% PVP, Proteinase K (50ug/ml)	Pervaiz *et al*., 2011
Legumes (*Cajanus*, *Cicer, Vigna*)	Leaf	100mM	20mM	4M	3%	2%	-	Agbagwa *et al*., 2012
Mangifera indica L	Leaf	100mM	20mM	3M	4 %	3%	0.025 g/ml PVP	Azmat *et al*., 2012
Chrysanthemum indicum	Seed and leaf	100mM	20mM	1.4M	2%	1%	-	Hasan *et al*., 2012
Gymnema sylvestre	Leaf	1000mM	500mM	4M	10%	N.M	-	Krishna *et al*., 2012
Abelmoschus esculentus	Leaf	100mM	20mM	1.4M	3%	0.006%	Tissue is ground in PVP before adding E.B.	Kumar *et al*., 2012
Mangroves sps.	Leaf	100mM	20mM	1.5M	2%	1%	-	Sahu *et al*., 2012
Boswellia serrata	Leaf	100mM	20mM	1.4M	2.5%	10 mM	1% PVP	Sharma & Purohit 2012
Reaumuria soongorica	Leaf	100mM	25mM	2mM	2%	5%	5% PVPP	Wang *et al*., 2012
Sorghum bicolor, Populus deltoids, Gossypium hirsutum, Triticum aestivum	Seed & leaf	100mM	20mM	1.2M	2%	0.1%	-	Xin & Chen 2012

Plant/Family	Tissue used	Extraction Buffer Composition					Additional components/ Features	References
		Tris HCl	EDTA	NaCl	CTAB	β-mercap		
Chenopodium album Lepidium latifolium	Plantlets	100mM	25 mM	1.5M	2.5%	0.4%	2%PVP	Akhtar *et al*., 2013
Morus sp.	Leaf	200mM	20mM	1.4M	2%	-	2%PVP, 5mM L-ascorbic acid, 4mM DIECA, 1% sodiummetabisulfite, 0.5% SDS.	Anuradha *et al*., 2013
Zingiberales sps.	Leaf	100mM	20mM	1.4M	2%	-	1% PVP used separately with E.B for grinding	Devi *et al*., 2013
Tropical plants (*Ficus carica, Hevea brasiliensis, Nicotiana tabacum, Carica papaya, Musa nana, Roystonea regia*,etc.)	Leaf	200mM	25mM	2 M	2%	-	2% PVPP, 1% sodium lauroyl sarcosine, 20 mM disodium tetraborate decahydrate	Huang *et al*., 2013
Cicer arietinum	Leaf	100mM	20mM	1.75 M	2%	0.5%	Tissue grinding using a genogrinder in a 96 well rack.	Kumar *et al*., 2013
Aconitum balfourii	Leaf	100mM	25mM	1.5 M	3%	0.2%	2.5% PVP	Sharma and Gaur 2014

Abbreviations: CTAB: Hexadecyltrimethylammonium bromide; DIECA: Diethylthiocarbamic acid; EDTA: Ethylenediaminetetraacetic acid; E.B: Extraction Buffer; HCl: Hydrochloric acid; LiCl: Lithium Chloride; NaCl: Sodium Chloride; PVP: Polyvinyl pyrollidone; PVPP: Polyvinyl polypyrollidone; SDS: Sodium dodecyl sulfate.

*The pH of Tris HCl and EDTA is maintained at 8, unless specified in the tables.
*Measurements given g/100ml of extraction buffer.
*N.M: not mentioned in the papers.

Table 2. Details of composition of SDS based extraction buffer used for DNA isolation from different plant species and tissues

	Tissue	Extraction Buffer Composition					Additional Components/ Features	References
		Tris HCl	EDTA	NaCl	SDS	B-mercap.		
Morus sp.	Leaf	12.11g	18.07g	29.20g	0.25g	-	-	Venkateswarlu *et al*., 2002
Triticum aestivum	Leaf	100mM	20mM	500mM	2%	0.1%	7 M urea	Nalini *et al*., 2003
Cocus nucifera	Solid endosperm	200M	70mM	2000mM	-	0.2M	PVVP, 20% SDS added separately with extraction buffer.	Angeles *et al*., 2005
Capsicum annuum	Fruit	100mM	50mM	500mM	20%	10mM	-	OgunKanmi *et al*., 2008
Brassica napus, Nicotiana tobaccum	Leaf	200mM (pH 7.5)	25mM	250mM	0.5%	-	-	Amani *et al*., 2011
Cicer arietinum, Trichosanthes dioica, Bacopa monnieri	Leaf	200mM	25mM	200mM	-	-	1% PVP, 10 % SDS added separately with extraction buffer.	Adhikari *et al*., 2012
Malus sp.	Seed	100mM	50mM	1.5M	1%	-	7.5 M Amm. acetate added to tissue homogenate.	Ansari and Khan 2012
Gossypium sps.	Root tips	30mM	10mM	100mM	-	-	200mM sucrose	Rao *et al*., 2012
		500mM (pH 9.2)	250mM	-	2.5%	-		
Cajanus cajan	Leaf	100mM (pH 7.4)	50mM	500mM	0.7%	-	52 mM sodium sulphite, 3.6 µg RNase A,36 µg Proteinase K	Singh *et al*., 2012
Eleusine coracana	Leaf	50mM	10mM	100mM	10%	N.M	PVP (N.M)	Gupta *et al*., 2013
Cowpea, soybean, maize, *Dioscorea* sp., cassava, mango, banana and okra	Seed, leaves, tubers, stems, tuberous roots	100mM	10mM	1 M	1%	1%	2% PVP 0.05 mg/ml proteinase K 4% (w/v) PEG	Sharma *et al*., 2013

Table 3. Comparison of the classical DNA isolation protocols

Step In Extraction Protocol	CTAB based methods				SDS based methods	
	Murray and Thompson	Rogers and Bendich	Saghai Maroof	Doyle and Doyle	Dellaporta	Aljanabi
Detergent Conc. in E.B	1%	2%	1%	2%	20%	20%
Organic Extraction -:						
Chemicals used	$CHCl_3$ / oct.	$CHCl_3$ / isoamyl alc.	$CHCl_3$ / oct.	$CHCl_3$ / isoamyl alc.	Phenol/$CHCl_3$	None
No. of times	>1	>1	Once	Once	Once	N.A
DNA precipitation -:						
Chemicals	CsCl gradients	CTAB ppt. buffer and ethanol	isopropanol	isopropanol	isopropanol	isopropanol
When	after org. ex.	before and after org. ex.	after org. ex.	after org. ex.	before org. ex.	N.A
Washing	removal of DNA with a syringe	80% EtOH	76 % EtOH and 10mM NH_4Ac	76 % EtOH and 10mM NH_4Ac	80% EtOH	70 % EtOH
Resuspension of DNA	N.A	0.1 X TE	(10mM NH_4Ac + 0.25 mM EDTA)	1 X TE	1 X TE	sterile d H_2O

these organic solvents. They examined the efficacies of protocols that involved use of NaOH, $NaOCl_4$, NaOCl and sorbitol and concluded that even though the DNA yield obtained by using phenol chloroform extraction was higher compared to others however the quality of DNA obtained was better using the other methods. Mostly the organic extraction is carried out using the supernatant derived after centrifugation of cell lysate. However, in some studies this step is carried out after the DNA pellet is suspended and dissolved in TE buffer (Hariprakash *et al*., 2010; Ansari and Khan, 2012; Azmat *et al*., 2012).

Precipitation: DNA is precipitated after organic extraction using ethanol or isopropanol in the presence of salts like sodium chloride, sodium acetate, potassium acetate or ammonium acetate, at -20°C for 1 hr to overnight. To reduce the binding and precipitation of residual contaminants with DNA, the time of incubation with isopropanol was decreased (Krizman *et al*., 2006; Srivastava *et al*., 2010). A temperature of 25°C was maintained for precipitation in the presence of isopropanol (Krizman *et al*., 2006). Isopropanol is found to be more effective for precipitation of DNA and has been more commonly used. However, the use of ethanol has been suggested to be better than isopropanol in precipitating DNA from seeds of apple (Ansari and Khan, 2012). Since polysaccharides have a similar solubility as DNA, they have a tendency to be co-precipitated with DNA in ethanol or isopropanol. The presence of salt (NaCl, NaAc., KAc.,NH_4Ac. etc.) increases their solubility in EtOH or isopropanol, thus enabling their removal once the DNA has been pelleted (Healey *et al*., 2014). Adhikari *et al*., 2011, used 2 M NaCl and ethanol for precipitation of DNA from the supernatant obtained after organic extraction, from leaves of chickpea and pointed gourd. 3 M Na acetate and chilled ethanol in defined proportionate volumes, have been used for precipitation of DNA from leaves of mango and cardamom (Azmat *et al*., 2012; Mathew *et al*., 2013). It has been observed that NH_4Ac. is capable of only partial precipitation of protein and polysaccharide components and hence was not found suitable for isolation of DNA from seeds of *Sorghum* (Chen *et al*., 2009). Some studies have reported the use of a CTAB-NaCl precipitation solution instead of or in addition to isopropanol. A CTAB precipitation solution (1% CTAB, 50mM Tris HCl (pH=8), 100mM EDTA) was used to precipitate DNA from tissues of medicinal plants (Moyo *et al*., 2008). Xin and Chen (2012), diluted the CTAB- DNA complex by the addition of a dilution buffer, resulting in a decrease of NaCl concentration from 1.2 M to 0.4 M which allows precipitation of CTAB-DNA complex . To reduce RNA contamination, Sharma and Purohit (2012), carried out DNA precipitation on diatomite suspensions.

Purification and resuspension: The precipitated DNA is washed with 70%-80% ethanol to remove salts and other water soluble impurities. The air or vacuum dried DNA pellet is then dissolved in 1 X TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8). An optional RNase treatment (at 37°C for 30 mins or more) can also be given to remove any contaminating traces of RNA.

Hariprakash *et al*. (2010), added a mixed solution of 2% polyethylene glycol and 1.5 mM NaCl, to the dissolved DNA pellet after RNase treatment. This was done to remove undissolved polysaccharide contaminants in the DNA. This was followed by organic extraction, precipitation and a final washing to remove PEG and NaCl.

Kit based DNA Isolation

There are a large number of commercial DNA isolation kits available in the market today. These methods are designed to remove contaminants and offer a simple, convenient procedure for rapid isolation of genomic DNA of high yield and purity from tissue samples. The use of these kits also averts the use of hazardous chemicals like phenol, chloroform for DNA isolation. Most modern kits are based on purification of DNA from crude cell lysate by selective binding to a support material. The support material is essentially chromatographic columns which are based on either anion exchange chromatography or adsorption based chromatography. The main steps in DNA isolation using kits are:

Cell lysis: Cells are broken down to release the DNA.

Binding: Genomic DNA gets selectively adsorbed on a synthetic column.

Washing: Contaminants are washed away.

Elution: DNA is eluted in a low salt buffer.

Anion Exchange Methods

These methods are based on electrostatic interactions between negatively charged phosphate groups of the DNA with positively charged immobilized surface molecules on the column. The DNA binds to the column under low salt concentrations and the other impurities are washed away using medium salt buffers. The bound DNA is then eluted using a high salt buffer. The eluted DNA is then precipitated in alcohol and can be used for further downstream applications.

Silica based Column Methods

These methods make use of spin columns made up of silica gel membrane, on which the DNA gets bound. The DNA is then eluted in the presence of high concentration of chaotropic salts. In these methods no alcohol precipitation is required and the eluted DNA is ready to use (www.qiagen.com). Sometimes there are problems of loss of DNA, due to subsequent column washes, particularly when only a small amount of tissue is available. These methods have been scaled upto a 96- well format to ensure simultaneous processing of many samples.

Paramagnetic Bead based Column Methods

These are based on reversible adsorption of DNA on paramagnetic beads (Czembor *et al*., 2014).The reagents provided with these kits are used with magnetic tools. The use of this kit averts the need for use of any organic solvents, repeated centrifugation or column separation (Tan and Yiap *et al*., 2009).

