INTERNATIONAL TRAINING COURSE ON MAINTENANCE, CHARACTERIZATION AND DUPLICATE IDENTIFICATION OF *Ipomoea batatas* COLLECTIONS

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IPOMOEA BATATAS GENETIC RESOURCES IN INDIA

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Introduction

Sweet Potato (*Ipomoea batatas* (L) Lam) of the family convolvulaceae is an important tuber crops grown in almost all parts of India. It is consumed mostly as a subsidiary food items. Sweet potato is cultivated in about 0.14 million hectares and has an annual production of 1.17 million tonnes, the productivity being 8.3 tha^{-1}. The major area under sweet potato in India is spread over to four states, Orissa, Bihar, Uttar Pradesh and West Bengal. The Consumption of this crop is high in the rural poor sections of society. The farmers grow several varieties or mixtures of varieties which form the building blocks of sweet potato improvement programme in India.

Collecting farmers varieties

The CTCRI has started collecting genetic resources of *I. batatas* from 1963 onwards. The dissemination of improved varieties often eliminates the traditional farmers varieties. So collection and conservation of farmer's variety is an essential objective of collection trips. On the basis of the phenotypic variability observed in farmer's varieties it is clear that a large amount of variability still exists in the farmer's varieties. The collection trips were made to the main areas of production. Vine cuttings of about 50 to 60 cms were taken from the collected accessions and distinct morphological character were recorded at the collection site itself. The collection teams often visited the market places of the villages and collected tubers also. The collection teams obtained details about the local name, peculiarities and uses of the particular accessions from the farmers. The local name often indicated the place of origin e.g. "Kanhangad local" means a variety belonging to the place Kanhangad. The name of the accessions also revealed the nature of

growth, appearance etc. A Kerala collection e.g. 'Mathapadappan' meant that the vines of the variety spreads like that of a cucurbitaceous plant. Another variety 'Anakomban' meaning similar to the tusk of an elephant i.e. the tubers were ivory coloured and elongated. Often, the same variety had different names at different places and this contributed to the assembling the duplicate accession. Accession like 'Bhadrakali chuvala' meant that the tubers were red in colour indicating the colour of the cloth dedicated to the local deity 'Bhadrakali' showing the local customs of workship of the farmers.

Present status

In India, the Central Tuber Crops Research Institute and its Regional Centre at Bhubaneswar and 12 other Centres under the All India Co-ordinated Project (AICRP) are engaged in the collection and conservation of the genetic resources of sweet potato. In addition to this, NBPGR, New Delhi and its Regional station at Amaravathy are also collecting and conserving sweet potato germplasm.

A total of 3095 germplasm accessions of sweet potato are maintained by different institutions in India (Table-2) The maximum number of accessions are maintained by CTCRI (869). Of these 547 are indigenous and 322 are from exotic sources. Five *Ipomoea* species are also maintained by CTCRI (Table-3a Table-3b). The Regional Centre of CTCRI maintains 227 accessions at Bhubaneswar of which the major portions (64.3%) forms the breeding materials. It also possesses 4 species of *Ipomoea* viz I. trifida, I. triloba, I. acqutica and I. nil. The percentage of holdings of various types of sweet potato accession at CTCRI is presented in Table-4. Among the total germplasm accessions, the percentage of native cultivars and land races are maximum (42.69%). The percentage of breeding liones is 19.46 where as the released varieties form only 8.0% of the total collections.

Characterisation and documentation

In CTCRI all the 869 accessions have been characterised for the important morphological trails (Table-6) and 764 have been characterised for 6 passport and 39

morphological descriptors and documented for which computerised data base is maintained (Rajendran, *et al* 1992). As biochemical analysis for starch, sugar and carotene contents of many of the genetic stocks is not complete, the update of the catalogue could not be done.

variability and association of characters.

Evaluation of 869 accession of germplasm revealed the spectrum of variability available for trails such as plant type, vine growth rate, petiole length number of storage roots and root yield per plant (Easwari Amma *et al* 1999).

The major portion of the genetic stocks were semi-compact types (44.0%). The spreading and extremely spreading plant types together constituted 40% of the collections and the compact type was only 16.0%.

The variability for vine growth rate among the accession was classified into three groups, slow, intermediate and fast, 45% of the accessions showed intermediate growth and were mostly high yielders.

Of the five groups under which petiole length was classified, the major portion of genetic stocks (55.0%) were with short petioles (10-15 cm). Only 6% of the accessions possessed long petioles and those with very long petioles (>25.0 cm) were extremely low. Of the long petioled types 45.0% were high yielders.

Number of storage roots per plant is a major yield component in sweet potato. The accessions having low and medium number of storage roots were nearly in equal proportion, 40.0% and 41.0% respectively. Genetic stocks with high number of storage roots (>4/plant) formed 10.0% of the collections.

The variability for root yield (Fig.-1) was categorised into 4 classes with scores '0' denoting accessions with no tubers roots, '1' with low yielders (>100g) '3' with

medium yielders (100-250g) and '5' with high yielders (>250g). Morthan 79.0% of the collections were low to medium yielders. The high yielders formed only 13.0% of the germplasm while 8.0% did not produce any tuberous roots.

Studies on the association between root yield and other morphological traits (Table-9) revealed that root yield was significantly associated with plant type, petiole length and number of roots per plant. Plant type inturn was highly associated with vine growth rate, petiole length and number of roots per plant. However, vine growth rate showed significant association only with plant type. In addition to root yield, petiole length was highly associated with number of roots per plant.

In general, sweet potato genetic stocks which possess semi-compact plant type, intermediate vine growth rate, long petioles and produce more number of storage roots were found to be high yielders. This shows that in addition to root yield and number of roots per plant, shoot characters such as plant type and petiole length should be given importance in the selection of high yielders from sweet potato germplasm.

