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In-Vitro Technology for Genetic Conservation of Banana—A Case Study

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The present communication deals with the various in-vitro *techniques attempted to conserve the germplasm of banana. The major aspects included are rapid clonal propagation through meristem culture of several cultivars belonging to different genomic constitutions, their medium-term storage under minimal growth condition and finally somatic embryogenesis. Rapid* in-vitro *multiplication of banana has been achieved by culturing the explants in Murashige and Skoog's semi-solid basal medium supplemented with low concentration of indole acetic acid and a fairly high level of benzyl amino purine. The genomic constitution of the cultivar on one hand as well as the concentration of benzyl amino purine in the medium on the other hand seem to control the rate of multiplication of shoot buds. Eventually, the multiplication rate was greatly accelerated by increasing the concentration of benzyl amino purine in the medium. However, the presence of one or two 'B' genomes which came from* Musa balbisiana *causes an increase in the rate of multiplication as compared to the cultivars possessing only 'A' genome, from* Musa acuminata. *Regarding the* in-vitro *conservation of banana germplasm, established shoot tip cultures could be successfully stored for over two years in the same container without any transfer to fresh medium by simply reducing the incubation temperature and light intensity from 30° to 15°C and 1000 lux respectively. The survival percentage of various cultivars at different stages of conservation have been critically analysed. The regeneration of somatic embryos through the induction of callus and their further studies have been briefly described in the last phase of the paper. The somatic embryos, when carefully analysed histologically, exhibited striking similarities with their zygotic counterparts.*

Conservation of germplasm of banana means collection, proper identification of genomic constitution of the accessions, multiplication and finally their maintenance in a disease-free condition at national, international and regional levels. There are some aspects like maintenance of genetic stability and disease indexing which are also closely associated with the term conservation of germplasm.

Both dessert and cooking bananas (*Musa* species) are considered as a major staple food in the tropical and sub-tropical parts of the world, particularly in Africa, Central America and South-East Asia. The commercially grown bananas have evolved by various mechanisms from two species of *Musa* viz., *M. acuminata* and *M. balbisiana* carrying the so called 'A' and 'B' genomes, respectively. The whole new range of edible cultivars are mostly triploids and sterile. As far as the conservation of banana germplasm is concerned, there are two possible approaches to achieve this goal. One approach is through *in-situ* conservation and the other is through *in vitro* conservation. For *in-situ* conservation, a number of attempts have been made to increase the multiplication rates using various conservation techniques (Barker, 1959; Hamilton, 1965; Ascenso, 1967). Barker (1959) obtained an increased rate of multiplication of banana by stripping the older leaf sheaths from the pseudostem, thereby exposing the corresponding buds which were then covered with soil. In the field condition, banana plants show a strong apical dominance which means, as long as the mother plant is present, it will suppress the development of its lateral shoots. Immediately after the removal of the mother plant, the lateral shoots start elongating. Using this property, De Langhe (1961) was able to increase the production of plants simply by destroying the apical bud of the mother plant. All these conventional approaches have some serious limitations and disadvantages. Some of these are: (1) plants kept in the field are fully exposed to diseases like 'bunchy top', 'panama disease', 'black sigatoka' etc. and also to natural calamities like drought, flood, etc; (2) the rate of multiplication is not sufficiently high to meet the demand of the growing world population; and (3) a large number of germplasm requires lot of space and manpower for proper conservation in the field.

To overcome these disadvantages, scientists in the late seventies adopted *in vitro* approaches by which banana germplasm can be conserved with high multiplication rate in a much smaller area (Cronauer and Krikorian, 1984; Banerjee and De Langhe, 1985; Jarret *et al.*, 1985; Wong, 1986; Banerjee *et al.*, 1986; Vuylsteke and De Langhe, 1985; Banerjee *et al.*, 1987, Banerjee and Sharma, 1988).

In the present paper, three different approaches will be discussed regarding the conservation of 15 commercial cultivars of banana and plantain belonging to five genomic constitutions (AA, AAA, AAAA, AAB and ABB). These are :

(a) shoot tip culture at normal temperature,

(b) shoot tip culture at reduced temperature, and

(c) somatic embryogenesis through callus induction.

MATERIALS AND METHODS

The cultivars of banana and plantain belonging to various genomic constitutions which have been used in the present investigation are listed in Table 1.

TABLE 1. LIST OF BANANA CULTIVARS WITH THEIR GENOMIC CONSTITUTIONS

Cultivar	Genomic constitution
Pisang Lilin	AA
Pisang Tongat	AAA
Musa acuminata	AAA
Dwarf Cavendish	AAA
Silk	AAB
Valeri	AAB
Mululou	AAB
Asamiensa	AAB
Ntanga	AAB
Agbagba	AAB
Pisang Abu Perak	ABB
Espermo	ABB
Saba	ABB
Monthan	ABB
IC-2	AAAA

Meristem culture technique

For isolation of shoot meristems of banana, all possible meristem bearing plant parts e.g. suckers, peepers, dormant eyes as well as the parental pseudostems were used. Cubes of tissues measuring 2-4 cm were first surface sterilised with 0.1 per cent mercuric chloride solution for 15 min. Tissues were then thoroughly washed with sterile distilled water and 1-2 mm^3 small shoot meristems with 1-3 leaf primordia were excised aseptically from the sterilised tissue. Explants were cultured on Murashige and Skoog's basal medium (Murashige and Skoog, 1962) containing 3 per cent sucrose and 10 mg/l ascorbic acid which was added to reduce the tissue and media blackening possibly caused by oxidation of polyphenols.

For shoot outgrowth, the basal medium was supplemented with 0.2 mg/l indole acetic acid (IAA) and 0.2 mg/l benzyl amino purine (BAP) and, for the proliferation of shoot buds, BAP level was raised to 2 mg/l keeping the IAA level constant. For the induction of roots, 0.2 mg of indole butyric acid (IBA) was used in the medium with half strength of macrosalts. The cultures were incubated at 25°C under 16 h photoperiod of 3000 lux light intensity.

Low temperature storage

For low temperature, rapidly proliferating established shoot tip cultures were used. Tissues were kept in MS semi-solid medium supplemented with 0.2 mg IAA and 2 mg BAP. Three different low temperatures (5°, 10°, and 15°C) were tried to find out an optimum temperature suitable for medium-term storage. A constant illumination of 1000 lux intensity was given to the cultures.

Somatic embryogenesis

For the induction of callus, thin meristematic layers from the rapidly proliferating meristem cultures were carefully excised and cultured on MS basal medium

supplemented with various concentrations of 2, 4-D and 2, 4, 5-T. After induction, the callus was periodically transferred to liquid medium supplemented with different concentrations of NAA and BAP, IAA and BAP or no hormone for somatic embryogenesis. Bipolar embryos were histologically analysed and compared with their zygotic counterparts following standard microtome technique.

RESULTS AND DISCUSSION

Regeneration of plants

Small explants consisting of 2-3 leaf primordia turned green within 2-3 weeks of inoculation. Depending upon the concentration of BAP used in the medium, two distinct types of growth responses were noticed. With 0.2 mg/l IAA and BAP, single shoot outgrowth from excised tip was observed in all the tested cultivars. In this medium, the outermost leaf primordium slowly opened and unfurled the lamina within four weeks. Gradually, the inner leaf primordia emerged. Rooting of these young shoots could be achieved within two weeks in root induction medium where IBA (0.2 mg/l) was added as the only growth regulator. After root induction, small plantlets were transferred to flasks containing MS semi-solid medium with half concentration of inorganic salts supplemented with no growth hormones. Plantlets grew fast in this last phase of development (Plate I Fig. 1). Finally they were transferred to soil with 70-80 per cent survival (Plate I Fig. 2).

Proliferation of shoot meristem

Proliferation of meristems could be achieved by culturing the isolated explants on MS semi-solid medium supplemented with IAA (0.2 mg/l) and BAP (2 mg/l). A sharp difference in the rate of proliferation as well as in the proliferative growth was noticed in different cultivars depending upon the genomic constitution. In the diploid (Pisang Lilin), triploid (Dwarf Cavendish) and tetraploid (IC-2) cultivars possessing only A genome, the rate of meristem proliferation was rather low which was manifested by the formation of a bunch of leafy shoots (Figs. 3 and 4) while the hybrid types with AAB (Asamiensa) and ABB (Bluggoe), the proliferation rates were high with the formation of fleshy, white bulbous structures each in turn, bearing a number of tiny meristems on their surface (Fig. 5). From the rates of proliferation recorded in each cultivar from first to fifteenth subculture, the cultivars with ABB genomic constitution exhibited very high rates which went upto 30-35 tips/explant. On the contrary, diploid, tetraploid and triploid *acuminata* types showed low rates ranging from 5-10 tips/explant. The AAB cultivars showed intermediate rates, usually ranging from 10-25 tips/explant. It was proved statistically by two-way Anova test that a high level of significant difference exists in the rates of proliferation between different cultivars in the same subculture and between subcultures of the same cultivar (Banerjee and Sharma, 1988).

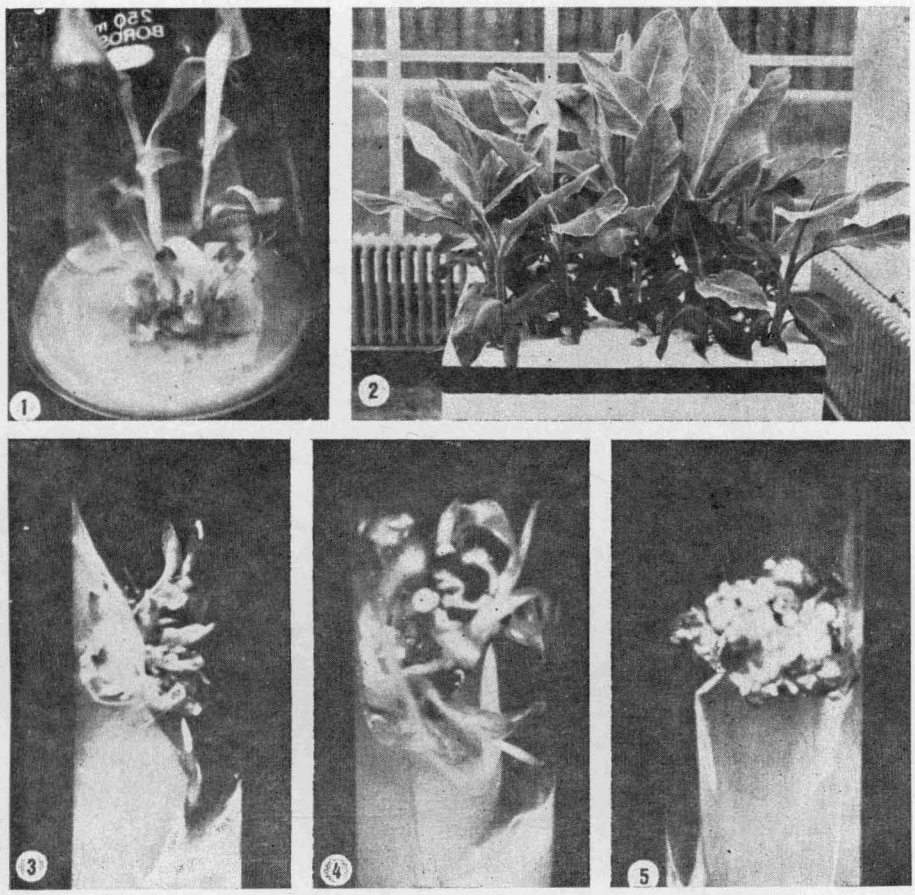

Plate I Fig. 1. Regeneration of rooted plantlets of *Musa* cv. Espermo (ABB).

Fig. 2. *In-vitro* plantlets transferred to soil.

Fig. 3. Proliferation of shoot-tips in *Musa* cv. Dwarf Cavendish (AAA) possessing only 'A' genome :

Fig. 4. Proliferation of shoot-tips in *Musa* cv. Pisang Linn (AA) possessing only 'A' genome

Fig. 5. Proliferation of shoot meristems in *Musa* cv. Espermo (ABB).

In spite of the significant difference in the proliferation rates between various genotypes, the regeneration potential for each cultivar did not show any significant decrease with increase in the age of culture. In other words, complete plantlets can be readily regenerated from each of these tiny meristem tips regardless of the age of the culture.

One very interesting feature that has been noticed regarding the influence of B genome on the acceleration of proliferative capacity was that the increase in dosage of B genome as in 'Bluggoe' leads to further acceleration of proliferative

capacity as compared to that of the cultivar 'Silk' (AAB), whereas in cultivars with A genome, the addition of A does neither retard nor accelerate the rates (Banerjee and Sharma, 1988).

As far as the ontogenesis of shoot meristem proliferation is concerned, these multiple buds developed from a uniform meristematic layer of cells which originated through rapid extension and subsequent flatening of the initial meristematic dome. These newly generated shoot primordia repeated the same ontogenetic pathway and ultimately gave rise to a number of primordia. This process continued as long as the tissues were kept in shoot proliferation medium (Benerjee *et al.*, 1986).

In-vitro storage

There are several possible approaches to achieve minimal growth of cultures. The most important ones are manipulation of culture medium, addition of growth retardants like ABA and incubation of cultures at reduced temperature and low light intensity. One of the principal objectives of storage experiments would be to slow down the growth rate and extend the subculturing intervals from normal 4-6 weeks to a much-longer period, for example, once a year or more.

In the present investigation, attempts have been made to store the meristem cultures of several cultivars of banana having various genomic constitutions at different reduced temperatures, e.g. 5°, 10°, 15°C. The cultures were kept in the shoot proliferation medium in presence of 1000 lux light intensity. The problem of medium dessication has been overcome by sealing the plugs with parafilm without any harmful effect.

One of the three reduced temperatures, (5°C) was found too cold for banana and as a result none of the cultivars survived beyond 6-8 weeks. At 10°C, the cultures survived longer but even this temperature was not very suitable for medium-term storage. The most satisfactory result was achieved at 15°C where the cultures remained healthy for 15-18 months depending on the genomic constitutions of the cultivars. Thus, it was observed that AAB and ABB cultivars were more tolerant to low temperatures than the cultivars with only A genome. After 14-16 months of continuous storage at 15°C, AAB and ABB cultivars showed a survival percentage of 66-100 per cent while the survival percentage of A type cultivars came down to 25-35 per cent.

In the last two decades, a number of research papers have been published on the progress of *in-vitro* storage of several plants, mostly temperate species, using different storage techniques. Medium term storage has been successfully achieved using low temperature incubation technique in various plant species, such as strawberry 6 years (Mullin and Schlegel, 1976), apple 12 months (Lundergan and Janick, 1979), potato (Westcott, 1981), *Lolium multiflorum* (Dale, 1980), *Trifolium pratens* (Cheyne and Dale, 1980), *Trifolium repens* (Bhojwani, 1981). Although reduced temperature in the range of 5°-10°C have been found suitable for temperate species, meristem cultures of tropical species like sweet potato

(*Ipomoea batatas*) show considerable chilling injury at these temperatures. However, satisfactory reduction in growth rate has been achieved at 15°-18°C (Alan, 1979). These results are in agreement with our results where meristem cultures of different genotypes of *Musa* suffer from chilling damage during incubation at 5°-10°C. However, some other tropical crops like *Colocasia esculantum* have been successfully stored *in-vitro* through meristem culture at 9°-10°C for three years (Staristsky *et al.*, 1986).

Somatic embryogenesis

In banana, the induction of callus is extremely difficult, simply because the explant tissue which may be leaf, shoot, root or even flower bud turns black when cultured on semisolid or liquid medium containing growth regulators. The blackening and subsequent killing of the explant is presumably caused by heavy amount of oxidised polyphenols released by the tissue itself. Addition of antioxidants in the medium does not help.

To overcome this problem of blackening, thin meristematic layers of cells were taken from the meristem cultures as explant for the induction of callus since meristem cultures after few passages show much less blackening. Several concentrations of 2, 4-D were tested for the induction of callus and the best results were obtained with the concentration 1.25 mg/1. The calli induced after 4-6 weeks exhibited slow growth initially. On prolonged incubation in the same medium, numerous globular proembryo-like structures were formed. The proembryos were sloughed off from the rest of the callus mass when transferred to liquid medium. After 2-3 weeks, these proembryos turned green but no leaf development was observed.

On the other hand, explants when cultured on MS semi-solid medium supplemented with 2, 4, 5-T (1.25 mg/1) produced numerous globular proembryoid on the surface of the callus after 7-9 weeks. Transferring them in liquid culture medium containing NAA (0.02 mg/l) and BAP (0.25 mg/l) yielded bipolar embryos within 2-3 weeks. In these somatic embryoids, both shoot and root development could be observed. Histological analysis of the somatic and zygotic embryos revealed some interesting results. The seed embryos were characteristically mushroom shaped where the cap-like structure was the haustorium which constituted the major part of the cotyledon. The stalk-like part represents the epicotyl-hypocotyl-radicle axis. The most interesting thing is that the shoot and root apical meristems always lie at an angle of more or less 90° which is a characteristic feature of the family *Musaceae*. The somatic embryos also revealed bipolarity with distinct root and shoot meristems connected by strands of vascular tissues. They are characteristically enclosed by an epidermal layer of cells. Moreover, the hook-like formation of shoot and root meristem axis was clearly observed in these embryoids (Banerjee *et al.*, 1987).

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In-Vitro Conservation of Some Endangered Plant Species of India

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Procedures for in-vitro *multiplication and short-term conservation of 3 endangered medicinal plants, viz.,* Saussurea lappa. Picrorhiza kurroa *and* Podophyllum hexandrum *are reported. While first two species were multiplied via shoot multiplication, the third species was multiplied* via *both shoot multiplication and somatic embryogenesis. On MS + BAP (5×10^{-6} M) + GA_3 (3×10^{-6} M) shoots of* S. lappa *proliferated at a rate of 3.5 fold every 3 weeks and 90 per cent cultures of these shoots rooted in MS + NAA (5×10^{-7} M).* P. kurroa *multiplied 36-fold every 30 days on MS + BAP (1×10^{-6} M). Similarly, shoots of* P. hexandrum *muliplied 8-fold in 4 weeks on MS + BAP (2×10^{-6} M). The callus derived from zygotic embryos of* P. hexandrum *on MS + BAP (2×10^{-6} M) + IAA (5×10^{-7} M) differentiated globular embryos. Further development of these embryos occurred on MS + NAA and the somatic embros germinated on MS with or without growth regulators. The shoot cultures and embryogenic calli stored at 5°C in dark for 7 to 12 months without any intervening subculture survived with 70 to 100 per cent viability.*

Indiscriminate exploitation of natural resources, destruction of natural habitats and spread of harmful chemicals are some of the human activities responsible for the deterioration of natural ecosystem. It is estimated that about 1500 plant species are threatened to extinction in India (Nayar and Sastry, 1987). Of these, about 100 species have been declared as rare plants. The growing awareness of dreadful consequences of uncontrolled destruction of plant types has led to the establishment of national and international bodies to examine the seriousness of the problem and suggest measures to control it. At this stage of belated realization of the problem, a two-pronged approach is required. Firstly, the natural ecosystem and habitats should be protected and preserved by establishment of natural reserves in order to check further loss of species due to human activities. Secondly, the taxa whose number has fallen to such critical level that they are to be regarded as threatened or endangered under natural habitat should be preserved under safe, artificial conditions which would also allow their rapid multiplication for reintroduction into the wild. Plant tissue culture fulfills the requirements of the second approach.

In tissue cultures genotypes can be maintained as actively prolifering cells, somatic embryos or shoots through regular subcultures. The necessity of frequent subculture may be avoided by storing the cultures under growth-limiting condi-

tions, such as low temperature (5°-12°C) and the application of growth retardants through the culture medium (Westcott, 1981; Henshaw and O'Hara, 1983). Alternatively, cultured cells may be stored for long-term at cryogenic temperature (Kartha, 1984; Withers and Williams, 1986). It is now well established that plant cells can withstand —196°C, and plants have been regenerated from tips of pea and strawberry cryopreserved for over two years.

Under an All India Co-ordinated Project, sponsored by the Department of Environment, possibilities of *in-vitro* conservations of some endangered plant species were examined. The paper describes our observation on *in-vitro* multiplication of three endangered species of medicinal important, viz., *Podophyllum hexandrum*, *Picrorhiza kurroa* and *Saussurea lappa*. Attempts have also been made to preserve the cultures of these species at 5°C in dark for short-term.

MATERIALS AND METHODS

Plant material

Seeds of *Saussurea lappa* C.B. Clarke were obtained through the courtesy of the Lahul Kuth Growers Co-operative Marketing Society Ltd., Manali. Live plants with mature fruits of *Podophyllum hexandrum* Royle, and vegetative parts of *Picrorhiza kurroa* Royle ex Benth. were collected from Valley of Flowers, Ghangarea, Hemkund and Kedarnath regions (Altitude 3000 m-5000 m) in Uttar Pradesh, India.

Initiation of aseptic cultures

Seed and vegetative parts were sterilized with 0.2 per cent solution of mercuric chloride for 5-10 min. and were washed thoroughly in sterile distilled water 2-3 times. The seeds and *ca* 5 mm long leaf, root, rhizome segments and nodal and terminal cuttings were planted aseptically on the nutrient medium. In the case of *P. hexandrum*, in addition, decoated seeds and excised embryos (*ca* 3 mm long) were also cultured. In the case of *S. lappa* cotyledons, leaf, hypocotyl and root segments from 15 days-old aseptic seedlings were used to initiate cultures.

Shoot multiplication

Approximately, 5 mm long shoots with 1 or 2 young leaves from aseptic cultures were used to multiply the shoots *in-vitro*.

Somatic embryogenesis

Approximately 150 mg (fresh weight) of embryogenic calli was used as inoculum.

Culture medium

Murashige and Skoog's (1962) medium containing 3 per cent sucrose gelled with 0.8 per cent agar, hereafter referred to as MS, was used as basal medium. Depending upon the experiment, the MS was supplemented variously with benzylamino purine (BAP), 2-isopentenyl adenine (2ip), kinetin (Kn), 2,4-dichlorophenoxyacetic

acid (2,4-D), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) and gibberellic acid (GA_3). All the constituents were adjusted to pH 5.8. The molten medium was dispensed in 150 mm × 25 mm tubes (20 ml/tube), and plugged with non-absorbent cotton wrapped in a layer of cheese cloth and autoclaved at 121°C for 15 min.

Culture conditions

P. hexandrum cultures were normally maintained at 25 ± 2°C in 16 h photoperiod with 9.5 W/m² irradiance provided by cool, white, fluorescent tubes (Phillips TL 40 W/54). Cultures of *S. lappa* and *P. kurroa* were kept under continuous light. For cold storage, cultures were kept at 5°C in dark in a refrigerator for various periods.

RESULTS

Saussurea lappa C.B. Clarke

Commonly known as 'Kuth', *S. lappa* (Asteraceae), grows as a perennial herb in the Western Himalayas at an altitude of 2500-3000m. The dried roots of Kuth are used to cure cough, fever, asthma, rheumatism and skin diseases. The roots also yield an essential oil (costus oil) which is used for blending in high class perfumery. Owing to over exploitation of its natural populations for commerce and declining area under cultivation, *S. lappa* has become a threatened species in this country. The Kuth growers are shifting to potato cultivation solely for economic reasons.

Seed germination

On MS basal medium 30 per cent seeds germinated. Onset of germination was observed within 3 days of culture and a 15-day old seedling had two dark green and spathulate cotyledons, a long hypocotyl and a tap root. Up to two plumular leaves developed after eight weeks.

