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SEED DRYING FOR GERMPLASM CONSERVATION

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Orthodox seeds can be dried to low moisture contents with considerable advantage to longevity. Seed drying involves the evaporation of moisture from seed surface into the outer atmosphere and is influenced primarily, by the gradient in relative humidity between the seed and drying environment. The various methods of drying seeds to reduce moisture content for subsequent storage were discussed. Sun-drying, although is the cheapest method usually practiced, affects seed quality and is impractical in humid tropics due to the prevailing high relative humidity. While using commercial seed dryers for artificial drying, proper control of the air temperature is essential to minimize seed deterioration. A low temperature-relative humidity drying is recommended to reduce the moisture content to 5±2 per cent for long-term conservation of the germplasm seeds. The relative advantages and the associated problems in drying seeds to such low moisture content are discussed.

Seeds of many diverse species can be dried to very low moisture contents with considerable advantage to subsequent longevity during storage (Roberts, 1979). It has been shown that, over a wide range of moisture contents, there is a negative logarithmic relationship between seed longevity and moisture content (Ellis, 1991). Moisture control, primarily by drying also provides an opportunity to prevent losses which occur during harvesting and subsequent handling, estimated to be about 10 per cent of the grain produced (Hall, 1980). Consequently, seed drying methods, have been the most important aspect of processing to prepare seeds for storage. However, seed drying needs considerable care because inappropriate methods can lead to unnecessary seed deterioration. Hence, it has been a subject of intensive investigations,

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especially in climates where the ambient relative humidity is high. In this paper, the principles and practices of seed drying are discussed with special reference to seed conservation in genebanks.

FACTORS AFFECTING SEED DRYING

Equilibrium moisture content

Seed drying involves the evaporation of moisture from the seeds into the outer atmosphere and is directly related to the equilibrium moisture content of the seeds. Under all storage conditions, the moisture content of the seeds comes to an equilibrium with the relative humidity of the surrounding atmosphere. For a given species, there is a definable equilibrium relationship between relative humidity and seed moisture content, although the biochemical composition of the seeds which can in turn be affected by the genotype and the growing environment has a slight effect on this relationship (Justice and Bass, 1978; Cromarty *et al.*, 1982).

The equilibrium relationship between seed moisture content and relative humidity at a constant temperature of 25°C in the mandate crops of ICRISAT is presented in Fig. 1. These data were obtained by allowing the seeds to equilibrate in environments with known relative humidity, obtained by saturated solutions of KOH or aqueous solutions of H_2SO_4 . The equilibrium curves (or sorption isotherms) have a characteristically sigmoid shape with three distinct phases which correspond to the three different states of seed hydration (Vertucci and Leopold, 1987). At the driest end, the curve represents the water that is tightly bound to the macromolecules and this kind of water cannot be easily removed without destruction of the seed tissues. The next phase of the curve is represented by water that is loosely bound which can be removed by forced drying. The upper part of the curve represents water that is loosely held by weak bonding and free water in the intercellular spaces, which can be easily removed by simple drying. The moisture equilibrium curves are only slightly influenced by temperature, moisture contents being a little lower in warmer temperatures than in cooler, and vice versa.

Temperature, air flow rate and vapour pressure

The rate of loss in seed moisture is influenced positively by temperature, air flow rate and the difference between initial seed moisture content and the equilibrium seed moisture content at the relative humidity of the drying environment (Cromarty, 1984). Since the rate of seed deterioration is influenced positively by temperature, the choice of suitable temperature for seed drying compromises the positive effects of temperature on both the rate of drying seeds and seed deterioration.

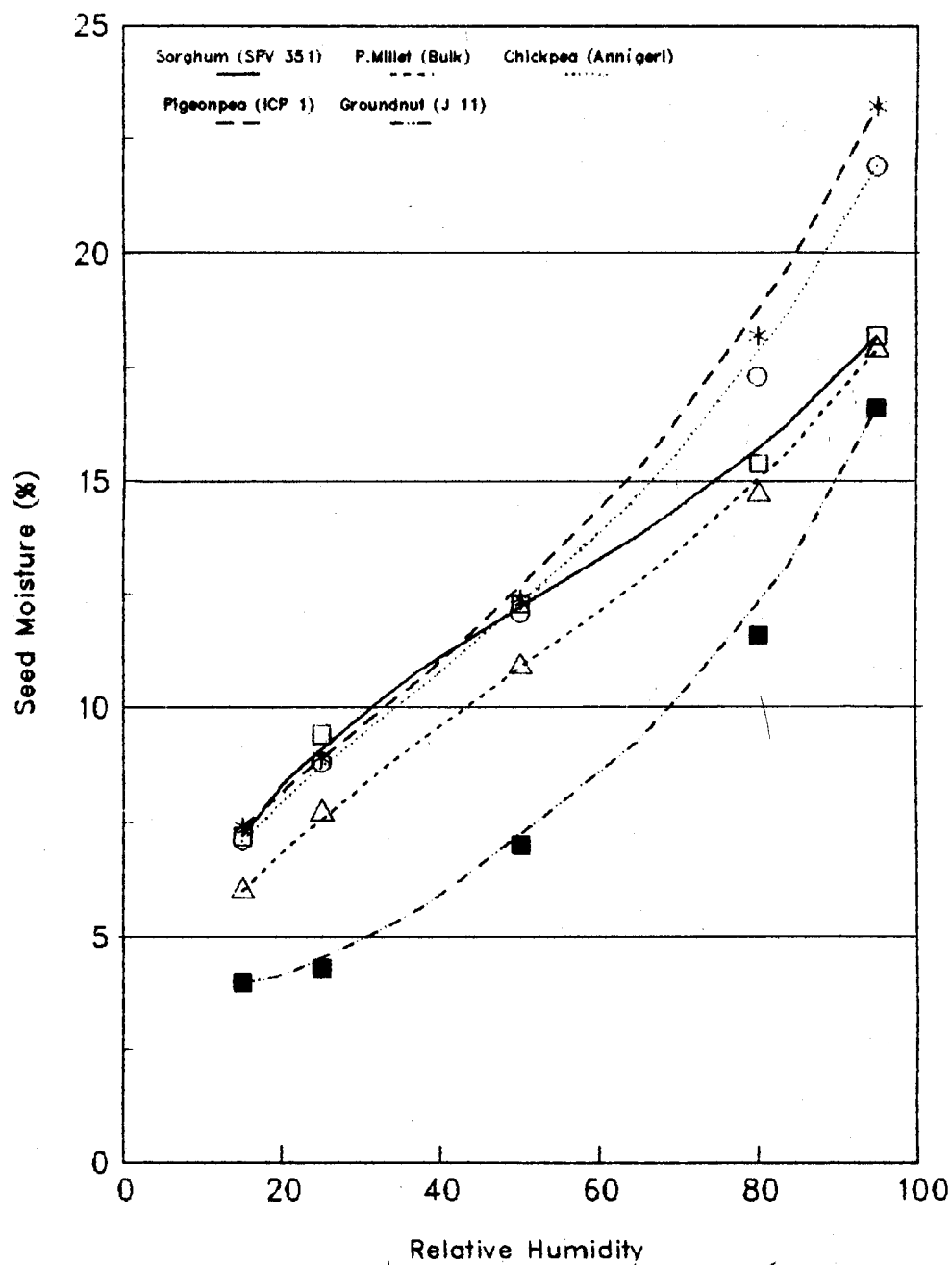


Fig.1. The equilibrium relationships between humidity (%) and seed moisture content (% wet wt.) at 25°C in sorghum, pearl millet, chickpea pigeonpea and groundnut. (N.K. Rao, unpublished)

The vapour pressure of the surrounding atmosphere should always be less than that inside the seed. If vapour pressure of the outer atmosphere becomes greater than that inside the seeds, the seed will gain moisture rather than lose it.

DRYING METHODS

Where the relative humidity of the ambient atmosphere is low, maturation drying reduces the moisture content of seeds sufficiently to avoid any significant losses in seed quality due to ageing under short- and medium-term storage conditions. However, in tropical and sub-tropical environments where the ambient relative humidity is high, and for long-term seed conservation where the moisture content recommended for storage is 5 ± 2 per cent (IBPGR, 1976), some post-harvest drying becomes necessary. Sun-drying and/or systems based on forced ventilation with heated air are generally used to reduce the seed moisture content.

Sun-drying

Sun-drying involves the heat of the sun and the evaporative effect of wind and is the cheapest method of drying, usually carried out by spreading the seeds in thin layers on a drying floor or by holding in cribs. Direct drying under sun is detrimental to seed quality, hence should be avoided. In groundnut, pods dried directly under the sun lost viability rapidly compared to pods dried under shade (Zade *et al.*, 1987; Nautiyal and Zala, 1991). Precautions are to be taken to prevent accidental mixing of accessions and to guard the seeds against rain, dust, insects and birds-which result in losses in quality and quantity. While drying on floor, the seeds have to be raked from time to time to prevent overheating, least it could lead to substantial losses in viability. Seeds may have to be removed at night times since the relative humidity increases markedly due to drop in air temperature. In humid tropics, sun-drying may be difficult or impractical due to the prevailing high ambient humidity and often there is a requirement for artificial drying.

Artificial drying

In artificial drying, the seeds are exposed to a current of heated air, usually at 40°C as with most commercial seed dryers. Heating relies on two physical effects: increase in temperature reduces the relative humidity of the air and increases the evaporation rate of the water from the seeds (Boxall and Calverley, 1985). While using this method, it is important to ensure that the air temperature does not rise above a relatively safe value. The use of heated air can reduce seed viability to some extent, especially when the initial moisture content is high. In fact, recommendations for maximum air temperature of heated air dryers vary with initial moisture content. Brandenburg *et al.*, (1961) provided

the general advise: if the initial moisture content is between 18 and 30 per cent, the air temperature should not exceed 32°C, if it is between 10 and 18 per cent, then air temperature of 40°C can be used. However, commercial recommendations, determined empirically in temperate countries to minimize damage during drying are unsuitable for poor quality seeds, heterogeneous seed lots and where the final moisture content aimed is as low as 5 per cent. Heated air drying was shown to induce dormancy in sorghum (Natile and Woodstock, 1967). When the grain is over heated during artificial drying, the quality also may be affected in a number of ways. For example, if the temperature is high and relative humidity of air low, moisture will be removed rapidly than water can diffuse from the inner layers of the seeds and a hardening or casing may be formed. The impervious layer will prevent free diffusion of moisture and will cause grain to become wrinkled, scorched and discoloured. Rapid drying can cause internal cracking as in rice, soybean and maize with serious implications for conservation (Boxall and Calverley, 1985). On the other hand, in humid tropics the reduction in relative humidity resulting from heating the air would not be sufficient to dry the seeds to low moisture contents needed especially for long-term conservation.

Low temperature drying

An alternative method which uses lower temperature (about 15°C) and an atmosphere with low relative humidity (10-15%) is recommended for drying seeds, especially for long-term conservation, where recommended seed moisture content is 5 ± 2 per cent (IBPGR, 1976). This requires the use of an air-dehumidifier with refrigeration to lower the temperature and remove the heat generated by dehumidifier. A seed drying cabinet manufactured to this design by Munters Ltd. (UK)* is being used effectively to dry the seeds of sorghum, pearl millet, chickpea, pigeonpea and groundnut for long-term conservation at ICRISAT. Larger seeds however, require substantial time to dry, hence a two stage drying procedure was suggested. In the first stage, drying could be carried out at 17°C with 40-45 per cent RH and in the second stage, seeds are placed in a dryer with good air circulation at about 30°C and 10-15% per cent RH (Cromarty *et al.*, 1982).

ADVANTAGE TO DRYING TO ULTRA-LOW MOISTURE CONTENTS

The advantage of dehydrating seeds to very low moisture contents is well known for several crops which produce orthodox seeds. For example, drying cereal seeds from 9 to 8 per cent moisture content

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doubles their storage life. Further decreases are shown to be even more beneficial, as drying from 5 to 4 per cent increases longevity by a factor of 3.7 (Roberts, 1989). Although little further desiccation of starchy seeds, the moisture content of many oily seeds could be further reduced to values between about 2 and 4 per cent with considerable benefit. For example, decreasing seed moisture content from 5 to 3.5 per cent in onion, and 2 per cent in groundnut increases longevity by a factor of 8 and 42, respectively (Ellis *et al.*, 1990b). Similarly, storage of *Brassica napus* seeds with 3.5 per cent moisture content instead of 5 per cent increases the half-viability period by 12 times (Ellis *et al.*, 1989). In view of the above, preservation of ultra-dry seeds is suggested to be a promising technique which might enable the cost of refrigeration reduced or even avoided. In this context, it is important to know whether extreme desiccation causes losses in quality of seeds or in viability and vigor.

PROBLEMS IN DRYING SEEDS TO LOW MOISTURE CONTENTS

Imbibition injury

Although there are essentially no reports on seed damage caused by drying to approximately 6 per cent moisture content, several workers have reported damage when seeds were dried to 5 per cent or lower (Toole and Toole, 1946; Roberts, 1959). Nutile (1964) found that sorghum seeds dried to 3 per cent moisture content showed more delayed germination and produced abnormal seedlings with damaged radicles, but the apparent injury was overcome by placing the seeds at 55 per cent relative humidity which increased the moisture content to 11 per cent. McCollum (1953) and Pollock and Manalo (1970) attributed the cotyledon cracking in garden pea to rapid imbibition of water during germination, since cracking was less when the seeds were humidified to about 12 per cent imbibition. Indeed much of the damage considered as desiccation damage was shown due to the stress caused by rapid uptake of water when dry seeds are placed in direct contact with water for germination (Ellis and Roberts, 1982; Ellis *et al.*, 1990a). Therefore, seeds can be dried to low moisture contents to prolong viability, but sensitive seeds require special handling before planting. Seeds must be allowed to equilibrate with normal atmospheric conditions, but in very dry arid areas, special humidification will have to be employed.

Hardseededness

In many species, the seed coat becomes impermeable to moisture ingress when dried to low moisture contents. This results in hardseededness which is particularly prevalent in the family

Leguminosae, but may also be seen in other families, for example, Malvaceae, Rosaceae, Convolvulaceae etc. When 100 pigeonpea accessions were screened, the percentage of hard seeds varied from zero to 71 per cent (Remanandan *et al.*, 1991). Two levels of hardseededness are recognized: as the seeds are dried to about 10-12 per cent moisture content, they become reversibly hardseeded, which soften when exposed to high relative humidity for a long time. When seeds are further dried to about 5 to 7 per cent moisture content, they tend to become irreversibly hardseeded, which are likely to be a problem for genebanks, especially in wild species of cultivated crops which have thicker seed coats (Ellis *et al.*, 1985). Unless specially treated, irreversible hardseededness by affecting plant stand when sown for regeneration, results in genetic shifts by selection of non-dormant types in heterogeneous germplasm accessions. However, hardseededness can be easily removed either by mechanical or acid scarification.

Cleavage damage

Considerable difficulty is encountered in soybeans and some genotypes of chickpea while the seeds are dried to low moisture contents. If rapidly dried, the seed coats crack and the cotyledons separate manifesting in what is known as cleavage damage (Rao *et al.*, 1990). To avoid this type of damage, soybean seeds are recommended to be dried in an environment with relative humidity of not less than 40 per cent (Cromarty *et al.*, 1982). In chickpea, the seeds have to be dried slowly, initially at about 40 per cent RH to an equilibrium moisture content of 8-9 per cent and then at 10-15 per cent RH for further reduction in moisture content (Rao *et al.*, 1990).

CONCLUSIONS

Seed drying, by removal of excess moisture in seeds reduces grain respiration and thereby slows deterioration. The growth of microorganisms and insects is also inhibited by drying. In many crops for general storage, the appropriate moisture levels recommended vary between 10 and 12 per cent, depending on crop. Organizations involved in seed production can plan their seed multiplication activity in areas where the crops attain harvest maturity when the ambient atmosphere is cool and dry. This enables production of good quality seeds with moisture content sufficient enough for direct short term storage, without recourse to postharvest drying. However, in humid tropics and in temperate climates, seed drying is imperative, and unless operated with care, it may adversely affect seed quality. Whatever method is used for drying, there is likely to be some effect on overall seed quality, since the same factors: temperature and time, which affect seed drying also contribute to seed deterioration. It is important that the choice of suitable drying

system should involve minimum seed deterioration during drying. Unfortunately, sun-drying and hot air drying, are by far the most common methods of drying seeds after harvest, although it is possible to reduce seed moisture content by other safer means. For example, substantial amounts of water can be removed without adversely affecting seed quality, by forced movement of dehumidified ambient air. Where ambient relative humidity is very low, drying rate can be maximised by increasing air flow rate in a thin layer drying without the need to heat the air. The IBPGR recommended conditions for drying (15°C and 10-15% RH) ensure minimum damage to the seeds, especially important while preparing seeds for long-term conservation in genebanks.

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VARIABILITY, INTER-RELATIONSHIPS AND UTILIZATION OF ANDIGENA POTATO GERMPLASM

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Tuber yield, its component and quality characteristics were studied in 108 genotypes of Andigena potato. Wide genetic variability was found for tuber number, tuber weight, tuber yield and dry matter, percentage vitamin C, proteins, phenols, total sugar and reducing sugars. The maximum variation was noted for reducing sugars and the minimum for dry matter. Tuber weight was more important than tuber number in determining tuber yield. Tuber yield and quality characteristics were not correlated. Promising genotypes have been identified for use in cultivation or breeding.

Among crop plants, potato produces highest energy and good quality protein per unit land area per day, which is similar to milk protein with EAA index : 76-79. Potato tuber is a rich source of vitamins, minerals and trace elements (Ross, 1986; Talburt and Smith, 1987; Woolfe, 1987) and can play a major role in alleviating energy and protein malnutrition in developing countries (Swaminathan and Sawyer, 1983).

High temperature, soon after harvesting, in sub-tropical countries requires cold storage of potatoes. Insufficient cold storage capacity in the country causes wide price fluctuations, uneven supply of potatoes over space and time and substantial losses due to rotting etc. (Nayar, 1986). To avoid this and to ensure higher per capita consumption, processing of potatoes is very important. Efforts to improve total food production have so far concentrated on breeding for crop yield and resistance to diseases. Hence, there is a need to put greater emphasis on initiating potato breeding programmes to improve tuber quality by isolating genotypes having the desirable attributes. To achieve this, wide genetic variability in the available

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germplasm is essential (Hawkes, 1990a). Due to its narrow genetic base and repeated cycles of inbreeding and selection *Tuberosum* (*Solanum tuberosum* subsp. *tuberosum* Hawkes) potatoes have limited genetic variability (Simmonds, 1962; Hawkes, 1990b). On the other hand *Andigena* (*Solanum tuberosum* subsp. *andigena* Hawkes) potatoes are storehouse of genetic variability (Simmonds, 1964; Ross, 1986; Hosaka and Hanneman, 1988; Birhman and Kaul, 1989 a, b; Waugh *et al.*, 1990). For formulation of an efficient breeding programme analysis of variability is vital (Hawkes, 1990 a). In the present study, genetic variability for tuber yield and quality traits and their inter-relationship was investigated in *Andigena* potatoes and the results are presented in this paper.

MATERIALS AND METHODS

One hundred and eight *andigena* (*Solanum tuberosum* subsp. *andigena* Hawkes) potato genotypes from four South American countries, viz., Argentina (5), Bolivia (11), Columbia (82) and Peru (10) were planted in a randomized complete block design with three replications at Central Potato Research Station, Modipuram, Meerut, UP. Each genotype was represented by one row of 15 plants per replication at a distance of 20 cm in rows kept 60 cm apart. Inorganic fertilizer was applied @ N : P : K :: 120 : 80 : 80 kg/ha. Half of the nitrogen and all phosphorus and potas were placed in bands before ridge making. The rest of the nitrogen was applied at the time of ridge making. Routine cultural practices were followed during the crop season.

Total tuber yield and tuber number per plant were recorded at harvest time. For tuber quality trait, a composite sample of 10 tubers was taken. Dry matter content was estimated by chopping and drying (at 90 °C for 48 hr.) 100g tuber tissue. For estimating vitamin C, true protein, phenols, total sugars and reducing sugars, methods were followed as described earlier (Rao, 1954; Swaminathan *et al.*, 1973; Spies, 1957., Dubois *et al.*, 1956; Nelson, 1944). Replication means were used for statistical and biometrical analysis. Analysis of variance was done as described by Snedecor (1968) for the randomized complete block design. Statistical parameters like measure of central tendencies, measure of dispersion and correlation coefficients were computed by the methods of Snedecor (1968).

RESULTS AND DISCUSSION

Intensive cultivation and repeated cycles of selection on regional basis have reduced the genetic variability of the *tuberosum* potatoes. On the other hand in its native place, South America, Andean potato germplasm lodges, absorbs, disseminates and perpetuates huge genetic variability (Brush *et al.*, 1981; Johns and Keen, 1986; Hawkes and Hjerting, 1989; Quiros *et al.*, 1990; Rabinowitz *et al.*, 1990). Results from the present investigations confirm

these findings (Fig. 1). Inter-genotypic variation was significant for all the traits (Table 1). All traits, except protein content and tuber weight, exhibited higher genetic variability in *andigena* potatoes to that previously recorded in *tuberosum* potatoes (Birhman and Kaul, 1989a; Talburt and Smith, 1987). The variation was maximum for reducing sugars and minimum for dry matter content.

Table 1. Variability parameters of 108 Andigena potato genotypes

Trait	Range	Mean $\pm$ SE	Coefficient of Variation
Tuber yield (g)	251 - 923	495 $\pm$ 11.9	25.05
Tuber number	11.7 - 38.3	20.9 $\pm$ 0.47	23.40
Tuber weight (g)	12.4 - 48.1	24.6 $\pm$ 0.68	28.78
Dry matter (%)	14.1 - 28.7	20.9 $\pm$ 0.27	13.57
Vitamin C*	7.0 - 17.0	11.6 $\pm$ 0.21	18.30
True protein*	565 - 1331	925 $\pm$ 19.3	21.73
Phenols*	18 - 105	63.7 $\pm$ 1.5	24.47
Total sugars*	149 - 1276	452 $\pm$ 18.2	41.69
Reducing sugars*	12 - 998	102 $\pm$ 14.2	144.43

*mg per 100 g fresh tuber weight

Yield is a polygenically controlled trait and many components add to this trait, the principal being tuber weight and number. Both these yield components exhibited considerable variability (Table 1). Tuber number exhibited low coefficient of variation. Though the mean tuber number was 20.9, it ranged between 11.7 to 38.3 in these genotypes. The least number of tubers were produced by JEX/A 676 and the maximum by JEX/A 578. Heaviest tubers were produced by JEX/A 324 and the lightest by JEX/A 314. Genotype JEX/A 789 had the least tuber yield and JEX/A 578 the maximum.

The overall variability in tuber dry matter content ranged from 14.1% to 28.7%, the lowest being in genotype JEX/A 653 and the highest in JEX/A 68. There was a large inter-genotypic variation in vitamin C content, the range being from 7 to 17 mg, least being in JEX/A 121 and the highest in JEX/A 226. Genotype JEX/A 584 had the lowest and genotype JEX/A 592 the highest protein content per 100g tuber tissue. In the genotypes, phenols per 100g tuber tissue varied from 18 to 105 mg with genotype JEX/A 695 having the least and JEX/A 102 the maximum. However, despite this large range, nearly 70 per cent genotypes fell in 62 to 66 mg range (Table 1). Reducing sugars followed by total sugars had the highest coefficient of variation.