Evaluation for pests and diseases

The sweet potato weevil *Cylas formiearius* Fab is the most important pest causing extensive damage and reduction in yield in sweet potato, though more than 100 species of pests are known attacking it. Evaluation of more than 750 accessions at CTCRI and 163 accessions at its RC Bhubaneswar showed that all the accessions were susceptable to weevil infestation. About 10.0% of the total accessions screened at CTCRI were less susceptible to weevil infestation with tuber damage rauging from 2-21%

A total of 983 accessions were screened for their reaction to various diseases under field conditions at CTCRI and its RC, Bhubaneswar. Out of the 822 accessions repeatedly screened at CTCRI only 29 were found to be completely free from all diseases and the rest were affected by one or more diseases. Among the virus diseases, 'chlorotic leaf spot' was seen in 498 accessions, 'ring spot' in 354 and 'leaf roll' in 73 accessions.

Besides this, other symptoms, prosumably produced by virus like diseases such as 'puckering' was seen in 305, "fan leaf" in 49, "inter venal chlorosis" in 26 and "yellow netting in 4 accessions (Table-7) Among the different fungal diseases "Chlorotic leaf distortion" (CLD) was seen in 400 accessions and 'Brown leaf spot' in 207 accessions.

Germplasm Utilization

In India so far 19 varieties have been released for cultivation by different Organisations out of which 9 are released by CTCRI (Table-5). The main breeding objectives are development of high yielding better quality varieties having early maturity and resistance to abiotic and biotic stresses. The Regional Centre of CTCRI at Bhubaneswar also developed salt tolerant and drought tolerant lines. The salt tolerant lines are being tested at salt affected areas. Efforts have also been made to develop high starch varieties and few lines have been isolated in this regard.

Conservation practices

In India, sweet potato germplasm collections are maintained as field gene bank. The germplasm accessions are replanted thrice in a year. Sweet potato is grown in India through out the year and it is conserved based on the agroclimatic conditions of the area. In places where the rainfall is not spread through out the year and sweet potato is grown under rainfed conditions the planting materials are conserved in the form of vines. Where the soil is sandly loam particularly in the Indogangetic plains, sweet potato is conserved in the form of tubers by spreading them under shade.

In-vitro conservation is the safest way to conserve the germplasm. At present a total of 114 accession are transferred to *in-vitro* form, 100 at CTCRI and 14 at RC, Bhubaneswar. Initial establishment of germplasm was done through meristem culture in Murashige and skoog (MS) media with 0.1 NAA, 0.1 um BA and 0.01 GA3. The accessions are then multiplied in MS medium without plant growth regulators. Cultivars are inoculated at 25-28^{0}C under 8 hours light (300) For slow growth manitol (3%) and sucrose (2%) are effective.

Future outlook and strategies

1. Identification of duplicates

As India is maintaining more than 3000 accessions many of which are duplicates identification of duplicates is essential in all centres. Identification duplicates should be based on morphological descriptors followed by biochemical means for high reliability.

2. Formation of Core collections.

Identification of duplicates makes the task of formation of core collections easy. As minimum number of accessions having the maximum extent of genetic diversity available in the original collections are retained in the core collection, the cost of maintenance comes down considerably and enough scope for utilization of genetic diversity remains.

3. Use of alternate strategies for germplasm maintenance

In-vitro conservation, cryopreservation, *insitu* conservation and botanical seed storage would be the other future strategies for sweet potato conservation.

Conclusion

In India simultaneous efforts to expand the genetic diversity of *Ipomoea batatas* by collection from indigenous and exotic sources and elimination of duplicates will be perused. In-vitro conservation of genetic resources needs further strengthening and the complementary conservation methods more research.

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Table 1 :Area Production and productivity of Sweet potato in India

Year	Area '1000 Ha	Production '1000 MT	Yield Kg /ha
1989-91	156	1265	8129
1995	138	1128	8167
1996	141	1174	8326
1997	141	1174	8326

Source: FAO production year book Vol. 51-1997

Table 2 : Sweet potato germplasm accessions maintained by different organization / centres in India

Organization/ Centres	Place and state	No. of accessions 1998	1996
CTCRI	Trivandrum, Kerala	869	807
RC OF CTCRI	Bhubaneswar, Orissa	239	253
RAU	Dholi, Bihar	802	709
APAU	Rajendra Nagar, Andhra Pradesh	95	85
BCKV	Kalyani, West Bengal	100	70
AAU	Jorhat, Assam	29	25
KKVP	Dapoli, Maharashtra	118	118
NEH Complex	Shillong, Meghalaya	31	31
BAU	Ranchi, Bihar	94	89
NDAU	Faizabad, Uttar Pradesh	63	3
GAU	Navasari, Gujarat	30	NA
IGKV	Jagadalpur Madhya pradesh	85	73
TNAU	Coimbatore, Tamil Nadu	540	NA
NBPGR	Amravathi, Maharashtra	NA	NA

Table 3 : Detailed sweet potato accessions maintained in CTCRI and its Regional Centre

Type of germplasm	No. of accessions
CTCRI	
Indigenous	547
Exotic	322
Ipomoea species (*I. aquatica, I. trifida, I.triloba, I.setosa, I. batatas var batata*)	5
REGIONAL CENTRE	
Land races	25
Breeding materials	146
CTCRI accessions	56
Ipomoea species *I. trifida germplasm* *I. triloba* *I. aquatica* *I. nil*	 80 2 1 7

Table 3a I. batatas genetic resources available at CTCRI

Indigenous Collections.

	State	No. of Accessions
1	Kerala	292
2	Orissa	48
3	Bihar	46
4	Maharashtra	27
5	Tamil Nadu	19
6	Madhya Pradesh	19
7	Karnataka	14
8	Nagaland	9
9	New Delhi	8
10	Assam	6
11	Andhra Pradesh	6
12	West Bengal	5
13	Uttar Pradesh	4
14	Tripura	2
15	Punjab	1
16	Sikkim	1
17	Goa	1
18	Unknown	39
	TOTAL	547

Table 3b. I. batatas genetic resources atailable at CTCRI

Exotic Collections

	Country	No. of Collections
1	CIP, Peru	85
2	Nigeria	77
3	China	43
4	Japan	32
5	U S A	12
6	Puerto Rico	5
7	Fiji	2
8	Sudan	1
9	Nairobi	1
10	Argentina	1
11	Newzealand	1
12	Unknown	62
	TOTAL	322

Table 4: Percentage of holding of native cultivars, breeding lines and released varieties of CTCRI sweet potato germplasm collection