Regenerations

Cotyledons, hypocotyl, root and leaf segments from 15-day old seedlings were used to examine their potentiality for regeneration. While cotyledons and leaf explants differentiated shoot buds, the hypocotyl and root explants exhibited only callusing.

The cotyledons cultured on MS + BAP (5×10^{-6} M) + IAA (1×10^{-7} M) enlarged and callused at the cut ends. The callus was creamish, hard and nodular. When the callus and the parent explants were separately transferred to fresh medium of the same composition, both of them differentiated shoot buds, with calli showing higher regeneration (50 per cent) than the cotyledon pieces (25 per cent).

The leaf pieces cultured on MS + BAP (5×10^{-6} M) + IAA (1×10^{-7} M)

enlarged and formed nodular callus at cut end within one week. By the end of the 4th week, 70 per cent of these cultures differentiated adventitious shoot buds (Fig. 1a). The maximum number of buds per 5mm² explant was 8.

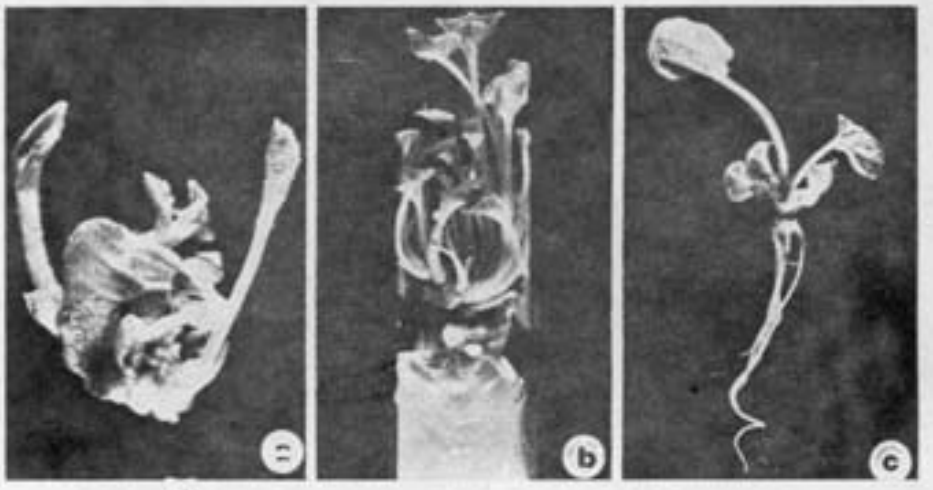

Fig. 1. *Saussurea lappa*

a : A 3-week old culture of leaf segment on MS + BAP (5×10^{-6} M) + IAA (10^{-5} M), showing differentiation of shoot buds from the cut end.

b : A cluster of shoots derived from a single adventitious shoot cultured on MS + BAP (5×10^{-6} M) + GA_3 (3×10^{-6} M) for 3 weeks.

c : An *in-vitro* multiplied shoot rooted on MS + NAA (5×10^{-7} M).

Shoot multiplication

The shoot buds differentiated from cotyledons and leaf pieces as terminal buds from aspectic seedlings were cultured on MS medium variously supplemented with growth regulators to achieve shoot multiplication. On MS + 3×10^{-6} M BAP, 3-4 healthy leaves developed and a hard nodular callus was formed at the basal cut end of the explant.

Shoot multiplication occurred in the combined presence of BAP (5×10^{-6} M) and GA_3 (3×10^{-6} M) (Fig. 1b). After one week of culture, a hard, slow growing callus developed at the basal cut end, which later differentiated shoot buds. On this medium, shoots have been multiplied for two years at a rate of 3.5 times every 3 weeks.

Rooting of shoots

Rooting of shoots occurred only in the presence of an auxin (Fig. 1c). NAA (5×10^{-7} M, 1×10^{-5}M, 1×10^{-6} M) induced rooting in 90 per cent cultures within 30 days. The roots were white, thick and hairy. The rooting was associat-

ed with callusing, the intensity of which was directly related to the concentration of the auxin. Thus, full plantlets have been established from *in-vitro* multiplied shoots.

Short term storage of shoot cultures

Seventy two *in-vitro* grown shoots were individually transferred to MS + BAP (5×10^{-6} M) + GA_3 (3×10^{-6} M) and shifted to cold storage after one week growth under culture room conditions (25°C, 16 h photoperiod). At monthly intervals, 5 cultures were withdrawn and placed under standard culture root conditions. The shoots cold stored up to 12 months remained fully viable. During cold storage, the original leaves dried and 1-2 new shoots with etiolated leaves had developed. The leaves turned green within a week after transfer to light. Individual shoots from these cultures exhibited a higher rate of multiplication (4.5 fold/4 week) under culture room condition than those not subjected to cold temperature.

Podophyllum hexandrum Royle

Commonly known as Himalayan May-apple, *P. hexandru* (Podophyllace) grow as a perennial rhizomatous herb in the inner ranges of Himalayas at an altitude of 2700 to 4200 m. The rhixome is a source of the resin (podophyllin) which contains the anticancerous principle, podophyllotoxin. While the resin is used for its purgative properties, the podophyllotoxin is used as a starting material for the production of certain semisynthetic anticancerous drugs. Resin from the Indian species yields higher amount of (40 per cent) podophyllotoxin, than the resin from the American species *P. peltatum* (10 per cent). Due to lack of organised cultivation and intense collection of its rhizome from the forests, *P. hexandrum* has become a threatened species.

Culture initiation

None of the vegetative parts responded in culture, except for young leaf segments showing little swelling on MS + 2,4-D (1×10^{-5} M) + BAP (1×10^{-6}). Similarly, the seeds and decoated seeds failed to show any change even after 8 weeks. The excised embryos, however, showed over 90 per cent germination within 7 days on MS basal medium. A 4-wk old seedling comprised two green cotyledons with free terminal lobes fused at the base forming a tube (CT) and a long tap root. The plumule situated at the base of the CT did not develop any leaf even after 3 subcultures. Even in nature the plant remains in seedling stage during the first year, and the first leaf emerges only during the second season (Badhwar and Sharma, 1963).

On MS + BAP (2×10^{-6} M) and MS + BAP (2×10^{-6} M) + IAA (5×10^{-7} M), the embryos showed expansion of cotyledons and slight elongation of the CT but root development was completely suppressed. Within 3 weeks the base of the CT became swollen and differentiated certain leafy structures on the surface

which later developed into normal shoots. In the presence of BAP and IAA, a nodular and compact callus also developed at the base of the CT. Shoots were subcultured for shoot multiplication and the calli were used to induce somatic embryogenesis.

Shoot multiplication

The adventitious shoot buds with one or two young leaves and a swollen base were excised from embryo cultures on MS + BAP and MS + BAP + IAA and transferred to MS supplemented with BAP alone at 3 different concentrations (2×10^{-6} M, 5×10^{-6} M, 1×10^{-5} M). In the first two weeks the petiole elongated, lamina expanded and the basal swelling enlarged. During the third week, white shoot buds emerged from the swollen base. Maximum proliferation of shoots (8 fold in 4 weeks) occurred in the presence of 2×10^{-6} M BAP (Fig. 2a).

Rooting of shoots

Satisfactory rooting of shoots is yet to be achieved. Only 25 per cent of the shoots developed 1 or 2 short, thick roots on MS (with 6 per cent sucrose) + 2×10^{-6} M BAP. Auxins, such as IAA, NAA and IBA were also tried for rooting but without any success.

Somatic embryogenesis

The calli from embryo cultures became friable and creamish, and in 70 per cent of the cultures abnormal looking leafy structures developed by the end of the fourth passage on MS + BAP + IAA. When transferred to MS + 2,4-D (1×10^{-5} M) + BAP (1×10^{-6} M), the calli turned slightly compact, light brown and differentiated a large number of globular embryos (Fig. 2b). On this medium proliferation of globular embryos continued but they failed to mature.

In order to induce further maturation, the globular embryos were transferred to MS supplemented with lower concentrations of 2,4-D or NAA ($1 \times 10^{-6} - 1 \times 10^{-5}$ M) and the cultures were maintained in dark. While the globular embryos proliferated on all the media, further maturation of embryo though heart, torpedo and dicotyledonous stages occurred only on media containing NAA (Fig. 2c). Maximum number of embryos (12/culture) were formed with 1×10^{-6} M NAA. Pluricotyledony, fused cotyledons and production of secondary embryos were common in these cultures.

Somatic embryos germinated on a range of media. On basal medium, a 4-wk old seedling possessed features similar to a zygotic seedling (Fig. 2d). Normal looking seedlings transferred to vermiculite : soil (1 : 1) mixture at 20°C in light remain and healthy up to 4 weeks, but none of the seedlings developed a plumular leaf.

Short-term Storage of Somatic Embryos

A preliminary study was undertaken on the storage of *Podophyllum* germplasm

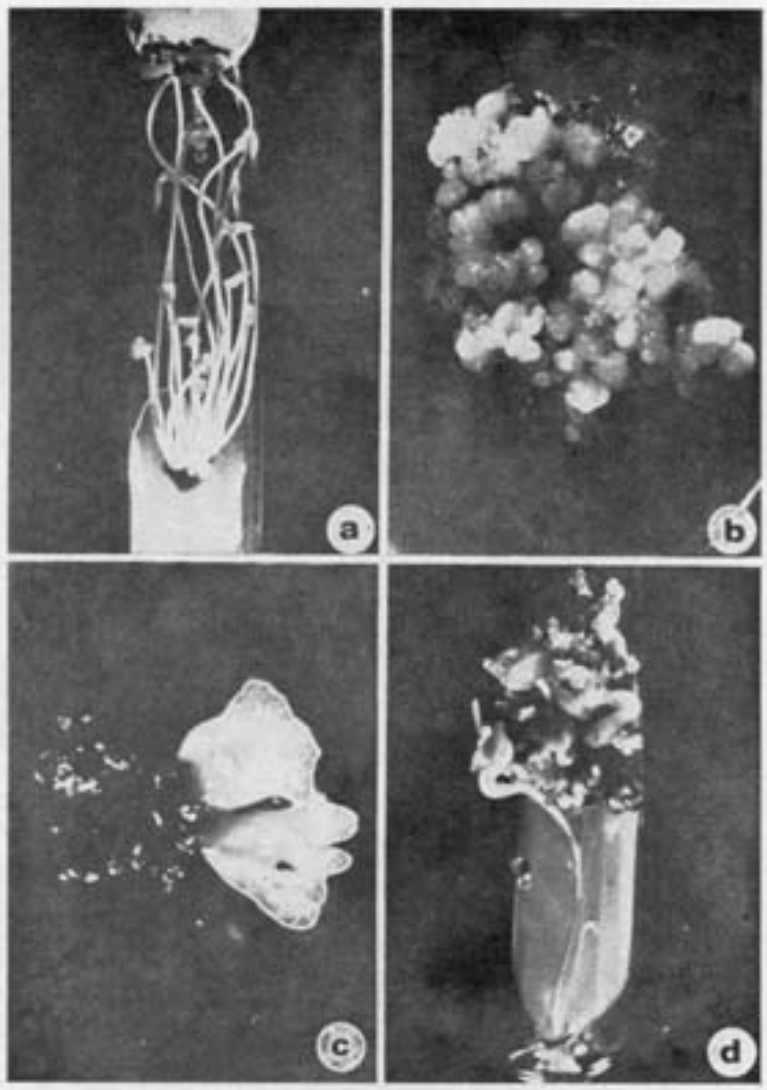

Fig. 2. *Podophyllum hexandrum*

a : A 4-week old shoot culture on MS + BAP (2×10^{-6} M).

b : Embryo callus cultured on MS + 2, 4—D (1×10^{-5} M) + BAP (1×10^{-6} M) for 4 weeks has developed numerous globular embryos.

c : Typical dicotyledonous somatic embryos attached to a piece of callus. Embryo maturation occurred on MS + NAA (2.5×10^{-6} M).

d : Somatic embryos germinated on MS basal seedling comprised two cotyledons whose base has fused to term a cotyledogary tube and a long tap root.

in the form of callus and somatic embryos at low temperature. Embryogenic calli cold stored for 7 months remained viable (95 per cent) and differentiated globullar embryos.

Picrorhiza kurroa Royal ex Benth.

Commonly known as Kutki, *P. kurroa,* (Scrophulariaceae) grows as a low hairy herb with a perennial woody rhizome, in the inner ranges of the Western Himalayas at an altitude of 3000-5000 m. The dried roots and rhizome of this plant are the source of a bitter tonic used in modern medicine as stomachic, purgative, and in dyspepsia and fever. Owing to over exploitation and the absence of systematic cultivation this species has become endangered. For the present study, the vegetative material was collected from Hemkund and Kedarnath regions of the Western Himalayas at an altitude of 4000 m.

Initiation of Aseptic Cultures

Surface sterilised terminal and nodal cuttings, measuring *ca* 2 cm, were cultured on MS, MS + BAP (5×10^{-6} M, 1×10^{-5} M, 5×10^{-5}) and MS + 2,4-D (1×10^{-6} M, 5×10^{-6} M). The sprouting of the cutting occurred only on BAP-containing media (Fig. 3A). On MS + BAP (5×10^{-6} M), the segments developed 2-3 leaves but the shoots failed to grow further. However, when these cuttings were transferred to MS with 3×10^{-6} BAP, the shoots resumed growth.

Shoot multiplication

The shoots developed in primary cultures on MS + 3×10^{-6} M BAP were excised and cultured on fresh media to test the effect of a range of concentrations of BAP, 2ip and kinetin on shoot proliferation. The cytokins were tried individually and in combination with IAA or GA_3. Shoot multiplication through forced axillary branching occurred in all the media. BAP at 1×10^{-6} M was found to be the best treatment (Fig. 3b). On an average, 12.6 shoots developed in 30 days on this medium. Higher levels of BAP were toxic. On MS + BAP (1×10^{-6} M) shoots have been multiplied for 14 months. At the end of each passage of 30 days, the long shoots were cut to prepared *ca* 2 cm long terminal and nodal cuttings (propagules) and individually transferred to fresh medium. Considering the number of propagules derived from each culture, a rate of 36-fold multiplication every 30 days was achieved.

Rooting of shoots

Attempts to root the shoots *in-vivo* were unsuccessful. However, 89 per cent of cultures on MS + 1×10^{-6} M NAA formed well developed roots (Fig. 3c), On this medium roots emerged within 20 days after culture and at the end of 30 days each shoot developed on an average 6 white stout roots. Increase in auxin level disturbed the health of the plantlet and in most cases induced callusing at the basal end of the shoot.

Short-term storage of shoot cultures

Individual shoots from MS + BAP (1×10^{-6} M) were transferred to fresh medium and after a week of acclimatisation under culture room conditions, they

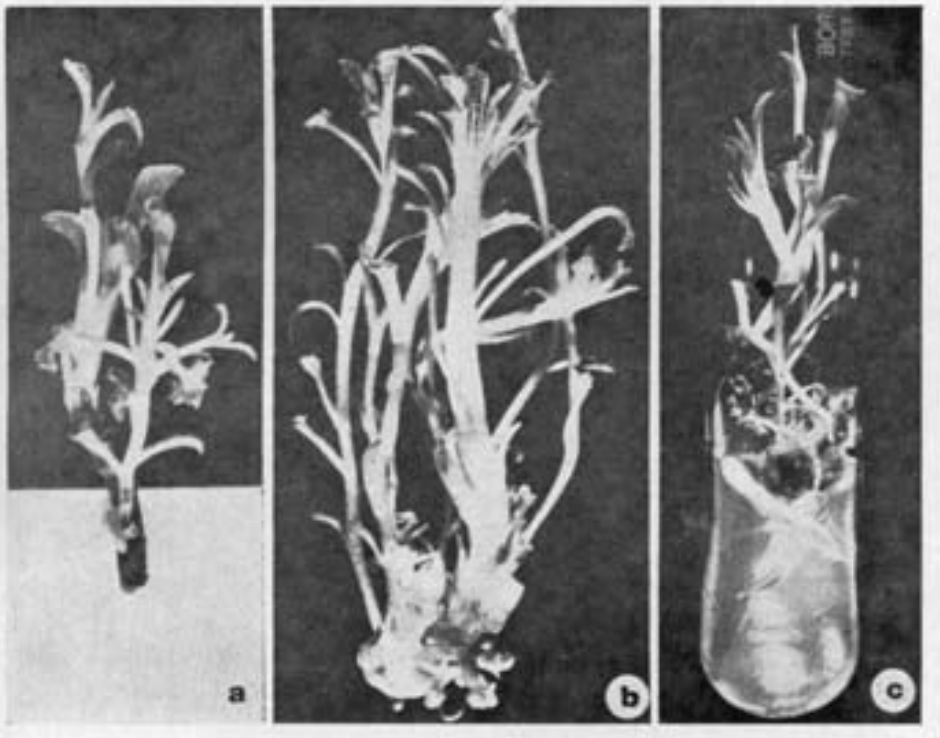

Fig. 3. *Picrorhiza kurroa*

a : Terminal shoot cutting cultured on MS + BAP (1×10^{-4} M) for 20 days, showing sprouting of terminal and an axillary bud.

b : A 15-day old proliferating shoot culture on MS + BAP (1×10^{-4} M).

c : An *in-vitro* formed shoot rooted on MS + NAA (1×19^{-4} M).

were transferred to cold storage conditions. The shoots received after 9 months of cold storage showed 70 per cent survival. A limited multiplication of shoots occurred at low temperature. When shifted to 25°C under 16 h light, viable cultures showed shoot multiplication comparable to the cultures maintained under culture room conditions throughout.

Shoot tip cultures

Shoot tips and globular embryos are the most suitable materials for long-term storage of plant materials at the super-low temperature (—196°C) of liquid nitrogen. However, cryopreservation of shoot tips for germplasm storage requires a protocol for high frequency plant regeneration from excised shoot tips. Therefore, an experiment was conducted to study the regeneration potential of shoot tips. Sub-millimeter shoot tips consisting of an apical dome and one or two leaf

primordia were excised from *in vitro* multiplied shoots, with the aid of a stereoscope, and cultured on MS, MS + 1×10^{-7} M IAA. Shoot generation occurred on all the three media, but MS + BAP (1×10^{-7} M) gave maximum regeneration (69 per cent).

DISCUSSION

In vitro multiplication of three important endangered medicinal plant species has been achieved. *Podophyllum hexandrum* has been multiplied through somatic embryogenesis and through shoot multiplication. *Saussurea lappa* and *Picrohiza kurroa* have been multiplied through shoot proliferation. Preliminary results with the storage of globular embryos of *Podophyllum* and shoot cultures of *Saussurea* and *Picrohiza* at low temperature are encouraging. Globular embryos and shoot tips will be subjected to cryopreservation for long term conservation of these species.

All the three species studied in the present work are new to tissue culture. Only one paper has been published on tissue culture of *P. kurroa* by Lal *et al.*, (1988). However, our observations are at variance with their results. This could be due to ecotypic differences in the plant materials used in the two studies. While we collected the plants from Hemkund-Kedarnath regions in Uttar Pradesh, Lal *et al.* (1988), obtained their material from Manikaran in Himachal Pradesh. Substantial ecotypic differences in *in vitro* response of *Trifolium repen* have been reported by Mahapatra and Gresshoff (1982).

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Use of Tissue Culture Technology in *Vanilla* and Possibilities of Germplasm Conservation

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Plantlets were produced from tip cultures of young aerial roots of Vanilla planifolia. *With the breakdown of the root cap, the cells of the quiescent centre divided forming a hemispherical mass of cells which developed either into a single shoot meristem or organized themselves into several meristems bearing leaf primordia. After a few leaves have been formed a root meristem differentiated establishing a well formed plantlet. Since the constituent cells of the root apical meristems are genetically stable, less differentiated and permit plant regeneration in high frequency, they form an ideal material for the long-term preservation of germplasm.*

Renewed interest in recent years in *Vanilla* (*Vanilla planifolia* (Salisb.) Ames (Family Orchidaceae), the natural source of the flavouring and spice, has led to increased interest in improved techniques of cultivation and propagation (Philip & Nainar, 1986, 1988a, b, c). *V. planifolia* produces numerous minute seeds but hardly 1-2 per cent of them germinate under natural conditions. Hence, the crop is commercially propagated by means of long-stem cuttings. Preservation of germplasm in such vegetatively propagated crops is highly taxing on manpower and land resources. Furthermore, a major threat to germplasm collections of *Vanilla* is a root-rot disease caused by *Fusarium batatatis* var. *vanillae* Tucker which often has a devastating effect and completely destroys the plantation.

Meristem culture is a proven technique for the production of disease-free plants and their clonal propagation. While meristem culture ensures short-term storage of germplasm, cryopreservation of meristem can offer an ideal and realistic method for the long-term storage of germplasm in a genetically stable and disease-free condition (Kartha, 1981). However, the current technique of meristem culture and cryopreservation often necessitates the removal of the shoot apex and in monopodial orchids like *Vanilla*, this causes a setback in the growth and development of the mother plant, limiting the availability of shoot meristems. Furthermore, shoot apical meristems are enclosed in many whorls of leaves and require cumbersome aseptic manipulations to dissect out the meristematic domes. Clearly, the development of *in-vitro* techniques of rapid and large scale multiplication of disease-free and genetically stable plants from apical meristems of aerial roots form one of the pre-requisites before an effective method of cryopreservation can be considered in *Vanilla*.

MATERIALS AND METHODS

Aerial root tips (2 cm long) were collected from seven-year old vines of *V. planifolia* grown in the Botanical Gardens of the University of Calicut, Kerala, India. They were surface sterilized with 0.5 per cent $HgCl_2$ for 10 minutes and washed three times with sterile water. The explants were incubated on filter paper bridges in tubes containing liquid MS (Murashige and Skoog, 1962) medium supplemented with IAA (0-10 mg l^{-1}) and 0.2 mg l^{-1} kinetin. The pH of the medium was adjusted to 5.8 prior to autoclaving and the cultures were maintained at 24 $\pm$ 2°C with continuous illumination (7000-8000 lux). After three months in MS medium when the root tips had swelled considerably, the tips (approx. ca 7 mm long) were excised under sterile conditions and planted on the solidified MS medium (0.8 per cent agar) supplemented with IAA (0-10 mg l^{-1}) and 0.2 mg l^{-1} kinetin in 100 ml Erlenmeyer flasks. Each experiment was performed with 40-60 root tips per culture treatment. At weekly intervals, five samples of the cultured root tips were removed from the medium and briefly washed in distilled water. The terminal region of the root tip (approx. 3 mm) was excised and fixed in FAA (formalin, acetic acid, alcohol) or Carnoy's fluid for 24 hours. After dehydration through tert-butyl alcohol series and embedding in paraplast, serial longitudinal sections were prepared at a thickness of 12 μm. Sections were stained with tannic acid and ferric chloride. Starch grains in sections were localized by the periodic acid Schiff reaction. Mitotic indices of cells were estimated from median longitudinal sections of 20 root tip meristems collected from cultures at weekly intervals.

For electron microscopy, 2 mm long root tips were cut and immediately fixed in 2.5 per cent gluteraldehyde (2 h., 0.05 M phosphate buffer, pH 7) at room temperature, followed by over night fixation with 2 per cent osmium tetraoxide at 4°C. After dehydration in graded ethanol series, the samples were pre-stained with 2 per cent alcoholic uranyl acetate, infiltrated with 99.5 per cent propylene oxide and embedded in ERL-4206 (Serva, Heidelberg). Ultrathin sections were post-stained with 0.3 per cent lead citrate (Reynolds, 1963) and examined under a Zeiss EM 109.

