

**Assessment of Variability and Standardization
of Protocol for Micropropagation in Spine
gourd (*Momordica dioica* Roxb.)**

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DOLI JAMATIA

Thesis
Doctor of Philosophy in Agriculture
(Horticulture)



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Department of Horticulture
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CERTIFICATE-I

Dated / /2016

This is to certify that **Miss Doli Jamatia** had successfully completed the Comprehensive Examination held on 5th July, 2014 as required by the regulation for degree of **Doctor of Philosophy in Agriculture (Horticulture)**.

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CERTIFICATE-II

Dated: / / 2016

This is to certify that the thesis entitled “**Assessment of Variability and Standardization of Protocol for Micropropagation in Spine gourd (*Momordica dioica* Roxb.)**” submitted for the degree of **Doctor of Philosophy in Agriculture** in the subject of **Horticulture**, embodies bonafied research work carried out by **Miss Doli Jamatia** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigations have been fully acknowledged. The draft of this thesis was also approved by the advisory committee on 13/06/2016.

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This is to certify that the thesis entitled “**Assessment of Variability and Standardization of Protocol for Micropropagation in Spine gourd (*Momordica dioica* Roxb)**” submitted by **Miss Doli Jamatia** to the Maharana Pratap University of Agriculture and Technology, Udaipur in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Agriculture** in the subject of **Horticulture** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on her thesis has been found satisfactory; we therefore, recommended that the thesis should be approved.

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CERTIFICATE-IV

Dated: / /2016

This is to certify that **Miss Doli Jamatia** of the Department of **Horticulture**, Rajasthan College of Agriculture, Udaipur has made all corrections and modifications in the thesis entitled “**Assessment of Variability and Standardization of Protocol for Micropropagation in Spine gourd (*Momordica dioica* Roxb.)**” which were suggested by the external examiner and the advisory committee in the oral examination held on / /2016. The final copies of the thesis duly bound and corrected were submitted on / /2016 are enclosed here with for approval.

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Assessment of variability and standardization of protocol for micropropagation in spine gourd (*Momordica dioica*)

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ABSTRACT

The present investigation entitled “Assessment of variability and standardization of protocol for micropropagation in spine gourd (*Momordica dioica*)” was carried out in two phases: *in-situ* variability study and to standardize the protocol for micropropagation in *Momordica dioica*. For *in-situ* study the variability was studied among thirty four randomly identified wild growing genotypes around Udaipur (Rajasthan). To standardize the micropropagation protocol, regeneration of plantlets from leaf and nodal explants were accomplished successfully using MS basal media supplemented with different growth regulators.

Analysis of variance for *in-situ* study revealed that mean squares due to genotypes were significant for all the traits except spine length in both the years, fruit specific gravity in the first year and chlorophyll content in second year thereby, showing considerable amount of variability for most of the characters and were amendable to improvement.

In terms of *per se* performance, genotype MPK-009 was superior for most of the characters followed by genotype MPK-006. These genotypes were identified as desirable ones in variability studies.

Magnitude of GCV and PCV was high for protein content, fruit weight, fruit volume, ascorbic acid and TSS. High heritability was recorded for TSS only. The high genetic gain was depicted by fruit weight, fruit volume, protein content and ascorbic acid.

When yield data are not available, the character like fruit weight may be considered as target character. In the present investigation it was positively and highly

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significant correlation with fruit length, fruit diameter, fruit volume and number of seeds per fruit at both levels in first year whereas fruit specific gravity and ascorbic acid at both levels in second year.

The regression analysis revealed that in *in-situ* observations of all the 8 characters contributed towards fruit weight was significant *i.e.*, 91.8 and 78.7 per cent variability respectively in both the years. The regression analysis revealed that contribution of all the 8 characters towards ascorbic acid was significant in second year only *i.e.*, 68.0 per cent variability.

The establishment of an efficient plant regeneration protocol in spine gourd using two types of explants viz., *in vivo* nodal and leaf as well as *in vitro* nodal and leaf explants, respectively, has been used in the present experiment. Among the different treatments of TDZ and BA, maximum shoot induction was obtained from 1.5 mg/L TDZ containing media which showed a maximum shoot induction of 71.6 per cent. In the second set of media, BA alone and in combinations with Kin and NAA were evaluated. Among all the treatments, the highest shoot induction response of 83.3 per cent was noticed in 1.5 mg/L BA supplemented media. Shoot regeneration rate of 100 per cent was obtained from the *in vitro* nodal segments in TDZ (0.5-2.5 mg/L) alone, BA (1.0-2.0 mg/L) alone, BA in combination with Kin (2.5+0.5 mg/L) and BA (2.5-3.0mg/L)+0.5 mg/L NAA fortified media. Among the various cold treated *in vitro* leaves, the leaves which were treated for 24 hr showed maximum bud breakage and highest shoot regeneration response (46.6 per cent) on media containing 3.0 mg/L TDZ. Comparison among all multiplication media, revealed that, 0.5 mg/L BA and in combinations with 1.0 mg/L Kin was found to be most suitable for shoot multiplication *i.e.*, 8.5 number of shoots per explant. Out of the different hardening media tested, cocopeat in combination with vermiculite achieved highest survival rate of plants *i.e.*, 73.3 per cent which was significantly different from others.

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1. INTRODUCTION

Spine gourd is a dioecious climber, with a glabrous stem, broad ovate leaves, entire deeply 3-5 lobed; yellow solitary flowers, ovoid or ellipsoid fruits, 2.5-6.3 cm long, shortly beaked, densely echinate with soft spines; seeds slightly compressed, 6-7 mm long, irregularly corrugated. Spine gourd is probably a native of India. Plants are distributed from Himalayas to Sri Lanka; upto an altitude of 1500 m. Plants of spine gourd are found naturally growing in hilly tracts of Rajmahal, Hazaribagh and Rajgir of Jharkhand and in wet hills of Maharashtra, Assam and West Bengal. It has prominent position among cucurbitaceous vegetables owing to its good nutritional and medicinal value, high keeping quality, ability to withstand long distant transportation, high market price and good export potential (Rasul, 2003). Although considered as an underutilized and minor cucurbitaceous vegetable, it is widely cultivated in Orissa, Maharashtra, Bihar and West Bengal and is slowly gaining popularity as a commercial vegetable crop, because of its rich taste and high nutritional value. Underutilized vegetables have immense potential for contribution to a particular pocket of food production because they are well adapted to existing as well as adverse climatic condition and are generally resistant to pests and pathogens. Underutilized vegetables are rich source of folic acid, which is commonly referred to as folate. They contain high amount of carotene and are cheap source of vitamins and minerals (Bhuiya *et al.* 1977). They possess several medicinal and curative properties *e.g.*, the decoction of leaves reduces fever, tuberous roots help in relieving headache, sweating stone formation and migraine while fruit is quite helpful in controlling diabetes and blood pressure. It is often cultivated for its fruits which are used as a vegetable. According to Pandey (2008), fruits are good sources of protein (3.1 g), iron (4.6 g), ascorbic acid (275.1 mg), carbohydrates (7.7 g), thiamine (45.2 g), calcium (33 mg), phosphorus (42 g), carotene (2700 IU), riboflavin (176.1 g), moisture (84.1 per cent) and niacin (0.59 g) respectively per 100 g of edible portion. Spine gourd grows in warm and humid weather and tuberous roots are planted in pits. The vines are trained on bowers and 5-10% of male parents are provided for good fruit set. Plantation is done at the onset of summer with the first shower, flowering starts in April and fruiting ends in October-November (Rahman *et al.* 2011). The plants remain dormant in winter, left *in-situ* and they over winter (Sadhu and Chakroborty, 1980; Nabi *et al.* 2002^a).

Being a minor vegetable, not much attention was paid towards it by researchers. Collection, evaluation of the existing germplasm and selection of better parents are the prerequisites for commencing a breeding programme in any crop like spine gourd. Besides, knowledge of genetic diversity and relationship among the sets of germplasm is critical for the plant improvement. For quick reference, variability based on morphological and physiological features can be readily used in preliminary screening. Genetic diversity in spine gourd based on morpho-physiological variation has been reported by (Rasul *et al.* 2004; Rasul and Okubo 2002). Because of its dioecious nature and poor seed germination the improvement of *Momordica dioica* Roxb. has a limited scope (Rashid 1976; Ali *et al.* 1991). Germplasm utilization of underutilized vegetable crops and devising vegetative propagation methods for selected high yielding genotypes have been the major constraints for popularizing this nutritious tropical vegetable among the farmers. To popularize this crop assessment of genetic diversity is a pre-requisite and for this a systematic morphological characterization to study the extant of variability and utilization of existing germplasm is necessary. Thus, the foremost objective of the agricultural scientists is to breed crop varieties or strains having higher yield potential, both in term of quality and quantity. The wide genetic variability that exists in the available genotypes, provides ample scope for further improvement. The correlation studies provide relative information on the nature and extent of relationship for bringing about improvement in yield and other traits. Correlation studies between yield and other traits of the crop will be of great interest to the breeders in planning the hybridization programme and evaluating the individual plants in segregating populations. It also provides an indication of effective selection of desirable traits towards the improvement of varieties. The knowledge on nature and magnitude of genetic variability existing in spine gourd will provide the foundation for designing breeding programme. However, reports on genetic diversity and morphological variation in spine gourd are scanty. Assessment of variation made on truly diverse germplasm provides an idea about the extent of genetic variation. Greater the genetic variability better the chances of improvement of the crop. As such, efforts may be concentrated on the selection of some characters which ultimately contribute towards yield. Therefore, it becomes very essential to find out yield contributing traits, their variability parameters and multiple regressions.

This popular vegetable has high demand in market but still remains underutilized and underexploited (Bharathi *et al.* 2007). Presently, its propagation entirely depends on underground tuberous roots, which occupy the valuable cultivated land for a long period *i.e.*, until next planting season. Maintaining tuber quality in field condition as well as to conserve it in storage is difficult. Vegetative mode of propagation through root tubers, lack of sufficient availability of root tubers to the farmers, very low percentage of seed germination (10%), hard seed coat, prolonged dormancy (4-5 months) and unpredictable sex ratio in seedling progeny are the major hurdles for large scale cultivation of spine gourd (Mondal *et al.* 2006). Male plants dominate natural populations and sex determination starts possibly only when the plants start flowering. Since fruits which are harvested from the female plants are the main edible portions of this species, it is desirable to have commercial fields with a large proportion of female plants. As the conventional methods of spine gourd propagation impose several limitations for large scale production of sex specific plants, an efficient clonal propagation is must. *In vitro* technology, especially plant tissue culture, offers many unconventional techniques for crop improvement. It can be employed as an alternative means for genetic upgrading, and its application largely depends on a reliable plant regeneration system. Meristematic explants may be ideal for use in transformation of experiments because meristematic cells and tissues are already programmed for shoot development (Siaram *et al.* 2003; Weber *et al.* 2003; Peddabonia *et al.* 2006). Micropropagation has been reported in shoot tip and nodal explants of Cucurbitaceae such as *Citrullus lanatus* (Barnes *et al.* 1978) & *Cucumis sativus* (Vasudevan *et al.* 2001; Ahmed and Anis 2005). Improved production technology for this crop is very much needed. Therefore, the proposed experiment was carried out to develop an efficient protocol for *in vitro* plant regeneration for large scale propagation.

Keeping in view the above said facts, an experiment entitled “Assessment of Genetic Variability and Standardisation of Protocol for Micropropagation in Spine gourd (*Momordica dioica* Roxb.)” was conducted at Department of Horticulture and Plant Tissue Culture Laboratory, Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur with the following objectives:

1. To study in-situ variability of spine gourd
2. To study the association between different traits of spine gourd
3. To standardize micropropagation protocol of spine gourd

2. REVIEW OF LITERATURE

Before aiming an improvement in yield, it is necessary to have the knowledge of relation of characters with yield. The genetic parameters such as genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance enable selection on a sound genetic basis, selection helps in improving the yield. However, selection based on yield alone is often misleading, because it is one of the complex characters being dependent on its component for its full expression. Hence, it is necessary to know how the change in environment will control the expression of the traits. For rational improvement of yield and its components, association of component characters with yield among the components themselves should be found out by estimating the correlation coefficients.

To increase germplasm usefulness for breeders, morphological characterization is needed. Morphological traits are useful in distinguishing between ecotypes and identified a high degree of phenotypic variability between populations of several genotypes. The morphological traits are valuable for the identification of distinct populations or genotypes for conservation, optimum sites for germplasm collection and ongoing changes in the pattern of diversity over time. Additionally, morphological traits are useful for the evaluation and utilization of genetic resources.

A comprehensive review of work on this topic in case of cucurbits have been collected from published sources and utilized for planning of present work. The pertinent literature has been reviewed under following heads:

1. Variability Parameters
2. Association Analysis
3. Micropropagation

2.1 Variability

Existence of genetic variability is a key factor to decide crop improvement. Evaluation of large number of germplasm is the first line of work to measure the extant of variability present in a population. The improvement in any crop is based on the extant of genetic variation and magnitude of available beneficial genetic variability. For finding the best genotype visually we go for morphological traits *i.e.*, phenotypic character, we choose easily observable characters like vine length, fruit

weight, fruit diameter, *etc.* Hence, an insight into the magnitude of variability present in the gene pool of a crop species is of utmost importance to a plant breeder for starting a judicious plant breeding program.

Some of the biometrical techniques commonly used to assess the variability in the plant breeding populations includes range, mean, standard deviation, variance, standard error, coefficient of variance, component analysis, D² statistics and metro glyph analysis are simple measures of testing variability. The simple measures of variability especially the coefficient of variation partitions the variation into phenotypic, genotypic and environmental components and determines the magnitude of these components for various traits. India being considered as the probable native of spine gourd, it has accumulated a wide range of variability providing good scope for improvement in yield and other characters of the crop through selection.

Horticultural crops are gaining importance throughout the world not only as food or economic crops but also for their importance as raw material for value added products and industrial uses. Cucurbits and gourds form an important and big group of vegetable crops belonging to family *cucurbitaceae*. The family is moderately large, comprising of 118 genera and 825 species in the world. From India 37 genera and about 100 species of cucurbits including wild and cultivated have been reported.

Pandey *et al.* (2002) studied genetic variability in sixty seven accession of pumpkin. The observations were recorded on biochemical and morphological characters viz., ascorbic acid content (mg/100gm), polar circumference of fruit (cm) and yield per plant (kg). Highly significant differences between genotype were recorded for all the characters. Maximum ranges of mean values were observed for equatorial circumference of fruit and minimum for number of fruits per plant. High degree of variability was observed for yield per plant, ascorbic acid and β -carotene content. Yield had significant and positive correlation with number of fruits per plant, equatorial and polar circumference of fruit. Ascorbic acid and β -carotene content had positive but non significant association with yield. Hence, number of fruits, equatorial and polar circumference of fruit can be considered as selection criteria for improving yield. Minor cucurbitaceous vegetables are considered as nutritious with high vitamins and minerals matter. The specific medicinal and curative properties are well appreciated. Spine gourd is one of the important members of the family *cucurbitacea* (Kumar 2003). Ram *et al.* (2004) had performed exploration and collection of kartoli

germplasm (*Momordica dioica*) to screen the total of twenty lines for high yield and fruit characters. The widest ranges were recorded for fruit length, while narrowest range observed for days to female flower anthesis. The characters like days to female flower anthesis, fruit set to edible maturity, plant height, fruit diameter, fruit length, number of fruits per plant and yield per plant expressed maximum variability at both phenotypic and genotypic level. The germplasm DR/05-08-02 with maximum yield was considered as a promising line, whereas DR/NKV-02-06-01 for smaller fruit size. The genetic improvement for higher yield could be achieved through clear understanding of the type and amount of variability present in genetic stocks. Knowledge of genetic diversity allows the breeder to select the suitable genetic base for breeding programme. Rakhi and Rajamony (2005) explored forty two landraces of culinary melons (*Cucumis melo* L.) for variability. Genotypic coefficient of variation and phenotypic coefficient of variation were highest for yield per plant followed by fruit weight, fruit per plant, keeping quality of fruits and 1000-seed weight indicating the scope for improvement of culinary melon through selection. The genetic variability for quality parameters were studied in thirty eight accessions of bitter gourd (*M. charantia*) for ascorbic acid content, carotenoid content and quantitative traits. The highest total carotenoid content (3.2 mg) was registered for DBTG-8, followed by DBTG-9 (3.0 mg), DBTG-101 (2.9 mg), DBTG-6 (2.8 mg), DBTG-4 (2.7 mg) and DBTG-2 (2.6 mg). DBTG-3, DBTG-8, DBTG-6 and DBTG-101 had small fruits, which were superior in terms of nutritive value. DBTG-8 was highly promising for nutritive value and fruit yield. Among the genotypes with large fruits, DBTG-4 was superior in terms of fruit yield, fruit length, fruit diameter, flesh thickness, ascorbic acid and total carotenoid contents as reported by Dey *et al.* (2005).

Pariari *et al.* (2005) evaluated the performance of twenty one cultivars of *T. dioica* collected from West Bengal, Bihar and Uttar Pradesh. Cultivars obtained from West Bengal BC-24 produced the greatest average vine length (6.10 m), number of primary branches per plant (7.19), number of fruits per plant (38.33) and yield per plant (1.72 kg). The other cultivars from West Bengal BC-65 produced the longest internodes *i.e.*, 8.21 cm. The average fruit length was greatest *i.e.*, 8.72 cm in BC-64, whereas the average fruit diameter was greatest *i.e.*, 3.84 cm in BC-5. BC-6 had the lowest average number of seeds per fruit (11.34). BC-53 registered the highest average fruit weight (49.98 g). The high-yielding accessions BC-24, BC-53 (1.53 kg

per plant) and BC-15 (1.38 kg per plant) were recommended for cultivation in West Bengal. BC-1, BC-5, BC-6, BC-7 and BC-34 were also suitable breeding materials for the improvement of the yield components of *T. dioica*. Variability studies have been reported in many cucurbits crop both utilized and underutilized one. Bharathi *et al.* (2006) studied the variability in spine gourd comprising of 32 genotypes. The study revealed that fruit volume exhibited high PCV estimate followed by number of fruits per plant, intermodal length, yield per plant, fruit length, node number on which the first flower appears, vine length and days to flowering. Good amount of heritability was observed for fruit volume, fruit weight and intermodal length.

Munshi *et al.* (2007) evaluated wild *Cucumis sativus* var. *hardwickii* germplasm for various parameters and observed highly significant variation for all characters namely mean fruit weight was 57.3 g with a range of 33.0 to 99.1 g, mean fruit number per plant 18.7 with a range of 11.0 to 27.9 and mean fruit yield per plant 663.7 to 1839.3 g. The evaluated results indicated that *Cucumis sativus* var. *hardwickii* possesses important and useful characters such as prolific fruit bearing with high numbers of laterals (10-15) which are of interest to breeders. The data presented suggested that the variability for fruit characteristics within the *Cucumis sativus* var. *hardwickii* germplasm collection is somewhat representative of the diversity within the species, and that variability for fruit morphologic characteristics is likely sufficient to provide the basis for the improvement of the cucumber crop. Singh *et al.* (2009) conducted an experiment to identify growth characteristics including propagation methods, best time of planting, productivity and the economics of spine gourd cultivation. The experiment consisted of eighteen genotypes collected from various naturally growing forest areas. Growth and yield attributes and their correlations were observed, genotypes AAI/S-1 and AAI/S-4 were found to be high yielding with an average yield of 606 g and 649 g/plant, respectively. All the genotypes were tested for nutritional value, but there was no significant difference among the genotypes. The average nutritional value per 100 g edible fruit was found to contain 84.1% moisture, 7.7 g carbohydrate, 3.1 g protein, 3.1 g fat, 3.0 g fibre and 1.1 g minerals. It also contained small quantities of essential vitamins like ascorbic acid, carotene, thiamin, riboflavin and niacin. This crop can provide additional nutrients and help the body to develop natural immunity from many common ailments. Variation in seed phenotypes were determined by Balkaya *et al.* (2009) in

one hundred and sixty populations of *Curbita maxima*, collected from different regions of Turkey. The experiential collection showed appreciable genotypic variation in seed shape, colour, brightness, seed dimensions and seed weight. Seed displaying a wide elliptic shape (62.5%) were the most common in collection. Most populations displayed cream seed colour (46.9%), followed by white (25.6%), brown (20.0%) and tawny (7.5%). The study demonstrated that substantial differences in seed dimensions exist in Turkish winter squash populations. Seed length ranged from 15.0 to 22.7 mm, 7.6-15.5 mm for seed width and 1.4-6.1 mm for seed thickness. For seed length to thickness ratio and seed width to thickness ratio, populations showed a range of 3.2-14.2 and 2.2-8.5 respectively. This seed variability evaluation can assist geneticists and breeders to identify the populations with unique characteristics for inclusion in future breeding activities. Variability studies in *Tricosanthes dioica* was performed by Khan *et al.* (2009). The experiment conducted with twenty four accessions revealed that high phenotypic and genotypic coefficients of variation. The accessions varied significantly for days required to first flowering, number of node at first harvest, internodal length at first harvest, vine length at first harvest, fruit length, fruit breadth, fruit weight, pulp weight, pulp seed ratio, number of fruits per plants, weight of fruits per plant, number of seeds per fruit, weight of seed per fruit and yield.

Bharathi *et al.* (2010) using twenty six accessions of spine gourd collected from Eastern India and studied the diversity and other genetic parameters and found that spine gourd collected from Eastern India showed immense variation and can be used as a potential source of germplasm for crop improvement. Phenotypic diversity analysis in pointed gourd (*T. dioica*) crop has been reported by Bharathi & Vishalnath (2010). The accessions showed a considerable level of variability for qualitative traits such as fruit colour (light green to dark green), fruit striping (striped to without stripe), fruit shape (round to oblong) and leaf surface (smooth to rough). The high level of phenotypic diversity observed among the accessions like fruit colour and shape would be helpful for the selection of genotypes with desired quality. In addition, the accessions displaying contrast characters can be used to study the genetics of trait expression. Jacobo *et al.* (2011) did chemical and physiochemical characterization of winter squash cv 'Cehualca' (*Cucurbita moschata* D.) which is a seasonal crop that has been used as food and animal feed including fiber, carotenoids, phenolic and mineral contents in the winter squash. The morphological analysis

showed that the squash 'Cehualca' did not have a homogeneous morphology. Data about their physical and physicochemical characteristics showed large variability. Also, high content of carotenoids and dietary fiber was observed in squash. The oil and total phenolic content was low in comparison with other fruits. The mineral content exceeded the values recommended to meet the nutritional needs of consumers, except for sodium (both in pulp and shell), potassium and magnesium in the shell.

Yadav and Kumar (2011) conducted an experiment on variability for yield advancement in bottle gourd [*Lagenaria siceraria* (Molina) Standl]". The observations were recorded on twelve characters of bottle gourd viz., days to fifty percent germination, days to first male flower anthesis, days to first female flower anthesis, node number to first male flower, node number to first female flower, vine length (m), number of nodes per vine, number of primary branches per plant, length of fruit (cm), weight per fruit (kg), number of fruits per plant and fruit yield per plant (Kg). The analyzed data revealed that among the 15 parents PSPL followed by d VRBG-88 and VRBG-105 gave maximum results for all the above studied characters and among the 36 F₁ hybrids, cross VRBG-44 x Pusa Naveen followed by VRBG-105 x Pusa Naveen and VRBG-44 x PSPL gave maximum fruit yield per plant. Similar genetic variability evaluations in bottle gourd genotypes were also reported by Sharma and Sengupta (2012) where they found the mean performance of the genotypes revealed a wide range of variability for all traits and the highest variation recorded for vine length in Narendra Sivani 342.52 cm, followed by Narendra Sankar Lauki 323.47 cm and Kashi Ganga 270.95 cm whereas the minimum vine length were recorded for Samrat 141.88 cm. The information regarding the components on which the selection pressure can be exercised most effectively for effective crop improvement was vine length. Bottle gourd (*Lagenaria siceraria*) germplasm collection and evaluation in Turkey were also performed by Yetisir *et al.* (2012).

Archana (2012) examined genetic variability in thirty one spine gourd for different traits with yield. Cataloguing of germplasm exhibited significance difference for all characters studied viz. early plant vigour, plant growth habit, leaf margin and flower colour in both male and female accessions, fruit shape, nature of spines, blossom end fruit shape, fruit skin colour fruit bitterness and seediness in female accessions. The quantitative characters studied were vine length, number of branches per plant, days to first flower opening and duration of the crop in both male and

female accessions, and also days to first harvest, days to last harvest, days from flowering to vegetable maturity, total number of harvests, fruit length, fruit girth, average fruit weight, number of fruits per plant, yield per plant, duration of the crop and shelf life in open condition in female accessions. Among these all the characters showed significant difference between the accessions except vine length. From the experiment the conclusion occurred that the highest yielding accession found to be MD 227 with an average yield of 110.06 g per plant. The same accession recorded the highest value for total number of fruits per plant (18.25) and total number of harvests (6.0). High genotypic and phenotypic coefficient of variation was observed for days from flowering to vegetable maturity, total number of harvests and fruit length. Yield exhibited genotypic correlation with number of branches per plant, total number of harvests, fruit length and number of fruits per plant.

