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Abiotic Stresses Tolerance and Nutrients Contents in Groundnut, Pearl Millet and Sorghum Mini Core Germplasm for Food and Nutrition Security

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Groundnut, pearl millet and sorghum germplasm were investigated to identify new sources of tolerance to low phosphorus (LP) and/or drought stress (WS) and to assess genotypic variation for iron (Fe) and zinc (Zn) contents. Experiments were conducted in lysimetre conditions in a randomized completely block design with 5 replications, 2 water and 2 phosphorus treatments. Genotypic variability was observed for morphoagronomic and nutrients traits investigated. Groundnut accessions ICG 3312, ICG 11855, ICG 10053, ICG 15232 and ICG 11088 revealed drought tolerant. The combined WS-LP effect (73%) was higher than individual WS (68%) and LP (49%) effects. LP significantly delayed heading and flowering dates, accessions IP1060, IP11405 and IP9000 (millet), and ISS1412, ISS2167, ISS376 and ISS738 (sorghum) showed less delay and LP tolerance. Fe and Zn contents under LP showed that accessions IP17775, IP5581, IP5153, IP1060, IP6517 and IP5438 of millet; ISS376, ISS1412, ISS2167, ISS242, ISS311 and ISS2151 of sorghum revealed high Fe and/or Zn concentrations.

Key Words: Crop improvement, Drought tolerance, Low phosphorus, Mini core collections, Nutrients contents

Introduction

Sustainable agriculture and malnutrition challenges require the use of genotypes tolerant to abiotic stress and highly rich in micronutrients. Detailed evaluation and characterization of materials conserved in genebanks could lead to select these genotypes. Mini core collections of groundnut, pearl millet and sorghum (Upadhyaya *et al*., 2002; Upadhyaya *et al*., 2009; Upadhyaya *et al*., 2011), representing diversity of global collections, are ideal material for extensive evaluation to select new crop varieties adapted to future conditions, improve productivity and reduce malnutrition. Several studies in India on pearl millet, sorghum and groundnut mini core collections under drought, soil nutrients deficiency, heat, salinity and/or photoperiod conditions contributed to select diverse sources of several traits used in breeding new and performant varieties (Upadhyaya *et al*., 2001; Upadhyaya *et al*., 2003; Upadhyaya *et al*., 2008, Upadhyaya *et al*., 2012; Yadav *et al*., 2017; Kumar *et al*., 2012). Accessions ICG 12625, ICG 442, ICG 2381, ICG 14710 and ICG 7963 of groundnut (Upadhyaya *et al*., 2014), IP 4066, IP 9496 and IP 9426 of pearl millet (Yadav *et al*., 2003), and IS 2205, IS 3946, IS 5094 and IS 12883 of sorghum (Upadhyaya *et al*., 2009; Mohankumar *et al*., 2013) were some of selected germplasm for release and/or used in breeding programs to improve productivity and nutrition quality in India. Climate shocks will likely have an overall negative effects on agricultural production in many African countries and regions, and this could lead to food insecurity and malnutrition exacerbation (IPCC, 2014). West Africa is known to be particularly vulnerable to climate change due to high climate variability, high reliance on rain-fed agriculture, and limited economic and institutional capacity to respond to climate variability and change (Nelson *et al*., 2009; Sultan and Gaetani, 2016). Pearl millet, sorghum and groundnut are most important crops for food security and nutrition in West African countries (Hausmann *et al*. 2011, Vigouroux *et al*., 2011, Hamidou *et al*., 2012, Hamidou *et al*., 2013, Hamidou *et al*., 2014.) In Sahelian areas of West African regions groundnut, pearl millet and sorghum cultivation play key roles in food and nutrition but abiotic stresses particularly drought, heat, low soil fertility and malnutrition are yield and nutritive quality limiting constraints. These stresses will become more severe in near future and require identification of genotypes tolerant to abiotic stresses and relevant traits useful in breeding programs for improving adaptation, production and/or nutrition

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quality of major crops cultivated in the Sahel (Sultan and Gaetani, 2016). Like in India, exploiting/investigating the mini core collections of groundnut, sorghum and pearl millet in Sahelian countries could lead to select genotypes tolerant to major abiotic stresses and highly rich in micronutrients for developing crop improvement strategies that favor adaptation to climate variability. This work aims to investigate response of groundnut, pearl millet and sorghum germplasm mini core collections (Upadhyaya *et al.,* 2002, 2009, 2011) to drought and low soil phosphorus stresses. The objectives were to (i) characterize these germplasms under drought and/or low phosphorus conditions for selecting high yielding genotypes, (ii) assess individual and combined effects of drought and low phosphorus stress on agronomic performance and nutrients content of the germplasm and (iii) identify tolerant genotypes and relevant traits for crop improvement.

Material and Methods

Three experiments were conducted at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Sahelian Centre (ISC) in Sadoré (45 km south of Niamey, Niger, 13^{o}N, 2^{o}E) during 2015 and 2016. Climatic data (temperature, humidity and ET through class A pan evaporation) were recorded daily from a meteorological station located close to the experiments. In addition, the temperature and relative humidity of the air were collected from a temperature and relative humidity recorder (Gemini Tinytag Ultra 2 TGU-4500 Data logger Ltd, Chichester, UK) located in the crop canopy.

One hundred and six (106) germplasm including 60 accessions of groundnut mini core collection (Upadhyaya *et al*., 2002), 25 accessions of pearl millet mini core collection (Upadhyaya *et al*., 2011) and 21 landraces of sorghum (Table 1) were assessed during rainy and off-seasons in lysimetre tubes (25 cm diameter, 130cm height). The lysimetric system was well described in our previous works (Beggi *et al*., 2014; Halilou *et al*., 2015). The soil used to fill the lysimetre tubes was collected from the farm in Sadoré station. The characteristics of the soil are: pH-H_2O (5.28), capacity of cation exchange (1.91 cmol+ kg^{-1}), organic matter (0.22%), total nitrogen (204.3 mg-N kg^{-1}), total phosphorus (26.25 mg-P kg^{-1}), phosphorus bray1 (1,83 mg-P kg^{-1}). Top soil (0-20cm) and deep soil (20-100cm) were collected separately from the farm. To mimic the field conditions, the lysimetre tubes were filled with deep soil (100 cm height) followed by top soil (20 cm height). The upper 10 cm of the tubes was left empty to allow the application of a layer of anti-evaporation beads and for watering. Three seeds were sown by hand and seedlings were thinned to one plant per tube at 14 days after sowing (DAS). The experimental design was a randomized complete block design with 5 replications, 2 water regimes and 2 phosphorus treatments. The water regimes were a full irrigation (WW) until harvest and a drought stress (WS) imposed from flowering (50%) to maturity times. WS was an intermittent drought consisting of cycles of drying (irrigation interruption) and re-watering (1000mL of water per tube) when the majority of WS plants showed clear wilting symptoms (Hamidou *et al*., 2012). Prior to imposing WS, the lysimetre tubes were water saturated, drained during 2 days to reach field capacity and the soil surface was covered with a 2cm thick layer of polyethylene beads to minimize soil evaporation (Ratnakumar *et al*., 2009). The two phosphorus treatments were HP treatment consisting of applying 7.5 g DAP $tube^{-1}$ in a circle 2–3 cm around the seedling area after emergence and the LP treatment consisted of no application of phosphorus in the tubes but supply urea (3.45 g applied in two doses) to compensate nitrogen input from DAP applied in HP tubes. DAP and urea were used in these experiments because they are the common fertilizers used by Sahelian farmers. The phenology (Heading, flowering and maturity times), growth (height), total transpiration (TTW) and transpiration efficiency (TE), micronutrients content (Fe, Zn), yield and its components were some of parameters measured. For TE measurement, two plants were left per tube after thinning. The day before water stress imposition, one of 2 plants of each tube was harvested, dried at 70^{o}C for 2 days and the initial biomass (IDM) was determined. During water stress period, transpiration was measured via a gravimetric procedure. Thus, lysimetre tubes were weighed regularly (twice per week). As there was no evaporation nor draining, the difference of consecutive lysimetre weights, plus water added after the previous weighing, was equivalent to the transpiration (Vadez *et al*., 2011a). The total transpired water (TTW) of WW and WS plants was determined for each individual plant. At maturity, plant of each cylinder was harvested, dried at 70^{o}C for 2 days for determining the final dry matter (FDM). So, TE was calculated as: TE = (FDM - mean IDM) /TTW. For the micronutrients (Fe, Zn) content

Table 1. Names and origins of groundnut, pearl millet and sorghum germplasm. ISS N^{o} = ICRISAT Niamey genebank sorghum accession number. RCA = Republique Centre Africaine

	Groundnut germplasm				Pearl millet germplasm		Sorghum germplasm (landraces)		
No	Name	Origin	Name	origin	Name	Origin	Local Name	ISS No	Origin
1	ICG 6813	India	ICG 15233	Unknown	IP19629	Niger	Yarimawa	ISS 242	Nigeria
2	ICG 15232	Unknown	ICG 11855	Bolivia	IP5455	Niger	Acualé	ISS 309	NIGER
3	ICG 36	India	ICG 7897	Zimbabwe	IP5719	Nigeria	Zermou FDawa	ISS 311	NIGER
4	ICG 6201	Unknown	ICG 5494	Sudan	IP5964	Senegal	98-884	ISS 333	Mauritanie
5	ICG 7906	Zimbabwe	ICG 3240	Unknown	IP5869	Senegal	98-972	ISS 337	Mauritanie
6	ICG 6394	Zimbabwe	ICG 1415	Senegal	IP1060	India	Betakatchi	ISS 376	Tchad
7	ICG 5195	Kenya	ICG 7181	USA	IP6517	Mali	Méré Rafa	ISS 378	Tchad
8	ICG 10384	Argentina	ICG 9666	Mozambique	IP5581	Niger	Socomba Tiudawa	ISS 705	niger
9	ICG 11542	Korea	ICG 3775	India	IP17532	Togo	Socomba Gogueize	ISS 738	niger
10	ICG 10920	Peru	ICG 334	China	IP19415	Chad	El Baoura	ISS 784	niger
11	ICG 9842	Peru	ICG 1703	Bolivia	IP5298	Niger	Takanda Bagaroua	ISS 862	niger
12	ICG 3992	Uganda	ICG 9777	Mozambique	IP6113	Niger	Jadawa Meguizawa	ISS 885	niger
13	ICG 6407	Paraguay	ICG 9809	Tanzania	IP5957	Senegal	Gadiabablé	ISS 1148	Mali
14	ICG 1711	Unknown	ICG 6703	Senegal	IP6278	Mali	Dérebléni	ISS 1350	Mali
15	ICG 15234	Unknown	ICG 5475	Malaysia	IP9000	Niger	Gadiabakoumaba	ISS 1412	Mali
16	ICG 7969	India	ICG 405	Unknown	IP5438	Niger	Kaurari K19-1	ISS 2110	Niger
17	ICG 6022	Cuba	ICG 2773	India	IP5711	Nigeria	Kaura Mai Sauri-	ISS 2148	Niger
18	ICG 4527	Sudan	ICG 12276	India	IP11405	BFaso	Botsotsoia	ISS 2133	Niger
19	ICG 1142	Benin	ICG 3746	Brazil	IP5185	Niger	Kaurari	ISS 2137	Niger
20	ICG 163	Unknown	ICG 4746	Malawi	IP17775	Togo	Tantagaria	ISS 2151	Niger
21	ICG 10053	Nigeria	ICG 81	Unknown	IP6057	RCA	Karangui	ISS 2167	Niger
22	ICG 1399	Malawi	ICG 14475	Mexico	IP8540	India			
23	ICG 1274	Indonesia	ICG 1823	Tanzania	IP5407	Niger			
24	ICG 1487	USA	ICG 115	India	IP5153	Niger			
25	ICG 10890	Unknown	ICG 11088	Taiwan	IP5261	Niger			
26	ICG 4670	Israel	ICG 3312	Argentina					
27	ICG 6375	Unknown	ICG 118	India					
28	ICG 10554	Peru	ICG 12988	Nigeria					
29	ICG 11109	China	ICG 862	India					
30	ICG 4911	Sudan	ICG 3027	Uganda					

of pearl millet and sorghum seeds, it was determined with XRF machine (Nielson *et al*., 1988).

Results

Genotypic Variation in Response to Drought and/or Low Phosphorus Stress

Groundnut Germplasm

Flowering and maturity dates recorded on the groundnut mini core subset ranged from 22 to 31 and 85 to 97 days after sowing (DAS) respectively which indicate large genotypic variation. ICG 6703, ICG 7181, ICG 3775, ICG 6407 and ICG 10890 were the top 5 earliest maturing accessions while ICG 3027, ICG 15234, ICG 3992, ICG 9777 and ICG 9842 were late maturing materials. Significant genotype and water treatment interaction (GxTrt) was observed on the total transpired water (TTW) from flowering to maturity. TTW increased significantly under WS compared to WW for all accessions except ICG 1399, ICG 5494, ICG 1274, ICG 11542 and ICG 1142 which showed significant transpiration decrease due to WS. ICG 7181, ICG 10384, ICG 7906, ICG 163 and ICG 1711 revealed the highest TTW increase. ICG 3312 and ICG 11088 showed high TTW under both WW and WS regimes. The highest TTW under WW was observed on ICG 3312, ICG 11088, ICG 3746, ICG 862, ICG 1274, ICG 11542 and ICG 1399 while ICG 7181, ICG 36, ICG 163, ICG 10384 and ICG 6703 showed the lowest TTW. Under WS, the genotypic variation on TTW revealed that ICG 11855, ICG 115, ICG 11088, ICG 15233 and ICG 3312 had highest TTW whereas ICG 1142, ICG 5494, ICG 9777, ICG 6703 and ICG 10890 had the lowest. Transpiration efficiency (TE) investigated under WW and WS showed significant ($P<0.001$) effect of water regime and WS decreased TE up to 78%. Significant ($P<0.001$) genotypic

variation was also observed under both WW and WS treatments, and ICG 9842, ICG 7181, ICG 3027, ICG 1274, ICG 14475 and ICG 4527 revealed high TE. Under WS, accessions with highest TE were ICG 3312 (1.85 g kg^{-1}), ICG 11088 (1.61 g kg^{-1}), ICG 13395 (1.50 g kg^{-1}) and ICG 4670 (1.31 g kg^{-1}) and revealed tolerant to drought stress. Genotypic variation was also observed on the branch number per plant, the pod weight per plant (Pwpt), haulm weight per plant (Hwpt and seeds weight per plant (Swpt) which ranged respectively from 4 to 10, 3.4g to 19.4g, 25 g to 55g and 1.5g to 15g. WS imposed at flowering time decreased Hwpt (30%), Pwpt (68%) and Swpt(71%). Accessions ICG 3312, ICG 118, ICG 10053, ICG 15232 and ICG 11088 showed high pods and seeds weights under both WW and WS conditions. Linear regression performed showed high correlation (r^2 = 0.73) between TE and Hwpt whereas low correlation (r^2 = 0.26) was observed between Pwpt and TE under WS conditions (Fig. 1). Low phosphorus stress (LP) decreased the pod number per plant (8%), the Pwpt (49%) and Hwpt (31%), the decrease due to combined WS and LP stress (WS-LP) was 16, 73 and 34% respectively. Under WS-LP conditions, ICG 4670 (4g) and ICG 3312 (8g) showed lowest pods weight while ICG 3399 (11g), ICG 115 (13g) and 3746 (18g) revealed the highest Pwpt.

Pearl Millet Germplasm

Phosphorus treatment has significant effect on tillering times, tillers number per plant, heading/booting time, flowering time and plant height of pearl millet accessions. Under HP, heading and flowering times ranged respectively from 43 to 65 DAS and 48 to 74 while under LP they ranged from 53 to 71 DAS and 57 to 75 DAS. LP delayed tillering, heading and flowering times while it decreased tillers number (30%) and plant height (22%). Heading and flowering delay due to LP ranged from 3 to 16 days and genotypic variation revealed that

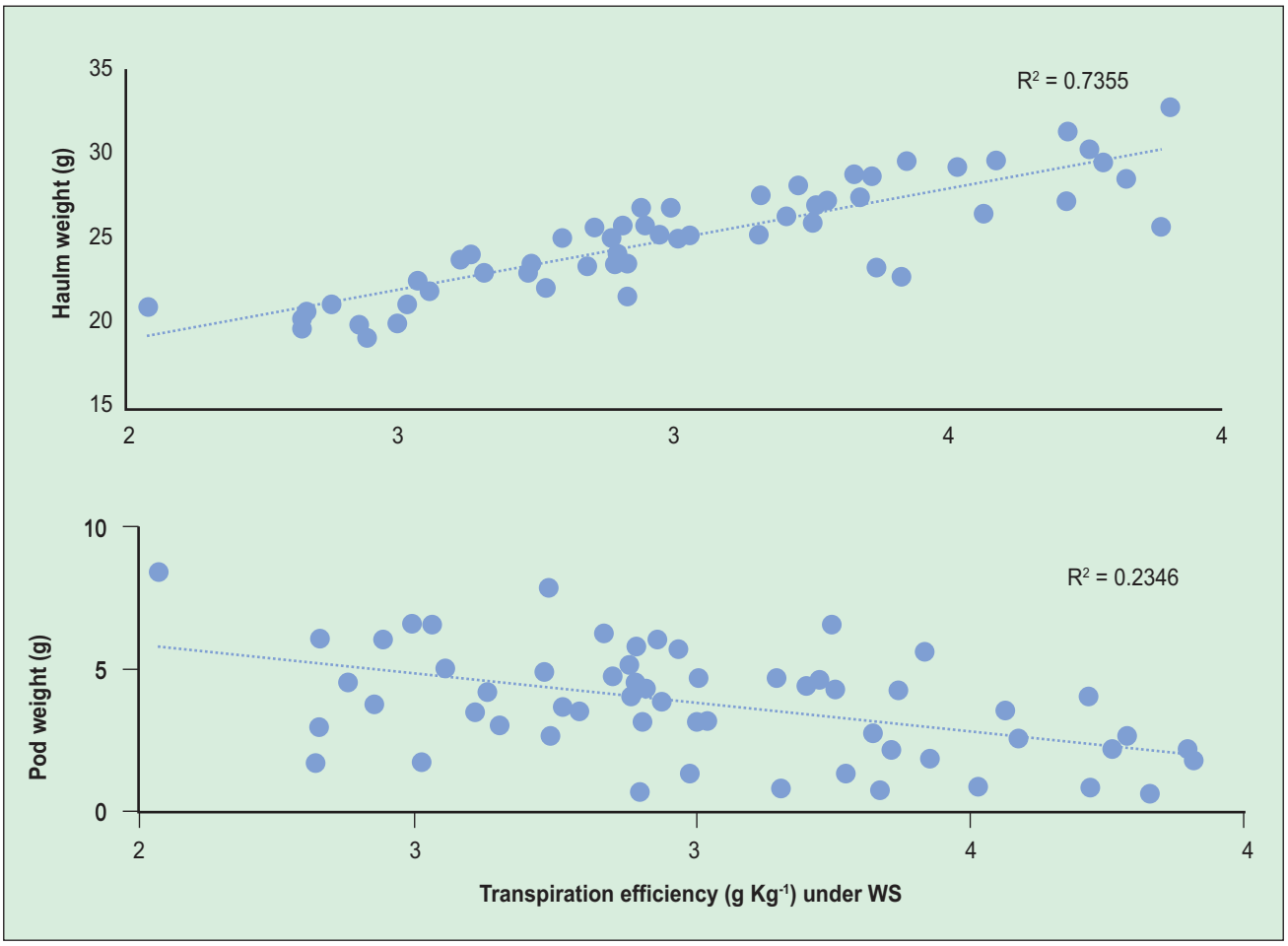

Fig. 1. Relationship between transpiration efficiency, pod and haulm weight per plant of groundnut germplasm under drought stress (WS) conditions

IP1060, IP11405, IP19629, IP5185, IP5455 and IP9000 had 4 days of delay whereas IP5298, IP5869, IP5964 and IP8540 delayed for 13 to 16 days. LP stress decreased the total biomass and panicle weight up to 37 and 56% respectively. The highest biomass (70g) was observed on IP19629, IP5298, IP5581, IP5719 and IP5407 whereas IP11405, IP17532, IP17775, IP5957 and IP6057 showed the lowest (35g). The panicle weight under LP revealed highest values (15g) for IP1060, IP11405, IP5957, IP5964 and IP9000 and lowest values (2.6g) for IP5153, IP5185, IP5438, IP5711 and IP6517.

Sorghum Germplasm

Genetic diversity was observed on the phenology of sorghum accessions. Significant genotype and phosphorus treatment interaction was observed on heading, flowering and maturity times. Under HP and LP conditions heading time ranged respectively from 39 to 80 DAS and 44 to 82 DAS. Variation on flowering time was from 41 to 81 under HP and 46 to 87 DAS under LP whereas maturity time varied under HP and LP from 61 to 93 DAS and 60 to 107 DAS, respectively. Phosphorus deficiency (LP) had no significant effect on days to flowering and maturity in accessions ISS 1148, ISS 1412, ISS 2133, ISS 2167, ISS 376, ISS 705 and ISS 738 while it delayed flowering and maturity dates in ISS 2110, ISS 2151, ISS 242, ISS 309 and ISS 337. Significant ($P<0.001$) genotypic difference was observed also on total biomass, panicle weight per plant (pawpt) and Swpt under both HP and LP treatments. Under LP, ISS 337 (80.6g), ISS 2110 (80.7g), ISS 2133 (81.6g), ISS 2151 (86.5g), ISS 1350 (94.4g) and ISS 2137 (97.8g) showed the highest biomass, the highest pawpt observed on ISS 1350 (27.5g), ISS 1412 (33.7g), ISS 2133 (32.5g), ISS 2137 (40g), ISS 2148 (27.6g) and ISS 337 (27.2g) while the highest Swpt was observed on ISS 2110 (21.7g), ISS 1350 (22g), ISS 1412 (24.5g), ISS 2133 (24.6g), ISS 2137 (30.3g) and ISS 2167 (22.4g).

Micronutrient Contents in Pearl Millet and Sorghum

Determination of Iron (Fe) and zinc (Zn) in seeds revealed significant ($P<0.001$) phosphorus treatment effect on Fe content in both pearl millet and sorghum (Fig. 2) while for Zn content the effect was significant ($P= 0.004$) only in sorghum. Fe decrease due to LP was 25 and 13% in pearl millet and sorghum, respectively, Zn decrease in sorghum was 11%. Genotypic variation in micronutrients contents showed that under HP conditions Fe content varied from 30 to 72 mg kg^{-1} in pearl millet, from 38 to 64 mg kg^{-1} in sorghum while under LP treatment Fe content ranged from 27 to 57 mg kg^{-1} in pearl millet and from 33 to 54 mg kg-1 in sorghum. IP5261 (58 mg kg^{-1}), IP5185 (63 mg kg^{-1}), IP5964 (64 mg kg^{-1}), IP17775 (66 mg kg^{-1}) and IP9000 (72 mg kg^{-1}) for pearl millet and ISS 885 (56 mg kg^{-1}), ISS 1148 (60 mg kg^{-1}), ISS 2110 (61 mg kg^{-1}), ISS 784 (61 mg kg^{-1}) and ISS 242 (64 mg kg^{-1}) for sorghum revealed the highest Fe concentrations. As for Zn content, the variation was from 38 to 96 mg kg^{-1} in millet and from 41 to 72 mg kg^{-1} in sorghum under HP while under LP it ranged from 47 to 94 mg kg^{-1} in pearl millet and from 38 to 75 mg kg^{-1} in sorghum. Accessions IP17775 (49 mg kg-1), IP17532 (51 mg kg-1), IP5581 (51 mg kg-1) and IP5153 (57 mg kg-1) for pearl millet and ISS 333 (50 mg kg-1), ISS 376 (52 mg kg-1), ISS 311 (53 mg kg-1) and ISS 1412 (54mg kg-1) for sorghum revealed the highest Fe contents under LP conditions. Highest Zn contents in pearl millet under LP were observed on IP1060 (77 mg kg-1), IP6517 (82 mg kg-1), IP5438 (84 mg kg-1) and IP17775 (94 mg kg-1) and in sorghum on ISS 2167 (54 mg kg-1), ISS 242 (54 mg kg-1), ISS 311 (54 mg kg-1) and ISS 2151 (75 mg kg-1).

Discussion

Drought and Low Phosphorus Tolerance in Groundnut

The genetic variability observed on TTW suggests different water requirement and use among the groundnut accessions. In response to drought stress, some accessions like ICG 7181, ICG 10384, ICG 7906, ICG 163 and ICG 1711 increased TTW while others like ICG 1399, ICG 5494, ICG 1274, ICG 11542 and ICG 1142 decreased TTW. This suggests different mechanism of response to drought. Indeed, TTW increase under drought stress indicates an increase of water lost while TTW decrease shows a reduction of water lost. Jayant *et al*., 2015 reported that under drought stress, prevention of water loss through aerial parts of the plant is considered as one of the most important mechanism to avoid drought. Transpiration reduction in response to drought led to dehydration avoidance due to lower stomatal conductance in order to conserve water (Mafakheri *et al.,* 2012). Accessions ICG 11855, ICG 115, ICG 11088, ICG 15233 and ICG 3312 showed the highest TTW under WS suggesting high stomatal conductance and intensive roots elongation to deeper part of the soil profile. High

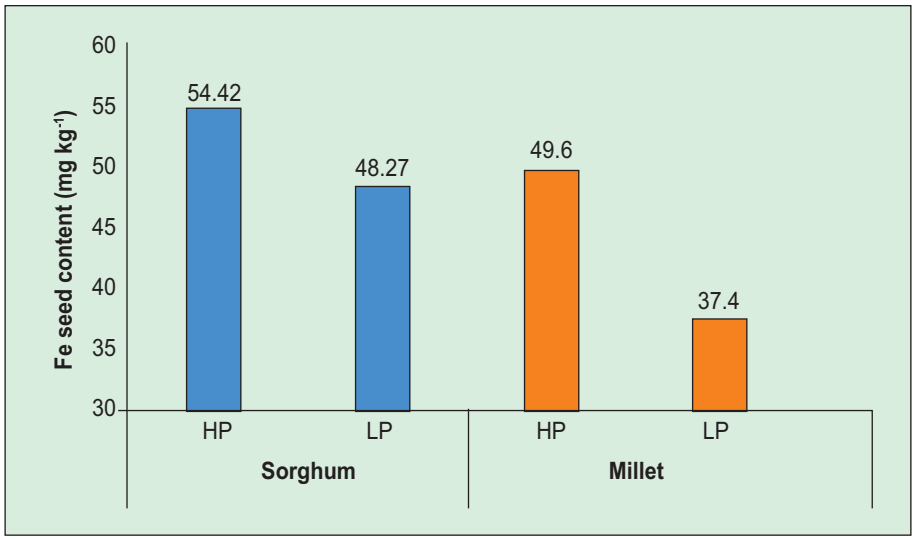

Fig. 2. Iron (Fe) content (mg kg-1) in pearl millet and sorghum seeds under high phosphorus (HP) and low Phosphorus (LP) conditions

transpiration under WS could reveal a drought tolerance mechanism by maintaining the turgor through osmotic adjustment, increase cell elasticity and decrease cell size as well as desiccation tolerance by protoplasmic resistance (Nemeskeri *et al.,* 2012; Jacqueline *et al*., 2016).

The genotypic variation observed in TE revealed the highest TE on ICG 3312 (1.85 g kg^{-1}), ICG 11088 (1.61 g kg^{-1}), ICG 13395 (1.50 g kg^{-1}) and ICG 4670 (1.31 g kg^{-1}) under WS. The TE, calculated as dry matter produced per kg of water transpired, was highly correlated (r^2 = 0.73) to haulm weight under WS suggesting that canopy development contributed to TE. This corroborates previous works which reported that high TE under drought stress could be explained by specific leaf area, high roots dry matter and/or chlorophyll content (Puangbut *et al*., 2009a, Puangbut *et al*., 2009b). Drought stress decreased Pwpt and Swpt, the genotypic variation observed revealed that accessions ICG 3312, ICG 11855, ICG 10053, ICG 15232 and ICG 11088 showed high Pwpt and Swpt under both WW and WS conditions. These accessions revealed high yielding and drought tolerant. They could be recommended for cultivation under rainfed environments and could be donors in groundnut improving programs for drought adaptation and productivity.

Low phosphorus (LP) stress had lower effect on Pwpt than WS. However, Pwpt decrease under WS-LP stress (73%) was higher than the decrease under individual WS (68%) and LP (49%) stress. ICG 3312 showed high Pwpt under WS and low Pwpt under WS-LP stress suggesting it was tolerant to drought but sensitive to combined WS-LP. These findings could indicate that phosphorus (P) is much critical in pods productivity under drought stress in groundnut. Authors reported that P nutrient improved root traits to enhance tolerance to water stress and reduce yield loss in chickpea (Jin *et al.,* 2012). ICG 115 which showed high TTW under WS conditions revealed high Pwpt under WS-LP stress suggesting a mechanism of roots elongation in deeper part of soil for exploring water and nutrients (P) for tolerance to WS-LP.

Low P Response and Nutrients Content in Pearl Millet and Sorghum

In pearl millet, LP stress induced significant delay in heading and flowering dates. However, a genetic variability was observed indicating that accessions IP1060, IP11405, IP19629, IP5185, IP5455 and IP9000 showed 4 days of delay whereas the delay was up to 16 days for IP5298, IP5869, IP5964 and IP8540. Beggi *et al.* (2014) reported a booting and flowering delay up to 2 weeks due to LP stress in pearl millet. The genetic diversity observed could be exploited in breeding for LP tolerance in pearl millet. Phenological delay has often been reported as an adaptive response of annual plants to P deficiency because it increases the duration of nutrient uptake (Nord and Lynch, 2008). However, under Sahelian conditions where mid-season and terminal drought occur frequently, a flowering delay in pearl millet would indeed increase the risk of the plant

encountering water deficient conditions. Our findings showed a biomass and panicle weight decrease due to LP stress. Accessions IP1060, IP11405 and IP9000 showed short delay and high panicle weight suggesting that no significant sensitivity of flowering time to LP stress could have benefits in grains productivity. In previous work (Beggi *et al.*, 2014) we found high correlations between flowering delay and grains yield in pearl millet and had also observed that genotypes with short flowering delay under LP stress showed less decrease of seeds size. Thus, IP1060, IP11405 and IP9000 revealed interesting materials potentially useful for low soil P conditions and in breeding program for improving tolerance to LP stress.

Low soil P affected also the micronutrients content of seed in pearl millet. Fe content deceased up to 25% while the effect was not significant for Zn content. Large genotypic variation was observed on Fe content which ranged from 27 to 57 mg kg^{-1}. Previous studies reported also a wide variability for Fe in pearl millet (Rai *et al.,* 2008; Rai *et al.,* 2012; Rai *et al.,* 2014; Elad *et al*., 2015; Yadav *et al.,* 2016; Yadav *et al.,* 2017). With high Fe concentration, IP5261 (58 mg kg^{-1}), IP5185 (63 mg kg^{-1}), IP5964 (64 mg kg^{-1}), IP17775 (66 mg kg^{-1}) and IP9000 (72 mg kg^{-1}) revealed new sources of high Fe concentration and could be useful for biofortification program in pearl millet. Fe concentration of those lines was less high than in IP 17602 (121 mg kg^{-1}), IP 17673 (109 mgkg^{-1}) and IP 9404 (108 mg kg^{-1}) reported by Yadav *et al*. (2017). This difference could be explained by the soil type and/or soil nutrient contents. Accessions IP17775 (49 mg kg^{-1}), IP17532 (51 mg kg^{-1}), IP5581 (51 mg kg^{-1}) and IP5153 (57 mg kg^{-1}) showed high Fe content under LP conditions. These accessions revealed promising materials for low soil P environments. Highest Zn contents under LP were observed on IP1060 (77 mg kg^{-1}), IP6517 (82 mg kg^{-1}), IP5438 (84 mg kg^{-1}) and IP17775 (94 mg kg^{-1}). Interestingly, IP17775 showed high Fe and Zn contents under LP stress suggesting it could contribute in food nutrition to combat Fe and Zn deficiency (Issoufou *et al*., 2013; Jakunti *et al*., 2016).

In Sorghum, large variation in phenology was observed. The flowering time varied from 41 to 81 DAS under HP and 46 to 87 DAS under LP whereas maturity time varied under HP and LP respectively from 61 to 93 DAS and 60 to 107 DAS. LP stress induced flowering and maturity delay on some accessions while it had no significant effect on others, which indicates genotypic variation in response to soil P deficiency. Delayed heading and flowering in west African sorghum panel under LP stress was reported previously (Leiser *et al*., 2014). The large delay in flowering under LP conditions indicates important growth differences which should permit a genetic study of adaptation to P-limited conditions. The absence of significant effect of LP stress on phenological traits of ISS 1148, ISS 1412, ISS 2133, ISS 2167, ISS 376, ISS 705 and ISS 738 could suggest tolerance to LP. Additional studies are required for confirmation and understanding the mechanisms underlying the non-significant effect of LP stress on the phenology plasticity of theses accessions. Under LP conditions, ISS 333 (50 mg kg^{-1}), ISS 376 (52 mg kg^{-1}), ISS 311 (53 mg kg^{-1}) and ISS 1412 (54mg kg^{-1}) showed high Fe content while high Zn concentration was observed on ISS 2167 (54 mg kg^{-1}), ISS 242 (54 mg kg^{-1}), ISS 311 (54 mg kg^{-1}) and ISS 2151 (75 mg kg^{-1}). ISS 311 with high Fe and Zn contents under LP stress could contribute in food nutrition to alleviate Fe and Zn deficiency but bioavailability of these micronutrients must be confirmed.

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Crop Interdependence, Adaptation to Climate Change and the Multilateral Systems of Access and Benefit Sharing: The Case of Nepal

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Improving farmers' access to more plant diversity is expected to be an effective strategy to respond to climate changes. Degrees of current crop interdependency were estimated based on the origin and pedigree analysis of modern varieties of rice, wheat and potato cultivated in Nepal. Geographical information system (GIS) was applied to identify germplasm from the global and national gene pools with respect to current and future climate analogue sites. In Nepal, 76% of 275 released varieties originated outside Nepal. Forty seven landraces originating in 12 countries were used to develop 20 mid- and high-hills rice cultivars and 35 landraces originating in 11 countries were used to develop 28 Tarai rice cultivars. Only exotic parents were used to develop 35 modern wheat varieties; 89 ancestors originated in 22 countries, mostly from the United States (13%), India (13%), France (12%), Argentina (6%), and Italy (6%). Only exotic parents were used to develop eight modern varieties of potato. Nepal is 95–100% dependent on foreign germplasm for varietal development. Using the Climate Analogue Tool (CAT), the analysis identified current, future, and past analogue sites within and outside Nepal, suggesting that there might be useful genetic materials that could be exchanged between such regions. To do so, Nepal has to be capable to make better use of the MLS-ITPGRFA. Right now more than 2500 genotypes of rice, wheat and potato are introduced annually for field evaluation in Nepal. This number could be increased with a fully operational MLS.

Key Words: Analogue sites, Genebank, Origin, Pedigree

Introduction

Nepal is agrobiodiversity rich country however; she depends very largely on agricultural plant genetic resources (APGRs) that originated elsewhere. Access to diverse APGRs are necessary for secure crop harvest. The strong interdependence of countries on APGRs is a key rationale for the creation of the multilateral system (MLS) of access and benefit sharing under the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). Most people are unaware of the current and future importance of interdependence and how the flow of genetic materials between countries contributes to national food security. Nepal is no exception in this regard. To effectively implement the MLS and facilitate exchange of genetic materials, it is important to generate empirical evidence of the extent to which Nepal is dependent on foreign-sourced APGRs for its agricultural research and development (including breeding) and, ultimately, food and nutrition security.

Over 4.6 million crop accessions are available in the public domain, and the CGIAR centres alone preserve more than 700 thousand accessions of crops and forage species collected from over 100 countries (Halewood *et al.,* 2013). These materials have been distributed mainly (more than 90%) through public research organizations, universities, regional organizations, germplasm networks, and genebanks; about 80% of distributed material goes to developing countries and countries with economies in transition (SGRP, 2011).

Climate change is one of the most pressing challenges facing the world; it has already had a profound impact on APGRs and the livelihoods of people, mainly smallholders living in marginal environments (FAO, 2011). Climate change may render locally available APGRs inadequate, thus underscoring the importance of access to other APGR sources (Esquinas-Alcazar, 2005; FAO, 2011; Fujisaka *et al.,* 2011). Novel strategies to conserve and use APGRs are likely required to strengthen farmers' capacities to adapt to climate change. The MLS of ITPGRFA could be of great help in putting these strategies into practice.

The use of "climate analogues" is one such strategy. Climate Analogue Tool (CAT) is an open-access

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tool developed by the program on Climate Change, Agriculture and Food Security in conjunction with the International Center for Tropical Agriculture and the Walker Institute (Ramírez-Villegas *et al.,* 2011). The tool can be used to identify future climate conditions at a particular location, sites that currently resemble these conditions, and locations that have or will have similar climate conditions. Based on careful analyses using the tool and data from actual conditions in farmers' fields, scientists can formulate possible intervention strategies, including identification of appropriate APGRs or development of new varieties for specific locations of interest (Vernooy *et al.,* 2015). Yield gap between research station and farmers' field is large and one of the reasons may be due to difference in climate (Joshi *et al.,* 2008a) between research station and farmers' field as shown in following equation.

Yield gap (Research station – Farmer's field) = Climatic differences (Research station – Farmer's field)

One of the notions of recommending variety based on analog site is to improve adaptation through reducing the climatic differences between research station and farmers' field. CAT helps to identify germplasm based on the analog sites for better adaptation and increasing options to diverse genotypes. The high degree of intra and inter-specific crop diversity may result in reducing the agricultural problems mainly related to abiotic and biotic stresses. In this paper, country dependency on foreign APGRs was reported based on the pedigree analysis and origin of modern varieties and ancestors. Analog sites and climate smart rice germplasm were identified from global and national gene pools using CAT.

Materials and Methods

Three crops (rice, wheat and potato) were chosen for detail study on germplasm flows into and out of the country. Information was gathered related to the reliance of APGR users in the country on APGR originating in other countries. Interviews and focus group discussions were organized. Crop dependency was estimated based on the origin of released varieties and area coverage, and origin of ancestors through pedigree analysis. Complete pedigree tree of each modern variety as in Fig. 1, was generated for knowing the origin of ancestors (Joshi, 2005; Joshi *et al.,* 2006). The numbers of native and

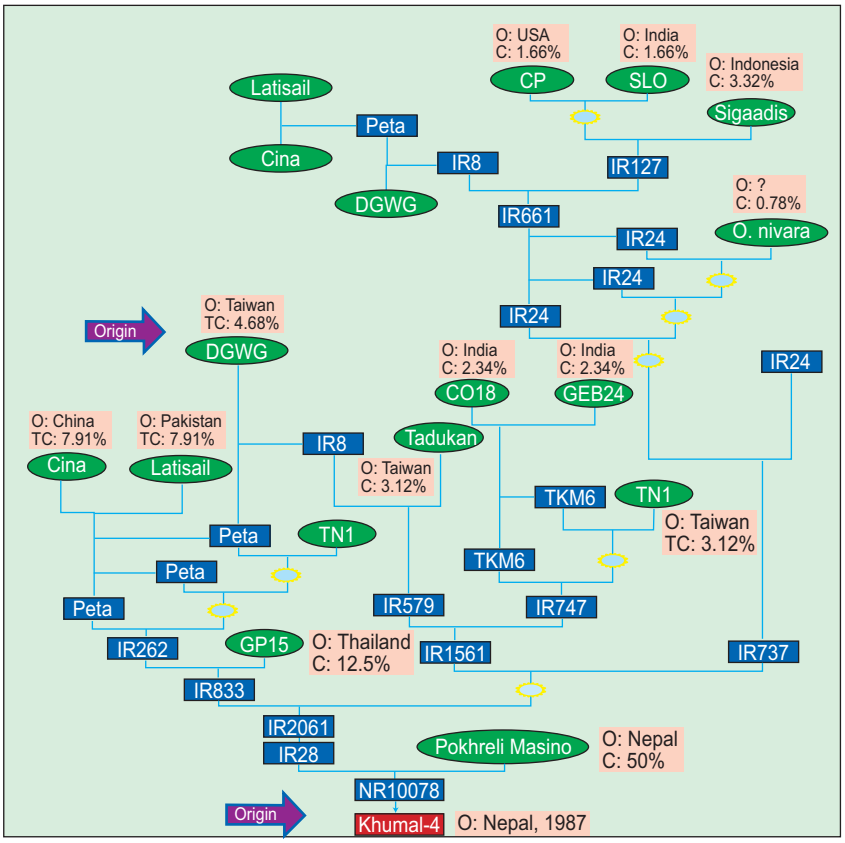

Fig. 1. Pedigree analysis and origin of ancestors of Khumal-4 rice variety for analyzing the interdependency (O, origin, TC, total contribution)

exotic parents used in developing a variety were counted as far back as possible. Detail pedigree analysis is described by Joshi *et al.* (2016).

For adaptation to climate change, we selected two districts for reference sites, representing mid hill (Begnas village of Kaski district) and Tarai (Kachorwa village of Bara district) region. The sites are rich in agrobiodiversity at species, variety and gene levels (Rana *et al.,* 2000), several of which are rare and localized or on verge of extinction (Chaudhary *et al.,* 2004; Joshi *et al.,* 2005). Rice was chosen for this study, as it is the most important staple crop grown by a large number of farmers in large area in Nepal.

We used online software called Climate Analogue Tool (CAT) to assess the current and future climates and identify analogues sites with the help of the reference sites and DivaGIS software to refine the maps produced using CAT (Joshi *et al.,* 2008b). CAT can identify two or more similar locations, and a location whose current situation resembles the past or future situation of another location. The matching sites were grouped into four categories based on likelihood or probability of matching: with 0.75-1 probability (highly matching sites), 0.5-0.75 probability (moderately matching sites), 0.25-0.5 (less likely matching sites), and 0-0.25 (no to very less likely matching sites). Based on the analog sites, rice accessions were identified from global and national gene pools for current and future use in these sites. Chaudhary *et al.* (2016) has described in detail the method of using CAT for identifying analog sites.

Results and Discussion

Germplasm flow

The import and export of germplasm in Nepal has not been systematized, and rules and regulations are not strictly followed. Import and export of germplasm is currently under the authority of the Seed Quality Control Centre and the National Plant Quarantine Office. However, a considerable amount of germplasm enters and leaves the country "informally." A large number of improved materials are being transferred from CGIAR centres' breeding programs to Nepal. Breeding programs and seed suppliers are independently collecting APGRs in Nepal as well from CGIAR centres and from India and China. Major crops introduced regularly from outside the country are rice, wheat, maize, potatoes, lentils, some vegetables, and some forage crops. Rice, wheat, and some vegetables are regularly sent to other countries, mainly Bhutan, India, and Kenya, for research and production. Some vegetables are regularly exchanged within countries of the South Asian Association for Regional Cooperation.

The National Rice Research Program (NRRP) has been acquiring International Network for Genetic Evaluation of Rice (INGER) nurseries of rice from IRRI for testing under different agro-ecological conditions; 14 nurseries are received annually, each consisting of 30–100 genotypes. In 2010, NRRP sent three rice varieties for INGER evaluation through IRRI. National Wheat Research Program receives more than 1000 genotypes annually. New trials and nurseries are added to address various biotic and abiotic stresses and find high-yielding lines. Similarly, Nepal receives more than 50 potato genotypes yearly from the CIP. In the 1990s, Nepal shared wheat genetic materials, particularly Helminthosporium Leaf Blight resistant germplasm, regionally and globally through CIMMYT. Indian wheat breeders, especially those in the eastern plains have used Nepalese lines extensively in their breeding programs, and Bhutan has used Nepalese cold-tolerant rice and wheat varieties.

Crop interdependency

Of the 254 crop varieties released for general cultivation, 185 (73%) originated outside the country and 52 were from CGIAR centres (Figure 2). Although Nepal is rich in rice diversity, 68% of rice varieties originated outside the country; for wheat and potato varieties that number increases to 80%.