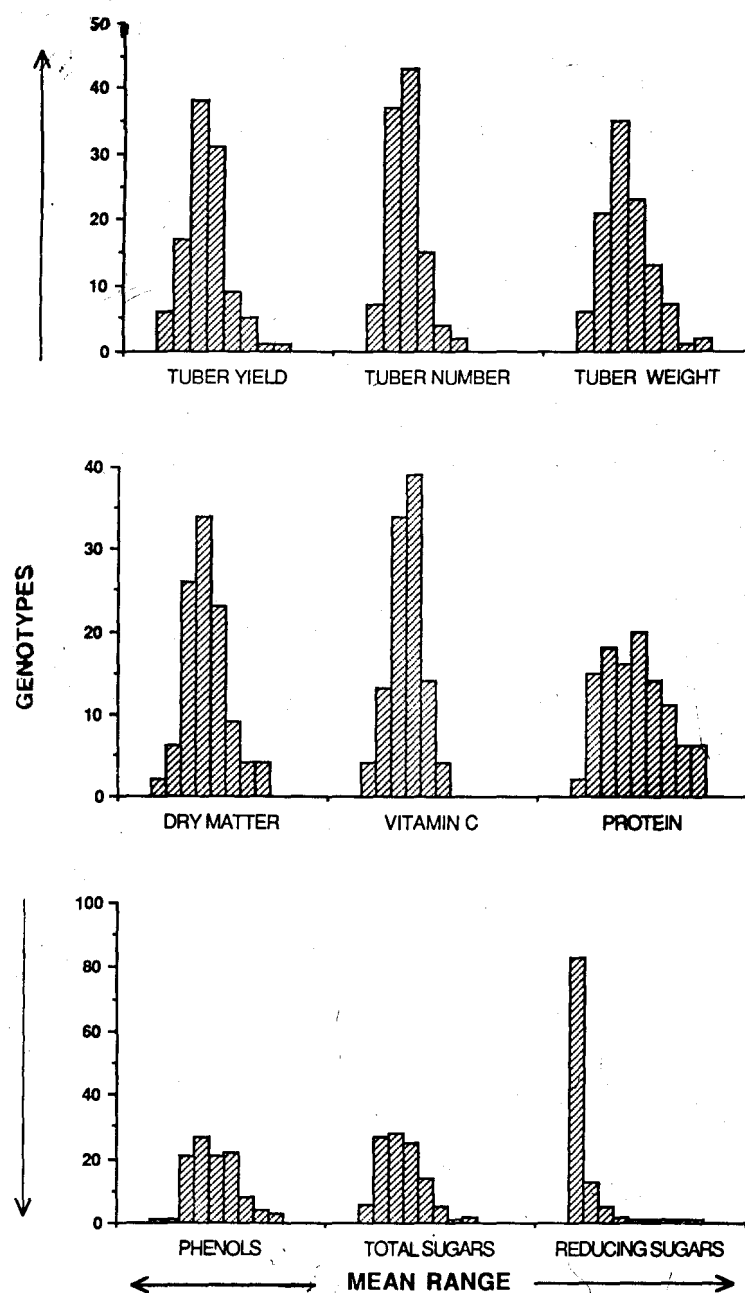


Figure 1 : Frequency distribution of metric characters in 108 Andigena potato genotypes (Histograms based on genotype means)

Maximum total sugars occurred in the tubers of genotype JEX/A 32 and the minimum in the tubers of the genotype JEX/A 701. The tubers of the genotype JEX/A 271 had the highest reducing sugars and of JEX/A 79 the least.

Tuber yield was significantly positively correlated with both of its components (Table 2; Maris, 1969; Sidhu and Pandita, 1979; Birhman and Verma, 1986). No significant association was observed between tuber yield and tuber quality traits (Killick, 1977; Gaur *et al.*, 1978; Singh *et al.*, 1988; Haynes *et al.*, 1989). Tuber number and tuber weight were negatively correlated with each other (Maris, 1969; Killick, 1977; Gaur *et al.*, 1978; Birhman and Verma, 1986; Singh *et al.*, 1988). While significant positive correlation existed between, dry matter and protein (Gaur *et al.*, 1978), total sugars and reducing sugars and phenols and reducing sugar, significant negative correlation was noted between tuber weight and dry matter (Gaur *et al.*, 1978) and dry matter and reducing sugars.

Percentage of dry matter in potato tubers is an indication of texture of cooked potatoes, mealiness and breakdown of tissues on cooking and is important for suitability for processing in different forms. Sugar content, especially the percentage of the reducing sugars, in the tubers is of great importance for potato processing. High sugar content causes discoloration of chips and dehydrated products. The amount of phenols in the tubers is regarded as the genetically most important factor for enzymic and non-enzymic browning of potatoes. The vitamin C and protein are nutritionally important (Hunius, 1981; Talburt and Smith, 1987; Woolfe, 1987). Existence

Table 2. Correlation coefficient in Andigena potatoes^a

Trait	TN	TW	DM	Vit. C	Protein	Phenols	TS	RS
TY	0.29**	0.63**	0.16	0.06	0.07	0.04	0.04	0.05
TN		-0.52**	0.08	0.10	0.05	-0.05	0.03	-0.01
TW			-0.20*	-0.02	-0.10	0.02	0.03	0.07
DM				0.06	0.25*	-0.10	-0.11	-0.21*
Vit C				0.04	0.15	0.10	-0.04	
Protein						-0.17	-0.09	-0.04
Phenols							0.07	0.19*
TS								0.56**
RS								

^aN = 108

TY = tuber yield; TN = tuber number; TW = tuber weight; DM = dry matter;

Vit C = vitamin C; TS = total sugars; RS = reducing sugars

* P < 0.05; **P < 0.01

of high inter-genotypic variability for tuber yield and its components and tuber quality characteristics in *Andigena* germplasm led to isolation of the promising genotypes for various economically important agronomic attributes (Table 3). The most promising genotypes were JEX/A nos. 68, 79, 175, 426, 592 and 695. These could be utilized in breeding and cultivation. Most important accessions for isolating promising genotypes were found to be PI nos. 225627, 225633, 230473, 243381, 243410, 243438, and 243471.

Potato tuber yield is a function of the tuber number and average tuber weight. Sidhu and Pandita (1979) and Lemaire and Caesar (1990) considered tuber number to be more important than average tuber weight in determining yield, whereas Maris (1969), Birhman and Verma (1986) and Joseph *et al.* (1992) considered the contrary to be true. Maris (1988) and Lynch and Kozub (1991) observed that while tuber number was more important for determining tuber yield in some of the progenies and environments, it was average tuber weight that was more important for tuber yield determination

Table 3. Promising *Andigena* potato genotypes

Traits	Genotypes (JEX/A Nos.) ^a
Tuber yield	37 (225627), 79 (230473), 578 (243436)
(> 700 g/plant)	592 (243438), 674 (243470)
Tuber number	18 (205624), 314 (243399), 426 (243410),
(< 30/plant)	578 (243436), 791 (280869)
Tuber weight	37 (225627), 57 (225632), 324 (243396),
(> 35 g)	496 (243423), 770 (258927)
Dry matter	68 (225633), 357 (243402), 426 (243410)
(< 27%)	455 (243417), 695 (243373)
Vitamin C	63 (225633), 68 (225633), 208 (243420),
(< 15 mg ^b)	218 (WRF 1299), 226 (243373)
Protein	275 (243381), 403 (243407), 592 (243438),
(< 1300 mg ^b)	653 (243452), 663 (243453)
Phenols	3 (Haw 2154), 10 (Haw 2154), 558 (243434),
(< 45 mg ^b)	695 (243471), 728 (246499)
Total sugars	69 (230471), 275 (243381), 391 (243486),
(9 < 200 mg ^b)	426 (243410), 701 (243471)
Reducing sugars	36 (225627), 79 (230473), 121 (233988),
(< 20 mg ^b)	359 (243404), 695 (243471)

^a Numbers in parenthesis are PI Numbers.

^b per 100 g fresh tuber tissue

in other genotypes and environments. The results from the present study suggest that tuber weight is more important in determining tuber yield in *Andigena* potatoes. Similarly, various authors have reported either positive, negative or absence of relationship between tuber number and tuber weight. A significant negative correlation was found between tuber number and tuber weight in the *Andigena* potato genotypes investigated presently. Strong and divergent variations in estimated values of correlation coefficients can be because the correlation coefficients between two traits constitute properties of not only the two traits but also of the population and environmental circumstances to which the individuals are subjected (Falconer, 1987).

Absence of undesirable association between tuber yield and tuber quality traits paves the way to combine these traits in all permutation and combination (Haynes and Haynes, 1990). Positive correlation between dry matter and protein, phenols and reducing sugars and total sugars and reducing sugars will help in identification of the desirable genotypes in a potato improvement programme. Negative correlation between tuber number and tuber weight will pose difficulty in simultaneous increase in tuber number and weight that is necessary for maximal yield gain. For enhancement of tuber yield, priority should be given to tuber weight because tuber yield has stronger association with tuber weight (standard partial regression coefficient = 1.1) than with tuber number (standard partial regression coefficient = 0.8). However, bigger tuber size will tend to reduce the dry matter content in tubers. This calls for optimization in selection for tuber number and weight.

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SOMATIC EMBRYOGENESIS IN *NARDOSTACHYS JATAMANSI* DC—AN ENDANGERED MEDICINAL HERB OF HIMALAYAS*

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Callus cultures of Nardostachys jatamansi DC (Family Valerianaceae) were established from petiole explants on a MS basal medium, supplemented with 3.0 mg/l α -naphthaleneacetic acid (NAA) and 0.25 mg/l kinetin (kn). Somatic embryogenesis was evident when the callus was sequentially subcultured through a series of media with gradually decreasing levels of auxin (from 3.0 mg/l down to 0.25 mg/l NAA) and a concomitant but gradual increase in the levels of cytokinin (from 0.25 mg/l to 2.0 mg/l in combination with 2.0 mg/l level of kn over a period of seven months. Fully developed somatic embryos were obtained when the concentration of NAA was reduced upto 0.25 mg/l in combination with 2.0 mg/l level of kn. The formation of plantlets via somatic embryogenesis in Nardostachys jatamansi is important in context with the conservation of this endangered medicinal and aromatic herb of the wet and high Himalayan ranges.

Nardostachys jatamansi DC., commonly known as Jatamansi or the Indian spikenard, is a primitive member of the family Valerianaceae. The plant has a restricted distribution from Garwhal Himalayas extending to Bhutan and Sikkim in the eastern Himalayas. It occurs on rocky edges and crevices at high altitudes ranges from 3,300m to 5,700m (Weberling, 1975). Jatamansi is a perennial rhizomatous herb with simple radical leaves and a compound dichasial inflorescence. The pentamerous sympetalous flowers give rise to indehiscent fruits that contain non-endospermous seeds. Nardostachys finds its use in medicine and perfumery, and place in Greek literature and holy Bible (Shah, 1982). The Indian

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Ayurvedic system of medicine recommends the use of *jatamansi* roots in various psychiatric disorders such as epilepsy and hysteria (Arora and Arora, 1963). The tranquilizing and anti-arrhythmic properties of the plant extract are attributed to the presence of a saturated bicyclic sesquiterpene ketone - jatamansone (Houghton, 1988) while the perfumery value of the oil is due to the presence of volatile iso-valeric esters and jatamanshic acid.

The widespread use of *jatamansi* in traditional medicines, perfumes, and incenses (Shah, 1982) has resulted in the indiscriminate exploitation of its wild populations. Very little efforts were made for its systematic cultivation. The plant is enlisted as an endangered species in Indian Plant Red Data Book (Jain and Sastry, 1984). Need for systematic approach towards conserving the natural populations of *jatamansi* and devising rapid propagation procedures for replenishment is, therefore, evident. Under the aegis of an All India co-ordinated project on the conservation of some endangered plant species through tissue culture (sponsored by the Department of Environment, New Delhi), the present work was undertaken to develop rapid *in vitro* propagation procedure in *jatamansi*. This communication enumerates the experimental protocol for the high frequency induction of somatic embryogenesis in petiole-derived calli of *N. jatamansi*. This is the first report on somatic embryogeny in a genus of the family Valerianaceae and this stresses upon the efficacy of sequential and gradual alterations in the levels of exogenously applied growth regulators for achieving morphogenesis in otherwise recalcitrant systems.

MATERIALS AND METHODS

Plants of *N. jatamansi* DC. ($2n=26$) were collected from Milam locality of Pithoragarh, Uttar Pradesh, and were maintained in a growth chamber (Percival Inc. USA, at 22°C, 70 per cent relative humidity-RH and 14 hr photoperiod 4000 lux). Petiole explants (2-3mm long) were excised and surface sterilized for 10 min. in a 2 per cent (v/v) solution of sodium hypochlorite. The basal medium (BM) consisted of Murahsige and Skoog's (1962) medium salts, 3 per cent sucrose, 100 mg/l myo-inositol and 0.7 per cent agar. For callus initiation and subsequent morphogenetic studies, various auxins namely IAA (Indole-3-acetic acid), IBA (Indole-3-butyric acid), NAA (α -naphthaleneacetic acid), or 2, 4-D (2,4-dichlorophenoxyacetic acid) and cytokinins such as kn (kinetin), BAP (6-benzyladenine), 2-iP (2-isopentyladenine) and Z (zeatin) were incorporated into the basal medium in concentration, combination and sequence as detailed in the results. The pH medium was adjusted to 5.8 ± 0.02 before autoclaving at 15 psi and 121°C for 15-20 minutes. Cultures were

incubated at $25 \pm 2^\circ\text{C}$ and 14/10 hr light/dark period (2000 lux) using cool, white, fluorescent lights.

Sequential subculturing

Experiments on sequential subculturing of callus on gradually decreasing auxin (NAA) and concomittantly increasing cytokinin levels were done using 18 hormonal combinations. These include 3.0, 2.0, 1.0, 0.5, 0.25 or 0.00 mg/l NAA with 0.25, 0.50, 1.0 or 2.0 mg/l of either kn (Medium No. 1-6) or BAP (Medium No.7-12) or 2-iP (Medium No. 13-18), respectively. At the end of each culture with the passage of 4-6 weeks duration on a particular medium, the callus was divided into three equal parts. One part was recultured on to the fresh medium of similar composition, second was placed on a hormone free BM and the third part was carried forward to the next medium in the sequence, i.e., from medium No. 1 (MS+ 3.0 mg/l NAA+ 0.25 mg/l kn) to medium No. 2 (MS+ 2.0 mg/l NAA+ 0.25 mg/l kn) and so on. In order to assess the effect on callus age on morphogenesis, the callus from the stock cultures was also directly transferred to the above mentioned 18 media at the time of each sequential subculture.

Histological studies

For histological observations, tissues were fixed in FAA (formalene: acetic acid : ethyl alcohol : : 95:5:5) and dehydrated through a graded tertiary butyl alcohol/xylene series. Observations were made on 10 μm thick serial sections which were double stained with saffranin and aniline blue. For Scanning Electron Microscopy (SEM), the embryogenic callus tissue was fixed in 4 per cent (v/v) glutaraldehyde, followed by 1 hr treatment with 1 per cent (w/v) osmium tetroxide in 0.1M phosphate buffer. Samples were then dehydrated in a graded ethanol series, critical point dried and gold - coated before viewing under a scanning electron microscope (JEOL 36, Japan)

RESULTS AND DISCUSSION

For callus initiation, the petiole explants were cultured on BM supplemented with 1.0 - 4.0 mg/l NAA, IAA, IBA or 2,4-D with or without 0.25 - 1.0 mg/l kn (Table 1). From amongst the four auxins, NAA was most effective in initiating callusing in *N. jatamansi*. While IAA and IBA could induce only a limited callus response at 4.0 mg/l level, supplementation of 2, 4-D in BM resulted in early browning and death of the explants. The optimum callusing occurred in medium having 3.0 mg/l of NAA in combination with 0.25 mg/l of kn. Calli obtained on medium having less than 3.0 mg/l NAA showed a tendency to develop small roots within 2 weeks of its initiation, while levels higher than 3.0 mg/l resulted in browning and senescence of the freshly induced callus. Presence of kn in the auxin-containing media, though not

Table 1. Efficacy of various auxins with or without Kn for inducing callus response from petiole explants in *N. jatamansi*

Kn (mg/l)	Auxin (mg/l)																
	IAA				IBA				NAA				2,4-D				
	0	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0.0	—	—	—	—	C ⁺	—	—	—	—	C ⁺	C ⁺	C ⁺⁺	C ⁺⁺	B	B	B	B
0.25	—	—	—	—	C ⁺	—	—	—	—	C ⁺	C ⁺	C ⁺⁺⁺	C ⁺⁺⁺	B	B	B	B
0.50	—	—	—	—	—	—	—	—	—	C ⁺	C ⁺	C ⁺⁺	C ⁺⁺	B	C ⁺	B	B
1.00	—	—	—	—	—	—	—	—	—	C ⁺	C ⁺	C ⁺⁺	C ⁺⁺	B	C ⁺	C ⁺	B

(—) No visible response; (C) Callusing; (R) Rooting from callus; (B) Browning and death; (+) Increasing number of + sign denotes the intensity of response and rate of subsequent growth

essential for initiating a callus response, improved upon the proliferation of the induced callus. Hence, a callus stock was developed and maintained through repeated subcultures on BM + 3.0 mg/l NAA + 0.25 mg/l kn. From third subculture onwards, callus tissue from stock cultures was used for organogenetic studies. For this, various single and double combinations of IAA, IBA or NAA (0.20-1.0 mg/l) with kn, BAP, Z or 2-iP (0.5-2.0 mg/l) were tested and the results are summarised in Fig. 1. Three distinct types of response were visible on these 140 medium combinations, i.e., continued callus proliferation only, callus growth was accompanied with differentiation of numerous small roots or the development of dark green nodular zones on the callus surface. The first two types of responses were prevalent in media having IAA, ABA or NAA alone or high levels of these auxin (>0.5 mg/l) with low level of kn, BAP, Z or 2-iP (< 1.0 mg/l). The appearance of green nodules was evinced by the presence of higher levels of these cytokinins (>1.0 mg/l) in all the auxin-containing medium. Histological examination of these green nodules revealed the presence of meristematic zones consisting of densely cytoplasmic cells with prominent nucleus and nucleolus. These zones were interspred with well-organised tracheids. The meristematic zones, however, remained quiescent and did not differentiate further into

mg.l ⁻¹		Kn					BAP				Z			2-iP		
		0	05	1.0	2.0	4.0	05	1.0	2.0		05	1.0	2.0	05	1.0	2.0
IAA	0	C	C	O	O	O	C	O	O	O	O	O	O	O	O	O
	0.2	R	R	O	O	O	O	O	O	O	O	O	O	O	O	O
	0.5	R	R	O	R	O	O	O	O	O	O	O	O	O	O	O
	1.0	R	R	R	R	O	C	O	O	O	O	O	O	O	O	O
IBA	0.2	R	R	O	O	O	O	O	O	O	O	O	O	O	O	O
	0.5	R	R	O	C	O	O	O	O	O	O	O	O	O	O	O
	1.0	R	R	R	O	O	O	O	O	O	C	O	O	O	O	O
NAA	0.2	R	R	O	O	O	C	O	O	O	O	O	O	O	O	O
	0.5	C	R	O	O	O	C	O	O	O	O	O	O	C	C	O
	1.0	C	C	O	O	O	C	C	O	O	O	O	O	C	C	O

Fig. 1. Morphogenetic responses of callus culture of *N. jatamansi* after 6 wks of growth on BM supplemented with double combinations of various auxins and cytokinins (C-callus proliferation; R-root formation; O-production of dark green nodules; - moderate response; - extensive response).

organised structures upon repeated subculturing onto the fresh medium of similar composition as the one on which these nodules were induced. Certain other manipulations in the medium like removal of growth regulators, lowering of nutrient strength of the BM to half and one fourth, and addition of 10-20 per cent (v/v) cococut milk, proved ineffective in inducing any organised development from these nodules.

Experiments were then initiated to assess the applicability of sequential culturing of calli through a graded series of auxin/cytokinin ratio for inducing organogenesis in *N. jatamansi*. Such an approach was based upon the inferences of the past studies (Steward *et al.*, 1967; Steward and Mapes, 1971; Krikorian and Kann, 1981; Srinivasan and Mullins, 1980) in *Asparagus*, *Hemerocallis* and *Vitis* spp., respectively. These workers emphasised the significance of balanced synergistic use of planned and sequential combination of auxin and cytokinin to foster organogenesis in otherwise recalcitrant cell cultures that exhibit arrest of growth at the early stages of differentiation, a situation very similar to that encountered in *N. jatamansi*. Hence, a graded series of decreasing NAA (3.0, 2.0, 1.0, 0.5, 0.25 and 0 mg/l) in combination with increasing cytokinin levels (0.25, 1.0, and 2.0 mg/l kn, BAP or 2-iP) were tried for sequential transfer of the callus cultures. Amongst these, only NAA/kn series proved effective in bringing about the somatic embryogenesis. The stock callus continued to proliferate on media 1 and 2, i.e., BM + 3.0 mg/l NAA = 0.25 mg/l kn and BM + 2.0 mg/l NAA + 0.25 mg/l kn (Fig. 2a, B). When calli were transferred from medium 2 to medium 3 i.e., BM + 1.0 mg/l NAA + 0.5 mg/l kn, dark green sectors became evident within 2 weeks of transfer (Fig. 1C). These green sectors organized into compact nodules on medium 4, i.e., BM + 0.5 mg/l NAA + 1.0 mg/l kn (Fig. 2D,d). On medium 5, i.e., BM + 0.25 mg/l NAA + 1.5 mg/l kn, numerous somatic embryos with distant radicular and plumular axes were produced (Fig. 2E,e). Further lowering of auxin/cytokinin ratio in the medium 6, i.e., BM + 0.25 mg/l NAA + 2.0 mg/l kn, allowed the embryo to plantlet conversion to take place with rapid development of cotyledonary leaves and new shoots (Fig. 1F). The plantlet conversion frequency ranged from 10-15 per cent on this medium. The root growth on this medium was slow. The radicular axis became flattened and recallused. Numerous secondary embryoids also originated from this freshly induced callus. The frequency of *in vivo* establishment of somatic embryo-derived plantlets under glass house conditions was very low (3-5%). This was possibly due to abnormal root development as mentioned above.

The calli that were recultured or maintained throughout the entire duration of the experiment on a particular medium in the sequence

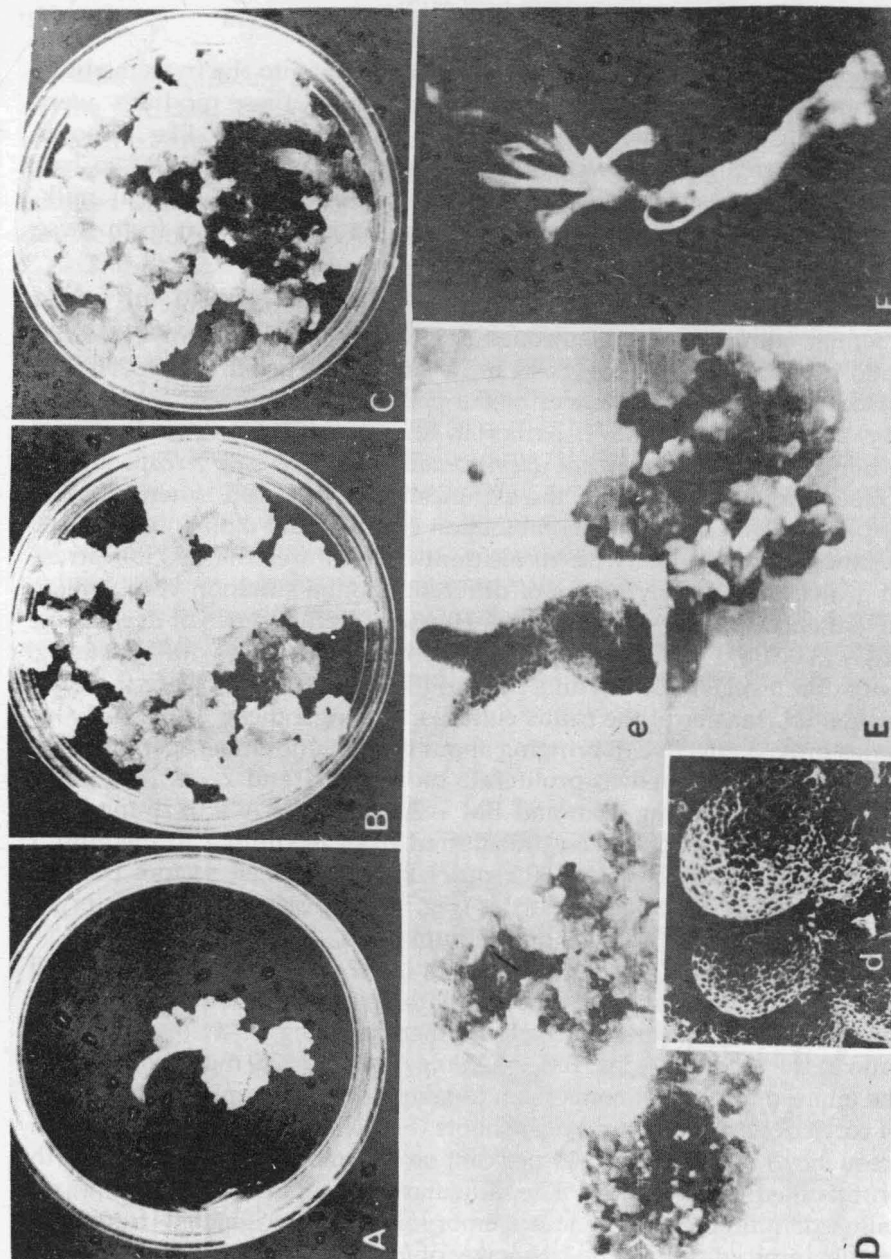


Fig. 2. Induction of somatic embryogenesis and plantlet development upon sequential subculturing of callus of *N. jatamansi* through a graded series of increasing NAA and concomitantly increasing levels of Kn in BM

A, Callus initiation on BM+3.0 mg/l NAA+0.25 mg/l Kn; B, Rapid callus proliferation on BM+2.0 mg/l NAA+0.25 mg/l Kn; C, Appearance of green compact regions on the callus on BM+1.0 mg/l NAA+0.5 mg/l Kn; D-d, Differentiation of dark green nodules on BM+0.5 mg/l NAA+1.0 mg/l Kn; E-e, Formation of complete embryoids on BM+0.25 mg/l NAA+1.5 mg/l Kn; F, Plantlet development on BM+0.25 mg/l NAA+2.0 mg/l Kn.

retained their respective stage of differentiation and did not grow into the next stage. Further, direct transfer of stock callus to Medium 4, 5, or 6 at the time of each sequential subculture also failed to evoke an embryogenic response, indicating thereby the embryogenesis obtained *via* sequential culture was not a function of callus age. Substitution of kn by BAP or 2-iP as a cytokinin source in the medium (Medium Nos. 7-18), at concentrations similar to that employed in NAA/kn series, did not allow differentiation to proceed beyond the green nodule stage.