Type of holding	Percentage
Native Cultivars	42.69
Breeding lines	19.45
Introductions	37.06
Released varieties	0.80

Table 5. Descriptive information available on the CTCRI germplasm

Information available	No. of accessions
Botanical seeds	500
Passport data	825
Farmers IK	371
Morphological characterisation	869
Molecular finger print	None
Evaluation data: agronomy	200
Pests (Weevil)	700
Diseases	822

Table 6: χ^2 Values for different characters in sweet potato germplasm

Characters	Root yield/ plant	Plant type	Vine growth rate	Petiole length	No. of roots/ plant
Root yield per plant		18.90*	6.50	7.90**	826.10**
Plant type			1002.8**	35.51**	29.28**
Vine growth rate				10.42	6.21
Petiole length					202.00**
No. of roots/ plant					

Table 7 : Germplasm accessions affected by different pests and diseases

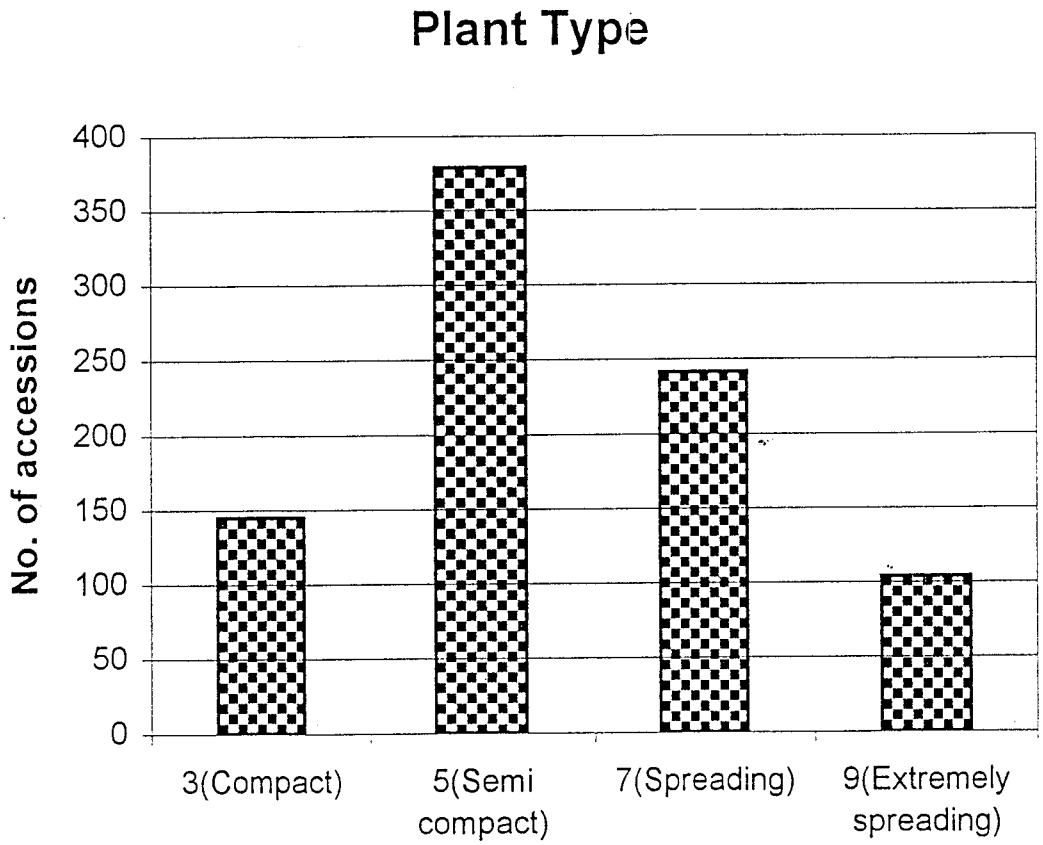
Pests	No. of accessions
Weevil	913

Diseases	No. of accessions
Infected	983
No disease	29

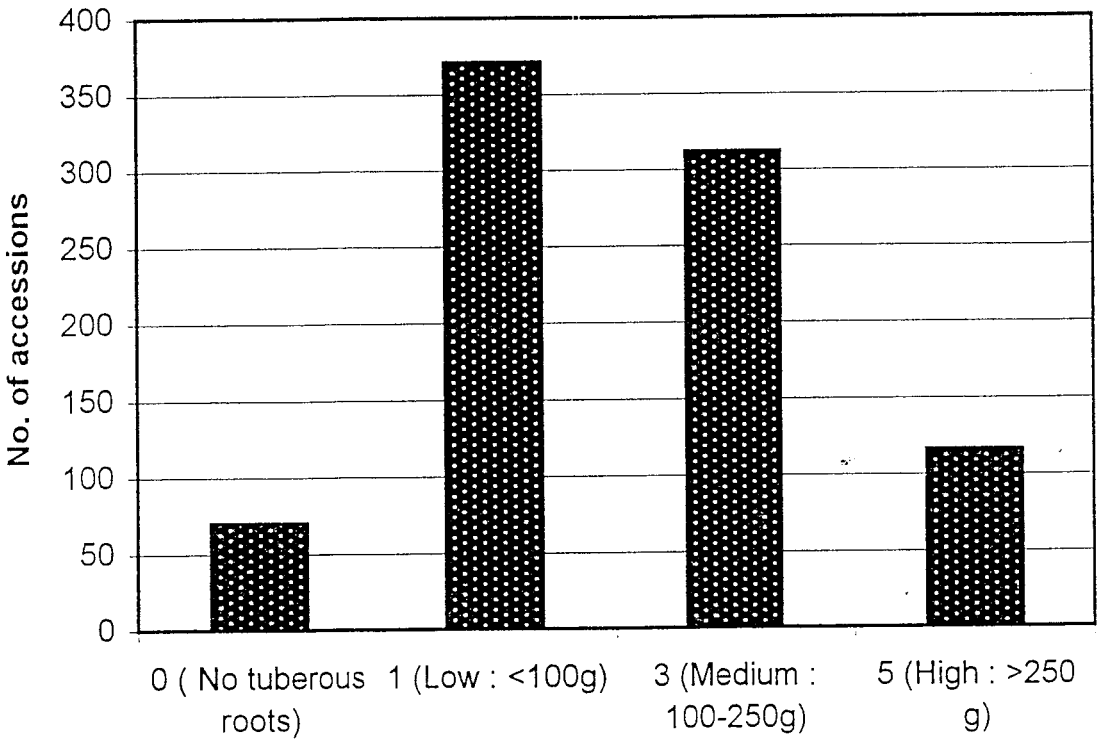
Diseases	No. of accessions
Viruses	
Chlorotic leaf spot	498
Ring spot	354
Leaf roll	73
Virus like diseases	
Puckering	305
Fan leaf	49
Intervenal chlorosis	26
Yellow netting	4
Fungal diseases	
Chlorotic leaf distortion(CLD)	400
Brown leaf spot	207

