IMPROVEMENT OF POINTED GOURD (*Trichosanthes dioica* Roxb.) THROUGH CLONAL SELECTION AND POLYPLOIDIZATION

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2000

Dedicated To

My

Beloved Parents

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CERTIFICATE

This is to certify that the work recorded in the thesis entitled **'IMPROVEMENT OF POINTED GOURD (*Trichosanthes dioica* Roxb.) THROUGH CLONAL SELECTION AND POLYPLOIDIZATION"** submitted by Shri Rajib Ghosh for the award of the Degree of Doctor of Philosophy in Vegetable Crops (Horticulture) of the Bidhan Chandra Krishi Viswavidyalaya, is a faithful and bonafide research work carried out under my personal supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

19.7.2000
Signature of the Advisor

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The 19th July..., 2000

Approval Sheet

Approval of Examiners for the award of the Degree of Doctor of Philosophy in Vegetable Crops (Horticulture).

We, the undersigned, having been satisfied with the performance of Sri Rajib Ghosh in viva voce examination, conducted today, the 25th Oct. 2000 recommend that the thesis be accepted for the award of the Degree.

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CHAPTER - I

INTRODUCTION

Introduction

Vegetables are important components of our balanced diet because of their contributions of vitamins, minerals, proteins, carbohydrates and fibres. Malnutrition of the huge Indian population is mainly due to inadequate intake of vegetables, the low cost protective foods. Although our country is the second largest producer of vegetables in the world, per capita availability of vegetables here is still far behind the recommended quantity of 280-300 g/caput/day. Furthermore, the alarming rate of the population growth will further worsen the situation. It has been estimated that by 2005 A. D., 120 m tonnes of vegetables will be required as against the present production of 84 m tonnes. Low productivity level of different vegetable crops is the main reason of such low availability of vegetables to our huge population. So genetic improvement plays the vital role in enhancing the yield potential of vegetable crops.

Among the different vegetable species grown in India, members of the family of Cucurbitaceae have enriched and diversified our diet for centuries. It is also the genetically most diverse group in plant kingdom as a family as well as individual crop. Not only many cultivars in these crops vary significantly in characteristics but also the same cultivar grown in different areas responds differently to the varying growing conditions.

Pointed gourd (*Trichosanthes dioica* Roxb.), also called "parwal" or "patal" is an important cucurbitaceous vegetable crop extensively cultivated in India particularly in Bihar, eastern Uttar Pradesh, West Bengal, Assam and Tripura, to some extent in Orissa, Madhya Pradesh, Maharastra and Gujarat. In the '*diara*' land of Bihar and eastern Uttar Pradesh the crop is rainfed. This perennial and dioecious vegetable crop is a highly accepted one, and is available for

nearly eight months of the year. It is called "King of gourds" because of its higher nutrient content than any other cucurbits. The green tender fruits of pointed gourd are consumed as vegetable. However, the tender shoots and leaves are used as potherbs particularly in the cookery of West Bengal. It is extremely nutritive, wholesome, easily digestible, diuretic and laxative. It also invigorates heart and brain and is useful in the disorders of the circulatory system. The fruits show some prospects in the control of certain cancer like conditions (Som *et al.*, 1993).

Improvement approaches of this highly priced vegetable crop could not be paved concertedly not only in West Bengal but also in the other parts of the country. For this reason, total area under pointed gourd over the country as well as the state is under several variable local cultivars which hinders the realization of yeild potential as well as quality parameters. Pointed gourd is essentially popagated vegetatively and naturally cross pollinated due to dioecious sexform. So improvement approaches for pointed gourd will be different than what would be applicable for other sexually propagated and cross pollinated vegetable crops. In this crop, the clones are heterozygous and therefore, diverse genotypically which provide ample scope for clonal selection. Obligatory outcrossing breeding system of this crop allows wide range of genetic recombinants with natural seed settings. Such occasional field grown seedling segregates also enrich the diversity in the farmers' field which is maintained indefinitely through vegetative propagation. However, creation of diversity in the clones is restricted in comparision to that in other sexually propagated vegetable crops. Assam - Bengal region is beleived to be the centre of origin of this crop which amply suggests the presence of diversity of pointed gourd in the eastern and north eastern India. Success in clonal selection depends mainly on the extent of the diversity of the available clones, proper characterization of the clones, assesment of variability present in the crop for different characters and determination of character associationships.

Value addition to the product through differential breeding approaches may be worthwhile in pointed gourd. Alteration of ploidy through the induction of tetraploidy and triploidy has been utilized commercially for the development of high quality varieties of two cucurbitaceous vegetable crops viz. water melon and musk melon. Such improvement approach has not so far been employed in pointed gourd having the same chromosome number (2n = 22) as that of water melon. Main disadvantage of induced polyploidy for practical utilization is the unstability of the higher ploidy level plants due to complex segregation. Such unstability in the plants of higher ploidy level due to imbalanced meiosis can be overcome in pointed gourd by rendering to vegetative propagation of the induced high ploidy level plants.

Keeping the necessity of initiating improvement approaches of pointed gourd, the highly priced vegetable crop in West Bengal and considering the effective breeding approaches for such asexually propagated, dioecious, perennial and cross pollinated crop, the present investigation was undertaken on two breeding approaches : Clonal selection and Polyploidization. Different aspects of these studies were:

1. Grouping and characterization of female clones through group means, multivariate analysis, isozyme pattern, petiole anatomy and stomatal features.
2. Genetic variability for different characters.
3. Character associationship study through genotypic and phenotypic correlation coefficients and path coefficient analysis.
4. Development and growing of the colchiploids.
5. Study of the colchiploids through morphological and reproductive features, stomatal features and pollen characters.

CHAPTER - II

REVIEW OF LITERATURE

Review of Literature

The literatures reviewed on different aspects for the present investigations were outlined under :

2.1 Biosystemics

2.2 Cytology

2.3 Origin, distribution, area, nutritional status and utilization.

2.4 Characterization

2.5 Genetic divergence

- 2.5.1 Multivariate analysis
- 2.5.2 Isozyme pattern for the characterization of genetic diversity
- 2.5.3 Petiole anatomy
- 2.5.4 Stomatal features

2.6 Genetic variability

2.7 Character associationship

2.8 Alteration of ploidy

2.9 Methods of ploidy determination

2.1 Biosystemics

The pointed gourd (*Trichosanthes dioica* Roxb.) belongs to the family Cucurbitaceae. There is controversy over the tribe to which genus *Trichosanthes* belongs. Jeffrey (1980) put the genus under the tribe Trichosantheae of the subfamily Cucurbitoideae. On the otherhand, Chakravarty (1982) kept this genus under the tribe Cucumerineae. Of the different species of *Trichosanthes*, the species under which the pointed gourd belongs (*Trichosanthes dioica* Roxb.) is principally the species of Indian distribution (Seshadri, 1986).

2.2 Cytology

Cytological studies have shown that *T. dioica* and *T. anguina* have 2n = 22 chromosomes (Singh and Roy, 1973; Sarkar and Dutta, 1988). Strong male determining role of Y chromosome was also evident (Seshadri, 1986).

2.3 Origin, distribution, area, nutritional status and utilization

Decandolle (1882) wrote in his book "Origin of Cultivated Plants" that the species of *Trichosanthes* were of old world origin, most probably from India, especially in case of pointed gourd *Trichosanthes dioica*. Although the centre of origin of *Trichosanthes* is not precisely known, most authors agree that India and Indo-Malayan region is its' original home (Seshadri, 1986). Chaudhury (1990) concluded that Assam-Bengal region was the primary centre of origin, although its wild forms are found throughout North India.

Pointed Gourd is widely cultivated in eastern part of India particularly in West Bengal, Assam, Bihar and Uttar Pradesh (Nath and Subramanyan, 1972). The area under pointed gourd cultivation is about 10,000 ha in Uttar Pradesh and 14,000 ha in North Bihar (Singh, 1989). Hundred gram of the fresh fruit contains 2.0g protein, ten times that of bottle gourd (0.2g), about four times that of ash gourd (0.4g), snake gourd (0.5g) and ridge gourd (0.5g) and beside these, vitamin A content (255 I.U.) is four and half times that of ridge gourd (65 I.U.); about three times that of pumpkin (84 I.U.) and hundred times that of the ashgourd and bottle gourd. As regards vitamin C, it is much higher (29 mg) as compared to any of the gourds mentioned before (Nath *et al.*, 1987). Pointed gourd is easily digestible, diuretic and laxative. It is also found very useful for heart and brain and very effective in curing disorders of the circulatory system (Chaudhury, 1990).

2.4 Characterization

Singh (1989) so far for the first time characterized pointed gourd clones and grouped on the basis of shape, size and striation of fruits as follows.

1. Plants bearing 10-13 cm long, dark green fruits with white stripes.
2. Plants bearing 10-16 cm long, thick dark green fruit with very faint pale green stripes.
3. Plants with small (5-8cm long) roundish dark green striped fruits.
4. Plants with small fruits tapering toward the ends, green and striped.

2.5 Genetic divergence

The germplasm is the reservoir of genetic diversity which is often exploited to meet the changing needs for developing improved varieties of a crop. It is also important that considerable variability for economic traits must exist in the the germplasm for profitable exploitation following selection or recombination breeding. Pointed gourd is a perennial, heterozygous and asexually propagated vegetable crop which exhibit an wide range of variation for utilization in clonal selection programme for its' improvement. However, it being an vegetatively propagated crop, the generation of variability through sexual means naturally is limited. It is therefore, essential to characterize the genetic divergence of the present clonal assemblage for selection of suitable and diverse clones for sustainable improvement programmes in pointed gourd. The importance of genetic diversity in the improvement of a crop is stressed in both self and cross pollinated crops (Griffing and Lindstrom, 1954; Matzinger *et al.*, 1962; Timothy, 1963; Fonseca and Patterson, 1968; Anand *et al.*, 1975).

2.5.1 Multivariate analysis through D^2 statistic : Among the several statistical method followed for measuring the divergence between the population, may it be sexual or clonal, multivariate analysis has been an useful tool in the quantitative estimation of genetic diversity. The importance of multivariate analysis has been greatly emphasised for assesment of genetic divergence in biological populations (Fisher, 1936; Smith, 1936).

Multivariate analysis by means of Mahalanobis's D^2 statistic (1936) is an useful tool in quantifying the divergence between the biological populations at genotypic level and to assess the relative contribution of different components to the total divergence both at inter and intra cluster levels (Murthi and Arunachalam, 1966; Ram and Panwar, 1970; Sachan and Sharma, 1971; Jatasra and Paroda, 1978). This multivariate analysis is effective because large amount of data about the germplasm can be reduced to manageable proportion (Malhotra and Singh, 1971; Katiyar and Singh, 1979). The procedure of characterizing genetic divergence has been succesfully utilized in other perennial and asexually propagated crop like mulberry (Gupta *et al.*, 1991, Tikader *et al.*, 1999) and asexually propagated annual flower crop like gladiolus (Raj and Mishra, 1999).

Works on genetic divergence in pointed gourd is limited. From a joint exploration in collaboration with NBPGR, IIVR(erstwhile PDVR) and Central Horticultural Experiment Station (CHES), Ranchi, 36 Genotypes/landraces were sampled from North Bihar and West Bengal. Ranges in magnitude of the characters were 3.67-45.6g for fruit weight, 10-30 for seeds/fruit, 0.30 to 0.48 cm for flesh thickness, fruit volume 15.4 ml to 66.0 ml, 2.71 to 4.10 cm for fruit diameter, 5.3 to 9.6 cm for fruit length, 0 to 14 for number of stripes/fruit and 0.4

to 3.20 cm for pedicel length (Gupta *et al.*, 1998). 167 genotypes were grouped into 16 clusters through non-hierarchial euclidian cluster analysis showing existence of high genetic diversity in the material and the characters, yield/plant, fruits/plant and fruit weight contributed maximum towards divergence (Ram *et al.*, 1998).

Dora *et al.* (1998) on the other hand, suggested that numerical taxonomic approach is relatively more potent in classification of biological entities as compared to D^2 statistic as in the former approach, a large cluster containing genotypically more or less similar individuals (at higher phenon level) help the breeders in choosing elite parents more precisely.

2.5.2 Isozyme pattern for the characterization : The term isozyme (syn. Isoenzyme) was first introduced by Markert and Moller (1959) to refer to multiple molecular forms of an enzyme with similar or identical substrate or enzymatic specificity, sharing a catalytic activity and derived within the same organism. Isozyme heterogeneity was discerned first in esterase and lactate dehydrogenase enzymes (Hunter and Markert, 1957).

In the isozymes, structural variation in the protein occurs with the retention of enzymatic activities. Isozyme may arise through the binding of a single polypeptide to varying of numbers of co-enzyme molecules (Jacobson, 1968), from allelic segregations at a single locus representating more subtle changes in the enzyme molecule (Pierce and Brewbaker, 1973), and due to mutations (Shannon 1968). Genetic control of isozyme polymorphism appears to be largely monogenic with the involvement of both structural and modifier loci, and in rare instances such genetic control is polygenic (Pierce and Brewbaker, 1973; Scandalios, 1974).

Enzymes can be separated into different molecular forms or isozymes by many biochemical methods, including chromatography, gel infiltration, sedimentation, electrophoresis and serological methods (Shannon, 1968). Of these, gel electrophoresis is the most powerful analytical techniques available to separate isozymes. In the undergoing theory of electrophoresis, direct current is used to separate the individual isozymes by taking advantage of the different and characteristic net charge of each isoenzyme. Thus electrophoresis is basically a process of forced diffusion within an electrical field (Pierce and Brewbaker, 1973). Protein molecules of the sample are moved through the medium (gel, paper, cellulose) by applying electrical gradient. Different proteins assume different charges, often with different net sign, at different pH levels, and their rates of migration through the gradient differs in proportion to their charge and molecular weight. This results in separation of the different proteins, i.e. isoenzymes into bands (visible finger prints or zymogram) which can be resolved by staining.

Population of purelines, highly self pollinated crops and clones refers to cultivars, lines or even accession of a collection. The characterization of the genetic variability of a population is an important task of the breeder to determine the extent of response in crop improvement. The agronomic characters involve high genotype x environment interaction hence, reliable estimates of population values for these characters can only be obtained from replicated multi-environment trials or from observation taken under standardised conditions. Again such phenotypic approach to characterizing and comparing populations does not provide genetic information (Crawford, 1983; Simpson and Withers, 1986). Therefore, isozymes are ideal biochemical/genetic markers to estimate genetic variability

and characterize plant populations. Advantage of isozymes as a marker are summerised (Pierce and Brewbaker, 1973; De la vega, 1993) : a) Isozymes as other biochemical markers are less affected, if at all, by environmental factors, b) many tissues (young leaf, cotyledon, mesocotyl, radicle, pollen, etc.), even morphologically indistinguishable ones (bark or root) can be assayed to produce isozyme fingerprints, c) phenotypic difference of electrophoretic band patterns are usually interpreted in terms of loci and alleles, d) alleles of different loci are distinguishable, e) allelic expression is generally co-dominant and free of epistatic interactions, f) enzymatic systems to be studied are usually chosen for technical reasons independent of their level of genetic variability and as a result of this, they can represent a random sample of the genome and g) allelic differences are always detected as mobility differences, independent of functioning and level of variability of each enzyme system.

In pointed gourd, population characterization or varietal identification has not so far been attempted through polymorphism at isozyme loci. However, isozyme/protein variations have been extensively used in genetic studies in other cucurbits particularly summer squash, *Cucurbita pepo* (Dvorak and Cernohorska, 1967; Denna and Alexander, 1975; Ignart and Weeden, 1984; Loy, 1972), cucumber (Wood, 1971; Isshiki *et al.*, 1992; Knerr *et al.*, 1995) and musk melon (Puchalski *et al.*, 1978; Berg-Vanden and Gabillard, 1994; Yadav *et al.*, 1998).

The enzyme system - peroxidases

Peroxidase isozyme, because of their common occurence and the ease of their detection, have been investigated more than any other plant isozyme. Peroxidases are a complex and heterogeneous group of enzymes which can utilize hydrogen peroxide to oxidise a wide

range of hydrogen donors such as phenolic substances, ascorbic acid, indole amines and certain inorganic ions especially the iodide ion (Saunders, 1964). The peroxidases are hemoproteins and extremely specific for their requirement for hydrogen peroxide (Scandalios, 1974).

2.5.3 Petiole anatomy

The tissues of petiole are comparable to primary tissues of the stem. There is close similarity between petiole and stem with regard to the structure of epidermis. The ground parenchyma of the petiole is like the stem cortex in arrangement of the cell and in number of chloroplasts, which are fewer than in the mesophyll of the leaf blade. The supporting tissue of the petiole is collenchyma or sclerenchyma and these may have both disposition and structure similar to those in the stem (Esau, 1991). So, petiole anatomy can well be regarded as stem anatomy. Taking into account the variability of vascular structure in the petiole, Howard (1979) proposed a classification relating the nodal structure at the level of the leaf gap to the vascular pattern in leaf base and successive higher level of the petiole and midrib. This approach to the study of the petiolar anatomy is important when the latter is used as a taxonomic character (Howard 1979; Dehgan, 1982). Information regarding the characterization of the genetic diversity in the light of petiole anatomy or stem anatomy is really meagre. Recently Mandal *et al.* (1999) characterized the genotypic specificity of *Catharanthus* in relation to leaf anatomy.

2.5.4 Stomatal features

The interests of stomata has arisen because of the widespread perception of their crucial role in the control of waterloss and of carbondioxide uptake, and because they provide a simple anatomical character that can be used in selection. It is clear from the

earlier reports that huge intraspecific genetic variation in various crops exists for different stomatal characters as presented in Table 1.

Table 1. Stomatal features of different crops.

Character and species	Range	Reference
Frequency/mm²		
Apple (abaxial)	350-600	Beakbane and Majumder, 1975
Barley (abaxial flagleaf)	39-96	Miskin and Rasmusson, 1970
Soybean (abaxial)	242-385	Ciha and Brun, 1975
Broad Bean (abaxial)	50-87	Singh *et al.*, 1982
Three cultigroup of cowpea (adaxial)	82.9-210.5	Hazra *et al.*, 1996
(abaxial)	163.6-350.6	-do-
Length (micron) Barley (abaxial, flag leaf)	40-56	Miskin & Rasmusson, 1970
Soybean (abaxial)	19.2-21.7	Ciha and Brun, 1975
Broad bean (abaxial)	24.9-26.6	Singh *et al.*, 1982
Three cultigroups of cowpea (adaxial)	28.4-36.0	Hazra *et al.*, 1996
(abaxial)	24.0-36.4	-do-

2.6 Genetic variability

Assessment of variability presents in the assemblage of the genotypes of a particular crop for different characters helps successful utilization of plant characters in developing suitable varieties through selection. The genotypic co-efficient of variation (GCV) is the measure of the range of genetic variability in the character, but with the help

of GCV, heritable variation can not be determined (Singh *et al.*, 1974). Burton (1952) suggested that genetic variability alongwith heritability should be considered for assesing the maximum and accurate effect of selection. Heritability is of interest to the plant breeder primarily as a measure of the value of selection for particular character in various types of progenies and as an index of transmissibility (Hayes *et al.*, 1955). Robinson *et al.* (1949) considered that additive genetic variance indicates the degree to which the progeny are likely to resemble the parent and define heritability as the additive genetic variance in per cent of total variance. However, broad sense heritability estimates include additive genetic variance as well as dominance and epistatis, and the estimates so obtained should be considered as maximum heritabilities. Johnson *et al.* (1955) suggested that heritability estimate in combination with genetic advance would be more reliable than heritability alone for predicting the effect of selection. Genetic advance as percentage of mean suggests the percentage advance of the concerned character if top 5% plants are selected from the population.