Somatic embryogenesis is a plastic response of cultured cells, controlled by numerous cultural and environmental variables (Ammirato, 1983, 1987; Ammirato and Steward, 1971; Tran Thann Van, 1981). Among others, the role of a proper balance between nature and concentration of auxin and cytokinin at different stages of somatic embryo development, i.e., initiation, maturation and hardening, has been frequently documented in a large number of plant species (Evans *et al.*, 1981; Rangaswamy, 1986). While auxins like 2,4-D, IAA and NAA have been shown to influence more directly the initial proliferation of embryogenically competent and committed tissue (Halperin, 1970; Fujimura and Komamine, 1980) and establishment of bipolar organisation (Ammirato and Steward, 1971; Christianson, 1987), cytokinins such as kn, BAP, Z and 2-iP are more critical requirements of embryo maturation and plantlet conversion phases (Kavathekar *et al.*, 1978; Krikorian and Kann, 1981). The present study substantiates the above inferences and also emphasises upon the importance of sequential change of hormonal regimes in the medium for the complete development of somatic embryos in *N. jatamansi*. It has been earlier enunciated that as in the case of sexual embryogenesis *in vivo* gradual shifts in nutritional/hormonal requirements also characterize the various developmental stages of somatic embryogenesis *in vitro*, (Steward *et al.*, 1967). Such gradual shifts in exogenously hormonal concentrations have proved successful in the complete differentiation of somatic embryos in many recalcitrant systems like *Panax ginseng* (Chang and Hsing, 1980) and *Hemerocallis* (Krikorian and Kann, 1981). The present study is of consequence for the rapid propagation of *N. jatamansi*. Efforts are now underway to enhance the embryo to plantlet conversion frequency with concomitant suppression of recallusing of the regenerated roots for better establishment of the plants in the soil.

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STRUCTURAL CHANGES ASSOCIATED WITH DORMANCY BREAKING TREATMENTS IN CARDAMOM

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Seeds of cardamom (Elettaria cardamomum Maton) variety Malabar were found to exhibit dormancy due to their hard seed coat. Experiments were conducted to study the effect of various physical and chemical treatments for breaking seed dormancy. Treatment of seeds with sulphuric acid (25 %) for ten minutes and absolute alcohol (80 %) for 30 minutes were found to be the best amongst all the treatments in breaking seed dormancy. Scanning electron microscopy of seed coat surface revealed the presence of mucilaginous layer over the control seed and irregular shape lesions of various sizes over the seed coat of treated seeds.

Germination in cardamom (*Elettaria cardamomum* Maton) is reported to be often poor, delayed and very irregular with some seeds taking over a year to germinate despite favourable conditions (Purseglove, 1972). Under natural conditions, the seeds remain dormant for considerable period of time (Abraham, 1958). The seeds of cardamom are aromatic, dark brown angled with thin mucilaginous aril, bulky white perisperm and small embryo. The hard stony seed coat is reported to be responsible for the delay in seed germination and hence any procedure which enables the seed to imbibe moisture, promotes germination (Kologi *et al.*, 1973; Reddy *et al.*, 1973). Therefore, an attempt has been made to soften the hard seed coat of cardamom by giving various physical and chemical treatments. Surface structural changes which led to increased per cent germination were studied using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Seeds of cardamom, variety Malabar (clone-37) obtained from National Research Centre for Spices, Calicut (Kerala) were used in the

present investigations. For conducting germination test, the seeds were surface sterilized with 0.1 per cent mercuric chloride for ten minutes, washed thoroughly 2-3 times with distilled water and then plated over two layers of moistened filter paper in a petriplate (11 cm diameter). Four replicates of 25 seeds each were used per treatment. All the petriplates were maintained in a germinator at 20°/30°C for 16/8 hr photoperiod. Triphenyl Tetrazolium Chloride (TTC) test for viability following various chemical treatments were carried out based on the earlier reports (Anonymous, 1978; Reddy *et al.*, 1973 and Kololgi *et al.*, 1973). Chemical treatments included acetic acid 25 per cent (10 min), nitric acid 25 per cent (10 min.), hydrochloric acid 50 per cent (10 min), sulphuric acid 25 per cent (10 min.), gibberellic acid 200 ppm (20 min), cellulase 1 per cent (20 min.), ethyl alcohol 80 per cent (30 min.) and potassium nitrate 0.2 per cent (30 Min.). The seeds were thoroughly washed under running tap water after each chemical treatment and then were plated for germination. Physical treatment was given by chipping the seeds at micropylar end and soaking in water for 6 days. All the experiments were repeated thrice and average value for percentage germination was recorded.

Since the cardamom seeds take two to three weeks for germination, a quick viability test was standardised to record the viability after various treatments. The seeds were pre-soaked in water for 18 hrs, then bisected and the half portion of seed containing embryo was put in 1 per cent solution of TTC for 8 hrs at 30°C. After washing, the seeds were observed for the development of red colour stain by embryo which indicated the viability of seed.

The seed coat surface structure studies were performed using scanning electron microscope Model Jeol JSM 840A. Treated and untreated seeds of various samples were cleaned and dried for 48 hrs. at room temperature. Selected seeds were cut with a sharp razor into halves at the raphe region. Both complete and cut seeds were mounted on brass stubs using silver adhesive and gold coated using ion sputter. The coated samples were examined at an accelerating voltage of 5 to 10 kv. The surface was uniformly scanned in all the samples and observations were recorded.

RESULTS AND DISCUSSION

The germination pattern of cardamom seeds after various treatments is depicted in Fig 1. Untreated (control) seeds showed 60 per cent germination whereas, the per cent germination was improved by 23, 28, 20, 35, and 32 per cent after treating with acetic acid, nitric acid, hydrochloric acid, sulphuric acid and ethyl alcohol, respectively (Table 1). However, the maximum increase in germination percentage was

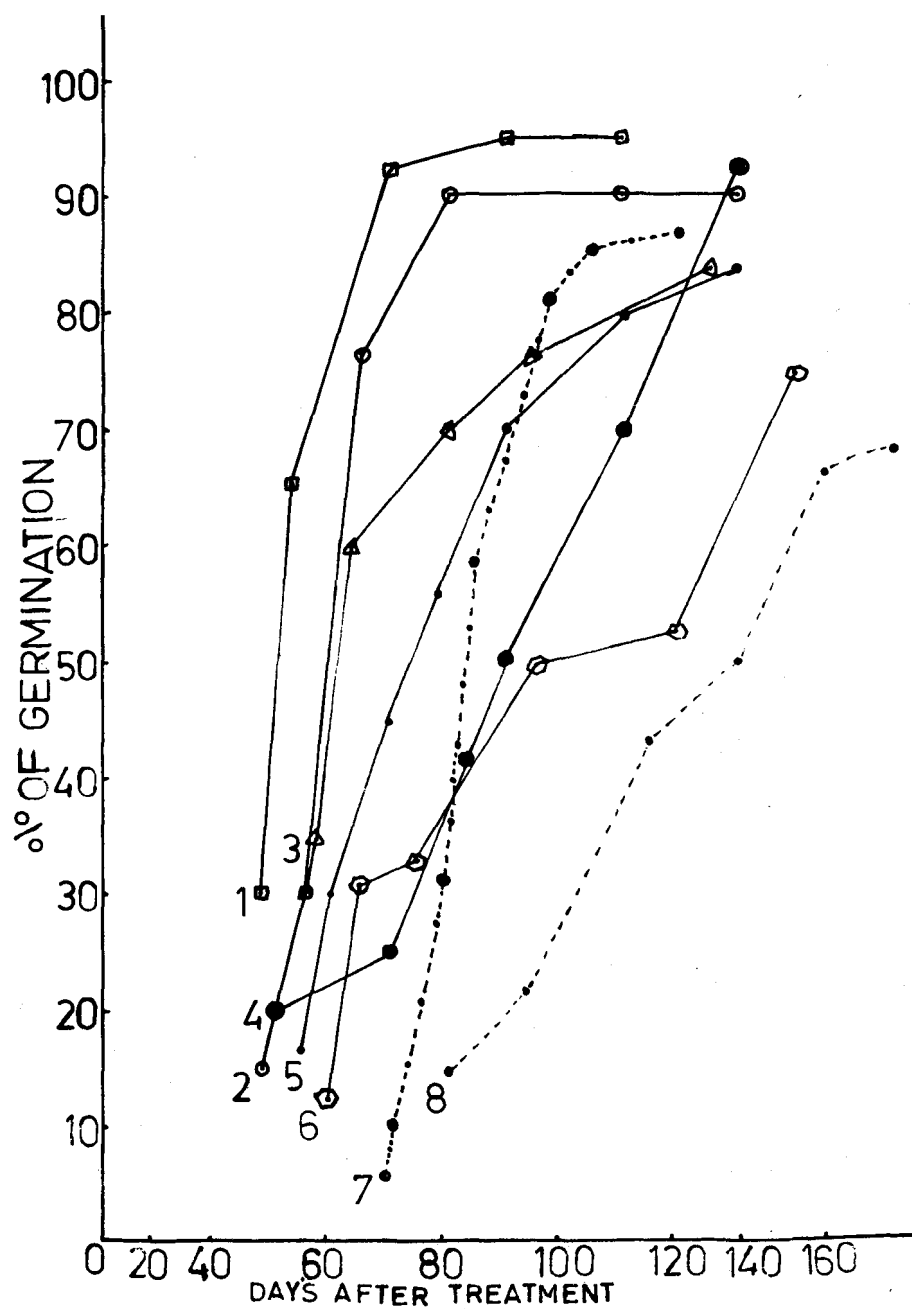


Fig. 1. Germination pattern of cardamom seeds after various treatments 1 = Control, 2 = AA 20, 3 = HNO₃ 10, 4 = HCl 10, 5 = Water washed (1 day), 6 = Water washed (6 days), 7 = KNO₃, 8 = H₂SO₄ + GA₃.

achieved with sulphuric acid and ethyl alcohol. Other treatments i.e., potassium nitrate, gibberellic acid and cellulase did not show any significant increase whereas, continuous washing of seeds with water for 6 days showed 15 per cent increase in germination over the control. Also there was a significant effect of acid treatment on speed of germination. Control seeds germinated after 135 days of plating, whereas the treated seeds germinated within 50 days of plating. Although acid treatment improved germination percentage and rate of germination, yet the seedlings treated with ethyl alcohol, potassium nitrate and the control showed higher vigour index (Table 1). Seeds chipped at the micropylar end did not show any germination.

Table 1. Percentage germination and vigour index of seeds under various treatments

Treatments	Germination (%)	Vigour Index (% germination x root length)
Dark treatment	62	80.4
Potassium nitrate	68	75.4
Gibberellic acid	62	78.5
Cellulase	65	77.8
Acetic acid	83	62.0
Sulphuric acid	95	59.5
Hydrochloric acid	80	60.6
Nitric acid	88	98.2
Absolute alcohol	92	184.0
Chipped seeds from one side	0	0
Low temperature treatment (4°C)	50	72.0
Water washing for 6 days	75	88.5
Control	60	79.7

*Average value of 3 replicates.

Difference between treatment and control highly significant ($P=0.01$) for all treatments except dark treatment, gibberellic acid and chipped seeds.

Result of the TTC test for viability showed a reddish-pink staining pattern in the embryo. Ninety five per cent of the seeds showed stain which closely paralleled with the viability of cardamom seeds after breaking dormancy. SEM studies were done to find out the micromorphological changes in the seed coat surface pattern after various treatments. Seed coat surface of control cardamom seeds was characterised by elongated hexagonal cells with indistinct cell boundaries. Thick deposition of mucilage in the form of fibres was observed along the cell boundaries (Fig. 2A). The seed coat surface of treated

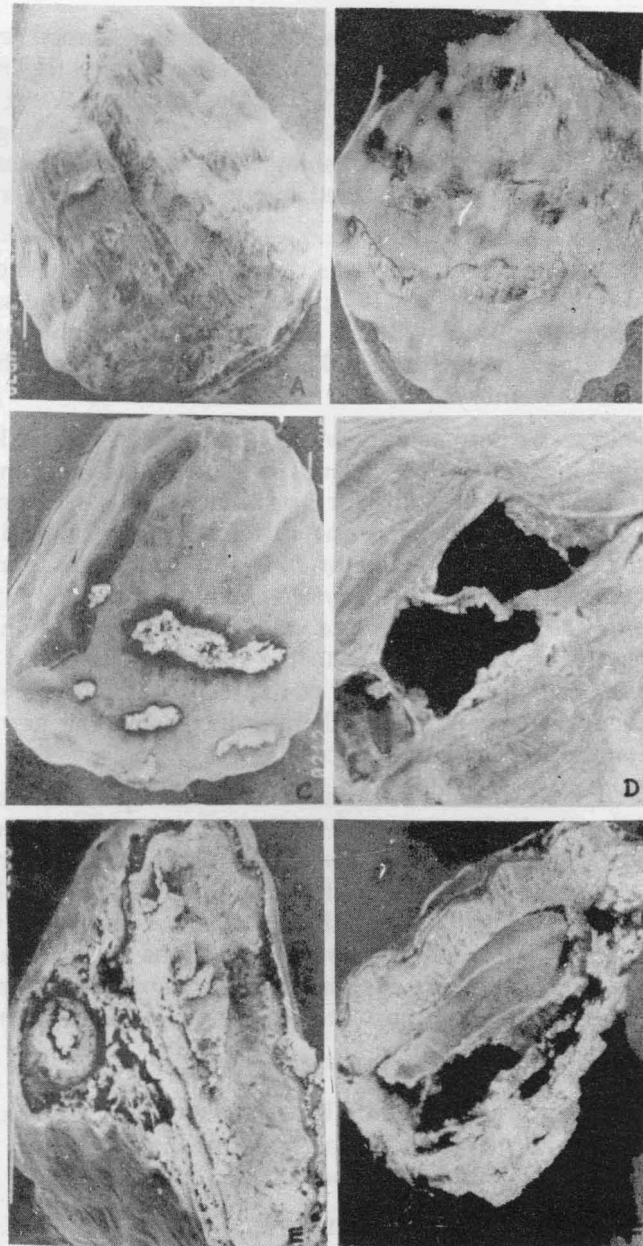


Fig. 2. A-F. SEM micrographs of cardamom seeds. A. Seed coat surface structure of normal seed. B. Seed coat surface of treated seeds, arrow showing lesions. C. Small pores on seed coat surface after treatment. D. Showing deep depressions on seed coat after treatment. E. Longitudinal section of normal seed showing embryo (arrow). F. Longitudinal section of water soaked seed arrow showing flaccid and distinct embryo. (A, B, C = 50x, D = 600x, E, F = 100x).

seeds was free of mucilage and possessed irregular shaped lesions of various sizes (Fig 2B). Small pores and depressions were often recorded on the surface of treated seeds (Fig. 2C,D). The surface of water washed seeds was completely free of mucilaginous white aril which might have been washed off during repeated washings. The embryo in water soaked seeds was found to be much enlarged due to imbibition in comparison to the control (Fig. 2E,F).

In cardamom, the dormancy was reported to be due to the impermeable seed coat (Kologi *et al.*, 1973; Reddy *et al.*, 1973). The seed coat was found to be very thick (28 μ m) and hard which might be causing the dormancy due to its impermeability to water, gases or mechanically a constraint to the embryo.

Increase in water permeability was found to be an essential prerequisite for breaking the dormancy and increasing seed germination in cardamom seeds. Water washing for 6 days increased seed germination percentage which may either be due to the softening of seed coat or washing off of germination inhibitor(s), if any. The increase in germination percentage after various physico-chemical treatments could be either due to the removal of extra deposition of mucilaginous aril present over the seed coat or due to the rupture of the seed coat. In both the cases, chemical component of the seed coat is involved which directly affects the water uptake, thereby increasing the germination percentage and speed of germination.

SEM seed coat surface studies revealed the presence of white mucilaginous aril which was completely removed in treated seeds. The mucilaginous aril is reported to play a vital role during germination of cardamom seeds. In the presence of water, the mucilage swells up by absorbing water and may become a diffusion barrier for oxygen (Sulikeri and Kologi, 1977, 1978). Various pores and lesions were found on the treated seeds which might be allowing the free imbibition of water and exchange of gases, thereby leading to increase in per cent germination.

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PATHOGENIC FUNGI INTERCEPTED IN INTRODUCED GERMPLASM

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During 1986-1990, a total of 2,31,740 introduced germplasm samples of seeds and other planting materials were examined for quarantine clearance. A number of economically important fungal pathogens were intercepted which include Peronospora manshurica (Naum) Syd. and Fusarium nivale (Fr.) Ces., which are yet not reported from India; Drechslera maydis (Nsik.) Subram. & Jain, Phoma betae Frank and Puccinia carthami Corda which are known to possess a large number/more virulent races; Botrytis cinerea Pers. ex Pers.; Fusarium solani (Mart.) Sacc. and Drechslera sorokiniana (Sacc.) Subram. & Jain, having a wide host range. A number of pathogenic fungi were also recorded on seeds of non-host crops.

Several pests and pathogens have got introduced and established in India from time to time along with imported germplasm. A number of destructive pathogens were intercepted in the introduced germplasm, some of which being new to India, viz., *Fusarium nivale*, *Peronospora manshurica* and *Uromyces betae* (Usha Dev *et al.*, 1989; Mukewar *et al.*, 1980 and Agarwal *et al.*, 1984). Pathogenic fungi intercepted on introduced planting material during 1986-1990 are reported and economic significance of certain important pathogens is discussed in this paper.

MATERIALS AND METHODS

During 1986-1990, a total of 2,31,740 introduced germplasm including 33,022 treated samples were received for quarantine clearance. Treated seeds were raised in the International Post-Entry Quarantine Nursery (IPEQN) and only healthy germplasm was released. All the untreated seeds and planting materials were examined visually and then

under stereobinocular microscope. The washing test was conducted for the detection of hazardous fungal pathogens which may be carried as surface contaminants. Apparently unhealthy looking seeds were incubated on standard moist blotters in plastic petriplates. Number of seeds placed on moist blotter varied as per the size of the seed sample. Observations for the associated pathogenic fungi were recorded on the eighth day. Observations on treated samples in IPEQN are reported separately.

RESULTS AND DISCUSSION

During visual examination, sclerotia of *Claviceps purpurea*, the ergot fungus were found mixed with seeds of *Agropyron* spp., wheat and oat. Observation of seeds under stereobinocular microscope revealed the presence of crusts of oospores of downy mildew fungus, *Peronospora manshurica* on soybean; teliospores of *Neovossia indica* (Karnal Bunt); *Tilletia caries* and *T. foetida* (Hill bunt) on wheat from a number of countries (Table 1).

Peronospora manshurica, a destructive pathogen with wide geographical distribution is not yet recorded from India. In USA alone, 32 races of this pathogen are known (Sinclair, 1982). The oospores of this fungus could retain viability for years, (Pathak *et al.*, 1978) and from quarantine view point, there is a zero tolerance for such pathogens (Neergaard, 1977). Teliospores of *Puccinia carthami* (= *Puccinia caleitrape* var. *centaurae*), (safflower rust) were detected in seeds received from Canada, Ethiopia and USA. This pathogen caused severe epiphytotics in USA in 1949 and 1950 (Thomas, 1952). The pathogen is known to have a number of races and has been recorded on *Carthamus tinctorius* L. and *C. oxycantha* M. Bieb. in India (Prasada and Chotia 1950). *Neovossia indica* detected in seeds of wheat from Mexico, Nepal, Pakistan and U.K., is a serious pathogen and European and Mediterranean Plant Protection Organization (EPPO) has declared it to be a pathogen of high quarantine importance (Anon. 1979); and recently USA has also listed it as a high risk pathogen. Sugarbeet rust, *Uromyces betae* which was detected in seed washing in samples from Denmark, Netherland, Hungary, Sweden, USSR and USA, is a widespread pathogen in Europe, Africa, Asia, Australia, North and South America and has not been reported so far from India. The pathogen can cause epidemics in new areas (Emdal and Foldo, 1979).

Observation of seeds incubated on moist blotter revealed large number of economically significant pathogens (Table 1). The snowmould fungus *Fusarium nivale* was detected in wheat seed from Mexico and U.K.; it is a serious pathogen of cereals and grasses and is so far not recorded from India. It is reported to cause total loss of winter wheat

Table 1. Pathogenic fungi recorded on introduced seed and other planting material (1986-1990)

Fungi intercepted	Crop(s)	Source(s)
<i>Alternaria brassicae</i> (Berk.) Sacc.	<i>Brassica</i> sp. Ethiopia, Taiwan, USA	Bangladesh, Sweden,
<i>A. crassa</i> (Sacc.) Rands	* <i>Solanum melongena</i>	Bangladesh
<i>A. padwickii</i> (Ganguly) M.B. Ellis	* <i>Coriandrum sativum</i>	Hungary
<i>A. zinniae</i> M.B. Ellis	* <i>Setaria</i> sp.	Korea
<i>Botrytis cinerea</i> Pers. ex Pers.	<i>Brassica</i> sp.	U.K.
	* <i>Eronimus</i> sp.	Germany
	* <i>Lycopersicon</i> <i>esculentum</i>	USA
	<i>Helianthus annuus</i>	USA
	<i>Glycine max</i>	Taiwan
	<i>Pisum sativum</i>	Sweden
	* <i>Psophocarpus</i> <i>tetragonolobus</i>	Papua New Guinea
	<i>Trifolium</i> spp.	Czechoslovakia
	<i>Triticum</i> spp.	U.K.
<i>Claviceps purpurea</i> (Fr. ex Fr.) Tul.	<i>Agropyron</i> spp.	USA
	<i>Avena sativa</i>	USA
	<i>Triticum</i> spp.	Hungary, Italy, USA
<i>Colletotrichum acutatum</i> Simmonds	* <i>Hibiscus</i> spp.	Bangladesh
	* <i>Capsicum</i> spp.	Taiwan
	* <i>Solanum melongena</i>	Bangladesh
<i>C. gloeosporioides</i> Penz.	* <i>Acacia</i> spp.	Australia
	<i>Capsicum</i> spp.	Bangladesh, Indonesia, Italy, Taiwan
	* <i>Cucurbita</i> spp.	Japan
	* <i>Corchorus</i> spp.	Bangladesh
	<i>Hibiscus</i> spp.	Bangladesh
	* <i>Leucaena</i> <i>leucocephala</i>	U.K., USA
	* <i>Melilotus alba</i>	USA
	* <i>Sesbania</i> spp.	Philippines
	<i>Stylosanthes</i> spp.	Australia, USA
<i>C. graminicola</i> (Ces.) Wils.	<i>Corchorus</i> spp.	Bangladesh
<i>Drechslera graminea</i> (Rabenh. ex Schlecht.) Shoemaker	<i>Avena sativa</i> <i>Hordeum vulgare</i>	USSR Egypt, Zimbabwe

Contd.

