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Genetic Variability, in Vitro Regeneration and Artificial Seed Production in Pointed Gourd (Trichosanthes Dioica ROXB.)

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GENETIC VARIABILITY, *IN VITRO* REGENERATION AND ARTIFICIAL SEED PRODUCTION IN POINTED GOURD (*TRICHOSANTHES DIOICA* ROXB.)

THESIS SUBMITTED FOR THE DEGREE OF
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Dedicated to my
Beloved
Parents and Daughters

DECLARATION

I do hereby declare that the thesis entitled **Genetic variability, *in vitro* regeneration and artificial seed production in pointed gourd (*Trichosanthes dioica* Roxb.)** is the results of my own investigation. I once again declare that the entire research work submitted for the degree of **Doctor of Philosophy** at the University of Rajshahi, Bangladesh, is original and has not been submitted before for any degree, diploma, or other similar title of any University.

Amalek.
12.12.07

December 2007 (Md. Abdul Malek)

CERTIFICATE

I hereby certify that the work embodied in this thesis has not been submitted in substance for any degree and has not been concurrently submitted in candidature for any degree.

12.12.07
(Md. Abdul Malek)
Candidate

This is to certify that the thesis entitled **Genetic variability, *in vitro* regeneration and artificial seed production in pointed gourd (*Trichosanthes dioica* Roxb.)** prepared by Mr. Md. Abdul Malek for the award of degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by him under my supervision. The work is original and to the best of my knowledge and belief, no part of the thesis has been submitted before for any degree, diploma, title or recognition.

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ABSTRACT

Pointed gourd is an under exploited important summer vegetable crop in Bangladesh. It is one of the most nutritive cucurbit vegetable holds a coveted position in the Bangladeshi market during summer and rainy season and available in the market up to the end of October beginning the lean period of vegetables. Being an important and nutritious vegetable it fails to draw due attention of the scientific community to launch research initiative towards improvement. The success of a crop improvement program largely depends on selection of desirable plants, which is possible if wide variation is present in the base population. Systematic characterization and evaluation of pointed gourd genotypes can be exploited to improve the genetic pattern for increasing yield potentialities through breeding. In these circumstances, 25 genotypes of pointed gourd were collected from different districts of the country to study the variability present in morphological and agronomical traits of the genotypes on all possible genetic parameters in order to address their merits in the gene pool configuration and breeding program.

In a study of genetic variability for fruit yield and its component characters 25 genotypes of pointed gourd (*Trichosanthes dioica* Roxb) were evaluated through genetic variance, heritability and genetic advance with the aim of obtaining information on the genetic architecture of the population and also to identify the best genotypes. Relationships between yield and yield contributing characters were studied in order to measure the magnitude of their association. In addition, to enhance breeding programme genetic diversity was also studied to address the range of genetic divergence of the individual genotype.

Ten quantitative characters were selected for the study of genetic variability and observed that there were significant variations among the genotypes for nodes per vine, vines per plant, vine length, fruits per plant, female flower length, fruit length, fruit width, fruit volume, fruit weight and fruit yield per plant. Wide variations were observed for all the characters except female flower length and fruit width. The environment affected these two characters, as genotypic and environmental variances were almost equal. The remaining characters were less influenced by the environment, as they showed less

environmental variances. While the other characters fruits per plant, fruit yield per plant, vines per plant, vine length, fruit length, fruit volume and fruit weight showed maximum range of variations indicated that remarkable variations were present in the genotypes. Considerable amount of genetic variability exists in respect of various characters in pointed gourd. Phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the characters studied. Similar to PCV, fruits per plant and fruit yield per plant showed high genotypic coefficient of variation. Moreover, the differences in GCV and PCV estimates were narrow, confirming the least environmental influence in attaining the observed variability. Therefore, fruits per plant, vine length and fruit yield per plant showed higher genotypic coefficient of variation offering ample scope for their improvement as being less affected by the environment. Highest genotypic coefficient of variability was recorded for fruit yield per plant holding the highest level of genetic variability providing enormous opportunity for the utilization of crop improvement.

Heritability estimates are useful in selection on the basis of the phenotypic performance of the quantitative characters. High estimates of broad sense heritability were observed for fruit volume, fruit weight, fruit yield per plant and vine length suggested that the selection based on phenotypic performance would be more effective. High heritability value along with high genetic advance as percent of mean provides most effective condition for selection. In the present study, the maximum genetic gain (expressed as % mean) was observed in fruits per plant and fruit yield per plant and both the characters showed high heritability value as well as high genetic advance as percent of mean. This suggested that selection for these two characters would be more fruitful.

Correlation studies revealed that fruits per plant had a positively and significantly correlated with fruit yield per plant both at genotypic and phenotypic level indicating fruit yield per plant could be improved by selection for this character. In most cases, genotypic correlation coefficient were higher than the corresponding phenotypic correlation coefficients suggesting the environmental influence reduce the relationship between yield and yield contributing characters in pointed gourd. Path coefficient analysis showed that fruits per plant and fruit volume had direct positive effects on fruit

yield per plant. This indicates that these two characters were the major contributors to fruit yield and they deserve priority in selection indices for yield improvement in pointed gourd. Based on the per se performance, inter-cluster distance and association among the traits, eight genotypes viz. AM-6 from cluster I, AM-10, AM-15 and MNI-12 from cluster II, MRM-28 from cluster III, MRM-23 from cluster IV and MHQ-197 and MHQ-198 from cluster V have been selected as best genotypes for variety improvement program in pointed gourd.

Under the study of *in vitro* regeneration, a protocol for multiple shoot propagation in pointed gourd (*Trichosanthes dioica*.Roxb) was successfully developed to facilitate further advance research on genetic improvement through application of biotechnological approaches. For direct regeneration the most suitable medium formulation was developed and highest percentage of shoot induction (93.86%), highest shoot number per explant (3.25) and longest shoot per explant (4.18 cm) were observed when nodal segment was cultured on MS medium supplemented with 2.0 mg/l BAP. Highest multiple shoot were also achieved in 2.0 mg/l BAP + 0.3 mg/l NAA from nodal segment. Half strength MS medium supplemented with 2.0 mg/l BAP was found best for multiple shoot induction from nodal segments of *in vitro* grown plants. The cultivar AM-8 was found better than AM-15 in regenerating performance. From the present investigation, it may be concluded that the nodal segments performed better than shoot tip explants for shoot induction, shoot number and shoot length. BAP was found superior to Kn and TDZ and in respect of hormonal combinations BAP and NAA proved superior to other combinations. In a comparative study, female genotypes responded better than the male genotypes for shoot regeneration. There was also a positional effect; nodal segment situated on the lower end of the vine was proved better than upper nodal segment for shoot regeneration. For indirect regeneration, MS medium supplemented with 0.5 mg/l NAA + 0.5 mg/l BAP was found most suitable in callus induction and plant regeneration. Among all the explants used in *in vitro* regeneration study, nodal segment of pointed gourd responded well for multiple shoot formation and internode appeared as the most suitable explant for callusing and plant regeneration.

Production of artificial seed has unraveled new vistas in plant biotechnology. The artificial seed technology is an exciting and rapidly growing area of research in plant cell and tissue culture. In the present investigation, shoot tips and nodal buds were successfully encapsulated by sodium alginate to perform as artificial seed beads. Artificial seeds developed from shoot tips and nodal buds were again subjected to hormonal treatments in order germination. Highest 95.00% shoot formation was obtained in MS medium containing 1.0 mg/l BAP from nodal segment followed by 90.00% shoot proliferation in 0.5 mg/l BAP and 1.0 mg/l BAP from nodal segment and shoot tips, respectively. The experimental results indicated that both cytokinin and auxin play an important role in germination of artificial seed being encapsulated by sodium alginate. Under the present study with limited experiments efforts have been made to establish the protocol for encapsulating the vegetative shoot for the production of artificial seed and their subsequent regeneration under the treatment of different hormonal formulations. It is the first report in Bangladesh to develop artificial seed production using vegetative tissue of the crop plants. It has opened a new area of advance research for developing the conservation strategies for plant genetic resources in the country.

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The following abbreviations have been used throughout the text

BAP	6- Benzyl amino purine
NAA	α-Naphthalene acetic acid
IAA	Indole 3-acetic acid
IBA	Indole 3-butyric acid
Kn	Kinetin (6-furfuryl amino purine)
2, 4-D	2,4-Dichlorophenoxy acetic acid
$HgCL_2$	Mercuric chloride
NaOH	Sodium hydroxide
min.	Minute
ml	Milliliter (s)
mm	Millimeter (s)
mg	Milligram (s)
mg/l	Milligram per liter
cm	Centimeter (s)
cm^2	Centimeter square
gm	Gram (s)
pH	Negative logarithm of hydrogen ion (H^+)
MS	Murashige and Skoog (1962)
HCl	Hydrochloric acid
Fig.	Figure (s)
½ MS	Half strength of MS medium
viz.	Videlicet = namely
KOH	Potassium hydroxide
e.g.	Exampli gratia = for example
et al.	*et alia* = and other people
%	Percentage
^0C	Degree Celsius
0.1N	0.1 Normal solution
i.e.	*id est* = that is
NaOCl	Sodium hypochlorite

Chapter I GENERAL INTRODUCTION

1.1 IMPORTANCE OF THE CROP

It is a popular, comparatively costly and delicious vegetable of higher demand. It is available in the market up to the end of October when there is a scarcity of vegetables. Keeping quality of this vegetable is considerably high which increases the scope of its export quality (Uddin, 2000).

The fruit is the edible part of the plant, which is cooked in various ways either in alone or in combination with other vegetables or meats. Fruits are a source of vitamin C and minerals (Gopalan *et al.*, 1982). It is rich in vitamins and contains 9.0 mg Mg, 2.6 mg Na, 83.0 mg K, 1.1 mg Cu and 17.0 mg S per 100g edible part (Singh, 1989).

Slices of fruits are used as vegetable curries before the seeds become hard. The leaves and tender shoots of the creeper may also be used for the preparation of soups for convalescents (Yawalker, 1969). It has also high industrial value as different types of jam, jelly and pickles can be made from this vegetable.

1.2 MEDICINAL VALUE OF THE CROP

Pointed gourd has a good medicinal value. It is easily digestible, diuretic and laxative, invigorates the heart and brain and is useful in disorder of the circulatory system (Yawalker, 1969). The decoction of the stalk is a good expectorant (Chauhan, 1989). It was reported that pointed gourd possesses the medicinal property of lowering the total cholesterol and blood sugar. These claims are supported by preliminary clinical trials with rats (Chandrasekar *et al.*, 1988) and rabbits (Sharma and Pant, 1988; Sharma *et al.*, 1988).

Additionally, the fruits and other plant parts such as the leaves and tender shoots are used as in the indigenous system of medicine since ancient times (Sharma *et al.*, 1989; Singh, 1989b). More recently, specific medicinal effects have been identified which include hypocholesterolemic, hypoglyceridemic and hypophospholidemic effects on normal and diabetic subjects (Sharma *et al.*, 1989; Mukherjee, 1996). Seeds of the plant are also found to possess antifungal and antibacterial activity and are widely used in the treatment of acid dyspeptic disease (Harit and Rathee, 1996).

1.3 ORIGIN AND DISTRIBUTION

Pointed gourd, native to India and Assam region is considered to the primary centre of its origin. The vegetable is widely cultivated in Assam, West Bengal, Bihar, Orissa and Uttar Pradesh. It is extensively grown in Bihar especially in Northern Bihar mostly on the bank of the Ganges and Eastern Uttar Pradesh where it is regarded as the king of the vegetable crops (Yawalker, 1969). The vegetable is grown almost every districts of Bangladesh especially in the Rajshahi, Bogra, Pabna, Jessore and Kustia (Rashid, 1993).

Trichosanthes is a large genus principally of Indo-Malayan distribution with about 44 species of which 22 occur in India. It is quite diverse in seed coat anatomy and poorly known taxonomically (Sheshadri, 1986). The centre of origin of *Trichosanthes* is not precisely known but most of the authors agree Indian or Indo-Malayan region as the original home.

Moluccas and the Philippine islands, is possibly of Sanskrit origin indicating its Indian nativity. De Candolle wrote that the species of *Trichosanthes* are all of old world and considered Indian origin as the most probable one, especially in the case of pointed gourd (*T. dioica.*) or parwal. The genus has undergone great changes in its species classification in the hands of several workers. The classification at species level was done mainly by Kundu and Chakravarty (Sheshadri, 1986). Kundu divided the genus into 2 sections:

Eutrichosanthes and *Pseudotrichosanthes*, former containing 23 species and latter three only. But Chakravarty described only 22 species and 3 species have been described in Flora Malayasiana and 4 under Flora of Japan (Sheshadri, 1986). But similarity or remoteness of these species with Indian one has not been studied. *Trichosanthes bracteata* is by far the commonest species in India, while *Trichosanthes cucumerina* extensive range. The third one, which has principal Indian distribution, is *Trichosanthes dioica.*

1.4 BOTANY AND CYTOLOGY

The vegetable belongs to the family Cucurbitaceae. It is a dioecious plant i.e.; male and female plant grows separately (Roy *et al.,* 1982). It is a diploid plant and its chromosome number is 22. Recently, a normal triploid plant of pointed gourd has

been found (Singh *et al.*, 1983). Pointed gourd is a creeper having climbing or trailing habit. Its flower bloom in the leaf axis. A tiny pointed gourd develops at the base of the female flower, which ultimately produce the edible fruit.

The plant is a perennial and grows as a vine. Roots are tuberous with long taproot system. Vines are pencil thick in size with dark green cordate simple leaves. Flowers are tubular white with 16-19 days initiation to anthesis time for pistillate flowers and 10-14 days for staminate flowers. Stigma remains viable for approximately 14 hours and 40-70% of flowers set fruit (Singh *et al.,* 1989).

Cytological studies have shown that *Trichosanthes anguina* and *Trichosanthes dioica* have 2n=22 chromosome, while polyploid series of 22, 44, 66 chromosomes have been recorded in *Trichosanthes palmate* (Sheshadri, 1986).

1.5 METHODS OF PLANTING

Pointed gourd is usually propagated through vine cuttings and root suckers. Propagation through seed is not desirable due to poor germination, cross pollinated nature of the crop, inability to determine the sex of plants before flowering, 50% male plants, slow growth of seedlings and seed propagated plant normally do not produce flower within one year, if flowering, it requires more than one year for fruit setting

Pointed gourd grows well in hot and moist climate (Bose and Som, 1993). It remains dormant during winter season and grows best in sandy loam soil. It requires perfect drainage and it is very much susceptible to water logging.

Vine cuttings of 60 cm long or more are taken from one year old plants in October when the plants complete fruiting and vines are mature. The crop is conventionally propagated by stem cuttings using 60-90 cm long segments from the basal portion of the vine. These cuttings are planted 15 cm deep, coiled in the shape of a ring, exposing both the ends (Tindall, 1983).

The cuttings are coiled in the shape of a ring and planted directly in the hills of prepared land or nursery and the nodes are covered by the soil. The rooted cuttings are planted in February–March in permanent places. At the time of planting it is to be ensured that 10-15% of the cuttings should be from male plants for adequate fruit set. A female: male ratio of 9:1 is optimum for ensuring maximum fruit set (Maurya *et al.*, 1985). Vines require trailing on some form of aerial support system to achieve

maximum fruit production (Prasad and Singh, 1987; Yadav *et al.,* 1989). In tropics, pointed gourd produces maximum yield for 3-4 years, after which yielding potential gradually declines (Samalo and Parida, 1983). The fruits are harvested when they are green and tender.

1.6 CULTIVATION IN BANGLADESH

Only 3-4 types of this crop are cultivated in the country. At present there is only two recommended variety in the country. Yield of pointed gourd in Bangladesh is very low. According to BBS (2002) annual total production and acreage is 39,000 M tons and 7155.96 hectares, respectively with an average yield of 5.45 tons/ha. Therefore, for the development high yielding variety systematic characterization and evaluation of existing cultivars is very much essential.

1.7 CONSTRAINTS IN BANGLADESH

Pointed gourd has a number of problems, related to yield and quality of fruit. Among them, low yields, small size fruit, poor fruit numbers per plant are common. In addition, lack of flowering synchronization of male and female plants, cumbersome hand pollination, presence of a large number of hard seeds in the fruit are considered to be important.

Improvement of this vegetable has not been attempted due to its dioecism and vegetative mode of propagation, or considered as a minor vegetable. The success of a crop improvement program depends on selection of desirable plants, which is possible if wide variation is present in the base population. At present there is less variability in pointed gourd. Only three or four fruit types are available in Bangladesh. There is short, medium and comparatively long fruit types are found in the market.

1.8 CROP IMPROVEMENT

1.8.1 **Conventional breeding**

Improvement of pointed gourd has not been attempted due to its dioecism and vegetative mode of propagation, or considered as a minor vegetable. The success of a crop improvement program depends on selection of desirable plants, which is possible if wide variation is present in the base population.

There is no high yielding variety of pointed gourd in the country. Therefore, it is urgent need to develop the high yielding variety. The high yielding variety may be

developed through the selection of desirable genotypes. For this purpose, an exploration team should be made for the collection of gemplasms of pointed gourd from the different cultivated area in Bangladesh. The collected germplasms were evaluated to identify the potential genotypes and trialed in different locations for the development of suitable varieties.

Pointed gourd is a diploid plant (2n=22). The variety of this vegetable can be developed through selection and hybridization. The seedless variety (triploid) can be developed through the following crossing program:

The diploid plant is to be treated with colchicines to develop tetraploid plant and this tetratploid will be crossed with diploid plant for developing triploid plant. This triploid plant produces seedless fruit. Recently, a triploid plant has been found (Sinha *et al.*, 1983). As the vegetable is mainly propagated asexually it is not needed to develop the triploid plant every year.

1.8.2 **Tissue culture and biotechnology**

Since pointed gourd (*Trichosanthes dioica*) is propagated solely by vegetative means; improvement of this vegetable through conventional breeding method is very difficult. Implementation and importance of tissue culture played a vital role in plant science. Micropropagation is a new advanced technology among the other traditional propagation. Rapid clonal multiplication of selective potential genotypes is a main objective of micro propagation. Traditional propagation is a slow process and in many cases it is infested by diseases and insects. Production of large number of plants per year is a very effective and powerful method.

Population growth of the country continues to grow at an alarming rate. This situation urgently needs to produce more food for the increased population of the country. Conventional plant breeding techniques have made considerable progress in the development of improved varieties but they have not been able to keep pace with the increasing food demand of the country. Tissue culture when integrated with conventional crop improvement techniques will be more efficient, environmentally compatible and ultimately cost effective utilization of resources for improved agricultural productivity. The neighboring countries have recently been taking the

advantages of this first growing science for the development of high yielding, disease resistant, stress tolerant and quality crop varieties.

Plant breeding is based mainly on genetic variability and plant selection. There are many ways to increase the genetic base of population. Sexual reproduction is nature's own way of broadening this base. It is the most effective way, but not the only tool available to the breeder. There are ways of bypassing sex. Applications and potential uses of these different approaches of creating genetic diversity are exemplified by the cell and tissue culture, meristem culture, callus culture, somatic fusion, molecular plant genetic engineering etc. The progress in biotechnology during the last decade has helped to generate genetic variation in a number of crop species including wheat, rice, cotton, *brassica*, tomato and potato.

The ability to readily regenerate plants from cultured tissues is an essential component of biotechnology for genetic manipulation and improvement of crops. During the last two decades, the techniques of plant cell and tissue culture have been considered as a powerful tool for crop improvement (Razdan and Cocking, 1981). There is a scope to generate variation in this crop through application of biotechnological techniques.

Plant tissue culture has also been utilized for rapid propagation of many vegetatively propagated crops (Hussay, 1986). So, there is a scope to propagate and generate variation in this crop through the application of tissue culture techniques. *In vitro* techniques has been developed as an upcoming and powerful tool to put forward as a possible means of propagation as well as increasing genetic variability for plant improvement and are being applied in different fields of agriculture, horticulture and forestry (Anisuzzaman *et al.*, 1993; Haider *et al.*, 1993).

Therefore, it is essential to improve this vegetable through *in vitro* culture technique for cultivation in the field on a commercial scale.

Chapter II GENETIC VARIABILITY IN QUALITATIVE CHARACTERS

2.1 INTRODUCTION

The two basic requirements of crop improvement are variation and selection. Variation in a character is a must for improvement in that character. Selection involves the identification and isolation of desirable plants. The progeny from selected plants may be released as a variety if they are found suitable and superior to the existing varieties. The selection of plants from a population is almost always based on their phenotypic appearance. Phenotype has heritable and non-heritable components. The value of progeny obtained from a selected plant, therefore, would largely depend upon the relative contributions by the heritable and non-heritable components to its phenotype. Clearly, the breeder should be thoroughly familiar with the laws of inheritance relative importance of the genotype and the environment in determining the concerned phenotype.

Some of the characters are little affected by other genes, i. e., the genetic background, or the environment. Such characters are generally governed by one or few genes (oligogenes) with large, easily detectable effects. The characters produced by oligogenes show distinct classes and are known as qualitative characters. On the other hand, the development of many characters is very much affected by the genetic background and, more particularly, the environment. These characters are governed by many genes (polygenes) with small individual and cumulative effects. The characters produced by polygenes are referred to as quantitative characters, because they do not show clear-cut classes and have to be studied by measurement. In crop improvement, both qualitative and quantitative characters are important. Many characters of economic value show the qualitative, while several others exhibit the quantitative mode of inheritance (Singh, 1990).

Qualitative and quantitative improvement of characters in pointed gourd is one of the basic requirements for developing a new variety. Earlier scientists were of opinion for high yielding exotic varieties and directly introducing them that might reduce the cost of production to a great extent. But experience has shown that the exotic varieties could not replace the indigenous ones in tropical regions because of their difficulties in adaptation to local cultural practices and environment (Bari *et al.*, 1988). Therefore, developing high yielding varieties suitable for local condition is the prime necessity in

order to boost up improvement of pointed gourd in the country. Breeding of high yielding pointed gourd varieties require information on the nature and magnitude of variation in the available materials, association of characters with yield and yield contributing characters.

Variation is the basis of improvement and germplasm represents the sum total of variability or hereditary materials or genes available in particular genus or species (Dandin, 1989). Germplasm is the basic foundation of crop improvement and its importance was realized as far back as 1989 (Boraiah, 1986). With the advent of last decade, the major breakthrough in the genetic improvement in crops has come through the utilization of germplasm.

Germplasm is the raw material of crop improvement and considered as the living museum of the sum total of variability. Scientists all over the world are realizing its importance for future needs. Collection, conservation and evaluation of the existing gene pool are the prime task of plant breeding for any crop improvement programme. Exploration of existing genetic variability by systematic exploration and collection of primitive land races, wild relatives, undomesticated forms and related weedy species are the urgent need of the day. Preservation of this natural wealth is more important as their existence is under threat. Cultivation of specific varieties and introduction of improved strains have resulted in the rapid elimination of primitive land races having wider adaptability. Extensive farming and over grazing have destroyed the natural undomesticated forms. As a result of above factors, the genetic erosion is faster than ever before. But the problems of the present day are more complex due to modernization and specific needs. To meet the demands of diverse nature, plant breeding programme requires wider spectrum of genetic base than ever before. Thinking over the future needs general awareness and concern have developed among the plant breeders all over the world to preserve the existing genetic wealth and prevent further loss of genetic resources. Till to day no work has been done regarding the characterization and evaluation of the germplasm of pointed gourd.

The conservation and utilization of genetic resources are the two vital components of varietal improvement programme. Without systematic evaluation on nature and magnitude of variation, existing strains, the available gene pool in the germplasm collection cannot be utilized to the full extent. The most promising source of genes,

controlling resistance to pest or tolerance to adverse conditions are likely to be found in minor varieties or special purpose types or primitive land races. The genetic evaluation of germplasm of pointed gourd was made according to IPGRI (International Plant Genetic Resources Institute) descriptors. The breeders are always looking into usefulness of morphological characters in breeding, as these are easy and cheap to measure, genetically correlated with yield and have a higher heritability (Gallais, 1984). Gross morphological studies helps to classify the material systematically. It also provides the information on specific gene sources for breeding programme and on the sum total of variability available in the genus, which fulfills the requirements of scientists working in various disciplines (Dandin and Jolly, 1986).

Higher range of variation in existing population offers better scope for selection of crop improvement. Pointed gourd is a dioecious and cross-pollinated perennial vegetable plant. As a result wide ranges of variation are observed among the species and also among the varieties of the same species in relation to morphological characters. Collection of genetic resources of pointed gourd by exploration or introduction indigenously or abroad and conserves it in the field genebank and its proper evaluation and characterization is the prime task of pointed gourd breeders for improvement its genetic pattern. The vegetable is grown almost every districts of Bangladesh especially in the Rajshahi, Bogra, Pabna, Jessore, and Kustia (Rashid, 1993). But the variability and the magnitude of diversity have not studied till today.

Therefore, the present study is intended to make an indepth study of variability present in morphological and agronomical traits of these pointed gourd cultivars on all possible genetic parameters in order to address their merits in the gene pool configuration and breeding programme.

2.2 MATERIALS AND METHODS

Twenty five genotypes of pointed gourd were collected during 2001-2002 from different parts of Bangladesh. The study was carried out at the experimental farm of Plant Genetic Resources Centre (PGRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur 1701 during the period from October 2001to August 2002.

The basal portion of the vine were coiled in the shape of a ring and planted in the experimental field of Plant Genetic Resources Centre, during rabi season on October 11, 2001. Sources or places of collection of these materials are shown in Table 2.1.

2.2.1 Experimental site and duration

The experiment was conducted at the experimental farm of Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur-1701 during October 2001to August 2002. The location of the site was 40 km North of Dhaka city with $23^0 59.323'$ N latitude and $90^0 24.845'$ E longitude and an elevation of 12 m from the sea level. The site was situated in the subtropical climate zone, characterized by rainfall during the month of May to September and scanty during the rest of the year.

2.2.2 Soil and climate

The soil of the experimental field belongs to the Grey Terrace soil type with silty clay texture. The soil was acidic in reaction with pH value of 5.5 (Sen and Noor, 2006). The average minimum and maximum air temperature during the cropping period were 20.53^0C and 30.22^0C respectively. The mean minimum and maximum relative humidity were 73.19% and 93.48% respectively and the total rainfall during the cropping period was 3825.9 mm.

The most important weather parameters such as air temperature, rainfall, relative humidity and sunshine for the years 2001-2002 and 2002-2003 have been taken year wise and presented as graphics in appendices 1, 2, 3, 4, 5, 6, 7 and 8.

2.2.3 Land preparation

The experimental plots were prepared with tractor ploughing followed by harrowing and laddering to bring the desired tilt. Raised beds with 20 cm height for each block within replications were made by spade and developed properly. Drains between blocks were made for immediate release of rainwater from the experimental field.

2.2.4 Time and method of planting

The seedlings of each genotype were planted on October 11, 2001 in the field maintaining 2.0 m spacing between the plants. The seedlings were distributed randomly in each replication and after planting, each pit of the block was irrigated by water.

2.2.5 Manure and fertilizer application

Chemical fertilizers were applied at the rate of 75 kg urea, 50 kg triple super phosphate (TSP), 62.5 kg muriate of potash (MP) and 5 kg zypsum per hectare. In addition, cow dung was applied at the rate of 2.5 tones per hectare. Cow dung, urea, TSP, MP and zypsum were applied during final land preparation.

2.2.6 Irrigation and drainage

One post planting irrigation was given by water cane to each pit to bring proper moisture condition of the soil to ensure uniform growth and development of the seedlings. A good drainage system was maintained for immediate release of rain water from the experimental field during the cropping period because the plant is very much susceptible to water logging.

2.2.7 Intercultural operation

Necessary intercultural operations were done during the crop period to ensure normal growth and development of the plants. The first weeding was done after 15 days of planting. The second weeding was done before flowering and no disease was found during the cropping period.

2.2.8 Sources of collection

Twenty five genotypes of pointed gourd were collected from different districts of Bangladesh for the study. Sources or places of collection of these genotypes including one male plant are shown in the following Table 2.1.

2.2.9 Data collection

Observations were recorded on the following 67 characters based on the descriptor list published by International Plant Genetic Resources Institute (IPGRI), (Anonymous, 1981). Some of the quantitative characters obtained were converted into qualitative descriptions according to Mahajan *et al.* (1999) and Van Rheenen (1981a).

A. Plant characters

1. Growth habit: Recorded at vegetative stage

1	viny
3	intermediate
5	bushy

2. Plant habit:

1	indeterminate
2	determinate

Table 2.1. Sources or places of collection of 26 pointed gourd genotypes

Sl. No.	Genotype	Source or place of collection
1	AM-2	Jessore
2	AM-3	Gaibandha
3	AM-6	Jessore
4	AM-8	Jessore
5	AM-10	Pabna
6	AM-14	Natore
7	AM-15	Pabna
8	AM-18	Rajshahi
9	AM-20	Rajshahi
10	AM-22	HRC, BARI, Joydebpur
11	MNI-11	Mymensingh
12	MNI-12	Mymensingh
13	MNI-13	Mymensingh
14	MNI-14	Mymensingh
15	MRM-23	Nawbabganj
16	MRM-24	Nawbabganj
17	MRM-25	Nawbabganj
18	MRM-28	Nawbabganj
19	MRM-30	Nawbabganj
20	MHQ-196	Jessore
21	MHQ-197	Jessore
22	MHQ-198	Jessore
23	MHQ-201	Jessore
24	MHQ-202	Jessore
25	MHQ-203	Jessore
26	AM-21 (Male plant)	Rajshshi

HRC: Horticulture Research Centre, BARI

B. Stem characters

1. Stem color: Recorded at vegetative stage

1	green
2	deep green
3	light green
4	white
5	light white
6	purple
7	light purple

2. Stem shape: As observed from cross-section

1	rounded
2	angular

3. Stem pubescence: Recorded at vegetative stage

0	none
3	low
5	conspicuous

4. Stem pubescence type: Recorded at vegetative stage

3	soft
5	intermediate
7	hard

5. Stem pubescence density: Recorded at vegetative stage

0	no hairs
3	sparse
5	intermediate
7	dense

6. Tendrils:

0	absent
+	present

C. Leaf characters

1. Leaf color: Recorded at vegetative stage

1	green
3	light green
5	intermediate
7	dark green

2. Leaf pubescence: Recorded at vegetative stage (both dorsal and ventral side)

0	none
3	low
5	conspicuous

3. Prominence of leaf veins: Recorded at vegetative stage

1	smooth
2	rugose (veins prominent)

4. Leaf blade tip angle: Observed at vegetative stage

1	very acute ($\leq 15^0$)
3	acute (about 45^0)
5	intermediate (about 75^0)
7	obtuse (about 110^0)
9	very obtuse ($>160^0$)

5. Leaf lobes: Recorded at vegetative stage

0	absent
3	shallow
5	intermediate
7	deep

6. Leaf margin: Recorded at vegetative stage

1	smooth
2	dented

7. Leaf type: Recorded at vegetative stage

3	soft
5	intermediate
7	hard

8. Leaf shape: Recorded at vegetative stage

1	ovate
2	orbicular
3	reniform
4	retuse
5	lobed

9. Leaf tip: Recorded at vegetative stage

1	pointed
2	blunt

10. Leaf size: As compared with that typical for crop type

1	small
2	medium
3	large

11. Leaf surface:

3	smooth
5	intermediate
7	rough

D. Flower characters

1. Node order of 1st female flower: Recorded from the basal node of the plant

1	≤ 5 th node
2	6-9 th node
3	10-13 th node
4	14-17 th node
5	> 17 th node

2. Flowering habit: Observed at flowering stage

1	monoecious: (♂, ♀) bearing staminate and pistillate flowers on the same plant
2	androecious: (♂) bearing only staminate flowers
3	gynoecious: (♀) bearing only pistillate flowers
4	dioecious: when staminate (♂) and pistillate (♀) flowers are produced on separate plants

3. Pedicel color: Observed at flowering stage

1	green
2	light green
3	dark green
4	purple
5	light purple

4. Flower color: Observed at flowering stage

1	white
2	yellow
3	orange
4	purple

E. Fruit characters

1. Node order of the fruit: Observed from the basal node of the plant

1	≤ 5 th node
2	6-10 th node
3	11-15 th node
4	16-20 th node
5	> 20 th node

2. Peduncle attachment: Observed at harvested stage

1	hard, not flared
2	hard, and flared
3	not flared, enlarged by hard cork
4	not flared, enlarged by soft cork

3. Fruit color: Recorded at harvested stage

1	green
2	light green
3	dark green
4	white
5	light white

4. Fruit shape: Recorded at harvested stage

1	ellipsoid
2	oblong ellipsoid
3	globular
4	stem-end tapered
5	blossom-end tapered

5. Fruit curvature: Recorded at harvested stage

1	none (fruit straight)
3	slightly curved
5	curved

6. Fruit stripes: Recorded at harvested stage

0	absent
3	low
5	intermediate
7	prominent

7. Fruit apex shape: Recorded at harvested stage

1	depressed
3	flattened
5	rounded
7	pointed

8. Stem end fruit shape: Recorded at harvested stage

1	depressed
3	flattened
5	rounded
7	pointed

9. Fruit skin texture: Recorded at harvested stage

1	smooth
2	grainy
3	finely wrinkled
4	shallowly wavy
5	netted
6	with warts
7	with spines

10. Fruit skin-hardness: Recorded at harvested stage

3	soft- easily marked by fingernail
5	intermediate- difficult to mark with fingernail
7	hard-impossible to mark with fingernail

11. Fruit ribs: Recorded at harvested stage

0	absent
3	superficial
5	intermediate
7	deep

12. Fruit flesh color: Observed at edible fruit size

1	white
2	green
3	yellow
4	orange
5	other (specify)

13. Color of ripening fruit: Recorded at ripening stage

1	yellow
2	red
3	orange
4	purple

F. Seed characters

1. Seed color: As observed according to the Royal Horticultural Society color chart

1	grayed-orange group (163-177)
2	brown group (200)

2. Seed surface:

1	smooth
2	wrinkled
3	slightly pitted
4	scaly
5	creased

3. Seed shape:

1	round
2	elliptical
3	ovoid
4	acorn
5	cone
6	deformed

4. Seed surface lustre:

3	dull
5	intermediate
7	glossy

G. Agronomic characters

Agronomic data were taken on the following 27 characters and converted into qualitative descriptions making frequency classes as follows according to Mahajan *et al.* (1999) with slight modifications.