A large number of studies are reported where comparisons between the manual and kit based methods of DNA isolation are reported (Coyle *et al*., 2003; Michiels *et al*., 2003; Tan *et al*., 2013). Michiels *et al*. (2003), reported a better quality and quantity of DNA from etiolated leaves of latex plants, isolated by the optimized CTAB method than that isolated by kit based method. Yadav *et al*., 2012 reported a greater quantity of DNA isolated from cotton leaves by kits as compared to that isolated by CTAB method. Application based superiority of DNA isolated from kit has been documented by Tefler *et al*. (2013), wherein it was purported that DNA isolated from needles of *Pinus radiata* through kits is more amenable for high throughput genotyping platforms in terms of SNP call quality. Moeller *et al*., 2014 showed the superiority of DNA isolated from two different kits over modified CTAB method in terms of yield and protein contamination. Karaslan *et al*. (2014) have compared six different commercial DNA isolation kits for DNA isolation from seeds and leaves of wheat, in terms of extraction efficiencies, cost and time effectiveness. There appears to be a lack of consensus in determining the superiority of kit or manual method over each other for DNA isolation in terms of quantity and quality. However, it can be safely concluded that there are major differences in the cost of processing/ sample through kit based and manual methods, with the cost being higher for kit based method. The estimated price for most kits is calculated to be ~$250 to $ 300+ for 50 reactions. Many approaches have been worked out to bring down this price. Some studies have recommended the use of both the methods, i.e. isolation by manual method and purification by kit based method, in order to reduce the cost involved in exclusive use of kits for DNA isolation (Sarwat *et al*., 2006; Tefler *et al*., 2013). Another approach to make the use of kits more cost effective is to buy the adsorbent

resins in bulk and pack them in ordinary class columns or syringes for use.

High throughput DNA Isolation

The development of high throughput DNA isolation methodologies has made the procedure of DNA isolation less time consuming and less cumbersome. DNA isolation has been scaled up to multiples of 96 well/sample format. Most of these methods utilize kits for DNA isolation and do not involve organic extraction as it is difficult to achieve phase separation in plates (Tibbits *et al*., 2006). These technologies utilize mechanical tissue grinders (Genogrinders) to handle a large number of samples simultaneously (Kumar *et al*., 2013; Sharma *et al*., 2013). Lemke *et al*. (2011) used a tissue lyser with tungsten carbide beads for DNA isolation from grapevine (*Vitis* sps.) in 96 column plates. DNA was isolated from lyophilized leaf tissue and seeds of *Sorghum bicolor* after grinding in a tissue lyser by placing a tungsten ball in a tube containing the tissue. A 96 well plate could be processed in less than 2 hrs using this method (Xin and Chen, 2012). Xu *et al*. (2005), described a high throughput method in rice which is capable of processing 384 samples in 2 hrs. A rapid and convenient method of DNA isolation from shoot and leaf tissues of transgenic plants was proposed by HwangBo *et al*. (2010) which uses a chelating resin Chelex 100 while homogenization of the tissue in an eppendorf tube followed by boiling and centrifugation, which yielded a PCR ready DNA preparation. High throughput has been achieved by using fully automated robotic workstations. Zhang *et al*. (2012) have elaborated a quick and simple method for megabase sized DNA isolation from plants, animals and insects. They have enunciated a LMP agarose gel based system for DNA isolation from intact nuclei or protoplasts. The protoplasts or nuclei are embedded in LMP agarose plugs and further purification is carried out in these plugs itself. Czembor *et al*., 2014 have described a state of the art DNA isolation procedure from wheat and barley leaves. They used a robotic workstation where grinding is achieved by a grinder after placing a stainless steel ball bearing in each tube containing lyophilized leaf sample. The processing capability of this system was 8 x 96 samples . The workstation is also equipped with a monochromatic plate reader for the purpose of DNA quantification. Moeller *et al*. (2014) used a Magna Cel paramagnetic cellulose DNA isolation method in conjunction with a Maxwell 16 robot unit which can carry out purification and elution of the DNA.

Conclusion

A detailed analysis of the papers published in the last decade or so, clearly reveals that the CTAB based DNA isolation protocols are more frequently used for different plant materials as compared to SDS based and other protocols. Apart from these detergents, different chemicals such as polyvinylpyrolidone (PVP), pectinases, proteinase K, sodium perchlorate, sodium acetate, ammonium acetate etc., are used in varying concentrations and at different steps for improving efficiency of the DNA isolation protocols. With the availability of a large number of commercial kits and high throughput sophisticated DNA isolation technologies, DNA isolation has now become much easier and feasible than ever before. There are protocols for DNA isolation available not only from new and freshly harvested plant tissues but also from ancient preserved herbarium specimens.

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SHORT COMMUNICATION

Morphological Characterization of Rice (*Oryza sativa* L.) Landraces of the Hilly Zone of Karnataka

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Rice landraces of hilly zone of Karnataka were characterized for distinctness and uniformity according to DUS test guidelines of PPV & FR authority. Grain characters *viz.,* hulled and non-hulled, grain length and width exhibited distinctness among the genotypes. The grain yield of *Bangaarugandu*, *Bagyajyothi*, *Ratnachudi*, *Balaji* and *Kempudoddi* landraces was more than check (*Jyothi*). Rice landraces of hilly zone of karnataka evaluated in this study were found to be distinct and uniform with respect to DUS traits and yield characters and hence may be considered for crop improvement programme for various agronomic and quality traits.

Key Words: Local genotypes, DUS test, Anthocyanin, Rice, Yield

India has rich and wide genetic wealth of rice cultivars. It has been estimated from various surveys that nearly 50,000 cultivars of rice is being grown in the country. From the dawn of agriculture, rice is being cultivated by farmers using manually selected seeds. Such an art is still in practice with the rice growing farmers. These varieties are referred to as landraces. The landraces are valuable as they possess a huge treasure of genetic material which may prove valuable in future crop improvement programmes. Landraces or local types have been used as the sources for the characters such as resistance to pest, disease, abiotic stresses and gene sources for some physico-chemical characters. Information regarding such aspects is incomplete and hence it is necessary to collect and conserve landraces of rice (Sinha and Mishra, 2012).

Various types of landraces of rice are available in the major rice growing regions of Karnataka and hence, their characterization and establishment of uniformity is essential to consider them as variety and can be protected under PPV & FR act. More than their registration as a variety, a better performing local type may be utilized in crop improvement programme as genetic resource.

Introduction of high yielding, semi-dwarf and fertilizer responsive verities of rice has ushered green revolution in India with accompanied loss of farmers own local genotypes (Srivastava and Jaffee, 1993). Characterization also serves an important operation of gene bank for better utilization of genotypes. Germplasm with unique traits can serve as good parent for hybridization programme. With the above back ground and scope a study was conducted to characterize the local rice genotypes for their distinctness and uniformity.

Seeds of 49 rice landraces were collected from Organic Farming Research Institute, UAHS, Shivamogga (Table 1). Landraces were evaluated according to 7 × 7 simple lattice statistical design with two replications during *Kharif*-2013 following 20 cm between rows × 15 cm between plant spacing. Recommended package of practices were followed to grow healthy crop. Ten plants were randomly selected from each landrace in each replication to record the observations on plant characters according to the national DUS test guidelines on rice (Shobarani *et al.*, 2004).

Forty-five plant morphological and 15 quantitative characters were recorded at various stages *viz.,* coleoptiles stage, booting stage, first spikelet of inflorescence just visible stage, half inflorescence emerged stage, beginning of anthesis stage, anthesis half way stage, milk development stage, dough development stage, ripening stage and caryopsis hard stage on single or group plants and measurement of single or group of plants. The data on yield and yield related parameters were analyzed using *SAS v9.2 software*.

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Table 1. Passport data of rice landraces considered in the present study

Code	Genotype	Passport details
L1	Bangaarasanna	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L2	Kempujaddu	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L3	Kiruvaani	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L4	Aaadribatta	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L5	Siggikaima	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L6	Beemassale	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L7	Mukkanna	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L8	Bagyajyothi	Basavaraj, A.S. (Farmar), Angerasu, Thirthahalli, Karnataka
L9	Kaalajeera	Basavaraj, A.S. (Farmar), Angerasu, Thirthahalli, Karnataka
L10	Aaravattelu	Basavaraj, A.S. (Farmar), Angerasu, Thirthahalli, Karnataka
L11	Puttabatta	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L12	Kaagisaale	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L13	Karijaadu	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L14	Barathanachudi	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L15	Rajbog	Basavaraj, A.S. (Farmar), Angerasu, Thirthahalli, Karnataka
L16	Raychursanna	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L17	Kichadisaayi	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L18	Sugandi	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L19	Kerekaaqlumuttiga	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L20	Bangaarugandu	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L21	Yelatakkigidda	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L22	Kempukaaru	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L23	Aaanekombinabatta	Basavaraj, A.S. (Farmar), Angerasu, Thirthahalli, Karnataka
L24	Sannakki	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L25	Gajagunda	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L26	Balaji	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L27	Holesaalachipiga	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L28	Dappabatta	Basavaraj, A.S. (Farmar), Angerasu, Thirthahalli, Karnataka
L29	Misebatta	Basavaraj, A.S. (Farmar), Angerasu, Thirthahalli, Karnataka
L30	Ippattu	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L32	Kanadathumba	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L32	Ratnachudi	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L33	Jeerigesamba	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L34	Kempudoddi	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L35	Mullubatta	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L36	Bilijaadu	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L37	Nirgulebatta	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L38	Naagabatta	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L39	Doddataikallu	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L40	Maranellu	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L41	Nettibatta	Basavaraj, A.S. (Farmar), Angerasu, Thirthahalli, Karnataka
L42	Buddabatta	Mruthyunjaya, H. (Farmar), Hugudi, Hosanagara, Karnataka
L43	Siddisanna	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L44	Gourisanna	Raju, H.R. (Farmar), Hugudi, Hosanagara, Karnataka
L45	Karimunduga	Basavaraj, A.S. (Farmar), Angerasu, Thirthahalli, Karnataka
L46	Kempudoddagidda	Basavaraj, A.S. (Farmar), Angerasu, Thirthahalli, Karnataka
L47	Bangarukaddi	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L48	Padmarekha	Kushi parivara (NGO), Thirthahalli, Shivamogga, Karnataka
L49	Jyothi (Check)	high yielding, local check variety

Various qualitative characters studied ware related to color of coleoptile, basal leaf sheath, ligule, stigma, lemma, palea, awns, sterile lemma and hulled grain. Similarly, anthocyanin color on leaves, leaf sheath, auricles, collar, lemma and stem were recorded visually by comparing with *Munson and Leaf color charts*. Lengths of the awns, hulled and non-hulled grain length and width were measured using Graph sheet. Pubescence on leaf blade and lemma were observed using light operated magnifying lances. Presence or absence of leaf auricles, collar, ligule, awns and secondary branching in panicle were recorded visually by observing individual plants. Male sterility in each genotype was estimated by observing microscopic fields of pollen grains stained with Acetocarmine. Angular characters such as culm attitude, flag leaf attitude, curvature of main axis of panicle and attitude of panicle branches were recorded by comparing with the photographs published in guidelines for conduct of test for DUS on rice (Shobharani *et al.,* 2004).

Quantitative characters like time of heading, number of panicles per plant, days to maturity, and test weight were measured manually. Whereas characters like, length and width of leaf blade, hulled and non hulled grains were measured using graph sheets and length of panicle using scale. A qualitative character of grain such as grain aroma was smelled by boiling the hulled grain taken in test tube using water bath. Amylase content was estimated according to Julino's standard method (Juliano, 1971) using commercial amylose as standard. Phenol reaction was estimated by following the Chang and Bordens method (Chang and Bardens,1965)

The estimates of genetic parameters such as mean, range, phenotypic coefficient of variability (PCV), genotypic coefficient of variability (GCV), heritability (h^2) and genetic advance over mean (GAM) are presented in Table 2. The PCV was found to be higher than GCV but the difference between these two was very less, may be due to the influence of genetic effect at the same time a comparable effect of environment. Hence selection of these characters and evaluation under one more location is advisable. Higher heritability coupled with higher GAM was recorded for eleven yields related characters inferring the role of additive genes in controlling such characters and hence selection for such characters is recommended for improvement of the traits.

The local rice genotypes in this study showed distinctness and uniformity for all the morpho-quantitative traits studied. Out of forty five morphological traits, color characters *viz.,* coleoptiles color, basal leaf sheath colour, leaf colour, leaf ligule color, stigma color, lemma and palea color and decorticated grain color were highly distinct and uniform. As majority of genotypes were found to possess purple coleoptiles (55.10%), green basal leaf sheath (67.35%), medium green leaf (40.82%), white ligule (87.76%), white stigma (48.98%), straw colored lemma and palea (89.80%), straw colored sterile lemma (81.63%), yellowish white awns in four out of ten genotypes which had awns. Decorticated grain color showed white (57.14%), dark brown (26.54%) and 8.16% of genotypes had variegated and dark brown each. The present findings are on par with the reports of Subbarao *et al*. (2013).