RESULTS

Morphology of the aerial root apex

V. planifolia produces two adventitious roots from each node with which it clings to the support (Fig. 1). The apical portion of an aerial root taken from a seven-year old vine in longisection is rather flat with a tightly adhering root cap (Fig. 5). Three promeristem layers, 2 to 5 cells wide, show continuity with (1) the pole of the central cylinder, (2) the cortex, and (3) the root cap and epidermis.

The root cap, at its deepest point, consists of approximately 12 layers of cells. The innermost layer, the cap meristem, consists of small densely cytoplasmic cells devoid of conspicuous starch grains and vacuoles. At about the fifth cell layer, cells increased in size and become highly vacuolated. These cells accumulate large

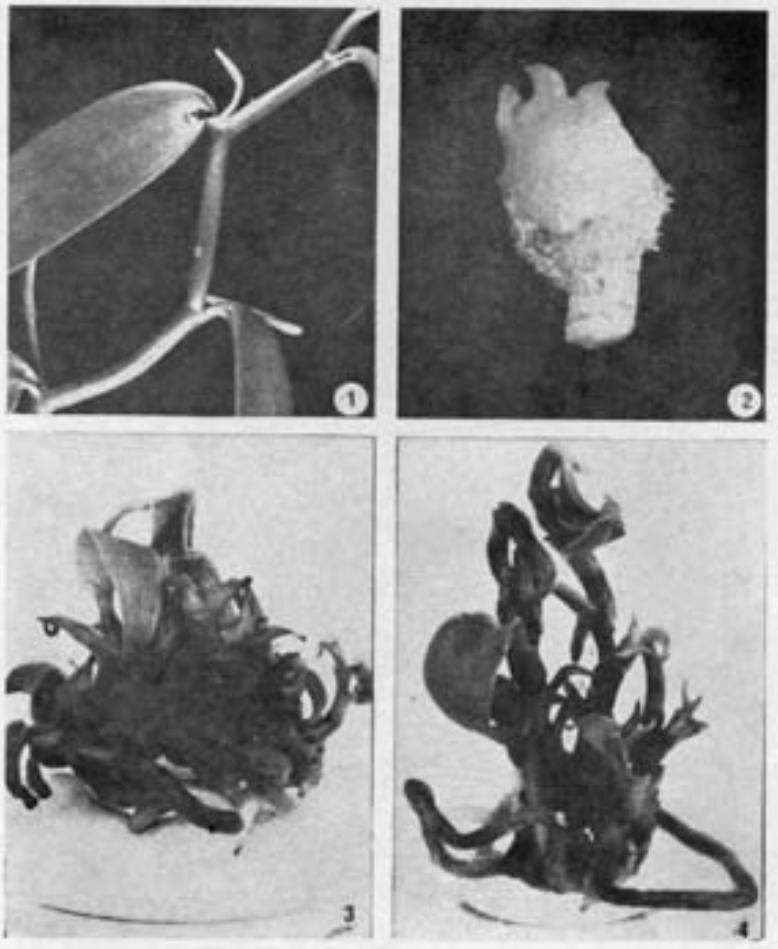

PLATE I

Fig. 1. An apical portion of *V. planifolia* vine with four nodes, young adventitious roots (arrow) and leaves. ×0.75.

2. 2 cm long aerial root tip excised from a young 6 cm long root after eight weeks in MS medium containing 2 mg l^{-1} IAA and 0.2 mg l^{-1} kinetin. ×5

3. Development of multiple shoots from swollen root tips subcultured on solidified MS medium after 12 weeks in culture. ×1.25

4. Multiple plantlets after 14 weeks in culture. ×1.25

amounts of amyloplasts in their plastids (Fig. 6). Abutting on the proximal surface of the root cap meristem is a small hemispherical group of 25-30 cells. The constituent cells of the hemisphere are relatively the smallest at the root apical region, nearly isodiametric, less densely cytoplasmic with no particular thickenings on their walls (Fig. 7). The nuclei in these cells, before transfer to the culture, medium, do not show mitotic figures in contrast to the neighbouring cells. These cells correspond closely to the quiescent centre as shown by Clowes (1971).

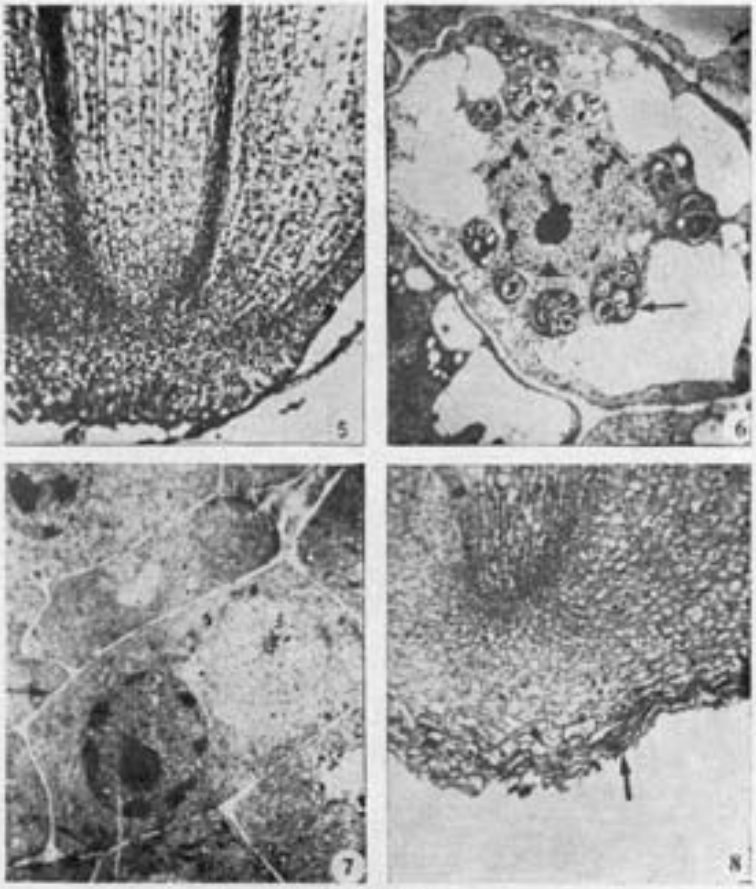

PLATE II

Fig. 5. Median longisection of a young aerial root tip of *V. planifolia* from a seven year-old vine showing the structure of the root meristem. ×126

6. A root cap cell showing multigranular amyloplasts (arrow) and large vacuoles. ×3000.

7. A quiescent centre cell. ×3000

8. Median longisection of a young aerial root tip after four weeks in MS medium containing 2 mg l^{-1} IAA and 0.2 mg l^{-1} kinetin showing remnants of the cap (arrow) and mitoses in the quiescent centre region. ×60

Organogenesis in-vitro

2 cm long root tips excised from aerial roots less than 15 cm long cultured on liquid MS medium supplemented with 2 mg l^{-1} of IAA and 0.2 mg l^{-1} of kinetin, swelled considerably within two months. After three months in culture, the swelled root cap tissue cracked, exposing small spherical bulges with surrounding dark sheets of necrotic material (Fig. 2). Median longisections of the root apices after two weeks in culture indicated that the root cap lysis began at the distal layer and progressed centripetally towards the meristem. Lysis began with the

breakdown of the starch granules in amyloplasts followed by the disintegration of the cytoplasm (Fig. 8).

Concomitant with these changes in the root cap, the cells of the quiescent centre increased in volume and divided at a rapid rate. The activation of the quiescent centre cells commenced with the breakdown of the distal half of the cap. In about four weeks in culture, lysis of the cap was complete and the number of cells in the quiescent centre region had increased considerably resulting in an increase in girth of the apex; the originally pointed apex now assumed a hemispherical outline.

The cells on the periphery of the hemispherical mound were relatively smaller, had dense cytoplasm and prominent nuclei, and accumulated small starch granules in their cytoplasm before dividing periclinally. Subsequent anticlinal and periclinal divisions in these cells resulted in a homogenous mass of densely stained cells which developed either into a single shoot meristem (Fig. 9) or organized themselves into several meristemoids (Fig. 10). The meristemoids thus produced were delimited by a thin layer of cells which were raised above the surface of the hemispherical mound giving it a corrugated appearance (Fig. 10). Each meristemoid enlarged further and their constituent cells continued to divide and form a shoot meristem on the flanks of which leaf primordia differentiated in an acropetal sequence. Within nine weeks in culture, a number of shoot apices each surrounded by a whorl of leaves were formed in the apical region of this mound of meristematic tissue (Fig. 11).

The new root was initiated only after four to five leaves had formed. The root emerged approximately at an angle of 120° with the mid-line of the plumule and the nodal plate (Fig. 12) by piercing through the surrounding tissue. However, none of the plantlets formed in liquid media emerged from the swollen root tip and required excision and transfer to a solid medium. When the swollen root tips were excised and grown in agar solidified media of the same constitution, they enlarged progressively and became further corrugated and covered with various outgrowths. Many new meristems developed, leading directly to shoots (Fig. 3) followed by roots. In about nine months, plantlets with well formed shoots, leaves and roots differentiated (Fig. 4) from each explant.

DISCUSSION

Potentialities of excised root cultures have been explored so far on only a very modest scale largely due to the difficulties encountered with majority of dicrty-ledonous species and with almost all monocotyledons. Root tissues are rarely used for *in-vitro* propagation purposes, because of contamination problems and the substantial disadvantages of the constituent cells of a differentiated root being of varying ploidy, resulting in uneven growth. Consequently, roots of very few species have been maintained in continuous cultures and in even fewer species have root buds been grown *in-vitro*. Shoot initiation from callus tissue derived from cultured roots of dandelion, tomato, *Isatis tinctoria*, *Atropa belladona* and

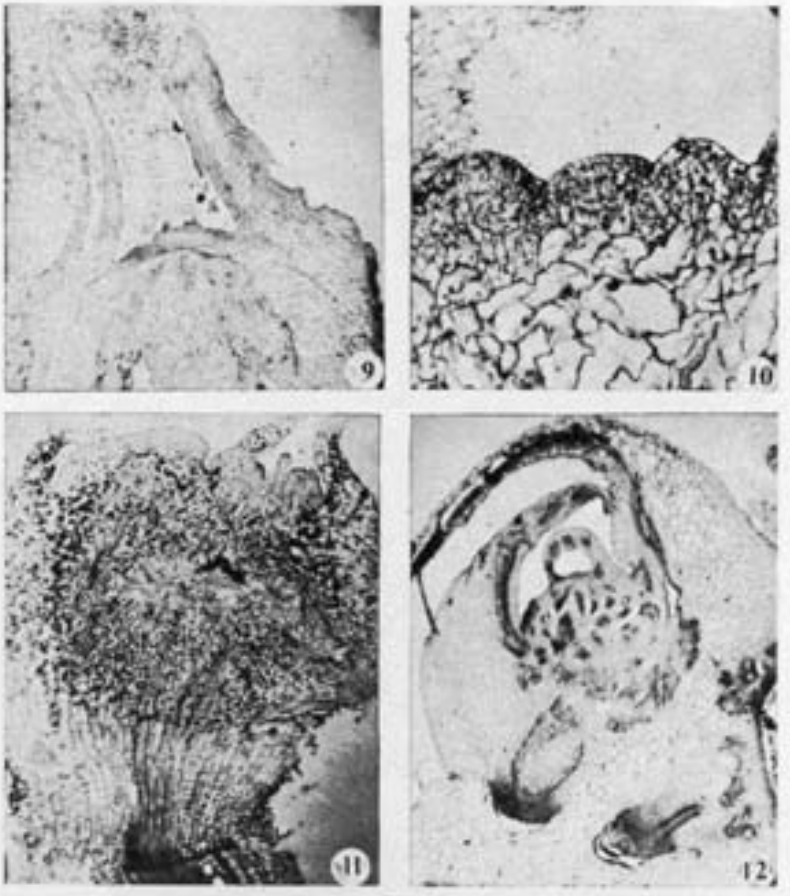

PLATE III

Fig. 9. Median longisection of a young aerial root tip after nine weeks in liquid MS medium showing differentiatton of a shoot meristem and a pair of leaf primordia from the root meristem. ×40

10. Some, showing meristemoids. ×40

11. Differentiation of multiple shoots from meristemoids formed at the distal end of the root tip after nine weeks in liquid MS medium. ×18.

12. Formation of plantlet by differentiating a root three weeks after transfer to solidified MS medium supplemented with 2 mg l^{-1} IAA and 0.2 mg l^{-1} kinetin. ×18

protocorm-like bodies from callus of cultured root tips of the orchids *Epidendrum obrienianum*, *Catasetum trulla* × *Catasetum* berthrand and *Oncidium varicosum* have been obtained. In all these reports, either the root apical meristem is not involved or its precise involvement in organogenesis is obscured due to the callus interphase.

The dual role of the root cap in graviperception and maintenance of the quiescent centre has been well documented (Barlow & Pilet, 1983; Wilkins, 1984).

Removal of the cap from actively growing roots of *Zea mays* have been observed to trigger off a series of changes, especially in the quiescent centre leading to the regeneration of a new cap (Barlow, 1974). In the present study on *Vanilla*, lysis of the cap cells began with the loss of its meristem and subsequent breakdown of starch in amyloplasts. As in decapped roots of *Zea mays*, degeneration of the cap cells of *Vanilla* roots in culture, stimulated growth and division of cells of the quiescent centre. However, in none of the 500 cultured root tips, sectioned at various stages of development and examined from day 3 to 90, was there any indication of a cap regeneration comparable to that of *Zea mays*. In contrast, the descendants of the quiescent centre either developed into a single shoot meristem or some of their peripheral cells organized themselves into meristemoids and then to entire plantlets indicating that a fundamental switch over in the organizational activity occurs so that formerly organized the root meristem now organize the shoot meristem.

Endogenous shoot buds without the callus interphase have been induced in cultured roots of a few taxa: A few such cases have been reported in ferns (Peterson, 1975) but with the exception of *Selaginella*, other examples have turned out to be more properly interpreted as the initiation of lateral buds from the derivatives of the root apical cells. The only reported case of root meristem transformation to shoot meristem in seed plants is for the orchid *Neottia nidus-avis* (Champagnat, 1971). However, there is little conclusive histological data on the fate of root apical meristem during transformation, although, in the case of *Neottia*, it was shown that the root meristem first differentiated into a protocorm from which a shoot primordium arose (Champagnat, 1971). Aerial root tips of *Vanilla* cultured in the present study, however, produced plantlets directly from the meristematic zone without a callus interphase, minimizing the possibility of induced epigenetic changes in the resultant plants. Using the methods reported in this paper, disease-free clonal material of *V. planifolia* could be easily produced for use in plantations. Furthermore, the root tips of *Vanilla* could perhaps be an ideal candidate for cryopreservation because of a small unit size, high regenerative potentiality, the constituent cells being vacuolated, meristematic, genetically uniform and without intercellular spaces.

ACKNOWLEDGEMENTS

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Cryopreservation Studies on Plant Germplasm

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Annual and biennial seed crops belonging to the orthodox crop species are generally amenable for preservation in seed 'Gene Banks' at low (+10°C) and ultra low (−20°C) temperatures. Until now it has proved to be the best means of germplasm storage all over the world. For several vegetatively propagated plant species, in-vitro *conservation techniques now offer new possibilities. Pollen preservation is yet another important method by which part of genome can be preserved, although it plays only a complementary role. However, recent researches in cryopreservation indicate that seeds, meristems, shoot tips, buds and both zygotic and somatic embryos as well as embryonic axes can be preserved and the trends indicate that perhaps these can be preserved for indefinite period without loss of viability or deterioration in qualiiy and genetic integrity. Cryopreservation work undertaken presently at National Facility Plant Tissue Culture Repository, (NFPTCR) using seeds, pollen, and* in-vitro *cultures has made significant progress and the information has been briefly presented in this paper.*

Several strategies for germplasm conservation have been employed by national and international organisations. These can be categorised under *in-situ* and *ex-situ* conservation. The former involves continuance of wild species in their natural habitats or biosphere reserves (genetic reserves) where process of evolution can continue, while *ex-situ* conservation involves maintenance of germplasm in the field by growing frequently for rejuvenation of seeds (Field Gene Banks), or holding germplasm in Seed Gene Banks, gardens, plantation, orchards or in green houses. Besides, *in-vitro* techniques developed recently offer potential for storage of meristem, shoot tips, buds and embryos under controlled physical and nutritional regime using minimal media, low temperature or through growth retardant over short, medium or long term periods. Cryopreservation techniques offer yet another strategy for the preservation of germplasm. However, practical considerations are important for adopting one or some of these methods for genetic conservation. Researches indicate that in *in-vitro* storage and cryopreservation provide useful adjuncts in the wake of specific problems associated with some plant species (Morel, 1975; D'Amato, 1975; Henshaw, 1975; Withers and Williams, 1985). Pollen preservation can help in crop breeding, in the development of haploids (inbreds) as well as dihaploids (genetically homozygous). Although it plays only a complementary role in the long term conservation strategy (Towill, 1985; Ganeshan, 1986a), yet it helps in conservation of germplasm.

Cryopreservation techniques provide alternative strategy for the conservation of seeds, pollen, embryos and *in-vitro* cultures at ultra low cryogenic temperature (Liquid nitrogen,—196°C). However, recalcitrant seeds are desiccation sensitive and are easily killed on dehydration below a critical value between 12-35 percent moisture content. Recalcitrant seeds are produced by some aquatic species, most of tropical fruits and many economically important species like coconut, oilpalm, jackfruit, mango, litchi, walnut, rubber and nutmeg. Partially desiccation tolerant species include tea, coffee, citrus and several others. According to Chin and Roberts (1980), although efforts have been regularly made for the storage of such species, however, none of the methods ensure long-term storability beyond four years without any loss of viability. Currently germplasm conservation work is being carried out intensively in several laboratories of the world (Plucknett *et al.*, 1987). India has also established its first National Plant Tissue Culture Repository and NBPGR already possesses a well established network (Paroda, 1988). The cryopreservation work being currently persued at the National Plant Tissue Culture Repository, NBPGR, is being presented here.

MATERIALS AND METHODS

In order to devise freeze preservation experiments, random samples drawn from seed lots of different orthodox species were used for present studies. The seed lots were dehydrated to 5-8 per cent moisture. The 100 grain weight (1000 grain weight in case of *Nicotiana tabacum*) was determined in different species/accessions. For long-term cryopreservation work (base collection), a minimum of 10,000 seeds were stored for cross pollinated crops (e.g. pearl millet) while about 5,000 seeds were stored for self pollinated crops. For determination of moisture content in orthodox seed species, ISTA rules (1985) were followed. Low constant temperature oven method was routinely followed for recalcitrant species. Seed lots were subjected to viability test and actual germination was used as a criterion of viability. In each case, 50 seeds in 2 replicates were germinated on top of filter paper discs. All desiccation sensitive and partially desiccation sensitive species were germinated in open trays or in polythene bags in mixture of 1 : 1 garden soil and sand, and were maintained at room temperature (25-32°C). Seedling vigour was tested for pearl millet, onion, *Brassica* and *Plantago*, etc., following the method suggested by Myhill and Konzak (1967). Shoot/root length was noted after a suitable germination period for different species.

(I) Cryopreservation Technique For Seeds

For conservation experiments, seeds were distributed in small capacity cryovials (1/1.8 ml) or in goblets (250 ml) made of polypropylene and were kept at different temperatures; (1) ambient, (2) —20°C and (3) —150° to —180°C (vapour phase of liquid nitrogen). The seeds of cocoa and pepper were simultaneously stored in closed petriplates subimbibed in different concentrations of abscicic acid (10^{-3}, 10^{-4} and 10^{-5} M) with a filter paper laid underneath and maintained at 5°C).

(II) Freeze Preservation Technique For Pollen

For pollen storage the material comprised of primitive landraces of maize (*Zea mays*) from Sikkim and North-eastern region of India as well as introduced germplasm of related genera and species, *Coix lacryma jobi*, *Chionachne*, *Zea parviglumis*, *Z. mexicana*, and *Z. diploperennis*. Pollen was collected in the early morning hours (between 8.30 a.m. and 11.00 a.m.) from actively dehiscing tassels. The *in-vitro* germination of pollen grains on semisolid medium and *in-vitro* pollination of silks (Mishra, 1984) was used as a criterion of viability. Water content was determined by drying to a constant weight at 80-90°C temperature and the percentage of water content of the pollen was determined. For freeze preservation of pollen, fresh samples of each species immediately after collection were dried over dry silica maintained in laboratory at ambient temperature for 3 hours before actually storing in cryovials. The loss of water was determined at the end of desiccation period by determining the decrease in weight. Stored seeds and pollen samples were thawed rapidly by immersing the storage vial in waterbath maintained at 40°C. Viability was tested following the same method as used before storage of these samples.

(III) *In-vitro* Freeze Preservation Technique

In order to set the *in-vitro* freeze preservation experiments, tobacco (*Nicotiana tabacum*) was taken up as a model system for meristem/shoot tip preservation. Meristem with 2-3 leaf primordia was excised aseptically from seedlings grown on MS basal medium supplemented with DMSO (5 per cent) for 48 hours. Cultures were further maintained at 5°C for 24 h. Meristems were transferred to 5 ml of chilled cryoprotectant free MS basal medium. 5 ml of 10 per cent PEG/DMSO was added dropwise to it over a period of 30 min. to a final concentration of 5 per cent and maintained further for 20 min. Meristem alongwith cryoptotectant solution were then stored aseptically in 1.8 ml cryotubes and frozen rapidly. After 24 hours cryotubes were thawed rapidly using water bath (40°C). One set of meristems was recultured on MS basal medium directly whereas the other set was washed repeatedly in sterile water before culturing. Controls were run using cryoprotectant alone without involving any freezing.

RESULTS AND DISCUSSION

Cryopreservation of orthodox seeds

All orthodox seed species under study possessed high viability (> 80 per cent) as indicated by the actual germination test at the time of storage (Table 1). Seedlots indicated a range of moisture content of 3.5 to 7.8 per cent. Seed samples were successfully stored for variable length of times as evident by the data available for well over two years (experiments still continuing). Pearl millet and onion seeds were tested periodically and all the samples were found to retain the initial viability, germination percentage and seedling vigour. Cryopreservation attempts on orthodox seeds have been undertaken only at a few selected laboratory, such as National Seed Storage Laboratory (NSSL), Fort Collins, Colorado, USA (Stanwood and Bass, 1981). At NSSL, seveal hundred accessions belonging to the orthodox

TABLE 1. VIABILITY, MOISTURE CONTENT AND STORABILITY OF DIFFERENT PLANT SPECIES CRYOPRESERVED IN LIQUID NITROGEN

Plant species	Initial Viability (%)	Moisture content (%)	Period of storage (days)
ORTHODOX SEED SP.			
Pearl millet			
Pennisetum typhoides var. BJ 104	80	6.7	900
MH 179	94	4.3	900
Onion			
Allium cepa var. Pusa Ratnar	96	7.8	933
Ratnar selection	96	7.69	933
Nicotiana			
Nicotiana tabacum var. NPN-190	98	4.53	520
PL- 5	97.8	4.5	520
Anand-119	100	4.5	520
Anand- 2	100	4.5	520
S- 20	98	4.4	520
Brassica			
Brassica juncea var Pusa Bold	93	4.4	507
Varuna	90	4.06	507
PR- 45	95	3.85	507
RLM-198	87	5.5	507
B. napus var. ISN-129	95	5.7	507
ISN- 11	90	5.19	507
706	91	3.53	507
BO- 54	90	3.99	507
ISN-106	91	4.0	507
B. carinata 1	90	4.95	507
Isabgol			
Plantago ovata var. Gujarat Isabgol-1	95	5.66	514
Sunflower			
Helianthus annuus var. Morden	96	6.4	518
Peredovik	94	6.5	518
Surya	95	6.63	518
*RECALCITRANT SEED SP.***			—
Theobroma cacao	95	41.3	—
Artocarpus heterophyllus	100	53.2	—
Piper nigrum	0*	31.9	—
Myristica fragrans	0	32.9	—
Camellia sinensis	100	30.0	—
Aegle marmelos	100	3.86	90

*10 per cent germination was noted in seeds plated on semi-solid medium (B_5 medium with 0.7 per cent agar) in test tubes maintained at 23°C constant under light.