1. Days to emergence: Number of days from planting to first seedling emergence.

1	≤ 5 days
2	6-10 days
3	11-15 days
4	16-20 days
5	> 20 days

2. Days to flower: Number of days from planting to 50% of the plants having female flowers.

1	very early (≤ 90 days)
2	early (91-115 days)
3	medium (116-140 days)
4	late (141-165 days)
5	very late (> 165 days)

3. Flowering to edible fruit size: Days required from opening of female flower to edible fruit size.

1	≤ 5 days
2	6-10 days
3	11-15 days
4	16-20 days
5	> 20 days

4. Leaf length (cm): Average length of 10 fresh leaves taken from the middle of the actively growing vine of the plant.

1	≤ 7.5 cm
2	7.6-9.0 cm
3	9.1-10.5 cm
4	10.6-12.0 cm
5	12.1-13.5 cm
6	>13.5 cm

5. Leaf width (cm): Average width of 10 fresh leaves taken from the middle of the actively growing vine of the plant.

1	≤ 5.0 cm
2	5.1-6.5 cm
3	6.6-8.0 cm
4	8.1-9.5 cm
5	> 9.5 cm

6. Leaf weight (g): Average weight of 10 fresh leaves taken from the middle of the actively growing vine from five plants.

1	≤ 0.5 g
2	0.51-1.0 g
3	1.1-1.5 g
4	1.6-2.0 g
5	> 2.0 g

7. Petiole length (cm): Mean length of 10 petioles. Observations were taken from the shoot tip of actively growing vine leaving 5 petioles.

1	very short (≤ 2.5 cm)
2	short (2.6-3.5 cm)
3	medium (3.6-4.5 cm)
4	long (4.6-5.5 cm)
5	very long (> 5.5 cm)

8. Petiole width (cm): Mean width of 10 petioles. Observations were taken from the shoot tip of actively growing vine leaving 5 petioles.

1	≤ 0.10 cm
2	0.11-0.20 cm
3	0.2.1-0.30 cm
4	0.31-0.40 cm
5	0.41-5.0 cm
6	> 5.0 cm

9. Female flower per leaf axil: Recorded at flowering stage.

1	1
2	2
3	>2

10. Female flower length (cm): Average length of 10 female flowers. Measured at flowering stage.

1	≤ 1.0 cm
2	1.1-1.5 cm
3	1.6-2.0 cm
4	2.1-2.5 cm
5	> 2.5 cm

11. Female flower width (cm): Average length of 10 female flowers. Measured at flowering stage.

1	≤ 0.30 cm
2	0.31-0.40 cm
3	0.41-0.50 cm
4	0.51-0.60 cm
5	> 0.60 cm

12. Style length (cm): Average length of 10 male flowers. Measured at flowering stage.

1	≤ 1.5 cm
2	1.6-2.0 cm
3	2.1-2.5 cm
4	2.6-3.0 cm
5	> 3.0 cm

13. Fruit length (cm): Mean length of 10 randomly selected fruits. Recorded at harvest stage.

1	very short (≤ 5.0 cm)
2	short (5.1-7.50 cm)
3	medium (7.60-10.0 cm)
4	long (10.1-12.5 cm)
5	very long (> 12.5cm)

14. Fruit width (cm): Mean width of 10 randomly selected fruits. Recorded at harvest stage.

1	≤ 2.0 cm
2	2.1-3.0 cm
3	3.1-4.0 cm
4	4.1-5.0 cm
5	> 5.0 cm

15. Fruit weight (g): Mean weight of 10 randomly selected fruits. Recorded at harvest stage

1	≤ 20.0 g
2	20.1-30.0 g
3	30.1-40.0 g
4	40.1-50.0 g
5	> 60.0 g

16. Fruit volume (cc): Mean volume of 10 randomly selected fruits. Recorded at harvested stage.

1	≤ 30.0 cc
2	30.1-40.0 cc
3	40.1-50.0 cc
4	50.1-60.0 cc
5	60.1-70.0 cc
6	> 70.0 cc

17. Fruits per plant: Recorded at final harvest.

1	≤ 40
2	41-65
3	66-90
4	91-115
5	116-140
6	> 140

18. Fruit yield per plant (kg): Recorded at final harvest.

1	very low (≤ 1.5 kg)
2	low (1.6-3.0 kg)
3	medium (3.1-4.5 kg)
4	high (4.6-6.0 kg)
5	very high (> 6.0 kg)

19. Seeds per fruit: Average seed number of 10 ripening fruits.

1	≤ 15
2	16-20
3	21-25
4	26-30
6	> 30

20. 100 Seed weight (g): Weight of 100 sun dried seeds.

1	≤ 5.0 g
2	5.1-7.5 g
3	7.6-10.0 g
4	10.1-12.5 g
5	> 12.5 g

21. Seed weight percentage per fruit: Weight of fresh seeds to the fruit weight and expressed as percent. Recorded from 10 randomly physiologically mature fruits.

$$\text{Seed weight percentage per fruit} = \frac{\text{Weight of seeds}}{\text{Fruit weight}} \times 100$$

1	≤ 5.0
2	5.1-7.5
3	7.6-10.0
4	10.1-12.5
5	> 12.5

22. Vines per plant: Recorded at final harvest.

1	≤ 5
2	6-7
3	8-9
4	10-11
5	> 11

23. Branch per vine: Recorded at final harvest.

1	≤ 1
2	2-3
3	4-5
4	> 5

24. Nodes per vine: Recorded at final harvest.

1	very few (≤ 50)
2	few (51-65)
3	intermediate (66-80)
4	high (81-95)
5	very high (> 95)

25. Nodes per 1m vine: Recorded at final harvest

1	≤ 5
2	6-10
3	11-15
4	> 15

26. Internode length (cm): Recorded at final harvest

1	short (≤ 7.50 cm)
2	medium (7.60-10.0 cm)
3	long (10.1-12.5 cm)
4	very long (> 12.5 cm)

27. Vine length (cm): Recorded at final harvest.

1	very short (≤ 2.5 m)
2	short (2.6-4.0 m)
3	intermediate (4.1-5.5 m)
4	long (5.6-7.0 m)
5	very long (> 7.0 m)

2.2.10 Harvesting and data recording

The fruits of the individual plot were harvested when they appeared to marketable size. Ten randomly selected fruits from each plot were harvested by hand and kept separately from each other by tagging. Data were recorded from these ten fruits.

2.3 RESULTS AND DISCUSSION

Breeding of high yielding pointed gourd varieties require information on the nature and magnitude of variation in the available materials. Therefore, in this context, 25 genotypes of pointed gourd were collected from different districts of the country and systematic characterization have been made to study the variability present in morphological and agronomical traits of these genotypes on all possible genetic parameters in order to address their merits in the gene pool configuration and breeding program. In this study, most of the characters showed variations among the pointed gourd genotypes. The harvested data were analyzed on the performance of their respective code values for individual character and their range of variations were segmented under appropriate frequency classes on counting the genotypic performances through frequency percentages. Qualitative descriptions and frequency distributions of different characters of 25 pointed gourd genotypes are presented in the Tables 2.2 to 2.25. The results of these characters were discussed under the following subheads:

2.3.1 Variation in plant characters

Growth habit: In the present study, no variation was observed in respect of this character. All the 25 genotypes had viny (creeping nature) growth habit.

Plant habit: Determinate type provides uniform edible size of the fruit and safe labor cost. However, indeterminate type of flowering had an advantage that it compensates the failure of fruit setting due to adverse effects of higher temperature or rainfall during fertilization. In the present study, all the genotypes had indeterminate plant habit.

2.3.2 Variation in stem characters

Stem color: The genotypes studied showed variations among themselves in stem color. Record of this trait revealed that 52% genotypes were green, 36% deep green and only 12% light green in color.

Stem shape: Usually round and angular shape stems are found in pointed gourd. In the present investigation, all the genotypes studied had angular stem shape and there was no variation in the character.

Stem pubescence: Variations was observed among the genotypes for this trait. The genotypes studied fell under three categories. 68% of the genotypes had low hairs and 32% had conspicuous hairs. None of the genotype was found to fall under frequency class 0.

Stem pubescence type: No difference was found among the genotypes for this character. The stem pubescence type for all the genotypes studied was soft.

Stem pubescence density: The genotypes studied showed variations among themselves in respect of this character. Record of this trait revealed that most of the genotypes (68%) had sparse hairs and 32% genotypes had dense hairs.

2.3.3 Variation in leaf characters

Leaf color: The genotypes of pointed gourd could be classified into green, light green and dark green for this character. In the present study, 80% genotypes was green, 4% was light green and 16% was dark green observed in leaf color character. Range of variation in leaf color indicates their different capacity in photosynthetic performance.

Table 2.2. Plant and stem characters in 25 pointed gourd genotypes

Genotype	Growth habit	Plant habit	Stem color	Stem shape	Stem pubescence	Stem pubescence type	Stem pubescence density
AM – 2	1	1	1	2	5	3	7
AM – 3	1	1	1	2	3	3	3
AM – 6	1	1	1	2	3	3	3
AM – 8	1	1	1	2	3	3	3
AM – 10	1	1	3	2	3	3	3
AM – 14	1	1	1	2	3	3	3
AM – 15	1	1	2	2	5	3	7
AM – 18	1	1	3	2	5	3	7
AM – 20	1	1	1	2	3	3	3
AM – 22	1	1	1	2	3	3	3
MNI – 11	1	1	3	2	3	3	3
MNI – 12	1	1	1	2	3	3	3
MNI – 13	1	1	1	2	3	3	3
MNI – 14	1	1	2	2	3	3	3
MRM – 23	1	1	2	2	3	3	3
MRM – 24	1	1	2	2	5	3	7
MRM – 25	1	1	2	2	3	3	3
MRM – 28	1	1	1	2	3	3	3
MRM – 30	1	1	1	2	3	3	3
MHQ –196	1	1	2	2	5	3	7
MHQ – 197	1	1	1	2	5	3	7
MHQ – 198	1	1	2	2	5	3	7
MHQ – 201	1	1	2	2	3	3	3
MHQ – 202	1	1	2	2	3	3	3
MHQ – 203	1	1	1	2	5	3	7

Leaf pubescence: In the present study, no variation was observed in respect of this character. Leaf pubescence was absent in all the genotypes studied.

Prominence of leaf veins: Depending upon the presence or absence of leaf veins, the genotypes are classified into two categories viz. smooth or rugose (veins prominent). All the accessions in the study were provided with rugose leaf veins.

Leaf lobes: In the present study, leaf lobes of all the genotypes were shallow.

Table 2.3. Frequency distribution of plant and stem characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Growth habit	1	viny	100
	3	bushy	0
	5	intermediate	0
Plant habit	1	indeterminate	100
	2	determinate	0
Stem color	1	green	52
	2	deep green	36
	3	light green	12
	4	white	0
	5	light white	0
	6	purple	0
	7	light purple	0
Stem shape	1	rounded	0
	2	angular	100
Stem pubescence	0	none	0
	3	low	68
	5	conspicuous	32
Stem pubescence type	3	soft	100
	5	intermediate	0
	7	hard	0
Stem pubescence density	0	none	0
	3	sparse	68
	5	intermediate	0
	7	dense	32

Leaf margin: No variation was observed among the genotypes for leaf margin and all the genotypes under study had dented leaf margin.

Leaf type: The leaf type of all the genotypes under study was hard.

Leaf shape: The genotypes studied for leaf shape showed 8% ovate, 76% orbicular and 16% reniform.

Leaf tip: No variation was found among the genotypes for this character and all the genotypes under study had pointed leaf.

Table 2.4. Leaf characters in 25 pointed gourd genotypes

Genotype	Leaf color	Leaf pubescence	Prominence of leaf veins	Leaf lobes	Leaf margin	Leaf type	Leaf shape
AM – 2	1	0	2	3	2	7	2
AM – 3	1	0	2	3	2	7	1
AM – 6	1	0	2	3	2	7	2
AM – 8	7	0	2	3	2	7	2
AM – 10	1	0	2	3	2	7	2
AM – 14	1	0	2	3	2	7	3
AM – 15	7	0	2	3	2	7	2
AM – 18	1	0	2	3	2	7	2
AM – 20	1	0	2	3	2	7	1
AM – 22	3	0	2	3	2	7	2
MNI – 11	1	0	2	3	2	7	2
MNI – 12	1	0	2	3	2	7	2
MNI – 13	1	0	2	3	2	7	2
MNI – 14	1	0	2	3	2	7	2
MRM – 23	7	0	2	3	2	7	3
MRM – 24	7	0	2	3	2	7	2
MRM – 25	1	0	2	3	2	7	3
MRM – 28	1	0	2	3	2	7	2
MRM – 30	1	0	2	3	2	7	3
MHQ –196	1	0	2	3	2	7	2
MHQ – 197	1	0	2	3	2	7	2
MHQ – 198	1	0	2	3	2	7	2
MHQ – 201	1	0	2	3	2	7	2
MHQ – 202	1	0	2	3	2	7	2
MHQ – 203	1	0	2	3	2	7	2

Leaf blade tip angle: In this study, different types of angle were observed for this character. The leaf blade tip angles of all the genotypes studied were 24% intermediate (about 75^0) and 76% obtuse (about 110^0). Acute leaf angle permits maximum sunlight penetration than horizontal or droopy leaf (Ashri, 1998).

Table 2.5. Frequency distribution of leaf characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Leaf color	1	green	80
	3	light green	4
	5	intermediate	0
	7	dark green	16
Leaf pubescence	0	none	100
	3	low	0
	5	conspicuous	0
Prominence of leaf veins	1	smooth	0
	2	rugose (veins prominent)	100
Leaf lobes	0	absent	0
	3	shallow	100
	5	intermediate	0
	7	deep	0
Leaf margin	1	smooth	0
	2	dented	100
Leaf type	3	soft	0
	5	intermediate	0
	7	hard	100
Leaf shape	1	ovate	8
	2	orbicular	76
	3	reniform	16
	4	retuse	0
	5	lobed	0

Leaf size: Among the genotypes studied, small, medium and large sizes of leaf were observed. Only 8% genotypes showed small leaf, 52% showed medium and 40% showed large size.

Leaf surface: In the present study, no variation was found in respect of this character. All the genotypes had rough leaf surface.

Tendrils: In the present study, tendrils were present in all the genotypes.

Petiole color: Wide variations were found for petiole color. Green color was observed in 72%, light green 24% and dark green in only 4% of the genotypes.

2.3.4 Variation in flower characters

Pedicel color: The pedicel color of the genotypes could be classified into five categories viz. green, light green, dark green, purple and light purple. In the present investigation, the pedicel color of all the genotypes was green.

Flower color: No variation was observed in respect of this character. The flower color of all the genotypes was white.

Flowering habit: Depending upon the nature of pollination, the genotypes of pointed gourd could be classified as monoecious, androecious, gynoecious, hermaphrodite and dioecious plant. But no variation was detected for this character and all the genotypes were dioecious.

Node order of 1st female flower: The genotypes studied were different among themselves for this attribute. Only 12% genotypes showed 1st female flower within node number 6 to 9, 76% within 10 to 13 and 12% within 14 to 17. None of the genotype was observed to include in frequency scale 1 (≤5 node number) and 5 (>17 node number). It is desirable to produce flower in the nodes near to the soil surface, which reduces biomass and facilitate to harvest (Ashri, 1998).

2.3.5 Variation in fruit characters

Node order of 1st fruit: Node order of 1st fruit varied from 8-24 node numbers. Among the genotypes studied, only 4% genotypes produced 1st fruit within 6 to 9 node number, 24% within 11 to 15, 64% within 16 to 20 and 8% with more than 20 node number. No genotype was observed to include in scale 1 (≤ 5 node number).

Peduncle attachment: No variation was found among the genotypes for this character. The peduncle attachment for all the genotypes under study had hard, not flared.

Fruit color: Marked variations were observed among the genotypes for this trait. The genotypes studied for fruit color fell under five categories. Only 4% genotypes showed white and light white, 36% showed green, 12% showed light green and 44% showed dark green.

Table 2.6. Leaf characters in 25 pointed gourd genotypes

Genotype	Leaf tip	Leaf blade tip angle	Leaf size	Leaf surface	Tendrils	Petiole color
AM – 2	1	7	3	7	+	1
AM – 3	1	7	2	7	+	2
AM – 6	1	7	3	7	+	2
AM – 8	1	7	3	7	+	1
AM – 10	1	7	2	7	+	2
AM – 14	1	7	1	7	+	1
AM – 15	1	7	2	7	+	1
AM – 18	1	7	2	7	+	2
AM – 20	1	5	1	7	+	2
AM – 22	1	7	3	7	+	1
MNI – 11	1	5	2	7	+	1
MNI – 12	1	7	2	7	+	2
MNI – 13	1	7	2	7	+	1
MNI – 14	1	7	2	7	+	1
MRM – 23	1	5	3	7	+	1
MRM – 24	1	7	2	7	+	1
MRM – 25	1	5	3	7	+	1
MRM – 28	1	5	3	7	+	1
MRM – 30	1	5	3	7	+	1
MHQ –196	1	7	2	7	+	1
MHQ – 197	1	7	3	7	+	1
MHQ – 198	1	7	2	7	+	1
MHQ – 201	1	7	2	7	+	1
MHQ – 202	1	7	2	7	+	3
MHQ - 203	1	7	3	7	+	1

Fruit shape: It is an important character for identification of the genotypes. In the present investigation, wide variations were observed for this character. Among the genotypes studied, 52% observed ellipsoid, 36% oblong ellipsoid, 4% stem end tapered and 8% stem end tapered and blossom end tapered fruit shape.

Table 2.7. Frequency distribution of leaf characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Leaf tip	1	pointed	100
	2	blunt	0
Leaf blade tip angle	1	very acute ($\leq 15^0$)	0
	3	acute (about 45^0)	0
	5	intermediate (about 75^0)	24
	7	obtuse (about 110^0)	76
	9	very obtuse ($>160^0$)	0
Leaf size	1	small	8
	2	medium	52
	3	large	40
Leaf surface	3	smooth	0
	5	intermediate	0
	7	rough	100
Tendrils:	0	absent	0
	+	present	100
Petiole color	1	green	72
	2	light green	24
	3	dark green	4
	4	purple	0
	5	light purple	0

Fruit curvature: It is another important character for variety selection. The genotypes studied showed variations among themselves in respect of this character. Record of this trait revealed that most of the genotypes (48%) were curved, 24% of the genotypes were straight and 28% were slightly curved.

Fruit stripes: Depending upon the presence or absence of stripes, the genotypes are classified into four categories having no stripes, low, intermediate and prominent. Among the genotypes, 12% found as intermediate and 88% prominent. None of the genotype was found to fall under frequency class 0 (absent) and 3 (low).

Stem end fruit shape: Marked variations were observed among the genotypes for this character. Among the genotypes studied 16% produced flattened, 64% rounded and 20% pointed shape. No genotype was found to fall under the category of depressed shape.

Table 2.8. Flower characters in 25 pointed gourd genotypes

Genotype	Pedicel color	Flower color	Flowering habit	Node order of 1st female flower
AM – 2	1	1	4	4
AM – 3	1	1	4	3
AM – 6	1	1	4	3
AM – 8	1	1	4	3
AM – 10	1	1	4	3
AM – 14	1	1	4	3
AM – 15	1	1	4	3
AM – 18	1	1	4	3
AM – 20	1	1	4	3
AM – 22	1	1	4	3
MNI – 11	1	1	4	3
MNI – 12	1	1	4	3
MNI – 13	1	1	4	3
MNI – 14	1	1	4	2
MRM – 23	1	1	4	3
MRM – 24	1	1	4	3
MRM – 25	1	1	4	3
MRM – 28	1	1	4	2
MRM – 30	1	1	4	2
MHQ –196	1	1	4	3
MHQ – 197	1	1	4	3
MHQ – 198	1	1	4	4
MHQ – 201	1	1	4	4
MHQ – 202	1	1	4	3
MHQ - 203	1	1	4	3

Fruit apex shape: Wide variations were found among the genotypes studied. The genotypes studied fell under four categories. 12% genotypes showed depressed, 56% showed flattened, 28% showed rounded and only 4% showed pointed shape.

Table 2.9. Frequency distribution of flower characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Pedicel color	1	green	100
	2	light green	0
	3	dark green	0
	4	purple	0
	5	light purple	0
Flower color	1	white	100
	2	yellow	0
	3	orange	0
	4	purple	0
Flowering habit	1	monoecious: (♂, ♀) bearing staminate and pistillate flowers on the same plant	0
	2	androecious: (♂) bearing only staminate flowers	0
	3	gynoecious: (♀) bearing only pistillate flowers	0
	4	dioecious: when staminate (♂) and pistillate (♀) flowers are produced on separate plants	100
Node order of 1st female flower	1	≤ 5	0
	2	6 - 9	12
	3	10 – 13	76
	4	14 – 17	12
	5	> 17	0

Fruit skin hardness: In the present study, soft fruit skin was found in 64% of the genotypes and the remaining 36% of the genotypes had intermediate fruit skin-hardness.

Fruit skin texture: In this character, finely wrinkled fruit skin texture was found in 64% of the genotypes and the remaining 36% of the genotypes had netted fruit skin texture.

Table 2.10. Fruit characters in 25 pointed gourd genotypes

Genotype	Node order of 1st fruit	Peduncle attachment	Fruit color	Fruit shape	Fruit curvature	Fruit stripes	Stem end fruit shape
AM – 2	4	1	3	2	1	7	5
AM – 3	4	1	2	6	5	5	7
AM – 6	4	1	1	1	5	7	5
AM – 8	3	1	3	1	5	7	5
AM – 10	4	1	3	2	3	7	5
AM – 14	3	1	5	1	5	5	5
AM – 15	3	1	3	1	5	7	5
AM – 18	4	1	1	6	5	7	7
AM – 20	4	1	4	2	1	7	3
AM – 22	4	1	3	1	3	7	5
MNI – 11	4	1	2	4	5	7	7
MNI – 12	4	1	3	2	3	7	5
MNI – 13	4	1	1	2	1	5	5
MNI – 14	3	1	1	1	1	7	7
MRM – 23	4	1	3	2	3	7	3
MRM – 24	4	1	3	2	1	7	5
MRM – 25	3	1	3	1	3	7	5
MRM – 28	3	1	1	1	3	7	5
MRM – 30	2	1	3	1	3	7	7
MHQ –196	4	1	1	2	5	7	3
MHQ – 197	5	1	2	1	5	7	3
MHQ – 198	4	1	1	1	5	7	5
MHQ – 201	4	1	1	2	5	7	5
MHQ – 202	4	1	1	1	1	7	5
MHQ - 203	5	1	3	1	5	7	5

Fruit ribs: Few variations were observed among the genotypes for this character. Most of the genotypes (96%) showed no ribs present on the fruit and only 4% genotypes showed superficial ribs on the fruit.

Table 2.11. Frequency distribution of fruit characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Node order of 1st fruit	1	≤ 5 node number	0
	2	6-10 node number	4
	3	11-15 node number	24
	4	16-20 node number	64
	5	> 20 node number	8
Peduncle attachment	1	hard, not flared	100
	2	hard, and flared	0
	3	not flared, enlarged by hard cork	0
	4	not flared, enlarged by soft cork	0
Fruit color	1	green	36
	2	light green	12
	3	dark green	44
	4	white	4
	5	light white	4
Fruit shape	1	ellipsoid	52
	2	oblong ellipsoid	36
	3	globular	0
	4	stem-end tapered	4
	5	blossom-end tapered	0
	6	stem end and blossom end tapered	8
Fruit curvature	1	none (fruit straight)	24
	3	slightly curved	28
	5	curved	48
Fruit stripes	0	absent	0
	3	low	0
	5	intermediate	12
	7	prominent	88
Stem end fruit shape	1	depressed	0
	3	flattened	16
	5	rounded	64

Fruit flesh color: No variation was found among the genotypes studied for this trait. The fruit flesh of all the genotypes was white in color.

Color of ripening fruit: Variations were observed among the genotypes for this character. 40% genotypes showed yellow, 28% showed red and 32% showed orange color of ripening fruits.

2.3.6 Variation in seed characters

Seed color: In the present study, variations were observed among the genotypes for seed color. The genotypes could be classified into two categories viz. grayed-orange group and brown group according to Horticultural Society color chart. Grayed-orange seed color was observed in 68% and brown in 32% of the genotypes.

Seed surface: The genotypes studied showed variations among themselves in respect of this character. Observations revealed that most of the genotypes (72%) were smooth, 16% were wrinkled and the remaining 12% of the genotypes were creased.

Seed shape: Little variation was observed in seed shape. Among the genotypes studied 24% observed round and 76% elliptical for this character.

Seed surface lustre: Wide variations were found among the genotypes studied for this trait. Dull seed surface was observed in 24% of the genotypes, 44% of the genotypes had intermediate seed surface lustre and the remaining 32% of the genotypes had glossy seed surface lustre.

2.3.7 Variation in agronomic characters

Remarkable variations were observed for all the agronomic characters in pointed gourd genotypes.

Days to emergence: Days to emergence varied from 7-21 days. There was early emergence of seedling in 24%, medium in 44%, late in 28% and very late in 4%. Early emergence was observed in AM-15, AM-18, AM-22, MNI-13, MNI-14, MHQ-196 and MHQ-203 indicated that these genotypes possessed the potentiality of early establishment of seedling.

Table 2.12. Fruit characters in 25 pointed gourd genotypes

Genotype	Fruit apex shape	Fruit skin hardness	Fruit skin texture	Fruit ribs	Fruit flesh color	Color of ripening fruit
AM – 2	1	3	3	1	1	2
AM – 3	7	5	5	1	1	1
AM – 6	3	5	5	1	1	1
AM – 8	3	3	3	1	1	1
AM – 10	5	5	5	1	1	2
AM – 14	3	3	3	1	1	1
AM – 15	5	5	5	1	1	3
AM – 18	5	3	3	1	1	2
AM – 20	3	3	3	3	1	1
AM – 22	3	5	5	1	1	1
MNI – 11	5	3	3	1	1	1
MNI – 12	3	3	3	1	1	3
MNI – 13	5	3	3	1	1	1
MNI – 14	5	3	3	1	1	1
MRM – 23	1	3	3	1	1	3
MRM – 24	1	3	3	1	1	2
MRM – 25	3	3	3	1	1	1
MRM – 28	3	3	3	1	1	3
MRM – 30	3	3	3	1	1	3
MHQ –196	3	5	5	1	1	2
MHQ – 197	3	3	3	1	1	2
MHQ – 198	3	3	3	1	1	2
MHQ – 201	3	5	5	1	1	3
MHQ – 202	3	5	5	1	1	3
MHQ - 203	5	5	5	1	1	3

Table 2.13. Frequency distribution of fruit characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Fruit apex shape	1	depressed	12
	3	flattened	56
	5	rounded	28
	7	pointed	4
Fruit skin hardness	3	soft-easily marked by fingernail	64
	5	intermediate- difficult to mark with fingernail	36
	7	hard-impossible to mark with fingernail	0
Fruit skin texture	1	smooth	0
	2	grainy	0
	3	finely wrinkled	64
	4	shallowly wavy	0
	5	netted	36
	6	with warts	0
	7	with spines	0
Fruit ribs	1	absent	96
	3	superficial	4
	5	intermediate	0
	7	deep	0
Fruit flesh color	1	white	100
	2	green	0
	3	yellow	0
	4	orange	0
Color of ripening fruit	1	yellow	40
	2	red	28
	3	orange	32
	4	purple	0

Days to flower: It is the important character for selecting earliness of the genotypes. Days to flower varied from 107 to 172 days in the study. Among the genotypes studied only 12% genotypes flowered early within 91-115 days and fell under frequency class 2; 24% flowered medium between 116 to 140 days under frequency class 3; 60% genotypes flowered late between 141 to 165 days under frequency class

4 and the remaining 4% genotypes flowered very late under frequency class 5. The genotype MRM-24 was found very late for days to flower.

Flowering to edible fruit size: It is also an important character for harvesting of suitable edible fruit. Most of the genotypes showed within the range of more or less early edible fruit size (11-15 days). Among them 56% fruit within 15 days and fell under frequency class 3, 40% between 16-20 days under frequency class 4 and 4 % with more than 20 days under frequency class 5.

Leaf length (cm): Variations were observed among the genotypes for this character. The genotypes studied fell under four categories. 4% genotypes fell in the frequency class 2 (7.6-9.0 cm), 64% fell in the frequency class 3 (9.0-10.5 cm), 20% fell in the frequency class 4 (10.6-12.0 cm) and only 12% fell in the frequency class 5 (12.1-13.5 cm). No genotype was found to fall under the frequency class 1 (≤ 7.5 cm) and 6 (> 13.5 cm).

Leaf width (cm): The genotypes studied showed variations among themselves in respect of this character. Record of this trait revealed that 20% of the genotypes fell in the frequency class 2 (5.1-6.5 cm), 40% of the genotypes fell in the frequency class 3 (6.6-8.0 cm) and 40% fell in the frequency class 4 (8.1-9.5 cm). No genotype was found to fall under frequency class 1 ((≤ 5.0 cm) and 5 (> 9.5 cm).

Leaf weight (g): The trait varied among the genotypes studied and 20% genotypes fell in the frequency class 2 (0.51–1.00 g), 44% in frequency class 3 (1.10–1.50 g) and 36% in frequency class 4 (1.60 – 2.00 g) for this character.

Petiole length (cm): Among the genotypes studied only 4% had very long petiole length, 20% had long petiole length, 60% had medium petiole length and the remaining 16% had short petiole length. The genotype MRM-23 had very long petiole.

Petiole width (cm): Most of the genotypes (72%) fell in the frequency class 3 (0.21-0.30 cm), 16% of the genotypes fell in the frequency class 2 (0.11-0.20 cm) and only 12% genotypes fell in the frequency class 4 (0.31-0.40 cm).