Shadique *et al.* (1986) studied the genetic variability in pointed gourd cultivars collected from different parts of eastern India and recorded high GCV for main creeper length, leaf number/plant, days from bud initiation to edible fruit maturity, fruit volume, seed number/fruit and fruit yield/plant. However, selection would be effective for fruit number/plant and fruit volume because of the revealation of high heritability coupled with higher genetic advance for these characters. Other such variability studies in pointed gourd indicated the registration of high magnitude of GCV, high broad sense heritability coupled with high genetic advance for shoot number/ plant, primary branches/plant, fruit number/plant, fruit length, fruit

weight, fruit volume, fruit yield/plant and skin thickness, suggesting the probable conditioning of these characters by additive gene action and hence, indicated good scope of improvement in these traits through clonal selection (Singh *et al.*, 1985; Singh *et al.*, 1986; Singh *et al.*, 1987; Sarkar, 1989 and Yadav *et al.*, 1998).

2.7 Character associationship

Indirect selection becomes imperative if the attribute in question has low heritability like yield in most of the cases and/or is not easily and precisely measurable. In such a condition, some criteria of early diagnosis have to be developed to rationalize the selection programme. The aim of correlation studies is primarily to know about the suitability of varioius characters for indirect selection because selection for one character results in correlated response for several other characters (Searle, 1965) resulting into changed patterns of variability (Waddington and Robertson, 1966). The studies on indirect selection response are, therefore, necessary for simultaneous and sequential improvement of component traits. Normally, in the study of correlated response the selection is directed towards better expression of the component character and its indirect effect is observed on yield. In random selections both the homozygous and heterozygous genotypes are equally likely to be retained in the selected groups. In the case the selection differential will be low, but the selection response may be high. The major factors which influence the correlated response are the environmental variations, intensity of selection and genetic constitution of the population i.e., whether the material under study are fixed genotypes or segregating populations (Falconer, 1981). In the totality of yield structure, it is obvious that each yield component could have either a direct or indirect effect on yield, but such causal relationship among yield and yield components

can not be unveiled through the study of linear correlations. In path analysis which was first applied in plants by Dewey and Lu (1959), the cause and effect relationship is well defined and it is possible to represent the whole system of variable in the form of a diagram known as path diagram which gives a much more realistic interpretation of characters involved.

Different studies of character associationship in pointed gourd are hereby reviewed. Fruit yield was positively and significantly correlated with fruits/plant and vine length (Singh *et al.*, 1986). From other study Singh *et al.* (1987) recorded strong and positive correlation between fruit yield/plant and length, diameter and weight of fruit. Significant positive correlation between yield/plant and fruit number/plant (Singh and Prasad, 1989) revealed the importance of fruit number/plant as a major yield component. Prasad and Singh (1990) reported that yield was positively correlated with fruit weight, seed number/fruit and late flowering. Similarly Sarkar *et al.* (1990) suggested fruit weight as the most important yield component followed by fruit diameter, primary branches/plant. In a recent study, high genotypic and phenotypic correlations were recorded between fruit yield/plant and fruit number/plant; negative correlations between yield and maturity traits, and negligible or near zero correlations of fruit yield with size of fruits, fruit weight, fruit length, fruit diameter, specific gravity of fruits, flesh thickness of fruits and seeds/fruit (Yadav *et al.*, 1998). In path coefficient analysis, Singh *et al.* (1993) suggested to consider fruits/plant, days to first picking, fruit weight and branches/plant as important fruit yield components. The above reports indicate that different fruit characters particularly number, length, and weight of fruit are the most important fruit yield attributing characters of pointed gourd.

2.8 Alteration of ploidy

In plants, the somatic chromosome number ranges from four (2n = 4) in *Haplopappus* (Compositae) to as many as 1260 in *Ophioglossum* (Pteridophyta). Constancy in the number of the genetic material is necessary for maintaining the indentity of the species. Sometimes variation in the gametic material occur involving some chromosomes or set in the course of evolution. Such type of change called alteration of ploidy level which causes an increase in the number of chromosome in multiples of the haploid number may be utilized in breeding to develop or impart certain desirable characters to the genotypes. Polyploidy which involves more than two genomes thereby, increase the dose of whole genome. The method of inducing polyploids in plants artificially through the application of colchicine was first demonstrated by Nebel (1937) in *Tradescantia* and subsequently by Derman (1938) in *Rhoeo*. Still it is the frequently used method of increasing the chromosome number of plant.

Colchicine is a poisionous chemical ($C_{22}H_{25}O_6N$) isolated from the seeds and bulbs of autumn crocus (*Colchicum autumnale*). It is readily soluble in water, alcohol and chloroform. It blocks spindle formation and thus inhibit the movement of sister chromatids to the opposite poles. The resulting restitution nucleus includes all the chromatids. As a result, the chromosome number of the cell is doubled.

Application of induced polyploidy has been referred in many vegetable crops but not yet in pointed gourd. However, in water melon belonging to same family Cucurbitaceae and having the same chromosome number as that of pointed gourd (2n = 22), induction of polyploidy has become a commercial method of breeding. In water

melon, autotetraploids itself have been developed as a variety (Karchi *et al.*, 1981), however, the tetraploids are commonly used to produce seedless triploid hybrids (Gray and Elmstrom, 1991; Gonzalez and Ayuso, 1992; Tan *et al.*, 1993; Earhart *et al.*, 1994). In musk melon also, tetraploidy and triploidy have been utilized to develop new variety (Nugent and Ray, 1992; Raamsdonk and Visser, 1992).

2.9 Methods of ploidy determination

a) The conventional method of ploidy determination has been by counting chromsomes of meristematic tissues (root tip cells) and of pollen or ovule before meiosis is complete. However, such cytological determination requires trained skills and technique.

b) Chloroplast number in the pair of stomatal guard cell (stomate) is an effective alternative method of ploidy determination. A positive correlation between the number of chloroplast in the guard cells and ploidy level exists in many plants (Butterfass, 1973). However, chloroplast in the guard cell can be viewed with or withoiut staining using bright field, phase contrast or fluroscence microscope, more preferably fluroscence microscope.

c) Stomatal dimension, particularly stomatal length have been commonly used as the alternative method for the determination of ploidy in plants. Increase in stomatal guard cell lengths with doubled ploidy level were reported in barley (Borrino and Powell, 1988), alfalfa (Bingham, 1968; Setter *et al.*, 1978). This method requires only a compound microscope, stage and ocular micrometer.

d) The effect of ploidy on stomatal frequency where stomatal frequency in higher ploidy plants tends to be much low than that of 2n species has also been documented in many genera like wheat (Dunstone *et al.*, 1973); *Bromus* (Tan and Dunn, 1975; Lea *et al.*, 1977) and *Vaccinium* (Chandler and Lyrene, 1982).

e) Pollen diameter is also a common alternative method for determining the ploidy level of plants (Ho *et al.*, 1990; Bamberg and Hanneman Jr., 1991).

f) Flow cytometry has become the recent alternative approach to chromosome counting by which nuclear DNA contents in plants is measured (De Laat *et al.*, 1987). In this method flow cytometer-cell sorter system is used.

CHAPTER - III

MATERIALS AND METHODS

Materials and Methods

Materials

Different experimental approaches under the broad area of investigations : **clonal selection and polyploidization** were undertaken under the present research programme. Field trials were carried out at Horticultural Research Station, Mondouri (located at $23^{0}5$ North latitude and 80^{0} East longitude and at an altitude of 9.75 m from the mean sea level) of Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya and AB Block farm, Kalyani (located at 23.5^{0}N, 89^{0}E and at 9.75 m above mean sea level) of Bidhan Chandra Krishi Viswavidyalaya, during the period from 1996-'97 to 1998-'99. Estimation of protein content in the fruits, petiole anatomy and stomatal study were carried out in the Department of Vegetable crops of the Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur and Isozyme pattern of the clones were determined in the laboratory of the Biotechnology unit, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur.

3.1 Grouping and Characterization of the female clones

Such studies were carried out from different angles mentioned hereunder.

3.1.1 Difference of means for the characters between groups

Materials : Sixty eight female clones of pointed gourd collected from the major pointed gourd growing areas of West Bengal, some parts of Bihar and Tripura comprised the materials of the study. Brief description of them along with the place of collection have been presented in Table 2.

Table 2. Brief description of the 68 female clones under study.

Collection	Place of collection	Fruit shape and size	Fruit skin colour/stripes
BC-S-1	Nadia, W.B.	Small, spindle shaped	Light green with white stripes
BC-S-2	Nadia, W.B.	Small, round shaped	Dark green with prominent stripes
BC-S-3	24 Pgs(N), W.B.	Small, oval shaped	Light green with faint stripes
BC-S-4	Nadia, W.B.	Small, spindle	Light green with faint stripes
BC-S-5	Nadia, W.B.	Small, round shaped	Dark green with white stripes
BC-S-6	24 Pgs (N), W.B.	Small, and spindle	Dark green with white stripes
BC-S-7	24 Pgs (N), W.B.	Small, oval shaped	Light green with faint stripes
BC-S-8	Teliamura, Tripura	Small, spindle shaped	Light green with ribs
BC-S-9	-do-	Small, and Cylindrical	Moderate green with faint stripes
BC-S-10	Bhagalpur, Bihar	Small, and round	Dark green with white stripes
BC-S-11	CoochBehar, W.B.	Small, spindle shaped	Light green with faint stripes
BC-S-12	Cooch Behar, W.B.	Small and round	Light green with faint stripes
BC-S-13	Kalyanpur, Tripura	Small, spindle shaped	Light green with shallow lines
BC-S-14	Kaloipara, Tripura	Small, spindle shaped	Light green with shallow lines
BC-S-15	Teliamura, Tripura	Small, spindle shaped	Light green with shallow lines
BC-SP-1	Nadia, W.B.	Spindle	Dark green with stripes
BC-SP-2	Teliamura, Tripura	Spindle	Light green with faint stripes
BC-SP-3	24 Pgs(N), W.B.	Spindle	Light green with faint
BC-SP-4	Nadia, W.B.	Spindle	Light green with faint stripes
BC-SP-5	CoochBehar, W.B.	Spindle	Light green with faint stripes
BC-SP-6	CoochBehar, W.B.	Spindle	Light green ribbed
BC-SP-7	Nadia, W.B.	Spindle	Dark green stripes
BC-SP-8	24 Pgs(N), W.B.	Spindle	Light green ribbed
BC-SP-9	South Dinajpur, W.B.	Spindle	Light green with faint stripes
BC-SP-10	24 Pgs(N), W.B.	Spindle	Dark green with stripes
BC-SP-11	Teliamura, Tripura	Spindle	Light green with faint stripes

BC-SP-12	Burdwan, W. B.	Spindle	Light green with faint stripes
BC-SP-13	S. Dinajpur, W.B.	Spindle	Light green with fait stripes
BC-SP-14	24 Pgs(N), W.B.	Spindle	Dark green with stripes
BC-SP-15	Nadia, W. B.	Spindle	Very dark green broad
BC-SP-16	Nadia, W. B.	Spindle	Very dark green broad
BC-SP-17	Nadia, W. B.	Spindle	Light green faint stripes
BC-SP-18	Nadia, W. B.	Spindle	Dark green with white stripes
BC-SP-19	Burdwan, W. B.	Spindle	Moderate green with stripes
BC-SP-20	Bhagalpur, Bihar	Spindle	Light green with fait stripes
BC-SP-21	Bhagalpur, Bihar	Spindle	Dark green with white stripes
BC-SP-22	Nadia, W. B.	Spindle	Light green with faint stripes
BC-SP-23	24 Pgs, W. B.	Spindle	Dark green with white stripes
BC-O-1	Nadia, W. B.	Oval	Light green with faint stripes
BC-O-2	Nadia, W. B.	Oval	Dark green with stripes
BC-O-3	24 Pgs. (N), W. B.	Oval	Dark green with stripes
BC-O-4	24 Pgs.(N), W. B.	Oval	Dark green with stripes
BC-O-5	24 Pgs. (N), W. B.	Oval	Moderate green with stripes
BC-O-6	Nadia, W. B.	Oval	Light green with stripes
BC-O-7	Nadia, W. B.	Oval	Light green with stripes
BC-O-8	24 Pgs.(N), W. B.	Oval	Light green with stripes
BC-O-9	24 Pgs. (N), W. B.	Oval	Light green with faint stripes
BC-O-10	Nadia, W. B.	Oval	Light green with faint stripes
BC-O-11	Nadia, W. B.	Oval	Light green with faint stripes
BC-O-12	Nadia, W. B.	Oval	Light green with faint stripes
BC-O-13	24 Pgs. (N), W. B.	Oval	Light green with faint stripes
BC-NC-1	24 Pgs. (N), W. B.	Near cylindrical	Light green with striped
BC-NC-2	24 Pgs. (N), W. B.	Near cylindrical	Moderate green with stripes
BC-NC-3	Nadia, W. B.	Near cylindrical	Light green with faint stripes
BC-NC-4	Nadia, W. B.	Near cylindrical	Light green with stripes
BC-NC-5	24 Pgs. (N), W. B.	Near cylindrical	Light green with faint stripes

BC-NC-6	24 Pgs. (N), W. B.	Near cylindrical	Dark green with white stripes
BC-NC-7	Nadia, W. B.	Near cylindrical	Light green with stripes
BC-NC-8	Bhagalpur, Bihar	Near cylindrical	Light green with stripes
BC-NC-9	Nadia, W. B.	Near cylindrical	Light green with stripes
BC-NC-10	Bhagalpur, Bihar	Near cylindrical	Dark green with stripes
BC-NC-11	Nadia, W. B.	Near cylindrical	Dark green with stripes
BC-NC-12	24 Pgs. (N), W. B.	Near cylindrical	Light green with faint stripes
BC-NC-13	24 Pgs. (N), W. B.	Near cylindrical	Dark green with prominent stripes
BC-NC-14	Nadia, W. B.	Near cylindrical	Light green with stripes
BC-NC-15	Ranchi, Bihar	Near cylindrical	Light green with stripes
BC-NC-16	24 Pgs. (N), W. B.	Near cylindrical	Light green with stripes
BC-NC-17	Nadia, W. B.	Near cylindrical	Light green with stripes

Methods : The assemblage of the clones were grown by vine cuttings at Horticultural research station, Mondouri, during 1996-'97 for broad grouping as per fruit shape and size. The 68 clones fell under four broad groups based on fruit shape and size.

Group 1 : 15 clones bearing small fruits of different sizes.

Group 2 : 22 clones bearing spindle shaped fruits.

Group 3 : 13 clones bearing oval shaped fruits.

Group 4 : 18 clones bearing near cylindrical fruits.

The clones belonging to these groups were grown separately by vine cuttings at spacing of 3 m × 60 cm in 3 m × 3m bed following randomised block design with three replications in 1997-'98 and 1998-'99 (October to August). Crop Husbandry were conventional and 10% male plants were ensured in the populations. Data on 17 characters recorded from three randomly selected plants from each plot (replication) of the total ten plants alloted for each genotype per replication (3 × 3 m plot) in only 1997-98 were utilized for the present study.

Observations recorded

1. **Vine lengths (cm) :** Vine length (main creeper length) of the clones was recorded at last fruit harvest.
2. **Internode lengths (cm) :** Internode lengths were taken randomly along the vines (basal, middle portion and top portion) and then averaged.
3. **Primary branches/plant :** The primary branches were taken at last harvest.
4. **Node at first flower :** Node at which the first female flower appeared were counted from the base of vine.
5. **Leaves/plant :** Total number of leaves in the plant (alongwith the leaves of the primary branches) were counted in the mid of the growing season and then averaged. Five fully developed leaves from the main creeper of each sampled plant per plot were taken for recording leaf characters.
6. **Leaf length (cm) :** It was the length from the point of petiole attachment to the tip of the lamina.
7. **Leaf width (cm) :** It depicted the maximum width of the leaf.
8. **Leaf dry weight (g) :** Five sampled leaves/replication were oven dried (excluding petiole) at $68^0 \pm 2^0$C for two days to take the dry weight.

Five labelled fruits of 12 days maturity from the periodical harvest of each sampled plant/plot were taken to record the observations on different fruits characters.

9. **Fruit length (cm) :** It was the length of the stem end and distal end and was taken by slide calipers.
10. **Fruit girth (cm) :** It was the maximum girth of the fruit and taken with the help of slide calipers.

11. **Fruit weight (g) :** Fruit weight was taken from fresh harvests.
12. **Fruit volume (cc) :** It was taken by water displacement method.

    The sampled fruit were then cut transversely into two for recording the following observations.
13. **Pulp content/fruit (g) :** Pulp content scooped from the two halves of a fruit constituted both immature seeds, placenta and mesocarp.
14. **Pericarp thickness (cm) :** After scooping the middle portion of the fruits i.e., pulp, the pericarp thickness from different positions of the fruit halves were taken with the help of slide calipers.
15. **Seeds/fruit :** Five randomly sampled over matured fruits (when seeds have become hardened inside the fruit) were employed to record this observation.
16. **Fruit number/plant :** Fruit number in the sampled plants per plot in each clone were recorded in all the periodical harvest upto August.
17. **Fruit yield/plant (g) :** It was the average weight of the periodical fruit harvest in the sampled plants.

3.1.2 Multivariate analysis through D^2 statistic

Materials : The same 68 clones were employed for the study.

Methods : Average data of the clones from 1997-'98 and 1998-'99 trails were utilized for this analysis. In 1998-'99, two additional characters were taken namely leaf area and protein content of fresh fruits. So altogether 19 characters namely vine length, internode length, primary branches/plant, node at first flower, leaves/plant, leaf length, leaf width, leaf area, leaf dry weight, fruit length, fruit girth, fruit volume, fruit weight, pulp content, pericarp thickness, seeds/fruit, protein content of the fresh fruit, fruits/plant and fruit yield/plant were employed for multivariate analysis using Mahalanobis's D^2 Statistic.

Observations : Recording of the observations of the 17 characters have already been discussed earlier. The remaining two are being mentioned below :

Leaf area : Five fully developed leaves that were taken from each sampled plant/replication for recording leaf length, leaf width and leaf dry weight were utilized to take the leaf area by the digital leaf area meter (Systronix Leaf Area Meter 211).

Protein content of the fresh fruits : The five sample fruits per replication in each clone for recording observations on fruit legnth, fruit girth, fruit weight, fruit volume, pulp content and pericarp thickness were oven dried after taking all the above observations. The ovendried sample of the fresh fruits were utilized to estimate total nitrogen on dry weight basis by Micro-kjeldahl method as described by Sadasivam and Manickam (1996). Nitrogen content on dry weight basis was later converted on the basis of fresh weight as per the weight of the 5 fruits that was taken previously. Nitrogen content on fresh weight basis was then multiplied by 6.25 to express the protein content of the fruit on fresh weight basis.

Preparation of reagent

a) Concentrated sulphuric acid (specific gravity 1.84) A. R.

b) Digestion mixture (K_2SO_4 : $CuSO_4$: Selenium dioxide in 10 : 1 : 1 ratio)

c) Standard sulphuric acid (N/10)

d) Mixed indicator solution : For mixed indicator solution, two parts of methyl red solution (0.2 g/10 ml ethanol) were added to one part of methylene blue (0.2 g/100ml) solution.

e) Boric acid solution : 40g boric acid (g.r.) was dissolved in 20 ml boiling water and then adding 5ml of mixed indicator solution and make upto 100 ml.

f) Sodium hydroxide solution (40%).