**Success not achieved, Experiments continued.

species (desiccation tolerant) have been cryoreserved for well over 8 years. It is considered that at the temperature of —196°C, most or all metabolic and cell division activities cease. The decline in viability at cryogenic temperature (—196°C) is argued to be well over several decades and it perhaps happens due to low kinetic energy of molecules and negligible diffusion characteristics (Mazur, 1976). Background irradiation during the storage period is thought to be the major potential source for damage to cells. Although cryopreservation had not been well studied in many systems, the conclusion is still that virtually no change should occur over a long period of time (Ashwood-Smith and Grant, 1977). Thus the potential of liquid nitrogen storage is considerable. It also provides better quality of seeds and improved maintenance of plant genetic resources much sought for crop improvement.

Cryopreservation of pollen

Pollen of maize landraces and allied genera/species used for freeze preservation studies showed very high moisture content (51 per cent). The pollen were desiccated to 12 per cent moisture level before storage without much loss of viability. Sufficient numbers and amount of pollen samples were stored for each genera/species and testing of viability was also undertaken after one year of storage in liquid nitrogen. *In-vitro* pollinations on maize cob silks gave a better estimate of viability as compared to *in-vitro* germination.

Reports on successful cryopreservation of pollen in liquid nitrogen have already appeared in the literature. Pollen of *Allium cepa, Carica papaya, Citrus limon, Vitis vinifera, Solanum melongena* and *Lycopersicon esculentum* were reported to be successfully cryopreserved for one or more years using freeze-preservation techniques (Ganeshan, 1986a, 1986b). Pollen banks have been established by various research organisations for the purpose of basic research in pollen biology, plant breeding and crop production programme. Yet the concept of pollen preservation for conservation of plant genetic resources is rather recent. Cryopreservation of pollen has several advantage, as it can facilitate wide hybridization between plants that are separated physiologically, geographically and also due to seasonal barriers. It would also help to conserve genetic diversity. Pollen can be used for the production of haploids to develop inbred lines rapidly as well as dihaploids, which can be of tremendous significance in plant breeding programme and genetical studies (Chandel *et al.*, 1988; Chandel and Pandey, 1990).

Cryopreservation of desiccation sensitive species

Majority of the recalcitrant seed species possess very high moisture (30-53 per cent). Also the literature on the seed germination of recalcitrant species is very scanty and this provided difficulty as no guidelines were available for standard germination procedures to be followed. Laboratory experiments were thus conducted for the standardization of germination for various desiccation sensitive/partially desiccation sensitive species presently being studied at NFPTCR.

Pepper seeds were extracted from berries on arrival of freshly harvested consignment. These showed very low germination only when plated on agar medium. Out of the berries received from CPCRI, Kasargoad, some showed drying and some already died before reaching the destination perhaps due to dehydration. Rest of the seed species showed very high germination.

Recalcitrant seeds were used for experimentation within few days after the freshly harvested lot became available. Storage of cocoa, jackfruit and pepper seeds wrapped in charcoal powder did not prove beneficial because of frequent fungal infection. Cacoa and pepper seeds were thus extracted from pods and berries, respectively just before cryopreservation experiments. Seeds of jackfruit were stored as such in polythene bags at 8-10°C.

In our preliminary studies, the seeds of several recalcitrant/partially desiccation sensitive species were not found amenable to LN_2 storage except bael (*Aegle marmelos*) seeds which behaved like orthodox seeds in the storage characteristics. Desiccation experiments conducted on jackfruit showed that reduction in moisture content by 10 per cent does not cause any damage, while it proved highly deterimental in case of cocoa.

Use of cryoprotectant in our experiments for seed storage in liquid nitrogen did not prove beneficial. The failure of LN_2 storage cannot be attributed to any single factor as the damage might have occurred at any stage during desiccation, cryoprotectant treatment, freezing, thawing or post-thaw treatment. It is evident that large size of recalcitrant seeds (oil palm, cocoa, jackfruit, mango, litchi, etc) itself may cause problem for efficient ingress of cryoprotectant into the seeds and also in precise control of freezing and thawing. The inter and intra-cellular ice crystal formation and changes in osmotic regulatory mechanism related to membrane composition and activity can result in nucleation leading to ice formation. This might cause death of embryos. Similarly, in sub-imbibed storage of pepper and cocoa seeds, fungal infection proved to be a constant menace which once set in a lot, could perhaps spoil the entire seed lot.

The review of the literature suggested that cryogenic storage so far has been restricted only to few plant species. Successful reports exist for *Anthurium* (Stanwood, 1986), coffee (Yilmaz, Pers Commu) and rubber (Normah and Chin, 1989).

Preservation of excised embryos have been reported from young coconut (Chin *et al.*, 1989) and *Veitchia* and *Howea* palm embryos (Chin *et al.*, 1988). Previously some of the tree species such as *Citrus* were considered to be recalcitrant, but recent researches have shown that lime and lemon belong to the orthodox group (Mumford and Grout, 1979).

Ultrastructural changes were observed in *Hevea* seeds stored at 10°C, 22°C and 27°C. Membrane degeneration appeared to be the most common feature of deterioration. At all storage temperatures, the plasmalemma was observed to be increasingly folded, disintegrated or withdrawn from the cell wall. The dissolution of the tonoplast was also widely observed (Normah and Chin, 1989). The reports on the cryopreservation of young coconut embryos have appeared recently (Chin

et al., 1988) where excised coconut embryos survived the cryopreservation treatment but only with callus formation from parts of embryos. In later experiments, success was achieved in cryopreservation of young coconut embryos with formation of organised growth after fifteen months of culture (Chin *et al.*, 1989). Further, it was highlighted that basic studies on the identification of causes of desication sensitivity in recalcitrant seeds and their intolerance to low temperatures of 10 to 15°C should also be given high priority. These factors are important since any long-term storage of recalcitrant seeds requires preventive measures for seed deterioration and viability loss. According to the above authors, alternatives to imbibed seed storage, such as partial drying or lowering moisture contents to just above a critical level in conjunction with fungicide treatments also need specific attention.

In-vitro freeze preservation

Meristem and shoot tip cultures initiated on tobacco (*Nicotiana tobacum*) on MS basal medium were observed periodically for growth and development. Meristem pretreated with 5% PEG (without LN_2 exposure) kept as control showed good growth, whereas in all other treatments, meristem slowly turned yellowish white and showed no further growth. It was found that PEG (5 per cent) was not toxic to meristem cultures while DMSO (5 per cent) was cytotoxic. Expriments were continued to bring improvement and refinement in the methods and protocols for successful storage.

Researches on cryopreservation of plant species were systematized only in the last decade, although the first success in the cryopreservation of a cell suspension was reported by Quatrano (1968) in flax at —50°C while another report was by Nag and Street (1973) who regenerated somatic embryos from a cell culture of carrot (*Daucus carota*) frozen at —196°C. Since then, there has been increase in number of species shown to be amenable to being exposed to low temperature with good percentage of recovery and survival. Cryopreservation process for *in-vitro* cultures can be divided into six stages; pregrowth, cryoprotection, freezing, storage, thawing, estimation of viability and cryoinjury (Wilkins and Dodds, 1983).

Cryopreservation researches have made considerable success in case of preservation of cells, suspension and protoplast cultures in plants. (Bajaj and Reinert, 1977; Withers 1980; Kartha, 1985). Cassava meristems have been also cryopreserved at CIAT, Cali, Columbia (Kartha *et al.*, 1982). Successful freeze preservation of meristems of strawberry (Kartha *et al.*, 1980) and apple (Caswell *et al.*, 1986) have been reported.

Cryogenic storage of semen, ova and fertilized eggs have been obtained in animal system. There are infact reports of cryogenic storage of complete human organs and also bodies (Mazur, 1970; Meryman, 1966; Mericka, *et al.*, 1988; Grischenko *et al.*, 1988). However, researches on basic aspects of cryobiology are required to obtain indepth knowledge of cryopreservation, particularly cryobiological aspects including physical and biochemical aspects of membrane integrity, osmotic regulatory mechanism, nucleation, cryoinjury, etc. Thus future research

efforts would be directed towards the solving of such problems so that effective and reliable methods of freeze preservation could be developed.

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Studies on Genetic Variability, Correlation and Path-Analysis for Some Qualitative and Quantitative Characters in Fenugreek Germplasm

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Eighty eight indigenous and exotic germplasm accessions of fenugreek (Trigonella foenum- graecum) *obtained from NBPGR, New Delhi, IGFRI, Jhansi and different parts of Haryana were evaluated for qualitative and quantitative attributes of grain yield. Considerable variability was present for component characters like plant height, branches, clusters, pods per plant, pod length, seeds per pod and days to maturity. These were correlated with grain yield and clusters per plant, plant height, pod length and 100-seed weight. A few lines, namely HFM 39, 65, 78, 187, 193 and IL 356 were better in seed yield and HFM 15, 65, 65-1, EC 18737, 26175 and NLM possessed the genetic marker traits and other desirable attributes. Their use in breeding programmes to develop ideal plant types in fenugreek has been discussed.*

Collection, maintenance and evaluation of germplasm for economically important traits is one of the most essential and useful step for initiating breeding programme for genetic improvement of any crop. In fenugreek, which has many diversified uses, reports on systematic evaluation of germplasm are very scanty. Accordingly, the available germplasm of 88 accessions of fenugreek maintained at Haryana Agricultural University was evaluated and the results are reported and discussed in this paper.

MATERIALS AND METHODS

The 88 fenugreek accessions collected from National Bureau of Plant Genetic Resources, New Delhi (11 accessions), Indian Grassland and Fodder Research Institute, Jhansi (8 accessions) and different parts of Haryana (69 accessions) were grown in *rabi* 1987-88 at the experimental farm of Department of Plant Breeding, Haryana Agricultural University, Hisar, in an augmented randomised block design with three checks, namely, Pusa Early Bunching, NIM and T8. Each accession was represented in a single row of 2.8 m length. The distances between and within rows were kept at 45 and 10 cm, respectively. Every eight accessions were followed by the three checks to constitute one block. Normal cultural practices were followed. Observations were recorded on five competitive and randomly selected plants from each cultivar including

checks on some qualitative and quantitative characters like colour of leaf and leaf margins, days to flowering, days to maturity, plant height, branch number, cluster number, pod number, pod length, seeds per pod, seed yield per plant and 100-seed weight.

Mean and range of variation for each character was worked out and germplasm lines were categorised as per their variation for leaf and other characters.

RESULTS AND DISCUSSION

Qualitative Characters

The observations on contrasting and distinguishing morphological attributes like colour of leaf and leaf margins were recorded. These characters could successfully be used as a marker trait for characterisation and identification of genotypes in fenugreek. Five types were observed with regard to leaf colour and leaf margins. Maximum germplasm accessions (63) possessed green or dull/dark green leaf with red margins; whereas minimum number of germplasm lines (2, 3 and 5) had bright green, green or light green leaf with green margins. The rest of the germplasm accessions (15) had purplish pigmented leaf and margins. On the basis of colour of leaf and leaf margins, the distinction between fenugreek genotypes can be made even at first solitary true leaf stage. It was observed that the first solitary leaf was green with red margins in the genotypes having green or dark green leaf with red margins; bright green or light green leaf with green leaf margins in the case of genotypes having bright green, green or light green with green margins and purplish pigmented leaf and margins in the case of genotypes having purplish pigmented leaf and margins.

Quantitative Characters

Mean, range and coefficient of genetic variation and number of accessions below/ at par/ above the best check are given in Table 1. The highest coefficient of variation (43.79%) was observed for seed yield/plant followed by branch number (38.65%), pod number (35.34%), cluster number (32.25%), 100-seed weight (21.45%), days to flowering (17.72%) and plant height (16.81%), whereas the lowest coefficient of variation was recorded for pod length (9.10%) followed by days to maturity (3.06%). The range of variation for days to flowering and maturity was from 46 (HFM 65-2) to 90 (HFM 169) and 140 (HFM 116, HFM 134, HFM 152) to 158 (EC 26175, EC 33904 and EC 33906), respectively. The variation from 37.6 cm (HFM 56) to 93.0 cm (IL 326) for plant height was recorded. The range for branch number was from 3.6 (HFM 107) to 25.0 (EC 33906), for cluster number from 12.0 (HFM 56) to 86.8 (HFM 39) and for number of pods from 13.4 (HFM 56) to 109.6 (HFM 16). The characters like seeds per pod and pod length ranged from 13.6 (HFM 23) to 20.6 (PLMe-46) and 9.1 (HFM 99) to 14.1 cm (HFM 15), respectively. The range of variation for 100-seed weight was from 0.6g (HFM 120) to 1.8 g (EC 26175). Seed yield is the ultimate objective in any crop and it was found to vary from 1.29 (HFM 187) to 16.0 g (HMF 187) in

TABLE 1. MEAN, RANGE AND COEFFICIENT OF VARIATION FOR VARIOUS CHARACTERS IN FENUGREEK

Sl. No.	Character	Mean value	Range of variation		Coefficient of variation (Cv%)	No. of accessions below/at par/above the best check variety				
			Minimum	Maximum		Below	At par	Above	Best	Mean value of best check
A	*Quantitative Characters*									
1.	Seed yield/plant (g)	6.92 ± 0.32	1.2 (HFM 56)	16.0 (HFM 187)	43.79	72	1	17	T8	9.1
2.	100-seed weight (g)	1.02 ± 0.02	0.6 (HFM 120)	1.8 (EC 26175)	21.45	75	6	9	NLM	1.3
3.	Days to maturity	148.63 ± 0.48	140 (HFM 116, 134 152)	158 (EC 26175, 33904, 33906)	3.06	34	2	54	NLM	147
4.	Days to flowering	66.75 ± 1.25	46 (HFM 65-2)	90 (HFM 169)	17.72	3	—	87	Pusa Early Bunching	50
5.	Seeds/pod	17.14 ± 0.25	13.6 (HFM 23)	20.6 (PLM 46)	13.67	53	2	35	T8	17.8
6.	Pod length (cm)	11.42 ± 0.11	9.1 (HFM 99)	14.1 (HFM 15)	9.10	59	2	29	T8	11.8
7.	Pods/plant	54.69 ± 2.03	13.4 (HFM 56)	109.6 (HFM 16)	35.34	71	—	19	T8	69.8
8.	Clusters/plant	47.64 ± 1.61	12.0 (HFM 56)	86.8 (HFM 39)	32.25	68	—	22	T8	58.4
9.	Branches/plant	9.03 ± 0.37	3.6 (HFM 107)	25.0 (EC 33906)	38.65	75	—	15	T8	11.3
10.	Plant height (cm)	66.28 ± 1.17	37.6 (HFM 56)	93.0 (IL 326)	16.81	68	—	22	Pusa Early Bunching	74.3
B.	*Qualitative characters*									
1	Colour of leaf and leaf margins.					63 germplasm accessions were of green or dull/dark green leaf with red margins (G, or DG + RM)				
						15 germplasm accessions were of purplish pigmented leaf and their margins (PP)				
						5 germplasm accessions were of light green leaf with green margins (LG + G)				
						3 germplasm accessions were of green leaf with green margins (G + G)				
						2 germplasm accessions were of bright green leaf with green margins (BG + G)				

G = Green, DG = Dull/dark green, RM = Red margins, PP = Purplish pigmentation, LG = Light green, BG = Bright green

TABLE 2. CORRELATION COEFFICIENT IN FENUGREEK GERMPLASM

	Branches/ plant	Clusters/ plant	Pods/ plant	Pod length	Seeds/ pod	Seed yield/ plant	100-seed weight	Days to flowering	Days to maturity
Plant height	— 0.0994	0.1884	0.1903	— 0.1358	0.0410	0.5003**	0.3488**	— 0.3813**	0.6264**
Branches/plant	—	0.6750**	0.6210**	0.2136*	0.1672	0.3710**	— 0.0464	0.3603**	0.0892
Clusters/plant	—	—	0.9261**	0.3330**	0.2113*	0.6846**	— 0.0063	0.0002	0.1865
Pods/plant	—	—	—	0.3554**	0.2011	0.6589**	— 0.0104	0.0315	0.1415
Pod length	—	—	—	—	0.3201**	0.3341**	0.0519	0.1413	— 0.0828
Seeds/pod	—	—	—	—	—	0.2944**	0.1249	0.0750	0.1674
Seed yield/plant	—	—	—	—	—	—	0.3652**	—0.2373*	0.4378**
100-seed weight	—	—	—	—	—	—	—	—0.4657**	0.4422**
Days to flowering	—	—	—	—	—	—	—	—	— 0.2189*

*P < 0.05; **P < 0.01

TABLE 3. DIRECT (DIAGONAL) AND INDIRECT EFFECTS OF GRAIN YIELD COMPONENTS IN FENUGREEK GERMPLASM

Characters	Plant height	Branches/ plant	Clusters/ plant	Pods/ plant	Pod length	Seeds/ pod	100-seed weight	Days to flower-ing	Days to maturity	Correlations with seed yield
Plant height	0.2880	— 0.0012	0.0810	0.0227	— 0.0219	0.0035	0.0684	0.0233	0.0365	0.5003**
Branches/plant	— 0.0286	0.0124	0.2903	0.0742	0.0344	0.0141	— 0.0091	— 0.0220	0.0052	0.3710**
Clusters/plant	0.0543	0.0084	0.4301	0.1107	0.0537	0.0178	— 0.0012	— 0.0000	0.0109	0.6846**
Pods/plant	0.0548	0.0077	0.3983	0.1195	0.0573	0.0170	— 0.0020	— 0.0019	0.0082	0.6589**
Pod length	— 0.0391	0.0026	0.1432	0.0425	0.1611	0.0270	0.0102	— 0.0087	— 0.0048	0.3341**
Seeds/pod	0.0118	0.0021	0.0909	0.0240	0.0516	0.0844	0.0245	— 0.0046	0.0098	0.2944**
100-seed weight	0.1004	— 0.0006	— 0.0027	—0.0012	0.0084	0.0105	0.1962	0.0284	0.0258	0.3652**
Days to flowering	— 0.1098	0.0045	0.0001	0.0038	0.0230	0.0063	— 0.0914	— 0.0611	— 0.0128	— 0.2373**
Days to maturity	0.1804	0.0011	0.0802	0.0169	— 0.0133	0.0141	0.0868	0.0134	0.0583	0.4378**

Residual effect : 0.2878

fenugreek germplasm lines studied. Pant *et al.* (1983, 1984); Raghuvanshi and Singh (1982); and Shukla and Sharma (1978) also reported a wide range of variability for yield and related characters in native and exotic collection of fenugreek.

Correlation and Path Coefficient Analysis

Correlation analysis (Table 2) revealed that all the characters, except days to flowering showed significant positive correlation with grain yield. Similar associations have also been recorded by various workers (Singh and Raghuvanshi, 1984; Pant *et al.*, 1984; Ivanova and Levandovskii, 1980). It was also interesting to note that branches, clusters, pods and pod length showed positive and significant association among themselves.

The direct and indirect effects of nine quantitative characters (Table 3) revealed that clusters/plant had the maximum direct as well as indirect effect on seed yield *via* pods/plant, branches/plant, pod length and seeds/pod. The other major components of the yield were plant height and 100-seed weight. Similar results have been reported by Singh and Raghuvanshi, (1984), Pant *et al.* (1984), Shukla and Sharma, (1978). Therefore, selection for yield improvement should be based on these characters.

On the basis of qualitative attributes, some of the lines, viz., HFM 65, HFM 15, EC 26175, EC 18820, and NLM may be utilized to study the inheritance of qualitative traits and in seed production programme. Some of the best genotypes like HFM 187 for high grain yield, EC 26175 for high grain weight, HFM 134 for early maturity, PLMe-46 for high seed number/pod, HFM 15 for longest pods, HFM 16 for highest pod number, HFM 39 for highest cluster number, EC 33906 for maximum branch number and IL 326 for tall plant types could be utilized in crossing programme to study the genetics of seed yield and other related characters and to evolve high seed yielding genotypes.

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Some Biochemical Changes in Relation to Loss of Seed Viability in Cauliflower Germplasm

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Nine cultivars of cauliflower were evaluated for seed longevity by accelerated ageing test (40°C, 90% RH). Six cultivars viz., Early Patna, Early Kunwari, Super Snow Ball, Alert, Poosi and Early Cauliflower were found to be good storer and capable of withstanding high storage temperature and relative humidity. Cultivars Aghani and Snow Ball were classified as moderate and Early Dawn as poor storer. The leaching of electrolytes, soluble sugars, amino acids and reduction of tetrazolium salt was more from seeds on deterioration. The quantum of leakage differ among different genotypes.

Cauliflower (*Brassica oleracea* var *botrytis* L.) is an important vegetable crop. The raising of seedlings and its successful establishment depend on the nature of seeds. The quality of seeds is often affected by the improper storage conditions, resulting in drop of germination potential and poor establishment of seedling in field and consequently low yield. Delouche and Baskin (1973) have developed accelerated ageing technique which is effective for evaluating relative storability of seed lots. At the same time certain biochemical changes were found to be associated with deterioration of seed such as excessive leaching of electrolytes, soluble sugars and free amino acids from the seeds (Givelberg *et al.*, 1984, and Doijode, 1985). Such an information is inadequate in the crop and an attempt was made to study seed longevity in different cauliflower germplasm and also to determine certain biochemical changes associated with seed deterioration.

MATERIALS AND METHODS

The experiment was conducted with nine cultivars of cauliflower both indigenous and exotic viz., Early Patna, Early Kunwari, Super Snow Ball, Alert, Poosi, Early Cauliflower, Aghani, Snow Ball and Early Dawn at the Indian Institute of Horticultural Research, Bangalore. These genotypes exhibited wide genetic variability for yield and its attributes. Accelerated ageing technique was employed for artificial ageing of seeds (Schoettle and Leopold, 1984), wherein seeds were exposed to 40° C and 90 per cent RH for three and six days.

Seeds were germinated by top paper method at 25°C in Cleland seed germinator. Seeds viability expressed as percentage of germination was recorded on hundred seeds each in three replications (ISTA Procedure, Anon, 1985). Seedling vigour

was compared using shoot length and dry weight on seven days old seedling. Based on percentage of germination, the relative storability of seeds was categorized as good (> 75), moderate (50-75) and poor (< 50). For biochemical analysis, seeds were surface sterilized with 0.1 per cent mercuric chloride, washed, dried and soaked in 25 ml sterile water for 18 hr at 25°C. Electrical conductivity of leachates was measured with conductivity meter. Soluble sugars were estimated as per the method of Dubois *et al.*, (1956) and free amino acids according to procedure of Lee and Takahashi (1966) in leachates. In seeds Dehydrogenase activitiy (DHA) was determined by estimation of formazan and expressed in terms of optical densitty at 480 nm (Kittock and law, 1968).

RESULTS AND DISCUSSION

Seed germination was significantly affected by accelerated ageing in different cauliflower cultivars and it was maximum (95%) in Early Patna and minimum (29%) in Early Dawn after six days of ageing (Table 1). Six cultivars viz., Early Patna, Early Kunwari, Super Snow Ball, Alert, Poosi and Early Cauliflower, showed good germination (above 75 per cent) after six days of ageing. These cultivars exhibit the capacity to tolerate high temperature and relative humidity during storage. Seeds of Aghani and Snow Ball were grouped as moderate and Early Dawn as poor storer.