Table 2.14. Seed characters in 25 pointed gourd genotypes

Genotype	Seed color	Seed surface	Seed shape	Seed surface lustre
AM – 2	165 A (1)	2	1	5
AM – 3	165 A (1)	1	1	5
AM – 6	200 C (2)	1	3	5
AM – 8	200 D (2)	2	1	5
AM – 10	200 C (2)	1	3	3
AM – 14	177 A (1)	1	3	7
AM – 15	200 B (2)	5	3	7
AM – 18	165 A (1)	1	3	5
AM – 20	200 A (2)	2	3	3
AM – 22	165 A (1)	1	3	5
MNI – 11	200 B (2)	1	3	3
MNI – 12	177 B (1)	5	3	3
MNI – 13	166 A (1)	1	3	3
MNI – 14	200 B (2)	5	3	3
MRM – 23	166 A (1)	1	3	7
MRM – 24	164 A (1)	2	1	5
MRM – 25	174 A (1)	1	3	5
MRM – 28	165 A (1)	1	1	5
MRM – 30	200 C (2)	1	3	5
MHQ –196	166 A (1)	1	3	7
MHQ – 197	165 A (1)	1	3	7
MHQ – 198	165 A (1)	1	3	5
MHQ – 201	165 A (1)	1	1	7
MHQ – 202	165 A (1)	1	3	7
MHQ - 203	165 A (1)	1	3	7

Grayed-orange group: 163-177 (1)
Brown group: 200 (2)

Table 2.15. Frequency distribution of seed characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Seed color	1	grayed-orange group (163-177)	68
	2	brown group (200)	32
Seed surface	1	smooth	72
	2	wrinkled	16
	3	slightly pitted	0
	4	scaly	0
	5	creased	12
Seed shape	1	round	24
	2	elliptical	0
	3	ovoid	76
	4	acorn	0
	5	cone	0
	6	deformed	0
Seed surface lustre	3	dull	24
	5	intermediate	44
	7	glossy	32

Female flower per leaf axil: It is one of the most important character because number of fruit per leaf axil is closely related with the number of female flowers per leaf axil. Plants producing three flowers per leaf axil could produce three fruits per leaf axil holding greater potential for high yield. Only 8% of the genotypes were provided with 3 female flowers per leaf axil, 40% had two female flowers and the remaining 52% had one female flower in the leaf axil.

Female flower length (cm): The genotypes showed variations among themselves in respect of female flower length. Only 8% genotypes fell in the frequency class 2 (1.10-1.50 cm), 24% fell in the frequency class in 3 (1.60-2.00 cm) and most of the genotypes (68%) fell in the frequency class 4 (2.10-2.50 cm) for this character.

Female flower width (cm): Variations were observed among the genotypes for this character. Among the genotypes studied 52% fell under frequency class 3 (0.41-0.50 cm) and 48% of the genotypes fell under frequency class 4 (0.51-0.60 cm). None of the genotypes were identified under frequency class 1 ($\leq$ 0.30 cm), 2 (0.31- 0.40 cm) and 5 (> 0.60 cm).

Table 2.16. Agronomic characters in 25 pointed gourd genotypes

Genotype	Days to emergence (days)	Days to flower (days)	Flowering to edible fruit size (days)	Leaf length (cm)	Leaf width (cm)
AM – 2	4	4	3	3	3
AM – 3	3	2	3	4	4
AM – 6	4	2	3	3	4
AM – 8	3	4	4	4	4
AM – 10	4	4	4	3	4
AM – 14	3	3	4	3	3
AM – 15	2	2	3	3	4
AM – 18	2	4	4	2	2
AM – 20	4	4	3	3	2
AM – 22	2	4	4	3	3
MNI – 11	3	3	3	3	2
MNI – 12	3	3	3	3	4
MNI – 13	2	4	3	3	3
MNI – 14	2	4	3	4	3
MRM – 23	4	3	3	5	3
MRM – 24	5	5	3	5	2
MRM – 25	4	4	4	3	3
MRM – 28	3	4	3	5	3
MRM – 30	3	4	5	3	2
MHQ –196	2	4	4	3	3
MHQ – 197	3	3	4	3	3
MHQ – 198	3	4	3	4	4
MHQ - 201	3	4	3	3	3
MHQ - 202	4	4	4	4	4
MHQ - 203	3	3	4	3	4

Table 2.17. Frequency distribution of agronomic characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Days to emergence	1	≤ 5 days	0
	2	6-10 days	24
	3	11-15 days	44
	4	16-20 days	28
	5	> 20 days	4
Days to flower	1	very early (≤ 90 days)	0
	2	early (91-115 days)	12
	3	intermediate (16-140 days)	24
	4	late (141-165 days)	60
	5	very late (> 165 days)	4
Flowering to edible fruit size	1	≤ 5 days	0
	2	6-10 days	0
	3	11-15 days	56
	4	16-20 days	40
	5	> 20 days	4
Leaf length (cm)	1	≤ 7.5 cm	0
	2	7.6-9.0 cm	4
	3	9.1-10.5 cm	64
	4	10.6-12.0 cm	20
	5	12.1-13.5 cm	12
	6	>13.5 cm	0
Leaf width (cm)	1	≤ 5.0 cm	0
	2	5.1-6.5 cm	20
	3	6.6-8.0 cm	40
	4	8.1-9.5 cm	40
	5	> 9.5 cm	0

Style length (cm): The trait varied among the genotypes studied. Only 4% of the genotypes fell in the frequency class 1 (1.60-2.00 cm), 88% genotypes in 3 (2.10-2.50 cm) and 8% genotypes in 4 (2.60-3.00 cm) for this character.

Fruit length (cm): Among the genotypes studied 12% had short fruit length, 52% had medium fruit length, 28% had long fruit length and only 8% had very long fruit length. Genotypes AM-18 and MRM-25 had very long fruit length.

Table 2.18. Agronomic characters in 25 pointed gourd genotypes

Genotype	Leaf weight (g)	Petiole length (cm)	Petiole width (cm)	Female flower per leaf axil	Female flower length (cm)
AM – 2	4	2	3	1	4
AM – 3	3	2	3	2	4
AM – 6	4	3	4	2	4
AM – 8	4	3	4	3	4
AM – 10	4	3	4	2	3
AM – 14	3	3	3	1	2
AM – 15	3	4	3	2	3
AM – 18	3	3	3	1	3
AM – 20	2	3	2	1	3
AM – 22	3	2	3	1	4
MNI – 11	3	3	3	1	4
MNI – 12	3	4	3	2	4
MNI – 13	4	3	3	1	3
MNI – 14	4	4	3	1	4
MRM – 23	3	5	3	2	4
MRM – 24	4	4	3	1	2
MRM – 25	2	2	2	2	4
MRM – 28	2	3	2	2	4
MRM – 30	2	3	3	3	4
MHQ –196	2	3	2	1	4
MHQ – 197	4	3	3	1	4
MHQ – 198	3	3	3	1	4
MHQ – 201	3	3	3	1	3
MHQ -202	3	4	3	1	4
MHQ - 203	4	3	3	2	4

Fruit width (cm): Most of the genotypes were found to similar in fruit width. 92% of the genotypes fell in the frequency class 3 (3.1-4.0 cm) and only 4% fell in the frequency class 2 (2.1-3.0 cm) and 4 (4.1-5.0 cm).

Table 2.19. Frequency distribution of agronomic characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Leaf weight (g)	1	≤ 0.5 g	0
	2	0.51-1.0 g	20
	3	1.1-1.5 g	44
	4	1.6-2.0 g	36
	5	> 2.0 g	0
Petiole length (cm)	1	very short (≤ 2.5 cm)	0
	2	short (2.6-3.5 cm)	16
	3	medium (3.6-4.5 cm)	60
	4	long (4.6-5.5 cm)	20
	5	very long (> 5.5 cm)	4
Petiole width (cm)	1	≤ 0.10 cm	0
	2	0.11-0.20 cm	16
	3	0.21-0.30 cm	72
	4	0.31-0.40 cm	12
	5	> 0.40 cm	0
Female flower per leaf axil	1	1	52
	2	2	40
	3	> 2	8
Female flower length (cm)	1	≤ 1.0 cm	0
	2	1.10-1.50 cm	8
	3	1.60-2.00 cm	24
	4	2.10-2.50 cm	68
	5	> 2.50 cm	0

Fruit weight (g): This character is directly related to fruit yield. The character varied among the genotypes studied and 4% genotypes fell in the frequency class 2 (20.1-30.0 g), 24% in 3 (30.1-40.0 g), 32% in 4 (40.1-50.0 g) and 40% in 5 (50.1-60.0 g) for this character. The genotypes AM-3, AM-10, AM-15, MNI-12, MNI-14, MRM-23, MRM-25, MRM-28, MHQ-202 and MHQ-203 were found to high fruit weight (>60.0 g).

Fruit volume (cc): The genotypes studied showed variations among themselves in respect of fruit volume. Record of this trait revealed that only 4% genotypes fell in the frequency class 2 (30.1-40.0 cc), 12% in 3 (40.1-50.0 cc), 24% in 4 (50.1-60.0 cc), 36% in 5 (60.1-70.0 cc) and the remaining 24% in 6 (>70.0 cc). The genotypes AM-3, AM-10, AM-15, MRM-23, MHQ-198 and MHQ-202 had more than 70.0 cc fruit volume.

Table 2.20. Agronomic characters in 25 pointed gourd genotypes

Genotype	Female flower width (cm)	Style length (cm)	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)
AM – 2	3	3	4	3	4
AM – 3	3	3	4	3	5
AM – 6	4	3	4	3	4
AM – 8	4	3	3	3	4
AM – 10	3	3	3	3	5
AM – 14	3	2	3	2	3
AM – 15	3	3	4	3	5
AM – 18	3	3	4	3	4
AM – 20	3	3	3	3	2
AM – 22	3	3	3	3	3
MNI – 11	3	3	4	3	4
MNI – 12	4	3	4	4	5
MNI – 13	4	4	3	3	3
MNI – 14	4	3	3	3	5
MRM – 23	4	3	4	3	5
MRM – 24	3	3	3	3	3
MRM – 25	4	3	4	3	5
MRM – 28	3	4	4	3	5
MRM – 30	4	3	4	3	4
MHQ –196	4	3	3	3	3
MHQ – 197	4	3	4	3	4
MHQ – 198	4	3	4	3	4
MHQ – 201	3	3	3	3	3
MHQ – 202	4	3	4	3	5
MHQ - 203	3	3	3	3	5

Table 2.21. Frequency distribution of agronomic characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Female flower width (cm)	1	≤ 0.30 cm	0
	2	0.31-0.40 cm	0
	3	0.41-0.50 cm	52
	4	0.51-0.60 cm	48
	5	> 0.60 cm	0
Style length (cm)	1	≤ 1.50 cm	0
	2	1.60-2.00 cm	4
	3	2.10-2.50 cm	88
	4	2.60-3.00cm	8
	5	> 3.00 cm	0
Fruit length (cm)	1	very short (≤ 5.0 cm)	0
	2	short (5.1-7.50 cm)	12
	3	medium (7.6-10.0 cm)	52
	4	long (10.1-12.50 cm)	28
	5	very long (> 12.50 cm)	8
Fruit width (cm)	1	(≤ 2.0 cm)	0
	2	(2.1-3.0 cm)	4
	3	(3.1-4.0 cm)	92
	4	(4.1-5.0 cm)	4
	5	(> 5.0 cm)	0
Fruit weight (g)	1	≤ 20 g	0
	2	20.1-30.0 g	4
	3	30.1-40.0 g	24
	4	40.1-50.0 g	32
	5	50.1-60.0 g	40
	6	> 60.0 g	0

Fruits per plant: This is the most important character for selection of fruit yield, which determines the yield of the genotypes. Higher number of fruits directly contributes to the yield. The genotypes showed higher levels of variation in magnitude for number of fruits per plant. Among the genotypes studied, 16% genotypes fell in the frequency class 1 (≤40 fruits), 32% in 2 (41-65 fruits), 12% in 3 (66-90 fruits), 20% in 4 (91-115 fruits), 16% in 5 (116-140 fruits) and the remaining 4% genotypes fell under frequency class 6 which showed more than 140 fruits per plant. Only the genotype AM-15 found as highest number of fruits per plant.

Fruit yield per plant (kg): This is another leading character bearing top priority in selection of the genotypes in any crop improvement program. Marked differences were observed among the genotypes for fruit yield per plant. Among the genotypes 44% showed low yield and fell under frequency class 2 (1.6-3.0 kg), 32% showed medium yield under frequency class 3 (3.1-4.5 kg), 16% showed high yield under frequency class 4 (4.6-6.0 kg) and only 8% showed very yield under frequency class 5 (> 6.0 kg). None of the genotypes were identified under frequency class 1 (≤ 1.5 kg).

Seeds per fruit: Significant variations were observed for this character. Among the genotypes, 48% fell under frequency class 2 (16-20 seeds), 36% fell under frequency class 3 (21-25 seeds), 8% fell under frequency class 4 (26-30 seeds) and the remaining 8% fell under frequency class 5 (>30 seeds). None of the genotype was identified under frequency class 1 (≤ 15 seeds).

100 Seed weight (g): The character varied among the genotypes studied and only 8% genotypes fell in the frequency class 2 (5.1-7.5 g) and 92% fell in the frequency class 3 (7.6-10.0 g). None of the genotypes were observed under frequency class 1 (≤ 5.0 g), 4 (10.1-12.5 g) and 5 (>12.5 g).

Seed weight percentage per fruit: Remarkable variations were observed for this character. Among the genotypes studied only 4% fell in the frequency class 1 (≤ 5.0%), 60% fell in the frequency class 2 (5.1-7.5%), 28% fell in the frequency class 3 (7.6-10.0%) and 8% fell in the frequency class 4 (10.1-12.5%). None of the genotypes were identified under frequency class 5 (> 12.5%).

Vines per plant: The genotypes studied showed variations among themselves in respect of this character. 20% genotypes fell in the frequency class 1 (0-5), 44% in 2 (6-7) and 32% in 3 (8-9), and only 4% in frequency class 4 (10-11). The genotype AM-15 had higher vine number per plant.

Branch per vine: The genotypes studied were different for this character. 8% genotypes showed 0-1 branch number per vine, 72% showed 2-3 branch number per vine and 20% showed 4-5 branch number per vine.

Table 2.22. Agronomic characters in 25 pointed gourd genotypes

Genotype	Fruit volume (cc)	Fruits per plant	Fruit yield per plant (kg)	Seeds per fruit	100 Seed weight (g)
AM – 2	5	1	2	3	3
AM – 3	6	4	3	2	3
AM – 6	5	4	4	2	3
AM – 8	4	5	4	2	3
AM – 10	6	5	5	5	3
AM – 14	3	4	3	3	3
AM – 15	6	6	5	4	3
AM – 18	4	4	3	3	3
AM – 20	2	5	3	2	3
AM – 22	4	2	2	3	2
MNI – 11	4	1	2	2	3
MNI – 12	5	5	4	5	3
MNI – 13	4	2	2	3	3
MNI – 14	5	3	3	4	3
MRM – 23	6	1	2	2	3
MRM – 24	3	1	2	2	2
MRM – 25	5	4	4	2	3
MRM – 28	5	3	3	3	3
MRM – 30	5	2	2	3	3
MHQ –196	4	2	2	2	3
MHQ – 197	5	2	2	3	3
MHQ – 198	6	2	3	3	3
MHQ – 201	3	2	2	2	3
MHQ – 202	6	3	3	2	3
MHQ – 203	5	2	2	2	3

Nodes per vine: Variations were observed among the genotypes studied for this character. 4% of the genotypes had very few node numbers (≤ 50), 60% had few node numbers (51-65), 24% had intermediate node number (66-80) and 12% had high node number per vine (81-95). The genotype MNI-13 had high node number per vine.

Table 2.23. Frequency distribution of agronomic characters in 25 pointed gourd genotypes

Character	Frequency class		Frequency (%)
Fruit volume (cc)	1	≤ 30 cc	0
	2	30.1-40.0 cc	4
	3	40.1-50.0 cc	12
	4	50.1-60.0 cc	24
	5	60.1-70.0 cc	36
	6	> 70.0 cc	24
Fruits per plant	1	≤ 40	16
	2	41-65	32
	3	66-90	12
	4	91-115	20
	5	116-140	16
	6	>140	4
Fruit yield per plant (kg)	1	very low (≤ 1.5 kg)	0
	2	low (1.6-3.0 kg)	44
	3	medium (3.1-4.5 k g)	32
	4	high (4.6-6.0 kg)	16
	5	very high (> 6.0 kg)	8
Seeds per fruit	1	≤ 15	0
	2	16-20	48
	3	21-25	36
	4	26-30	8
	5	> 30	8
100 Seed weight (g)	1	≤ 5.0 g	0
	2	5.10-7.50 g	8
	3	7.60-10.00 g	92
	4	10.10-12.50 g	0
	5	> 12.50 g	0

Nodes per 1m vine: The genotypes studied were different among themselves for nodes per 1m vine. 40% genotypes showed 6 to 10 nodes per 1m vine and 60% showed 11 to 15 nodes per 1m vine of pointed gourd.

Internode length (cm): Variations were observed in the genotypes for this character. 44% of the genotypes had medium internode length, 48% long internode length and only 8% had very long internode length. The genotypes AM-15 and MNI-12 had very long internode.

Vine length (m): Among the genotypes studied 12% had short vine length, 48% had medium vine length, 32% had long vine length and only 24% had very long vine length. The genotypes MRM-24 had very long vine in length.

Table 2.24. Agronomic characters in 25 pointed gourd genotypes

Genotype	Seed weight percentage per fruit	Vines per plant	Branch per vine	Nodes per vine	Nodes per 1m vine	Internode length (cm)	Vine length (cm)
AM – 2	2	1	2	2	3	2	3
AM – 3	2	3	1	3	3	2	3
AM – 6	2	3	2	2	2	3	3
AM – 8	2	2	2	2	2	3	4
AM – 10	3	2	2	2	2	3	3
AM – 14	4	2	3	3	3	2	4
AM – 15	3	4	2	3	2	4	4
AM – 18	2	2	2	2	2	3	4
AM – 20	4	3	2	2	3	2	3
AM – 22	2	1	2	2	3	2	2
MNI – 11	3	1	2	1	3	2	2
MNI – 12	3	2	2	2	2	4	4
MNI – 13	2	3	2	4	3	2	4
MNI – 14	3	3	2	3	2	3	4
MRM – 23	1	1	2	4	3	3	5
MRM – 24	3	2	3	4	2	3	5
MRM – 25	2	3	3	3	3	2	4
MRM – 28	2	2	3	2	3	3	3
MRM – 30	2	1	3	3	3	3	3
MHQ –196	2	2	2	2	3	2	3
MHQ – 197	2	3	2	2	2	3	3
MHQ – 198	3	3	2	2	3	2	3
MHQ – 201	2	2	1	2	2	3	2
MHQ – 202	2	2	2	2	3	3	3
MHQ - 203	2	2	2	2	3	2	3

Table 2.25. Frequency distribution of agronomic characters in 25 pointed gourd genotypes

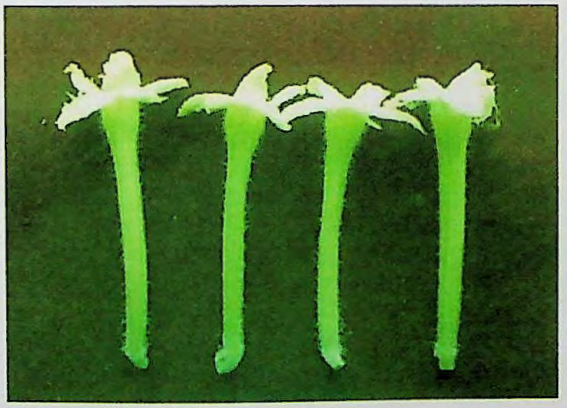
Character	Frequency class		Frequency (%)
Seed weight percentage per fruit	1	≤ 5.0 %	4
	2	5.10-7.5 %	60
	3	7.6-10.0 %	28
	4	10.1-12.5 %	8
	5	> 12.5 %	0
Vines per plant	1	≤5	20
	2	6-7	44
	3	8-9	32
	4	10-11	4
	5	>11	0
Branch per vine	1	≤1	8
	2	2-3	72
	3	4-5	20
	4	> 5	0
Nodes per vine	1	very few (≤ 50)	4
	2	few (51-65)	60
	3	intermediate (66-80)	24
	4	high (81-95)	12
	5	very high (> 95)	0
Nodes per 1m vine	1	≤ 5	0
	2	6-10	40
	3	11-15	60
	4	> 15	0
Internode length (cm)	1	short (≤ 7.5 cm)	0
	2	medium (7.6-10.0 cm)	44
	3	long (10.1-12.5 cm)	48
	4	very long (>12.5 cm)	8
Vine length (m)	1	very short (≤ 2.5 m)	0
	2	short (2.6-4.0 m)	12
	3	medium (4.1-5.5 m)	48
	4	long (5.6-7.0 m)	32
	5	very long (>7.0 m)	8

Vegetative stage of pointed gourd

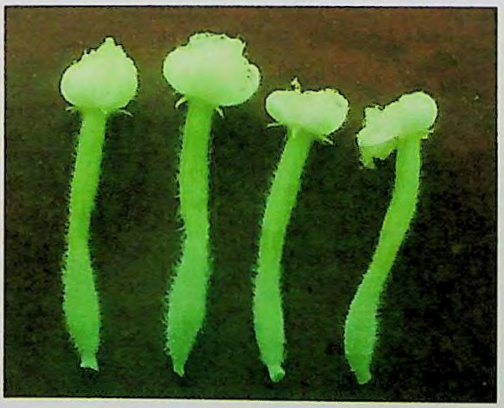
Pointed gourd plant with fruits

Two fruits in a leaf axil

Three fruits in a leaf axil

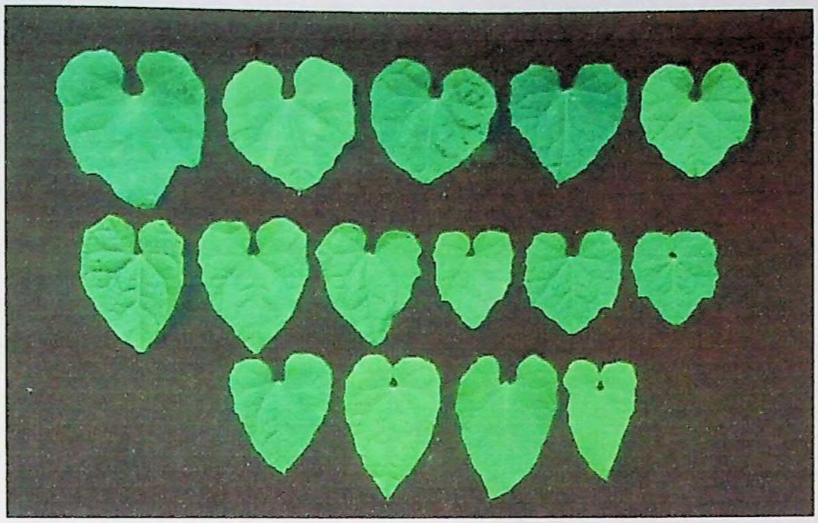


Male flower of pointed gourd



Female flower of pointed gourd

Fig. 1. Morphological study in pointed gourd



Variability in leaf color and shape

Variability in fruit color and shape

Fig. 2. Variability in leaf and fruit of pointed gourd

2.4 SUMMARY

A both qualitative and quantitative variations present in the characters of pointed gourd is one of the basic requirements for developing a new variety. Therefore, 25 genotypes of pointed gourd were characterized to study the variability present in morphological and agronomical characters of these genotypes.

Most of the qualitative characters studied showed variations among the pointed gourd genotypes. The characters such as stem color, stem pubescence, stem pubescence density, leaf color, leaf shape, leaf size, leaf blade tip angle, petiole color, node order of first female flower, node order of first fruit, fruit color, fruit shape, fruit curvature, fruit stripes, stem-end fruit shape, fruit apex shape, fruit skin-hardness, fruit skin texture, fruit ribs, color of ripening fruit, seed color, seed surface, seed shape and seed surface luster showed variations among the genotypes. While the other characters viz. growth habit, stem shape, stem pubescence type, leaf pubescence, prominence of leaf veins, leaf lobes, leaf margin, leaf type, leaf tip, leaf surface, tendrils, pedicel color, flower color, flowering habit, peduncle attachment and fruit flesh color showed no variations among the pointed gourd genotypes.

On the contrary, wide variations observed for all the quantitative characters in pointed gourd genotypes especially in fruit length, fruit weight, fruit volume, fruits per plant, fruit yield per plant, vines per plant, nodes per vine and vine length which are considered as important yield and yield contributing characters for improvement of this vegetable.

Chapter III GENETIC VARIABILITY IN QUANTITATIVE CHARACTERS

3.1 INTRODUCTION

Presence of genotypic variation is the basis of any crop improvement programme. In any breeding programme for improving the genetic pattern of plant depends upon the nature and magnitude of variability and the extent to which the desirable characters are heritable (Dudley and Moll, 1969). Phenotypic variability is the observable variation present in a character of a population. It includes both genotypic and environmental components of variation and as a result, its magnitude differs under different environmental conditions. Genotypic variation, on the other hand is the component of variation, which is due to the genotypic differences among the individuals within a population, and is the main concern of the plant breeder. The genotypic variability shown by the characters can be measured from the genetic coefficient of variation. Nevertheless, the genetic coefficient of variation alone is not sufficient to determine the amount of variation that is heritable (Swarup and Chaugale 1962). Quantitative or component characters of yield are governed by a large number of genetic factors that are also largely influenced by the environment. However, it is difficult to estimate whether the observed variation of a particular character is heritable or due to environment. Therefore, breeder requires knowledge on the nature and extent of variation present in the available materials. A large number of scientific works have been done by many scientists in various crops to measure the genetic variability (Singh *et al.*, 1985; Singh *et al.*, 1986; Singh and Prasad 1989; Prasad and Singh 1991a; Prasad and Singh 1991b; Singh *et al.*, 1993; Umesh *et al.*, 1995; Prasad *et al.*, 1999 and Behera *et al.*, 2003 in pointed gourd, Prasad and Prasad 1979 in bottle gourd, Mangal *et al.*, 1981 and Shafiullah *et al.*, 2003 in bitter gourd, Dahiya *et al.*, 1989 in round melon, Prasad and Singh 1989 in ridge gourd, Rana *et al.*, 1986 in pumpkin, Abusaleha and Dutta 1990a; Prasad and Singh 1992 and Islam *et al.*, 1993 in cucumber and Hossain *et al.*, 2000 in sweet gourd).

Yield whether it is grain yield or fruit yield is a complex character and governed by the number of component characters. But a breeder is always concerned with the selection of superior genotypes where performance is dependent on the phenotypic expression. Often selection based on phenotypic performance does not lead to expected genetic advance mainly due to the presence of genotype x environment

interaction as well due to the undesirable association between component characters at the genetic level. For rational approach towards the improvement of yield, selection has to be made for the components of yield. Therefore, the knowledge of association of component characters with yield is of great importance to plant breeders, as it helps in their selection with more precision and accuracy. The degree of relationship and association of these components with yield can be measured by the correlation coefficient studies. Genotypic correlation is the inherit association between two variables and is the main concern of the plant breeder. Estimation of genetic association along with phenotypic correlation not only displays a clear picture of the extent of inherent association but also indicates how much of this phenotypically expressed correlation is influenced by the environment.

Identification of important yield components helps in selection of high yielding cultivars. The efficiency of selection mainly depends on the direction and magnitude of association between yield and its components. But selection based on correlation without taking into consideration the interactions between the component characters may sometimes prove misleading (Codawat, 1980). Path coefficient analysis developed by Wright (1921) provides an exact picture of the relative importance of direct and indirect effects of each of the component characters towards yield.

Simple correlation measures only the mutual relationship, by which it becomes difficult to understand the direct and indirect effects of the components on the end product. Wright (1921, 1923) has developed an entirely different technique, which has proved extremely effective for many problems to theoretical genetics and for practical application in breeding as well as in the statistical analysis of cause and effect relationship in a system of correlated variables. Path coefficient analysis further permits the partitioning of the correlation coefficients into components of direct and indirect factor of association and provides an effective tool in finding out the direct and indirect contribution of different contributing characters towards yield. This will be helpful in effective selection for simultaneous improvement of the component characters towards yield. Many researchers worked with different crops to find out the relationship of yield and its component characters for selecting the characters more accurately towards the improvement of yield in pointed gourd (Singh *et al.*, 1993; Prasad and Singh 1990; Sarkar *et al.*, 1999 and Behera *et al.*, 2003), bottle gourd

(Tyagi, 1972 and Rahman *et al.*, 1986), pumpkin (Doijode and Sulladmath 1986; Saha *et al.*, 1992; Mangal *et al.*, 1979; Rana, 1982, and Gopalakrishna *et al.*, 1980), bitter gourd (Ramchandran *et al.*, 1978 and Lawande and Patil 1989), cucumber (Patil, 1988), snake gourd (Thamburaj *et al.*, 1978 and Raj *et al.*, 1984), summer squash (Pandita *et al.*, 1989), water melon (Zhang and Wang 1989), ribbed gourd (Abusaleha and Dutta 1990b) and in sweet gourd (Hossain *et al.*, 2000)

Information on genetic divergence among the plant materials is vital to a plant breeder for an efficient choice of parents for hybridization. Improvement in yield and quality is normally achieved by selecting genotypes with desirable character combinations existing in the nature or by hybridization. The parents identified on the basis of divergence analysis would be more promising. Thus, the genetic diversity plays an important role in plant breeding for any varietal improvement programme. The knowledge of genetic divergence of available genetic materials is essential to identify diverse genotypes for the purpose of hybridization programme. D^2 statistic proposed by Mahalanobis (1936) widely used for quantifying the genetic divergence among the population. The genotypes grouped together are less divergent than the ones, which are placed in different clusters. The clusters, which are separated by the greatest statistical distance, show the maximum divergence. This technique provides the information on magnitude of genetic diversification at intra and inter-cluster levels, which helps the breeder in the selection of genetically divergent parents for exploitation in hybridization programme. However, such information is not available in pointed gourd. But a number of scientists worked on genetic divergence in many other crops viz. in pumpkin (Masud *et al.*, 1995 and Rashid, 2000), in sponge gourd (Masud *et al.*, 2001), in ridge gourd (Masud *et al.*, 2003a), in sweet gourd (Masud *et al.*, 2003b), in upland cotton (Singh and Gupta, 1968), in green gram (Gupta and Singh, 1970), in sorghum (Govil and Murty, 1973), in sugarcane (Singh and Singh, 1979), in bread wheat (Dasgupta and Das, 1982), in rice (Singh *et al.*, 1986), in sugar beat (Kapur *et al.*, 1987), in opium poppy (Saini and Kaicker, 1987), in black gram (Dasgupta and Das, 1991), in winter wheat (Shoran and Tandon 1995), in mungbean (Ali *et al.*, 1996), in groundnut (Chowdhury and Uddin 1994), in chickpea (Kumar, 1997), in rice bean (Singh *et al.*, 1998), in soybean (Kumar and Nadarajan 1994) and in oat (Singh and Mishra 1996).

In Bangladesh yield of pointed gourd is not encouraging due to lack of research initiative towards developing improved variety. On this context, it is essential for characterization of existing land races of pointed gourd and launching breeding program for developing improved variety to produce maximum fruit yield per plant. In this circumstance, 25 pointed gourd germplasm were collected from different districts to study the genetic pattern for increasing yield potentialities through breeding. Systematic evaluation and characterization of pointed gourd genotypes that can be exploited for improving the genetic pattern for increasing yield potentialities through breeding. It is established that the success of any breeding programme depends upon the nature and magnitude of genetic variability presence in the existing germplasm materials. Fruit yield of pointed gourd is a complex trait contributed by a large number of component characters. For improving this traits breeder requires information on nature and extent of genetic variation, heritability and genetic advance, association of component characters with fruit yield and direct and indirect contribution of different contributing characters towards yield and the magnitude of genetic divergence existing in the gene pool. But till today no such information is available regarding these aspects in respect of yield and its component traits of available germplasm materials in Bangladesh.

Therefore, in the present investigation an attempt was made to indepth study on

i. nature and magnitude of genetic variability in the genotypes
ii. heritability and genetic advance
iii. association between fruit yield and its component characters and
iv. genetic divergence and clustering the genotypes based on genetic divergence

3.2 MATERIALS AND METHODS

Twenty five genotypes of pointed gourd were collected from the field of Plant Genetic Resources Centre (PGRC) during 2002-2003. The study was carried out at the experimental farm of Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur-1701 during October 2002 to August, 2003.