Procedure : 200mg of ground fruit sample was taken in a micro kjeldahl flask, 1 ml of concentrated sulphuric acid and 5g of digestion mixture were added to it. Digestion was carried out till the digested material become colourless or light green in colour. The flask was then cooled for a while. A 50 ml conical flask containing 10 ml of 4% boric acid solution and indicator was taken. The digest was transferred with two or three washings into the vaccum mantle of the micro distillation unit. 50 ml of NaOH (40%) solution was added with 10-15 ml of distilled water. A 100 ml conical flask containing 5 ml of 4% boric acid and indicator solution was placed under the tube of a condenser so that distillate may be collected. The distillation operation was continued for 20 minutes so that 20 ml of distillate (evolved ammonia) may be collected in the receiving conical flask. A blank was also caried out side by side. After each distillation the distillation appratus was washed with distilled water. The distillate was titrated with N/10 H_2SO_4 and the % of total nitrogen was calculated as per –

$$N(\%) = \frac{(T-B) \times f \times 0.014}{W} \times 100$$

Where, T = Burette reading of the sample in ml

B = Burette reading of blank in ml

f = Normality factor of H_2SO_4 used for filtration (0.01)

0.014 = m.e. weight of nitrogen.

100 = decimal to percentage conversion factor.

3.1.3 Analysis of diversity through isozyme pattern

Peroxidase isozyme pattern of 20 random clones, five each from the four broad groups based on fruit shape and size (mentioned earlier) were determined by Gel electrophoresis following the method of Vallejos (1983).

Clonal materials :

BC-S-1, BC-S-6, BC-S-8, BC-S-10, BC-S-13 of group 1, BC-SP-2, BC-SP-5, BC-SP-12, BC-SP-16, BC-SP-21 of group-2, BC-0-1, BC-0-3, BC-0-6, BC-0-8, BC-0-13, of group-3, BC-NC-1, BC-NC-7, BC-NC-12, BC-NC-14, BC-NC-18 of group-4 of 1998-'99 field trials.

Methods

Reagents

Phosphate buffer pH 7 : This extraction buffer was prepared in the following manner –

Preparation of X : 3.14 g $NaH_2PO_4 . 2H_2O$ in 100 ml water

Preparation of Y : 3.62 g $Na_2HPO_4 . 2H_2O$ in 100 ml water

39 ml of X + 61 ml of Y = 100 ml pH 7 phosphate buffer

Solution A : It was prepared by adding 14.6 g Acrylamide and 0.4 g Bisacrylamide in 50 ml water.

Solution B : It was prepared by adding 18.17 g TRIS (Hydroxy methyl amino methane) in 75 ml of water. pH was adjusted to 8.8 by adding HCl.

Solution C : It was prepared just before use by adding 150 mg APS (Ammonium per sulphate) in 10 ml of water.

Tank buffer (10X) : This buffer solution of pH 8.3 was prepared by adding 1.2 g TRIS and 5.8 g glycine in 200 ml water.

Procedure

Gel casting

1. Glass plates and spacers were cleaned properly, dried and then assembled. Steel grip was used around the edges to prevent leakage.
2. A sufficient volume of gel mixture was prepared by mixing the followings for 7.5% gel.
   Solution A : 7.5 ml, Solution B : 3.75 ml, Solution C : 1.5 ml, TEMED (Tetramethyl ethylene diamine) : 50 µl, and water : 17 ml.
3. The solutions were mixed gently and carefully and the gel solution was poured in the chamber between glass plates. A layer of water was kept on the top of the gel and left to set for 1 hour.

Extraction of sample for electrophoresis

1. Five actively growing leaves of same maturity per 20 random clones were sampled from the field in the morning, put in polyethylene packets and immediately kept in a thermoflask with ice inside it.
2. Leaf pieces weighing 1g were sampled from 5 leaf explants per clone.
3. The 1g leaf sample was macerated and homogenised in a pre-chilled mortar and pestle using 1 ml phosphate buffer at 0-4^{0}C temperature condition around the mortar and pestle by keeping ice.
4. The homogenate was centrifuged at 20000 rpm at 4^{0}C for 30 minutes by refrigerated centrifuge.
5. The supernatant was collected and stored at 0^{0}C.

Loading of supernatant in the gel lane

The dye was prepared by mixing Bromophenol blue and glycerol in 1 : 1 ratio. 25 µl of supernatant was mixed with 10 µl of dye. The 35 µl of sample and dye mixture was loaded in each lane.

Electrophoresis run

The electrophoresis apparatus was kept inside the refrizerator at 0-4°C temperature condition. Current was flowed and continued at 20-22 mA, 120 voltage. The gel was run for 2-3 hour. A pre-run of 20-22 mA was given for 30 minutes to elute the samples and then continued for 3 hours.

Staining of the gel

1. The staining solution was prepared by adding 50 mg O,-dianisidine in 100 ml of distilled water. Before adding water, the stain was first dissolved in few drops of acetic acid.
2. After completion of the run, the gel was removed from electrophoresis apparatus and glass plates.
3. The gel was first immersed in staining solution. Then 1 ml hydrogen peroxide (H_2O_2) was added. The gel in a petridish immersed in the staining solution and H_2O_2 was kept in dark for 1 hour.

Observation of banding pattern

The peroxidase enzyme absorb the dye (O-dianisidine). Hydrogen peroxide (H_2O_2) act as inducer to start the reaction for colour precipitation. The isozymes in form of bands in the gel were seen reddish brown. For destaining, the gel was placed in a petridish with distilled water. The banding pattern in the gel was examined through diffused illumination by fluroscent tube light as both very deep and faint bands were present.

Estimation of protein following the method of Lowry *et al.* (1951).

Materials

Reagent A : 2% sodium carbonate in 0.1 N sodium hydroxide.

Reagent B : 0.5% copper sulphate ($CuSO_4$, 5 H_2O) in 1% potassium sodium tartarate.

Reagent C : Alkaline copper solution (50 ml of A and 1 ml of B prior to use).

Reagent D : Folin-ciocalteau reagent.

Protein solution (stock standard) : Accurately weighed 50 mg bovine serum albumin was dissolved in double distilled water and volume was made upto 50 ml in a standard flask.

Working standard : 10 ml of stock solution was diluted to 50 ml with distilled water in a standard flask. 1 ml of this solution contain 200 µg protein.

Buffer solution : Tris-HCl buffer of pH 6 8.

Procedure

Extraction of protein from the leaf samples

500 mg of actively growing leaf explants from the clones were ground well with a mortar and pestle in 5 ml of the buffer, centrifuged at 12000 rpm for 30 minutes and supernatant was used for protein estimation.

Estimation of protein

1. 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard were pipetted out into a series of test tubes.
2. 0.1 and 0.2 ml of leaf sample extract were pipetted in two other test tubes.
3. In all the test tubes volume was made to 1 ml. A tube with 1 ml of distilled water served as blank.
4. 5 ml of Reagent C was added to each test tube including the blank, mixed well and allowed to stand for 20 minutes.
5. 0.5 ml of Reagent D was added to each test tube, mixed well and incubated well at room temparature in the dark for 30 minutes.

Blue colour was developed due to reduction of phosphomolybdic and phosphotungstic components of Folin-Ciocalteau reagent by the amino acids, tyrosine and tryptophan present in protein and colour development was due to direct reaction of the protein with the alkaline cupric tartarate.

6. Optical density reading was taken at 660 nm in spectrophotemeter.
7. Standard curve was prepared, amount of protein in the leaf sample was calculated and expressed in mg/g leaf sample.

3.1.4 Petiole anatomy

Material : The same 20 random clones of 1998-'99 field trials employed for the study of isozyme pattern were also utilized for studying the petiole anatomy.

Method : Five random petioles per clone were and hand sectioned transversely with the help of a sharp blade. Five thin sections per clone were selected, single stained with saffranin, mounted on a glass slide in a drop of saffranin and petiole anatomy was studied under the microscope - (Olympus, KIC 29285). Features and dimensions of some internal structures determined were :

1. Length and width of epidermal cell layer (micron).
2. Number of layers of hypodermal collenchyma cells.
3. Length and width of hypodermal collenchyma cells (micron).
4. Number of vascular bundles in the cross sections.
5. Length and width of the vascular bundles (micron).

3.1.5 Stomatal features

Materials : The same 20 clones of field trials 1998-'99 employed for the study of isozyme pattern and petiole anatomy were also utilized for this study.

Method : Five fully expanded leaves were sampled in each clone. Stomatal characters were examined following peel method. Five peels from each two of the leaf surfaces were finally utilized to record the following stomatal characters in each clone.

1. Stomatal frequency per mm^2 in upper and lower leaf surface.
2. Stomatal length (micron) in upper and lower leaf surface.
3. Stomatal width (micron) in upper and lower leaf surface.

Longer axis of the stomata were measured and recorded as stomatal length. Similarly, region of maximum width of the stomata was recorded as stomatal width. For recording stomatal frequency, five counting in different field of vision comprising 25 countings per clone were taken. For stomatal length and width three readings per field of vision comprising 75 reading per clone were taken.

3.2 Genetic variability for different characters and character associationship studies

Average data of the 68 clones from 1997-'98 and 1998-'99 trials for the 19 characters (mentioned earlier in multivariate analysis) were analysed for the study.

3.3 Development and growing of the colchiploids

The freshly harvested seeds from the randomly harvested ripe fruits from different clones were sown in the plastic pot trays in both 1997 and 1998. Aquous solution of colchicine (Sigma, USA) at 0.2% concentration were soaked in cotton and applied to the dome shaped shoot apex of the just emerged seedlings having cotyledonary leaves intact in following three methods.

T_1 : 12 hours soaking in two consecutive days at day time (5.0 to 11 A.M.) under dark and humid condition.

T_2 : 12 hours soaking in one day at day time (5.0 A.M. to 5 P.M.) under dark and humid condition.

T_3 : 12 hours soaking continuously at night followed by next morning (6 P.M. to 6 A.M.).

100 seedlings were employed per treatment in both the years and treatments were given in a span of one month. After treatment duration, tip of the seedlings were washed thoroughly with running tap water.

The colchicine treated seedlings were grown in the field till October 1997 and 1998 as single vine without allowing any primary branches and diploid shoot regenerated from the base. In October 1997 and 1998, each colchiploid seedling was vegetatively propagated by single vine cutting. Different characters recorded in the colchiploids are mentioned below.

1. **Morphological and reproductive features :** The colchiploid seedlings of 1997 which were later propagated by single vine cutting in October of that year were grown upto August 1998 and subsequently colchoploid seedlings of 1998 were grown upto August 1999 and subsequently propagated vegetatively. The morphological and reproductive features of the 25 colchiploids were taken on the basis of one season growth (October, 1997 to August, 1998 for 1997 colchiploids and October 1998 to August 1999 for 1998 colchiploids). Observations recorded were vine length (cm), internode length (cm), primary branches/plant, leaves/plant, leaf length, leaf width, node to first flower and fruits plant stomatal features.

2. **Stomatal features :** Five fully expanded leaves from each of the 25 colchiploid seedlings of 1997-'98 growth and 1998-'99 growth were sampled and stomatal characters were examined under microscopes

using peel method as done for diploid clone. Different stomatal features examined were

a) Stomatal frequency/mm^2 of upper and lower leaf surface.

b) Stomatal length (micron) of both upper and lower leaf surface.

c) Stomatal width (micron) of both upper and lower leaf surface.

3. Features of pollen characters : Of the total 25 colchiploids in 1997 and 1998, 23 were male. Two flowers from each of these 23 male colchipoids were utilized for this study. Composite pollens from the two flowers per male colchiploids were stained with 1% acetocarmine solution and the following pollen chatacters were studied under microscope.

1. Viable pollen percentage/mm^2 : The viable pollens took stain and were counted in 10 field of vision under 40 x magnification.
2. Pollen diameter : Diameter of two pollens per field of vision (total 20 readings per male colchiploid) were taken and expressed in micron.

Ten random diploid male clones kept in the field for pollen donor were utilized to study the same features of the pollen as done in colchiploids.

3.4 Preparation of 1% acetocarmine solution

Materials	:	Carmine	1 g
		Glacial acetica acid	45 ml
		Distilled water	55 ml

Preparation : Distilled water was added to the glacial acetic acid to form 40% acetic acid solution. The solution was kept in a conical flask and heated to boiling. Carmine was added to the boiling solution slowly with stirring by glass rod. Gentle boiling was on till the carmine

dissolved. The acetocarmine was cooled down to room temperature filtered and stored in the glass bottle.

3.5 Statistical and biometrical analysis

The observations recorded on the various characters for the present investigation were subjected to the following statistical analysis.

3.5.1 Analysis of variance

Differences between clones for different characters were tested for significance using analysis of variance. Analysis of variations was done on the basis of the following model.

$$Y_{ij} = m + g_i + r_j + e_{ij}$$

where Y_{ij} = Phenotypic observation in i-th clone and jth replication

m = general mean

gi = effect of i-th clone

rj = effect of j-th replication

e_{ij} = random error associated with i-th clone and j-th replication.

Structure of analysis of variance

ANOVA

Source	d.f.	M.S.	Expected M.S.	F
Clone	(t – 1)	Mt_{11}	$\sigma^2 e_{11} + r \sigma^2 g_{11}$	Mt_{11} / Me_{11}
Replication	(r – 1)	Mr_{11}	–	
Error	(r – 1) (t – 1)	Me_{11}	$\sigma^2 e_{11}$	

where, t = number of clones

r = number of replications.

Standard error (S.E.), Standard error of difference between two means (S.Ed.) and critical difference (C.D.) were calculated as follows –

$$S.E. = \sqrt{(MSe/r)} \text{ and } S.Ed. = \sqrt{2(MSe/r)}$$

where, Mse = Error mean square

r = number of replications

C.D. = S.Ed. x t (table 't' at error degrees of freedom).

Coefficient of variation (C.V.) was calculated as follows –

$$C.V.(\%) = \frac{\sqrt{\text{Environmental variance}}}{\bar{X}} \times 100$$

where, Environmental variance = Mse ; X = General mean for the character. Significance of the difference of mean for the characters between the broad groups based on fruit shape and size were tested through 't' test as –

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

where, $\bar{X}_1$ and $\bar{X}_2$ = two group means

$$S_1^2 = \frac{\Sigma(x_{i1} - \bar{X}_1)^2}{n_1 - 1}$$

$$S_2^2 = \frac{\Sigma(x_{i2} - \bar{X}_2)^2}{n_2 - 1}$$

The calculated value of 't' was compared with the tabulated value of 't' for significance at $n_1 + n_2 - 2$ degrees of freedom.

Standard deviations were calculated as per the statistics –

$$\sqrt{\frac{\Sigma (Y_i - \bar{Y})^2}{n - 1}}$$

where, Y_i = Variables i.e. observations

$\bar{Y}$ = Mean

n = Number of observations

3.5.2 Component of variances

Considering that all the clones tested were uniform genetically, the expected mean sum of squares for error (MSe) will be purely a random environmental variance. The mean sum of squares between genotypes (clones) will consist of the variances (i) attributable to genotypic differences of the clones and (ii) due to environmental variation along the individuals of each genotypes. So, the different variances will be as follows –

$$\text{Error variance} = \sigma^2 e_{11} = Me_{11}$$

$$\text{Genotypic variance} = \sigma^2 g_{11} = (Mt_{11} - Me_{11})/r$$

$$\text{Phenotypic variance} = \sigma^2 p_{11} = \sigma^2 g_{11} + \sigma^2 e_{11}$$

where, Mr_{11}, Mt_{11} and Me_{11} stand for mean sum of squares due to replication, genotype and error, respectively.

The genotypic (GCV) and phenotypic (PCV) coefficients of variation were calculated by the formulae given by Burton (1952) and Burton and De Vane (1953) –

$$GCV = \frac{\text{Genotypic standard deviation}}{\text{Grand mean}} \times 100$$

$$PCV = \frac{\text{Phenotypic standard deviation}}{\text{Grand mean}} \times 100$$

Heritability in broad sense (H) was estimated by the formula suggested by Hanson *et al.* (1956) –

$$H(\%) = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100$$

The expected genetic advance (GA) was calculated as per Johnson *et al.* (1955 a) $GA = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times K \times \sigma p$

where, σp = Phenotypic standard deviation

k = Selection differential, a constant, 2.06 for 5% selection intensity (Lush, 1943).

3.5.3 Analysis of covariance

Analysis of covariance was estimated for all the characters in their all possible combinations taking two variables at a time. The structure of covariance table was as follows –

ANCOV

Source	d.f.	M.P.	Expected M.P.	F
Clones	(t – 1)	Mt_{12}	$\sigma e_{12} + r\,\sigma\, g_{12}$	Mt_{12} / Me_{12}
Replication	(r – 1)	Mr_{12}	–	
Error	(t – 1) (r – 1)	Me_{12}	σe_{12}	

where, r and t are number of replications and clones, respectively.

Genotypic covariance = $\sigma g_{12} = (Mt_{12} - Me_{12})/r$

Phenotypic covariance = $\sigma p_{12} = \sigma g_{12} + \sigma e_{12}$

Phenotypic and genotypic correlation coefficients for all possible combinations were worked out by employing variance and covariance table as suggested by Aljibouri *et al.* (1958) and Computation was done as follows –

$$r\,(xy) = \frac{COV.\,xy}{\sqrt{Var\,(x)\,.\,Var\,(y)}}$$

where, r (xy) is the correlation between characters x and y

Cov. xy is the covariance between x and y

Var (x) is the variance of x

Var (y) is the variance of y

3.5.4 Path coefficient analysis

Path coefficients were calculated to estimate the direct and indirect effects of the characters as per Dewey and Lu (1959). The following set of simultaneous equations were formed and solved for

estimating various direct and indirect effects.

r1y = p1y + r12 p2y + r13 p3y + ... r1I pIy
r2y = r21 pIy + p2y + r23 p2y + ... r2I pIy
.
.
.
.
rIy = rI1 p1y + r12p2y + r13p3y + ... pIy

where, rIy to rIy = correlation coefficients between causal factors 1 to I and dependent character y.

r12 to rI-1, I = correlation coefficients among causal factors.

pIy to rIy = Direct effects of characters 1 to I on character y.

Residual effect which measures the contribution of the characters not considered in the causal scheme was obtained as –

Residual effect (PRy) = $\sqrt{1 - R^2}$

whose, $R^2 = P^2iy + 2 \sum \sum Piy\ Pjy\ rij$

3.5.5 Multivariate analysis

D^2 - statistic (Mahalanobis, 1936)

D^2-statistic was used for assessing the genetic divergence between the clonal populations. The generalised distance between any two populations is defined by

$D^2 = (\lambda_{ij})\ d_i\ d_j$ where,

(λ_{ij}) is the reciprocal matrix to the common dispersion matrix and d_i is the difference between the mean values of the two population for the i-th character. This quantity is estimated by the D^2-statistic (Majumder and Rao, 1958) as

$$D^2 = (s^{ij})\ d_i\ d_j$$

where, (s^{ij}) is the sample estimate of (λ^{ij}) and d_i of δ_i. Since the formula for computation requires the inversion of the matrix, transformation of the original correlated, unstandardized character means to standardized uncorrelated variables was done to simplify the computational procedure. This transformation was effected by pivotal condensation method (Rao, 1952).

Determination of group constellations or clusters

The grouping of the populations was done as per Tocher's method described by Rao (1952). The criterion used in clustering by this method was any two clones belonging to the same cluster should at least, on an average, show a smaller D^2 value than those belonging to different clusters.