Seedling vigour in terms of shoot length and dry weight were reduced on ageing of seeds. This was more conspicuous in moderate and poor storer cultivars (Table 2). Pesis and Ng (1983) also observed that longer period of ageing resulted in greater decline in seed quality.

The leaching of electrolytes increased with increase in duration of ageing in all cultivars (Table 3). Similarly, there was excessive leaching of soluble sugars and free amino acids from seeds with the advance in ageing. However, the quantum

TABLE 1. SEED VIABILITY IN DIFFERENT GERMPLASM OF CAULIFLOWER FOLLOWING AGEING TREATMENT

Cultivars	Ageing period (days)		
	0	3	6
Early Patna	100 (88.2)	97 (79.6)	95 (77.9)
Early Kunwari	100 (88.2)	97 (79.6)	91 (72.3)
Super Snow Ball	100 (88.2)	95 (77.9)	89 (71.2)
Alert	100 (88.2)	82 (64.9)	83 (66.0)
Poosi	100 (88.2)	87 (68.8)	83 (66.1)
Early Cauliflower	100 (88.2)	89 (71.3)	79 (64.4)
Aghani	100 (88.2)	85 (67.9)	74 (59.2)
Snow Ball	95 (77.9)	51 (45.3)	55 (47.7)
Early Dawn	100 (88.2)	89 (70.3)	29 (32.7)

CD at 5% = 6.7 (Figures in parenthesis are angular transformed values).

TABLE 2. SEEDLING VIGOUR IN DIFFERENT GERMPLASM OF CAULIFLOWER FOLLOWING AGEING

Cultivars	Shoot length (cm)			Dry weight (mg)		
	0	3	6	0	3	6
Early Patna	3.3	3.6	3.6	2.85	2.75	2.16
Early Kunwari	3.2	3.6	3.0	2.63	2.29	1.86
Super Snow Ball	2.8	3.3	3.9	3.14	3.06	2.49
Alert	2.6	2.9	3.4	2.90	2.58	2.05
Poosi	3.4	3.3	3.8	3.81	4.48	3.38
Early Cauliflower	3.2	3.4	3.4	3.00	3.25	2.64
Aghani	3.5	3.4	3.7	3.05	2.97	3.00
Snow Ball	3.0	3.1	2.6	3.84	3.03	3.31
Early Dawn	3.8	3.2	2.7	4.55	3.45	3.67
C D at 5%		0.58			0.57	

TABLE 3. LEACHING OF METABOLITES FROM SEEDS OF DIFFERENT CAULIFLOWER GERMPLASM DURING AGEING.

Cultivars	E C (μ mhos)			Soluble Sugars (mg/g)			Free amino acids (mg/g)		
	0	3	6	0	3	6	0	3	6
Early Patna	241	474	718	2.06	7.20	7.95	1.29	5.62	11.40
Early Kunwari	199	211	226	0.10	0.78	1.40	0.26	1.05	1.09
Super Snow Ball	185	255	298	0.80	1.46	3.05	0.57	1.92	2.47
Alert	246	296	378	1.64	3.52	4.75	1.52	3.17	3.98
Poosi	269	297	354	1.86	2.01	3.35	1.16	2.52	3.22
Early Cauliflower	193	203	237	0.99	1.33	2.05	0.95	1.25	1.26
Aghani	221	284	316	0.80	1.22	2.90	0.78	2.10	2.46
Snow Ball	342	587	859	4.95	8.00	13.60	2.54	7.90	11.90
Early Dawn	189	209	282	0.23	0.71	1.25	0.28	0.95	0.85
CD at 5%		54			0.59			0.36	

of leachates varied with the genotypes. Dehydrogenase activity also decreased with the ageing of seeds (Table 4), which coincided with loss of seed vigour and viability.

The above results suggest that seed longevity differs among cultivars. Certain genotypes have capacity to withstand high storage temperature and relative humidity and thus exhibit high longevity as well as high seedling vigour. In general longer period of ageing resulted in greater decline in seed quality. There was uneven leaching of metabolites in different genotypes and some genotypes have been found to be more sensitive to ageing than others (Pesis and Ng, 1983). This excessive leaching of metabolites was attributed to the deterioration of cell membrane (Powell and Matthews, 1977 and Schoettle and Leopold, 1984). Since the damage to cell membrane was more at high temperature and relative

TABLE 4. CHANGES IN DEHYDROGENASE ACTIVITY (OD 480) IN DIFFERENT CAULIFLOWER GERMPLASM AFTER AGEING.

Cultivars	Ageing period (days)		
	0	3	6
Early Patna	0.30	0.08	0.12
Early Kunwari	0.48	0.09	0.09
Super Snow Ball	0.55	0.12	0.13
Alert	0.38	0.21	0.20
Poosi	0.37	0.10	0.09
Early Cauliflower	0.46	0.20	0.20
Aghani	0.53	0.15	0.18
Snow Ball	0.31	0.14	0.14
Early Dawn	0.20	0.10	0.11
CD at 5%		0.086	

humidity, therefore it is advisable to protect the seeds by storing them at low temperature and low moisture for high quality of seeds in terms of high viability and seedling vigour.

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Preserving Viability and Fertility of Tomato and Egg Plant Pollen in Liquid Nitrogen

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Tomato (Lycopersicon esculentum *Mill cv. 'Arka Saurabh')* & *Egg plant* (Solanum melongena *L. cv. 'Arka Kusumakar')* *pollen cryopreserved over extended durations remained viable after retrieval from a cryopreserved state, inducing fruit and seed set equivalent to controls. Studies carried out at the experimental farm of IIHR showed that pollen stored under cryogenic conditions for a duration of 6 years could be successfully utilized by the breeder concerned, to produce seeds in the absence of fresh pollen normally collected during the current season. Varietal crosses involving frozen pollen did not deteriorate in their performance and capability to induce viable seed among the fruits set.* In vitro *pollen germination profiles though recorded, a considerable decline in egg plant does not appear to reflect the true performance potential of frozen pollen in the field. However, in tomato,* in vitro *pollen germination results appear to be in good fit with the results obtained using stored pollen in field pollinations. From this study it was concluded that it is advantageous to preserve pollen of these crops over extended durations in liquid nitrogen, since pollen retains its capability to fertilize and induce normal seed set. The study also establishes the fact that pollen preservation for genetic conservation in these crops could be accomplished, besides other conventional methods of germplasm storage involving seeds.*

Vegetable crop breeders require pollen at frequent intervals for affecting fertilization in their crop breeding programmes. Often, it has been felt that raising a crop exclusively for its pollen attributes and use as a pollen parent could be dispensed off, provided viable pollen could be cryopreserved for extended durations. The possibility of preserving valuable crop germplasm through pollen storage has been well recognised throughout the world. Pollen storage often enables crossing of different genotypes which are asynchronous in flowering. Protocols offering viable pollen after collection for several days to several years at different times and at different locations holds promise to accelerate the breeding programme efficiency since crossing could be accomplished almost round the year, irrespective of seasonal variation. Stored pollen could be effectively transported under refrigeration to several locations for breeding purposes, and thus enhances the output of seed production units. Besides, a genome could be preserved partially through pollen in a small cryovials. Conservation of a vast range of genetic diversity comprising wild species and landraces, sampled through pollen can be easily accomplished.

The 'pollen cryobanks' could form an integral part of the Gene bank, or repository.

Protocols for pollen preservation should be applicable to a wide range of germplasm. Extremely variable and cultivar dependent results were often encountered when attempts were made to preserve pollen for short durations, manipulating the two key factors that govern pollen viability; low temperature and relative humidity. Therefore, attempts were made to cryopreserve vegetable pollen world wide in crops like capsicum (Barnabas, 1984; Kristof and Barnabas, 1986), Onion (Ganeshan, 1986) tomato and eggplant (Alexander and Ganeshan, 1988). The present report establishes the viability state of cryopreserved tomato and egg plant pollen after a duration of 6 years, with fertility levels estimated under field conditions, in terms of fruit and seed set.

MATERIALS AND METHODS

(i) *Pollen collection and cryostorage*

Tomato (*Lycopersicon esculentum* Mill Cv. 'Arka Saurabh') and Egg plant (*Solanum melongena* L. Cv. 'Arka Kusumkar') cultivars released and popularised by IIHR, Bangalore were taken up for pollen cryostorage and viability assessment studies. Pollen extraction was accomplished by carefully clipping the anthers from healthy flowers at the time of dehiscence, and placing it in clean pertri-dishes in a desicator containing activated silicagel under ambient conditions. The anthers dehisce after a duration of 30-45 minutes, releasing pollen when tapped gently over a clean butter paper, pure pollen is thus collected and transferred into gelatin capsules which are in turn fastened using a high grade cellophane adhesive tape and enclosed in small laminated pouches with capsules are sealed airtight, using a heat sealer and then stacked at the bottom of the cannisters and lowered gradually into a liquid nitrogen cryoflask. Periodical refilling of cryoflask with liquid nitrogen ensures complete immersion of pollen samples. The cannisters are capped with perforated lids, to avoid samples from floating out into the cryoflask while refilling is done.

(ii) *Viability assessment*

Tomato pollen was germinated *in vitro* by the hanging drop technique (Stanley and Linskens, 1974). Egg plant pollen was germinated *in vitro* by the improved cellophane method developed in this laboratory. Egg plant pollen fails to germinate in hanging drops, since pollen sinks to the bottom of the drop. Hence, a different *in vitro* procedure (improved cellophane method) was developed. For both crops, the medium consisted of a carbohydrate source (15 per cent sucrose) supplemented with 300 ppm of Calcium nitrate, 200 ppm of Magnesium sulphate, 100 ppm of Potassium nitrate and 100 ppm of Boric acid. The preparations were incubated at 25° $\pm$ 2°C for a duration of 4-6 hours, after which staining was accomplished using the versatile stain (Alexander, 1980). Three replicates each of fresh and cryostored pollen were made out when viability was assessed. Pollen

grains whose tube lengths were greater than the grain diameter were considered as germinated. More than 300 pollen grains per replicate were scored, using a binocular microscope and percentage germination was calculated.

In order to ensure viability of cryostored pollen, samples retrieved from a cryogenic state to ambient temperature were dusted on stigmas of excised pistils (Alexander, 1987) to study the pollen tube growth on styles. This was carried out prior to actual pollinations with cryostored pollen.

Pollinations with cryostored pollen (inter-varietal crosses) were carried out on healthy plants grown in pots. 'Arka Vikas' served as the female parent for tomato while 'Arka Shirish' served as female parent for egg plant. Flowers were emasculated a day prior to pollination and covered with butter paper bags, in order to avoid contamination. Cryostored pollen was applied on the receptive stigma the next day, and then immediately covered. For controls, stigma of emasculated flowers were applied with freshly collected pollen. Fruit and seed set was recorded with crosses made using fresh and cryostored pollen. Atleast 5 effective pollinations, using cryostored pollen were accomplished for each crop, from which fruit and seed set data was generated.

RESULTS AND DISCUSSION

In vitro pollen germination showed significant reduction after 6 years of cryostorage, both in tomato & egg plant (Fig. 1). More than 60 per cent germination

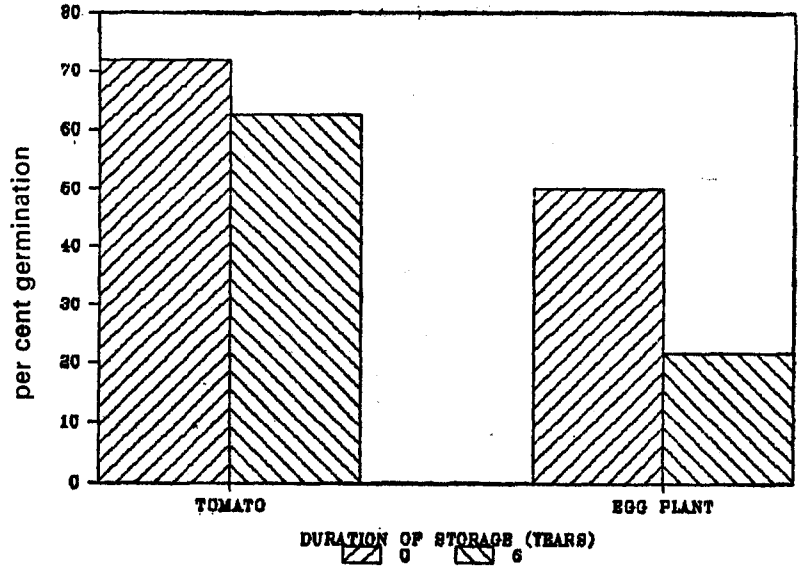

Fig. 1. Viability of Tomato and Egg Plant.

could be recovered in tomato (SE ± 2.36 & 0.64 for fresh and cryostored pollen). While only ca 35 per cent of egg plant pollen could germinate (SE ± 1.32 & 0.46 for fresh and cryostored pollen), in the media where fresh pollen germinated profusely. The trend in reduction was more drastic in egg plant than in tomato. However, this trend appears to have not much significance on observing the performance of cryostored pollen *in vivo* and controlled pollinations. Pollen tube growth was found to be normal on excised stylar material, in both the crops. In tomato controlled pollinations with cryostored pollen could induce ca. 50 per cent fruit and seed set, whereas cryostored egg plant pollen induced ca. 66 per cent seed set of control (Table 1). Seeds set through cryostored pollen germinated normally, with the vigour and vitality equivalent to seeds set through pollinations carried out with fresh pollen.

TABLE 1. CONTROLLED POLLINATIONS WITH CRYOSTORED POLLEN

Crop/Cultivar	Parents		Number of flowers pollinated	Number of fruits set	Number of Seeds/Fruit recovered
	Female	Pollen			
TOMATO :					
'Arka Saurabh'	'Arka Vikas'	× 'Arka Saurabh' (Control)	6	6	165
	'Arka Vikas	× 'Arka Saurabh' (6 years cryostored)	6	3	84
EGG PLANT :					
'Arka Kusumakar	'Arka Shirish'	× 'Arka Kusumakar' (Control)	6	6	840
	'Arka Shirish'	× 'Arka Kusumakar' (6 years cryostored)	6	4	738.25

The foregoing results indicate that it is possible to accomplish long-term cryogenic preservation of tomato and egg plant pollen retaining pollen fertility over extended durations. Although *in vitro* studies predict stored pollen viability, the capacity of cryostored pollen to fertilise and induce normal fruit and seed set could be established only by controlled pollinations. This is especially true in case of egg plant, where *in vitro* studies have indicated a poor viability of cryostored pollen, but when used in controlled pollinations under *in situ* conditions, fertility rates had not declined to a great extent, as shown by a good fruit and seed set. Poor germination rates were recorded for egg plant pollen cryostored for shorter durations. It is not uncommon, however, where a good fruit and seed set may occur even though the results of *in vitro* pollen germination assay indicate low viability (Stanley and Linskens, 1974). Maximum fruit and seed set was obtained with stored pollen viability as low as 6 per cent (Olmo, 1942) in grapes. In onion, with a germination of ca. 50 per cent for 1 year cryostored pollen, a high recovery of fruit and seed set was obtained by using the same in controlled pollinations (Ganeshan, 1986).

In conclusion, the present report emphasizes the role of cryogenic temperature for preserving fertility of tomato and egg plant pollen over prolonged durations. The technique is very simple, and could be effectively used in genebanks for preserving haploid male germplasm. Vegetable crop breeders can exploit such a facility for the requirement of specific pollen parents in their breeding programmes. Infact, breeders can preserve their pollen stocks in cryogenic containers and utilise cryostored pollen in hybridization schedules, as per their choice, and effectively manage their crop improvement strategies.

ACKNOWLEDGEMENTS

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Genetic Divergence in Fababean

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Fababean germplasm including 84 exotic lines from West Germany and 9 indigenous lines, were evaluated for ten metric traits of economic importance. The lines could be grouped into six clusters using D^2 and metroglyph analysis. The grouping parttern revealed by the metroglyph analysis and D^2 analysis had 55.9% resemblance. The comparative efficiency of these two methods to classify the germplasm collection has been discussed.

Fababean (*Vicia faba* L.), a winter food legume grown sporadically as a minor vegetable crop in Himalayan region possesses high genetic potential to produce seed yield greater than cereal crops (Bean, 1967). Considering its exceptional high productivity, it is necessary to undertake breeding work is order to develop high yielding varieties. For a successful hybridization programme, the parents need to be selected from the diverse groups so as to generate greater variability. Many of the lines might be similar to each other with respect to their genetic constitution. However, only the crosses of genetically divergent parents give highly heterotic F_1 and broad-spectrum variability in segregating generations (Arunachalam, 1981). Therefore, it becomes imperative to classify the germplasm into various groups so as to select diverse parents from these groups for hybridization programme. Keeping this in view, the present investigation was planned to evaluate 93 fababean germplasm lines and classify them into various distinct groups using metroglyph analysis (Anderson, 1957) and D^2 statistic (Mahalanobis, 1949).

MATERIALS AND METHODS

The experimental material comprised 84 exotic lines from West Germany and 9 indigenous lines of fababean. These were sown in randomized block design with three replications. Each genotype was grown in single, 3 m long rows. The spacing between rows and between plants was 50cm and 20cm, respectively. Observations on ten randomly chosen plants per row were recorded for days to flowering, days to maturity, plant height, branches/plant, internode length, clusters/plant, pods/plant, seeds/pod, 100-seed weight and seed yield/plant.

The mean values were used for metroglyph analysis as suggested by Anderson (1957) and branches/plant and seed yield were used as x- and y-coordinates, respectively. For D^2-analysis. the original mean values were transformed to standardized uncorrelated variables by Pivtol Condensation method (Rao, 1952).

Genetic distances between genotypes were assessed using Mahalanobis's (1949) D^2-statistic and the material was grouped into clusters according to Tocher's method (Rao, 1952).

RESULTS AND DISCUSSION

Analysis of variance of means revealed significant differences amongst the genotypes for all the characters. The phenotypic coefficient of variation for branches/plant (47 per cent), clusters/plant (87 per cent), seed yield/plant (104 per cent) and pods/plant (108 per cent) revealed that three traits were highly variable.

Based on metroglyph analysis the germplasm was classified in six clusters (Table 1). All exotic lines except three were grouped in cluster I and were low yielding with poor branching. However, the differences among the genotypes within cluster were noted for days to flowering, days to maturity, clusters/plant, pods/plant and seed weight. Cluster II had two exotic and one indigenous genotypes which were characterized by tall stature medium-branching and low expression for other characters. Clusters III and IV possessed 3 and 4 genotypes,

TABLE 1. CLUSTERING PATTERN OF 93 FABABEAN LINES.

Cluster No.	Metroplyph method		Tocher method	
	Number of accessions	Genotypes	Number of accessions	Genotypes
I	81	2, 3, 4, 6, 7, 8, 10, 12, 13, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 32, 33, 34, 36, 37, 39, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 59, 60, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73, 74, 80, 83, 89, 91, 92, 93, 94, 95, 96, 98, 100, 101, 103, 106, 107, 108, 109, 110, 111, 112, 116, 117, 118, 119, 121, 123, 128	55	2, 3, 6, 7, 8, 10, 12, 13, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 32, 33, 34, 36, 37, 39, 42, 55, 70, 71, 80, 83, 89, 91, 92, 95, 96, 100, 101, 109, 110, 111, 112, 113, 116, 117, 118, 119, 121, 123, 129*, 130*
II	3	7, 113, ML*	27	45, 46, 47, 48, 49, 50, 51, 52, 54, 56, 57, 59, 60, 63, 64, 65, 66, 67, 68, 73, 74, 93, 94, 98, 106, 107, 128
III	3	129*, 130*, 134*	7	5, 44, 131*, 132*, 133*, 134*, DM-I*
IV	4	131*, 132*, 133*, DM-1*	2	72, 108
V	1	5	1	ML*
VI	1	HL*	1	HL*
Resemblance (%)				55.9% of metroglyph method

*Indicates indigenous genotypes.

respectively, which were indigenous and had medium seed yield, early maturity, number of high clusters and pods per plant. There was only one exotic line in cluster V and one indigenous line in cluster VI. Both these genotypes showed high manifestation for clusters/plant, pods/plant, seeds/pod and seed yield.

Based on generalized distance (D), 93 lines were grouped in six clusters (Table 1). The intra-cluster distance varied from 0 to 5.26, while inter-cluster distance ranged from 8.09 to 20.55 (Table 2). Intra-cluster distances were smaller than the inter-cluster distances which revealed that the genotypes within clusters had greater similarity. High inter-cluster distances between cluster I and III, I and VI, and IV and VI indicated greater divergence between the genotypes belonging to these clusters. Cluster I accounted for about 87 per cent of total germplasm and about 96 per cent of exotic lines. This showed that the exotic lines had greater similarity amongst themselves presumably due to being from the same source.

TABLE 2. INTRA- AND INTER-CLUSTER DISTANCES (D)

Cluster	I	II	III	IV	V	VI
I	4.85	14.22	18.43	9.30	11.61	20.55
II		5.00	9.19	8.09	9.84	11.43
III			5.26	12.15	9.83	8.21
IV				4.53	8.38	15.11
V					0.00	12.82
VI						0.00

Cluster means (Table 3) showed appreciable variation among the six clusters grouped according to D^2 analysis. The differences among cluster means, though observed for all characters, were more pronounced for days to maturity, plant height, branches/plant, clusters/plant, pods/plant, seeds/pod and seed yield/plant. The cluster II showed the highest mean seed weight. The genotypes in cluster III had higher number of branches, clusters and pods, seed weight and seed yield.

The utility of classifying germplasm for selection of diverse parents for hybridization has long been appreciated (Bhatt, 1970; Arunachalam, 1981). However, choice of method for classification continues to be an inportant issue. In the present study, the clustering pattern obtained with metroglyph analysis revealed 55.9 per cent resemblence with that of D^2 analysis. Of six clusters, only cluster I and II, in both approaches, seemed to possess some similar genotypes. The precise classification based on metroglyph technique is possible only when the maximum variability is explained by the two characters plotted as x- and y-coordinates. Obviously, these two characters should be chosen judiciously. The results of the present investigation revealed that this technique had been quite useful in grouping the material. Although D^2 statistic is quantitative measure of divergence, yet grouping pattern is arbitrary, subjective and changeable under the influence of environment (Singh and Gupta, 1979).

TABLE 3. CLUSTERS MEANS FOR VARIOUS CHARACTERS

Clusters	Days to flowering	Days to maturity	Plant height (cm)	Branches/ plant	Inter-node length (cm)	Clusters/ plant	Pods/ plant	Seeds/ pod	100-seed weight (g)	Seed yield (g/plant)
I	84.9	165.5	77.3	2.0	2.1	8 8	11.2	2.4	32.9	5.9
II	85.5	146.2	74.6	1.9	2.0	8.3	10.0	2.3	33.8	6.0
III	78.4	128.1	57.1	4.8	3.0	36.4	55.9	2.8	33.0	25.4
IV	84.5	158.5	67.8	1.7	1.7	5.8	11.4	2.0	29.8	3.4
V*	75.0	123.0	44.1	3.0	2.7	20.7	31.3	2.3	27.0	13.5
VI*	91.0	127.0	62.4	3.0	2.8	34.0	65.3	3.0	26.8	49.9

*Clusters with single genotypes

It was noticed that the lines of the clusters I and VI (D = 20.55) I and III (D = 18.43) and IV and VI (D = 15.11) showed the maximum distances. Obviously, the crosses between genotypes in these clusters might be useful for fixing transgressive segregants. However, the selection of more than one genotype from large clusters such as cluster I poses specific problems. In that context apart from high genetic divergence, the performance of genotypes for characters such as plant type, disease resistance etc. should be given due consideration. In the present study, genotypes, VH5, VH7, VH72, VH129, HL, LM-I and ML appeared to be desirable for further fababean improvement programme. Inclusion of these parents in multiple crossing programme of diallel selective mating is expected to prove more rewarding.