Table 8: Recommended Sweet potato cultivars in India

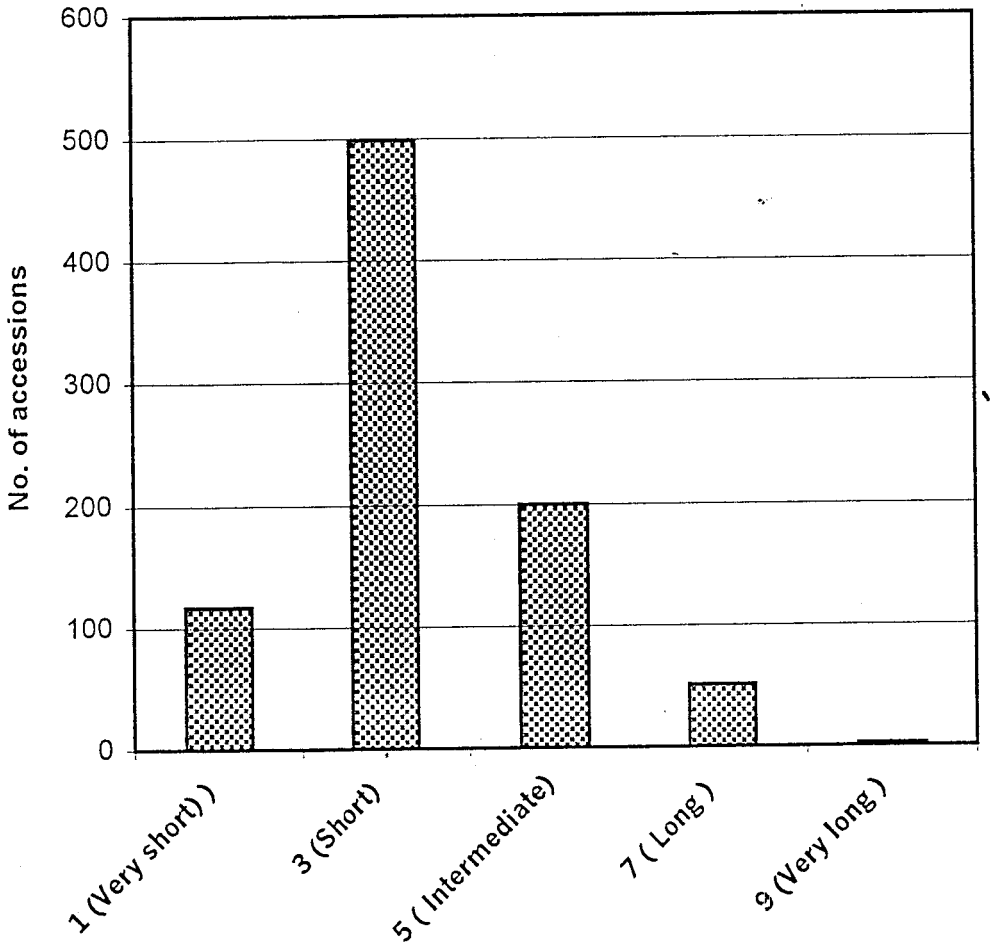
Cultivar	Yield (t/ha)	Skin colour	Flesh colour	Year of release
H-41	20-30	Pink	White	1971
H-42	20-25	Pink	White	1971
VL-sakarkand	20	Purple	Light yellow	1974
Co-1	20-30	Light pink	White	1976
Co-2	25-30	Light pink	White	1980
Co-3	25-30	Light red	Orange	1980
Rajendra Sakarkand-5	24-30	White	White	1985
H-268 (Varsha)	20-25	Pink	Light yellow	1987
Sree Nandini	20-25	White	White	1987
Sree Vardhini	20-25	Pink	Orange	1987
Samrat	20-28	Dark brown	White	1987
Rajendra Sakarkand-43	20-25	Brown	White	1994
Rajendra Sakarkand-35	20-30	Brown	White	1994
Kiran	22-30	Brown	Orange	1994
Rajendra Sakarkand-47	25-32	Red	White	1997
Sree Rethna	20-26	Purple	Orange	1997
Sree Bhadra	20-27	Pink	Cream	1997
Gouri	20-25	Purple	Deep orange	1998
Sankar	14-20	Red	White	1998


Plant Type
No. of accessions
400
350
300
250
200
150
100
50
0
3(Compact)
5(Semi compact)
7(Spreading)
9(Extremely spreading)