Table 1. *Contd.*

Fungi intercepted	Crop(s)	Source(s)
<i>D. maydis</i> (Nisikado) Subram. & Jain	* <i>Eleusine</i> spp. <i>Setaria</i> spp. <i>Sorghum vulgare</i> * <i>Stylosanthes</i> spp.	Bhutan, Nepal Korea USA Australia, USA
<i>D. oryzae</i> (Van Breda de Haan) Subram. & Jain	* <i>Eleusine</i> spp. <i>Oryza sativa</i>	Bhutan Nigeria, USA
<i>D. sacchari</i> (Butler) Subram. & Jain <i>D. sorghicola</i> (Lef. & Shrew.) Richardson & Fraser	* <i>Panicum</i> spp. * <i>Setaria</i> spp. * <i>Setaria</i> spp. <i>Sorghum vulgare</i>	USA Australia Korea USA
<i>D. sorokiniana</i> (Sacc.) Subram. & Jain	<i>Avena sativa</i> * <i>Capsicum</i> spp. * <i>Lycopersicon</i> <i>esculentum</i> * <i>Medicago</i> spp. <i>Phaseolus aureus</i> * <i>Panicum</i> spp. <i>Solanum melongena</i> <i>Sorghum vulgare</i>	Hungary, USA, USSR Hungary Taiwan Nigeria Iran USA Bangladesh USA
<i>Fusarium culmorum</i> (W.G. Smith) Sacc.	<i>Avena sativa</i> * <i>Caesalpinia</i> spp. * <i>Vigna unguiculata</i>	USA U.K. Australia
<i>F. nivale</i> (Fr.) Ces.	<i>Triticum</i> spp.	Mexico, U.K.
<i>F. oxysporum</i> Schlecht. ex Fr.	<i>Vigna unguiculata</i>	USA
<i>F. solani</i> (Mart.) Sacc.	* <i>Acacia</i> spp. <i>Allium cepa</i> * <i>Allizzia coribaea</i> <i>Beta vulgaris</i> <i>Capsicum</i> spp. * <i>Callendra</i> <i>lalothyosus</i> * <i>Corchorus</i> spp. <i>Coriandrum sativum</i>	Australia, U.K. Egypt U.K. USA Bulgaria, Hungary, Taiwan, USA Nigeria Bangladesh Hungary

Contd

Table 1. *Contd.*

Fungi intercepted	Crop(s)	Source(s)
	<i>Cucumis melo</i>	USA
	* <i>Daucus carota</i>	USA
	<i>Datura alba</i>	Hungary
	<i>Dolichos</i> spp.	USA
	* <i>Enterolobium cyclocarpus</i>	USA
	* <i>Ecballium elaterium</i>	Japan
	* <i>Geranium</i> spp.	Holland
	<i>Gossypium</i> spp.	Australia
	* <i>Guazuma ulmifolia</i>	U.K.
	* <i>Helianthus annuus</i>	Italy
	* <i>Lagenaria</i> spp.	U.K.
	* <i>Lavandula vega</i>	Bulgaria
	* <i>Leucaena</i> spp.	U.K.
	<i>Lycopersicon esculentum</i>	Taiwan, USA
	* <i>Medicago</i> spp.	Argentina, Australia, USA
	* <i>Mentha</i> spp.	USA, Vietnam
	* <i>Ocimum</i> spp.	Germany
	* <i>Paspalum vaginatum</i>	West Australia
<i>Fusarium solani</i> (Mart.) Sacc.	<i>Pisum sativum</i>	Netherland, Sweden
	* <i>Pistacia</i> spp.	Australia
	* <i>Quinoa</i> spp.	Nepal
	<i>Setaria</i> spp.	Argentina
	* <i>Solanum melongena</i>	Bangladesh
	* <i>S. tuberosum</i>	Bangladesh
	* <i>Samanea saman</i>	U.K.
	* <i>Tagetes</i> spp.	Netherland
	<i>Trifolium</i> spp.	Australia
	<i>Vigna unguiculata</i>	USA
	<i>Zea mays</i>	USA
<i>Neovossia indica</i> (Mitra) Mundkur	<i>Triticum aestivum</i>	Mexico, Nepal, U.K., Pakistan
<i>Peronospora manshurica</i> (Naum.) Syd.	<i>Glycine max</i>	Brazil, Belgium, Italy, Japan, S. Korea, Poland, Nigeria, Taiwan, Thailand, Zimbabwe
<i>Phoma betae</i> Frank	<i>Beta vulgaris</i>	Germany, Hungary, Sweden, USA
<i>Phoma lingam</i> (Tode ex Fr.) Desm.	<i>Brassica</i> spp.	Canada, U.K.

Contd.

Table 1. Contd.

Fungi Intercepted	Crop(s)	Source(s)
<i>Puccinia carthami</i> Schw.	<i>Carthamus tinctorius</i>	Canada, Ethiopia, USA
<i>P. helianthi</i> Schw.	<i>Helianthus annuus</i>	Australia, Argentina, Bulgaria, Canada, Hungary, Italy, Spain, USA
<i>Stenocarpella macrospora</i>	<i>Zea mays</i>	USA
<i>Tilletia caries</i> (DC.) Tul.	<i>Triticum</i> spp.	Australia, Iran
<i>T. foetida</i> (Wallr.) Liro.	<i>Triticum</i> spp.	Poland, Syria, USA
<i>Uromyces betae</i> Kickx.	<i>Beta vulgaris</i>	Denmark, Netherland, Hungary, Sweden, USSR, USA
<i>Ustilaginoides virens</i> (coothe) tak	<i>Oryza sativa</i>	Philippines, S. Korea

*Fungi not yet recorded as seed-borne in respective crops (Richardson, 1990)

and rye in parts of USSR, Japan, Canada and Central Europe (Blomquist 1970, Jamalainen, 1970). Fungicide resistant strains are also reported in *F. nivale* (Olvang, 1984). The grey mould fungus *Botrytis cinerea* was detected on wheat, winged bean and *Eronymus* spp. *B. cinerea* has a wide host range. It has caused heavy losses to chickpea crop in states of Bihar, Haryana, Punjab and U.P. (Lambat *et al.*, 1985), though it is considered as a common saprophyte in European countries.

A large number of or more virulent races are known to exist in certain economically important pathogens such as *Drechslera maydis*, *D. sorokiniana*, *Fusarium oxysporum*, *Phoma betae*, *Tilletia caries* and *T. foetida* which were also intercepted on various crops. *Fusarium solani* and *D. sorokiniana* that are known to possess a wide host range were recorded on planting materials of a variety of crops. A number of economically significant pathogens were recorded on seeds of non-host crops, such as, *Alternaria crassa* on *Solanum melongena*, *A. padwickii* on *Coriandrum sativum*, *A. zinniae* on *Setaria* spp. and *D. maydis* on *Stylosanthes* spp., (Richardson, 1990). Such association, often overlooked, can also bring in certain dangerous pathogens. The repeated interception of pathogens

not known to occur in India or those that possess physiological races or have a wide host range, stress the need to conduct critical seed health test during quarantine processing of exotic germplasm materials.

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ANALYSIS OF GENETIC DIVERSITY IN INDIGENOUS AND EXOTIC MACROSPERMA LENTILS

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Forty two genotypes of large seeded (macrosperma) lentils, both indigenous and exotic, were evaluated to study the nature and magnitude of genetic diversity by using Mahalanobis's D^2 statistic and canonical analysis. All the genotypes were grouped into 14 clusters. Days to flowering and maturity, seeds per pod and seed yield per plant were important characters in differentiation among the germplasm entries from different geographical regions. Genotypes originating from a particular geographical region grouped into the same or different clusters. Based upon the clustering pattern and performance of genotypes in different clusters, a breeding programme can be initiated for the genetic improvement of macrosperma lentils by crossing L-2961 with L-3991-24 and L-1646 and selecting the recombinants combining the higher seed yield and earliness in the segregating generations.

There is existence of two complexes within the cultivated lentils; the small-seeded (microsperma) and large-seeded (macrosperma). The small-seeded lentils, insensitive to environmental stresses and photoperiod are grown east of Pakistan during winter. The large-seeded lentils, adapted to long days, sensitive to environmental stresses are grown in Afghanistan westward during spring. The latter possesses useful genetic traits such as large-seed size, upright and taller growth habit. So far, *microsperma* attempts have only been made to improve the genetic potential of *microsperma* lentils by introgressing certain desirable characters from *macrosperma* gene pool. However, efforts have not been made to improve the *macrosperma* lentils alone by combining genes scattered in the *macrosperma* gene complex. Hence, there is an urgent need to undertake suitable breeding programme to seek the genetic upgradation of *macrosperma* lentils. Precise information on the nature and degree of genetic diversity present in *macrosperma* lentils

could help in the choice of desirable parents for evolving superior varieties (Moll *et al.*, 1962; Miller and Marani, 1963). Based upon the consideration, multivariate analysis by means of Mahalanobis's D^2 -statistic has been used in identifying genotypes to initiate an appropriate breeding strategy to develop suitable varieties in *macrosperma* lentils.

MATERIALS AND METHODS

The materials for the present study comprised 42 *macrosperma* genotypes. Of these, 18 and 24 were of indigenous and exotic origin, respectively. These genotypes were grown in a randomized complete block design with three replications at the Experimental Farm, Himachal Pradesh Krishi Vishwa Vidyalaya, Palampur, located in north-western Himalayas (32°6'N, 76°3'E, 1290m above msl). Each entry was grown in a single 1.5m long row with inter-row distance of 45 cm. The plant to plant distance was 2-3cm. Days to 50 percent flowering and maturity were recorded. Five plants were selected at random in each plot for recording observations on first pod height (cm), Plant height (cm), primary branches per plant, flower clusters per plant, pod clusters per plant, pods per cluster, seeds per pod, pods per plant, seeds per plant, 100-seed weight (g), harvest index (%) and seed yield per plant (g). Protein content (%) was estimated from the pooled sample derived from five plants. The estimation of protein ($N\% \times 6.25$) content was done by conventional micro-kjeldahl method.

Analysis of variance and covariance were done on the basis of mean values of each plot. A simultaneous test of significance of the differences in the mean values of the 15 variables for 42 genotypes was applied using the wilk's criterion as described by Rao (1952). The computerised D^2 values were arranged in an ascending order. Treating D^2 as the square of a generalised distance, all the genotypes were grouped into a number of clusters following the method described by Tocher (Rao, 1952). After finding out the clusters, the square root of the average D^2 between any two clusters was used to represent the relative disposition of the clusters diagrammatically. The data were also subjected to canonical analysis (Rao, 1952).

RESULTS AND DISCUSSION

The analysis of variance revealed that the entries differed among themselves for each of the 15 characters studied (Table 1). Wilk's criterion (λ) also revealed highly significant differences among them for the aggregate of all characters ($\chi^2 = 1641.84$ for df. 615). D^2 values were computed which ranged from 11.47 (LG-171 and L-1633) to 631.31 (L-4163 and L-2297). After computing the individual D^2 values, 42 genotypes were grouped into fourteen clusters such that within individual cluster they had smaller D^2 values among themselves than those belonging to any two different clusters

Table 1. Analysis of variance for 15 characters

Characters	Mean sum of squares (MSS)	
	Genotypes	Error
Days to flowering	1197.27*	10.18
Days to maturity	11.13*	0.58
First pod height	18.57*	6.78
Plant height	26.76*	9.34
Primary branches/plant	2.47*	1.06
Flower clusters/plant	641.16*	299.77
Pod clusters/plant	258.63*	147.81
Pods/cluster	0.22*	0.14
Pods/plant	526.49*	238.93
Seeds/pod	0.16*	1.74
Seeds/plant	1020.67*	315.34
100-seed weight	1.14*	0.72
Harvest index (%)	119.22*	52.76
Protein (%)	7.49*	0.41
Seed yield/plant	0.76*	0.34

*Significant at $P = 0.05$

Degrees of freedom: Genotypes = 41; Error = 82

Note: MSS for replications was non-significant for all traits

(Table 2). The cluster II included the highest number of genotypes (7), followed by V(5), II and III (4), VI and XIII (3) and VII to XII (2). Cluster XIV had one genotype. The group constellation obtained by D^2 analysis could also be confirmed by canonical analysis. The small discrepancy in the relative disposition of entries in the chart could possibly be due to the fact that the first two canonical roots accounted for only 78 per cent of the total variation. To obtain an explicit two dimensional representation, contribution of the first two canonical roots should be more than 95 per cent.

The composition of each cluster in *macroserma* lentils revealed that genotypes originating from a particular geographical region were not only grouped into the same cluster, but also into different clusters (Table 2). Of 42 genotypes, 16 of near Mediterranean countries were distributed in 10 clusters, 20 of south east Asia in 11, 3 of Asia Minor in 3 and 3 of New World in 3. The results could not establish a parallelism between geographical and genetic diversity. The explanation for grouping genotypes with the same geographical origin into different cluster may be due to different genetic background and wide divergence in features, which might have resulted on account of free exchange of materials among different regions of the world

**Table 2. Distribution of *macrosperma* genotypes
in relation to their origin**

Cluster	Total genotypes in the cluster	Genotypes	Origin
I	4	L-2979 LG-171, L-1633 and L-1644	Asia Minor South-east Asia
II	7	L-2114 L-178 and L-3991-11 L-24123, L-24118, L-4026 and L-2117	Asia minor South-east Asia Near Mediterranean
III	4	L-3991-25 and L-4076 L-4047 L-4224	South-east Asia New World Near Mediterranean
IV	3	L-3038 L-4228 LH-82	New World Near Mediterranean South-east Asia
V	5	L-4152 and L-416 L-3355, L-3284 and L-3293	South-east Asia Near Mediterranean
VI	3	HPL-4 and L-4163 L-4266	South-east Asia Near Mediterranean
VII	2	L-1646 and L-3991-24	South-east Asia
VIII	2	L-2297 L-3034	Asia minor Near Mediterranean
IX	2	L-4048 and LL-4163	South-east Asia
X	2	L-3991-29 and PL-81-3	South-east Asia
XI	2	L-3046 and L-165446	Near Mediterranean
XII	2	K-303 L-3286	South-east Asia Near Mediterranean
XIII	3	Precoz L-3991-30 L-3304	New World South-east Asia Near Mediterranean
XIV	1	L-2961	Near Mediterranean

either for direct introduction or for breeding puposes. Genetic drift and selection in different environments could be other important factors contributing to the divergence. Therefore, the selection of genotypes for hybridization with a view to get the maximum chance of identifying transgressive segregants of practical utility should be baed on genetic rather than geographic diversity. Suryawanshi *et al.* (1985) and Sharma and Luthra (1987) reported similar results for hybridization in *microserma* lentils.

The inter and intra-cluster distances (D) are given in Table 4 and Fig. 1. The intra-cluster distances ranged from 4.89 (cluster V) to 10.70 (cluster XIII). The inter-cluster distance ranged from 6.35 (cluster VI and XIII) to 23.17 (cluster VI and VIII). The dispersion matrix used for D² analysis was also utilized for canonical analysis. The first canonical root (λ_1) accounted for 63.5 percent of the variation, while the second root (λ_2) for 15.0% (Table 3). The coefficients of the first two canonical vector, Z₁ and Z₂, indicated that days to flowering and maturity, seeds/pod and seed yield/plant constituted the major axis of differentiation. Besides days to flowering, plant height and protein (%) constituted the second axis of differentiation. Characters such as days to flowering and maturity are related to the photoperiodic response, seem to have contributed to the genetic divergence under natural selection. Similarly, first pod height, plant height and protein (%) appear to have contributed towards genetic divergence through both natural and human selection.

The highest inter-cluster value (23.17) indicated that cluster VI comprising three genotypes, viz., L-4266, L-4163 and HPL-4, were the most divergent

Table 3. Coefficients of first two canoical vectors

Vector	Characters							
	Dys to flower- ing	Days to maturity	First pod height	Plant height	Primary branches/ plant	Flower clusters/ plant	Pod cluster plant	
Z ₁	0.901	0.236	0.042	-0.052	-0.062	-0.068	-0.121	
Z ₂	0.072	-0.322	0.029	0.106	0.045	0.069	0.016	
	Pods/ cluster	Pods/ plant	Seeds/ pod	Seeds/ plant	100-seed weight	Harvest index (%)	Protein (%)	Seed yield plant
Z ₁	0.001	-0.082	0.144	-0.197	0.009	0.065	0.044	0.176
Z ₂	0.011	-0.022	-0.029	0.013	0.005	0.061	0.926	-0.088

$$\lambda_1 = 63.369; \lambda_2 = 15.027$$

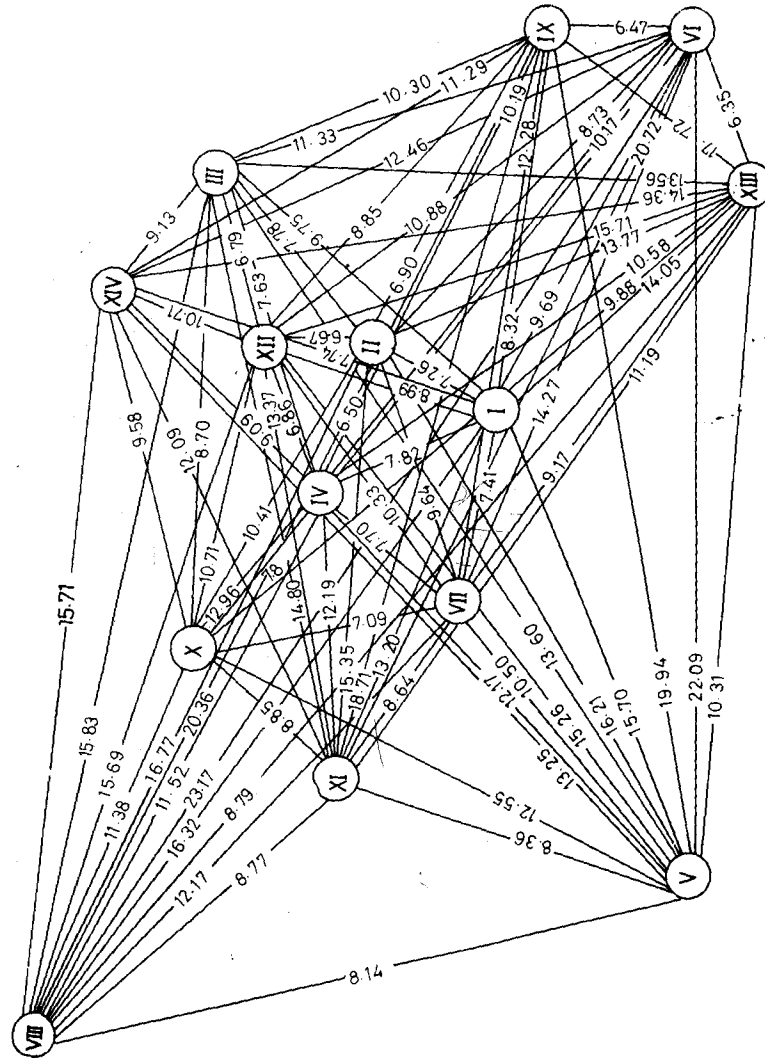


Fig. 1 Spatial distribution of different clusters and their inter-relationships based on D values

Table 5. Cluster means for 15 traits in macrosperma lentil

Cluster	Trait														
	Days to flower ing	Days to matur- ity	First pod height (cm)	Plant height (cm)	Primary bran- ches/ plant	Flower clus- ters/ plant	Pod clus- ters/ plant	Pods/ clus- ter	Pods/ plant	Seeds/ pod	Seeds/ plant	100- seed weight (g)	Protein (%)	Harvest index (%)	Seed/ yield/ plant (g)
I	128.30	183.58	11.96	25.46	3.37	50.68	21.70	1.87	28.43	1.21	34.97	2.89	33.44	23.51*	1.41
II	132.69	181.33	12.37	26.54	4.40	46.03	19.49	2.01	26.78	1.03	28.67	3.10	42.08**	26.02	1.38
III	124.33	181.67	13.19	26.84	3.82	40.73	24.12	2.18	31.25	1.36	44.54	2.24	40.84	28.47**	1.42
IV	121.33	180.99	10.72	26.61	5.32**	52.43	25.91	1.65**	34.41	1.00*	38.33	2.78	30.08	25.83	1.30
V	88.21*	181.73	12.01	24.19	3.29	38.39	19.99	1.97	27.36	1.33	37.99	2.72	30.35	26.41	1.27
VI	148.71**	183.67	12.34	25.39	4.30	42.48	13.87	1.95	20.32*	1.22	23.77	3.00	28.20	26.56	1.18
VII	111.48	148.50*	9.23*	23.06	3.11*	26.12*	15.80	2.31	24.15	1.25	29.45	2.69	28.67	24.61	1.13*
VIII	89.26	178.33	10.2	21.55*	4.17	28.51	11.71*	1.78	20.50	1.26	16.73*	3.00	36.20	24.78	1.18
IX	143.11	181.83	15.15	28.60	3.71	48.85	15.51	2.41**	22.67	1.36	27.61	2.10	27.03*	25.42	1.17
X	116.97	182.67	11.81	31.67**	3.41	42.10	22.51	2.11	29.23	1.05	38.13	3.08	31.50	26.56	1.56
XI	91.20	184.00	11.76	28.31	3.46	33.12	28.47	1.78	25.50	1.28	29.31	4.06**	33.80	23.53	1.90
XII	131.26	180.00	11.16	27.15	3.92	40.11	26.78	2.00	33.83	1.33	49.08	1.95	40.89	26.73	1.21
XIII	102.48	178.78	10.24	29.38	3.33	45.84	30.26**	1.97	38.13	1.19	30.90	2.74	33.90	25.37	1.60
XIV	118.37	185.33**	16.10**	26.03	4.57	73.77**	25.43	2.10	64.43**	1.83**	75.40**	1.93*	35.43	25.60	2.13**

*Minimum and **maximum value

Table 4. Intra-and inter-cluster average divergence ($\sqrt{D^2}$) values of 14 clusters

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
I	6.21	7.26	9.75	7.82	15.70	9.69	7.41	16.32	8.25	10.25	13.20	8.99	14.05	7.74
II		5.44	7.78	6.50	16.21	8.73	9.64	16.77	6.90	10.41	15.35	6.67	13.77	9.80
III			5.38	7.63	13.60	11.35	9.39	15.83	10.30	18.70	14.80	6.79	13.56	9.13
IV				4.92	12.17	12.96	7.70	11.52	10.19	7.84	12.19	6.86	10.58	9.09
V					4.89*	22.09	10.50	8.14	19.94	12.55	8.36	15.26	10.31	13.25
VI						5.69	14.27	23.17**	6.47	15.05	20.72	10.88	6.35*	12.46
VII							5.17	12.17	12.28	7.09	8.64	10.33	9.17	7.13
VIII								5.53	20.36	11.38	8.77	15.69	8.79	15.71
IX									5.33	13.89	18.71	8.85	17.24	11.29
X										6.18	8.85	10.17	9.88	9.58
XI											6.89	13.37	11.19	12.09
XII												7.42	15.71	10.17
XIII													10.70**	14.36
XIV														—

* Minimum and ** maximum value.

from cluster VIII having L-2297 and L-3034 genotypes. Although the genotypes of cluster VI and VIII had the maximum diversity, yet their performance for seed yield and its various components was poor (Table 5). Hence, these genotypes may not be direct choice for the hybridization programme. In autogamous species, while considering genetic diversity for the selection of the parents to be included in hybridization programme, yielding potential of clusters should also be considered. The only genotype L-2961 of cluster XIV had the highest flower clusters/plant, pods/plant, seeds/plant, seeds/pod and seed yield. However, it had the maximum first pod height and late maturity, which are not desirable traits for the improvement of *macroserma* lentils. So, these traits should be complemented with the desirable traits of other clusters. Genotypes L-3991-24 and L-1646 of cluster VII were desirable for the early maturity and the lower first pod height. The genetic diversity between these two clusters was substantial ($\sqrt{D^2} = 12.46$). The genetic upgradation of *macroserma* lentils seeks to require high yield with early-medium maturity. Based upon this consideration, a breeding programme for the genetic amelioration of *macroserma* lentils could be envisaged by crossing genotypes of cluster XIV (L-2961) with the genotypes of cluster VII (L-3991-24 and L-1646), and selecting the transgressive segregants of practical utility. Useful heterobeltiosis may also be obtained by intercrossing genotypes of clusters VI and VIII, which have otherwise shown poor expression. It is also worthwhile to attempt crosses between clusters having better *per se* performance and average genetic diversity i.e. genotypes of cluster XIV with cluster XI, or cluster XI with cluster III.