CHAPTER - IV

RESULTS AND DISCUSSION

Results and Discussions

4.1 Grouping and characterization of the female clones

The highly heterozygous female clones of pointed gourd are perpetuated uniformly and indefinitely in the farmers' field due to vegetative mode of propagation of this perennial vegetable crop as a convention. Despite the existence of wide clonal variation (Plate 1) very little work has so far been done for grouping, characterization and assesment of genetic diversity. The first experimental approach aimed at these aspects from different angles.

4.1.1 Differences of means for the characters between groups

Singh (1989) first assigned the groups of pointed gourd clones based on fruit shape and size. In this approach the idea of the basic grouping has been elaborated by cosidering 17 growth and fruit characters and at the same time by taking the help of basic statistics to determine the significance of differences of mean for the characters between groups.

The clones fell under four basic groups :

Group 1 : Plants bearing small sized fruits, fruit-shapes were mostly oval and tapering, very small spindle-shaped fruit bearing clones were also kept in this group.

Group 2 : Plants bearing spindle-shaped fruits.

Group 3 : Plants bearing oval-shaped fruits.

Group 4 : Plants bearing near cylindrical fruits.

Of the 68 female clones under study, 15 belonged to Group 1, 22 to Group 2, 13 to Group 3 and 18 to Group 4. Four basic fruit colours were recorded namely :

Photo 1 Variability in fruit shape and size

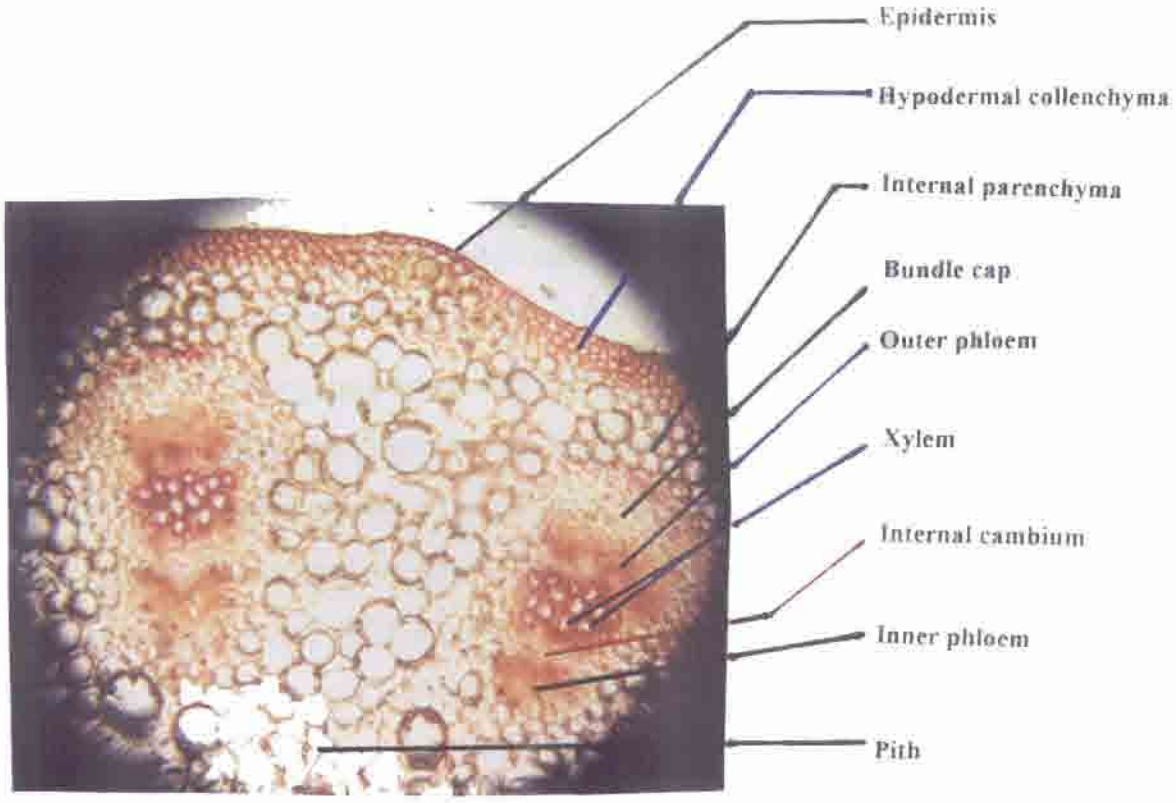

Photo 2 Transverse section of petiole (5 X 10 X)

a) Dark green with white stripes

b) Dark green with faint pale green stripes

c) Pale green fruit with inconspicuous stripes.

d) Pale green fruits with very shallow line in place of stripes.

Characterization

Group 1 : This group was characterized by the highest mean length of both vine (214.1 cm) and internode (6.07 cm). Range for these characters was also very high, particularly vine length (85.33 cm to 536.9 cm). In this group first flower appeared in the highest of 13.8 nodes (Table 3). Group mean for leaf number was the highest (34.28) but leaf length (5.45 cm), leaf width (6.15 cm) and leaf dry weight (0.15 g) was the lowest among the groups. All the fruit characters namely fruit length (5.17 cm), fruit girth (8.11 cm), fruit volume (22.53 cc), fruit weight (19.43 g). Pulp content (7.02g) and seed number/fruit (7.65) showed the lowest magnitude, however, pericarp thickness of the fruit (0.37 cm) was *at per* with that of Group 2. Average fruit number/plant was second high (24.57) but mean fruit yield of this group was the lowest due to low fruit weight. High range was observed for both fruit number/plant (15.11-40.16) and fruit yield/plant (272.5-1179.12 g).

Group-2 : This group revealed the mean growth characters quite in line with those of Group 1 (internode length 6.06 cm, primary branches/plant 3.81, node to first flower 13.73, leaves/plant 31.53, leaf length 6.02 cm, leaf width 6.41 cm, leaf dry weight 0.19 g) excepting vine length which was markedly low (173.37 cm). This group registered highest mean fruit length (6.93 cm) and fruit number/plant (25.89) and second high fruit yield/plant (648.85 g). Magnitude of all the other fruit characters lay in between those of Group 3 and 4, viz. fruit girth (9.28 cm), fruit volume (30.50 cc), fruit weight (25.58g), pulp content (8.54 g), pericarp thickness (0.37 cm) and seed number/fruit (10.16).

Table 3. Group means of the female clones for growth, fruit characters and yield (range in the parenthesis)*

Characters	**Group 1** plant bearing small sized fruits of different shape	**Group 2** plants bearing spindle shaped fruits	**Group 3** plants bearing oval shaped fruits	**Group 4** plants bearing nearly cylindrical fruits
Growth characters				
1. Vine length (cm)	214.10 (85.33-536.90)	173.37 (90.51-331.46)	170.95 (92.16-301.06)	154.87 (88.16-245.30)
2. Internode length (cm)	6.07 (2.96-9.26)	6.06 (2.93-11.16)	5.76 (3.23-6.03)	5.64 (4.03-8.93)
3. No of primary branches /plant	3.61 (1.81-5.02)	3.81 (2.02-5.73)	3.34 (2.43-4.66)	4.81 (2.08-9.01)
4. Node number at first flower	13.81 (7.06-24.32)	13.73 (7.00-20.33)	12.42 (6.08-18.43)	11.09 (5.56-21.33)
5. Leaves / plant	34.28 (16.68-49.66)	31.53 (13.66-69.34)	32.27 (24.13-59.32)	27.91 (19.63-36.66)
6. Leaf length (cm)	5.45 (4.8-6.10)	6.02 (4.93-7.42)	6.02 (5.14-8.06)	6.14 (4.76-8.56)
7. Leaf width (cm)	6.15 (5.03-7.16)	6.41 (5.13-8.07)	7.14 (6.67-8.46)	7.05 (5.26-8.46)
8. Leaf dry weight (g)	0.15 (0.08-0.31)	0.19 (0.07-0.51)	0.26 (0.06-0.43)	0.23 (0.07-0.40)
Fruit characters and yield				
1. Fruit length (cm)	5.17 (3.37-5.92)	6.93 (5.13-9.32)	6.84 (5.12-9.06)	6.61 (5.82-7.56)
2. Fruit girth (cm)	8.11 (6.13-9.96)	9.28 (7.06-11.21)	10.12 (9.07-11.60)	9.41 (7.44-10.93)
3. Fruit volume (cc)	22.53 (14.20-29.06)	30.50 (14.46.30)	36.06 (19.21-59.86)	31.15 (24.63-44.96)
4. Fruit weight (g)	19.43 (14.23-29.08)	25.58 (14.21-38.40)	30.15 (19.46-15.96)	25.19 (19.03-32.16)
5. Pulp content / fruit (g)	7.02 (4.93-10.36)	8.54 (4.16-12.03)	9.63 (6.42-11.53)	8.47 (6.41-10.43)
6. Pericarp thickness (cm)	0.37 (0.27-0.51)	0.37 (0.25-0.48)	0.45 (0.29-0.71)	0.43 (0.27-0.94)
7. Seed number /fruit	7.65 (4.96-15.02)	10.16 (6.22-15.00)	11.34 (6.13-17.76)	9.22 (6.06-17.40)
8. Fruit number /plant	24.57 (15.11-40.16)	25.89 (10.23-50.23)	22.10 (9.63-31.52)	22.23 (9.93-40.56)
9. Fruit yield /plant (g)	528.07 (272.50-1179.12)	648.85 (201.20-1091.29)	703.26 (241.93-1395.76)	583.52 (198.16-1183.03)

*** based on data from 1997-98 field trial.**

Group 3 : This group was characterized by low vine length (170.95 cm) and internode length (5.76 cm) and lowest number of primary branches/ plant (3.34). Mean node number to first flower (12.42), leaves per plant (32.27) and leaf length (6.02 cm) were much closer to those of Group 2. Leaf width (7.14 cm) and leaf dry weight (0.26 g) was the highest among the four broad groups of the clones. Magnitude of almost all the fruit characters excepting fruit length and fruit number were the highest in this group (Table 3) viz., fruit girth (10.12 cm), fruit volume (36.06 cc), fruit weight (30.15 g), pulp content (9.63 g), pericarp thickness (0.45 cm) and seed number/fruit (11.34). Highest mean fruit yield/plant (703.26 g) was recorded in this group.

Group 4 : This group was characterized by lowest vine length (154.87 cm) and internode length (5.64 cm) and highest mean primary branches/ plant (4.81) and leaf length (6.14 cm). Leaf width and leaf dry weight was second high among the groups (Table 3). In this group, first flower appeared in the lowest of 11.09 nodes. For fruit characters mean of this group lay in between Group 2 and Group 3 with inclination towards Group 2. Mean fruit number of this group (22.23) was almost the same to that of Group 3 (22.1), but, mean fruit yield of this group (583.52 g) was much lower than that of Group - 3 (703.26 g) mainly due to low fruit weight.

Significance of mean difference and relative position of the groups

Mean differences for growth characters were most of the time not significant mainly due to revealation of huge ranges for the characters indicating the presence of the clones in the groups having overlapping characters. However, all the growth characters excepting one (internode length) were significantly different between Group 1 and Group 4 (Table 4). Leaf length was significantly different between Group

Table 4. Significance of the mean difference between the groups for different characters (actual 't' values given)

Characters	Group 1-2	Group 1-3	Group 1-4	Group 2-3	Group 2-4	Group 3-4
Growth characters						
1. Vine length (cm)	1.33	1.19	2.14*	0.08	0.08	0.72
2. Internode length (cm)	0.01	0.53	0.88	0.38	0.65	0.29
3. No. of primary branches /plant	0.64	0.88	2.40*	1.47	2.26	2.79
4. Node number at first flower	0.06	0.92	2.05*	0.93	2.16*	1.09
5. Leaves / plant	0.82	0.58	2.49*	0.20	1.25	1.53
6. Leaf length (cm)	2.75**	2.49*	2.81**	0.01	0.51	0.42
7. Leaf width (cm)	0.99	4.95**	3.49**	2.80**	2.36*	0.33
8. Leaf dry weight (g)	0.44	0.55	0.17	2.08*	1.01	1.00
Fruit characters and yield						
1. Fruit length (cm)	5.48**	5.38**	6.66**	0.23	1.44	0.94
2. Fruit girth (cm)	3.35**	5.59**	4.24**	2.31	0.38	2.33
3. Fruit volume (cc)	3.17**	4.49 **	5.02**	1.65	0.27	1.70
4. Fruit weight (g)	2.52*	4.60**	3.83**	1.92*	0.05	2.48
5. Pulp content / fruit (g)	2.38*	3.91**	2.73**	2.01*	0.13	2.36*
6. Pericarp thickness (cm)	0.13	2.08*	1.02	2.75**	1.33	0.44
7. Seed number /fruit	2.77**	3.42**	1.83*	1.09	1.06	2.01*
8. Fruit number /plant	0.41	0.88	0.80	1.13	1.15	0.04
9. Fruit yield /plant (g)	1.53	1.75*	0.65	0.58	0.83	1.19

*** and ** denote significance at 0.05 and 0.01 probability level, respectively.**

1 and 2, 1 and 3, and 1 and 4. Similarly leaf width was also significantly different between Group 1 and 3, 1 and 4, 2 and 3 and 2 and 4. Mean difference of most of the fruit characters were significant between Group 1 and 2, 1 and 3 and 1 and 4. Thus Group 1 (clones having small sized fruits) appeared on distinctly different group than the other three clonal groups. Significant mean difference also existed between Group 2 and 3 for few growth and fruit characters namely leaf width, leaf dry weight, fruit weight, pulp content and pericarp thickness (Table 4). Mean fruit characters of Group 2 and 4 were not significantly different and at the same time only two growth characters viz., node to first flower and leaf width registered significant difference between these two groups. Interestingly, no group means for the growth characters were significantly different between Group 3 and 4. However, mean of only three fruit characters namely, fruit weight, pulp content and seed number/fruit were significantly different between Group 3 and 4.

From the study of the group means and their significance some distinct pictures emerged :

1) Group 1 (clones having small sized fruits of different shapes) and group 4 (clones bearing near cylindrical fruits) exhibited most conspicuous distinctness between themselves.

2) Distinctness of Group 2 and 4 based on fruit shape and size has been questioned due to revealation of no significant mean difference between these two groups for any of the fruit characters.

3) In all the four groups, ranges for the characters were very high revealing marked intra group variation and at the same time presence of clones having overlapping characters.

4.1.2 Multivariate analysis using Mahalanobis's D^2 statistics

In the earlier characterization study through the significance of group mean difference for 17 growth and fruit characters individually, it revealed huge intra group variability and overlapping nature of the characters in the clones. In this situation, multivariate analysis by means of Mahalanobis's D^2 statistic would be a powerful statistical tool in quantifying the degree of divergence between the clonal populations and to asses the relative contributions of different component character to the total divergence.

The present study aimed at analysing the genetic divergence of 68 female clones of pointed gourd on the basis of two years average data (1997-'98, 1998-'99) on 19 growth, fruit, quality characters and yield. Pointed gourd being basically a vegetatively propagated crop, generation of variation through natural means is limited. It was therefore essential to characterize the genetic divergence of the present clonal assemblage to select suitable and diverse clones for sustainable improvement programmes. The analysis of variance for 19 characters revealed highly significant difference among the clones for all the characters excepting protein content/100 g of fruit (Table 10). The D^2 values were computed for all possible 2278 pairs of comparisions [n x (n-1)/2]. On the basis of divergence, the 68 female clones under investigation have been grouped into 16 clusters. High range for most of the characters in the four broad groups of earlier study (Table 3) was mirrored by the revealation of large number of clusters in the grouping by multivariate analysis. Cluster 1, the largest one contained 18 clones, on the other hand cluster 7, 11 and 15 contained single clone each (Table 5). The next high of 9 clones were grouped together under cluster 4. Most of the clusters contained by few (1-3) clones. In the comparision of clustering pattern and grouping of the genotypes on the basis of fruit shape and size, the following pictures emerged :

Table 5. Clustering pattern of the 68 female clones determined through multivariate analysis*

Cluster	Number of clones under the cluster	Name of the clones under the cluster
1	18	BC-NC-7, BC-NC-8, BC-NC-16, BC-O-3, BC-S-10, BC-NC-13, BC-SP-2, BC-SP-12, BC-NC-14, BC-SP-6, BC-NC-11, BC-O-8, BC-NC-17, BC-S12, BC-O-2, BC-O-4, BC-O-7, BC-NC-2.
2	8	BC-S-1, BC-SP-21, BC-SP-20, BC-SP-19, BC-NC-15, BC-SP-4, BC-NC-NC-9, BC-NC-10
3	6	BC-S-5, BC-O-5, BC-O-6, BC-NC-4, BC-SP-5, BC-SP-3
4	9	BC-S-7, BC-S-9, BC-S-15, BC-S-12, BC-S-3, BC-SP-11, BC-SP-23, BC-NC-3, BC-S-14
5	2	BC-S-11, BC-S-8
6	5	BC-SP-15, BC-SP-17, BC-SP-13, BC-SP-1, BC-SP-7
7	1	BC-SP-8
8	3	BC-S-13, BC-S-6, BC-O-13
9	4	BC-SP-14, BC-SP-16, BC-NC-6, BC-O-10
10	2	BC-S-4, BC-SP-18
11	1	BC-SP-10
12	2	BC-O-9, BC-O-1
13	2	BC-O-11, BC-NC-1
14	2	BC-NC-5, BC-SP-22
15	2	BC-O-12, BC-SP-9
16	1	BC-NC-12

Notations associated with the name of the clones :
S : Clones bearing small sized fruits, SP : Clones bearing spindle shaped fruits, O : Clones bearing oval shaped fruits, NC : Clones bearing near cylindrical fruits. * Based on the average of 1997-98 and 1998-99 field trials .

1) In most of the cases, fruit shape and size did not interfere clustering pattern through multivariate analysis. As for example, of the 18 clones under cluster 1, eight were with near cylindrical fruits (Group 4), 5 with oval-shaped fruits (Group 3), 3 with spindle-shaped fruits (Group 2) and 2 with small fruits (Group 1). Similarly, of the 9 clones under cluster 4, 6 were with small fruits (Group 1), 2 with spindle-shaped fruits (Group 2), and 1 with near cylindrical fruits (Group 4).

2) Clones having same fruit shape and size also clustered together. As for example, In cluster 5, both the clones under it were having small sized fruits (Table 5). Similarly, all the 5 clones under cluster-6 fell under group 2 according to fruit-shape and size (clones bearing spindle-shaped fruits) and both the clones under cluster 12 bore oval-shaped fruits (Group 3). From the emergence of such two opposite pictures, implication of the broad grouping based on fruit shape and size could not altogether be ignored. Grouping of the pointed gourd genotypes into high of 16 clusters were also reported earlier (Ram *et al.*, 1998). Works on genetic divergence in pointed gourd is very much limited. In other report (Gupta *et al.*, 1998), high range in magnitude of characters were recorded which coroborated to the present findings. In the present study, no close correspondence was evident between the geographical distribution of the clones and their genetic divergence through multivariate analysis which agreed well to the earlier observations on different sexually propagated vegetable crops like cowpea (Hazra *et al.*, 1993), brinjal (Doshi *et al.*, 1998), etc. This showed that geographical diversity was not adequate as an index of genetic diversity. However, in some cases the clones collected from the same geographic region clustered together which suggested that although geographical distribution was not the sole criterion of genetic diversity, the importance of the former would be traced. In pointed gourd, where

generation of variability is limited due to indefinite propagation of the heterozygous clones through vegetative means, genotype x environment interactions causing genetic diversity must be taken into consideration.