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Collection of Germplasm Resources in North-Eastern Rajasthan

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The arid/desertic region of Rajasthan is endowed with rich genetic diversity in cultivated and wild plant species. The land race variability is of considerable interest, as it is atuned to dry conditions and adapted under agro-ecological niches with acute water stresses. In order to capture this unique genetic diversity, an exploration trip to north-eastern Rajasthan was undertaken in March-April 1986. The area surveyed comprised several districts of Rajasthan and also adjacent Haryana state. In all 290 population samples in different crops were collected. The details of which are discussed here in this paper.

Considerable genetic diversity was collected in wheat, barley, Brassicae, *chickpea and pigeonpea from diverse agro-ecological niches. In chickpea, variations occurred for plant type and seed; being dwarf, bushy to tall, erect types; pod size and colour also varied. Occasionally double pods/peduncle types also occurred in some landraces. Pigeonpea exhibited variation for dwarf types with small, light yellow, brown, black and mottled grain types. The frequency of occurrence of tall types was more in case of pigeonpea cultivars. In wheat, predominant local varieties grown included deshi gehun, deshi kanak, dhauli kanak, safed kanak, pili kanak and kharchia. The latter type being well known for its adaptability to salanity. In oilseed crops,* Brassica juncea *and* B. campestris var. yellow sarson *and var. brown* sarson *were prevalent. Taramira* (Eruca sativa) *exhibited rich genetic variation in plant type, branching pattern, pigmentation, fruiting habit, pod size, shape, grain size and colour. The response to drought and hardiness showed high tolerance. Variation exhibited in case of brinjal germplasm was interesting particularly, both spiny and non spiny forms with oblong or round, small to large size fruits occurred. Rich variability was observed in musk melon and* Cucurbita moschata. *Among wild types,* Balanites aegyptiaca *and wild bitter ground* (Momordica balsamina) *were important.*

The germplasm diversity in crop plants adapted to arid/desertic climatic conditions of Rajasthan and adjoining Haryana state is of immense significance in crop breeding programme as it provides opportunity for identifying drought hardy/tolerant genotypes in different crop plant species. With the sole aim of collecting landrace variability, an exploration trip was planned and carefully executed. The details are discussed briefly in this paper.

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MATERIALS AND METHODS

The diverse agro-ecological habitats and microniches were extensively surveyed covering in all about 125 collection sites spread in 12 districts of Rajasthan as well as in two adjoining districts of Haryana state. Districts of Bikaner, Churu, Sikar, Jhunjhunu, Nagour, Jodhpur, parts of Jaipur, Sri Ganganagar, northern Ajmer, and western Pali were extensively explored. The variability collected was of significant importance in *T. durum*, *T. aestivum*, barley, chickpea, pigeonpea, *Eruca sativa* and several vegetable crops. At each collection site, random samples were drawn to obtain reasonable estimates of natural population. The collection route is given in Fig. 1 and details of collection are summarized in Table 1.

Fig. 1. Exploration and germplasm collection from N. E. Rajasthan and Haryana, (March-April, 1986)

RESULTS AND DISCUSSION

The major collection sites were marked by distinct ecology, habitats, rainfall pattern and precipitation regime. It was observed that rainfall gradually decreased from east to north west and vegetation pattern also varied accordingly. Based on the nature of soil and vegetation, Rajasthan could be divided in three major regions; (i) fertile eastern Rajasthan, (ii) arid western Rajasthan, and (iii) semi-arid middle Rajasthan. From above regions comprising several districts, 290 samples of germplasm in pulses (120) and other crops (170) were collected. The major collection comprised of *Hordeum vulgare* (47), *Triticum aestivum/T. durum* (37), *Cicer arietinum*

TABLE 1. GERMPLASM DIVERSITY COLLECTED

State/District	Crop group	Crops	Number of accession
(I) *Rajasthan*			
Pali (2)	Cereals	Barley (47), Maize (1), *Triticum aestivum* (37), *T. durum* (1)	86
Jodhpur (6) Nagour (1)	Pulses	Chickpea (104), Cowpea (2), Lentil (4), Mungbean (1), Pigeonpea (9)	120
Bikaner (11)	Oilseeds	Castor (2), Rapeseed mustard (23), *Eruca sativa* (16)	41
Sri Ganganagar (27) Churu (5)	Vegetables	Chilli (1), Eggplant (6), Fenugreek (14) Garlic (2) Onion (3), Radish (4), Tomato (2)	32
Jhunjhunu (14)		Bittergourd (1), *Solanum xanthocarpum* (1), *Vicia sativa* (1), *Balanites* sp. (1)	4
Sikar (16)	Miscellaneous	Cotton (1), Cumin (2), *Lepidium sativum* (1), Muskmelon (2), *Plantago ovata* (1)	7
Jaipur (23) Alwar (16)			
II *Haryana*			
Mahendragarh (2) Gurgaon (2)			
Total collections			290

(104), *Cajanus cajan* (9), *Brassica* sp. (23), *Eruca sativa* (16), *Trigonella foenum graecum* (14), *Solanum melongena* (6), *Allium cepa* (5), *Raphanus sativus* (4), *Lycopersicon esculentum* (2) and *Cucumis melo* (2). Wild *Momordica balsamina* was also collected from natural/disturbed habitats.

Observations on ecology and habitats

The extreme arid climate of Rajasthan desert is characterized by high solar radiation, seasonally and diurnally fluctuating temperatures and precarius, erratic low annual rainfall which varies from year to year without following any trend. The low atmospheric humidity, powerful hot and cold winds, sand storms, dust storms, dew, mist, fog, frost, evaporation far exceeding precipitation accompanied with high isolation, all tend to modify the ecosystem (Mulay and Joshi, 1964). Based upon the physiology, the nature of the soil substratum varied from place to place in the desertic region of Rajasthan. The desert soil partly being derived from material formed by physical disintegration of the local rocks and partly from blown sand. The desert is characterized by wind blown sand dunes of various types, besides the occurence of saline and sporadic aquatic habitats. The salt content of the desert soils is not so high as to be toxic to plant growth; being

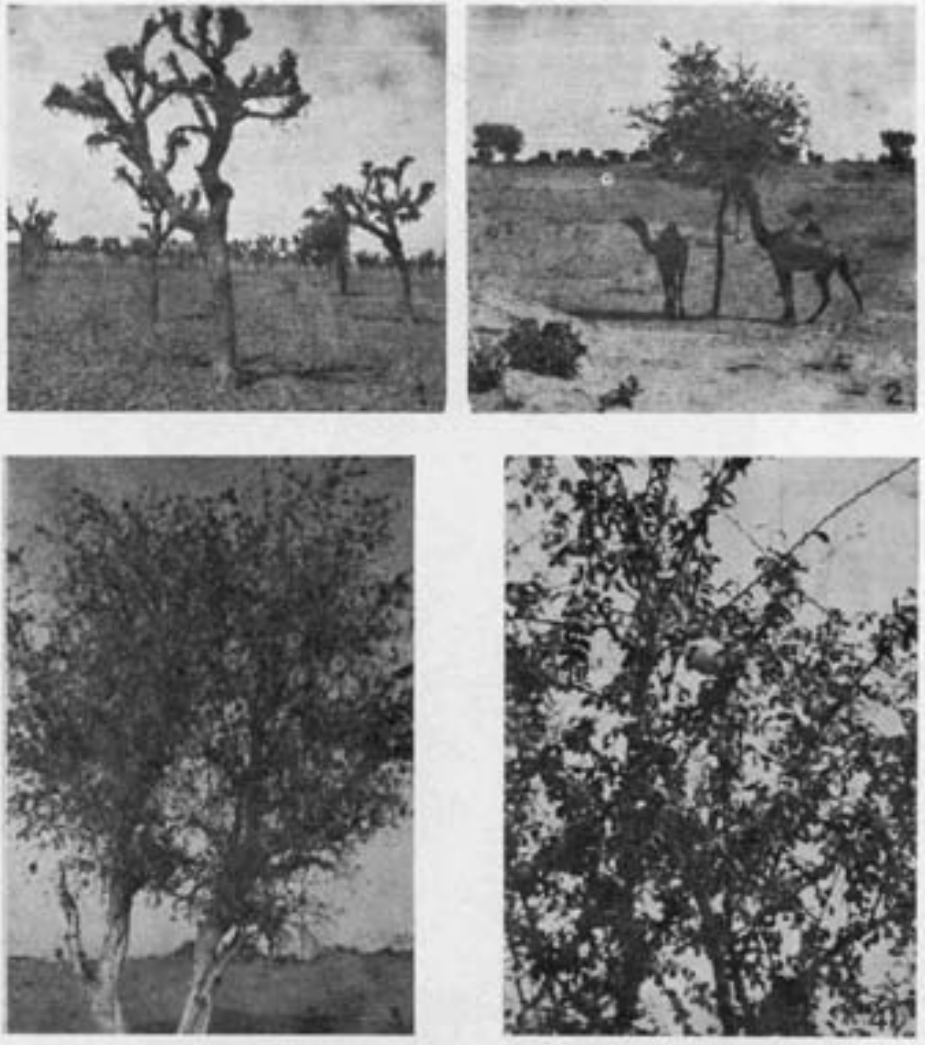

Plate I Fig. 1. Desert ecology; excessive lopping of *Prosopis cineraria* (A life saving fodder tree)

Fig. 2. *Acacia arabica*—Excess camel pressure ruins vegetation

Fig. 3. *Tecomela undulata* (Rohiro)—threatened desert tree

Fig. 4. *Balanites aegyptiaca* (Hingota)—Valuable tree species; fruits contain diosgenin.

present in that moderation to impact a certain amount of impermeability to the soil to keep the moisture in the region of the plant roots for a longer time. The subsoil is present in variable depths in different parts of the region explored.

Plate II Fig. 1. Landrace diversity in wheat
Fig. 2. Variability in musk melon germplasm
Fig. 3. *Cordia myxa*—A wild tree species with edible fruits

Observations on vegetation

The excessive and indiscriminate cutting, lopping, stripping and amputating the branches of the sparsely natural occurring trees of *Prosopis cineraria, Acacia* species, *Balanites aegyptiaca,* etc. have caused considerable damage to the developing vegetation (Plate 1). The impact on vegetation of the indiscriminate grazing and trampling by domestic animals like camels, large flocks of goats, sheeps, and donkeys and other burrowing animals have all resulted in the destruction of ground vegetation inhabiting wild species. Despite hazardous climate, unproductive soils and intense pressure, vegetation still covers a lot of land surface of Rajasthan. The flora comprises xerophytic shrubs, such as *Zizyphus nummularia, Capparis decidua, Calotropis procera,* etc. spread in descrete and discontinuous patches of plant communities. It consists of ephemerals, grasses, perennial herbs, under shrubs, dwarf trees and badly mutilated trees.

Observations on crop diversity

The predominant crops prevalent in *Rabi* season in the area explored comprised mainly wheat (*Triticum aestivum* and *T. durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*), rapeseed mustard (*Brassica* species), and taramira (*Eruca sativa*). In some localized pockets, cultivation of pigeonpea (*Cajanus cajan*) was also observed. Similarly, cultivation of fenugreek (*Trigonella foenum graecum*) and lentil (*Lens culinaris*) in the districts of Alwar, Jaipur, Jhunjhunu and Sikar was predominant. Chickpea exhibited considerable variability largely grown in the rainfed areas of Sri Ganganagar, Hanumangarh upto Rawatsar and only scantly upto Sardar Shahar.

Observations on genetic variability

In Phagi and Chaksu areas (Distt. Jaipur) cultivation of local wheat called Safed kanak was observed followed by barley, rapeseed and mustard. Local landraces of *durum* wheat prevalent in the area were also collected particularly from Chaksu and Dhansa region. Rajasthan region holds rich genetic variability in chickpea and a wide spectrum of landraces/forms are still in the cultivation. Crop is chiefly grown under rainfed conditions and to a limited extent under canal irrigation. Two types of cultivated chickpea are recognised; deshi (small seeded, angular and variable colour forms) much prevalent in north-west region; kabuli types mainly confined to irrigated tracts being invariably round, large seeded and slightly tinged in some cases. The latter form is mainly cultivated in Sri Ganganagar district. In majority of the areas explored, it was found that local landraces were still predominant due to their better adaptations and moderately good grain yields. In some areas, improved chickpea varieties were also tried, but these could not replace the old cultivars due to poor adaptability, low grain yield, susceptibility to drought and water stress conditions. In the areas explored, chickpea cultivation was predominant in Bikaner, Churu, Sikar, Jhunjhunu, Jaipur, Alwar and Sri Ganganagar region. Variations largely occurred for plant type; both dwarf bushy to moderately tall, erect types occur under cultivation. Genetic

polymorphism for pigmentation of stem was observed in cultivars. The pod, grain size and colour were also observed to be variable; light yellow to deep purplish pigmented pods with single, occasionally double pods/peduncle occurred in the populations of some fields. Invariably pigmented type was linked with brown seed. While greenish yellow plant type possessed dull yellow to bright yellow grains of variable size. A few cultivars with white seeds were also collected. Variations were predominant for their root system and also for water requirement/uptake.

Among other crops, pigeonpea exhibited interesting variability particularly in the Chaksu/Phagi region of Jaipur and Alwar districts. Both dwarf to tall types with small, light yellow, brown and mottled grained types were found as mixed culture (crop) with pearlmillet and sorghum. *Trigonella* is grown extensively in this region particularly as cattle fodder and feed crop. Tall erect types were predominant. Important oil seed crops included *Brassica juncea*, *B. campestris* var. *yellow sarson* and taramira (*Eruca sativa*), the latter is grown extensively and exhibited significant genetic variations for plant type, branching patterns, pigmentation, fruiting habit, pod size, shape and colour as well as grain size and colour. The crop exhibited remarkable plasticity for adaptation to soil conditions, drought hardiness as well as resistance to diseases and pests.

Extensive surveys in the Jodhpur-Pali region showed predominance of *Kharchia* landrace of wheat (*T. aestivum*). This race is well adapted to the high concentration of salinity and gives fairly high grain yields even under adverse conditions. In Sikar district, interesting variability in wheat landraces (Plate II, Fig. 1) was observed. The variation was conspicuous, as these landraces were very tall with long ear, black purplish glumes and long purplish black awns. The grains were very bold, amber coloured; the crop is primarly grown under rainfed condition (Plate I).

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Multiple Resistance in Indigenous Rice (*Oryza sativa* var *indica*) Germplasm

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Some indigenous landrace diversity of rice collected from diverse agro-ecological habitats and microniches with predominant tribal and ethnic population were screened against bacterial leaf blight (Xanthomonas campestris *pv.* oryzae *Dye* et al), *brown plant hopper* (Nilaparyata lugens *Stal*) *and white backed plant hopper* (Sogatella furcifera *Horvath*) *prevalent in the field. The local landraces adapted to this region, namely*, Agiyasal, Agyasar, *Akkapm,* Badshahbhog, Jhili *and* Karrigurmatia *were resistant to all the above mentioned pathogens and pests. The study on inheritance showed that genic control was under single gene pair (monogenic).*

Rice (*Oryza sativa* var. *indica*) is considered to be the host of over 60 diseases and about 100 insects/pests. Some of these are of major international importance. The most prominent and devastating among these are the bacterial blight, brown plant hopper and white backed plant hopper. It has been observed that chemical control is expensive and in some cases futile. Therefore, in rcent years, major thrust has been focused for incorporating genes for disease and insect/pest resistance in most of the national/international programmes. Germplasm collection, screening and conservation, thus became one of the most essential component of rice improvement programme. The screening of germplasm for identification of resistant donors has now been assigned high priority to pave the way for further utilization, both by conventional methods as well as by new biotechnological techniques. Raipur Agricultural University, located at Raipur, Madhya Pradesh, has built a very comprehensive collection of well over 18,000 rice accessions. The present study was undertaken to screen some of the indigenous rice cultivars/landraces against bacterial leaf blight (BLB), brown plant hopper (BPH) and white backed plant hopper (WBPH). Nature of resistance was also studied in some of the cultivars.

MATERIALS AND METHODS

Among the rice germplasm maintained in field gene bank (*ex-situ*) at Raipur, Madhya Pradesh, 200 accessions were found tolerant to BLB in preliminary field

*Part of the screening carried out by the author at International Rice Research Institute, Manila, Philippines.

evaluation during the year 1982. These were screened further at International Rice Research Institute, Manila, Philippines, for bacterial leaf blight as per the technique described by Kaufman *et al.* (1973) and against BPH and WBPH as per technique suggested by Athwal *et al* (1971). Standard evaluation system of IRRI (1980) was used for assessment of damage, only six cultivars (Table 1) which were found resistant to all the three biotic stresses (BLB, BPH and WBPH) were further utilized for genetic study. These were crossed with cv. Taichung Native-1 and their F_1 and F_2 generations were screened along with parents. 10 F_1's and more than 500 F_2 seedlings per cross were utilized for screening and study.

TABLE 1. RICE CULTIVARS SHOWING MULTIPLE RESISTANCE

Name of the cultivar	M.P. Acc. No.	Disease score SES 0-9 scale		
		*1 Bacterial leaf blight	*2 Brown plant hopper	*3 White backed plant hopper
Agiyasal	A 330	1	1	1
Agyasar	A 518	1	1	1
Akkapm	A 551	1	3	1
Badshahbhog	B 2486	1	3	1
Jhili	J 273	1	3	1
Karrigumatia	K 1393	1	1	1

*1 Philippine pathotype group I
*2 Philippine biotype I
*3 Philippine biotype

RESULTS AND DISCUSSION

The studies on the inheritance of bacterial leaf blight (BLB) showed that all the six cultivars under study indicated monogenic pattern of genetic control (Table 2). Monogenic dominant nature of gene has been reported by several earlier workers (Devdath, 1970; Murthy and Khush, 1972; Singh *et al.*, 1983). Allelic test earlier conducted on these cultivars possess *xa-4* dominant gene for resistance (Sidhu *et al.*, 1978). These cultivars were also resistance at Raipur centre, when screened under field conditions. This suggested that perhaps *xa-4* gene predominantly occurs in the landraces being cultivated in Madhya Pradesh region of India. However, it needs to be fully established through more comprehensive studies. The present virulent race of bacterial leaf blight can be checked through the introduction of improved varieties possessing *xa-4* gene for resistance. The cultivars identified for resistance gene *xa-4* at Philippine and India also exhibited resistance at Japan. In view of the above fact, these cultivars/landraces have assumed international importance for overcoming the problem of bacterial leaf blight epidemics.

TABLE 2. F_1 AND F_2 REACTION TO RACE I OF BACTERIRL LEAF BLIGHT FROM THE CROSSES OF TAICHUNG NATIVE 1/RESISTANT CULTIVARS

Cross	F_1	F_2		X^2 3 : 2
		Resistant	Susceptible	
T.N.1/Agiyasal	Resistant	710	218	1.23
T.N.1/Agyasar	Resistant	734	369	1.77
T.N.2/Akkapm	Resistant	663	243	2.03
T.N.1/Badshahbhog	Resistant	787	242	2.68
T.N.1/Jhill	Resistant	643	242	2.60
T.N.2/Karrigumatia	Resistant	637	209	9.030

BHP reaction indicated that *Agiyasal*, *Agyasar*, *Akkapm*, *Badshahbhog* and *Karrigurmatia* possess single dominant gene for resistance (Table 3). The single dominant gene conferring resistance to this pest has also been reported by earlier workers (Athwal *et al*, 1971; Chen and Chang, 1971; Choi *et al*, 1979; Krishna and Seshu 1984,; Lakshminarayan and Khush 1977; Martines and Khush, 1974).

TABLE 3. F_1 AND F_2 REACTION TO BIOTYPE I OF BROWN PLANT HOPPER FROM THE CROSSES OF TAICHUNG NATIVE 1/RESISTANT CULTIVARS

Cross	F_1	F_2		X^2 3 : 1
		Resistant	Susceptible	
T.N.1/Agiyasal	Resistant	804	256	0.40
T.N.1/Agyasar	Resistant	760	251	0.026
T.N.1/Akkapm	Resistunt	773	263	0.082
T.N.1/Badshahbhog	Resistant	682	222	0.06
T.N.1/Jhili	Susceptible	208	533	3.75
T.N.1/Karrigurmatia	Resistant	682	223	0.06

TABLE 4. F_1 AND F_2 REACTION OF WHITE BACKED PLANT HOPPER FROM THE CROSSES OF TAICHUNG NATIVE 1/RESISTANT CULTIVARS

Cross	F_1	F_2		X^2 3 : 1
		Resistant	Susceptible	
T.N.1/Agiyasal	Resistant	487	224	2.51
T.N.1/Agyasar	Resistant	433	142	0.014
T.N.1/Akkapm	Resistant	655	229	0.34
T.N.1/Badshahbhog	Resistant	630	231	1.44
T.N.1/Jhili	Resistant	587	221	2.26
T.N.1/Karrigurmatia	Resistant	352	144	3.44

Nature of resistance to WBPH indicated that a single dominant gene conferred resistance in all the cultivars under study (Table 4). Several workers earlier reported monogenic nature of gene control for WBPH (Angeles *et al.*, 1981; Hernandes and Khush, 1981; Khush and Virmani, 1985 and Krishna, *et al.*, 1984).

The present study suuggested that many indigenous landraces collected from Madhya Pradesh possess multiple resistance and the resistance is monogenically inherited. Further study on allelic test for BPH and WBPH will suggest whether the genes observed in the present study are same or different from earlier reported genes. After allelic test, the desired resistant genes can be utilized in further breeding programme. Among 200 landraces screened, six landraces with multiple resistant could be identified. This clearly emphasizes the need and urgency for very comprehensive evaluation and screening of indigenous diversity in *Oryza* species for identifying resistant genetic resources to diseases and pests. The incorporation of the resistance derived from such well adapted landraces will be of considerable significance in rice crop improvement. The present study is an attempt to investigate the genetics of resistance to BLB, BPH and WBPH in a small number of landraces from Central India. Further, studies would be required to screen entire germplasm against other pests and diseases.

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Diversity and Evolutionary Relationship Among Four Cultivated and Two Wild Species of Vigna

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Cultivated Vigna *species*; mung *bean* (V. radiata (*L.*) *Wilczek*), urad *bean* (V. mungo (*L.*) *Hepper*) *and their wild occurring putative progenitors*; V. radiata *var.* sublobata *Verdc. and* V. mungo L. *var.* silvestris *Lukoki, Marechal and Otoul respectively were studied along with rice bean* (V. umbellata (*Thunb.*) *Ohwi and Ohashi*) *and* V. trilobata *Ait. The data from experimental biology demonstrated discrete variation among variable populations of different* Vigna *species. Four distinct groups emerged showing that all the four cultivated species are distinct and have diverse and independent origin, while var.* silvestris *showed continuous variation with* urad *bean suggesting close affinity and direct lineage from above putative progenitor.*

Mung bean (*V. radiata* (L.) Wilczek, *urad* bean (*V. mungo* (L.) Hepper, rice bean (*V. umbellata* (Thunb.) Ohwi and Ohashi and *V. trilobata* Ait. are cultivated in India as pulse and forage crops. While the two putative progenitors, *V. radiata* var. *sublobata* Verdc. and *V. mungo* L. var. *silvestris* Lukoki marechal and Otoul are predominently wild and occur in natural/disturbed habitats. The *mung* bean and *urad* bean have evolved directly from above wild types respectively (Lukoki *et al.*, 1980; Chandel, 1980, 1981, 1984; Chandel *et al.*, 1984.)