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ISOZYME POLYMORPHISM AND GENETIC DIVERSITY IN GARLIC (*ALLIUM SATIVUM* L.) GERMPLASM

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Twenty-three accessions of garlic (Allium sativum L.) collected from five diverse agro-climatic regions of India differing for various agro-morphological traits were analysed for isozyme polymorphism. Esterase, superoxide dismutase and catalase were the enzymes studied. Esterase and superoxide dismutase showed polymorphism to some extent. However, catalase was observed to be monomorphic. The study indicated that there was less genetic diversity among the garlic germplasm analysed. Broader groupings of the accessions was possible even though the enzyme systems studied were not ideal for 'fingerprinting' of germplasm. The implications of the findings with reference to genetic diversity in garlic germplasm and the usefulness of the isozyme technique for germplasm characterisation has been discussed.

Garlic (*Allium sativum* L.) is an important spice and is widely cultivated in India and China. It is an essentially vegetatively propagating species. The source of genetic variation is obviously through somatic mutations, which may include both natural and induced types. Evolution of new cultivars in garlic has mainly been through selection and introduction from other agro-climatic regions. Therefore, the new cultivars are expected to differ from one another for few genes only. The present study was undertaken to evaluate the genetic diversity prevalent in the garlic accessions and to characterise the germplasm using the technique of electrophoresis of isozymes.

Isozyme electrophoresis has been applied in a number of crop species to evaluate the genetic diversity and to characterise the cultivars. Systematic and evolutionary studies were made in 29 genera using estimates of isozyme variation. Further, in 40 different genera, isozymes were used for cultivar identification (Simpson and Withers, 1986). Such

studies in *A. sativum* are very scanty, however studies on alcohol dehydrogenase, isocitrate dehydrogenase, phosphoglucomutase and phosphoglucoisomerase in 188 accessions in *A. cepa* and 29 in *A. fistulosum* have been reported. This underlines the need to survey isozyme variation in the germplasm collections so that the exact nature of cultivar diversification can be understood.

MATERIALS AND METHODS

Twenty-three accessions of garlic (*A. sativum* L.) from 750 germplasm collections held at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India were chosen for the study (Table 1). All

Table 1. Source of *A. sativum* accessions analysed

Accession Numbers	Place of Collection
IC 35280, IC42870, IC 48628, IC 48649, IC 48661, IC 49410	Maharashtra
IC 49343, IC 49350, IC 49351, IC 49356, IC 49358, IC 49359, IC 49371, IC 49373 IC 49400, IC 49405	Himachal Pradesh
IC 35231, IC 35334, IC 35341	Rajasthan
IC 19084	Orissa
IC 35315	Madhya Pradesh
IC 2053, IC 12370	Not known

accessions were being maintained by clonal multiplication and no heterogeneity within a collection was apparent. This was further evident from the analysis of individual plants within a collection. Hence, young leaves from a single plant per accession were considered for the present analysis.

The cloves of each accession were planted in pots filled with 1:1 mixture of compost and soil. First half emerged leaf at 30 days of sprouting was collected over ice and used for isozyme analysis. The leaves were first powdered after freezing in liquid nitrogen. The powdered sample before thawing was homogenised with acetone to remove chlorophyll and other secondary metabolites. The homogenate was filtered through Whatman No. 1 filter paper. The residue on the filter paper was air-dried and stored at -10°C till use. The samples were homogenised in prechilled pestle and mortar over ice in an extraction buffer comprising 0.1 M tris-Cl pH 7.4, 17 per cent sucrose and 0.1 per

cent each of ascorbic acid and cysteine-HCl. The sample to buffer ratio was maintained at 1:5 for all the samples. The homogenates were centrifuged at 12,000x g for 15 min. at 4°C in a refrigerated centrifuge and the clear supernatant was collected. The protein content of each sample was estimated by Folin-Ciocalteu reagent method (Lowry *et al.*, 1951). The protein content of the extracts was equalised by the addition of extraction buffer. The tracking dye, bromophenol blue (to a final concentration of 30% per cent) and sucrose (to a final concentration of 0.002%) were added to the extract and stored frozen till use. Anodic vertical slabs gel electrophoresis was performed on polyacrylamide gels according to Davis (1964). For each sample 60 µg protein equivalent was loaded on the gels. On completion of electrophoresis, the gels were incubated in the respective reaction mixtures of different enzymes. The enzyme localisation procedures were followed according to Brewbaker *et al.*, (1968) for esterase, Thorup *et al.* (1961) for catalase and Beauchamp and Fridovich (1971) for superoxide dismutase. The gels on optimum staining were fixed in 7 per cent acetic acid. The relative migration velocity (Rf) was calculated for each band and zymograms were constructed from the Rf values.

RESULTS AND DISCUSSION

The investigations compared a total of three gene loci (one for each enzyme). Since the total genome size in *Alliums* is very large, it is necessary to study more enzyme systems so as to compare the maximum possible amount of hereditary material. The present study adds to the genetic information available about the genus *Allium* in general and *A. sativum* specifically. The zymograms for esterase, catalase and superoxide dismutase drawn to scale with Rf values are depicted in figure 1.

Isozyme polymorphism

Garlic is an essentially vegetatively propagated species. Only one report was cited on collection of fertile flowering garlic types from the former Soviet Union (Kotlinska *et al.*, 1990). Therefore, the main source of genetic variation available in garlic has so far been by accumulation of natural mutations. The rate of somatic mutations in nature is less than 0.01 per cent. The propagation of mutations expressing in the form of changed morphological traits and/ or biochemical parameters would further be reduced. Hence, the proportion of detectable variations in such obligate vegetatively propagated crops like garlic, where the chances of diversification through natural/artificial hybridization and recombination is less, would naturally be limited.

The lack of substantial polymorphism for allo/iso-enzymes of esterase, catalase and superoxide dismutase among the twenty-three acces-

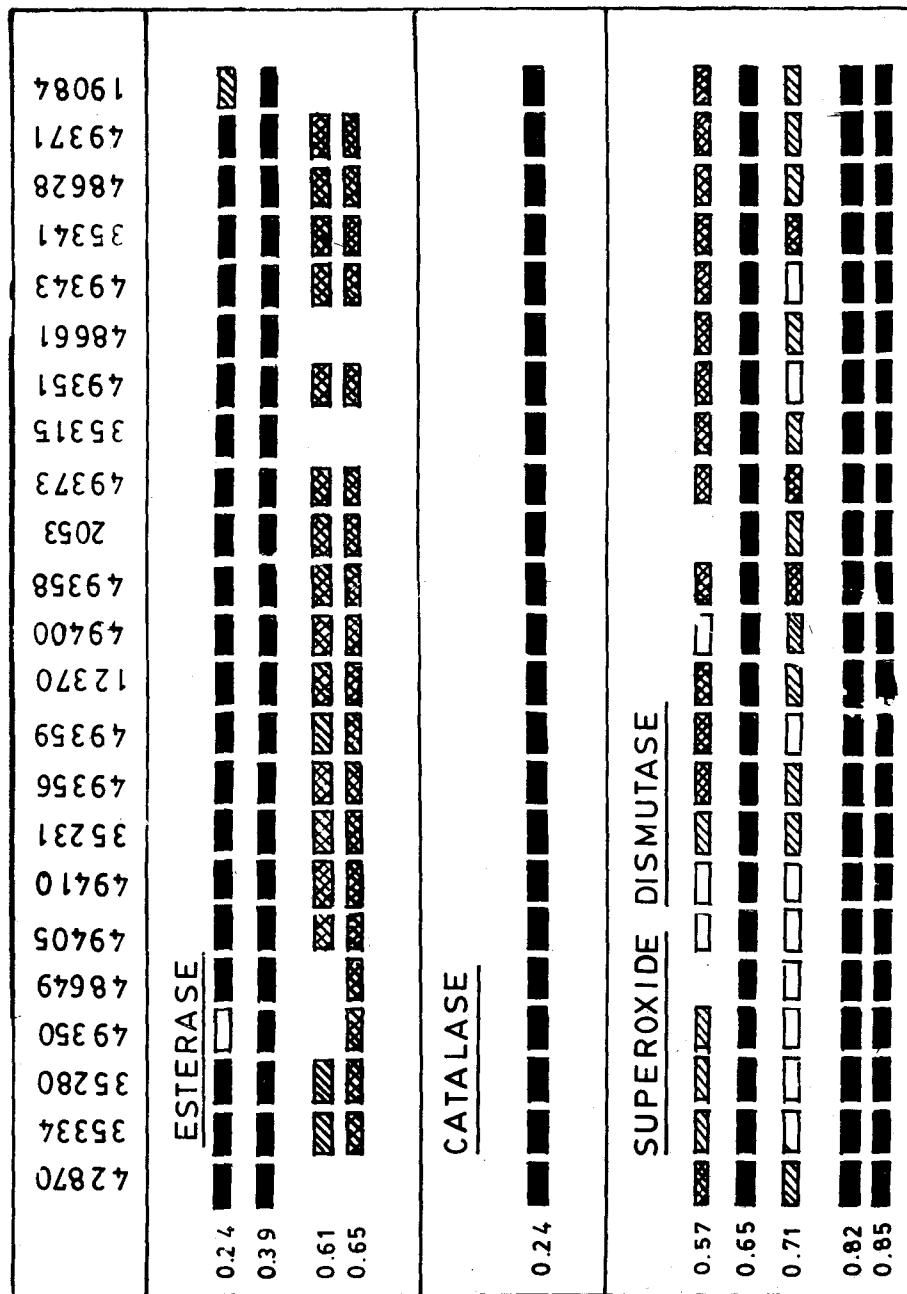


Fig. 1. Profiles of Isozymes in the garlic accessions.

sions of garlic are evident from Fig.1. Catalase was found to be monomorphic as only one band was observed in all the accessions. On the contrary, polymorphism was observed for the other two enzymes esterase and superoxide dismutase.

For esterases, differences were noticed for the fast migrating bands with Rfs 0.61 and 0.65. The slow moving bands at Rfs 0.24 and 0.39 were monomorphic and are probably two different isozymic forms. But the two fast migrating bands possibly represent the allelic forms of the third isozymic form of esterase. In the present study, a total of three isozyme phenotypes for esterases were observed. However, there were pronounced differences in the intensity of fast moving bands. These might be useful for germplasm characterisation as there was consistency in the results. In case of superoxide dismutase, polymorphism was prevalent only for the slowest migrating band (Rf 0.57). Further, differences among the accessions in the intensity were noticed for the band at Rf 0.71. The remaining three bands (Rfs 0.65, 0.82 and 0.85) did not differ even for intensity of staining in any of the accessions.

The present results are interesting considering the lack of variation for IDH and PGI among 1293 plants of *A. cepa* and *A. fistulosum* (Peffley and Orozco-Castillo, 1987). Further, in *A. cepa*, an open pollinating seed propagated species, no variation was observed for PGM by these workers in contrast to *A. fistulosum* where polymorphism for PGM was reported. This investigation stresses the fact that lack of variations for two or more enzyme systems do not necessarily mean lack of genetic differences between the accessions studied. On the other hand, it calls for studying different sets of isozymes so that extent of similarities and differences between the genotypes compared, can be estimated. Germplasm characterisation is, therefore, a continuous process whereby it is essential to utilize all the available techniques so that maximum amount of total genomic DNA can be sampled.

Study of isozyme polymorphism has been used as a technique to determine genetic diversity in crop plants such as barley (Clegg *et al.*, 1984; Jana and Pietrzak, 1988), pearl millet (Gepts and Clegg, 1989) and common beans (*Phaseolus vulgaris* L.) (Singh *et al.*, 1991). Extensive studies of polymorphism in isozymes give reliable estimates of genetic relationship among the genotypes compared. Another important application of isozyme studies is in cultivar identification. For unambiguous identification of cultivars, it is essential to use as many enzyme systems as possible. The data on enzyme systems which are monomorphic in a given set of cultivars is also essential. Considering these factors as requirements for identification of cultivars, a system has been developed which helps in classifying the cultivars in case the extent of isozyme limited polymorphism prevalent is lesser. This study indicated that

polymorphism prevalent for the isozymes analysed. Although it is not possible to differentiate cultivars with the prevalence of limited polymorphism, the study would be useful for broader classification of the collections based on the similarities. It was not possible to clearly identify any of the 23 accessions of garlic analysed based on only one isozyme pattern. However, when both esterase and SOD profiles were considered, three collections, namely, IC 49350, IC 48649 and IC 2053 could be distinguished from others. For SOD, the accessions IC 48649 and IC 2053 had similar isozyme profiles but for IC 49350 the pattern was different. On the other hand for esterases, the banding pattern was different for IC 48649 and IC 2053 while IC 49350 and IC 48649 had same pattern. This combination of enzyme bands was not found in any of the other accessions. In case of SOD, polymorphism was noticed only for slowest migrating band (R_f 0.57). A total of two isozyme phenotypes were present for SOD among the 23 accessions. The other four bands (R_f s 0.65, 0.71, 0.82 and 0.85) were monomorphic in the materials studied. However, differences in the intensity of staining were present for the bands at R_f 0.57 and 0.71.

The remaining 20 accessions could be classified into two groups. The first group included four accessions, namely, IC42870, IC 35315, IC 48661 and IC 19084 and 16 remaining accessions were in the second group as they had similar patterns for esterase and SOD. However, when differences in the intensity of bands were considered, the second group could be subdivided into four groups (Table 2). The accession IC 494000 of the first sub group was seen to be different from others as the first SOD band at R_f 0.57 was very faint. The number of accessions in other sub groups are mentioned in Table 2.

A comparison of the accessions under each group with their place of collection indicated that all the ten accessions from Himachal Pradesh

Table 2. Classification of 23 garlic accessions based on the banding pattern for esterases and superoxide dismutases

Group 1	Group 5	IC49343
IC49350	Sub group 1	Sub group 4
Group 2	IC 49400	IC 12370
IC 48649	Sub group 2	IC 35231
Group 3	IC 49405	IC 35341
IC 2053	IC 49410	IC 48628
Group 4	Sub group 3	IC 49356
IC 42870	IC 35334	IC 49358
IC 35315	IC 35280	IC 49371
IC 48661	IC 49359	IC 49373
IC 19084	IC 49351	

and three from Rajasthan were in group V. Even though, further classification of these collections based on the intensities of each band was attempted, barring in few cases like IC 49400 and IC 49405 where substantial differences existed in stainability of one or more bands no unambiguous identification of accessions was possible. It is evident that the accession from Maharashtra were more diverse compared to accessions from other states. These results probably indicate large number of duplicates in germplasm collections at NBPGR.

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PERFORMANCE AND POTENTIAL OF JOJOBA IN THE INDIAN ARID REGIONS

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Jojoba (*Simmondsia chinensis* (Link.) Schneider) was introduced in India in sixties because of its adaptability in deserts and high economic potential mainly as a substitute to sperm whale oil. Since then, its performance in the experimental area has been observed for its survival and potential in the Indian arid region. The studies revealed great variation in the plant habit, form, growth pattern, reproductive potential and seed yield among and within various introduced accessions. Of them, EC 33198 introduced in the late sixties has been identified as suitable variety for this region.

Jojoba (*Simmondsia chinensis* (Link.) Schneider) is an evergreen, hardy shrub native to the Sonoran desert of Mexico. It can withstand drought, tolerate salinity and has been reported to adapt to wide range of temperatures, rainfall and habitat conditions (Anon, 1975). Its suitability to areas where no agricultural crop can thrive along with its valuable liquid wax rich seeds render it potential, economically viable and an attractive agricultural investment for such areas (Bhatia and Gulati, 1981). The economic potential of jojoba was known in the early sixties, yet it received widespread attention only the chemical similarity between its oil and the sperm whale oil was ascertained coinciding with the placement of sperm whale in the endangered list (Princen, 1983). Since then, worldwide efforts have been put into the cultivation, breeding, harvesting and processing of jojoba.

BOTANY AND USES

Jojoba (pronounced as ho-ho-ba) also called as bushnut, bucknut, deernut, pignut, sheepnut, goat berry, coffee berry, wild

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hazel or quinine plant is considered to be the only arid adapted species of widely distributed family, Buxaceae, though it is also considered to be a member of a unique monotypic family Simmondsiaceae (Brooks, 1978).

Jojoba is an evergreen, compact, perennial shrub with greyish green leaves and tap root system. In the past, its oil was used by native North American Indian tribes to treat sores, cure stomach problems and restore hair. As a food, seeds were used raw or roasted, ground with water and sugar to make various beverages, like a coffee substitute. Besides its similarity to sperm whale oil, the oil of jojoba has an added advantage of having fewer impurities, odourless nature, greater shelf life and thus has a great potential as a lubricant and chemical intermediate in industrial processes. The oil can be hydrogenated to form a hard, crystalline wax, to be used in manufacture of polish waxes, carbon paper, impregnated heat resistant paper containers. The reaction of oil with sulphur chloride to form a rubbery compound known as factice is used in varnishes, rubber, adhesives, etc. Owing to its remarkable resistance to degradation and odourless nature it has a wide potential in the pharmaceutical and cosmetic industry.

ORIGIN, DISTRIBUTION AND CULTIVATION

The origin of jojoba remains obscured, but its growth in natural stands has been noticed to be linked with winter-spring rains, thus indicating its origin in the Mediterranean type of climate along the Pacific coast (Brooks, 1978). At present, the widest distribution of jojoba is in the islands of Gulf of California, coastal Sonora in Mexico, the mountains around Salton sea basin, southern portions of San Diego, and Tucson in Arizona. The species extends from 600-1300 m. elevation above sea level on well drained dry slopes, mostly in areas receiving 19 to 50 cm annual rainfall. The topographical effects on micro-environmental conditions, particularly soil moisture, influence the distribution of jojoba. These effects are more pronounced during early seedling and flowering stages. Otherwise, the plant has a high degree of physiological tolerance to drought and high temperatures. However, during severe drought the leaves may be shed. Jojoba has a medium water requirement throughout the year (Sherbrooke and Hasse, 1974). In all habitats, including coastal and inland desert areas, plants remain physiologically active during the entire year reflecting its potential to maintain a positive carbon balance even under conditions of severe drought.

Jojoba has been extensively cultivated in many arid regions of the world resulting in its spread in over fifty thousand hectares of land worldwide mainly in Israel, Mexico, Costa Rica and Australia (Anon.

1975). Other countries with similar climates are already in the process of introduction and many countries including India have taken up its cultivation.

PLANT INTRODUCTION IN INDIAN ARID REGION

Jojoba was first introduced in Indian arid region in 1965 (Harsh *et al.*, 1987). Simultaneously, augmentation of jojoba from its native habitats was made by the National Bureau of Plant Genetic Resources, New Delhi and in the process several accessions were introduced mainly from USA. The prime objective was to assess their suitability under the Indian arid conditions at one of its Regional Stations at Jodhpur. This region is characterized by scanty and erratic rainfall concentrated in monsoon (July to September), winters devoid of rains, frequent droughts, high temperatures, intense sunshine, frost, high wind velocity, poor soil conditions viz., low content of organic matter and minerals coupled with soil salinity and alkalinity. The live plants of various accessions of jojoba being maintained at NBPGR Regional station, Jodhpur were raised from seeds obtained from different countries, mainly USA (Table 1) and those multiplied from selections made in due course. In the early years of introduction, adaptability evaluation was the main criteria and the growth parameters were not recorded. But of late, these parameters, viz., morphology of the vegetative and reproductive parts, the flowering

Table 1. Accessions of jojoba introduced at NBPGR Regional Station, Jodhpur

Year	Number of Accessions	
	Introduced	Surviving (EC number)
1966	1	1 (33204)*
1967	2	2 (33205*; 33198***)
1969	7	2 (52039*; 52040*)
1972	5	2 (99689*; 99690*)
1977	3	1 (124381)***
1980	9	1 (134349)**
1981	1	Nil —
1983	2	Nil —
1984	2	Nil —
1985	1	Nil —
1987	1	1 (171269)*
1988	7	6 (253492***; 267778*; 267779*; 267780***; 267781**; 267783*)
1989	4	4 (272472*; 272473**; 279585**; 279586*)
Total introduction	45	22

* = Male, ** = Female, and *** = Male and Female.

and fruiting behaviour and the seed yield were recorded at periodic intervals on plants of various age groups. Attempts were also made to obtain hybrids between the highest yielding female accession identified and the various male accessions being maintained.

PLANT GROWTH AND HABIT

Observations of the initial growth rate of jojoba revealed it to be a slow starter with an average increase of only 5-20 cm per year. Striking variation in plant height was observed within and between accessions in the field. The variation, though evident in the early stages of plant growth was more conspicuous in the age group of 8-10 years. Among the various accessions introduced, the maximum number of plants of accession EC 33198 of different ages were available in the field. These plants were observed for studying the variation. Maximum variability in the plant height was observed in the age group of 9 years (Fig. 1). The variation was also observed in plant canopy in all the accessions. It ranged from 50 to 412 cm (Table 2). At the initial stages of plant growth the ratio of plant height to canopy was found to be 1:1 but at later stages it changed due to greater increase in canopy over height.

Plant habit ranged from tall erect to short bushy, with the greater representation of the latter with spheroid, compact and loose forms (Plate 1 A & B). Several environmental factors as plant density, light intensity, pruning, etc., affect the form of the bush. The branching pattern observed in all the accession was basically a main shoot of 15-20 cm supporting a number of primary, secondary and tertiary branches. The leaves, in general, are simple, greyish green, thick, leathery, coriaceous arranged in pairs on the primary, secondary and tertiary branches. Variation in leaf size, shape, colour, thickness and pubescence was observed. Such variations in the growth habit, form and leaf shape have also been recorded by Yermanos (1974) and Brooks (1978). However the variation occurs throughout the population in such a way that no distinct subspecies is recognizable.

FLOWERING AND FERTILIZATION

All plants of jojoba in the field are dioecious. Female flowers are pale green, usually single, while male flowers are small, yellow and in clusters. The flowers are borne on leaf axils. Normally, one flower on an alternate leaf axil develops into a seed while others remain dormant. However, flowering on all leaf axils has also been observed in a few plants. Flowering, in general, was observed in all surviving accessions at the age of 3-4 years in November-December and the fruits matured between April-May (Table 2). Approximately, 10 flowers per branch for female, and 20-30 clusters/branch for male flowers were observed. Flowering was mostly observed on peripheral tertiary branches. The

EXPLORATION FOR IDENTIFICATION OF 'BAEL' GERMPLASM FROM EASTERN PART OF UTTAR PRADESH AND ADJOINING BIHAR

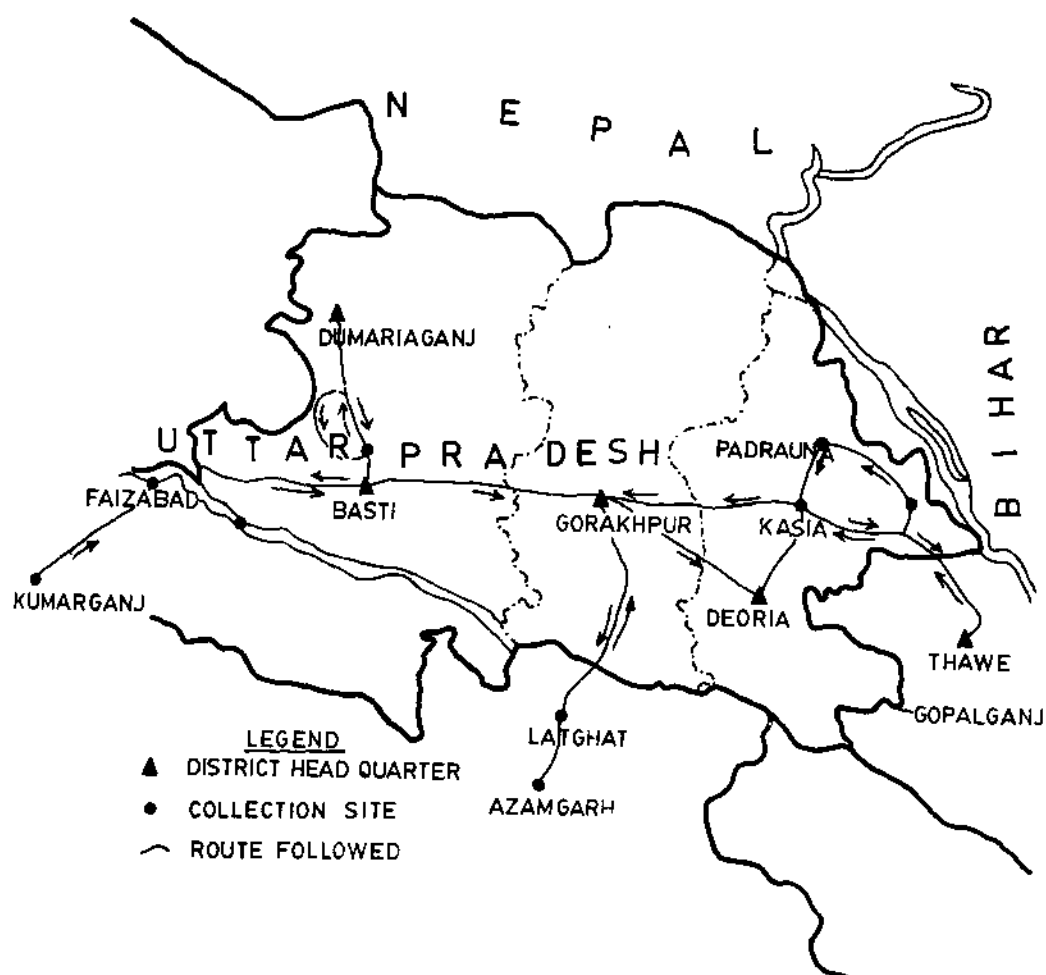


Fig. 1. Height of individual jojoba plants (EC 33198) of different ages.