Twenty improved rice varieties adapted to the mid- and high hills of Nepal and released between 1967 and 2002 were analyzed regarding their ancestors and origin. A total of 47 ancestors (landraces) originating in 12 countries, mainly in Asia, were used to develop these rice cultivars. Most ancestors were from India and Taiwan followed by China and Nepal. Only four landraces, Jarneli, Jumli Marshi, Pokhreli Masino, and Ghankdruk Local from Nepal were used in developing these cultivars, although 2000 landraces have been reported to exist (Mallick, 1981). Among the ancestors, 48.94% were of the long-grained indica ecotype and 23.40% short-grained japonica; 97.87% were *Oryza sativa* species and 2.13% were *O. nivara*.

A complete pedigree tree for one common rice variety, Khumal-4, is depicted in Fig. 1. A total of 13 landraces originating in eight countries were used to develop Khumal-4, indicating the dependency on

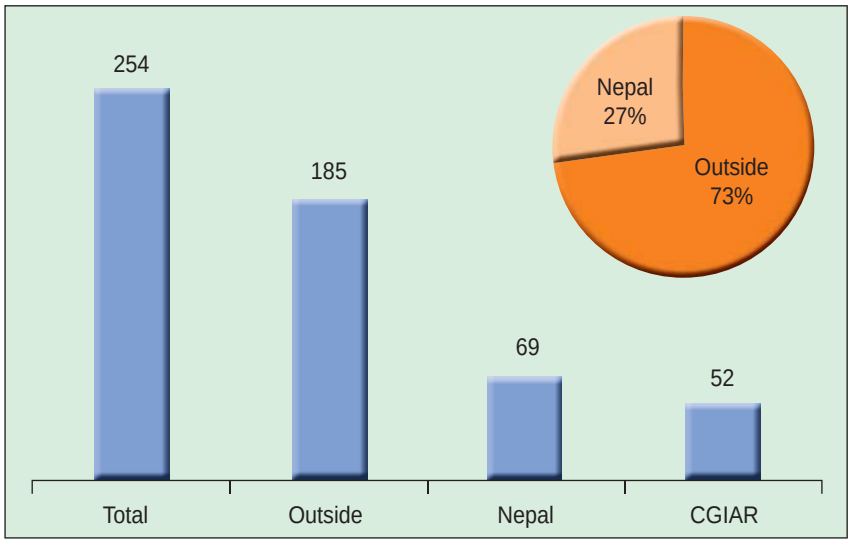

Fig. 2. Origin of crop varieties released in Nepal

foreign genetic materials. The highest proportion of genetic material comes from a landrace originating in Nepal. Two landraces originating in Malaysia and Taiwan were used in breeding Masuli, which has been the most popular variety in the last few years.

The 35 varieties of wheat released in Nepal originated in India (16 varieties), Mexico (14), Nepal, and Kenya. In Nepal four cultivars were bred and developed using foreign landraces. The genetic contribution of landraces from these countries was 25%, 25%, and 50%, respectively (Figure 3). A total of 89 ancestors from 22 countries were used to develop these cultivars (Figure 4). Most were from India followed by the United States and Kenya. Pedigree analysis of modern wheat varieties in Nepal shows that all ancestors and landraces were from other countries and international organizations, empirical evidence of Nepal's dependency on foreign PGRs for wheat research and development. Only exotic ancestors were used for developing 35 modern wheat varieties.

Only exotic parents have been used to develop the eight modern varieties of potato in Nepal. One variety was developed in Nepal using foreign landraces, but most varieties are from CIP and India. Most of the ancestors used in developing these potato varieties were from Germany. For example, 14 landraces originating in Germany were used to produce Janak Dev.

For generations, farmers have been relying on each other to conserve, use, and improve plant genetic resources (PGRs) and associated knowledge and skills

Table 1. Frequency of native and exotic parents used in developing Nepal's major crop varieties and area coverage by improved varieties

Crop	No. varieties	Native parents	Exotic parents	Area coverage, %
Rice				92
Hill set	20	4	43	
Terai set	28	1	34	
Wheat	35	0	87	97
Potato	8	0	All	85

to ensure on-farm diversity as a strategy to secure livelihoods (FAO, 2011). In addition to the unconscious actions of farmers, scientists have been making deliberate efforts to create diversity and develop biotic and abiotic stress-resistant, high-yielding varieties by manipulating genetic materials from around the world (Zeven and De Wet, 1982; Ramirez-Villegas *et al.*, 2013). No country in the world is self-sufficient in APGRs in terms of meeting domestic needs and international market demand (IPGRI, 1996; Boring, 2000).

Interdependence on PGRs at all levels, from local to global scales, is increasing and becoming more complex as a result of globalization and easier means of transportation. For example, some south and central African countries rely on external sources for over 80% of the germplasm they use (Palacios, 1998; Ramirez-Villegas *et al.*, 2013). Similarly, forage grasses originating in sub-Saharan Africa cover about 90% of all land under forage grasses worldwide (Boonman, 1993); alfalfa (*Medicago sativa*), a forage legume species, alone covers 79 million ha of land (Putnam *et al.*, 2007).

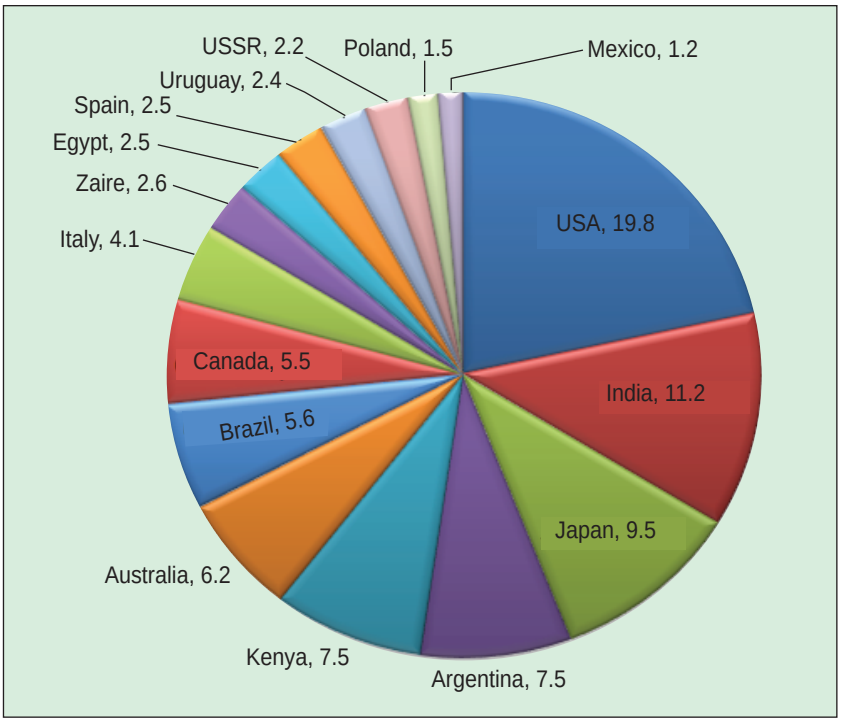

Fig. 3. Country-wise cumulative contribution of ancestors to Nepalese wheat varieties

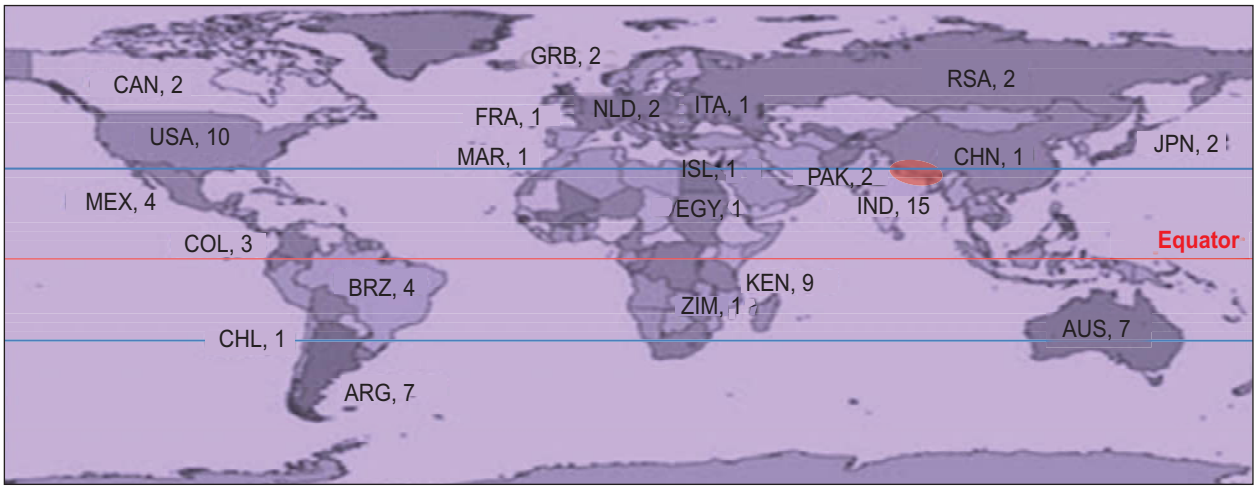

Fig. 4. Origin of ancestors of 35 modern wheat varieties released in Nepal (figure after country code is number of ancestors originated from this country and used in Nepalese wheat varieties)

Analog Sites and Climate Smart Rice Germplasm

We found a number of locations around the world that match at present, will match in future, and matched in the past the current conditions at the reference sites. Highly matching analogous sites for Begnas are found in some parts of China, and for Kachorwa such sites are found in Bihar, Jharkhand, Madhya Pradesh, and Uttar Pradesh, India. Current analogous sites for Begnas and Kachorwa in Asia are listed in Table 2. Current analogous sites for Kachorwa, Bara are depicted in Fig. 5.

The national genebank (the National Agriculture Genetic Resources Centre), an agency in the public domain, preserves more than 11 000 accessions of various crop species (1141 species of rice alone) that were once grown or are currently grown in the country. Similarly, 2839 accessions from various parts of the country have

Table 2. Locations in Asia that could be analogous to sites in Begnas and Kachorwa

Probability of matching	Analogue sites of Begnas	Analogue sites of Kachorwa
Highly likely (0.75–1)	Wucunxiang, China	Bariarpur Kuntari, Bihar, India; Tati, Jharkhand, India; Dindori, Madhya Pradesh, India and Sisotar, Uttar Pradesh, India
Moderately likely (0.5–0.75)	Gowaryo, Pakistan; Kerman and Horozygon, Iran; Hainana Sheng, China; Salavan, Laos	Shandong Sheng, China; Indus river side Pakistan; Henan Sheng, China; Vietnam; Pichaguntrahalli, Karnataka, India; Gorja, Pakistan; Kerman, Iran; Baldwyn, Saudi Arabia
Less likely (0.25–0.5)	Most of the Asian region	Most parts of Asia
Unlikely (0–0.25)	Zhejiang, China; Ayni, Tajikistan; Victoria, Sri-Lanka; Shirmine, Japan	Gifu-shi, Gifu-ken, Japan; Meghalaya Kynshi, India; Changsha Shi, Changsha Xian, China; Hasalaka Road side, Ulpathagama, Sri Lanka; Chatkal, Kyrgyzstan; and many others

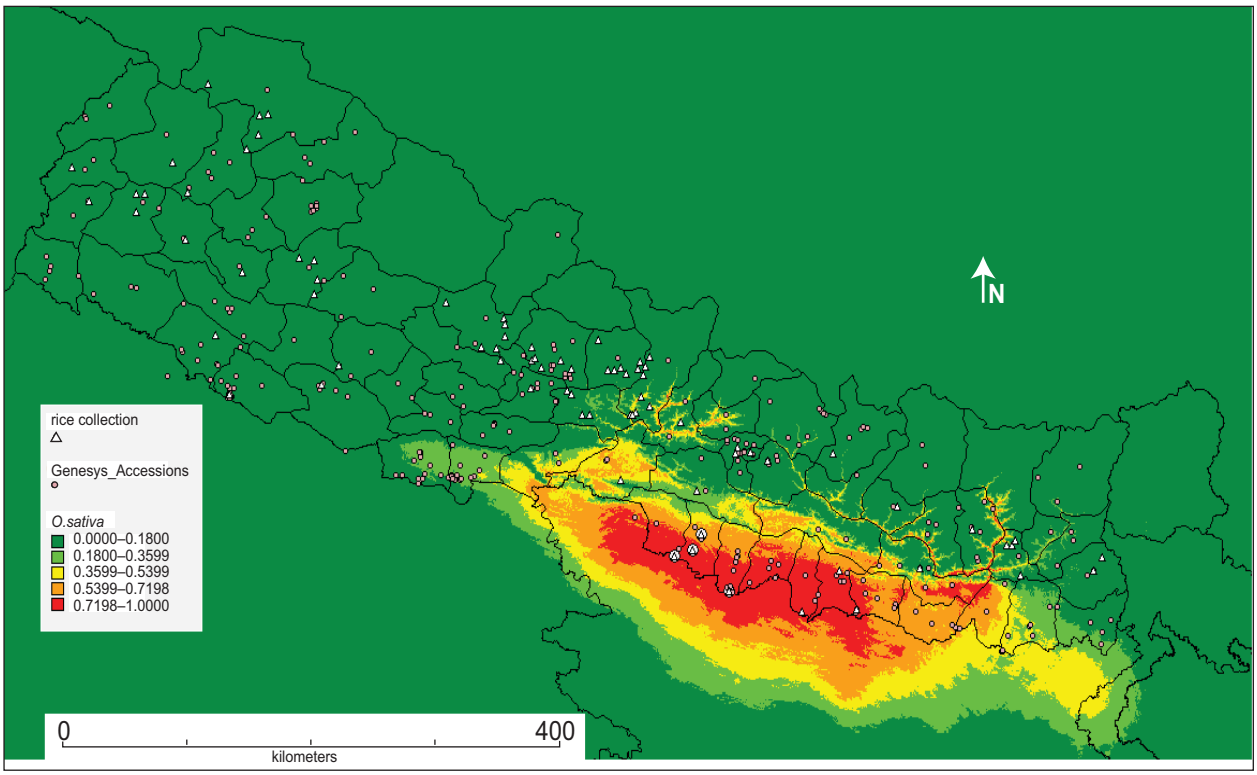

Fig. 5. Nepalese rice collection map from GeneSys database and analog sites of Kachorwa Bara, over layered for identification of rice germpalsm for analog sites

already been added to the global gene pool, mainly via the International Rice Research Institute, other CGIAR centres, and the United States Agency for International Development. A total of 100 rice accessions from the Kachorwa site can be found in Genesys and 15 in the National Agriculture Genetic Resources Centre.

Using rice as example for Begnas site, Kaski, we developed a potential flow chart to identify and/or develop climate smart rice germplasm (Fig. 6). In this process, the first step is to identify the analogue sites of Begnas using the three types of CAT relations, i.e. current-current, current-past and current-future. Potentially climate smart rice germplasm for Begnas includes germplasm found in all the analogue sites of Begnas. Potentially useful rice germplasm currently grown in analogue sites of Begnas based on the current-current analysis could be directly introduced for cultivation in Begnas. Such germplasm could also be identified in the National Genebank collection considering appropriate collection years and sites.

The concept of climate smart plant breeding is depicted in Fig. 7 based on the analog sites. With the concept of analogue sites, the breeding station and testing sites should focus on developing climate smart varieties. Based on the future climate of target site, breeder can select parental lines or landraces from analogue sites.

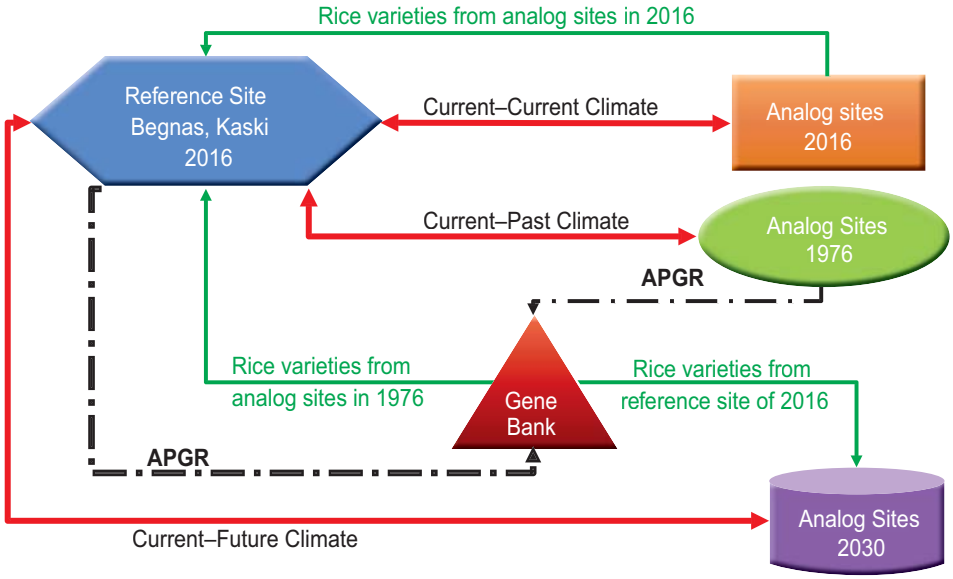

Fig. 6. Steps for identification of climate smart rice germplasm

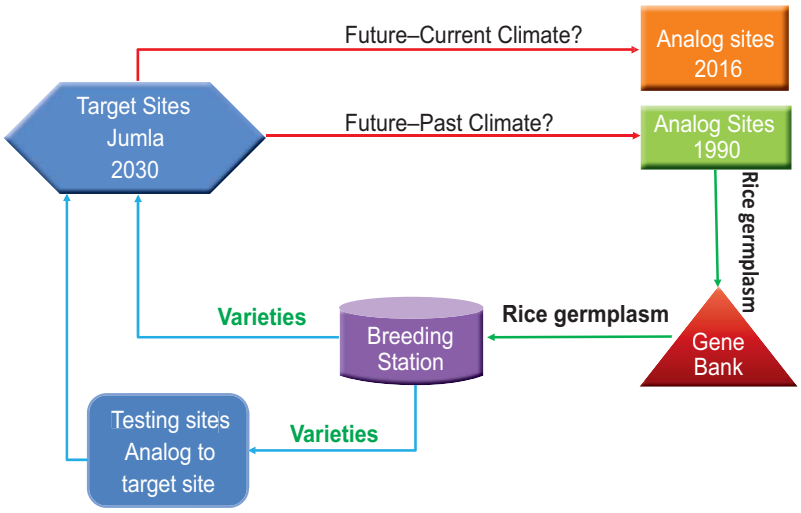

Fig. 7. Conceptual framework for climate smart plant breeding

Such landraces can directly be collected on-farm or searched for in the Genebank. After developing new genotypes, these need to be tested in similar sites of future climate of target site. Alternatively, given that genetic changes in stored materials of the Genebank are negligible and such old collections can be introduced in sites that are of similar climate as the collections sites during the time of collection.

In the future, we intend to identify more precisely which analogue sites could be used to develop location-specific strategies to adapt to climate change and build the resilience of farmers. Field-testing of novel genetic material will be the ultimate test of the utility of the CAT.

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Plants of Nutrimental and Ethno-Veterinary Therapeutic Potential Value in Bahraich (Uttar Pradesh), India

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The present ethno-medicinal investigation was undertaken for the documentation of information on uses and conservation of ethno-medicinal plants being used in various veterinary ailments. Out of 134 families with 600 genera and 1,027 plant species found in Bahraich the plants of 57 families represented by 106 genera and 132 plant species were used in various veterinary ailments.

The study showed that Papilionaceae is represented by nine genera and ten species; Asteraceae is represented by seven genera and nine species whereas Cucurbitaceae by six genera and seven species; Poaceae by four genera and seven species; Caesalpinaceae by four genera and six species; Apiaceae by four genera and species each; Solanaceae by three genera and six species; Acanthaceae and Lamiaceae by three genera and species each; Moraceae by two genera and five species; Brassicaceae and Amaranthaceae by two genera and four species each; Menispermaceae and Liliaceae by two genera and three species each; Meliaceae, Sapindaceae, Anacardiaceae, Rosaceae, Lytharaceae, Apocyanaceae, Asclepiadaceae, Piperaceae, Euphorbiaceae and Zingiberaceae by two genera and two species each; Mimosaceae, Combritaceae, Rubiaceae, Dioscoreaceae and Cyperaceae by one genera and two species each; where as rest twenty eight families, viz., Annonaceae, Fumariaceae, Cappariadeace, Carryophyllaceae, Malvaceae, Linaceae, Oxalidaceae, Rutaceae, Mitaceae, Leaceae, Myrtaceae, Punicaceae, Cactaceae, Plumbaginaceae, Oleaceae, Buddlejaceae, Cuscucataceae, Bignoniaceae, Verbenaceae, Plantagenaceae, Nyctagenaceae, Chenopodiaceae, Polygonaceae, Louraceae, Loranthaceae, Cannaceae, Agavaceae and Araceae are found to be represented with single genera and species each.

Key Words: Bahraich, Ethno-veterinary use, Plant diversity

Introduction

Plants have a significant contribution towards the wealth of a country. During recent years, exploration of our plant wealth and its economic utilization have rightly been given due importance. The value of medicinal plants to the mankind is very well proven since Vedic period. The allopathic method of treatment is advancing day-by-day but still about 64% of world population depends on the traditional method of medicinal system for improving their health problems (Farnsworth, 1994). Out of 64 % of the world population, the major traditional users belong to the areas of rural and tribal areas of the developing countries. People of these regions are using neighbouring plant species for treatment of minor and major diseases either by their choice, or economic reasons or lack of access to other expensive treatments (Prance, 1991; Qureshi *et al.,* 2006). The use of these neighbouring plants for overcoming the health realted issues has started from many ancient eras in different part of the world. As these plants are being used from many past centuries, the knowdge of people has enhanced *per se* in the response of plants to respective diseases (Sen *et al.,* 2011). Harshberger (1895) and Jain (1995) were the first who perform study on application of neighbouring plants as per the pre-historic knowledge base for medicinal use and such study comes under ethnobotany or ethnomedicine. Over 2,500 medicinal plants are introduced to the modern world and still much more are yet to be explored (Huxley, 1984).

India and China are two largest countries in Asia which have the richest array of registered and relatively well-known medicinal plants. Nature has been a source of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Unique geography, climate and environmental conditions of India, make it richest source of medicinal plants (Kshirsagar and Singh, 2000). About 65% of total population depend on such traditional knowledge for healthcare (Timmermans, 2003) while of the rural population, this amounts to 85% (Jain, 1994) in India. Our country is known for its rich ancient cultural practices and about 300 tribal communities consisting

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of 53 million populations are still dependent on such source for health care (Reddy *et al*., 2010). Roughly the amount of plants enegaged in such activities by these people ranges from 7000-7500 (Matthews, 2005; Mao *et al*., 2009; Survase and Raut, 2011). Unani, Siddha and Amchi medicine systems also provide a valuable source of knowledge of medicinal plants by prescribing about 700, 600 and 600 species, respectively (Joy *et al*., 1998; Ahmad *et al*., 2006; Samy *et al*., 2008; Sen *et al*., 2011) for healthcare uses. Plant produces a diverse range of bioactive molecules making them a rich source of different type of medicines. Ethno-botanical and ethno-medicinal studies are today recognized as the most viable method of identifying new medicinal plants or refocusing on those earlier reported plants for bioactive constituents. However, many plants having medicinal value have been documented in our ancient literature but still there is a lack of documentation of plants which are being used by the tribal people (De *et al*., 2010; Sen *et al*., 2011; Kumar, 2013; Bajpai *et al*., 2016). Lack of proper documentation and oral communication of such knowledge base from one to another generation are forcing towards a need for scientific exploration and documentation of medicinal knowledge before it faces the risk of extinction (Sajise, 1995; Murthy, 2012).

The study area is blessed with several floras by nature and it is referred as natural paradise and is very rich in ethnic and floristic diversity. Due to vast area of natural forests, Bahraich is well known as *City of Forests*. The rich biodiversity of Bahraich district of Uttar Pradesh has provided an initial advantage to its inhabitants for observing and scrutinizing the rich flora for developing their own traditional knowledge in curing various ailments of men as well as animals. The primitive tribals acquired the knowledge of economic and medicinal properties of many plants by trial and error methods and they are the store house of such valuable knowledge. This accumulated knowledge is passed on from one generation to the other by oral tradition without any written document. The people of the region are rich in ethno-medicinal knowledge owing to their close affinity with the surrounding vegetation. Many plant species of immense medicinal value are abundantly found in the district. Medicinal plants form the basis of traditional or indigenous systems of healthcare are being used by most remotely located dwellers of the area. Religious inspiration, inaccessibility and lack of medical facilities in the villages seem to be the cause of depending on these medicinal plant species. Remedies based on these plants often have negligible side effect and due to relatively high costs of allopathic medicines, traditional herbal medicine have become an affordable choice for the poor people in rural areas. Traditional system of medicine is a wise practice of indigenous knowledge system, which has saved the lives of poor people in the region. There is great traditional knowledge hidden among the tribes and rural people of the district which can be used for human welfare.

Keeping the aforesaid view, the rich ethno-medicinal practices of the area have already received considerable scientific attention and the ethno-medicinal practices have been documented. The present work was undertaken to document and analyse various traditional herbal method of treatment for various ailments in the rural areas of Bahraich district not only for human beings but also for the domestic animals.

Study Area

Bahraich is known as '*City of Forest'* because of its natural beauty and rich phytodiversity. It is located between 28.24 and 27.4 latitude and 81.6 N to 81.3 E longitude, having area about 4696.8 sq. km. in which 95,040 ha land is covered by dense natural forests. Bahraich has international border with Nepal on the northern part. North-Eastern and Western part of the district is Tarai which is covered by dense natural forests. The climate is hot and humid, maximum and minimum temperature ranges between 44^{o} to 5^{o}C where as average rainfall is 1,125 mm. Soil is very fertile and is composed of Gangetic alluvium of Saryu and Ghagra. In aspect of botany, this area is very interesting. In north, the Himalaya rise as a virtual wall beyond the snow line. Above the alluvial plain lies the Tarai strip, a seasonally mashy zone of sand and clay soils. As the northern Tarai region has higher rainfall than the plains and the falling rivers from the Himalaya slows down thereby spreading out in the flatter Tarai zone with the deposition of fertile silt which makes it reproductive land during the monsoon season and receding in the dry season. Thus, Tarai region has higher water level and is characterized by moist sub-tropical condition and a luxuriant turnover of green vegetation all the year around. This Tarai belt is well blessed and inhabited by tribal community inside the forest as well as around the forest area and is a natural paradise for ethno-botanical, mycological, plant pathological as well as work related

with wildlife alone or interdisciplinary work. The general vegetation of the area is tropical deciduous type. However, some of the trees are evergreen and semi-evergreen. "Katarniaghat Wildlife Sanctuary" is main point of attraction and specialty of the district Bahraich. Aforesaid ideal environmental factors support the luxurious growth of biodiversity.

Methodology

The present study was based on the field survey of Bahraich district of Uttar Pradesh. The voucher specimens of ethno-medicinal importance were collected, pressed, dried, preserved and mounted (Jain and Rao, 1976) with their ethno-therapeutic data and identified through the available taxonomic literature manuals and floras (Duthie, 1994 and Hooker, 1872-1897). The information was collected from herbal practitioners or local healers and other experienced persons. They were interviewed for local names, plant part used, method of preparation of medicine, dosages and their mode of administration. The specimens were maintained in the Herbarium of the Postgraduate Department of Botany. Information was validated using literatures (Yineger *et al*., 2007; Pande *et al*., 2007; Phondani *et al*., 2010; Tarik *et al*., 2014; Verma, 2014; Eshetu *et al*., 2015; Narayana and Narasimharao, 2015 and Mall and Tripathi, 2017) and findings were incorporated. The plants used in the treatment of various ailments are enumerated with correct botanical name followed by vernacular names and family as well as plant parts used and mode of administration in respect to simple preparation as well as compound preparation of medicine.

Result and Discussion

The perusal of the Table 1 and Figure 1 revealed that 17 plant species help in curing diarrhoea and dysentery whereas 16 plants are being used for wounds, 14 plant species for removal of ectoparasites while in case of indigestion and gas problem,10 species play effective role in curing up. 9 plant species is being used in foot and mouth diseases and skin infection while 8 plant species to expel internal parasites. In case of

Table 1. Plants with ethno-veterinary uses in different ailments and their mode of use

S.No.	Disease/ Disorder	Plant species	Family	Part used	Method of use
1	Anorexia	*Trachyspermum ammi* Spr.	Apiaceae	Seeds	Seeds of *Trachyspermum ammi* and bark of *Terminalia chebula*, Rhizome of *Cuminum cyminum* (ajwain), seeds of *Raphanus sativus*is grinded and mixed with black salt are applied to eat in anorexia when animal stops eating fodder.
		Terminalia chebula Retz.	Combretaceae	Bark	
		Cuminum cyminum L.	Apiaceae	Rhizome	
		Raphanus sativus L.	Brassicaceae	Seeds	
2	Anthrax	*Abrus precatorius* L.	Papilinoidea	Stem bark	Stem bark of *Abrus precatorius* (Rosery pea) along with leaves of *Vitex negundo*, tubers of *Curculigo orchioides* each 50 gm and 15 gm *Piper nigrum* and *Allium sativum* are grained and boiled in water so as to prepare decoction. The decoction is given orally twice in a day for a weak to cure anthrax, a serious disease that effect sheep and cows and sometimes people and can cause death.
		Vitex negundo L.	Lamiaceae	Leaves	
		Curculigo orchioides Gaertn.	Hypoxidaceae	Tubers	
		Piper nigrum L.	Piperaceae	Seeds	
		Allium sativum L.	Amaryllidaceae	Rhizome	
3	Analgesic	*Cuscuta reflexa* Roxb.	Cuscutaceae	Bark	*Cuscuta reflexa* Roxb., amarbel is used in bone fracture and lockjaw as an analgesic.
4	Anti-diuretic	*Coriandrum sativum* L.	Apiaceae	Seed	The seed powder of *Coriandrum sativum*, dhania (Apiaceae) is mixed with paste of leaves of *Lawsonia inermis* along with small amount of water is given twice a day for a weak to animal to cure loose motion.
		Lawsonia inermis L.	Lythraceae	Leaves	
				Leaves	The decoction of leaves and roasts are given to buffaloes orally twice in a day for a weak which works as anti-diuretic.
				Seed	The powder of seed mixed with water is administered orally twice in a day till cure when saliva comes from mouth due to food poisoning.
5	Antihelminthic	*Achyranthus aspera* L.	Amaranthaceae	Whole plant	The whole plant of *Achyranthus aspera*, latjeera is grinded to make paste and given orally along with sugar to buffaloes, cow, goat, sheep as anthelmintic and easy delivery.
		Azadirachta indica A. Juss.	Meliaceae	Leaf	One hundred gm leaf paste of *Azadirachta indica* A, Juss., neem is administered twice in a day for about five days to treat cough, lever diseases and as anthelmintic.

S.No.	Disease/ Disorder	Plant species	Family	Part used	Method of use
6	Appetizer	*Cannabis sativa* L.	Cannabinaceae	Leaf	Dried leaf powder of *Cannabis sativa*, bhang given orally works as appetizer.
		Cassia fistula	Caesalpiniaceae	Pods	The paste of pods along with wheat bread is given twice in a day to cattle in the case of indigestion
				Leaves	The paste of leaves is mixed along with mustard oil and given twice in a day for a weak to improve appetite
				Leaves	The young leaves are cooked and given as purgative.
				Pods	The paste of ripe pods is also administered along with water for purgative purpose. The pod is directly used.
7	Alimentary disorder	*Piper longum* L.	Piperaceae	Fruit	Powder of fruit of *Piper longum*, pipli, pippali, pipar, piplamul is mixed with water and applied to drink orally till cure when saliva comes from mouth due to poisoning.
		Prunus persica Betsch.	Rosaceae	Leaf	Leaf paste is externally used to cure germs and wounds.
		Trachyspermum ammi Sprague	Apiaceae	Seeds	Seeds and rhizome of *Zingiber officinale, Ferula asafoetida* and fruit of *Piper nigrum* are mixed and grinded with water and the paste is used to cure blot which results gloating of stomach.
		Vigina radiata L. Wiliz.	Papilinoideae	Seeds	Two hundred fifty gm seed powder is mixed with hundred ml *Arachis hypogea* oil and given twice a day for a weak so as to cure cattle suffering from cough and cold.
		Arachis hypogea L.		Oil	
		Curcuma domestica L.	Zingiberaceae	Rhizome	Rhizome of *Curcuma domestica*, *Zingiber officinale* and bulb of *Allium sativa,* seeds of *Trachyspermum ammi* and *Brassica juncea* is milled and mixed with jaggery of *Saccharum officinarum* is provided to animal to eat for curing abdominal pain.
		Zingiber officinal Roscoe	Zingiberaceae	Rhizome	
		Allium sativa L.	Amaryllidaceae	Bulb	
		Trachyspermum ammi Sprague	Apiaceae	Seeds	
		Brassica juncea L. Vassili Matveievitch Czernajew	Brassicaceae	Seeds	
		Saccharum offinarum L.	Poaceae	Jaggery	
8	Bone fracture	*Achyranther aspera* L.	Amaranthaceae	Root	A piece of fresh root of *Achyranthus aspera*, latjeera is grounded and the paste is applied so as to cure bone fracture.
		Agave americana L.	Agavaceae	Leaf fibres	Leaf fibres of *Agave americana*, ramban are used to tie the fractured bone.
		Amaranthus sp. L.	Amaranthaceae	Leaves	*Amaranthus* sp., chaulai is being used in bone fracture and wounds.
		Calerbrookia oppositifolia Sm.	Lamiaceae	Leaves	*Calerbrookia oppositifolia,* shamber, bhirmoli is being used in bone fracture, internal injury, sprain and muscular pull.
		Cuscuta reflexa Roxb.	Cuscutaceae	Thin vine	*Cuscuta reflexa*, Amarbel is used in bone fracture and lockjaw.
		Lannea coromandelica Houtt. Mess.	Anacardiaceae	Vegetative part	*Lannea coromandelica*, Mohin is used in bone fracture.
		Litesea glutinosa Lour Robinson.	Lauraceae	Vegetative part	*Litesea glutinosa*, Maida is used in stomach disorder and is bone fracture.
9	Blot	*Basella alba* L.	Basellaceae	Vegetative part	The fresh vegetative paste and the flowers of *Acmella caulirriza* are made in to paste and mixed together is squeezed. The filtrate solution is given thrice in a day orally to cure blot in cattle, sheep goat and equine.
		Acmella caulirriza L.	Asteraceae	Flowers	
		Lycopersicum esculentum L.	Solanaceae	Fruit	The fruit applied to cure blot where gloating of stomach take place.
		Trachyspermum ammi Spr.	Cucurbitaceae	Seeds	Seeds of *Trachyspermum ammi*, rhizome of *Zingier officinale*, *Ferula asafoetida* and fruit of *Piper nigrum* are mixed and grinded with water and the paste is used to cure blot which results gloating of stomach.
		Zingiber officinale Roscoe	Zingiberaceae	Rhizome	
		Piper nigrum L.	Piperaceae	Fruit	
		Ferula asafoetida L.	Apiaceae	Rhizome	
10	Boils	*Bauhinia vahlii* Weight and Arnott.,	Caesalpiniaceae		*Bauhinia vahlii* is used in hoof disease, boils pimples, carbuncle and post calving care.
		Dioscorea bulbifera L.	Dioscoreaceae		*Dioscorea bulbifera* is used in ear diseases, pimples and boil.

S.No.	Disease/ Disorder	Plant species	Family	Part used	Method of use
11	Broken horn	*Curcuma longa* L.	Zingiberaceae	Rhizome	Fine paste of rhizome is mixed with pure mustard oil of *Brassica nigra*, Black mustard. It is applied on the mischief parts of cattle horn.
		Brassica nigra L.	Brassicaceae	Oil	
		Curcuma domestica L.	Zingiberaceae	Rhizome	The rhizome paste is applied externally to the broken horn.
		Lens culinaris Medik.	Papilinoideae	Seed	*Lens culinaris* Medik., Lentil, Masoor is used to remove sterility and cure of broken horn.
		Tagetus erecta L.	Asteraceae	Leaves	The fresh leaves are gound to make paste. The paste is squeezed, the juice so obtained is applied extremely in broken horn where there is shelling off the outer of horn and conconitant bleeding.
12	Burn	*Capsicum annum* L.	Solanaceae	Fruit	*Capsicum annum*, Mircha is being used in burn.
		Lathyrus sp. L.	Papilinoideae		*Lathyrus sp.*, Sweet pea is used when there is burn.
		Solanum tuberosum	Solanaceae	Underground stem	*Solanum tuberosum*, Aalu is used in burns
		Triticum aestivum L.	Poaceae	Seeds	Seeds are grinded and a paste is made. It is applied externally on burns on the skin.
13	Carbuncle	*Bauhinia vahlii* weight and Arnott.	Caesalpiniaceae	Flowers	Flower is used in hoof disease, boils pimples, carbuncle and post calving care
14	Cold	*Datura* metel L.	Solanaceae	Fruits	About 100 gm of ripe fruits of *Datura* metel, Hindu dhatura are made into paste along with water and given to cattle twice a day for a weak to cure cold.
		Ocimum sanctum L.	Lamiaceae	Leaves	Three hundred and fifty gm fresh leaves (Shyamatulsi) are boiled in 500 ml water for preparation of decoction. The decoction so obtained is given to cattle twice in a day to cure cough and cold.
		Triticum aestivum L.	Poaceae	Seeds	Seeds made into powder and administered orally to cow to cure common cold and dysentery.
		Vigna radiata R.	Papilinoideae	Seeds	Two hundred fifty gm seed powder of *Vigna radiata* R. Witezek, Mung bean is mixed with hundred ml *Arachis hypogea* oil and given twice a day for a weak so as to cure cattle suffering from cough and cold.
		Arachis hypogea L.		Oil	
15	Cough	*Allium cepa* L.	Liliaceae	Bulb	The paste of onion, *Allium cepa*, Pyaz, bulb mixed with mustered oil given orally thrice for three weeks for the treatment of cough.
		Dendrocalamus strictus Roxb. Nees	Poaceae	Leaves	Green leaves of *Dendrocalamus strictus* (Roxb.) Nees., Bans, Bamboo, Calcutta bamboo are grinded with seeds of *Hordeum vulgare* and is administered along with water to cattle to cure cough.
		Hordeum vulgare L.	Poaceae	Seeds	
		Ocimum sanctum L.	Lamiaceae	Leaves	Three hundred and fifty gm fresh leaves of (Shyamatulsi) is boiled in 500 ml water for preparation of decoction. The decoction so obtained is given to cattle twice in a day to cure cough and cold.
		Linum usitatissimum L.	Linaceae	Seeds	*Linum usitatissimum*, alsi is used in dysentery, cold, cough and as tonic.
		Oryza sativa L.	Poaceae	Seeds	Seeds of *Oryza sativa*, dhan are boiled with water and the extract is applied orally to cure cough.
		Saccharum officinarum L.	Poaceae	Poaceae	*Saccharum* sp., ganna is used when there is cough.
		Tribulus terrestris L.	Zygophyllaceae	Leaves	Juice of fresh leaves of *Tribulus terrestris*, Bindii, Devil's thorn is given to animal in case of colic and chronic cough.
		Vigna radiata (L.) Wiliz.	Papilinoideae	Seed	Two hundred fifty gm seed powder of *Vigna radiata*, Mung bean is mixed with hundred ml *Arachis hypogea* oil and given twice a day for a week to cure cattle suffering from cough and cold.
		Arachis hypogaea L.		Oil	
16	Cataract	*Ampelocissus latifolia* (Roxb.) Planch	Vitaceae		*Ampelocissus latifolia*, Wild grape is reported to be used in cataract.
		Tridex procumbens L.	Asteraceae	Leaves	Used in cataract.

S.No.	Disease/ Disorder	Plant species	Family	Part used	Method of use
17	Constipation	*Ficus religiosa* L.	Moraceae	Stem bark	The paste of stem bark of *Ficus religiosa*, Peepal is given against constipation.
		Plambago zeylanica L.	Plumbaginaceae		Used in constipation.
18	Continual release of urine	*Cucumis melo* Duth and Full.	Cucurbitaceae	Fruit	Used in continual release of urine, heat stroke, indigestion.
19	Conjunctivitis	*Cucurbita maxima* Duch ex Lam.	Cucurbitaceae	Fruit	Used in conjunctivitis.
		Cynodon dactylon Pers.	Poaceae	Leaves	One tea spoonful leaf juice of *Cynodon dactylon* Pers., Doob ghas is dropped in each eye in morning and evening for three days or so for the treatment of conjunctivitis.
20	Chicken pox	*Phyllanthus emblica* L.	Eupharbiaceae	Fruit	Fruit is used in chicken pox.
		Plantago ovata Forssk.	Plantaginaceae	Seed husk	Used in chickenpox and to expel internal parasites.

curing bone fracture, cough and cold, food poisioning, retention of placenta, eye problems and stomachache, Seven plant species are being used up. Six plant species are revealed to be effective against mastitis, tympany, maggots wound, fever, lockjaw (tetanus) and haematuria. Five plant species are related to overcome the sun burn, four plant species were revealed to overcome the problems of rheumatism and lactation. Also, four plant species are used for easy delivery, snake bite, galactogogue, flatulence, eczema, to induce fertility, in loose motion and broken horn. Three plant species are used to overcome dyspepsia, arthiritis, hoof diseases, sun stroke, neck rose, to stop bleeding, ear disease and debility. In case of anthelmentic, as epitizer, boils, blot, cataract, conjuctivitis, chiken pox, colic disorder, dysentery, frequent loose motion, food suffering from infection, internal injury, itching, jaundice, lactation, liver disease, pimples, swelling, twitching, vomiting, in dog bite, to remove sterility, in bronchitis and as anti-diureatic, 2 plant species are being used up where as in case of anthrax, anorexia, burn, chronic cough, constipation, cold cough, carbuncle, darissa, dysurea, dyptheria, fascioptasis, febnifuge, hepatitis, internal heat, intestinal disorder, iritation, mange, mouth blisters, muscular pull, neck injury, post calvin care, paralysis, pneumonia, as purgative, ranikhet disease, render pest, sprain, scabies, shoulder injury, stomach mange and ulcer, throat swelling, as tonic, tonsils, tongue sore, udder problem, urinary troubles, yolk galls andyolk sore, only one plant species were found to be being used in ailments.

Out of 134 families having 600 genera and 1027 plant species are found in Bahraich (Saini, 2005). Of these the plants of 57 families represented by 106 genera of 181 plant species are found to be being used in various veterinary ailments. Acanthaceae is being represented by 3 genera and 5 species; Asteraceae by 7 genera and 9 species, Amaranthaceae by 2 genera and 4 species; Apiaceae by 4 genera and species each; Brassicaceae by 2 genera and 4 species; Caesalpinaceae by 4 genera and 6 species; Cucurbitaceae by 6 genera and 7 species; Moraceae by 2 genera and 5 species; Lamiaceae by 3 genera and species; Papilionaceae is represented by 9 genera and 10 species; Menispermaceae by 2 genera and three species, Poaceae by 4 genera and 7 species; Solanaceae by 3 genera and 6 species; while Meliaceae, Sapindaceae, Anacardiaceae, Rosaceae, Lytharaceae, Apocyanaceae, Asclepiadaceae, Piperaceae, Euphorbiaceae and Zingiberaceae by 2 genera and 2 species each; Mimosaceae,Combritaceae, Rubiaceae, Dioscoreaceae and Cyperaceae by 1 genera and 2 species each; where as rest 28 families, *viz*., Annonaceae, Fumariaceae, Cappariadeace,Carryophyllaceae, Malvaceae, Linaceae, Oxalidaceae, Rutaceae, Mitaceae, Leaceae, Myrtaceae, Punicaceae, Cactaceae, Plumbagenaceae, Oleaceae, Buddlejaceae, Cuscucataceae, Bignoniaceae, Verbenaceae, Plantagenaceae, Nyctagenaceae, Chenopodiaceae, Polygonaceae, Louraceae, Loranthaceae, Cannaceae, Agavaceae and Araceae are found to be represented with single genera and species each (Fig. 2).