The divergence within the cluster (intracluster distance) indicates the divergence among the genotypes falling in the same cluster. On the other hand, intercluster divergence suggests the distance (divergence) between the genotypes belonging to different clusters. The data suggested medium and consistent level of intracluster divergence in all the clusters (D^2 27.67-34.77) excepting the lowest of 16.52 among the clones falling in cluster 5. Inter cluster distances were in most of the cases not very high and at the same time quite consistent (Table 6). Pointed gourd is perennial in growth habit and remain in the field for about eight months which might have caused the mutual balancing of the characters in the clones operative during such prolonged cropping duration. Vine growth of pointed gourd is not interruped by the flushes of fruiting rather, vine growth takes a spurt between two distinct fruiting flushes. Such growth habit leaves ample scope for mutual balancing of the characters. However, maximum intercluster distance existed between cluster 5 and 15 (D^2 120.75) followed by between cluster 12 and 16 (D^2 105.28) and between cluster 4 and 15 (D^2 101.72). If fruit shape and size of the clones were taken into consideration, it was evident that the clusters showing high distance between them contained clones having different fruit shape and size. Cluster 5 containing two clones bearing small sized fruits were most divergent than cluster 15 containing 2 clones bearing oval shaped and spindle-shaped fruits. Similarly cluster 12 containing two clones bearing oval shaped fruits were highly divergent than cluster 16 containing single clone with cylindrical fruits. Cluster 4 which was also widely divergent as compared to cluster 15 contained 6 clones out of total 8 in this cluster with small sized fruits (Group 1,

Table 6. Average intra and inter cluster D^2 distance

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	**28.55**															
2	38.63	**29.76**														
3	36.46	48.06	**28.72**													
4	36.52	49.98	38.27	**27.67**												
5	57.37	74.27	49.70	39.88	**16.52**											
6	40.29	57.77	40.51	46.84	53.41	**29.51**										
7	35.76	41.20	42.24	42.40	66.23	50.56	**0.00**									
8	54.64	76.99	52.05	50.20	47.39	42.80	64.01	**32.12**								
9	52.62	53.40	65.09	72.54	94.33	55.94	56.22	78.51	**30.20**							
10	40.99	57.12	40.77	50.32	62.99	36.53	54.90	46.24	54.93	**29.32**						
11	46.82	56.20	51.29	62.72	77.87	40.43	46.94	59.41	37.66	43.41	**0.00**					
12	75.68	98.40	73.49	76.54	69.39	54.09	87.07	42.20	86.57	58.08	66.50	**31.08**				
13.	51.62	54.33	56.53	71.05	91.92	53.38	58.31	74.67	37.18	43.09	40.48	84.08	**27.50**			
14	44.53	48.75	54.85	60.45	83.56	55.62	59.27	68.51	47.15	40.06	52.82	82.83	37.20	**30.86**		
15	81.92	83.53	92.72	101.72	120.75	76.72	83.93	98.65	43.35	76.82	55.03	97.17	53.54	70.70	**34.77**	
16	48.91	42.39	61.36	67.45	94.34	67.54	54.65	87.36	46.51	59.21	59.85	105.28	42.28	41.14	73.54	**0.00**

Bold values in the diagonals denote intra cluster D^2 distance.

based on fruit size and shape). So broad grouping based on fruit shape and size must be considered alongwith the clustering pattern of multivariate analysis to have a clear picture on genetic divergence of pointed gourd.

From the cluster mean value (Table 7) it was clear that among the cluster showing wide divergence, the cluster means were substantially high for the characters, viz., vine length, fruit length, fruit weight, fruit volume, seeds/fruit, fruits/plant and yield/plant. Prominent contribution of these characters towards divergence of the clones find support from the earlier report (Ram *et al.*, 1998). From the point of view of high fruit length, weight and yield/plant, cluster-15 and cluster-11 was deemed best for selecting diverse and desirable clones. Clones belonging to these clusters were BC-SP-10 (cluster-11) and BC-0-12 and BC-SP-9 of cluster-15 (Table 5). These promising and genetically diverse clones may as such be utilized as clonally selected varieties. However, these diverse clones may be utilized in homosexual crossing as described in giant spine gourd (*Momordica cochinchinensis*), the other very important dioecious, perenial and vegetatively propagated cucurbit (Anonymous, 1995). In this method (Ali *et al.*, 1991; Rajput *et al.*, 1994), foliar sprays with 300-600 ppm $AgNO_3$ or silver thiosulphate are applied at preflowering stage to induce bisexual flowers in the female clones. Intercrossing between the diverse female clones utilising the advantage of induced hermaphordite flowers, F_1 population of broad and desirable genetic base can be established for the selection of diseriable female homosexual hybrid seedlings which can be maintained clonally.

Table 7. Clusterwise mean values for different characters

Cluster	Vine length (cm)	Internode length (cm)	Primary branches / plant	Node at first flower	Leaves per plant	Leaf length (cm)	Leaf width (cm)	Leaf dry weight (g)	Leaf area (cm^2)	Fruit length (cm)	Fruit girth (cm)	Fruit volume (cc)	Fruit weight (g)	Pulp content (g)	Pericarp Thickness (cm)	Seeds / fruit	Protein content (g)	Fruits per plant	Yield / plant (g)
1	155.75	6.05	4.4	10.69	30.03	5.92	6.20	0.15	38.99	7.15	9.58	31.18	27.95	9.12	0.44	13.33	1.73	25.67	683.81
2	118.85	5.81	2.40	8.94	22.16	5.86	5.78	0.20	41.11	7.54	9.63	29.94	25.12	9.36	0.42	9.83	1.79	24.37	653.05
3	156.38	6.24	4.88	13.63	30.31	6.63	7.11	0.18	53.3	7.45	9.76	35.64	29.42	9.97	0.40	11.72	1.79	25.04	626.29
4	177.26	5.58	4.09	12.56	34.81	5.45	5.87	0.23	35.11	6.32	9.07	29.24	26.52	7.81	0.37	9.57	1.79	35.44	722.91
5	235.21	6.35	3.38	13.52	42.66	6.1	5.76	0.12	39.28	5.73	7.68	20.73	16.63	5.33	0.33	8.42	1.8	25.92	440.43
6	305.26	4.70	4.5	13.84	39.85	5.53	6.24	0.14	49.99	13.99	10.40	20.73	36.83	9.14	0.37	15.21	1.85	41.95	883.26
7	132.13	4.93	4.40	9.77	25.80	5.13	4.83	0.16	28.20	9.23	9.97	36.83	30.90	11.60	0.21	13.17	1.87	26.32	895.86
8	223.3	4.73	4.32	13.3	38.43	5.99	6.48	0.19	38.29	5.3	9.33	30.90	23.11	9.94	0.46	12.01	1.82	41.92	1067.50
9	163.37	5.33	3.89	10.95	32.96	5.92	6.11	0.19	43.56	7.74	9.81	32.47	25.24	10.01	0.41	14.25	1.83	20.21	678.06
10	146.97	5.61	3.06	15.97	36.93	6.36	6.6	0.34	51.85	6.72	10.16	43.06	28.61	10.45	0.49	8.2	1.8	21.5	662.03
11	165.43	4.00	2.67	9.07	33.03	5.33	5.70	0.12	31.87	9.13	8.67	42.31	37.27	13.13	0.39	12.93	1.77	33.47	1067.52
12	113.18	3.93	3.69	10.6	26.68	5.71	6.3	0.24	50.55	7.01	10.1	38.73	31.66	11.1	0.49	15.95	1.95	26.58	757.86
13	117.8	4.68	5.62	6.4	31.13	6.6	5.76	0.21	51.63	7.25	10.58	43.31	35.53	11.73	0.44	10.91	1.87	17.3	548.00
14	156.85	6.4	3.76	10.85	28.66	6.00	5.8	0.28	38.93	7.53	10.22	42.65	37.16	11.0	0.53	15.93	1.85	23.95	912.42
15	276.16	6.98	4.35	12.55	28.63	5.96	6.5	0.24	37.05	8.95	10.63	48.3	34.15	11.61	0.45	15.91	1.83	46.63	1570.2
16	210.33	6.63	4.00	9.97	28.70	5.23	6.17	0.12	39.13	7.07	8.17	30.90	29.40	7.20	0.49	10.23	1.97	36.53	780.43

4.1.3 Isozyme pattern for characterization of genetic diversity

General unerstanding lay on interdependence of phenotypic diversity, genotypic variation and maintenance of enzyme polymorphism, i.e. functional differences among allozyme variants. It is widely accepted that genetic variation of loci encoding for soluble enzymes is ubiquitons in natural populations in most of the clones (Koehn, 1977). Genetic control of isozyme polymorphism appears to be largely monogenic with the involvement of structural and modifier loci and in rare instances such genetic control is polygenic (Pierce and Brewbaker, 1973, Scandalios, 1974). Study on genetic diversity in terms of isozyme variation have been extensively used in different cucurbits, like summersquash, *Cucurbita pepo* (Dvorak and Cernohorska, 1967; Denna and Alexander, 1975, Ignart and Weeden, 1984; Loy, 1972), cucumber (Wood, 1971; Isshiki *et al.*, 1992; Knerr *et al.*, 1995) and musk melan (Puchalski *et al.*, 1978; Berg-vanden and Gabillard, 1994; Yadav *et al.*, 1998).

In pointed gourd clonal diversity has not so far been studied through such enzyme polymorphism. In the present peroxidase enzyme polymorphism study, casting of gel, loading of sample supernatant in the gel lane and electrophoretic run flatly standardised for other crops have been employed for this crop. Leaf explants of all the twenty clones under four broad groups based an fruit shape and size exhibited a total of eight bands of peroxidases having almost similar relative mobility values. The relative mobility of the eight peroxidases, i.e., the distance travelled by the isozyme was recorded on the graph paper as Rm values :

$$Rm = \frac{\text{distance travelled by a band}}{\text{distance travelled by tracking dye}}$$

Rm values of the eight bands are furnished below :

Band No.	Rm value
1	0.075
2	0.112
3	0.156
4	0.200
5	0.237
6	0.350
7	0.387
8	0.500

The representative banding pattern of the four groups were shown in Plate 4.

From the banding pattern of the peroxidases in the clones belonging to four groups based on fruit shape and size, the following picture emerged.

1. No specific peroxidases were absent so that associationship of specific isozymes with the clonal group could not be established.
2. Band 1 (Rm = 0.075), Band 2 (Rm = 0.112), Band 3 (Rm = 0.156), Band 7 (Rm = 0.387) and Band 8 (Rm = 0.500) were conspicuous in all the clonal groups which indicated that these peroxidases could not be employed to distinguish the clones and clonal group.
3. Band 4 (Rm = 0.200), Band 5 (Rm = 0.237) and Band 6 (Rm = 0.350) though present in all the clonal groups, yet showed some variations with regard to the intensity of the bands. As for example, band 4 was inconspicuous in Group 1, conspicuous in Group 2 and 3, while this band in Group 4 was more conspicuous group 1 but less than

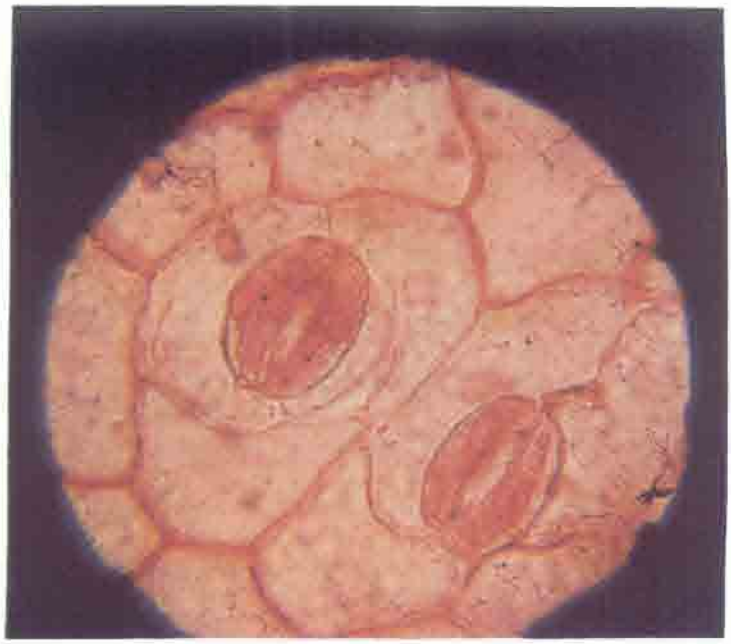

Photo 3 Stomata of upper leaf surface (5X 100X, Total 1100 times magnification)

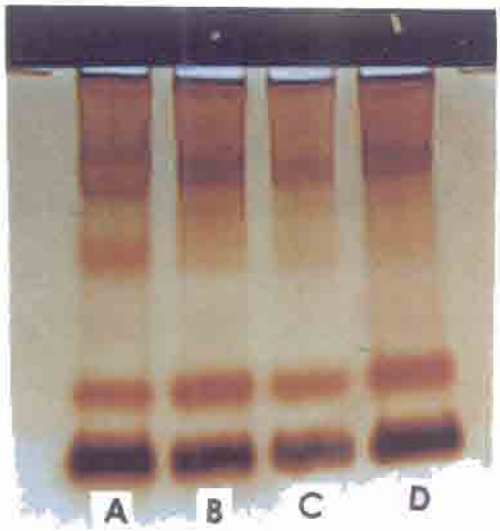

Photo 4 Representative peroxidase isozyme pattern in four clonal groups
(A: Group 1, B : Group 2, C : Group 3, D : Group 4)

Group 2 and 3. Band 5 and 6 was conspicuous than in Group 1, 2 and 4 but inconspicuous in Group 3.

4. Standardization of the method for this isozyme study in pointed gourd is needed for precision and accuracy in terms of expression of polymorphism.

4.1.4 Petiole anatomy of the clones under four groups

The petiole of pointed gourd was solid due to the integrity of pith. The petiole was wavy in outline and showed distinct ridges and furrows. Tranverse section of the petiole showed the following plan of arrangement of tissues from the peripheri to the centre in all the 20 random clones belonging to 4 broad groups based on fruit shape and size. (Plate 2).

A. Epidermis : It constituted the outer most cell layer of petiole. The epidermis was continuous, single layered consisting of compact-set tubular cells. Long and multicellular hairs developed from the epidermis. Mean epidermal cell length and width did not vary much among the four groups (Table 8). However, highest mean cell length of 15.77 micron was recorded in Group 2 (spindle shaped fruit) followed by 15.54 micron in the clones of Group 4 (nearly cylindrical fruit), 15.38 micron in Group 3 (oval shaped fruits) and 15.21 micron in Group 1 (small sized fruits). Similar trend was recorded for mean cell width of the epidermal cells where the highest cell width of 11.33 micron was recorded in Group 2 and lowest of 10.16 micron was recorded in Group 1. It is to be mentioned here that clones under Group 2 showed maximum mean fruit length of 6.93 cm and those under Group 1 showed the lowest length of 5.17 cm (Table 3). Such anatomical relationships with fruit and other economic characters need much detailed investigations for the establishment of this proposition.

Huge intragroup variations for cell length (e.g. range of 7.66-27.53 micron in Group 2) and cell width (range of 5.47-24.31 micron in Group 2) may pose some restrictions in ascertaining the interrelationship between anatomy and reproductive characters.

B. Cortex : It was differentiated in the following regions

a) Hypodermis : It lay below the epidermis and was made up of collenchyma cells, 3.16-5 (average 4) cell layers in thickness. The collenchyma cells generally formed a continuous band without interruption by the parenchyma cells. Mean hypodermal cell length also did not vary much among the groups (Table 8). From the study of group means it was evident that Group 2 with maximum epidermal cell dimensions recorded minimum mean hypodermal collenchyma cell length (16.35 micron). Maximum variation (range 7.62-47.58 micron) was also recorded in the clones belonging this groups for mean length of hypodermal cells. Highest mean cell length of 17.52 micron was recorded in Group 4 (clones bearing near cylindrical fruits). Mean width of the hypodermal cells also did not vary much among the groups ranging from 13.83 micron in Group 1 to 14.05 micron in Group 2. Conspicuous intragroup variation was also exhibited for the mean dimensions of hypodermal collenchyma cells.

b) Internal parenchyma : Just internal to hypodermis 3-4 layers of parenchymatous zones comprising of big parenchyma cell occured. This internal parenchyma appeared to be limited by a layer of closely packed cells.

C) Stele : It was found composed of the following tissues.

a) Bundle cap : Few layers of elongated of parenchyma cells of the outer most part of phloem appeared to have become thick-walled and occured as peri-vascular fibres.

Table 8. Features of petiole anatomy of the clonal groups (average data from 5 random clones in each group)*

Clonal group based on fruit shape and size	Epidermal cell layer		Hypodermal collenchyma cells			Vascular bundles		
	Mean cell length (micron)	Mean cell width (micron)	Mean number of cell layers	Mean cell length (micron)	Mean cell width (micron)	Mean number of vascular bundles in the cross section	Mean length of vascular-bundles (micron)	Mean width of vascular bundle (micron)
Group 1 : Plants bearing small fruits of different size	15.21 (9.93-24.81)	10.16 (5.34-15.48)	4.0 (3.16-4.88)	17.13 (7.53-25.32)	13.83 (7.44 - 20.13)	7.14 (6.21-8.33)	370.23 (250.42-530.17)	224.66 (114.56-300.14)
Group 2 : Plants bearing spindle shaped fruits.	15.77 (7.66-27.53)	11.33 (5.47-24.31)	4.00 (3.26-5.00)	16.35 (7.62-47.58)	14.05 (7.58-44.16)	6.83 (6.17-8.25)	331.36 (200.72-450.16)	200.53 (140.34-300.17)
Group 3 : Plants bearing oval shaped fruits	15.38 (11.82-20.33)	10.76 (7.48-15.42)	4.12 (3.36-5.17)	17.49 (9.87-25.44)	14.01 (9.88-24.67)	6.75 (6.19-7.35)	328.12 (190.66-400.28)	231.14 (140.62-298.86)
Group 4 : Plants bearing near cylindrical fruits	15.54 (10.54-29.48)	10.83 (7.16.20.19)	4.26 (3.31-5.62)	17.52 (7.81-29.56)	13.92 (7.87-22.56)	7.03 (6.66-7.96)	324.37 (202.68-401.07)	202.16 (150.74-260-53)

Ranges given in parenthesis. * Group based on fruit shape and size.

b) Vascular budles

(i) Number : Mean number of vascular bundles in the cross section was almost same in all the groups (6.8-7.1).

(ii) Dimensions : Variation for mean length of vascular bundles among Group 2, 3 and 4 were minimum (324.37 micron - 331.36 micron) but, that of Group 1 was conspicuously high (370.23 micron). Upper range of the mean length of the vascular bundles in the clones of Group 1 was also the highest (530.17 micron). Mean width of vascular bundle in Group 1 was also high (224.66 micron) compared to Group 2 (200.53 micron and Group 4 (202.16 micron). Though huge intragroup variations existed for the dimensions of vascular bundles among all the groups, aparent associationship of high vascular tissues in the low yielding clones of Group 1 needs further investigations to unveil the intricate relationship between the dimension of vascular system i.e., transport system of the plants with the partitioning of photosynthates in the clones.

(iii) Arrangement : In relation to the arrangement of vascular tissues in the stem the vascular bundles may be colateral, bi-colateral or concentric (Fahn, 1990). In pointed gourd the arrangement is bi-colateral where phloem occured on both side of the xylem.