Table 2. Some phenological observations on different accessions of jojoba plants of various age groups

Accession number	Year of trans-plantation	Plant height (cm)	Plant canopy		First flowering	First fruiting
			X ₁	X ₂		
EC 33205	1967	200	297	318	1970	Nil (Male)
EC 33198	1970	232	412	397	1972	1974
EC 33198	1974	202	185	215	1978	1979
EC 33198	1976	160	158	153	1981	1982
EC 33198	1978	146	169	152	1982	1983
EC 33198	1982	93	100	96	1986	1986
EC 33198	1983	108	97	99	1986	1987
EC 33198	1984	69	65	58	1987	1987
EC 99690	1972	182	180	205	1977	1979
EC 99691	1972	150	205	158	1978	1979
EC 124381	1978	170	163	163	1985	1985
EC 134349	1982	90	50	88	1988	—



Plate I. Plant forms of jojoba. A. Bushy B. Erect

ovary is tricarpeal, but generally only one carpel develops into seed. Occasionally, more than one ovule develops to form 2-3 seeded fruits. Male plants flowered earlier to females. Also, in the population of different accessions, the number of male plants was higher than females. Pollen loss and reduction in seed yield was also observed. To avoid this, a number of planting patterns like alternate rows of male and female plants, transplantation of three saplings together and later removing the males were tried but no method could be standardized. Thus, the hand pollination by dusting the desired pollen on female flowers was practised to increase the pollen availability to female flowers. Male plants, however, tended to survive stresses at early stages more effectively than females as also observed in natural populations (Gentry, 1958). In Arizona, male plants were reported to outnumber females by more than 4 to 1 in natural stands. In California, the sex ratio was reported to be nearly equal. Yermanos (1977) concluded that the data collected on male-female ratio over the first three years of growth show that there is a significant deviation from a 1:1 ratio in favour of males. However, it has also been reported that if flowering data collection was continued for an additional year, this ratio moves closer to 1:1. Heavy flowering and fruiting was observed in plants when supplementary irrigation was applied at the onset of flowering.

SEED YIELD

The most common fruiting pattern in jojoba is of a single fruit on each alternate node (Plate II A). However, a few plants with tendency to develop more than one fruit per node have also been observed (Plate II B & C). Multiple flowering leads to the formation of such fruits as shown in Plate II D. This is a very desirable trait as these plants have great potential for enhancing the seed yield per plant and also facilitate harvesting (Yermanos, 1974). Variation in flowering in natural populations has also been reported in Arizona and California. While in the former, female flower buds remain dormant at every alternate node, in the latter flowering is observed at every node. This reduces the yield potential of Arizona stock. It is desirable to select the all node bearing stocks. Data on seed yield per plant as observed over the years in the field revealed that all accessions have fruited after 5-6 years (Table 2). Seed yield was only 50-200 g in the first few years of fruiting, which increased to 2-3 kg in plants of 8 years. Variability in seed yield per plant was noted in plants of same accession of the same age. Maximum fruit yield of 8.3 kg/plant was noted in accession EC 33198, which incidentally is also the earliest introduction in this region (21 years old) (Fig. 2). Fruits are usually acorn like capsules, bearing mostly one seed. However, 2, 3, or 4 seeded fruits have also been observed. Seed length varied from 1-1.5 cm.

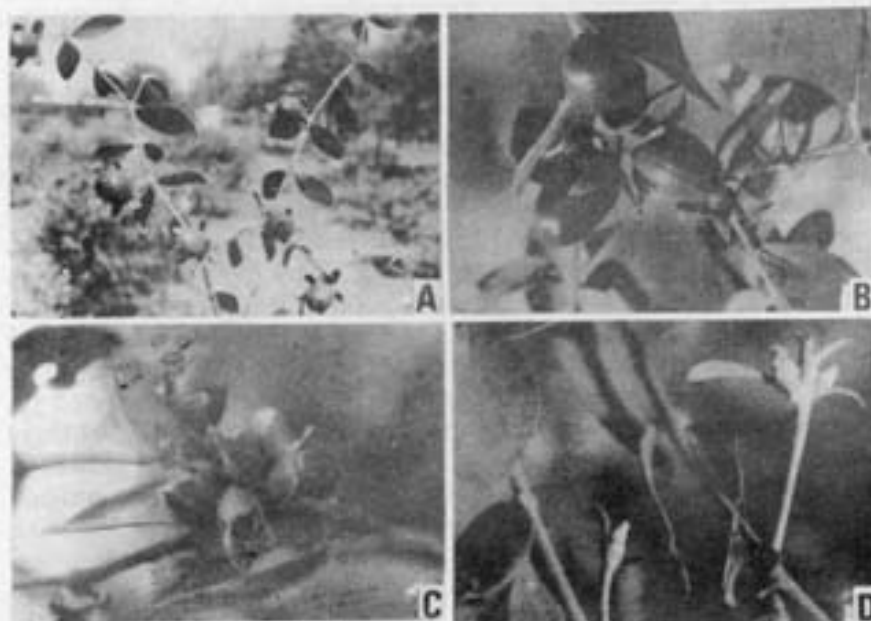


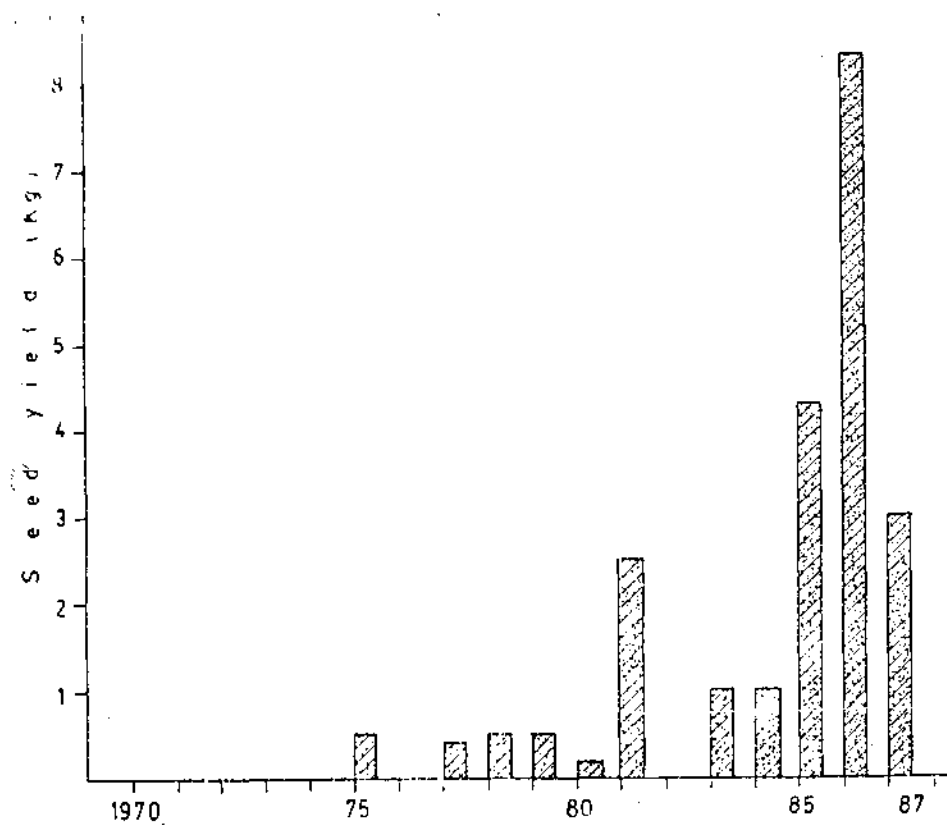
Plate II. Flowering and fruiting in jojoba. A. Fruiting at alternate nodes. B. and C. Fruits in clusters. D. Multiple female flowering.

PROMISING ACCESSIONS AND PLANT IMPROVEMENT

Among the accessions introduced, one female plant (EC 33198 from USA) was identified as the best seed yielding accession (Chopra and Mital, 1979). Thus, attempts were made to multiply the germplasm. The selected material of EC 33198 was used to obtain crosses with pollen of male plants of accessions EC 33204, 52039, 99690 and 99692 (all from USA) in the flowering season of 1979. The plant canopy was divided into four equal parts. Controlled pollination of the equal number of desired female flowers was carried out in the each of the four parts with pollen from the known male plants, making sure that no contamination occurred either before or after pollination. Flowers were monitored closely at weekly intervals to follow success of fruit formation. Some success was achieved and seeds collected. The plants raised from these seeds were found to perform better, flower earlier, i.e., within three years of establishment and produce bold seeds. The observations recorded on the plants obtained from crossed material are summarised in Table 3.

Table 3. Some phenological observations on crossed material of jojoba

Male accession crossed with female accession EC 33198	Year of trans- planting	Plant height (cm)	Plant canopy		First flower- ing	First fruit- ing
			X_1	X_2		
EC 33205	1982	176	183	192	1985	1985
EC 52039	1982	167	165	151	1985	1985
EC 99690	1982	133	154	150	1985	1985
EC 99692 (control)	1982	131	140	143	1985	1985

**Fig. 2. Seed yield of an individual plant of jojoba, EC 33198 over years**

CONCLUSIONS

The success of jojoba for the past two decades suggests its suitability especially of accession EC 33198 (ex USA) for the Indian arid region. However, information on its production potential, economic viability and returns, land utilization, market, etc. under different agroclimatic conditions needs to be collected. Though this work has been initiated in India, the results are yet to be consolidated. Therefore, careful investigation, planning and future strategies need to be formulated before mass-scale introduction of jojoba be carried out in wastelands or barren regions not fit for agricultural crops in Indian deserts. Work should also be done for early identification of sex by detailed cytochemical, biochemical or anatomical studies. Hybridization work must be given top priority as it would aid in production of high yielding varieties. Vegetative propagation and extraction and utilization of jojoba oil needs to be tried as it has direct relevance to the adoption of jojoba farming by the local farmers and agriculturists.

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CRYOPRESERVATION OF DESICCATED SEEDS OF NEEM (*AZADIRACHTA INDICA* A. JUSS.) FOR GERMPLASM CONSERVATION

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Among tree species, immensely valued for timber and medicine, neem (Azadirachta indica) is extremely important. Neem seeds, inspite of possessing some typical features of recalcitrant seeds like short life, high moisture content (about 46%) at the time of shedding and possessing endocarp which played a significant role in germination and desiccation responses, showed orthodox nature in terms of desiccation and freezing sensitivity. Seeds showed desiccation tolerance upto 4 per cent and freezing tolerance at 196°C for seeds desiccated between 18.5 per cent and 4 per cent moisture level. Cryopreservation, the technique of storage of germplasm at the ultra low temperatures of liquid nitrogen (196°C) can thus be utilized for long term conservation of neem germplasm.

Neem (*Azadirachta indica* A. Juss.) is a valuable arboreal species found extensively in tropical regions of India. FAO panel of experts on Forest Gene Resources in their recommendations regarding collection, evaluation, and conservation of forest species, have selected neem for urgent action in India. So far, very limited information is available on germination, viability and storage of neem seeds. Neem seeds are reported to be short-lived (Ezumah, 1986; Troup, 1986; Maithani *et al.*, 1989) mainly due to their high moisture levels which causes deterioration in them (Troup, 1986). Attempts at storing seeds under different temperature and storage conditions have been successful only for 16 weeks at room temperature (Ezumah, 1986) and 6 months at 15°C (Maithani *et al.*, 1989). Storage temperatures of 5-11°C were found unfavourable (Ezumah, 1986; Maithani *et al.*, 1989). Controversial re-

ports exist regarding the classification of neem seeds either as orthodox or recalcitrant. Roberts (1983) had earlier designated neem as recalcitrant. In a later report (Holden & Williams, 1984), it was found that viability of seeds is drastically reduced on desiccating the intact neem seeds and endocarp affected the germination responses. Neem seeds have been, however, categorised as orthodox, based on electrical conductivity measurements of fresh seeds (Khare *et al.*, 1989). Present studies were, therefore, undertaken to characterise neem seeds as orthodox or recalcitrant based on the germination, desiccation and freezing sensitivity studies.

MATERIALS AND METHOD

Seeds were extracted from the ripe yellow fruits following the method described earlier (Radhamani *et al.*, 1990). Moisture content of the seeds was determined (after removing the endocarp) by low constant temperature oven method (Anon., 1985) 103 ($103 \pm 2^\circ\text{C}$ for 17 h) and expressed as percentage of fresh weight. Germination was carried out by the method reported earlier (Radhamani *et al.*, 1990). Seedling vigour was determined by average root lengths of seedlings. Seeds were desiccated to different levels by two methods, (1) over silica gel in a desiccator at room temperature and (2) in a seed drier (Model MVE 50 SP., Bry Air India Ltd.) supplying forced air at 20-25 per cent relative humidity and 20°C . Desiccated seeds were immediately plunged in liquid nitrogen after packing in polypropylene cryovials. After 24 hours, seeds were retrieved and thawed by immersion in a water bath maintained at $37-38^\circ\text{C}$.

RESULTS AND DISCUSSION

Average moisture content of neem seeds was 46 ± 3.7 per cent. This value is much higher than that reported earlier (Maithani *et al.*, 1989; Khare *et al.*, 1989). Moisture content of endocarp alone was 12.01 per cent. In view of inhibitory role of endocarp (Radhamani *et al.*, 1990), freshly harvested seeds were always germinated after removing their endocarp and the germinability ranged from 90 to 100 per cent. Fresh seeds could be desiccated much faster in seed drier than in desiccators over silica gel (Table 1) and within each of the methods of drying, excised seeds (without their endocarp) lost moisture much faster than the intact seeds especially when desiccation was prolonged. Excised seeds lost considerable moisture (about 43% of fresh weight) after 24 hours of desiccation in a seed drier, whereas, those maintained in silica gel took 144 hours to lose same amount of moisture. Intact seeds could also be desiccated to this low level but only after 161 hours of desiccation over silica gel.

Table 1. Desiccation of intact and excised seeds of neem for varying periods using seed drier and silica gel

Hours of desiccation	Desiccation in seed drier (% moisture lost in seeds)*		Desiccation over silica gel (% moisture lost in seeds)*	
	intact	excised	intact	excised
1	1.6 ± 0.04	6.9 ± 0.04	1.8 ± 0.06	2.3 ± 0.10
4	8.5 ± 0.05	9.9 ± 0.04	2.9 ± 0.10	3.0 ± 0.15
7	11.5 ± 0.04	16.3 ± 0.09	4.3 ± 0.51	10.2 ± 0.88
15	17.1 ± 0.10	36.4 ± 1.1	9.5 ± 1.1	25.1 ± 2.3
24	19.3 ± 0.41	43.3 ± 1.3	12.1 ± 2.1	30.2 ± 2.2
43	—	—	15.0 ± 2.2	32.5 ± 3.1
72	—	—	28.0 ± 1.8	35.2 ± 2.1
144	—	—	35.9 ± 1.1	42.0 ± 1.8
161	—	—	41.9 ± 2.1	42.0 ± 1.9

* Average values of three replicates (% of fresh wt.).

Excised seeds desiccated upto about 21 per cent moisture content maintained their original high viability (Fig. 1). Seeds desiccated further to 13.5 per cent showed the first sign of desiccation injury. Three hours of rehydration of these seeds over 100 per cent relative humidity at room temperature could, however, recover its original germinability. Seeds desiccated to 4 per cent moisture level showed a fall in germination values to 65 per cent, which could be recovered to a maximum of 70 per cent by rehydration for three hours. In all the subsequent experiments, prehumidification of seeds for 3 hours was routinely done before sowing for germination.

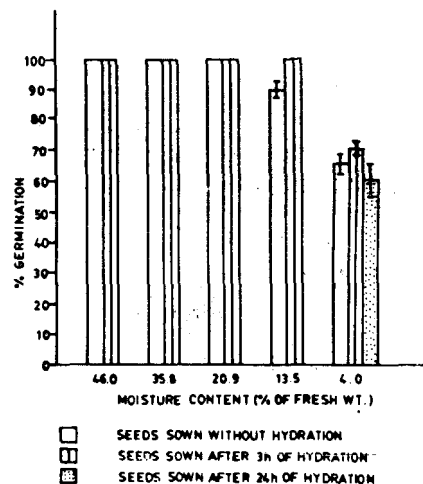


Fig. 1. Extent of desiccation tolerance of excised neem seeds and recovery of desiccated seeds following rehydration at 100 % RH at room temperature for various periods.

Intact seeds desiccated to different moisture levels on sowing for germination developed cracks in their endocarp, indicating initiation of germination by 6 days of sowing in 10 to 30 per cent on the seeds (Table 2). However, these seeds showed further growth only in seeds with

Table 2. Effect of presence or absence of endocarp on germination of neem seeds desiccated to different levels in intact state

Moisture content of seeds (% of fresh wt.)	Endocarp retained/removed	Observations recorded after 6 days of sowing for	
		per cent Germination	Average root length (cm.)
46.9	Retained	5* (30)	1.2
	Removed	95	3.65
32.8	Retained	0* (20)	-
	Removed	95	3.5
28.3	Retained	0 (10)	-
	Removed	90	4.6
22.4	Retained	0 (10)	-
	Removed	70	2.1
12.7	Retained	0 (0)	-
	Removed	50**	2.05

*Final germination percentage increased to 25.

**Final germination percentage increased to 65.

Values in parenthesis are % of intact seeds that showed only cracks in endocarp

higher than 32 per cent moisture level. A part of the seeds sample at each of the desiccation levels sown after removal of the endocarp showed much better germinabilities and seedling vigour; seeds possessing about 28 per cent moisture and higher, showed germination values of 90 per cent and higher and seedlings with more than 3.5 cm roots and those possessing 22.4 per cent and 12.7 per cent moisture showed 70 per cent and 65 per cent germination and 2.1 and 2.05 cm roots, respectively. Thus, in neem seeds, endocarp was found to influence the germination and desiccation responses. It delayed the loss of moisture during desiccation and also the uptake of water during imbibition on sowing the seeds for germination. Neem seeds, in the absence of their endocarp, showed desiccation tolerance upto 4 per cent moisture level—extent found in the orthodox seeds. However, it was accompanied by 25-30 per cent loss in viability. Intact seeds on desiccation to about 13-15 per cent moisture showed lower germination values even when sown without their endocarp in comparison to the seeds desiccated to the same level in excised state from the beginning. This was due to the comparatively longer time taken by the intact seeds to achieve this level of moisture which could have caused some physiological deterioration.

Excised seeds possessing moisture contents of about 27 per cent and above on exposure to liquid nitrogen for 24 h showed total loss of viability (Fig. 2). Only seeds desiccated to 18.5 per cent and lower upto 4 per cent moisture content survived liquid nitrogen exposure for 24 h. High survival values between 57-65 per cent were, however, obtained only for seeds possessing moisture contents between 13.5 to 4 per cent.

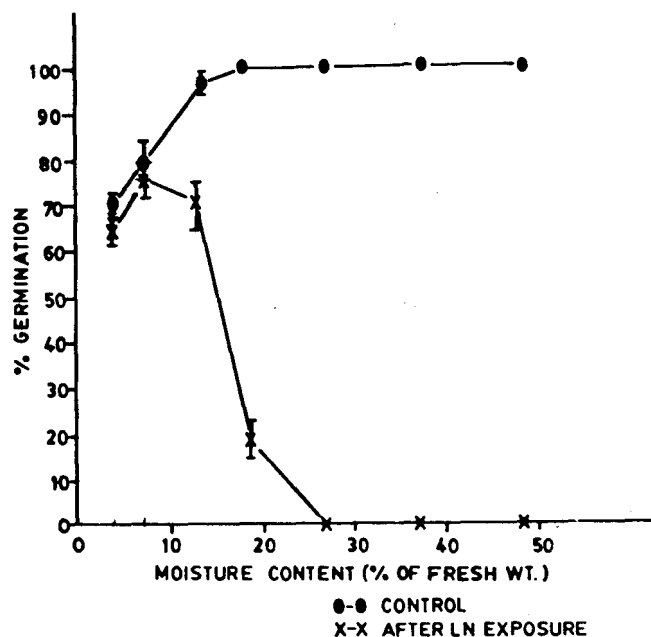


Fig. 2. Germinability of excised seeds of neem after desiccation and liquid nitrogen exposure

Based on desiccation and freezing sensitivity, neem seeds could be clearly categorised as orthodox in nature. It was in the presence of endocarp that the seeds showed recalcitrant nature since desiccated seeds showed germination only when endocarp was removed. Cryopreservation of neem seeds is thus feasible using the simple procedure of desiccation of seeds to 13.5 per cent and lower and freezing them rapidly in liquid nitrogen. This can ensure long term conservation of neem germplasm for future use.

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ETHNOBOTANICAL STUDIES IN ABUJHMARH (BASTAR), INDIA

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The maria and muria tribal communities living in Abujhmarh area in Narayanpur taluka of Bastar, Madhya Pradesh (India) mostly depend on the natural resources of their neighbouring forests for food, medicine and for other material culture and local rural technology. They use many plants in their religious ceremonies also. The Abujhmarh area is ethnobotanically under-explored. This paper deals with the ethnobotany of 35 plants from there. The information was derived from the specimens of plants and plant parts collected by the author during several explorations in the area (1986-1990) and by having interviews and discussions with the tribals. Vernacular names are also mentioned. A survey was conducted among medicinal plant sellers and local people of the area and 5 recipes and their use in fertility cult, menstrual problems, birth control and in deriving stimulation etc. were discussed.

Ethnobotany deals with the studies among the tribals and rural people for recording their unique knowledge about plant wealth and for search of new resources of herbal drugs, edible plants and other aspect of plants. It also includes the impact of man on his plant surroundings and the attitude of people towards plants. Plants have always been important to man. They provide oxygen, shelter, clothings and directly or indirectly food. Perhaps as a consequence of his dependence on plants, man has incorporated them into his language, religion, art, drama and recreation. For this reason, evaluation of the relationships between a group of people and their surroundings vegetation can provide useful cultural insights. The multiple uses for which plants are employed by *Abujhmarhia* tribals are often not known outside their community. With the advent of civilization and rapid spread of modern technology, the *Abujhmarhias* also, like other tribals, are changing very fast from their primitive cultural systems. Before the customs, traditions are replaced by modern acculturation, it was thought necessary to

record the uses of plants and plant parts in the folklore and folklife among *Abujhmarhias*.

LOCATION AND AREA OF STUDY

The *Abujhmarh* (Abujh = dark, unknown; marh = plateau) area is a mass of rugged hills in the south-west portion of Narayanpur taluka of the old princely state of Bastar (17° 45' - 20° 30' N and 80° 15' - 82° 15' E) and now the southernmost Jagdalpur (Bastar) district of Madhya Pradesh. The region is conspicuous because it is wholly comprised of high ridges and deep valley. From the west boundary of the district, it continues eastwards and descends clearly to Narayanpur-Kondagaon plateau in the east along Narayanpur and Chhotedungar. *Abujhmarh* offers unusual opportunity for ethnobotanical studies as these tribals largely depend upon the surrounding plant resources for their food, medicine and other material culture and for their existence.

The climate is generally warm, wet and humid in this region with average annual rainfall of 152 cm. Commercially valuable teak (*Tectona grandis* L.) and sal (*Shorea robusta* Gaertn. f.) forests were found in this area. The commonest species in these forests are *Madhuca indica* J.F. Gmel., *Lagerstroemia indica* L., *Pterocarpus marsupium* Roxb., *Grewia subinaequalis* DC, *Holarrhena antidysenterica* (L) Wall., *Diospyros melanoxylon* Roxb., *Hardwickia binata* Roxb., *Anogeissus latifolia* Wall., *Bambusa arundinacea* Willd. and *Boswellia serrata* Roxb.