About 60 percent of the world's population is using the traditional medicines for health care which not only includes rural areas but even in developing and developed countries, where use of modern medicine is predominant. The traditional method of medicine

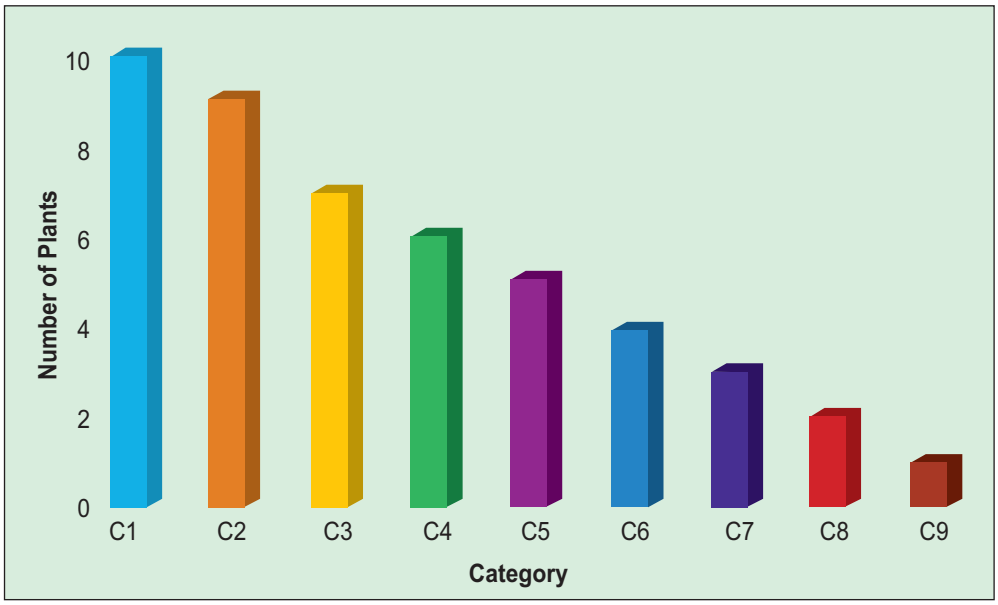

Fig. Number of plants having ethnoveterniary importance of different species (C1-Papilionaceae; C2-Asteraceae; C3-Cucurbitaceae and Poaceae; C4-Caesalpineceae and Solancaeae; C5-Moraceae and Acanthaceae; C6- Amarthanceae, Apicaceae, Brassicacae; C6-Lamiaceae and Menispermaceae; C7-Meliaceae, Sapindaceae, Anacardiaceae, Rosaceae, Lythraceae, Apocyanaceae, Asclepiadaceae, Piperaceae, Euphorbiaceae and Zingiberaceae; C8-Mimosaceae, Combritaceae, Rubiaceae, Dioscoreaceae and Cyperaceae; and C9-Annonaceae, Fumariaceae, Cappariadeace,Carryophyllaceae, Malvaceae, Linaceae, Oxalidaceae, Rutaceae, Mitaceae, Leaceae, Myrtaceae, Punicaceae, Cactaceae, Plumbagenaceae, Oleaceae, Buddlejaceae, Cuscucataceae, Bignoniaceae, Verbenaceae, Plantagenaceae, Nyctagenaceae, Chenopodiaceae, Polygonaceae, Louraceae, Loranthaceae, Cannaceae, Agavaceae and Araceae)

involves the use of medicinal plants, minerals, and organic matter. Application of plants in the medicinal field is not a new thought but rather an inherited process. Indian system of medicine had derived many of their curative tools from these plants. About 45,000 plant species are present in India having a high concentration in the regions of Eastern Himalayas, Western Ghats and Andaman & Nicobar Islands. Roughly 3,000 plants have been well documented of being used as medicine for various diseases and related problems but for traditional practitioners this amount is of greater than 6,000. India is one such country which is the largest producer of medicinal herbs and so it may be called as botanical garden of the world (Mall and Tripathi, 2017).

This study showed that the study area is rich in plants having ethno-medicinal importance which may help in overcoming various diseases and its related issues. Though there is availability of modern medicinal system for treatment for various diseases but the local people are still dependent on the traditional medicinal sytem. The knowledge of traditional healthcare is limited to traditional healers, who are residing in rural areas.Thus there is a need for preserving this traditional knowledge in a form of documentation before it is too late. This study also enhances the need for further investigation on aspects like biochemical and pharmaceuticals as one of the major problems with herbal medicines is that their active ingredients are not very well known. Hence, if active component and molecular interaction is known of these plants, it will help in analysing the theraupetic efficacy of various medicines being used. It is also important to pay attention on preserving the rich diversity of nature bestowed on the study area for conservation of mankind and for sustainance of life on the earth.

Conclusion

This study showed that there is wide scope for further scientific work as still there are some plants unexplored for their medicinal value. Information on Ethno-medicinal plants may provide a base to explore the new compounds associated with phyto-chemistry and pharmacology. It is even important to consider that the floristic diversity and natural beauty of the study area is the most precious gift that nature has bestowed on our planet Earth and thus one must pay attention to the growing medicinal

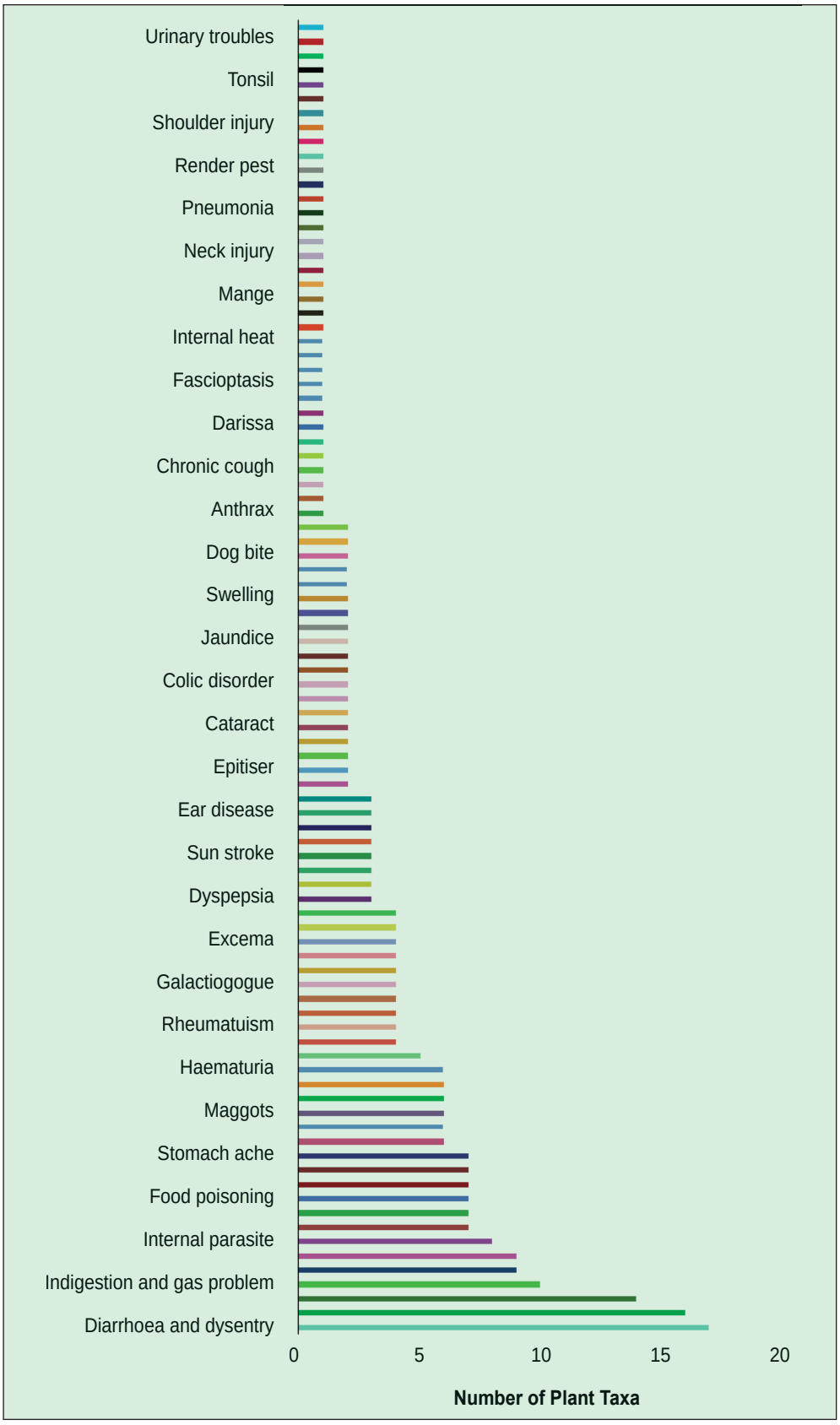

Fig. 1. Diseseas cured by various plants species

plants in these areas for its sustainable exploitation, cultivation and even its conservation for the sake of mankind as it is well known that "Nature Protects if She is Protected".

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Identification of High-Risk Agro-Ecological Regions using Species Distribution Modeling of Priority Invasive Species in Sri Lanka

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Mimosa pigra, Annona glabra, Lantana camara, Prosopis juliflora and *Parthenium histerophorus* are considered as priority invasive plants of Sri Lanka that cause considerable impact on agriculture and food security. The potential areas of these species are not available for land managers for timely control and management measures. This study modelled the suitable climate area for these invasive species under current climate scenarios using Maxent model. The resulting models were found to have good discrimination ability of presence and absence, with very high AUC values. The study identified highly responding variables for the model prediction of each species. Maxent predictions were overlaid on agro-ecological map of Sri Lanka and high-risk agro-ecological regions were identified for the five species considered. Resulting maps can be a vital tool for land managers to take information based decisions to control and manage invasive species.

Key Words: Invasive species, Maxent, Species distribution modeling

Introduction

Invasive species are a major threat to earth's biodiversity. An understanding of the current and potential distribution pattern of an invasive species is fundamental for managing invasive alien species (Taylor, 2012; Ward, 2007). Forecasting the potential areas of occupancy of invasive species is important to environmental planners for risk assessment and also to prepare long term management strategies (Taylor, 2012). Maxent (Phillips *et al*., 2006) is one of the accurate, increasingly popular and globally accepted machine-learning techniques (Graham and Hijmans, 2006; Ramirez-Villegas and Bueno-Cabrera, 2009) for presence-only data (Baldwin, 2009). Predicting and quantifying the actual or the potential areas of occupancy of invasive species facilitates environmental planners to take timely control, eradication or containment actions (Gormley *et al*., 2011) to manage the issue.

The aim of this study is to predict the potential distribution of five high priority invasive plants in Sri Lanka and identify agro ecological regions that could be under threat due to future invasion of these species.

Material and Methods

Study Species

Study focused on five priority invasive plant species in Sri Lanka *viz*., *Mimosa pigra, Annona glabra, Lantana camara, Prosopis juliflora* and *Parthenium histerophorus*. The ecological and economic damage caused by these species is significant for ecosystem health especially in production landscapes and protected areas.

Species Distribution Data

Field surveys of data collection were conducted at several locations of Sri Lanka. Occurrence data of species distribution was collected using random sampling. Primary data was sustained by geo-referenced secondary data which were collected from various reliable sources, such as study reports, expert consultations etc.

Environmental Data

Fine resolution (30 sec.), current bioclimatic data freely available at 'worldclim' database were selected for the study. All variables were plotted using 'get value' and 'hist' functions in R programme to visualize the range of values of each variable. Variables with higher value ranges comparatively were selected as the subset of variables for model run (Table 1). The selected variables were supplemented with 'yearly average aridity index' since this parameter showed a strong impact on the model prediction.

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Table 1. Selected parameters and their codes

Parameter	Code
Mean Diurnal Range (Mean of monthly (max temp - min temp))	bio_2
Isothermality (BIO2/BIO7) (* 100)	bio_3
Temperature Seasonality (standard deviation *100)	bio_4
Temperature Annual Range (BIO5-BIO6)	bio_7
Annual Precipitation	bio_12
Precipitation Seasonality (Coefficient of Variation)	bio_15
Yearly Average Aridity Index	ai_yr

Table 2. Contribution of environmental variables for *Mimosa pigra* model in Sri Lanka

Variable	Percent Contribution	Permutation Importance
ai_yr	53.4	59.4
bio_2	37.4	18.8
bio_4	6.8	18.1
bio_3	2.4	3.6
bio_7	0	0
bio_15	0	0
Bio_12	0	0

Running the Model in Maxent

Maximum Entropy Modeling version 3.3.3k was employed for the study (Phillips *et al*., 2009). Cumulative output format was selected instead of the default logistic output. Random test percentage was set to 25% enabling the model automatically set all presence points into training and test samples. Other relevant default settings of the maxent software were applied. Model was run for each species separately with the selected subset of variables. The discrimination capacities of these distributional models were determined by calculating the area under the receiver operating characteristic curve (AUC) criterion.

The areas of predictions were visualized for each species using 'minimum training presence' as the cut off level. Occurrences of each species were overlaid on the prediction image. Maxent prediction of each species was overlaid on agro-ecological map of Sri Lanka.

Results and Discussion

All models were found to have a high predictive power or good discrimination ability with high AUC_{test} values of 0.888, 0.989, 0.910, 0.821 and 0.998 for *M. pigra, A. glabra, L. camara, P. juliflora* and *P. histerophorus,* respectively (Fielding and Bell, 1997; Araujo *et al.,* 2005). AUC value is generally considered as a good statistical measure of evaluating the discrimination ability of species distribution models.

In *M. pigra* model, analysis of jackknife tests (Fig. 1) revealed that yearly average aridity index (ai_yr) which is an indicator for the degree of dryness significantly affected the model prediction. The percent contribution of each variable to the final model of *M. pigra* (Table 2) also showed significantly high contribution of aridity index. Therefore, suitable conditions for *M. pigra* will depend on the level of dryness caused by the evaporation or more combined effect associated with the variables.

Similarly, temperature seasonality (bio_4), isothermality (bio_3), Annual Precipitation (bio_12) and Isothermality (bio_3) contributes significantly in *A. glabra*, *L. camara*, *P. juliflora* and *P. histerophorus* models, respectively.

Areas predicted as suitable for *M. pigra, A. glabra, L. camara, P. juliflora and P. histerophorus* at minimum

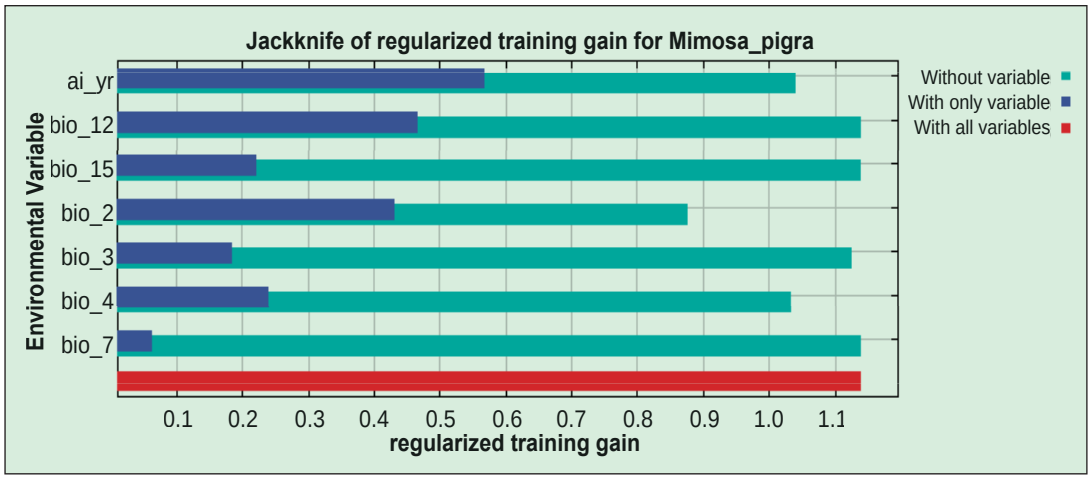

Fig. 1. Results of Jackknife test of variable importance in the regularized training gain for *Mimosa pigra* model

training presence threshold level are shown in Fig. 2. The predicted range of *M. pigra* lies mainly in the central and Eastern parts of Sri Lanka whereas the predicted range of *A. glabra* is limited only to the South-western coastal areas. The prediction of *L. camara* lies mainly in South-eastern part of the country. The predicted range of *P. juliflora* lies only in the two relatively dry areas located in the North-western and South-eastern Sri Lanka, however, a greater predicted area could be observed in South-eastern coastal areas. The predicted range of *P. histerophorus* lies mainly in the Jaffna peninsula. The Central and Southern parts of Sri Lanka are predicted as not suitable for this species distribution.

Localities where the study species has not been recorded may not be directly causing significant differences to the model prediction if the study area is large enough to capture the environmental variability of the study species. Natural barriers, soil type, predators and competition by closely related species limit the distribution of species and therefore, omission of one or several of these factors can over-estimate the predictions (Xavier and Van Zonneveld, 2010). Alternatively, the threshold value which is set for suitable area prediction is an arbitrary value (Liu *et al.,* 2005), hence the prediction of potential area could change with the selected threshold value.

Basically, there are three climatic zones in Sri Lanka, *viz.*, wet zone, dry zone and intermediate zone and yet again topographically the country is divided into three regions, up country, mid country and low country. Forty six unique ago-ecological zones can be identified in Sri Lanka based on features such as topography, soil type, land use, rainfall etc. (Punyawardena, 2008). Fig. 3 shows Maxent prediction for *M. pigra* overlaid on agro-ecological map of Sri Lanka. Thirty four agro-ecological zones out of 46 are vulnerable to *M. pigra* invasion. Spreading in a wide range of agro ecological zones indicates that this species has adaptations to survive in a wide range of climatic conditions. Southern coastal belt, South-eastern intermediate and wet zones are most vulnerable for *L. camara* invasion in this climatic regime. The potential distribution of this species also overlaps with many number of agro ecological zones. Since *M. pigra* and *L. camara* are widespread, they could be very aggressive invaders and need considerable effort for controlling measures. Potential distribution of *A. glabra*, *P. julifora* and *P. histerophorus* overlap with few agro ecological zones indicating that these species have rather narrow range of climatic preferences in this climate regime.

Maps produced in this study can be a useful tool for protected area managers to take information based

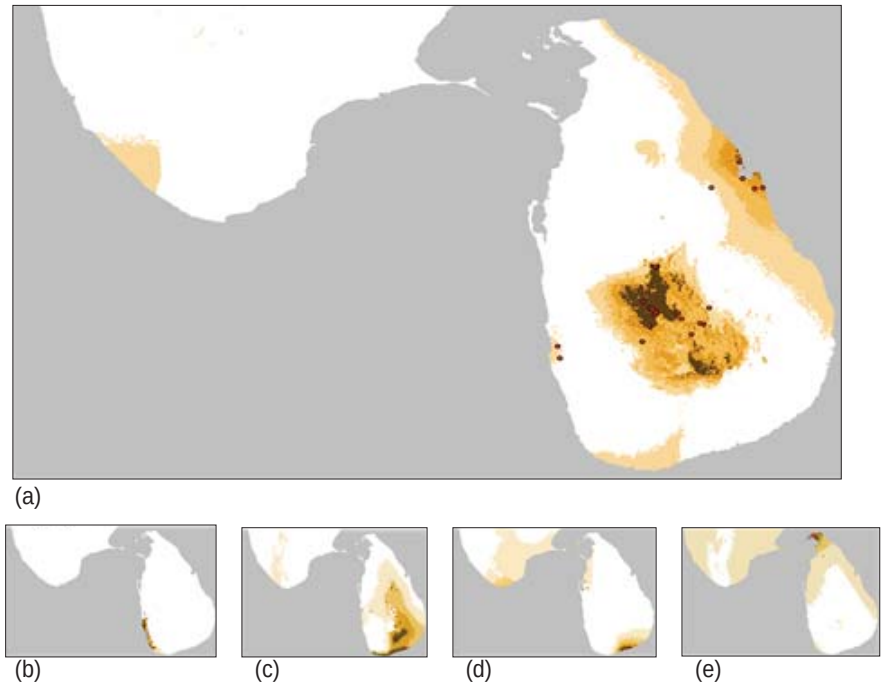

Fig. 2. Predicted areas of *Mimosa pigra* (a), *Annona glabra* (b), *Lantana camara* (c), *Prosopis juliflora* (d) and *Parthenium histerophorus* in Sri Lanka at minimum training presence (cumulative threshold).
Red dots indicate occurrence records of the species in Sri Lanka

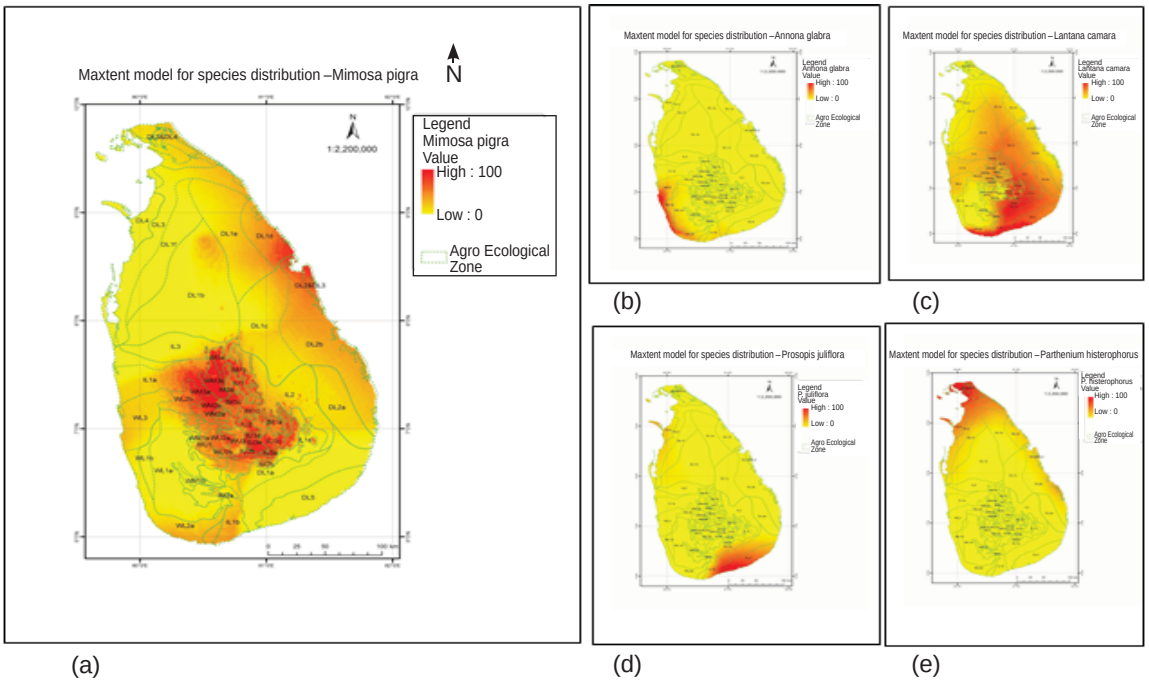

Fig. 3. Maxent prediction overlaid on agro-ecological map of Sri Lanka for *Mimosa pigra* (a), *Annona glabra* (b), *Lantana camara* (c), *Prosopis juliflora* (d) and *Parthenium histerophorus* (e) in Sri Lanka

decisions. Also they can be used in public awareness campaigns so as to enlist the help of local communities in the management of existing infestations and the prevention of further invasion.

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Evaluation of Indian Potato Germplasm for Iron and Zinc Content

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Micronutrient deficiency affects more than half of the world's population especially in developing countries. Amongst micronutrients iron and zinc are the two major elements for human nutrition and their deficiency is commonly observed. Biofortification of food crops is an emerging area of research to overcome such mineral deficiencies. Potato has a priority claim for biofortification as it yields more nutritious food per unit area and time and may contribute significantly in meeting nutritional requirements of the population. Keeping this in view a breeding programme is initiated for which germplasm characterization was done. Forty-six potato germplasm with varying degree of flesh colour from white to yellow to violet were evaluated for iron and zinc content in raw and peeled tubers. The iron content ranged between14.90-67.13 mg/ kg (ppm) dry weight and zinc between 2.78-35.40 mg/kg (ppm) dry weight basis in tuber flesh. Significant and positive correlation between these two nutrients suggests that breeding varieties with high content of both these nutrients is feasible. Carotenoids content enhances the bioavailability of the micro nutrients but lack of correlation between tuber flesh colour index and nutrient concentration in the present study indicated that these traits are independent. The genotypes CP 1239, CP1435, CP1812, CP1983, CP1978, CP2067, CP2414, CP3443, CP3772 and CP4242 are promising as parental material for developing iron and zinc rich potato varieties.

Key Words: Micronutrient content, Potato germplasm, Tuber flesh

Introduction

Potato (*Solanum tuberosum* L.) is the third most important food crop following rice and wheat (Camire *et al*., 2009) and is increasing its hold in developing countries because of its price stability, high yield capacity and better nutritional properties (White *et al.*, 2012). The ability of potatoes in providing food and nutrition security has been given prime importance in today's era. China, the largest potato producer and holding 20 percent of the world total potato production has declared potato as food crop and targeted 50 percent of its extra food demand during the next 20 years to be met from this crop (http://www.cipotato.org/press-room/press- releases/feeding-the-future). India has the next rank and constitutes 10 percent of the global share. The per capita consumption of potatoes in India has reached to 20 kilogram (CPRI Vision document, 2050) but this is quite low than the world average of 32.60 kilogram (FAOSTAT, 2013). In India potatoes are consumed as vegetable crop and are considered as seasonal staple food crop at times of glut in the market (Scott and Suarez, 2011). The vegetarian pattern of food culture in India will keep potatoes as an important part of their diet even with rise in income (Pingali, 2006). Micronutrient deficiency contributes high rate of infant, children and women's mortality. The dietary intake of iron and zinc is often insufficient in diet based on cereals rich foods. Globally 3500 million people are vitamin A, iodine, iron or zinc deficient (Pfeiffer and McClafferty, 2007) causing hidden hunger. The role of iron is of oxygen carrier in human and is incorporated into the heme complex. Iron deficiency is prevalent in both developed and developing regions and the typical deficiency symptoms are pale skin, general fatigue, stunting of growth and vulnerability to infections. Zinc is part of several key enzymes and its deficiency is prevalent in the deprived populations feeding mainly on plant based foods causing poor brain development, hair loss, lesions on skin, diarrhoea, wastage of body tissues, weak eyesight, memory loss, taste and olfactory abnormalities.

Potato, declared as the hidden treasure, (International Year of Potato, 2008) is an important source of carbohydrates, vitamins, minerals and high biological value protein. Biofortification is the technique of breeding new varieties of better food value i.e. dense nutrients and phytonutrients concentrations. It is the genetic modification of plants aimed to enhance the concentration of some specific nutrient elements. To target the potato crop for nutrient biofortification, characterisation of germplasm is the basic step to

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know the range and nature of variations available. The literature cites significant variations (Burgos *et al*., 2007) with high heritability i.e. more genotypic influence on mineral content than environment for iron and zinc micronutrient concentrations. In addition to content, bioavailability of minerals is very important for absorption of nutrient element. This depends on the chemical form the nutrients is present and the presence of promoter and anti-nutrient factors. The absorption percent of potato iron is 10 percent and 25 percent of potato zinc (Bonierbale, 2014). This absorbed iron and zinc constitutes 50 and 40 percent, respectively of estimated average requirement. The low content of phytates and oxalates (inhibitor) while vice versa high ascorbic acid, b-carotene, protein cysteine and amino acids (promoter) content along with significant associations among both the elements strengthens the concept of iron and/or zinc biofortified potato varieties. Keeping this in view forty-six diverse flesh coloured potato germplasm of tuberosum background were analysed for iron and zinc content to find the variations for the traits and, thereby, genotype selection for mineral biofortification.

Materials and Methods

Freshly harvested tubers of forty-six potato germplasm (Table 1) were collected from the field germplasm repository of Central Potato Research Station, Kufri, Himachal Pradesh. The samples were derived from the plants grown under standard recommended production practice and non nutrient limiting conditions in summer season of 2015. The plot size was 4.8 m^2 with 4 rows of 2 m each and the row to row and plant to plant distance was 60×20 cm. The soil of the experimental field was acidic (pH 6.1) in reaction and the available micronutrients was high. All the samples (5 tubers per replication) were prepared in three replications. The samples were peeled (0.5 mm peel removed using standard food grade stainless steel knife), oven dried at 70 °C and finely powdered samples were analyzed for their iron and zinc content after acid digestion (nitric and perchloric) with an atomic absorption spectrophotometer (Model-Shimazdu-AA700). Data of the collected parameters were subjected to analysis of variance. Correlations among traits were studied using Pearson's correlation coefficient. The dendrogram was prepared in software XLSTAT version 2017 by calculating pair-wise genetic dissimilarity index following the method of Euclidean distance using Ward's minimum variance method (Ward, 1963).

Results and Discussion

Variations for Iron and Zinc Content

The Analysis of variance for iron and zinc content in varied coloured potato germplasm depicted significant variations ($p < 0.05$). The range and mean values (given in parenthesis) for iron content were 14.90-67.13 ppm (32.44 ppm; Fig .1). In the previous reports the iron content in Indian potato varieties varied between 17.75 to 40.74 ppm and the average content was 27.53 ppm in dried tuber flesh (Dalamu *et al*., 2016) while in Indian

Table 1. List of germplasm and the source countries

CP Numbers (Variety Name)	Source region
CP 1497 (2814a(1), CP 1500 (2183c(2), CP 1527 (2403a(1), CP 1547 (Pentland Envoy), CP 1616 (835a(4)	United Kingdom
CP 1549 (Aspotet), CP 1555 (Pimpernelx 42No.174)	Norway
CP 2062 (Serrana), CP 2067 (ASN69-1), CP 2132 (Tollocan), CP 2378 (POOS.16), CP 2413 (Piratini), CP 2414 (CEZ69.1), CP 2416 (MEX 750826), CP 3443 (CIP 380474.6), CP 3479 (TS-10),CP 3486 (TS-15), CP 3493 (P-55.7), CP 3577 (E86.300), CP 3756 (LB-X), CP 3762 (CIP 392657.8), CP 3768 (CIP 393280.82), CP 3772 (CIP 393382.44), CP 3781 (CIP 385556.4), CP 3802 (WilaYari)	Peru
CP 1435 (Tedria), CP 1544 (Asoka), CP 1546 (Sirtema), CP 1978 (Amalfy), CP 3535 (Arinda)	Netherlands
CP 3167 (Perkoz)	Poland
CP 2012 (Rosita)	Mexico
CP 3917 (Hermes)	Austria
CP 1012(Unknown), CP 1239 (Unknown), CP 1315 (Unknown), CP 1317 (Unknown)	Unknown
CP 4242 (Bora Valley)	Korea
CP 1604 (B5052-7), CP 1817 (B 6038-1), CP 1890 (B 5698-8), CP 1945 (K 194-3), CP 3973 (Russet Burbank)	United States of America
CP 1812 (HYB.NO.20078), CP 1983 (49.540/2)	Germany
CP 1701 (Belle De Founteny)	France

potato genotypes constituting varieties and advanced stage hybrids the content ranged from 21 to 53 ppm (Trehan and Sharma, 1996).In reference to global scenario, CIP, Peru potato varieties had more narrower range of 11-30 ppm iron content (Bonierbale, 2014). Thus, the present germplasm collection had better iron content than those of varieties and advanced hybrids as till now the potato breeding was mainly focused on quantitative improvement. With the growing consumer awareness and preference, trend has been shifting towards quality breeding with respect to nutrient value along with yield advantage. The best performing genotypes for iron content in the descending order of content were CP 1435 (67.13 ppm), CP 3443 (62 ppm), CP 3772 (55.73 ppm), CP 1239 (52 ppm) and CP 2067 (50.20 ppm).

Zinc content varied from 2.78-35.40 ppm with mean value of 14.29 ppm (Fig 1). CP 3443, CP1978, CP1435, CP2067, CP1812 and CP1983 had the highest zinc content of 35.40, 27.18, 27.13, 26.71, 25.53 and 25.11 ppm, respectively. Indian potato varieties had mean zinc content of 15.29 ppm (9.54 to 21.00 ppm on dry weight basis in potato flesh; Dalamu *et al*., 2016) while the varieties and advanced hybrids had zinc content of 10 to 18 ppm (Trehan and Sharma, 1996). Similarly CIP potato varieties had 8-25 ppm zinc content (Bonierbale 2014). Though, the mean content of zinc in varieties and germplasm are somehow equivalent but the high zinc containing genotypes viz., CP 3443, CP1435 and CP2067 also having high iron content are the potential genotypes for breeding mineral rich potato varieties.

Variations for Tuber Flesh Colour Index

Xanthophylls group of carotenoids impart yellow colour to potato tuber flesh and has antioxidative properties and prevention of age-related macular degeneration. The flesh colour represented in three-digits code where the first digit is for predominant flesh colour, second digit for secondary flesh colour and the third digit for the distribution of the secondary flesh colour (Fig. 2a; Kumar *et al*., 2005) where 100 represents white flesh with no secondary colour, 200 is cream with no secondary colour, 300 is yellow-cream with no secondary colour, 400 is yellow with no secondary colour, 500 is red with no secondary colour and 600 is violet flesh with no secondary colour. The majority of germplasm lies in the range of white to yellow flesh colour (Fig. 2b) except for CP 4242, a purple/violet fleshed genotype. The CP Nos. 1547, 1544, 3768, 1527, 1435, 1317, 1978, 1604, 1239 and 2416 had yellow flesh colour.

Association among Iron, Zinc and Tuber Flesh Colour Index

The significant correlation between zinc content and iron content (r =0.69) insights the feasibility of breeding genotypes with both high iron and zinc content, however, this study relates to only one location and environment thus necessitates validation of association under varied edaphic and environmental conditions. Lack of significant correlation between tuber flesh colour index and iron and zinc content indicates that mineral content and flesh colour are independent traits. Yellowness of tuber flesh colour is an indicator of xanthophylls content and also reflects higher iron bioavailability (Bonierbale *et al.*, 2011). Thus, selection of yellow tuber fleshed germplasm with high iron content is the selection yardstick for breeding iron rich genotypes alongwith yield advantage, agronomic superiority and wider adaptability.

Cluster Analysis Based on Iron and Zinc Content

Cluster analysis of 46 germplasm based on iron and zinc content was performed by Ward's minimum variance method and a dendrogram was constructed as depicted in Figure 3. All the germplasm were resolved into three major clusters.

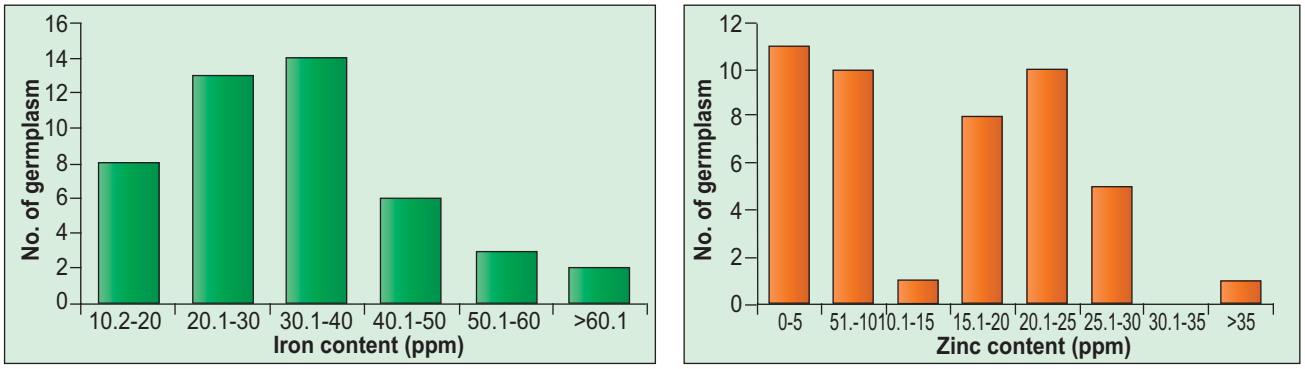

Fig. 1. Frequency distribution of Iron and Zinc content in 46 potato germplasm

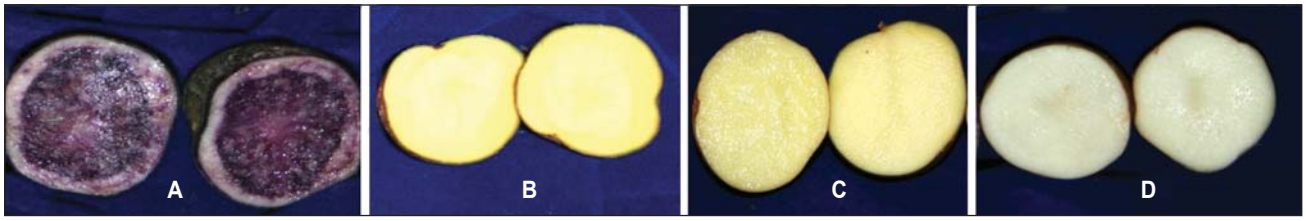

Fig. 2a. Potato tuber flesh colour (A) Violet/purple (B) Yellow (C) Cream (D) White

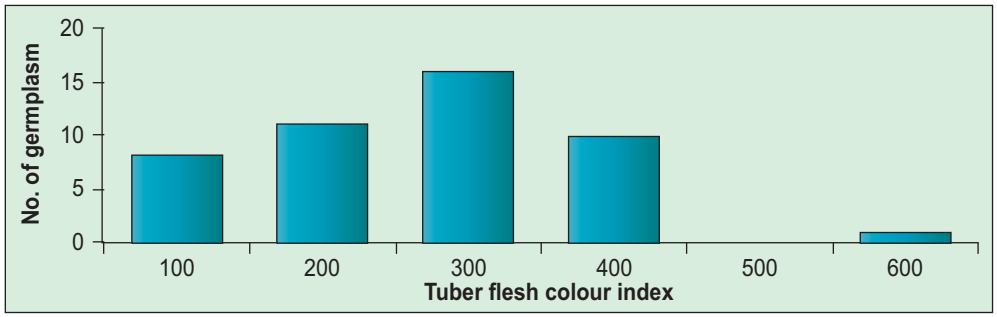

Fig. 2b. Frequency distribution of Tuber flesh colour index in 46 potato germplasm

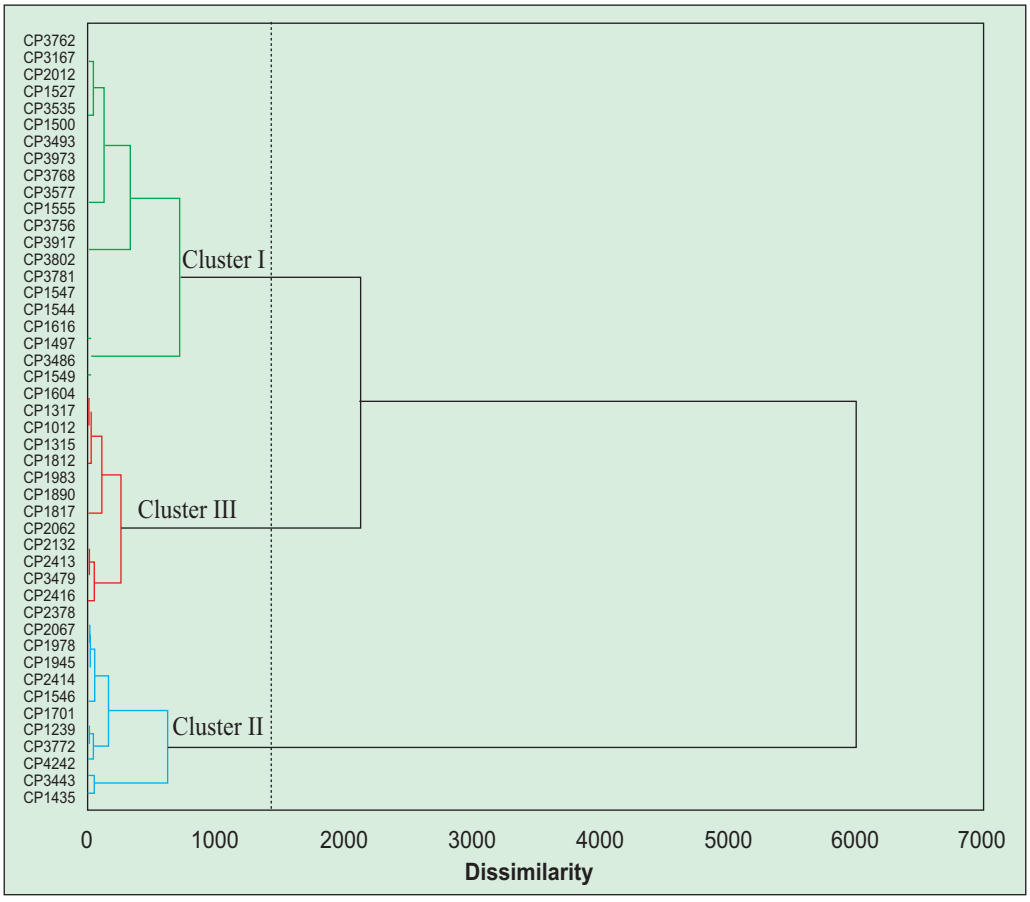

Fig. 3. Clustering of 46 potato germplasm based on Euclidean Distance

Cluster II constituting genotypes CP Nos. 1435, 2414, 4242, 3772, 1945, 1978, 2067, 1701, 1546, 3443 and 1239 had the highest iron (50 ppm) and zinc content (24.56 ppm) while cluster I comprising the maximum number of genotypes (21) had the least iron and zinc content of 24.53 and 5.14 ppm, respectively.

Conclusion

Genotypes CP 1239, CP1435, CP1812, CP1983, CP1978, CP2067, CP2414, CP3443, CP3772 and CP4242 with desirable zinc and iron content individually or in both were identified which will be useful for future breeding programme to develop nutritional superior potatoes.

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Genetic Differentiation of within and among the Genome Groups of Banana (*Musa* spp.) Cultivars of Northeast India

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Genetic variation of 27 banana (*Musa* spp.) cultivars collected from North-eastern region of India belonging to five different genome groups (AA, BB, AAA, AAB and ABB) were studied using RAPD markers. Altogether 30 RAPD markers were tested, out of which nine were successful in generating consistent PCR products. Amongst the 99 PCR products generated, 7.07% were monomorphic and 92.93% were polymorphic bands. High level of genetic diversity values were observed comprising expected genetic heterozygosity (Ht) of 0.24, homozygosity (Hs) of 0.07, genetic differentiation (Gst) of 0.67 and gene flow (Nm) of 0.23. Analysis of molecular variations (AMOVA) also revealed high level of genetic variation among the cultivars of different genomic groups (70%) and within the same genome group (30%). Among the five genome groups studied, AA showed the highest genetic variation. The dendrogram and principal component analysis based on RAPD profiles revealed the successful differentiation and clustering of banana cultivars based on their genome groups except in one cultivar (Noneh-laphu, AA) which was clustered with cultivars belonging to BB group.

Key Words: Genome, Genetic differentiation, Musa, North-eastern region of India, RAPD

Abbreviations: RAPD: randomly amplified polymorphic DNA; UPGMA: unweighted pair group method with arithmetic averages; PIC: Polymorphism information content; AFLP: amplified fragment length polymorphism; CTAB: cetyltrimethyl ammonium bromide

Introduction

Bananas (*Musa* spp.), consisting of both dessert and cooking types, are giant herbaceous monocotyledonous plants that belong to the family Musaceae of the order Zingeberales (Simmonds and Shepherd, 1955). The Musaceae family consist of two genera *Musa* and *Ensete* where *Musa* differs from *Ensete* in producing suckers around the parent plants (Simmonds and Shepherd, 1955; Ħakkinen, 2013). The present day edible and seeded bananas are believed to be derived from hybridization between wild diploid of *M. acuminata* (AA genome) and *M. balbisiana* (BB genome) contributing various levels of ploidy and genomic constitution such as AA, BB, AB, AAA, AAB, ABB, ABBB, etc. (Simmonds and Shepherd, 1955). The North-eastern region (NER) of India has been considered as a rich hub of natural banana diversity where *M. balbisiana* from Indian subcontinent meet *M. acuminata* from South-East Asia (Molina and Kudagamage, 2002). However, the vast genetic resource of wild and cultivated banana in the region remains relatively unexplored and untapped for scientific studies.