Table 2. Estimates of mean, range, variability, heritability and genetic advance for 14 characters in local rice genotypes

S. No.	Character	Mean± SE	Range	PCV (%)	GCV (%)	H^2 Broad Sense (%)	GAM (%)
1	Days to 50% flowering	108.10 ± 0.29	86.15 - 131.00	11.19	11.19	99.94	31.83
2	Plant height	137.57 ± 3.64	94.66 - 162.60	11.74	11.44	94.92	31.84
3	Panicle length	22.99 ± 1.31	17.90 - 27.00	9.83	08.01	66.52	18.77
4	Number of tillers	13.91 ± 1.01	10.50 - 20.50	16.94	15.30	81.59	39.25
5	No. productive tillers	12.19 ± 1.34	7.80 - 16.20	18.87	15.32	65.93	35.82
6	Panicle exertion	9.05 ± 0.65	6.35 - 12.80	18.29	16.83	84.62	45.05
7	No of spikelets /panicle	97.57 ± 7.02	75.00 - 188.00	19.94	18.60	86.98	49.23
8	No of grains per panicle	67.80 ± 6.98	43.87 - 98.35	19.95	16.56	72.11	40.45
9	Panicle fertility (%)	71.12 ± 6.63	47.10 - 90.16	14.23	10.73	56.96	23.89
10	Test weight	22.65 ± 0.84	17.05 - 33.61	19.02	18.65	96.15	52.17
11	Straw yield / plant	42.19 ± 1.97	31.5 - 67.00	19.43	18.86	94.22	52.10
12	Days to maturity	144.46 ± 0.74	123 - 160.00	12.66	12.65	99.83	25.47
13	Harvest index	0.71 ± 0.04	0.46 - 0.94	19.33	18.29	89.44	49.95
14	Grain yield / plant	21.67 ± 0.61	10.1 - 32.70	17.70	17.47	97.49	49.12

Anthocyanin coloration in various plant parts was also distinct across the genotypes where, 22.45% of genotypes had leaf anthocyanin color while leaf sheath anthocyanin color is present only in 12.25% of genotypes. Similarly anthocyanin coloration of collor was absent in majority of genotypes (91.84%). In lemma anthocyanin coloration of area below apex was recorded in 57.14, while apex coloration noted in 58.40% of genotypes. Anthocyanin coloration in nodes was observed in only 16.33 % of genotypes with strong intensity. Density of pubescence on leaf was medium (36.73%), weak (30.61%), strong (22.45%) and very strong only in rest (10.21%) of genotypes. Similarly pubescence of spikelets among the genotypes was strong (44.90%), medium (42.86%) and weak (12.24%).

Culm attitude was observed as erect in 67.35%, semi-erect in 30.61% and horizontal only in 2.04 % of genotypes. Flag leaf attitude was semi-straight in most of the genotypes (65.31%). Curvature of main axis was drooping-deflexed (44.90%), straight (28.57%), deflexed-drooping (18.37%) and only 8.16 % of genotypes had semi-straightness. Similarly attitude of panicle branches were erect in majority (65.31%) of genotypes. On the basis of panicle exertion character, most of the genotypes were found to be well exerted (65.31 %) followed by mostly exerted (20.41%) and partly exerted (14.28%).

Among the fifteen quantitative characters studied, all showed distinctness and uniformity over the genotypes. Most of the genotypes exhibited, long leaf blade with medium width (67.35%), medium days to heading (55.11%), thick stem (40.81%), short stem length (46.94%), medium panicle length (69.38%), medium number of panicles per plant (59.19%) and late to maturity (53.06%). Quantitative traits of grains *viz.,* length and width of both hulled and non hulled, shape and test weight were recorded as, short grain length in 89.79% of genotypes while broad width in 46.94% of genotypes whereas, decorticated grain length was, short (67.35%), medium (28.57%) and long in 4.08% of genotypes. Similarly, width of decorticated grain was broad (55.11%), medium (38.77%) and narrow in 6.12% of genotypes. Decorticated grain shape was short bold in majority of genotypes (89.79%) whereas, test weight of grain showed five distinct classes like, medium (53.06%), low (24.49%), very high (10.21%) and very low in 8.16% of genotypes.

Chemical character *viz.,* Aroma, amylase content and gelatinization tempateture showed less variation across the genotypes evaluated where, none of the genotype had aroma and all had low amylase content. While gelatinization temperature was low in majority of genotypes (83.67%) and rest had exhibited medium temperature (16.33%). All the above results were on par with the characterization studies conducted by Sinha and Mittra (2013).

Thus, all the forty nine local genotypes of rice considered in the present study were found to be distinctive on the basis of 45 qualitative and 15 quantitative characters. The local rice genotypes possess high amount of genetic variability for the yield and its associated traits. We suggest distinct, uniform and better performing genotypes may be used as genetic resource for crop improvement in agronomic and nutritional characters.

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Plant Germplasm Registration Notice*

The Plant Germplasm Registration Committee of ICAR in its XXXVIth meeting held on February 6th, 2017 at the National Bureau of Plant Genetic Resources, New Delhi approved the registration of following 30 germplasm lines out of 68 proposals considered. The information on registered germplasm is published with the purpose to

disseminate the information to respective breeders for utilization of these genetic stocks in their crop improvement programmes. Upon request, the developer(s)/author(s) is/are obliged to distribute the material for crop improvement programme of National Agricultural Research System.

1. NABIMG-9-Blue; BW/2*/PBW621 (IC0620914; INGR17001), a Wheat (*Triticum aestivum*) Germplasm with Blue Grain (aleurone) Color

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Anthocyanins are normal constituents in colored fruits and vegetables. These can act as antioxidants and help in prevention of cardiovascular diseases (Kris-Etherton *et al*., 2004), diabetes (Patel *et al*., 2013), inflammation, cancer (Arts *et al*., 2005), obesity (Tsuda *et al*., 2003) and aging (Chen *et al*., 2013). Common wheat cultivars across the world are white/amber in color. Nutraceutical colored wheat, rich in anthocyanin is quite uncommon. The grains of developed NABIMG-9 line are blue in color due to anthocyanins in the aleurone layer.

Donor line for blue grain trait was imported from Wheat Genetic and Genomic Resource Center (WGGRC) through NBPGR (EC866732-28H129, BW). This line was backcrossed once with high yielding Indian cultivar

Table 1. Stability of blue color observed in different years and locations

Name	Pedigree	Grain color 2013-2014 (NABI)	Grain color 2013-2014 (Kelong)	Grain color 2014-2015 (NABI)	Grain color 2014-2015 (Kelong)	Grain color 2015-2016 (NABI)
NABIMG-9	28H129/ 2*PBW621	Blue	Blue	Blue	Blue	Blue
PBW621	Recipient parent	Amber	Amber	Amber	Amber	Amber
BW EC866732)	Donor parent	Blue	Blue	Blue	Blue	Blue

Table 2. Yield and thousand kernel weight observed in different years and locations

Name	Yield 2013-2014 T/ha	TKW (g) 2013-2014	Yield 2014-2015(NABI)	TKW (g) 2014-2015 (NABI)	Yield 2015-2016 (Chaparchiri) Late sowing	TKW (g) 2015-2016 (Chaparchiri)
NABIMG-9	4.44	45.8	3.08	39.78	2.81	36.25
PBW621	6.1	42.8	5.15	41.2	2.93	36.25
BW EC866732)	2.9	29.6	2.68	27.23	1.14	26.0

Table 3. Agronomic traits of colored wheat line and high yield parent

Name	Plant height	Spike length	Splikelets per spike	Anthocyanin content (mg/kg)
NABIMG-9	111.6	7.6	17.2	114.1
PBW621	94.2	7.8	18	2.8

Compiled and edited by Anjali Kak and RK Tyagi, Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012.

PBW621and followed by production of the stable blue colored line (F_9 generation). Developed colored wheat line has satisfactory yield potential and regional adaptation.

Morpho-agronomic characteristics: Stability of grain color was monitored for several generations. NABIMG-9 showed stable blue color in the seeds. Its yield and thousand kernel weight (TKW) were significantly higher than donor wheat cultivar. This line can be used directly for development of colored wheat products, alternately it can be used in future breeding programs.

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Kris-Etherton PM, GR Beecher, TD Etherton, MD Gross, CL Keen and M Lefevre (2004) Bioactive compounds in nutrition and health-research methodologies for establishing biological function: The antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. *Annual Review of Nutrition*. **24**: 511-538.

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2. NABIMG–10–Purple (BW/2*PBW621) (IC0620915; INGR17002), a Wheat (*Triticum aestivum*) Germplasm with Purple Grain (pericarp) Color

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Anthocyanins are normal constituents in colored fruits and vegetables. These can act as antioxidants and help in prevention of cardiovascular diseases (Kris-Etherton *et al*., 2004), diabetes (Patel *et al*., 2013), inflammation, cancer (Arts *et al*., 2005), obesity (Tsuda *et al*., 2003) and aging (Chen *et al*., 2013). Common wheat cultivars across the world are white/amber in color. Nutraceutical colored wheat, rich in anthocyanin is quite uncommon. The grains of developed NABIMG-10 line are purple in color due to anthocyanins in the pericarp layer.

Donor line for purple grain trait was imported from Wheat Genetic and Genomic Resource Center (WGGRC) through NBPGR (EC866732-28H129, BW). This line was backcrossed once with high yielding Indian cultivar PBW621and followed by production of the stable purple colored line (F_9 generation). Developed colored wheat line has satisfactory yield potential and regional adaptation.

Morpho-agronomic characteristics: Stability of grain color was monitored for several generations. NABIMG-

Table 1. Stability of purple color observed in different years and locations

Name	Pedigree	Grain color 2013-2014 (NABI)	Grain color 2013-2014 (Kelong)	Grain color 2014-2015 (NABI)	Grain color 2014-2015 (Kelong)	Grain color 2015-2016 (NABI)
NABIMG-10	28H129/ 2*PBW621	Purple	Purple	Purple	Purple	Purple
PBW621	Recipient parent	Amber	Amber	Amber	Amber	Amber
BW EC866732)	Donor parent	Purple	Purple	Purple	Purple	Purple

Table 2. Yield and thousand-kernel weight observed in different years and locations

Name	Yield 2013-2014 T/ha	TKW (g) 2013-2014	Yield 2014-2015 (NABI)	TKW (g) 2014-2015 (NABI)	Yield 2015-2016 (Chaparchiri) Late sowing	TKW (g) 2015-2016 (Chaparchiri)
NABIMG-10	4.44	45.8	4.69	39.48	2.53	45.25
PBW621	6.1	42.8	5.15	41.2	2.93	36.25
BW EC866732)	2.9	29.6	2.68	27.23	1.14	26.0

Table 3 Agronomic traits of colored wheat line and high yield parent

Name	Plant height	Spike length	Splikelets per spike	Anthocyanin content (mg/kg)
NABIMG-10	84.2	7.4	16	65.4
PBW621	94.2	7.8	18	2.8

10 showed stable purple color in the seeds. Its yield and thousand kernel weight (TKW) were significantly higher than donor wheat cultivar. This line can be used directly for development of colored wheat products, alternately it can be used in future breeding programs.

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Kris-Etherton PM, GR Beecher, TD Etherton, MD Gross, CL Keen and M Lefevre (2004) Bioactive compounds in nutrition and health-research methodologies for establishing biological function: The antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. *Annual Review of Nutrition*. **24**: 511-538.

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3. NABIMG-11-Black (BW/2*PBW621) (IC0620916; INGR17003), a Wheat (*Triticum aestivum*) Germplasm with Black Grain Colour; (purple pericarp + blue aleuron)

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Anthocyanins are normal constituents in colored fruits and vegetables. These can act as antioxidants and help in prevention of cardiovascular diseases (Kris-Etherton *et al.,* 2004), diabetes (Patel *et al*., 2013), inflammation, cancer (Arts *et al.,* 2005), obesity (Tsuda *et al*., 2003) and aging (Chen *et al*., 2013). Common wheat cultivars across the world are white/amber in color. Nutraceutical colored wheat, rich in anthocyanin is quite uncommon. The grains of developed NABIMG-11 line are black in color due to anthocyanins in the aleurone and pericarp layers.