Veena *et al.* (2012) evaluated thirty-eight advanced lines of cultivated cucumber (*Cucumis sativus* L.) for variability, heritability and genetic advance for yield and contributing traits. Genotypic coefficient of variation and phenotypic coefficient of variation were highest for node at first female flower appearance followed by node at first male flower appearance, yield per plant, seed cavity breadth, average fruit weight and number of fruits per plant. Thus indicating that these characters had additive gene effect and therefore, they are more reliable for effective selection. Similar kinds of experiment work were also conducted using cucumber genotypes (*Cucumis sativus* L.) by collecting data on morphological features, yield and components of yield of cucumber which includes total fruit yield per pickling, fruit number per pickling, branch number per plant, plant height, distance between internode, length of branches, shoot diameter, leaf length, leaf width, fruit diameter, fruit length, plant vigor and fruit number per node. Mean comparison showed that the genotypes showed a broad phenotypic variation for studied traits, such that total fruit yield per pickling ranged from 474.3 g (Gohar) to 338.3 g (Tornado) have been confirmed by Golabadi *et al.* (2012) and concluded that the result of the said research could be compromised from a broad genetic diversity among selected germplasm. It seems that there is a high variation for fruit number per pickling among evaluated genotypes. Genotypes with high total fruit yield per pickling also showed high fruit number per pickling with the exception of genotype Green Majic with low total fruit yield per pickling. To give attention to other traits in this genotype revealed that fruit

length, fruit diameter and fruit number per node were low that caused yield reduction. Fruit number was found to be more stable measure of productivity than fruit weight for cucumber. Female clones of teasle gourd (*Momordica dioica*) were evaluated by Naik *et al.* (2012) for variability. Higher genotypic coefficients of variation were observed for all characters except fruit length at marketable stage. Total sugar in mesocarp, total sugar in exocarp, reducing sugar in mesocarp, ascorbic acid in exocarp, ascorbic acid in mesocarp, total soluble solids (TSS) in exocarp, β -caroten in exocarp, acidity in mesocarp, β -carotene in mesocarp, TSS in mesocarp, acidity in exocarp showed high heritability coupled with high genetic advance indicating that these traits were under the additive gene control and simple selection can be used for further improvement in these traits of teasle gourd. Variability studies have also been reported in minor crop like Egyptian sweet melon (*Cucumis melo* var. *aegyptiacus*) by (Ibrahim 2012). Ranges, mean and standard deviation and coefficient of variation among genotypes revealed the presence of genetic variation for all the studied traits. As the genetic potential of a germplasm remains unknown until it is systematically evaluated and screened only then it may become an important gene source for utilization in breeding programme. Evaluation of germplasm ultimately an improving yield and quality yield as such affected by a large number of characters, hence selection based on morphological traits plays a major role in variability studies.

Assessment of genetic variation for both quantitative and qualitative characters for *Cucurbita maxima* have also been reported by (Onyishi *et al.* 2013) and revealed that the accessions showed uniform spreading growth and great diversity in qualitative characters such as shape, colour and skin texture. The significant variations observed in the traits could be as a result of the genotypic differences existing among the accessions which are an indication that the characters are genetically controlled. Ara *et al.* (2013) assessed the performance of thirty four snake gourd genotypes for evaluate their performance for various quantitative as well as qualitative characters. All the qualitative characters viz., fruit colour, fruit shape, lamina type, fruit stripe, skin texture and collection site showed wide variation among the genotypes studied. Wide range of variability were also noted with respect to days to first male flower anthesis, days to first female flower anthesis, vine length, node number at first female flower, number of shoot per plant and days to first harvest. However, with regard to quantitative character like number of fruits per plant, the

highest number (45.6) was obtained from the genotype TAISD028 which was statistically similar to TAISD032 (44.4) while the lowest (15.99) was from check. Similarly, the genotype TAISD028 gave the highest yield (61.05 t/ha) and the check had the lowest yield (21.86 t/ha). Kumar *et al.* 2013 studied the genetic variability in sponge gourd (*Luffa cylindrica* Roem.). They observed significant variations for all the characters in all the genotypes used in the experiment. Highest genotypic and phenotypic variations were observed for total yield per vine followed by number of seeds per fruit, average weight of fruit and total soluble solids. Number of seeds per fruit, average weight of fruit and specific gravity showed high heritability with high genetic advance.

Singh *et al.* (2014) studied genetic architecture and association analysis in twenty five bitter gourd (*Momordica charantia* L.) landraces for ten quantitative traits, so as to identify promising traits on which basis selection can be made and conceded that high phenotypic and genotypic coefficients of variation were found for fresh fruit yield per plant followed by fruit length, fruit width and number of fruits per plant, indicating high genetic variability in these traits. They concluded that the emphasis should be given to improve fruit yield per plant in bitter gourd, focus should be given on fruit weight, fruit length, fruit diameter and plant height as it has positive correlation with fruit yield per plant. Variability in bitter gourd has also been confirmed by Rashmi and Sreelathakumary (2015). In respect of vegetative characters, ample variability was observed as evident from the wide range obtained for days to seedling emergence, vine length and internodal length. Among the genotypes evaluated, MC 9 was the earliest to emerge (6.00), MC 1 was the most vigorous registering the highest values for vine length and MC 20 for internodal length. The variation in vine length might be due to the specific genetic constitution, inherent character and vigour of different genotypes. Significantly maximum number of primary branches was recorded by MC 32 and was the earliest to harvest. Maximum fruit length, fruit girth, average fruit weight and yield per plant were recorded in MC 20. Highest number of fruits per plant was recorded in MC 10. MC 32 had higher number of seeds per fruit while MC 24 recorded higher seed weight. This confirms that the fruit yield is a complex trait and is the ultimate expression of many component characters in bitter gourd. Abd El-Hamed *et al.* (2015) has reported genetic diversity and relationship in squash using morphological and chemical

analysis. Germplasm characterization is an important link between the conservation and utilization of plant genetic resources. A collection of thirteen summer, spaghetti and acorn squash (*Cucurbita pepo* L.) and one winter squash (*Cucurbita moschata* L.) germplasm accessions were screened using morphological (leaf area, fruit weight, fruit length, fruit firmness), chemical [soluble solids content (S.S.C.), ascorbic acid]. A wide range of variability among genotypes was recorded for morphological and chemical characteristics. The percentages of variation were 217%, 532%, 175%, 166%, 148%, 520% for leaf area, fruit length, fruit weight, fruit firmness, S.S.C, ascorbic acid content, respectively. Both significant positive and negative correlations have been found between the morphological and chemical characteristics.

2.2 Correlation:

The concept of correlation was given by Galton (1889) and later extended by Fisher (1918) and Wright (1921). Correlation analysis is a biometrical technique to find out the nature and degree of association among various traits. Therefore, information on association among yield and quality characters are of vital importance in plant breeding programme. An understanding and knowledge of the interrelationships among characters are important to aid selection and set limits of each economic character that a breeder can choose without adversely affecting another important character. Improving in crop programme, it is essential to have simultaneous progress of more than one character like yield, which is influenced by many other traits. Lerner (1958) stressed the importance of correlation of the various characters with yield. This is because of linkage between physiological and relationship of genes governing various characters. Hence, knowledge of correlations between different economic characters is of importance in selection programme. Positive correlations make simultaneous improvement in two or more attributes possible, whereas, negative association indicates the need to compromise between desirable characters.

Many researchers have studied correlation in various cucurbitaceous crops. Highly significant correlation of fruit yield were observed with number of fruits per plant in pointed gourd (Singh *et al.* 1993), number of fruits per plant, number of internode, length of fruits, diameter of fruits and specific gravity in ivy gourd (Sarnaik *et al.* 1999), fruit weight, fruit diameter and number of primary branches per plant in spine gourd (Sarkar 1999) and fruit size in pointed gourd (Srivastava *et al.* 2005).

Puzari (1999) studied the correlation between fresh weight (FW) of fruit and seed content of spine gourd for twenty fruits of (*M. cochinchinensis* Roxb). He found that apart from seed : FW fruit ratio, all the other parameters were positively correlated to fruit FW. There was a positive correlation between the number of seeds/fruit and seed : FW fruit ratio. Seed FW and DW were also positively correlated with seed : FW fruit ratio. Hazra *et al.* (2003) evaluated sixty-eight diverse female clones of *T. dioica* for growth, morphological, yield and quality characters and their relationship through correlation studies. Most of the character pairs showed negligible or insignificant correlation that might have resulted due to simultaneous vegetative and reproductive growth in the plant. Only fruit number per plant had significant positive correlation with yield, whereas fruit weight showed highest positive direct effect on yield. However, from the overall study, most of the fruit characters, viz., fruit weight, pulp content of fruit, fruit number per plant and fruit volume and growth traits, such as leaves per plant and leaf length, were identified as important yield contributors. Correlation studies has also been explored in thirty strains of long melon (*Cucumis melo* var. *utilissimus*) by Mishra *et al.* (2005) for nine quantitative traits. Yield per plant had the positive and significant correlation with days to flower and highly significant association also observed for number of nodes per vine, width of fruit, length of fruit and number of fruit per plant. Therefore, the studies on correlation is directed that maximum emphasis should be given on number of fruits per plant while making selection.

Yadav *et al.* (2005) collected twenty-six diverse genotypes of chow-chow (*Sechium edule*) which is the only single seeded cucurbit, from different growing areas of Arunachal Pradesh and West Bengal. Variability in terms of fruit shape, size, colour and presence of spine was observed in different genotypes. Most of the genotypes collected from Meghalaya had high fruit polar diameter. Fruit weight was lowest in the genotype Sikkim collection-3 (79.3 g) and highest in Meghalaya collection-5 (734.3 g). Genetic and phenotypic coefficients of variation were high for fruit equatorial diameter (top) and fruit weight, indicating chances of improvement of these characters through simple selection. Correlation studies showed that all the characters showed positive and significant correlation with fruit weight. Therefore, emphasis should be given on fruit weight for further selection programme. Singh *et al.* (2007) studied twelve cultivars of pointed gourd (*Trichosanthes dioica*), collected

from different places, for seven characters (vine length, number of days for first female flower appearance, node number for first female flower appearance, number of fruits per plant, fruit length, fruit diameter and fruit weight). Correlation showed that the number of fruits per plant, fruit length, fruit diameter and fruit weight were major contributing factors towards yield. Selection based on these characters can be effective for developing high yielding cultivars.

Genetic variability, inter-association and effect relationship in ash gourd (*Benincasa hispida* L.) was standardized by Parkash (2008). The results revealed that genotypic coefficient of correlations were found higher than the phenotypic ones. Fruit weight, fruit diameter, number of fruits per plant and fruit length exhibited significant positive correlations with fruit yield. Hence, by increasing fruit weight and fruit number, fruit yield can be increased. Correlation only measures the degree of association among different component characters but does not imply any cause and effect relation. Rahman *et al.* (2011) evaluated eleven genotypes of sweet gourd (*Momordica subangulata* ssp. *renigera*) for fourteen quantitative and two qualitative components. Correlation studies in relation to various characters revealed that total fruit yield per vine was positively and significantly correlated to fruit weight, fruit length, fruit diameter, fruit girth and protein content. Days to first female flower appearance and pedicel length. Among the biochemical traits, protein content showed significant and positive correlation with fruit weight, fruit length, fruit diameter, fruit girth and total fruit yield/vine. Character association and path analysis have been reported in other crop like snake gourd (*Trichosanthes anguina* L.) by Rana and Pandit (2011). Correlation findings revealed that fruit yield had significant positive correlation with total number of fruits, total number of primary branches and total number of nodes. The highest direct positive effects were recorded for days to first female flower opening and it can be considered as key factor in improving snake gourd yield. Husna *et al.* (2011) examined variability and correlation among different characters of thirty one bottle gourd genotypes and observed great deal of significant variation for all the characters among the genotypes. High genotypic co-efficient of variation (GCV) was observed for yield per plant, fruit weight whereas low genotypic co-efficient of variation was observed for fruit breadth. In all cases, phenotypic variances were higher than the genotypic variance. Differences between genotypic and phenotypic co-efficient revealed that the major portion of the phenotypic variance

was genetic in nature. Correlation studies revealed that highest significant association of yield per plant with reproductive characters were number of fruit per plant followed by fruit weight both at genotypic and phenotypic level. Considering all the characters the G4 (BD-4580), G31 (BD-8948), G26(BD-4560) and G28 (BD-4569) were selected for future breeding programme. Singh *et al.* (2011) studied correlation and path coefficient analysis in spone gourd (*Luffa cylindrica* Roem). They observed fruit yield per plant was found significantly and positively correlated with fruit length, fruit width, fruit weight and number of fruits per plant, while it was negatively correlated with days to first male flower, days to first female flower and days to first harvest. Rabbani *et al.* (2012) collected sixty genotypes of ridge gourd from different locations of Bangladesh for evaluating variability, character association and genetic diversity. Seventeen traits were studied, of these eight showed high heritability over (80%). Correlation coefficient studied indicated that yield per plant had highly significant positive relationship with the number of fruits per plant ($r = 0.874^{**}$), average fruit weight ($r = 0.707^{**}$), vine length at harvest ($r = 0.359^{**}$) and length of fruit ($r = 0.334^{**}$). But on the other hand it revealed that the sex ratio and circumference of fruit exhibited negative association with yield. The said findings of yield significantly and positive correlation with number of fruits per vine both at genotypic and phenotypic levels in ridge gourd is also corroborated by Rani (2012). Evaluation of thirty sponge gourd (*Luffa cylindrica* Roem) genotypes including Pusa Chikni (Natioanl Check) best suited to high yield and marketable trait for eleven traits has been done by Pandey *et al.* (2012). Days to first marketable fruit harvest exerted significant and positive association with days taken for anthesis of first female flower. The path coefficient analysis revealed average fruit weight, number of fruit per plant and days to first marketable fruit harvest had positive direct effect on marketable fruiting yield per plant. They concluded that an ideal plant with days to first marketable fruit harvest, days taken for anthesis of first female flower, days taken for anthesis of first male flower, higher average fruit weight and maximum number of fruit per plant might be helpful in improving marketable fruit yield per plant in sponge gourd for formulating desirable plant with high yield with superior marketable characters. Singh *et al.* (2012) studied the character association in ridge gourd [*Luffa actangula* (Linn). Roxb.]. The experiment revealed that fruit yield per plant was found significantly and positively correlated with fruit length, fruit diameter and fruit weight.

Padmaraja *et al.* (2014) conducted a research to elicit information on the nature of variability of snake gourd genotypes. Results indicated that genotypic correlations were higher than phenotypic correlations indicating highly heritable nature of the characters like yield per plant showed positive and significant correlation with vine length, productive length of vine, number of fruits per plant, mean fruit weight both at phenotypic and genotypic levels. In all the instances however, more reliance may be placed on the genotypic correlations. Since, these association characters are in desirable direction, selection for these traits may improve the yield per plant. Basumatary *et al.* (2014) conducted assessment of correlation studies in spine gourd (*Momordica dioica* Roxb.). The correlation trial revealed highly significant and positive correlation of yield per plant with number of primary branches, internodes length, fruits per plant, fruit diameter and single fruit weight. Days to 50% flowering exhibited significant positive correlation with total soluble sugar (%). Simple selection for those component characters will be helpful for making considerable improvement in yield. The results were also in confirmation with the works of Aliya *et al.* (2014) who also carried out correlation studies to find out character association and contribution in spine gourd for twelve quantitative characters namely vine length (m), number of stems per plant, days to first female flower appearance, first female flowering node, days to first fruit harvest, days to last fruit harvest, fruiting period (days), fruit length (cm), fruit width (cm), fruit weight (g), number of fruits per plant and fruit yield per plant (kg) for identification of the potential selection indices. Correlation analysis revealed that fruiting period, number of fruits per plant not only had positively significant correlation with fruit yield but also had positively high direct effect on it and are regarded as the main determinants of fruit yield. Days to first fruit harvest had positively moderate direct effect on fruit yield and their associations were negatively significant, days to last fruit harvest had negatively high direct effect on fruit yield and its association found to be significant positively, hence restricted simultaneous selection can be made for days to first fruit and days to last fruit harvest. Panigrahi *et al.* (2015) examined correlation coefficient in ivy gourd for various yield attributing traits and they explored that fruit yield had highly significant positive correlation with vine length, fruit diameter, average fruit weight, number of fruits per plant and fruit volume, hence selection based on these characters would be more rewarding. Koppad *et al.* (2015) studied correlation among the physiological growth attributing characters in ridge gourd (*Luffa actangula* Roxb.)

with reference to yield. Correlation coefficient analysis revealed that leaf area during 45 and 90 DAS (0.5350 and 0.4689, respectively), leaf area index during 45 DAS (0.5714), Absolute Growth Rate of leaf at the period of 45 to 90 DAS (0.7765), Crop Growth Rate of leaf at the period of 45 to 90 DAS (0.6095) had positive significant correlation with the total fruit yield per vine but net assimilation rate of vine at the period of 45 to 90 DAS (-0.4796) had negative significant correlation.

2.3 Micropropagation

In 1902, the German Botanist Gottlieb Hebarlandt developed the concept of *in vitro* cell culture. *In vitro* literally means in glass and refers to the use of tissue culture techniques under carefully controlled laboratory conditions. Therefore, *in vitro* vegetative multiplication refers to the rapid propagation of a specific clone so that the genetic makeup is unaltered and the basic biological, physiological and horticultural characteristics are preserved (Chee *et al.* 1984). High multiplication ratio can be achieved by micropropagation technique, which enables rapid multiplication of disease pest free and elite plants within short space and time. Micropropagation has been reported in shoot tip and nodal explants of cucurbitaceae such as *Citrullus lanatus* (Vasudevan *et al.* 2001; Ahmad and Anis 2005), *Trichosanthes dioica* (Kumar *et al.* 2003). However, *Momordica* species regeneration via direct organogenesis is still limited due to low regeneration frequency in *M. charantia* (Wang *et al.* 2001) and *Momordica dioica* Roxb. (Thiruvengadam and Jayabalan 2001). *In vitro* plant regeneration has been found to depend on many factors, of which most important are: genotype, explants, composition of the basic medium, growth regulators, gelling agent, light intensity and quality, photoperiod, temperature, cultivation vessels and vessel covers (Reed, 1999).

2.3.1 Conventional propagation methods in spine gourd

In nature, seed of spine gourd rest in dormancy, hence, freshly extracted seeds cannot germinate and should not be sown for 5-6 months. The major problem of seed propagation is 1:1 ratio of male and female plants and delay in fruiting. Ali *et al.* (1991) observed enhanced germination of spine gourd seeds at 30°C when the seed coat was removed. Spine gourd is successfully propagated by tuberous roots as tubers do not have dormancy and plant raised through tubers are healthy but getting huge planting material is a major drawback for cultivation of this crop. Ahmed *et al.* (1992)

obtained highest percentage of rooting and number of roots per cutting comprising one axillary bud. Tripathy *et al.* (1993) reported that terminal cutting produced more nodes per plant and leaves, leaf area and intermodal length than basal cuttings. Panda *et al.* (1994) obtained enhanced sprouting of tuberous rooted cuttings (87.5%) by dipping cutting in 1 % thiourea. Propagation of *M. dioica* Roxb. through stem cuttings is a new approach in cucurbit. For raising plants through this method, cuttings are made from terminal portions (Sahu *et al.* 1995), however, did not observe any significant effects due to type of cutting (basal or terminal). Sprouting of stem cuttings is achieved by using growth regulators such as NAA and IBA in various concentrations (1, 2, 4, 5, 10, 15 and 20 mg/L). Higher rate of sprouting and vegetative propagation is possible by treating stem cuttings with IBA compared to NAA. IBA at 10 mg/L was efficient in improving the sprouting and survival of the plant (Shiragave and Chavan 2004). Moreover, a huge number of stem cuttings are required for mass propagation of plants which is an obstacle as number of mother plant need to be vanish. Another vegetative means of this crop propagation is grafting. Spine gourd was grafted on rootstock of *Cucurbita moschata*, *Cucurbita trifolia* or F₁ hybrids of *Cucurbita maxima* × *C. moschata*. The rootstocks have marked influence on scion reported by (Mian & Morokuma, 1992). All the growth parameters of *Momordica dioica* at both early vegetative and flower initiation stages were strongly influenced by grafting Mian *et al.* (1993). Through grafting unproductive surplus male plants can be converted to bearing female plants.

2.3.2 *In vitro* tissue culture of Spine gourd

Plant regeneration from *in vitro* culture can be achieved by either organogenesis or embryogenesis. *In vitro* shoot regeneration is greatly affected by genotype, explants source, plant growth regulators and culture conditions (Yildiz and Ozcan 2002). Knowledge on the effect of explants source on regeneration is very important. In this case, earlier studies have been demonstrated by various researchers *i.e.* nodes (Nabi *et al.* 2002^a; Mustafa *et al.* 2013), shoot and axillary bud (Thirivengadam *et al.* 2006 and Rekha *et al.* 2013^a). Nabi *et al.* (2002^a) reported that cotyledon of spine gourd showed the best performance among the four explants viz. node, shoot tip and leaf. They found combination of 1.0 mg/L BA and 0.10 mg/L NAA were most suitable in callus induction followed by 0.20 mg/L NAA. The highest number of multiple and tallest shoots were obtained on MS fortified with 1.0

mg/L BA and 0.10 mg/L NAA. However, for rooting, half strength MS supplemented with IBA proved to be better than IAA. Deokar *et al.* (2003) for *in vitro* culture of spine gourd evaluated four type of explants viz. shoot tip, axillary bud, cotyledon and hypocotyls and found that axillary buds were the best sources of explants. They concluded that the best treatment for crop establishment were MSHP + Ads + BA + 10 mg/L + IBA at 5 mg/L + gelrite and best treatment for the induction and development of roots (87% roots developed) were MSHP + Ads + IBA at 1.0 mg/L. Pawar *et al.* (2004) obtained optimum culture media and culture conditions for *in vitro* propagation of spine gourd using axillary bud explants. They investigated for shooting high-phosphate MS media containing Ads at 80 mg/L, mesoinositol at 100 mg/L, 3% sucrose and 0.8% agar supplemented with BA at 0, 10, 8, 6 and 4.0 mg/L and with IBA at 0, 5, 4, 3 and 2.0 mg/L were found optimum. For rooting MS media containing 0.8% agar and 5% sucrose supplemented with NAA or IBA at 3 mg/L, with or without activated charcoal at 0.2%. Media supplemented with BA at 10 mg/L and IBA at 5.0 mg/L produced the highest shoot number and optimum establishment. Maximum rooting was obtained in media supplemented with NAA at 3.0 mg/L and 0.2% activated charcoal.

Ghive *et al.* (2006) reported that the axillary bud was a better explant than the shoot tip for micropropagation. They used 3 local genotypes of *M. dioica* Roxb., comprising 2 females (AKSG-5 and AKSG-35) and one male genotype (AKSGM-1). In their study, they used MS basal medium containing Ads (70 or 80 mg/L) and supplemented with BA (1.0 mg/L) and NAA (1.0 mg/L) had the maximum multiple shoots (more than 5 per culture) with better growth response. Plantlet regeneration from the cell suspension cultures of spine gourd (*Momordica dioica* Roxb.) through somatic embryogenesis was reported by Thiruvengadam *et al.* (2007). Petiole derived embryogenic calli were cultured on MS medium augmented with 4.5 μ M 2,4-D and 10% coconut milk. The maximum frequency of somatic embryos (36.6%) was observed on MS supplemented with 2.2 μ M 2,4-D for three weeks of culture. Ontogenic studies of somatic embryos revealed that the cell destined to become somatic embryos divided into spherical proembryos and then progressed to globular, heart and further differentiated into torpedo and cotyledonary stages within 5 weeks. Embryo development was asynchronous and strongly influenced by the 2,4-D concentration. The MS liquid medium augmented with 2.2 μ M 2,4-D and 0.5 μ M L-

glutamine was effective to achieve high frequency embryo induction (44.4%). The cotyledonary-stage somatic embryos were transferred to MS liquid medium with no plant growth regulators to achieve complete maturation within 7 days. Lack of 2,4-D in suspension increased somatic embryo maturation with decreased abnormalities. Sucrose was found to be the best carbon source for callus induction, embryo maturation and embryo germination. Relatively, only few numbers of embryo developed into root/shoot when transferred to 1/10 MS solid medium containing 0.5 μ M ABA, 2% (w/v) sucrose and 0.2% (w/v) gelrite. About 115 of somatic embryos germinated into morphologically normal fertile plants within 2 weeks. Regenerated plants were successfully hardened, with a survival rate of approximately 60% and established in the field. This regeneration protocol assured successful embryo induction, maturation and plantlet conversion.