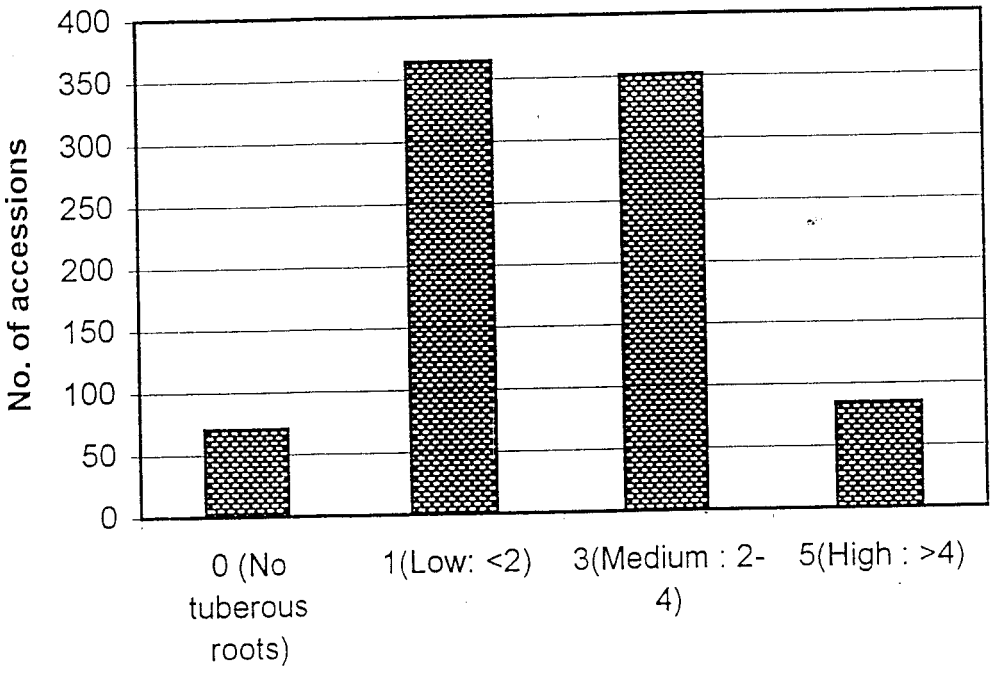
Storage Root Yield/plant



Petiole Length



No. of Storage Roots/plant



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Quarantine materials introduced to the gene bank

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Sweet potato is a vegetatively propagated crop. It is generally propagated through vine cuttings or roots. Sexual seeds are also used for its improvement. Vine cuttings, roots or sexual seeds can be the genetic material of introduction from outside. But the introduction of genetic materials in the form of vine cuttings or roots is dangerous for vegetative crop like sweet potato. Because disease like viruses are easily spread through the vine cuttings or roots. So *in vitro* movement of germplasm contained indexing and in vitro propagation together provide a novel and safer quarantine system (Wither, 1989) . Tissue culture materials consists of small asceptic plantlets growing on a synthetic nutrient is an ideal method. The other safe method of introduction of genetic materials is in the form of seeds. Seeds if properly treated have minimum chance of spreading pests and diseases. In this publication information on the handling procedures to be followed for the quarantine materials to gene bank either in the form of *in vitro* plantlets or seeds is discussed.

In vitro plantlets :

After receiving the plantlets the first step is not to open and remove the plantlets from the tube. If the plantlets in the test tube turn yellow then place it under a diffused sunlight for a week in a clean room.

Transfer of *in vitro* plantelts :

Maximum care must be taken for handling the introduced material which will be done in clean condition. Plantlets from test tube can be transferred in two ways (Dodds et al. 1997)

* Transfer to planting mix
* Micropropagation

Transfer to planting mix :

Following steps should be followed while transferring the plantlets to planting mix.

1. Mix peat moss and sand (1:2 volume)
2. Fill the pots with peat moss/sand mix, cover them with alluminium foil and sterilize for 1 hr.
3. Wash your hands with strong soap and 1% calcium-hypochlorite solution and then rinse in 70% alcohol.
4. Take the pots and the *in vitro* culture to a clean bench in asceptic condition
5. Irrigate the pot with a small amount of water
6. Make a hole in the centre of the peat moss/sand mix with a clean stick or pencil.
7. Disinfect the outside of the test tube using piece of cotton or cloth moistened in 70% alcohol before removing the plantlets.
8. Remove the parafilm and plastic cover from the test tube.
9. Gently pull the plantlets with the agar out of the test tube using sterilized forceps.
10. Wash the agar from the roots by gently immersing them several times in sterilized water avoiding wetting the rest of the plantlets.

11. Plant each plantlet alongwith roots and one or two nodes in the holes in the potting mix.
12. Place the sterilized sand around the plantlet and press lightly to keep it strait in the pot.
13. Place the pot into a humid chamber for 48 hours. Remove the pots from the chamber to facilitate root development.
14. Keep the pot in a clean place under 14-16 hours illumination at 25 to 27°C.
15. Irrigate the plants with demineralized or rain water.
16. When roots are well established provide supplementary nutrient in the irrigation water.
17. Gradually expose the plants to the normal atmosphere for short periods each day.
18. Once the plants are established transfer it to a larger pots

Micropropagation

Following steps must be followed in case of micropropagation of the plantlets.

1. Prepare the nutriative growth medium based on medium to sweet potato micropropagation.

Medium to sweet potato micropropagation

The medium will be prepared based on the salts Murashige-Skoog (1962). Additional nutrients should be added to 1 litre of Murashige-Skoog medium.

Preparing the medium to sweet potato micropropagation.

* Calcium Pantothenate	2 ppm
* Gibberellic acid	20 ppm
* Ascorbic acid	100 ppm
* Calcium nitrate	100 ppm
* Putrescine HCL	20 ppm
* L-Arginine	100 ppm
* Coconut milk	1%
* Sucrose	5%
* Agar or	0.7%
*Phytagel/Gelrite	0.25%

2. Dispense 4 cm^3 of the above medium in each test tube. Cap the tubes with plastic caps or cotton wool plugs and autoclave them for 15 minutes. Keep the test tubes vertical while the agar sets.

3. Disinfect the outside of the test tube using piece of cotton or cloth moistened in 70%.

4. Remove the parafilm and plastic cover from the test tube

5. Gently pull the plantlets with the agar out of the test tube using sterilized forceps.

6. Transfer the plantlets from the test tube to sterile petridishes and make nodal cuttings of a 0.2-0.5 cm stem segement with an axillary bud using sterile scalpel and forceps.

7. Place two nodal cuttings on the agar surface with their axillary buds pointing upwards in a test tube.

8. Close the test tubes, seal with parafilm, label and keep in a clean area under 3000 lux illumination at 25-27°C for 14-16 hours each day.

9. Nodal cuttings grow into a new plantlet within 2-4 weeks which is readyfor transplanting to pots as perviously described or for future micropropagation.

Seeds

The following steps must be followed after receiving the genetic materials in the form of seeds.