Donor line for black grain trait was imported from Wheat genetic and genomic resource center (WGGRC) through NBPGR (EC866732-28H129, BW). This line was back crossed once with high yielding Indian cultivar PBW621 and followed by production of the stable black colored line (F_9 generation). Developed colored wheat line has satisfactory yield potential and regional adaptation.

Morpho-agronomic characteristics: Stability of grain color was monitored for several generations. NABIMG-

Table 1 Stability of black color observed in different years and locations

Name	Pedigree	Grain color 2013-2014 (NABI)	Grain color 2013-2014 (Kelong)	Grain color 2014-2015 (NABI)	Grain color 2014-2015 (Kelong)	Grain color 2015-2016 (NABI)
NABIMG-11	28H129/ 2*PBW621	Black	Black	Black	Black	Black
PBW621	Recipient parent	Amber	Amber	Amber	Amber	Amber
BW (EC866732)	Donor parent	Black	Black	Black	Black	Black

Table 2 Yield and thousand-kernel weight observed in different years and locations

Name	Yield 2013-2014 T/ha	TKW (g) 2013-2014	Yield 2014-2015 (NABI)	TKW (g) 2014-2015 (NABI)	Yield 2015-2016 (Chaparchiri) Late sowing	TKW (g) 2015-2016 (Chaparchiri)
NABIMG-11	4.44	45.8	4.71	37.26	3.285	32.5
PBW621	6.1	42.8	5.15	41.2	2.93	36.25
BW (EC866732)	2.9	29.6	2.68	27.23	1.14	26.0

Table 3 Agronomic traits of colored wheat line and high yield parent

Name	Plant height	Spike length	Splikelets per spike	Anthocyanin content (mg/kg)
NABIMG-11	92.6	7.1	15.6	198
PBW621	94.2	7.8	18	2.8

11 showed stable black color in the seeds. Its yield and thousand kernel weight (TKW) were significantly higher than donor wheat cultivar. This line can be used directly for development of colored wheat products, alternately it can be used in future breeding programs.

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Kris-Etherton PM, GR Beecher, TD Etherton, MD Gross, CL Keen and M Lefevre (2004) Bioactive compounds in nutrition and health-research methodologies for establishing biological function: The antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. *Annual Review of Nutrition*. **24**: 511-538.

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4. DDW 42 (IC0621692; INGR17004), a Wheat (*Triticum durum*) Germplasm with High Yellow Pigment Content (High Beta-carotene)

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High yellow pigment (YP) content, a desirable trait for end-use quality in durum wheat is being targeted particularly under durum breeding programs worldwide. This is a precursor of vitamin A, which is an essential ingredient for human health. The Beta-carotene also enhances the end product quality and aroma and thus preferred by the people in the market. For this purpose, DDW 42 (coming out of cross HI 8713/DBP 01-9// PDW 233), one of the promising durum line developed at ICAR-IIWBR, Karnal was evaluated at four locations each in central zone (CZ) and peninsular zone (PZ) along with two check varieties (AKDW 2997-16 and HI 8627) and 22 other lines during 2015-16under All India Coordinated NIVT-5B trial.

DDW 42 was ranked first at all the tested locations for YP content and was better in yield levels as compared to check varieties. Mean of YP content for DDW 42in CZ, was recorded as 8.2ppm, whereas it was 3.3ppm and 6.2ppm for check varieties namely AKDW 2997-16 and HI 8627 respectively. Similarly under PZ, mean YP content of DDW 42, AKDW 2997-16 and HI 8627 were noted as 8.1ppm, 3.3ppm and 5.6ppm, respectively (Table 1).

The highest YP content in DDW 42 was to tune of 9.4 ppm at Powarkheda in CZ and 9.0 ppm at Bagalkot in PZ. At national level DDW 42, AKDW 2997-16 and HI 8627 recorded 8.2, 3.4 and 6.0 ppm, respectively.

Table 1. Yellow pigment content and yield of proposed DDW 42 and checks over locations during 2015-16

Zone	Location	Yellow pigment content (ppm)			% Superiority over AKDW 2997-16	% Superiority over HI 8627	Yield (q/ha)		
		DDW 42	AKDW 2997-16 (C)	HI 8627 (C)			DDW 42	AKDW 2997-16 (C)	HI 8627 (C)
CZ	Dhanduka	**8.4**	4.1	6.8	104.9	23.5	25.6	23.4	29.3
	Indore	**7.4**	2.1	5.3	252.4	39.6	38.1	35.5	34.3
	Kota	**7.6**	2.9	5.6	162.1	35.7	33.6	53.7	49.0
	Powarkheda	**9.4**	4.2	7.0	123.8	34.3	45.1	34.8	33.6
	Mean	**8.2**	3.3	6.2	148.5	32.3	35.6	36.9	36.6
PZ	Dharwad	**8.3**	3.8	6.8	118.4	22.1	21.4	22.2	19.3
	Bagalkot	**9.0**	3.8	6.0	136.8	50.0	27.8	28.7	21.0
	Niphad	**7.2**	2.8	5.1	157.1	41.2	21.7	21.8	18.9
	Pune	**8.0**	2.9	4.6	175.9	73.9	-	-	-
	Mean	**8.1**	3.3	5.6	145.5	44.6	23.6	24.2	19.7
Mean (National)		**8.2**	3.4	6.0	141.2	36.7	30.5	31.4	29.3

Table 2. Other salient characteristics of the genotype DDW 42

Trait	Entry		Check varieties			
	DDW 42		AKDW 2997-16		HI 8627	
	CZ	PZ	CZ	PZ	CZ	PZ
Yellow pigment content (ppm)	**8.2**	**8.1**	3.3	3.3	6.2	5.6
Grain yield q/ha	**40.4**	23.6	40.0	24.2	37.0	19.7
Protein content (%)	**13.7**	**14.1**	13.2	13.6	13.6	13.7
Sedimentation value (ml)	**37**	**36**	33	34	31	31
Thousand grain weight (gm)	**45.0**	37	44.0	37	48.0	39
Test weight (kg/hl)	**85.0**	83.3	85.0	83.2	86.3	84.7
Other morpho-agronomic traits						
Days to heading (days)	79	68	70	61	75	71
Days to maturity (days)	122	108	123	108	124	110
Plant height (cm)	78	68	79	66	86	70
Grain appearance	6.6	6.8	6.4	6.3	7.0	6.6
Yellow berry incidence (%)	**4**	9	10	7	5	9
Threshability	Ey- M	Ey	M	Ey	M	Ey- M
Grain texture	SH	SH- H	SH	H	SH	H

Besides, the other salient features including quality attributes and agro-morphological traits of DDW 42 were found in acceptable range (Table 2) and thus it would serve as potential donor having improved background.

The new durum wheat genotype (DDW 42) possessed high yellow pigment as it showed 141.2% and 36.7% superiority for YP over check varieties (AKDW 2997-16 and HI 8627) respectively at national level, was also good for other desirable agro morphological and nutritional attributes and thus, DDW 42 would serve as a potential donor source to be utilized in future breeding programs aimed to improve YP content of durum varieties.

5. DDW 150 (IC0621693; INGR17005), a Wheat (*Triticum aestivum*) Germplasm with High Tolerance to Heat Stress

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The present climatic conditions pose serious challenge to wheat production in form of abiotic stress conditions. Among abiotic stresses, terminal heat stress during grain filling stage is of major concern as it reduces in grain size that ultimately result in yield losses. The wheat genotype DBW 150 developed from cross DBW 16/ GW 322 was identified as highly heat stress tolerant genotype based on physiological trait characterization. Both the parental lines are high yielding released cultivars (DBW 16 released for irrigated late sown conditions of the NWPZ and GW 322 for irrigated timely sown conditions of the central zone) of wheat being grown in respective zone. Moreover, production conditions (late sown of NWPZ and timely sown of CZ) of both the genotypes have almost similar maturity period and the wheat crop is exposed to terminal heat stress. This way parental adaptability to withstand in the stress condition has been combined in DBW 150.

The genotype DBW 150 was evaluated in coordinated yield trial of AICW&BIP during 2013-14 (NIVT-3) under irrigated late sown condition across different zones of India. The genotype was promoted to advanced varietal trial (AVT) for irrigated late sown condition of NWPZ during 2014-15. Simultaneously, it was evaluated in multi-locational heat tolerance trial (MLHT) that comprised AVT final year entries alongwith checks. DBW 150 was initially evaluated in 2014-15 in MLHT-1 in which it showed promising performance for heat stress tolerance as indicated by very low HSI (heat susceptibility index) score during 2014-15. It was promoted to MLHT 2 during 2015-16 and evaluated for various heat stresses related traits under multi-locational evaluation (Table 1).

This potential genotype possessing heat stress tolerance as indicated by lower value of HSI (0.35) compared to check varieties in the MLHT (Table 1). It also showed lowest reduction (4.99%) in grain yield under late sown conditions.

Under coordinated yield trials also this genotype showed comparable performance to check varieties for yield and other agronomic characteristics thereby showing its suitability as promising genotype for late sown conditions possessing high degree of tolerance to heat stress.

The centre-wise HSI pooled over two years also indicated suitability of DBW 150 under heat stress environment and HSI of 8 heat stress locations (Durgapura, Dharwad, Hisar, Indore, Faizabad, Kanpur, Pantnagar and Sagar) was less than 1.0 (indicator of heat tolerance). As compared to four checks namely DBW 107, IC 138852, UAS 360 and UAS 361, the proposed entry DBW 150 showed tolerance to heat stress at all above 08 locations covering major wheat growing zones and thus was found better as evident by HSI and reduction in yield. The ideal locations for heat tolerance included Sagar, Niphad, Junagarh, Indore, Hisar, Durgapura and

Table 1. Performance of DBW 150 for heat stress parameters in MLHT under AICW&BIP (2015-16)

Traits	Entry	Checks in MLHT (2015-16)			
	DBW 150	DBW 107	UAS 360	UAS 361	IC 138852
Heat susceptibility index (HSI)	**0.35**	1.35	0.92	1.12	1.54
Reduction in yield under late sown (%)	**4.99**	19.27	13.08	15.93	21.99

Table 2. Location-wise heat sensitivity index (HSI) and yield reduction (%) of DBW 150 at different locations in MLHT (pooled over years)

Centre	HSI	Yield Reduction (%)	Centre	HSI	Yield Reduction (%)
Sagar	-0.87	-10.7	Durgapura	0.64	15.5
Dharwad	0.49	11.8	Hisar	0.80	21.1
Kanpur	-0.31	-5.8	Pantnagar	-0.01	-0.1
Indore	0.71	17.0	Faizabad	0.84	22.8

Table 3. Mean performance of DBW 150 and checks for agronomic characteristics during 2014-15

Traits	DBW 150	WH 1021 ©	WH 1124 ©	HD 3059 ©	DBW 90 ©
Days to heading	81	82	80	82	80
Days to maturity	125	124	123	124	123
Plant height (cm)	89	90	86	89	85
1000-grains weight (g)	33	33	35	37	35

Dharwad and at above all these hot spot locations, the proposed genotype has showed superiority over at least one or more check entries.

The genotype DBW 150 will serve as potential donor parent for transferring heat stress tolerance coupled with high yield level in wheat improvement programmes for climate resilience.

6. FLW10 (IC0621833; INGR17006), a Wheat (*Triticum aestivum*) Germplasm with Resistance to Yellow Rust Carrying *Yr10*+ in WH542 Background

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Rusts are economically the most important diseases of wheat as they pose major threat to wheat production in most of the wheat growing areas of the world. The most effective and environmentally sound strategy to manage these diseases is through the deployment of resistant cultivars (Bhardwaj *et al*., 2016). A large number of wheat cultivars have become susceptible to new pathotypes of rusts particularly yellow rust (Prashar *et al*., 2007). At Regional Station ICAR-IIWBR, Shimla FLW10 was developed from the cross WH542/Moro. Moro, a yellow rust differentials, possesses *Yr10* which provides resistance to all the yellow rust pathotypes reported in India. The *Yr10* gene in Moro is in winter wheat background and cannot be utilized directly in breeding programme, hence it has been transferred into spring wheat variety WH542 for facilitating the wheat breeders in NWPZ, NEPZ and NHZ to develop wheat genotype resistant to yellow rust.