The basal portion of the vine were coiled in the shape of a ring and planted in the experimental field of Plant Genetic Resources Centre during rabi season on October

22, 2002. The vines of each plant were supported by bamboo trail for their steady growth.

3.3 EXPERIMENTAL DESIGN AND LAYOUT

The experiment was laid out in a Randomized Block Design with three replications. Each block within the replication consisted of 27 genotypes (including two male plants). The vines of each genotype were planted in the field maintaining 2.0 m spacing between the plants. The genctypes were randomly distributed to each pit within the blocks in each replication. The blocks were raised 20 cm above the ground level to safe the crop from water logging.

3.4 DATA COLLECTION

The following quantitative characters were taken for the study of genetic variability:

1. Nodes per vine: Average number of nodes of 5 randomly selected vines. Recorded at final harvest.
2. Vines per plant: Recorded at final harvest.
3. Vine length (m): Mean length of 5 randomly selected vines. Recorded at final harvest.
4. Fruits per plant: Recorded at final harvest.
5. Female flower length (cm): Mean length of 10 randomly selected female flowers. Recorded at flowering stage.
6. Fruit length (cm): Mean length of 10 randomly selected fruits. Recorded at harvested stage.
7. Fruit width (cm): Mean width of 10 randomly selected fruits. Recorded at harvest stage.
8. Fruit volume (cc): Mean volume of 10 randomly selected fruits. Recorded at harvested stage.
9. Fruit weight (g): Mean weight of 10 randomly selected fruits. Recorded at harvested stage.
10. Fruit yield per plant: Weight of fruits per plant. Recorded at harvested stage.

3.5 STATISTICAL ANALYSIS

The collected data were analyzed following the biometrical techniques of analysis developed by Mather (1949) based on mathematical model of Fisher *et al.* (1932). Mean and standard error were worked out by the method of analysis of variance used for randomized block design. The techniques of analysis of data used are described under the following sub-heads:

A. Estimation of genetic parameters

i) Mean

It is the arithmetic mean or average and computed by dividing the sum of all observations in a sample by their number. It was computed by using the following formula:

$$\text{Mean} = \frac{\sum_{i=j}^{n} x_i}{N}$$

Where, x = individual reading recorded on each plant

N = number of observation

i = 1, 2, 3 .....n

Σ = summation

ii) Range

It is the difference between the lowest and highest values present in the observation included in the study. In this study, lowest and highest values are shown to represent the range of variation.

iii) Standard error

It is the measure of uncontrolled variation present in a sample. It is estimated by dividing the estimate of standard deviation by the square root of the number of observations in the sample, and is denoted by SE. Thus,

$$SE = \frac{SD}{\sqrt{N}}$$

Where, SE = standard error

SD = standard deviation

N = number of observation

iv) Analysis of variance

Variance analysis is a measure of dispersion of a population or sample. So, for testing the significant differences among the population or samples, the analysis of variance is necessary. It is expressed as the sum of square of deviations of all observations of a sample from its mean and divided by the degree of freedom (n-1).

It was computed by using formula following Singh and Chaudhary (1985) as follows:

$$\delta^2 = \frac{\sum_{i=1}^{n} x_i^2 - (\sum_{i=1} x_i)^2/n}{n-1}$$

Where, δ^2 = variance

x = individual reading

n = total number

Σ = summation

i = 1, 2, 3.....n

n-1 = degrees of freedom

In analysis of variance, the fixed model was used and the expected mean sum square (EMS) was determined as follows:

Analysis of variance

Source of variation	d.f	MS	EMS
Replication	R-1	MSr	$\delta^2 e + \delta^2 r$
Genotype	G-1	MSg	$\delta^2 e + \delta^2 g$
Error	(R-1) (G-1)	MSe	$\delta^2 e$
Total	GR-1	MSt	-

Where, R = replication

G = genotype

MSr = mean square of replication

MSg = mean square of genotype

MSe = mean square of error

MSt = mean square of total

$\delta^2 g$ = variance due to genotype

$\delta^2 e$ = variance due to environment

v) Estimation of variance components

Genotypic and phenotypic variances were estimated by the following formula as suggested by Singh and Chaudhary (1985):

$$\delta^2 g = \frac{MSv - MSe}{r}$$

$$\delta^2 p = \delta^2 g + MSe$$

Where, $\delta^2 g$ = genotypic variance

$\delta^2 p$ = phenotypic variance

MSe = genotypic mean sum of squares

MSv = error mean sum of squares

r = number of replications

vi) Estimation of genotypic and phenotypic coefficient of variation

Genotypic and phenotypic coefficients of variation were estimated as per Singh and Chaudhary (1985) by the following formulae:

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\delta^2 g}{\overline{X}} \times 100$$

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\delta^2 p}{\overline{X}} \times 100$$

Where, $\delta^2 g$ = genotypic variance

$\delta^2 p$ = phenotypic variance

$\overline{X}$ = population mean

vii) Estimation of heritability

The broad sense heritability of a character was calculated by using the following formula as suggested by Singh and Chaudhary (1985):

$$\text{Heritability in percentage (broad sense)}, h^2 b = \frac{\delta^2 g}{\delta^2 p} \times 100$$

Where, $\delta^2 g$ = genotypic variance

$\delta^2 p$ = phenotypic variance

viii) Estimation of genetic advance

Genetic advance was estimated adopting the formula as suggested by Singh and Chaudhary (1985).

$Gs = H.K.\ \delta p$

$$\text{Genetic advance expressed as percentage of mean} = \frac{Gs}{\overline{X}} \times 100$$

Where, H = heritability in broad sense

δp = phenotypic standard deviation

K = selection differential in standard unit (value of K at 5 percent selection intensity = 2.06)

$\overline{X}$ = population mean

ix) Estimation of genotypic and phenotypic correlation coefficients

Estimation of genotypic and phenotypic correlation coefficients was estimated by the formula suggested by Miller *et al.* (1958).

$$\text{Genotypic correlation, } r_g = \frac{Cov.g_{12}}{\sqrt{\delta^2 g_1 x\ \delta^2 g_2}}$$

Where, $Cov.g_{12}$ = genotypic covariance between the traits x_1 and x_2

$\delta^2 g_1$ = genotypic variance of the trait x_1

$\delta^2 g_2$ = genotypic variance of the trait x_2

$$\text{Similarly, phenotypic correlation, } r_{ph} = \frac{Cov.\ Ph_{12}}{\sqrt{\delta^2 ph_1 x\ \delta^2 ph_2}}$$

Where, Cov. ph_{12} = phenotypic covariance between the traits x_1 and x_2.

$\delta^2 ph_1$ = phenotypic variance of the trait x_1

$\delta^2 ph_1$ = phenotypic variance of the trait x_2

x) Path coefficient analysis

The components of genotypic correlation coefficients of different yield component characters with fruit yield were separated into direct and indirect effects through path coefficient analysis. The analysis requires the solution of a set of linear equations as suggested by Dewy and Lu (1959). Assuming three independent (x_1,

x_2 and x_3) and one dependent variables (x_4) the relationship between them can be represented as follows:

$p_{14} + r_{12}.p_{24} + x_{13}. p_{34} = r_{14}$

$r_{12}. p_{14} + p_{24} + r_{23}. p_{34} = r_{24}$

$r_{13}. p_{14} + r_{23}. p_{24} + p_{34} = r_{34}$

Where, p_{14}, p_{24} and p_{34} = path coefficient of the variables x_1, x_2 and x_3 on variable x_4, respectively.

r_{14}, r_{24} and r_{34} = correlation coefficient of x_1, x_2 and x_3 with x_4, respectively.

B. Estimation of genetic divergence

a) Analysis of data

Mean data of the characters were subjected to both univariate and multivariate analysis. Univariate analysis of the individual character (analysis of variance) was done by computer using MSTAT-C software. The test of significance was done by F-test. Mean, range and co-efficient of variation (CV %) was also estimated using MSTAT-C Multivariate analysis was done by computer using GENSTAT 5.13 and Microsoft Excel XP software through four techniques *viz,* principal component analysis (PCA), principal coordinate analysis, cluster analysis and canonical vector analysis.

1. Principal component analysis (PCA)

The technique PCA was used to examine the inter relationship among 10 quantitative characters. The principal components were computed from the correlation matrix (obtained from sum of squares and products matrix of the characters) and genotypes scores (obtained from the first components and the succeeding component with latent roots greater than unity). The latent roots are called 'Eigeen values'. The first component has the property of accounting for maximum variance. The PCA displays most of the original variability in a smaller number of dimensions, since it finds linear components of a set of variate that maximize the variation contained within them. Contributions of the different characters towards divergence are discussed from the latent vectors of the first two principal components.

2. Principal coordinate analysis (PCO)

PCO was used to calculate the inter genotype distance and it gave the minimum distance between each pair of the N points using similarity matrix through the use of all dimensions of P (Digby *et al.* 1989).

3. Cluster analysis (CA)

Cluster analysis was performed by D^2 analysis (originally outlined by Mahalanobis, 1928, 1936 and extended by Rao, 1952), which divides the genotypes based on the data set into more or less homogenous groups. D^2 is the sum of squares of differences between any two populations for each of the uncorrelated variables (obtained by transforming correlated variables through Pivotal condensation method). Clustering was done using non-hierarchical and hierarchical classification. D^2-statistic is defined by

$$D^2 x = \sum_{i}^{p} \sum_{j}^{p} (\lambda^{ij})\, d_i d_j$$

Where, x = number of metric traits in point

p = number of populations or genotypes

λ^{ij} = the matrix reciprocal to the common dispersion matrix

$d_i d_j$ = the differences between the mean values of the two genotypes for the i^{th} and j^{th} characters, respectively.

In simpler form D^2 statistic is defined by the formula

$$D^2 = \sum_{i}^{x} d_i^2 = \sum_{i}^{x} (y^j{}_i - y^k{}_j) \qquad (j \neq k)$$

Where, y = uncorrelated variable (character) which varies from i= 1 to x

x = number of characters

Superscripts j and k to y = a pair of any two genotypes

Cluster analysis was performed by computer software Genstat 5.13, which used algorithm to search for optimal values of the chosen criterion. The algorithm did some initial classification of the genotypes into required number of groups and then repeatedly transfers genotypes from one group to another so long as such transfer improved the value of the criterion. When no further transfer could be found to

improve the criterion, the algorithm switched to a second stage, which examined the effect of swooping of two genotypes of different groups, and so on.

4. Canonical vector analysis (CVA)

CVA complementary to D^2 statistic is a sort of multivariate analysis where canonical vectors and roots representing different axes of differentiation and the amount of variation accounted by each of such axes, respectively are derived. Canonical vector analysis finds linear combination of original variability that maximize the ratio of between groups to within groups variation, thereby giving functions of the original variables that can be used to discriminate between the groups. Thus, in this analysis, a series of orthogonal transformations sequentially maximize the ratio of among groups to within group variations.

b) Computation of average intra-cluster distances

The average intra-cluster distance for each cluster was calculated by taking all possible D^2 values within the members of a cluster obtained from PCO. The formula used to measure the average intra-cluster distance was:

Intra-cluster distance = $\Sigma D^2/n$

Where, ΣD^2 is the sum of distances between all possible combinations (n) of the genotypes included in a cluster.

The square root of the D^2 represents the distance (D) within cluster.

c) Cluster diagram

A cluster diagram was drawn using the values (D) of inter and intra-cluster distances. The diagram represented the pattern of diversity among the genotypes and relationships between different genotypes included in the clusters.

d) Selection of genotypes for future hybridization programme

Divergence analysis is usually performed to identify the diverse genotypes for hybridization purposes. The genotypes grouped together are less divergent among themselves than those, which fall into different clusters. Clusters separated by the largest statistical distance (D^2) express the maximum divergence among the genotypes included into these different clusters.

Singh and Chaudhary (1985) stated the following points should be considered while selecting genotypes for hybridization:

i. Choice of cluster from which genotypes are selected for use as parents
ii. Selection of particular genotype(s) from the selected cluster(s).
iii. Relative contribution of the characters to the total divergence.
v. Other important characters of the genotypes (*per se* performance).

3.6 RESULTS AND DISCUSSION

In the present study, 25 genotypes of pointed gourd were evaluated to find out the extent of genetic variability for yield and its component characters through genetic variance, heritability and genetic advance. Relationship between yield and its component characters were studied. In addition, for identifying the genetically diverse parents in hybridization programme multivariate analysis was also studied. Mean values of important quantitative characters are given in appendices 9, 10 and 11. The results are discussed under the following subheads:

3.6.1 Variations among the genotypes

Analysis of variance showed highly significant variations among the characters. This indicated that considerable amount of genotypic differences existed in the characters under study (Table 3.1). Singh and Prasad (1989) reported significant differences among the cultivars for the characters viz. number of nodes, number of shoots, number of fruits, fruit width, fruit volume and fruit yield per plant in pointed gourd. Highly significant results due to genotypes were also observed by Prasad and Singh (1991a), Prasad and Singh (1991b), Prasad *et al.* (1999) and Prasad and Singh (1994) for fruit number, fruit weight, fruit yield per plot, fruit length, node number, vine length, shoot number and seed number in pointed gourd. Similar results were reported by Singh *et al.* (1985) and Singh *et al.* (1992) in pointed gourd for vine length, fruit number, fruit length, fruit diameter, fruit weight and fruit yield.

3.6.2 Mean, range and genotypic and phenotypic variance

Wide variations were observed for all the characters except female flower length and fruit width (Table 3.2). These two characters exhibited the minimum range of 1.39-2.44 cm and 2.88-4.06 cm with a general mean of 2.08±0.05 and 3.69±0.06 indicating narrow range of variability. These characters were affected by the environment as

genotypic and environmental variances were almost equal. The remaining characters were less influenced by the environment, as they showed less environmental variances.

The range of variation was maximum in fruits per plant (32-159), fruit yield per plant (1.12-7.24), vines per plant (4-10), vine length (3.53-7.50 m), fruit length (7.72-12.17 cm), fruit volume (38.02-77.55 cc) and fruit weight (29.06-58.41 g), indicating remarkable variations were present among the genotypes.

The fruit yield per plant exhibited the maximum range of variation (1.12-7.24) with a mean of 3.09±0.30. The general mean value for fruits per plant was 80.00±7.65, but the range varied from 32-159. Characters, which showed high range of variation, should be given priority in the selection (Vijay, 1987). While the range of variation was low for the characters indicated narrow range of variability among the genotypes and suggested that selection would not be effective for these traits.

3.6.3 Genotypic and phenotypic coefficient of variation

Coefficient of variability is the relative measure of dispersion (Singh *et al.*, 1992). Genotypic coefficient of variations was computed to find out the amount of variation caused by genotypic and environment (Srivastava and Srivastava, 1976). Sufficient amount of genetic variability is prerequisite for starting a breeding programme in any crop. The results of this investigation indicated that considerable amount of genetic variability exists in respect of various characters in pointed gourd and therefore, selection should be effective in improving the yield.

In the present investigation, phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the characters studied (Table 3.3). These findings are in agreement with those reported by Singh *et al.* (1992) in pointed gourd, Masud *et al.* (1998) in pumpkin, Singh *et al.* (1977) in bitter gourd. The range of phenotypic coefficient of variation was 10.45-50.45. Higher values of phenotypic coefficient of variation were observed in fruits per plant (48.84) and fruit yield per plant (50.45). Similar to PCV, fruits per plant (47.17) and fruit yield per plant (48.34) showed high genotypic coefficient of variation. The fruits per plant, vine length and fruit yield per plant showed higher genotypic coefficient of variation which offered scope for their improvement as they were less affected by the environment. Moreover,

the differences in PCV and GCV estimates were narrow, confirming the least environmental influence in attaining the observed variability. These results are in agreement with that of Saha *et al.* (1992) and Masud *et al.* (1998) as they estimated higher levels of variability in pumpkin. Burton and de Vane (1953) and Singh *et al.* (1985) opinioned that the higher GCV estimates could be of potential indicator for effective selection. Highest genotypic coefficient of variability was recorded for fruit yield per plant, which indicated the possibilities of utilization of the variation for improvement. Similar results were reported by Singh *et al.* (1992) and Masud *et al.* (1998) in pointed gourd and pumpkin, respectively. High genetic coefficient of variation (GCV) estimate was also observed in pointed gourd for the characters such as length of vine, number of nodes per plant and number of fruits per plant (Behera *et al.,* 2003). Higher magnitude of genetic variance suggested the presence of high genetic variability. These results are in agreement with Singh *et al.* (1977) in bitter gourd and Singh (1983) in pointed gourd. The characters showing low genotypic coefficient of variation value indicated that they were more influenced by the environment. The GCV values for remaining characters were more or less low, which showed the low genetic variability as they were more influenced by the environment and limited scope for improvement through selection for these characters. Genotypic coefficient of variation was lower than the corresponding phenotypic one, which indicated the larger influence of environment. This finding is in full agreement with Masud *et al.* (1998) in pumpkin.

Table 3.1. Analysis of variance for yield and yield contributing characters in pointed gourd

Source of variation	d.f.	Nodes per vine	Vines per plant	Vine length (m)	Fruits per plant	Female flower length (cm)	Fruit length (cm)	Fruit width (cm)	Fruit volume (cc)	Fruit weight (g)	Fruit yield per plant (kg)
Block	2	338.440	2.893	0.147	569.053	0.012*	0.085	0.006	0.153	1.192	1.287
Genotype	24	390.608**	6.463**	3.825**	4390.808**	0.218**	3.510**	0.282**	362.109**	206.418**	6.856**
Error	48	44.468	1.435	0.236	103.039	0.034	0.399	0.082	6.514	4.418	0.176

** Significant at 1% level

Table 3.2. Mean, range and phenotypic and genotypic variances for different characters of pointed gourd

Character	Mean ± SE	Range	Phenotypic variance	Genotypic variance	Environmental variance
Nodes per vine	64.12 ± 2.28	49-95	159.85	115.38	44.47
Vines per plant	7.05 ± 0.29	4-10	3.11	1.68	1.44
Vine length (m)	5.16 ± 0.23	3.53-7.50	1.43	1.20	0.24
Fruits per plant	80.15 ± 7.65	32-159	1532.30	1429.26	103.04
Female flower length (cm)	2.08 ± 0.05	1.39-2.44	0.10	0.06	0.03
Fruit length (cm)	10.16 ± 0.22	7.72-12.17	1.44	1.04	0.40
Fruit width (cm)	3.69 ± 0.06	2.88-4.06	0.15	0.07	0.08
Fruit volume (cc)	61.18 ± 2.19	38.02-77.55	124.29	118.00	6.79
Fruit weight (g)	46.35 ± 1.64	29.06-58.41	70.32	66.23	4.10
Fruit yield per Plant (kg)	3.09 ± 0.30	1.12-7.24	2.45	2.25	0.20

3.6.4 Heritability and genetic advance

The genotypic coefficient of variation does not offer full scope to estimate the variation that is heritable and therefore, estimation of heritability becomes necessary. Heritability estimates are useful in selection on the basis of the phenotypic performance of the quantitative characters. The characters with high heritability value could be improved straight way through selection since they are less affected by the environment. The degree of success of a selection programme also depends upon the magnitude of heritable variation.

In the present study, estimates of broad sense heritability were very high for fruit volume (94.56 %) followed by fruit weight (94.18 %), fruit yield per plant (93.28 %), vine length (83.30 %), fruit length (72.31 %) and nodes per vine (72.18 %) which indicated the further substantiate the scope of genetic improvement for these

Table 3.3. Phenotypic and genotypic coefficients of variation, heritability in broad sense and genetic advance for different characters in pointed gourd

Character	PCV	GCV	Heritability (h^2b)	Genetic advance	GA as % of mean
Nodes per vine	19.72	16.75	72.18	18.80	29.32
Vines per plant	25.01	18.36	53.87	1.96	27.75
Vine length	23.23	21.20	83.30	2.06	39.87
Fruit per plant	48.84	47.17	93.28	75.22	93.85
Female flower length	14.86	11.94	64.51	0.41	19.75
Fruit length	11.80	10.04	72.31	1.79	17.58
Fruit width	10.45	6.98	44.60	0.35	9.61
Fruit volume	18.26	17.75	94.56	21.76	35.56
Fruit weight	18.09	17.56	94.18	16.27	35.10
Fruit yield per Plant	50.45	48.34	91.80	2.96	95.41

PCV= Phenotypic coefficient of variation; GCV= Genotypic coefficient of variation

characters. Similar results for fruit weight and fruit yield have been reported by Singh *et al.* (1992), Sachan and Tikka, (1971) and Singh *et al.* (1977) in pointed gourd, water melon and bitter gourd, respectively. Singh and Prasad (1989) and Masud *et al.* (1998) reported high heritability for number of fruits per plant, fruit weight and fruit yield in pointed gourd and pumpkin, respectively. Behera *et al.* (2003) also reported high heritability for length of vine, number of nodes per plant and number of fruits per plant in pointed gourd. High estimates of heritability for above mentioned traits suggested that the selection based on phenotypic performance would be more effective (Johnson *et al.*, 1995a and Singh *et al.*, 1992).

The remaining characters viz. fruit width (44.60 %), vines per plant (53.87 %) and female flower length (64.51 %) showed low and moderate level of heritability. These three characters had also low genotypic coefficient of variation (6.98, 18.36 and 11.94, respectively). This suggested that fruit width, vines per plant and female flower

length were markedly influenced by the environment. In pointed gourd, high heritability also observed by Singh *et al.* (1985) for fruit length, fruit diameter, fruit weight and yield per plant.

Although estimates of heritability are useful to plant breeder as they provide basis of selection, more reliable conclusion can be made when heritability is considered in conjunction with genetic advance. Johnson *et al.* (1955a) suggested that heritability and genetic advance when calculated together are more useful for predicting the resultant effect of selecting the best individual than heritability and genetic advance calculated alone. High heritability value along with high value of genetic advance as percent of mean is most effective condition for selection (Gandhi *et al.*, 1964). In the present study, the maximum genetic gain of 95.41% (expressed as % mean) was observed in fruit yield followed by fruits per plant (93.85%). Characters like fruit yield and fruits per plant showed high heritability value as well as high value of genetic advance as percent of mean (Table 3.3). Such condition arises due to the action of additive genes (Panse, 1957). This suggested that selection for these two characters would be more fruitful. These characters were less influenced by environment and simply inherited by a few additive genes. It suggests that selection of these characters could be more straightforward and effective (Masud *et al.*, 1998). These findings are in full agreement with earlier reports for yield in winter melon (Sachan and Tikka, 1971), number of fruits per plant and yield in bitter gourd (Srivastava and Srivastava, 1976), fruits per plant, yield per plant and fruit weight in pumpkin (Masud *et al.*, 1998) and number of fruits per plant and fruit yield in pointed gourd (Singh *et al.*, 1992). Additionally, high heritability coupled with high genetic advance in pumpkin was reported for fruits per plant (Mangal *et al.*, 1979 and Rana *et al.*, 1986). These findings were supported by the present study. Further more, heritability and genetic advance patterns for fruits per plant and yield were corroborated with those of Saha *et al.* (1992) and Doijode and Sulladmath (1986) in pumpkin.

There are other characters like fruit volume, fruit weight, vine length, fruit length and nodes per vine, which showed high value of heritability but low value of genetic advance as percent of mean. This is because of nonadditive gene action, which includes dominance and epistasis (Liang and Walter, 1968). Since higher heritability

coupled with high genetic advance indicates the importance of the character, the improvement can be made by repeated selection for these characters. Similar results were obtained by Singh and Prasad (1989) and Singh *et al.* (1985) in pointed gourd. High heritability estimates with high genetic advance for fruits per plant was also reported by Mangal *et al.* (1979) and Rana *et al.* (1986) in pumpkin and their findings were in the same trend with the present study. Further, heritability and genetic advance pattern for fruit weight and yield per plant were matched with those reported by Saha *et al.* (1992) and Doijode and Sulladmath (1986) in pumpkin.

Low heritability along with low genetic advance was observed in fruit width (44.60% and 9.61%), vines per plant (53.87% and 27.75%) and female flower length (64.51% and 19.75%) supporting the polygenic inheritance of these traits. Similar results obtained by Masud *et al.* (1998) for fruit diameter (34.15% and 11.63%) in pumpkin. Hence, direct selection for these characters would not be beneficial (Singh *et al.*, 1977).

Therefore, it can be concluded that fruits per plant and fruit yield having high heritability associated with high genetic advance and also high GCV confirmed additive gene action suggesting effective selection could be made for these characters.

3.6.5 Correlation studies

Relationship between yield and its component characters were studied at genotypic and phenotypic levels. Genotypic correlation coefficients between fruit yield and its component characters are presented in Table 3.4. In general, genotypic correlation coefficients were higher than their corresponding phenotypic correlation coefficients indicating that there is a strong inherent association between the characters studied, the phenotypic expression of the correlation being reduced under the influence of the environment. The magnitudes of genotypic correlation coefficients were observed higher than that of corresponding phenotypic correlation coefficients for most of the character associations (Table 3.4). Higher genotypic correlations than phenotypic ones were possibly due to modifying or masking effect of environment in the expression of the different characters (Nandpuri *et al.*, 1973). Higher and wider genotypic correlations than phenotypic correlations have been reported by Sarkar *et al.* (1999) in pointed gourd (*Trichosanthes dioica* Roxb.), Johnson *et al.* (1955b) in soybean [*Glycin max* (L.) Merr.], Mital *et al.* (1969) in cluster bean [*Cymopsis tetragonoloba*

(L.) Taub.] and Sharma and Swarup (1964) in cabbage (*Brassica oleracea* L. var. *capitata* L.). Tyagi (1987) and Dhanda *et al.* (1984) also reported higher magnitude of genotypic correlation coefficients over phenotypic ones between yield and its yield contributing characters.

Higher genotypic correlation coefficients than phenotypic correlation coefficients between various pairs of characters have also been observed in different species of cucurbit vegetables like pumpkin (Doijode and Sulladmath, 1986), muskmelon (Swamy *et al.*, 1984), water melon (Hossain, 1989), cucumber (Nishi and Kurigama, 1963) and bitter gourd (Singh *et al.*, 1977). On the other hand, nodes per vine with fruit length and fruit volume, vines per plant with fruit width and fruit volume, vine length with fruit length and fruit volume, fruits per plant with female flower length, female flower length with fruit length and fruit width and fruit length with fruit width had higher phenotypic correlation coefficients than corresponding genotypic correlation coefficients indicating remarkable environmental influence on the association of these characters.

It appears that fruits per plant was highly significant and positively correlated with fruits yield per plant at both genotypic and phenotypic level (Table 3.4) indicated that the yield per plant could be improved by selection for this character. These results are in consonance with the finding of Singh *et al.* (1993), Singh and Prasad (1989), Singh (1983) and Behera *et al.*, (2003) in pointed gourd, Panwar *et al.* (1977) in sponge gourd and Rana (1982) in pumpkin. The estimates of heritability, genotypic and phenotypic coefficient of variation and the expected genetic advance was also higher for these characters. This suggested that direction for these two characters would be beneficial for the improvement of yield. Significant positive correlation between yield per plant and fruits per plant was reported in summer squash (r_g = 0.678) by Pandita *et al.* (1989). Tyagi (1972) also observed significant positive correlation between fruit length and yield in bottle gourd (r_g=0.426). Moreover, Swamy *et al.* (1984) reported significant positive correlation between fruit yield and seed weight per fruit (r_g=0.589) in muskmelon. The correlation between vines per plant and fruit yield per plant (0.458, 0.504), fruit volume and fruit yield (0.398, 0.413) and fruit weight and fruit yield (0.484, 0.504) were also positive and significant at both genotypic and phenotypic level. Sarkar *et al.* (1999) also reported that fruit weight, fruit diameter

and number of primary branches per plant were positively and significantly correlated with yield per plant at genotypic and phenotypic levels. Highly significant positive genotypic and phenotypic correlations were observed between nodes number per vine (0.837) and vine length (0.849), respectively.

Nodes per vine showed nonsignificant negative correlation with vines per plant (-0.017, -0.141), fruits per plant (-0.111, -0.182), female flower length (-0.270, -0.390) and fruit length (-0.067, -0.075) both at genotypic and phenotypic level except fruit volume (0.016, -0.008). Negative correlation observed between nodes per vine and fruit volume only at the genotypic level. Similarly, vines per plant showed nonsignificant negative correlation with female flower length (- 0.118, - 0.305) and fruit length (-0.095, -0.220). Moreover, fruits per plant exhibited nonsignificant negative correlation with female flower length (-0.147, -0.136).

For interrelationship between yield components, a positive and significant association was observed between vines per plant and fruits per plant (0.486, 0.547) at genotypic and phenotypic level. Female flower length showed positive and highly significant correlation with fruit length (0.596, 0.544), fruit volume (0.575, 0.629) and fruit weight (0.497, 0.514). Female flower length also showed significant and positive correlation with fruit width (0.459) only at phenotypic level. Fruit length was positively and significantly correlated with fruit volume (0.588, 0.613) and fruit weight (0.597, 0.609). Fruit width showed positive and highly significant correlation with fruit volume (0.646, 0.836) and fruit weight (0.681, 0.855). A very strong positive and significant correlation was observed between fruit volume and fruit weight (0.941, 0.953). Singh and Prasad (1989) also reported highly significant positive correlation between fruit volume and fruit weight (0.636) and significant negative correlation in fruit width and nodes per vine (0.624). Contrarily, nodes per vine showed nonsignificant positive correlation with fruit width (0.067, 0.004) at both genotypic and phenotypic levels.

Table 3.4. Correlation coefficients among yield and yield contributing characters in pointed gourd

Character		Vines per plant	Vine length	Fruits per plant	Female flower length	Fruit length	Fruit width	Fruit volume	Fruit weight	Fruit yield per plant
Nodes per vine	r_p	-0.017	0.837**	-0.111	-0.270	-0.029	0.067	0.016	0.124	-0.048
	r_g	-0.141	0.849**	-0.182	-0.390	-0.075	0.004	-0.008	0.142	-0.106
Vines per plant	r_p		0.086	0.486*	-0.118	-0.095	0.129	0.039	0.001	0.458*
	r_g		0.112	0.547**	-0.305	-0.220	0.116	0.021	-0.002	0.504**
Vine length	r_p			0.165	-0.372	-0.049	0.067	0.016	0.184	0.195
	r_g			0.170	-0.472	-0.031	0.139	0.004	0.207	0.208
Fruits per plant	r_p				-0.147	0.090	0.031	0.068	0.161	0.917**
	r_g				-0.136	0.126	0.064	0.079	0.181	0.924**
Female flower length	r_p					0.596**	0.459*	0.575**	0.497**	0.019
	r_g					0.544**	0.286	0.629**	0.514**	0.030
Fruit length	r_p						0.314	0.588**	0.597**	0.247
	r_g						-0.032	0.613**	0.609**	0.282
Fruit width	r_p							0.646**	0.681**	0.264
	r_g							0.836**	0.855**	0.358
Fruit volume	r_p								0.941**	0.398*
	r_g								0.953**	0.413*
Fruit weight	r_p									0.484*
	r_g									0.504**

*, ** Significant at 5% and 1% level, respectively.

3.6.6 Path coefficient analysis

Association of characters determined by correlation coefficients may not provide an exact picture of the relative importance of direct and indirect influence of each of the yield components towards yield. As a matter of fact, in order to find a clear picture of the inter-relationship between fruit yield and other yield components, direct and indirect effects were worked and using path analysis at genotypic level which also measured the relative importance of each factor.

The results of the path analysis (Table 3.5) revealed that fruits per plant had the highest positive direct effect (0.9112) on fruit yield followed by fruit volume (0.2215). Srivastava and Srivastava (1976) reported that fruits per plant had high positive direct effect on yield (0.9030) in pointed gourd. Positive and high direct effects were also noted for fruit volume, fruit weight, fruit diameter and number of fruits per plant on fruit yield in pointed gourd (Sarkar *et al.*, 1999). High positive direct effect of fruits per plant on fruit yield was reported in pumpkin by Masud *et al.* (1998) and Saha *et al.* (1992). Moreover, similar results observed in muskmelon by Kalloo and Sidhu (1982). It appeared that fruits per plant and fruit volume were the major component traits for fruit yield in pointed gourd. Fruits per plant also showed the highest significant positive genotypic correlation with fruit yield, which was obtained merely because of a considerably high direct effect of fruits per plant on yield. This result suggests that an increase in fruits per plant will increase the fruit yield per plant. The direct effects of vine length and female flower length on fruit yield were negative. But these two characters had positive and nonsignificant genotypic correlation with yield, which might be due to indirect effect of these characters on yield.