Seed scarification

Sweet potato seed has hard seed coat or germination the seed coat must be scarfieid with conc. H_2SO_4 .The procedure is to place the seeds in a beaker or any suitable glass container. Pour the conc. H_2SO_4 very slowly and carefully in the container immersing the seeds up to three quarter mark of the container or beaker. Stir the seeds with a glass rod and keep it for an hour or the time indicated on the seed package. Then decant the used H_2SO_4 into another glass container and wash the acid off the seeds in running water.
After rinsing, the seeds must be kept in the beaker for one day to observe the cracking or swelling. If no apparent cracking is observed repeat the scarification procedure (Saladaga et al., 1991).

Preparation of seed bed

Prepare the seed bed or plastic bags with friable soils. Fertilizer can be mixed with the soil if it is found necessary.

Sowing and propagation

Sow the scarified seeds in plastic bags or seed bed. After 45 days or more when seedlings attain 45 cm length it is ready for transplanting.

Transplanting in field

Prepare the field for transplanting like any cultivation of sweet potato. Uproot seedlings of 40-50 cm length. Cut the vine into two halves of 20 cm length (basal and terminal portion). Plant both the portions on the ridges of 60 cm apart. Grow the seedlings for 90, 100 and 120 days after planting. Select the best clones based on tuber yield, DM, reaction to diseases and quality characters. Label the selections and maintain them in field gene back (FGB).

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RAPID MULTIPLICATION METHODS OF SWEET POTATO: STORAGE ROOT SPROUTING, SINGLE-NODE, MICRO-CUTTINGS AND MINI-STORAGE ROOTS

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Introduction

The lack of efficient formal and informal seed production systems has limited the diffusion of new and improved varieties by providing only limited amounts of healthy planting materials. To speed up varietal introduction and diffusion rapid multiplication is important. Rapid propagation methods are very useful in the breeding programmes. If a promising clone is isolated, these will enable the breeder to skip two or three seasons and test the variety at the earliest in the field for advance and ON FARM trials reducing the time lag encountered between the seedling and testing stage. These methods can be applied effectively for the supply of disease free planting material for commercial planting. Regeneration of healthy plants can be achieved in sweet potato by the rooting of leaves, leaf-blades (laminae) petiole pieces, inflorescence axils, micro-cuttings, single and half-nodes and by sprouting of storage and thickened roots (mini-storage roots).

Conventional propagation of sweet potato

Sweet potato planting materials usually consists of vine cuttings or sprouts from tubers. The use of vine cuttings is the recommended commercial methods of propagating sweet potato. Vine cuttings of 20-40 cm long having 3 to 5 nodes are ideal. The use of vine cuttings from the top are preferred to those from the middle and basal portions of the vine (stem). Generally the vine cuttings obtained from the first and secondary nurseries are more vigorous and produce maximum yield.

Nursery plot (technique) : Nurseries are commonly established at the time of harvest utilizing vine cuttings/healthy tubers at the time of harvest. To plant one hectare of land about 100 m^2 of primary nursery area and about 80 kg of medium sized weevil-free tubers (120-150 g weight) are required. Vine cuttings of 25-30 cm long obtained from the nursery plot are used to establish the field or can be multiplied in an area of 500 m^2 to plant one hectare of land.

Rapid multiplication methods

Sweet potato can be rapidly multiplied by sprouting of storage and mini storage roots, by rooting of single leaves, shoot tips, micro cuttings, petiole pieces and nodes or by tissue culture. By employing some of these techniques 800 cuttings could be produced from 30 days old plant in 60 days and 64000 to 80000 cuttings in seven months period (Martin 1987).

Production of sprouts from tubers

This method of producing vines for planting is recommended in situations where the non-growing season is either too long or too severe for a nursery plot to be maintained. This is the standard method of producing planting materials in the sub-tropical and temperate regions. The method involves essentially causing the tubers to produce sprouts by growing them in moist beds of sand/soil, removing (pulling) the sprouts at intervals and planting them in the field. The tubers are placed in the beds covered lightly with soil and kept watered. The first batch of sprouts can be pulled 3-4 weeks after bedding followed by pulling at weekly intervals. Farm yard manure and urea is applied along with periodical irrigation to promote growth of sprouts.

In order to maximize the production of sprouts, the tubers to be bedded (planted) should be cut transversely into pieces so as to minimize proximal dominance. To improve

the production of sprouts the tuber pieces are treated with growth regulators. The treatments include dipping in ethtephon at 1500 ppm (Tomkins et al 1973) in 12% dimethyl sulphoxide (DMSO) for 20 minute (Wheatly 1969); treating with carbondioxide gas at 30°c for three days before bedding (Su et al 1965); prior heating of tubers at 43 °c for 26 hours also increases the production of sprouts. the beds are maintained at 28 °c - 30 °c by controlled heating practices.

Successive planting methods : This can be adopted when there is shortage of planting material, by planting a portion of the field with the available vines. Vine-cuttings are repeatedly taken from the established plants until the entire field has been planted. This strategy can be combined with either the nursery plot or the tuber sprouting methods already described. The main disadvantage of this system is that plants in he field are in different ages and may mature at different times. this system can be used in traditional production of sweet potato since harvesting is usually done piece meal .

Sprouts from cut-pieces of tubers/non-marketable tubers

The results of the use of non-marketable (full/broken undersized tubers) tubers for raising vines in the primary nursery and subsequent multiplication of these vines in the secondary nursery for the production of planting material for commercial cultivation is presented in Table - 1. It is indicated that there was no significant difference between the use of undersized full/broken non-marketable tubers of weight range from 25-75 g and good quality marketable tubers of 100-125 g size for raising nursery for the commercial production of planting material. (Mohankumar & Potty 1996).

Production of planting materials from mini-storage thickened roots

For the production of planting materials mini-roots/ thickened roots can be used successfully. Studies at CTCRI indicated that thickened roots of the variety OP-2 having a length of 25-30 cm when planted in sandy beds inside the glass house could

produce 15 sprouts and 250 vine cuttings (25 cm long) per root with an yield of 120.0 g within a period of 100 days. (Kamalam, 1978).