FLW10 was tested at seedling stage under controlled conditions against the virulent and prevalent pathotypes of yellow rusts, it gave immune (0;) type of reaction (Table 1). When tested for adult plant reaction (APR) to yellow rust, FLW10 was found to be completely resistant FLW10 was also tested for its response to brown and black rusts and it was found to be resistant to black rust. The rust resistance genes like *Yr*9/*Lr*26/*Sr31* and *Yr10* has been confirmed in FLW10.

Plants of FLW10 have waxiness on leaf sheath, peduncle and glumes. This line is short statured (75cm), medium duration (118 days) with amber coloured grains having thousand-grains weight of 39.7g under Karnal conditions.

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Table 1. Seedling resistance tests (2013-2016) of FLW10 to most virulent pathotypes of yellow and black rusts

	Yellow rust pathotypes								Black rust			
Pathotype	38S102	47S102	47S103	46S102	46S103	46S119	78S84	110S119	34-1	40A	40-1	117-1
Score	0;	0;	0;	0;	0;	0;	0;	0;	0;	2-	2-	2-

7. FLW16 (IC0621834; INGR17007), a Wheat (*Triticum aestivum*) Germplasm with Resistance to Yellow Rust Carrying *Yr5* in UP2338 Background

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), is a destructive disease of wheat (*Triticum aestivum*) worldwide. Growing resistant cultivars are the most preferred method to control the disease. However, wheat cultivars usually become susceptible within a few years of release due to rapid evolution and/or increase of previously rare races. Only few *Yr* genes provide complete resistance to all the known pathotypes in Indian sub-continent (Prashar *et al*., 2007). The *Yr5* gene confers resistance to all races of the stripe rust pathogen of wheat in. FLW16 has been developed from cross UP2338/TSA(*Yr*5) at ICAR-IIWBR regional station Shimla through limited backcross followed by pedigree selection. TSA (*Triticum spelta album*) is a *spelt* wheat genotype. The transfer of unexploited *Yr5* in UP2338 background would facilitate development of yellow rust resistant wheat genotypes suitable for yellow rust prone wheat grown zones of Northern India.

In seedling resistance test FLW16 was found to be immune (0;) against the most virulent and prevalent pathotypes of yellow rust (Table 1). Its adult plant reaction (APR) shows complete resistance to yellow rust when tested under polyhouse conditions at Shimla during the years 2013, 2014 and 2015. It was also found to be moderately resistant to black rust. FLW16 carries *Lr26*, *Sr31*, *Yr9* and *Yr5* genes for wheat rust resistance.

The plants of FLW16 have light waxiness on leaf sheath and peduncles. It has plant height of 78cm with maturity duration of 120 days, amber coloured grains and thousand grains weight of 38.1g under Karnal conditions.

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Table 1. Seedling resistance tests (2013-2016) of FLW16 to most virulent pathotypes of yellow and black rusts

	Yellow rust pathotypes								Black rust			
Pathotype	38S102	47S102	47S103	46S102	46S103	46S119	78S84	110S119	34-1	40A	40-1	117-1
Score	0;	0;	0;	0;	0;	0;	0;	0;	0;	2-	2-	2-

8. FLW 21 (IC0621836; INGR17008), a Wheat (*Triticum aestivum*) Germplasm with Resistance to Yellow and Brown Rusts Carrying *Yr15+Lr24* in Background of UP2338

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Common wheat (*Triticum aestivum* L.) is the most important food crop directly linked to food security in India. The rust pathogens of wheat are shifty and capable of acquiring virulence against resistance genes, and are able to spread rapidly between widely separated wheat production areas. Deployment of highly effective and durable rust resistance genes is considered eco-friendly and sustainable management (Bhardwaj *et*

Table 1. Seedling resistance tests (2013-2016) of FLW21 to most virulent pathotypes of yellow and brown rusts

Pathotypes	Yellow rust								Brown rust							
	38S102	47S102	47S103	46S102	46S103	46S119	78S84	110S119	12-5	77A	77-2	77-5	77-7	77-8	77-10	104-2
Score	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;

al., 2016). The genes normally act independently and when multiple resistance genes in the same genotype are challenged with a pathotype avirulent to all, the strongest resistance phenotype is normally manifested (Knott, 1989). However, combination of two or more resistance genes sometimes results in a lower or more resistant infection type (IT) than is conditioned by the most effective gene (Samborski and Dyck, 1982).

At ICAR-IIWBR regional station FLW21 was developed with highly effective resistance genes for yellow, brown and black rusts for utilization in wheat breeding programme for all Zones of India. FLW21 is derived from double cross UP2338/Centurk//UP2338/CN25087(*Yr15)*. Centurk is a popular winter wheat variety from Nebraska, USA and carries linked genes *Lr24/Sr24*. Line CN25087 carrying *Yr15* originates from *T. dicoccoides*. Use of FLW21 will facilitate wheat breeders in developing rust resistant genotypes for all wheat growing zones in India.

FLW21 showed immune (0) reaction in seedling resistance test with most virulent pathotypes of brown and yellow rusts (Table 1). FLW21 also showed resistance to black rust. Adult plant reaction of FLW21 showed resistance against all the three rusts. The rust resistance genes postulated in FLW21 are *Lr24/Sr24*, *Lr26*/*Sr31*/*Yr*9 and *Yr15*. The plants of FLW21 have waxiness on leaf sheath, peduncle and glumes. The plants are semi-erect with average plant height of 91cm, maturity duration of 122 days with thousand-grain weight of 37.3g.

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9. FLW 22 (IC0621837; INGR17009), a Wheat (*Triticum aestivum*) Germplasm with Resistance to Brown (*Lr*28) and Yellow Rusts (*YrChina84*) in the Background of WH542

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Among several constraints towards realizing the potential yield in wheat, the rust diseases pose major threat to wheat production worldwide including India. All the three rusts of wheat, stripe rust caused by *Puccinia striiformis,* leaf rust incited by *Puccinia triticina* and stem rust caused by *Puccinia graminis* Pers. f.sp. *tritici* are occurring in designated wheat zones in India and cause significant losses in wheat production. Wheat rusts have been controlled effectively with the deployment of resistant wheat cultivars for the last several decades (Tomar *et al*., 2014).

Keeping in view the need to utilize diverse rust resistance gene the genotypes FLW22 was developed at IIWBR regional station, Shimla through pedigree method. Developed through double cross WH542/*Lr28*//WH542/China84-40022. China84-40022 possesses undesignated stripe rust resistance gene *YrChina84*. *YrChina84* provides resistance against all the pathotypes of yellow rust found in India. Use of FLW22 in wheat breeding programme will diversify resistance gene for brown and yellow rust in India.

Table 1. Seedling resistance tests (2013-2016) of FLW22 to most virulent pathotypes of yellow and brown rusts

Pathotypes	Yellow rust								Brown rust							
	38S102	47S102	47S103	46S102	46S103	46S119	78S84	110S119	12-5	77A	77-2	77-5	77-7	77-8	77-10	104-2
Score	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;

In seedling resistance test, FLW22 showed immune (0) reaction against virulent pathotypes of yellow and brown rusts (Table 1). It also exhibited resistance against all the three rusts in adult plant reaction (APR) tests during three years i.e., 2013 to 2015. FLW22 has been confirmed to carry *Lr28*, *Lr26*/*Sr31*/*Yr9* and *YrChina84* genes which provide resistance to all the three rusts of wheat.

Plants of FLW22 have slight waxiness on leaf sheath, peduncle and glumes. It has semi-erect growth habit with average plant height of 90cm and average maturity duration of 118 days, amber coloured grain with soft texture having thousand-grain weight of 37.4g.

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10. FWW2 (IC0621838; INGR17010), a Wheat (*Triticum aestivum*) Germplasm with Resistance to Brown Rust carrying *Lr19+Lr24* in the Background of PBW343

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Rusts are important diseases of wheat (*Triticum aestivum* L.) throughout the world. The preferred way of controlling the disease is through the use of resistant varieties. There are several genes that can express resistance to this disease. However, changes in pathogen virulence can render them useless for breeding after some time (Bhardwaj *et al*., 2016). The emergence of the Ug99 group of stem rust races (TTKSK, TTKSF, TTKST, TTTSK) has reaffirmed the need to deploy diverse and effective resistance sources to safeguard wheat production (Pretorius *et al*., 2000). Keeping in view the need to deploy diverse resistance gene against brown and black (including Ug99) rusts the genotype FWW2 was developed for utilization by wheat breeders in India.

FWW2 is derived from cross PBW343/PH137 wherein PH137 contributes rust resistance genes *Lr19/Sr25* and *Lr24/Sr24*. FWW2 having two gene based resistance would be useful in diversifying resistance for brown and black rusts in all wheat growing zone of India.

Seedling resistance test against most virulent and prevalent pathotypes under controlled conditions showed that FWW2 is immune (0) to brown rust (Table 1). It also showed resistance against black rust. The adult plant reaction (APR) tests for three years (2013 to 2015) showed resistance to brown and black rusts. FWW2 carries *Lr19/Sr25, Lr24/Sr24*, *Lr26*/*Sr31/Yr9* genes for resistance to wheat rusts. *Sr25* also confers resistance to Ug99 virulences of stem rust (Singh *et al*., 2015). The plants of FWW2 have waxiness on sheath of flag leaf, peduncle and glumes. It has semi-erect growth habit with average plant height of 85cm and matures in 118 days having amber coloured grains with thousand-grain weight of 40.6g.

Table 1. Seedling resistance tests (2013-2016) of FWW2 to most virulent pathotypes of brown and black rusts

	Brown rust pathotypes							Black rust			
Pathotypes	12-5	77-2	77-5	77-7	77-8	77-10	104-2	34-1	40A	40-1	117-1
Score	0;	0;	0;	0;	0;	0;	0;	0;	2-	2-	2-

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11. Local Wheat Hango (LWH) (IC0621839; INGR17011), a Wheat (*Triticum aestivum*) Germplasm Universally Susceptible to Yellow, Brown and Black Rusts

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The bread wheat germplasm 'Local Wheat Hango (LWH), was collected from village Hango in Kinnaur district of Himachal Pradesh in the year 2002. This local wheat variety is susceptible to all the three rusts of wheat under field condition. The susceptible reaction of LWH was examined in comparison with another susceptible wheat variety 'Agra Local'. Agra Local has been used as universally susceptible variety since last about eighty years. Of late, Agra Local was observed to reveal resistant response against three pathotypes of *Puccinia triticina* (Datta *et al*., 2008) causing brown rust of wheat. In addition, few yellow rust pathotypes also exhibited low sporulation on it. This feature of Agra Local showed that it was not 'universally susceptible' to all pathotypes of brown, black and yellow rusts.

The germplasm LWH was tested for its response against all pathotypes of brown, black and yellow rusts. The susceptible reaction of LWH to all existing pathotypes of the three rusts revealed its universally susceptible nature. Thus, LWH can be safely used in genetic studies on resistance to all the three rusts of wheat (Bhardwaj *et al*., 2006).

The plants of this genotype have pink coleoptiles, erect habit, light green foliage and average plant height of 88cm. The auricles lack pigmentation, but are pubescent. The ear is clavate having short awns; outer glumes have no pubescence; lemma has square shape shoulder, medium beak length and strong beak curvature. There is slight waxiness on leaf sheath and peduncle. Grains are amber coloured with soft texture. Seeds are small, elliptical shaped with deep crease.

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12. DWRB137 (IC0620682; INGR17012), a Barley (*Hordeum vulgare* L.) Germplasm Highly Resistant to Stripe Rust (*Puccinia striiformis* f. sp. hordei) at Seedling and Adult Plant Stages Coupled with Short Plant Height

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DWRB137 (DWR28/DWRUB64) is an advance six-row feed barley strain, which is directly utilizable genetic resource for stripe rust with desirable plant height. It was evaluated for disease reactions at multi-locations for consecutive three years viz. *rabi*, 2013-14 to 2015-16, under Initial Barley Disease Screening Nursery (IBDSN) and National Barley Disease Screening Nursery (NBDSN), respectively. During *rabi*, 2013-14, the genotype DWRB137 was evaluated by code BK1321 at five locations namely Bajaura, Durgapura, Dhaulakuan, Ludhiana and Karnal in IBDSN. The genotype DWRB137 exhibited highly resistant response (0 reaction) for yellow rust under artificial disease epiphytotic conditions (Anonymous, 2014). Whereas, the recent check varieties of NWPZ namely BH902 and BH946 exhibited susceptible yellow rust reactions of 30S and 10S, respectively (Table 1). During *rabi*, 2014-15 and 2015-16, DWRB137 was again evaluated for disease resistance under artificial inoculations in NBDSN. DWRB137 reconfirmed resistance response to yellow rust (0 reaction) during both the seasons at all the centers (Anonymous, 2015; Anonymous, 2016). In seedling resistance test (SRT) DWRB137 showed resistant reactions for all the barley stripe rust races *viz.* 57, 24, M, G and Q consecutively for two years *i.e. rabi*, 2014-15 and 2015-16.