Fruit length and fruit width though had positive direct effect (0.0374, 0.0906) on fruit yield but the genotypic correlation with yield was nonsignificant. The direct effects of fruit length and fruit width on fruit yield were diluted mainly due to negative indirect effect via female flower length (-0.0380). Consequently, such anomalous situation suggested that a restricted simultaneous selection model could be followed to nullify the undesirable indirect effects to make proper use of the direct effects (Saha *et al.*, 1992). These characters showed no remarkable positive indirect effect via remaining characters on yield.

Though vine length (-0.1115) and female flower length (-0.0699) showed negative direct effect on fruit yield, they had positive but nonsignificant correlation with fruit yield, which possibly might be due to positive indirect effects of these traits via fruits per plant (0.1549) and fruit volume (0.1393). Nodes per vine (0.1227), vines per plant (0.0072), fruit volume (0.2215) and fruit weight (0.0693) had positive direct effect on fruit yield but the characters like vines per plant, fruit volume and fruit weight showed positive and significant correlation with fruit yield. Direct effect, total indirect and largest indirect effect through angle variable is shown in Fig. 3.

It clearly indicates that the fruits per plant and fruit volume are the most important components of fruit yield. Therefore, emphasis should be given on fruits per plant followed by fruit volume in selecting good genotype for improvement of yield in pointed gourd.

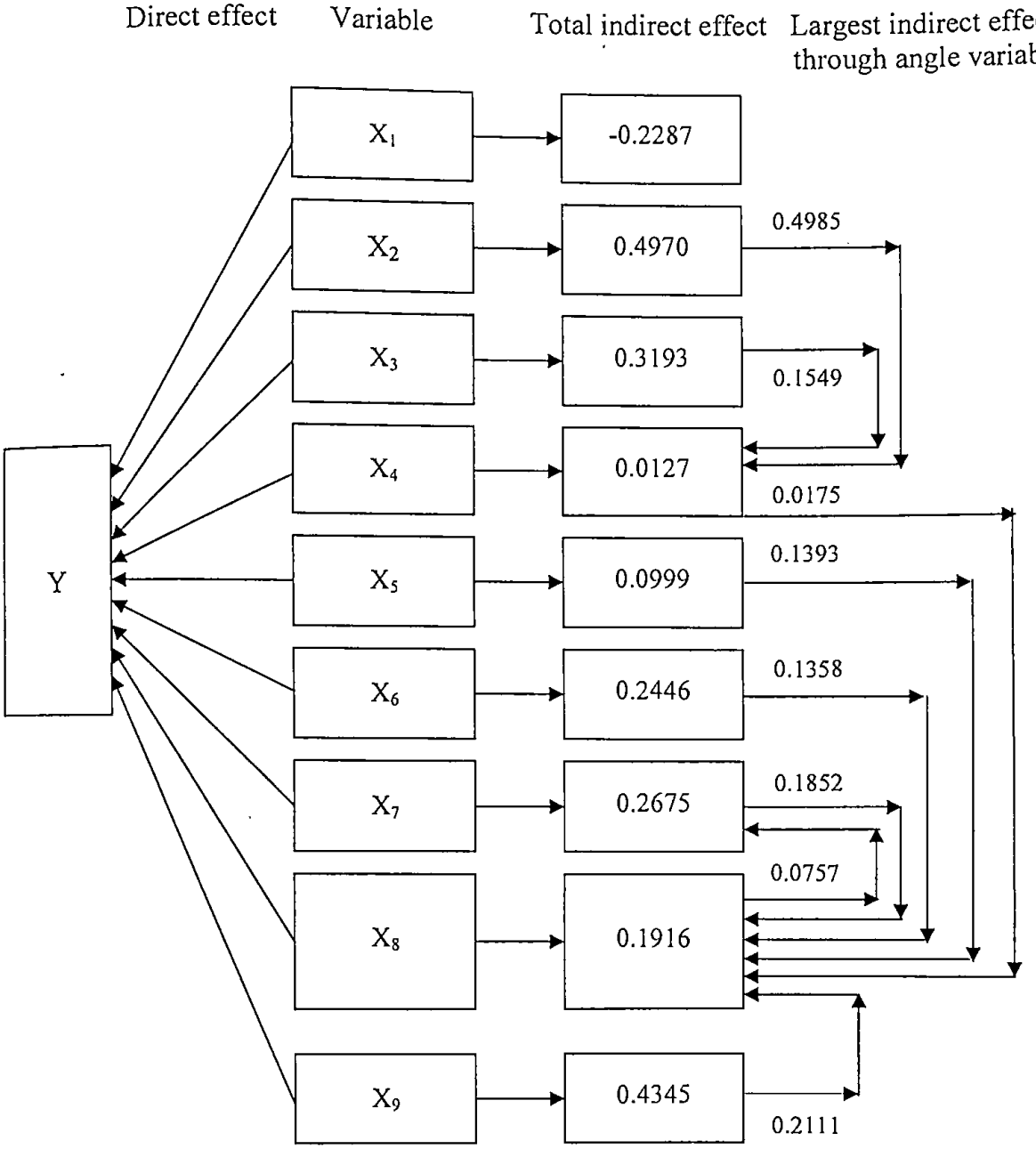
The estimated residual effect was 0.154 indicating that about 85 percent of the variability in fruit yield was contributed by ten characters studied in path analysis. This residual effect towards yield in the present study might be due to many reasons such as other factors like environmental factor and sampling errors, which are not included in the investigation (Sengupta and Kataria, 1971). Within the scope of the path analysis carried out in the present study it is therefore, suggested that the fruits per plant is the main component of fruit yield should be given high priority in the selection programme.

Table 3.5. Path coefficients of nine yield contributing characters on fruit yield in pointed gourd

Character	Nodes per vine	Vines per plant	Vine length	Fruits per plant	Female flower length	Fruit length	Fruit width	Fruit volume	Fruit weight	r_g with fruit yield
Nodes per vine	**0.1227**	-0.0010	-0.0947	-0.1659	0.0273	-0.0028	0.0004	-0.0018	0.0098	-0.106
Vines per plant	-0.0172	**0.0072**	-0.0125	0.4985	0.0213	-0.0082	0.0105	0.0047	-0.0001	0.504 **
Vine length	0.1041	0.0008	**-0.1115**	0.1549	0.0329	-0.0012	0.0126	0.0009	0.0143	0.208
Fruits per plant	-0.0223	0.0039	-0.0189	**0.9112**	0.0095	0.0047	0.0058	0.0175	0.0125	0.924 **
Female flower length	-0.0478	-0.0022	0.0526	-0.1239	**-0.0699**	0.0204	0.0259	0.1393	0.0356	0.030
Fruit length	-0.0092	-0.0016	0.0035	0.1148	-0.0380	**0.0374**	-0.0029	0.1358	0.0422	0.282
Fruit width	0.0005	0.0008	-0.0155	0.0583	-0.0199	-0.0012	**0.0906**	0.1852	0.0593	0.358
Fruit volume	-0.0009	0.0002	-0.0004	0.0719	-0.0439	0.0229	0.0757	**0.2215**	0.0661	0.413 **
Fruit weight	0.0174	-0.0001	-0.0231	0.1649	-0.0359	0.0227	0.0774	0.2111	**0.0693**	0.504 **

Bold figures indicate the direct effects
Residual effect (R) = ± 0.154

*, ** Significant at 5% and 1% levels, respectively.



X_1 = Nodes per vine
X_2 = Vines per plant
X_3 = Vine length
X_4 = Fruits per plant
X_5 = Female flower length
X_6 = Fruit length
X_7 = Fruit width
X_8 = Fruit volume
X_9 = Fruit weight
Y = Fruit yield

Fig. 3. Path diagram of yield component characters on fruit yield

3.6.7 Genetic divergence

Genetic diversity is one of the important tools to quantify genetic variability in both cross and self-pollinated crops (Griffing and Lindstorm, 1954; Murty and Arunachalam, 1966; Gaur *et al.*, 1978). The quantification of genetic diversity through biometrical procedures has made it possible to choose genetically diverse parents for a successful hybridization programme (Rao, 1952 and Jain *et al.*, 1975). Tomooka (1991) reported that evaluation of genetic diversity is important to know the source of gene for a particular trait within the available germplasm. High yielding parents with greater genetic diversity are required to develop productive hybrids. For identifying genetically diverse parents for hybridization, multivariate analysis (Mahalanobis' D^2 statistic) has been used in pointed gourd. It is a powerful tool for quantification of genetic divergence among the parents.

Genetically diverse and geographically isolated lines express a wide range of variation when brought together. Mahalanobis (1936) generalized distance estimated by D^2 statistic, which has been used as an efficient tool in the quantitative estimation of genetic diversity and a rational of potential parents for a breeding program.

In this context, a number of pointed gourd genotypes were collected from different districts of Bangladesh to study with different parameters in order to find their genetic divergence and scope for improvement.

3.6.7.1 *Cluster analysis*

Based on the degree of divergence, 25 genotypes of pointed gourd were grouped into five clusters (Table 3.6). Cluster V had the maximum number of genotypes (9) followed by cluster II (7), III (4), IV (3) and I (2). The clustering pattern of the genotypes revealed that the genotypes collected from the same district did not form single cluster. The genotypes belonging to same districts were grouped into different clusters (Table 3.6). The genotypes of Mymensingh and Nawabganj district were distributed in four different clusters in spite being collected from the same district. This shows that geographic diversity is not always related to genetic diversity and therefore it is not adequate as an index of genetic diversity. In addition, this may be due to free exchange of plant material among the different regions and as a consequence the characters constellation that might be associated with particular region has not lost their individuality under human interferences. Similar opinions

were also suggested by Anand and Rawat (1984) in brown mustard, Mannan *et al.* (1993) in pani kachu, Patel *et al.* (1989) in safflower and Singh and Singh (1979) in okra. Shanmugam and Rangasmy (1982) reported that falling of materials of same origin into different clusters was an indication of broad genetic base of the genotypes belonging to that origin. Therefore, genotypes originating at same place may have different genetic architecture or vice-versa. Moreover, Masud *et al.* (1995) in pumpkin, Masud *et al.* (2001) in sponge gourd, Masud *et al.* (2003a and 2003b) in ridge gourd and sweet gourd, Chowdhury *et al.* (1998) in soybean, Bhadra and Akhtar (1991) in mung bean, Natarajan *et al.* (1988) in green gram and Anand and Rawat (1984) in brown mustard found no relationship between geographic distribution and genetic diversity of the crop. The results however, suggested that geographic isolation is not the only factor causing genetic diversity and this point should be considered in selecting parents for hybridization.

3.6.7.2 *Canonical variate analysis*

The intra and inter-cluster D^2 values have been presented in Table 3.7. The inter-cluster D^2 values varied from 17.389 to 135.327 exhibited wide diversity in the genotypes studied. The maximum inter-cluster divergence was observed between genotypes of cluster II and IV (135.327) followed by genotypes of cluster II and V (103.227), I and IV (83.266). Intermediate or moderate inter-cluster distance was observed between genotypes of cluster III and IV (48.261), IV and V (44.209), I and II (42.445). The genotypes in these clusters may serve as potential parents and crossing between the genotypes may result in heterotic expression for yield components. Arunachalam *et al.* (1984) and Ramanujam *et al.* (1974) reported that heterosis could be exploited by using genetically diverse genotypes.

Parents belonging to the maximum divergent clusters were expected to manifest maximum heterosis in crossing and also wide variability in genetic architecture. It is expected that the crosses involving parents belonging to the medium divergent cluster may also exhibit significant and positive heterosis for yield and some of its components (Ramanujam *et al.*, 1974 and Mian and Bhal, 1989). The least genetic distance at inter cluster level was observed between III and V (19.847) and I and III (17.389) followed by I and V (35.605) and II and III (35.820) indicating that the genotypes of these clusters were genetically close. The intra-cluster divergence varied from 4.887 to 6.489, the maximum being in cluster III that comprised four genotypes (Table 3.6).

Table 3.6. Distribution of 25 genotypes of pointed gourd in five clusters

Cluster	Number of genotypes	Name of genotypes with sources of collection
I	2	AM-3 (Gaibandha), AM-6 (Jessore)
II	7	AM-8 (Jessore), AM-10 (Pabna), AM-15 (Pabna), AM-18 (Rajshahi), AM-20 (Rajshahi), MNI-12 (Mymensingh), MRM-25 (Nawabganj)
III	4	AM-14 (Natore), MNI-14 (Mymensingh), MRM-28 (Nawabganj), MHQ-202 (Jessore)
IV	3	MNI-13 (Mymensingh), MRM-23 (Nawabganj), MRM-24 (Nawabganj)
V	9	AM-2 (Jessore), AM-22 (HRC, Joydebpur), MNI-11 (Mymensingh), MRM-30 (Nawabganj), MHQ-196 (Jessore), MHQ-197 (Jessore), MHQ-198 (Jessore), MHQ-201 (Jessore), MHQ-203 (Jessore)

Table 3.7. Average intra (bold) and inter-cluster distance (D^2) for 25 pointed gourd genotypes

Cluster	I	II	III	IV	V
I	**4.887**	42.445 (6.515)	17.389 (4.170)	83.266 (9.125)	35.605 (5.967)
II		**6.106**	35.820 (5.985)	135.327 (11.633)	103.227 (10.160)
III			**6.489**	48.261 (6.947)	19.847 (4.455)
IV				**5.384**	44.209 (6.649)
V					**4.992**

Bold figures indicate intra-cluster distance
Figures in the parenthesis indicate D value

The cluster means of ten characters for 25 genotypes of pointed gourd are shown in Table 3.8. Differences in cluster means existed for almost all the characters. Cluster I composed of two genotypes namely, AM-3 and AM-6 were collected from Jessore district. The cluster I had the highest mean value for fruit length (10.98 cm), fruit weight (52.03 g), fruit volume (71.80 cc) and vines per plant (9). The second highest values for fruits per plant (104) and fruit yield per plant (4.05 kg). The second rank in yield ability of this cluster might be due to the highest fruit length (10.98 cm), fruit weight (52.03 g), fruit volume (71.80 cc), vines per plant (9) and the second highest of fruits per plant (104). Prasad *et al.* (1993) and Masud *et al.* (1995) also reported the similar findings relating to fruit bearing in cucumber and pumpkin, respectively. Cluster II was comprised seven genotypes namely, AM-8, AM-10, AM-18, AM-20, MNI-12 and MRM-25 collected from Jessore, Pabna, Rajshahi, Mymensingh and Nawabganj districts, respectively. This cluster had the highest cluster mean value for seeds per fruit (23), fruits per plant (130) and fruit yield per plant (4.88 kg). The high yielding ability (4.88 kg) of this cluster might be due to the highest fruits per plant (130), seeds per fruit (23), higher number of vines per plant (8) and vine length (5.60 m). It ranked second in fruit length (10.31 cm), vines per plant (8) and vine length (5.60 m). This cluster also possessed genotypes of early flowering (139 days) associated with highest number of fruits per plant (130) and fruit yield per plant (4.88 kg).

The high yielding ability (4.88 kg) of this cluster might be due to the highest fruits per plant (130), seeds per fruit (23), higher number of vines per plant (8) and vine length (5.60 m). It ranked second in fruit length (10.31 cm), vines per plant (8) and vine length (5.60 m). This cluster also possessed genotypes of early flowering (139 days) associated with highest number of fruits per plant (130) and fruit yield per plant (4.88 kg).

Cluster III had four genotypes namely, AM-14, MNI-14, MRM- 28 and MHQ-202 was collected from Natore, Mymensingh, Nawabganj and Jessore districts. None of the ten characters had the highest mean value under this cluster.

Table 3.8. Cluster means for 10 characters of pointed gourd genotypes

Character	Cluster				
	I	II	III	IV	V
Days to flower	110.00	139.00	143.00	152.00	143.00
Fruit length (cm)	10.98	10.31	10.28	9.31	10.08
Fruit weight (g)	52.03	47.87	48.01	45.47	43.44
Fruit volume (cc)	71.80	60.71	62.08	57.76	59.86
Seeds per fruit	18.00	23.00	23.00	19.00	20.00
Vines per plant	9.00	8.00	7.00	6.00	6.00
Nodes per vine	59.00	63.00	65.00	88.00	58.00
Vine length (m)	4.74	5.60	5.25	7.14	4.20
Fruits per plant	104.00	130.00	80.00	41.00	49.00
Fruit yield per plant (kg)	4.05	4.88	3.17	1.69	2.24

Cluster IV had three genotypes namely, MNI-13, MRM-23 and MRM-24, which were collected from Mymensingh and Nawabganj districts. This cluster had the genotypes of late flowering (152 days) but highest nodes per vine (88) and longest vine length (7.14 m) with fewer fruits per plant (41) that caused the lowest yield per plant (1.69 kg). Prasad *et al.* (1993) reported grouping of the lowest number of fruits per plant in one cluster, which caused lower yield in cucumber.

Cluster V was comprised of nine genotypes, which was highest in number among the clusters. The genotypes fall in cluster V were AM-2, AM-22, MNI-11, MRM-30, MHQ-196, MHQ- 197, MHQ- 198, MHQ-201, and MHQ- 203 that were collected from Jessore, Horticulture Research Centre (HRC), Joydebpur, Mymensingh, Nawabganj and Jessore districts. None of the characters were achieved the highest cluster mean. This cluster got second position only in days to flower (143 days).

The clustering pattern of D^2 analysis in Table 3.6 has followed the similar trend of distribution of the genotypes in PCA (Fig. 4). The D^2 and PCA were found to be alternative methods in giving the information regarding the clustering pattern. Moreover, the PCA provides information on contribution of the characters towards divergence.

3.6.7.3 *Contribution of the characters towards divergence of the genotypes*

The contributions of the ten characters towards divergence are presented in Table 3.9. The results of PCA revealed that in vector II (Z_2), the important characters responsible for genetic divergence in the second axis of differentiation were vine length (0.4838), nodes per vine (0.3636), seeds per fruit (0.3299), fruits per plant (0.3016), vines per plant (0.2943), fruit yield per plant (0.1710) and days to flower (0.1259) while rest of the characters played a minor role in the second axis of differentiation. Masud *et al.* (1995) observed that fruits per plant and fruit yield per plant played a minor role in the second axis of differentiation in pumpkin. Singh and Singh (1979) also reported that days to flower and fruit yield per plant contributed maximum in okra while Mathew *et al.* (1986) reported that fruits per plant contributed maximum in *Cucumis melo* towards total divergence and suggested to give considerable weightage on these characters to improve yield.

In vector I (Z_1) which was the major axis of differentiation, days to flower (0.3477) and nodes per vine (0.0152) played a major role while rest of the characters showed negative values under this study indicated the minor role of those traits in genetic divergence in the major axis of differentiation. Masud *et al.* (2003a and 2003b) reported that days to opening of first male and female flower and fruit length played a significant role towards genetic divergence in the second axis of differentiation.

3.6.7.4 *Selection of parents*

The crosses involving parents belonging to the intermediate or medium divergent clusters were expected to manifest significant and positive heterosis and also wide variability in genetic architecture. Ramanuajam *et al.* (1974) and Singh and Jain (1970) reported heterosis in F_1 generation of mung bean, which was due to genetic diversity of parents. Mian and Bhal (1989) also reported that parental clusters separated by medium D^2 values exhibited significant positive heterosis for seed yield and some of its components in chickpea. Thus, wide or medium diverged genotypes

Table 3.9. Latent vector for 10 characters of 25 pointed gourd genotypes

Character	Vector-I	Vector-II
Days to flower	0.3477	0.1259
Fruit length	-0.2871	-0.3932
Fruit weight	-0.4231	-0.2061
Fruit volume	-0.4037	-0.3243
Seeds per fruit	-0.2324	0.3299
Vines per plant	-0.2442	0.2943
Nodes per vine	0.0152	0.3636
Vine length	-0.0974	0.4838
Fruits per plant	-0.3551	0.3016
Fruit yield per plant	-0.4530	0.1710

Table 3.10. Eight selected genotypes with their agronomic performances

Genotype	Cluster	Days to flower	Nodes per vine	Vines per plant	Vine length (cm)	Fruits per plant	Fruit length (cm)	Fruit volume (cc)	Fruit weight (g)	Fruit yield (kg)
AM-6	I	112	52	9	4.93	112	10.52	69.04	49.43	4.18
AM-10	II	148	61	6	5.15	131	9.73	71.43	57.86	5.98
AM-15	II	114	68	10	6.19	159	10.33	73.52	55.04	7.09
MNI-12	II	123	64	7	6.21	125	10.42	64.17	50.27	4.75
MRM-23	III	139	87	5	7.25	38	10.87	77.55	58.41	2.11
MRM-28	IV	144	56	7	4.26	83	10.95	61.93	50.79	3.28
MHQ-197	V	128	55	9	4.44	55	10.33	62.08	44.60	2.20
MHQ-198	V	147	53	8	4.13	62	10.74	70.86	49.92	2.75

(if crossed) will result in high heterosis and produce new recombinants with desired characters. Based on the per se performance, inter-cluster distance and association among the traits, eight genotypes viz. AM-6 from cluster I, AM-10, AM-15 and MNI-12 from cluster II, MRM-28 from cluster III, MRM-23 from cluster IV and MHQ-197 and MHQ-198 from cluster V have been selected as the best genotypes for variety improvement program in pointed gourd (Table 3.10). Therefore, crosses between the genotype of cluster I with that of cluster II, IV and V, cluster II with that of cluster IV and V are expected to produce new recombinant with desired traits in pointed gourd.

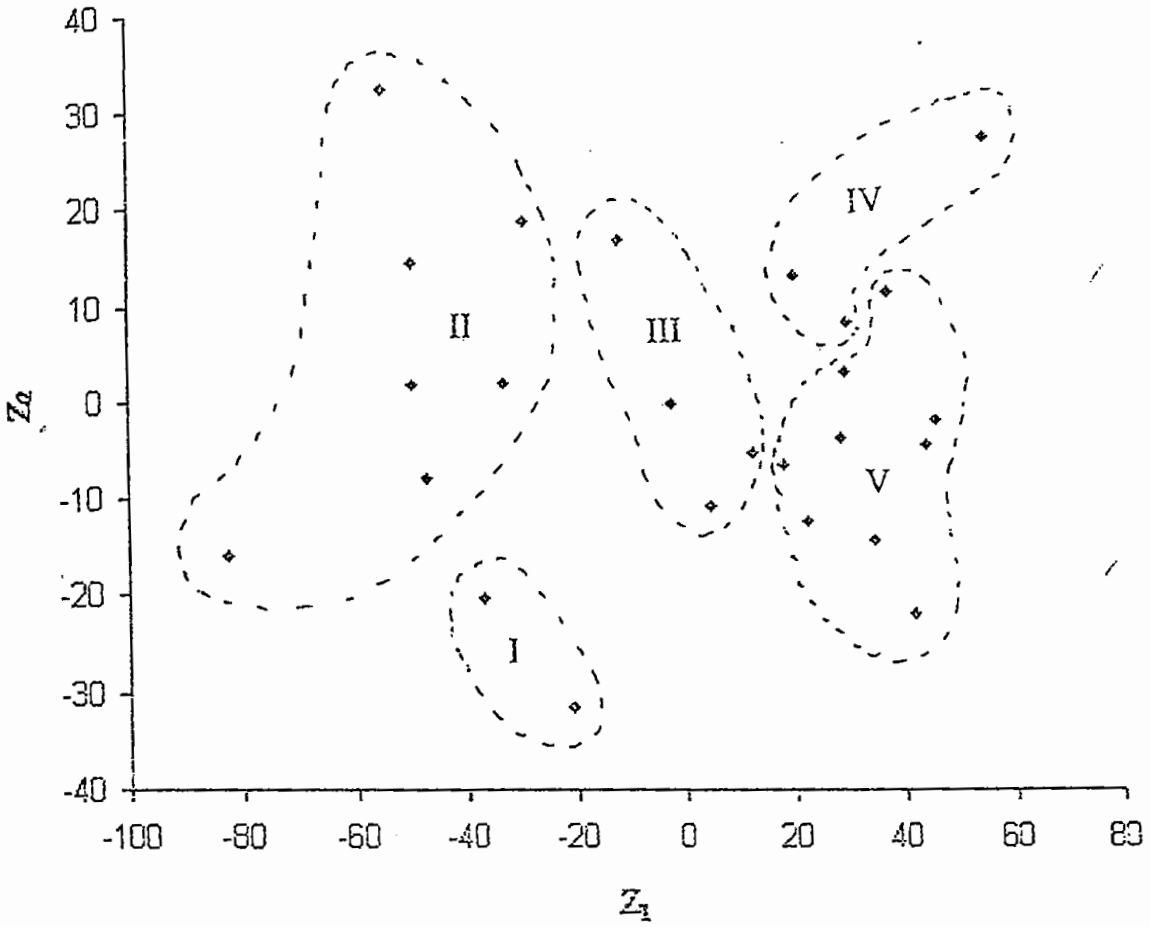


Fig. 4. Scatter diagram of 25 pointed gourd genotypes on the basis of principal component score supper imposed with clustering

3.7 SUMMARY

In a study of genetic variability for fruit yield and its component characters 25 genotypes were evaluated through genetic variance, heritability and genetic advance with the aim of obtaining information on the genetic architecture of the population and also to identify the best genotypes. Relationships between yield and yield contributing characters were studied. In addition, in order to identify the genetically diverse parents for exploitation in hybridization programme genetic diversity was also studied.

Variation among the genotypes

It was observed that there were significant variations among the genotypes for nodes per vine, vines per plant, vine length, fruits per plant, female flower length, fruit length, fruit width, fruit volume, fruit weight and fruit yield per plant.

Mean, range and genotypic and phenotypic variance

Wide variations were observed for all the characters except female flower length and fruit width. The environment affected these two characters, as genotypic and environmental variances were almost equal. The remaining characters were less influenced by the environment, as they showed less environmental variances. While the other characters fruits per plant, fruit yield per plant, vines per plant, vine length, fruit length, fruit volume and fruit weight showed maximum range of variations indicated that remarkable variations were present among the genotypes.

Genotypic and phenotypic coefficient of variation

Considerable amount of genetic variability exists in respect of various characters in pointed gourd. Phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the characters studied. Similar to PCV, fruits per plant and fruit yield per plant showed high genotypic coefficient of variation. Moreover, the differences in PCV and GCV estimates were narrow, confirming the least environmental influence in attaining the observed variability. Therefore, fruits per plant, vine length and fruit yield per plant showed higher genotypic coefficient of variation which offered scope for their improvement as they were less affected by the environment. Highest genotypic coefficient of variability was recorded for fruit yield per plant, which indicated the possibilities of utilization of the variation for improvement.

Heritability and genetic advance

Heritability estimates are useful in selection on the basis of the phenotypic performance of the quantitative characters. High estimates of broad sense heritability were observed for fruit volume, fruit weight, fruit yield per plant and vine length suggested that the selection based on phenotypic performance would be more effective. High heritability value along with high genetic advance as percent of mean is most effective condition for selection. In the present study, the maximum genetic gain (expressed as % mean) was observed in fruit yield and fruits per plant. The characters such as fruit yield per plant and fruits per plant showed high heritability value as well as high genetic advance as percent of mean. This suggested that selection for these two characters would be more fruitful.

Relationship between the characters

Correlation studies revealed that fruits per plant had a positively and significantly correlated with fruit yield per plant both at genotypic and phenotypic level indicating that fruit yield per plant could be improved by selection for this characters. There was also significant relationship between yield and its component characters. In most cases, genotypic correlation coefficient were higher than the corresponding phenotypic correlation coefficients suggesting the environmental influence reduce the relationship between yield and yield contributing characters in pointed gourd. Path coefficient analysis showed that fruits per plant and fruit volume had direct positive effects on fruit yield per plant. This indicates that these two characters were the major contributors to fruit yield and they should be included in selection indices for yield improvement in pointed gourd.

Genetic divergence

Based on the per se performance, inter-cluster distance and association among the traits, eight genotypes viz. AM-6 from cluster I, AM-10, AM-15 and MNI-12 from cluster II, MRM-28 from cluster III, MRM-23 from cluster IV and MHQ-197 and MHQ-198 from luster V have been selected as the best genotypes for variety improvement program in pointed gourd.

Chapter IV *IN VITRO* REGENERATION IN POINTED GOURD

4.1 INTRODUCTION

Pointed gourd (*Trichosanthes dioica* Roxb.) locally known as 'patal' is an under exploited important summer vegetable in Bangladesh. *T. dioica* is native to the Indian subcontinent. It is one of the most nutritive cucurbit vegetables holds a coveted position in the Bangladeshi market during summer and rainy season. It is a perennial crop highly accepted due to its availability for eight months in a year (February-September). Being very rich in protein and Vitamin A, it has certain medicinal properties and many reports are available regarding its role in circulatory system especially in lowering blood sugar and serum triglycerides (Sheshadri, 1990). The fruits are easily digestible and diuretic in nature. It is also known to have antiulcerous effects (Som *et al.*, 1993).

Traditionally pointed gourd is multiplied through stem cuttings and root cuttings. Propagation through seeds is not desirable due to poor germination and imbalanced male-female ratio. Seed based populations have a tendency to give more male than female plants and in some cases the ratio goes up to 85:15 (Som *et al.*, 1993), limiting their use as their utility ends with pollination. Improvement of this vegetable crop has not been attempted adequately, because of its dioecious nature and its vegetative mode of propagation. Moreover, less attention has been given to tissue culture of pointed gourd than its closely related taxa such as. cucumber and melon (Dong and Jia, 1991). Maintaining stem and root cuttings quality in field conditions as well as to conserve it in storage is difficult. Additionally, due to dioecy and resulting cross-pollinated, the maintenance of true to type plant is another major problem. Stem and root cutting are labor intensive and also requires bulk amount of vines per roots, which restricts their multiplication at commercial level. *In vitro* multiplication of elite clones will be an attractive approach in order to meet the requirement of quality propagules at large scale for commercial cultivation. The technique of *in vitro* culture aims primarily to solve two problems, i. e., to keep the culture free from microbes and secondary to ensure desired development in the cells and organs in suitable nutrient media and environmental conditions (Raghuvanshi, 2001). Micropropagation ensures rapid clonal multiplication through culturing of adventitious shoot, bulbs, protocorm and axillary buds.

Callus is an actively dividing non-organized tissue of undifferentiated and differentiated cells often developing from injury (wounding) or in tissue culture. An exogenous supply of growth regulators is often recommended to initiate callus from different explants. Exogenous supply of auxin and often in combination with cytokinin to medium are essential for callus induction but their requirements depends strongly on the genotype and endogenous hormone content of the explant (Pierik, 1987). Rao and Lee (1986) reported that intermediate level of auxins and cytokinins in the medium usually promote callusing. However, many other factors like genotype, composition of the nutrient media, physical growth factors such as light, temperature, moisture, etc. are important for callus induction (Pierik, 1987).

Many of the problems of inducing callus from plant tissue may be over come by using parts of freshly germinated plantlets ensuring tissue fragments composed of cells with high potential (Yeoman and Forche, 1980). Only younger parts of the growing shoots were selected as explants source to induce callus and shoot formation in this investigation. Many reports are available on callus initiation from herbaceous and vegetable crop plant species (Yamada *et al.*, 1967; Matsuoka and Hinata, 1983; Antonioli *et al.*, 1983 and Misra *et al.*, 1983). Most of the workers reported that younger and fresh plant parts comparatively produce more callus in culture.

There have not been many studies on *in vitro* regeneration of pointed gourd in Bangladesh or in neighboring countries. So, adequate information on this aspect is not available. Therefore, considering the above situations and scope to improve this important vegetable the present study has been designed to establish an efficient *in vitro* regeneration technique for pointed gourd with the following objectives:

i) To develop protocol for *in vitro* plant regeneration of pointed gourd
ii) To identify the suitable explants for *in vitro* propagation of pointed gourd
iii) To establish a protocol for efficient induction of callus and it's regeneration

4.2 MATERIALS AND METHODS

Young shoot tip, nodal segment and cotyledon of mature and immature seed after removing of seed coat of pointed gourd were considered as explant of this study. The explants were collected from actively growing plants of the experimental field, Plant Genetic Resources Centre (PGRC), Bangladesh Agricultural Research Institute

(BARI), Joydebpur, Gazipur 1701. Leaf, internode, cotyledon and root were used as the explant of *in vitro* grown plantlets for callus formation and plant regeneration.