Regeneration of plants from shoot-tips, shorter and micro-cuttings of vine (stem)

Shoot tips of 2-5 cm long from mature plants were kept inside mist chamber 98% of them established and developed as new plants having profuse shoots and growth.

Five hundred pieces of single and half-nodes of five cultivars were planted in sandy beds inside the mist chamber. the highest percentage of new plants developed was observed in 75 OP-1 followed by 76 OP-217. (Tables 2,3). Mean number of normal planting materials (25 cm long cuttings) obtained per plant of 75 OP-1 both from half and single nodes along with yield at 105 DAP are presented in Table 4. The percentage of establishment inside the glass house, mist chamber and field varied in different varieties.

Regeneration of plants from leaves, leaf-blades, petiole pieces and inflorescence axils

The petiole pieces (2 cm long) and leaf-blades of sweet potato variety S-4-16 when planted in sandy beds they produced callus and new plantlets profusely. From a single leaf with petiole of a spreading variety S.4-16, sixteen plants and 320 vine cuttings (25 cm long) could be obtained within 60 days (Kamalam, 1978).

Plants developed from leaf blade rooting

Five hundred leaf blades (laminae) each of the nine varieties of sweet potato were planted flat in sandy beds inside the glass house. Frequent watering was done at an interval of 4 hours during day time. After 15 days, observations on callus formation and root formation were taken. After one month, observations on the number of new plants produced by the leaf-blades of each variety were also taken. The data are presented in Table 5. It was found that leaf blades of different varieties vary in their capacity of

producing new plants. The highest percentage (85%) of new plants from leaf blades was obtained in S.28-5 and S-31-43. The maximum percentage of callus formation on the 15th day was observed in S-18-101 whereas profuse rooting was observed in S-31-43 (80%).

Plants from petiole pieces

One hundred petiole pieces (2 cm long each) each of 5 sweet potato varieties were planted inside the mist-chamber. After one month, observation were taken on the number of petioles produced callus and new plants. The data of percentage of establishment and plants developed from petiole-pieces are presented in Table 6. The petiole pieces of 76 OP-217 and 76 OP-219 showed the highest percentage of establishment producing roots and shoots The petiole pieces produced callus and from the callus new plants developed profusely. The plants developed from petiole pieces develop as normal plants and produce vines and tubers.

Regeneration of plants from Inflorescence axils

One hundred and fifty inflorescence axils were planted inside the mist chamber. After 30 days 32.8% of them produced new plants. (Table 7).

The results of the experiments conducted for six seasons indicated that sweet potatoes can be multiplied successfully by rooting of shoot tips, single and half-nodes, leaves and leaf-blades and petioles. Yields upto 300.0 g were obtained from plants developed from shoot tips and leaf-blades. Cultivar differences in rooting, establishment, production of shoots and yield were observed. (Kamalam, 1981).

Phytomodels

The Phytomodels eg. rooted leaves, petioles, laminae provide simple and easy to handle mechanisms for the study of physiological, pathological and biochemical problems. They can be used in the mutation breeding programmes to elimiante diplontic selection and making the segregation true mutants easier.

Table 1 : Effect of planting material on tuber and vine yield of sweet potato

Treatment	Size group of tubers (g)	Yield t/ha	
		Tuber	Vine
Full tuber above 50 g	51-75	8.3	35.8
Full tubers below 50 g	25-50	8.5	35.1
Tuber pieces of 50 gm	51-75	8.3	35.8
Tuber pieces below 50 gm	25-50	8.4	35.1
Standard tuber of 100 g and above	100-125	8.5	3.5
Treatment	NS		

Table 2- Percentage of plants developed from the petiole pieces in the mist chamber after one month

Petiole pieces of the varieties planted (%)	Percentage of plants developed from petiole pieces after one month (%)
76-OP-219	62.0
75-OP-1	51.5
H-478	43.8
76-OP-217	68.0
H-268	38.0

Table 3 - Percentage of new plants developed from half-nodes of sweet potato of different clones in the mist chamber

Half nodes of varieties planted	Percentage of new plants developed from half-nodes after one month (%)
76- OP-219	52.8
75-OP-1	68.0*
H-478	60.0
76- OP-217	62.6*
H-268	32.8

* The highest no. of plants obtained from half nodes of 75 OP-1 followed by 76-OP-217. The lowest was obtained in H-268.

Table 4. Mean number of normal planting materials obtained per plant both from half and single nodes along with yield at 105 DAP of Sweet Potato

Variety - 75-OP-1			
Plants developed from Half node		Plants developed from single node	
Mean no. of normal planting materials per plant of ½ - node (No.)	Mean tuber yield per plant of ½ - node (g)	Mean no. of normal planting materials obtained from single node (g)	Mean yield per plant of single node (g)
150.0	105	138	200.0

Table 5. Percentage of rooting & Callus formation of the leaf blades of different clones of sweet potato

Leaf blades of clones	Observations on 15 days after planting		Observations at one month stage after planting
	Percentage of callus formation (%)	Percentage of blades produced roots (%)	
S-35-3	25	Nil	32
S-30-27	20	15	48
H-42	20	20	46
S-18-101	80	30	80
H478	25	20	75
S-31-43	82	80	85*
S-28-5	25	75	85*
S30-X-1	Nil	65	65
OP-3	60	75	70

The highest percentage (85%) of the new plants from leaf-blades was obtained in S-31-43, S-28-5, S-18-10 and H-478 followed by OP-3. The maximum percentage of callus formation on the 15th day was obtained in S.31-43 & S-18-101. Profuse rooting on 15th day was observed in S-31-43 followed by S-28-5

Table 6. Percentage of new plants developed from full nodes of different clones

Full of nodes of varieties planted	Percentage of planted developed from full nodes after one month (%)
76- OP-219	92.0
75-OP- 1	98.6
H-478	93.8
76- OP-217	93.5
H-268	60.4

Table 7. Percentage of plants developed from the inflorescence axils of sweet potato

No. of inflorescence axils planted	Percentage of plants devloped
150	32.8%

MULTIVARIATE ANALYSIS OF MORPHOLOGICAL DATA

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1. Introduction

Multivariate statistical analysis is concerned with data that consists of sets of measurements on a number of individuals or objects. The sample data may be vine length, number of branches, vine girth, number of leaves per branch etc. of some plants of sweet potato drawn randomly from a population. The measurements made on a single individual can be assembled into a column vector. We think of entire vector as an observation from a multivariate population or distribution. It is helpful in visualizing the data and understanding the methods to think of each observation vector as constituting a point in a Euclidean space, each coordinate corresponding to a measurement or variable.