PEOPLE AND LOCAL INHABITANTS

Any ethnobotanical study is incomplete without a reference to the local inhabitants. *Abujhmarh* is a place of *maria* and *muria* tribals. These tribals are generally farmers in the aboriginal areas which have poorer villages. This area is very sparsely inhabited by aboriginal people in spite of being most difficult to access in Bastar because its inhabitants - the *abujhmarhia* tribes are still in the stage of shifting cultivation on the steep slopes of the hills. These aboriginals are Hill-Marias and Bison-Horn-Marias. The diet of these tribals consists of three meals of very simple kind. The breakfast is finger-millet (*Eleusine coracana*) cooked with a thin gruel called *pej*. The mid-day meal is practically the same supplemented with boiled *mahua* (*Madhuca indica*). The evening meal consists of finger millet or coarse rice with salt in it. The food is supplemented by chicken on festive occasions. They have rich folklore transmitted orally from generation to generation. Thus a wealth of information exists among them on utility of plants.

In India, Dr. E.K. Janaki Ammal initiated researches on ethnobotany

and studied subsistence food plants of certain tribals of south India and better prospects of *Dioscoreas* in India but good scientific base have appeared in the last two decades leading to publication of regional enumerations consisting of about 3000 plants used as medicines, fuel, food, fodder, fibre or house building, musical instruments, narcotics, beverages, in material culture and for magico-religious purposes (Agrawal and Saha, 1968; Jain, 1963, 1980, 1991). Several studies on the plants used in veterinary medicines have also been made. But considering the vast size of the country with varied flora and many kinds of tribes, these studies seem to be meagre and this is particularly in Bastar tribals. The only existing account of the ethnobotany of this region are those of Jain (1964, 1965), Umesh Chandra and Mehra (1983) and Roy and Chaturvedi (1987).

METHODOLOGY

The general procedure for gathering data was the same as described by Jain (1963). It consists of data obtained from informants by interviewing them in detail or also by witnessing the uses of plant or plant parts during stays in several tribal villages. The voucher specimens collected have been identified (Chopra *et al.*, 1956; Anon., 1946-1976). In addition to this, the author has employed different models of approach for field study. In this, following points were taken into consideration : (a) the dress, expression, speech, behaviour etc. are the factors which help the respondent infer whether you are one of them or an outsider, (b) let the respondent speak out as much as possible keeping your verbal expression to the minimum. However, certain words/phrases which help the continuity of their replies and keep them on the right track, will be useful, (c) make the respondent feel that you are not merely eliciting something from them but you are actually sharing or ready to share in their problems, (d) help the respondent understand before they reply to the queries, that (i) problems exists around them, (ii) the key solution of the problems is with them only. The surveyor can help them trace the key and guide them to the right direction, (e) it is not advisable to side with one group or the other if the groups begin to emerge during discussion. Be neutral, and (f) the interview should be informal one and not question-and-answer type. Still better, if the respondent knows the least that he is being interviewed.

During field work, local tribals or informants of the area were requested to accompany to the forest and to identify the plants/plant parts they use. Sometimes plants are collected from one locality and queries were made from adjacent tribal villages familiar with the plants.

RESULTS AND DISCUSSION

(a) Food: Although rice constitutes the major foodgrain of the *Abujhmarhias*; bulbs, rhizomes, leaves of various native plants supplement their food. But generally they boil rice and **kosara-ragi** (*Eleusine coracana*) and make a paste (known as **pej**). They take it in the morning and then they carry it in **tumba** (dried bottlegourd - *Lagenaria siceraria* Standl.) urns used to store liquor **pej** to the forest where they roam during day time. They use bulbs of *Amorphophallus* spp. also in their food.

(b) Medicine: The tribals or aboriginal people generally reside in very remote areas where practically no organized medical aid is available. They use herbal medicines in the ailments. The knowledge of herbal medicines is limited to a few members of the community. Some of the plants employed by the *Abujhmarhias* in medicine are enumerated in this paper. In these enumerations, the species are alphabetically arranged. The letters Ha, G, H, E in parenthesis indicate Halbi, Gondi, Hindi and English names respectively. This follows a brief notation of uses, as revealed by local tribals and informants consulted during the study. Collector's name (Umesh Chandra = UC), collector numbers, plant families are recorded after scientific names. The location of the collection is given after the notation of uses.

Abelmoschus manihot (L) Medic., UC 3145, Malvaceae, Wild okra (E), **Banramkalia**, **Bhursakanda** (G); decoction of root if taken by impotent person, stimulation is derived. Gudadi

Achyranthus aspera L., UC 3142, Amaranthaceae, **Agya** (Ha); when women insert roots in vagina, it has abortifacient effect. Garhbengal

Andrographis paniculata Nees, UC 3152, Acanthaceae, **Bhuilimb** (G), **Chirayata** (G); the plant is febrifuge, the leaves are pounded and made into small pills, two pills per day are reported to cure fever. Gudadi (near Orachha)

Bryonopsis laciniosa (L) Naud. UC 3172, Cucurbitaceae, **Shivlingi** (H). the powdered seeds are given to barren women to help conception. Ranwahi (near Bhanupratappur)

Casearia elliptica Willd. UC 3174, Samydaceae, **Swarnmool** (Ha, H); the bark of the root is considered to be very good tonic for anaemic condition. Chhotedungar

Chloroxylon swietenia DC, UC 3167, Meliaceae, **Nadbirla** (G), **Bhirra** (H); leaves are crushed and applied to cattles to heal wounds. Bastanar

Citrus aurantifolia (Christm.) Swingle syn. *C. medica* var. *acida*. UC 3159, Rutaceae, **Kagzi Nimbu** (H); juice of one lemon is added to half kg of unboiled milk and is given to lessen diarrhoea. Chhotedungar

Cochlospermum religiosum (L.) Alston, UC 3162, Bixaceae, **Kayari** (G), **Harsingar** (Ha) **Gongal** (H); bark of the tree is pounded and applied to broken limbs, particularly in cattles. Pandaripani (Narayanpur)

Cuminum cyminum L., UC 3144, Umbelliferae, Wild cumin (E), **Banjeer** (Ha); the fruit is pungent, hot and sweet, is reported to be stomachic. Kusumkasha

Euphorbia prostrata Ait., UC 3153, Euphorbiaceae, **Dudheli** (Ha); when taken as vegetable, it enhances lactation. Three doses are sufficient. Geedam (near Dantewada)

Gardenia turgida Roxb. UC 3169, Rubiaceae, **Pendra-ki-jadi** (G); decoction of the roots of *Gardenia turgida* and *Ficus glomerata* is said to cure sugar (Diabetes) in urine when urine is dark and reddish. Ranwahi

Glossogyne bidens (Retz.) Alston, UC 3158, Compositae, **Jangali Dhanja** (Ha); the whole plant is crushed, mixed with sweet and applied to aching teeth for relief; also the pounded roots applied in snake-bite and scorpion sting. Bastar

Helicteres isora L., UC 3163, Sterculiaceae, **Marodphali** (H,G); the fruits are used to relieve stomach disorders. Kusumkasha

Leucas aspera Spr., UC 3175, Labiateae, **Tamba**, **Gubibuta** (Ha); half headache, juice of the root is administered in corresponding nostril. Chhotedungar

Mucuna prurita Hook. f., UC 3143 Papillionaceae, **Baidhok**, **Kawanch** (H, Ha); seeds are given for improving retention of semen. Ranwahi

Ocimum sanctum L., UC 3164, Labiatae, **Memri** (G), **Tulsa** (H); decoction of the whole plants applied to kill ticks in hen. Ranwahi

Petunga dhana Buck. Ham. ex C.B. Clark var. *dalzellii* C.B. Clark, UC 3147, Rubiaceae, **Bhojraj** (G) and

Petunga nagpurens, UC 3148, Rubiaceae, **Tejraj** (G); decoction of leaves and roots when taken, stimulation derived. Ranwahi

Polygonum plebejum R. Br., UC 3161, Polygonaceae, **Kati Bhaji** (Ha); eaten as vegetable, the plant is believed to promote lactation. Garhbengal

Pongamia pinnata L., UC 3160, Polygonaceae, **Karanj** (Ha); used for treating eczema, the seed oil is applied after bathing. It is applied to burns and itches also. Chhotedungar, Narayanpur

Scoparia dulcis L., UC 3165, Scrophulariaceae, **Mithipatti** (H), **Ghodatulsi** (H, Ha); the leaves are powdered and made into pills, three pills are taken daily in weakness of semen. Jagdalpur

Semecarpus anacardium L., UC 3149, Anacardiaceae, **Idumaraam**, **Biba** (G), **Bhilawa** (H); the fruits heated, an oil oozes out and is applied

to cuts and other foot injuries, also in ache of waist and hand joints. Orachha

Shorea robusta Gaertn. f., UC 3171, Dipterocarpaceae, **Sargi** (G), **Sal** (H); the fruits are pounded and the paste is given for treating diorrhoea. Chhotedungar

Smilax prolifera Roxb., UC 3166, Liliaceae, **Mutrilaha** (G), **Ramdatoon** (H); children who urinate in the night are cured if they employ leaves of this plant in their food and branches as tooth brush. Gudadi (near Orachha)

Sterculia urens Roxb., UC 3156, Sterculiaceae, **Gulu** (H), **Karat** (Ha); the bark is pounded and given to women before childbirth to facilitate delivery. Ranwahi

Tamarix dioica Roxb., UC 3155, Tamaricaceae, **Gajri** (G), **Jhau** (H); a few leaves are cooked with rice and given to children to relieve cough, also decoction of leaves for cure of enlarged spleen in children as well as adults. Chhotedungar

Tectona grandis L., UC 3170, Verbanaceae, **Sagwan** (H); oil obtained by distillation of wood chips are reported to cure eczema and ringworm, ashes of wood applied to swollen eyelids. Narayanpur

Tephrosia purpurea Pers., UC 3151, Papilionaceae, **Chiktibuta** (Ha), **Bajradanti** (H); this species with *Asparagus* spp. is an ingredient for medicine used in treating impotency, roots crushed and the paste is given in snake-bite. Ranwahi

Terminalia belirica Roxb., UC 3146, Combretaceae, **Baheda** (H); it is used in piles, diorrhoea and headache etc., as purgative also. Chhotedungar

Thespesia populnea Soland ex Correa, UC 3168, Malvaceae, **Paraspipar** (Ha); barks leaves and flowers are pounded into paste and used in skin infection, scabies, ringworm and eczema etc. Kusumkasha

Urtica dioica L. UC 3150, Urticaceae, **Bichchhu-buti** (H, Ha); an infusion of it, is administered to control excessive menstrual flow. Narayanpur, carhbengal

Wrightia tinctoria R. Br., UC 3157, Apocynaceae, **Indrajau** (G); fresh leaves when chewed - relief in toothache, decoction of leaves is taken as stomachic. Kusumkasha

Xeromphis spinosa (Thunb.), UC 3154, Rubiaceae, **Dhudhributa** (Ha); roots are used for treating gonorrhoea and disease of urinary tract. Orachha

As per the local medicinal plant sellers and the local people of the area, the plants and plant parts were mixed according to following recipes and are given in various treatments.

Recipes 1: Inflorescence of *Crocus sativus* L., UC 3177, Iridaceae, **Kesar** (H); inflorescence of *Thespesia populnea* Soland ex Correa, UC 3168, Malvaceae, **Paraspipar** (Ha); *Terminalia belirica* Roxb., UC 3146, Combretaceae, **Baheda** (H); *Emblica officinalis* Gaertn., UC 3188, Euphorbiaceae, **Amla** (H); *Anogeissus latifolia* Wall., UC 3179, Combretaceae, **Dhawada** (G); *Elletaria cardamomum* Maton, UC 3180, Zinziberaceae, **Chhoti elaichi** (H); *Syzygium aromaticum* (L) Merr. & L.M. Perry, UC 3183, Myrtaceae, **Laung** (H); *Styrax officinalis* L., UC 3182, Styraceae, **Silajeet** (H); and *Piper nigrum* L., UC 3184, Piperaceae, **Black pepper** (E) - all are pounded and a mixture powder is obtained. One teaspoonful of this powder if taken daily with water for six months, barren women will redevelop menstruation, and can produce child.

Recipes 2 : Decoction of roots of *Gardenia turgida* Roxb., UC 3169, Rubiaceae, **Pendra** (G); and fruits of *Ficus racemosa* L., syn. *F. glomerata* Roxb., UC 3185, Moraceae, **Gular** (H) are used in diabetes.

Recipes 3: Leaves of *Petunga dhana* Buch. Ham. ex C.B. Clarke var. *dalzellii* C.B. Clarke, UC 3147, Rubiaceae, **Bhojraj** (G); is pounded with sugar. If the paste is taken, high stimulation is derived.

Recipes 4: *Alpinia officinarum* Hance, UC 3186, Zinziberaceae, **Kalanjan** (G); *Myristica fragrans* Houtt, UC 3187, Myristicaceae, **Jaiphal** (H); *Zingiber officinale* Rose, UC 3188, Zinziberaceae, **Adrak** (H) - all are mixed and pounded in equal quantity. The mixture helps in curing acute cough.

(c) Other uses: Women use *Carthamus tinctorius* L., UC 3189, Compositae, **Kusum** (H), Safflower (E) oil in their dark hairs. Women of *Abujhmarh* - their arm and faces are heavily tattood. The dyes used for tattooing are of plant origin. They use *Madhuca indica* J.F. Gmel., UC 3190, Sapotaceae, **Mahua** (H) cups for sipping **pej** etc. Mats used are made of bamboos (*Melcoanna* spp. and *Arundinaria* spp.).

It can be well established from the above enumerations and from work published in the past that *Abujhmarh* has a good treasure of folklore uses of plants. The author has only mentioned those plants which came into his notice during exploration expeditions to Bastar district in the years 1986 to 1990. Many other folklore uses of plants are to be found out. The only difficulty in this work is that the *Abujhmarh*ias do not easily yield the secrets of the plants, they use for medicinal and other purposes; they have a traditional notion that if they do so, the curing properties of the plants will vanish. They yield those secrets only

to their kith and kin and at the last stages of their life. While interviewing the tribals, an effort was made not to make these discussions/queries monotonous for the informants and on certain occasions, the author encouraged them to ask for treatment of any diseases; what tribals of their villages did for these diseases and then what they did, was discussed. In majority of cases, the prescription was known to several tribals for common ailments such as cuts, pains, fever but for serious ailments and complicated diseases, the village medical man alone was considered competent. This person is generally the most respected and rather indispensable man of the society. He is usually jealous of his knowledge. Animal sacrifice and exorcism still play a role in tribals of *Abujhmarh*. Propitiation of God or the spirit whose ill-will has caused the sickness, are always an essential part of treatment. Also all the herbs used in prescription must be considered ineffective without accompanying oracles. These oracles have to be recited by the so called medicine man at the time of administering it. They have no prescription for controlled diet during sickness. They are usual in diet. The medicine men generally don't tell about the medicine considering it as trade secret but in case the approach is good and in case, they have been given a leading role in discussions, generally they spoke out without more reservations but to few ailments, they don't tell at all. The work for exploring *Abujhmarh* requires much patience and technique to win the confidence of the people. No doubt, if systematic work is done, many valuable results can be expected but before the time lapses, the work needs to be expedited.

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HODGSONIA MACROCARPA—A NON-CONVENTIONAL EDIBLE FAT AND PROTEIN SOURCE

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Among large number of cucurbits found in North-East India, the roasted seed kernels of Hodgsonia macrocarpa (Bl.) Cogn. has been a valuable source of fat (62.71 per cent) and protein (31.21 per cent) to the tribals. The quality of oil in respect of saturated and unsaturated fatty acids is comparable to the palm oil. The identification of staminate and pistillate plants at seedling stage in order to establish populations in the right proportion is the major obstacle in its commercialisation. Further research efforts on this cucurbit would be required.

Hodgsonia macrocarpa (Bl.) Cogn. syn. *H. heteroclita* Hook f. & Thomas, is a semi-woody cucurbitaceous perennial dioecious climber growing upto 25-30 m. It is believed to have its origin in North East India, China (Western Yunan), and Malaysia (Hu, 1964; Zaven and Zhukovsky, 1975). It grows widely upto 1500 m. altitude in hills of Meghalaya, Mizoram, Sikkim, Nagaland, North-Cachar Hills (Assam), Manipur etc. Its various vernacular names are Kadam seed (English), Thibe (Garó), Soh-mynthar (Khasi), Khaum (Mizo), Kat'hin-pot (Sikkim), Kathai (Manipuri), The bou-lata (Assamese), Til lau (Bengali) etc.. The climber is much branched and spreads on lofty trees. It bears leathery, 3-5 deeply lobed leaves 15-20 cm in diameter on 5 to 10 cm long petioles. The plant begins to flower in February-March and continues throughout the summer. The fruits ripen in autumn and winter (November-December). The staminate flowers borne on axillary racemes and the pistillate usually solitary appear on separate plants. The fruit is pomiform, 6-20 cm long and 20 to 25 cm in diameter, globose, reddish-brown (on maturity) and 12-grooved covered with reddish or grey hairs. The outer rind of fruits is thin but hard containing inedible spongy and juicy pulp embedded with 4-6 ellipsoid flattish seeds (5-8 cm x 2-4 cm) covered with woody wrappings (2-3 layers). The inside kernel of seed is slightly bitter, but is eaten after roasting or baking.

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with woody wrappings (2-3 layers). The inside kernel of seed is slightly bitter, but is eaten after roasting or baking.

The til lau (*Hodgsonia* sp.) kernels are eaten after roasting and in some areas oil is extracted for food and for burning in light lamps while leaves and young stems have been used as medicine for nose complaints and for relief of fever (Hu, 1964). Besides North-east India, it is widely grown/cultivated in hills of Upper Burma (Myanmar), Bangladesh, Vietnam, Java, Sumatra and Thailand.

MATERIALS AND METHODS

The ripe fruits of *Hodgsonia macrocarpa* were collected from Mizoram, Nagaland and Meghalaya in the year 1990-91. The seed kernels were taken out and supplied to the Biochemistry Unit at NBPGR, New Delhi and at IARI, New Delhi for chemical analysis and determination of fatty oil composition. The oil content was estimated by NMR and protein by Kjeltac Auto 1030 Analyser. The fatty acid composition was determined by HPLC method. The related information on cultivation and local use were also collected from tribal farmers. The seeds were grown at Barapani Farm (Meghalaya). However, all the plants turned out to be male, hence no fruits were produced.

RESULTS AND DISCUSSION

The results of chemical analysis revealed that the seeds contained 49.27 per cent kernel which showed the following per cent nutrient composition, viz., water, 2.48; fat, 62.71; protein, 31.25; fibre, 2.41 and Ash 1.15. Further analysis of fatty acid composition in the oil revealed palmitic acid (17.28%), stearic acid (9.36%), oleic acid (21.76%), linoleic acid (33.90%), hexadecenoic acid (7.25%), arachidic acid (6.86%) and myristic acid (3.56%). The results of fatty acid composition and oil content of present investigation are not in agreement with earlier reports (Arora and Hardas, 1976; Anon. 1959). This may be due to different source of material or the methodology of analysis. Hu (1964) compiled results of various reports indicating oil range from 57.4 to 80 per cent; specific gravity (at 15°C) 0.9164 — 0.998; saponification value 198.9-203.9; and iodine value from 63.6 — 107.3. The light yellow coloured oil had a melting point at 25°C. Comparison of fatty acid composition of some dietary fats (Vles and Gottenbos, 1989) with that of til lau show that its fatty acids are next to coconut and almost similar to palm oil (Table 1). Though the ratio of saturated and unsaturated acids is comparatively high, a desirable character of deep frying oils (Lapedes, 1977), and it also contains a good amount (56 %) of unsaturated fatty acids—linoleic and oleic. The high amount of linoleic acid (33.9 %) is useful as it is considered to be essential in human diets. It is also a precursor of

arachadonic acid being essential for normal growth and maintenance of skin (Lapedes, 1977).

Table 1. Comparison of fatty acid composition (%) of some important dietary fats with *Hodgsonia macrocarpa*

Source (oil)	Saturated	Unsaturated				Total	A/B
		Mono unsaturated	Poly-unsaturated				
			Linoleic Acid	Others			
	A	B ₁	B ₂	B ₃	B(B ₁ +B ₂ +B ₃)		
Coconut	90	8	2	0	10	9.00	
Palm	50	39	10	1	50	1.00	
Olive	17	73	10	0	83	0.20	
Groundnut	18	52	30	0	82	0.22	
Maize	15	30	55	0	85	0.18	
Soybean	16	24	53	7	84	0.19	
Sunflower	12	21	67	0	88	0.14	
Safflower	10	15	75	0	90	0.11	
<i>Hodgsonia</i>	56	10	34	0	44	1.27	
Rape seed	6	67	17	10	94	0.06	

Nowhere in the North-east region, til lau is cultivated on commercial basis. A few plants of *Hodgsonia* are planted by farmers around their dwellings. These are usually grown from seeds in places where minimum temperature does not fall below freezing. However, survival rate of 50 per cent from cuttings and 90 per cent through layering has been obtained by chinese botanists (Hu, 1964). It was reported that a single plant is able to produce 40 to 100 fruits which can yield upto 10 kg of oil and at this rate it was found that 15 plants of til lau can produce the amount of oil equivalent to that obtained from the produce of rapeseed on one hectare area. In spite of above, it could not become a commercial oilseed crop because of the difficulties involved in the early identification and establishment of staminate and pistillate plants in the right proportion. Systematic and vigorous research programmes are required for exploitation of this cucurbit.

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VARIABILITY AND POTENTIALS OF IDENTIFIED GERMPLASM IN BAEI (*AEGLE MARMELLOS* CORR.)

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In an exploration for 'bael' (Aegle marmelos Corr.) germplasm from the tarai belt of eastern Uttar Pradesh and adjoining area of Bihar, twenty four genotypes including three wild types were identified which showed wide range of variation for thorniness on the stem, fruit shape, skull thickness and pulp characteristics, viz., colour, sweetness, taste and presence of aroma. These genotypes also exhibited a wide range of variability for all the 6 quantitative characters recorded. The mean values were also high for number of fruits per tree, fruit yield and number of seed per fruit. Among these, six genotypes were identified as promising based on yield potential and fruit quality. These have been suggested for direct utilization and also use in improvement programmes. Three wild genotypes having small size non-edible fruits showed very high bearing (upto 3000 fruits per tree) and thus may be useful as suitable donor parents for high bearing potential.

Bael (*Aegle marmelos* Corr.) is known for its medicinal properties, nutritive values and religious importance. It is one of the most common fruit tree, native to India. Many of the spiritual writings have a mention of bael (Singh and Ray, 1984) probably due to great therapeutic value of various parts of its tree. The ripe fruit is delicious, good for processing and also used in diarrhoea and dysentery. Various chemical constituents, viz., alkaloids, coumarins and steroids have been isolated and identified from its leaves, wood, root and bark (Ray, 1985). It has ability to tolerate arid conditions (Chundawat, 1990) as well as high rainfall. However, during explorations, no systematic plantation was observed. It was growing mainly on roadside, forests, threshing yard, scattered planting in orchard, backyard of houses and religious places.

Although known varieties were not available yet different local types/landraces, viz., Mirzapuri (Singh, 1961), Kagji Gonda (Chundawat, 1990)

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are being grown at different places with considerable variability in qualitative and quantitative characters. Few superior types reported by the farmers are under threat of erosion. Therefore, there is an urgent need to identify the germplasm, augment/collect and conserve for improvement and for future uses.

MATERIALS AND METHODS

An exploration for bael germplasm was undertaken in parts of Faizabad, Basti, Gorakhpur, Deoria and Azamgarh districts of Uttar Pradesh and Gopalganj district of Bihar (Fig. 1) located between 82°-85° E longitude and 25°-27° N. latitude and twenty four genotypes including three wild types were identified. Among these, six located in Basti, thirteen in Deoria, two each in Gorakhpur and Azamgarh district of Uttar Pradesh and one in Gopalganj district of Bihar. The exploration was undertaken when fruits were physiologically mature and ripening started. The method of random sampling from a population and biased sampling after gathering information about a particular genotype was followed (Sinha, 1981). In general, germplasm were sampled from seedling origin trees having desirable qualitative parameters, viz., papery scull (paper type), less seeded, less mucilage content, attractive pulp colour, aromatic, sweet taste and soft pulp. Five fruits were randomly collected and observations for qualitative traits were recorded. The yield of the fruits per tree were calculated on the basis of average weight of the fruit. Range, mean and standard error for six quantitative characters were also computed.