(iv) Distribution : The vascular bundles were arranged like a ring and embedded within the parenchymatous ground tissuess. No variation was observed in the distribution of vascular tissues within the body of petiole among the clones belonging to 4 broad groups.

(v) Sequence of tissues in vascular bundles : Each vascular bundle consisted of two patches of phloem and one patch of xylem and tissues in between appeared to be cambium. So, the sequence of tissues was : outer phloem, outer cambium, xylem, inner cambium and inner phloem. In the xylem vessel, both metaxylem (longer vessels) and protoxylem

(smaller vessels) were present. The outer cambium was composed of several layer of cells while the inner cambium was comparatively few layered.

From the present study few prominent observations have been emerged.

1) In the transverse section of petiole there existed no variation whatsoever in the plan of arrangement of tissues from the peripheri to the centre.
2) Variation, though meagre, existed in mean dimensions of epidermal and hypodermal cells among the clonal groups. However, large intragroup variations indicated by huge ranges suggested the existence of clones having overlapping characters in the groups.
3) The clonal groups bearing the longest fruit also recorded longest epidermal cell. This associationship need to be established through some more detailed studies.
4) Highest dimensions of vascular bundles was recorded in the lowest yielding clonal group which needs further investigations in the light of source-sink relationships.

However, it was apparent from the study that increased conducting tissues in pointed gourd did not necessarily mean improved partitioning of photosynthates for the production of high yield.

4.1.5 Stomatal features of the clones under four groups

The interest in stomata has arisen because of the widespread perception of their crucial role in the conrol of waterloss (hence of draught tolerance) and carbon dioxide uptake (hence of productivity), and because they provide a simple anatomical character that can be used in selection (Jones, 1987).

The continuity of epidermis (leaf, petiole, stem etc.) is interrupted by minute openings. These are intercellular spaces each of which is limited by two specialized cells called guard cells. The guard cells, together with opening between them constitute the stoma. The guard cell of the stomata of pointed gourd appeared to be levelled relative to other epidermal cells. Of the four main types of stomata distinguished in dicotyledons on the basis of the arrangement of epidermal cells neighbouring guard cells (Metcalfe and Chalk, 1979), stomata of leaves of pointed gourd appeared to be **Anomocytic** type (Plate 3) where the guard cells were surrounded by a certain number of cells that did not differ in size and shape from the other epidermal cells. Stomata were present in both lower and upper leaf surfaces as mostly found in the plants with Platesynthetic capacity and plants living in full sun environments (Mott *et al.*, 1982). In the present study taking the twenty clones under study as a whole without considering their group constellation based on fruit shape and size, huge intraspecific variation existed for different stomatal characters as presented in Table 9, viz., stomatal frequency/mm^2 in upper leaf surface (11.86-43.13), stomatal frequency /mm^2 in lower leaf surface (32.72-118.84), stomatal length in upper leaf surface (16.72-24.74 micron), stomatal length in lower leaf surface (12.69-21.92 micron), stomatal width in upper leaf surface (10.16-18.48 micron), stomatal width in lower leaf surface (8.62-14.78 micron). This findings of wide intraspecific (interclonal) genetic variation for stomatal characters agreed well to the earlier studies in different crops like apple (Beakbane and Majumder, 1975), barley (Miskin and Rasmussan, 1970), soybean (Ciha and Brun, 1975), broad bean (Singh *et al.*, 1982) and cowpea (Hazra *et al.*, 1996). Existence of variation in the group means also for the different stomatal characters in the present study suggested the provision of ample scope to effect selection on the

Table 9. Stomatal features of the clonal groups (average data from 5 random clones in each group)*

Clonal group based on fruit shape and size	Mean stomatal frequency/mm^2		Mean stomatal length (Micron)		Mean stomatal width (Micron)	
	Upper leaf surface	Lower leaf surface	Upper leaf surface	Lower leaf surface	Upper leaf surface	Lower leaf surface
Group 1 : Plants bearing small fruits of different size	27.55 (17.22-39.61)	68.35 (44.78-118.84)	20.29 (17.53-24.74)	17.35 (12.69-21.92)	14.07 (10.16-18.48)	12.94 (9.87-14.78)
Group 2 : Plants bearing spindle shaped fruits.	20.12 (12.19-31.03)	60.65 (32.72-72.34)	19.13 (17.58-22.48)	18.25 (15.64-21.73)	13.26 (10.24-17.51)	10.87 (8.62-12.66)
Group 3 : Plants bearing oval shaped fruits	16.25 (11.86-22.39)	63.99 (49.94-75.78)	17.16 (16.72-21.81)	16.96 (15.18-20.34)	12.58 (10.17-16.76)	10.36 (9.78-13.18)
Group 4 : Plants bearing near cylindrical fruits	24.68 (12.05-43.13)	64.94 (32.97-93.04)	17.06 (17.19-21.63)	16.91 (14.28-20.28)	11.66 (10.23-16.48)	10.54 (9.76-12.86)

Ranges given in parenthesis. * Group based on fruit shape and size.

stomatal characters. However, such variation is expected to have been confounded by large environmental component of variation, as stomatal frequencies can change more than two fold in response to radiation (Gay and Hurd, 1975), in response to water status (Rawson *et al.*, 1980), or according to developmental stage (Meidner and Mansfield, 1968). So genetic worth of the present wide clonal variation for the stomatal characters need to be fortified with the exclusion of environmental component of variation from the observations.

It was interesting to note from the group means of stomatal characters and yield (Table 3 and 9) that mean stomatal characters of the clones belonging to low yielding group (Group 1, plants bearing small fruits of different size and shape) were the highest viz. stomatal frequency/mm^2 (27.55 in upper leaf surface and 68.35 in lower leaf surface), stomatal length (20.29 micron in upper leaf surface and 17.35 micron in lower leaf surface which was second high), stomatal width (14.07 micron in upper leaf surface and 12.94 micron in lower leaf surface). In contrast, stomatal characters in the clones belonging to the highest yielding group (Group 3, plants bearing oval-shaped fruits) were comparatively much low and in some of the cases the lowest viz. stomatal frequency/mm^2 (16.25 in upper leaf surface and 63.99 in lower leaf surface), stomatal length (17.16 micron in upper leaf surface and 16.96 micron in lower leaf surface), stomatal width (12.58 micron in upper leaf surface and 10.36 micron in lower leaf surface). This findings indicated that the relationship between total leaf pore area (consequence of stomatal frequency, length and width) with net photosynthesis and ultimately yield was not straight foreward as expected that higher carbondioxide assimilation due to higher total pore area in the leaf would increase the yield. Earlier studies in different crops indicated that although production of dry matter often limits crop yields, high

Photosynthetic rate per unit area do not generally lead to high yield because on a crop basis, many other crop factors such as carbohydrate partitioning and leaf area development are involved and may compensate for changes in assimilation per unit leaf area (Dusntone *et al.*, 1973; Jones, 1983). Imbalanced partitioning of photosynthates in the clones of Group 1 was aparent due to revealation of higher vine length, internode length and leaves/plant (Table 3). From the present investigations, it was also established that the clones of this low yielding group contained the highest dimension of conducting tissues in the petiole (Table 8). There were reports that fewer and less dense stomata increased net photosynthesis in French bean and maize (Izhar and Wallace, 1967; Heickel, 1971). It has also been suggested that lower stomata frequency leads to higher stomatal conductance (Das and Kundragrami, 2000), and low stomatal number also reported to be desirable for higher productivity (Chaudhury and Tikhotiker, 1986). Hazra *et al.* (1996) recorded strong negative correlation between stomatal frequency/mm^2 in upper leaf surface and pod yield/plant in cowpea.

The present investigations on the stomatal features in the clones of pointed gourd clearly depicted some inference and arosed some questions.

1) Huge genetic variation existed in the clones under study at the disposal of breeder which can be used as selection indices.

2) Clones of Group 1 (Plants bearing small fruits of different size and shape) appeared as a distinct group with the revealation of highest stomatal frequency, length and width in both the leaf surfaces.

3) Straight forward relationships between stomatal characters and yield could not be established, rather low stomatal frequency, length and width registering low pore area per unit leaf area was aparently desirable for high productivity.

4) Parallelism between high pore area/unit leaf area (due to high frequency, length and width) and high dimension of conducting tissues (vascular bundles) was recorded in the clones of low yielding group (Group-1, Plants bearing small sized fruits).

4.2 Genetic variability for different characters

Analysis of variances for the nineteen growth and reproductive characters namely, vine length, internode length, primary branches/plant, node at first flower, leaves/plant, leaf length, leaf width, leaf dry weight, leaf area, fruit length, fruit girth, fruit volume, fruit weight, pulp content/fruit, pericarp thickness, seeds/fruit, protein content/100g of fruit, fruits/plant and fruit yield/plant based on the average data of 1997-'98 and 1998-'99 revealed the signifiance of all these characters in the clones execepting protein content/100 g of fruit (Table 10) which showed the existence of significant difference among the clones for 18 characters. Wide phenotypic variation in term of variance of range of the clonal means did not necessarily indicated the variability at genotypic level. For this reason, estimates for the coefficients of genotypic and phenotypic variations (GCV and PCV), heritability in broad sense (H), and genetic advance as percentage of mean (GA) for these 19 characters over the 68 clones irrespective of the broad grouping were computed and presented in Table 11. In each characters excepting protein content/100 g of fruit, the estimates of GCV and PCV corresponded well which revealed that environment did not influence much on the expression of these characters. On the other hand, wide gap between the estimates of GCV and PCV for protein content of fruit indicated huge environmental influence behind the expression of this character. High GCV (31.91-42.26%) was recorded for vine length, node at first flower, leaf dry weight, fruit length, fruits/plant, and fruit yield/plant and moderate GCV (20.84-28.17%) was registered for fruit weight,

Table 10. Variance analysis for different characters over 68 clones*

Characters	Mean sum of squares		Standard error (S.E.)	Critical difference		Coefficient of variation (%)
	Genotype (clone)[1]	Error[2]		(P = 0.05)	(P = 0.01)	
Vine length (cm)	11067.16**	356.372	10.893	30.214	39.693	10.58
Internode length (cm)	5.04**	0.158	0.229	0.635	0.832	7.24
Primary branches / plant	3.74**	0.172	0.239	0.663	0.871	10.47
Node at first flower	40.86**	0.128	0.206	0.572	0.750	3.13
Leaves per plant	223.71**	4.219	1.186	3.285	4.318	6.43
Leaf length (cm)	1.67**	0.088	0.171	0.476	0.624	5.07
Leaf width (cm)	1.96**	0.061	0.142	0.395	0.519	4.06
Leaf dry weight (g)	0.021**	0.0008	0.016	0.045	0.059	14.57
Leaf area (cm^2)	319.79**	3.092	1.015	2.813	3.696	4.21
Fruit length (cm)	19.66**	1.593	0.728	2.019	2.653	16.41
Fruit girth (cm)	23.08**	1.079	0.599	1.661	2.183	10.80
Fruit volume (cc)	216.63**	5.186	1.314	3.643	4.787	6.54
Fruit weight (g)	122.17**	7.153	1.544	4.278	5.622	9.00
Pulp content / fruit (g)	10.73**	1.099	0.605	1.677	2.204	10.58
Pericarp Thickness (cm)	0.032**	0.0017	0.023	0.065	0.086	9.86
Seeds / fruit	57.74**	2.133	0.843	2.336	3.071	11.83
Protein content (g)[3]	1.16	0.875	0.541	NS	NS	51.11
Fruits per plant	344.13**	11.536	1.960	5.433	7.141	11.49
Yield / plant (g)	325400.81**	4410.51	38.342	106.242	139.623	8.65

*** Based on average of 1997-98 and 1998-99 field trials.**

1. d.f 67.

2. d.f 134.

3. fresh weight basis per 100 g of fruit.

NS = Not significant. ** Significant (P = 0.01).

internode length, primary branches/plant, leaves/plant, leaf area, fruit girth, fruit volume and pericarp thickness. These findings proved the existence of justifiable genetic distance among the clones under study, a fact indicative of the efficacy of these clones in clonal selection of pointed gourd. From such a study employing the clonal collections from different part of eastern India. Shadique *et al.* (1986) also recorded high GCV for main creeper length, leaf number/plant, days from bud initiation to edible fruit maturity, fruit volume, seed number/fruit and fruit yield/plant which agreed well to the findings of the present investigations.

However, the genotypic co-efficient of variation helps to measure the range of genetic variability in the character and provides a measure to compare the genetic variability present in various characters, but with the help of GCV alone the heritable variation can not be measured (Singh *et al.*, 1974). Heritability of the character is of interest of the plant breeder as an index of transmissibility of the character to the progeny (Hayes *et al.*, 1955). High heritability in broad sense (74.49-99.06%) was recorded for all the characters excepting protein content/fruit. This broad sense heritability values were likely to be overestimated because it was not possible to exclude the variations of the different genetic components and their interactions in the present study. To asses the maximum effect of selection, genetic advance need to be computed because high heritability values does not necessarily mean increased genetic gain for the concerned character in the next generation (Johnson *et al.*, 1955). Genetic advance as per cent age of mean (Table 11) indicated few significant trends. It was not necessarily true that the characters showing high heritability would always exhibit high genetic advance. For this reason leaf length and leaf width exhibited high broad sense

Table 11. Grand mean (GM), component of variances (G.C.V., P.C.V), heritability in broad sense (H%) and genetic advance as percentage of mean (GA%) for different characters over 68 clones*

Characters	G.M.	G.C.V.	P.C.V.	H%	GA%
Vine length (cm)	178.38	33.49	35.12	90.92	65.79
Internode length (cm)	5.49	23.22	24.33	91.14	45.69
Primary branches/plant	3.96	27.52	29.46	87.36	53.02
Node at first flower	11.41	32.28	32.44	99.06	66.21
Leaves per plant	31.92	26.79	27.55	94.54	53.67
Leaf length (cm)	5.85	12.41	13.42	85.69	23.67
Leaf width (cm)	6.07	13.11	13.72	91.21	25.78
Leaf dry weight (g)	0.194	42.26	44.69	89.37	82.28
Leaf area (cm^2)	41.80	24.57	24.93	97.15	49.90
Fruit length (cm)	7.69	31.91	35.87	79.08	58.44
Fruit girth (cm)	9.61	28.17	30.17	87.17	54.19
Fruit volume (cc)	34.80	24.12	24.99	93.14	47.96
Fruit weight (g)	29.71	20.84	22.69	84.27	39.41
Pulp content (g)	9.90	18.09	20.95	74.49	32.16
Pericarp Thickness (cm)	0.418	24.04	25.98	85.59	45.82
Seeds / fruit	12.34	34.88	36.83	89.67	38.04
Protein content (g)	1.83	16.84	53.77	9.79	10.84
Fruits per plant	29.55	35.63	37.45	90.57	69.85
Yield / plant (g)	767.25	42.61	43.49	96.04	86.06

G.C.V. = Genotypic coefficient of variation, H = Heritability in broad sense.
P.C. V. = Phenotypic coefficient of variation, GA = Genetic advance.
*** Based on average of 1997-98 and 1998-99 field trials.**

heritability of 85.69% and 91.21%, respectively but their genetic advance was low, 23.67% and 25.78% respectively. Such an association of high heritability and low genetic advance was attributable to non-additive actions of the polygenes for the conditioning of the character (Liang and Walter, 1968). However, complementality of heritability and genetic advance as suggested by Hanson (1961) was revealed in most of the characters. According to Panse (1957), if the expressivity of a charracter is governed by the additive action of polygenes, high heritability coupled with high genetic advance may be expected for the concerned characters. In the present investigation, high heritability coupled with high genetic advance was recorded for vine length, internode length, primary branches/plant, node at first flower, leaves/plant, leaf dry weight, leaf area, fruit length, fruit girth, fruit volume, fruit weight, pericarp thickness, fruits/plant and fruit yield/plant which was in agreement with the earlier reports (Shadique *et al.*, 1986; Singh *et al.*, 1985; Singh *et al.*, 1986; Singh *et al.*, 1987; Sarkar, 1989 and Yadav *et al.*, 1998). It was therefore, logical to infer that in respect of these characters registering high GCV, heritability and genetic advance, clonal selection should be effective and satisfactory for practical purpose.

4.3 Character associationship

4.3.1 Correlations : The study of correlationship will help to know suitability of various characters for direct selection because selection for one or more traits results in correlated responses in several other traits (Searle, 1965). Knowledge of such correlationships is very much important for indirect selection involving the characters that are not easily measured and for those that exhibit low heritability, and it might be easier to increase yield by increasing the smallest yield component in otherwise good cultivar (Grafius, 1959). In the present study, magnitude of genotypic correlation co-efficients were higher than

phenotypic correlation co-efficient for all the pair of characters under study (Table 12 and 13). and in most of the cases wide gap was recorded between the two estimates of correlation coefficients which indicated the influence of environment on the correlated response of the pair of characters as proposed by Falconer (1981). The phenotypic correlation coefficients (Table 12) being the observed relationships between the pair of characters under study have been utilized to record the observations in this aspect.

Most of the characters did register either insignificant or negligible correlations among themselves which might have resulted due to simultaneous vegetative and reproductive growth in the plant over long period of time which developed internal balancing and mutual cancellation of the characters. Prolonged period for the partitioning of photosynthates to different vegetative and reproductive sinks due to perenniality in growth habit of pointed gourd might have also contributed towards un-correlated responses of the pairs of characters under study. The present observation find ample support from the earlier observation (Yadav *et al.*, 1988) where negligible or near zero correlations were recorded for good number of the pairs of characters.