Knowledge on the genetic diversity and genetic relationships among the various wild and cultivated bananas will provide valuable information on the genetic status and can be utilized for breeding and sustainable production. The use of morphological data for determination of ploidy levels and genome classification in banana has been supplemented with the use of molecular data (Pollefeys *et al.,* 2004; Pachuau *et al.,* 2014). Among the molecular markers, RAPD, AFLP and microsatellites are frequently used for the characterization of banana (Wong *et al.,* 2002; Onguso *et al.,* 2004). The use of RAPD makers has the advantage of being efficient and inexpensive without requiring prior knowledge of the genome (Bhat *et al.,* 1995). It requires very small amount of genomic DNA without the need for blotting and radioactive detection and are moderately reproducible (Williams *et al.,* 1990; Cipriani *et al.,* 1996; Aagaard *et al.,* 1998). Thus, the present study was undertaken to analyze the genetic variation of banana cultivars of NER of India using RAPD markers.

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Materials and Methods

A total of 27 banana cultivars which were collected from different parts of the NER state of Manipur and previously classified into five genome groups (AA, AAA, BB, ABB and AAB) by Pachuau *et al*. (2014) were used in the RAPD analysis. The details of the cultivars are given in Table 1.

Genomic DNA Isolation

Genomic DNA was extracted from the young cigar leaf tissues using modified CTAB method (Thangjam *et al.*, 2003). The leaves were grounded in 700 µl extraction buffer (1M Tris HCl, pH 8.0; 5M NaCl; 0.5 M EDTA; 10% sodium dodecyl sulphate) and the mixture was treated with 5 µl of proteinase K (20 mg/ml) for 1 h at 36°C. To this cell lysate, 500 µl of CTAB buffer [10% CTAB; 1M Tris HCl pH 8.0; 0.5M EDTA; 5M NaCl; 5% PVP (Polyvinyl pyrolidine] was added and incubated for 2 h at 65°C. The suspension was extracted thrice with equal volume of phenol, chloroform, isoamyl alcohol (24:25:1). The DNA was precipitated using isopropanol, sodium acetate and 70% ethanol; 10 µL RNase (10µg/ml) was added to each 50 µL DNA and incubated for 30 min at 37°C. Purity and quantity of DNA were measured by using Biophotometer plus (Eppendorf, Germany) by taking their absorbance at 260 nm and 280 nm and verified by running on 0.8% agarose electrophoresis to check the quality of DNA along with 1kb plus DNA ladder.

RAPD-PCR and Data Analysis

PCR of the isolated DNA from the 27 banana samples were carried out in a 25µl volume reaction. Each reaction tube contained 50 ng of genomic DNA, 2.5 mM $MgCl_2$, 0.5 mM dNTP mix, 2.5 mM buffer, 1U

Table 1. Details of banana (*Musa* spp.) cultivars from Manipur state of North-eastern India used for genetic diversity study

S. No.	Collector No.	Local cultivar name	Collection site (place, district)	Scientific name	Genome group*
1.	TRSMN- 01	Ragunshen-masung-ahumnaibi	Khongsang, Tamenglong	*Musa* sp.	ABB
2.	TRSMN- 02	Heijao-angouba	Keithelmanbi, Imphal West	*Musa* sp.	ABB
3.	TRSMN- 03	Champa-colla- manbi	Jiribam, Imphal East	*Musa* sp.	AAB
4.	TRSMN- 04	Champa-colla-angangbi	Kangbai, Churachandpur	*Musa* sp.	ABB
5.	TRS MN- 05	Teralaphoi	Pangai, Imphal East	*Musa* sp.	ABB
6.	TRS MN- 06	Luine	Noneh, Imphal West	*M. balbisiana*	BB
7.	TRS MN- 07	Champa-colla	Khumbong, Imphal west	*Musa* sp.	AAB
8.	TRSMN- 08	Korbot	Khuga river, Bisnupur	*M. acuminata*	AA
9.	TRSMN- 09	Noneh-laphu	Noneh, Imphal West	*M. acuminata*	AA
10.	TRSMN- 10	Morpi	Kangbai, Churachandpur	*Musa* sp.	ABB
11.	TRS MN- 11	Hei	Khumbong, Imphal West	*Musa* sp.	ABB
12.	TRSMN- 12	Grandnaine	Keithelmanbi, Imphal West	*M. acuminata*	AAA
13.	TRSMN- 13	Jahaji	Keithelmanbi, Imphal West	*M. acuminata*	AAA
14.	TRSMN- 14	Changbi-mara-chatpi	Sekmai, Imphal East	*M. acuminata*	AA
15.	TRS MN- 15	Hangou	Pangei, Imphal East	*Musa* sp.	ABB
16.	TRSMN- 16	Masung-ahum-naibi	Mayai Leikai, Thoubal	*Musa* sp.	ABB
17.	TRSMN- 17	Meitei-hei	Pangei, Imphal East	*Musa* sp.	ABB
18.	TRSMN- 18	Des-laphu	Sekmai, Imphal East	*M. acuminata*	AAA
19.	TRS MN- 19	Yensang-chabi	Jiribam, Imphal East	*Musa* sp.	ABB
20.	TRSMN- 20	Morshell	Noneh, Imphal West	*Musa* sp.	ABB
21.	TRSMN- 21	Nagunsen	Tipaimukh, Churachandpur	*Musa* sp.	AAB
22.	TRSMN- 22	Chingchup	Bamdyar, Imphal West	*M. acuminata*	AAA
23.	TRSMN- 23	Changbi	Yurembam, Imphal West	*M. balbisiana*	BB
24.	TRSMN- 24	Mizoram-laphu	Monlom, Churachandpur	*M. acuminata*	AAA
25.	TRSMN- 25	Kharam-laphu	Kharam, Imphal West	*M. balbisiana*	BB
26.	TRSMN- 26	Utteibi	Bamon Leikai, Thoubal	*Musa* sp.	ABB
27.	TRSMN- 27	Ngalai	Mata, Churachandpur	*Musa* sp.	ABB

* based on Pachuau *et al.* (2014)

Taq polymerase (Bangalore Genei, India) and 10 pmol of each primer. The RAPD primers (UBC Set # 5) were obtained from University of British Columbia, Vancouver, Canada. The amplification was carried out using a thermal cycler (Applied Biosystems Geneamp PCR System 9700) programmed at 94°C for 4 min., followed by 35 cycles consisting of 94°C for 1 min., 36°C for 50 sec. and 72°C for 2 min. The final extension step was programmed at 72°C for 5 min. Amplified PCR products were separated by electrophoresis along with 100bp standard (100 bp DNA ladder) on 1.8% agarose gel prepared in 1X TAE buffer. DNA banding profiles were visualized and photographed using Gel Documentation System (G:BOX Chemi XT4 version 1.2.5.0, Syngene).

The amplified DNA fragments were treated as a separate character and scored as a discrete variable, using 1 to indicate presence and 0 for absence. Accordingly, rectangular binary data matrix was generated from the RAPD profiles. The efficiency of each primer were studied by evaluating band characteristics such as number of bands (NB), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB).

Polymorphism information content (PIC_i) of a band was calculated according to Anderson *et al.* (1993) as follows:

$$PIC_i = 1 - \sum_j f_{ij}^2 \sum_j f_{ij}^2$$

Where f_{ij} is the frequency of the j^{th} pattern of the i^{th} band (dominant markers have two patterns for a band as being present and absent). Then, the PIC of each primer was calculated as:

$$PIC = 1/n \sum_{i=1}^{n} PIC\ i \sum_{i=1}^{n} PIC\ i$$

where n is the NPB for that primer.

Resolving power (Rp) was calculated according to formula of Prevost and Wilkinson (1999):

$$RP = \sum_{i=1}^{n} IBi \sum_{i=1}^{n} IBi$$

Where Informativeness of a band (BI_i) was calculated as:

$$BI_i = 1 - (2 \times | 0.5 - p)$$

where *p* is the proportion of the all accessions containing the band.

Further we calculated mean resolving power (MRP) for each primer as:

$$MRP = 1/n\ RP$$

Marker index (MI) was calculated as product of PIC and effective multiplex ratio (EMR), which is defined as the product of the fraction of polymorphic loci and the number of polymorphic loci.

MI of each primer was calculated according to Milbourne *et al.,* (1997) as

$$MI = PIC^* EMR$$

Genetic Diversity Analyses

Considering different genomes as a group, a total of five groups were used in the genetic analysis. It was carried out using software PopGene (version 1.32) (Yeh *et al.,* 1997). Nei's genetic distance (Nei, 1978) was calculated by using the programme RAPDDIP, a part of PopGene, between all the studied groups of banana. Shanon Population Index was also calculated; the larger the index, larger is the genetic diversity.

Total heterozygosity (Ht) is the expected heterozygosity, calculated as 1 minus the sum of the average allele frequencies over populations. G_{st}, a measure of population genetic differentiation was calculated (Nei, 1978) as:

$$G_{st} = \frac{Ht - HsHt - Hs}{Ht \quad Ht}$$

Where Hs and Ht are the expected heterozygosity within subpopulations and for the total population.

Effective migration rates (Nm) were estimated based on genetic differentiation (G_{st}) ($Nm=0.5(1-G_{st})/G_{st}$) inbreeding rates (McDermott and McDonald, 1993). An analysis of molecular variance (AMOVA) was performed by GenAlEx ver. 6.501 (Peakall and Smouse, 2006) to assess the overall distribution of molecular variance among and within different banana groups.

Genetic Relationships, Cluster Analysis and Principal Component Analysis

A pairwise comparison of the 27 samples was done based on simple matching (SM) matrix to show the similarity coefficient between the samples. A dendrogram, summarizing the genetic relationships among all samples was constructed using the same matrix employing UPGMA method. Principal component analysis (PCA) was carried out to assess the genetic distances among the groups. These analyses were conducted using the software NTSYS-pc version 2.20a (Numerical Taxonomy and Multivariate Analysis for Personal Computer, Rohlf, 2000).

Results

A total of 30 UBC-RAPD primers (set # 5) were initially screened with the DNA isolated from 27 banana samples for their amplification. Out of the 30 primers only nine primers (UBC-414, UBC-417, UBC-425, UBC-433, UBC-438, UBC-445, UBC-447, UBC-449 and UBC-450) produced clear and reproducible banding patterns in all the banana samples and two primers (UBC-440 and UBC-441) produced monomorphic bands thus, nine primers were used for further experiments. Fig. 1 shows the amplification pattern of 27 samples using UBC-438 primer.

Genetic Variation Revealed by RAPD Analysis

The nine UBC-RAPD primers generated a total of 99 bands ranging from 250-3000 bp molecular weight in the 27 tested samples (Table 2). The primer UBC-438 produced the highest number of bands (17) whereas UBC-417 generated the lowest number (6) of scorable bands. Out of the 99 bands, 92 were found polymorphic (92.93%). Polymorphic information content (PIC) was found highest in primer UBC-433 (0.59) while UBC-417 has least PIC value of 0.38. The resolving power (Rp) which indicates ability to distinguished groups was found highest in UBC-425 (6.2) and the lowest in UBC-433 (1.00). The MRP ranged from 0.25 – 0.83. MI, which is the discriminating power for a marker was found highest in UBC-438 (7.48) and lowest in UBC-433 (1.06). Among the nine primers, 5 primers (UBC-414, UBC-438, UBC-445, UBC-425 and UBC- 447) showed 100% polymorphism.

Analysis of Genetic Diversity within and among Genome Groups

The total heterozygosity was calculated as 0.245, a rich genetic variation among sample which is evident from a high value of G_{st}, *i.e.* 0.676, indicating a very great genetic differentiation. Effective migration rates (Nm) was observed to be 0.238, depicting a very limited gene flow (Table 3).

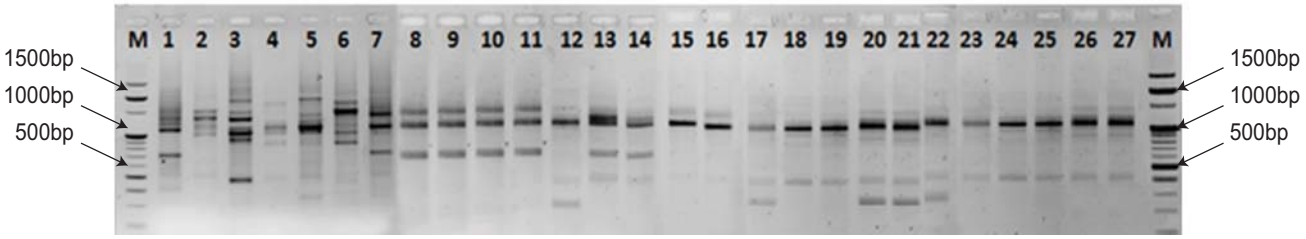

Fig. 1. RAPD-PCR profile of the 27 banana cultivars of Manipur using UBC primer #445.
M: 100bp marker; 1: Korbot; 2: Noneh-laphu; 3: Changb-imara-chatpi; 4: Luine; 5: Changbi; 6: Kharam-laphu; 7: Grandnaine; 8: Jahaji; 9: Des-laphu; 10: Chingchup; 11: Mizoram-laphu; 12: Champa-colla-manbi; 13: Champa-colla; 14: Nagunen; 15: Ragunshen-masung-ahum-naibi; 16: Heijao-angouba; 17: Champa-colla-angangbi; 18:Teralaphoi; 19: Morpi; 20: Hei; 21: Hangou; 22: Masung-ahum naibi; 23: Meitei-hei; 24:Yensang-chabi; 25: Morshell; 26: Utteibi; 27: Ngalai

Table 2. Details of RAPD–PCR amplification obtained from the 27 banana cultivars

UBC- RAPD Primer set #5	Sequence (5′→3′)	Band size (bp)	Total band (No.)	PMB	% PM	PIC	R_P	MRP	EMR	MI
UBC-414	AAGGCACCAG	500-2000	9	9	100	0.39	3.0	0.33	9	3.51
UBC-417	GACAGGCCAA	600-1600	6	3	50	0.38	2.5	0.83	1.5	0.57
UBC-425	CGTCGGGCCT	300-3000	14	14	100	0.46	6.2	0.44	14	6.44
UBC-433	TCACGTGCCT	800-2000	5	3	60	0.59	1.0	0.33	1.8	1.06
UBC-433	TCACGTGCCT	800-2000	5	3	60	0.59	1.0	0.33	1.8	1.06
UBC-438	CGTCGGGCCT	300-3000	17	17	100	0.44	5.1	0.3	17	7.48
UBC-445	TAGCAGCTTG	250 -2000	16	16	100	0.32	4.8	0.3	16	5.12
UBC-447	CAGGCTCTA	500-2000	10	10	100	0.50	3.04	0.30	10	5.00
UBC-449	GAGGTTCAAC	400-1500	10	9	90	0.50	2.5	0.27	8.1	4.05
UBC-450	CGGAGAGCCC	400-1600	12	11	91.6	0.52	2.88	0.25	10.08	5.24
			99	92†	92.93*					

*Mean; †Total
PMB: Polymorphic band; % PM: Percentage of polymorphic band; PIC: Polymorphic information content; ABA: Average band; Rp: Resolving power; EMR: Effective multiplex ratio; MI: Marker index.

Analysis of Molecular Variance (AMOVA) among the Groups

The genetic variation generated by the nine RAPD markers within the five banana groups in the present study were 52.53% in AA group, 24.24% in BB group, 10.10% in AAB group, 4.04% in AAB group and 23.3% in ABB group (Table 4). Among the groups, AA exhibits the highest variability with 52.53% of percentage polymorphic band (PPB), 1.52 allelle number (*Ae*), 1.3 effective allele number (*Ao*), 0.18 Nei's genetic diversity (*He*) and 0.28 Shannon index (I). The least variability was found in AAB group with 4.04 % of PPB, 1.04 allelle number (*Ae*), 1.02 effective allele number (*Ao*), 0.01 Nei's genetic diversity (*He*) and 0.02 Shannon index (I). The estimates of Nei's genetic identity and genetic distance of pairwise comparison between the groups pairs (Table 5) showed that the highest identity existed between AA and BB group with a value of 0.84 and the least identity exhibited between AAB and BB group (0.69). The overall genetic variation was done in the present study using analysis of molecular variance (AMOVA) among five groups. The significant levels of differentiation (Φ_{PT}) was calculated with 1000 permutation among the groups and it showed that the genetic differentiation among the banana cultivars belonging to the five genome groups was statistically significant (P<0.001) (Table 6). Of the total molecular variance, 70% variation was found in among the groups and 30% variation within the populations (Fig. 2).

Genetic Relationships, Cluster Analysis and Principal Component Analysis

A pairwise comparison of the 27 samples shows that the similarity coefficient ranged from 0.55 between the cultivars 'Kharam-laphu' and 'Champa-colla-manbi' to 1.0 between 'Jahaji' and 'Grand naine'. The dendrogram obtained by using (UPGMA) revealed clustering of the 27 banana cultivars into three major clusters (Fig. 3). The first major cluster has two of the cultivars belonging to AA genome (Korbot and Changbi-mara-chatpi) while

Table 3. Details of genetic differentiation obtained from RAPD analysis of the 27 banana cultivars

Samples	Primers	Overall, Ht	Overall, Hs	Overall, Gst	Overall Nm
27	9	0.245†±0.025*	0.0793†± 0.003*	0.676 *	0.238*

*Standard deviation; † Mean

Overall Ht: heterozygosity; Hs: expected within population heterzygosity; G_{st}: genetic differentiation; Nm: gene flow from Gst.

Table 4. Analysis of genetic variation generated by RAPD markers in the 27 banana cultivars belonging to 5 genome groups

Genome group	Percentage polymorphic band (PPB)	Allele number (Ao)	Effective allele number (Ae)	Nei's gene diversity (He)	Shannon index (I)
AA	52.53	1.52	1.30	0.18	0.28
BB	24.24	1.24	1.17	0.09	0.14
AAA	10.10	1.10	1.06	0.03	0.05
AAB	4.04	1.04	1.02	0.01	0.02
ABB	23.30	1.23	1.09	0.05	0.09

Table 5. Measure of Nei's (1972) genetic identity (above diagonal) and genetic distance (below diagonal) between the 27 banana cultivars belonging to the 5 genome groups studied

Genome group	AA	BB	AAA	AAB	ABB
AA	****	0.84	0.80	0.77	0.80
BB	0.16	****	0.74	0.69	0.74
AAA	0.21	0.29	****	0.77	0.78
AAB	0.25	0.37	0.25	****	0.80
ABB	0.22	0.29	0.24	0.22	****

Table 6. Details of AMOVA between the banana groups generated from the RAPD profiles of banana cultivars studied

Source	df	SS	MS	Est. var.	Percent	PT	p value
Among genome groups	4	217.293	54.323	10.598	70	0.703	0.001
Within genome groups	22	98.410	4.473	4.473	30		

df: degree of freedom; SS: sum of square; MS: mean of square; Est.var: estimated variance; percent: percentage of variance.

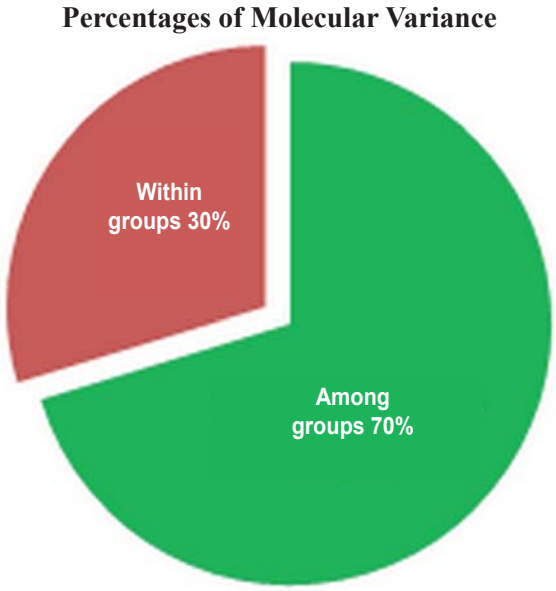

Fig. 2. Distribution of molecular variance within and among the genome groups obtained from the RAPD data

the second major cluster has been grouped into three minor clusters with the first minor cluster comprises of five triploid (AAA) cultivars ('Grandnaine', 'Jahaji', 'Des-laphu', 'Chingchup' and 'Mizoram-laphu') which are all AAA genome group, the second minor cluster comprises three AAB cultivars ('Champa-colla-manbi', 'Champa-colla' and 'Nagunsen') and the third minor cluster with 13 ABB cultivars ('Ragunsen-masung-ahum-naibi', 'Heijao-angouba', 'Champa-colla-angangbi', 'Teralaphoi', 'Morpi', 'Hei', 'Hangou', 'Masung-ahum-naibi', 'Meitei-hei', 'Yensang-chabi', 'Morshell', 'Utteibi' and 'Ngalai').

The third major cluster was subdivided into two clusters- the first minor cluster represented a lone AA genome cultivar ('Noneh-laphu') while the second cluster has three cultivars of BB genome ('Luine', 'Changbi' and 'Kharam-laphu').

The principal component analysis represented by the 2-D projection diagram (Fig. 4) was also in accordance with the dendrogram, projecting 'Noneh-laphu' (AA) near to BB genome group cultivars ('Changbi', 'Luine', 'Kharam-laphu') and far from other AA genome cultivars- 'Korbot' and 'Changbi-mara-chatpi'.

Discussion

In the present study the selected nine RAPD markers generated a total of 99 bands from the 27 selected banana cultivars, with an average of 14.14 bands per primer. Out of the 99 bands amplified, 7.07% were monomorphic and 92.93% were polymorphic bands. The generation of high level of polymorphic bands indicated the existence of a large genetic variability in the banana samples studied. Among the primers, UBC-438 produced the highest number of bands (17) and was the best in terms of detecting polymorphism (100%) with the Rp of 5.1 and MI of 7.48 respectively.

Analysis of the RAPD profiles revealed a high degree of genetic diversity as evident from the difference between the values of expected heterozygosity (Ht)

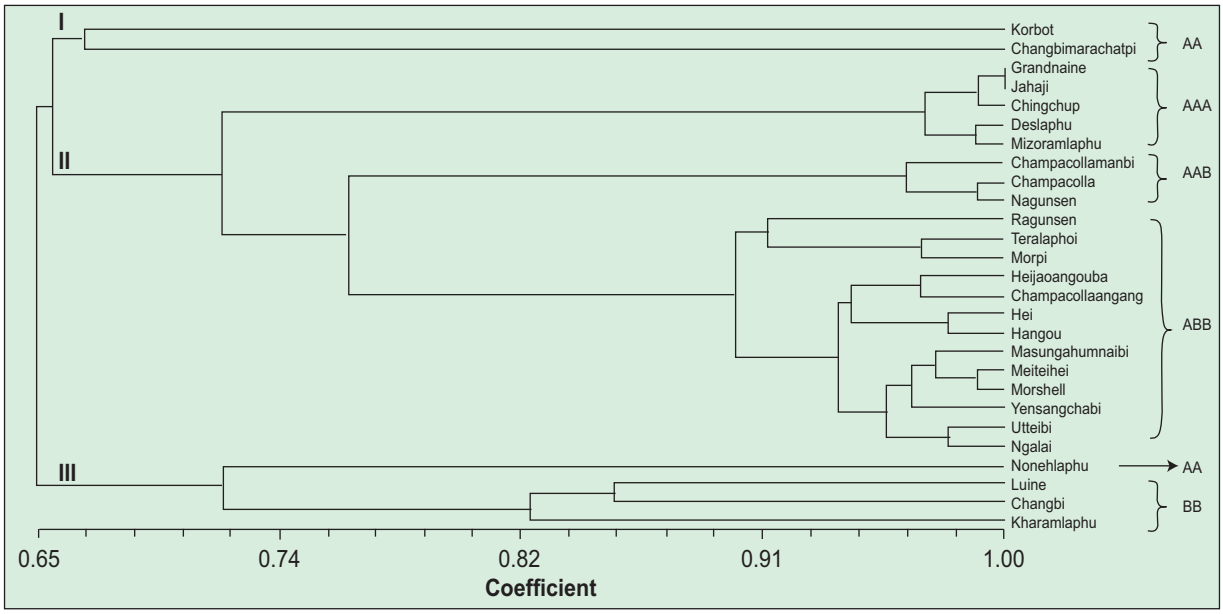

Fig. 3. Dendrogram obtained from the analysis of 5 different genome groups using RAPD data from 27 banana cultivars

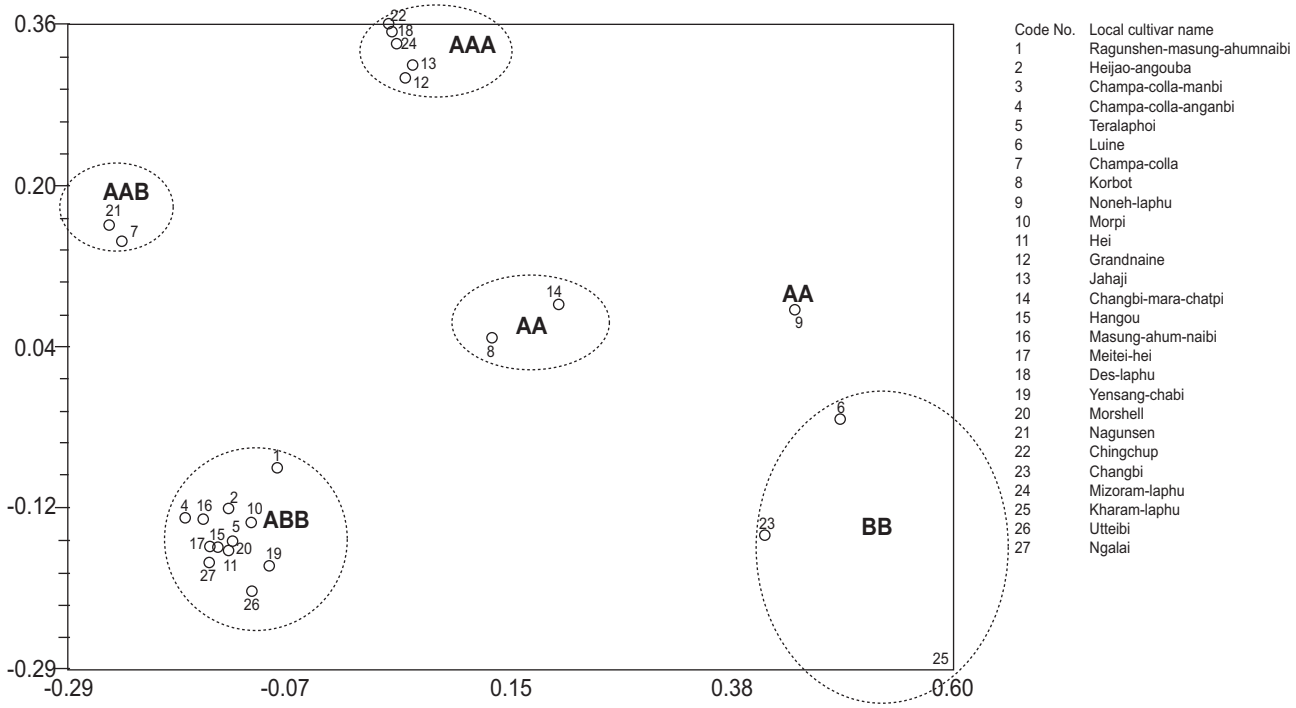

Fig. 4. 2-D view of Principal component analysis (PCA) based RAPD data using similarity (SM) coefficient of the different 27 banana cultivars

with 0.24 and homozygosity (Hs) of 0.07. The genetic differentiation (G_{st}) value of 0.67 and gene flow (Nm) value of 0.23 revealed the existence of high genetic differentiation and low gene flow among the cultivars of different genome groups studied. Similar observation has been reported by Resmi *et al.,* (2016) in banana cultivars from Southern India with a G_{st} value of 0.69. Lamare and Rao (2015) reported higher proportion of genetic variation among 25 *M. acuminata* genotypes from Meghalaya state of NER of India with a G_{st} value of 0.451. The source of difference may be attributed to its parthenocarpic nature and wide range of ecological condition within the distribution area and its adaptability to various environmental conditions. Studies on 40 cultivars of *M. balbisiana* by Oreiro *et al.* (2006) also revealed high levels of genetic differentiation with Ht values of 0.411. The analysis of molecular variance showed that high level of genetic variation occurred among the groups (70%) than within the groups (30%). Among the five genome groups studied, AA group showed the highest genetic variation with high Shannon index (I) value of 0.28, Nei's gene diversity (He) 0.18, effective allele number (Ae) 1.30 and PPB 52.5%. Similar observation has been reported by Resmi *et al.* (2011; 2016). Creste *et al.* (2003) postulated that high level of genetic diversity in AA diploid cultivars may be because of limited fertility and heterozygosity for chromosome structural abnormalities, maintained by vegetative propagation. Grapin *et al.* (1998) also reported that heterozygous condition mostly occurred in microsatellite loci in cultivated diploids. Ge *et al.* (2005) reported that heterozygosity may have occurred due to somatic mutation that accumulates genetic variation. Nei's genetic identity showed that the highest similarity occurred between the two wild types (AA groups and BB groups) with a value of 0.84 and lowest between BB groups and AAB groups (0.69). Similar observation has been made by Mukundakumar *et al.* (2013) in wild *Musa* with high level of genetic diversity (H=0.47, Shannon index=0.66, Ae No.=1.89). In the present study, analysis of the genetic relationship between the 27 cultivars based on RAPD markers revealed that, with the exception of 'Noneh-laphu' (AA) which is grouped along with BB group, all the remaining cultivars were clustered according to their genome groups which is not conforming to the findings of Pillay *et al.* (2000). The inclusion of the A genome containing cultivar, 'Noneh-laphu' (AA) within cluster of BB genome and its wide separation from other AA genome group cultivars is, however, explained by the fact that 'Noneh-laphu' may have undergone repeated selection/mutation that resulted in genome far from its ancestral diploid progenitors

(Bhat and Jarret, 1995; Resmi *et al.*, 2016). This finding is supported by the fact that Noneh laphu shares many similar morphological characters like plant height, male bud and presence of seed in fruit compared to remaining AA genome group cultivars like 'Korbot' and 'Changbi-mara-chatpi'.

The present study showed the usefulness of RAPD markers for the analysis of genetic variability, the distribution of variability and the genetic relationship present within the *Musa* germplasm of NER of India, which is an important region of banana diversity and origin. The ability of this marker to differentiate and analyse the cultivars of A and B genomes efficiently will be useful for characterization and understanding of the potential banana genetic resources for breeding and conservation programmes.

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National Herbarium of Cultivated Plants: A Resource for Study of Crop Genepools

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National Herbarium of Cultivated Plants (code 'NHCP') at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India occupies an important place among 25 major Indian herbaria. NHCP presently holds collection of 22,778 herbarium specimens representing 265 families, 1500 genera and 4,156 species (besides ~6,000 images maintained as virtual herbarium). Besides, seed and carpological samples/economic products are represented as complementary collection. Variability is depicted in crop plants as cultivars, primitive types/ landraces, crop wild relatives (CWR)/weedy types, wild/semi-domesticated forms and taxa introduced for breeding purpose, and potential species. These features make the NHCP unique among other herbaria in India. The NHCP with significant holdings representing over 500 crop taxa under genera: *Oryza*, *Sorghum*, *Vigna*, *Cajanus/Atylosia*, *Allium*, *Abelmoschus*, *Solanum*, *Cucumis*, *Trichosanthes*, *Piper*, *Curcuma*, *Rosa*, *Prunus*, etc. and over 550 crop wild relatives serves as a resource for study of crop genepools.

Key Words: Crop Genepools, National Bureau of Plant Genetic Resources, National Herbarium of Cultivated Plants, NHCP, Virtual Herbarium

Introduction

Global herbarium resources consist of approximately 4,000 recognised herbaria collectively holding over 35,00,00,000 herbarium specimens. India represents over 3.5 million herbarium specimens including over 23,000 type specimens (source: http://sciweb.nybg.org/science2/IndexHerbariorum.asp). An ideally dried herbarium with character representation (vegetative characters: roots, tubers, bulbs and rhizome, leaf, stipule, spine, bark, etc. and floral characters: inflorescence, flower- spathe, scape, stamen, sepal, petal/tepals; and fruit characters: pericarp, placentation, seed) are good resources for taxonomic studies (Lawrence, 1951; Davis and Heywood, 1963; Holmgren and Holmgren, 1998). Besides, information on plant species with respect to the area of availability, variability pattern, flowering/fruiting time, threat status and endemism, other ecological features, economic uses, indigenous traditional knowledge (ITKs), etc. gathered from herbarium data serves as resource for basic and applied research, referral use and for educational programme.

Major global herbaria are committed to provide herbarium resources accessible to users with holdings including large representation of vascular plants especially those with major economic value (BM, E, K, P, MO, S, B, UC/JEPS), and yet some others focus on regional flora (F, PE, CAL); only few are rich in representation of cultivated plants (only for cultivated ornamentals). Among the cultivated plant herbaria, The Gatersleben Herbarium (GAT) located in the Department of Genebank of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) is one of the largest specialized herbaria which serves as a source of reference and working material for the reproduction of accessions maintained in genebank and other institutional research programme. Over 4.30 lacs specimens of cultivated plants and its wild relatives, seed and fruit collection (about 1 lac samples) and the spike collection (55,000 samples) are well represented. The holding include mainly the vascular plant species of Europe and the Mediterranean and the temperate region, eastern and middle Asia, Mongolia and Cuba.

The herbarium of cultivated plants at ICAR-National Bureau of Plant Genetic Resources, New Delhi, also known as National Herbarium of Cultivated Plants (code 'NHCP') occupies an important place among the 25 major Indian herbaria (Singh, 2010; Nayar *et al.*, 2014). The NHCP is listed in the Index Herbariorum which is a global directory of public herbaria in different regions (Holmgren and Holmgren, 1998; http://sciweb.nybg.org/science2/IndexHerbariorum.asp). It holds significant collections mainly of cultivated taxa and wild relatives/weedy relatives of both native and exotic origin, and taxa of potential value identified through plant genetic resource (PGR) programme. Besides, seed and carpological samples/economic products of plant genetic

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resources (PGR) relevance serve as complementary collection. Digitization herbarium images of selected specimens serve as an important resource for research activities at the institute.

The NHCP differs in its mandate from the general herbaria across the country in representing wide range of variability in crop plants depicted as cultivars, primitive types/ landraces, wild/semi domesticated forms and crop wild relatives (CWR)/ weedy types and also the minor economic species collected from different agro-ecological regions of India under various PGR programmes. In addition herbarium specimens of exotic germplasm introduced under various research programme, local flora of Delhi, weed flora, vouchers of research material deposited are well represented. It intends to serve as a reference collection for identification, taxonomic study and for teaching.

Historical Perspective

Taxonomy of cultivated plants has largely been a neglected field (Huaman and Spooner, 2002). Traditional herbaria rarely focus on herbarium collections depicting variability within cultivars, primitive forms, landraces, and as also obsolete cultivars. Keeping this in view the Herbarium of Plant Introduction Division of Indian Agricultural Research Institute was reportedly set up in 1948. During 1948-1974 nearly 5,000 specimens were added through collections made under various genetic resource programmes *viz*. evaluation, breeding and plant introduction. The herbarium was rechristened in 1985 as National Herbarium of Cultivated Plants with its location at the ICAR-National Bureau of Plant Genetic Resources, New Delhi. To lay thrust on building-up of infrastructure facilities work was taken up in project mode in 1985 under institutional project entitled "Establishment, build-up and maintenance of herbarium and seed museum of cultivated plants" under the leadership of Dr. E Roshini Nayar as the curator of NHCP.

Specialised training attained by the scientific staff of the herbarium at various national and international herbaria such as the Royal Botanic Gardens at Kew, and New York Botanical Garden and Arnold Arboretum in USA and Wealth of India Herbarium, New Delhi and Botanical Survey of India and Forest Research Institute, Dehradun and National Botanical Research Institute, Lucknow, Uttar Pradesh facilitated in upgradation of infrastructure. The insect-pest- and dust-free storage cabinets gradually replaced the traditional pigeon hole cabinets. With addition of five new-space saver compactors during 2004-2008, the capacity of herbarium has increased to up to 40,000 specimens.

National Herbarium of Cultivated Plants

Build-up of Herbarium Specimens

The National Herbarium of Cultivated Plants (NHCP) presently has 22,778 herbarium specimens representative of 265 families, 1500 genera and 4,156 species (as on August, 2017) of important taxa of plant genetic resource (PGR) relevance including over 500 crop taxa and 550 species of crop wild relatives (CWR)/weedy relatives (Pandey *et al*., 2015a). Additionally, the seed and the economic botany museum provide a reference to collection of crop, wild and weedy plants. Build-up of material is through specimens/ seeds collected during explorations in different agro-ecological zones of India, material introduced from abroad under various research/ breeding/selection programmes and also vouchers deposited of the systematic studies on crop-groups. Presently there is thrust on target collections towards landraces, CWR and with thrust in collections from unrepresented areas (as neglected regions, across eco-geographical regions, tribal/ north-eastern region).

To achieve the targeted collection, gaps are revisited from time to time. Some important works have served as baseline for build-up of material in the NHCP: cultivated taxa (and variability within them) of crop/ economic species (Ambasta *et al.*, 1986; Nayar *et al*., 2003), wild relatives of major crop taxa (Arora and Nayar, 1984; Pandey *et al*., 2005) and wild edible and economic taxa (Arora and Pandey, 1996).

System of arrangement of herbarium specimens differs from that of the other herbaria; specimens are arranged by families, then by genera and then by species; all in an alphabetical order. This was found more convenient for wide use by PGR workers, para-botanists and non-taxonomists and beginners. For efficient access to use the resources, documentation of the herbarium holdings as soft data, images as virtual herbarium, Index Cards and inventory of digitised taxa (Nayar *et al*., 2011) can be referred. Facilities such as net-house to grow out for identification and teaching purpose, experimental area for study on selected taxa and raising material received as vegetative propagules/ seed and are also available. In addition, standardization of methodology for economic/eco-friendly storage, specialized groups such as landraces (variation), difficult

groups (succulents, large fruited types, aquatic plants, plants tending to leaf fall on drying), etc. (Pandey *et al*., 2013) is in progress. Guidelines for effective use of the herbarium for consultation/visit to NHCP and identification/authentication of species are well in place (Pandey, 2015a, b).

Current Holdings

Year-wise holdings of herbarium specimens maintained in the NHCP (as on August 31, 2017) are given in Figure1).

Some Significant Collections

Besides herbarium specimens of cultivated plants, some neglected groups: less-known domesticated species *viz*. *Moghania vestita* (Soh-phlong), *Digitaria* (Raishan), *Coix lacryma-jobi* in north-eastern hills, and others such as *Malva verticillata, Inula racemosa, Hodgsonia heteroclita*, *Brachiaria mutica*, *Aisandra butyracea* (Cheura), *Adansonia digitata* (Gorakh imli), *Setaria glauca*, *Momordica dioica*, *Allium* spp., rice bean, winged bean, *Vigna vexillata*, and taxa of potential/commercial value are represented.

Important taxa of crop wild relatives (CWR) maintained in the NHCP include: *Oryza*, *Sorghum*, *Vigna*, *Cajanus/Atylosia*, *Solanum*, *Abelmoschus*, *Cucumis*, *Luffa*, *Allium*, *Trichosanthes*, *Sesamum*, *Curcuma*, *Piper*, *Amaranthus*, *Melilotus*, *Medicago* and *Trifolium*. Some specialty collections include wild *Vigna* from north-western Himalaya, fodder grasses and forage legumes from north-western areas and peninsular India, and wild *Allium* from high altitude areas of western and eastern Himalaya; and wild Triticeae from western Himalaya.

Type collections of newly described taxa by ICAR-NBPGR under genera *viz. Curcuma*, *Abelmoschus*, *Vigna*, *Cucumis*, *Herpetospermum*, *Momordica*, etc. are some valuable collections maintained in NHCP. *Eragrostiella bifaria* (Vahl) Bor (HS3007) collected from the Delhi Ridge area (10.9.1939 by Dr. HB Singh), *Indigofera viscosa* (HS5390- the oldest herbarium specimen collected in 1933 from Baluchistan, now in Pakistan), *Vicia hyaeniscyamus* (HS8539- exotic material raised in Plant Quarantine Experimental Fields, NBPGR, Delhi), rare/ endangered taxa- *Cycas beddomei*, *Podophyllum hexandrum* (anti-cancer plant), etc. form some significant holdings.

Herbarium specimens of PGR of exotic origin include those prepared under the Plant Introduction (PI) Scheme operational in the Botany Division of the IARI commenced functioning in 1946. Some important genera/crop groups represented under this category included *Oryza* from Philippines; *Avena* from Australia; *Brassica* from Canada; *Medicago* from Portugal, Australia; *Trifolium* from Australia, Portugal, UK; *Vicia* from Australia; *Solanum* from USA and Sri Lanka; *Lycopersicon* from South America; others such as *Amaranthus*, chenopods from USA; dwarf peaches from Australia and *Agathis*, *Calluna*, *Corynocarpus* from New Zealand (Nayar *et al.*, 2011). Virtual herbarium specimens of 298 taxa representing 482 exotic germplasm accessions bearing Exotic Collection (EC)

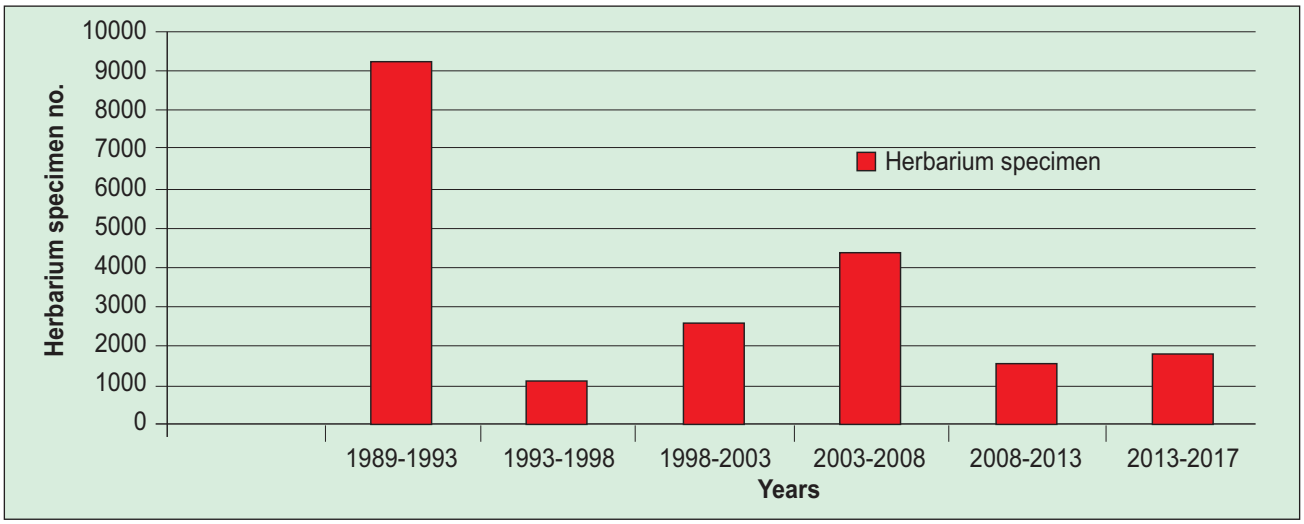

Fig. 1. Year-wise additions of herbarium specimens, seed samples and economic products maintained in the NHCP (August, 2017)

numbers have been uploaded in website for wider use (http://192.168.5.92/NHCP/Advancesearch.aspx).