The experiments were conducted in the Tissue Culture Laboratory, Biotechnology Division, BARI, Joydebpur, Gazipur 1701 and Plant Biotechnology Laboratory, Institute of Biological Sciences (IBSc.), University of Rajshahi, Rajshahi.

4.2.1 Preparation of Murashige and Skoog (MS) stock solution

Chemical composition of Murashige and Skoog (1962) is tabulated in the appendix 12.

Basal stock solution major salt (MS-I) of 10 X

The required quantities of salts were weighed and added successively to 750 ml of distilled water in a 1000 ml beaker. The stock solution was prepared at 10 times the final strength of the medium and it was made finally 1000 ml of solution.

Minor salt (MS-II) of 100 X

The required quantities of minor salts were weighed and added successively to 750 ml of distilled water in a 1000 ml beaker. The stock solution was maintained at 100 times the final strength of the medium and it was made finally 1000 ml of solution.

Iron EDTA (MS-III) of 100 X

The required quantities of Na_2EDTA (Ethylenediaminetetra acetic acid, disodium salt) and $FeSO_4$, $7H_2O$ were weighed and dissolved in 750 ml of distilled water in a 1000 ml beaker by heating in hot plate stirrer until the salts dissolved completely and final volume was made up to one litre by addition of distilled water. The mixture was then poured into an amber color bottle for storage.

Organic (MS-IV) of 100 X

The required quantities of organic constituents and vitamins were weighed and dissolved in 750 ml distilled water in a 1000 ml beaker. The stock solution was prepared at 100 times the final strength of the medium and finally made 1000 ml of solution. All the stock solution was prepared separately, leveled and kept in the refrigerator at 9 ± 1^0C temperature for readily use.

4.2.2 Plant growth regulators

Different cytokinins (BAP, Kn and TDZ) and auxins (IBA, IAA, NAA and 2, 4-D) were used in the study.

4.2.3 Preparation of growth regulators' stock solution

The following growth regulators were used in the experiments:

a) Cytokinin

1. 6-Benzylaminopurine (BAP)
2. Kinetin (Kn)
3. TDZ (Thidiazuron)

b) Auxin

1. Indole-3-butyric Acid (IBA)
2. Indole-3-acetic Acid (IAA)
3. α-Naphthalene acetic acid (NAA)
4. 2, 4 Dichloro phenoxy acetic acid (2, 4-D)

To prepare the stock solution of any of these growth regulators, 5.0 mg of the solid growth regulator was placed in a clean test tube and dissolved in 1 ml of specific solvent shown below:

Growth regulators	Solvents
BAP	1 N HCl
Kinetin	1 N NaOH
NAA	1 N NaOH
IAA	1 N NaOH
IBA	1 N NaOH
2, 4-D	50% Ethanol

The solution was poured in a measuring cylinder and made up to the marked volume of 50 ml by further addition of distilled water and stored in a refrigerator at $9\pm1^{0}C$ temperature for a specific time to use as stock solution.

4.2.4 Preparation of Culture Media

The medium contained Murashige and Skoog's (MS) inorganic salts and vitamins. To prepare one litre of the MS medium the following steps were taken:

i) 30g (3%) of sucrose was dissolved in 750 ml distilled water in a one litre beaker.

ii) 100 ml stock solution of macro-nutrients (MS-I), 10 ml stock solution of micro-nutrients (MS-II), 10 ml stock solution of FeEDTA (MS-III) and 10 ml stock solution of organic (MS-IV) nutrients were added to this sucrose containing 750 ml distilled water and mixed properly using magnetic stirrer.

iii) Different concentrations of hormonal supplements were added to the solution either singles or in combinations required as per treatment.

iv) The pH of the medium was checked with a pH meter and adjusted to 5.7 ±0.1 with addition of 0.1N sodium hydroxide (NaOH) or 0.1N hydrochloric acid (HCl) as necessary.

v) Finally, the volume of whole solution was made up to 1000 ml by adding distilled water.

vi) To make the media solid, 8.0 g (0.8%) of high brand agar was added to the medium and the mixture was heated in a microwave oven for 10-12 minutes until the agar dissolved completely.

vii) 10 ml prepared medium was dispensed into culture tubes while medium still hot. The culture tubes were plugged with aluminum foil and marked with a glass marker pen to indicate specific hormonal supplement.

viii) The culture tubes were sterilized at 1.09 kg cm^2 pressure at 121^0C for 15-20 minutes in an autoclave.

ix) After autoclaving, the culture media were taken out and allowed to cool and solidify.

4.2.5 **Precautions for aseptic condition**

To maintain and ensure aseptic conditions precautions will be taken in every step:

All inoculation and aseptic manipulation were carried out in a laminar air flow cabinet, which was usually switched on half an hour before use. The cabinet was wiped with 70% ethyl alcohol to reduce the chances of contamination.

The inoculating instruments like scalpels, forceps etc. were pre-sterilized by autoclaving and second sterilization was done by using hot bead sterilizer. Other required materials like distilled water, hard board etc. were sterilized by autoclaving. Hands were properly washed with soap before starting work in laminar air flow cabinet. Hands were also sterilized by spraying 70% ethyl alcohol. Surgical operations were taken care of as usual to obtain possible contamination free condition. During the period of inoculation, operation hands were rubbed with 70% alcohol frequently and wiped the base of the laminar airflow cabinet for maintaining clean condition.

4.3 CULTURE TECHNIQUES

The culture techniques are shown below:

4.3.1 Collection of explant

Explants were collected from the experimental field of Plant Genetic Resources Centre (PGRC), BARI, Joydebpur, Gazipur 1701. Two cultivars (AM-8 and AM-15) were used as the source of explant. For establishing the plant in the media, the tender and actively growing vine and cotyledon of mature and immature fruits of pointed gourd were taken.

4.3.2 Preparation of explant

Different types of explants namely shoot tips and nodal segments were excised from tender and actively growing vine of the plant. Actively growing shoot tips (1-1.5 cm) containing leaf primordial with 2-3 nodes and single nodal segment (1-1.5 cm) were taken.

Fourteen to seventeen day old fruits of pointed gourd having mature seeds with solid cotyledons and eight to twelve day old fruits having soft seed coat and semi solid cotyledons were collected from the same experimental field. The fruits were cut and the seeds were removed from the fruits with scalpel. After that the seed coats were removed carefully with the help of forceps and only the cotyledons were taken in a beaker.

4.3.3 Washing and surface sterilization of explant

Prepared explants were taken in a beaker and thoroughly washed in running tap water for 5-10 minutes. The explants were surface sterilized with detergent for 15 minutes in a beaker containing tap water and finally washed 3-4 times with distilled water. The

explants were transferred in autoclaved plastic pot with 0.1% mercuric chloride ($HgCl_2$) and with few drops of Tween 20 and finally treated for 5-7 minutes for surface sterilization. The surface sterilized explants were then washed inside the clean bench 4-5 times with sterile distilled water to remove all traces of $HgCl_2$.

Prepared cotyledons explants were surface sterilized gently with distilled water for 3-4 times. The surface sterilized explants of cotyledons were dipped into 70% ethyl alcohol for 30 seconds. Finally, the cotyledons were washed 3 times with sterilized distilled water inside the clean bench and these were ready to inoculate into the culture media.

4.3.4 **Inoculation**

Shoot tips and nodal segments were prepared on the laminar air flow cabinet aseptically from the sterilized explant using a fine sterile forceps and scalpels. The excised explants and prepared solid and semi-solid cotyledons were then inoculated on to each culture test tubes containing MS media with various concentrations and combinations of hormonal supplements for *in vitro* multiple shoot regeneration.

4.3.5 **Incubation**

For growth and development of cultures, the temperature was set 25 ± 1^0C at a light intensity of 2000-3000 lux from fluorescent tubular lamps and the photoperiod was maintained generally 16 hours light and 8 hours dark (16 L/8 D) and relative humidity 60-70%.

4.3.6 **Subculture**

Successful shoot formations become evident when small green leaves began to emerge. It is the first sign of regeneration. These tiny leaves when developed in their actual shape they were transferred into fresh media containing the same hormonal combination or best one among them for further proliferation and development. Subculture was carried out regularly at an interval of 3-4 weeks.

4.3.7 **Shoot induction**

The regenerated multiple shoots were removed carefully from the culture tubes and placed on a hard sterile paper. Each shoots and nodal explants were cut from the basal end and transferred into new media at different combinations of hormonal supplements for further multiple shoot induction.

4.3.8 Root induction

The proliferated multiple shoots were cut down from the base and individual shoots were placed in half strength MS medium containing various concentrations of NAA.

4.3.9 Callus induction

The *in vitro* grown shoots of 3-5 cm in length were rescued aseptically from the culture vessels and inter-nodes, leaves and roots were cultured on freshly prepared medium containing different concentrations and combinations of hormonal supplements for callus induction.

4.3.10 Transfer to pots

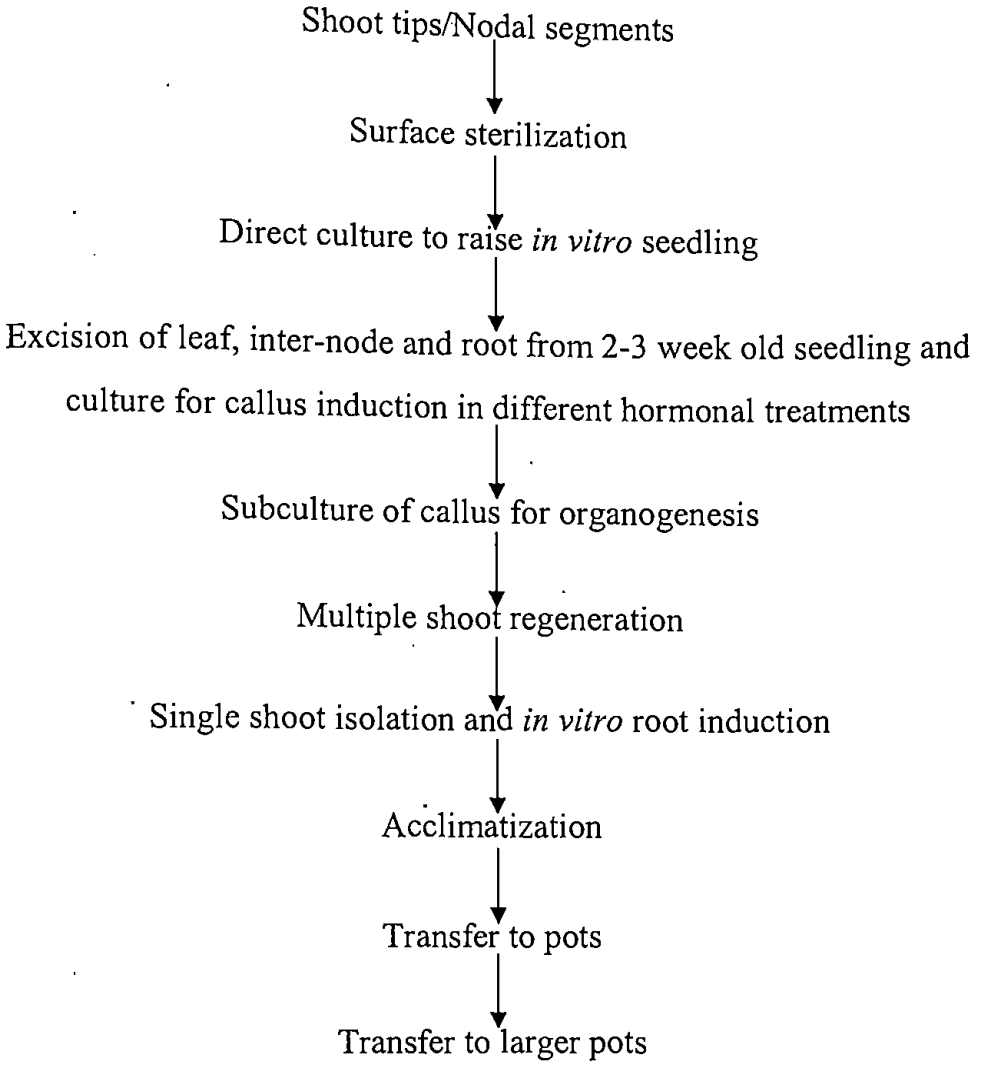
The regenerated healthy rooted plantlets (when attained a 2-3 leaf stage and had a well developed root system) were replaced from culture room and kept in room temperature (20-25^{0}C). The plantlets were removed from the culture tubes and washed all the adhering media carefully so that the roots damage was minimum. The washed plantlets were transplanted into small plastic pots with wet potting soil (A mixture of sand, soil and rice bran ash at the ratio of 1:1:1).

The transplanted plantlets were covered with a transparent polyethylene bag (size 12 x 20 cm) to maintain high humidity. The plantlets were watered once or twice in a week and kept in covered condition for two weeks.

4.3.11 Acclimatization

After 2-3 weeks the covered polyethylene was removed and the transplanted plantlets were kept in the net house. Watering was done as when necessary. As the root system generally does not take nutrient from the soil, nutrient uptakes discontinue. Periodic application of macro and microelements as a foliar spray or along with the soil application were necessary for the plantlets until well established. After that the healthy plants were transplanted in 9"x 12" earthen pots.

4.3.12 Flow chart for direct and indirect plant regeneration



4.3.13 Data to be recorded

The data were recorded on the following parameters and the methods for data record are given below:

i. Percentage of explant induced shoot

The generated explant that showed proliferations were counted after required days of culture. Percentage of explant induced to develop shoots were calculated using the following formula:

$$\% \text{ Explant induced shoot} = \frac{\text{Number of explant induced shoot}}{\text{Total number of cultured explant}} \times 100$$

ii. Number of shoots per explant

Number of shoots per explant was counted after required days of culture. Arithmetic mean was calculated using the following formula:

$X = \Sigma x_i/n$

Where, X = Mean number of shoots per explant

Σ = Summation

x_i = Number of shoots per explant

n = Number of observations

iii. **Days to shoot induction**

When the explant showed first greenish color shoot that day was counted for days to shoot induction and the mean was calculated.

iv. **Days to root induction**

When the first root formed from shoot that day was counted for days to root induction and the mean was calculated.

v. **Number of roots per shoot**

The number of roots in each shoot is recorded and the mean was calculated.

4.4 STATISTICAL ANALYSIS

The experiments were followed Completely Randomized Design (CRD). The data were subjected to mean value plus Standard Error (S. E). The experiments for callus formation and plant regeneration were not followed any design. The mean data were used in these experiments.

4.5 RESULTS AND DISCUSSION

Development of efficient and reproducible regeneration protocol from cells or tissues is a pre-requisite for the successful application of recent cellular manipulation techniques for the improvement of crop plants. In this direction, the choice of explants is of cardinal importance and makes an absolute difference between success and failure in inducing regeneration *in vitro*. The prime objective of this study was to get true to type plants using shoot tips and nodal portions as explants. Also the establishment of aseptic culture is must in any *in vitro* study.

In most of the cucurbits the root induction was achieved on either basal MS medium alone or with very low level of auxin (Mythili and Thomas, 1999). The rooted plantlets were transferred to pots for acclimatization and hardening and finally they were ready to transplant in the field for further growth.

The significant aspects of this work is that as compared to conventional root cutting, it gives a much higher multiplication rate from small portion of tissue with true to type plants. Another point is that shoots continue to elongate even after transferring them to root regenerating medium. Different experiments were conducted with a view to find out optimum culture condition for shoot regeneration from cultured explants.

4.5.1 Direct regeneration in pointed gourd

4.5.1.1 Shoot induction and proliferation

In this experiment, different concentrations of cytokinins and auxins were used singly or in combinations to investigate the induction of shoot and its subsequent regeneration. Shoot tips, nodal segments and cotyledon were used as explant for shoot regeneration. These explants were cultured onto the MS (Murashige and Skoog, 1962) agar gelled medium supplemented with different concentrations and combinations of two cytokinins (BAP and Kn) and three auxins (NAA, IBA and IAA). Effect of TDZ (Thidiazuron) was also evaluated. Data on shoot induction percentage, days to shoot induction, number of shoot per explant and shoot length per explant were collected after 4 weeks of culture. The results are presented according to types of explants used under separate tables.

4.5.1.1.1 *Single hormonal effect on nodal segment*

In the present study, nodal segments of actively growing vine of the plant was cultured in MS medium supplemented with different concentrations of BAP (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l), Kn (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) and TDZ (0.1, 0.2, 0.3, 0.4, and 0.5 mg/l) alone. Initiation of shoot in most of the treatments was observed within three weeks of culture. Shoot proliferation began from 8 days and continued to 16 days. Data were recorded after 4 weeks of culture. Results obtained on morphogenic response of the cultured explants are shown in Table 4.1. Morphogenic responses of the explants were found to vary with different hormonal formulations present in the culture media. Shoot proliferation was noticed in both cultivars (AM-8 and AM-15) of pointed gourd.

The varietals effect was observed at varied concentrations of BAP, Kn and TDZ. In case of AM-8, shoot induction ranged from 22.41 to 93.86%. Significant and highest percentage of shoot induction (93.86%) was observed in 2.0 mg/l BAP followed by 1.5 mg/l BAP when the nodal explants cultured in MS medium supplemented with BAP alone. The lowest percentage of shoot induction (22.41%) was observed in the

medium containing 0.5 mg/l TDZ. Days to shoot initiations were observed earlier in 2.0 mg/l BAP and 2.5 mg/l BAP. It was observed that different treatments responded differently in shoot regeneration. Shoot number per explant was recorded highest (3.25) in the medium containing 2.0 mg/l BAP followed by 1.5 mg/l BAP (3.00). The MS medium supplemented with different concentrations of hormones showed different results for shoot length. The longest shoot per explant (4.18 cm) was produced by the treatment 2.0 mg/l BAP followed by 1.5 mg/l BAP for the same cultivar. The shoot length per explant was observed minimum (0.65 cm) in the treatment 0.5 mg/l TDZ. In AM-8 cultivar, 2.0 mg/l BAP was statistically significant

Table 4. 1. Effect of different concentrations of BAP, Kn and TDZ on shoot induction and proliferation from nodal segment of AM-8 and AM-15 cultivar

Treatment (mg/l)		AM-8				AM-15			
		Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)	Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)
Control		0	0	0	0	0	0	0	0
BAP	0.5	75.00	10	1.75	3.20	60.00	10	2.71	3.45
	1.0	80.91	10	2.75	3.19	66.66	10	2.75	3.61
	1.5	82.31	10	3.00 *	3.57	85.71 **	9	3.00	3.81 *
	2.0	93.86 **	8	3.25 **	4.18 **	91.66 **	9	3.50 **	3.97 **
	2.5	57.14	8	2.00	3.55	50.00	9	2.50	3.72
Mean		77.84	9.20	2.55	3.53	70.80	9.40	2.89	3.71
S.E (±)		5.376	0.438	0.258	0.161	6.996	0.219	0.153	0.079
Kn	0.5	55.14	9	2.00	2.94	50.27	9	2.00	2.50
	1.0	57.43	9	2.25	3.00	55.43	9	2.15	3.00 *
	1.5	68.00 *	9	2.20	3.00	61.20 *	9	2.50 *	3.00 *
	2.0	75.15 **	9	2.50 **	3.46 **	69.38 **	9	3.00 **	3.15 **
	2.5	40.00	9	1.75	2.91	46.00	9	1.80	2.42
Mean		59.14	9.00	2.14	3.06	56.45	9.00	2.29	2.81
S.E (±)		5.367	0.000	0.112	0.090	3.677	0.000	0.189	0.132
TDZ	0.1	25.86	16	1.00	0.66	21.23	16	1.00	0.70
	0.2	23.81	16	1.00	0.87	25.00	16	1.22	0.78
	0.3	28.88	16	1.50 *	0.89	27.81	16	1.40 *	0.81
	0.4	40.00 **	15	1.75 **	1.38 **	37.00 **	15	1.70 **	1.35 **
	0.5	22.41	15	0.75	0.65	24.41	15	0.80	0.69
Mean		28.19	15.60	1.20	0.89	27.09	15.60	1.22	0.86
S.E (±)		2.814	0.219	0.164	0.118	2.405	0.335	0.139	0.110

*, ** Significant at 5% and 1% levels, respectively.

from other treatments for shoot induction percentage, shoot number per explant and shoot length per explant.

Significant difference was also observed in AM-15 cultivar for all the characters studied. Highest percentage of shoot proliferation (91.66%) was observed in the medium containing 2.0 mg/l BAP followed by 85.71% in 1.5 mg/l BAP. The lowest percentage of shoot induction (21.23%) was recorded in 0.2 mg/l TDZ. Maximum number of shoots (3.50) per explant was observed in 2.0 mg/l BAP followed by treatment 1.5 mg/l BAP (3.00) and 2.0 mg/l Kn (3.00). These results were in agreement with the findings of Uddin (2000) in pointed gourd and Shibli and Aajlouni (1996) in cucumber. The hormonal concentrations also responded differently on shoot length in AM-15 cultivar. Longest shoot per explant 3.97 cm) was found in the medium containing 2.0 mg/l BAP followed by 1.0 mg/l BAP (3.81 cm). The shortest shoot length (0.69 cm) per explant was recorded in the treatment of 0.5 mg/l TDZ. In AM-15 cultivar, 2.0 mg/l BAP also showed significant and best results for shoot induction percentage, shoot number per explant and shoot length per explant.

4.5.1.1.2 *Single hormonal effect on shoot tip*

The effects of different concentrations of BAP, Kn and TDZ on shoot tip explants are presented in Table 4.2. Shoot induction completed within 8 to 16 days of culture. The range of shoot induction percentage was achieved 20.16 to 84.71% when shoot tip explant of AM-8 cultivar cultured in MS medium supplemented with different concentrations of BAP, Kn and TDZ. Highest shoot induction percentage (84.71%) was found in 2.0 mg/l BAP followed by 1.5 mg/l BAP (75.00%). The lowest percentage of shoot induction was observed in 0.5 mg/l TDZ (20.16%). Highest number of shoot per explant (2.50) was recorded both in 2.0 mg/l BAP and 1.5 mg/l BAP followed by 2.0 mg/l Kn (2.37). Different shoot length was also found at different types of hormones as well as explants of the cultivars. The cultivar AM-8, showed longest shoot (4.55 cm) in 2.0 mg/l BAP followed by 1.5 mg/l BAP (3.89 cm). The lowest shoot length per explant (0.64 cm) was observed in 0.5 mg/l TDZ.

Culture of shoot tip of AM-15 cultivar also showed variation in shoot induction percentage, shoot number per explant and shoot length per explant. In AM-15 cultivar, highest percentage of shoot (70.30%) was recorded in 2.0 mg/l BAP

followed by 1.5 mg/l BAP (59.22%) and the lowest shoot induction percentage was observed in 0.5 mg/l TDZ (18.00%). In case of shoot number per explant, highest shoot number (3.00) was achieved in 2.0 mg/l BAP followed by 0.5 mg/l BAP, 1.0 mg/l BAP, 1.5 mg/l BAP and 2.5 mg/l BAP. Longest shoot (4.35 cm) was observed in 2.0 mg/l BAP followed by 0.5 mg/l BAP (3.20 cm). The lowest shoot length was found in 0.3 mg/l TDZ and 0.5 mg/l TDZ (1.00 cm). Debnath *et al.* (2000) reported similar type of results in pointed gourd.

Table 4. 2. Effect of different concentrations of BAP, Kn and TDZ on shoot induction and proliferation from shoot tip of AM-8 and AM-15 cultivar

Treatment (mg/l)	AM-8				AM-15			
	Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)	Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)
Control	0	0	0	0	0	0	0	0
BAP 0.5	64.29	10	2.00	2.73	55.00	9	2.00	3.20
1.0	71.43	10	2.25	3.73	58.10	9	2.00	3.19
1.5	75.00	8	2.50 **	3.89	59.22	9	2.00	2.30
2.0	84.71 **	8	2.50 **	4.55 **	70.30 **	9	3.00 **	4.35 **
2.5	62.50	8	2.00	3.59	42.15	9	2.00	3.16
Mean	71.58	8.80	2.25	3.69	56.95	9.00	2.20	3.24
S.E (±)	3.576	0.438	0.100	0.261	4.037	0.000	0.178	0.291
Kn 0.5	45.26	8	1.80	2.15	40.78	8	2.00 *	2.14
1.0	50.33	8	2.00	2.54	42.82	8	2.00 *	2.31
1.5	52.60	8	2.00	2.55	45.18	8	2.00 *	3.11 **
2.0	60.15 **	8	2.37 **	2.97 **	54.50 **	8	2.00 *	3.18 **
2.5	32.11	8	1.50	2.63	33.20	8	1.00	2.20
Mean	48.09	8.00	1.93	2.56	43.29	8.00	1.80	2.58
S.E (±)	4.167	0.000	0.127	0.117	3.082	0.000	0.178	0.205
TDZ 0.1	21.00	16	1.20	0.68	20.00	15	1.00	1.10
0.2	23.57	16	1.00	0.75	22.10	15	1.00	1.00
0.3	30.32 **	16	1.28	0.77	25.30 *	15	1.00	1.17
0.4	33.00 **	16	1.59 **	1.41**	30.35 **	15	2.00 **	1.50 **
0.5	20.16	16	0.72	0.64	18.00	15	1.00	1.00
Mean	25.61	16.00	1.15	0.85	23.15	15.00	1.20	1.15
S.E (±)	2.297	0.000	0.129	0.126	1.939	0.000	0.178	0.082

*, ** Significant at 5% and 1% levels, respectively.

Table 4. 3. Effect of different concentrations and combinations of BAP with IBA, NAA, IAA and Kn on shoot induction and proliferation from nodal segment of AM-8 and AM-15 cultivar

Treatment (mg/l)	AM-8				AM-15			
	Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)	Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)
Control	0	0	0	0	0	0	0	0
BAP+IBA								
2.0 + 0.1	62.00	10	2.50	3.08	60.00	10	2.00	3.00
2.0 + 0.2	80.00 **	10	2.75 **	3.70 **	74.00 **	10	2.68 **	3.60 **
2.0 + 0.3	60.00	10	2.25	3.10	63.00	10	2.00	3.00
2.0 + 0.4	55.56	10	2.70 **	2.93	52.56	10	2.40 *	2.67
2.0 + 0.5	50.00	10	2.50	3.10	50.00	10	2.00	3.00
Mean	61.51	10.00	2.54	3.18	59.91	10.00	2.21	3.05
S.E (±)	4.527	0.357	0.079	0.119	3.796	0.000	0.125	0.135
BAP+NAA								
2.0 + 0.1	71.43	10	2.25	3.25	38.24	9	2.25	3.25
2.0 + 0.2	81.82 *	10	3.32 *	4.30 *	52.00	9	3.25	3.26
2.0 + 0.3	88.78 **	10	4.00 **	4.88 **	84.85 **	9	4.00 **	4.30 **
2.0 + 0.4	72.50	10	3.20	3.85	82.93 *	9	3.50 *	3.95 *
2.0 + 0.5	53.33	10	1.75	3.26	34.29	9	1.78	3.55
Mean	73.57	10.00	2.90	3.90	58.46	9.00	2.95	3.66
S.E (±)	5.347	0.178	0.358	0.279	9.653	0.000	0.366	0.183
BAP+IAA								
2.0 + 0.1	63.64	8	2.25	3.73	56.20	8	2.17	3.00
2.0 + 0.2	62.50	8	2.75	3.68	60.30	8	2.46	3.23
2.0 + 0.3	66.67	8	3.00*	3.80	62.48	8	2.51	3.40
2.0 + 0.4	81.33**	7	3.25**	4.65**	78.21**	7	3.00**	4.00**
2.0 + 0.5	60.00	7	2.50	3.80	58.70	7	2.34	3.60*
Mean	66.82	7.60	2.75	3.93	63.17	7.60	2.49	3.44
S.E (±)	3.381	0.335	0.158	0.162	3.484	0.219	0.124	0.152
BAP+ Kn								
2.0 + 0.5	61.34	8	3.00 *	3.56 *	56.60	8	2.80 *	3.12 *
2.0 + 1.0	72.00 **	8	3.50 **	3.85 **	70.00 **	8	3.00 **	3.25**
2.0 + 1.5	60.00	8	2.45	3.24	62.87*	8	2.16	3.14*
2.0 + 2.0	56.00	8	2.00	2.65	50.00	8	2.00	2.60
2.0 + 2.5	42.35	8	2.00	2.82	45.40	8	2.10	2.22
Mean	58.33	8.00	2.59	3.22	56.97	8.00	2.41	2.86
S.E (±)	4.287	0.00	0.262	0.199	3.935	0.00	0.182	0.176

*, ** Significant at 5% and 1% levels, respectively.

4.5.1.1.3 *Combined effect of hormones on nodal segment*

The effect of different concentrations and combinations of BAP with NAA, IBA, IAA and Kn were also evaluated on shoot induction and proliferation of pointed gourd (Table 4.3). In both cultivars (AM-8 and AM-15) the best results were recorded on shoot induction percentage, shoot number per explant and shoot length per explant in MS +

2.0 mg/l BAP + 0.3 mg/l NAA which is statistically different from each other when the nodal segment used as explants.

The cultivar effect was also observed at different concentrations and combinations of BAP with IBA, NAA, IAA and Kn when the nodal segment cultured in the MS basal media. In case of AM-8, highest 88.78% of the nodal segment explants developed shoots in MS medium supplemented with 2.0 mg/l BAP + 0.3 mg/l NAA followed by 2.0 mg/l BAP + 0.4 mg/l IAA (81.33%). The best result was also observed in 2.0 mg/l BAP + 0.3 mg/l NAA for shoot number per explant (4.00) and shoot length (4.88 cm) followed by 2.0 mg/l BAP + 1.0 mg/l Kn for shoot number (3.50) per explant and 2.0 mg/l BAP + 0.4 mg/l IAA for shoot length (4.65 cm) per explant.

In case of AM-15 cultivar, the highest shoot regeneration (84.85%), maximum shoot number per plant (4.00) and longest shoot (4.30 cm) were recorded in MS + 2.0 mg/l BAP + 0.3 mg/l NAA followed by MS + 2.0 mg/l BAP + 0.4 mg/l NAA for shoot induction percentage (82.93%) and shoot number per explant (3.50) and 2.0 mg/l BAP+0.4 mg/l IAA for shoot length per explant (4.00 cm).

4.5.1.1.4 *Combined effect of hormones on shoot tip*

The effect of different concentrations and combinations of BAP with IBA, NAA, IAA and Kn also observed when the shoot tip explants cultured in MS basal medium. The results are presented in Table 4.4. The shoot induction percentage, number of shoot and shoot length per explant was found to vary in MS medium supplemented with different concentrations of BAP with IBA, NAA, IAA and Kn.

In case of AM-8, percentage of shoot proliferation ranged from 40.00 to 78.19%. Highest percentage of shoot (78.19%) proliferation was observed in the medium containing 2.0 mg/l BAP + 0.3 mg/l NAA followed by 2.0 mg/l BAP with 0.4 mg/l IAA (72.00%). The lowest percentage of shoot formation (40.00%) was observed in the media having 2.0 mg/l BAP + 2.5 mg/l Kn. The maximum number of shoots per explant (3.80) was recorded in the treatment 2.0 mg/l BAP + 0.3 mg/l NAA followed by 2.0 mg/l BAP + 0.4 mg/l IAA (3.60). In case of shoot length, the longest shoot (4.10 cm) was found in 2.0 mg/l BAP + 0.4 mg/l IAA followed by 2.0 mg/l BAP + 0.3 mg/l NAA.