Karim and Ullah (2011) tested four explants viz. internodes, nodes, leaves, shoot tips and cotyledons for *in vitro* culture of spine gourd. They observed better performance of cotyledon explants resulted for best callus, shoot and root induction of spine gourd over other plants parts *via* callus formation. Cotyledons showed higher percentage of callus induction (98.33%) at 1.0 mg/L BA in 15 days whereas callus initiation were induced by 1.0 mg/L BA supplemented with 0.50 mg/L NAA from internode explants within shortest time (14 days). Proliferated calli were further cultured on MS medium for shoot initiation. The cotyledon explants had produced highest frequency of shoot formation (89.67%). MS medium containing 1.0 mg/L BA resulted maximum number of shoots (5.33%) and longest shoots (0.9 cm). The shoots were subcultured on MS medium supplemented with IBA and IAA for rooting and sufficient roots were induced on half MS medium containing only 0.30 mg/L IBA. An efficient *in vitro* regeneration protocol using petiole as explants derived callus of male and female plants of spine gourd were reported by Thiruvengadam *et al.* (2012). They reported plant regeneration from alginate-encapsulated shoot tips explants of spine gourd. Shoot tip explants excised from *in vivo* proliferated shoots were encapsulated in calcium alginate beads. A gelling matrix of 3% sodium alginate and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were found most suitable for formation of ideal calcium alginate beads. Maximum response (100%) for conversion of encapsulated shoot tip explants into plantlets were obtained on 0.7% agar solidified full-strength MS medium containing 0.50 μ M BA. Rekha *et al.* (2013^b) revealed the use of spine gourd seed in medium containing MS

supplemented with medium 3.0 mg/L BA to produced *in vitro* plantlets and axillary buds were excised and cultured on MS media. Mature seeds were used for the regeneration of *M. dioica* Roxb. has also been confirmed by Arekar *et al.* (2012). The decoated seeds of *M. dioica* Roxb. were cultured on MS medium supplemented with various combinations of auxins (α -naphthaleneacetic acid) and cytokinins (N6-benzyl adenine). MS basal medium supplemented with 4.44 μ M and 8.88 μ M N6 - benzyl adenine (BA) gave rise to maximum number of shoots in 7-8 weeks. *In vitro* grown shoots were sub cultured on MS medium supplemented with different concentrations of indole-3-butyric acid (IBA) for root initiation. MS medium with 0.049 μ M indole-3-butyric acid (IBA) showed rooting in 45 days. The regenerated plantlets were successfully hardened in vermiculite. Further increase in the concentration of BA failed to achieve multiple shoots. The media used for shoot multiplication favored shoot elongation. Raju *et al.* (2014) studied the effect of amino acids in combination with cytokinin for the maturation and regeneration of embryogenic callus in spine gourd. They observed highest percentage (80%) embryogenic callus on MS medium fortified with 2.0 mg/L each of 2,4-D and BA in tendrill explants. Maximum numbers of shoots (8 shoots/embryogenic calli) were observed on MS medium supplemented with 4.0 mg/L BA in combination with 2.0 mg/L L-glutamic acid from embryogenic calli derived from tendrill explants. All shoots were rooted on MS medium augmented with IBA 3.0 mg/L.

(a) Establishment of *in vitro* culture through nodal explant

Nodal part is the choicest explant for *in vitro* culture for most researchers. Various reports on *in vitro* use of nodal explant had been documented (Thiruvengadam *et al.* 2006 and Mustafa *et al.* 2013). Karim and Ahmed (2010) cultured node of spine gourd showed highest capacity of producing multiple shoots at 1.0 mg/L BA + 0.10 mg/L NAA in shortest time (15 days). More recent studies on high frequency plantlet regeneration from nodal segment culture of female by Shekhawat *et al.* (2011) observed bud breaking on MS basal agar-gelled medium +2.0 mg/L BA + 0.10 mg/L IAA. The cultures were amplified by passages on MS medium supplemented with 1.0 mg/L BA +0.10 mg/L IAA. Further, shoot amplification (29.2 shoots per vessel) was achieved by subculturing of *in vitro* regenerated shoot clump on MS medium +0.50 mg/L BA + 0.10 mg/L IAA. Rai *et al.* (2012) used two spine gourd genotypes RSR/DR15 (female) and

DR/NKB-28 (male) developed through enhanced axillary proliferation from nodal segments and maximum shoot proliferation of 6.2 shoots per explants with 100% shoot regeneration frequency obtained from the female genotypes on MS media supplemented with 0.9 μ M BA and 200 mg/L casein hydrolysate (CH). While from the male genotype they observed optimum shoot regeneration frequency (86.6%) and 6.4 shoots per explants on MS medium supplemented with 2.2 μ M BA. CH induced vigorous shoots, promoted callus formation and proved inhibitory for shoot differentiation and shoot length, especially in explants from male genotype. Rooting was optimum on half-strength MS medium (male 92.8%, female 74.6%) containing 4.9 μ M IBA. Mustafa *et al.* (2013) cultured the nodal region on MS media supplemented with 2.0 mg/L BA + 2.0 mg/L L-Glutamic acid, the explants produced little amount of callus and shoot buds. The shoot buds on successive subcultures for twice on the same medium produced multiple shoots. Shoot proliferations were further continued even after six months. Debnath *et al.* (2013) established high frequency *in vitro* regeneration of female *Momordica dioica* Roxb. from callus derived from nodal explants and cultured on MS supplemented with 2.0 mg/L 2,4-D and 0.5 mg/L BA/coconut milk (CM) (15% v/v) produced multiple shoot buds when they were cultured on MS containing 4 mg/L BA and CM (15% v/v). Further shoot multiplication (86 ± 3.44) were achieved by subculturing of regenerated shoot clump. For rooting micropropagated shoots were subsequently transferred to half strength MS medium containing 0.50 mg/L IBA with 100% success.

(b) Establishment of *in vitro* culture through leaf explants

It is well established that a precise level of cytokinin would play a critical role in shoot organogenesis in various plants. Report on leaf explants for *in vitro* culture of spine gourd by different researchers is very scanty. Nabi *et al.* (2002^a) used leaf explants for *in vitro* culture of spine gourd. They observed swelling of the leaf in MS supplemented with 2.0 mg/L BA + 0.10 mg/L NAA. A high callus growth were obtained in the MS augmented with 2.0 mg/L BA + 0.20 mg/L NAA and 3.0 mg/L BA + 0.20 mg/L NAA. However, leaf explants had showed no response to shoot initiation. Efficient spine gourd plant regeneration via organogenesis were established by Devendra *et al.* (2009) using leaf derived calli. The maximum morphogenic callus induction rate (80%) observed from leaf explants by culturing in MS with 1.0 mg/L 2,4-D + 2.0 mg/L BA. Callus size and fresh weight increased

substantially through subculturing and highest percentage of shoot regeneration (70%) and highest mean number of shoots (12.33) per culture obtained from 1.5 mg/L BA + 1.50 mg/L Kn. Regenerated shoots were rooted in MS medium supplemented with 1.0 mg/L BA. About 70% of regenerated plantlets survived. Leaf explants has been also used by Karim & Ullah (2011) for *in vitro* culture but doesn't observed any significant result and Swamy *et al.* 2015. Leaf derived callus of *Momordica dioica* Roxb. was tested for regeneration by inoculating on MS medium supplemented with different combinations and concentration of 3.0 mg/L BA + 0.5 mg/L NAA. After 3 weeks of cultures were observed proliferated callus this callus was turned to white friable and green compact callus in combination with high concentration with cytokinin 1 mg/L BA. After sub culture of same callus in 3.0 mg/L BA + 0.5 mg/L NAA concentration were observed that 80% of regenerated plantlet was found. With low concentration of cytokinin 0.5 mg/L BA no callus induction were observed.

3. MATERIALS AND METHODS

Investigations were carried out to study the *In-situ* variability in spine gourd (*Momordica dioica* Roxb.) around nearby villages of Udaipur, Rajasthan. In addition, micro-propagation protocol standardization was conducted in Plant Tissue Culture Laboratory, MBBT, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur during 2013-2015.

The materials used and the methodologies followed are explained in detail here under in two groups:

Variability study

3.1 Experimental site

Spine gourd is underutilized vegetable and it is wildy grown in forest of southern Rajasthan. Accordingly, area around Udaipur (Udaipur, Badgaon, Girwa and Mavli Tehsils) surveyed and 34 *in-situ* genotypes were selected for present investigation. This area is situated between 24.35'to 27.00' North latitude and 73.68' to 74.00' East longitude. It has an average elevation of 598 m above the mean sea level.

3.2 Experimental materials

For *in-situ* study, the material comprised of 34 selected plants identified from nearby forest locations of Udaipur, Rajasthan. Plants were identified from area having high density of spine gourd plants. Plant density was selectively higher in the rocks (Plate 1b) and fencing of cactus (Plate 1a). Each plant was assigned a name where, MPK abbreviation stands for Maharana Pratap Kikoda (Table 1).

For *in-situ* study, five fruits were collected from each selected plant for data recording during July 2013 to September 2014.

Table 3.1 Detail of experimental material.

S.N	LOCATION	NAME	S.N	LOCATION	NAME
1.	KEWADA	MPK-001	18.	BHIMAL	MPK-018
2.	KEWADA	MPK-002	19.	BHIMAL	MPK-019
3.	KEWADA	MPK-003	20.	BHIMAL	MPK-020
4.	KEWADA	MPK-004	21.	GADWADA	MPK-021
5.	KEWADA	MPK-005	22.	LAKADWAS	MPK-022
6.	AAWRA	MPK-006	23.	BAGDADA	MPK-023
7.	AAWRA	MPK-007	24.	BAGDADA	MPK-024
8.	AAWRA	MPK-008	25.	BAGDADA	MPK-025
9.	AAWRA	MPK-009	26.	PAROLA	MPK-026
10.	AAWRA	MPK-010	27.	NAYA KHERA	MPK-027
11.	MANMATHARA	MPK-011	28.	BUJRA	MPK-028
12.	MANMATHARA	MPK-012	29.	NOHRA	MPK-029
13.	MANMATHARA	MPK-013	30.	JHAMARKOTRA	MPK-030
14.	MANMATHARA	MPK-014	31.	JHAMARKOTRA	MPK-031
15.	MANMATHARA	MPK-015	32.	JHAMARKOTRA	MPK-032
16.	BHIMAL	MPK-016	33.	JHAMARKOTRA	MPK-033
17.	BHIMAL	MPK-017	34.	JHAMARKOTRA	MPK-034

3.3 Observations recorded

In *in-situ* study, it was very complicated to select the plant of similar age and environment, therefore observations were recorded for such characters that were relatively less affected by these factors and are easily assessable and available at the time of data recording. For each collection five fruits were harvested at horticultural maturity stage in two consecutive years *i.e.*, 2013 & 2014. The procedures of recording observations were as follows:

1. **Vine length:** Length of the main vine was measured in meter for the selected plant from base to tip of the main vine of the plant with the help of measuring tap.
2. **Fruit length:** Fruit length was recorded for five randomly selected green fruits from each selected plant at horticultural maturity stage. It was measured in millimeter from base to tip of the fruit with the help of Vernier Calipers.
3. **Fruit diameter:** Maximum fruit diameter was recorded in millimeter by Vernier Calipers.
4. **Fruit weight:** The fruits used for measuring fruit length and diameters were weighed on digital balance and average fruit weight was worked out and expressed in gram.
5. **Pedicle length:** Pedicle length of above selected fruits were measured in millimeter from base to tip attached to the fruit with the help of measuring scale and average was taken
6. **Fruit tip length:** For above fruits, fruit tip length was measured in millimeter and average was taken.
7. **Spine length:** From each selected fruits spines were selected for measuring spine length. The length was measured in millimeter and average was taken for each genotypes.
8. **Fruit volume:** Volume of above five fruits was measured by water displacement method. For this purpose, the fruits were dipped in full filled jar of water and the water displaced by the fruit was collected and measured in cc by graduated glass jar and average volume of fruit was calculated and expressed in cc.

9. **Fruit specific gravity:** The specific gravity of these fruits was worked out by dividing the weight of fruit by volume of same fruits and was expressed as gram per cubic centimeter (g/cc).
10. **Number of seeds per fruit:** Seeds were extracted from above selected fruits and counted. Finally average number of seeds per fruit was worked out.
11. **TSS (Total soluble solids):** Juice was extracted from each fruit with the help of pestle and mortar and total soluble solids of the juice was determined by using “Hand Refractometer” of 0-53 per cent range at room temperature and values obtained were corrected at 20°C (A.O.A.C, 1995).
12. **Moisture (%):** Electronic moisture analyzer (Sartorius, Germany, Model-MA 100) was used to determine moisture content of five selected fruits (A.O.A.C, 2007). The instrument is recommended for quick and reliable determination of the moisture content of materials of liquid, pasty and solid substances based on the method of thermogravimetry. Thermogravimetry is the process of determining the loss of mass that occurs, when a substance is heated. In this process the sample is weighed before and after heating and the difference between two weights was calculated as moisture content. Following procedure was adopted to determine the moisture content of fruit.
 - (a) Instrument was allowed to heat up for one hour.
 - (b) 1-5 g of fruit sample was placed in the instrument.
 - (c) Temperature was adjusted at 105°C for drying the sample.
 - (d) Automatic computation of the moisture per cent was displayed after completion of drying the sample.
13. **Chlorophyll content:** The chlorophyll content of above selected plants leaves was determined at fruiting stage. Leaves were washed with distilled water and cleaned with blotting paper. Leaves were cut in small pieces and dip in 100% acetone (in 50 ml conical flask) for 12 hours and kept in dark. The intensity of absorbance was recorded at 663 and 645 nm.

The chlorophyll content was expressed in terms of mg/g tissue:

$$(a) \text{ Chlorophyll 'a' mg/g tissue} = [12.7(A_{663}) - 2.69(A_{645})] \times \frac{\text{Volume of extract}}{(1000 \times \text{Weight of sample})}$$

$$(b) \text{ Chlorophyll 'b' mg/g tissue} = [22.9(A645) - 4.68(A663)] \times (\text{Volume of extract}) / (1000 \times \text{Weight of sample})$$

$$(c) \text{ Total chlorophyll mg/g tissue} = [20.2(A645) - 8.02(A663)] \times (\text{Volume of extract}) / (1000 \times \text{Weight of sample})$$

- 14. Ascorbic acid (mg/100g):** The ascorbic acid content of fruits was determined by diluting known volume of pulp with 3 percent metaphosphoric acid as buffer and titrating against 2,6-dichlorophenol indophenols dye solution until the stable faint pink colour was obtained (A.O.A.C, 2007). The results were expressed as mg ascorbic acid/100g of fruit pulp.

Standardization

Standardization of 2,6-dichlorophenol indophenols dye was done by titrating it against standard ascorbic acid in 100 ml of 3 per cent metaphosphoric acid. Five ml ascorbic acid solution (aliquot) was used for titration.

The ascorbic acid was expressed in terms of mg/100g of fruit pulp.

$$\frac{0.5 \text{ mg} \times \text{standard reading (ml)} \times 100 \times 100}{\text{Sample reading (ml)} \times 15 \text{ ml} \times \text{weight of sample (g)}}$$

- 15. Protein content (mg/100g edible portion):** Total protein content was determined by Lowry's method (Lowrey *et al.* 1951). To 0.5 ml of aliquot from respective extracts, 3.0 ml of alkaline copper sulphate solution was added with shaking and allowed to react standing at room temperature for 10 minutes. To each tube, 0.5 ml of Folin-Ciocalteu reagent was added and shaken and incubated in dark at room temperature for 30 minutes in order to complete reaction. Absorbance of the blue colour was measured at 620 nm against a blank. A standard curve was prepared using bovine serum albumin (1.0 mg/ml) as standard protein.

3.4 Statistical Analysis

The plot means were subjected to analysis of variance, mean analysis and coefficient of variation analysis Completely Randomized Design (CRD) was employed. The procedures of analysis of these parameters were as follows:

3.4.1 Mean ($\bar{X}$):

$$\bar{X} = \sum_{i=1}^n X_i / n$$

Where,

X_i = Value of i^{th} observation

n = Number of observations

3.4.2 Coefficient of variation:

Coefficient of variation will be calculated as:

$$CV = SD / \bar{X} \times 100$$

Where,

CV = Coefficient of variation $\bar{X}$ = Population mean

3.4.3 Standard deviation (SD):

$$SD = \sqrt{\frac{\sum_{i=1}^n X_i^2 - \frac{(\sum_{i=1}^n X_i)^2}{n}}{n-1}}$$

Experimental Design (CRD)

The Skeleton of ANOVA and procedure of calculation is shown in Table 3.2

Table 3.2 Analysis of variance for CRD

SOURCE	DF	SS	MS	F	EMS
GENOTYPE	G-1	$\sum_{i=0}^g \frac{(\sum_{j=1}^r X_{ij})^2}{r} - \frac{(\sum_{i=1}^g \sum_{j=1}^r X_{ij})^2}{gr}$	MSG		
ERROR	G Z(R-1)	TOTAL SS – GENOTYPE SS	MSE		$\Sigma^2 + R$ $\Sigma^2 G$
TOTAL	GR-1	$\sum_{i=1}^g \sum_{j=1}^r X_{ij}^2 - \frac{(\sum_{i=1}^g \sum_{j=1}^r X_{ij})^2}{gr}$			S^2

Where,

X_{ij} = Value of i^{th} genotype in j^{th} replications
 g = Number of genotypes
 r = Number of observations for i^{th} genotype

Critical differences:

To compare the mean of two genotypes, CD was used

$$\text{CD at 5\%} = \sqrt{\frac{2\text{MSE}}{r}} \times t \text{ at 5\% and error degree of freedom}$$

$$\text{CD at 1\%} = \sqrt{\frac{2\text{MSE}}{r}} \times t \text{ at 1\% and error degree of freedom}$$

3.4.4 Variability parameters

- a) **Genetic variance:** It is the variance contributed by genetic causes or the genetic occurrence of difference among the individuals due to their genetic makeup. It was calculated by using the formula.

$$V_g = \frac{MSV - V_E}{r} = \frac{b' - c'}{r}$$

Where,

V_g = Genotypic variance,
 MSV = mean square for varieties,
 V_E = Error mean square and
 r = Number of replication

- b) **Phenotypic variance:** It is the sum of variance contributed by genetic causes and environmental factors and was computed as:

$$V_p = V_g + V_e = V_g + C'$$

Where,

V_p = Phenotypic variance,
 V_g = Genotypic variance and
 V_e = Error variance.

- c) **Genotypic coefficient of variation (GCV):** The magnitude of genetic variation existing in a character was estimated by the formula given by Burton (1952).

$$GCV = \frac{\sqrt{V_g}}{\bar{X}} \times 100$$

Where,

V_g = Genotypic variance and

$\bar{X}$ = General mean of the character under study.

- d) Phenotypic coefficient of variation (PCV):** The magnitude of phenotypic variation existing in a character was estimated by the formula given by Burton (1952).

$$PCV = \frac{\sqrt{V_p}}{\bar{X}} \times 100$$

Where,

V_p = Phenotypic variance and

$\bar{X}$ = General mean of the character under study.

- e) Heritability:** Heritability in the broad sense was calculated by the formula given by Burton and Devane (1953).

$$h^2 = \frac{V_g}{V_p} \times 100$$

Where,

h^2 = Heritability (Broad sense)

V_g = Genotypic variance and

V_p = Phenotypic variance.

- f) Expected genetic advance:** It was measured by formula proposed by Lush (1949).

$$GA = \frac{V_g}{V_p} \times \sqrt{V_p} \times K$$

$$= \frac{V_g}{\sqrt{V_p}} \times K$$

Where,

GA = Genetic advance,

V_g = Genotypic variance,

V_p = Phenotypic variance and

K = Selection differential (constant) *i.e.*, 2.06 at 5% selection intensity (Allard, 1960).

g) Genetic gain: It was calculated by using the following formula suggested by Johnson *et al.* (1955).

$$\text{Genetic gain} = \frac{GA}{\bar{X}} \times 100$$

Where,

GA = Genetic advance and

$\bar{X}$ = General mean of the character under study.

3.4.5 Association analysis:

Simple correlation coefficient (r) was calculated by using the following formula suggested by Singh and Choudhary (1977).

$$r = \frac{Cov_{xy}}{\sqrt{V_x V_y}}$$

Where, x and y are two characters under consideration.

Genotypic and phenotypic correlation coefficients were calculated using the genotypic and phenotypic variance (V_g and V_p) and covariance (CoV_g and CoV_p) in the formula suggested by Fisher (1954) and Al-Jibouri *et al.* (1958).

3.4.5.1 Genotypic correlation coefficient :

$$r_{(g)} = \frac{Cov_{xy(g)}}{\sqrt{V_{x(g)} V_{y(g)}}}$$

3.4.5.2 Phenotypic correlation coefficient :

$$r_{(p)} = \frac{Cov_{xy(p)}}{\sqrt{V_{x(p)} V_{y(p)}}}$$

Where,

$r_{(g)}$	=	Genotypic correlation coefficient
$r_{(p)}$	=	Phenotypic correlation coefficient
$Cov_{xy(g)}$	=	Genotypic covariance
$Cov_{xy(p)}$	=	Phenotypic covariance

$V_{X(g)}$ = Genotypic variance for character x.

$V_{Y(g)}$ = Genotypic variance for character y.

$V_{X(p)}$ = Phenotypic variance for character x.

$V_{Y(p)}$ = Phenotypic variance for character y.

The significance of correlation was tested using the formula

$$t = \frac{r}{\sqrt{1-r^2}} \times \sqrt{n-2}$$

Where,

r = Correlation coefficient and

n = Total number of observations.

The calculated values of 't' were tested against the table values of 't' with (n-2) d.f. at 5 and 1% levels of significance.

3.6 Regression coefficient:

The degree of dependence of one variate on the other is measured by regression coefficient (b). Thus, regression coefficient of Y on any number of X is the measure of change in Y for a unit change in independent variables (s) (Steel and Torrie, 1980). To describe the dependence of variable Y on several X variables can be depicted as:

$$Y = a + b_1 X_1 + b_2 X_2 + \dots + b_K X_K$$

Where,

Y = the dependent variable

$X_1, \dots, X_K$ = the independent variables viz., fruit length, fruit diameter, pedicel length, fruit specific gravity, TSS and vine length.

a = intercept

$b_1, \dots, b_K$ = partial regression coefficients

Analysis of variance for regression

The variance of dependent variable Y can be partitioned into two parts namely, the variance due to regression on X and the variance due to deviation from the regression on X (Table 3.3).

Table 3.3 Analysis of variance for overall regression.

SOURCE	DF	SS	MS	FCAL
REGRESSION	K	$\sum_{i=1}^n (\bar{Y}_i - \bar{Y})^2$	MS_B	
ERROR	N-K	TOTALSS- REGRESSIONSS	MS_E	
TOTAL	N-1	$\sum_{i=1}^n (Y_i - \bar{Y})^2$	MS_T	

Here,

K	=	number of independent variables
n	=	number of observations (paired)
Y_i	=	i^{th} observation of dependent variable
$\bar{Y}$	=	mean dependent of variable
$\hat{Y}$	=	estimated value of dependent variable

The regression coefficient was further carried out for significant character only.

3.7 Micropropagation:

3.7.1 Plant materials

Present investigation was conducted on *Momordica dioica* Roxb. On the basis of morphological characters well established mother plant growing at Horticulture Farm in RCA were selected for isolation of nodal and leaf explants. Regenerated plants were also used for obtaining *in vitro* nodal and leaf explants.

3.7.2 Chemicals

All chemicals used for present study were of Analytical grade. Prepared MS medium (Hi-Media laboratories Pvt. Ltd., Mumbai) and agar (Hi-Media laboratories Pvt. Ltd., Mumbai) was used and following phytohormones were added in the media. Media used in present study is described in tables (Table 3.4 to 3.6).

Auxins:

- (a) Indole -3- butyric acid (IBA)
- (b) Indole acetic acid (IAA)
- (c) α -naphthalene acetic acid (NAA)

Cytokinins:

- (a) 6-benzyl amino purine (BAP)
- (b) Kinetin (Kin)
- (c) Thidiazuron (TDZ)
- (d) Zeatin

3.7.3 The major equipment used includes

pH meter

pH is the negative logarithm of hydrogen ion concentration. Measurement of pH (Thermoorion) is based on ion exchange in between hydrated layers formed on

glass surface. Change in ion exchange results in voltage different causing current flow. The current intensity gives the value of p^H

Electronic Balance

A manual top loading balance (Contech) used for quick weighing and for analytical purposes. This is a single pan balance capacity 200-300 g, sensitivity 0.1-200 V 50 H₂ AC mains. Precision of ± 0.005 g, weighing range 0.1-200 g, and digital read out was used for making stock solutions of growth regulators and for other fine weighing.

Glasswares

The glassware's used for all the experiments were either of Hitech, Borocil or Corning makes. Media preparation bottles (200 and 400 ml), Microsyrienges, Sigma bottles, Jam bottles, test tubes, Beaker, measuring cylinders (25, 100, 500 and 1000 ml) were used for media preparation and inoculation. The glassware first rinsed with chromic acid followed by lab clean and thorough washing with tap water until the detergent was removed completely. Finally they were rinsed with sterile double distilled water and allowed to oven dry at 100°C for 24 hours.