Significant positive phenotypic correlations were recorded between vine length and node at first flower, leaves/plant; between node at first flower and leaves/plant; between leaves/plant and fruits/plant; between leaf length and leaf width, leaf area, fruit weight and pericarp thickness; between leaf width and leaf area; between leaf dry weight and pericarp thickness; between leaf area and fruit girth and fruit volume; between fruit girth and fruit volume; between fruit volume and fruit weight, pulp content and pericarp thickness; between fruit weight, pulp content and pericarp thickness (Table 12). Fruit yield/plant was correlated positively and appreciably with fruit volume, fruit weight

Table 12. Phenotypic correlations among different characters[1]

Characters	Vine length	Internode length	Primary branches/pl	Node at first flower	Leaves per plant	Leaf length	Leaf width	Leaf dry weight	Leaf area	Fruit length	Fruit girth	Fruit volume	Fruit weight	Pulp content	Pericarp Thickness	Seed / fruit	Protein content	Fruits per plant	Yield / plant
Vine length	1.000	0.200	0.200	0.312**	0.762**	-0.154	-0.105	-0.109	-0.209	0.150	0.024	0.106	0.036	0.004	-0.118	0.107	-0.073	0.262*	0.123
Internode length		1.000	0.212	-0.046	0.009	-0.028	-0.033	-0.201	0.054	-0.008	0.030	-0.014	-0.061	-0.044	-0.069	0.052	-0.033	0.128	-0.034
Primary branches/pl			1.00	0.009	-0.073	0.211	0.097	0.166	0.124	0.039	0.007	-0.058	-0.050	0.001	0.087	0.016	0.065	-0.050	-0.093
Node at first flower				1.000	0.481**	0.103	0.005	0.056	0.109	0.176	0.221	0.078	-0.004	0.140	-0.043	-0.038	-0.051	0.074	0.091
Leaves per plant					1.000	-0.130	-0.165	-0.027	-0.229	0.087	0.076	-0.004	-0.054	0.006	-0.113	0.065	-0.134	0.236*	0.112
Leaf length						1.000	0.589**	0.222	0.586**	-0.041	0.126	0.260	0.264*	0.230	0.466**	-0.061	-0.085	-0.379**	-0.084
Leaf width							1.000	0.133	0.584**	0.016	0.117	0.105	0.118	0.138	0.242*	-0.189	-0.082	-0.325**	-0.080
Leaf dry wt								1.000	0.118	-0.067	-0.042	0.074	0.188	0.105	0.336**	-0.096	0.160	-0.189	-0.070
Leaf area									1.000	0.088	0.332**	0.247*	0.202	0.196	0.219	-0.101	0.075	-0.490**	-0.146
Fruit length										1.000	0.190	0.159	0.110	0.141	-0.100	0.096	0.000	0.003	0.034
Fruit girth											1.000	0.514**	0.421**	0.577**	0.130	0.178	-0.067	0.177	-0.047
Fruit volume												1.000	0.842**	0.687**	0.298*	0.233	0.050	-0.197	0.200
Fruit wt													1.000	0.621**	0.407**	0.218	0.033	-0.193	0.219
Pulp content														1.000	0.175	0.183	-0.060	-0.156	0.202
Pericarp thickness															1.000	0.008	0.051	-0.299**	-0.040
Seed /fruit																1.000	-0.011	0.085	0.069
Protein content																	1.000	-0.106	-0.034
Fruits/plant																		1.000	0.265*
Fruit yield /plant																			1.000

1 **Based on average of 1997-98 and 1998-99 trials.**

* **Significant at P = 0.05.**

** **Significant at P = 0.01.**

Table 13. Genotypic correlations among different characters[1]

Characters	Vine length	Internode length	Primary branches /pl	Node at first flower	Leaves per plant	Leaf length	Leaf width	Leaf dry weight	Leaf area	Fruit length	Fruit girth	Fruit volume	Fruit weight	Pulp content	Pericarp Thickness	Seed / fruit	Protein content	Fruits per plant	Yield / plant
Vine length	1.000	0.223	0.009	0.325	0.801	-0.168	-0.112	-0.121	-0.222	0.395	0.037	0.113	0.041	0.007	-0.124	0.280	-0.136	0.278	0.309
Internode length		1.000	0.225	-0.048	0.008	-0.014	-0.015	-0.214	0.053	-0.015	-0.036	-0.018	-0.066	-0.050	-0.070	0.147	-0.0276	0.134	-0.109
Primary branches/pl			1.00	0.009	-0.078	0.220	0.091	0.170	0.127	0.105	0.060	-0.060	-0.049	-0.002	0.091	0.047	0.204	-0.050	-0.193
Node at first flower				1.000	0.484	0.115	0.003	0.056	0.109	0.376	0.232	0.078	-0.003	0.143	-0.044	-0.083	-0.216	0.076	0.212
Leaves per plant					1.000	-0.138	-0.171	-0.027	-0.232	0.289	0.079	-0.004	-0.054	0.004	-0.114	0.149	-0.463	0.241	0.251
Leaf length						1.000	0.651	0.244	0.633	0.094	0.141	0.279	0.286	0.249	0.511	-0.166	-0.363	-0.414	-0.164
Leaf width							1.000	0.145	0.719	0.033	0.128	0.109	0.123	0.138	0.260	-0.425	-0.270	-0.344	-0.155
Leaf dry wt								1.000	0.122	-0.247	-0.043	0.074	0.196	0.111	0.342	-0.219	0.530	-0.190	-0.189
Leaf area									1.000	0.193	0.347	0.249	0.209	0.200	0.224	-0.226	0.361	-0.503	-0.346
Fruit length										1.000	0.408	0.323	0.317	0.408	-0.333	0.141	-0.374	0.026	0.211
Fruit girth											1.000	0.539	0.447	0.609	0.139	0.260	-0.126	-0.186	-0.73
Fruit volume												1.000	0.855	0.697	0.301	0.328	0.133	-0.201	0.366
Fruit wt													1.000	0.640	0.415	0.402	0.166	-0.197	0.527
Pulp content														1.000	0.180	0.4257	-0.176	-0.161	0.481
Pericarp thickness															1.000	-0.008	0.266	-0.305	-0.087
Seed /fruit																1.000	-0.182	0.205	0.332
Protein content																	1.000	-0.396	-0.302
Fruits/plant																		1.000	0.416
Fruit yield /plant																			1.000

1. Based on average of 1997-98 and 1998-99 trials.

and pulp content of the fruit, but only fruit number/plant registered significant positive correlation with fruit yield. Different earlier studies also indicated the implications of the fruit characters namely fruits/plant, fruit weight, fruit length, fruit diameter and seed number/fruit as the highly correlated characters of fruit yield/plant. (Singh *et al.*, 1986, Singh *et al.*, 1987, Singh and Prasad, 1989, Prasad and Singh, 1990, Sarkar *et al.*, 1990, Yadav *et al.*, 1998).

4.3.2 Path coefficients : The association between pairs of characters comprises of a complicated pathway involving various other attributes. Direct contribution of component characters to fruit yield/plant and the indirect effects which this might have through their relationship with each other were separated through path analysis. The path coefficient analysis using the phenotypic correlation coefficients among the pairs of characters (Table 14) showed that the fruit character namely, fruit weight had the highest positive direct effect on yield (0.326) followed by pulp content of fruit (0.202), fruit volume (0.121) and leaves/plant (0.103). Singh *et al.* (1998) also recorded that fruit weight had high positive direct effect on yield. These characters excepting leaves/plant also registered appreciable positive correlation with fruit yield/plant. High positive indirect effects on fruit yield/plant exerted by fruit weight was *via* pulp content of fruit, by pulp content of fruit and fruit volume was *via* fruit weight (Table 14). So these fruit characters emerged as highly interrelated characters which influenced fruit yield/plant concertedly. Fruits/plant showing significant positive correlation with fruit yield/plant, could not exert any positive direct effect on fruit yield/plant. However, importance of fruit number/plant as a prime fruit yield component can not be overlooked. High residual effect in the present path analysis (0.767) might have occured due to registration of poor correlations among the pairs of characters under study.

Table 14. Path coefficient analysis using phenotypic correlation coefficients

Characters	Vine length	Internode length	Primary branches	Node to first flower	Leaves per plant	Leaf length	Leaf width	Leaf dry weight	Leaf area	Fruit length	Fruit girth	Fruit volume	Fruit weight	Pulp content	Pericarp Thickness	Seeds / fruit	Protein content	Fruits per plant	Phenotypic correlation with yield
Vine length	**-0.060**	-0.006	0.000	0.009	0.079	0.014	-0.003	0.011	-0.004	0.000	-0.006	0.013	0.012	0.001	0.003	-0.002	-0.001	0.064	0.123
Internode length	-0.012	**-0.031**	0.001	-0.001	0.001	0.003	-0.001	0.021	0.001	0.000	-0.008	0.002	-0.020	-0.010	0.002	-0.001	-0.001	0.031	-0.034
Primary branches / pl	0.000	-0.007	**0.006**	0.000	-0.008	-0.018	0.003	-0.017	0.002	0.000	-0.002	-0.007	-0.016	0.000	-0.002	0.000	0.001	-0.012	-0.093
Node at first flower	0.019	0.001	0.000	**0.029**	0.050	0.009	0.0000	-0.005	0.002	0.000	-0.057	0.009	-0.001	0.034	0.001	0.001	-0.101	0.018	0.091
Leaves/plant	-0.046	0.000	0.000	0.014	**0.103**	0.012	-0.005	0.003	-0.004	0.000	-0.019	0.000	-0.018	0.001	0.003	-0.001	-0.002	0.058	0.112
Leaf length	0.009	0.001	0.001	0.003	-0.013	**-0.090**	0.019	-0.023	0.011	0.000	-0.033	0.031	0.086	0.055	-0.012	0.002	-0.001	-0.093	-0.084
Leaf width	0.006	0.001	0.001	0.000	-0.017	-0.053	**0.032**	-0.014	0.012	0.000	-0.030	0.013	0.038	0.033	-0.006	0.004	-0.001	-0.080	-0.080
Leaf dry wt	0.007	0.006	0.001	0.002	-0.003	-0.020	0.004	**-0.104**	0.002	0.000	0.011	0.009	0.062	0.025	-0.008	0.002	0.003	-0.046	0.070
Leaf area	0.013	-0.002	0.001	0.003	-0.024	-0.053	0.022	-0.012	**0.018**	0.000	-0.085	0.030	0.066	0.047	-0.005	0.002	0.001	-0.120	-0.146
Fruit length	-0.009	0.000	0.000	0.005	0.009	0.004	0.001	0.007	0.007	**-0.003**	-0.049	0.019	0.036	0.034	0.002	-0.002	0.000	0.001	-0.034
Fruit girth	-0.001	-0.001	0.000	0.006	0.006	-0.012	0.004	0.004	0.006	0.000	**-0.257**	0.082	0.137	0.138	-0.003	0.004	-0.001	-0.044	-0.047
Fruit volume	-.006	0.000	.000	0.002	0.000	-0.24	0.003	-0.008	0.005	0.000	-0.132	**0.121**	0.275	0.165	-0.007	-0.005	0.001	-0.048	0.200
Fruit wt	-.002	0.002	.000	0.000	-0.006	0.024	0.004	-0.020	0.004	0.000	-0.108	0.102	**0.326**	0.149	-0.010	-0.004	0.001	-0.047	0.219
Pulp content	0.000	0.001	0.000	0.004	0.001	-0.021	0.004	-0.011	0.004	0.000	-0.148	0.083	0.202	**0.240**	-0.004	-0.004	-0.001	-0.038	0.202
Pericarp thickness	0.007	0.002	0.001	-0.001	-0.012	-0.042	0.008	-0.055	0.004	0.000	-0.034	0.036	0.133	0.042	**-0.025**	0.000	0.001	-0.073	-0.040
Seeds /fruit	-0.006	-0.002	0.000	-0.001	0.007	0.007	-0.006	0.010	-0.002	0.000	-0.046	0.028	0.071	0.044	0.000	**-0.020**	0.000	0.021	0.069
Protein content	0.000	-0.001	0.000	-0.005	-0.007	-0.018	0.003	-0.011	0.004	0.000	-0.111	-0.082	0.230	0.127	-0.006	-0.003	**0.001**	-0.063	0.034
Fruits/plant	0.004	0.001	0.000	-0.001	-0.014	0.008	-0.003	-0.019	0.001	0.000	0.017	0.006	0.011	-0.014	-0.001	0.000	0.015	**-0.026**	0.265

Residual effect = 0.7673, Bold diagonals denote direct effects.

From the studies of character associationship through correlations and path analysis at phenotypic level the characters namely leaves/plant, leaf length, fruit number/plant, fruit weight, pulp content of fruit and fruit volume emerged as the most important fruit yield attributing characters of pointed gourd. From the earlier studies of genetic variability these characters appeared to have been controlled by the additive action of polygenes thereby, showing their reliability as selection indices for clonal selection in pointed gourd.

4.4 Application of induced polyploidy in pointed gourd

The phenotypic variation due to the increased chromosome number (tetraploid : four homologous sets, triploid : three homologous sets.) adds much to their morphological and physiological values which may have high value in vegetable breeding. Though application of induced polyploidy has been referred in many vegetable crops, commercialization of such breeding approach has been accomplished in few vegetable crops including watermelon and musk melon (Karchi *et al.*, 1981; Gray and Elmstrom, 1991; Gonzalez and Ayuso, 1992; Tan *et al.*, 1993; Earhart *et al.*, 1994; Nugent and Ray, 1992; Raamsdonk and Visser, 1992). Application of induced polyploidy in breeding pointed gourd has been investigated in the present study.

4.4.1 Development of colchiploids : Seeds of pointed gourd are recalcitrant in nature hence, lose viability very soon. For this reason, fresh seeds extracted from fully ripe fruits were used for the development of seedlings to induce polyploidy in them by colchicine treatment. After extraction, the seeds were thoroughly washed to remove the adhering placenta on them. It took 7-10 days for germination. Colchicine at the concentration of 2% was applied to the seedling apex within 3-5 days of their emergence (Plate 5). In all the three methods of

Photo 5 Seedling stage for colchicine treatment

Photo 6 Consequence of colchicine treatment :
Some what normal growth and slight chlorosis

Photo 7 Consequence of colchicine treatment :
Severe tip distortion and simultaneous regeneration of shoot from the base.

colchicine application of seedlings, three basic consequences after 10 days of application were met with in both 1997 and 1998 which has been summarised in Table 15.

In most of the cases growth of the seedlings were severely checked alongwith bursting of axillary buds, chlorosis and thickening of the tip of the seedlings (Plate 8). Affected seedlings in 1997 treatment was 65% in T_1, 43% in T_2, and 62% in T_3 which were 62, 51 and 60 per cent respectively in 1998 treatment. In another consequence, severe tip distortion of the seedlings and simultaneous regeneration of the shoots from the base (Plate 7) occured in 40, 55 and 32 percentage of seedlings under T_1, T_2 and T_3 respectively in 1997; and 35, 46, and 36 per cent in T_1, T_2 and T_3 respectively in 1998.

Very few colchicine treated seedlings (5, 2 and 6 per cent under T_1, T_2 and T_3 respectively in 1997 and 3, 3, 4 per cent under T_1, T_2 and T_3 respectively in 1998) showed somewhat normal growth accompanied by slight leaf chlorosis and less growth of axillary buds (Plate 6). Of the three methods of developing colchiploids in the present investigation, T_3 was found the best to develop the highest percentage of normal colchiploid seedling. So it was suggested to treat the dome shaped shoot apex of the seedlings at cotyledonary stage with 0.2% aquous solution of colchicine soaked in cotton for 12 hors continously at night followed by morning time (6 pm to 6 am) to develop colchiploids in pointed gourd.

Consequences of colchicine application after 30 days of treatment has been presented in Table 16. Most of the colchicine treated seedlings (94 per cent in 1997 treatment and 95 per cent in 1998 treatment) showed abnormality in growth coupled with vigorous regeneration of diploid shoots from the base which were rejected and

Table 15. Consequence of colchicine treatment to the seedlings (observation after 10 days)

Treatment	Percentage of plants showing (May-June 1997)*		
	Severe check in growth, bursting of axillary buds, chlorosis and thickening of tip	Severe tip distortion and simultaneous regeneration of shoot from the base	Somewhat normal growth, slight chlorosis and less growth of axillary buds
T_1	65	40	5
T_2	43	55	2
T_3	62	32	6
June-July 1998*			
T_1	62	35	3
T_2	51	46	3
T_3	60	36	4

T_1 : Shoot apex treatment at the cotyledonary stage with 0.2% aquous solution soaked in cotton for 12 hours in two consecutive days at day time (5.0 A. M. to 11 A. M) under dark and humid condition .

T_2 : Shoot apex treatment at the cotyledonary stage with 0.2% aquous solution soaked in cotton for 12 hours in one day at day time (5.0 AM to 5.0 P.M.) under dark and humid condition .

T_3 : Shoot apex treatment at the cotyledonary stage with 0.2% aquous solution soaked in cotton for 12 hours continuously at night followed by morning time (6 P.M. to 6 A. M.).

* 100 seedlings were employed per treatment in both the years and treatments were given in the span of one month .

Table 16. Consequence of colchicine treatment to the seedlings (observation after 30 days)

Treatment	Percentage of plants (May-June 1997 treated) showing	
	Normal appearance and growth which were transplanted in the field	Abnormality (check in growth and tip distortion) coupled with vigorous regeneration of diploid shoots from the base which were not transplanted in the field
T_1	5	95
T_2	3	97
T_3	9	91
Percentage of plants (June-July 1998 treated) showing		
T_1	4	96
T_2	4	96
T_3	6	94
Total number of expected colchiploids transplanted in the field	1997	1998
	17	14

not transplanted in the field for further study. Very few colchiploids (17 per cent from 1997 treatment and 14 per cent from 1998 treatment) showed normal appearence in growth which were transplanted in the field for further study.

4.4.2 Growing and study of the colchiploids : Of the total 31 normal colchiploids developed during 1997 and 1998 treatment, six subsequently died after transplanting. The following studies were carried out on the 25 colchiploid plants which were subsequently propagated vegetatively using vine cutting from each seedling.

a) Morphological and reproductive features : Data on different morphological and reproductive characters of the colchiploids and their comparisons with respect to the mean data on the same characters of the diploid clones have been presented in Table 17. The colchiploids were very slow in growth and much reduced in size (Mean vine length 115.95 cm as compared to 178.38 cm of the diploids), having less internode length (3.84 cm compared to 5.49 cm in diploids), marginally higher primary branches/plant (4.04 as against 3.96 in the diploids), less leaves/plant (26.68 as against 31.92 in the diploids), less leaf length (5.16 cm as against 5.85 cm in the diploids) and higher leaf width (6.26 cm compared to 6.07 cm in the diploids).

Pointed gourd is a dioecious crop and strong male determining role of Y chromosome was evident (Seshadri, 1986). For this reason there is always probability of getting 50 per cent male seedlings from the seeds of the natural settings. Of the 25 colchiploids, 23 were male and rest 2 were female. The colchiploids were highly shy flowering and mean node to first flower (male and female flower in respective colchiploids) was 22.36 compared to 11.41 node to first flower (female flower in the female clone) in the diploid clones. Two female colchiploids produced

Photo 8 Consequence of colchicine treatment :
Severe check in growth, bursting of axillary buds (excised), chlorosis and thickening of tip.

Photo 9 Aborted female flower of female colchiploid

Normal female flower of diploid

Table 17. Morphological and reproductive features of the 25 colchiploids

Colchiploids	Vine length (cm)	Internode length (cm)	Primary branches /plant	Leaves/plant	Leaf length (cm)	Leaf width (cm)	Node to first flower	Fruits/plant
Col –1	135.3	4.1	4	29	4.92	5.84	22	Male
Col –2	119.6	3.8	3	28	5.27	5.75	24	Male
Col –3	65.7	3.1	3	22	4.34	5.66	19	Male
Col –4	142.8	4.2	4	32	5.46	5.87	26	Male
Col –5	50.3	2.3	3	22	3.91	5.18	18	Male
Col –6	53.7	2.6	3	22	3.81	4.86	16	Male
Col –7	115.3	4.5	4	24	5.35	6.15	18	Male
Col –8	99.2	4.1	5	23	5.16	6.22	18	Male
Col –9	97.4	3.5	4	25	4.82	5.64	21	Male
Col –10	128.3	3.8	4	30	5.44	6.33	26	Male
Col –11	79.4	3.6	3	21	3.68	4.97	14	Male
Col –12	154.2	4.2	5	34	4.32	5.65	22	Male
Col –13	162.8	4.3	5	34	5.26	36.36	24	Male
Col –14	108.5	4.2	4	24	5.52	6.77	19	Male
Col –15	104.4	3.7	4	25	5.36	6.83	21	Male
Col –16	125.3	3.6	5	32	5.68	7.16	25	Male
Col –17	104.5	3.4	5	28	4.94	6.23	22	Male
Col –18	133.6	4.2	4	29	4.75	5.65	24	Male
Col –19	145.2	4.3	4	31	5.17	5.93	19	Male
Col –20	88.3	3.3	3	25	5.43	6.24	23	Male
Col –21	102.8	4.2	3	23	4.26	5.75	16	Male
Col –22	78.4	2.8	3	29	5.53	6.43	24	Male
Col –23	122.5	3.5	4	31	5.42	6.35	22	Male
Col –24	162.5	4.3	5	34	5.65	7.36	28	Female no set[1]
Col –25	148.7	4.4	4	32	5.38	6.52	27	Female no set[1]
Mean colchiploids*	**115.95**	**3.84**	**4.04**	**28.68**	**5.16**	**6.26**	**22.36**	**Nil**
Mean diploids**	**178.38**	**5.49**	**3.96**	**31.92**	**5.85**	**6.07**	**11.41**	**29.55**

* The colchiploid seedlings of 1997 and 1998 were grown in the field till October months as single vine without allowing any primary branches and shoot regenerated from the base. In October 1997 and 1998, the cholchiploids were propagated by single vine cutting. So the features were taken from 1997 – 98 and 1998 – 99 growth of the vegetatively propagated cholchiploids.