There are about 22 species of *Vigna*, both cultivated and wild that occur in India (Arora, 1984). *V. radiata* var. *sublobata*, *V. mungo* var. *silvestris*, *V. vexillata* and *V. umbellata* var. *major* and var. rumbaiya have been reported to occur sympatrically in sub-Himalayan region (Chandel, 1981) and some of these even show a very wide distribution occurring in the Western Ghats, Eastern Ghats, Central Deccan Plateau and North-Western Himalaya extending their distribution and adaptive range from Shiwalik hills, to Kamaon/Garhwal Himalaya, Chota Nagpur Plateau, Sikkim, North Bengal and North-Eastern Region of India (Arora, 1984). The distribution of some of these has also been documented recently from South-East Asia and Far East (Annishetty and Moss, 1987). Ironically, the taxonomic status of several Asian *Vigna* species is still in a confused state and needs critical studies (Verd-Court—personal communication, 1970). The present investigation is in the series of the studies to derive evidences from experimental biology. The genetic diversity and pattern of genetic variation (continuous or discrete) among the populations of

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six *Vigna* species would provide strong support about the evolutionary inter-relationships.

MATERIALS AND METHODS

The experiments were conducted on four culivatad *Vigna* species and two wild sub-species comprising variable populations from diverse agro-ecological habitats. The material consisted of *V. radiata* var. *sublobata* (6), *V. radiata* (2), *V. mungo* (2), *V. mungo* var. *silvestris* (8), *V. umbellata* (8) and *V. trilobata* (11). The material was grown during rainy season (July-November, 1980) at IARI farm. Each strain had 3 m long row and plants spaced 8-10 cm. Five randomely selected plants in each populations were scored for five important morphological characters. The data were averaged and plotted using metroglyph (Fig. I). Stipule index and cotyledon leaf index were used as ordinate and abcissa respectively and five characters, viz. cotyledon stalk, Ist leaf index, depth of hilum, length and density of hairs on pods were represented as rays emerging from each of the glyphs.

The interspecific crosses including reciprocals were also attempted between *V. radiata* and var. *sublobata*, *V. mungo* and var. *silvestris* (manuscript under preparation).

RESULTS

The careful observations on six *Vigna* species for germination habit and morphological characters showed that *urad* bean, *mung* bean and wild types, var. *sublobata*, var. *silvestris* and *V. trilobata* possessed epigeal germination habit and rice bean (*V. umbellata*) showed hypogeal germination. The comparative morphology of stipule showed that *V. mungo* and its wild type var. *silvestris* possess narrow linear stipules like that of *V. umbellata*. The hilum is also deep, furrowed and concave in structure. In contrast to above, *V. radiata* and its wild form *V. radiata* var. *sublobata* have broad stipules like that of *V. trilobata*. The hilum is long, linear and stretched like that of *V. umbellata*. These morphological similarities provide indications that they have close affinity. The present investigation provides experimental evidence that these are quite distinct species as they form independent groups. It is certain that they have not evolved from same common ancestor. Although sympatric variation pattern might have provided opportunities for occasional natural crossing and gene exchange. The apparent homology in morphological variation might have also been perforced by the ecological conditions.

A comparative study of the morphological characters exhibited conspicuous variation for cotyledon leaf stalk (0.05-1.3 cm), cotyledon leaf index (0.8-4.8 cm), first leaf index (0.7-4.8 cm), stipule index (1.2-5.5 cm), depth of hilum (7-50 mm), length of hairs on pods (short-long), and density of hairs on pod (very sparse-dense). The character association analysis of the *Vigna* species based on above morphological characters indicated existence of four broad groups (Fig I); group I comprised 54 plants belonging to *V. trilobata* which showed association of long cotyledonary leaf stalk (0.5-1.3 cm), broad cotyledonary leaf pair (0.8-1.7 cm.),

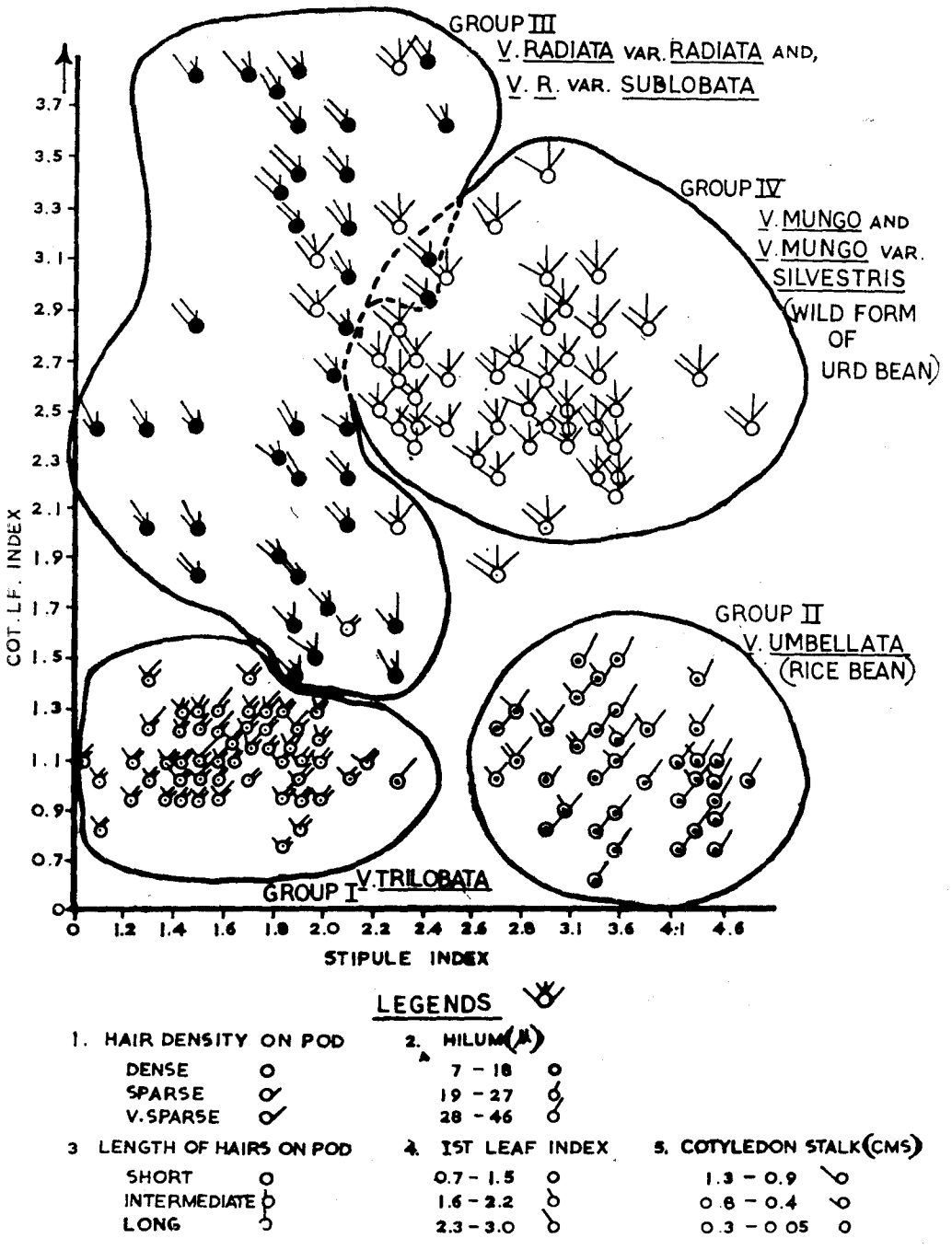

Fig. 1 Metroglyph showing inter relationships among *Vigna* species

and first leaf index (0.7-1.3 cm.), short and broad stipule (1.2-2.0 cm) very much raised hilum (19-35 mm.) and short sparse hairs on pods.

Group II consisted of 39 plants identified as *V. umbellata*. They possessed long stalk (0.6-1.3 cm), broad cotyledonary leaf pair (0.8-1.6 cm) and first trifoliate lateral leaflet (1.1-1.7 cm), narrow long stipule (2.5-4.5 cm), hilum not much raised (7-16 mm.), short and very sparse hairs on pods.

Group III comprised forty glyphs identified as *V. radiata* and its wild form *V. radiata* var. *sublobata*. They are more dispersed, showing some intermingling with *V. mungo* var. *silvestris* in one direction and *V. trilobata* on the other. The plants in this group have short cotyledonary stalk (0.05-0.4 cm), long and narrow cotyledonary leaf index 1.5-4.6 cm, first trifoliate leaf of moderate size (1.5-3.5 cm), short and broad stipule (1.3-2.5 cm), not much raised hilum (7-18 mm) and long, moderately dense hairs on pods.

Group IV comprised 50 plants which can be identified as *V. mungo* and its wild putative progenitor *V. mungo* var. *silvestris*. They have short coteledonary leaf stalk (0.05-0.2 cm), long and narrow coteledonary leaf (1.7-3.6 cm), first trifoliate leaf moderately long (1.8-3.5 cm), long and narrow stipule (2.0-5.5 cm), raised hilum (2.8-5.0 mm). and long dense hairs on pods.

DISCUSSION

The study of genetic variation showed the occurrance of intermediate forms between *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris* for some morphological characters studied in the present studies. This suggests the possible natural chance outcrossing allowing introgression and restricted gene flow between the two wild putative progenitors with their respective cultigen species. It appears from the natural populations that outcrossing perhaps also occurred occasionally between the wild form of *V. radiata* var. *sublobata* and *V. radiata* var. *setulosus*, and *V. trilobata* as well as with wild forms of *V. umbellata* (var. *major* and *rumbaiya*). Normally, these species do not intercross in nature although occasional but restricted outcrossing has been documented (Chandel, 1984; Chandel *et al.*, 1988). Morphologically, the *Vigna* species were earlier grouped into three taxonomic complexes. First group included *V. trilobata* and *V. aconitifolia*; second complex consists of *V. radiata* var. *sublobata*, *V. mungo* and *V. mungo* var. *silvestris*, and third complex included *V. umbellata* and *V. angularis*. The species within each complex can be intercrossed and fertile hybrids are reported to have been obtained with *V. radiata* and *V. mungo* with *V. umbellata* or with *V. trilobata* (Dana, 1966a, 1966b; Ahn and Hartmann, 1978; Chandel, 1984). In some crosses, the F_1 hybrids are reported to be sterile despite the facts that 6-10 bivalents, out of the possible eleven were observed during meiosis (Dana, 1966a, 1966b). The detailed studies on Asian *Vigna* species (Chandel, 1980, 1984; Chandel *et al.*, 1984; Lukoki *et al*, 1980) have established conclusively the origin and evolution of *mung* bean and *urad* bean through extensive evidences from taxonomy, biochemical studies such as chromatography of leaf phenolics and protein gel electrophoresis, scanning electron microscopy of seed coat pattern and hilum structures. The quantitative

data derived from independent sources was used in numerical taxonomy for drawing dendrograms each arriving at the same conclusion, (Chandel *et al.*, 1984). It was concluded that *V. radiata* and *V. mungo*, the two cultivated species in India, have independent lineage and were domesticated from two very distinct taxa namely, *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris* respectively. The evidences were also supported by cytological relationship, crossing and hybridization studies, experimental biology, archaeology and phytogeographic distribution of cultivated types, their wild ancestral forms and weedy relatives. The possible area of domestications in Indian gene centre were also propounded (Chandel, 1984).

The present studies also indicated distinctness of different taxa under genus *Vigna* particularly *V. radiata* var. *sublobata*, *V. mungo* var. *silvestris*, *V. trilobata* and *V. umbellata*. The results further render support to previous investigations. The work is being intensified on all cultivated and related wild Asian species of *Vigna* using isozymes as well as cytological techniques such as DNA quantification and banding technique to elucidate relationships with other wild forms occuring in India. The evidences are likely to provide strong support and hopefully would pave the way for new opportunities in uitlization of wide genepool, particularly allowing inter-specific hybridization resulting in the improvement of cultivated *Vigna* species.

The study of the genetic variation and inter-relationship among large number of species of *Vigna* will be immensely valuable and rewarding as these species possess various useful agronomic traits, such as yellow mosaic virus disease resistance (*V. umbellata*), wide range of adaptability of *V. trilobata*, protein quality of *V. mungo* and balanced amino acids of *V. umbellata*. Such genepool offer tremendous opportunities in conventional breeding as well as in the application of biotechnology for crop improvement.

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Short Communication

Study of Duplicates in the Germplasm

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Small samples of germplasm with identical names, grain features, maturity, and other morpho-agronomic features and from same origin were screened for bacterial blight and white backed planthopper at IRRI, Philippines. Variable reaction for the disease and pest was observed within the identical names. The collections screened, therefore, were not duplicates.

In the cultivated rice (*Oryza sativa* L.), enough genetic variability is present to serve most of the needs of rice breeders. The genetic wealth of rice germplasm is threatened by rapid spread of new improved varieties. It became important, thus, to collect and conserve rice varieties of an obscure nature and of unknown genetic potential. Rice researchers in the tropics often have difficulty in maintaining a large collection in the absence of storage facilities. It is often suggested to reject the duplicates within the collection. Duplicate samples are discarded generally when two collected samples have (i) similar names, (ii) identical grain features, maturity, and other morpho-agronomic features, and (iii) the same or neighbouring places of origin (Chang *et al.*, 1972).

Zonal Agriculture Research Station, Raipur has a huge collection of about 19,000 indigenous rice germplasm, many of them having similar names. A small sample from this germplasm comprising entries with similar names, maturity, grain features and same origin were screened for bacterial blight (BB, *Xanthomonas campestris* pv. *oryzae*) and white backed planthopper (WBPH, *Sogatella furcifera*) at International Rice Research Institute, Philippines. The screening procedure for BB and WBPH were followed, using the method suggested by Kauffman *et al.* (1973) and Athwal *et al.* (1971), respectively.

The screening results for BB and WBPH are presented in Table 1. Fourteen accessions of *Badshah bhog* were evaluated. Among these, B 248 and B 1209 were rated resistant for BB and one accession B 189 was resistant for WBPH. Similar to this, other accessions of *Bhata dudgi*, *Chhatri*, *Dubraj*, *Gurmatia* and *Jhilli* showed variable reaction for this disease and pest.

The above screening was only in a few local types, and against only BB and WBPH. But more variable and differential reactions may be expected if the material is evaluated for other general traits, and for various pathotypes and/or biotypes of diseases and pests. Thus, the results suggested that the land races

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TABLE 1. SCREENING RESULTS OF BACTERIAL BLIGHT AND WHITE BACKED PLANTHOPPER

Landraces/ cultivar	MPRRI Acc. No.	Reaction to*	
		BB	WBPH
Badshah bhog	B 54	S	S
Badshah bhog	B 189	S	R
Badshah bhog	B 220	S	S
Badshah bhog	B 227	S	S
Badshah bhog	B 236	S	S
Badshah bhog	B 248	R	S
Badshah bhog	B 466	S	S
Badshah bhog	B 670	S	S
Badshah bhog	B 799	S	S
Badshah bhog	B 973	S	S
Badshah bhog	B 1005	S	S
Badshah bhog	B 1209	S	S
Badshah bhog	B 1322	S	S
Badshah bhog	B 1899	S	S
Bhata Dudgi	B 1899	S	S
Bhata Dudgi	B 2177	R	S
Chhatri	C 90	S	S
Chhatri	C 364	R	S
Dubraj	D 12	S	S
Dubraj	D 61	R	S
Dubraj	D 341	S	S
Dubraj	D 422	S	MR
Dubraj	D 1026	S	S
Gurmatia	G 123	S	R
Gurmatia	G 185	S	R
Gurmatia	G 245	S	S
Gurmatia deshi	G 123	S	R
Jhilli	J 7	R	R
Jhilli	J 107	S	R
Jhilli	J 273	R	R
Jhilli	J 274	R	MR
Jhilli	J 388	S	R
Jhilli parag	J 105	S	S

*R—Resistant
MR—Moderatel resistant
S—Susceptible.

collected from different fields and/or villages some times may have the identical names and morpho-agronomic features, but genetically they may not be the same. Therefore, the duplicates should not be discarded, unless evaluated thoroughly for different traits reactions.

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Short Communication

Evaluation of Wheat Germplasm Against Yellow Rust and Powdery Mildew of Wheat at Keylong

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The eroding genetic base of the cultivated wheats has been of much concern in recent years. Wheat being a staple food for 35 per cent of the world population, the International Board for Plant Genetic Resources (IBPGR) has set high priority for the collection, evaluation, documentation and utilization of wheat genetic resources (Crostan and Williams, 1981). Although a reasonably good level of rust resistance in our present day wheat cultivars has been obtained, resistance to powdery mildew is very rare. Sources of resistance to yellow rust (*Puccinia striiformis* West.) and powdery mildew (*Erysiphe graminis* f. sp. *tritici*) of wheat, which are known to reduce wheat yields, have to be identified and utilized to avoid losses.

Keylong (Lahaul-Spiti valley) in Himachal Pradesh is a 'hot spot' for both these diseases and thus is an ideal place for screening wheat germplasm. This paper reports our findings on evaluation of a comprehensive collection of 2211 accessions of wheat germplasm (Table 1) under natural field conditions at Keylong.

Each accession was planted in one metre row at the Punjab Agricultural University, Research Farm, Gumrang (Keylong) in May 1987, where the incidence of yellow rust and powdery mildew was very high and this facilitated screening of the germplasm. The data on the incidence of yellow rust and powdery mildew were recorded at the time of maximum disease development in September 1987 according to the modified Cobb scale and a scoring scale of 0-9 given by Saari and Prescott (1975), respectively. Accessions having 0-ts of rust severity were rated as resistant whereas for powdery mildew accessions which were completely free from the disease were marked as resistant.

A majority of the accessions of *Triticum aestivum*, *T. durum*, triticale, rye and *T. carthlicum* evaluated gave resistant reaction to yellow rust (Table 1). In comparison, the susceptible accessions had a very high rust severity. Resistance to powdery mildew was identified only in a very few accessions of *T. aestivum* and *T. durum* thus showing that these were the most susceptible species. However, triticale, rye and *T. carthlicum* were the most resistant species, Resistance was also found in several other species with only one or two accessions in our collection. Overall, a very high proportion (86.5%) of the accessions evaluated were resistant to yellow rust, whereas only 18.6 percent accessions were resistant to powdery mildew.

TABLE 1. EVALUATION OF WHEAT GERMPLASM FOR RESISTANCE TO YELLOW RUST AND POWDERY MILDEW OF WHEAT AT KEYLONG

Species	No. of accessions evaluated	No. (%) resistant accessions	
		Yellow rust	Powdery mildew
T. aestivum	1366	1132 (82.8)	84 (6.1)
T. durum	495	450 (90.9)	3 (0.66)
Triticale	331	325 (98.1)	321 (98.7)
Secale cereale	4	3 (75.0)	3 (75.0)
Barley	8	3 (37.5)	3 (37.5)
T. monococcum	1	1 (100)	0 (0)
T. carthlicum	3	2 (66.6)	2 (66.6)
T. compactum	1	1 (100)	0 (0)
T. dicoccum	2	2 (100)	0 (0)
Total accessions	2211	1919 (86.5)	416 (18.6)

This study shows that excellent sources of resistance to yellow rust and powdery mildew are present in wheat germplasm which are worth exploiting. According to Kumar (1988), out of 23 samples of yellow rust collected from Lahaul-Spiti district (H. P.) and analysed, 18 sample were found to be of race K (47S 102), 2 of race A (70S4), 2 of race G (4S0) and 1 of race 24 (0S0-1). It is, therefore, presumed that the material evaluated at Keylong possesses resistance to race K which is the most virulent and widely prevalent race in the northern zone. As resistance to race K is very rare in our cultivated wheats, these resistant sources are highly desirable and worth utilizing. The utilization of cultivated cereals, such as rye and barley to improve wheat may be self-defeating because it potentially enhances the overall genetic vulnerability of the three cereal crops (Gill *et al.*, 1983). However, of the 35 genes for resistance to brown rust identified in wheat, two genes i.e. Ir 25 and Ir 26 were transferred from rye (Sharma and Gill, 1983). The most promising new CIMMYT wheat lines "Veery" and "Bob white" also owe their resistance to yellow rust to chromosome 1 of rye involved in 1B/1R translocation. Resistance to both yellow rust and powdery mildew in one host is highly desirable. The best accessions of *T. aestivum*, *T. durum*, triticale, rye and *T. carthlicum* free from both the diseases can be utilized for improving disease resistance in bread wheat. Tomerlin *et al.* (1984) have also shown *T. carthlicum* and *T. monococcum* to be resistant to powdery mildew. However, confirmation of the sources of resistance and cataloguing of genes for resistance should precede any attempt to transfer the resistance into cultivated wheats.

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Short Communication

Screening of *Sem* and Tomato Germplasm Against Root-knot Nematode (*Meloidogyne incognita*)

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National Bureau of Plant Genetic Resources, New Delhi is maintaining a large number of exotic and indigenous collections in *sem* (*Lablab purpureus*) and tomato (*Lycopersicon esculentum*) including some wild species which have already been evaluated for various agro-botanical characters. Therefore, to supplement the catalogues further with additional information, all the available collections of *sem* and tomato were screened against root-knot nematode (*Meloidogyne incognita*), which is a serious pest of both the crops resulting in considerable damage alone or in association with other microorganisms.

All the available collections/lines of *sem* (1361 accessions) and tomato (2167 accessions) were screened against *M. incognita*. In the preliminary screening trial, 20 days old seedlings of tomato (raised in nematode free soil) and *sem* seeds were planted in root-knot infested soil (having inoculum level of 5 larvae/g of soil) contained in earthen pots. After 45 days, roots were gently washed and number of galls per plant were counted under stereobinocular microscope. Collections/lines showing escape or resistance were retested for second season by inoculating each plant of *sem* and tomato with 1000 freshly hatched larvae of *M. incognita* under controlled conditions. Observations were recorded after 45 days of inoculation. In the collections/lines where galls were not observed, roots were stained in acid fuchsin lactophenol and checked under stereobinocular microscope to confirm the absence of root-knot nematode in the roots. Pusa Early Dwarf in tomato and IC-46126 in *sem* were used as controls. Results of the final screening are presented in Table 1.

TABLE 1. EVALUATION OF *sem* AND TOMATO COLLECTIONS/LINES AGAINST *Meloidogyne incognita*

Crop	Category	Collections/lines
Sem	Resistant	IC-770/V-16, IC-77002/Sel 68
	Moderately resistant	IIHR-164, IC-46158
Tomato	Resistant	EC-129606, EC-129606/P-1
	Moderately resistant	EC-486, EC-493, EC-8974, EC-12527, EC-27412, EC-65975, IC-76991, IC-76993, IC-76995, IC-76996, IC-76997, and La Bonita

An interesting observation made was that, in moderately resistant lines, the larvae had penetrated the roots and were in different stages of development but

no adult female could be seen even after 45 days of inoculation, suggesting thereby that either the development of the nematode is slowed down or it is unable to complete its life-cycle due to unfavourable host.

Amongst resistant/moderately resistant collections of *L. esculentum*, yield based on ten marketable fruits was recorded as follows : EC-129606 (860 g); EC-129606/P-1 (910 g), EC-27412 (1200 g) and IC-76996 (710 g). Remaining collections found moderately resistant gave yield less than 400 g. Wild species of tomato *L. hirsutum* (EC-486) and *L. peruvianum* (EC-65975) produced 20 g and 22 g fruits, respectively. These studies show that in root-knot infested soils, EC-129606/P-1 can be grown without much loss in their yield.