Table 3.4 List of various plant regeneration media for shoot induction

MEDIUM	PLANT GROWTH REGULATORS (MG/L)				
	TDZ	BA	KIN	ZEA	NAA
SHOOT INDUCTION					
MS	0.5	-	-	-	-
MS	1.0	-	-	-	-
MS	1.5	-	-	-	-
MS	2.0	-	-	-	-
MS	2.5	-	-	-	-
MS	3.0	-	-	-	-

MEDIUM	PLANT GROWTH REGULATORS (MG/L)				
	TDZ	BA	KIN	ZEA	NAA
MS	-	0.5	-	-	-
MS	-	1.0	-	-	-
MS	-	1.5	-	-	-
MS	-	2.0	-	-	-
MS	-	2.5	-	-	-
MS	-	3.0	-	-	-
MS	-	0.5	0.5	-	-
MS	-	1.0	0.5	-	-
MS	-	1.5	0.5	-	-
MS	-	2.0	0.5	-	-
MS	-	2.5	0.5	-	-
MS	-	3.0	0.5	-	-
MS	-	-	-	0.5	-
MS	-	-	-	1.0	-
MS	-	-	-	1.5	-
MS	-	-	-	2.0	-
MS	1.0	0.5	-	-	-
MS	2.0	0.5	-	-	-
MS	3.0	0.5	-	-	-

MEDIUM	PLANT GROWTH REGULATORS (MG/L)				
	TDZ	BA	KIN	ZEA	NAA
MS	1.0	-	-	-	-
MS	2.0	-	-	-	-
MS	3.0	-	-	-	-
MS	-	1.0	0.5	-	-
MS	-	1.5	0.5	-	-
MS	-	2.0	0.5	-	-
MS	-	1.0	-	-	0.5
MS	-	1.5	-	-	0.5
MS	-	2.0	-	-	0.5

Table 3.5 List of various plant regeneration media for shoot multiplication

MEDIUM	PLANT GROWTH REGULATORS (MG/L)	
	BA	KIN
SHOOT MULTIPLICATION		
MS	0.5	-
MS	1.0	-
MS	1.5	-
MS	2.0	-
MS	2.5	-
MS	3.0	-
MS	0.5	0.5
MS	1.0	0.5
MS	1.5	0.5
MS	2.0	0.5
MS	2.5	0.5
MS	3.0	0.5
MS	0.5	1.0
MS	1.0	1.0
MS	1.5	1.0
MS	2.0	1.0

MS	2.5	1.0
MS	3.0	1.0

3.7.4 General procedure of plant tissue culture

3.7.4.1 Preparation of stock solution

For preparation of 1000 ppm stock solution, 100 mg auxin or cytokinin was dissolved in 1N NaOH and thereafter volume was raised up to 100 ml by adding sterile double distilled water. For getting a particular concentration of phytohormone in required volume of medium, the quantity of stock solution of phytohormone required to be added was calculated by the following formula.

$$V_1 = N_2 V_2 / N_1$$

V_1 = Volume of stock solution

V_2 = Required volume of medium

N_1 = Concentration of stock solution *i.e.*, 1000 ppm

N_2 = Required concentration of phytohormone in the medium

Table 3.6 List of various plant regeneration media for root induction

MEDIUM	PLANT GROWTH REGULATORS (MG/L)		
	IAA	IBA	NAA
ROOT INDUCTION			
MS	0.1	-	-
MS	0.2	-	-
MS	0.3	-	-
MS	0.4	-	-
MS	0.5	-	-
MS	0.6	-	-
MS	0.7	-	-
MS	0.8	-	-

MEDIUM	PLANT GROWTH REGULATORS (MG/L)		
	IAA	IBA	NAA
MS	0.9	-	-
MS	1.0	-	-
MS		0.1	-
MS		0.2	-
MS		0.3	-
MS		0.4	-
MS		0.5	-
MS		0.6	-
MS		0.7	-
MS		0.8	-
MS		0.9	-
MS		1.0	-
MS			0.1
MS			0.2
MS			0.3
MS			0.4
MS			0.5
MS			0.6
MS			0.7
MS			0.8
MS			0.9
MS			1.0

3.7.4.2 Media composition and culture medium

All the media composition tried in the present investigation had the MS basal medium (Murashige and Skoog, 1962). MS basal media fortified with different concentrations and combinations of plant growth regulators were used (Table 3.4, 3.5 and 3.6).

For preparation of one liter MS medium, 4.41 g prepared powder of MS medium with agar (from 0.4-0.8 % as per optimum for medium) were dissolved in one litre double distilled water. There after required quantity of fresh stock solution of phytohormone was added to create a desired concentration of phytohormone in the medium. The p^H of medium was adjusted to 5.8 ± 0.2 with digital pH meter using 1N NaOH or 1N HCL solution prior to autoclaving.

3.7.4.3 Storage of stock solution and culture medium

Proper storage is very essential and the initial precautions which need a lot of attention. Stock solutions of growth regulators, antibiotics and other reagents were stored in refrigerator at temperature range of 2-8°C. Autoclaved culture media were kept at low temperature of $9.0 \pm 2^\circ\text{C}$ in the incubator. The medium prepared was not used immediately, rather it was kept for 3-4 days and monitored for any sort of contaminations and then it was used within a week of its preparation.

3.7.4.4 Procedure of aseptic condition

The medium was poured in Sigma bottles or in test tubes. The Sigma bottles or test tubes were autoclaved at 15 psi at 121°C temperature for 15 minutes. After autoclaving these sterilized media bottles or test tubes were cooled and kept at $25 \pm 2^\circ\text{C}$ temperature in growth chamber for gelling of the medium.

Elimination of unwanted microorganisms, contaminants, is a prerequisite in tissue culture studies. All culture vessels, glassware, media and instruments were sterilized properly before use. Highly aseptic conditions were maintained by performing all operations in laminar airflow cabinet. Clean glassware were rinsed with distilled water and sterilized by autoclave at 121°C at 15 psi for 15 min. Scalpels, forceps, petri plates, centrifuge tubes, micro-tips and Whatman filter papers were autoclaved at 121°C at 15 psi for 15 minutes. Before starting any operation, the floor/walls of the laminar airflow were sterilized with 70 per cent ethanol and UV light exposure for 40 minutes.

3.7.4.5 Surface sterilization of explants

Explants were selected from mother plants of spine gourd growing at Horticulture Farm. Nodal and *in vivo* leaf explants were thoroughly washed in sterilized distilled water containing 1-2 drops of Tween-20 and then rinsed five times with sterilized distilled water. The explants were disinfected by treating with 0.4% Bavistin (Carbendazim 50% WP, BASF, India) for 30 minutes, with constant swirling at 110 rpm on shaker. Thereafter, the solution was drained and explants were rinsed properly 4-5 times with sterilized distilled water than explants were disinfected by treating with freshly prepared 0.1% mercuric chloride (HgCl₂) solution for 3-4 minutes (leaf explants) and 7-8 minutes (nodal explants) with constant swirling at 110 rpm on shaker and then rinsed 3-4 times with distilled sterilized water.

3.7.4.6 Culture conditions

After inoculation cultures were kept in culture room having 25±2°C temperature. Cultures were exposed to 16/8 h light / dark period. All the cultures were maintained at 25±1°C under 16 hrs/ 8 hrs light/dark regime provided with photon flux at 30µ mol m⁻² s⁻¹ by cool white fluorescent lamps.

3.8 Plant regeneration

3.8.1 Nature of explants

Two types of explants *i.e.*, surface sterilized *in vivo* nodal and leaf explants and *in vitro* explants were used in present study.

3.8.2 Shoot regeneration

The explants were implanted on medium with the help of alcohol (95 per cent) sterilized hands and surgical forceps in the laminar airflow bench. Surface sterilized *in vivo* nodal explants (2-3 cm) were placed on various medium for shoot induction. Surface sterilized *in vivo* leaves were cut into approximately 0.5-1.0 cm² size and inoculated in different concentration of media. For *in vitro* explants (nodal and leaf) *in vitro* regenerated plants used to isolate nodal segments (2-3 cm) and leaf segments (0.5- 1.0 cm²) for culture. As soon as bud induction was started and data was observed after 1 weeks on shoot induction media.

$$\% \text{ Shoot induction} = \frac{\text{No. of explants showing shoot induction}}{\text{Total no. of explants cultured}} \times 100$$

Ultimately, the regenerated shoots were transferred to the shoot multiplication

medium to allow maximum proliferation. To avoid necrosis in growing tissues, subcultures were done in about 3 weeks intervals. After 3 weeks, data were recorded for number of shoots per explants and mean length of regenerated plants.

3.8.3 Root regeneration and hardening

The regenerated shoots were transferred to the rooting media in test tubes (one shoot in each bottle). After a week of root initiation, the plants were maintained until they were reached 2.0-3.0 cm height.

Rooted plantlets were washed in running tap water to remove solid media and transferred to plastic pots containing a mixture of sterilized hardening media (various ratio of cocopeat, vermiculite, vermicompost and sand) kept in culture room for at least 2 week. The plants were covered by polythene bags with optimum humidity.

3.8.4 Observations recorded

All culture bottle/test tubes in each experiment were examined every day carefully. Following observations were recorded for experiments:

- 1. Shoot induction from explants (%):** Shoot induction was counted after 1 week.
- 2. Days to shoot induction:** Days to shoot induction was counted from inoculation day to shoot initiation.
- 3. Number of shoots per explants:** Number of shoots per explants was counted after 3 weeks.
- 4. Shoot length (cm):** Length of longest shoot of each explants was recorded after 3 weeks. It was measured in cm from the base to the tip of the shoot with the help of measuring scale.
- 5. Root induction (%):** Root induction was recorded after 3 weeks.
- 6. Days to root initiation:** Days to root induction was counted from inoculation day to root initiation.
- 7. Number of roots per explants:** Number of roots per explants was counted after 3 weeks.

8. **Root length (cm):** Length of longest root of each explants was recorded after 3 weeks. It was measured in cm from the base to the tip of the shoot with the help of measuring scale.
9. **Survival of regenerated plants (%):** Survival of plants was counted after 3 weeks of hardening.

3.8.5 Statistical analysis for micropropagation

Statistical analysis for the data was performed with JMP software version 8 (SAS, 2009) using Turkey Kramer HSD test (Oneway Anova, summary to fit, analysis of variance followed by comparisons for all pairs) for determining significant differences among treatments at $p \geq 0.05$ level

4. EXPERIMENTAL RESULTS

The present study was undertaken with the objective to study the *in-situ* variability in spine gourd around Udaipur region and to standardize the protocol for micropropagation in *Momordica dioica* Roxb. For *in-situ* study the variability for different characters was assessed with the help of genotypic and phenotypic coefficient of variation, heritability and genetic gain. Apart from this, character association was also studied to workout relationships between different characters. To standardize the micropropagation protocol, regeneration of plantlets from leaf and nodal explants were accomplished successfully using MS basal media supplemented with different growth regulators. The results of the present investigation were divided into two parts:

4.1 *In-situ* studies

For this study, 34 genotypes were randomly selected from different locations around Udaipur (Rajasthan) and observations were recorded for various characters viz., vine length, fruit length, fruit diameter, fruit weight, pedicel length, fruit tip length, spine length, fruit volume, fruit specific gravity, number of seeds per fruit, moisture, TSS, ascorbic acid and protein content. Vine length of spine gourd was not subjected to analysis of variance as it was based on single observation.

The results obtained are presented under following heads:

4.1.1 Analysis of variance and mean value

4.1.2 Variability parameters

4.1.3 Correlation coefficient

4.1.4 Regression analysis for fruit weight, protein and ascorbic acid

Table 4.2 Analysis of variance over the environments for different characters

SN	CHARACTERS	ENVIRONMENT	GENOTYPE	G X E	POOL ERROR
		[1]	[33]	[33]	[272]
1	FRUIT TIP LENGTH (MM)	0.0340	0.0563**	0.0073	0.01472
2	SPINE LENGTH (MM)	0.1699**	0.0057	0.0054	0.005897
3	FRUIT VOLUME (CC)	0.0029	107.6988**	9.2454**	4.406
4	NO. OF SEEDS/FRUIT	163.8118**	254.5825**	6.6118	19.38
5	MOISTURE (%)	27.0325	413.4784**	5.9194	29.76
		[1]	[33]	[33]	[136]
6	CHLOROPHYLL CONTENT (MG/100G)	0.7733*	0.2207*	0.0995	0.1228

*,** significant at 5 per cent and 1 per cent level of significance.

4.1.1 Analysis of variance and mean value

The mean squares due to genotypes were significant for all the traits in both the years except for spine length in both the years, fruit specific gravity in first year and chlorophyll content in second year (Table 4.1). The Bartlett's test showed homogeneity of the error variance for six characters only viz., fruit tip length, spine length, fruit volume, number of seeds, moisture and chlorophyll content. All these characters had significant differences between genotypes over the years except for spine length. Between years difference was significant for spine length, number of seeds per fruit and chlorophyll content whereas G×E was significant for fruit volume only. The mean values and genetic parameters are discussed accordingly:

4.1.1.1 Vine length (m): Vine length was non replicated in both the years. Therefore, only mean value and CV was calculated (Appendix-I). The pool mean value for vine length was 5.13. The genotype MPK-009 showed longest pool vine length of 11.3 m followed by genotype MPK-006 *i.e.*, 9.2 m. The pool coefficient of variation for vine length was 35.73.

4.1.1.2 Fruit length (mm): The error variances of both the environments were not homogenous, therefore pool analysis was not performed. The mean square due to genotypes was significant in both the years. The mean fruit length in first year was 37.66 mm and in second year was 36.78 mm (Appendix-II). Fruit length ranged from 26.00 mm to 53.20 mm in the first year and 28.60 mm to 51.81 mm in the second year. Highest value was recorded for MPK-009 (53.20 mm) and MPK-006 (51.81 mm) in the first and second year, respectively. The genotypes at par to MPK-009 in first year were MPK-006 (52.40 mm), MPK-010 (46.00 mm), MPK-022 (45.70 mm), MPK-027 (44.09 mm) and MPK-016 (44.00 mm). The genotypes at par to the best genotype MPK-006 in the second year were MPK-009 (46.31 mm), MPK-016 (45.84 mm), MPK-010 (45.48 mm), MPK-022 (44.24 mm), MPK-011 (42.60 mm) and MPK-003 (41.33 mm).

4.1.1.3 Fruit diameter (mm): The error variances of both the environments were not homogenous. The mean square due to genotypes was significant in both the years. The value of fruit diameter ranged between 17.80 mm (MPK-030) to 31.20 mm (MPK-009) in the first year and between 18.00 mm (MPK-030) to 30.24 mm (MPK-003) in the second year (Appendix-II). In both the years genotype MPK-030 showed lowest fruit diameter. The genotypes at par to the higher value genotype *i.e.*, MPK-009 were MPK-006 (30.00 mm), MPK-010 (27.60 mm), MPK-022 (27.51), MPK-017 (26.80 mm), MPK-008 (26.40 mm) and MPK-026 (26.20 mm) in first year and MPK-009 (29.87 mm), MPK-006 & MPK-010 (both having value of 27.78 mm), MPK-001 & MPK-026 (both having fruit diameter of 26.00 mm) and MPK-020 (25.01 mm) in the second year.

4.1.1.4 Fruit weight (g): The error variances in first and second year were not homogenous. The mean fruit weight in the first year was 8.88 g and in the second year 8.63 g (Appendix-III). Fruit weight was ranged between 4.46 g (MPK-033) to 19.34 g (MPK-009) in the first year and 5.05 g (MPK-21) to 22.92 g (MPK-006) in the second year. The MPK-009 was followed by MPK-006 (19.20 g), MPK-017 (14.38 g), MPK-

022 (14.36 g) and MPK-019 (12.03 g). The second year best genotype was followed by MPK-009 (15.87 g), MPK-003 (13.21 g), MPK-022 (12.63 g), MPK-016 (12.00 g) and MPK-010 (11.95 g).

4.1.1.5 Pedicel length (mm): The error variances in first year and second year were heterogeneous and mean square due to genotypes was significant. The maximum pedicel length was recorded for MPK-005 (Y1= 37.00 mm and Y2= 31.40 mm) in both the years and MPK-013 also exhibit maximum pedicel length *i.e.*, 31.40 mm in the second year (Appendix-III). The best genotype was closely followed by MPK-014 (35.80 mm), MPK-026 (28.80 mm), MPK-025 (28.60 mm), MPK-012 (28.40 mm) and MPK-013 (27.00 mm) in the first year and MPK-012 (30.00 mm), MPK-015 (26.20 mm), MPK-028 (26.00 mm) and MPK-025 (24.00 mm) in the second year, respectively. The minimum pedicel length was recorded for MPK-003 (9.20 mm) in first year and MPK-006 (10.20 mm) in second year.

4.1.1.6 Fruit tip length (mm): The error variances of both the environments were homogenous (Bartlett 0.23). Therefore, pool analysis of variances was performed and difference between genotypes over the years was significant. Over the years the highest fruit tip length were observed in MPK-025 (5.20 mm) followed by MPK-030 (5.10 mm), MPK-001, MPK-021 and MPK-032 (all having value of 4.90 mm) respectively (Appendix-IV). The minimum fruit tip length was recorded in MPK-006 (2.20 mm)

4.1.1.7 Spine length (mm): There was no difference between genotypes in both the years. The error variances in first and second year were homogenous. Over the years spine length was ranged from 1.60 mm (MPK-019 & MPK-032) to 2.50 mm (MPK-009) with a mean spine length of 1.98 mm (Appendix-IV). Genotypes exhibiting at par values to MPK-009 were MPK-012, MPK-013, MPK-024 (all 2.40 mm), MPK-010, MPK-015 (both 2.30 mm), MPK-011, MPK-034 (both 2.20 mm) and MPK-007, MPK-020 (both 2.10 mm).

4.1.1.8 Fruit volume (cc): The error variances of both the environments were homogenous and genotypic and G×E mean squares were significant. In both the years difference between genotypes were significant. Over the years the highest fruit volume were observed in MPK-006 (20.90 cc) followed by MPK-009 (16.80 cc), MPK-019 (15.70 cc), MPK-022 (15.60 cc), MPK-010 (14.00 cc), MPK-017 (12.17

cc), MPK-20 (12.40 cc) and MPK-018 (12.00 cc) (Appendix-V). The minimum fruit volume was recorded in MPK-033 (5.50 cc).

4.1.1.9 Fruit specific gravity (g/cc): The difference between genotypes was significant only in second year. The error variances of both the environments were not homogenous, therefore means of each year were concluded separately. The fruit specific gravity ranged from 0.60 g/cc (MPK-024) to 1.91 g/cc (MPK-005) and 0.66 g/cc (MPK-005) to 1.05 g/cc (MPK-006) in the first and second year, respectively (Appendix-V). The highest fruit specific gravity was recorded 1.91 g/cc (MPK-005) and 1.05 g/cc (MPK-006) in first and second year, respectively.

4.1.1.10 Number of seeds per fruit: The error variances of both the environments were homogenous. The mean square due to genotypes and years were significant. Over the years the range for number of seeds per fruit was from 10.40 (MPK-001) to 30.70 (MPK-010) with a mean number of seeds per fruit *i.e.*, 19.86 (Appendix-VI). The genotypes at par to the best genotype were MPK-022 (29.90), MPK-009 (28.80), MPK-006 (27.30), MPK-018 (25.50), MPK-013 (25.30), MPK-002 (24.30), MPK-020 (23.90) and MPK-031 (23.40).

4.1.1.11 Moisture (%): The error variances in first and second year were homogenous and difference between genotypes over the years and in individual year was significant. Over the years the range for moisture percentage was 57.4 per cent (MPK-032) to 81.67 per cent (MPK-034) with a mean moisture percent of 70.16 per cent (Appendix-VI). The genotypes at par to the best performer were MPK-011 (80.72 per cent), MPK-023 (80.24 per cent), MPK-027 (79.06 per cent), MPK-025 (77.59 per cent), MPK-001 (75.67), MPK-024 (74.80 per cent) and MPK-006 (73.89).

4.1.1.12 TSS (%): There was significant difference between error variance of first year and second year therefore means of each year are discussed separately. The range of TSS was ranged from 3.54% (MPK-006) to 8.98% (MPK-001) in first year and from 3.82% (MPK-006) to 8.62 (MPK-009) in second year (Appendix-VII). The genotype MPK-001 was followed by MPK-009 (8.68%), MPK-029 (8.64%), MPK-007, MPK-011, MPK-015, MPK-025 (all 8.60%) and MPK-025 (8.40%) in first year. In case of second year the genotype MPK-009 was closely followed by MPK-011, MPK-015 (both 8.38%), MPK-001 (8.34%), MPK-029 (8.30%) and MPK-007

(8.20%). The genotype MPK-006 showed lowest TSS in both the years (Y1= 3.54% and Y2= 3.82%).

4.1.1.13 Ascorbic acid (mg/100g): The range of ascorbic acid was 132 mg/100g (MPK-001) to 378.40 mg/100g (MPK-006 and MPK-013, respectively) in first year and from 132 mg/100g (MPK-022, MPK-025 and MPK-030, respectively) to 404.80 mg/100g (MPK-009) in second year (Appendix-VII). The genotype at par to the best performer were MPK-009, MPK-034 (both 352.00 mg/100g), MPK-016, MPK-027 (both 334.40 mg/100g), MPK-003 and MPK-015 (both 308.00 mg/100gm) and MPK-002 (264.00 mg/g). In case of second year the genotype MPK-009 was closely followed by MPK-006 (396.00 mg/100g), MPK-013 (378.40 mg/100g), MPK-003 (352.00 mg/100g) and MPK-034 (343.20 mg/100g).

4.1.1.14 Protein (g/100g): The error variance was not homogenous in both the years therefore means of each year are considered separately. The mean protein content in first year was 2.28 g/100 g and in second year was 2.42 g/100 gm (Appendix-VIII). Protein content ranged from 1.02 g/100g to 5.68 g/100g in the first year and 1.32 g/100g to 4.91 g/100g in the second year. Highest value was recorded for MPK-015 (5.68 g/100g) and MPK-009 (4.91 g/100g) in the first year and second year, respectively. The genotypes at par to MPK-015 in the first year were MPK-004 (4.42 g/100g), MPK-003 (4.26 g/100g) and MPK-001 (4.14 g/100g). The genotypes at par to MPK-009 in the second year were MPK-015 (4.57 g/100g), MPK-006 (4.02 g/100g), MPK-001 (3.98 g/100g) and MPK-002 (3.44 g/100g).

4.1.1.15 Chlorophyll (mg/100g): The error variances were homogenous. The mean square due to genotypes was significant in first year only. The mean chlorophyll content in first year was 0.95 mg/100g and in second year was 1.07 mg/100g. Over the years chlorophyll content was ranged from 0.59 mg/100g (MPK-031) to 1.52 mg/100 g (MPK-019) with a mean chlorophyll content of 1.01 mg/100g (Appendix-VIII). Genotypes exhibiting at par values to MPK-019 were MPK-018(1.37 mg/100g), MPK-020 (1.30 mg/100g), MPK-016 (1.27 mg/100g), MPK-014 (1.22 mg/100g), MPK-008 (1.16 mg/100g) and MPK-009 (0.15 mg/100g).

4.1.2 VARIABILITY PARAMETERS

The variability can be measured by range but it indicates only extremes therefore, it is not a true measure of variability. This can be measured with the help of

variance. The variation among genotypes may occur due to genotypic and environmental influence. In systematic study where same set of genotypes is repeated in different environments, the environmental effect can be separated upto certain extent but in case of *in-situ* study it is not applicable. However variability among plants at different locations is environmental or genotypic in nature. But on account of non-availability of any simple method of variability measurements for such situations, the variability parameters viz., GCV, PCV and genetic gain as suggested by Burton (1956) and Johnson *et al.* (1995) were used. Variability parameters computed for different characters are presented in Table 4.3.

4.1.2.1 Genotypic and phenotypic coefficient of variation

Table 4.3 revealed that for all the traits in both the years, phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV). Further the value of GCV and PCV change simultaneously in all the characters and in both the years.

Among all the traits magnitude of GCV and PCV was high for protein content (54.32% & 33.83% and 65.29 & 53.07%), fruit weight (39.95% & 41.65% and 49.01% & 47.91%), fruit volume (32.24% & 31.70% and 30.02% & 37.17%), ascorbic acid (29.19% & 35.87% and 43.15% & 43.66%) and TSS (27.27% & 22.55% and 31.14% & 24.52%) respectively in first and second year. The magnitude of GCV and PCV was moderate for pedicel length (26.61% & 21.57% & 35.54% & 43.05%), number of seeds per fruit (25.74% & 21.71% and 35.24% & 30.22%) and fruit tip length (16.98% & 12.32% and 35.87% & 33.61%) respectively in both the years. It was low for fruit length (17.11% & 14.93% and 21.14% & 17.90%), fruit diameter (13.68% & 12.63% and 18.05% & 16.01%) and moisture content (9.14% & 7.86% and 12.35% & 10.83) respectively in both the years. The magnitude of PCV was moderate for fruit specific gravity (21.92%) in second year. The chlorophyll content showed higher PCV in both the years and low GCV in both the years.