DWRB137 exhibited the desired plant height of 78 cm (62-93), which is highly desirable as barley is a lodging prone crop. Average days to heading, maturity and 1000-grain weight for DWRB137 were recorded as 82 days (67-93), 127 days (118-135) and 44 g (36-49), respectively in AVT-TS-FB-NWPZ coordinated trial, during *rabi*, 2015-16. The grains are hulled, elongated and light yellow in colour.

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Table 1. Multi-location adult plant resistance (APR) for stripe rust reactions (artificial inoculations) of DWRB137 and checks over three years

Year	Location	DWRB137	Checks (NWPZ)		Check (CZ)	Infector
			BH902	BH946	BH959	
First year (2013-14)	Bajaura	0	30S	0	0	100S
	Dhaulakuan	0	0	0	0	100S
	Durgapura	0	0	10S	30S	100S
	Ludhiana	0	0	0	0	40S
	Karnal	0	10MS	0	10MR	80S
Second Year (2014-15)	Durgapura	0	tMR	10MR	40MS	100S
	Bajaura	0	0	10S	0	100S
	Almora	0	5S	10MS	20MS	60S
Third Year (2015-16)	Bajaura	0	20S	10MR	20S	100S
	Ludhiana	0	10S	0	0	60S
	Durgapura	0	10MS	5S	10S	100S
	Karnal	0	5MR	0	5MS	60S
	Jammu	0	10S	5MR	0	80S
	Almora	0	20S	5S	10S	80S
Three years HS		0	30S	10S	40MS	100S

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13. DQL-2105-1 (IC0621103; INGR17013), a Maize (*Zea mays L.*) Germplasm with Moderately Resistant to Maydis Leaf Blight (MLB) (disease mean score 2.13 on the scale of 1-5). Moderately Resistant to Turcicum Leaf Blight (TLB) (disease mean score 2.58 on the scale of 1-5). Tryptophan Content 0.69% in Protein

Ramesh Kumar[1], Jyoti Kaul[2], KS Hooda[1], Dharam Paul[1], Vinay Mahajan[1], Sunil Neelam[4], Chikkappa G Karjagi[3], Bhupender Kumar[3], Ganapti Mukri[2], SB Singh[5], Harleen Kaur[6], N Mallikarjuna[7], R Devlash[8], Avinash Singode[9], Nirupma Singh[2], R Ambika Rajendran[2], Abhijit Kumar Das[1], Yathish KR[1], Vishal Singh[1], Usha Nara[6], Vinod Kumar[2], Khushbu Jain[2] and DS Olakh[1]

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Maize is the third most important cereal crop of the country after rice and wheat and is valued as food, feed, fodder and industrial raw material. In view of maize being produced under very diverse ecology in our country, development of high yielding hybrids with in-built resistance and tolerance to diseases, pests and various climatic stresses; and development and fine-tuning of production ecology are our top priorities.

Maydis Leaf Blight (MLB) and Turcicum Leaf Blight (TLB) are the most important diseases of India. MLB is also known as Southern Maize Leaf Blight. It is caused by the fungi *Bipolaris maydis.* This foliar disease is reported from most of the maize growing regions. It is a most serious disease in warm and wet temperate and tropical areas. 70% of the yield losses have been reported in the maize growing areas due to this disease. Symptoms and severity of this disease depends on the pathogen race and host germplasm. TLB is also known northern corn leaf blight. This disease is caused by the fungi *Exserohilum turcicium*. This foliar disease occurs in high humidity and moderate temperature areas. It also results in 70% loss in the yield.DQL 2105-1 is a late maturing quality protein maize line moderately resistant to Maydis Leaf Blight and Turcicum Leaf Blight. It is derived from HQPM-7, a quality protein maize population.

Morpho-agronomic characteristics: DQL 2105-1 is a late maturing quality protein maize inbred line with moderately resistant to Maydis Leaf Blight and Turcicum Leaf Blight, medium ear placement, straight attitude of lateral branches, sparse spikelets of tassel, yellow, flint and conico-cylindrical kernels. This line is derived from HQPM-7, a quality protein maize population by following pedigree breeding. Under hot spot locations (Delhi, Ludhiana and Karnal) for Madyis Leaf Blight and Turcicum Leaf Blight, the line displayed a mean disease score of 2.13 and 2.58 while the susceptible check 3.98 and 4.67 and resistance check 1.58 and 1.83, respectively. Hence, this QPM line was found to be superior for traits viz; MLB, TLB and Tryptophan. The inoculation procedure followed as given by Sekhar and Kumar, 2012 on the rating scale of 1-5.

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14. DQL 2048 (IC0621104; INGR17014), a Maize (*Zea mays L.*) Germplasm with Moderately Resistant to Maydis Leaf Blight (MLB) (disease mean score 2.65 on the scale of 1-5). Moderately Resistant to Turcicum Leaf Blight (TLB) (disease mean score 2.33 on the scale of 1-5). Tryptophan content 0.71% in Protein

Ramesh Kumar[1], Jyoti Kaul[2], KS Hooda[1], Dharam Paul[1], Vinay Mahajan[1], Sunil Neelam[4], Chikkappa G Karjagi[3], Bhupender Kumar[3], Ganapti Mukri[2], SB Singh[5], Harleen Kaur[6], N Mallikarjuna[7], R Devlash[8], Avinash Singode[9], Nirupma Singh[2], R Ambika Rajendran[2], Abhijit Kumar Das[1], Yathish KR[1], Vishal Singh[1], Usha Nara[6], Vinod Kumar[2], Khushbu Jain[2] and DS Olakh[1]

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DQL 2048 is a medium maturing quality protein maize line moderately resistant to Maydis Leaf Blight and Turcicum Leaf Blight. It is derived from HQPM-1, a quality protein maize population.

Morpho-agronomic characteristics: DQL 2048 is a medium maturing quality protein maize inbred line with moderately resistant to Maydis Leaf Blight and Turcicum Leaf Blight, medium ear placement, straight attitude of lateral branches, dense spikelets of tassel, yellow and flint kernels. This line is derived from HQPM-1 a quality protein maize population by following pedigree breeding. Under hot spot locations (Delhi, Ludhiana and Karnal) for Madyis Leaf Blight and Turcicum Leaf Blight, the line displayed a mean disease score of 2.65 and 2.33 while the susceptible check 3.98 and 4.67 and resistance check 1.58 and 1.83, respectively.Hence, this QPM line was found to be superior for traits viz; MLB, TLB and Tryptophan.The inoculation procedure followed as given by Sekhar and Kumar, 2012 on the rating scale of 1-5.

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Meena Sekhar and Sangeet Kumar (2012) Inoculation methods and disease rating scales for maize disease.Technical bulletin, Directorate of Maize Research, Pusa Campus New Delhi–110012. Pp. 22-28.

15. PDL-1 (IC0621470; INGR17015) a Lentil (*Lens culainaris* Medikus) Germplasm with Drought Tolerance

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16. SGS 389 (IC0621690; INGR17016), a Sorghum (*Sorghum bicolor* (L.) Moench) Germplasm with Six Stamens and Two Gynoecia and Twin Seeds

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The sorghum [*Sorghum bicolor* (L.) Moench] spikelet normally contains one gynoecium and three stamens. During the germplasm evaluation study performed at Indian Grassland and Fodder Research Institute (IGFRI) in the rainy season of 2012, a novel genotype (identity SGS 389) bearing six stamens and two gynoecium was observed which is a trait reported here for the first time. Critical examination of all the plants of the genotype revealed that this unique trait had 100% expressivity and 100% penetrance. The panicles of 60 plants were enveloped from heading to completion of flowering stage. During the rainy season of 2013, self seeds of each plant were planted in paired rows of 4 m length. No within line and between line variation was observed for the unique trait among the self progenies (Joshi *et al*., 2013; Joshi *et al*., 2015). The panicles of 15 plants were enveloped from heading to completion of flowering stage in each row. Rigorous examination of sessile spikelets in individual plants of each selfed progeny during the rainy season of 2014 revealed that the novel trait is stably inherited, suggesting its genic control. Interestingly, all the spikelets having six stamens and two gynoecia also produced two seeds instead of one, in contrast to the normal sorghum generating one seed per spikelet. Thus production of two seeds per spikelet indicates that all the six stamens and two gynoecia were fertile.

In sorghum deviations from normal floral structures *viz*., spikelet shoots, scaly proliferation, polyembryony and fertile pedicellated spikelets have been observed. However, these deviations are governed by environmental factors and are not hereditary (Stephens, 1936). Furthermore, reports on development of more than one seed/spikelet are available in some American genotypes but these instances are very rare, moreover the spikelet contained one fertile flower in each cases (Karper, 1931). However, the novel genotype reported in the present study is unique as it produces twin seeds but the spikelet bears six stamens and two gynoecia. The novel trait reported in the present study is genetically controlled and stably inherited over the years and generations (Joshi *et al*., 2015). Both way (direct and reciprocal) crosses were attempted between the novel genotype and the normal genotype (three stamens and one gynoecium) to understand the genetic control of the trait. To the best of our knowledge it is the first report on a sorghum genotype having six stamens and two gynoecia. The reported genetic stock seems to be a homeobox mutant. It is extremely useful in deciphering the genetic control and regulatory mechanism of floral structure development and embryogenesis in sorghum in particular as well as grasses in general.

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17. JP-96 (IC0621469; INGR17017), a Castor (*Ricinus communis* L.) Germplasm with Pistillate Line; Good Combiner

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Castor (*Ricinus communis* L.) is an important industrial non-edible oilseed crop grown in various countries throughout the world. Castor oil is used for number of industrial products. During the year 2014-15, the area under castor crop in India was 10.99 lakh ha, which contributed 13.51 lakh metric tonne production with a productivity of 1229 Kg/ha. Gujarat is the leading state in the country with respect to area, production and

productivity of castor covering an area of 7.34 lakh ha with the production to 10.69 lakh tonne and average productivity of 1454 Kg/ha (Anonymous, 2015). Genetic diversity of parents is a prerequisite for the development of high yielding hybrids in castor. Efforts initiated at Main Oilseeds Research Station, Junagadh Agricultural University, Junagadh to enhance the diversity of pistillate lines using diverse parents led to identification of the genetic stock JP-96 in a segregating population of the cross JP-66 x 454 with the unique trait of a single male flower at the tip of the spike. Heritability of the trait was observed more than 90% in the population of nearly 80-100 plants every year. This pistillate line was utilized by many centres for hybrid development and gave good hybrids. SHB-896, one of the hybrids from Sardarkrushinagar was performing well in State and AICRP trials having JP-96 as female parent. The proposed stock has other distinct morphological characters like green stem, triple bloom, long (65-75cm), compact spike with spiny capsules (Table 1). The genetic stock has a potential to use in the heterosis breeding as a diverse source of pistillate line and will also be further useful in the study of sex polymorphism in castor and development of genetic stocks for distinct sex expression.