In *sem*, moderately resistant lines IIHR-164 and IC-46158 were found to be promising lines with respect to green pod weight, number of clusters, number of pods and seed yield per plant.

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Short Communication

Fungi Recorded on Exotic Rice Germplasm

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A large number of germplasm samples of rice are being exchanged throughout the world for utilization in crop improvement programmes and such exchange has an inherent risk of introducing exotic pests and pathogens as well as virulent races/ strains of the existing ones. Seed-borne pathogens of major importance in rice seed are *Pyricularia oryzae, Drechslera oryzae, Alternaria padwickii, Fusarium monilforme, Fusarium graminearum, Tilletia barclayana, Cercospora oryzae, Balansia oryzae, Rhizoctonia solani* and *Ustilagenoidea virens* (Richardson, 1978).

Pathogens intercepted during quarantine processing at Quarantine Laboratory of NBPGR in the past include *Alternaria padwickii* in germplam from Philippines and USA; *Drechslera oryzae* in germplasm from Netherlands, Nigeria, Philippines, UK, USA, Zambia and Zimbabwe; *Tilletia barclayana* and *Ustilagenoidea virens* in germplasm from Philippinnes (Anonymous, 1987).

During the early part of 1988, a consignment of rice germplasm comprising of 1024 samples was received from International Rice Research Institute, Philippines. Each sample consisted of about 200-300 seeds and these were individually examined for fungal mycoflora by 'Blotter Method' wherein 25 seeds per sample were plated due to less number of seeds in each sample.

A total of 11 fungi were found to be associated with these germplasm samples out of which *Alternaria padwickii, Drechslera oryzae, Curvularia* spp., *Fusarium monliforme, F. solani* and *Gerlachia oryzae* are of economic importance and *Gerlachia oryzae* has been intercepted for the first time.

Alternaria padwickii was predominant fungus found to be associated with 35.5 per cent samples and range of infection varied from 4-64 per cent. This was followed by *D. oryzae* and *G. oryzae* with 4-32 per cent and 4-20 per cent infection range, respectively, in 5.3 per cent samples, and *Nigrospora* spp. in 0.4 per cent samples with 4-36 per cent infection range. Infection due to *Fusarium semitectum, F. solani, F. equiseti* and *Alternaria longissima* were almost neglible, infection ranging from 0.4-1.6 per cent only.

Drechslera oryzae, the causal organism of brown spot disease, was responsible for the heavy crop losses in 1942, wherein more than 2 million people died of starvation. Heavy losses due to this pathogen were also reported from Nigeria, Japan and Surinam (Ou, 1985). Pathogenic variability in *D. oryzae* has been reported from Japan, Pakistan and India (Sakamoto, 1934; Nawaz and Kausar, 1962; Misra and Chatterjee, 1963).

Alternaria padwickii, the causal organism of stack burn, has caused considerable losses in Kerala and West Bengal (Rangaswami, 1975). Mathur *et al.* (1972) observed upto 80 per cent seed infection of *A. padwickii* in seed samples collected from 11 different countries in Africa and Asia. Similarly, high percentage (64 per cent) seed infection by *A. padwickii* was observed in the present study.

Gerlachia oryza which causes leaf scald was recorded in more than 2 per cent samples. Leaf scald had reached epidemic proportions in Japan in 1967 and now it is a serious threat in other parts of Asia and Africa (Lamey and Williams, 1972). Yield losses upto 30 per cent have been reported due to this pathogen from Bangladesh (Bakr and Miah, 1975).

Fusarium moniliforme, the casual organism of bakane disease was intercepted in 2.3 per cent seed samples and the range of infection varied from 4-12 per cent. In India, yield losses due to this disease are 1/3rd than what have been reported in other parts of the world (Ou, 1985).

Although, almost all these pathogens recorded during the present studies are known to occur in India, new races can get introduced which may pose serious threat to rice cultivation in the country. Ae such we must take all required precautions including seed treatment so that only healthy germplasm material is introduced for our crop improvement programmes.

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Short Communication

Evaluation of Wheat Germplasm Against the Cereal Cyst Nematode (*Heterodera avenae* Woll.)

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The cereal cyst nematode, *Heterodera avenae* Woll. was proved to be the causal organism for 'Molya' disease of wheat and barley in Rajasthan (Vasudeva, 1958). Since then this nematode has been reported from all the wheat growing regions in India including Rajasthan, Punjab, Haryana, Delhi, Himachal Pradesh and Jammu & Kashmir. Use of resistant cultivars is one of the most effective and economical ways of reducing this nematode in soil. Resistance in wheat against *H. avenae* is known so far only in two cultivars, namely, 'Loros' from Denmark (Nielsen, 1966) and 'Katyil' from Australia (Brown and Young, 1982).

To find out a source of resistance, screening of a large number of germplasm collections having greater genetic diversity is essential. With this objetives a total of 365 wheat collections available with NBPGR were screened for resistance against *H. avenae*. In the preliminary screening, seeds were sown in nematode infested soil, contained in plastic tubes (with inoculum level of 5 cysts/tube). Each cyst contained an average of 220 eggs. The experiment was terminated 95 days after planting. Roots were gently washed with water to remove soil and examined for white females or juveniles after staining with acid fuchsin-lacto-phenol. The collections were categorised as : resistant-no female/cyst; moderately resistant-upto 5 cyst/plant and susceptible-more than 5 cysts/plant. During the second season, all the moderately resistant and resistant collections were further tested by inoculating 500 freshly hatched larvae/plant. The experiment was terminated after 95 days and roots and soil were examined for presence of larvae/females/cysts, if any. Susceptible varieties, Kalayansona and C-306, were used as controls. The results of final screening are given in Table 1.

TABLE 1. SCREENING OF WHEAT GERMPLASM AGAINST *Heterodera avenae*

Category	Accession Number
Resistant	NC-55673, NC-57587, NC-59603, NC-60222, NC-64221
Moderately resistant	IC-26787, IC-28676, IC-31616, IC-47483, NC-55583, NC-55739, NC-57515, NC-57573, NC-57575, NC-57586, NC-59140, NC-59565, NC-59566, NC-59582, NC-59590, NC-59594, NC-59597, NC-60201, NC-60207, NC-60215, NC-62308

Out of 365 collections, only five viz., NC-55673, NC-57587, NC-59603, NC-60222 and NC-64221 were found to be free from nematode attack. NC-60222 is a semi-dwarf, while others have moderate yield potentiality. Amongst the moderately resistant collections, NC-57575 has high tillering and NC-57586, NC-59590, NC-59582 have higher yield potential.

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Short Communication

Exploration and Collection of *Porteresia coarctata* (Roxb.) Tateoka from Orissa Coast, India

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Porteresia coarctata (Roxb.) Tateoka formerly known as *Oryza coarctata* (Roxb.) has recently attracted the attention of rice researchers for its tolerance to soil salinity. This species is grown in estuaries and tidal swamps in the deltas of rivers in India, Bangladesh, Pakistan, Burma and Malaysia. In India, it has been reported from the states of Karnataka, Tamilnadu, Orissa and West Bengal. With a view to collect; conserve and evaluate the genetic diversity of this species, the delta region of Baitarani and Brahmani rivers of Orissa (India) were explored.

Geographical location and habitat

The explored area is known as Bhitarkanika and is the deltaic region of the confluence of rivers Brahmani, Baitarani and Dhamra. The area is located between 20°04′ and 20°08′ north latitude and 86°45′ and 87°05′ east longitude. The species grows in the muddy banks of the ramifying creeks, channels and distributaries of these rivers. Vast stretches of this species were seen in the zone that gets submerged during high tides. The associated grass species were *Myriostachya whitiana* (Nees ex steud.) Hookf, and *Cyperus exaltatus* Retz. The vegetation on the banks was typical mangroove as noticed from the species *Xylocarpus granatum* Koen., *Kandellia candel* (Linn.) Druce, *Aegiceras corniculatum* (Linn.) Blanco, *Sonneratiaa petala* Buch.—Ham and *Rhizophora mucronata* Peir, with predominance of crocodiles amidst the thickests of this grass.

Collection

The habitats were explored during November 1987 when flowering period of this species was almost over and the spikelets were mostly shed. The plant height, culm diameter, leaf length and width, exertion, panicle length, and length and width of spikelets were measured (Table 1). The soil was collected from the habitat of the species and tested for its electrical conductivity and pH which were found to be 4.61 mmhos and 7.0, respectively.

The sprouting plants as well as adult plants along with their rhizomes were collected and brought to the CRRI Campus, Cuttack and were potted. All these plants, withered and appeared to be dead. However, fresh plantlets appeared from the underground rhizomes and soon get established.

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TABLE 1. VARIABILITY WITHIN *Porteresia coarctata* (*Roxb.*)

Character (unit)	Mean	Range
Plant height (cm)	86.10	70–110
Leaf length (cm)	43.30	38.6 –47.5
Leaf width (cm)	0.83	0.7 – 0.9
Flag leaf length (cm)	14.92	11.0 –18.8
Flag leaf width (cm)	0.41	0.35– 0.45
Culm diameter (cm)	0.34	0.3 – 0.4
Panicle length (cm)	17.90	16.3 –19.5
Exsertion (cm)	0.94	0.2 – 1.6
Spikelet length (cm)	1.20	1.1 – 1.3
Spikelet width (cm)	0.30	0.2 – 0.3
Glume length (cm)	0.53	0.4 – 0.7
Grains per panicle (nos)	25.20	15.0 – 36
Spikelet sterility (%)	40.74	27.7 – 46.8

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Short Communication

Collecting Cotton in Bhutan

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Bhutan, situated in the Himalayan ranges lies to the north of West Bengal and Assam, and west of Sikkim between longitude 89° and 92° east and latitude 28° north of equator. It is a hilly country interspersed with mountains and valleys, varying in altitudinal ranges and climatic diversity. The temperature ranges from mild in foot hills to icy cold on high altitudes. The congenial edaphic and climatic factors in foot hills have circumscribed the cotton cultivation upto an altitude of 800-1000 metres only. It is said that about 20-25 years back, cotton was frequently cultivated in the eastern parts of Bhutan in the districts of Samdrup Jongkar, Shumar, Tashigang, Mongar, Shemgarg and Chirang. However, the area under cotton has since then reduced considerably first due to uneconomic return from this crop and secondly due to competition from better remunerative crops like chillies. In 1978, under the Indo-Bhutanese agreement at the inviation of Royal Government of Bhutan, the author was deputed to look into the declining trend of cotton growing and to suggest ways of improvement in its cultivation. Extensive touring indside the cotton growing districts of Bhutan was undertaken and the following diverse type of cotton material was collected.

Gossypium barbadense

About three metres tall perennial plants with many monopodial branches bearing three lobed leaves and three loculed small bolls, were found growing in back yards of houses and in gardens. This type of cotton is being grown for lint used for worship. The plants were free from sucking pests like jassids (*Amrasca* spp.), but their bolls were damaged (50 per cent incidence) by boll worms. These perennial plants were the constant source of bollworm for other cotton species. Seeds of this cotton were naked, 7-8 in number per loculus, with average 100 seed weight around 9.5 g, lint 36.6, per cent fibre short (25 mm) and coarse (5.32 micronaire value and 209 millitex). The perennial *barbadense* plants were found in districts of Samdrup, Jongkar, Sumar and Tashigang. The origin and antiquity of perennial *barbadense* could not be ascertained. However, it is felt that it could be an introduction from African countries.

Gossypium hirsutum

Two annual upland types belonging to *G. hirsutum* were found in cultivation. One of them in Yalang village of Shumar district at a height of 1000 m, in a flat land was characterised by essentially sympodial habit with 7th first fruiting node number. The plants were free from sucking pests like jassids even without plant

protection measures. The bolling potential was quite high (about 40-50 bolls/ plant) with about 2.5 g seed per boll. Bolls were 3-5 loculed with 6-7 seeds per loculus. The ginning out-turn of this type was 35.8 per cent. The fibre was short and comparatively coarse. The other type was found in cultivation in a village in Tashigang District. Its fibre was shorter and more coarser than former type with lower ginning (31.3 per cent). Both upland types mentioned above have probably been introductions from northern India belonging to race *latifolium* of *G. hirsutum.*

Gossypium arboreum

Cotton belonging to *Gossypium arboreum* was extensively grown in the districts of Samdrup, Jonkar, Shumar, Tashigang, Mongar, Shemgang and Chirang about 20-25 years back. However, its cultivation has declined to a considerable extent. Four different types of samples were collected from villages Yalang, and Yajor of district Shumar and also from two different villages in distrtcts of Mongar and Tashigang. The plants of all four types were annual, essentially sympodial with deeply laciniated five lobed leaves, yellow petals with red spot. Bolls were mostly three loculed with ten seeds per loculus with average seed cotton of 2.25 g per boll. Fibre length ranged from 17 mm to 20 mm. The fibre was coarse with micronaire value of 5.85 to 7.50, and millitex 230 to 295. The most distinguishable feature was the value of ginning out-turn. The two samples viz. A_1 (from village Yajur, district Sumar) and A_2 (from Mongar) were characterised by low G.O.T. (31.0 per cent) while the other two samples viz. A_3 (village Yalang, district Sumar) and A_4 (from Tashing) were high ginner (37.4 to 39.8 per cent). The incidence of boll worms in these two types was also comparatively lower than A_1 and A_2. Though all the *arboreum* types have shown high degree of resistance to boll worms showing upto 2.0 per cent incidence as compared to 44-50 per cent in *G. hirsutum* types. The four types are currently being studied in detail for their behaviour for boll worm incidence under Delhi conditions where they are being maintained in *arboreum* germplasm collection.

The four *arboreum* types could be classified in two groups. The first group with low G.O.T. may represent race *burmanicum* while the other group comprising A_3 to A_4 of high ginner types may represent race *cernuum* which might have gone from Assam area of India.

The *arboreum* germplasm collected from Bhutan may be of great significance from boll worm resistance point of view. The germplasm not only needs thorough collection but also evaluation and conservation to make the full realization of the potential of this landrace diversity.

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Short Communication

In-vitro Germination of Oospores of *Sclerospora graminicola*, the Green-ear Pathogen of Pearl Millet

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The green-ear disease of pearl millet caused by *Sclerospora graminicola* (Sacc.) Schroet. is seed transmitted as oospores on/with seed and/or mycelium in the embryo (Arya and Sharma, 1962; Shetty *et al.*, 1977). It is generally agreed that the soil-borne oospores of the pathogen provide major source of perpetuation of disease and primary infection of plants in the field (Suryanarayana, 1962). It has been shown that leaf debris containing oospores, when added to the soil, resulted in higher disease incidence as compared to when no infected debris was added to soil (Siddiqui and Gaur, 1978). However, role of oospores in causing primary infection has been questioned by several research workers because it has not been possible to germinate the oospores convincingly under laboratory conditions. Pande (1972) reported oospore germination in the laboratory, however, her findings have not been confirmed. It was communicated that oospores of *S. graminicola* were germinated in the laboratory at ICRISAT, Hyderabad (Dr. S. D. Singh Personal communication, 1989). Attempts were, therefore, made for *in-vitro* germination of *S. graminicola* oospores at the quarantine laboratory of the National Bureau of Plant Genetic Resources, New Delhi.

Bits from infected dry material of pearl millet containing numerous oospores were taken on a microscopic slide in about 5 drops of Chlorax (Sodium hypochlorite con tain-ing 1.0 per cent available chlorine). Immediately, the leaf bits were teased under a stereoscopic binocular microscope with the help of forceps and dissecting needle to release oospores in the chlorax solution. The oospores were allowed to remain in the solution for 10 minutes after which these were picked up with the help of a capillary tube and placed in sterilised water on another slide. These oospores were subsequently transferred to the third slide having sterilised water so as to remove unwanted plant debris. Thus, more than 10 oospores were placed on each slide. These slides were then placed in plastic Petridishes containing 3 layers of well moistened blotter papers and incubated at 22°C ($\pm$ 2°C).

These slides were examined under a compound microscope after every 24 hours. Initiation of germination was observed in a number of oospores after 5 days of incubation in the form of a small hyline protrusion from the wall of oospore. By the 8th day, most of the oospores had germinated producing hyaline long aseptate germ tubes (Fig. 1). In some cases, the germ tube showed branching at the tip. The experiment was repeated thrice with 5-6 replications each time to confirm the observations. Further investigations on various aspects of oospore germination and infection of seedlings by germinating oospores are in progress.

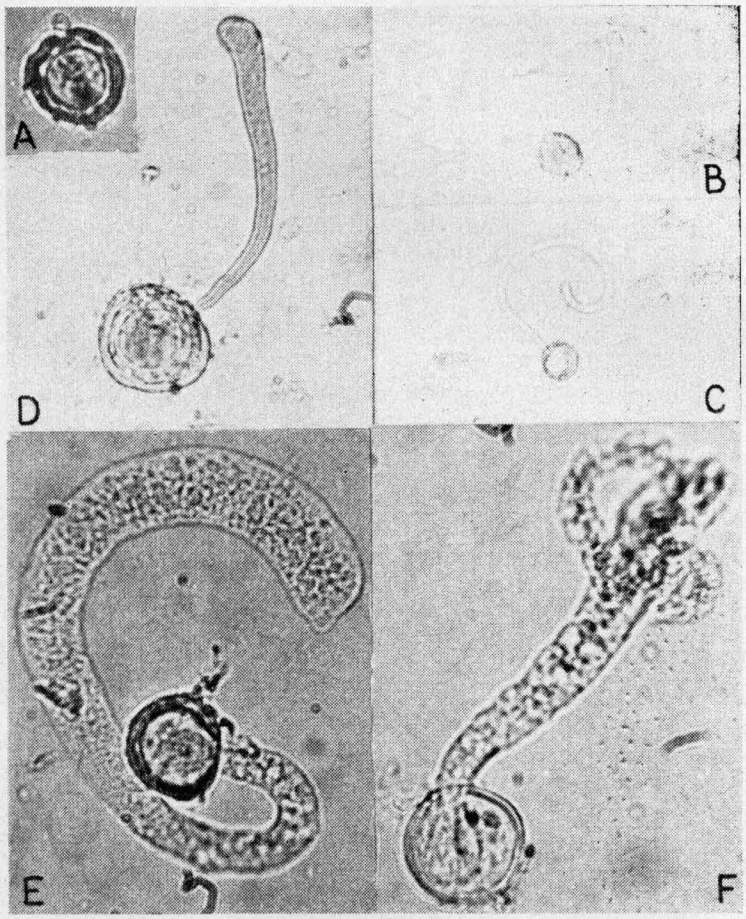

Fig. 1. Germination of oospores of *Sclerospora graminicola*
A. Initiation of germ tube emergence (X 875)
B & C. Germ tube elongation (X 400)
D. Germ tube elongation under high power (X 940)
E. An advanced stage showing aseptate germ tube (X 875)
F. Germ tube showing branching at the tip (X 1000)

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Indian J. Pl. Genet. Resources 2(2) : 187-188, 1989

Book Review

Gene Banks and the World's Food.

D. L. Plucknett, N. J. H. Smith, J. T. Williams and N. Murthi Anishetty. 1987 Princeton University Press, Princeton, New Jersey. 247 pp.

'Plant Genetic Resources' is a contentious and much debated topic in international fora. It is a sign of recognition of the importance of germplasm and signifies a major departure in public thinking over the past two decades. Thanks to the devastating sourthern corn leaf blight of the 1970s in the U. S. the need for developing a broader genetic base of our crop cultivars was highlighted. The authors, serving at advisory/managerial level of the International Board for Plant Genetic Resources, have a thorough and intimate knowledge of various issues related to germplasm resources. They have made an excellent effort in bringing out this timely publication and presenting the subject in its proper perspective.

The book, divided into ten chapters, begins with a discussion on the erosion of genetic diversity leading to genetic uniformity of present day crop cultivars. Historic Irish potato famine as well as some of the lesser-known casualities have been clearly shown to have resulted from the narrow genetic variability of the then prevalent cultivars. The constant race of plant breeders against ever evolving pests and pathogens, rapid turnover of varieties and the paramount importance of quality seeds in sustaining agricultural productions form the subject of second chapter. While discussing the controversy about exploitation by multinational seed companies, of genetic resources, mostly concentrated in the third world countries, the authors opine that private seed companies can play a healthy role in agriculture.

In the third chapter, the pivotal role played by various Botanical Gardens in introduction and dissemination of plants and the yeoman services of famous plant explorers in gathering and preserving global genetic wealth and the evolution of modern gene banks are beautifully recounted.

Working of genebanks and various problems in their smooth operation are dealt with in chapter 4. The authors make a very pertinent observation that pre-requisites, such as uninterrupted power supply, proper voltage and timely servicing of equipment for running gene banks are often difficult to ensure in the third world countries. The significant contributions of gene banks towards rehabilitation of germplasm in Ethiopia, Kampuchea and Nicaragua are also highlighted. Importance of biotechnology in germplasm evaluation and utilisation, and the use of tissue culture method of conservation are briefiy reviewed in chapter 5.

Current status of germplasm collection and holdings of major crops and their wild relatives in various gene banks all over the world are presented in chapter 6. Redundancy in collections and need for a thorough evaluation of accessions are stressed. The major efforts of IBPGR in the establishment of gene banks in the

third world countries and in creating a better international network are highlighted. Profits realised from germplam resources and the importance of wild species as donors of genes for disease/pest resistance and tolerance to adverse soil/weather conditions are documented in chapters 7 and 8.

The case study of rice variety IR 36, presented in chapter 9 best illustrates the whole theme of the book. The need for cooperation among countries for comprehensive collection and exhaustive evaluation of germplasm, the cental role of international organizations in generating primary breeding material and in coordinating various aspects of varietal development involving several nations are epitomised in IR 36 story.

The final chapter is addressed to administrators and policy makers. Presenting the controversy surrounding ownership and exploitation rights of genetic resources, the authors have outlined the experiences of various nations in bilateral, regional and international cooperation in exchange of germplasm. Emphasising the need for continuous financial commitment for running gene banks, the authors suggest that an international organisation, free from political bias, is the best custodian of global plant wealth for the present and posterity. Finally, they envisage gene bank facilities for animals and micro-organisms.

The book, written in simple terms, gives a highly readable account of germplasm resources today. Science policy makers, administrators as well as politicians will find this very valuable as it points to the political and financial commitments esential for the establishment and maintenance of gene banks. It is a must read book for the students and specialists for it gives the history of germplasm resources and gaps in their collection and exploitation. It will also appeal to common man and is recommended for general reading.

However, the book suffers from deficiencies in certain sections. For example, while cereals, millets, pulses, oilseed crops, vegetable and commercial crops have been well covered in discussions of germplasm holdings and utilization, hardly any mention has been made about fruit crops. In view of the nutritional and economic importance of fruit crops, a detailed species-wise treatment of holdings of fruit crops in clonal repositories in different countries would have greatly enhanced the value of the book. Further, *in-vitro* methods are being increasingly applied for the conservation of vegetatively propagated material including fruit plants. A greater emphasis on this topic was necessary in chapter 5. Finally, only a passing reference has been made about cryopreservation. Acknowledging the problems of running gene banks in the third world countries, it is perhaps time to look for cryogene banks. Cryopreservation is now feasible for many crops with orthodox seeds. Besides, cryopreservation is technically less complicated and cost-effective. A separate chapter on cryopreservation would have really completed the story of gene banks and greatly benefitted the readers.

But for these ommissions, the treatment is scholarly and the book is worth possessing for ready reference.

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