Table 4. 4. Effect of different concentrations and combinations of BAP with IBA, NAA, IAA and Kn on shoot induction and proliferation from shoot tip of AM-8 and AM-15 cultivar

Treatment (mg/l)	AM-8				AM-15			
	Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)	Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)
Control	0	0	0	0	0	0	0	0
BAP+IBA								
2.0 + 0.1	59.24	10	2.13	3.00	56.48	10	2.00	2.70
2.0 + 0.2	70.32 **	10	2.58 **	3.40 **	68.00 **	10	2.56 **	3.12 **
2.0 + 0.3	57.10	10	2.23	3.14 *	60.20 *	10	2.00	2.80
2.0 + 0.4	53.44	10	2.20	2.70	50.26	10	2.00	2.30
2.0 + 0.5	48.00	10	2.00	2.46	47.60	10	2.00	3.00 *
Mean	57.62	10.00	2.22	2.94	56.50	10.00	2.11	2.78
S.E (±)	3.311	0.000	0.086	0.147	3.250	0.000	0.100	0.127
BAP+NAA								
2.0 + 0.1	56.10	8	2.00	2.90	58.00	8	2.00	3.55
2.0 + 0.2	71.40 *	8	2.55	3.00	61.20	8	3.00	3.20
2.0 + 0.3	78.19 **	8	3.80 **	3.60 **	75.67 **	8	4.00 **	4.00 **
2.0 + 0.4	70.24 *	8	2.51	3.15	64.00	8	2.00	3.65
2.0 + 0.5	51.00	8	1.42	2.65	52.00	8	3.00	3.21
Mean	65.38	8.00	2.45	3.06	62.17	8.00	2.80	3.52
S.E (±)	4.733	0.000	0.352	0.141	3.506	0.000	0.334	0.133
BAP+IAA								
2.0 + 0.1	57.30	8	2.54	3.10	40.27	8	2.12	3.10
2.0 + 0.2	55.60	8	2.47	3.25	50.00	8	2.23	3.15
2.0 + 0.3	62.40	8	2.65	3.35	72.35 **	8	2.14	3.40
2.0 + 0.4	72.00 **	8	3.60 **	4.10 **	65.64 *	8	3.32 **	3.98 **
2.0 + 0.5	58.50	8	2.31	3.30	41.24	8	2.30	3.18
Mean	61.16	8.00	2.71	3.42	53.90	8.00	2.42	3.36
S.E (±)	2.622	0.000	0.204	0.156	5.794	0.000	0.202	0.145
BAP+ Kn								
2.0 + 0.5	54.83	8	2.60	3.42 **	52.20	8	2.00	3.19 **
2.0 + 1.0	66.00 **	8	3.40**	3.44 **	64.40 **	8	3.00 **	3.24**
2.0 + 1.5	60.87 *	8	2.15	3.17	57.81 *	8	2.10	3.00 *
2.0 + 2.0	53.40	8	2.00	2.32	46.38	8	2.00	2.40
2.0 + 2.5	40.00	8	2.00	2.00	40.60	8	2.30	2.20
Mean	55.02	8.00	2.43	2.87	52.27	8.00	2.28	2.80
S.E (±)	3.914	0.000	0.238	0.266	3.734	0.000	0.168	0.190

*, ** Significant at 5% and 1% levels, respectively.

In AM-15, shoot proliferation ranged from 40.60-75.67%. Highest percentage of shoot (75.67%) proliferation was observed in the medium containing 2.0 mg/l BAP + 0.3 mg/l NAA followed by 2.0 mg/l BAP with 0.3 mg/l IAA (72.35%). The lowest percentage of shoot formation (40.60%) was observed in 2.0 mg/l BAP + 2.5 mg/l Kn. The maximum number of shoots per explant (4.00) and longest shoot (4.00 cm) were

recorded in the treatment 2.0 mg/l BAP + 0.3 mg/l NAA followed by 2.0 mg/l BAP + 0.4 mg/l IAA (3.32, 3.98 cm). The smallest shoot was observed in 2.0 mg/l BAP + 2.5 mg/l Kn for AM-8 in this study. Lauzer and Vieth (1990) reported that shoot tip explants of *Cyndara scolymus* (Green Glove) seedlings showed the best shoot regeneration and multiplication on MS medium supplemented with BAP and NAA. Effectiveness of BAP + NAA and Kn + NAA for *in vitro* shoot regeneration and multiplication from shoot tip culture was reported in several other plants (Conver and Lits, 1987; Tokuhara and Mii, 1993). These results are in partially agreement with our results because they reported that shoot tip explants were found best in their studies.

The results on different concentrations of BAP and Kn and combinations of BAP with IBA, NAA, IAA and Kn from shoot tips and nodal segments indicated that nodal segments showed better performance for shoot induction percentage, number of shoot per explant and shoot length per explant than shoot tip explants. The results also indicated that the nodal explants were more capable of producing multiple shoots compared to those of the shoot tip explants. The greater responsiveness of nodal explants over shoot apices can be attributed to the absence of apical dominance and the presence of axillary buds at a more advanced stage of development. It may be mentioned here that the shoot apex displays apical dominance, which might result from auxin produced at the terminal bud. Due to apical dominance, the lateral bud formation is suppressed. In apple (Hutchinson, 1981) and thornless blackberry (Zimmerman and Broome, 1980) nodal segments proved to be good explants for micro propagation over shoot tips. The results reported here indicate that nodal segment was more suitable for shoot regeneration and multiplication and also maximum shoot elongation. These results were in agreement with the findings of Debnath *et al.* (2000) and Uddin (2000) in pointed gourd. Zaman *et al.* (1992) demonstrated similar effects of BAP on shoot elongation in nodal segments culture of mulberry genotypes. The similar result has also been described in nodal segments culture of *Verbena* spp. (Hosoki and Katahira, 1994).

The regeneration of multiple shoots varied both with the type of explants and kind of supplements used. Performance of the nodal explants for multiple shoot regeneration was better than shoot tips culture. Sikdar *et al.* (2003) also observed similar results and they reported that performance of the nodal explants for multiple shoot

regeneration in bitter gourd was better than shoot tip. It has also been observed that with the increasing of BAP up to 2.0 mg/l, the number of shoots increased and then decreased at high BAP concentration (2.5 mg/l). The ability of BAP to induce axillary branching is well documented (George, 1993). The effectiveness of BAP was proved to be superior with respect to shoot proliferation reported by Awal *et al.* (2005). In general, herbaceous plants are highly responsive to BAP treatments and most of the cultures produce robust well formed shoots suitable for further shoot proliferation (Debergh and Zimmerman, 1991).

4.5.1.1.5 *Hormonal effect on cotyledon*

The response of cotyledon explants from mature and immature seed of pointed gourd cultured in MS media supplemented with BAP (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) and NAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) are shown in Table 4.5. The cotyledon obtained from mature seed when used as the explant produced roots and shoots. In cultivar AM-15, the highest percentage of shoot (96.00%) and highest number of shoot per explant (2.55) were obtained in MS medium supplemented with 1.0 mg/l BAP followed by 1.0 mg/l NAA (82.00%, 2.30) and the lowest percentage of shoot induction (40.00%) recorded in 2.5 mg/l BAP. While in cultivar AM-8, the highest percentage (95.00%) of cotyledons from mature seed produced shoots in 1.0 mg/l BAP followed by 1.0 mg/l NAA (80.00%). The maximum number of shoot (1.75) and longest shoot (3.20 cm) were observed in 1.0 mg/l BAP followed by 1.0 mg/l NAA (1.67, 3.00 cm). The shoot formation was higher in cultivar AM-15 due to use of BAP. The experimental results proved that BAP has a positive effect on shoot induction. Shoot development occurred within 14 days in 1.0mg/l BAP whereas it took 17 days in 1.0 mg/l NAA. Similar results were reported by Hoque *et al.* (1998) in pointed gourd. They also observed roots and shoots in hormone free MS medium which is not similar to the results observed in this experiment.

Semi-solid cotyledon obtained from immature seed did not produce any root and shoot. The cell mass of immature cotyledon may not be treated as a suitable explant for regeneration.

Table 4. 5. Effect of different concentrations of BAP and NAA on shoot induction and proliferation from cotyledon of mature and immature seed of AM-8 and AM-15 cultivar

Explant	Treatment (mg/l)	AM-8				AM-15			
		Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)	Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)
Cotyledon (Mature seed)	Control	0	0	0	0	0	0	0	0
	BAP 0.5	48.00	14	1.40	2.30	65.00	14	2.20 *	3.72 **
	1.0	95.00 **	14	1.75 **	3.20 **	96.00 **	14	2.55 **	3.81**
	1.5	45.00	14	1.45	2.80	55.00	14	2.00	3.10
	2.0	42.00	14	1.00	2.00	41.00	14	1.50	2.00
	2.5	38.00	14	1.00	1.80	40.00	14	1.00	1.45
	Mean	53.60	14.00	1.32	2.42	59.40	14.00	1.85	2.82
	S.E (±)	9.374	0.000	0.128	0.230	9.176	0.000	0243	0.420
Cotyledon (Mature seed)	Control	0	0	0	0	0	0	0	0
	NAA 0.5	62.00	17	1.54	2.22	68.00	17	2.10	3.31 **
	1.0	80.00 **	17	1.67 **	3.00 **	82..00 **	17	2.30 **	3.57 **
	1.5	53.00	17	1.38	2.20	56.00	17	2.27	2.56
	2.0	40.00	17	1.15	1.35	47.00	17	2.00	2.20
	2.5	40.00	17	1.00	1.25	43.00	17	1.48	2.10
	Mean	55.00	17.00	1.35	2.00	59.20	17.00	2.03	2.74
	S.E (±)	6.717	0.000	0.109	0.287	6.383	0.000	0.132	0.264
Cotyledon (Immature seed)	Control	0	0	0	0	0	0	0	0
	BAP 0.5	0	0	0	0	0	0	0	0
	1.0	0	0	0	0	0	0	0	0
	1.5	0	0	0	0	0	0	0	0
	2.0	0	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0	0
Cotyledon (Immature seed)	Control	0	0	0	0	0	0	0	0
	NAA 0.5	0	0	0	0	0	0	0	0
	1.0	0	0	0	0	0	0	0	0
	1.5	0	0	0	0	0	0	0	0
	2.0	0	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0	0

*, ** Significant at 5% and 1% levels, respectively.

4.5.1.2 Shoot induction and multiplication

Multiplication of regenerated shoot is the prime objective of the *in vitro* plant regeneration or multiplication of plant species. The hormonal concentrations had a significant effect on multiple shoot regeneration. In this experiment, various concentrations of BAP were used along with control in ½ strength MS basal medium

to study their effect on shoot induction and multiple shoot formation from shoot tips and nodal segments. Results are presented in Table 4.6 and 4.7.

In both cultivars AM-8 and AM-15, the best result was recorded in ½ MS + 2.0 mg/l BAP when nodal segment used as explants (Table 4.6). In AM-8 cultivar, significantly highest percent of the explants developed shoot (98.00%), maximum number of multiple shoot per explant (5.50) and the longest shoot per explant (6.55 cm) were observed in 2.0 mg/l BAP when the nodal segment cultured in the medium followed by 1.5 mg/l BAP (95.00%, 4.00 and 5.05 cm, respectively). The lowest percentage of shoot induction (80.00%) was found in 0.5 mg/l BAP for the AM-8 cultivar. In case of AM-15, percentage of the nodal explants produced shoots (95.00%), the number of multiple shoots per explant (5.14) and the shoot length (5.76 cm) per explant were also recorded highest in the same treatment followed by 1.5 mg/l BAP (93.00% for percentage of shoot induction, 4.50 for shoot number per explant and 4.97 cm for shoot length per explant.). When shoot tip explants cultured in ½ MS media supplemented with 2.0 mg/l BAP, the highest shoot induction percentage (88.00%), maximum number of shoot (4.25) and the longest shoot (4.85 cm) were observed in AM-8 cultivar (Table 4.7) followed by ½ MS + 1.5 mg/l BAP (81.00%, 3.51 and 4.35 cm for shoot induction percentage, shoot number per explant and shoot length per explant, respectively). The lowest percentage of shoot induction (70.00%) was found in 0.5 mg/l BAP. The cultivar AM-15 showed highest shoot induction percentage (85.00%), shoot number per explant (4.00) and shoot length per explant (4.42 cm) in 2.0 mg/l BAP which was less compared to AM-18 for all the characters studied. It was observed that MS medium without hormone had no response in shoot regeneration for all the explants of the cultivars.

Table 4. 6. Effect of different concentrations of BAP in half strength MS medium on shoot induction and multiplication from nodal segment of *in vitro* plantlets of AM-8 and AM-15 cultivar

Cultivar	Treatment (mg/l)	Shoot induction (%)	Days to shoot induction	Shoot number per explant	Shoot length per explant (cm)
AM-8	Control	0	0	0	0
	BAP				
	0.5	80.00	11	3.50	4.10
	1.0	85.00	11	3.67	4.17
	1.5	95.00 *	11	4.00	5.05 *
	2.0	98.00 **	11	5.50 **	6.55 **
	2.5	90.00	11	3.52	3.50
	Mean	89.60	11.00	4.03	4.67
	S.E (±)	2.920	0.000	0.336	0.474
AM-15	Control	0	0	0	0
	BAP				
	0.5	82.00	12	3.00	4.20
	1.0	84.00	12	3.20	4.28
	1.5	93.00 *	12	4.50 *	4.97 *
	2.0	95.00 **	12	5.14 **	5.76 **
	2.5	85.00	12	4.00	4.10
	Mean	87.80	12.00	3.96	4.66
	S.E (±)	2.322	0.000	0.356	0.281

*, ** Significant at 5% and 1% levels, respectively.

Table 4. 7. Effect of different concentrations of BAP in half strength MS medium on shoot induction and multiplication from shoot tip of *in vitro* plantlets of AM-8 and AM-15 cultivars

Cultivar	Treatment (mg/l)	Shoot induction (%)	Days to shoot induction	Shoot number per explant	Shoot length per explant (cm)
AM-8	Control	0	0	0	0
	BAP				
	0.5	70.00	11	3.00	3.40
	1.0	74.00	11	3.20	3.45
	1.5	81.00 *	11	3.51	4.35 *
	2.0	88.00 **	11	4.25 **	4.85 **
	2.5	78.00	11	3.15	3.30
	Mean	78.20	11.00	3.42	3.87
	S.E (±)	2.748	0.000	0.199	0.277
AM-15	Control	0	0	0	0
	BAP				
	0.5	71.00	12	2.80	3.25
	1.0	75.00	12	3.00	3.30
	1.5	80.00 *	12	3.60 *	3.80
	2.0	85.00 **	12	4.00 **	4.42 **
	2.5	75.00	12	3.38	3.40
	Mean	77.20	12.00	3.35	3.63
	S.E (±)	2.308	0.000	0.191	0.196

*, ** Significant at 5% and 1% levels, respectively.

4.5.1.3 Rooting of *in vitro* grown shoot

Easy and high frequency rooting is very important for establishment of *in vitro* regenerated plantlets. For successful micro propagation, healthy and strong root system is required. Response of different concentrations of NAA in half strength MS medium for *in vitro* adventitious root formation is presented in Table 4.8. The highest percentage of root formation (90.00%) and number of root per explant (11) were found in 0.5 mg/l NAA which was significantly different from other treatments. But longest root per explant (7.25 cm) was observed in 0.4 mg/l NAA. Early root induction was obtained from the treatment of 0.4 mg/l NAA and 0.5 mg/l NAA. This result was in agreement with the findings of Mamun Hossain *et al.*, 1996 and Uddin, 2000 working on pointed gourd.

Table 4.8. Effect of NAA in half strength MS medium on rooting of induced shoots in pointed gourd

Treatment (mg/l)	Days to root initiation	Root induction (%)	Root number per explant	Root length (cm)
Control	0	0	0	0
NAA 0.1	18	20	6	6.10
0.2	18	34	8	6.40
0.3	18	64	8	7.00*
0.4	15	78*	9	7.25**
0.5	15	90**	11**	7.14*
Mean	16.80	57.20	8.40	6.77
S. E (±)	0.657	11.792	0.726	0.201

*, ** Significant at 5% and 1% levels, respectively.

4.5.1.4 Comparative performance of male and female plant

In a comparative study of male and female genotypes, only nodal segments was used as explant and cultured on MS medium supplemented with different concentrations of BAP (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) and Kn (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l). Data were recorded after 4 weeks of culture. Morphogenic *in vitro* responses of the explants were found to vary with hormonal concentrations present in the culture media (Table 4.9). Shoot proliferation was observed in all the treatment formulations. The female plant showed the best results on shoot induction percentage and shoot number per explant in 2.0 mg/l BAP when different concentrations of BAP and Kn used singly in this experiment without any combination. In case of shoot induction

percentage, female genotype (AM-8) produced highest shoot induction (93.86%) than that of male genotype (80.00%) when the nodal explants cultured in MS medium containing 2.0 mg/l BAP (Table 4.9). For shoot number per explant, the female genotype showed also better results (3.25) than the male genotype (2.30) in 2.0 mg/l BAP. The MS basal medium along with 1.5 mg/l Kn and 2.0 mg/l Kn had also better effect on female genotype than that of male one for shoot number per explant. In this case, female genotype produced higher shoot number per explant (2.50) than male genotype (1.90) in 2.0 mg/l Kn. Moreover, shoot induction percentage (75.15%) also found better in female genotype than the male genotype (63.00%) when the explant cultured on basal medium supplemented with 2.0 mg/l Kn. These results were in agreement with the findings of Mythili and Thomas (1999) and Uddin (2000) in pointed gourd.

Table 4. 9. Effect of different concentrations of BAP and Kn on shoot induction of male (AM- 21) and female (AM-8) genotypes from nodal segment of pointed gourd

Treatment (mg/l)	AM-8 (Female plant)			AM-21 (Male plant)		
	No. of explant cultured	Shoot induction (%)	Shoot number per explant	No. of explant cultured	Shoot induction (%)	Shoot number per explant
Control	0	0	0	0	0	0
BAP						
0.5	20	75.00	1.75	20	65.47	1.50
1.0	20	80.91	2.75	20	68.75	2.00
1.5	20	82.31	3.00 *	20	75.00 *	2.10
2.0	20	93.86 **	3.25 **	20	80.00 **	2.30 **
2.5	20	57.14	2.00	20	50.00	2.20 *
Mean		77.84	2.55		67.84	2.02
S E (±)		5.376	0.258		4.578	0.120
Kn						
0.5	20	55.14	2.00	20	44.23	1.10
1.0	20	57.43	2.20	20	56.65	1.00
1.5	20	68.00 *	2.50 **	20	58.00 *	1.40
2.0	20	75.15 **	2.50 **	20	63.00 **	1.90 **
2.5	20	40.00	1.75	20	48.00	1.00
Mean		59.14	2.19		53.97	1.28
S E (±)		5.367	0.129		3.069	0.153

*, ** Significant at 5% and 1% levels, respectively.

4.5.1.5 Comparative performance of upper and lower nodal segments

In another comparative study of upper and lower nodal segments, the explants were cultured on MS medium supplemented with different concentrations of BAP (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) and Kn (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l). Data were recorded after 4 weeks of culture. Morphogenic *in vitro* performances of the explants were found to vary with hormonal concentrations present in the culture media (Table 4.10). Shoot proliferation was observed in all the treatment formulations. At the concentration of BAP (2.0 mg/l) and Kn (2.0 mg/l), lower nodal segment produced higher percentage of shoot (91.66% and 69.38%) than the upper nodal segment (Table 4.10). In all concentrations, lower nodal segment showed better results for percentage of shoot induction, shoot number per explant and shoot length per explant than upper nodal segments. Percentage of shoot induction was decreased at 2.5 mg/l BAP. Similar results were also reported by Uddin (2000) in pointed gourd. Lee *et al.* (1995) investigated *in vitro* propagation of various musk melons (*Cucumis melo*) cultured from nodal explants. They found node from the upper part of the stem shooted well and shooting efficiency decreased with nodes taken from the lower part of the stem. These findings are reverse in the present study for percentage of shoot induction in pointed gourd.

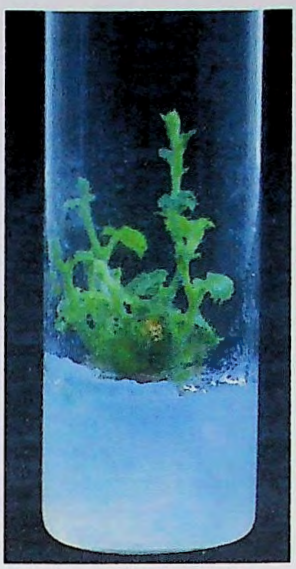
Table 4. 10. Effect of different concentrations of BAP and Kn on shoot induction (%) and shoot number from upper and lower nodal segment of AM-15 cultivar

Treatment (mg/l)		Upper nodal segment				Lower nodal segment			
		Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)	Shoot induction (%)	Days to shoot initiation	Shoot number per explant	Shoot length (cm)
Control		0	0	0	0	0	0	0	0
BAP	0.5	50.00	12	1.30	2.12	60.00	9	2.71	3.45
	1.0	53.00	12	1.70	2.23	66.66	9	2.75	3.61
	1.5	56.00 *	12	2.00 *	2.28	85.71 **	9	3.00	3.81*
	2.0	62.00 **	12	2.15 **	2.55 **	91.66 **	9	3.50 **	3.97 **
	2.5	44.00	12	1.52	2.14	50.00	9	2.50	3.72
Mean		53.00	12.00	1.73	2.26	70.80	9.00	2.89	3.71
S.E (±)		2.683	0.000	0.138	0.069	6.996	0.219	0.153	0.079
Kn	0.5	43.00	12	1.12	2.00	50.27	9	2.00	2.50
	1.0	49.00	12	1.50	1.92	55.43	9	2.15	3.00
	1.5	50.00	12	1.47	2.00	61.20	9	2.50	3.00
	2.0	54.00 **	12	2.18 **	2.38 **	69.38 **	9	3.00 **	3.15 **
	2.5	32.00	12	1.40	2.00	46.00	9	1.80	2.42
Mean		47.00	12.00	1.53	2.06	56.45	9.00	2.29	2.81
S.E (±)		2.011	0.000	0.156	0.072	3.677	0.000	0.189	0.132

*, ** Significant at 5% and 1% levels, respectively.

A

B



C

D

E

Fig. 5. Direct regeneration of pointed gourd from nodal explant

A. Initiation of shoot

B. Elongated shoot

C. Multiple shoot

D. Rooted plantlet

E. Established plant

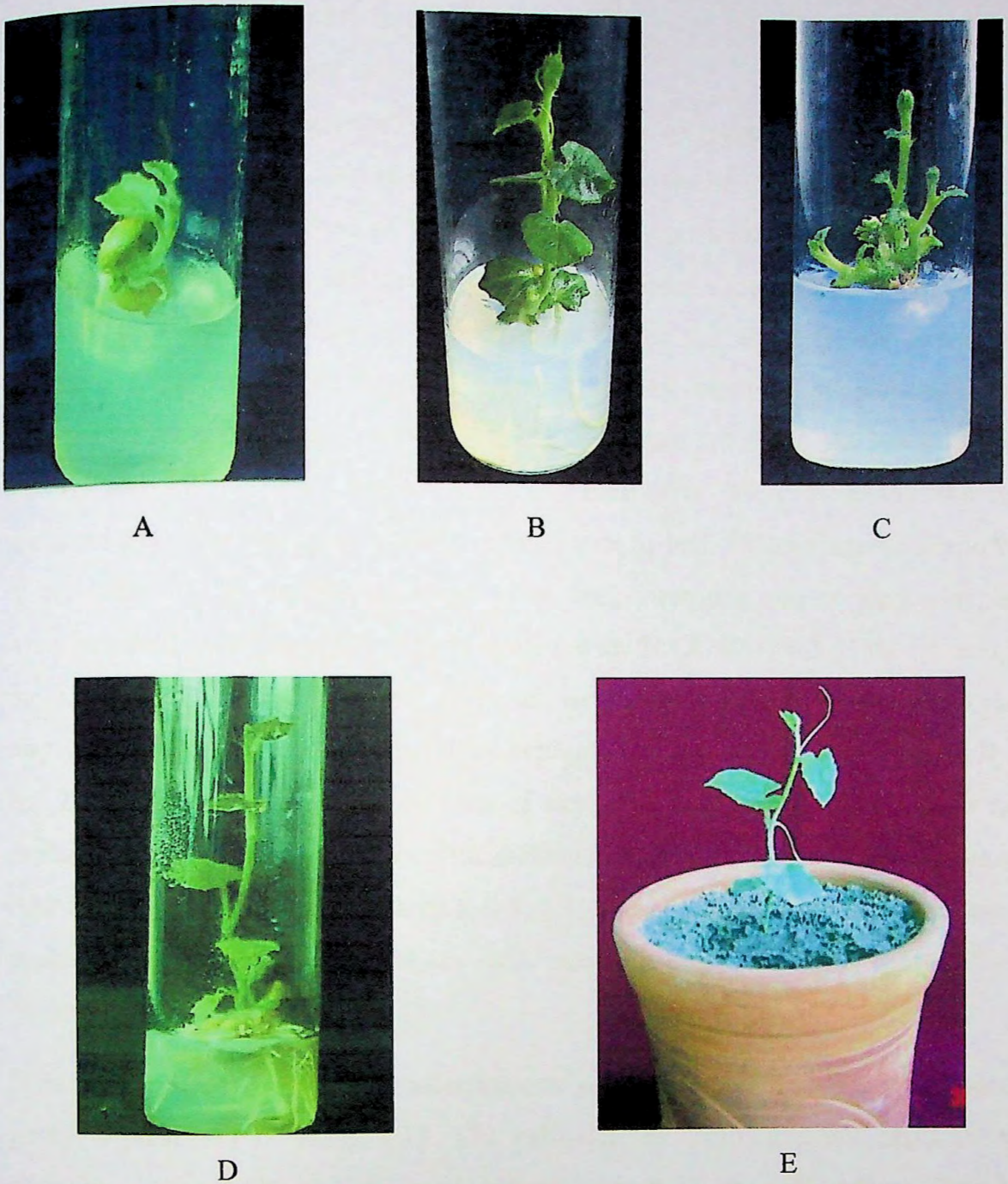


Fig. 6. Direct regeneration of pointed gourd from cotyledon explant

A. Initiation of shoot

B. Elongated shoot

C. Multiple shoot

D. Rooted plantlet

E. Established plant in earthen pot

4.5.2 Indirect regeneration in pointed gourd

4.5.2.1 *Callus induction and shoot regeneration*

Response of different explants of *in vitro* plantlets of pointed gourd such as leaf, internode, cotyledon and root at different concentrations of growth regulators viz. 2, 4-D, NAA and combinations of BAP with NAA are presented in Table 4.11. These explants were cultured in MS medium without hormonal supplements remained fresh for long time without showing any signs of callus. All the explants except root produced callus when the explants cultured in MS medium supplemented with different concentrations of 2, 4-D (0.1, 0.5, 1.0, 1.5 and 2.0 mg/l) and NAA (0.1, 0.5, 1.0, 1.5 and 2.0 mg/l) and combinations of BAP with NAA. However, the roots remained fresh in culture media for long time. 80% of leaf, 88% of internode and 92% of cotyledon explants produced callus when leaf, internode and cotyledon explants were cultured in MS medium supplemented with NAA (0.1, 0.5, 1.0, 1.5 and 2.0 mg/l). Similarly, 88%, 84% and 92% of leaf, internode and cotyledon explants respectively, produced callus when these explants cultured in 2, 4-D (0.1, 0.5, 1.0, 1.5 and 2.0 mg/l) and 68%, 80% and 60% of explants produced callus when the same explants cultured in MS medium with different concentrations and combinations of BAP (0.5 and 1.0 mg/l) and NAA (0.1, 0.5, 1.0, 1.5 and 2.0 mg/l). However, internode produced roots when cultured in MS medium supplemented with different concentrations of NAA and 2, 4-D.

Effect of different hormonal concentrations on morphogenic response of pointed gourd is presented in Table 4.12. The calli derived from different explants were morphologically different. The calli derived after 8-11 days of culture. The calli induced from leaf were soft, creamy and friable, inter-node calli were white and loose and cotyledon calli were creamy and soft. The auxin 2, 4-D at 0.1-2.0 mg/l concentrations resulted in callus growth after 8-11 days of culture. The optimum level of callus induction was found when leaf explants were cultured in MS medium supplemented with 0.5 mg/l 2, 4-D. Average callus formation was observed in 0.1 mg/l 2, 4-D and 1.0 mg/l 2, 4-D. But 2, 4-D at the highest concentrations (1.5 mg/l and 2.0 mg/l) produced minimum amount of callus. Optimum level of callus induction was produced by internode explant when cultured in 0.5 mg/l 2, 4-D followed by average level of callus produced in 1.0 mg/l 2, 4-D and 1.5 mg/l 2, 4-D. Lowest

Table 4.11. Effect of different explants of pointed gourd in MS medium supplemented with 2, 4-D, NAA and combinations of BAP and NAA on callus induction

Explant	Number of explant cultured	No. of responsive explants for callus induction			% of explants forming callus		
		NAA	2, 4-D	BAP + NAA	NAA	2, 4-D	BAP + NAA
Leaf	25	20	22	17	80	88	68
Inter-node	25	22	21	20	88	84	80
Cotyledon	25	23	23	15	92	92	60
Root	25	NR	NR	NR	NR	NR	NR

NR: No response

Table 4.12. Effect of different hormonal concentrations for callus and shoot induction on different explants of *in vitro* grown plantlets of pointed gourd

Concentrations of growth regulator (mg/l)	Morphogenic responses of explants			
	Leaf	Internode	Cotyledon	Root
2, 4-D				
0.1	++ C, SS Creamy, Friable	+ C Loose, white	++ C, Creamy, Soft	NR
0.5	+++ C, Creamy, Friable	+++ C Loose, white	++ C, Creamy, Soft	NR
1.0	+ + C, Creamy, Friable	++ C Loose, white	+++ C, Creamy, Soft, MS	NR
1.5	+ C, Creamy, Friable	++ C Loose, white	++ C, Creamy, Soft	NR
2.0	+ C, Creamy, Friable	+ C, Loose, white	+ C, Creamy, Soft	NR
NAA				
0.1	+ C, Creamy	+++ C, MS Loose, white	++ C, Creamy	NR
0.5	++ C, Creamy	++ C, MS Loose, white	++ C, Creamy	NR
1.0	++ C, Creamy	++ C, MS Loose, white	++ C, Creamy	NR
1.5	++ C, Creamy	+ C, Profuse root	++ C, Creamy	NR
2.0	NC	+ C, Profuse root	+ C, Creamy	NR

+ C=Minimum callus, ++ C=Average callus, +++ C=Optimum callus, SS=Single shoot, MS=Multiple shoot, NR=No response

amount of callus observed in 0.1 mg/l 2, 4-D and 2.0 mg/l 2, 4-D. Cotyledon had also produced different level of callus in different concentrations of 2,4-D (0.1, 0.5, 1.0, 1.5 and 2.0 mg/l). Optimum level of callus was found in 1.0 mg/l 2, 4-D and average amount of callus observed in 0.1 mg/l 2, 4-D, 0.5 mg/l 2, 4-D and 1.5 mg/l 2, 4-D. Minimum amount of callus was also found in 2.0 mg/l 2, 4-D. No callus was observed

at different concentrations of 2, 4-D when the root explant cultured in MS medium. Masum (1999) investigated different concentrations of 2, 4-D for callus production in ginger (*Zingiber officinale* Rosc.) and reported that MS medium supplemented with 3.0 mg/l 2, 4-D was the most suitable treatment for callus induction. Nanda Lal and Pramvir Singh Ahuja (1996) reported that MS medium supplemented with 0.5-2.0 mg/l 2, 4-D induced callus from shoot cuttings and leaf explants of *Picrorhiza heerroa* Royle ex Benth. within two weeks.

Effect of NAA at various levels of concentrations (0.1- 2.0 mg/l) for callus formation is also presented in Table 4.12. Callus formation completed within 10 days of culture. The average level of callus induction was observed when the leaf explants cultured in MS medium supplemented with 0.5 mg/l NAA, 1.0 mg/l NAA and 1.5 mg/l NAA. Lowest amount of callus was observed in 0.1 mg/l NAA. Internode explants produced optimum level of callus at the lowest concentrations of NAA (0.1 mg/l) and average level of callus observed in 0.5 mg/l NAA and 1.0 mg/l NAA. The minimum amount of callus with profuse root was achieved in 1.5 mg/l NAA and 2.0 mg/l NAA. The average level of callus was found when cotyledon explant cultured in MS medium supplemented with 0.1 mg/l NAA, 0.5 mg/l NAA, 1.0 mg/l NAA and 1.5 mg/l NAA and minimum amount of callus was observed at the highest concentrations of NAA (2.0 mg/l). No callus was observed at different concentrations of NAA when the root explant cultured in MS medium.