RESULTS AND DISCUSSION

Considerable variability was observed in the germplasm for various characters viz., thorniness of the stem, tree height, time of initiation of ripening etc. Diversity was also noted in different morphological characters of fruits such as shape, scull thickness, pulp colour, pulp taste and mucilage content in the pulp (Table 1). The range, mean and C.D. of 24 genotypes for six quantitative characters are presented in table 2. Range for fruit length varied from 5.5 cm (MR/RD 887) to 16.5 cm (MR-889 and MR-890), fruit circumference from 12.5 cm (MR-888 and MR/RD-871) to 56.5 cm (MR-889), average fruit weight from 0.08 kg. (MR/RD-877) to 3.5 kg (MR-889), seeds per fruit from 46 (MR/RD-876) to 120 (MR/RD-877), number of fruits per tree from 60 (MR 883) to 3000 (MR/RD-877) and fruit yield per tree from 126 kg (MR 883) to 754.4 kg (MR/RD 875). Variances were high for number of fruits per tree and fruit yield. Considerable variance was also found for number of seeds per fruit and fruit circumference. Six genotypes were identified as promising for fruit size, (medium to large); scull thickness (thin and papery), and soft, deep yellow colour pulp having sweet and highly flavoured taste and less content of mucilage. Among these, MR/RD-876 (identified in Deoria district) showed its superiority in comparison to all other genotypes having very thin (papery) scull which can even be pressed by the finger at maturity. The pulp of this genotype is highly sweet with pleasant aroma, less quantity of mucilage and few number of seeds (46 per fruit). The tree is less thorny, with spreading branches and drooping growth habit.

The genotype identified at Tiwaripatti (MR-889) in Deoria district bore largest fruit size (length 16.5cm, circumference 56.5cm, and average weight 3.5kg). The pulp of this fruit is deep yellow, highly sweet and aromatic. Other four genotypes (MR/RD-869, MR/RD-870, MR/RD-872, MR/RD-874) identified as promising are located in Basti district of Uttar Pradesh. The genotype MR/RD-874 bore medium size fruit having 14.5 cm length, circumference 31.5 cm and average weight 16.5 kg. The fruits were of superior quality with less amount of mucilage and seeds in the pulp. It yielded 709.5 kg per tree and was the second highest yielding accession. Early maturing genotype MR/RD-869 has larger fruits, deep yellow sweet pulp, and paper scull. The genotype MR/RD-872 bore flattened round fruits. The scull of the fruit is papery and contain deep yellow pulp with moderate number of seeds (72/fruits). The tree has tall upright growth habit and less number of thorn on branches. However, landrace MR/RD-870 has medium size fruits. The scull of the fruit is papery, yellow colour, aromatic pulp having less mucilage.

Three wild genotypes (MR/RD-877, MR/RD-879, MR/RD-893) bearing small sized non-edible types were also identified. It was reported that fruits

Table 1. Characterization of Bael germplasm

Fruit traits									
Shape	Scull thickness	Pulp colour	Pulp taste	Mucilage	Tree height	Thorniness	First maturity	Organoleptic test/ Remarks	
MR/RD 869	Round	Thin	Deep Yellow	Highly sweet	Less	Tall	Few	Early March	Papery type, best quality
MR/RD 870	Round	Thin	Deep Yellow	Highly sweet	Less	Tall	Medium	Early March	Papery type, good quality
MR/RD 871	Round	Thin	Yellow	Highly sweet	Less	Medium Tall	Medium	Mid March	Papery type, good quality
MR/RD 872	Flattened	Thin	Deep Yellow	Highly sweet	Less	Med. tall	Few	Mid March	Papery type, best quality
MR/RD 873	Round	Thin	Deep Yellow	Highly sweet	Less	Med. tall	Medium	Mid March	Papery type, best quality
MR/RD 874	Round	Thin	Deep Yellow	Highly sweet	Less	Tall	Medium	Early March	Papery type, best quality
MR/RD 875	Oval	Thin	Light Yellow	Less sweet	Less	Tall	Medium	nd of March	papery type, good quality
MR/RD 876	Round	Thin	Deep Yellow	Highly sweet	Less	Medium tall	Few	Mid March	Papery type, best quality Very thin scull
MR/RD 877	Oval	Thick	Light Yellow	Bitter	High	Tall	Many	End of March	Hard scull-wild
MR/RD 878	Pyriiform	Medium	Deep Yellow	Medium sweet	Tall	Medium	Many	End of March	Hard scull, good quality
MR/RD 879	Round	Thick	Light Yellow	Bitter	High	Tall	Many	Mid March	Hard scull-wild
Contd.									

Contd.

Table 1. *Contd.*

Fruit traits									
Shape	Scull thickness	pulp colour	Pulp taste	Mucilage	Tree height	Thorniness	First maturity	Organoleptic test/ Remarks	
MR 880	Round	Thin	Light Yellow	Sweet	Less	Medium	Early March	Papery type, better quality	
MR 881	Round	Thin	Deep Yellow	Sweet	Less	Medium	Early March	Papery type, better quality	
MR 882	Round	Thin	Deep Yellow	Highly sweet	Less	Medium	Mid March	Papery type best quality	
MR 883	Oval	Thin	Deep Yellow	Highly sweet	Medium	Medium	Late March	Papery type, good quality	
MR 884	Oval	Thin	Deep Yellow	Highly sweet	Medium	Medium	Late March	Papery type, best quality	
MR 886	Round	Thin	Deep Yellow	Highly sweet	Medium	Medium	Late March	Papery type, best quality	
MR 887	Round	Thin	Deep Yellow	Sweet	Medium	Tall	Late March	Papery type, best quality	
MR 888	Oval	Thin	Deep Yellow	Sweet	Less	Medium	Late March	Papery type, best quality	
MR 889	Oval	Thin	Deep Yellow	Highly Sweet	Less	Tall	Late March	Papery type, best quality	
MR 890	Oval	Medium	Deep Yellow	Sweet	Less	Medium	Mid March	papery type, good quality	
MR 891	Round	Thin	Deep Yellow	Sweet	Less	Medium	Mid March	Papery type, good quality	
MR 892	Round	Thin	Deep Yellow	Highly sweet	Less	Medium	Mid March	Papery type, good quality	
MR 893	Oval	Thick	Orange	Bitter	High	Tall	End of March	Hard scull-wild	

Table 2 : Mean values and Range for six quantitative characters in bael

Accession number	Fruit length (cm)	Fruit circumference (cm)	Single fruit weight (Av. kg.)	No. of seeds/ fruit	No. of fruits/ tree	Fruit yield (kg.)
MR/RD 869	16.5	51.5	2.60	50	130	338.00
MR/RD 870	12.5	36.0	1.65	90	150	247.50
MR/RD 871	11.5	12.5	1.45	90	140	203.00
MR/RD 872	15.5	48.5	2.20	72	120	264.00
MR/RD 873	16.0	52.5	2.90	63	70	203.00
MR/RD 874	14.5	31.5	1.65	61	430	709.50
MR/RD 875	14.5	15.5	1.84	90	410	754.40
MR/RD 876	15.5	48.5	2.20	46	120	264.00
MR/RD 877	5.5	26.5	0.08	120	3000	240.00
MR/RD 878	11.5	36.5	0.90	95	200	180.00
MR/RD 879	9.0	22.0	0.28	74	610	170.80
MR 880	11.8	34.9	1.45	72	250	362.50
MR 881	10.0	25.5	0.70	96	400	280.00
MR 882	12.1	33.0	1.40	63	280	392.00
MR 883	16.5	42.1	2.10	52	60	126.00
MR 884	15.5	32.7	1.40	75	150	210.00
MR 886	14.5	30.5	1.10	72	310	341.00
MR 887	15.5	31.5	1.45	112	390	565.50
MR 888	15.8	12.5	1.00	105	380	380.00
MR 889	16.5	56.5	3.50	65	90	315.00
MR 890	16.5	32.5	1.80	92	210	378.00
MR 891	16.0	33.0	1.90	85	225	427.50
MR 892	14.5	29.5	2.40	82	130	312.00
MR 893	10.5	27.5	0.30	112	1250	375.00
Range	5.5 16.5	12.5 56.5	0.08 3.50	46.00 120.00	60.00 300.00	126.00 754.00
Mean ± SE	13.65 ± 0.59	33.46 ± 2.44	1.60 ± 0.17	83.50 ± 4.11	396.04 ± 124.05	345.36 ± 31.92
C.D.	1.72	7.10	0.49	11.96	360.98	92.88

of these genotypes causes unconsciousness, even if the smallest fruit is consumed. The genotype (MR/RD-877) is of spreading drooping habit with maximum number of fruits (about 3000 nos. per tree) which may be utilized as donor parent to increase the number of fruits. Another accession (MR/RD-879) is of tall growing habit and bore fruits having length 9.0cm, circumference 22.0cm and average weight 0.2.kg, whereas MR/RD-893 had fruits with 10.5 cm length, circumferences 27.5 cm and average weight 0.3kg.

The existence of variability for various traits can be utilised in the breeding programme of bael. Six lines described with desirable traits can be utilised in selection and further improvement of this fruit. Lines having few seeds per fruit, which is a desirable trait can be exploited directly.

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VARIABILITY AND ASSOCIATION STUDIES IN HOPS (*HUMULUS LUPULUS* L.)

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Hops are used by the brewing industry to impart desirable, bitter and aromatic taste and sparkle to the beer. Being photo-controlled plant, its cultivation is restricted to about 32 countries having temperate climate. Hops got established as a new cash crop in Kashmir and some parts of Himachal Pradesh (Bakshi and Atal, 1985). Different varieties were successfully cultivated on large scale. The present study was undertaken to determine the variability parameters, heritability and correlation between various quantitative traits of 28 genotypes.

Twenty-eight entries comprising 24 hop genotypes bearing pedigree Nos. RRL(H) 17 x Q57-2, RRL(H) 17 x Q63-8, RRL(H) 17 x Q63-14, RRL(H) 9 x Q49-8, RRL(H) 17 x Q57-6, RRL(H) 9 x Q49-1, RRL(H) 9 x Q57-14, RRL(H) 7 x Q57-1, RRL(H) 7 x Q57-2, RRL(H) 7 x Q57-3, RRL(H) 9 x Q18-9, RRL(H)6, RRL(H)54, RRL(H)20-13, RRL(H)4-60, RRL(H)4-111, PL208, PL246, PL320, PL324, PL433, PL442, PL576, PL597, and four standard varieties like *Late Cluster*, *Talisman*, *Comet*, *Hybrid-2* were incorporated in this study. These entries were grown in randomised block design with four replications at the experimental field of Regional Research Laboratory, Srinagar Branch, in the year 1987. Each plot consisted of five 10m long rows, spaced 2 m apart. Plant to plant distance was also kept 2m. The plants were trained in upright system with 4 bines per plant under 5.18 m high trellis. Observations were recorded on 10 randomly selected plants per plot in the year 1988 and 1989. The parameters included days to maturity, days to bines reaching top wire, length of laterals (cm), cone width (cm), number of cones per node on lateral, strig length (cm), number of floral axis on cone strig, oil (ml/100g), alpha acids (%), 100 cone weight (g). The pooled data for two years were subjected to analysis of variance (Panse & Sukhatme, 1957). Phenotypic, genotypic coefficient of variation; heritability and correlations were worked out as per standard procedure.

A wide range of variation was observed in number of characters. The range of alpha acids varied from (3.0-12.2) per cent with a mean value of 7.83 per cent. The weight of 100 cones and length of laterals varied from 50.0-122.0 g with an average value of 90.0 g and 51.8-146.92 cm with a mean value of 101.10 cm, respectively. It takes an average of 52.37 days for the bines to reach to top wire and 64 days for cones to mature from the date of burr initiation. The genotypic coefficient of variation was maximum for alpha acids (41.10), minimum for maturity days of cones (5.56) and days for bines to reach to top wire (3.94) (Table 1). Characters showing low genotypic coefficient of variation indicate

Table 1. Mean, range, coefficient of variability and heritability in hops

Characters	Mean	Range	Co-efficient of variability		Heritability (%)
			Pheno- typic	Geno- typic	
Maturity days	64.0	57-72	10.49	5.56	28.08
Bines reaching top wire (days)	52.37	49-56	5.96	3.94	43.56
Length of lateral (cm)	101.10	51.80-146.92	33.0	32.98	99.90
Cone width (cm)	2.28	1.90-2.90	5.63	11.28	51.47
Number of cones/node on laterals	7.25	4-10	28.70	28.40	97.73
Strig length (cm)	2.38	1.8-2.9	16.94	15.42	82.85
No. of floral axis on cone strig	9.37	7.0-12.80	21.53	21.29	97.77
Oil (ml/100 g)	0.57	0.32-0.87	42.75	31.47	54.17
Alpha acids (%)	7.83	3-12.20	41.21	41.10	99.45
100 cone weight (g)	90.00	50-122	25.73	24.58	91.22

that they were more influenced by the environment. Also little difference was observed between phenotypic and genotypic coefficient of variability in the alpha-acids, number of floral axis on cone strig, number of cones per node, 100 cone weight and length of laterals indicating that these characters respond less to environmental factors. The percentage of heritability in the broad sense was the highest for length of laterals (99.90), alpha-acids (99.45), number of floral axis on cone strig (97.77), number of cones per node on laterals (97.73) and 100 cone weight (91.22), and the lowest for maturity days of cones (28.08),

Table 2 : Correlation coefficients of various characters in hops

Characters	Bines reach- ing top wire (days)	Cone width (cm)	100 cone weight (g)	Number of cones/node on laterals	Length of laterals (cm)	Strig length (cm)	Number of floral axis on cone strig	Alpha acids (%)	Oil (ml/100g)
Maturity days	-0.1138	-0.1028	-0.0826	-0.0567	0.1967	0.4531	0.5970	0.1585	-0.2768
Bines reaching top wire (days)		-0.3664	0.0204	-0.6307	0.6604*	-0.1609	-0.3186	-0.4008	0.1666
Cone width (cm)			-0.2572	0.1807	-0.2881	-0.2879	-0.3914	-0.3974	0.0202
100 cone weight (g)				-0.0561	0.0543	0.2973	-0.0251	-0.5958	0.1409
No. of cones/node on laterals					-0.3921	0.0328	0.1724	0.6291	-0.6565*
Length of laterals (cm)						-0.3297	-0.4737	-0.7077*	-0.0291
Strig length (cm)							0.7541*	0.0069	-0.869
No. of floral axis on cone stig								0.2334	0.0171
Alpha acids (%)									0.0054

*Significant at 5%

and days to reach to top wire (43.56). Characters having high heritability value could be directly improved by selection since they are less affected by environment. Simple correlation was worked out between various characters. Most of the correlations were not significant. However positive and significant correlation was obtained between length of laterals and days for bines to reach to top wire ($r=0.6604$), strig length and number of floral axis on cone strig ($r= 0.7541$). A negative and non-significant correlation was observed between 100 cone weight and alpha acid content ($r= -0.5958$) which confirms earlier findings (Haunold *et al.*, 1983). It has also been reported that high alpha acid content and high cone yield are independent genetic traits (Haunold *et al.*, 1982; Romanko *et al.*, 1979). A negative and significant correlation was obtained between oil (ml/100g) and number of cones per node on laterals ($r=0.7077$) (Table 2). The negative correlation may be attributed to size and number of cones per node. With the absence of significant correlation, it would be possible to select lines in these crosses which are high yielding and are of superior quality.

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JERUSALEM ARTICHOKE — A POTENTIAL EDIBLE TUBER YIELDING PLANT

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Jerusalem artichoke (*Helianthus tuberosus* L.) — a tuberous under shrub of family Compositae is a native of North America. The name Jerusalem Artichoke, is considered to be a correction of the Italian Girasoli articoea, sunflower artichoke. According to Gray (1877), it originated in the valley of Mississippi from the species of sunflower, *H. doronicoides* Lam. This plant is cultivated in Europe, few parts of Asia in temperate regions of southern hemisphere. In India, it is found growing in W. Bengal, Assam, Maharashtra, Uttar Pradesh and Andhra Pradesh. It is well adapted to rich sandy or light loam and alluvial soils but does not thrive in wet and heavy soils. Although perennial in nature, it is treated as an annual under cultivation. This species is reported to have 13-18 per cent carbohydrate and 1.5-2.0 per cent protein (Anon, 1986) and is an economically useful species (Uphalf, 1959; Hedrick, 1981 and Singh *et al.*, 1983).

Tubers have multiple uses; as food, fodder, source of levulose and alcohol etc. Tubers can be eaten raw or boiled. They can be pickled, made into chips or ground into flour. Green tops and stems of young plants are used as feed for cattle. Tubers are used as feed for stock. Green stalks after treatment with soda chlorine process gives a pulp which is suitable for manufacture of certain type of papers. Tubers are rich in levulose which is used as a sweetening agent for diabetic patients. The carbohydrate portion of tuber is made up of insulin, hydrolysis of which will yield high fructose syrup which is good for diabetics. Tubers can be utilized for preparation of industrial alcohol through fermentation. Flowers of the plant are very beautiful, therefore it is grown as an ornamental also.

In order to assess the growth and tuber yield potential of exotic material, four exotic accessions of *Helianthus tuberosus* namely, EC 243070, EC 243071, EC 243073 and EC 243074 were introduced from France and

were evaluated for tuber yield and other growth characteristics during 1990 at the NBPGR Experimental Farm, Issapur. Healthy tubers were planted in two 4m long rows spaced 50 cm apart in the month of March. The distance between tubers was kept at 20 cm. The tubers were planted about 6 cm deep in well prepared beds. The tubers sprouted in 10-15 days. Two weeding were done in the early stages of growth. Irrigations were given as and when required. Earthing was done when the plants attained one foot height. The crop matured within six months and tubers were ready for harvesting when plants started drying up. It was observed that the tubers shrivelled up on exposure and cannot be kept out for more than a few weeks. Therefore, for storage it is advisable to leave tubers in soil until required.

The observations on various plant characters, namely plant height, stem thickness, branch number, leaf length, leaf width, tuber length, tuber width and tuber yield were recorded on ten randomly selected plants in each accession. The data revealed a good range of variability for most of the characters studies (Table 1). The range of variability for plant heights was 1.98-2.19 m, maximum being shown by EC 243071. Stem thickness ranged from 1.72-2.02 cm, the maximum being shown by EC 243071. Number of branches per plant varied from 11.0-12.2 maximum being shown by EC 243070. The leaf length and leaf width varied from 10.6-13.7 cm and 4.60-6.26cm respectively. The maximum being exhibited by EC 243074. The range variation in tuber length and tuber width was 8.0-20.5 cm and 2.6-7.5 cm respectively, the highest being recorded for EC 243073. Tuber yield per plot (4cm row) varied from 1.75-9.0 kg, the highest being recorded for EC 243073 followed by EC 243071.

Table 1. Variability for different characters in exotic accessions of Jerusalem artichoke

Accessions	Plant height (cm)	Stem thickness (cm)	Number of branches per plant (cm)	Leaf length (cm)	Leaf width (cm)	Tuber length (cm)	Tuber width (cm)	Tuber yield per 4 m row plot (kg)
EC 243070	219.0	1.99	12.2	11.7	6.12	8.0	2.6	1.75
EC 243071	208.2	2.02	12.0	10.8	5.00	14.2	4.4	4.75
EC 243073	226.2	1.94	11.4	10.6	4.60	20.5	7.5	9.00
EC 243074	198.0	1.72	11.0	13.7	6.26	11.3	3.8	2.50

The multiple uses, high tuber production potential and rapid propagation without much agricultural inputs qualify this species for economic use. Thus, it offers a good scope for cultivation in marginal areas and wastelands. Well planned efforts are required to be made to introduce more exotic germplasm and collect locally adapted tubers to build up the germplasm collection. This will enable extensive screening on the material so as to isolate promising desirable types for different eco-geographic situations. Efforts in this direction are going on in a phased manner.

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GENETIC DIVERGENCE IN SUGARCANE GERMPLASM

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Kovvur - 534 350

Fifty three sugarcane clones were evaluated for sugar yield and its components under coastal conditions of Andhra Pradesh with a view to identify elite donors for future breeding programme for east coast zone. Data recorded on 12 characters in each clone were subjected to Mahalanobis' D^2 analysis. The clones were grouped in various clusters by Tocher's method (Rao, 1952). These 53 clones were grouped into 12 clusters (Table 1). Cluster I comprised the maximum number (24) of clones followed by seven in cluster II. Clones Co 8348, Co 8354, Co 8356, Co 8361 and Co 8365 formed separate clusters (VIII to XII). Inter-cluster distances varied from 7.05 between cluster II and III to 20.17 between clusters III and XII. Clusters II, III, IV and XI were quite close to each other. Intra-cluster distances ranged from 0.00 to 6.96. The highest value was observed for cluster VII followed by cluster I and VI.

Cluster means (Table 2) indicated that cluster III had the highest stalk yield and sugar yield and acceptable juice quality (18.84% sucrose at 12 months), whereas cluster IX had the lowest stalk yield and sugar yields. This deviation was mainly attributed to number of millable canes (NMC) per plot. Comparatively lower stalk yield in cluster X was compensated with the best CCS per cent resulting in almost similar sugar yields in cluster III and X. Cluster VI comprised clones with the maximum mean sucrose per cent in juice but poor mean stalk yield. In cluster XII (Co 8365) stalk diameter and single stalk weight were the highest but NMC was low whereas in cluster VII-stalk diameter and single stalk weight were the lowest having the highest NMC.

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Table 1. Distribution of 53 sugarcane clones in different clusters

Cluster	No. of clones	Clones
I	24	Co 6907, Co 7219, Co 7542, Co 7704, Co 7907, Co 8013, Co 8014, Co 8021, Co 8343, Co 8351, Co 8352, Co 8357, Co 8358, Co 8363, Co 8364, Co 8366, Co 8367, Co 8368, Co 85032, Co 85041, Co 85268, Co 86047, Co 87045, Co 87263.
II	7	Co 7643, Co 8025, Co 8342, Co 8344, Co 8346, Co 8347, Co 85035
III	3	Co 7805, Co 8345, Co 8362
IV	4	Co 671, Co 8353, Co 8359, Co 8369
V	3	Co 6304, Co 85033, Co 85044
VI	2	Co 8020, Co 85045
VII	2	Co 7901, Co 85036
VIII	1	Co 8348
IX	1	Co 8354
X	1	Co 8356
XI	1	Co 8361
XII	1	Co 8365

The use of genotypes from clusters III and XII, III and IX, and VII and IX with the maximum diversity among themselves may yield better recombinants. Clusters VII and XII were complementary to each other for cane yield components and crossing among the clones from these two clusters will be advantageous for improving stalk yield and hence sugar yield of the progenies when the sucrose percent is maintained at parental level. As also advocated by Singh and Singh (1981), Gill and Tripathi (1983) and Hooda *et al.* (1990). A hybridization programme among clones belonging to different clusters having maximum distances will be fruitful to generate variability particularly for stalk yield components which will increase the selection efficiency among the progenies.

Table 2. Cluster means for 12 characters (stalk yield and quality) in sugarcane

Cluster	Germi- nation (%)	NMC	Stalk diameter (cm)	Stalk length (cm)	Single stalk weight (kg)	Stalk yield/ plot (kg)	Brix (%)	Sucrose (%) 10 mon.12 mon.	Purity (%)	CCS (%)	Sugar yield/ plot (kg)
I	53.1	111	2.46	274	0.95	103.2	22.03	18.13	19.54	88.68	13.52
II	65.5	151	2.41	267	0.88	130.6	21.10	17.91	18.87	89.10	13.13
III	69.2	156	2.65	286	1.10	170.6	20.85	17.87	18.84	89.63	13.30
IV	38.3	92	2.88	286	1.34	122.2	21.04	16.91	18.89	89.79	13.21
V	37.6	145	2.53	249	0.95	135.6	22.17	19.04	19.50	87.94	13.45
VI	43.5	73	2.30	230	0.76	54.7	23.00	20.16	20.33	88.40	14.04
VII	56.5	176	2.15	248	0.67	116.1	21.36	14.82	19.26	90.12	13.45
VIII	92.7	137	2.60	283	1.00	137.0	18.81	13.51	16.10	83.55	10.96
IX	58.3	56	2.80	108	0.90	51.6	21.36	17.33	18.80	88.00	12.98
X	39.7	106	2.65	304	1.50	158.8	22.45	18.89	20.17	89.86	14.06
XI	44.0	126	2.50	283	1.06	133.5	22.37	15.70	20.27	90.64	13.69
XII	28.0	63	3.05	324	1.50	92.8	23.07	15.78	20.24	87.73	13.84

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