4.1.2.2 Heritability

The high estimates for heritability was recorded for TSS only (84% & 76%) in both the years. The heritability was moderate for fruit length (65.55% & 69.60%),

fruit weight (66.44% & 75.59%), fruit volume (68.28% & 72.70%), fruit diameter (57.46% & 62.24%), moisture per cent (54.72% & 52.75) and number of seeds per fruit (53.34% & 51.64%) in both the years. It was moderate for ascorbic acid (67.52%) in second year and protein content (69.23%) in first year. The heritability estimates were low for pedicel length (28.49% & 36.82%), fruit tip length (22.42% & 13.44%) and chlorophyll (17.07% & 14.45), respectively in first & second year and fruit specific gravity (23.56%) in second year.

4.1.2.3 Genetic gain

The high genetic gain was depicted by protein (93.10%) in first year only, fruit weight (67.08% & 74.61%), fruit volume (54.88% & 55.67%), protein content and ascorbic acid (40.67% & 60.73%) in both the year. Moderate genetic gain was revealed for TSS (49.19 & 42.71%), number of seeds per fruit (38.72 & 32.15%), pedicel length (29.26 & 26.96%), fruit length (28.54 & 25.67%) and fruit diameter (21.37 & 20.53%) in both the years. The numerical values of these parameters were low for fruit tip length, moisture, fruit specific gravity and chlorophyll content.

4.1.3 Correlation analysis

For the identification of suitable plant type, complete information regarding nature and extent of association of various morphological characters would be very useful. The information regarding association among correlated characters and association of correlated characters with other characters would also be helpful in this concern.

In the present investigation correlation was estimated for all the traits in both the years except for vine length and chlorophyll content of leaf. The correlations between all possible combinations between characters were calculated at phenotypic and genotypic level in both the years. In the present investigation since yield data were not available therefore, estimation of direct correlation was not possible thus only mutual correlation between various attributes was worked out, which is presented in (Table 4.4 and 4.5). When yield data are not available, the character like fruit weight may be considered as target character. Perusal of correlation tables indicated that the magnitude of genotypic correlation coefficient was higher for most of the character pairs than their respective values of phenotypic correlation

coefficient, which may be ascribed to the low effect of environment on the character expression.

4.1.3.1 Correlation for fruit weight

In the first year fruit weight indicated positive and highly significant correlation with fruit length ($rg=0.87^{**}$ & $rp=72^{**}$), fruit diameter ($rg=0.95^{**}$ & $rp=79$), fruit volume ($rg=0.96^{**}$ & $rp=0.76^{**}$) and number of seeds per fruit ($rg=0.70^{**}$ & $rp=0.45^{**}$) at both genotypic and phenotypic levels.

In second year fruit weight possessed positive and highly significant correlation with fruit length ($rg=0.85^{**}$ and 0.69^{**}), fruit diameter ($rg=0.78^{**}$ and 0.65^{**}), fruit volume ($rg=0.98^{**}$ & $rp=0.90^{**}$), fruit specific gravity ($rg=0.73^{**}$ & $rp=0.53^{**}$), number of seeds per fruit ($rg=0.52^{**}$ & $rp=0.42^{**}$) and ascorbic acid ($rg=0.59^{**}$ & $rp=0.45^{**}$) at both levels.

Fruit weight showed negative association with fruit tip length (Y1 $rg=-0.57^{**}$ & Y1 $rp=-0.22$ and Y2 $rg=-0.44^{**}$ & Y2 $rp=-0.17$), TSS (Y1 $rg=-0.21$ & Y1 $rp=-0.17$ and Y2 $rg=-0.16$ & Y2 $rp=-0.09$) in both the years and pedicel length (Y1 $rp=-0.15$) at genotypic level only in first year and (Y2 $rg=-0.40^{*}$ & $rp=-0.16$) at both levels in the second year.

Correlation among 6 characters which were positively correlated with fruit weight revealed that fruit length, fruit diameter, fruit volume and number of seeds per fruit showed mutual correlation in both the years except for number of seeds per fruit with fruit specific gravity in second the year. Other than the above correlations fruit length is positively correlated with number of seeds per fruit (Y1 $rg=0.58^{**}$) at genotypic level only in first year, (Y2 $rg=0.54^{**}$ & Y2 $rp=0.35^{**}$) and ascorbic acid (Y2 $rg=0.53^{**}$ & Y2 $rp=0.36^{**}$) respectively at both levels in the second year; fruit diameter with ascorbic acid (Y2 $rg=0.56^{**}$ & $rp=0.39^{**}$) at both levels in second year and protein content (Y2 $rg=0.55^{**}$) at genotypic level only in second year.

Fruit tip length which showed negative correlation with fruit weight also exhibited negative correlation with positively correlated characters with fruit weight viz., fruit volume (Y1 $rg=-0.46^{*}$ & Y1 $rp=-0.20$ and Y2 $rg=-0.47^{**}$ & Y2 $rp=-0.17$), number of seeds per fruit (Y1 $rg=-0.36^{*}$ & Y1 $rp=-0.15$ and Y2 $rg=-0.41^{*}$ and Y2 $rp=-0.14$), ascorbic acid (Y1 $rp=-0.10$ and Y2 $rg=-0.63^{**}$ & Y2 $rp=-0.14$) and protein (Y1 $rg=-0.14$ & Y1 $rp=-0.11$ and Y2 $rg=-0.51^{**}$ & Y2 $rp=-0.20$) respectively, in both

first and second year except with fruit specific gravity (Y2 r_p =-0.05 at phenotypic level only) in second year only. TSS which showed negative correlation with fruit weight also possessed negative correlation with positively correlated characters with fruit weight viz., fruit length (Y1 r_g =-0.08 & Y1 r_p =-0.06 and Y2 r_g =-0.11 & Y2 r_p =-0.08), fruit diameter (Y1 r_g =-0.10 & Y1 r_p =-0.03 and Y2 r_p =-0.02 at phenotypic level only), fruit volume (Y1 r_g =-0.20 & Y1 r_p =-0.12 and Y2 r_g =-0.18 & Y2 r_p =-0.14) and number of seeds per fruit (Y1 r_g =-0.32 & Y1 r_p =-0.21 and Y2 r_g =-0.28 & Y2 r_p =-0.18) in both the years except with fruit specific gravity (Y2 r_g =-0.16 & Y2 r_p =-0.08) in second year only.

4.1.3.2 Correlation of correlated characters with other characters

Total 6 characters out of 14 showed correlation with fruit weight. The correlation of these characters with other characters exhibited that fruit length and fruit diameter showed negative association with pedicel length (Y1 r_g =-0.17 & Y1 r_p =-0.00 and Y2 r_g =-0.17 & Y2 r_p =-0.09) and (Y1 r_g =-0.09 & Y1 r_p =-0.01 and Y2 r_g =-0.30 & Y2 r_p =-0.12), respectively at both levels in both the year. Other than above correlations fruit length and fruit diameter also showed positive association with moisture (Y1 r_g =0.27 & Y1 r_p =0.19 and Y2 r_g =0.13 & Y2 r_p =0.08) and (Y1 r_g =0.08 & r_p =0.07 and Y2 r_g =0.18 & Y2 r_p =0.12), respectively at both levels in both the years.

Pedicel length possessed negative association with fruit volume (Y1 r_g =-0.20 & Y1 r_p =-0.09 and Y2 r_g =-0.27 & Y2 r_p =-0.07), number of seeds per fruit (Y1 r_g =-0.33 & Y1 r_p =-0.23 and Y2 r_g =-0.23 & Y2 r_p =-0.05), moisture (Y1 r_g =-0.09 & Y1 r_p =-0.01 and Y2 r_g =-0.09 & Y2 r_p =-0.05) and protein (Y1 r_g =-0.10 & Y1 r_p =-0.04 and Y2 r_g =-0.12 & Y2 r_p =-0.06) respectively at both levels in both the years except for ascorbic acid (Y1 r_g =-0.16 & Y1 r_p =-0.10 at both levels in first year and Y2 r_g =-0.03 at genotypic level only in second year). Pedicel length showed positive association with TSS (Y1 r_g =0.17 & Y1 r_p =0.10 and Y2 r_g =0.16 & r_p =0.10) at both levels in both the years.

Number of seeds indicated high degree of significant and positive correlation with fruit volume (Y1 r_g =0.66** & Y1 r_p =0.44** and Y2 r_g =0.57** & Y2 r_p =0.44**) in both the years.

Ascorbic acid showed significant and positive association with fruit volume (Y2 rg=0.57 ** & Y2 rp=0.42*) at both levels in second year only, fruit specific gravity (Y2 rg=0.50**) at genotypic level only in second year, number of seeds (Y2 rg=0.45**) at genotypic level in second year.

Protein showed significant and positive association with fruit volume (Y2 rg=0.45**), ascorbic acid (Y2 rg=0.64**), fruit diameter (Y2 rg=0.55**) and protein (Y2 rg=0.40*), respectively at genotypic level in second year only.

4.1.4.1 Regression analysis for fruit weight

Table 4.6a Analysis of variance of overall regression analysis for fruit weight during first year

SN	SOURCE	DF	SS	MS	FCAL	R ²
1	REGRESSION	8	419.24	52.40**	34.81	0.918
2	RESIDUAL	25	37.63	1.50		

** Significant at 1 per cent level of significance

To workout the effect of various characters on fruit weight, overall regression analysis was used in both the years. In first year, the mean square due to regression was significant and R² was 0.918 (Table 4.6a). The intercept (Table 4.6b) was -12.71 ± 2.13. Similarly, in second year the mean square due to regression was significant and R² was 0.787 (Table 4.6c). The intercept (Table 4.6d) was -12.36±4.48.

Table 4.6b Partial regression coefficients for fruit weight during first year.

SN	CHARACTERS	COEFFICIENTS	VALUE	SE
1	INTERCEPT	A	-12.71**	2.13

2	FRUIT LENGTH	B1	0.03	0.06
3	FRUIT DIAMETER	B2	0.83**	0.14
4	PEDICEL LENGTH	B3	-0.75	0.39
5	FRUIT SPECIFIC GRAVITY	B4	1.13	1.08
6	TSS	B5	-0.04	0.13
7	ASCORBIC ACID	B6	0.00	0.00
8	PROTEIN	B7	-0.41	0.18
9	VINE LENGTH	B8	0.55**	0.15
R			0.958	1.22
R²			91.8 PER CENT	

**** - Significant at 1 per cent level of significance.**

Table 4.6c Analysis of variance of overall regression analysis for fruit weight during second year

SN	SOURCE	DF	SS	MS	FCAL	R ²
1	REGRESSION	8	357.46	44.68**	11.55	0.787
2	RESIDUAL	25	96.65	3.86		

** - Significant at 1 per cent level of significance.

Table 4.6d Partial regression coefficients for fruit weight during second year

SN	CHARACTERS	COEFFICIENTS	VALUE	SE
1	INTERCEPT	A	-12.36	4.48
2	FRUIT LENGTH	B1	0.31	0.09
3	FRUIT DIAMETER	B2	0.24*	0.18
4	PEDICEL LENGTH	B3	-0.72	0.81
5	FRUIT SPECIFIC GRAVITY	B4	6.92	5.03
6	TSS	B5	-0.32	0.29
7	ASCORBIC ACID	B6	0.00	0.00
8	PROTEIN	B7	0.43	0.53
9	VINE LENGTH	B8	0.06	0.23
R			0.887	1.96
R ²			78.7 PER CENT	

* - Significant at 5 per cent level of significance.

The analysis of variance of overall regression analysis in *in-situ* study revealed significant effect of 8 characters on fruit weight in first and second year, respectively. The R value revealed simple correlation between dependent variable *i.e.*, fruit weight and joint effect of independent variables *viz.*, fruit length, fruit diameter, pedicel length, fruit specific gravity, TSS, ascorbic acid, protein and vine length showed significant effect on the dependent variable *i.e.*, fruit weight. Higher R^2 value indicates that the model is well fitted. The R^2 value revealed that 91.8 and 78.7 per cent variation for fruit weight, respectively was governed by the above independent characters *viz.*, fruit length, fruit diameter, pedicel length, fruit specific gravity, TSS, ascorbic acid, protein and vine length. In first year, the regression coefficient was significant positive for fruit diameter (0.83 ± 0.14) and vine length (0.55 ± 0.15). The character fruit diameter and vine length had significant influence on fruit weight whereas the rest of the characters had no influence on dependent variable *i.e.*, fruit weight. The character fruit diameter (0.24 ± 0.18) showed significant positive partial regression coefficient for fruit weight in second year also. The character fruit diameter only had significant influence on fruit weight whereas the rest of the characters had no influence on dependent variable *i.e.*, fruit weight.

4.1.4.2 Regression analysis for ascorbic acid

To workout the effect of various characters on ascorbic acid, overall regression analysis was used in both the years. The analysis of variance of overall regression analysis for ascorbic acid revealed that mean square due to regression was non-significant in first year. The value of R^2 was 0.419 (Table 4.7a) and partial regression coefficient was non-significant for all the independent characters.

Table 4.7a Analysis of variance for overall regression analysis for ascorbic acid during first year

SN	SOURCE	DF	SS	MS	FCAL	R^2
1	REGRESSION	8	75265.32	9408.16	2.25	0.419
2	RESIDUAL	25	104313.48	4172.53		

Table 4.7b Partial regression coefficients for ascorbic acid during first year

SN	CHARACTERS	COEFFICIENTS	VALUE	SE
1	INTERCEPT	A	325.63	162.27

2	FRUIT LENGTH	B1	4.76	3.39
3	FRUIT DIAMETER	B2	-0.16	10.96
4	FRUIT WEIGHT	B3	4.33	10.49
5	PEDICEL LENGTH	B4	-27.23	21.65
6	FRUIT SPECIFIC GRAVITY	B5	66.43	56.78
7	TSS	B6	-8.17	6.77
8	PROTEIN	B7	15.12	10.40
9	VINE LENGTH	B8	15.50	9.29
R			0.647	64.59
R ²			41.9 PER CENT	

The analysis of variance of overall regression analysis in *in-situ* study revealed non-significant effect of 8 characters on ascorbic acid in first year. The R value revealed non-significant correlation between dependent variable *i.e.*, ascorbic acid and joint effect of independent variables *viz.*, fruit length, fruit diameter, fruit weight, pedicel length, fruit specific gravity, TSS, protein and vine length. The lower R value indicates model is not well fitted. The R² value revealed that 41.9 per cent variation for ascorbic acid in first year was governed by the above independent characters *viz.*, fruit length, fruit diameter, fruit weight, pedicel length, fruit specific gravity, TSS, protein and vine length. Above all the independent characters none of them showed significant partial regression coefficient for ascorbic acid in first year. All the independent characters showed no effect on dependant variable *i.e.*, ascorbic acid.

Table 4.7c Analysis of variance of overall regression analysis for ascorbic acid during second year

SN	SOURCE	DF	SS	MS	FCAL	R ²
1	REGRESSION	8	160435.61	20054.45**	6.63	0.680
2	RESIDUAL	25	75619.72	3024.78		

** - Significant at 1 per cent level of significance.

In second year, the analysis of variance of overall regression analysis showed that mean square due to regression was significant. The value of R^2 was 0.680 (Table 4.7c) .The value of intercept was -142.03 ± 140.33 (4.7d) and all the independent characters had non-significant partial regression coefficient for ascorbic acid.

Table 4.7d Partial regression coefficients for ascorbic acid during second year

SN	CHARACTERS	COEFFICIENTS	VALUE	SE
1	INTERCEPT	A	-142.03	140.33
2	FRUIT LENGTH	B1	-0.80	3.17
3	FRUIT DIAMETER	B2	1.04	5.41
4	FRUIT WEIGHT	B3	0.92	5.59
5	PEDICEL LENGTH	B4	32.96	22.08
6	FRUIT SPECIFIC GRAVITY	B5	313.56	132.16
7	TSS	B6	-21.23	7.27
8	PROTEIN	B7	36.40	13.27
9	VINE LENGTH	B8	16.00	5.83
R			0.284	54.99
R^2			68.0 PER CENT	

The analysis of variance of overall regression analysis in *in-situ* study revealed significant effect of 8 characters on ascorbic acid in second year. The R value revealed that significant correlation between dependent variable *i.e.*, ascorbic acid and joint affect of independent variables *viz.*, fruit length, fruit diameter, fruit weight, pedicel length, fruit specific gravity, TSS, protein and vine length. The higher R^2 value indicates model is well fitted. The R^2 value revealed that 68.0 per cent variation for ascorbic acid in second year was governed by the above independent characters *viz.*, fruit length, fruit diameter, fruit weight, pedicel length, fruit specific gravity, TSS, protein and vine length. Above all the independent characters were non-significant partial regression coefficient for ascorbic acid in second year.

4.1.4.3 Regression analysis for Protein

Table 4.8a Analysis of variance of overall regression coefficients for protein during first year

SN	SOURCE	DF	SS	MS	FCAL	R ²
1	REGRESSION	8	19.772	2.47	1.73	0.358
2	RESIDUAL	25	35.532	1.42		

Table 4.8b Partial regression coefficients for protein during first year

SN	CHARACTERS	COEFFICIENTS	VALUE	SE
1	INTERCEPT	A	-6.17	2.98
2	FRUIT LENGTH	B1	-0.07	0.06
3	FRUIT DIAMETER	B2	0.54	0.18
4	FRUIT WEIGHT	B3	-0.39	0.17
5	PEDICEL LENGTH	B4	-0.51	0.39
6	FRUIT SPECIFIC GRAVITY	B5	1.19	1.05
7	TSS	B6	0.06	0.12
8	ASCORBIC ACID	B7	0.00	0.00
9	VINE LENGTH	B8	0.17	0.17
R			0.598	1.19
R ²			35.8 PER CENT	

To find out the effect of various characters on protein, overall regression analysis was worked out in both years. In both the years, the mean square due to

regression was non-significant for all the traits and R^2 was 0.358 (Table 4.8a) and 0.536 (table 4.8c), respectively.

Table 4.8c Analysis of variance of overall regression analysis for protein during second year

SN	SOURCE	DF	SS	MS	FCAL	R^2
1	REGRESSION	8	15.279	1.91	3.61	0.536
2	RESIDUAL	25	13.202	0.52		

Table 4.8d Partial regression coefficients for protein in second year

SN	CHARACTERS	COEFFICIENTS	VALUE	SE
1	INTERCEPT	A	2.35	1.83
2	FRUIT LENGTH	B1	-0.33	0.04
3	FRUIT DIAMETER	B2	0.10	0.69
4	FRUIT WEIGHT	B3	0.05	0.73
5	PEDICEL LENGTH	B4	-0.36	0.29
6	FRUIT SPECIFIC GRAVITY	B5	-4.03	1.75
7	TSS	B6	0.18	0.10
8	ASCORBIC ACID	B7	0.00	0.00
9	VINE LENGTH	B8	0.00	0.08
R			0.732	0.72
R^2			53.6	

The analysis of variance of overall regression analysis in *in-situ* study revealed all 8 independent characters viz., fruit length, fruit diameter, fruit weight, pedicel length, fruit specific gravity, TSS, ascorbic acid and vine length contributed non-significant effect on protein. The R value revealed no correlation between dependent variable i.e., protein and joint effect of independent variables viz., fruit length, fruit diameter, fruit weight, pedicel length, fruit specific gravity, TSS, ascorbic acid and vine length. The R^2 value revealed i.e., only 35.8 and 53.6 per cent towards the

variation for protein content in first and second year, respectively. All the above independent characters were non-significant partial regression coefficient for protein in both first year and second year, respectively. All the above independent characters had no effect on dependent variable *i.e.*, protein in both the years.

4.2 *In vitro* plant regeneration system

Plant regeneration system was established from two types of explants *i.e.*, surface sterilized *in vivo* nodal and leaf explants of field grown mature female plants and *in vitro* nodal and leaf explants were also used in present study.

4.2.1 Optimization of plant growth regulators for shoot induction (*in vivo* nodal explants)

The MS basal media supplemented with various combinations and concentrations of PGRs were studied for plant regeneration from nodal explants of *M. dioica* Roxb. Different sets of media were formulated with different combinations and concentrations of PGRs with the objective to cover all the combinations of PGRs that have been tried in previous reports and included the other potential possible combinations. In the first set of media for *in vivo* nodal explants, MS basal supplemented with TDZ and Zeatin and in the second set, media consisting of BA alone and in combination with Kin and NAA were evaluated.

Table 4.10 Effect of TDZ and Zeatin on shoot induction from *in vivo* nodal explants of *M. dioica* Roxb.

PGR (MG/L)	% SHOOT INDUCTION	MEAN SHOOT LENGTH (CM)
CONTROL	30.0±2.88C	0.35±0.09BCD
TDZ		
0.5	51.6±1.66B	0.50±0.05ABC
1.0	58.3±4.4B	0.58±0.08A
1.5	71.6±1.66A	0.52±0.01AB
2.0	55.0±2.88B	0.49±0.04ABC
2.5	51.6±1.66B	0.30±0.02CD
3.0	50.0±1.66B	0.28±0.02D

ZEATIN		
0.5	20.0±2.88CDE	0.15±0.01DE
1.0	23.3±1.66CD	0.17±0.00DE
1.5	26.6±1.66C	0.15±0.00DE
2.0	13.3±1.66DE	0.03±0.00E
2.5	11.6±1.66DE	0.02±0.00E
3.0	8.3±1.66E	0.02±0.00E

Data were observed after 1 week of culture. Values are the mean of 3(n=3). Mean and SE followed by different letters are significant different at $p \leq 0.05$ Turkey Kramer HSD test.

A single micro shoot emerged from each nodal explant after about 4-5 days on various shoot induction media (Plate 7a-b). Micro shoots of 0.5-1.0 cm long developed from each of these nodal explants within a week. In the first set of media combinations (TDZ and Zeatin), a varied level of shoot induction response was noticed. The regeneration response from explants grown on media containing TDZ was significantly different from those grown on media containing Zeatin. Among the treatments, maximum shoot induction was obtained from 1.5 mg/L TDZ which show maximum 71.6 per cent shoot induction (Table 4.10).

Table 4.11 Effect of BA alone and in combination with Kin and NAA on shoot induction from *in vivo* nodal explants of *M. dioica* Roxb.

PGR (MG/L)	% SHOOT INDUCTION	MEAN SHOOT LENGTH (CM)
CONTROL	30.0±2.88EFG	0.35±0.09BCD
BA		
0.5	45.0±2.88CD	0.28±0.00BCDE
1.0	80.0±2.88A	0.41±0.09BC
1.5	83.3±3.33A	0.45±0.01AB
2.0	63.3±1.66B	0.22±0.01CDEF
2.5	61.6±4.40B	0.21±0.01CDEF
3.0	51.6±1.66BC	0.20±0.02DEF

BA+KIN		
0.5+0.5	5.0±2.88I	0.03±0.018F
1.0+0.5	10.0±2.88HI	0.20±0.04DEF
1.5+0.5	10.0±2.88HI	0.15±0.02DEF
2.0+0.5	28.3±3.33EFG	0.14±0.01EF
2.5+0.5	33.3±1.66DEF	0.13±0.00EF
3.0+0.5	13.3±1.66HI	0.11±0.06EF
BA+NAA		
0.5+0.5	16.6±1.66GHI	0.07±0.00F
1.0+0.5	18.3±1.66GHI	0.06±0.00F
1.5+0.5	20.0±0.00FGH	0.15±0.01DEF
2.0+0.5	38.3±1.66CDE	0.30±0.04BCDE
2.5+0.5	40.0±2.88CDE	0.61±0.02A
3.0+0.5	41.0±1.66CDE	0.30±0.09BCDE

Data were observed after 1 week of culture. Values are the mean of 3(n=3). Mean and SE followed by different letters are significant different at $p \leq 0.05$ Turkey Kramer HSD test.

The highest mean shoot length of 0.58 cm was achieved by 1.0 mg/L TDZ and induced little white watery callus at the cut ends of nodal explants within 2-3 days of culture. Different concentrations of Zeatin were found least effective for shoot induction ranging from 8.3 to 26.6 per cent with mean shoot length ranging from 0.02 cm to 0.17 cm. Whereas, control *i.e.*, without PGRs, it gave 30.0 per cent of shoot induction response with mean shoot length of 0.35 cm which is more effective than other different concentrations of Zeatin. More than 50 per cent shoot induction was recorded on media supplemented with TDZ (0.5-3.0 mg/L) which indicated that TDZ play a crucial role in shoot induction of *M. dioica* Roxb.

In second set of media for nodal explants, MS basal media supplemented with BA alone and in combinations with Kin and NAA were evaluated. After 4-5 days on various shoot induction medium, one small micro shoot was emerged from the nodal explants. A varying degree of shoot induction response was observed. Shoot induction from nodal explants was found significantly different in media with BA alone (Plate

7a-b). Among all the treatments, the highest shoot induction response of 83.3 per cent was noticed at 1.5 mg/L BA (Table 4.11). About 20-40 per cent shoot induction was recorded on media supplemented with different combinations and concentrations of BA and NAA. The highest mean shoot length of 0.61 cm was achieved by 2.5 mg/L BA and in combination with 0.5 mg/L NAA. The different combinations and concentrations of BA and Kin showed very little shoot induction ranging from 5.0-33.3 per cent with lowest mean shoot length of 0.03 cm.