Table 1. Chief botanical and morpho-agronomic traits of JP-96

Sr.	Character	Description
1	Internode type	Elongated
2	Leaf shape	Flat
3	Leaf lascination	Shallow
4	Petiole length	Long (31-40 cm)
5	Petiole surface	Smooth
6	Spike shape	Cylindrical
7	Spike compactness	Compact
8	Capsule spininess	Dense
9	Capsule length (cm)	2-3
10	Location of branch	Basal/ all over
11	Branching pattern	Divergent
12	Seed shape	Oval
13	Seed coat colour	Dark brown/ Blackish
14	Seed mottling	High
15	Seed caruncle	Small
16	Plant height (cm)	70-80
17	Length of primary spike (cm)	65-75
18	Number of nodes upto primary spike	16-18
19	Number of capsules on primary spike	70-80
20	Days to 50% flowering	66-75
21	Days to maturity of primary spike	130-140
22	Number of effective spikes per plant	5-7
23	100-seed weight (g)	33-35
24	Oil content (%)	48-49

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18. PT-141 (IC0621691; INGR17018), a Toria (*Brassica rapa* var. *toria*) Germplasm with Earliness of Maturity; Earliness of Flowering

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Amongst three ecotypes of *Brassica rapa* namely toria, yellow sarson and brown sarson, former, due to its shorter maturity duration, has been traditionally grown as catch crop between rainy (*kharif*) and winter (*rabi*) season crops. Maturity duration of existing toria varieties ranges from 90-95 days. Any source of extra earliness of flowering and maturity in toria will help to develop varieties combining these unique features, which will pave the way for their diversification to new growing situations and regions.

A local germplasm of toria collected from Chamba block of District Tehri, Uttarakhand showed variability for earliness of flowering and maturity. This collection after purification and selection for earliness of flowering and maturity, and uniformity for height and associated characters gave rise to an extra early maturing line of toria named PT-141(Ram Bhajan *et al*., 2013).

Morpho-agronomic characteristics: PT 141 is very early in flowering initiation (~18 days) as well as maturity (56-60 days) (Table-1). Flowering period is considerably reduced which increases synchrony both within and between racemes of a plant. It is known that earliness is associated with increased photoperiod insensitivity (day neutrality) in different crop plants including oilseeds (Singh and Sharma, 1996). Earliness, or day neutrality, of varieties also increases their adaptability.

Extra earliness of flowering/maturity of PT 141 will enable to design a plant type of toria which is high yielding, short stature and extra early maturing. Extra early maturity of this line make it more suitable for use

Table 1. Main features of PT-141 vis-à-vis of PT-303 of toria

Characters	PT-141	PT-303 (check)	SEm±	CV (%)
Altitude of collection site (m)	2229	-	-	-
Days to flowering initiation	18	36	1.61	5.61
Days to maturity	56	95	1.70	6.55
Plant height (cm)	30.8	122	4.45	12.22
Length of main raceme	19.48	49.04	1.29	5.73
Primary branches/plot	3.80	4.93	0.30	9.53
Secondary branches/plot	5.00	6.15	0.49	10.24
Siliqua length (cm)	5.40	5.59	0.21	6.99
Seeds/siliqua	10.60	15.61	0.41	4.61
Siliquae on main raceme	18.00	26.32	0.94	7.14
Siliquae/plant	69.60	131.00	3.72	13.25
1000-seed weight (g)	2.08	2.87	0.10	7.25
Seed yield/plant (g)	2.11	4.22	0.28	12.22

as catch crop and regain the toria area declined in the recent past mainly due to introduction and large scale cultivation of long duration high yielding varieties of rice, in the major rice growing belt of northern India.

Associated characters and cultivation practices: PT 141, besides being early in flowering and maturity, it is dwarf statured than the check variety PT-303 (Table 1) and exhibits satisfactory growth, seed and siliquae set under delayed sowing in the month of November as well. However, sowing after September delays its maturity duration.

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19. CR 143-2-2 (IC0513420; INGR17019), a Rice (*Oryza sativa)* Breeding Line Tolerant to both Vegetative and Reproductive Stage Drought Stress

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The breeding line (CR 143-2-2) was developed at ICAR-NRRI, Cuttack by making a cross between Bala × Lalnakanda 41 following pedigree selection method. This showed tolerance to vegetative as well as reproductive stage drought stress consistently for several years after rigorous screening under simulated moisture stress in the field in NRRI Main Institute and its substation CRURRS, Hazaribag. Under simulated stress during vegetative

Table 1. DUS Characteristics of Rice Germplasm 'CR 143-2-2'

Characteristics	CR 143-2-2 (IC0513420)
Blade colour	Dark Green
Blade pubescence	Pubescent
Basal leaf sheath colour	Pale green
Ligule shape	2-cleft
Ligule length	0.9
Ligule colour	White
Collar colour	Pale green
Auricle colour	Pale green
Internode colour	Green
Flag leaf angle	Descending
Culm angle	Intermediate
Culm strength	Strong (No lodging)
Panicle exsertion	Well exserted
Panicle axis	Droopy
Panicle type	Intermediate
Secondary branching	Heavy
Apiculus colour	Straw
Stigma colour	White
Leaf length (cm)	33.6
Leaf width (cm)	1.4
Culm length (cm)	94.3
Culm diameter(cm)	0.5
Number of EBT	8
Panicle length (cm)	25
Awning	Absent
Sterile lemma colour	Straw
Grain length (mm)	8.3
Grain breadth (mm)	3.2
l/b ratio	2.59
Grain shape	Medium
Maturity duration (days)	115
Yield (t/ha)	3.0 (normal) ; 1.6-2.0 (under stress)
Drought scoring (in SES scale)	1

and reproductive stage, CR 143-2-2 was observed to be tolerant with least leaf death score of SES "0" and "1", had high and stable grain yield of around 2.0 t/ha with lowest reduction in yield (10%), highest drought index (90.3)/lowest drought susceptibility index (0.47), high water retention capacity (>70%), highest grain filling (68%) with less impaired translocation at 10-13% soil moisture content and soil moisture tension of -55 to -60 kPa during the stress period (Ramakrishnayya and Swain, 2005; Swain *et al*., 2013). This line also has considerably produced good yield under moderate stress and control conditions (Verulkar *et al*., 2010). It has high *per se* performance for more than one root traits under control and stress conditions. Based on its stable performance, it is being used as a tolerant check in all drought related experiments across the globe. Many breeding lines have been derived taking it as a donor parent and are under study for developing drought tolerant varieties.

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20. COMS 14A & COMS 14B (IC0612955 & IC0612956; INGR17020), a Rice (*Oryza sativa)* CMS line/Maintainer Line with Higher Rate of Medium Slender Grain Type. Good Cooking Quality

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Wild Abortive (WA) cytoplasm was found to be the most stable and about 95% of rice hybrids in the world were based on this single source of cytoplasm for sterility (Virmani, 1998). The major constraint to the popularization of hybrid rice in India is its poor grain quality, though the yield potential is 20% higher than that of inbred varieties. Developing new CMS lines in the background of agronomically adapted and popular varieties is the need of the hour. ADT 43, a derivative of IR50/Improved White Ponni, is a short-duration and popular variety with good grain quality. ADT 43 was crossed with IR69616 A in the hybrid rice breeding programme at Department of Rice, Tamil Nadu Agricultural University, Coimbatore. The hybrid was evaluated in the test-cross nursery during *kharif*, 2000. The hybrids IR69616 A/ADT43–showed a maintainer reaction with 100% pollen sterility at the flowering stage. The male parent, ADT 43 was used as recurrent parent in the backcrossing program. New CMS lines developed through substitution backcrossing have been reported by Ingale *et al.* (2004). After repeated backcrossing of six generations (Rabi 2003), new CMS line, COMS14 A (IR69616 A/ADT43) was developed with better floral characteristics.

This CMS line is of the duration of 115 days with semi dwarf plant type. The panicle exertion percentage is 75.00% with stigma exertion of 75.4% and wide angle of glume opening which makes the line with higher out crossing potential highly amenable for commercial exploitation (Manonmani *et al*., 2012). The grain quality of the CMS line is highly preferable with intermediate amylose content, soft gel consistency and intermediate Gelatinization Temperature. Grain type of the line is medium slender with 1000 grain weight of 15 grams. This type of grain character is highly suitable for market demands of south India. This line was also tested for its physiological efficiency in expressing the yield potential in many of the cross combinations (Malarvizhi *et al*., 2001). The stability of the line was tested across the locations viz., Chinsurah, West Bengal (Nath *et al*., 2016) and Madurai proved that it maintains the 100 % sterility. This new line can be further exploited to develop medium slender rice hybrid with higher yield potential.

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21. COMS 24A & COMS 24B (IC0612957 & IC0612958; INGR17021), a Rice (*Oryza sativa)* Germplasm with CMS Line/Maintainer Line with Higher Rate of Stigma Exsertion. Long Slender Grain Type. High Out Crossing Rate

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Hybrid rice varieties demonstrated 15-20% higher yield comparing to the inbred varieties. The first attempt in India for hybrid rice research was initiated as early as 1987 with the introduction of two CMS lines such as V20A and Zhen Shan 97A from IRRI. Another two CMS lines namely IR58025A and IR62829A were introduced from IRRI in 1991 and the development of new CMS lines in well adapted and high yielding improved varietal background was initiated in 1994. In the initial phases of hybrid rice technology in India, the number of promising hybrids was negligible mainly because of non-adaptable CMS lines that were used in developing hybrids resulted in hybrid impurity and un acceptable grain quality. Therefore, an attempt was carried out with the objective to develop adaptable CMS line with high out crossing rate to develop superior rice hybrids. Keeping this mandate, a new CMS line was developed from the culture CB 97036. The culture CB 97036 was used as a male parent with IR 79156 A and the hybrid was found to be 100% sterile. The hybrid was examined for its floral traits and was found to be promising for its out crossing traits and hence it was back crossed to its recurrent parent, CB 97036 for five generations. Each generation the plants were carefully chosen with good floral traits with complete sterility.

Morpho-agronomic characters: This CMS line is with the flowering duration of 120 days, semi-dwarf plant height (76 cm). This CMS line, COMS 24 A was evaluated along with other CMS lines and was found to be promising for its characters viz., pollen sterility (%), glume angle, panicle exertion, stigma exertion and natural out crossing (Manonmani *et al*., 2012). This line was utilized for developing hybrid combinations with many restorers. The line was evaluated under multi-locations at Coimbatore, Madurai and Aduthurai (Mahalingam *et al*., 2013) and it had reported a stable sterility.

Associated characters and cultivation practices: This line was found to be promising for its seed produceability (Anon, 2014). Staggering recommended for this A & B line is 0, +3, +6 and +9. Other package of practices is as like that of other CMS line seed multiplication. One of the hybrid combination, TNRH 206 (TNAU CMS 24 A/CB 206 R) maturing in 130-135 days was found to be superior in yield and grain quality traits. This hybrid was recommended for on farm testing (Anon, 2012).

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22. DML 339 (IC0612721; INGR17022), a Maize (*Zea mays* L.) Germplasm with Resistance to Charcoal Rot (disease mean score 2.8 on the scale of 1-9). Mean Anthesis Silking Interval of 2.0 days

and

23. DML 1019 (IC0612704; INGR17023), a Maize (Zea mays L.) Germplasm with Resistance to Charcoal Rot (disease mean score 2.3 on the scale of 1-9) in QPM background. Mean Tryptophan (0.90%) and Lysine (3.73%)

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The charcoal rot caused by *Macrophomina phaseolina* is the devastating component of post flowering stalk rot (PFSR) complex which may cause 25 to 32 % yield loss in maize. With availability of different types of corns *viz.*, normal, quality protein maize (QPM), sweet corn, popcorn and babycorn, the availability of resistance sources for major diseases in their genetic background is always preferable. Therefore, the study was carried out to identify stable sources of charcoal rot resistance by multi-environments screening of 137 inbred lines (135 test entries and two checks) including QPM, popcorn and normal under artificially created epiphytotics at hot-spot locations. The resistance sources were identified in normal and QPM genetic backgrounds, however the almost all popcorn lines were sowing high susceptibility for the target disease on 1-9 rating (Sekhar & Kumar 2012: Anonymous 2016; Kumar *et al*., 2016). The two inbred lines, one each in normal (DML339), and QPM (DQL1019), developed at ICAR-IIMR, from selfing of desirable plants in NZBOPH (12) & HQPM-1, respectively followed with ear to row selection continuously for six generations were identified resistant to charcoal rot disease (Table 1). They were registered as unique germplasm for charcoal rot resistance in normal and QPM genetic backgrounds.