Herbarium specimens gathered during special programmes: Grassland Survey Scheme of PL-480 (1960-70s); Flora of Karnataka Project (1980s); Project on Collection of Fodder Grasses and Forage Legumes from North-western region (1948-86); National Agricultural Technology Project (NATP) on Plant Biodiversity (1999-2005); Subproject on Biosystematics of the Genera- *Vigna, Cucumis*, and *Abelmoschus* under National Agricultural Innovation Project (NAIP) (2011-14); Herbarium of Dr YS Rao's Collection on Aquatic Plants (1948-1986) have significantly contributed to NHCP holdings.

Seed collection (3,087) and economic products (694 samples) are maintained as complementary collection in NHCP (Table 1). Seed samples are maintained in plastic boxes in dry form depicting species under genus: *Oryza*, *Vigna*, *Phaseolus, Pisum*, *Cicer, Solanum, Moringa, Cucurbita, Capsicum, Trichosanthes*, *Momordica*, *Allium*, *Luffa, Brassica*, *Sesamum, Ricinus*, *Gossypium, Crotalaria,* etc. Economic botany collection is maintained as bulky herbarium (wet preservation or dry form). Some significant ones include- *Lagenaria siceraria* (90 cm long fruit; dry fruits as decorative items); five taxa of *Luffa* (dry fibrous fruit), *Entada phaseoloides* (pod 60 cm; seed; stem); *Moringa oleifera* (fruit and seed collection of cultivated and wild types); *Musa balbisiana* (inflorescence and fruits); *Juglans regia* and *Prunus amygdalus* (fruits), coconut (fruit, coir); arecanut (fruit, plates made from spathe); *Ravenala madagascariensis* (inflorescence); *Trapa bispinosa* (dry fruit); *Diospyros* (fruits of four species); *Aleurites moluccana* (nuts); *Glycyrrhiza glabra* (roots), *Moghania vestita* and *Pachyrrhizus erosus* (tubers), sorghum and okra (landrace diversity as inflorescence/ fruit); *Gossypium arboreum* (bolls of different races); *Triticum* and related taxa (species diversity as spike); *Curcuma*, ginger (rhizome); *Pandanus odoratissimus* (male inflorescence); *Sassurea lappa* (rootstock); *Tecomella undulata* (bark), *Commiphora mukul* (gum crystals), *Saraca indica* (fruits); *Garcinia gummi-gutta* and *G. indica*–kokam (fruits) and *Terminalia* (species diversity as fruit).

Virtual Herbarium

Digital images are present as virtual herbarium in alphabetical order in family, genus and species folders. These images have been labeled with unique identity numbers (as of herbarium specimens) and linked to database. Digital images for over 4,000 species (~ 6,000 images) of crop plants and their wild relatives and potentially useful plants are available for use. Digitization process involves scanning of authenticated/identified specimens/taxa, linking with digital images of reference herbarium specimen(s); working out 'spot' characters for identification of species (closer view, if needed). Digital scans (jpeg images) with good resolution (300 dpi for close up of parts-seed, trichomes, and 600-1200dpi for micro details) not only facilitate fast access of material for identification but drastically reduce chances of damage due to routine handling.

Significant Achievements

Taxonomic and Systematic Study: Taxonomic and systematic studies on native crop taxa viz. *Oryza*, *Vigna, Crotalaria*, *Cajanus/Atylosia*, *Macrotyloma, Sesamum*, *Abelmoschus*, *Luffa*, *Trichosanthes, Allium, Moringa*, and *Ocimum* have helped in better understanding the identity/relationship of taxa under these crop genera of PGR relevance (Nayar, 2015). Taxa of dubious identity are studied in detail for further validation using evidences from biochemical and molecular tools under inter-institutional collaborative research works. Many important wild relatives of crop plants in the Indian region have been enumerated in the publication by Pradheep *et al*. (2014). Based on characters of taxa studied, field identification keys are prepared for genera *Vigna*, *Crotalaria*, *Allium*, *Trichosanthes*, *Luffa* and other taxa (Pandey and Pandey, 2005; Pandey and Nayar, 1994, Pandey and Bhatt, 2008; Pandey *et al.*, 2014a, 2014c).

During systematic study undertaken in NHCP not only voucher materials have been added but also significant information was generated. Material and information on newly described taxon [*Herpetospermum operculatum*- Pradheep *et al*., 2014; *Trichosanthes* - Pradheep *et al.,* 2015); study on extended distribution (Pradheep *et al*., 2013; Pandey *et al*., 2015a, Pradheep and Soyimchiten, 2016; Soyimchiten *et al.*, 2016; Pandey *et al*., 2017 in press); and diversity and ecogeographic analysis of important taxa/crops such as maize, paddy, moringa, *Luffa*, *Ocimum*, *Juglans* (Pandey *et al.*, 2014a, b; Semwal *et al*., 2014; Malav *et al.*, 2015; Soyimchetan and Pradheep, 2016; (Pradheep *et al.*, 2011) are of greater important knowledge base available for use.

Study on domestication trends: Crop taxa important for Indian region- *Luffa*, *Moringa*, *Ocimum*, *Crotalaria*, etc. were studied for available diversity in the region through field and study of herbarium resources. Range of diversity available here facilitates study of trends of domestication thereby facilitated highlighting changes in character during this process (Pradheepet al., 2011; Pradheep *et al*., 2015; Pandey *et al*., 2016).

Potential species of PGR value identified for new uses/new records included- *Crotalaria tetragona* (tum thang, Bhatt *et al*., 2009a), *Bidens pilosa* (Bhatt *et al*., 2009b), *Plukenetia corniculata* (meetha patta), *Ziziphus nummularia* (ber), *Hodgsonia heteroclita*, *Abelmoschus tetraphyllus* (Sukhlai) (Pandey *et al*., 2010, 2011a, b; Pandey *et al.,* 2014; Pradheep *et al.*, 2015b; Semwal *et al*., 2014; Pandey *et al.,* 2015; Rathi *et al*., 2016). Eco-geographic study on taxa of PGR relevance done through resources available in NHCP has supported other research programmes in the institute and has facilitating viewing new dimensions to the existing crop genepool (Pradheep *et al.*, 2011).

Development of Protocol for Safe Preservation: Protocols are being developed for difficult-to-store groups, pest-free storage, ideal storage conditions for families which are sensitive to pest damage and through standardization of use of low temperature (-20°C) using deep freezer, dusting of naphthalene powder, etc. Difficult-to-represent taxa like bulbous group, tuberous/rhizomatous taxa, *Musa, Agave,* etc. are being work out for representation in NHCP. Routine drying techniques using modified methods and microwave drying techniques for difficult material are being further refined based on traditional methods (Jain and Rao 1977; Pandey *et al*., 2006a; Pandey *et al*., 2016; Pandey *et al*., 2013). Keeping in view the material used for advance studies such as biosystematic study, biochemical/ phytochemical and molecular study the efforts have been on minimal use of hazardous chemicals for maintaining the specimens. To minimize use of contact poisons/ chemicals, and insect repellants (naphthalene balls), deep freezing methods are preferred.

Significant Documents: Several publications in the form of books, manuals, chapters, and research papers on new geographical distribution have been brought out. Some significant ones include: Wild Relatives of Crop Plants of India (Arora and Nayar, 1984), Wild Edible Plants of India (Arora and Pandey, 1996), Wild Relatives of Crop Plants- Collection and Conservation (Pandey *et al*., 2008), Genetic Resources of Rosaceae of India (Pandey *et al*., 2007) and Guidelines for Use of NHCP (Nayar *et al.,* 1999-2005) (krishikosh.egranth.ac.in/.../1/2035781; krishikosh.egranth.ac.in/.../1/.../1/8.pdf) and Importance of Voucher Specimens of Introduced Germplasm (Nayar *et al*., 2003; 2014).

The work done in project mode on 'Genetic Resources Study of Economically Important Plant Families- Cucurbitaceae, Malvaceae, Rosaceae and Poaceae' during 1984-1995 served as base line for many taxonomic works undertaken in NHCP. Study of crop taxa of Indian region (Asiatic *Vigna*, *Crotalaria*, *Allium*, *Prunus* and wild Triticeae); and check-lists of Indian Crop Plants and Crop Wild Relatives pin-pointed gaps in collection and prioritisation for build-up holdings (Arora and Nayar, 1984; Pandey and Nayar, 1994; Nayar *et al*., 2003; Nayar, 2015).

Services, Teaching and Linkages: Besides providing the expert consultation service in field of taxonomy, NHCP is actively involved in providing technical input on identification/ authentication, validation of taxa of PGR relevance; it also provides hands on exercise on herbarium procedures to large number of collage and school students and researchers especially working in fields of pharmacy, pathology, entomology, breeding, etc.. It is linked to the other fields of science especially for seeking identification/authentication of material conserved, as host- plant species relationship, introduced germplasm, weed science, agronomy etc. For the benefit of different users seeking services provided by the NHCP, special guidelines are in place (Pandey *et al*., 2015; Annexure 1, 2). Since 1999 this facility is available for teaching courses on taxonomy, ethnobotany and economic botany with PG School, ICAR-Indian Agricultural Research Institute, New Delhi.

The NHCP maintains links with many ICAR institutes, State Agriculture Universities and traditional universities, Botanical Survey of India (BSI), Forest Research Institute (FRI), and Herbarium Cryptogamae Indiae Orientalis (HCIO- a national-fungal herbarium facility at IARI, New Delhi), and the Herbarium of Wealth of India, CSIR-NISCAIR, New Delhi.

The NHCP accepts unique and unrepresented genetic resources (as herbarium specimens, seed samples and economic products) and encourages users for depositing vouchers for future reference.

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Enhancement of Maize Allelic Diversity using Wild Relative Teosinte (*Zea mays* ssp. *Parviglumis*)

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Teosinte (*Zea mays ssp. Parviglumis*), a wild relative of maize was assumed to be a potential donor for novel gene(s) for diversification and enhancement of maize germplasm. Subsequently teosinte was crossed with three maize inbred lines Tarun 83, DMRHyd 1284 and Pant 12K 53 followed by one generation of back crossing and two generation of selfing. The teosinte derived BC_1F_3 lines from all the three crosses were evaluated during spring season 2016. Analysis of variance revealed significant variance for most of the characters in all the three teosinte derived maize lines. Variability parameters revealed low variations for maturity traits namely days to 50% anthesis and silk emergence, days to 50% leaf senescence and anthesis-silking interval. Plant height exhibited wide range of variation and in general plant height of teosinte derived lines was more than the plant height of maize lines. Grain yield/plot was the most responsive trait because of the maximum variance observed in teosinte derived lines. Other traits exhibiting fairly high diversification in teosinte derived lines were cobs/plot followed by tassel branches and flag leaf length. The results, therefore, indicate that teosinte can be used effectively for domestication of wild alleles and diversification of maize germplasm. In the present investigation, only few morphological traits were analysed, however, teosinte can also be used for diversification of abiotic and biotic stress tolerance.

Key Words: Allelic diversity, Maize, Teosinte, Wild Relative

Introduction

Maize (*Zea mays* ssp. *mays*) is an important cereal worldwide, used primarily as food, feed and to create a variety of food and non-food products, such as corn flour, corn meal, sweeteners, corn oil, starch and ethanol. Racial diversity has always been the basis for productivity enhancement to support continuous increasing demand of maize. However, selective breeding amongst few promising has accelerated genetic erosion, a phenomenon that is obvious globally as well as locally. Despite being one of the species with greater genetic diversity, molecular analysis of the maize genome suggests that a single domestication event reduced diversity when compared with wild relative (Vigouroux *et al*., 2002; Warburton *et al*., 2008). Most maize commercial varieties in the world has limited genetic diversity, whereas, today the germplasm base in maize breeding programs is relatively narrow (Tarter *et al*., 2004; Le Clere *et al*., 2005; Liu *et al*., 2016). Domestication and breeding bottlenecks have resulted in genome-wide reductions in genetic variation in maize relative to teosinte (Tenaillon *et al*., 2004). Additional studies indicated that approximately 2-4% of genes were targets for artificial selection during domestication and breeding (Wright *et al*., 2005; Hufford *et al*., 2012), which implies that about 500 to 1,000 genes were critical during the evolution of modern maize, and are prime subjects for evolutionary and agronomic research. Large differences in plant morphology between maize and teosinte make phenotypic comparisons difficult. Photoperiod sensitivity, ear morphology, and kernel traits are among the most distinguishing characters between maize and teosinte (Iltis, 2000). In spite of larger genetic polymorphism in teosinte, limited efforts have been made to generate the genetic resources and tap the allelic diversity of teosinte for diversification and maize germplasm enhancement (Liu *et al.*, 2016). To enhance and broaden genetic base of maize germplasm, there is need to explore potential of teosinte in maize breeding programme. With elementary initial knowledge, the present investigation was planned to utilize teosinte in crossing programme for domesticating wild alleles for enhancing genetic diversity in maize.

Materials and Methods

With initial observations on teosinte (*Zea mays ssp. Parviglumis*), the present investigation was planned to diversify maize inbred lines through teosinte hybridization. Consequently, three inbred lines of maize namely Tarun ⊗ 83-1-3-2-⊗-1-2-1 (Tarun83), DMR Hyd

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–1284-⊗-1-1-1 (DMRHyd 1284) and Pant 12K/NAIP II/53⊗-⊗-1-1-1 (Pant12K53) were selected and raised in crossing block of Maize Breeding plots during *kharif* 2013 at Norman E. Borlaug, Crop Research Centre of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. India. Teosinte was also sown in the same field 20-25 days earlier than the maize sowing. Maize inbred lines were used as seed parent and at the time of silk emergence hand pollination was made by collecting pollen in tassel bag from teosinte. The F_1s were backcrossed followed by self-pollination to generate BC_1F_2 and BC_1F_3 populations in subsequent years. Since the purpose of this programme was to develop improved inbred lines, therefore selection during the back crossing and selfing was exercised. In total 88 teosinte derived maize lines, comprised of 40 BC_1F_3 lines of Tarun 83, 24 BC_1F_3 lines of each of the DMRHyd 1284 and Pant12K 53 along with three parental lines, were evaluated during *Spring* 2016 in a Randomized Block Design replicated thrice. Each line was sown in 3 m row spaced 75 cm and plant to plant distance was kept to be 20 cm. Irrigations were stopped at pre-flowering stage and allowed the crop to grow naturally under existing conditions of low moisture and high temperature. Observations were recorded on days to 50% anthesis, days to 50% silking, anthesis silking interval (ASI), days to 75% leaf senescence, plant height, tassel length, number of branches/ tassel, flag leaf length (cm), flag leaf width (cm), number of cobs/plot and grain yield/plot. Traits related to fodder i.e. basal branching, plant height, leaf length and width, biomass production per plant were recorded. Data were analyzed using Windostat to determine statistical significance.

Results and Discussion

Analysis of variance revealed significant variance for all the characters in all the three teosinte derived maize lines except for ASI and flag leaf width in teosinte derived lines of DMRHyd 1284 (Table 1). Significant variance, therefore, indicates reshuffling of maize genome due to introgression and recombination of alleles from teosinte. Teosinte derived maize lines were observed to be phenotypically different in terms of more height, tassel shape and size, and many plants bears multiple ear. Silk colour and purple tinge on glume, the two prominent characters of teosinte, were observed in most of the teosinte derived lines of all the three crosses. Thus, morphological variation amongst the teosinte derived lines indicates wide range of allelic variation in the maize genome.

Days taken to 50% anthesis varied from 71.0 to 86.7 days with general mean of 73.9 days and mean of Tarun 83 of 82 days. The PCV and GCV for anthesis duration were 5.3% and 4.5% (Table 2). Minimum days required for silk emergence was 71.0 whereas it was maximum of 89.7 days in teosinte derive maize lines whereas the maize line Tarun 83 exhibited 50% silk emergence in 85.7 days. The overall mean for days to silk emergence was 75.9 days with PCV and GCV of 5.8% and 4.5%, respectively. Anthesis-silking interval (ASI) for Tarun 83 was 3.7 days with overall mean of 2.0 days. ASI varied from 0.7 day to 4.0 days with PCV of 65.6% and GCV of 27.8%. The overall mean for days to senescence was 114.5 whereas Tarun 83 took 117 days in senescence. Minimum and maximum days to senescence were 109.0 and 119.0 days with PCV and GCV of 2.4% and 2.1%, respectively. Twenty five BC_1F_3

Table 1. Analysis of variance for different characters in teosinte derived maize lines

S. No.	Character	Mean square (MS)		
		Tarun 83	DMRHyd 1284	Pant12K 53
1.	Days to 50 % anthesis	37.8**	31.3**	69.2**
2.	Days to 50% silk emergence	46.5**	41.9**	123.1**
3.	Anthesis - Silking Interval (ASI)	2.4*	1.9	12.5**
4.	Days to 75% leaf senescence	19.9**	11.1**	22.2**
5.	Plant height (cm)	895.6**	1198.8**	2525.4**
6.	Tassel length (cm)	30.7**	49.3**	17.8*
7.	Branches/tassel	44.9**	50.9**	33.8**
8.	Flag leaf length (cm)	12.1**	27.1**	70.1**
9.	Flag leaf width (cm)	0.6**	0.4	0.9**
10	Cobs/plot	49.5**	17.9**	35.7**
11.	Grain yield/ plot (g)	99298.5**	82160.0**	114078.0**

*, **-denotes 5% and 1% level of probability, respectively.

Table 2. Genetic variability parameters for different traits of teosinte derived maize lines of Tarun 83

S. No.	Character	Mean		Range	PCV (%)	GCV (%)
		Tarun 83	General mean			
1.	Days to 50% anthesis	82.0	73.9	71.0-86.7	5.3	4.5
2.	Days to 50% silking	85.7	75.9	71.0-89.7	5.8	4.9
3.	Anthesis - Silking Interval (ASI)	3.7	2.0	0.7-4.0	65.6	27.8
4.	Days to 75% leaf senescence	117.0	114.5	109.0-119.0	2.4	2.1
5.	Plant height (cm)	105.0	131.6	105.0- 171.7	14.1	12.6
6.	Tassel length (cm)	24.0	28.6	21.5- 36.1	12.7	10.3
7.	Branches/tassel	14.1	18.0	11.1- 26.9	23.4	20.5
8.	Flag leaf length (cm)	25.6	25.8	16.5- 35.9	16.8	12.2
9.	Flag leaf width (cm)	3.9	3.7	2.9- 4.9	9.64	4.7
10.	Cobs/plot	4.7	9.3	2.3-18.0	45.2	42.9
11.	Grain yield/ plot (g)	120.0	309.3	80.0-820.0	58.9	58.7

lines (including maize line) evaluated from each of the other two crosses namely DMRHyd 1284 and Pant12K 53 had by and large similar pattern of observations for days to anthesis, days to silk emergence, ASI and days to senescence as noted for BC_1F_3 lines derived from crossing Tarun 83 with teosinte (Table 3, 4). The PCV and GCV for days to anthesis, days to silk emergence and days to senescence are quite low yet results indicate diversification of maize lines due to wide range of observed variation. Some of the teosinte derived maize lines are significantly lower in maturity traits while some others are significantly higher than the maize parental lines. Low variability in maturity traits is inevitable since selection was exercised for relatively uniform maturity during backcrossing and selfing. ASI is one of the important parameter that determines productivity under normal as well under stress environment. Minimum ASI (<4 days) ensures complete pollination and seed setting whereas frequency of partial seed setting or even no seed setting at all increases with increasing ASI. In the tesosinte derived lines, ASI was normal or within the limit except in teosinte derived maize lines of Pant12K 53 where general mean for ASI was 3.1 days and range was varied from 3.1 to 9.0 days. Observations on ASI across the three teosinte derived maize populations indicates Pant12K 53 as relatively more sensitive to environmental stress as indicated by 8 days differences in days to anthesis and days to silk emergence.

Leaf senescence is another physiology related parameter that is associated with terminal drought or heat stress. Parental mean, general mean, range, PCV, GCV for days to 75% leaf senescence were 117.0, 114.5, 109.0-119.0, 2.4%, 2.1% in Tarun 83, 116.0, 116.3, 113.0-119.3, 2.0, 1.5 in DMR Hyd 1284 and 118.0, 117.7, 108.7-123.0, 2.6%, 21% in Pant12K 53-teosinte lines, respectively.

Plant height is one of the traits that have drastic effect when maize and teosinte is crossed. Tarun 83 maize line had plant height of 105.0 cm whereas in Tarun 83-teosinte lines, plant height varied from 105.0 to 171.7 cm with general mean, PCV and GCV of 131.5 cm, 14.1% and 12.6%, respectively. Plant height of DMRHyd 1284 was 69.7 cm whereas its derived lines varied in plant height from 69.7 to 174.3 cm with mean of 125.2 cm. PCV and GCV for plant height were 17.0% and 15.5%,

Table 3. Genetic variability parameters for different traits of teosinte derived maize lines of DMRHyd 1284

S. No.	Character	Mean		Range	PCV (%)	GCV (%)
		DMRHyd 1284	General mean			
1.	Days to 50 % anthesis	86.3	74.1	70.3-86.3	5.1	3.9
2.	Days to 50% silk emergence	89.3	75.5	70.7-89.3	5.6	4.6
3.	Anthesis - Silking Interval (ASI)	3.0	1.6	0.3-3.3	79.1	29.2
4.	Days to 75% leaf senescence	116.0	116.3	113.0-119.3	2.0	1.5
5.	Plant height (cm)	69.7	125.2	69.7-174.3	17.0	15.5
6.	Tassel length (cm)	13.8	27.6	13.9-36.6	16.0	14.0
7.	Branches/tassel	10.3	20.7	10.3-30.3	22.0	18.7
8.	Flag leaf length (cm)	25.5	29.5	24.9-34.3	13.6	7.9
9.	Flag leaf width (cm)	3.1	3.8	3.1-4.7	15.0	4.5
10.	Cobs/plot	5.7	9.6	5.7-14.7	28.1	24.0
11.	Grain yield/ plot (g)	69.7	371.4	69.7-680.0	44.6	44.5

Table 4. Genetic variability parameters for different traits of teosinte derived maize lines of Pant12K 53

S. No.	Character	Mean		Range	PCV (%)	GCV (%)
		Pant12K 53	General mean			
1.	Days to 50 % anthesis	86.0	77.7	71.3-87.0	6.6	6.0
2.	Days to 50% silk emergence	94.7	80.8	72.7-94.7	8.3	7.7
3.	Anthesis - Silking Interval (ASI)	8.33	3.1	3.1-9.0	72.5	62.92
4.	Days to 75% leaf senescence	118.0	117.7	108.7-123.0	2.6	2.1
5.	Plant height (cm)	98.0	120.2	48.0-158.7	26.1	23.1
6.	Tassel length (cm)	24.4	26.6	21.0-29.7	13.2	6.2
7.	Branches/tassel	18.6	18.1	10.3-23.9	23.7	15.3
8.	Flag leaf length (cm)	17.0	25.7	15.9-33.8	21.9	17.0
9.	Flag leaf width (cm)	3.4	3.5	2.6-4.7	18.5	13.1
10.	Cobs/plot	1.3	6.8	1.3-15.7	53.7	48.8
11.	Grain yield/ plot (g)	15.0	269.4	15.0-670.0	72.5	72.3

respectively. In case of Pant12K 53-teosinte lines, plant height varied from 108.7 to123.0 cm with general mean of 118.0 cm and mean plant height of Pant12K 53 of 117.7 cm. Variations in plant height in teosinte derived maize lines along with PCV and GCV observed in the investigation clearly indicates the diversification in plant height when compared with the height of maize line. In addition, teosinte derived maize lines have become sturdier than the maize plant.

Tassel length and branching are also influenced under stress environment and in the investigation variable response has been observed. In all the three teosinte derived maize populations, tassel length and tassel branches showed wide range of variations along with moderately high PCV and GCV. General mean for tassel length and range of variations were 28.6 cm, 21.5- 36.1 cm, and 27.6, 13.9-36.6 cm, and 26.6, 21.0-29.7 cm in teosinte derived maize lines of Tarun 83, DMRHyd 1284 and Pant12K 53. Number of branches/ tassel in teosinte derived lines from Tarun 83 varied from 11.1- 26.9 with parental mean and general mean of 14.1 and 18.0. DMRHyd 1284 possessed tassel branches of 10.3 whereas its derived lines varied in tassel branching from 10.3 to 30.3 with general mean of 20.7. Teosinte derived lines of Pant12K 53 had average of 18.1 branches each tassel whereas minimum and maximum number of tassel branches were noted to be 10.3 and 23.9. Tassel branches in Pant12K 53 were 18.6.

Flag leaf length and width are again physiologically important parameters. Both the parameters exhibited variation in teosinte derived maize lines. Tarun 83 had flag leaf length of 25.6 cm whereas its derived lines varied in flag leaf length from 16.5 to 35.9 cm with overall mean of 25.8 cm. PCV and GCV for flag leaf length were 16.8% and 12.2%, respectively. Parental mean, general mean, range, PCV and GCV for flag leaf length in DMRHyd 1284 and its derived lines were 25.5 cm, 29.5 cm, 24.9-34.3 cm, 13.6% and 7.9%, respectively. Pant12K 53-teosinte lines possessed flag leaf length from 15.9 to 33.8 cm with mean of 25.7 cm while parental line Pant12K 53 had flag leaf length of 17 cm. The PCV and GCV were 21.9% and 17.0% in teosinte derived lines of Pant12K 53.

Number of cobs/plot depends on the plant ability to bear one or many cobs. Effective cobs/plot reduced under abiotic stresses including high plant density. During the stress, many plants remain barren either due to no seed set because of increased ASI or there is no emergence of cob or reduced number of cobs per plant. Cobs/plot was noted to be one of the diverse parameters in teosinte derived maize lines. Tarun 83 had 4.7 cobs/plot whereas teosinte derived lines of Tarun 83 possessed minimum of 2.3 cobs/plot to maximum of 18.0 cobs/plot with overall mean of 9.3 cobs. The high range of cobs/plot is also reflected in the form of PCV and GCV which were recorded to be 45.2% and 42.9%, respectively. DMRHyd 1284-teosinte lines had range of variation from 5.7 to 14.7 cobs/plot with overall mean of 9.6. Cobs/plot in parental line DMRHyd 1284 was observed to be 5.7. The PCV and GCV for cobs/ plot were 28.1and 24.0%, respectively. Pant12K 53 had least cobs/plot (1.3) whereas its teosinte derived lines had average cobs/plot of 6.8 with range from 1.3 to 15.7. PCV for cobs/plot was 53.7% whereas GCV was 48.8%. Such a diverse phenomenon for cobs/plot across the three teosinte derived lines indicates diversification through allelic reshuffling between maize and teosinte genomes. Grain yield/plot was observed to be the most diversified trait as indicated by high range of variation from minimum of 80.0g to maximum of 820.0g with

high PCV and GCV of 58.9% and 58.7%, respectively in teosinte derived lines of Tarun 83. Overall yield/plot was 309.3 g whereas Tarun 83 had per plot yield of 120 g. The inbred line DMRHyd 1284 had plot yield of 69.7g whereas average grain yield of teosinte derived lines of DMRHyd 1284 was 371.4g with range of plot yield from 69.7 to 680.0 g. The PCV and GCV for yield/plot were 44.6% and 44.5%, respectively. Grain yield/plot in case of inbred line Pant12K 53 was 15.0 g whereas overall mean was 269.4 g with variation in yield from minimum of 15.0 to maximum of 670.0 g. Highest PCV and GCV of 72.5% and 72.5%, respectively were observed for yield/plot in Pant12K 53 derived lines.

The contribution of wild species to breeding cultivated species is well known. However, the characterization of adapted and wild populations is a fundamental aspect to increase the introduction of favorable genes in breeding programs (Laborda *et al*., 2005). While investigating hybrids between maize and perennial teosinte, Srinivasan and Brewbaker (1999) noted range of variations for many traits. Vigouroux *et al*., (2002) used SSR to identify genes of agronomic importance in maize under the influence of selection in the process of domestication. Lubberstedt *et al*., (1998) studied microsatellite variation in maize and suggested their use in detecting genetic variability in associated genotypes, which can be applied to maize and teosinte. In order to examine a broad range of genetic diversity for maize and shed light on the genetic basis of agronomic and domestication traits, Liu *et al*., (2016) developed near-isogenic introgression lines from ten teosinte accessions in the B73 background and determined genetic variation for flowering time and genetic architecture of traits specifically targeted by domestication. Further, they opined that introgression populations offer novel tools for QTL discovery and validation, as well as a platform for initiating fine mapping.

In the investigation, maize-teosinte crosses and backcrosses were made successfully. BC_1F_3 lines of three crosses exhibited significant variance for most of the characters. Mean, range, PCV and GCV for different characters analysed across the three populations indicated that teosinte derived lines were diversified probably because of the allelic reshuffling between maize and teosinte genome. Thus, teosinte can be used effectively for diversification as well enhancement of maize germplasm and domestication followed by selective breeding led narrow genetic base can be broaden using teosinte.

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Biosecurity Policies Influencing International Exchange of PGR

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Biosecurity policies play an important role in facilitating transboundary exchange of plant genetic resources (PGR) by preventing introduction of pests into new geographical areas. International exchange of PGR has contributed significantly towards crop improvement and increased crop production. However, a number of pests have also moved across the countries along with planting material. Most countries regulate the import of germplasm because of the pest risk posed by such imports. With the coming of the two international Agreements - Convention on Biological Diversity (CBD) in 1992 and World Trade Organization (WTO) in 1995, international exchange of PGR has been altered. Under WTO, the aim is to promote trade by undertaking quarantine and influencing trade policies while CBD aims at protection and conservation of biodiversity. A number of national regulations related to biosecurity and related issues have an impact on safe international movement of PGR. Even the PGR being exchanged under the International Treaty on Plant Genetic Resources of Food and Agriculture are governed by the respective national biosecurity regulations of the nations. The various national regulations provide a fragmented legislative system which needs to be harmonized and integrated to holistically deal with biosecurity while complying with international norms. The establishment of National Agricultural Biosecurity Authority and the Biosafety Regulatory Authority of India and the enactment of the Seed Bill needs to be taken up to have a holistic approach in dealing with biosecurity. Also, there is a need to support research, training, capacity-building, networking and information sharing activities at both national and regional levels.

Key Words: Biosecurity, Holistic, Plant pests, Policies, Regulation

Introduction

Biosecurity literally means safety of living things. Technically, it is a strategic and integrated approach that encompasses the policy and regulatory frameworks to analyze and manage risks in the sectors of food safety, animal life and health, and plant life and health, including associated environmental risk (http://www.fao.org). Hence, it broadly covers food safety, zoonoses, the introduction of animal and plant diseases/pests, the introduction and release of LMOs or GMOs and the introduction and management of invasive alien species.

Globalization and climate change have impacted human and plant life and our environment in unprecedented ways. With the advent of WTO and the liberalization of trade in agriculture since 1995, new avenues for growth and diversification of agriculture have emerged while bringing in many new challenges. There is an increased risk of introduction of exotic pests, including weeds, in the country with the potential to cause serious economic loss. Climate change has the potential to alter the habitat of known pests and even cause introduction of new pests. The emergence and spread of transboundary diseases such as the Ug_{99} stem rust of wheat and rice blast pose new threats. Also, there is an increasing threat of bioterrorism. Because of these factors, biosecurity has emerged as one of the most urgent issues facing countries requiring them to foster policies and develop technological capabilities to prevent, detect, and respond to such threats.

This paper primarily deals with the regulations impacting phytosanitary aspects of biosecurity with a focus on dangers from exotic pests, status of national policies, identifies important issues that need to be addressed and the need for developing a holistic biosecurity policy to address them.

Plant Biosecurity Policies in India

Plant quarantine is the government endeavor enforced through legislative measures to regulate the introduction of planting materials, plant products, soil, living organisms, etc. in order to prevent inadvertent introduction of pests (including fungi, bacteria, viruses, nematodes, insects and weeds) harmful to the agriculture of a country/ state/ region, and if introduced, prevent their establishment and further spread. The devastating effects resulting from diseases and pests introduced along with international movement of planting material, agricultural produce and products are well documented (Gupta *et*

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al., 2005; Khetarpal and Gupta, 2008; Khetarpal *et al*., 2009; Dubey and Gupta, 2016) which clearly demonstrate that introduction and establishment of quarantine pests including weeds into new areas can severely damage the crop production and economy of a region/ country.

After the Second World War, FAO convened an International Plant Protection Convention (IPPC) in 1951, to which India became a party in 1956 along with Australia, Sri Lanka, UK, Netherlands, Indonesia, Portugal and Vietnam. At present, there are 183 contracting parties of the IPPC (as on May 28, 2017). The IPPC aims to develop international cooperation among various countries to prevent the introduction and spread of regulated pests along with international movement of plants and planting material (http://www.ippc.org) and requires that each country establish a national plant protection organization to discharge the functions specified by it.

With the coming of the two international Agreements of Convention on Biological Diversity (CBD) in 1992 and World Trade Organization (WTO) in 1995, international exchange of agricultural commodities has taken a different turn. Under WTO, the aim is to promote trade by undertaking quarantine and influencing trade policies while CBD aims at protection and conservation of environment and biodiversity. Also, the International Treaty on Plant Genetic Resources for Food and Agriculture was adopted in 2001 for establishing a global system to provide farmers, plant breeders and scientists' access to plant genetic materials. The Treaty facilitates access to the genetic materials of 64 crops in the Multilateral System for research, breeding and training for food and agriculture. India is a signatory and has ratified all three international instruments impacting transboundary movement of plants/ planting material and needs to comply with the requirements under each. Even the PGR being exchanged under the Treaty are governed by the respective national biosecurity regulations of the nations.

A number of national regulations related to biosecurity or issues related to it have an impact on safe trade and exchange of PGR. The chronology of notification of Indian Acts and Policies related to plant biosecurity and the provisions in brief covered therein are given in Table 1.

Infrastructure

In India, agriculture is in the State list of the Constitution and crop production, including plant protection from indigenous pests, is the responsibility of State Governments. The Central Government (Ministry of Agriculture and Farmers Welfare) supplements States' efforts in pest surveillance and management by disseminating innovative pest management technologies. Plant quarantine and locust control in the scheduled desert area, being inter-state and international subjects, are managed by the Government of India.

The Department of Agriculture Cooperation and Farmers Welfare (DAC&FW) through its Directorate of Plant Protection, Quarantine and Storage (DPPQS) implements schemes of plant protection and quarantine through Central Integrated Pest Management Centers (CIPMCs), Plant Quarantine Stations (PQS) at different airports, seaports and land frontiers, Locust Warning Organization circle offices and Central Insecticides Laboratory (CIL) and Regional Pesticides Testing Laboratories (RPTLs), Central Insecticide Board and Registration Committee (CIB&RC) and the National Institute of Plant Health Management (NIPHM).

Plant quarantine regulations are implemented under the Destructive Insects and Pests Act, 1914 and the Plant Quarantine (Regulation of Import into India) Order, 2003. Other regulations relevant to biosecurity are the Environment (Protection) Act, 1968, the Biological Diversity Act, 2002, and the Insecticides Act, 1968. Plant quarantine regulations aim to prevent introduction of exotic pests, diseases and weeds into India, during the import of agricultural commodities, in accordance with the WTO-SPS Agreement. Phytosanitary Certificates (PSCs) are issued for exports as per the International Plant Protection Convention (IPPC) 1951 of the FAO and post-entry quarantine (PEQ) inspections undertaken for imports.

In all, two categories of materials are being imported under the PQ Order, 2003: (a) bulk consignments for consumption and sowing/ planting, and (b) samples of germplasm in small quantities for research purposes. The PQS under the DPPQS undertake quarantine processing and clearance of consignments of the first category (http://www.plantquarantineindia.nic.in). The five major Plant Quarantine are fully equipped and were modernized under an FAO-UNDP funded project.

Table 1. Indian Regulations related to Plant Biosecurity from the turn of the 20th Century

Year	Legislation	Key features related to plant protection
1914	Destructive Insects and Pests (DIP) Act	• Enacted as the first quarantine law in India after the British ordered compulsory fumigation of imported cotton bales to prevent Mexican cotton boll weevil (*Anthonomus grandis*) ordered in 1906 • Prohibits or regulate the import into India or any part thereof or any specific place therein or any article or class of articles specified therein • Prohibits or regulate the export from a State or the transport from one State to another State in India of any plants and plant materials, diseases or insects, likely to cause infection or infestation • Authorizes the State Govt. to make rules for detention, inspection, disinfection/ disinfestation or destruction of any pest or class of pests or of any article or class of articles for which center has issued notifications • Revised and amended several times over the years with provisions for domestic quarantine of nine pests
1966	Seeds Act	• Act was passed by the parliament in 1966 and the Seed Rules were framed under it in 1968 • Legislative measures promulgated to ensure high quality of seed in the market place • Act provides for a system of notification of kinds or varieties of seeds • Seed (Control) Order, 1983 seeks to control and regulate seed production and distribution • Seed declared as an essential commodity under the Essential Commodities Act, 1955 • Periodically revised with the changing national and international scenario • A relevant aspect to be kept in mind is that the authorities created under the Act are entitled to act only in the case of seeds sold for agricultural purposes and not for human consumption
1968	Insecticides Act	• The Act and the rules framed thereunder seek to ensure the availability of quality, safe and efficacious pesticides to the farming community and to manage risks to human health and the environment. • Regulates import, manufacture, sale, distribution and use of insecticides with a view to prevent risk and promote safety measures • Registration committee registers insecticides after verifying claims regarding efficacy and safety
1984 1989	Plants Fruits and Seeds (Regulation for Import into India) Order Revised PFS Order	• No consignment would be imported into India without a valid import permit issued by the competent authority for a. bulk consignments – the Plant Protection Advisor to the Govt. of India b. importing germplasm of agri-horticultural crops including transgenic material for research- the Director ICAR-NBPGR c. forest plants – the Forest Research Institute, Dehradun d. remaining plants of economic and general interest- the Botanical Survey of India, Kolkata • No consignment can be imported unless accompanied by an official Phytosanitary Certificate issued by an official agency of the exporting country • Seeds/ planting materials requiring isolation growing under detention, to be grown in an approved post-entry quarantine facility • Import of soil, earth, sand, compost, plant debris accompanying seeds/ planting materials is not permitted. Hay, straw or any other material of plant origin would not be used as packing material • Special conditions for import of plants, seeds for sowing, planting and consumption have also been mentioned under Schedule II (Clause 4) of the Order • This was repealed after PQ Order 2003 came into force.
1986	Environment Protection Act	• Aims to protect and improve quality of environment • Provides for management and handling of hazardous wastes, use, import/ export and hazardous micro-organisms, genetically engineered organisms or cells • Both LMOs and invasive alien species (IAS) are covered under the EPA; but, it does not state in clear terms the modality for restriction.
1988	New Policy on Seed Development	• Came into force with an objective to provide the Indian farmers with the best genetic material available anywhere in the world to increase agricultural productivity, farm income and export earnings • Encourages an export-oriented horticultural industry the government wanted to provide best seed or other planting material like bulbs, cuttings, saplings and bud woods freely to the farming community for export promotion • Aimed at liberalization of imports along with streamlining of plant quarantine procedures and encouragement to domestic seed industry through incentives
2001	Protection of Plant Varieties and Farmers Rights Act	• Has set up a Plant Varieties and Farmers' Rights Protection Authority • Allows the registration of new plant varieties within a specific list of genera and species, as well as farmers' varieties • Accords specific privileges to researchers/ breeders while respecting quarantine regulations

Contd.

Year	Legislation	Key features related to plant protection

2002	Biological Diversity Act	• Addresses the access to genetic resources, associated knowledge and equitable sharing of benefits arising there from to the country
		• To safeguard the interest of the people of India few exceptions are:
		  • Use by vaids and hakims
		  • Free access to the Indian citizens to use within the country for research
		  • Collaborative research involving biodiversity subject to policy guidelines and approval
		• The Act does not restrict movement of pests within the country nor has any provision to deal with invasive alien species (IAS). In fact, no mention is made of these species throughout the legislation.

2002	National Seeds Policy	• The Seeds Act, 1966, Seeds Control Order and NPSD, 1988 promote and regulate the seed industry • Encompasses quality assurance mechanisms along with facilitation of seed industry • Thrust areas include quarantine of imported seeds and planting material and compliance to biosafety • A specified quantity of imported seeds to be sent to National Genebank, ICAR-NBPGR

2003	Plant Quarantine (Regulation for Import into India) Order and its amendments	• Notified in compliance to the Sanitary and Phytosanitary Agreement of WTO
		• Pest risk analysis made a precondition for all imports
		• Gives schedules for import of various plants and planting materials based on degree of risk involved with additional declarations and special conditions
		  • prohibited plant species are plants/planting materials and countries from which import is prohibited (Schedule IV)
		  • restricted species are plants and plant materials the import of which into India is restricted and permissible only with the recommendation of an authorized institution (Schedule V)
		  • species requiring additional declarations and special conditions: where no recommendation is required from issuing authorities (Schedule VI)
		  • plant material imported for consumption or industrial processing permissible on the basis of a phytosanitary certificate, an inspection conducted by inspection authority and treatment as may be required (Schedule VII)
		  • List of prohibited weed species given in Schedule VIII
		  • List of nine regulated pests under domestic quarantine, now notified under Schedule XIII as regulated non-quarantine pests (http://agricoop.nic.in/sites/default/files/WTO1032016.pdf)
		• Director, ICAR-National Bureau of Plant Genetic Resources empowered to deal with import of PGR for research purposes.
		• The order also regulates the import of GMOs of plant origin for the purpose of agricultural research or experimentation. Such imports would require an import clearance by Review Committee on Genetic Manipulation (RCGM) before the import permit is issued by the Director, ICAR-NBPGR. However, the order does not cover imports of GMOs for commercial purposes, which are governed by separate clearances.

2015-20	Foreign Trade Policy	• Provides policy measures for enhanced market access across the world and diversification of export markets. • To increase India's percentage share in global trade special initiatives identified for market diversification, technological upgradation, support to stakeholders in Agriculture among other sectors • Vishesh Krishi and Gram Udyog Yojana (VKGUY) (Special Agriculture and Village Industry Scheme) has the objective to promote exports of agricultural products, minor forest products, Gram Udyog products and forest-based products.
2010	Seed Bill (draft)	• More emphasis given to seed quality including health aspects • Any traded seed should be identified in terms of the variety to which it belongs, meet the minimum prescribed standards for germination, genetic and physical purity, maximum standard in seed health and an acceptable level of agronomic performance • Certification of seed standards is mandatory and done by accredited Seed Testing Laboratories • Deals with the import of seeds and it provides for the compulsory registration of all imported seeds (although the government may allow the import of an unregistered seed for research purposes). Further, all imports of seeds "shall be subject to the provisions of the Plant Quarantine (Regulation of Import into India) Order, 2003, made under the Destructive Insects and Pests Act, 1914
2013	Biotechnology Regulatory Authority of India Bill (draft)	• The Bill sets up an independent authority, the Biotechnology Regulatory Authority of India (BRAI), to regulate organisms and products of modern biotechnology. • BRAI to regulate the research, transport, import, containment, environmental release, manufacture, and use of biotechnology products. • Regulatory approval by BRAI would be granted through a multi-level process of assessment undertaken by scientific experts. • BRAI would certify that the product developed is safe for its intended use, however, all other laws governing the product would continue to apply.

Contd.

Year	Legislation	Key features related to plant protection
2013/ 2016	Agricultural Biosecurity Bill (draft)	• Provides for establishment of an Authority/ Board for prevention, control, eradication and management of pests and diseases of plants, animals and unwanted organisms for ensuring agricultural biosecurity. • Most international obligations of India for facilitating imports and exports of plants, plant products, animals, animal products, aquatic organisms and regulation of agriculturally important microorganisms • Provisions for effective domestic quarantine incorporated • Regulates introduction of new or beneficial organisms in the country • Regulates impact of transgenic material with respect to sanitary and phytosanitary matters • Empowers the quarantine officers for search and seizure of material

ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) undertakes the quarantine processing of all plant germplasm and transgenic planting material under exchange for which it has well- equipped laboratories, green house complex and a Containment Facility has also been established for processing transgenics (Khetarpal *et al.*, 2006). ICAR-NBPGR also provides the necessary back-up research. ICAR-NBPGR also has a well-equipped quarantine station at Hyderabad, which mainly deals with the export samples of International Crop Research Institute for Semi-arid Tropics (Chakravarty *et al*., 2005).