4.2.2 Optimization of plant growth regulators for shoot induction (*in vitro* nodal explants)

The MS basal media supplemented with various combinations and concentrations of PGRs were studied for plant regeneration from *in vitro* nodal explants of *M. dioica* Roxb. Different sets of media were formulated with different combinations and concentrations of PGRs. In first set of media for *in vitro* nodal explants, MS basal media supplemented with TDZ and Zeatin and in the second set MS basal media consisting of BA alone and in combination with Kin and NAA were used for evaluation in the experiment.

After 2-3 days on various shoot induction medium, more than one small micro shoot emerged from each nodal explants. In one week's duration, micro shoots of 0.5-1.5 cm length were developed from nodal explants on various shoot induction media. In the first set of media combinations *i.e.*, TDZ and Zeatin, a varied level of shoot induction response was noticed. Different concentrations of TDZ were found more effective than Zeatin for shoot induction from *in vitro* nodal explants. Shoot regeneration rate of 100 per cent was obtained from the *in vitro* nodal segments from TDZ at (0.5-2.5 mg/L). About 68.3-96.6 per cent shoot induction was also observed from different concentrations of Zeatin treatments (Plate 8). MS basal media free from PGRs *i.e.*, control also gave more than 66.6 per cent shoot induction which indicates that *in vitro* nodal explants have more potential for plant regeneration as compare to *in vivo* nodal explants of *M. dioica* Roxb. (Table 4.12).

Table 4.12 Effect of TDZ and Zeatin on shoot induction from *in vitro* nodal explants of *M. dioica* Roxb.

PGR (MG/L)	% SHOOT INDUCTION	NO. OF SHOOTS/EXPLANT	MEAN SHOOT LENGTH (CM)

CONTROL	66.6±1.66C	0.68±0.01G	0.43±0.01DE
TDZ			
0.5	100.0±0.00A	1.16±0.12E	1.89±0.02A
1.0	100.0±0.00A	1.76±0.03D	1.96±0.02A
1.5	100.0±00.00A	2.63±0.03B	0.97±0.02A
2.0	100.0±0.00A	2.86±0.10AB	0.79±0.03C
2.5	100.0±0.00A	2.93±0.06A	0.54±0.02D
3.0	96.6±3.33A	2.23±0.06C	0.50±0.02D
ZEATIN			
0.5	75.0±2.88BC	0.76±0.01FG	0.38±0.01E
1.0	80.0±0.00B	0.83±0.03FG	0.43±0.01DE
1.5	95.0±2.88A	0.98±0.01EF	0.38±0.02EF
2.0	96.6±1.66A	0.96±0.01EF	0.36±0.02EF
2.5	80.0±2.88B	0.83±0.01FG	0.33±0.00EF
3.0	68.3±1.66C	0.68±0.01G	0.27±0.00F

Data were observed after 1 week of culture. Values are the mean of 3(n=3). Mean and SE followed by different letters are significant different at $p \leq 0.05$ Turkey Kramer HSD test.

The maximum number of shoots/explant *i.e.*, 2.93 were recorded from media containing 2.5 mg/L TDZ, followed by 2.86 from 2.0 mg/L TDZ whereas, minimum number of shoots/explant *i.e.*, 0.68 was recorded from media fortified with 3.0 mg/L Zeatin and also from control (PGRs hormone free). More than one micro shoots were induced from MS basal media supplemented with TDZ alone whereas single shoot was induced from media supplemented with Zeatin alone. The longest mean shoot length of 1.96 cm was recorded from 1.0 mg/L TDZ followed by 1.89 cm from 0.5 mg/L TDZ which is statistically similar. The shortest mean shoot length of 0.27 cm was recorded from 3.0 mg/L Zeatin supplemented MS basal media.

Another shoot induction media consisting of BA alone and in combinations with Kin and NAA were also studied. Among the various media concentrations and

combinations tested, shoot regeneration rate of 100 per cent was obtained from the *in vitro* nodal segments raised on 1.0-2.0 mg/L BA alone, 2.5 mg/L BA and in combination with 0.5 mg/L Kin and 2.5-3.0 mg/L BA in combinations with 0.5 mg/L NAA respectively (Table 4.13).

Table 4.13 Effect of BA alone and in combination with Kin and NAA on shoot induction from *in vitro* nodal explants of *M. dioica* Roxb.

PGRS (MG/L)	% SHOOT INDUCTION	NO. OF SHOOT/EXPLANT	MEAN SHOOT LENGTH (CM)
CONTROL	66.6±1.66E	0.68±0.01D	0.43±0.01FGH
BA			
0.5	96.6±3.33AB	0.98±0.04ABCD	0.82±0.02BCD
1.0	100.0±0.00A	1.03±0.01ABC	0.90±0.00ABCD
1.5	100.0±0.00A	1.13±0.07AA	1.01±0.00AB
2.0	100.0±0.00A	1.08±0.08AB	1.06±0.39A
2.5	90.0±2.88ABCD	0.91±0.01BCD	0.96±0.04ABC
3.0	80.0±4.40D	0.81±0.04DE	0.50±0.01FG
BA+KIN			
0.5+0.5	78.3±1.66DE	0.83±0.01CDE	0.29±0.01H
1.0+0.5	88.3±1.66ABCD	0.88±0.01BCDE	0.40±0.02FGH
1.5+0.5	93.3±4.40ABC	1.00±0.07ABCD	0.46±0.00FGH
2.0+0.5	96.6±1.66AB	1.00±0.07ABCD	0.48±0.02FGH
2.5+0.5	100.0±0.00A	1.00±0.00ABCD	0.60±0.03EF
3.0+0.5	86.6±4.40BCD	0.88±0.04BCDE	0.55±0.11EFG
BA+NAA			
0.5+0.5	66.6±3.33E	0.68±0.01E	0.34±0.00GH
1.0+0.5	81.6±1.66CD	0.81±0.01DE	0.41±0.01FGH
1.5+0.5	88.3±1.66ABCD	0.88±0.01BCDE	0.43±0.02FGH
2.0+0.5	98.3±1.66AB	0.95±0.05ABCD	0.60±0.03EF
2.5+0.5	100.0±0.00A	1.00±0.00ABCD	0.71±0.03DE
3.0+0.5	100.0±0.00A	1.00±0.00ABCD	0.76±0.03CDE

Data were observed after 1 week of culture. Values are the mean of 3(n=3). Mean and SE followed by different letters are significant different at $p \leq 0.05$ Turkey Kramer HSD test.

The maximum number of shoots/explant *i.e.*, 1.13 were recorded from media augmented with 1.5 mg/L BA alone followed by 1.08 from media *i.e.*, 2.0 mg/L TDZ. The longest mean shoot length of 1.06 cm was recorded from 2.0 mg/L sole BA while the shortest mean shoot length of 0.29 cm was recorded from 0.5 mg/L BA + 0.5 mg/L Kin which is significantly different from other. More than 66.6 per cent shoot induction was observed for other tested treatments also including MS basal free from PGRs *i.e.*, control. This experiment reveals that *in vitro* nodal explants have higher regeneration capacity than *in vivo* nodal explants.

4.2.3 Shoot induction from *in vivo* leaf explants

Leaf explants of *M. dioica* Roxb. were collected from field growing female plant. Surface sterilized leaf explants of 0.25-0.5 cm² were cultured on MS basal media supplemented with various concentrations of plant growth regulators alone and in combinations, for shoot regeneration. Callusing was observed and there was no

Table 4.14 Shooting response of *in vivo* leaf explants of *M. dioica* Roxb. on MS basal media supplemented with various combinations and concentrations of BA, NAA, Kin and TDZ

PGRS (MG/L)	CALLUS INDUCTION
CONTROL	+C
BA+NAA	
1.0+0.5	++C
1.5+0.5	++C
2.0+0.5	+++C
BA+KIN	
1.0+0.5	-C
1.5+0.5	-C
2.0+0.5	-C
TDZ+BA	
1.0+0.5	+++C

1.5+0.5	+++C
2.0+0.5	+++C
TDZ	
1.0	+C
1.5	++C
2.0	++C

Data were observed after 3 weeks of culture. -C = No callusing, +C = Low callusing, ++C = Medium callusing and +++C = High callusing.

shoot bud formation in any of the treatments (Table 4.14). Frequency of callus formation was seen highest from TDZ in combinations with BA whereas, callus formation was not seen from media containing 1.0 mg/L BA in combinations with 0.5 mg/L Kin.

4.2.4 Shoot induction from *in vitro* leaf explants

The leaves generated *in vitro* were used as a source of explants. The cut pieces of leaves were excised and culture in various media for shoot regeneration response. The cultures were observed constantly for any response and results were recorded after 4 weeks of culture (Table 4.15). Formation of callus alone was observed and there was no shoot formation in any one of the concentrations and combinations of plant growth regulators. Callus induction was highest for TDZ in combinations with BA whereas, BA in combination with Kin showed zero callus formation.

Table 4.15 Shooting response of *in vitro* leaves of *M. dioica* Roxb. on MS basal media supplemented with various combinations and concentrations of BA, NAA, Kin and TDZ

PGRS (MG/L)	CALLUS INDUCTION
CONTROL	+C
BA+NAA	
1.0+0.5	+C
1.5+0.5	++C
2.0+0.5	++C
BA+KIN	
1.0+0.5	-C

1.5+0.5	+C
2.0+0.5	++C
TDZ+BA	
1.0+0.5	++C
1.5+0.5	+++C
2.0+0.5	+++C
TDZ	
1.0	+C
1.5	++C
2.0	+++C

Data were observed after 3 weeks of culture. -C = No callusing, +C = Low callusing, ++C = Medium callusing and +++C = High callusing.

4.2.5 Effect of cold treatment on shoot regeneration from *in vitro* leaf on TDZ supplemented MS basal media

In earlier experiments, both *in vivo* and *in vitro* leaf explants were incapable of producing any shoot buds. Therefore, in this experiment *in vitro* leaves were cold treated for (0-72 hr) for shoot regeneration on TDZ supplemented MS basal media and data was recorded after 4 weeks of culture. Among the various cold treated *in vitro* leaves, the leaves which were treated for 24 hr was found to induce maximum bud breakage (Plate 9). The highest shoot regeneration response of 46.6 per cent was highest for 24 hr cold treated *in vitro* leaves on media containing 3.0 mg/L TDZ with highest number of shoots/explant *i.e.* 1.6 and with highest shoot length of 0.08 cm followed by 43.3 per cent shoot regeneration on media containing 4.0 mg/L TDZ (Table 4.16). The lowest shoot regeneration response of 5.0 per cent was observed for 72 hr cold treatment on media supplemented with 5.0 mg/L TDZ with minimum number of shoots/explant as well as lowest mean shoot length. Among the various treatments 12 hr cold treated *in vitro* leaves and control (hormone free) did not produce any shoot bud.

4.2.6 Optimization effect of plant growth regulators on shoot multiplication of *M. dioica* Roxb.

After shoot induction of *M. dioica* Roxb. on shoot induction media, the shoots were multiplied and regenerated *in vitro* by repeated transfer of micro shoots. For this, cultures were repeatedly transferred to MS basal medium containing different concentrations of BA and in combinations with Kin.

After 3 weeks of culture number of shoots/explants and mean shoot length was recorded. Significant differences were observed among the treatments on multiple shoot proliferation. Comparison among all media, revealed that, BA and Kin combinations at 0.5 mg/L BA and in combinations with 1.0 mg/L Kin was found to be most suitable for shoot multiplication *i.e.*, 8.5 (Plate 10a to 10c) which is significantly different from other medium followed by medium 1.5 mg/L BA and in combinations with 0.5 mg/L Kin *i.e.*, 8.0 while minimum number of shoots/explant were obtained from concentration of 3.0 mg/L BA *i.e.*, 4.3 which is significantly different and also produce little watery callus at the base of the explant. The longest mean shoot length was achieved by the MS basal medium supplemented with 2.0 and 2.5 mg/L BA *i.e.*, 4.9 cm while, minimum shoot length was found significantly different in the medium with higher concentration of BA 3.0 mg/L and in combinations with 1.0 mg/L Kin *i.e.*, 1.9 cm. The control *i.e.*, PGRs free hormone medium also showed 1.15 number of shoots/explant with mean shoot length of 2.3 cm (Table 4.17). Shoot multiplication experiment concluded that lower concentrations of BA and in combinations with Kin was effective for shoot multiplication of *M. dioica* Roxb.

4.2.7 Optimization effect of plant growth regulators for root induction of *M. dioica* Roxb.

Successful micropropagation depends on healthy and strong root system. *In vitro* multiple shoots were excised and cultured on MS basal medium fortified with various rooting PGRs IAA, IBA and NAA. Response of different concentrations of IAA, IBA and NAA on *in vitro* root formations was observed from the cut portion of the shoot after 10-16 days of inoculation. After 3 weeks of culture on the rooting medium the frequency of rooted shoots, total number of roots and root length for each explants were recorded. Of the three auxins used, IAA was found best for root induction Plate (11a & 11b). The optimal rooting response of 95.0 per cent which is significantly different from others was recorded at 0.7 mg/L IAA (Table 4.18) followed by 91.6 per cent root induction at 0.6 mg/L IAA. Whereas, minimal rooting frequency of 18.3 per cent was noticed at 1.0 mg/L IAA. Maximum number of

roots/explant in present investigation *i.e.*, 7.3 was observed in MS medium augmented with 0.8 mg/L NAA followed by 7.0 roots/explant in the MS medium augmented with 0.7 mg/L NAA, which were found on par with each other and induced basal callus formation. While, lowest number of roots/explant *i.e.*, 0.5 was noticed with increased concentration of IAA at 0.9 and 1.0 mg/L concentration. The longest length of root *i.e.*, 3.3 cm was recorded for MS basal supplemented with 0.7 mg/L IAA followed by 3.0 cm at 0.6 mg/L IAA which were statistically similar. Whereas, shortest length of root *i.e.*, 0.2 cm was recorded at 0.9 and 1.0 mg/L IBA concentration respectively. Rooting frequency was found to increase with an increase in the concentration of IAA upto 0.7 mg/L IAA and then slowly decline. Moderate to profuse rooting was also observed in other treatments.

Table 4.19 Effect of different hardening media on % survival of regenerated plants of *M. dioica*

HARDENING MEDIA	% SURVIVAL OF PLANTS
COCOPEAT+VERMICULITE (1:1)	73.3±1.66A
COCOPEAT+VERMICOMPOST (1:1)	63.3±1.66AB
COCOPEAT (SINGLE)	60.0±2.88B
COCOPEAT+SAND (1:1)	41.6±1.66C
VERMICULITE+VERMICOMPOST (1:1)	30.0±2.88D

Data were observed after 3 weeks of culture. Values are the mean of 3(n=3). Mean and SE followed by different letters are significant different at $p \leq 0.05$ Turkey Kramer HSD test.

4.2.8 Effect of different hardening media on rooted shoots of *M. dioica* Roxb.

Hardening media play an important role for establishment of successful plant material. Therefore, the rooted plants were taken out from the culture vessels, washed thoroughly with sterile water in order to remove the adhered nutrient agar, and potted in bottles containing hardening media. Initially plants were kept in plastic boxes and covered to maintain high humidity and supplied with MS nutrient solution at 3-4 days interval. Different hardening media *i.e.*, cocopeat alone and in combinations with vermiculite, vermicompost, sand and vermicompost in combination with vermiculite in the ratio of 1:1 were tested. Of the different hardening media tested cocopeat in combination with vermiculite achieved highest survival rate of plants 73.3 per cent (Plate 12) which is significantly different from others followed by cocopeat and in combinations with vermicompost which is 63.3 percent whereas, the lowest survival rate of plants was recorded from Vermiculite and in combinations with vermicompost *i.e.*, 30.0 per cent (Table 4.19). More than 50 per cent survival of plants were also recorded for cocopeat without any combinations.

5. DISCUSSION

Momordica dioica Roxb. known as kankoda or kikoda, is a highly nutritious valuable wild edible underutilized vegetable with domestication potential. Commercial cultivation of spine gourd not picking up mainly due to lack of awareness among the people about this potential tropical vegetable. Underutilized vegetables have immense potential for contribution to a particular area of food production because they are well adapted to existing as well as adverse environmental conditions and are generally resistant to pest and pathogens. Vegetables are generally low in energy and dry matter content, but immensely important as a source of protective nutrients, especially vitamins, minerals and phytochemicals. Vegetables are rich source of dietary fibre. The concept of dietary fibre has attracted global attention and nutritional benefits of dietary fibre (protection against certain type of cancer, lowering blood pressure and cholesterol, etc.) are now well accepted. Fruits of spine gourd possess many medicinal properties. Fruits are diuretic, alexiteric stomachic and laxative. The fruit is cooked in a small amount of oil and consumed for treating diabetes (Bawara *et al.* 2010).

In Rajasthan, it is not cultivated but grown wild in between the rocks, field boundaries and available as wild creeper on cactus plant, *Pongamia pinnata*, *Butea monosperma*, etc. This state possesses diverse germplasm of spine gourd which will provide immense scope to popularize the underutilized vegetable through breeding programme.

For the success of any breeding programme variability in a population is prerequisite. The cheaper, easy and early resulting procedure is selection of desirable genotypes directly from naturally grown plants. Therefore, characterization and knowledge of available variability help in formulating the breeding programme for obtaining the improved types. Less attention has been paid towards its utilization, documentation and variability studies in these landraces. Therefore, intensive research is required for improving this crop as there are no prominent varieties reported except Indira Kankoda 1 (RMF-37). In view of this, the present investigation was conducted to assess the variability in spine gourd genotypes found around Udaipur, Rajasthan. The current study was carried out in two phases *i.e.*, *in-situ* variability and micropropagation of this crop as the crop faces much hurdles in propagation through

conventional techniques. In *in-situ* variability was studied among wild growing genotypes around Udaipur and in case of micropripagation, plant tissue culture experiments were conducted in Plant Tissue Culture Laboratory, Department of Molecular Biology and Biotechnology, RCA, MPUAT, Udaipur. The results obtained have been discussed in this chapter under following heads:

5.1 *In-situ* variability

In *in-situ* study, it was very difficult to select the plant of same age and size therefore, observations were recorded only for such characters that were relatively less affected by these factors and are easily assessable and available at the time of data recording.

Analysis of variance revealed that mean square due to genotypes was significant for all the traits in both the years except for spine length in both the years, fruit specific gravity in first year and chlorophyll content in second year. The Bartlett's test showed homogeneity of the error variance for six characters only viz., fruit tip length, spine length, fruit volume, number of seeds, moisture and chlorophyll content. Therefore for these characters results are discussed over the environments and for rest of the characters in individual environment. It exhibited that experimental material possesses considerable variability for all traits.

Perusal of mean values of traits in *in-situ* experiment revealed that genotype MPK-009 was superior for most of the characters viz., for fruit length in first year, fruit diameter in both the years, fruit weight in first year, TSS, ascorbic acid, protein content and chlorophyll respectively in second year only followed by genotype MPK-006 which was superior for the traits viz., fruit length, fruit weight respectively in second year, fruit volume in both the years, ascorbic acid in first year and fruit specific gravity in second year only. Furthermore, MPK-005 was found to be good for pedicel length in both the years and fruit specific gravity in first year. Other genotypes that could be identified for highest number of seeds per fruit were MPK-010 in both first and second year, respectively, MPK-034 for moisture in both the years. Genotype MPK-009 exhibited longest vine length in both first year and second year, respectively followed by MPK-014. These genotypes could be identified as desirable ones in variability studies.

Variability can be measured by range and variance but characters with different unit of measurement cannot be compared. To get comparative values variance is converted in coefficients. Magnitude of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) varied simultaneously which imply the uniform response of different traits to different environmental conditions, where magnitude of PCV was higher than GCV. This is expected because of the fact that phenotypic coefficient of variation includes environmental component also. Comparatively wide differences between PCV and GCV for most of the characters indicate a degree of environmental control for these traits. This is in confirmation with the findings of (Rakhi and Rajamony 2005). They observed wide differences between PCV and GCV for vine length, number of primary and secondary branches and leaf petiole length. The high magnitude of variability in the present study may be due to the fact that the genotypes representing different agro climatic conditions.

Magnitude of GCV and PCV was high for protein content followed by fruit weight, fruit volume, ascorbic acid and TSS in both the years. The magnitude of GCV and PCV was moderate for pedicel length, number of seeds per fruit and fruit tip length respectively in both the years. The magnitude of GCV and PCV was low for fruit length, fruit diameter and moisture in both the years. The chlorophyll content showed higher PCV in both the years and low GCV in both the years. Our findings of highest GCV and PCV for protein content are also in agreement with the findings of Rahman *et al.* (2011). The highest phenotypic coefficient of variation (PCV) for fruit weight, fruit volume and highest genotypic coefficient of variation (GCV) for fruit weight in spine gourd have also been reported by (Bharathi *et al.* 2006 & Naik *et al.* 2012). Kumar *et al.* (2013) also have been reported high GCV and PCV for fruit weight in sponge gourd (*Luffa cylindrica* Roem). Highest GCV for fruit weight have also been reported in other cucurbits viz., *Benincasa hispida* (Parkash 2008), *Lagenaria siceraria* (Husna *et al.* 2011) and *Cucurbita moschata* (Onyishi *et al.* 2013). Low GCV and PCV for fruit length and fruit diameter in the present study are also in agreement with the findings of Golabadi *et al.* (2012) and low phenotypic variance for fruit diameter (Basumatary *et al.* 2014).

In the present investigation traits like protein, fruit weight, fruit volume, ascorbic acid and TSS recorded higher estimates of genetic parameters, thereby indicating that selection can be made efficiently for these traits. Selection of these

traits offers good opportunity for improvement of this crop and probability of getting high performance recombinant segregants will be more.

According to Burton (1952), estimates of genotypic coefficient of variation together with high heritability estimates provide better knowledge regarding the efficiency of selection programme because estimates of GCV and PCV alone do not assess the amount of heritable variation, which can in turn be estimated by heritability. A character possessing high genotypic coefficient of variation with high heritability will be valuable in a selection programme.

The heritability estimate gives an idea about the proportion of observed variability, which is attributed to genetic difference. Results indicated that heritability in *in-situ* study was recorded high for TSS only (84% & 76%) in both first year and second year, respectively. Thus, suggesting that the highly heritable traits were least affected by environmental variation and selection based on phenotypic performance would be reliable. The present results are in contrast with the findings of Rahman *et al.* (2011) in sweet gourd (*Momordica subangulata* ssp. *renigera*). The heritability estimates were moderate for fruit length (65.55% & 69.60%), fruit weight (66.44% & 75.59%), fruit volume (68.28% & 72.70%), fruit diameter (57.46% & 62.24%), moisture per cent (54.72% & 52.75) and number of seeds per fruit (53.34% & 51.64%) in both the years. It was moderate for ascorbic acid (67.52%) in second year and protein content (69.23%) in first year. Rakhi and Rajamony (2005) reported high heritability for fruit length and fruit weight in *Cucumis melo*. Good amount of heritability for fruit weight, fruit volume and fruit length and spine length have also been reported by Barathi *et al.* (2006) and fruit weight in sweet melon (Ibrahim 2012). High heritability for fruit weight and fruit length in bitter gourd was also confirmed by Singh *et al.* (2014). The heritability estimates were low for pedicel length (28.49% & 36.82%), fruit tip length (22.42% & 13.44%) and chlorophyll (17.07% & 14.45%), respectively in first and second year and fruit specific gravity (23.56%) in second year. Heritable variation can be found out with greater accuracy when heritability in conjunction with genetic advance studies. In the present investigation PCV values were higher than GCV for most of the traits suggesting that these traits could not be utilized for selection programme as values highly variable in E1 and E2.

Shift in the mean towards superior side of base population mean due to some selection pressure is termed as genetic advance and when as per cent of mean, it is

referred as genetic gain. Heritability estimates together with genetic advance provides better response during selection than either of the parameters alone (Johnson *et al.* 1955).

In *in-situ* study, high genetic gain was recorded for protein content (93.10%) in first year only, fruit weight (67.08 & 74.61%), fruit volume (54.88% & 55.67%) in both the years respectively, and ascorbic acid (60.73%) in second year. Moderate genetic gain was revealed for TSS (49.19 & 42.71%), number of seeds per fruit (38.72% & 32.15%), pedicel length (29.26 & 26.96%), fruit length (28.54% & 25.67%) and fruit diameter (21.37% & 20.53%). The numerical values of these parameters were low for pedicel length, moisture, fruit specific gravity and chlorophyll content.