The measurement and analysis of dependence between variables, between sets of variable, and between variable and sets of variables, are fundamental to multivariate analysis. A number of statistical problems arising in multivariate population are straight forward analogs of problems arising in univariate populations.

Conservation of germplasm resources is basic to crop improvement programmes. However, to understand usable variability, grouping or classification of genetic stocks based on suitable scale is quite imperative. Divergence analysis has been widely used by researchers in biological sciences for differentiating between two or more populations/groups on the basis of multivariate measurements. Genetic diversity assessments of agricultural species traditionally are based on differences in morphological and agronomic characteristics. Multivariate statistical techniques can be used to overcome the complexity of the data, which will facilitate the description and interpretation of accessions across environments. Classification and ordination statistical techniques are two major sets of multivariate techniques used for these purpose.

2. Classification methods

The objective of this method is to classify accessions into cluster with more or less similar value for all the descriptors. Two well known classification methods are : Cluster analysis and Discriminant analysis.

2.1 Cluster analysis : Clustering is the technique of partitioning a set of accessions into cluster such that accessions within a cluster are homogenous and between clusters, they are heterogeneous. The main aim of any numerical cluster analysis is to find discontinuity in the data sets by means of a clustering strategy that groups the items.

Steps involved :

- Applying multivariate analysis of variance technique to test the significance of different groups.
- Computation of inter-group distances which can be determined by one of the several "Distance Statistics".

The generalized D^2 - statistic developed by Mahalanobis can be used to measure the divergence between genetic groups. It is defined as

$$\mathbf{D^2_p = d'S^{-1}d}$$

where '**d**' is the difference in mean vectors of the two groups and '**S**' is the pooled variance - covariance matrix of **p** traits. The test of significance can be determined using F - test given by

$$F = \frac{n_1 n_2 (n_1 + n_2 - p - 1)}{(n_1 + n_2)(n_1 + n_2 - 2) p} D^2_p$$

with p and $(n_1 + n_2 - p - 1)$ degree of freedom

where n_1 : number of individual in group 1

n_2 : number of individual in group 2

p : number of characters observed.

- Cluster formation : Based on the degree of divergence (D^2 values) between any two genotype, a logical grouping of genotypes with low D^2 values can be arrived at. Since the range of D^2 values varies enormously from one set of varieties to another, clustering is purely arbitrary.

General guidelines for group formation may be laid down as under:

- The limit for divergence within groups should be based upon the range of all $\frac{n(n-1)}{2}$ D^2 values.
- Divergence between groups (inter-cluster average D^2) should not be less than within groups (intra-cluster average D^2 values).
- In case of maximum overlapping , determination of group constellation might become a case of common sense.
- Ideally, the divergence within a cluster must not decrease on considering some additional characters.
- A recurrent exercise of grouping of genotypes leads to perfection in technique of cluster formation.

Clustering can be hierarchical or non-hierarchical.

Hierarchical Clustering method:

The methods starts with a dissimilarity matrix and fuse the two individuals which have the smallest dissimilarity between them to form a group within the two members. Next, the group - individual dissimilarity between this new group and all the remaining individuals is calculated. This set of dissimilarity is added to the matrix of dissimilarities among the remaining individuals to form a new dissimilarity matrix. The procedure is repeated and another fusion is made. The procedure terminates when all

the individuals are in one group. The method for computing group - individual and group - group dissimilarity is called Clustering strategy.

Non-hierarchical Clustering method :

All non-hierarchical methods operate by guessing a group and employing some method or algorithm to improve the classification. This procedure is repeated until no further improvement in the classification occurs. This algorithm requires both the criteria for evaluating the improvement in grouping when individuals are transferred from one group to another and a procedure for determining how individual should be reallocated to improve the classification.

[Note: SPSS package provides both the algorithm for clustering]

2.2 Discriminant Analysis :

Linear discriminant analysis is the most widely used method of classification. The formal purpose of such analysis is to assign distinct set of items to one or several groups based on a set of measurements. The method allocates new items to previously defined groups by determining mathematical discriminant functions (linear combinations of original variables) that minimize the chance of misclassification.

3. Ordination methods

Ordination methods summarize multidimensional data by producing a low dimensional space in which similar accessions are close together and dissimilar ones are far apart. Spatial representation in two or three dimensions will reflect the relationship in higher dimensions with minimum distortion.

- **Principal Component Analysis/Canonical vector analysis**

These are linear combination of random or statistical variables which have special properties in terms of variances. In effect, transforming the original vector variable to the vector of principal components amounts to rotation of co-ordinate axes to a new co-ordinate system that has inherent statistical properties. The principal components turn

out to be the characteristic vectors of the covariance matrix. In many exploratory studies the number of variables under study is too large to handle. Since it is of interest to study the variation a way of reducing the number of variables to be treated is to discard the liner combinations which have smaller variation and to study only those with large variance.

Another multivariate statistic used frequently is

4. Hotelling's T^2 statistic / Generalized T^2 statistic

This is the multivariate analogue of the univariate 't' given by

$$T^2 = N (x - \mu) S^{-1} (x - \mu)$$

where x is the mean vector of sample of N and S is the sample covariance matrix. μ is the population mean vector.

This is used in situations

- Testing the hypothesis that mean vector is a given vector.
- To test that mean of one normal population is equal to the mean vector of other.

Conclusion

Plant germplasm collections have grown fairly large in size. Developing procedures for reducing the size of a collection to manageable and accessible level is becoming are of the most important uses in the management and utilization of germplasm collection. The D^2 and Canonical analysis are useful to attain a meaningful classification or grouping of germplasm collections.

Modern computers make multivariable methods feasible when large number of variables are investigated. Because hardware and software development is so explosive and programmes require specialized knowledge, packages of statistical programmes are available for most of the methods.

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