Gaps in the National Plant Biosecurity System

I Legislation: *Enactment of Agricultural Biosecurity Bill 2013/ 2017*

The Destructive Insects & Pests Act (DIP Act), 1914 and the PQ Order 2003 cover import of plants/ plant products based on risk assessments and management there is a need to strengthen them with respect to the following:

- The DIP Act is an old legislation and subsidiary to the Sea and Customs Act which does not give direct powers to the Plant Quarantine Officers to deport or destroy or confiscate the consignment or lodge complaints under the Indian Penal Code; powers for seizures/ detention or forfeiture/ confiscation of infected/ infested material need to be incorporated; punishment or penalty on the importers or custom house clearing agents or other defaulters for violation of the Acts needs to be introduced and the provisions for regulating or prohibiting plants need to be strengthened. Also, the Act contains several definitions which need revision and updation.
- Although the DIP Act has the provisions for domestic plant quarantine, there are no supporting clauses for its enforcement resulting in ineffective enforcement.

The enactment of the Agricultural Biosecurity Bill 2013/ 2017 would take care of the above issues.

II Infrastructure: *Establishment of new plant quarantine stations with adequate facilities, manpower to regulate imports through notified points of entry, pest monitoring and control*

Government of India has notified more than 130 International Entry Points which are being managed by only 57 functional Plant Quarantine Stations. There is an urgent need for new PQ Stations with good infrastructure and trained technical personnel. The existing PQ Stations are required to be strengthened with additional human and infrastructural resources in view of the rapid increase in the quantum of international trade.

Further, strengthening of technical human resources is required for pest monitoring and surveillance, pesticide registration and testing systems, etc. The five major Plant Quarantine Stations need to be strengthened/ established to act as referral centres for rapid and accurate diagnosis of plant pests/ pathogens.

III Capacity Building and Modernization: *Development of an Integrated Information Management System*

There is a need to improve the information management system used by the plant and animal quarantine services to meet organizational and client needs by way of free exchange of information. The Plant Quarantine Information System recently launched by the DPPQS, is a good step in this direction. The PQIS accessible from http://plantquarantineindia.nic.in provides an efficient and effective service for the stakeholders such as importers, exporters, individuals and the Government. It facilitates importers to apply online for Import Permit, Import Release Order and Exporters to apply online for Phytosanitary Certificate. Exporters and importers can view the status of their application online and access history of his application during the entire life cycle of

the application. This will help in bringing transparency in functioning.

Establishment of Biosecurity and Trade Unit

There is an urgent need to establish a Biosecurity and Trade Unit with various Cells namely;

- ***Sanitary and Phytosanitary Cell*** dealing with requirements for import of plants and planting material in the SPS-WTO regime, scientific justification to facilitate pest/ disease free trade in agricultural commodities, fulfill the obligations of the SPS Enquiry Point, examine the WTO-SPS notifications from other countries, preparing India's SPS notifications for WTO, preparing India's market access claims, bilateral agreements with countries etc. (Khetarpal and Gupta, 2002).
- ***Risk Analysis Unit for Import and Export and Market Access Cell*** to undertake science based pest risk analysis for the application of SPS measures consistent with the WTO-SPS Agreement and IPPC international standards on pest risk analysis (Gupta and Khetarpal, 2004). The unit would also organize trainings for capacity building in plant quarantine, develop national standards and harmonize with the international standards.
- ***Integrated Pest Surveillance and Rapid Response Cell*** to ensure early detection of introduced pests, to provide reliable data for Pest Risk Analysis and monitoring pest status in an area to support market access through "pest-free areas". There is a need to conduct regular surveys to watch the introduction, establishment and spread of pests & diseases. In case of any eventuality, a Rapid Response Team would be constituted to check and control the spread of the disease
- ***Centralized Biosystematics Cell*** needs to be established for identification of pests/ diseases faster to enhance the decision making by the operational staff at port of entries. The unit would comprise experts in the fields of entomology, plant pathology including nematodes and weed science with supporting staff for identification of the pests/diseases. The ICAR-DARE Bureaux for microorganisms and insects (arthropods) would be utilized as resource base for this purpose. They would use the latest tools such as molecular techniques, digitized keys and remote microscopy for speedy and accurate identification of pests.
- ***Emergency Action and Biosecurity Disaster Management Cell*** to be developed as an integrated unit with specialized experts for undertaking biosecurity risk assessment, draw out Emergency Action Plan to combat pest menace in the event of any pest epidemic by integrating the various control techniques so as to timely manage the pest incidence and avoid crop loss/yield. It could have four wings dealing with plants, farm animals, living aquatic resources and agriculturally important micro-organisms.
- ***Human Resource Development Cell*** for regular trainings on sanitary and phytosanitary issues for identification of pest diagnostics, sampling, international standards/ guidelines etc. is required. This Cell would also organize trainings, seminars, workshops etc. on sanitary and phytosanitary issues and standards along with related standard operating procedures, at domestic and international level. Besides, the Cell would also sensitize policy makers/ implementers, administrators, politicians, stakeholders (village farmers, farmers owning large organized farms), general public to the importance of food safety, good agricultural practices, plant quarantine requirements through seminars, symposia, workshops and mass media programmes is required.

IV *Strengthening Research Back-up*

- Classification of the threat agents including insects, mites, micro-organisms and bio-weapons challenging the biosecurity of agriculture based on perceived risk levels.
- Increased use of radiation and other frontier techniques as effective mitigation treatment.
- Development of user-friendly serological/molecular diagnostic protocols/kits for prognostic detection of exotic pests and their variants and also for the detection of GMOs/ LMOs.
- Development of digitized biosystematic keys for identification of pests.
- Epidemiological studies including survey and surveillance of diseases/pests to prepare database on endemic pests, identify pest free areas and target IPM for reducing threats.
- Developing models for risk analysis for exotic pests, diseases and invasive weeds.

- Developing standard operating procedures through relevant Handbooks/Manuals for survey and surveillance and monitoring major diseases/pests including invasive weeds.
- Studies on epidemiology of economically important pests and diseases vis-à-vis climate change.
- Studies on factors affecting likelihood of survival of pests under different conditions of transport, modes of dispersal, distribution of hosts/ alternate hosts at destination, potential for establishment, reproductive strategy and method of pest survival, potential vectors and natural enemies of the pest in the area.
- Simulated evaluation of mitigation options to deal with epidemics/ pandemics.
- Need for special focus on management of indigenous minor diseases, pests and invasive weeds with a potential to impact food security, environment including biodiversity, and trade.
- Development of national dynamic biosecurity database of pest, diseases, invasive weeds and their management.

Need for Integrated National and Regional Agricultural Biosecurity

Internationally, the Agreement on the Application of Sanitary and Phytosanitary Measures of the WTO, governs SPS measures in relation to international trade. The Codex Alimentarius Commission (Codex), the IPPC and the Office International des Epizooties (OIE) provide international standards for food safety, plant health, and animal health, respectively. Further, the Cartagena Protocol of Convention on Biological Diversity (CBD) applies to the transboundary movement, transit, handling and use of Living Modified Organisms (LMOs). Guidelines on the management of invasive alien species have been developed under the SBSTTA (Subsidiary Body on Scientific, Technical and Technological Advice) of CBD (Rana *et al.*, 2004, Gupta *et al*., 2009). This group of international agreements, organizations and programmes are part of a loose international framework for biosecurity, and reflects the sectorial approach to regulate this area.

FAO has recognized the growing importance of biosecurity, and made it one of its sixteen Priority Areas for Inter-disciplinary Action. Biosecurity was also included in the Medium Term Plan which aims at "promoting, developing and reinforcing policy and regulatory frameworks for food, agriculture, fisheries and forestry" (http://www.fao.org.COAG/2003/9.htm).

Models to rationalize regulatory functions among sectors in the quest for improved effectiveness and efficiency have appeared in a number of countries. New Zealand has a Biosecurity Act since 1993 and a Biosecurity Minister and Council since 1999. In US, agricultural biosecurity is looked after by the Animal and Plant Health Inspection Service (APHIS) headed by an administrator under the US Department of Agriculture (USDA). In Australia, biosecurity is the charge of the Department of Agriculture, Fisheries and Forestry (DAFF) looking after plant and animal biosecurity policy development, risk management measures and international trade. The quarantine machinery of New Zealand, USA and Australia have been totally transformed and upgraded in their own ways to meet the challenges of their national biosecurity and safe trade.

Presently in India, agricultural biosecurity is managed on a sectoral basis through the development and implementation of separate policy and legislative frameworks (e.g. for animal and plant life and health). Sectoral agencies organize their work without much attention to the other sectors and no attention is paid to the interdisciplinary nature of biosecurity. In a modern national system, there is need for a more harmonized and integrated approach, with competent authorities responsible for different sectors of agricultural biosecurity working together towards common goals. Sectoral policies, laws and regulations can be harmonized to avoid contradictions, overlaps and gaps. This encompasses the joint setting of biosecurity priorities and allocation of resources, joint planning of activities, and integrated systems for monitoring and review of outcomes. An integrated agricultural biosecurity system would present a single interface to exporters/ importers and allows for sharing of resources among the sectoral agencies (Khetarpal and Gupta, 2007).

Accordingly, there is a need to establish a national mechanism under whose umbrella all the related issues are dealt with in a comprehensive manner to achieve national agricultural biosecurity, protect animal and plant life and health and environment. There is a need for synergy of expertise from various organizations under the Ministries of Agriculture and Farmers Welfare, Environment Forests and Climate Change, Commerce & Industry, Rural Development, Health and Family Welfare,

Science and Technology, Home Affairs, Defence, and National Disaster Management Authority. Integration of the system is required at the top level for taking prompt decisions and conveying firm policy guidelines for managing risk of entry, establishment or spread of pests and diseases and undertaking action plans to regulate them effectively. The integrated system would facilitate (a) timely handling of calamities, (b) appropriate utilization of resources, and (c) establishment of a systems approach to achieve desired results. Integration would imply sharing of national facilities and expertise across different disciplines and institutions like ICAR, CSIR, and SAUs etc. In the light of the various issues highlighted, there is an urgent need for a coordinated effort to have an effective biosecurity system in the country.

The challenges for implementation of the Biosecurity regime in India are immense, given the size and geographical variations within the country. Lack of trained manpower and the resources for scientific research are additional challenges that loom large. In some of the other countries that have undertaken a similar exercise, there is a suggestion to consolidate existing legislation and create a single agency to deal with Biosecurity concerns. However, this approach needs more careful consideration in the Indian context. The motivations behind the existing legal framework and the focus of work of the respective institutions differ vastly. Besides, the Biosecurity concerns do not necessarily override the pre-existing purposes behind the sectoral legislative instruments as listed in Table 1. An altogether new legal framework harmonized with these pre-existing legislations, with a mechanism tailored to carry out the task of promoting biosecurity within their respective mandates, could perhaps be a more effective approach.

Presently, at the national level efforts are being made to develop a coherent biosecurity strategy for the country by the formulation of a comprehensive Agricultural Biosecurity Bill in 2013. DAC&FW has initiated the establishment of a National Agricultural Biosecurity System with a National Agricultural Biosecurity Centre at DPPQS and a National Agricultural Biosecurity Network with the stakeholders from various ministries, ICAR, SAUs and state departments (Swaminathan, 2008). Also, the re-drafting of the Biosecurity Bill in 2017 by the DAC&FW to address the issue of national biosecurity in a holistic manner are some of the important steps in the right direction.

Besides, effective regional collaborative efforts are needed to deal with all known or perceived agricultural biosecurity threats. India has land contiguity with many neighbouring countries and close proximity to the Island nations of Sri Lanka and Maldives. As a result any new pest incursions in these neighbouring countries may eventually enter into India as pests do not recognize national boundaries. The cooperation with neighbouring countries and harmonization of phytosanitary measures in the South Asia will go a long way in protecting the biosecurity of the Region as well as the Nation. India being a lead country in the region, should take the initiative for identifying the potential pest threats to the region. Further, in the event of any new pest incursions in the neighbouring countries which may be of potential concern to India's biosecurity, the NPPO of India may need to take a proactive role by providing necessary assistance to the neighbouring countries in containment and eradication (Satyanarayan and Satyagopal, 2013). This is also required to fully utilize the available resources at the regional level both in terms of infrastructure and expertise.

The key areas for regional collaboration include harmonizing regulations, simplifying quarantine procedures, risk analyses, research and human resource development (HRD). The regulatory cooperation would be for joint deliberation on policy issues at international fora. The quarantine procedural collaboration includes identification of expertise/ accredited labs/ post-entry quarantine facilities for testing special crops/ pests in a network mode and countries with contiguous borders could develop programmes for eradication/ declaration of pest free areas to facilitate exports from the region. Also, each country to freely share information on respective surveillance programmes to enable development of common pest risk analyses for crops/ pests of quarantine significance and also deal with common biosecurity threats and emergencies (Gupta and Dubey, 2016). The areas of collaborative research and HRD also need to be identified based on gap analysis and suitable collaborative programmes developed. Development of database on pest status in countries of the region in addition to regular trainings to strengthen research capabilities need to be conducted.

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Conservation of Forest Genetic Resources: Need and Challenges

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Threats to Forest Genetic Resources (FGRs) continue at an unprecedented rate and almost 20% of the FGRs are facing different categories of threats to their existence. There is an urgent need to address the issue to arrest degradation of natural populations of forestry species and improve status of regeneration from continuously increasing biotic pressure, assess conservation status of FGRs and develop a prioritized action plan as well as to prepare an elaborate inventory of the wild populations of FGR. Before embarking on a comprehensive programme for conservation of FGRs, there are many issues that need to be addressed, like inadequate knowledge about the genetic diversity of priority forest species; limited knowledge about conservation biology, population dynamics, reproductive biology and fruit/seed production; irregularity in flowering and fruiting; variability in seed production among species and regions- the causes of these variations are often uncertain; determining the seed storage behaviour of a species; devising appropriate methods of conservation for species producing intermediate and recalcitrant seeds. Under a programme for conservation of FGRs of Uttarakhand, *ex situ* conservation of valuable germplasm of economically important forestry species as well as the ones facing threats to their existence, is envisaged. The paper presents the entire programme and the approaches to successfully conserve the precious genetic resources through seeds.

Key Words: Conservation, Challenges, Forest Genetic Resources, Seeds, Storage physiology

Introduction

Forest loss and degradation remain major global concerns despite the enormous efforts to achieve sustainable forest management. There is also increasing awareness of the critical values that forest genetic diversity provide per se and as means to confront global challenges, such as degradation of forests and climate change (www.fao.org/nr/cgrfa). Among the different components of biodiversity, genetic diversity is the building block of the evolutionary process. Conservation of Forest Genetic Resources, therefore, should accommodate evolutionary concepts to ensure continuous adaptation under changing environments (Eriksson *et al*., 1993). Several global and regional threats to forest ecosystems are contributing to profound changes in the pattern of distribution of tree genetic diversity (Koskela and Amaral, 2001).

Increased use of forest resources and a shrinking forest land base threaten the sustainability of forest genetic resources and highlight the importance of conservation and sustainable management of these resources. As forest trees are normally the keystone species of forest ecosystems, their continued existence is essential for many floral and faunal associations in these ecosystems. The major challenges include population decline and population structure changes due to forest removal and conversion of forest land to other uses, forest fragmentation, forestry practices, climate change, disease conditions, introduced pests, atmospheric pollution, and introgressive hybridization. Forest genetic resource conservation and resource use should be considered complementary rather than contradictory to each other (Rajora and Mosseler, 2001). Genetic diversity provides the fundamental basis for evolution of forest tree species. This diversity has enabled forests and trees to adapt to changing and adverse conditions for thousands of years, and has resulted in a unique and irreplaceable portfolio of forest tree genetic resources. Nevertheless, the vast majority of forest genetic diversity remains unknown (www.fao.org/nr/cgrfa).

Threats to Forest Genetic Diversity

Conservation aims at maintaining as much as of genetic variation of the original sources as possible. Protection or conservation measures are essential for securing a sustained supply of genetic variation. Effective conservation of genetic variability is dependent on thorough knowledge of the species in respect of occurrence, mode of reproduction, breeding system, genetic structure and the number of other features related

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to them. Five major causes of biodiversity loss have been identified globally: (1) Habitat loss, fragmentation and degradation, (2) Unsustainable use of ecosystem and over-exploitation of resources, (3) Invasive alien species, (4) Pollution, and (5) Climate change (Sinha, 2011).

Conservation and Management of Forest Genetic Resources in India

About 80 percent of forest genetic resources of India are found in ten bio-geographic zones in various forests in the country. Remaining 20 percent of plant genetic resources are found in sacred grooves, pasture lands, community lands, agricultural lands, urban areas, botanical gardens, etc. National Bureau of Plant Genetic Resources (NBPGR) in the country is mandated with planning, execution and coordination of all activities concerned with the germplasm collection, introduction, quarantine, evaluation, conservation and documentation of agriculture, horticultural crops, aromatic and medicinal plants and agroforestry species at national level. Despite vast FGRs, and its great importance, there is no separate Bureau of Forest Genetic Resources for exploration, collection, documentation, characterization, evaluation and conservation. As of now, there is no concerted and coordinated effort for the conservation and documentation of forest genetic resources in India (Kumar *et al*., 2016).

The National Forest Policy (1988) has conservation as its basic objective, that emphasizes conserving the natural habitat of the country by preserving natural forests and their vast variety of flora and fauna that represents the biological diversity and genetic resources of the country. The aim of genetic resources conservation is to secure the adaptability of population and species in changing environment by maintaining a sufficient level of genetic variability. This requires provenance trials and progeny tests to complement new research approaches.

Provenance trials : Provenance trials help in exploration of genetic resources called genecological exploration. Through genecological exploration, patterns of ecological and phenotypic variation within the natural range of species are studied, leading to provenance seed collection and provenance evaluation. Provenance trials have been established for species like teak, eucalyptus, casuarinas, acacias, neem, shisham, pines, melias, etc.

Seed orchards: Seedling seed orchards and clonal seed orchards are established for production of genetically improved seeds for operational planting programme and are a good form of *ex-situ* conservation.

***Ex-situ* conservation of FGRs in gene banks, *in vitro* banks and cryobanks:** Forest Genetic Resources of economically and ecologically important plant species have great importance in the genetic improvement programmes to inculcate desired traits like improved productivity, quality, drought/ cold/ salt tolerance, disease resistance, etc. before further genetic erosion, FGRs of priority species must be conserved in gene banks (at 0 to -20^{o} C temperature) for medium/ long term conservation, *in vitro* culture and cryopreservation of forestry species is also lacking. Therefore, there is a need to carry out studies on the short, medium and long term storage of germplasm of the priority species using seed, tissues, organ, embryo and pollen.

Need for FGR Conservation

The forest genetic resources are facing several types of threats due to adverse abiotic and biotic stresses; habitat degradation, destruction, grazing, over-exploitation, forest fires, climate change and invasive alien species, etc., resulting in damage to forest ecosystem as well as loss of biodiversity. When genetic variation is lost through habitat destruction succeeding generation are more exposed to adverse conditions such as atmospheric pollution, climate change, pests and diseases, etc. in addition, illegal trade of forest germplasm poses problem of losing valuable resources permanently. A long-term, multi-faceted approach of conservation of forest genetic resources has to be evolved and followed in the country.

Constraints in FGR Conservation

There are many constraints in the field of conservation of FGRs, which make priority-setting one of the most important tasks not only of conservation work but also of research. These are :

- State-wise inventories of FGRs, with quantitative assessment of population/threat status not available.
- Conservation of FGRs only incidental under 'tree-centric' forest management/PA management; or under Preservation Areas/Sacred Groves, etc.
- Inadequate knowledge about the population parameters and genetic diversity of priority forest species.

- Limited knowledge about conservation biology, population dynamics, habitat ecology, seed storage physiology of most of the important FGR species.
- *Ex situ* conservation efforts (Germplasm Banks/ Seed Banks) limited to only a few native species viz. teak, neem, shisham, babool, melia, and bamboos. Whereas major emphasis of such conservation being on exotic tree species like eucalypts, poplars, *casuarina, Jatropha*, rubber, and *Acacia auriculiformis*, etc.
- No designated national agency for undertaking programmes on conservation, management and development of FGRs. Agencies like Botanical Survey of India (BSI), ICAR-National Bureau of Plant genetic Resources (ICAR-NBPGR), National Biodiversity Authority (NBA), GB Pant Institute, National Bio-resource Development Board, IIRS and FSI working on FGRs within their limited mandate.

Forest Genetic Resources Conservation and Development Program for North-Western Himalayas (Uttarakhand)

Uttarakhand being a part of Indian Himalayan region (IHR) with forest cover of 24,992 sq km (46.73 % of its total geographical area) (ISFR 2015) is home to vast variety and unique range of floral and faunal diversity of India as the state is uniquely endowed with a diverse assemblage of natural ecosystems. Its diversity under 1,503 genera and 213 families of flowering plants, including 93 endemic species is harboured in various vegetation types, ranging from sub-tropical forests in upper Gangetic Plain and Shiwaliks zone in the south to arctic-alpine vegetation of trans-Himalayan cold desert in the north in Uttarakhand. Major natural vegetation entails of pines, oaks (*Quercus* spp.), rhododendrons, walnut (*Juglans regia*) hill bamboos, etc. Below the snow line, the vegetation consists of forests of spruce (*Picea* spp.), fir (*Abies* spp.), cyprus, juniper (*Juniperus* spp.) and birch (*Betula* spp.)

A Research Programme on Creation of Centre of Excellence of FGRs is being implemented at Forest Research Institute, Dehradun. Its pilot phase (2016-2020) with special focus on the exploration and conservation of Forest Genetic Resources of North-West Himalayas, is being implemented. This programme will built the foundation for Creation of Centre of Excellence on Forest Genetic Resources of India in Indian Council of Forestry Research and Education.

The major activities of the programme include:

Documentation of FGRs

Field surveys would be conducted to document the diversity and population of about 250 species of FGRs. Species distribution records of FGRs would be extracted from herbaria and Working Plans, based on these information eco-distribution maps of the species would be prepared upon completion of these.

FGR Seed and Germplasm Storage

Collection of seed/ germplasm of prioritized FGR species and also pollens for *in vitro* storage, would be done. Seed extraction, cleaning, grading is being done and passport data will be maintained. Quality of the seed lot is evaluated. Sensitivity of the seed to desiccation is being studied using IPGRI-DFSC (2002) protocol, after which storage protocols for medium to long-term conservation will be developed (processed samples will be stored in the Genebank of NBPGR for long-term conservation). Protocols for storage of pollens of critically endangered species would also be attempted.

Protocols would be developed for *in vitro*, minimum growth, embryo culture storage for recalcitrant seeds of priority FGRs.

FGR Characterization

Under this component evaluation and molecular characterization for biochemical traits and tolerance to disease and pests is being assessed. Genetic diversity studies of FGRs of conservation concern/high commercial value would be conducted through advanced molecular techniques.

FGR Conservation

For this, Regional Conservation Assessment and Management Plan (CAMP) Workshops would be held to assess the threat status of FGRs based on the field surveys and population studies. Also Field Gene Banks of priority FGRs species would be established, preferably in their natural zone of occurrence.

Conclusion

The pilot programme on Conservation of FGRs of the fragile ecosystems in North-Western Himalaya would enrich the team in such work, this would gradually be taken up for FGRs of other biogeographic regions of the country. Conservation of FGR aims to maintain the evolutionary processes of forests and the diverse

genepools they contain for present and future use. To make conservation or sustainable forest management successful a broad planning process involving different stakeholders within and outside the forest sector, needs to be evolved. The capacity of national institutions to carry out FGR conservation should be strengthened, and FGR conservation programmes should be included in national plans/policies for forestry and biodiversity conservation.

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Role of Viral Diagnostics in Quarantine for Plant Genetic Resources and Preparedness

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International exchange of plant genetic resources (PGR) and trade play an important role in the long-distance dissemination of destructive viruses/strains. The ICAR-NBPGR has been empowered for quarantine processing of imported PGR including transgenics meant for research purposes. Adopting a workable strategy of post-entry quarantine growing/inspection followed by use of combination of detection techniques viz., electron microscopy, ELISA and RT-PCR, 45 viruses of great economic and quarantine importance were intercepted in imported PGR in the last three decades (1985-2016). The interceptions include 19 viruses not yet reported from India viz., *Barley stripe mosaic virus*, *Bean mild mosaic virus, Bean pod mottle virus*, *Broad bean mottle virus, Broad bean stain virus, Broad bean true mosaic virus*, *Cherry leaf roll virus*, *Cowpea mottle virus, Cowpea severe mosaic virus,* Dioscorea latent virus, *Garlic virus C*, High plains virus, *Maize chlorotic mottle virus*, *Pea enation mosaic virus*, *Peanut sunt virus*, *Pepino mosaic virus, Raspberry ringspot virus, Tomato ringspot virus* and *Wheat streak mosaic virus*. Besides, 21 viruses not known to occur on particular host(s) in India were intercepted and 23 viruses were intercepted in germplasm imported from CGIAR centres. Fourteen viruses including nine viruses not reported from India have been intercepted in transgenic germplasm. Even though some of the intercepted viruses are not known to occur in India, their potential vectors exist and also congenial conditions to multiply, disseminate and spread destructive exotic viruses/strains. India also need to establish a strong network of interconnected accredited laboratories able to quickly diagnose new viruses/strains; enhance surveillance capacity and develop early warning systems. This makes it imperative that India should put in place a "National Plant Pests Diagnostic and Certification Network" to enhance preparedness.

Key Words: Diagnostics, Plant genetic resources, Quarantine, Viruses

Introduction

The global movement of plant genetic resources (PGR), agri-horticultural produce and products has the potential of introducing new pests including viruses which may pose potential risk to the agriculture of the importing country. The unrestricted exchange of seed lots has been attributed to the worldwide distribution of many economically important viruses such as *Bean common mosaic virus*, *Soybean mosaic virus*, *Pea seed-borne mosaic virus*, *Wheat streak mosaic virus*, *Peanut mottle virus,* etc. A number of exotic plant viruses have been introduced into India along with imported planting material causing serious crop losses. These included *Banana bunchy top virus* (BBTV), *Peanut stripe virus* etc. BBTV was introduced and spread into India from Sri Lanka in 1940 (Magee, 1953). The international spread of BBTV is primarily through infected planting material (Wardlaw, 1961). In India, an annual loss of Rs.40 crore due to BBTV has been estimated in Kerala alone. These introductions highlight the fact that increased pace of international travel and trade, had exposed countries to the danger of infiltration of exotic viruses harmful to the agriculture.

The National Plant Protection Organizations are responsible for protecting their countries from the entry of new viruses and for eradicating those that have recently arrived and are still sufficiently confined for their elimination to be realistic. The strategies for biosecurity from plant viruses include stringent quarantine measures for the imported material and domestic quarantine/ use of certified virus-free seed and other planting material within the country. As per norms a workable sample of the bulk samples of seed lots/ vegetative propagules/ tissue culture-raised plants need to be inspected and tested. The detection of viruses is then carried out by the approved/ available techniques. Availability of rapid, reliable, robust, specific and sensitive methods for detection and identification of viruses determine the success of the process.

The ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), the nodal institution for management of PGR has been empowered under the

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Plant Quarantine (Regulation of Import into India) Order 2003 (hereinafter referred to as PQ Order), under the Destructive Insects and Pests Act, 1914, to undertake quarantine processing of germplasm including transgenic planting material imported for research purposes into the country by both public and private sectors. It has well-equipped laboratories and post-entry quarantine (PEQ) green house complex to undertake the task. A National Containment Facility of level-4 (CL-4) has been established to ensure that no viable biological material/ pollen/ pathogen enters or leaves the facility during quarantine processing of transgenics. At ICAR-NBPGR, adopting a workable strategy using post-entry quarantine (PEQ) growing/inspection, Electron Microscopy (EM), Enzyme-linked Immunosorbent Assay (ELISA), and Reverse-transcription Polymerase Chain Reaction (RT-PCR), a number of viruses have been intercepted in germplasm imported from many countries including Consultative Group on International Agricultural Research (CGIAR) centres (Khetarpal *et al*., 1992; Khetarpal *et al*., 1994; Khetarpal *et al*., 2001; Khetarpal *et al*., 2006; Chalam and Khetarpal, 2008; Chalam *et al*., 2005a; Chalam *et al*., 2005b; Chalam *et al*., 2007; Chalam *et al*., 2008b; Chalam *et al*., 2009b; Chalam *et al*., 2014b; Chalam *et al*., 2017; Kumar *et al*., 1991; Parakh *et al*., 1994; Parakh *et al*., 2005; Parakh *et al*., 2006; Parakh *et al*., 2008; Parakh *et al*., 2009).

The present paper discusses the interception of 45 plant viruses in quarantine processing of germplasm including transgenics imported during the last three decades (1985-2016).

Materials and Methods

Testing methods for detection of viruses in imported germplasm (seeds, *in vitro* cultures, vegetative propagative material etc.) including transgenics during quarantine as given below were employed.

Visual Inspection

All the accessions (seeds, *in vitro* cultures, vegetative propagative material etc.) were examined visually. In case of seeds, shrivelled, small sized and seeds showing mottling, necrosis, split seed coat symptoms and other discolourations were discarded and incinerated.

Growing-on Test at ICAR-NBPGR, New Delhi

Apparently healthy looking seeds of legume germplasm belonging to 25,499 accessions were sown during respective *Kharif* and *Rabi* seasons in an insect-proof PEQ greenhouse in soil beds during 1985-2016. Each accession was grown in one-metre row with 15-20 plants per row. Seedlings were observed regularly after emergence till flowering. The accessions showing virus-like symptoms were further subjected to Electron microscopy (EM), Enzyme-linked immunosorbent assay (ELISA) and Reverse-transcription- polymerase chain reaction (RT-PCR), if required.

Growing-on Test of Transgenics in Containment Facility at ICAR-NBPGR, New Delhi

The transgenic germplasm belonging to 13,648 accessions were grown in containment facility. The accessions showing virus-like symptoms were further subjected to EM, ELISA and RT-PCR, if required.

Post-entry Quarantine Inspection at Indenter's Site

The germplasm other than legumes were released on an undertaking by the indenter that material will be grown in isolation under the supervision of a plant pathologist. The transgenics were released on an undertaking by the indenter that material will be grown in containment facility approved by the Department of Biotechnology, Ministry of Science and Technology, Government of India under the supervision of a plant pathologist. The accessions showing virus-like symptoms were further subjected to EM, ELISA and RT-PCR, if required.

Electron Microscopy (EM)

Two mg tissue from leaf samples showing prominent viral symptoms during growing-on test and *in vitro* cultures were taken and homogenized in 0.07M phosphate buffer, pH 6.5. Ten µl leaf extracts of each accession were deposited for one min on carbon-coated grids. The grids were then washed with 10 drops of distilled water and stained with 2% uranyl acetate. Excess of stain was removed by touching the edge of the grid with a piece of filter paper and the grids were examined under Transmission Electron Microscope (JEOL-JEM 1011).

Enzyme-linked Immunosorbent Assay (ELISA)

The leaf samples showing virus-like symptoms of different crops, representative healthy-looking samples and *in vitro* cultures were tested by adopting the classical ELISA protocol (Clark and Adams, 1977) using polyclonal antiserum against 52 viruses viz., *Alfalfa mosaic virus* (AMV), *Arabis mosaic virus* (ArMV),

Barley stripe mosaic virus (BSMV), *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Bean mild mosaic virus* (BMMV), *Bean pod mottle virus* (BPMV), *Bean yellow mosaic virus* (BYMV), *Broad bean stain virus* (BBSV), *Broad bean true mosaic virus* (BBTMV), *Broad bean mottle virus* (BBMV), *Broad bean wilt virus* (BBWV), *Carnation latent virus* (CLRV), *Cherry leaf roll virus* (CLRV), *Cowpea aphid-borne mosaic virus* (CABMV), *Cowpea mild mottle virus* (CPMMV), *Cowpea mosaic virus* (CPMV), *Cowpea mottle virus* (CPMoV), *Cowpea severe mosaic virus* (CPSMV), *Cucumber mosaic virus* (CMV), Dioscorea latent virus (DLV), Dioscorea mosaic virus (DMV), *Garlic common latent virus* (GarCLV), *Garlic virus* C (GarV-C), *Grapevine fanleaf virus* (GFLV), High plains virus (HPV), Iris yellow spot virus (IYSV), *Leak yellow stripe virus* (LYSV), *Maize chlorotic mottle virus* (MCMV), *Maize dwarf mosaic virus* (MDMV), *Onion yellow dwarf virus* (OYDV), *Pea enation mosaic virus* (PEMV), *Pea seed-borne mosaic virus* (PSbMV), *Peanut mottle virus* (PeMoV), *Peanut stripe virus* (PStV), *Peanut stunt virus* (PSV), *Pepino mosaic virus* (PepMV), *Raspberry ringspot virus* (RRSV), *Red clover vein mosaic virus* (RCVMV), *Shallot latent virus* (SLV), *Shallot yellow stripe virus* (SYSV), *Southern bean mosaic virus* (SBMV), *Soybean mosaic virus* (SMV), *Tobacco necrosis virus* (TNV), *Tobacco rattle virus* (TRV), *Tobacco ringspot virus* (TRSV), *Tobacco streak virus* (TSV), *Tomato aspermy virus* (TAV), *Tomato black ring virus* (TBRV), Tomato mosaic virus (ToMV), *Tomato ringspot virus* (ToRSV) and *Wheat streak mosaic virus* (WSMV) (Source: Agdia Inc., USA; AC Diagnostics, USA; Adgen, UK; Bioreba, Switzerland; Biorad, USA; DSMZ Germany; Loewe Phytodiagnostica, Germany; Neogen USA; Sanofi Pasteur, France). Double Antibody Sandwich-ELISA (DAS-ELISA) was used. The leaf samples were ground in phosphate buffered saline -Tween 20, pH 7.4 + 2% polyvinyl pyrrolidone (PVP) for all except SBMV for which samples were ground in sodium sulfite + sodium azide + ovalbumin + PVP + Tween 20 buffer, pH 7.4. The extracts (1:10 w:v) were centrifuged at 3000 rpm for 3 min., and supernatant was collected. The microtiter plates of Costar (USA) were used. Immunoglobulins (IgG) to different viruses and enzyme conjugate were used at 1:100 or 1:200 dilution each as per the Company's direction. The positive and negative controls for each of the viruses used were the lyophilized leaf tissue (Source: Agdia Inc., USA; AC Diagnostics, USA; Adgen, UK; Bioreba, Switzerland; Biorad, USA; DSMZ Germany; Loewe Phytodiagnostica, Germany; Neogen USA; Sanofi Pasteur, France). The enzyme substrate used was *p*-nitro phenyl phosphate (1 mg/ml). The optical density (OD) values of samples were recorded at 405 nm using ELISA plate reader (TECAN Sunrise, Austria) at a time interval of 15 min., 30 min., 1 h, 1 h 30 min., and 2 h after incubation. A sample was considered as infected if its OD value was more than twice that of negative control (Chalam and Khetarpal, 2008; Chalam *et al*., 2005a; Chalam *et al*., 2007; Chalam *et al*., 2008b; Chalam *et al*., 2009b; Chalam *et al*., 2014b; Chalam *et al*., 2016b).

Reverse-transcription Polymerase Chain Reaction (RT-PCR)

RNA was extracted from samples using RNeasy ® Plant Mini Kit (Qiagen), following protocol provided by the manufacturer. Concentration (ng/µl) of isolated RNA was measured by Thermo Scientific Nanodrop Reader. Synthesis of cDNA was performed with Thermo Fischer cDNA Kit. For PCR reaction of viral cDNA, 5 µl of RT reaction was used as template. The 20 µl PCR reaction was prepared including 3 µl sterile water, 1 µl forward primer (5 µM), 1 µl reverse primer (5 µM), 5 µl cDNA template and 10 µl of 2X Go Taq master mix (Promega M7123) for each sample. PCR reactions for negative control were also prepared without adding the cDNA template. The lid of thermo cycler was kept at 99°C and pre- heating was also given. The PCR products were separated by electrophoresis of 20 µl of amplified product on 1.5% TAE agarose gel stained with ethidium bromide at 80 volts for 45 min. and image was visualized and captured by Gel documentation system (BioRad). RT-PCR was used wherever the ELISA results were ambiguous/inconclusive (Arif *et al*., 20012; Chalam and Khetarpal, 2008; Chalam *et al*., 2004; Chalam *et al*., 2008a; Chalam *et al*., 2012a;. Chalam *et al*., 2012b; Chalam *et al*., 2016b).

All plant residues and plant samples used for testing were incinerated as per quarantine procedure.

Results and Discussion

During the last three decades (1985-2016), by adopting a workable strategy such as PEQ growing in PEQ greenhouses/ containment facility and inspection, PEQ inspection at indenter's site, EM, ELISA and RT-PCR, 45 viruses of great economic and quarantine importance have been intercepted in exotic germplasm including

transgenics (Table 1). The interceptions include 19 viruses not yet reported from India viz., BSMV, BMMV, BPMV, BBMV, BBSV, BBTMV, CLRV, CPMoV, CPSMV, DLV, GarV-C, HPV, MCMV, PEMV, PepMV, PSV, RpRSV, ToRSV and WSMV. Besides, 21 viruses not known to occur on particular host(s) in India have been intercepted and these are also of quarantine significance for India. Two viruses viz., ArMV and RCVMV are present in India but with restricted distribution in India. ArMV is reported to occur only on rose in Palampur, Himachal Pradesh and RCVMV is reported only on pea from Faizabad, Uttar Pradesh. Twenty three viruses have been intercepted in germplasm imported from CGIAR centres. Fourteen viruses including nine viruses not reported from India have been intercepted in transgenic germplasm. Majority of the accessions were found infected by more than one virus (Chalam 2016; Chalam 2014; Chalam *et al*., 2005a; Chalam *et al*., 2007; Chalam *et al*., 2008b; Chalam *et al*., 2009a; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2012b; Chalam *et al*., 2012c; Chalam *et al*., 2012d; Chalam *et al*., 2012e; Chalam *et al*., 2013b; Chalam *et al*., 2013c; Chalam *et al*., 2013d; Chalam *et al*., 2014a; Chalam *et al*., 2014b; Chalam *et al*., 2014c; Chalam *et al*., 2015a; Chalam *et al*., 2015b; Chalam *et al*., 2016a; Chalam *et al*., 2016b; Chalam *et al*., 2017; Chalam and Khetarpal 2008; Khetarpal *et al*., 1992; Khetarpal *et al*., 1994; Khetarpal *et al*., 2001; Khetarpal *et al*., 2003; Kumar *et al*., 1991; Parakh *et al*., 1994; Parakh *et al*., 2005; Parakh *et al*., 2006; Parakh *et al*., 2008; Parakh *et al*., 2009; Prasada Rao *et al*., 1990; Prasada Rao *et al*., 2004; Prasada Rao *et al*., 2012; Singh *et al*., 2003).

Out of 45 viruses detected, 35 viruses either by virus species name or as genus including 26 as virus species viz., AMV, ArMV, BBSV, BBMV, BBTMV, BBWV, BSMV, BYMV, CLRV, CMV, GFLV, HPV, PEMV, PepMV, PStV, PSV, RpRSV, TAV, TBRV, TNV, TRV, TRSV, ToMV, ToRSV, TSV, WSMV and nine as genus *viz., Comovirus* (BPMV, CPMV and CPSMV), *Carmovirus* (BMMV, CPMoV) *Potyvirus* (BCMV, BCMNV, CABMV, SMV), have been mentioned in the PQ Order and require an additional declaration from exporting country that the consignment is free from one or more of these viruses on case to case basis, hence these intercepted viruses are of high quarantine significance for India.

All the plants showing virus-like symptoms were thus checked for the presence of virus and when found infected were uprooted and incinerated. A total of 226 accessions belonging to *Capsicum annuum* (47); *Glycine max* (64); *Phaseolus vulgaris* (1); *Pisum* spp. (24); *Solanum lycopersicum* (21), *Vigna catjung* (1); *V. radiata* (1); *V. subterranea* (2); *V. unguiculata* (46); *Zea mays* (19 including four accessions of transgenic *Z. mays*) were rejected due to presence of viruses of quarantine significance for India. Harvest from virus-free plants was released to the indenters.

Among the viruses intercepted 19 are not known to occur in India and their potential vectors exist and so also the congenial conditions for them to multiply, disseminate and spread the destructive exotic viruses/ strains. The risk of introduction of 45 viruses or their strains into India was thus eliminated. If not intercepted, some of these viruses could have been introduced into the country and caused economic yield losses. Besides, the harvest obtained from virus-free plants also ensured conservation of virus-free exotic germplasm in the National Genebank.

In order to highlight the importance of interception of viruses in exotic germplasm an example of *Bean pod mottle virus* (BPMV) is given below:

BPMV, not reported from India, was detected and intercepted in exotic germplasm of *Glycine max* from USA (Chalam *et al*., 2014b). BPMV is seed-transmitted at a rate of 0.037 to 0.1% in *G. max*. It causes yield-reductions ranging from 3 to 52.4% and seed coat discolouration. Seed coat mottling and dark pigments have often been problematic for soybean farmers and industry as it reduces consumer acceptance (Giesler *et al*., 2002). If not intercepted, BPMV could have been introduced into the country and established as favourable environmental conditions are available in India. If introduced, it would have caused an annual yield loss of Rs.2632.5 million with the incidence of 1%. The Possible yield losses were calculated based on soybean production (10.53 m t) and minimum support price (Rs. 2500/q) during 2014-15 (Anonymous, 2016).

The present findings thus highlight the importance of plant quarantine in minimizing/ eliminating the risk of introducing destructive exotic viruses along with seeds/ *in vitro* cultures of germplasm including transgenics.