To get promising results through selection the estimates of heritability and genetic gain should be considered simultaneously. Among *in-situ* characters fruit weight showed high heritability with high genetic gain in both first and second year, respectively, fruit volume and ascorbic acid showed moderate heritability with moderate genetic gain in both the years. TSS showed high heritability with moderate genetic gain and number of seeds showed moderate heritability with moderate genetic gain. Protein exhibited moderate heritability with high genetic gain in first year. Therefore, these traits can be improved through direct selection (Panse, 1957). The present investigation results are in accordance with the findings of Naik *et al.* (2012). They reported high heritability with moderate genetic gain for TSS in mesocarp and ascorbic acid in mesocarp indicating that these traits were under the additive gene control and simple selection can be used for further improvement in these traits in *Momordica subangulata* blume. Subsp. *renigera*. Moderate heritability with moderate genetic gain for ascorbic acid in *Cucurbita moschata* have been confirmed by Pandey *et al.* (2002). The present findings of high heritability with high genetic advance for fruit weight was also in accordance with the findings of Bharathi *et al.* (2006) in spine gourd, Munshi *et al.* (2007) in *Cucumis sativus* var. *hardwickii* R. (Alef) germplasm and also have been reported in bottle gourd by Husna *et al.* (2011).

The overall discussion on variability parameters from the present investigation revealed that the characters viz., fruit weight, fruit volume, TSS, ascorbic acid and protein possessed substantial to higher genetic variability in the genotypes studied. This variability was due to the moderate to high differences in the genetic makeup of

genotypes and was controlled by additive gene effects. Further these above mentioned parameters also exhibited higher genetic gain and heritability revealed response to selection. Therefore, selection practiced on these parameters will be useful tools for improvement in this crop.

The economically important characters like fruit yield are not absolute characters but depend on number of traits. To identify component characters, correlation is an important tool. In case of *in-situ* study it was not possible to workout direct correlation for fruit yield as it was not available, so only mutual association among characters was worked out. When yield data are not available, the character like fruit weight may be considered as target character.

In the present investigation correlation was estimated for all the traits in both the years except for vine length, spine length, chlorophyll content and fruit specific gravity in first year. The correlations between all possible combinations among the characters were calculated at phenotypic and genotypic level in both the years (Table 4.4 to 4.5). Perusal of correlation tables indicated that the magnitude of genotypic correlation coefficient was higher for most of the character pairs than their respective values of phenotypic correlation coefficient, indicating a strong inherent association of the characters under study with a probable influence of environment on the expression.

The magnitude and direction of genotypic and phenotypic correlation coefficients varied together for all the characters. Positive correlation makes simultaneous improvement in two or more attributes possible whereas, negative association indicates the need to compromise between desirable characters. Fruit weight indicated positive and highly significant correlation with fruit length, fruit diameter, fruit volume and number of seeds per fruit at both genotypic and phenotypic levels in first year, fruit specific gravity and ascorbic acid at both levels in second year. Parkash (2008) also confirmed the positive association of fruit weight with fruit length and fruit diameter in *Benincasa hispida* L. Earlier reports on positive and highly significant association of fruit weight with number of seeds have been confirmed by Hanumegowda *et al.* (2012) in ridge gourd (*Luffa actangula*). Similar results exhibiting highly significant and positive correlation of fruit weight with fruit length and fruit diameter in spine gourd have been reported by (Aliya *et al.* 2014 and Basumatary *et al.* 2014). Panigrahi *et al.* (2015) have also reported the positive

association of fruit weight with fruit volume at both genotypic and phenotypic levels in *Coccinia grandis* (L.) Viogt.

Fruit weight showed negative association with fruit tip length, TSS in both the years and pedicel length at genotypic level only in first year and at both levels in the second year.

Correlation among 6 characters having positive correlation with fruit weight revealed that fruit length, fruit diameter, fruit volume and number of seeds per fruit showed mutual correlation in both the years except for number of seeds per fruit with fruit specific gravity in second year only. The results on mutual correlation between fruit length and fruit weight has also been confirmed by Singh *et al.* (2011) in sponge gourd. Fruit length is positively correlated with number of seeds per fruit at genotypic level only in first year and ascorbic acid respectively at both levels in the second year; fruit diameter with ascorbic acid at both levels in second year and protein content at genotypic level only in second year. The positive relation between fruit length and number of seeds per fruit in the present study is in accordance with (Hanumegowda *et al.* 2012 and Singh *et al.* (2014). Both they have reported positive correlation between fruit length with number of seeds per fruit in *Luffa actangula* (L) Roxb and *Momordica charantia* respectively. These above discussed parameters indicated that they have mutual association with each other. Thus, selection practiced for the improvement in one character will automatically result in the improvement in the other.

Fruit tip length which showed negative correlation with fruit weight also exhibited negative correlation with positively correlated characters with fruit weight viz., fruit volume, number of seeds per fruit, ascorbic acid and protein respectively, in both first and second year except with fruit specific gravity at phenotypic level only in second year. TSS which showed negative correlation with fruit weight also possessed negative correlation with positively correlated characters with fruit weight viz., fruit length, fruit diameter, fruit volume and number of seeds per fruit in both the years except with fruit specific gravity in second year only. The present finding of fruit length showed negative correlation with TSS is also in agreement with the findings of Panigrahi *et al.* (2015) in *Coccinia grandis* (L) voigt.

Pedicle length possessed negative association with fruit volume, number of seeds per fruit, moisture and protein (Y1 r_g = -0.10 & Y1 r_p = -0.04 and Y2 r_g = -0.12 & Y2 r_p = -0.06) respectively at both levels in both the years except for ascorbic acid (Y1 r_g = -0.16 & Y1 r_p = -0.10 at both levels in first year and Y2 r_g = -0.03 at genotypic level only in second year). Pedicle length showed positive association with TSS at both levels in both the years.

Number of seeds indicated significant and positive correlation with fruit volume at both levels in both the years.

Ascorbic acid showed significant and positive association with fruit volume at both levels in second year only, fruit specific gravity at genotypic level only in second year, number of seeds at genotypic level in second year.

Protein showed significant and positive association with fruit volume, ascorbic acid, fruit diameter and protein respectively at genotypic level in second year only. The positive association between protein and ascorbic acid in the present study was in agreement with the findings of Basumatary *et al.* (2014). They also confirmed that ascorbic acid had positive correlation with protein content in spine gourd fruit.

If the genetic correlation is high, the two characters can be regarded as being substantially the same, and if there are no special circumstances affecting the heritability or the intensity of selection, it will make little difference in which environment the selection is carried out (Falconer, 1981).

Based on nature and magnitude of association, the characters like fruit length, fruit diameter, fruit volume, number of seeds and ascorbic acid content which showed positive correlation with fruit weight, these characters can be effectively used for improvement of important trait *i.e.*, fruit weight.

The analysis of variance for partial regression analysis in *in-situ* study revealed significant effect of 8 characters on fruit weight in first and second year, respectively. The R^2 value revealed that 91.8 and 78.7 per cent variability for fruit weight respectively was governed by the above independent characters. In first year, the regression coefficient was significant positive for fruit diameter (0.83) and vine length (0.55). Similarly in second year significant positive effects were noticed for fruit diameter only (0.24).

The analysis of variance for partial regression analysis in *in-situ* study revealed non-significant effect of 8 characters on ascorbic acid in first year whereas, significant effect of 8 characters on ascorbic acid in second year. The R^2 value revealed that 68.0 per cent variability for ascorbic acid in second year was governed by the above independent characters. All the characters were non-significant partial regression coefficient for ascorbic acid in both first year and second year, respectively.

The analysis of variance for partial regression analysis in *in-situ* study revealed all 8 characters contributed non-significant *i.e.*, only 35.8 and 53.6 per cent towards the variability of protein content in first and second year, respectively. All the characters were non-significant partial regression coefficient for protein in both first year and second year, respectively.

5.2 Micropropagation

Spine gourd is a dioecious cucurbitaceous climber. Their fruits contain high amount of proteins, calcium, phosphorus, iron and the highest amount of carotene among the cucurbitaceous vegetable. In addition, this species is valued for several medicinal and curative properties. This useful vegetable has high demand in the market but still remains underutilized and unexploited mainly due to its vegetative mode of propagation and dioecious nature. Conventional commercial propagation of spine gourd mainly depends on tuberous roots (Nabi *et al.* 2002^a), followed by stem cuttings and seeds. Commercial multiplication using tuberous roots and stem cuttings is critically limited due to inadequate availability of tuberous roots and late availability of stem cuttings in fruiting season. Tuberous roots have low multiplication rate (Mondal *et al.* 2006) and occupy valuable cultivable land until next planting season. Difficulties in propagation through seeds include dormancy and unpredictable sex ratio in seedling progenies. As the conventional methods of spine gourd propagation impose several limitations for large scale propagation of sex specific plants, an efficient clonal propagation is a must. An *in vitro* propagation system offers unlimited availability of planting material early in the planting season. The application of micropropagation is well established for large scale propagation of many crops and cucurbitaceous vegetables including *Momordica* species (Ahmed & Anis, 2005; Sultana & Bari Mia, 2003).

5.2.1 Effect of TDZ and Zeatin on bud breakage from *in vivo* nodal explants

Culture initiation of spine gourd from field explants proved rather difficult due to heavy fungal contamination and sensitivity of explants to the reagents such as mercuric chloride solution. Nodal explants collected from *in vivo* grown plants were cultured on MS media supplemented with TDZ and Zeatin. The response varied with the nature of explants used and their type and concentrations of growth regulators. MS media supplemented with 1.5 mg/L TDZ was found more suitable for the bud breaking (71.6 per cent) and single shoots were regenerated from each node on this medium and lowest shoot regeneration of 8.3 per cent was recorded for Zeatin at 3.0 mg/L. The highest shoot length of 0.58 cm was observed for TDZ at 1.0 mg/L whereas, shortest shoot length of 0.02 cm was recorded for Zeatin at 2.5 mg/L and 3.0 mg/L respectively. TDZ was found to be more effective in shoot regeneration compared to Zeatin (Table 4.10) The present investigation is in contrast to the earlier report on *M. dioica* Roxb. by Thiruvengadam *et al.* (2012). The effectiveness of TDZ over other cytokinins have also been reported in other cucurbits such as *Cucurbita pepo* (Pal *et al.* 2007), *Cucumis sativus* (Selveraj *et al.* 2006) and *Momordica charantia* (Thiruvengadam *et al.* 2010). The frequency of shoot regeneration is comparatively low in MS supplemented with different concentrations of Zeatin. No previous reports have been found regarding the effect of TDZ & Zeatin on bud breakage of *in vivo* nodal explants of *M. dioica* Roxb.

5.2.2 Effect of BA alone and in combination with Kin and NAA on bud breakage from *in vivo* nodal explants

The nodal shoot segments proved to be most suitable explants material for regeneration of shoots in *Momordica* species (Agarwal & Kamal, 2004 and Sultana *et al.* 2005). The stems of *M. dioica* Roxb. are soft and delicate, very sensitive to physical handling and surface sterilization, hence extra care must be taken while selecting the explants and surface sterilizing agents. During surface sterilization, the solution of mercuric chloride can enter into vascular tissues which cannot be removed even by repeated washing with autoclaved water. Therefore, after sterilization both the cut end portions of the nodes *i.e.*, proximal and distal portion of 0.5 cm long was removed by cutting the explants inside laminar hood using sterile blade to minimize toxicity caused by mercuric chloride. A range of combinations of different plant growth regulators were tried to obtain shoot induction. The maximum response of bud

break was noticed from 1.5 mg/L BA alone *i.e.*, 83.3 per cent whereas, minimum bud break was noticed from BA 0.5 mg/L and in combinations with 0.5 mg/L Kin *i.e.*, 5.0 per cent (Table 4.11). BA alone was found to be most suitable for bud breakage of single shoot from each node rather than in combinations with Kin and NAA. The present findings are nearly similar with that of the findings of Hoque *et al.* (1995) who achieved shoot regeneration rate of 88.0 per cent from hypocotyls. The earlier reports on BA alone showed highest frequency of shoot formation (89.67 per cent) and maximum number of shoots/explant (5.33) with longest shoots (0.9 cm) from cotyledon explants of *M. dioica* Roxb. at 1.0 mg/L were confirmed by Karim & Ullah (2011). As the concentration of BA increase above 2.0 mg/L, the intensity of shoot induction decreased. This finding is also on par with the findings of Rai *et al.* (2012) for nodal explants. Moderate shoot induction of 20-40 per cent was noticed for media containing different concentrations of BA and in combinations with NAA. The successful combination of BA and NAA have been reported for spine gourd regeneration by Hoque *et al.* (1995) but these promoted callus formation in spine gourd. In the present study the combinations of BA and Kin showed very little shoot regeneration response as compared to BA alone and in combinations with NAA.

5.2.3 Effect of TDZ and Zeatin on bud breakage in *in vitro* nodal explants of *M. dioica* Roxb.

This experiment was carried out with *in vitro* regenerated nodal explants cultured on MS fortified with various concentrations of TDZ and Zeatin. In this experiment, culture initiation of *M. dioica* Roxb. from *in vitro* explants proved easier than culture initiation from field plants as the explants are already well established in aseptic condition. Multiple shoots were obtained from *in vitro* nodal explants cultured on shoot induction medium whereas only single shoot developed on each node of *in vivo* grown nodal explants. Shoot regeneration rate of 100 per cent was obtained from the *in vitro* nodal segments in the present study on MS media augmented with 0.5-2.5 mg/L TDZ and decreased to 96.6 per cent when concentration of TDZ was increased to 3.0 mg/L (Table 4.12). Earlier, the shoot regeneration rate of 100 per cent using nodal segments of *M. dioica* Roxb. was achieved by Rai *et al.* (2012). The maximum number of shoots/explant of 2.93 was recorded from 2.5 mg/L TDZ whereas, the longest mean shoot length of 1.96 cm was observed from media fortified with 1.0 mg/L TDZ. The shoot regeneration rate of 68.3-96.6 per cent was also

recorded from MS media fortified with different concentrations of Zeatin. TDZ was found to be more suitable for shoot induction than Zeatin. The present study revealed that *in vitro* generated nodal explants performed better than *in vivo* nodal explants for shoot induction and the number of days taken for shoot induction was minimum as well. The reason behind this is that the *in vitro* regenerated plantlets are already adapted and established under aseptic culture conditions.

5.2.4 Effect of BA alone and in combination with Kin and NAA on bud breaking from *in vitro* nodal explants of *M. dioica* Roxb.

MS basal media fortified with BA alone and in combination with Kin and NAA were tested for shoot induction from *in vitro* generated nodal explants. In this experiment minimum days were taken for shoot induction as compared to *in vivo* grown explants. More than one micro shoot emerged from a single node after 3-4 days of culture. Shoot regeneration frequency of 100 per cent was recorded from 1.0-2.0 mg/L BA alone, 2.5 BA mg/L in combinations with 0.5 Kin and 2.5-3.0 BA mg/L and in combinations with 0.5 NAA respectively (Table 4.13). Shoot regeneration rate of 88 per cent for spine gourd was achieved by Hoque *et al.* (1995) from hypocotyls. In the present study, the maximum number of shoots/explant *i.e.*, 1.13 was recorded from the media containing BA 1.5 mg/L whereas; longest mean shoot length of 1.06 cm was recorded from BA 2.0 mg/L alone. All the treatments containing different concentrations and combinations of Plant growth regulators were found capable of producing more than 50 per cent shoot regeneration from *in vitro* nodal explants. A higher shoot regeneration potential of *M. dioica* Roxb. was reported from female embryo explants (76.4 per cent, 22.44 shoots/explant) by Hoque *et al.* (2007). Arekar *et al.* (2012) found that MS medium supplemented with BA and combinations of BA and NAA showed shoot as well as callus initiation.

5.2.5 Effect of TDZ alone and BA in combinations with NAA and Kin on shoot induction from *in vivo* leaf explants

In vivo leaf explants do not show any shoot bud formation when cultured on MS basal media fortified with TDZ alone and BA in combinations with NAA and Kin. Leaf explants cultured on hormone free medium *i.e.*, control showed no response. TDZ in combination with BA have induced more callus development in explants (Table 4.14). Both TDZ and BA have been used often to induce shoot

organogenesis in various plant species (Nugent *et al.* 1991, Arene *et al.* 1993 and Isac *et al.* 1994). Swelling of the leaf was reported by Nabi *et al.* (2002^a) in MS media supplemented with 2.0 mg/L BA in combinations with 0.1 mg/L NAA and high callus growth in 2.0 mg/L BA in combinations with 0.2 mg/L NAA. Similarly, MS fortified with BA 2.0 mg/L in combinations with NAA 0.2 mg/L and BA 3.0 mg/L in combinations with NAA 0.2 mg/L also showed swelling of the leaf (Nabi *et al.* 2002^b). Callus induction from leaf explants was also reported by Karim & Ahmed (2010) and Karim & Ullah (2011) in MS media augmented with BA 1.0 mg/L in combinations with 0.5 mg/L NAA. Whereas, there was no callus formation in MS fortified with BA 1.0-3.0 mg/L in combination with 0.5 mg/L Kin. The use of leaf derived calli for shoot regeneration in *M. dioica* Roxb. was also reported by Swamy *et al.* (2015). They obtained high frequency of regeneration on MS media fortified with 2.0 mg/L to 4.0 mg/L BA in combination with 0.5 mg/L NAA.

5.2.6 Effect of TDZ alone and BA in combinations with NAA and Kin on shoot induction from *in vitro* leaf explants

In earlier investigations *in vivo* leaf explants failed to produce shoot buds in all the treatments. Therefore, we used *in vitro* leaf explants for shoot regeneration on MS basal media augmented with TDZ alone and BA in combinations with NAA and Kin. Similarly, in this experiment also we failed to achieve the shoot regeneration from *in vitro* leaf explants (Table 4.15). No earlier reports were found for shoot regeneration from *in vitro* leaf explants in *M. dioica* Roxb. However, efficient plant regeneration via organogenesis for *M. dioica* Roxb. using leaf derived calli was reported by Devendra *et al.* (2009). They concluded that leaf explants were more responsive than nodal explants in terms of callus induction for subsequent regeneration.

5.2.7 Effect of cold treatment on shoot regeneration from *in vitro* leaf on TDZ supplemented MS basal media

From the earlier two experiments, it was evident that leaf explants (*in vivo* and *in vitro*) failed to induce any shoot buds in different treatments provided with different concentrations and combinations of plant growth regulators. Therefore, we tried to see the effect of cold treatment (0-72 hr) on shoot regeneration from *in vitro* leaves on TDZ supplemented MS basal media. In contrast to this experiment no earlier reports were found in *M. dioica* Roxb. The present experiment revealed that

cold treatment on *in vitro* leaves has the capacity to induce shoot bud formation on TDZ supplemented MS basal media. The results showed that from various cold treated *in vitro* leaves, the leaves which were treated for 24 hr showed maximum bud breakage. The highest shoot regeneration response of 46.6 per cent was observed in 24 hr cold treated *in vitro* leaves on media containing 3.0 mg/L TDZ with highest no. of shoots/explant *i.e.*, 1.6 and with highest shoot length of 0.08 cm (Table 4.16). Shoot regeneration from petiole derived callus on TDZ augmented media was also confirmed by Thiruvengadam *et al.* (2012). The lowest shoot regeneration response of 5.0 per cent was recorded from 72 hr cold treated *in vitro* leaves on media fortified with 5.0 mg/L TDZ. No earlier reports have been confirmed for shoot induction from the cold treated *in vitro* leaves of *M. dioica* Roxb.

5.2.8 Effect of different concentration of BA and in combinations with Kin on *in vitro* shoot multiplication of *M. dioica* Roxb.

After excision of the *in vitro* raised shoots when the regenerated micro shoots were cultured on the fresh medium, the shoot number increased significantly. A delay of 5-10 days in subculturing of micro shoots did not pose any problem with regard to the multiplication rate or the quality of shoots obtained. However, small basal callus developed on some explants during repeated subculturing. The increased shoot number, due to repeated transfer of the micro shoots, may be owing to suppression of apical dominance during subculture that induced basal dominant meristematic cells to form new shoots (Shekhawat & Shekhawat 2011). Significant differences were observed among the treatments with regards to multiple shoot proliferation. In the present study, 0.5 mg/L BA and in combinations with 1.0 mg/L Kin was found to be most suitable for shoot multiplication *i.e.*, 8.5 which is significantly different from other medium followed by medium 1.5 mg/L BA in combination with 0.5 mg/L Kin *i.e.*, 8.0 while minimum number of shoots/explant were obtained from a concentration of 3.0 mg/L BA *i.e.*, 4.3 which is significantly different. Further increase in the concentration of BA in combinations with Kin reduced the number of shoots/explant. The longest mean shoot length in the present investigation was achieved by the MS basal medium supplemented with 2.0 and 2.5 mg/L BA *i.e.*, 4.9 cm while minimum shoot length was found significantly different in the medium with higher concentration of BA 3.0 mg/L and in combinations with 1.0 mg/L Kin *i.e.*, 1.9 cm. More than 4-5 shoots/explant were obtained from all other treatments (Table 4.17).

The results of the present investigation *i.e.*, a maximum number of 8.5 shoots/explant is nearly similar with the findings of Hoque *et al.* 1995. They obtained a maximum number of 8.8 shoots/explant when 1.5 mg/L BA and 0.1 mg/L NAA was added to the medium. The highest number of shoots (8.5) obtained in the present study was found close to the lowest number of shoots (8.6) obtained by Nabi *et al.* (2002^a). The variation might be due to the different treatment concentrations, combination and different genotypes used. Karim & Ahmed (2010) obtained 5.1 shoots per nodal explant by somatic embryogenesis. Earlier, the highest number of shoots/explant *i.e.*, 29.2 with a longest mean shoot length of 13.6 was achieved by Shekhawat *et al.* (2011) using nodal explants, 6.2 number of shoots/explant from nodal explants (Rai *et al.* 2012) which is nearly similar to our findings *i.e.*, 8.5 shoots/explant. MS media containing high cytokinin 4.0 mg/L BA along with coconut milk (15 per cent v/v) induced maximum number of adventitious buds and shoots of (22.2) as reported by (Debnath *et al.* 2013) from callus derived nodal explants and maximum number of shoots (*i.e.*, 8.0/embryogenic callus) derived from tendril explants was also reported by Raju *et al.* (2014).

5.2.9 Effect of PGRs on root induction in regenerated shoots of *M. dioica* Roxb.

In vitro grown multiple shoots were excised and culture on MS supplemented media with different concentrations of IAA, IBA and NAA. The results of the present investigation showed significant results on the root induction, number of roots/explant and mean root length. Most of the shoots produced roots within 10-16 days of culture. Among the various media tested, MS media supplemented with 0.7 mg/L IAA was found to be more suitable for the *in vitro* rooting *i.e.*, 95 per cent with longest mean root length of 3.3 cm (Table 4.18). Rooting frequency increased with the increase in the concentration of IAA upto 0.7 mg/L and then rooting frequency slowly decline further with the increase in IAA concentration. The effectiveness of IAA on rooting has been reported by Thiruvengadam *et al.* (2006) in *M. dioica* Roxb. Moderate to profuse rooting (40-91.6 per cent) occurred from all other media with varying average number of roots and root length. The highest rooting frequency of 74.6 per cent from female shoots has also been reported earlier by Rai *et al.* (2012) in half strength MS basal media with 9.9 µM IBA. In the present investigation a maximum number of 7.3 roots/explant was obtained in MS fortified with 0.8 mg/L NAA and induced basal callus formation. These results are nearly similar with the findings of Rai *et al.*

(2012). They obtained 7.4 roots per shoot in half strength MS basal media containing 24.6 μ M IBA, Karim & Ullah (2011) obtained 13 roots per shoot, Shekhawat *et al.* (2011) 6 roots per shoot and Thiruvengadam *et al.* (2012) 12 roots per shoot. Earlier reports on basal callus formation from NAA supplemented rooting media were confirmed by Rai *et al.* (2012). Nabi *et al.* (2002^a) reported 2.8 roots per shoot on MS media supplemented with 0.2 mg/L IBA, which is very less as compared to our findings (7.3 roots/explant). The minimum number of roots/explant *i.e.*, 0.5 was recorded from MS basal media fortified with 0.9 and 1.0 mg/L IAA respectively.

5.2.10 Effect of different hardening media on *in vitro* regenerated plants

Different hardening media *i.e.*, cocopeat, cocopeat in combinations with vermiculite, vermicompost, sand and vermicompost in combination with vermiculite in the ratio of 1:1 were tested. Significant differences were observed among all the treatments on per cent survival of plants. Among the different hardening media, maximum survival per cent of 73.3 was noticed when hardening media with equal proportion of cocopeat and vermiculite was used followed by, 63.3 per cent in hardening media containing equal proportion of cocopeat and vermicompost. The lowest survival per cent of 30.0 per cent was recorded when vermiculite and vermicompost of equal proportion were used (Table 4.19). Minimum yellowing was observed from hardening media containing vermiculite in combination with vermicompost. During the hardening process plants developed an efficient root system, fully expanded leaves and were found to be photosynthetically active. The use of sterilized soil for hardening media has been reported by Nabi *et al.* (2002^a). They obtained a per cent survivability of 83.33 than in normal soil and also confirmed that addition of soil from its native habitat during hardening could increase the survival chances in the field. The potting mixture containing cocopeat and perlite showed 84 per cent survival by (Rai *et al.* 2012) and per cent survival of 68-72 was obtained in *M. dioica* Roxb. from vermiculite which is nearly similar to the present findings *i.e.*, 73.3 per cent as reported by Arekar *et al.* (2012). Water logging during hardening showed negative effect on the growth of the plantlets which have been also confirmed by Shekhawat *et al.* (2011).