Morpho-agronomic and associated characteristics: DML 339 (IC no. 0612721/ INGR17022) is a late maturing charcoal resistant line in normal maize

Table 1. Stable sources of resistance against charcoal rot disease in maize

	Inbred name	Rabi 2012-13 (Hyderabad)-E1	Kharif 2013 (Delhi)-E2	Kharif 2013 (Hyderabad)-E3	Kharif 2015 (Delhi)-E4	Mean	Remarks	DTA	DTS	ASI
	DML 339	2.8	2.4	2.1	2.7	2.5	R	58.5	60.5	2.0
	DQL 1019	2.3	2.2	2.5	2.7	2.4	R	61.5	64.5	3.0
	DQL 1005	2.5	2.0	3.0	3.2	2.7	R	59.0	61.0	2.0
	DML33	2.3	2.8	3.0	5.4	3.4	MR	59	61.5	2.5
	DML 289	3.0	2.3	2.8	4.7	3.2	MR	53.5	57.5	4.0
	DML50	5.0	2.4	4.8	3.7	4.0	MR	51.0	55.5	4.0
	DQL 1022	4.3	1.8	4.5	3.9	3.6	MR	63.0	65.5	2.5
	DML315	4.1	3.1	4.0	2.8	3.5	MR	55.5	59.0	3.5
	CM117-4(RC)	3.1	2.7	3.0	2.4	2.8	R	57.5	60.5	3.0
	WOSC (SC)	7.0	7.0	7.6	7.2	7.2	S	57.0	60.5	3.5
		1.2	1.3	1.3	1.8	1.4	-	2.3	3.0	1.3
LSD (*P*=0.01)										

E*= Environments, Days to anthesis (DTA), Days to silking (DTS), ASI: Anthesis silking Interval, C = Check; Rating scale: 1.0-3.0 resistance (R); 3.1-5.0 moderately resistance (MR), 5.1-7.0 moderately susceptible (MS) and ≥7.1 susceptible (S).

genetic background on scale of 1-9 (Table 1), with low Anthesis Silking Interval (ASI) i.e. 2.0 days, medium ear placement, semi erect leaves, dense spikelets of tassel, yellow and semi-dent kernels. This line has good productivity and hence can be a better female parent in hybrids breeding programme.

DQL 1019 is a late maturing quality protein maize (QPM) showing resistance to charcoal rot disease on scale of 1-9. This line has low Anthesis Silking Interval (ASI) i.e. 3.0 days, medium ear placement, drooping leaf attitude of blade, yellow kernels, and medium cob length. This line has good productivity and hence can be a better female parent in hybrids breeding programme.

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24. IML-PFSR-R3 (IC0593934; INGR17024), a Maize (*Zea mays* L.) Germplasm Resistant to Multiple Disease Post Flowering Stalk Rots, Turcicum Leaf Blight and Maydis Leaf Blight. Stiff and Stay Green Character of Stalk. High Oil Content > 5.0 (5.53%).

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IML–PFSR-R3: Pedigree - (JCY3-7-1-2- 3b-1-1-2-3-1-1-1-1) is inbred line, source of resistance to Post flowering stalk rots of maize caused by *Macrophomina phaseolina* and *Fusarium moniliforme* (Rakshit *et.al* 2011). The primary source of this material is JCY series (pools) from PAU Ludhiana. From this material this inbred line was developed at IIMR New Delhi for desirable character following pedigree breeding methodology. The source material (pools) was received from PAU Ludhiana under collaborative programme. The development of inbred line and multi location evaluation of the genotype was done in hot spot locations at Hyderabad, Udaipur, Delhi and Ludhiana against PFSR from 2010 to 2013 under artificial epiphytotic condition. Maintenance and multiplication of inbred line was done at Indian Institute of maize research New Delhi and winter nursery Hyderabad.

Morpho-agronomic characteristics: Plant exhibited stay-green character, stiff, strong stem, purple brace root, with light purple silk, sparse spikelets, normal silk, Plant length – long, ear conico cylindrical; Grains are semi dent, yellow round in shape with desirable plant type and good agronomic traits like; optimum ear placement, stiff stalk, good pollen shed. The inbred was consistently resistant against PFSR. The disease reaction recorded from 1.0 to 4.2 on 1-9 rating scale (1 is highly resistant, 9 is highly susceptible) across the location during 4 years of testing. Other additional characteristic feature that the high oil content i.e. 5.59 % (dry weight basis).

Stem is green, stiff robust. Resistant against PFSR which is an economically important disease causes reduction of 18.7% in cob weight and 11.2%in 1000-grain weight in infected plants (Cook 1978). The losses due to this disease in India have also been calculated to range from 10 to 42% (Payak and Sharma 1978; Desai *et al.,* 1991; Harlapur *et al.,* 2002).

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25. OMU 005 (IC0503186; INGR17025), a Jute (*Corchorus olitorius* L.) Germplasm with Super Fibre Wedge Length and Diameter

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Due to limited variability of morpho-agronomic characters in jute, fibre anatomy characters are gaining importance in selection of superior genotypes, as many of these characters have high correlation with fibre yield (Satya *et al*., 2011). The jute fibre is a phloic fibre being arranged in a triangular wedge like shape in the bark which grows longitudinally in the stem. As fibre yield cannot be directly evaluated from a plant that is allowed to form seed, the fibre wedge related characters are highly important for non-destructive sampling and selection of superior genotypes with high fibre yield. However, there is little information on variability of fibre wedge characters in jute and no genetic source is identified for high fibre wedge length and diameter in tossa jute.

We have identified a tossa jute mutant genotype OMU 005 (National ID IC0503186, Registration No. INGR17025) superior for two characters, fibre wedge length (1.25 mm) and fibre wedge diameter at base of the wedge (0.61 mm). The genotype consistently outperformed popular cultivar JRO 524 for these two characters during 2012-2015 (Table 1).

Morpho-agronomic characteristics: Morpho-agronomic characterization revealed that the mutant (OMU 005) has plant height: 3.09 m; basal diameter: 1.0 cm; dry fiber percentage: 9.5% and harvest index: 24.7. Plant bears numerous green cylindrical dehiscence capsules having 3 locules filled with small chocolate brown colored seeds with 1.93 gm per 1000 seed weight. Seed was found to be non-dormant with high germination rate (98%).

Table 1. Mean performance of OMU 005 and JRO 524 for fibre yield related anatomical traits over four years (2012-2015)

Anatomical traits	OMU 005	JRO 524
Bark cross-section length (mm)	1.55	1.07
Fibre wedge length (mm)	1.25	0.89
Fibre wedge diameter at base (mm)	0.61	0.37
Fibre bundles / wedge at wedge base	9.59	7.01
Number of fibre cells / bundle	25.10	17.75

Source: ICAR-CRIJAF Annual report 2014-15 (Satpathy *et al.,* 2015)

Associated characters and cultivation practices: The genotype OMU 005 also has high bark cross section length and number of cells/fibre bundle. It also bears high number of fibre bundles per fibre wedge. Cultivation practice during experimentation followed standard crop management practices of cultivated jute species. At molecular level, OMU 005 could be differentiated from other mutant genotypes with closest relative being OMU 009 (Satya *et al*., 2014).

It responses well to standard cultivation practice of tossa jute with seed rate of 300-400 g/ha, row spacing of 30 cm and fertilizer requirement of N:P:K (60:40:40), and harvesting at 120 days after sowing. Flowering is asynchronous, initiates under short day (from September onwards) and seeds are harvested during November-December.

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26. OMU 007 (IC0503703; INGR17026), a Jute (*Corchorus olitorius* L.) Germplasm with Superior Bark Cross Section Length

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As jute fibre is formed in the secondary phloem (bast), bark cross section length (bark diameter) length is an important indicator of fibre yield in jute, which also shows high correlation with basal diameter, another widely used selection criterion for jute genetic improvement (Satya *et al*., 2011). However, basal diameter includes both bast and core diameter, while fibre is only formed in the bast region. Thus bark diameter (representing only the bast region) seems to be a better criteria for selection while targeting fibre yield improvement. Importance of using such anatomical attributes in *tossa* jute (*C. olitorius* L.) fibre improvement had been realized long back, but such traits could not be targeted due to unavailability of suitable genetic source.

OMU 007 is a mutant line originating from JRO 632 that shows considerable higher bark diameter (1.65 mm) than other mutant genotypes and the commercial popular variety JRO 524 (1.07 mm).

Morpho-agronomic characteristics: Morpho-agronomic characterization revealed that the mutant OMU 007 is a dwarf one with plant height: 2.10 m; basal diameter: 1.4 cm; dry fiber percentage: 5.8% and harvest index: 30.3. The mutant bears numerous large and leathery tobacco shaped leaves and green cylindrical non-dehiscence capsules having small chocolate brown colored seeds with 1.41 gm per 1000 seed weight. Seed found non-dormant and can immediately germinate after sowing without any treatment.

Associated characters and cultivation practices: OMU 007 also shows superiority for other fibre anatomy traits such as fibre-wedge length, fibre wedge diameter, number of fibre bundles/fibre wedge and number of fibre cells per fibre bundle over popular variety JRO 524 (Table 1). Molecular genetic studies established this genotype to be distinct from JRO 524 and also form other mutant genotypes available at ICAR-CRIJAF germplasm repository (Satya *et al*., 2014). Using 23 SSR markers, OMU 007could be differentiated from other mutant genotypes at a distance of D = 0.88 in a dendrogram, with closest relative being OMU 001 and OMU 004.

Table 1. Mean performance of OMU 007 and JRO 524 for fibre yield related anatomical attributes during 2012-2015

Anatomical traits	OMU 007	JRO 524
Bark cross-section length (mm)	1.65	1.07
Fibre wedge length (mm)	1.16	0.89
Fibre wedge diameter at base (mm)	0.55	0.37
Fibre bundles / wedge at wedge base	8.69	7.01
Number of fibre cells / bundle	26.49	17.75

Source: ICAR-CRIJAF Annual report 2014-15 (Satpathy *et al*., 2015)

It responses well to standard cultivation practice of tossa jute with seed rate of 300-400 g/ha, row spacing of 30 cm and fertilizer requirement of N:P:K (60:40:40), and harvesting at 120 days after sowing. Flowering is asynchronous, initiates under short day (from September onwards) and seeds are harvested during November-December.

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27. OMU 018 (IC0503297; INGR17027), a Jute (*Corchorus olitorius* L.) Germplasm with High Number of Fiber Cells/Fibre Bundle

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Jute fibre is formed as bundle of fibre cells in the secondary phloem of the bark. each bundle comprises of numerous cells and these bundles are connected to each other providing the meshy structure of jute fibre. Thus number of fibre cells per fibre bundle is an important criterion influencing fibre yield of jute (Satya *et al*., 2011). More number of fibres in a bundle increases fibre bundle strength, a desirable character for spinning and developing geotextiles. Importance Despite being an important character, breeding for higher number of fibre cells per fibre bundle could not be targeted due to non-availability of suitable source genotype.

While screening tossa jute mutants for anatomical traits correlated with fibre yield for a period of four years, OMU 018 a mutant genotype was identified that shows considerable higher number of fibre cells/fibre bundle (28.39) compared to other genotypes and including popular variety JRO 524 (17.75).

Morpho-agronomic characteristics: Morpho-agronomic characterization revealed that the mutant (OMU 018) is a tall plant with height: 3.02 m; basal diameter: 1.2 cm; dry fiber percentage: 6.8% and harvest index: 27.8. The mutant bears numerous red colored cylindrical non-dehiscence capsules having small deep brown colored seeds with 1.96 gm per 1000 seed weight. Seed found non-dormant and can immediately germinate after sowing without any treatment.

Associated characters and cultivation practices: The genotype also shows superiority for other fibre anatomy traits such as bark-cross section length, fibre-wedge length, fibre wedge diameter, and number of fibre bundles/fibre wedge (Table 1). Molecular genetic studies established this genotype to be distinct from JRO 524 and also form other mutant genotypes available at ICAR-CRIJAF germplasm repository (Satya et al., 2014). It responses well to standard cultivation practice of tossa jute with seed rate of 300-400 g/ha, row spacing of 30 cm and fertilizer requirement of N:P:K (60:40:40), and harvesting at 120 days after sowing. Flowering is asynchronous, initiates under short day (from September onwards) and seeds are harvested during November-December.

Table 1. Mean performance of OMU 018 and JRO 524 for fibre yield related anatomical attributes from 2012-2015

Anatomical traits	OMU 018	JRO 524	SD
Number of fibre cells / bundle	28.39	17.75	4.25
Bark cross-section length (mm)	1.39	1.07	0.28
Fibre wedge length (mm)	1.15	0.89	0.24
Fibre wedge diameter at base (mm)	0.51	0.37	0.09
Fibre bundles / wedge at wedge base	8.35	7.01	1.12

Source: ICAR-CRIJAF Annual report 2014-15 (Satpathy *et al*., 2015)

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