The results of combinations of BAP and NAA on callus formation are presented in Table 4.13. The effect of different concentrations and combinations of BAP and NAA on callus induction was also found. The average amount of creamy callus was observed in combination of 0.5 mg/l BAP + 0.5 mg/l NAA and 1.0 mg/l BAP + 0.5 mg/l NAA when leaf explants cultured in the medium. The minimum amount of callus was found in 0.5 mg/l BAP + 0.1 mg/l NAA, 0.5 mg/l BAP + 1.0 mg/l NAA, 0.5 mg/l BAP + 1.5 mg/l NAA, 0.5 mg/l BAP + 2.0 mg/l NAA, 1.0 mg/l BAP + 0.1 mg/l NAA, 1.0 mg/l BAP + 1.0 mg/l NAA, 1.0 mg/l BAP + 1.5 mg /l NAA and 1.0 mg/l BAP + 2.0 mg/l NAA.

The optimum amount of white callus was observed in 0.5 mg/l BAP + 0.5 mg/l NAA and 1.0 mg/l BAP + 0.5 mg/l NAA along with root and multiple shoot when internode explants used in the culture media. The minimum amount of callus induction was

found in 1.0 mg/l BAP + 0.1 mg/l NAA and 1.0 mg/l BAP + 1.0 mg/l NAA. But the treatments 0.5 mg/l BAP + 0.1 mg/l NAA, 0.5 mg/l BAP + 1.0 mg/l NAA, 0.5 mg/l BAP + 1.5 mg/l NAA and 1.0 mg/l BAP + 1.5 mg/l NAA produced minimum callus with some roots. The internode explants produced profuse roots only without formation of callus in 0.5 mg/l BAP + 2.0 mg/l NAA and 1.0 mg/l BAP + 2.0 mg/l NAA. Creamy callus was observed in all the concentrations of 2, 4-D, NAA and the combinations of BAP with NAA when the leaf and cotyledon explants cultured in MS medium. Cotyledon explants produced average amount of callus by the combination treatments of 0.5 mg/l BAP + 1.0 mg/l NAA, 1.0 mg/l BAP + 0.1 mg/l NAA and 1.0 mg/l BAP + 0.5 mg/l NAA. Minimum amount of callus was developed by all other treatments. No root was observed in case of leaf and cotyledon explants. In case of root explant no callus was also observed at different concentrations and combinations of BAP and NAA.

Table 4.13. Combined effect of growth regulators for callus formation and shoot regeneration from different explants of *in vitro* grown plantlets of pointed gourd

Concentrations of growth regulator (mg/l)		Morphogenic responses of explants			
		Leaf	Internode	Cotyledon	Root
BAP	NAA				
0.5	0.1	+ C, Creamy	+ C, White, Root	+ C, Creamy	NR
0.5	0.5	+ + C, Creamy	+ + + C, White, Root and MS	+ C, Creamy	NR
0.5	1.0	+ C, Creamy	+ C, White Root	+ + C, Creamy	NR
0.5	1.5	+ C, Creamy	+ C, White, Root	+ C, Creamy	NR
0.5	2.0	+ C, Creamy	Profuse root only	+ C, Creamy	NR
1.0	0.1	+ C, Creamy	+ C, White	+ + C, Creamy	NR
1.0	0.5	+ + C, Creamy	+ + + C, White Root and MS	+ + C, Creamy	NR
1.0	1.0	+ C, Creamy	+ C, White	+ C, Creamy	NR
1.0	1.5	+ C, Creamy	+ C, White, Root	+ C, Creamy	NR
1.0	2.0	+ C, Creamy	Profuse root only	+ C, Creamy	NR

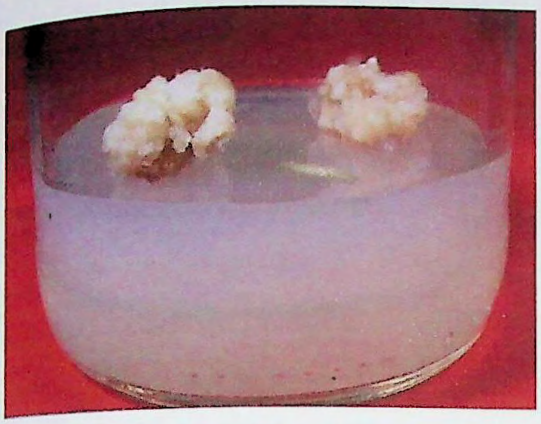
+ C= Minimum callus, ++ C = Average callus, +++ C = Optimum callus, MS = Multiple shoot, NR= No response

In another experiment, 2, 4-D and NAA were used singly and in combination of BAP and NAA on different explants of *in vitro* grown plantlets of pointed gourd. The effect of different concentrations of 2, 4-D and NAA used alone and combination of BAP and NAA on shoot regeneration from leaf and internode derived callus are presented in Table 4.14. Leaf explants produced good callus growth with multiple shoot formation when cultured in the medium containing 0.1 mg/l 2, 4-D. Different concentrations of 2, 4-D (0.5, 1.0, 1.5 and 2.0 mg/l) except 0.1 mg/l produced only callus without showing shoot when the *in vitro* leaf explants were cultured in the medium. The multiple shoots were achieved only in 0.1 mg/l 2, 4-D when *in vitro* leaf used in culture media. The internode explants produced callus as well as multiple shoot only in treatment 1.0 mg/l NAA. The other concentrations of NAA (0.5, 1.0, 1.5 and 2.0 mg/l) produced callus and root. The leaf explants showed no shoot in the medium supplemented with the same concentration of NAA.

The combination effects of growth hormones also responded differently for shoot regeneration in different explants. These morphogenic results are shown in Table 4.14. Internode explants derived callus showed multiple shoot when cultured on MS medium supplemented with 0.5 mg/l BAP + 0.5 mg/l NAA and 1.0 mg/l BAP + 0.5 mg/l NAA. No shoot formation was achieved in all treatments except the above two treatments. Highest shoot number (7.00) was observed in 0.5 mg/l BAP + 0.5 mg/l NAA followed by 1.0 mg/l BAP + 0.5 mg/l NAA when internode derived callus cultured in the medium.

Table 4.14. Effect of different concentrations of 2, 4-D and NAA and combination of BAP with NAA on shoot regeneration and proliferation from leaf and internode derived callus

Treatment (mg/l)		Days to shoot induction		Shoot number per culture		Shoot length (cm)		Leaf number per shoot	
		Leaf	Internode	Leaf	Internode	Leaf	Internode	Leaf	Internode
2, 4-D									
0.1		28	-	2.0	-	5.80	-	9.00	-
0.5		-	-	-	-	-	-	-	-
1.0		-	-	-	-	-	-	-	-
1.5		-	-	-	-	-	-	-	-
2.0		-	-	-	-	-	-	-	-
NAA									
0.1		-	-	-	-	-	-	-	-
0.5		-	-	-	-	-	-	-	-
1.0		28	-	3.00	-	6.50	-	10.00	-
1.5		-	-	-	-	-	-	-	-
2.0		-	-	-	-	-	-	-	-
BAP	NAA								
0.5	0.1	-	-	-	-	-	-	-	-
0.5	0.5	-	35	-	7.00	-	6.25	-	8.10
0.5	1.0	-	-	-	-	-	-	-	-
0.5	1.5	-	-	-	-	-	-	-	-
0.5	2.0	-	-	-	-	-	-	-	-
1.0	0.1	-	-	-	-	-	-	-	-
1.0	0.5	-	30	-	3.00	-	5.70	-	7.20
1.0	1.0	-	-	-	-	-	-	-	-
1.0	1.5	-	-	-	-	-	-	-	-
1.0	2.0	-	-	-	-	-	-	-	-



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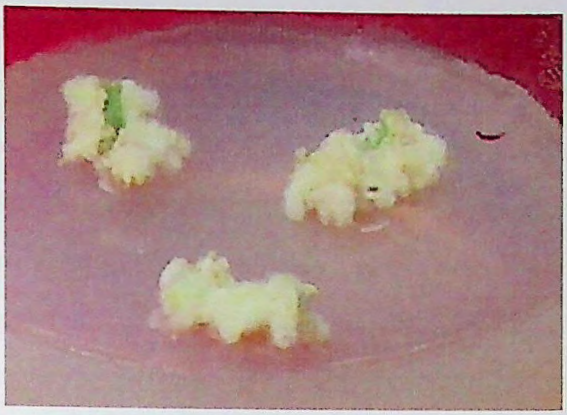
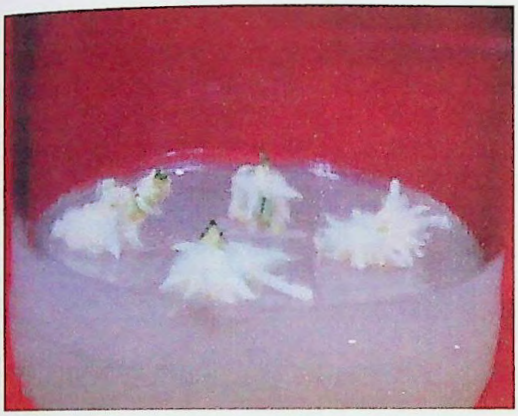


B



C

D

E

F

G

Fig. 7. Indirect regeneration of pointed gourd.

A. Callus of leaf
B. Callus of internode
C. Callus with root
D. Callus of cotyledon
E. Shoot derived from callus
F. Multiple shoot with root
G. Established plant in earthen pot

5.2 SUMMARY

Under this study, a protocol for multiple shoot propagation in pointed gourd (*Trichosanthes dioica* Roxb) was successfully developed to facilitate further advance research on genetic improvement through application of biotechnological approaches. For direct regeneration the most suitable medium formulation was developed and highest percentage of shoot induction (93.86%), highest shoot number per explant (3.25) and longest shoot per explant (4.18 cm) were observed in 2.0 mg/l BAP followed by 1.5 mg/l BAP when the nodal explants cultured on MS medium supplemented with BAP alone. Highest multiple shoot were achieved in 2.0 mg/l BAP + 0.3 mg/l NAA from nodal segment. Half strength MS medium supplemented with 2.0 mg/l BAP was also found best for multiple shoot induction from nodal segments of *in vitro* grown plants. The cultivar AM-8 was found better than AM-15 in regenerating performance. From the present investigation, it may be concluded that the nodal segments performed better than shoot tip explants for shoot induction, shoot number and shoot length. BAP was found superior to Kn and TDZ and in respect of hormonal combinations of BAP and NAA was proved superior to other combinations.

Different concentrations of 2, 4-D, NAA (0.1, 0.5, 1.0, 1.5 and 2.0mg/l) and combinations of BAP with NAA were added to MS medium to investigate their callus induction potentiality. MS medium supplemented with 0.5 mg/l NAA + 0.5 mg/l BAP was found most suitable in callus induction and plant regeneration. Among all the explants used in *in vitro* regeneration study, nodal segment of pointed gourd responded well for multiple shoot formation and internode appeared as the most suitable explant for callusing and plant regeneration. In comparative study, female genotypes responded better than the male genotypes for shoot regeneration. On the other hand, lower nodal segment situated on the lower end was found better than upper nodal segment for shoot regeneration.

Chapter V ARTIFICIAL SEED PRODUCTION

5.1 INTRODUCTION

Plant propagation using artificial or synthetic seeds developed from somatic but not zygotic embryo opens up new vistas in plant species. Artificial seeds make a promising technique for propagation of transgenic plant conservation, non-seed producing plants, polyploids with elite traits and plant lines with problems in seed propagation. Being clonal in nature the technique cuts short laborious selection procedure of the conventional recombination breeding and can bring the advancements of biotechnology to the doorsteps of the farmer in a cost effective manner (Saiprasad, 2001). Micropropagation techniques will ensure abundant supply of the desired plant species. In some crop species seed propagation has not been successful. This is mainly due to heterozygosity of seed, minute seed size, presence of reduced endosperm and the requirement of seed with mycorrhizal fungi association for germination (e.g. orchids), and also in some seedless varieties of crop plants like grapes, watermelon and tomato. Pointed gourd and some other species can be propagated by vegetative means. Traditionally pointed gourd is cultivated through stem and root cuttings. Propagation through seeds is not desirable due to poor germination, imbalanced male-female ratio and seed propagated plant normally do not produce flower within one year, if flowering, it requires more than one year for fruit setting. However, *in vivo* vegetative propagation techniques are time consuming and expensive and the propagules carry the inborn diseases and pest from the mother plant to the seedlings. Development of artificial seed producing technology is currently considered as an effective and efficient alternate method of propagation in several commercially important agronomic and horticultural crops. It has been suggested as a powerful tool for mass propagation of elite plant species with high commercial value (Saiprasad, 2001).

Artificial seed technology involves the production of tissue derived somatic embryos encased in a protective coating. Artificial seeds have also been often referred to as synthetic seeds. These synthetic seed would also be a channel for new plant lines produced through biotechnological advances to be transferred directly to the green house or field. This synthetic seed production technology is a high volume, low cost production technology. High volume propagation potential of somatic embryos

combined with formation of synthetic seeds for low cost delivery would open new vistas for clonal propagation in several commercially important crop species.

The artificial seeds can be used for specific purposes, notably multiplication of non-seed producing plants, ornamental hybrids (currently propagated by cuttings) or the propagation of polyploid plant with elite traits. The artificial seed system can also be employed in the propagation of male or female sterile plants for hybrid seed production. Cryopreserved artificial seeds may also be used for germplasm preservation, particularly in recalcitrant species (such as mango, jackfruit, cocoa and coconut), as these seeds will not undergo desiccation. Furthermore, transgenic plants, which require separate growth facilities to maintain original genotypes may also be preserved using somatic embryos. Artificial seed produced in tissue culture are free of pathogens. Thus another advantage is the transport of pathogen free propagules across the international borders avoiding bulk transportation of plants, quarantine and spread of diseases. Synthetic seed techniques can be especially useful in increasing propagates of a new sexual or somatic hybrid or a plant free from pathogens or even in case of a genetically engineered plant.

Plant tissue culture techniques are now being widely used in plant propagation and crop improvement. Recently, somatic embryogenesis and the encapsulation of somatic embryos have been attempted in several plants (Redenbaugh, 1985). Encapsulation of embryos as well as axillary buds to produce synthetic seeds could serve as a substitute for the seeds produced *in vivo*.

Recently, production of synthetic seeds by encapsulating somatic embryos has been reported in few species. One prerequisite for the application of synthetic seed technology in micro-propagation is the production of high quality, vigorous somatic embryos that can produce plants with frequencies comparable to natural seeds. Inability to recover such embryos is often a major limitation in the development of synthetic seeds. Synthetic seed technology requires the inexpensive production of large numbers of high quality somatic embryos with synchronous maturation. Encapsulation and coating systems, though important for delivery of somatic embryos are not the limiting factors for development of synthetic seeds.

In order to make the system economically viable many workers have encapsulated the somatic embryos in an alginate matrix to prepare synthetic seeds (Kitto and Janik, 1985a, 1985b; Redenbaugh *et al.*, 1986). In some instances, instead of embryos axillary buds also have been encapsulated and germinated (Bapat *et al.*, 1987). This study includes a report of successful micro propagation through shoot tip and nodal bud culture in pointed gourd (Malek *et al.*, 2007 and Mamun Hossain *et al.*, 1996). The present study describes the production of artificial seeds from encapsulation of shoot tip and nodal bud through solid cultures. The result indicates that this method provides a mechanism for handling tissue cultured plants in a manner similar to that of seed grown ones. Recent advances in the area have revealed that besides somatic embryos, encapsulation of cells and somatic tissues obtained following tissue culture techniques has become popular as a simple way of handling cell and tissue, protecting them against strong external gradients and as an efficient delivery system (Datta *et al.*, 1999).

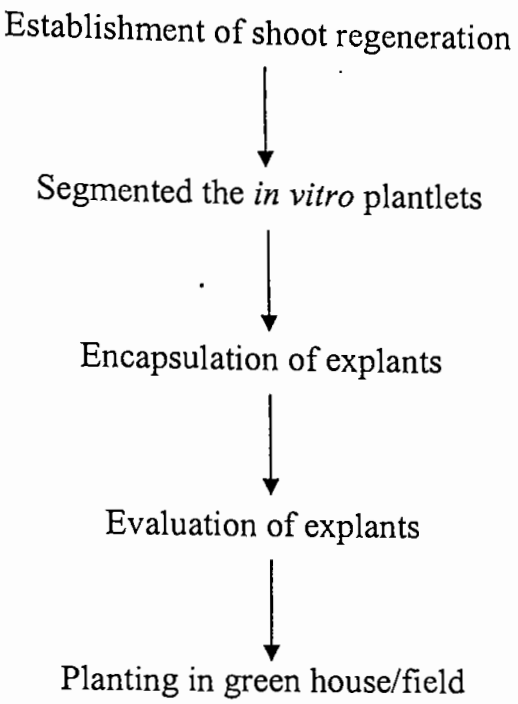
Besides rapid and mass propagation of plants, the artificial seed technology has added new dimensions not only to handling and transplantation but also for conservation of endangered and desirable genotypes. Many facets of artificial seed production by encapsulating the PLBs (protocorm-like bodies) have been intensively investigated in the terrestrial and fast disappearing orchid, namely, *G. densiflorum*. With the ultimate objective of conservation of orchid taxon; PLB production, encapsulation, *in vitro* and *in vivo* germination of the artificial seeds had been achieved (Datta *et al.*, 1999).

The concept of somatic seed formation has been extended to cover the possibilities of production of artificial seeds through encapsulating all types of regenerative tissues for developing true parent plants. The main objective of this investigation was to develop a procedure for the encapsulation shoot tips and nodal segments of pointed gourd to the production of synthetic seeds, as a new propagation method. Under the present initiative attempts were taken to develop the revenue for the production of artificial seeds. By encapsulating highly regenerative shoot tips and nodal segments of pointed gourd genotypes were used in the present study.

5.2 MATERIALS AND METHODS

Shoot tips and nodal segments, 3-4 mm long were aseptically excised from *in vitro* cultured plants regenerated by the method described by Maruyama (1996).

5.2.1 Flow chart for production of artificial seed



Sodium alginate beads were produced by encapsulation according to the method of Kinoshita and Saito (1990). The methods involved in this investigation can be described under the following heads:

5.2.2 Culture media preparation

Culture media were prepared following MS (Murashige and Skoog, 1962) media preparation technique and 8 gram agar was added to each 1000 ml solution and then autoclaved for 20 minute under 121^0 C.

5.2.3 Preparation of Alginate and $CaCl_2$ solution

5.2.3.1 *Media preparation*

200 ml MS medium was prepared 8.0 gm (4%) sucrose was first added to 150 ml of MS solution and then different concentrations and combinations of BAP (0.5, 1.0, 1.5 and 2.0 mg/l) and NAA (0.5, 1.0, 1.5 and 2.0 mg/l) was also added to it. After mixing the solution it was filled up to 200 ml.

5.2.3.2 *Alginate solution*

20 ml of above mentioned solution (MS + Sucrose + hormones) was taken and 0.8 gm (800 mg) of sodium alginate was added and taken in a small beaker (50 ml beaker). With a small piece of glass rod efforts were made to mix the alginate in solution.

Alginate was partially dissolved and it was then kept aside. During autoclaving alginate was completely dissolved.

5.2.3.3 *$CaCl_2$ solution*

50 ml of above mentioned solution (MS + Sucrose + hormones) was taken in a small beaker. An amount of 0.7 gm (700 mg) $CaCl_2$ was added to it and dissolved. Out of 200 ml, 70 ml (50+20) was used during the preparations of alginate and $CaCl_2$ solution and another 130 ml was remained reserved. After autoclaving it was used during washing the encapsulated explants.

5.2.4 **Autoclaving**

a. Culture media
b. Alginate solution
c. $CaCl_2$ solution
d. Several petridishes
e. Remaining 130 ml (MS + Sucrose + hormones) solution

5.2.5 **Encapsulation**

Sodium alginate beads were produced by encapsulation according to the method of Kinoshita and Saito (1990). Only *in vitro* grown plants were used for this experiment. Explants were taken in an autoclaved petridishes and shoot tips and nodal segments were cut carefully removing the internodal zones. The excised shoot tips and nodal segments with active buds were placed to the beaker containing sodium alginate solution. The explants were dipped in alginate solution and kept inside the solution for about 30 minutes. The dipping explants were taken by a forceps and placed to the beaker of $CaCl_2$ solution. During picking up the explants, the forceps also took some addition of alginate solution together with the explants. After 30 minutes each explants become a hard ball encoded by alginate. They were then washed well with remaining solution of MS + Sucrose + hormones (BAP, NAA and combinations of BAP and NAA).

After washing, the explants were cultured in culture tubes containing MS medium supplemented with different concentrations of BAP (0.5, 1.0, 1.5 and 2.0 mg/l) and NAA (0.5, 1.0, 1.5 and 2.0 mg/l) and combinations of BAP and NAA. The germination response of the encapsulated shoot tips and nodal buds were scored after

three weeks of culture. For each treatment 20 shoot tips and nodal buds were tested and all the experiments were conducted under controlled conditions of light (1000 lux), temperature (25±2^{0}C) and relative humidity (50-60%).

5.3 RESULTS AND DISCUSSION

Under artificial seed production study, explants were taken from the living *in vitro* plants. Two genotypes of pointed gourd (AM-8 and AM-15) were used to exhibit different level of morphogenic growth response in tissue culture media. The explants taken from a particular plant part is also equally important in artificial seed production. Different concentrations of auxin and cytokinin were used singly or in combinations to investigate the induction of shoot regeneration. These hormonal concentrations were tested in MS basal media (Murashige and Skoog, 1962). Shoot tips and nodal buds were used as explants for this investigation. Different concentrations of BAP and NAA were used singly or in combinations to investigate the initiation of shoot and its subsequent regeneration. The encapsulated shoot tips and nodal segments explants were cultured for shoot regeneration. Encapsulated explants (synthetic seeds) were cultured in the MS agar gelled media supplemented with different concentrations and combinations of BAP (0.5, 1.0, 1.5 and 2.0 mg/l) and NAA (0.5, 1.0, 1.5 and 2.0 mg/l). Data on percentage of shoot forming explants and days to shoot proliferation were recorded after 4 weeks of culture. The results are presented according to types of explants used under separate heads.

5.3.1 **Artificial seed regeneration derived from shoot tip**

In the present investigation, encapsulated shoot tips of in vitro grown plants were used as explants and cultured in MS media supplemented with different concentrations and combinations of BAP (0.5, 1.0, 1.5 and 2.0 mg/l) and NAA (0.5, 1.0, 1.5 and 2.0 mg/l). Shoot proliferations began from 7-9 days. Data were recorded after 4 weeks of culture. Results obtained on morphogenic response of the cultured explants are shown in Table 5.1. Morphogenic responses of the encapsulated explants were found to vary with hormonal formulations present in the culture media. Shoot regeneration was observed for all the treatments and genotypes (AM-8 and AM-15) of pointed gourd studied. In case of AM-8, shoot proliferation ranged from 30.00-85.00%. Highest percentage (85.00%) of shoot proliferation was observed in MS medium containing 1.0 mg/l BAP followed by MS medium containing 1.0 mg/l BAP + 1.0 mg/l NAA (75.00%). The lowest percentage (30.00%) of shoot formation was observed in MS + 2.0 mg/l NAA and MS + 1.0 mg/l BAP + 2.0 mg/l NAA.

Table 5.1 Effect of different concentrations and combinations of BAP and NAA on artificial seed proliferation from shoot tip explants

Treatments (mg/l)	AM-8		AM-15	
	Days to shoot proliferation	% of shoot forming explants	Days to shoot proliferation	% of shoot forming explants
BAP 0.5	7	75	7	80*
BAP 1.0	7	85**	7	90**
BAP 1.5	7	70	7	72
BAP 2.0	7	55	7	58
Mean	7.00	71.25	7.00	75.00
S E (±)	0.000	6.250	0.000	6.757
NAA 0.5	8	65*	8	70*
NAA 1.0	8	70**	8	75**
NAA 1.5	8	45	8	47
NAA 2.0	8	30	8	30
Mean	8.00	52.50	8.00	55.50
S E (±)	0.000	9.242	0.000	10.460
BAP + NAA				
1.0 + 0.5	8	55	8	60
1.0 + 1.0	8	75**	8	80**
1.0 + 1.5	8	50	8	55
1.0 + 2.0	8	30	8	35
Mean	8.00	52.50	8.00	57.50
S E (±)	0.000	9.242	0.000	9.242
NAA + BAP				
1.0 + 0.5	9	50	9	60
1.0 + 1.0	9	75**	9	80**
1.0 + 1.5	9	50	9	55
1.0 + 2.0	9	40	9	40
Mean	9.00	53.75	9.00	58.75
S E (±)	0.000	7.465	0.000	8.260

*, ** Significant at 5% and 1% levels, respectively.

In case of AM-15, shoot proliferation ranged from 30.00-90.00%. Among the different concentrations and combinations of BAP and NAA, the highest 90.00% of shoot formation was observed in MS medium containing 1.0 mg/l BAP followed by 80.00% shoot proliferation that was obtained in MS + 0.5 mg/l BAP and MS + 1.0 mg/l BAP + 1.0 mg/l NAA. The lowest percentage (30.00%) of shoot formation was observed in MS + 2.0 mg/l NAA.

5.3.2 Artificial seed regeneration derived from nodal segment

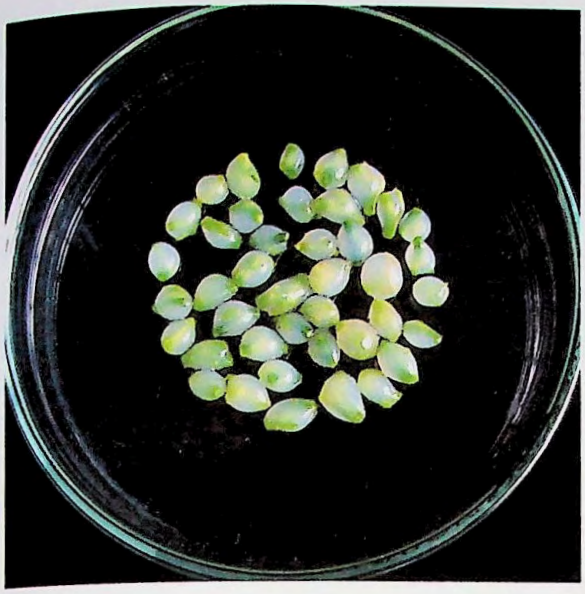
In this experiment, nodal segments of *in vitro* grown plants were used as explants and cultured in MS media supplemented with different concentrations and combinations of BAP (0.5, 1.0, 1.5 and 2.0 mg/l) and NAA (0.5, 1.0, 1.5 and 2.0 mg/l). Shoot proliferations began from 5-8 days. Data were recorded after 4 weeks of culture. Results obtained on morphogenic response of the cultured explants are shown in Table 5.2. Morphogenic responses of the encapsulated explants were found to vary with hormonal formulations present in the culture media. Shoot regeneration from nodal bud was also observed in all hormonal formulations. In case of AM-8, shoot proliferation ranged from 35.00-90.00%. Highest percentage (90.00%) of shoot proliferation was observed in MS medium containing 1.0 mg/l BAP followed by MS medium containing 0.5 mg/l BAP and 1.0 mg/l BAP + 1.0 mg/l NAA (80.00%). The lowest percentage (35.00%) of shoot formation was observed in MS medium having 2.0 mg/l NAA and 1.0 mg/l BAP + 2.0 mg/l NAA.

In case of AM-15, shoot proliferation ranged from 30.00-95.00%. Among the different concentrations and combinations of BAP and NAA, the highest 95.00% of shoot formation was observed in MS medium containing 1.0 mg/l BAP followed by 90.00% shoot proliferation in MS medium containing 0.5 mg/l BAP. The lowest percentage (30.00%) of shoot formation was observed in MS medium having 1.0 mg/l BAP + 2.0 mg/l NAA. The experimental results indicated that different concentrations and combinations of both cytokinin and auxin play an important role in germination of artificial seeds being encapsulated by sodium alginate. In the present study, MS + 1.0 mg/l BAP gave the best results in both explants as well as genotypes of pointed gourd. In the present investigation, it was observed that a good number of shoot proliferations could be established in *Trichosanthes dioica* Roxb.

Table 5.2 Effect of different concentrations and combinations of BAP and NAA on artificial seed proliferation from nodal segment explants

Treatments (mg/l)	AM-8		AM-15	
	Days to shoot proliferation	% of shoot forming explants	Days to shoot proliferation	% of shoot forming explants
BAP 0.5	5	80	5	90**
BAP 1.0	5	90**	5	95**
BAP 1.5	5	70	5	75
BAP 2.0	5	65	5	70
Mean	5.00	77.50	5.00	82.50
S E (±)	0.000	5.204	0.000	5.951
NAA 0.5	7	65*	7	70*
NAA 1.0	7	70*	7	75*
NAA 1.5	7	55	7	60
NAA 2.0	7	35	7	35
Mean	7.00	56.25	7.00	60.00
S E (±)	0.000	7.739	0.000	8.897
BAP + NAA				
1.0 + 0.5	7	60	7	60
1.0 + 1.0	7	80**	7	85**
1.0 + 1.5	7	40	7	50
1.0 + 2.0	7	35	7	30
Mean	7.00	52.50	7.00	55.00
S E (±)	0.000	9.242	0.000	10.408
NAA + BAP				
1.0 + 0.5	8	55	8	65
1.0 + 1.0	8	80**	8	85**
1.0 + 1.5	8	50	8	55
1.0 + 2.0	8	40	8	45
Mean	8.00	55.00	8.00	61.25
S E (±)	0.000	7.359	0.000	7.465

*, ** Significant at 5% and 1% levels, respectively.



A

B

C

D

E

Fig. 8. Artificial seed regeneration from nodal explant of pointed gourd.

A. Encapsulated seed in sodium alginate bead

B. Germination of encapsulated seed

C. Shoot induction of encapsulated seed

D. Multiple shoot from encapsulated seed

E. Rooted multiple shoot of encapsulated seed

5.4 SUMMARY

The artificial seed technology is an exciting and rapidly growing area of research in plant cell and tissue culture and unraveling new vistas in plant biotechnology. The idea of artificial seeds was first conceived by Murashige (1978) which was subsequently developed by several investigators. Initially, the development of artificial seed had been restricted to encapsulation of somatic embryos in a protective jelly.

It has been suggested that most suitable encapsulating agent is sodium alginate due to its solubility at room temperature and its ability to form completely permeable gel with calcium chloride (Sharma *et al.,* 1992). Alginate is one of the most commonly used polymers for immobilization of plant cells and production of manufactured seeds because it is available in large quantities, is inert, nontoxic, cheap and easily handled (Endress, 1994). However, studies on *in vitro* germplasm conservation using alginate encapsulation techniques have been reported in only a few species.

Under the present study, encapsulation of shoot tip and nodal segment was successfully developed towards formation of artificial seed with sodium alginate in pointed gourd plant. Artificial seeds developed from shoot tips and nodal segments were subjected to the germination or regeneration study under the treatments of different hormonal formulations. For germination of seeds, encapsulated shoot tips and nodal segments (synthetic seed) were cultured in MS basal medium containing different concentrations and combinations of BAP and NAA to induce shoot proliferation. Among the explants, nodal segments also performed well for shoot proliferation and artificial seed production. In case of AM-15, the highest 95.00% of shoot formation was observed from nodal segments in MS medium containing 1.0 mg/l BAP followed by 90.00% shoot proliferation that was observed in MS medium containing 0.5 mg/l BAP. While in AM-8, highest 85.00% of shoot formation was observed in MS medium containing 1.0 mg/l BAP followed by 75.00% shoot proliferation which was obtained in MS +1.0 mg/l BAP +1.0 mg/l NAA when shoot tips cultured in the medium. The experimental results indicated that the genotype AM-15 showed better results for artificial seed regeneration. The results also exhibited that cytokinin play an important role in germination of artificial seed being encapsulated by sodium alginate.

Under the present study with limited experiments efforts have been made to establish the protocol for encapsulating the vegetative shoot for the production of artificial seed and their subsequent regeneration under the treatment of different hormonal formulations. It is the first report in Bangladesh to develop artificial seed production using vegetative tissue of the crop plants. It has opened a new area of advance research for developing the conservation strategies for plant genetic resources in the country.

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