6. SUMMARY

The present investigation was carried out to elicit information on variability parameters, association among various traits and to standardized micro propagation protocol in spine gourd. The current study was carried out in two phases *i.e.*, *in-situ* variability and micropropagation of this crop as the crop faces much hurdles in propagation through conventional techniques. In *in-situ* study, variability was studied among wild growing genotypes around Udaipur and in case of micropropagation, plant tissue culture experiment were conducted at Plant Tissue Culture Laboratory, Department of Molecular Biology and Biotechnology, RCA, MPUAT, Udaipur.

Observation were recorded on fifteen traits in *in-situ* study *viz.*, fruit length, fruit diameter, fruit weight, pedicel length, fruit tip length, spine length, fruit volume, fruit specific gravity, number of seeds per fruit, moisture, TSS, ascorbic acid, protein, chlorophyll and vine length (non-replicated). Observations were recorded for two consecutive years on each genotype and five collected fruits from each genotype were used as replication. The observations were analysed using Completely Randomized Design (CRD). The important findings of the studies were as follows.

1. Analysis of variance for *in-situ* study revealed that mean squares due to genotypes were significant for all the traits except spine length in both the years, fruit specific gravity in the first year and chlorophyll content in second year thereby, showing considerable amount of variability for most of the characters and were amendable to improvement.
2. The Bartlett's test showed homogeneity of the error variance for six characters only *viz.*, fruit tip length, spine length, fruit volume, number of seeds, moisture and chlorophyll content. All these characters had significant differences between genotypes over the years except for spine length. Between years difference was significant for spine length number of seeds per fruit and chlorophyll content whereas G×E was significant for fruit volume only.
3. Perusal of mean values of traits in *in-situ* experiment revealed that genotype MPK-009 was superior for most of the characters *viz.*, for fruit length in first year, fruit diameter in both the years, fruit weight in first year, TSS, ascorbic acid, protein content and chlorophyll respectively in second year only followed by genotype MPK-006 which was superior for the traits *viz.*, fruit

length, fruit weight respectively in second year, fruit volume in both the years, ascorbic acid in first year and fruit specific gravity in second year only. Furthermore, MPK-005 was found to be good for pedicel length in both the years and fruit specific gravity in first year. Other genotypes that could be identified for highest number of seeds per fruit were MPK-010 in both first and second year, respectively, MPK-034 for moisture in both the years. Genotype MPK-009 exhibited longest vine length in both first year and second year, respectively followed by MPK-014. These genotypes could be identified as desirable ones in variability studies.

4. The magnitude of GCV and PCV changed simultaneously in all the characters. Among all the traits magnitude of GCV and PCV was high for protein content, fruit weight, fruit volume, ascorbic acid and TSS. The magnitude of GCV and PCV was moderate for pedicel length, number of seeds per fruit and fruit tip length. It was low for fruit length, fruit diameter and moisture content.
5. High heritability was recorded for TSS only. The heritability estimates were moderate for fruit length, fruit weight, fruit volume, fruit diameter, moisture per cent and number of seeds per fruit. It was moderate for ascorbic acid in second year and protein content in first year. The heritability estimates were low for pedicel length, fruit tip length and fruit specific gravity respectively.
6. The high genetic gain was depicted by fruit weight, fruit volume, protein content and ascorbic acid. Moderate genetic gain was revealed for TSS, number of seeds per fruit, pedicel length, fruit length and fruit diameter in both the years. The numerical values of these parameters were low for fruit tip length, moisture, fruit specific gravity and chlorophyll content.
7. When yield data are not available, the character like fruit weight may be considered as target character. In the present investigation it was positively and highly significant correlation with fruit length, fruit diameter, fruit volume and number of seeds per fruit at both levels in first year, fruit specific gravity and ascorbic acid at both levels in second year. Correlation among 6 characters having positive correlation with fruit weight revealed that fruit length, fruit diameter, fruit volume and number of seeds per fruit showed mutual correlation in both the years except for number of seeds per fruit with

fruit specific gravity in second year. Among quality parameters, ascorbic acid showed significant and positive association with fruit volume at both levels in second year only, fruit specific gravity at genotypic level only in second year, number of seeds at genotypic level in second year. Protein showed significant and positive association with fruit volume, ascorbic acid, fruit diameter and protein respectively at genotypic level in second year only.

8. The analysis of variance for partial regression analysis in *in-situ* study revealed significant effect of 8 characters on fruit weight in first and second year, respectively. The R^2 value revealed that 91.8 and 78.7 per cent variability for fruit weight respectively was governed by the above independent characters. In first year, the regression coefficient was significant positive for fruit diameter (0.83) and vine length (0.55). Similarly in second year significant positive effects were noticed for fruit diameter only (0.24). The analysis of variance for partial regression analysis in *in-situ* study revealed non-significant effect of 8 characters on ascorbic acid in first year whereas, significant effect of 8 characters on ascorbic acid in second year. The R^2 value revealed that 68.0 per cent variability for ascorbic acid in second year was governed by the above independent characters. Regression analysis for protein revealed that all the 8 characters was non-significant and contributed *i.e.*, only 35.8 & 53.6 per cent towards the variability of protein content in first and second year, respectively.
9. Plant regeneration system was established from two types of explants *i.e.*, *in vivo* nodal and leaf explants of the field grown mature female plants and also from well established *in vitro* nodal and leaf explants.
10. *In vivo* nodal explants were subjected to shoot induction in two different sets of media. Among the different treatments of TDZ and BA, maximum shoot induction was obtained from 1.5 mg/L TDZ containing media which showed a maximum shoot induction of 71.6 per cent. In the second set of media, BA alone and in combinations with Kin and NAA were evaluated. Among all the treatments, the highest shoot induction response of 83.3 per cent was noticed in 1.5 mg/L BA supplemented media.

11. Shoot regeneration rate of 100 per cent was obtained from the *in vitro* nodal segments in TDZ (0.5-2.5 mg/L) alone, BA (1.0-2.0 mg/L) alone, BA in combination with Kin (2.5+0.5 mg/L) and BA (2.5-3.0mg/L)+0.5 mg/L NAA fortified media.
12. *In vivo* as well as *in vitro* leaf explants were not responsive to any of the treatments in our experiment beside callus formation.
13. Among the various cold treated *in vitro* leaves, the leaves which were treated for 24 hr showed maximum bud breakage and highest shoot regeneration response (46.6 per cent) on media containing 3.0 mg/L TDZ.
14. Comparison among all multiplication media, revealed that, 0.5 mg/L BA and in combinations with 1.0 mg/L Kin was found to be most suitable for shoot multiplication *i.e.*, 8.5 number of shoots per explant. The longest mean shoot length was achieved by the MS basal medium supplemented with 2.0 and 2.5 mg/L BA *i.e.*, 4.9 cm.
15. Highest frequency of root induction *i.e.*, 95.0 per cent was obtained from the regenerated shoots with longest root length of 3.3 cm on root induction medium (MS basal media supplemented with 0.7 mg/L IAA) and further proliferation of roots *i.e.*, maximum number of roots/explant of 7.3 were accelerated on MS medium augmented with 0.8 mg/L NAA which showed little callusing at the base.
16. The rooted plants were potted in bottles containing hardening media. Out of the different hardening media tested, cocopeat in combination with vermiculite achieved highest survival rate of plants *i.e.*, 73.3 per cent which is significantly different from others.

Table 4.4 Correlation coefficients between different characters during first year or kharif 2013

Characters		Fruit diameter (mm)	Fruit weight (g)	Pedicle length (mm)	Fruit tip length (mm)	Fruit volume (cc)	No. of seeds/fruit	Moisture (%)	TSS (°Brix)	Ascorbic acid (mg/100gm)	Protein (g/100g)
Fruit length	G	0.90**	0.87**	-0.17	-0.35*	0.85**	0.58**	0.27	-0.08	0.25	0.09
	P	0.76**	0.72**	-0.00	-0.13	0.64**	0.36	0.19	-0.06	0.15	0.06
Fruit diameter	G		0.95**	-0.09	- 0.45**	0.93**	0.61**	0.08	-0.10	0.12	0.23
	P		0.79**	-0.01	-0.23	0.71**	0.36*	0.07	-0.03	0.08	0.20
Fruit weight	G			-0.15	- 0.57**	0.96**	0.70**	-0.01	-0.08	0.26	0.12
	P			0.03	-0.22	0.76**	0.45**	-0.03	-0.02	0.22	0.07
Pedicle length	G				-0.02	-0.20	-0.33	-0.09	0.17	-0.16	-0.10
	P				-0.04	-0.09	-0.06	-0.01	0.10	-0.10	-0.04
Fruit tip length	G					-0.46*	-0.36*	0.33	0.14	0.37*	-0.14

	P					-0.20	-0.15	0.11	0.13	-0.10	-0.11
Fruit volume	G						0.66**	0.02	-0.20	0.05	-0.00
	P						0.44**	-0.01	-0.12	0.05	0.01
No. of seeds/fruit	G							-0.10	-0.32	0.30	-0.09
	P							-0.02	-0.21	0.13	-0.03
Moisture	G								0.07	0.10	-0.08
	P								0.05	0.01	-0.02
TSS	G									-0.14	0.08
	P									-0.04	0.05
Ascorbic acid	G										0.32
	P										0.16

* - Significant at 5 per cent level of significance. ** - Significant at 1 per cent level of significance. G & P indicates genotypic and phenotypic correlations.

Table 4.5 Correlation coefficients between different characters during second year or kharif 2014

Characters		Fruit diameter (mm)	Fruit weight (g)	Pedicle length (mm)	Fruit tip length (mm)	Fruit volume (cc)	Fruit sp. gravity (g/cc)	No. of seeds/fruit	Moisture (%)	TSS (°Brix)	Ascorbic acid (mg/100g)	Protein (g/100g)
Fruit length	G	0.75**	0.85**	-0.17	-0.47**	0.82**	0.68**	0.54**	0.13	-0.11	0.53**	0.30
	P	0.57**	0.69**	-0.09	-0.20	0.63**	0.31	0.35*	0.08	-0.08	0.36*	0.18
Fruit diameter	G		0.78**	-0.30	-0.25	0.74**	0.72**	0.41*	0.18	0.01	0.56**	0.55**
	P		0.65**	-0.12	-0.03	0.60**	0.31	0.21	0.12	-0.02	0.39*	0.27
Fruit weight	G			-0.40*	-0.44**	0.98**	0.73**	0.52**	0.10	-0.21	0.59**	0.40*
	P			-0.16	-0.17	0.90**	0.53**	0.42*	0.09	-0.17	0.45**	0.25
Pedicle length	G				0.30	-0.27	-0.61	-0.23	-0.09	0.16	-0.03	-0.12
	P				0.12	-0.07	-0.18	-0.05	-0.05	0.10	0.03	-0.06
Fruit tip length	G					-0.47**	0.12	-0.41*	0.26	0.30	-0.63**	-0.51**
	P					-0.17	-0.05	-0.14	0.04	0.13	-0.14	-0.20
Fruit volume	G						0.60**	0.57**	0.02	-0.18	0.57**	0.45**
	P						0.13	0.44**	0.05	-0.14	0.42*	0.28
Fruit sp. gravity	G							-0.20	-0.35*	-0.16	0.50**	0.03
	P							0.15	-0.15	-0.08	0.21	0.00
No. of seeds/fruit	G								-0.01	-0.28	0.45**	0.10
	P								0.03	-0.18	0.26	0.08
Moisture	G									0.05	0.00	0.02
	P									0.01	-0.02	0.11
TSS	G										-0.28	0.14
	P										-0.20	0.11
Ascorbic acid	G											0.64**
	P											0.32

* - Significant at 5 per cent level of significance. ** - Significant at 1 per cent level of significance. G & P indicates genotypic and phenotypic correlations.

Table 4.16 Effect of cold treatment on shoot regeneration from *in vitro* leaf explants *M. dioica* Roxb. on TDZ supplemented MS basal media

PGR (MG/L)	% SHOOT INDUCTION	NO. OF SHOOTS/EXPLANT	MEAN SHOOT LENGTH (CM)
CONTROL	0.0±0.00H	0.0±0.00H	0.0±0.00H
TDZ (12H COLD TREATMENT)			
1.0	0.0±0.00H	0.0±0.00H	0.0±0.00D
2.0	0.0±0.00H	0.0±0.00H	0.0±0.00D
3.0	0.0±0.00H	0.0±0.00H	0.0±0.00D
4.0	0.0±0.00H	0.0±0.00H	0.0±0.00D
5.0	0.0±0.00H	0.0±0.00H	0.0±0.00D
TDZ (24H COLD TREATMENT)			
1.0	18.3±1.66FG	0.4±0.16CDEF	0.04±0.01BC
2.0	38.3±1.66BC	0.7±0.08BCDE	0.06±0.00AB
3.0	46.6±1.66A	1.6±0.15A	0.08±0.00A
4.0	43.3±1.66AB	1.5±0.24AB	0.07±0.00AB
5.0	33.3±1.66CD	1.4±0.51AB	0.04±0.01BC
TDZ (48H COLD TREATMENT)			
1.0	13.3±1.66G	0.2±0.01EF	0.02±0.01CD

2.0	16.6±1.66FG	0.3±0.01DEF	0.03±0.00CD
3.0	26.6±1.66DE	0.4±0.05CDEF	0.05±0.01ABC
4.0	31.6±1.66CD	1.3±0.12AB	0.06±0.00ABC
5.0	23.3±1.66EF	1.0±0.01ABCD	0.04±0.00BC
TDZ (72H COLD TREATMENT)			
1.0	16.6±1.66FG	0.3±0.06DEF	0.03±0.00CD
2.0	21.6±1.66EF	1.1±0.06ABC	0.04±0.00BC
3.0	31.6±1.66CD	1.3±0.12AB	0.05±0.00ABC
4.0	18.3±1.66FG	0.3±0.01DEF	0.03±0.00CD
5.0	5.00±2.88H	0.0±0.04EF	0.00±0.00D

Data were observed after 4 weeks of culture. Values are the mean of 3(n=3). Mean and SE followed by different letters are significant different at $p \leq 0.05$ Tukey Kramer HSD test.

Table 4.17 Effect of different concentrations of BA and in combination with Kin on *in vitro* shoot multiplication from regenerated shoots of *M. dioica* Roxb.

PGRS (MG/L)	NO. OF SHOOTS/EXPLANT	MEAN SHOOT LENGTH (CM)
CONTROL	1.15±0.02H	2.3±0.28DEF
BA		
0.5	4.4±0.03FG	3.4±0.47ABCDEF
1.0	4.9±0.15EFG	3.5±0.55ABCDE

1.5	5.3±0.92DEFG	4.0±0.31ABC
2.0	5.8±0.70CDEFG	4.9±0.05A
2.5	5.4±0.10CDEFG	4.9±0.04A
3.0	4.3±1.03G	3.4±0.07ABCDEF
BA+KIN		
0.5+0.5	6.3±0.29ABCDEFGG	4.0±0.26ABC
1.0+0.5	7.6±0.76ABC	4.4±0.29AB
1.5+0.5	8.0±0.66AB	4.5±0.20AB
2.0+0.5	7.2±0.10ABCD	3.9±0.42ABCD
2.5+0.5	6.6±0.10ABCDE	3.6±0.51ABCDE
3.0+0.5	6.1±0.04ABCDEFGG	3.4±0.34ABCDEF
0.5+1.0	8.5±0.22A	3.4±0.39ABCDEF
1.0+1.0	6.6±0.05ABCDEF	2.9±0.07BCDEF
1.5+1.0	6.3±0.15ABCDEFGG	2.7±0.34CDEF
2.0+1.0	6.1±0.07BCDEFG	2.4±.37CDEF
2.5+1.0	5.9±0.01BCDEFG	2.1±0.15EF
3.0+1.0	5.1±0.10DEFG	1.9±0.10F

Data were observed after 3 weeks of culture. Values are the mean of 3(n=3). Mean and SE followed by different letters are significant different at $p \leq 0.05$ Tukey Kramer HSD test.

Table 4.18 Root induction from regenerated shoots of *M. dioica* Roxb. on MS basal media supplemented with different concentrations of IAA, IBA and NAA

PGR (MG/L)	% ROOT INDUCTION	NO. OF ROOTS/EXPLANT	MEAN ROOT LENGTH (CM)	FREQUENCY OF CALLUSING
CONTROL	0.0±0.00P	0.00±0.00L	0.00±0.00K	
IAA				
0.1	51.6±1.66FGH	1.3±0.06HIJKL	0.9±0.03FGHI	
0.2	53.3±2.88FGHI	1.7±0.06GHIJK	1.7±0.15CDE	
0.3	55.0±1.66FGH	2.0±0.05FGHIJK	2.1±0.10BC	
0.4	65.0±2.88DEF	2.3±0.16EFGHIJK	2.2±0.04B	
0.5	88.3±1.66AB	3.1±0.10DEFGH	3.0±0.06A	
0.6	91.6±1.66AB	3.3±0.07DEFG	3.0±0.02A	
0.7	95.0±2.88A	4.0±0.04CDE	3.3±0.07A	
0.8	56.6±3.33EFGH	1.0±0.10IJKL	1.3±0.03EF	
0.9	35.0±2.88KLMN	0.5±0.04KL	0.8±0.05GHI	
1.0	31.6±3.33LMNO	0.5±0.01KL	0.5±0.03IJ	
IBA				
0.1	36.6±1.66JKLM	2.0±0.06FGHIJK	0.9±0.01FGHI	
0.2	38.3±1.66IJKLM	2.3±0.22EFGHIJK	0.9±0.02FGHI	
0.3	48.3±4.40GHIK	3.2±0.26DEFGH	1.0±0.11FG	
0.4	60.0±0.00EFG	4.3±0.26BCD	1.5±0.14DE	
0.5	61.6±1.66EFG	6.3±0.19A	1.5±0.23DE	
0.6	71.6±1.66CDE	6.6±1.09A	2.1±0.04BC	
0.7	41.6±4.40HIJKLM	4.3±0.22BCD	0.8±0.13GHI	
0.8	38.3±4.40IJKLM	2.7±0.64DEFGHIJ	0.7±0.03GHIJ	

0.9	20.0±2.88NO	1.0±0.10IJKL	0.2±0.02JK	
1.0	18.3±1.66O	1.0±0.41JKL	0.2±0.00JK	
NAA				
0.1	26.6±1.66MNO	1.4±0.18HIJKL	0.5±0.03HIJ	-
0.2	31.6±1.66LMNO	1.7±0.13GHIJKL	0.6±0.04GHIJ	-
0.3	31.6±1.66LMNO	2.9±0.15DEFGHI	0.7±0.01GHI	-
0.4	46.6±3.33GHIJ	3.8±0.46CDEF	0.7±0.00GHI	+
0.5	48.3±4.40GHIJKL	3.9±0.08CDE	0.8±0.11GHI	+
0.6	56.6±4.40EFGH	5.6±0.34ABC	0.9±0.05FGHI	+
0.7	61.6±4.40EFG	7.0±0.04A	1.0±0.11FGH	++
0.8	81.6±4.40ABC	7.3±0.91A	2.0±0.00BCD	++
0.9	78.3±1.66BCD	6.0±0.05AB	0.8±0.03GHI	++
1.0	58.3±1.66EFG	4.0±0.15CDE	0.8±0.01GHI	+++

- = absent , + = Low callusing, ++ = Moderate callusing and +++ = High callusing. Data were observed after 3 weeks of culture. Values are the mean of 3(n=3). Mean and SE followed by different letters are significant different at $p \leq 0.05$ Tukey Kramer HSD test.

Table 4.1 Mean square for different characters recorded in *in-situ* study under individual environment

SN	Characters	Environment	Genotypes	Error	χ^2 (Bartlett's test)
			[33]	[136]	[1]
1	Fruit length (m)	E1	229.4565**	21.18	
		E2	164.0504**	12.79	8.56**
2	Fruit diameter (mm)	E1	56.1136**	7.024	
		E2	45.9675**	4.827	4.75*
3	Fruit weight (g)	E1	69.2396**	6.167	
		E2	68.7864**	4.05	5.97*
4	Pedicel length (mm)	E1	2.1883**	0.7099	
		E2	1.2493**	0.3098	22.74**
5	Fruit tip length (mm)	E1	0.0356**	0.01412	
		E2	0.0280**	0.01532	0.23
6	Spine length (mm)	E1	0.0045	0.005324	
		E2	0.0066	0.006471	1.29
7	Fruit volume (cc)	E1	59.9137**	4.944	
		E2	57.0305**	3.868	2.04
8	Fruit specific gravity (g/cc)	E1	0.2998	0.1987	
		E2	0.0555**	0.02294	134.79**
9	No. of seeds/fruit	E1	142.9512**	20.66	
		E2	118.2431**	18.1	0.59
10	Moisture (%)	E1	241.4379**	33.28	
		E2	177.9599**	26.24	1.91
11	TSS (Brix°)	E1	14.8397**	0.825	
		E2	10.2406**	0.3506	24.18**

12	Ascorbic acid (mg/100g)	E1	27208.9091**	5062	
		E2	35765.9621**	3046	8.68**
13	Protein (g/100gm)	E1	8.3815**	0.6642	
		E2	4.3145**	0.9468	4.25*
			[33]	[68]	[1]
14	Chlorophyll content (mg/100g)	E1	0.2206*	0.1324	
		E2	0.0996	0.1131	0.42

* - significant at 5 per cent level of significance. ** - significant at 1 per cent level of significance.

Table 4.3 Variability parameters for various characters

SN	Characters	Environments	GCV	PCV	h ²	GG
1	Fruit length	E1	17.11	21.14	65.55	28.54
		E2	14.93	17.90	69.60	25.67
2	Fruit diameter	E1	13.68	18.05	57.46	21.37
		E2	12.63	16.01	62.24	20.53
3	Fruit weight	E1	39.95	49.01	66.44	67.08
		E2	41.65	47.91	75.59	74.61
4	Pedicel length	E1	26.61	49.86	28.49	29.26
		E2	21.57	35.54	36.82	26.96
5	Fruit tip length	E1	16.98	35.87	22.42	16.56
		E2	12.32	33.61	13.44	9.31
6	Fruit volume	E1	32.24	39.02	68.28	54.88
		E2	31.70	37.17	72.70	55.67
7	Fruit sp. gravity	E1	11.10	30.28	13.45	8.39
		E2	10.64	21.92	23.56	10.64
8	No. of seeds/fruit	E1	25.74	35.24	53.34	38.72
		E2	21.71	30.22	51.64	32.15
9	Moisture	E1	9.14	12.35	54.72	13.92

		E2	7.86	10.83	52.75	11.76
10	TSS	E1	27.27	31.14	76.70	49.19
		E2	22.55	24.52	84.54	42.71
11	Ascorbic acid	E1	29.19	43.15	45.75	40.67
		E2	35.87	43.66	67.52	60.73
12	Protein	E1	54.32	65.29	69.23	93.10
		E2	33.83	53.07	40.64	44.43
13	Chlorophyll	E1	17.65	42.73	17.07	15.03
		E2	16.21	37.43	14.45	13.03

Table 4.9 Visual traits of 34 genotypes in *Momordica dioica* Roxb.

SN	Genotype	Fruit colour	Fruit shape	Fruit surface
1	MPK-001	Light green	Oblong round	Spiny
2	MPK-002	Light green	Oblong	Spiny
3	MPK-003	Yellowish green	Oblong	Slightly spiny
4	MPK-004	Green	Oblong round	Slightly spiny
5	MPK-005	Green	Oblong	Spiny
6	MPK-006	Light green	Oblong	Spiny
7	MPK-007	Light green	Round	Spiny
8	MPK-008	Green	Oblong	Spiny
9	MPK-009	Light green	Oblong	Spiny
10	MPK-010	Light green	Oblong	Spiny
11	MPK-011	Dark green	Oblong	Spiny
12	MPK-012	Dark green	Oblong	Spiny
13	MPK-013	Light green	Oblong	Spiny
14	MPK-014	Light green	Cylindrical	Spiny
15	MPK-015	Green	Oblong	Spiny
16	MPK-016	Dark green	Oblong	Spiny

17	MPK-017	Dark green	Oblong	Spiny
18	MPK-018	Dark green	Oblong	Spiny
19	MPK-019	Dark green	Oblong	Slightly spiny
20	MPK-020	Dark green	Oblong	Spiny
21	MPK-021	Light green	Oblong	Spiny
22	MPK-022	Dark green	Oblong	Spiny
23	MPK-023	Dark green	Round	Spiny
24	MPK-024	Light green	Oblong	Spiny
25	MPK-025	Light green	Oblong	Spiny
26	MPK-026	Light green	Round	Spiny
27	MPK-027	Dark green	Oblong	Spiny
28	MPK-028	Dark green	Cylindrical	Spiny
29	MPK-029	Dark green	Oblong	Spiny
30	MPK-030	Light green	Oblong	Spiny
31	MPK-031	Light green	Oblong	Spiny
32	MPK-032	Light green	Oblong	Slightly spiny
33	MPK-033	Green	Oblong	Spiny
34	MPK-034	Dark green	Oblong	Spiny

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