V. Conclusions and Future Perspectives

Due to the exchange of PGR and liberalized trade under WTO there is an increasing possibility of introduction of exotic viruses if stringent quarantine measures are not

Table 1. Plant viruses intercepted in germplasm imported into India for research purposes during 1985-2016

S. No.	Virus intercepted	Host	Source of import	Reference
1	[cd]*Alfalfa mosaic virus* (AMV)	*Glycine max*	AVRDC (Taiwan), IITA (Nigeria), Brazil, Canada, Costa Rica, Myanmar, Sri Lanka, USA	Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008; Parakh *et al*., 2005; Parakh *et al*., 2008
		Phaseolus vulgaris[c]	CIAT (Colombia), Canada, Kenya, Nepal, Russia	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		Pisum sativum[c]	ICARDA (Syria), Russia, Spain, USA	Chalam *et al*., 2014b
		Vicia faba	ICARDA (Syria), Spain	Chalam *et al*., 2009a; Chalam *et al*., 2014b
		Vigna mungo[c]	Bhutan	Chalam *et al*., 2014b
		V. radiata[c]	IITA (Nigeria), Japan	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata[c]	CIAT (Colombia), IITA (Nigeria), Italy, USA	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008
2	[bcde]*Arabis mosaic virus* (ArMV)	*G. max*[ce]	Canada, Costa Rica, USA[e]	Chalam *et al*., 2014b; Chalam 2016; Chalam *et al*., 2016a
		V. unguiculata[c]	IITA (Nigeria), Italy	Chalam 2016; Chalam *et al*., 2016a
3	[acde]*Barley stripe mosaic virus* (BSMV)	*Hordeum vulgare*	USA	Chalam and Khetarpal 2008; Khetarpal *et al*., 2006
		Triticum aestivum[f]	USA[f]	Chalam 2014; Chalam 2016; Chalam *et al*., 2013b; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2015b; Chalam *et al*., 2016b
		Zea mays[f]	Philippines[f], France[f], USA[f]	Chalam 2014; Chalam 2016; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2016a
4	[c]*Bean common mosaic virus* (BCMV)	*G. max*[c]	AVRDC (Taiwan), IITA (Nigeria), Brazil, Canada, Costa Rica, Thailand, USA	Chalam *et al*., 2014b; Chalam *et al*., 2016; Chalam and Khetarpal 2008; Parakh *et al*., 2005; Parakh *et al*., 2008
		P. vulgaris	CIAT (Colombia), CIS, Hungary, Kenya, Nepal, Russia, USA	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008; Khetarpal *et al*., 1994, Khetarpal *et al*., 2001
		V. radiata	AVRDC (Taiwan), Japan, USA	Chalam and Khetarpal 2008; Chalam *et al.,* 2014b; Khetarpal *et al.,* 2001
		V. subterranea[c]	Ghana	Chalam *et al*., 2014b
		V. unguiculata	AVRDC (Taiwan), IITA (Nigeria), Guyana, Italy, USA	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2016a;
5	[c]*Bean common mosaic necrosis virus* (BCMNV)	*G. max*	Costa Rica	Chalam *et al*., 2016a
		P. vulgaris	CIAT (Colombia), Kenya, Russia	Chalam *et al*., 2007; Chalam *et al*., 2014b
		V. marina[c]	AVRDC (Taiwan)	Chalam *et al*., 2014b

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
6	[ae]*Bean mild mosaic virus* (BMMV)	*G. max*[e]	Canada, Colombia, USA[f]	Chalam, 2016; Chalam *et al*., 2016a; Chalam *et al*., 2017
		P. vulgaris	USA	Chalam 2016; Chalam *et al*., 2017
7	[a]*Bean pod mottle virus* (BPMV)	*G. max*	USA	Chalam *et al*., 2014b
		P. vulgaris	USA	Chalam *et al*., 2016
		V. unguiculata	IITA (Nigeria), Italy	Chalam, 2016; Chalam *et al*., 2016a
8	[de]*Bean yellow mosaic virus* (BYMV)	*G. max*[e]	IITA (Nigeria), Canada, Myanmar, USA[e]	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008; Parakh *et al*., 2005
		P. vulgaris	CIAT (Colombia), Nepal	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		P. sativum	Spain, USA	Chalam *et al*., 2014b
		V. faba	ICARDA (Syria), Bulgaria, Spain	Chalam *et al.,* 2007; Chalam *et al.,* 2009a; Chalam *et al.,* 2014b; Chalam and Khetarpal 2008; Khetarpal *et al.,* 2001
9	[ad]*Broad bean stain virus (*BBSV*)*	*G. max*	AVRDC (Taiwan), Costa Rica	Chalam, 2016; Chalam *et al*., 2016a
		V. radiata	AVRDC (Taiwan)	Chalam, 2016; Chalam *et al*., 2016
		P. sativum	Spain	Chalam *et al*., 2014b
		V. faba	ICARDA (Syria), Bulgaria	Chalam *et al*., 2007, Chalam *et al*., 2009b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008; Khetarpal *et al*., 2001
10	[ad]*Broad bean true mosaic virus (*BBTMV*)*	*P. vulgaris*	USA	Chalam 2016; Chalam *et al*., 2017
11	[ad]*Broad bean mottle virus (*BBMV*)*	*P. vulgaris*	AVRDC (Taiwan)	Chalam, 2016; Chalam *et al*., 2017
12	[cd]*Broad bean wilt virus* (BBWV)	*G. max*[c]	Canada, Costa Rica, USA	Chalam *et al*., 2014b; Chalam *et al*., 2016a
		P. vulgaris[c]	CIAT (Colombia)	Chalam *et al*., 2014b
		P. sativum	USA	Chalam *et al*., 2014b
		V. faba	ICARDA (Syria)	Chalam *et al*., 2009a; Chalam *et al*., 2014b
13	[ade]*Cherry leaf roll virus* (CLRV)	*G. max*[e]	AVRDC (Taiwan), Sri Lanka, Thailand, USA[e]	Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008
		P. vulgaris	CIAT (Colombia)	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata	IITA (Nigeria)	Chalam, 2016; Chalam *et al*., 2016a

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
14	[c]*Cowpea aphid-borne mosaic virus* (CABMV)	*G. max*[c]	AVRDC (Taiwan), IITA (Nigeria), Myanmar, Sri Lanka, Thailand, USA	Chalam *et al*., 2014b; Chalam and Khetarpal 2008; Parakh *et al*., 2005; Parakh *et al*., 2008
		V. radiata[c]	AVRDC (Taiwan)	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata	IITA (Nigeria), Eritrea, Guyana, USA	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008; Khetarpal *et al*., 2001
15	[c]*Cowpea mild mottle virus* (CPMMV)	*G. max*	AVRDC (Taiwan), USA	Chalam *et al*., 2014b
		P. sativum[c]	Spain	Chalam *et al*., 2014b
		V. faba[c]	ICARDA, Syria	Chalam *et al*., 2014b
		V. radiata	AVRDC (Taiwan)	Chalam *et al*., 2014b
16	[c]*Cowpea mosaic virus* (CPMV)	*G. max*[c]	AVRDC (Taiwan), Canada, Costa Rica,USA	Chalam, 2016; Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam *et al*., 2016a
		V. radiata[c]	AVRDC (Taiwan), USA	Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008
		V. unguiculata	IITA (Nigeria)	Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata ssp. sesquipedalis	Philippines	Chalam, 2016; Chalam *et al*., 2016a
17	[a]*Cowpea mottle virus* (CPMoV)	*V. unguiculata*	Philippines	Khetarpal *et al*., 2001
		V. subterranea	Ghana	Chalam *et al*., 2014b
18	[ae]*Cowpea severe mosaic virus* (CPSMV)	*G. max*[e]	AVRDC (Taiwan), Canada, Costa Rica, USA[e]	Chalam *et al*., 2014b; Chalam *et al*., 2016a
		V. radiata	Australia	Chalam *et al*., 2014b
		V. unguiculata	Belgium	Chalam *et al*., 2014b
19	[d]*Cucumber mosaic virus* (CMV)	*Capsicum annuum*	Republic of Korea	Chalam *et al*., 2016a
		Cucumis sativus	Brazil	Chalam, 2016; Chalam *et al*., 2017
		G. max	AVRDC (Taiwan), IITA (Nigeria), Brazil, Canada, Myanmar, Sri Lanka, USA	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008; Parakh *et al*., 2005; Parakh *et al*., 2008
		P. vulgaris	CIAT (Colombia)	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		Solanum lycopersicum	Netherlands, Republic of Korea, Thailand, USA	Chalam *et al*., 2016a
		V. faba	ICARDA (Syria)	Chalam *et al*., 2014b
		V. serratifolia	ICARDA (Syria)	Chalam *et al*., 2014b
		V. radiata	USA	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata	IITA (Nigeria), Belgium, USA	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
20	[a]Dioscorea latent virus (DLV)	*Dioscorea nipponica*	USA	Chalam *et al*., 2016a
21	*Garlic common latent virus* (GarCLV)	*Allium longicuspis*	USA	Parakh *et al*., 2009
		A. sativum	USA	Parakh *et al*., 2009
22	[a]*Garlic virus C* (GarV-C)	*A. sativum*	USA	Parakh *et al*., 2009
23	[cd]*Grapevine fanleaf virus* (GFLV)	*G. max*[c]	AVRDC (Taiwan), Canada, Costa Rica, USA	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2016a
		V. mungo[c]	Bhutan	Chalam *et al*., 2014b
		V. radiata[c]	AVRDC (Taiwan)	Chalam *et al*., 2016a
24	[ade]High plains virus (HPV)	*Z. mays*[e]	France[f], Thailand, USA[f]	Anonymous, 2017; Chalam, 2014; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2014a; Chalam *et al*., 2014c; Chalam *et al*., 2016a; Chalam *et al*., 2017
25	[ae]*Maize chlorotic mottle virus* (MCMV)	*Z. mays*[e]	Puerto Rico[f], Thailand, USA[f]	Anonymous, 2017; Chalam, 2016; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2013b; Chalam *et al*., 2013c; Chalam 2014; Chalam *et al*., 2016a; Chalam *et al*., 2017
26	[ce]*Maize dwarf mosaic virus* (MDMV)	*T. aestivum*[cf]	USA[f]	Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam 2014; Chalam *et al*., 2016b; Chalam *et al*., 2017
		Z. mays[e]	Colombia, France[f], Kenya, Mexico, Philippines[f], Sudan, USA[f]	Chalam, 2016; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2016a; Chalam *et al*., 2017; Chalam and Khetarpal, 2008; Khetarpal *et al*., 2006
27	[ad]*Pea enation mosaic virus* (PEMV)	*G. max*	Costa Rica	Chalam, 2016; Chalam *et al*., 2015a; Chalam *et al*., 2017
		P. vulgaris	Nepal	Chalam *et al*., 2014b
		P. sativum	Spain, USA	Chalam *et al*., 2014b
		V. faba	ICARDA (Syria)	Chalam *et al*., 2014b
		V. unguiculata	Italy	Chalam, 2016; Chalam *et al*., 2017
28	[c]*Pea seed-borne mosaic virus* (PSbMV)	*P. sativum*	AVRDC (Taiwan), Australia, Bulgaria, Colombia, Eritrea, Germany, Nepal, Netherlands, Russia, Spain, Syria, USA	Khetarpal *et al*., 2001; Parakh *et al*., 2006; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. faba[c]	ICARDA (Syria), Bulgaria, Spain	Kumar *et al*., 1991; Khetarpal *et al*., 2001; Chalam *et al*., 2007, Chalam *et al*., 2009a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
29	[c]*Peanut mottle virus* (PeMoV)	*Arachis hypogaea*	Indonesia, Malawi, Philippines, Uganda, USA	Prasada Rao *et al*., 2004; Khetarpal *et al*., 2006; Chalam and Khetarpal 2008; Chalam *et al*., 2017
		G. max[c]	AVRDC (Taiwan), Sri Lanka	Chalam *et al*., 2014b
		V. radiata[c]	Sri Lanka	Chalam *et al*., 2014b

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
30	[bcd]*Peanut stripe virus* (PStV)	*A. hypogaea*	China, Japan, Myanmar, Philippines, USA	Prasada Rao *et al*., 1990; Prasada Rao *et al*., 2004; Prasada Rao *et al*., 2012; Khetarpal *et al*., 2006; Chalam and Khetarpal 2008; Chalam *et al*., 2017
		G. max[c]	China	Prasada Rao *et al*., 2012; Chalam *et al*., 2017
31	[ad]*Peanut stunt virus* (PSV)	*G. max*	AVRDC (Taiwan), Canada, Costa Rica	Chalam, 2016; Chalam *et al*., 2016; Chalam *et al*., 2017
		P. vulgaris	USA	Chalam, 2016; Chalam *et al*., 2015a; Chalam *et al*., 2017
		V. radiata	AVRDC (Taiwan), Costa Rica	Chalam *et al*., 2016
		V. unguiculata	Italy	Chalam, 2016; Chalam *et al*., 2016a
32	[ad]*Pepino mosaic virus* (PepMV)	*C. annuum*	Republic of Korea	Chalam *et al*., 2016a
		S. lycopersicum	Netherlands, Thailand, USA	Chalam *et al*., 2016a
33	[ade]*Raspberry ringspot virus* (RRSV)	*G. max*[e]	AVRDC (Taiwan), Costa Rica, Sri Lanka, Thailand, USA[e]	Chalam, 2016; Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam *et al*., 2017; Chalam and Khetarpal 2008
		V. unguiculata ssp. *sesquipedalis*	Philippines	Chalam, 2016; Chalam *et al*., 2016a
34	[bc]*Red clover vein mosaic virus* (RCVMV)	*P. vulgaris*[c]	USA	Chalam *et al*., 2014a ; Chalam *et al*., 2016a
35	[c]*Southern bean mosaic virus* (SBMV)	*G. max*[c]	AVRDC (Taiwan); IITA (Nigeria), Thailand, USA	Chalam *et al*., 2014b; Chalam and Khetarpal 2008; Parakh *et al*., 2005; Parakh *et al*., 2008
		P. vulgaris[c]	CIAT (Colombia)	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata	Belgium, USA	Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
36	[cde]*Soybean mosaic virus* (SMV)	*G. max*[e]	AVRDC (Taiwan), IITA (Nigeria), Australia, Brazil, Canada, Costa Rica, Hungary, Nigeria, Sri Lanka, Thailand, USA[e]	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2009b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008; Khetarpal *et al*., 1992; Khetarpal *et al*., 2001; Parakh *et al*., 1994; Parakh *et al*., 2005; Parakh *et al*., 2008; Singh *et al*., 2003
		P. vulgaris[c]	CIAT (Colombia), Russia	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal, 2008
		V. unguiculata	IITA (Nigeria)	Chalam, 2016; Chalam *et al*., 2016a

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
37	[cd]*Tobacco necrosis virus* (TNV)	*P. vulgaris*	Nepal	Chalam *et al*., 2014b
		P. sativum[c]	Spain, USA	Chalam *et al*., 2014b
		V. faba[c]	ICARDA (Syria)	Chalam *et al*., 2014b
38	[cd]*Tobacco rattle virus* (TRV)	*P. vulgaris*[c]	CIAT (Colombia)	Chalam *et al*., 2014b
39	[d]*Tobacco ringspot virus* (TRSV)	*C. annuum*	USA	Chalam *et al*., 2016a
		G. max	AVRDC (Taiwan); IITA (Nigeria), Brazil, Myanmar	Chalam and Khetarpal 2008; Chalam *et al*., 2014b; Parakh *et al*., 2005; Parakh *et al*., 2008
40	[cde]*Tobacco streak virus* (TSV)	*C. annuum*	Republic of Korea	Chalam *et al*., 2016a
		G. max[e]	AVRDC (Taiwan), Brazil, Sri Lanka, Thailand, USA[e]	Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		Helianthus annuus	Spain	Prasada Rao *et al*., 2012
		P. vulgaris[c]	CIAT (Colombia), Nepal	Chalam *et al*., 2014b
		P. sativum[c]	USA	Chalam *et al*., 2014b
		S. lycopersicum[c]	Netherlands, Thailand, USA	Chalam *et al*., 2016a
		V. faba[c]	ICARDA (Syria)	Chalam *et al*., 2014b
		V. serratifolia[c]	ICARDA (Syria)	Chalam *et al*., 2014b
		V. radiata	AVRDC (Taiwan)	Chalam *et al*., 2014b
		V. unguiculata[c]	CIAT (Colombia), IITA (Nigeria)	Chalam, 2016; Chalam *et al*., 2014b
41	[d]*Tomato aspermy virus* (TAV)	*C. annuum*	Republic of Korea	Chalam *et al*., 2016a
		S. lycopersicum	Netherlands, Republic of Korea, Thailand, USA	Chalam *et al*., 2016a
		P. vulgaris	CIAT (Colombia)	Chalam *et al*., 2014b
42	[cd]*Tomato black ring virus* (TBRV)	*C. annuum*	Republic of Korea	Chalam *et al*., 2016a
		G. max[c]	AVRDC (Taiwan), Brazil, Canada, Costa Rica, Sri Lanka, USA	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2016a
		P. vulgaris[c]	CIAT (Colombia), Brazil, Canada, Kenya, Russia, USA	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata[c]	CIAT (Colombia), IITA (Nigeria), USA	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
43	[d]Tomato mosaic virus	*C. annuum*	Republic of Korea	Chalam *et al*., 2016a
		S. lycopersicum	Netherlands, Thailand, USA	Chalam *et al*., 2016a

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
44	[ade]*Tomato ringspot virus* (ToRSV)	*G. max*[e]	AVRDC (Taiwan), Canada, Costa Rica, Sri Lanka, Thailand, USA[e]	Chalam, 2016; Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam *et al*., 2017; Chalam and Khetarpal 2008
		V. radiata	AVRDC (Taiwan)	Chalam *et al*., 2017
		V. unguiculata	IITA (Nigeria), Italy	Chalam, 2016; Chalam *et al*., 2016a; Chalam *et al*., 2017
45	[adf]*Wheat streak mosaic virus*	*T. aestvum*[f]	USA[f]	Chalam, 2014; Chalam, 2016; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam 2013b; Chalam *et al*., 2013d; Chalam *et al*., 2016a; Chalam *et al*., 2016b
		Z. mays[f]	France[f], Philippines[f], Puerto Rico[f], South Africa[f], USA[f]	Chalam 2014; Chalam 2016; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2013b; Chalam *et al*., 2013d; Chalam *et al*., 2016a

[a]Virus not reported from India
[b]virus present in India but with restricted distribution, hence it is of quarantine significance for India
[c]Virus present in India but not recorded on the particular host on which it is intercepted, , hence it is of quarantine significance for India
[d]Virus listed as regulated pest in the Plant Quarantine (Regulation of Import into India) Order 2003
[e]Virus intercepted in transgenics also
[f]Virus intercepted in only transgenics
AVRDC = Asian Vegetable Research Development Center- The World Vegetable Center; IITA = International Institute of Tropical Agriculture; ICARDA = International Center for Agricultural Research in the Dry Areas; CIAT = Centro Internacional de Agricultura Tropical
Note: Viruses in italics are characterized and listed in the ninth report of the International Committee on Taxonomy of Viruses (ICTV); viruses not in italics are those not characterized completely but listed in the ninth report of the ICTV (Andrew *et al*., 2012).

followed. The introduced viruses could get established as virus vectors are present in the country. There is a need to strengthen the domestic quarantine system to prevent the spread of viruses with limited distribution within the country. Besides, there are tremendous opportunities to the growers for enhancing export of agricultural commodities if they meet the international quality standards by overcoming phytosanitary constraints, which may involve virus-free areas for production. Various government agencies involved in international and domestic quarantine, certification of seed and other planting material need to work in complete coordination and there is a need to create enough awareness among general public, customs officials and private stakeholders on importance of both international and domestic quarantine.

Virus detection and diagnosis are crucial for application of mitigation strategies and for exchange of PGR and safe trade. There is a need for *accredited diagnostic laboratories* at central and state level for accurate and quick detection and identification of viruses and there is a need for National Certification Programme for Seed Health in line with National Certification System for Tissue Culture-raised Plants (NCS-TCP), Department of Biotechnology, Govt. of India and need to review seed certification standards.

Establishment of a *National Repository of Diagnostics for Viral Diseases* including antisera Bank, database of primers, seeds of indicator hosts, virus reference collections (lyophilized positive controls), user-friendly diagnostics such as lateral flow strips/ dip sticks which can detect multiple viruses, multiplex RT-PCR protocols, loop mediated isothermal amplification, helicase dependent amplification protocols for detection of viruses in the field and at ports of entry, microarrays, DNA barcoding and ultimately, a cost-effective *national biosecurity chip* for diagnosis of all current threats to crop plants would be the backbone for strengthening the programme on biosecurity for plant viruses. Also, *South Asia Regional Working Group of Detection and Identification of Plant Viruses* thus need to be formed among SAARC countries for sharing knowledge and facilities.

Database on all viral diseases, including information on host range, geographical distribution, strains, etc. should be made available for its use as a ready reckoner

by the scientists, extension workers and quarantine personnel. Plant viruses of quarantine significance for India in cereals (Chalam *et al*., 2005b), grain legumes (Chalam *et al*., 2012d) and edible oilseeds (Chalam *et al*., 2013a) have been published. The Crop Protection Compendium of CAB International, UK, is an useful asset to scan for global pest data (CAB International, 2007).

Preparation of *authentic reports of new viruses* and deposition of reference cultures in the National Repositories may be made mandatory. The exports may suffer due to wrong identification of viruses and reporting the same as new reports.

Need to develop *rapid response teams* at state level to deal with the sudden outbreak of viral diseases.

Develop the *web-based information portal* for management of plant viruses and regulations including database on taxonomists and dignosticians.

Revisit the national regulatory framework and and develop a mechanism for supply of virus-free seeds/ plants/ planting material within the country, be it seed distribution meant for multilocation testing under All India Coordinated Research Projects of ICAR, inland supply of germplasm by ICAR-NBPGR or seed distribution by the National/ State Seed Corporations/ private organizations. Also, need to monitor the movement of vegetatively propagated material and tissue culture-raised plants across the states. Requirement for treatment of germplasm/ commercial seed/ other material being distributed across the states to be made mandatory. Also, strengthen state certification mechanism to ensure the supply of virus-free nursery material.

Strengthen Plant Quarantine Stations dealing with bulk samples in terms of manpower, infrastructure (well equipped laboratories, treatment facilities and greenhouses) and expertise with special emphasis on advanced techniques for detection of viruses, their strains in bulk samples through regular trainings. ICAR-NBPGR has Organized Training course on "Diagnostic methods for detection and identification of pests of seed and other planting material and their management" for plant quarantine officials from DPPQS in 2011 (Gupta et al., 2011). Recently during 2015-17, ICAR-NBPGR has organized 11 Training Workshops on "Biosafety and transboundary movement of living modified organisms (LMOs)" for >300 officials of customs and plant quarantine (Bhalla *et al*., 2017). Need to organize such workshops to create *awareness on plant quarantine issues* among stakeholders such as customs officials, other airport officials, travelers, scientists and other stakeholders with diverse kind of teaching material both print and audio-visual for wider use (Chalam *et al*., 2009c).

There is an urgent need to develop a *National Plant Pests Diagnostic and Certification Network* (NPPDCN) (Fig. 1) linking the research laboratories with seed/ vegetative planting material testing laboratories and quarantine stations, which would be the backbone for strengthening the programme on biosecurity from plant pests including viruses.

The NPPDCN as proposed in Fig. 1 can be a store-house of information on pests (fungi, bacteria, viruses, nematodes, insect pests and weeds), diagnostics procedures, national data base on state-wise endemic pests, policies, and related issues. The quality of seed and other planting material will be further enhanced only if seed and other planting material health is integrated into certification procedures. We have the potential for strengthening the system but need to focus in a pragmatic manner.

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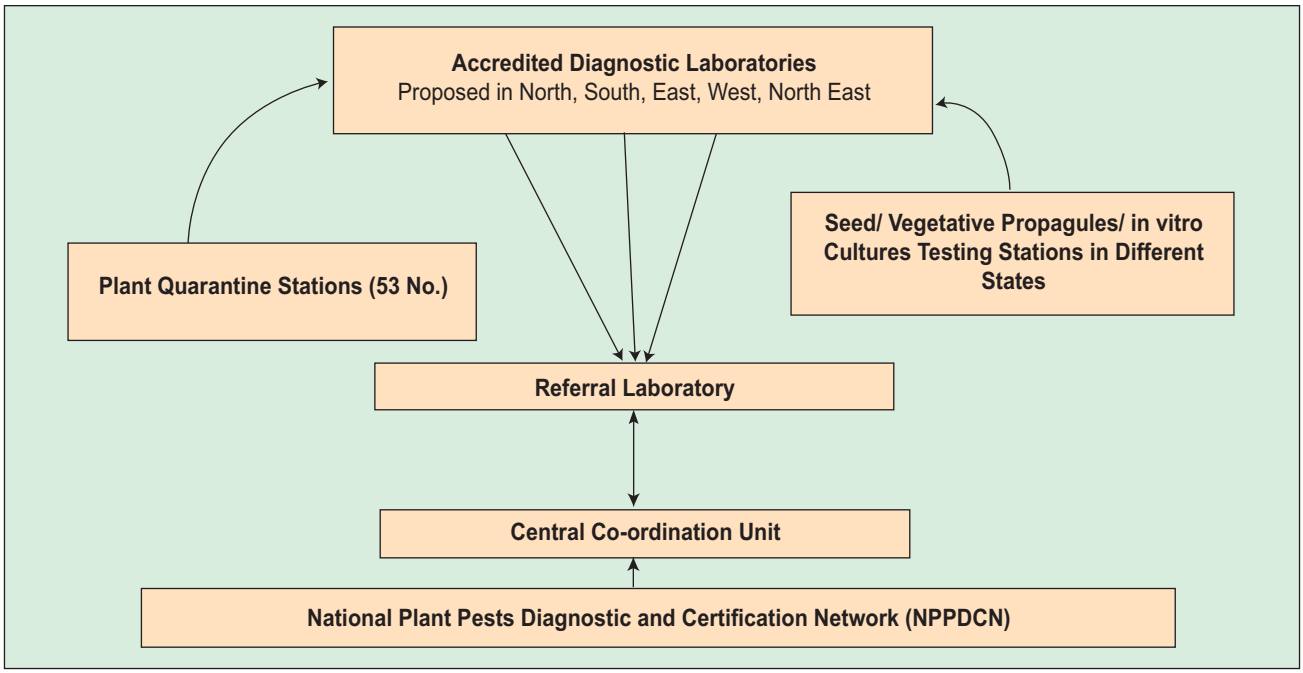

Fig. 1. Proposed Flow Chart of Networking by National Plant Pests Diagnostic and Certification Network (NPPDCN)

Source: Adapted from Chalam *et al*., 2016b

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Genetic Diversity among Wild *Grewia tenax* Accessions Collected from Kachchh Region of Gujarat, India

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The germplasm of *Grewia tenax* (Phalsa cherry or white cross-berry) as fruits were collected from arid region of Kachchh, Gujarat during the year 2015. The collected germplasm (27 accessions) were germinated and evaluated in both laboratory and nursery at ICAR-CAZRI, RRS, Kukma-Bhuj, Gujarat. Data were recorded on its germination and growth parameters. Statistical analysis revealed the presence of high coefficient of variation for morphological parameters. Range of variation for important traits of *G. tenax viz.*, plant height (cm), number of fruits per 100 g, 1000-seed weight (g) and fruit thickness (mm) were observed with range (20.8 to 78.5, 690 to 1800, 60.3 to 150.7 and 4.0 to 6.7) and mean (46.8, 971.6, 113.8 and 5.1) value, respectively. Range of value of Pearson's correlation coefficient varied from – 0.85 between NF and TSW to 0.83 between PL and leaf width. Cluster analysis revealed the presence of three major clusters at coefficient value of Euclidian distance of 6.0. PCA analysis revealed the presence of 75.59% variability among *G. tenax* genotypes through first five most informative components and it also confirmed the clustering pattern obtained through cluster analysis.

Key Words: Cluster analysis, Correlation, Genetic diversity, *Grewia tenax*, PCA analysis

Introduction

Grewia tenax popularly called white cross-berry is most common in arid and semi-arid plains, lowlands and lower mountains up to a maximum altitude of 1,250 m a.s.l. and in regions with mean annual rainfall of 200-1000 mm in sandy, rocky and lateritic soils. It is widely distributed in the lowlands and mountains of Arabian countries, Egypt, Iran, Afghanistan, Sudan, Pakistan, Srilanka and India (Alrikain, 2004; Gebauer *et al.,* 2007; Sohail, 2009). In India, wild genotypes of *G. tenax* are commonly grown naturally in rocky and gravelly piedmont plains of arid and semi-arid areas of Gujarat, Rajasthan, Andhra Pradesh, Haryana, Karnataka, Maharashtra and Punjab (Sharma and Patni, 2012; BSI, 2014). In India about 120.4 mha area has been classified as degraded and wasteland category, of which major part lies in western part of Rajasthan, and Kachchh and Saurashtra regions of Gujarat. About 16% (3,129 thousand ha) of total geographical area (19,683 thousand ha) of Gujarat state is under degradation. As Tropic of Cancer passes through the Rann of Kachchh, Gujarat, the region experiences intensely hot and arid climate (Priyanka *et al.,* 2015). These areas are enriched with thorny forest, which include species of trees, shrubs and herbs. The important under-utilized shrubs of this region are *Acacia senegal* (Mimosaceae), *Commiphora wightii* (Burseraceae) *Euphorbia caducifolia* (Euphorbiaceae), *Grewia villosa* (Tiliaceae), *Salvadora oleoides* and *S. persica* (Salvadoraceae), *Premna resinosa* (Verbenaceae), and *Ziziphus nummularia* (Rhamnaceae). Among these, *G. tenax* has much potential value as food, fodder and medicine, yet to be exploited/utilized.

G. tenax is a typical tropical plant species which can tolerate seasonal drought and withstand temperatures of more than 50°C (Gebauer *et al.,* 2007). The plant is not only adapted to high temperatures, salinity and dry conditions, but also has deep roots which can stabilize sand dunes (Saied *et al.,* 2007). It is closely related to Phalsa (*G. asiatica*), popularly eaten fruit in North India. *G. tenax* has often been cited as a prime candidate plant for domestication as a useful horticultural plant (Gebauer *et al.,* 2007). It plays an effective role in rehabilitation of wastelands (Teketay, 1996) and requires only 200 mm of annual rainfall. Being tolerant to drought and soil salinity, *G. tenax* perform well in Kachchh region of Gujarat where due to arid climate that poses a major problems in establishment of any species in this region. Hence, this paper is an attempt to assess the genetic diversity in *G. tenax* on basis of morphological and pomological characteristics, and it would also help in developing adequate phenotypic markers for identification of promising genotypes and germplasm management.

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Materials and Methods

Experimental Site

The location of the study area was nursery and farm area of ICAR-CAZRI (Central Arid Zone Research Institute), Regional Research Station, Kukma-Bhuj, Gujarat (India) (latitude 23°21'19" - 23°21'33" N, longitude 69°79'72" - 69°78'78"E). The climate of the Kachchh region is hot arid with a maximum temperature of 48°C during summer and minimum 2°C during winter. The average rainfall of the region is 326 mm and most of rainy days occur during July to September with high evapo-transpiration (1500-2000 mm per year).

Field Survey and Collection

Field surveys were conducted in arid region of Kachchh, Gujarat for the occurrence of *G. tenax* during the months of March to October 2015. The 27 germplasm accessions of *G. tenax* as fruits collected from different sites were brought to laboratory and morphological parameters of 50 fruits from each accession such as fruit weight (g), diameter (mm), length (mm) etc. as well as qualitative traits *viz*., fruit morphology, colour and fruit lobe were measured. Length, width and diameter of fruits were measured with a digital Vernier caliper using method described by Gogu *et al.* (2009).

Nursery and Field Evaluation

The 27 germplasm accessions were evaluated in both laboratory and nursery. Germination studies were conducted by sowing seeds of collected accessions in polythene bags (size: 15 x 10 cm), filled with sand, soil and farm yard manure in 1:2:1 ratio. Seeds were also sown in portray having coco-peat, vermiculite and perlite (2:1:1 ratio) as supportive materials. Two seeds were sown in each bag and portrays compartment and kept in shade net house (50% sun light) and were irrigated with normal tap water daily until seeds germinated. Different growth parameters such as plant height (cm), number of branches per plant, stem girth (mm), leaf length (mm), leaf width (mm), petiole thickness (mm) were estimated at monthly interval in the nursery as well as in one year old plants at field.

Data Analysis

Data recorded were subjected to statistical analysis such as descriptive statistics, Pearson's correlation and multivariate analysis by using the software package "PAST3" (Hammer *et al.,* 2001).

Results and Discussion

Genetic diversity study of *G. tenax* genotypes collected from Gujarat arid zone were subjected to morphological and pomological characterization.

Range of Variation

Range of variation for 27 *G. tenax* genotypes (Table 1) revealed that maximum amount of variation present in the material. Coefficient of variation (CV) for quantitative traits such as DTG, GP, NL, NB, PH and PL was found to be 69.3, 67.0, 36.8, 35.4, 35.2 and 34.8 percent, respectively. Range of variation for important traits *viz.,* plant height (cm), leaf length (mm), number of fruits per 100 g, 1000-test weight (g) and fruit thickness (mm) was observed with range (20.8 to 78.5, 21.4 to 55.9, 690 to 1800, 60.3 to 150.7 and 4.0 to 6.7) and mean (46.8, 35.1, 971.6, 113.8 and 5.1) value, respectively. The maximum number of fruits per 100 g was obtained on genotype CZBGT-3 (1800) followed by CZBGT-6 (1573.33) and CZBGT-31 (1305), whereas minimum was recorded in CZBGT-10 (690). Maximum 1000-test weight (g) was observed in germplasm CZBGT-19 (150.7), closely followed by CZBGT-1 (150.0) and CZBGT-29 (146.7), while minimum was found in CZBGT-3 (60.3). Understanding of nature and amount of variability present in genotypes of crops might be useful in improvement of traits of existing germplasm which could be used in breeding programs of that crop. Furthermore, this kind of evaluation will provide information about uniqueness and distinctness of genotypes, which is of vital importance in optimal and effective conservation of genotypic variability (Bisrat *et al.,* 2000). Genetic diversity is best estimated if agro-morphological, biochemical and marker studies are used together (Lattoo *et al.,* 2008).

Pearson's Correlation Analysis

Correlation analysis is an important statistical tool which provides the relationship between two random variables whether they are dependant or independent. Pearson's correlation matrices of quantitative traits of 27 *G. tenax* genotypes are presented in Table 2. Range of value of correlation coefficient varied from –0.85 between number of fruits per 100 g and TSW to 0.83 between petiole length and leaf width.

Cluster Analysis

Dendrogram was generated for 14 quantitative traits of 27 *G. tenax* genotypes by using Euclidian distance

based UPGMA method of cluster analysis (Fig. 1). The coefficient of Euclidean genetic distance ranged from 0.0 to 6.2, which indicated the presence of considerable amount of genetic variability among *G. tenax* genotypes. It formed three major clusters at coefficient value of Euclidian distance of 6.0 with two sub-clusters each, excluding third cluster, where genotype CZBGT-31 grouped alone. In general, the pomological traits viz., petiole length, petiole thickness, fruit length, thickness, width and thousand seed weight played a major role

Table 1. Range of variation in plant and fruit characteristic of 27 *Grewia tenax* genotypes collected from arid Kachchh of Gujarat

Traits	Min.	Max.	Mean	SE (±)	SD	Skewness	Kurtosis	CV (%)
Day to germination	13.7	99.3	31.0	4.1	21.5	1.8	3.2	69.3
Germination %	0.0	45.0	15.0	1.9	10.1	1.3	1.9	67.0
Plant height (cm)	20.8	78.5	46.8	3.2	16.4	0.4	-0.8	35.2
No. of branches	2.0	9.0	4.0	0.3	1.4	1.6	4.7	35.4
Stem girth (mm)	1.5	7.9	4.5	0.3	1.5	0.0	-0.1	33.2
No. of leaves	17.3	87.7	37.8	2.7	13.9	1.8	5.5	36.8
Leaf length (mm)	21.4	55.9	35.1	1.5	7.7	1.3	2.3	22.0
Leaf width (mm)	20.9	61.6	34.3	1.8	9.2	1.6	3.3	26.8
Petiole length (mm)	6.1	23.9	12.2	0.8	4.2	1.2	1.5	34.8
Petiole thickness (mm)	0.4	1.3	0.8	0.0	0.2	1.0	0.9	26.0
No. of fruits /100 (g)	690.0	1800.0	971.6	49.7	258.3	1.8	3.6	26.6
1000-seed weight (g)	60.3	150.7	113.8	5.0	25.9	-0.3	-0.9	22.7
Fruit thickness (mm)	4.0	6.7	5.1	0.1	0.6	0.1	1.0	11.3
Fruit length (mm)	5.5	12.0	7.6	0.4	2.1	0.7	-1.2	27.1
Fruit width (mm)	5.0	13.3	8.9	0.4	2.1	0.0	-0.6	23.4

Table 2. Pearson's correlation coefficient for quantitative traits of 27 *Grewia tenax* genotypes

Traits	DTG	GP	PH	NB	SG	NL	LL	LW	PL	PT	NF	TSW	FrT	FrL
Day to germination (DTG)														
Germination % (GP)	0.16													
Plant height (cm) (PH)	-0.31	-0.09												
No. of branches (NB)	-0.08	-0.28	-0.29											
Stem girth (mm) (SG)	-0.40*	0.06	-0.14	0.27										
No. of leaves (NL)	-0.34	0.06	0.13	0.13	0.59**									
Leaf length (mm) (LL)	-0.40*	0.22	0.15	0.05	0.39*	0.33								
Leaf width (mm) (LW)	-0.21	0.24	0.00	0.10	0.45*	0.36	0.50**							
Petiole length (mm) (PL)	-0.29	0.18	0.02	0.05	0.51**	0.52**	0.39*	0.83**						
Petiole thickness (mm) (PT)	-0.14	0.31	0.30	-0.26	0.08	-0.07	0.35	0.30	0.01					
No. of fruit/100 (g) (NF)	0.03	-0.03	-0.22	0.13	-0.30	-0.32	0.06	-0.12	-0.24	-0.23				
1000-seed weight (g) (TW)	-0.15	0.03	0.24	-0.02	0.48*	0.40*	0.14	0.21	0.30	0.12	-0.85**			
Fruit thickness (mm) (FrT)	-0.09	0.03	0.17	-0.22	0.02	0.15	-0.02	0.21	-0.07	0.30	-0.27	0.23		
Fruit length (mm) (FrL)	-0.29	0.03	0.10	0.17	0.43*	0.45*	0.50**	0.42*	0.71**	-0.09	-0.15	0.21	-0.45*	
Fruit width (mm) (FRW)	0.16	-0.01	0.01	-0.31	-0.25	-0.30	-0.22	-0.22	-0.50**	0.25	-0.17	0.15	0.55**	-0.81**

*Significant at $p < 0.05$ / **Significant at $p < 0.01$

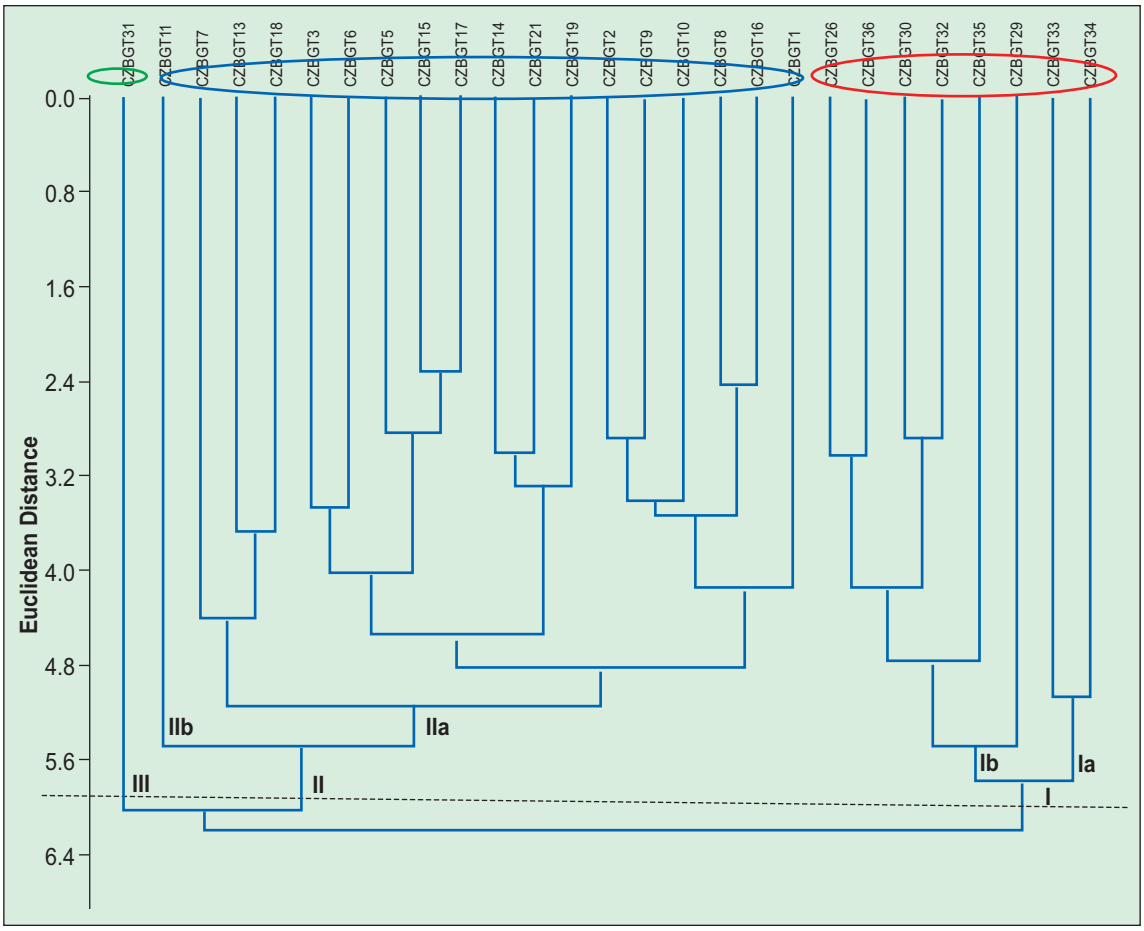

Fig. 1. Dendrogram of 27 *Grewia tenax* genotypes based on UPGMA method of cluster analysis using Euclidian distance

in grouping of genotypes into different clusters. At coefficient value of Euclidian distance of 5.6, Cluster-I formed two sub-clusters Ia and Ib with two (CZBGT-34 and CZBGT-33) and six (CZBGT-29, CZBGT-35, CZBGT-32, CZBGT-30, CZBGT-36 and CZBGT-26) genotypes, respectively. These genotypes exhibited better in length and thickness of petiole and fruit than other genotypes. At coefficient value of Euclidian distance of 5.4, Cluster-II formed two sub-clusters named IIa and IIb with 17 and single (CZBGT-11) genotypes, respectively. These genotypes showed comparatively low in length and thickness of petiole and fruit to differentiate from genotypes under Cluster-I. Further, the genotype present in Cluster-III, CZBGT-31 showed intermediate in above characters, which clearly separated it from Cluster-I and II. This kind of grouping pattern on basis of pomological traits were reported in cluster analysis in horticultural crops viz., Apricot (Mratinic *et al.*, 2011; Kumar *et al.*, 2015), turmeric (Bahadur and Meena, 2016) and wild hip rose (Verma *et al.*, 2015). The pairs of genotypes present in sub-cluster, Ib [(CZBGT-30 and CZBGT-32); (CZBGT-26 and CZBGT-36)] and in sub-cluster, IIa (CZBGT-15 and CZBGT-17; CZBGT-5), (CZBGT-8 and CZBGT-16), (CZBGT-2 and CZBGT-9) and (CZBGT-14 and CZBGT-21; CZBGT-19) showed least variability between them. The maximum genetic distance (8.96) observed between genotypes, CZBGT-14 and CZBGT-34 followed by CZBGT-34 and CZBGT-13 (8.53) on basis of Euclidean distance, whereas, minimum between CZBGT-15 and CZBGT-17 (2.32).

Principal Components Analysis

The PCA analysis performed on 14 quantitative traits of 27 *G. tenax* genotypes revealed that the first five most informative components accounted for 75.79% variance (Table 3). Almost, similar amount of variability revealed in PCA analysis study on Apricot with > 80% (Mratinic *et al.*, 2011) and 73.44% (Kumar *et al.*, 2015) in first 4 and 3 PC components and 85% in fist 4 PC components in wild hip rose (Verma *et al.*, 2015). The variability contributed by PC axis-I was 28.81% followed by PC axis-II (18.21%), III (11.34%), IV (9.12%) and V

Table 3. The cumulative % variance of first five Principal components obtained through PCA analysis of 27 *Grewia tenax* genotypes

Principal component	% Variance	Cumulative % Variance
I	28.81	28.81
II	18.20	47.01
III	11.34	58.35
IV	9.12	67.47
V	8.32	75.79

(8.32%). The characters such as petiole length (0.40), fruit length (0.38), stem girth (0.35), number of leaves (0.34), leaf width (0.34), leaf length (0.30) and thousand seed weight (0.23) were major contributors for 28.81% variability of PC axis-I. The fruit thickness (0.45), fruit width (0.42), petiole thickness (0.36) and thousand seed weight (0.33) were responsible for 18.21% variability in PC axis-II, whereas, the characters such as germination percentage (0.42), petiole thickness (0.41), number of fruits (0.38) and leaf length (0.35) were accountable for 11.34% of variability in PC axis-III. In general, the pattern obtained through cluster analysis confirmed by two-dimensional scatter plot of PCA ordination using the first two most informative PC axes obtained in the present study through PCA analysis (Fig. 2). Nevertheless, several years of studies must be conducted before parental selection for a possible plant breeding. The PCA analysis provided a simplified classification of the *G. tenax* genotypes for collecting and improvement. This scatter plot also shows geometrical distances among genotypes that reflects similarity among them in terms of traits measured.

The morphological and pomological diversity study on 27 genotypes revealed the presence of considerable amount of genetic variability among them. Multivariate analysis was found useful for detection of phenotypic and pomological differences among the *G. tenax* genotypes. Principal component analysis revealed that traits related to petiole length, fruit length, fruit thickness, number of fruits, thousand seed weight, stem girth, leaf length and width accounted for distinct variability among different genotypes. Identification and description of the genetic variability in *G. tenax* genotypes are preliminary requirements for the exploitation of its useful traits in

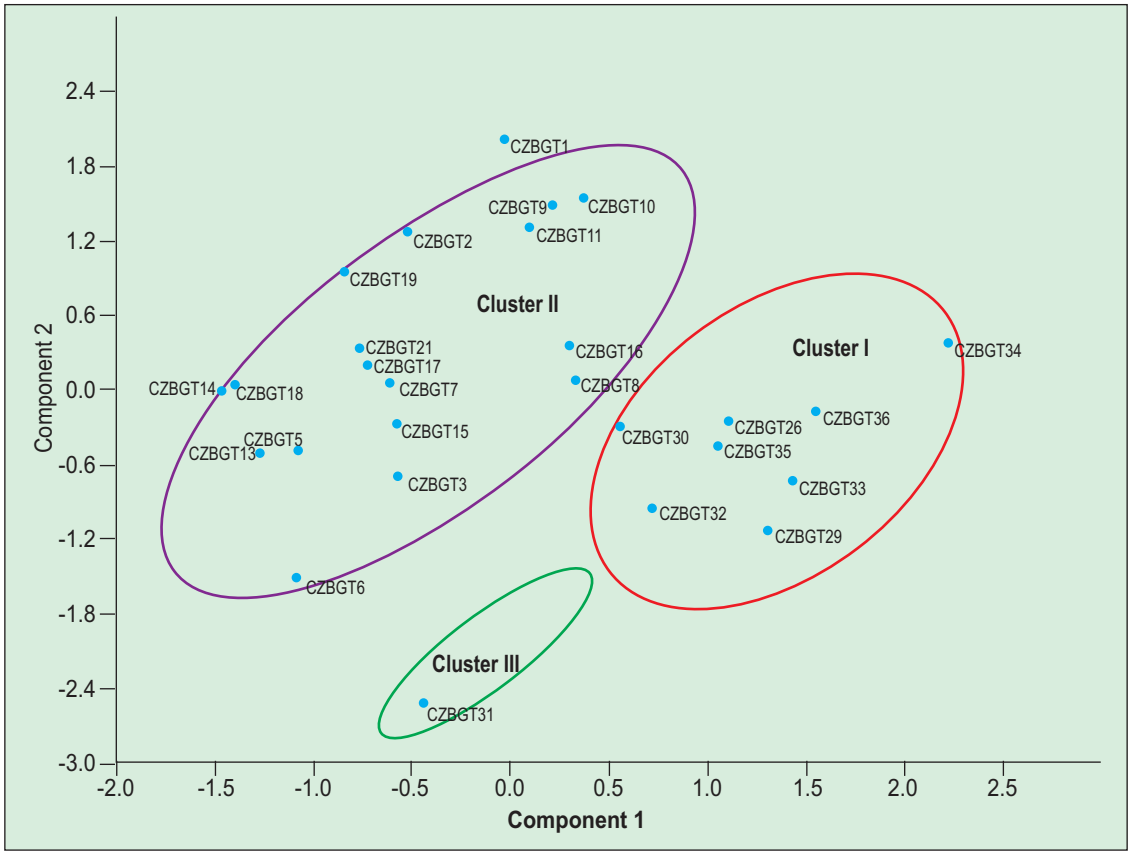

Fig. 2. Two-dimensional scatter plot of PCA ordination using the first two most informative PC axes obtained for 27 *Grewia tenax* genotypes through PCA analysis

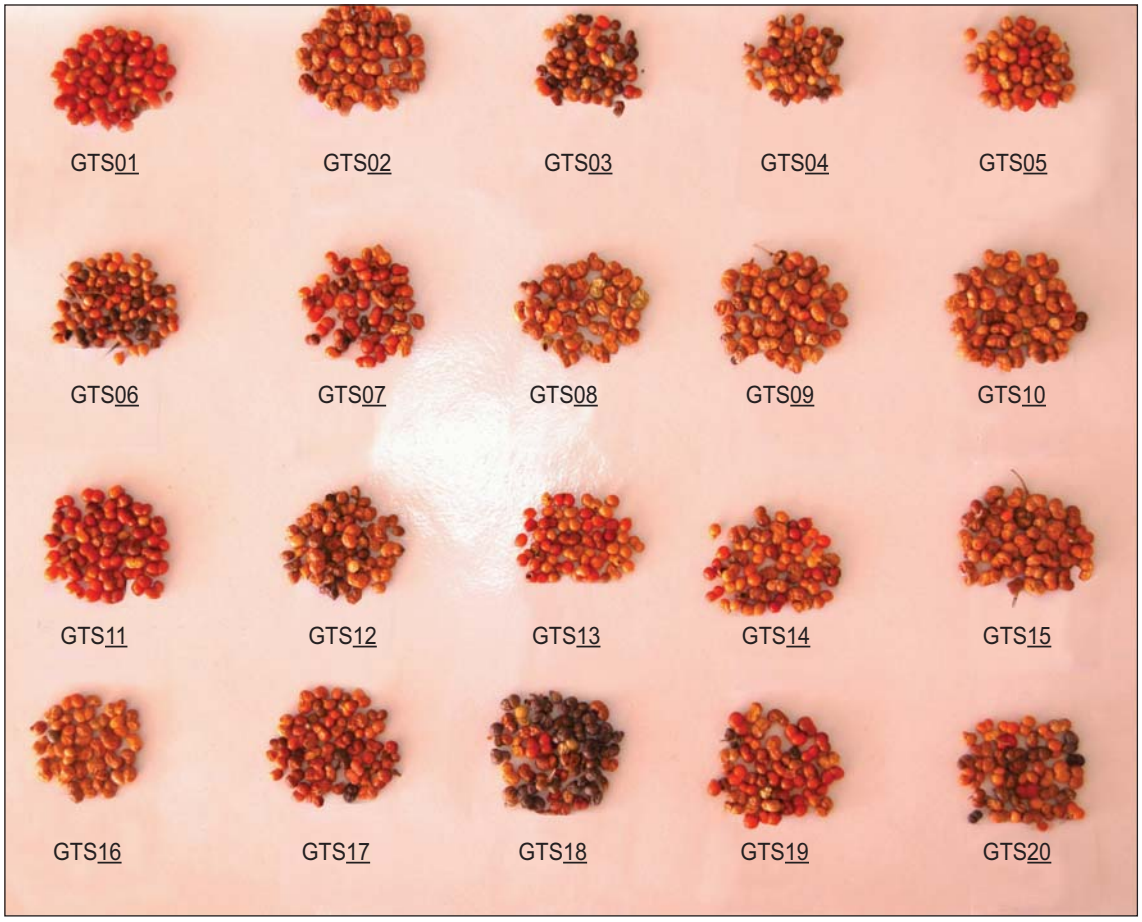

Fig 3. Morphological variation in collected *Grewia tenax* seeds

introducing them into Alternate Land Use system and into rangelands of hot arid regions of India for food and fodder security of the rural inhabitants.

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SNP Marker Based Genetic Diversity and Population Structure Study of Rice Germplasm of Arunachal Pradesh

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Rice genetic resource is the primary source for rice breeding. It also makes a concrete contribution to global wealth creation and food security. India is one of the centers of origin for rice and the nutritional security of the North-Eastern (NE) states of India predominantly depends on rice. The NE region especially Arunachal Pradesh is considered to be one of the hot pockets of rice genetic resources in the world and also has diverse rice growing conditions as compared to other parts of the country. Genetic diversity of 662 rice accessions originating from the state of Arunachal Pradesh was assessed using 36 single nucleotide polymorphism (SNP) markers distributed across all 12 chromosome of rice genome. These SNP markers generated a total of 36 allelic combinations. Polymorphic Information content (PIC) ranged from 0.006 to 0.375 and major allele frequency ranged from 0.51 to 0.99. Similarly, heterozygosity and gene diversity ranged from 0.003 to 0.162 and 0.006 to 0.49, respectively. A picture of genetic relatedness was inferred using Neighbor joining tree, which grouped all the genotypes in to three major clusters. AMOVA analysis showed that, among population 31% whereas, among individual 51% diversity. Population structure from K=1 to K=20 were tested and Δk was found maximum at three population (K=3). Principal Coordinate Analysis (PCoA) showed that rice germplasm were intermixed as per Neighbour joining tree but showed three major groups based three population structure information. Hence, PCoA supports the population obtained from the model based approach. This study shows that large diversity exists in the rice germplasm of Arunachal Pradesh and can be utilized for trait-specific breeding.

Key Words: Genetic diversity, Population structure, Rice, SNP markers

Introduction

Rice (*Oryza sativa*) feeds more than 50% of the world's population and is one of the most important crops in the world. Rice accounts for 21, 14 and 2% of global energy, protein and fat supply respectively (Kennedy *et al.,* 2003). Being a model organism with fully sequenced genome, various genomic approaches have been used to study its domestication, adaptive selection, and the history of crop improvement (Wing *et al.,* 2005, Zhang *et al.,* 2008). Rice genetic resource is the primary material for rice breeding and makes a concrete contribution to global wealth creation and food security (Zhang *et al,.* 2011). Numerous studies on genetic structure of rice cultivars at a local scale (within a country) have also been conducted (Gao *et al*., 2005, Barry *et al*., 2007, Thomson *et al.,* 2009, Zhang *et al.,* 2007, Li *et.al.,* 2014). Such local-scale studies not only provide a detail view of rice genetic diversity within a country, but it also gives a better understanding of complex interaction between rice genetic diversity and human cultivation practices (Thomson *et al.,* 2009), and for formulating *in situ* conservation strategies (Barry *et al.,* 2007). India is one of the centres of origin for rice. Phylo-geographical and archaeological evidence suggest that rice was domesticated about 10000 years ago from its wild ancestor *O. rufipugon* in the region south of Himalayan mountain range, likely in the present day Eastern and North Eastern India, extending eastward to Nepal, Myanmar and Thailand to Southern China (Chang, 1976; Khush 1997; Londo *et al.,* 2006) the eastern Himalayan region of North-east India, a geographical area of over 255000 Km^2 consisting of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura states is home to a large number of indigenous rice varieties (Choudhury *et al.,* 2013). The state of Arunachal Pradesh is situated on the eastern most corner of India sharing international borders with Bhutan, China and Myanmar. It has a geographical area of 83,743 sq. km. The state has a population density of 17 persons per sq. km, and around 68.8% of the population is tribal (Govt. of India, Statistics, 2015). The tribal communities of Arunachal Pradesh are of Tibeto-Burman linguistic origin, and there are as many as 21 tribes and 50 sub-tribes. The topography of the

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state is mostly hilly terrain and the agro climate varies from tropical to alpine (Roy *et al.,* 2016). The farmers majorly follow traditional shifting cultivation (locally known as jhum) and with little sedentary agriculture. Shifting or 'slash-and-burn' cultivation is the earliest form of agriculture and is still practiced in vast areas of the state. About 76 % of the total cropped area in the state is under jhum cultivation. However rice productivity in this state is low, 2065kg/ha, as rice is cultivated in high altitudes, plateaus, terraces and river beds. Despite having low yield potential, rice landraces grown in the mountains under jhum cultivation system possess many important biotic, abiotic and quality traits which can be important source for crop improvement programmes. Thus, keeping in view the above points, the genetic diversity of rice germplasm of Arunachal Pradesh was assessed using SNP markers, the most advanced set of markers reported till now.

Materials and Methods

Plant Materials

Six hundred and sixty two seed samples of Arunachal Pradesh were procured from Indian National Genebank, National Bureau of Plant Genetic Resources (NBPGR),New Delhi. The details of each accession alongwith passport data (national ID i.e. Indigenous Collection (IC) number) were identified from the online available data of NBPGR.

DNA Extraction from Rice Seed

Total Six to nine seeds of each accession were dehusked and used for DNA isolation using QIAGEN DNeasy plant mini kit. Kernels were ground into fine powder using tissue lyser (Tissue lyser II Retsch, Germany) with a tissue lyser adapter set (QIAGENq). DNA extraction procedure was as per manufacturer's protocol.

Genotyping of Rice Accessions using SNP Markers

Genomic DNA of all the 662 accessions was diluted to prepare working stocks of 10 ng/µl. The Sequenom MassARRAY system uses matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer for accurate detection of SNPs in a high-throughput manner (www.sequenom.com). Sequenom MassARRAY multiplex assays were designed for 36 SNPs (iPLEX gold chemistry), representing conserved single-copy rice genes (Singh *et al.,* 2007), taking three genes per rice chromosome. The unlinked SNP markers located on short arm, centromeric region and long arm of all 12 rice chromosomes were developed and used for diversity analysis (Table 1). The 36-plex assays were designed and validated by Sequenom Corporation (San Diego). The 30-mer pre-amplification primers and variable length genotyping primers generated by the Assay Design 3.1 software were procured and used for the validation of SNPs according to the Sequenom user manual. MassARRAY Typer 3.4 Software was used for the visualization of SNPs and allele calling.

Genetic Diversity and Phylogenetic Analyses

The major allele frequency, gene diversity, heterozygosity and PIC for each locus were calculated for SNP markers using Power Marker 3.5 (Liu and Muse, 2005). In addition, principal component analyses, genetic distances (Nei *et al*., 1983) across the genotypes and neighbour-joining (NJ) tree were calculated using Power Marker 3.5 (Liu and Muse, 2005). The dissimilarity matrix generated by Power Marker was used to construct un-weighted neighbour joining tree using DARwin software 5.0.158 (Perrier and Jacquemoud-Collet, 2006). Software STRUCTURE V2.3.1 was applied to infer historical lineages that show clusters of similar genotypes (Pritchard *et al.*, 2000). The membership of each genotype was run for range of genetic clusters from value of K= 1 to 20 with the admixture model and correlated allele frequency. For each K it was replicated 3 times. Each run was implemented with a burn-in period of 100,000 steps followed by 100,000 Monte Carlo Markov Chain replicates (Pritchard *et al.*, 2000). Ln(PD) derived for each K and then plotted to find the plateau of the ΔK values (Evanno *et al.* 2005). To calculate final population structure online available programme "structure harvester" (Earl and vonHoldt, 2012) was used (http: //taylor0. biology.ucla.edu). The proportion of the genome of an individual that belongs to each inferred population (admixture) was also estimated.

Results and Discussion

Statistical Analysis

Genetic diversity of Arunachal Pradesh rice germplasm collection (662 accessions), available at National Gene Bank, NBPGR, New Delhi was estimated using 36 SNP markers. These unlinked SNP markers located on the short arm, centromeric region and long arm of all 12 rice chromosomes were developed and used for diversity analysis.SNP is bi-allelic in nature therefore; total 72 alleles were amplified with an average of 2 alleles per

Table 1. List of primers used for characterization of 662 rice germplasm of Arunachal Pradesh along with their physical position on chromosome and primer sequences

Primer ID	Physical Position	Amplification primer-1	Amplification primer-2
01-2916-1_C_156	25381654	ACGTTGGATGGGGTTTGCATGTTAATAGGG	ACGTTGGATGCCGAATCTCTATCAAGGAAG
01-608-4_2_375	3421011	ACGTTGGATGAGGACCATCTTCTTGCACTG	ACGTTGGATGCCATTTGCAAGGCCCATTTC
01-6351-1_C_202	40914292	ACGTTGGATGGTTGGAACACATGATTTCAC	ACGTTGGATGATCTCTTTGGACAGAGTCCC
02-267	1570149	ACGTTGGATGGTCAATCTTGCAGGAGTTGG	ACGTTGGATGTGGCTCCTCTTCTCCGGTCT
02-3029-1_C_474	18821156	ACGTTGGATGTGTCTGCAATAACTTGTGCC	ACGTTGGATGAAATCAGCTGCAGCATTACC
02-4333-1_2_293	28688819	ACGTTGGATGGGAATGTTTAGTTTTGAGG	ACGTTGGATGTGTAGGTGCTACTTGCTTCC
03-1691-1_C_373	10849512	ACGTTGGATGAACAACGCCAGGAACATCAC	ACGTTGGATGAAGCGGCTCAAGGTACAATC
03-3478-1_C_206	22815422	ACGTTGGATGCCTGCAGCAAACGCCAATTT	ACGTTGGATGTCAGGTAACCGATCGATTTG
03-4660-1_2_355	31020366	ACGTTGGATGCTCCCATCCTAGTATCCATC	ACGTTGGATGTGCCTTCTCTTACAGGTTCC
04-1801-20_2_428	11859836	ACGTTGGATGCCCTCAAAAAAAAGTTGTAAG	ACGTTGGATGCAGTAAATTTCCAGGGAGATA
04-19-4_C_240	225838	ACGTTGGATGTCTACACATTAGCTCGCTGG	ACGTTGGATGACAGTAACCACAATATGCCG
04-3787-3_1_358	25211800	ACGTTGGATGTTATCTCTGCTTGCTCGCTC	ACGTTGGATGAAGTATCTGCCCCAAGTGAC
05-2692-1_2_109	18783426	ACGTTGGATGGAACTTTACTCTCAGTACA	ACGTTGGATGTGGTTTGATGAGTCGTTTGC
05-4192-1_C_280	28065769	ACGTTGGATGAGTTTGTTGACAGCAGAACC	ACGTTGGATGTAGCTTACTAGTTCATGTG
05-48-1_C_279	287362	ACGTTGGATGCAGAGATGTCTGTTGTTAGC	ACGTTGGATGCAACCAGGGATACAATATGAC
06-1256-1_C_147	7573979	ACGTTGGATGCACGTGCCTATGATTAGCAG	ACGTTGGATGGATCGTTTACTTCTTTGCCC
06-1776-1_1_501	11093772	ACGTTGGATGGGGCCAATTTGCTTAGTGC	ACGTTGGATGAGCATAAGGTATTAAAGTC
06-2509-1_C_497	15737387	ACGTTGGATGCCTTCGCGCTTGCAATTTGG	ACGTTGGATGAAATCAGCACGCGTCAACAC
07-2904-39_C_299	19160255	ACGTTGGATGAATGGTGGTGTATCTTGAGC	ACGTTGGATGGGTGTGACTTCTCATGACAG
07-293-12_1_368	1859603	ACGTTGGATGCACTAATTCTTGGTATTATGG	ACGTTGGATGTCAATGTGTTCTCACAGACC
07-4304_new			
08-2765-2_C_360	18084851	ACGTTGGATGTCCCTCCATGTTGTGAGTTC	ACGTTGGATGCTTGCAAGAGACATCCAAGA
08-4218-5_C_129	27692470	ACGTTGGATGGGTGGACAAAGATAAGGAAG	ACGTTGGATGGACTGGAAATATACTCCCTC
08-847-6_C_113	5399913	ACGTTGGATGCCCAACGTATTAATGGCAAC	ACGTTGGATGGCTGTGTAGTAATTTGCCTG
09-209	1297966	ACGTTGGATGGAGGCAAAAGGCAAACCGAC	ACGTTGGATGGACTTGAGCGAGTCGATGTC
09-2107-5_C_145	13705487	ACGTTGGATGTGACCACACCACACAAACAC	ACGTTGGATGGGGATTTGCGGTTTTTGGAC
09-2716-4_C_457	19541336	ACGTTGGATGTGAGCCACAGATTCCCTTTC	ACGTTGGATGCTCGAGTAATTCAAAACCAC
10-1192-7_C_178	8122635	ACGTTGGATGCTTTGCTACGGATAAAATG	ACGTTGGATGTCATGCAAATACAGACATGG
10-188-1.	1218215	ACGTTGGATGGCGCCAGTGTATGGAAAAAG	ACGTTGGATGGTCCATAACATCATGGACTC
10—2723	20696970	ACGTTGGATGCCCACAATGAGATGCAGATG	ACGTTGGATGAGACAAAATGCAACACTCCG
11-1849.	11974790	ACGTTGGATGCGCCACTCTTCCTGATTTAG	ACGTTGGATGACAGATACGGGAGGCATTTC
11-3935.	28434679	ACGTTGGATGATCCCTGAGACTTTGGATGG	ACGTTGGATGCCAACTTGAATGTCCATTCC
11-522-1_C_214.	3033366	ACGTTGGATGCTACATGGTATCAGATACCG	ACGTTGGATGAGAAGCGAACGCGGAAAAAG
12-1794	11215946	ACGTTGGATGGTGAGCCCCAAAAGTTGGTG	ACGTTGGATGTAAGGTCCAGTTTGCTTGGT
12-3200-2_C_389	21396181	ACGTTGGATGGCTCAAACCTAGCAATAACTG	ACGTTGGATGCCTCCTTCCTACAAGTTTAA
12-400	2160546	ACGTTGGATGCCAATAGAGTCCATCTCAGC	ACGTTGGATGGCACGAGGATTTAAGACAGC

locus in all tested rice accessions. The Polymorphic Information content (PIC) ranged from 0.006 for marker 04-19-4_C_240 to 0.375 for marker 01-608-4_2_375 (Table 2). Major allele frequency ranged from 0.51 for marker 01-608-4_2_375 to 0.99 for 04-19-4_C_240. Similarly, heterozygosity and gene diversity ranged from 0.003(04-19-4_C_240.) to 0.162 (10-1192-7_C_178) and 0.006 (04-19-4_C_240.) to 0.49 (01-608-4_2_375.) with mean values 0.07 and 0.29, respectively (Table 2).The amount of genetic diversity and the population structure for the popular rice genotypes of India are extremely important to know in terms of the extent of genetic variability. This is also useful in order to develop effective breeding strategies for broadening the genetic base of commercial varieties, to identify molecular tags, and for germplasm conservation (Upadhyay *et al.*, 2012). Average PIC has been reported to 0.23 in this study which is lower to those compared to studies done by Lu *et al.*, 2005; Pervaiz *et al.*, 2009 and Lin *et al.*, 2012. This could be due to SNP markers used in the present study. Mean PIC was reported to be high (>0.5) by Anupam *et al.* (2017) for the state of Tripurawith trait linked markers. The value of gene diversity in the present study is reported to be 0.29 which is low as compared to the results obtained by Anupam *et al*., 2017

Table 2. List of SNP primers used for genotyping of 662 rice germplasm of Arunachal Pradesh along with major allele frequency, gene diversity, heterozygosity and PIC

S. No.	Primer ID	Major allele frequency	Gene diversity	Heterozygosity	PIC
1	01-2916-1_C_156	0.5383	0.4971	0.0781	0.3735
2	01-608-4_2_375	0.5116	0.4997	0.1435	0.3749
3	01-6351-1_C_202	0.8803	0.2107	0.0534	0.1885
4	02-267	0.6859	0.4309	0.1313	0.3380
5	02-3029-1_C_474	0.6269	0.4678	0.1419	0.3584
6	02-4333-1_2_293	0.7952	0.3257	0.0383	0.2726
7	03-1691-1_C_373	0.7374	0.3873	0.0689	0.3123
8	03-3478-1_C_206	0.5340	0.4977	0.1422	0.3738
9	03-4660-1_2_355	0.7926	0.3287	0.0736	0.2747
10	04-1801-20_2_428	0.8112	0.3063	0.1373	0.2594
11	04-19-4_C_240	0.9968	0.0064	0.0032	0.0064
12	04-3787-3_1_358	0.8793	0.2122	0.0575	0.1897
13	05-2692-1_2_109	0.9040	0.1736	0.0323	0.1585
14	05-4192-1_C_280	0.7090	0.4127	0.0879	0.3275
15	05-48-1_C_279	0.5646	0.4916	0.1417	0.3708
16	06-1256-1_C_147	0.9916	0.0166	0.0076	0.0165
17	06-1776-1_1_501	0.6209	0.4708	0.1404	0.3600
18	06-2509-1_C_497	0.9107	0.1627	0.0376	0.1495
19	07-2904-39_C_299	0.9084	0.1664	0.0275	0.1526
20	07-293-12_1_368	0.8734	0.2211	0.0253	0.1967
21	07-4304_new	0.5190	0.4993	0.0471	0.3746
22	08-2765-2_C_360	0.9164	0.1532	0.0175	0.1415
23	08-4218-5_C_129	0.5180	0.4994	0.1095	0.3747
24	08-847-6_C_113	0.6868	0.4302	0.1146	0.3377
25	09-209	0.8683	0.2288	0.0416	0.2026
26	09-2107-5_C_145	0.6907	0.4272	0.0879	0.3360
27	09-2716-4_C_457	0.9525	0.0906	0.0245	0.0865
28	10-1192-7_C_178	0.6396	0.4610	0.1625	0.3548
29	10-188-1.	0.7936	0.3276	0.1040	0.2740
30	10—2723	0.9245	0.1397	0.0826	0.1299
31	11-1849.	0.8152	0.3013	0.1102	0.2559
32	11-3935.	0.9375	0.1172	0.0324	0.1103
33	11-522-1_C_214.	0.9127	0.1593	0.0379	0.1466
34	12-1794	0.8950	0.1880	0.0396	0.1703
35	12-3200-2_C_389	0.9799	0.0393	0.0048	0.0386
36	12-400	0.8386	0.2707	0.0779	0.2340
	Mean	0.7822	0.2950	0.0740	0.2395

(0.7) where they have used PCR based allele specific markers for generation of genetic diversity.

Hierarchical Cluster Analysis

All 36 SNP loci were scored across all 662 rice germplasm. Genetic distance was calculated and NJ tree was constructed using dissimilarity matrix. Unrootedtree as was generated to find genetic relationships among the rice germplasm. All germplasm were grouped into three major clusters. Cluster 1 having maximum genotypes got grouped into subclusters. Cluster 3 is the smallest comprising of only four genotypes (Fig. 1.). Garris *et al.* (2005) observed clear distinction of wild, *indica*, *japonica* and basmati types showing their independent evolution by genetic relationships. Similarly, Travis *et al.* (2015) studied genetic diversity among 511 cultivars from Bangladesh and North-East India using a 384-SNP microarray assay and identified 191, 229 and 142 SNPs clearly distinguish *indica*, *japonica* and *aus* accessions, respectively, and the *aus* group has been further resolved into two subpopulations *aus*1and *aus*2. However, our results do not correspond to the study conducted by

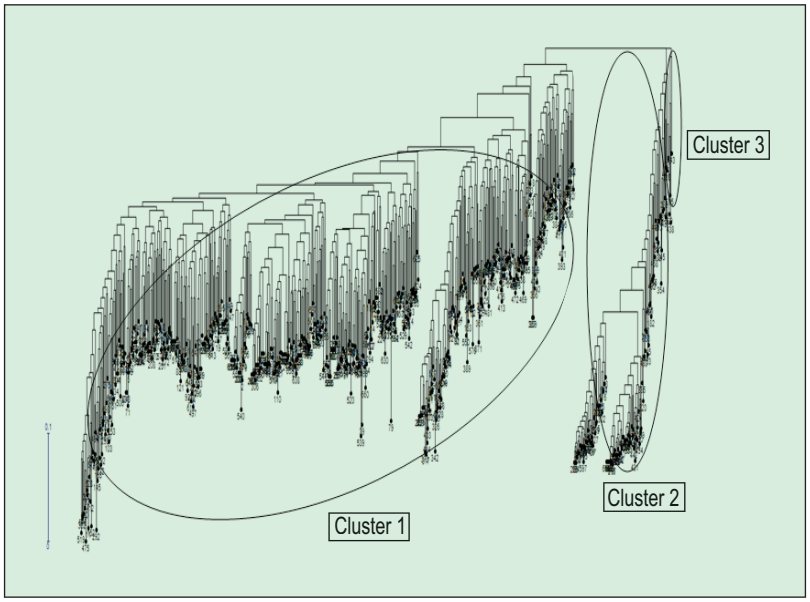

Fig. 1. NJ tree constructed for Arunachal Pradesh rice germplasm based on SNP data

Garris *et al.* (2005) and Travis *et al.* (2015). But our study provides a useful pool of SNP markers with uniform coverage throughout the rice genome. We have characterized 36 SNPs that were genotyped in 662 rice accessions. Our genomic diversity and structure analyses have included old and modern local cultivars, germplasm attempting to uncover a maximum spectrum of variability and occurrence of three different gene pools identified by structure analysis. This allowed us to reconstruct the genetic relationships and genetic diversity of the rice germplasm of Aunachal Pradesh.

Model Based Population Structure

To study the population structure, a model based programme STRUCTURE was used to determine genetic relationship among individual rice germplasm. This model assumes that the number of populations was k and the loci were independent and at Hardy–Weinberg equilibrium. Ln(PD) derived Δk was plotted against the K to determine the number of populations using a software "Structure harvester" available online. At K=3 maximum Δk was found, hence, this was considered as the number of population for the state of Arunachal Pradesh. Population structure divided 662 accessions into 3 populations (Fig. 2). Population1 (pop1), population2 (pop2) and population3 (pop3) contained 378, 203 and 81 germplasm, respectively. Further, based on the membership fractions, rice germplasm under different populations were categorized as pure or admixture. The accessions with the probability more than ≥0.80 score was considered as pure and less than 0.80 as an admixture. Pop1 showed 250 pure (66%) and 128 (34%) admixed individuals, pop2 showed 122 (60%) pure and 81 (40%) admixed individuals and pop3 showed 69 (85%) pure and 12 (15%) admixed individuals (Fig. 3). The relatively small value of alpha ($\alpha = 0.12$) in present study reveals that, only few individuals were admixed. Alpha value approaching zero indicates that most individuals in the study are from separate populations (Li *et al.*, 2014) whereas; an alpha value greater than 1 indicates that most of accessions of populations are admixed (Ostrowski *et al.*, 2006).

AMOVA and PCoA of Clusters Obtained Using Hierarchical Approach

AMOVA for the 662 accessions was performed based on the three clusters obtained using hierarchical cluster analysis. The three populations showed 0% variance at population level, whereas, 79% variance was recorded among individuals and 21% variance within individuals (Fig. 4; Table 3). PCoA based on hierarchical clusters showed intermixing of the three groups across the coordinates (Fig. 5; Table 4). The first three axes explained 37.8% of cumulative variation.

AMOVA and PCoA of Populations Obtained Using Model Based Approach

AMOVA was performed on three populations obtained using a model based approach. Among three populations 31% variance was recorded, whereas, among individuals,

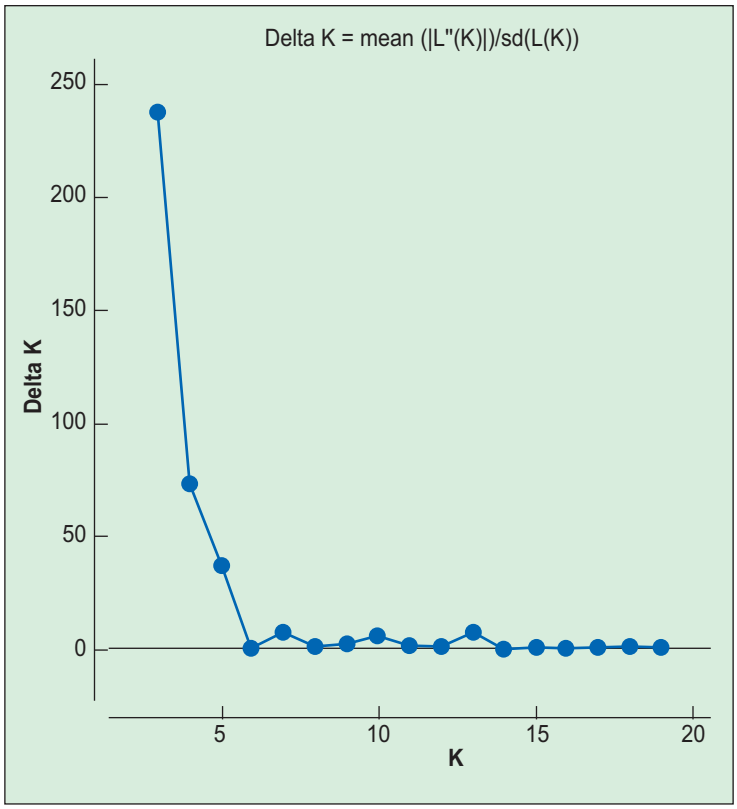

Fig. 2. Estimation of population using LnP(D) derived Δk for k from 1 to20 using SNPs marker data

51% variance and within individuals, 18% variance was found (Fig. 6; Table 5).PCoA revealed that large genetic diversity exists in rice germplasm of Arunachal pradesh. The first three axes explained 37.92% of cumulative variation (Fig. 7; Table 6). In PCoA, rice germplasm were labelled with three different colours which represent the three populations obtained from population structure. The pop1 and pop2 showed intermixing among themselves whereas pop3 occupied a distinct distribution between co-ordinates one and four. It showed very less intermixing with the other two populations. The above study shows that there is a lot of diversity prevailing in Arunachal Pradesh thus enhancing the belief that India has a rich resource of diverse germplasm.

Evaluation of accessions is important to discover useful sources and predict the genetic potential and breeding value of the currently underutilized materials in genebanks. Phenotypic evaluation has been successful in identifying beneficial materials for simple traits in genebanks. When an accession is identified as containing desirable traits, such as biotic or abiotic stress tolerance, the underlying alleles or haplotypes need to be transferred into elite germplasm for trait improvement with the help of molecular evaluation (Wang *et al*., 2017). Hence, molecular characterization of germplasm can provide the raw material in this regard. Despite the advancement in genomics, the Indian rice collection remained uncharacterized at the molecular level, with respect to parameters such as genetic diversity and

Table 3. Summary of AMOVA table (Hierarchical Cluster based method)

Summary AMOVA Table					
Source	df	SS	MS	Est. Var.	%
Among Pops	2	26.564	13.282	0.013	0%
Among Indiv	659	7183.956	10.901	4.808	79%
Within Indiv	662	850.500	1.285	1.285	21%
Total	1323	8061.020		6.106	100%

Table 4. Percentage of variation explained by the first 3 axes using SNP markers in principal coordinate analysis (Hierarchial Cluster based method)

Axis	1	2	3
%	22.89	8.74	6.25
Cum %	22.89	31.63	37.88

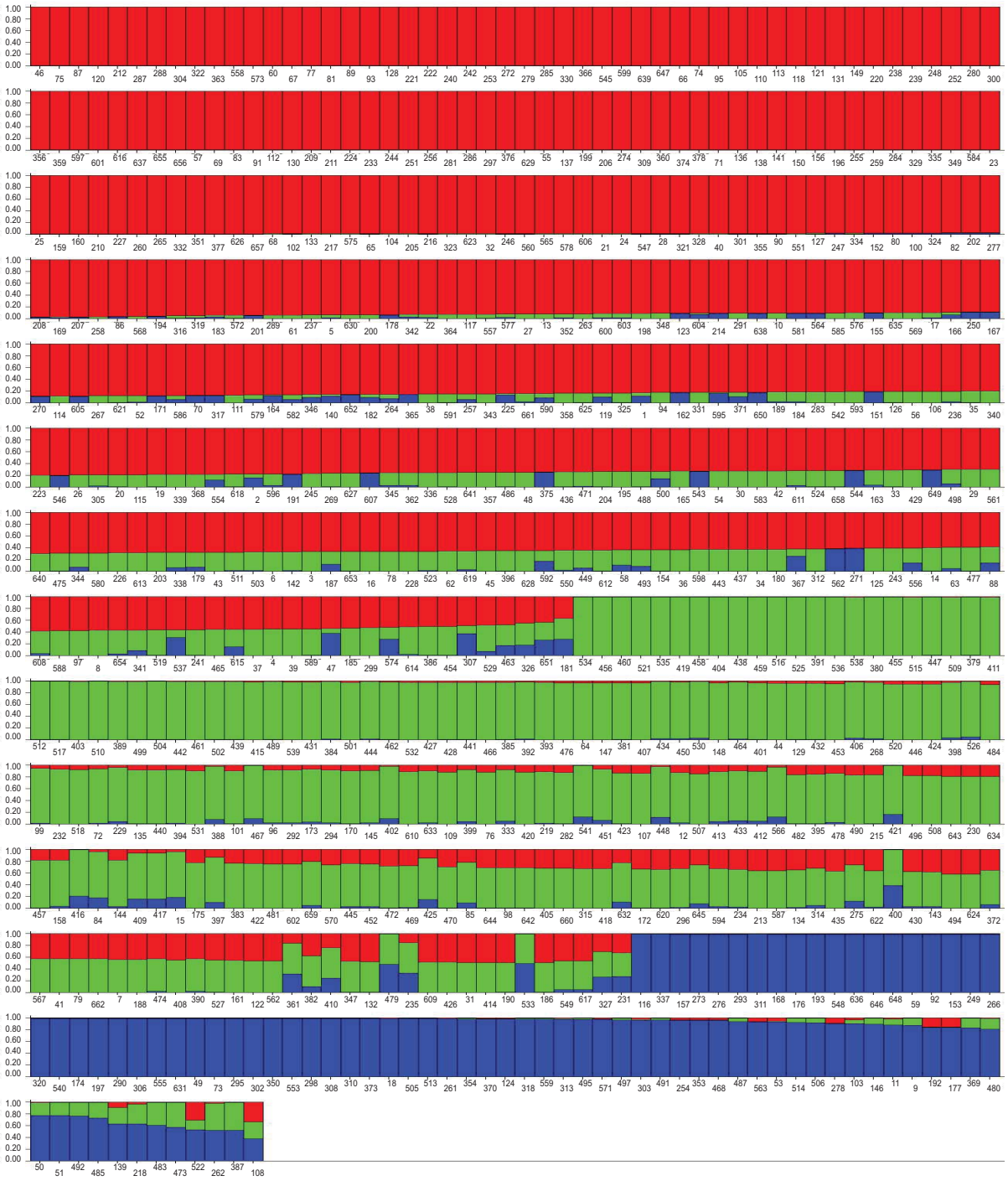

Fig. 3. Model based clustering of Arunachal Pradesh rice germplasm based on SNP (K=3) markers

population structure. This has been the major limiting factor in their utilization and development of improved cultivars.

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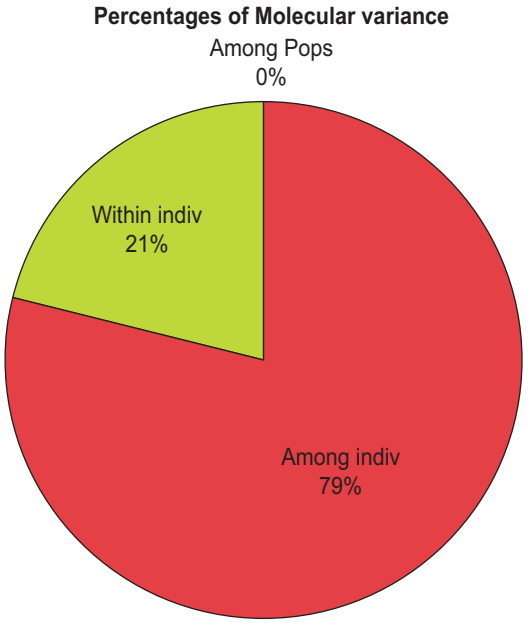

Fig. 4. Analysis of Molecular Variance (AMOVA) of 662 rice germplasm based on hierarchical cluster based method

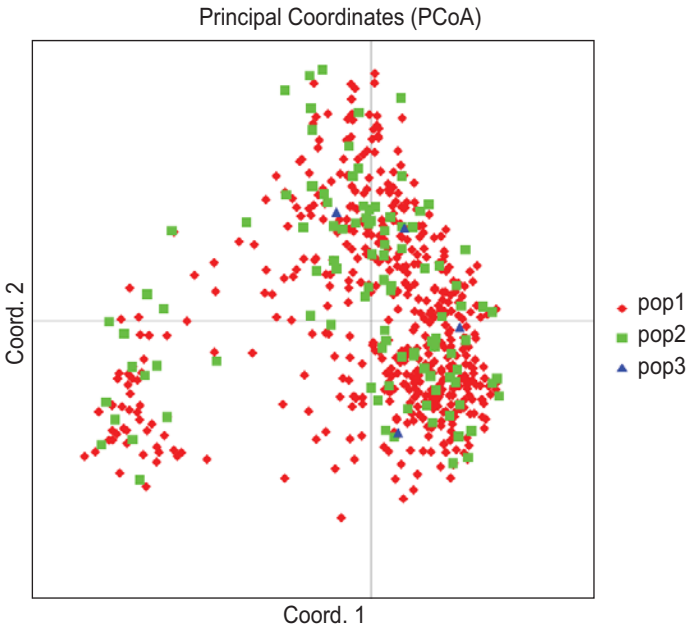

Fig. 5. Principal Coordinate Analysis (PCoA)of 662 rice germplasm based on hierarchical cluster based method

Table 5. Summary of AMOVA table (Model Based approach)

Source	df	SS	MS	Est. Var.	%
Among Pops	2	1667.178	833.589	2.206	31%
Among Indiv	659	5533.448	8.397	3.552	50%
Within Indiv	662	855.500	1.292	1.292	18%
Total	1323	8056.126		7.051	100%

Table 6. Percentage of variation explained by the first 3 axes using SNP markers in Principal Coordinate analysis(Model based approach)

Axis	1	2	3
%	22.91	8.76	6.25
Cum %	22.91	31.67	37.92

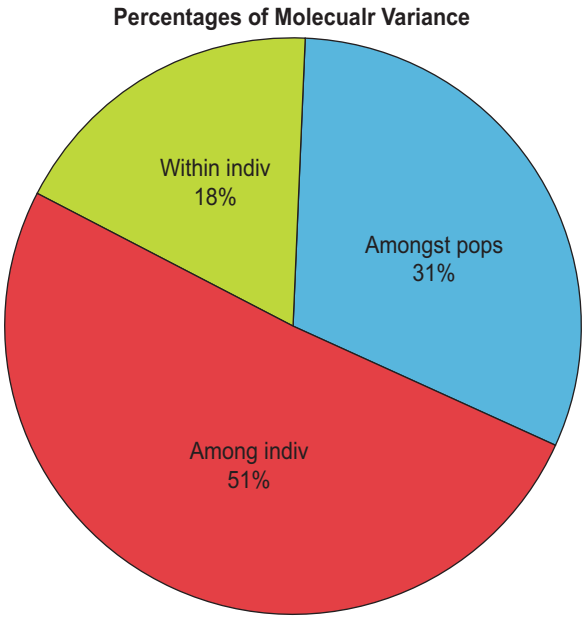

Fig. 6. Analysis of Molecular Variance (AMOVA) of 662 rice germplasm based on population structure based method

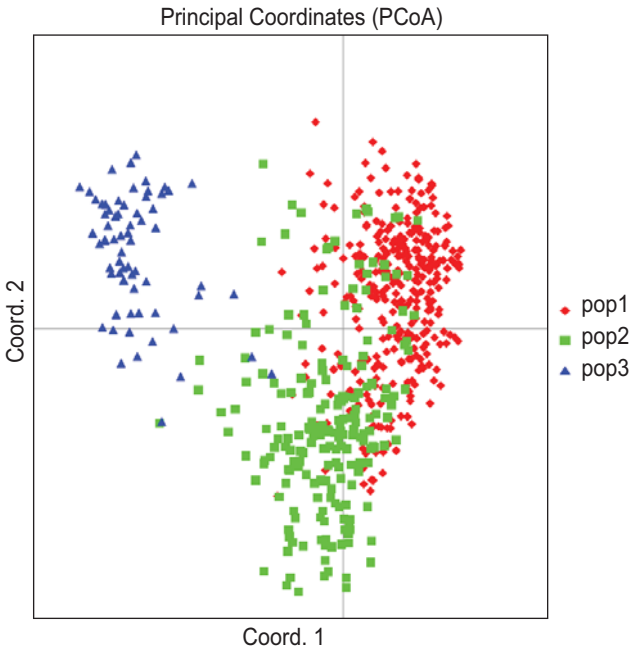

Fig. 7. Principal Coordinate Analysis (PCoA) of 662 rice germplasm based on population structure based method

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