** Grand mean from 68 clones of 1997 – 98 and 1998 – 99 field trials.

1. Only two female cholchiploids produced very few aborted flowers which did not set fruits following hand pollination with the pollens from male cholchiploids as well as normal diploids.

very few aborted female flowers (Plate 9) which did not set fruit following hand pollination with the pollens from male colchiploids as well as normal diploids.

b) Stomatal features : Different stomatal features of the colchiploids namely frequency/mm^2, length and width and the significance of their mean difference with respect to those of the diploid clones have been presented in Table 18. Mean stomatal frequency of both the leaf surfaces of the colchiploids were significantly less than the diploid clones. In the upper leaf surface, mean stomatal frequency/mm^2 in the colchiploids was 12.04 as against 22.15 in the diploids. Similarly in the lower leaf surface, mean stomatal frequency/mm^2 was 30.29 while that of the diploids was 64.48. It was interesting to note that revealation of stomatal frequency/mm^2 in both the leaf surfaces of colchiploids was almost half of that was found in the diploid. The effect of ploidy on stomatal frequency has been documented in many crops where stomatal frequency in the higher ploidy plants tended to be much low than that of the diploid species (Dunstone *et al.*, 1973; Tan and Dunn, 1975; Lea *et al.*, 1977; Chandler and Lyrene, 1982). The findings of the investigations agreed well to the earlier studies and suggested the use of stomatal fequency as a ploidy determining charcter of pointed gourd.

Stomatal dimensions (length and width) of the colchiploids were significantly higher than those of the diploid clones. Mean stomatal length of the upper and lower leaf surfaces of the colchiploids were 30.69 and 21.24 micron respectively as compared to 18.41 and 17.36 micron respectively in the diploid clones. Mean stomatal width of the upper and lower leaf-surfaces of the colchiploids were 17.38 and 15.76 micron, respectively as against 12.89 and 11.17 micron respectively in the diploid clones. Stomatal dimensions particularly stomatal length

have been commonly used as an alternative method for determination of ploidy in plants, and increase in stomatal length with doubled ploidy level were reported in many crops (Butterfass, 1973; Borrino and Powel, 1988; Bingham, 1968; Setter *et al.*, 1978). Findings of the present investigations in agreement with earlier reports clearly sugested the use of stomatal dimensions as a method for determination of ploidy in pointed gourd.

c) Pollen features : The male flowers in the male colchiploids were deformed in shape (Plate 10) and longer than the normal male flowers with spreading petals of the diploid male clones (Plate 11). Pollen production in the male flowers of the colchiploids was much less than what was found in the diploid male clones. Pollen viability was determined by the application of acetocarmine.

i) **Viable pollen percentage/mm^2 :** Mean viable pollen percentage in the diploid clones was significantly higher (94.3%) than that of the male colchiploids (58.83%). Range of viable pollen percentage in the diploid clones was much lesser then the range found in the male colchiploids (Table 19). This findings suggested imbalanced meiosis in the higher ploidy level plants which resulted the production such low percentage of viable pollens in the male colchiploids. Such decreased pollen viability per unit area may be utilized in determining the induced higher ploidy level in the plants.

ii) **Pollen diameter :** Mean pollen diameter in the diploid male clones was significantly less (56.33 micron) than the average pollen diameter of the male colchiploids in the present study (60.06 micron). So increased pollen diameter may be regarded as an effective alternative in determining the higher ploidy levels in

Photo 10 Deformed male flower of male colchiploid

Photo 11 Normal male flower with spreading petal of male diploid

Table 18. Features of stomatal frequency and dimensions in the diploid clones and colchiploids.

Genotypes of the ploidy level	Stomatal frequency/mm^2		Stomatal length (micron)		Stomatal width (micron)	
	Upper leaf surface	Lower leaf surface	Upper leaf surface	Lower leaf surface	Upper leaf surface	Lower leaf surface
Diploid clone[1]						
Mean	22.15	64.48	18.41	17.36	12.89	11.17
Range	11.86-43.13	32.72-118.84	16.72-24.74	14.28-21.92	10.16-18.48	8.62-14.78
Standard deviation	8.75	23.43	2.71	2.83	3.01	1.78
Colchiploids[2]						
Mean	12.04	30.39	30.69	21.24	17.38	15.76
Range	9.52-16.22	24.92-36.17	25.61-37.56	17.52-25.17	14.18-20.32	12.78-17.76
Standard deviation	2.06	4.18	3.44	2.36	1.73	1.45
Significance of mean difference[3]	** (12.22)	** (22.75)	** (17.96)	** (5.56)	** (7.89)	** (8.56)

1. **Data from 20 random diploid clones (five random clones from 4 groups).**
2. **Data from 25 colchiploid seedlings of both 1997 and 1998 plantings.**
3. **Significance of mean difference was determined by 't' test (actual 't' values in parenthesis).**

** **Significant at P = 0.01.**

Table 19. Features of pollen characters in the male diploids and male colchiploids

Genotypes of the ploidy level	Viable pollen percentage /mm^2	Pollen diameter (micron)
Diploid clones[1]		
Mean	94.30	56.33
Range	89.16-98.22	45.30-62.56
Standard deviation	2.79	4.61
Colchiploids[2]		
Mean	58.83	60.06
Range	42.28-76.81	50.16-75.21
Standard deviation	11.16	7.72
Significance of mean difference[3]	** (42.53)	** (6.75)

1. **Data from 10 random diploid male clones in the field.**
2. **Data from 23 male colchiploids.**
3. **Significance of mean difference (actual 't' in parenthesis).**

** **Significant at P = 0.01.**

pointed gourd as suggested by Ho *et al.*, (1990) and Bamberg and Hanneman Jr. (1991) in other crops. Higher range in pollen diameter in the male colchiploids (50.16-75.21 micron) also suggested the production of pollens having different ploidy level in the colchiploids (Table 19).

From the present investigations on induced polyploids in pointed gourd, it appeared that application of such alteration of ploidy as a breeding method did not hold promise in improvement of pointed gourd due to the following reasons -

1) Pointed gourd is basically a perennial and vegetatively propagated vine crop which leads to natural regeneration of the shoots from the fleshy root as well as base of the plant in higher propensities. This regeneration of the shoots from the base is triggered due to check in apical dominance following colchicine treatment.

2) Development of very low per cent age colchiploids capable of normal growth following the present method of colchicine treatment. Overwhelming growth of diploid shoots from the base of the colchiploid virtually stagnates the growth of the tetraploid vine unless such diploid shoot are excised at regular intervals to allow the growth of a colchiploids as single vine.

3. As a matter of probability 50 per cent colchicine treated seedlings would be male, though much excess was recorded in the present study. Male colchiploids is generally of no use in breeding pointed gourd.

4. Infertility of the female colchiploid ultimately suggested such alteration of ploidy to be an ineffective method of pointed gourd improvement.

CHAPTER - V

SUMMARY AND CONCLUSION

Summary and Conclusion

Different experimental approaches under the broad area of investigation : **clonal selection and polyploidization** were carried out under the present research programme. Results are being summarised under respective headings.

Grouping and characterization of the female clones : Such studies were carried out from different angles as mentioned below :

Differences of means for the characters between the groups : The 68 female clonal assemblage in the present study fell under four groups based an fruit shape and size.

Group 1 : Plants bearing small sized fruits, fruit-shapes were mostly oval and tapering, very small spindle-shaped fruit bearing clones were also kept in this group.

Group 2 : Plants bearing spindle-shaped fruits.

Group 3 : Plants bearing oval-shaped fruits.

Group 4 : Plants bearing near cylindrical fruits.

In all the four group ranges for the 17 characters were very high revealing marked intragroup variation. However, clones of the Group 1 and those of Group 4 exhibited most conspicuous distinctness between themselves. Distinctness of Group 2 and 4 based on such grouping of clones has been questioned due to revealation of no significant mean difference between them for any of the fruit characters.

Multivariate analysis using Mahalanobis's D^2-statistic : Quantification of the degree of divergence between the 68 clonal populations employing 19 characters revealed 16 clusters. Cluster 1, the largest one contained 18 clones however, most of the clusters contained few (1-3) clones. In most of the cases, fruit shape and size

did not interfere clustering pattern through multivariate analysis. However, some clones having the same fruit shape and size clustered together which indicated some implications of the earlier grouping based on fruit shape and size.

Medium and consistent level of intra and inter cluster divergence might have been caused due to mutual balancing of the characters during prolonged cropping duration.

Isozyme pattern for the characterization of the diversity : Such study exhibited a total of 8 bands of peroxidases having almost similar relative mobility values in all the clones belonging to four groups under study. Band 1, 2, 3, 7 and 8 were conspicuous in all the groups. The other three bands of peroxidases (4, 5 and 6) though present in all the clonal groups, yet showed some variations with regard to the intensity of the bands.

Petiole anatomy : Arrangement of tissues in the transverse section of petiole from the peripheri to centre were : epidermis, cortex (collenchymatous hypodermis and internal parenchyma) and stele (bundle cap and bicolateral vascular bundles). Highest mean epidermal cell length was recorded in the clones of Group 2 (spindle shaped) which produced the highest fruit length. Number of vascular bundles was almost same in all the groups, as well as arrangement which was bicolateral in all the clones. Mean dimension of the vascular bundles was markedly high in the clones of low yielding group (Group 1).

Stomatal features : Stomata of the leaves of pointed gourd appeared to be anomocytic type. Stomatal frequency was much higher in the lower leaf surface than the upper leaf surface of the leaves. The stomatal characters viz., stomatal frequency/mm^2, stomatal length and stomatal width in both the leaf surfaces presented wide interclonal and intergroup

variations which indicated the possibility of using the stomatal characters as selection indices. Clones of Group 1 appeared as distinct group with the revealation of highest stomatal frequency, length and width in both the leaf surface.

Genetic variability for different characters

The study through the coefficient of variations, heritability in broad sense and genetic advance as percentage of mean suggested the following reliable characters for effective clonal selection viz, vine length, internode length, primary branches/plant, node at first flower, leaves/plant, leaf dry weight, leaf area, fruit length and fruit yield/plant. Expression of these characters was presumed to have been controlled by additive action of the polygenes.

Character associationship

Most of the 19 characters did register either insignificant or negligible correlations among themselves. Fruit yield/plant was correlated positively and appreciably with fruit volume, fruit weight and pulp content of the fruit, but only fruit number/plant registered significant positive correlation with fruit yield.

The path coefficient analysis using the phenotypic correlation coefficients showed that fruit weight had the highest positive direct effect on yield followed by pulp content of the fruit, fruit volume and leaves/plant.

From the studies of correlations and path analysis, the characters viz., leaves/plant, leaf length, fruit number/plant, fruit weight, pulp content of fruit and fruit volume emerged as the most important fruit yield attributing character of pointed gourd.

Indentification of the promising clones

The promising, high yielding and genetically diverse clones which may be utilized as clonally selected varities were BC-SP-10 (cluster 11) and BC-O-12 and BC-SP-9 of cluster 15.

Application of induced polyploidy

Treatment of the dome shaped shoot apex of the 3-5 days old seedlings at the cotyledonary stage with 0.2% aquous solution of colchicine soaked in cotton for 12 hours continuously at night followed by the morning time (6pm - 6am) to develop colchiploids in pointed gourd was found the best. Very few colchiploids showed normal appearence in growth which were transplanted in the field for following further studies.

a) **Morphological and reproductive features :** The colchiploids were very slow in growth and much reduced in size having less internode length, marginally higher primary branches/plant, less leaves/plant and less leaf length. Of the 25 colchiploids under study, 23 were male and 2 female. The colchiploids were very shy flowering in nature. The two female colchiploids produced very few aborted female flowers which did not set fruit following hand pollination.

b) **Stomatal features :** Mean stomatal frequency of both the leaf surfaces of the colchiploids were significantly less than the diploid clones. On the other hand, stomatal dimensions of the colchiploids were significantly higher than those of the diploid clones.

c) **Pollen features :** Pollen production in the male flowers of the colchiploids was much less than what was produced by the diploid male clones. Pollen viability percentage of the diploid clones was significantly higher than that of the male colchiploids. Mean pollen diameter in the diploid male clones was significantly less than the average pollen diameter of the male colchiploids.

It appeared from the present investigation that alteration of ploidy as a breeding method did not hold promise in the improvement of pointed gourd.

CHAPTER - VI

FUTURE SCOPE OF RESEARCH

Future scope of research

1. Initiation of clonal selection based on the identified growth and reproductive characters with the inclusion of tolerance to *Phytophthora* blight.

2. Study on the stomatal characters in view of Photosynthetic rate per unit area, dry matter partitioning and fruit yield.

3. Study on the vascular bundles of the petioles or stem in the light of source-sink relationships.

4. Study on the induction of hermaphrodite flowers in the female clones with the application of growth substances and other chemicals with a view to developing high yielding homosexual hybrids.

5. Investigations on the extent of natural parthenocarpy and at the same time, induced parthenocarpy with the pollen stimulus of other cucurbits.

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APPENDIX

Appendix I. Meteorological data* of the experimental period

Year / Month	Temperature ^{0}C		Rainfall (mm)	Relative Humidity (%)	
	Max	Min		Max	Min
1996					
Oct	31.5	22.4	133.9	95.45	62.4
Nov.	30.5	16.9	5.7	96.03	49.23
Dec	26.0	11.4	0.00	94.64	44.03
1997					
Jan	24.38	10.71	24.3	95.19	47.45
Feb	27.0	15.01	61.7	96.0	46.7
March	32.7	21.2	111.3	95.8	49.0
April	31.4	21.1	59.2	82.3	61.1
May	35.1	24.5	184.3	93	56.52
June	35.16	25.5	97.5	94	67
July	35.2	25.3	399.8	98	89
August	32.6	25.5	293.5	97	73
September	32.8	25.2	246.3	98	78
Oct	31.19	21.7	19.1	97.1	61.4
Nov	30.4	18.5	2.2	93.9	56.1
Dec	24.4	13.1	9.6	97.6	60.6
1998					
Jan	22.8	11.2	89.9	98.3	61.1
Feb	27.9	15.9	39.2	96.8	52.7
March	0.4	17.8	199.6	95.03	49.2
April	30.03	23.22	76.9	94.4	62.0
May	35.6	25.1	61.5	89.96	62.4
June	36.2	27.02	119.2	92.87	66.22
July	33.7	24.5	190.8	94.54	77.03
August	33.2	26.5	217.3	96.29	79.67
September	32.7	25.5	273.4	97.56	79.30
Oct	33.4	24.4	133.1	97.06	69.61
Nov	30.0	19.9	156.4	98.26	68.10
Dec	27.3	12.2	0.00	99.80	53.51
1999					
Jan	25.9	9.9	0.00	99	47.5
Feb	30.0	14.5	0.00	98.3	43.7
March	34.6	20.01	6.2	93.9	36.9
April	37.3	25.5	1.4	93.1	49.1
May	34.98	25.31	158.0	91.77	62.33
June	34.22	25.79	277.5	93.53	74.53
July	32.52	26.02	635.1	97.12	82.16
August	32.28	25.95	251.3	97.61	79.54
September	31.50	25.27	353.2	98.30	84.75
Oct	32.07	24.06	414.9	99.39	73.07

*** Average monthly mean.**

Appendix II. Mean performance of the 68 female clones *

Sl.No	Clones	Vine length (cm)	Internode length (cm)	Primary branches/ plant	Node at first flower	Leaves per plant	Leaf length (cm)	Leaf width (cm)	Leaf dry weight (g)	Leaf area (cm^2)
1	BC-S-1	93.27	4.77	2.83	7.80	18.77	5.97	7.10	0.20	53.13
2	BC-S-2	110.33	3.80	4.13	13.00	26.17	5.40	6.10	0.19	36.17
3	BC-S-3	200.47	5.47	4.00	12.00	33.70	6.20	6.00	0.30	43.23
4	BC-S-4	103.77	4.30	3.10	12.97	36.07	6.43	6.07	0.39	42.60
5	BC-S-5	185.43	5.37	6.07	18.67	33.33	6.33	6.20	0.11	42.83
6	BC-S-6	277.10	3.43	3.90	14.93	50.67	5.50	5.63	0.29	30.17
7	BC-S-7	146.97	4.30	3.97	10.67	28.93	5.03	5.30	0.20	31.60
8	BC-S-8	293.40	5.67	4.00	12.97	43.83	6.03	6.20	0.12	35.80
9	BC-S-9	141.80	7.15	5.00	11.87	25.97	6.13	6.13	0.18	45.43
10	BC-S-10	151.27	5.73	2.87	9.60	29.37	5.27	6.20	0.15	29.70
11	BC-S-11	177.03	7.03	2.77	14.07	41.50	6.17	5.33	0.12	42.77
12	BC-S-12	165.17	5.00	3.73	9.93	29.47	5.50	6.70	0.13	34.10
13	BC-S-13	252.13	5.77	313	10.97	39.37	5.40	6.70	0.20	25.40
14	BC-S-14	155.10	5.47	3.00	8.43	37.93	4.63	6.30	0.21	25.30
15	BC-S-15	200.10	6.43	3.33	9.27	30.90	4.40	4.97	0.20	24.97
16	BC-SP1	295.37	4.87	4.43	18.87	41.93	5.83	6.40	0.16	49.87
17	BC-SP2	143.80	6.00	3.00	13.60	30.53	5.43	5.40	0.23	36.07
18	BC-SP3	176.83	7.00	4.03	12.10	30.17	6.03	7.17	0.09	58.50
19	BC-SP4	118.43	3.63	2.83	7.03	20.57	5.57	5.70	0.12	33.20
20	BC-SP5	158.40	8.47	5.03	15.60	29.30	6.70	6.33	0.18	55.07
21	BC-SP6	102.33	6.07	5.43	9.00	25..57	5.13	5.43	0.23	36.10
22	BC-SP7	318.63	5.03	4.40	12.47	45.30	5.60	7.63	0.23	62.23
23	BC-SP8	132.13	4.93	4.90	9.77	25.80	5.13	4.83	0.16	28.20
24	BC-SP9	258.93	8.17	5.90	7.97	29.30	6.43	6.23	0.18	35.93
25	BC-SP10	165.43	4.00	2.67	14.80	33.03	5.33	5.70	0.12	31.87
26	BC-SP11	153.77	6.97	5.80	11.07	35.27	5.50	5.33	0.29	36.00
27	BC-SP12	195.47	5.73	4.80	9.97	29.63	5.20	5.13	0.13	31.83
28	BC-SP13	162.27	5.07	4.97	13.97	34.93	5.50	5.43	0.10	40.00
29	BC-SP14	185.63	4.80	2.80	6.10	24.20	5.37	5.57	0.22	35.10
30	BC-SP15	277.07	5.83	4.70	9.97	38.00	5.40	5.63	0.11	48.30
31	BC-SP16	198.67	6.37	4.87	11.93	26.87	5.17	5.17	0.20	37.90
32	BC-SP17	154.37	2.73	3.90	13.93	39.10	5.33	6.13	0.11	49.57
33	BC-SP18	190.17	6.93	3.03	18.97	37.80	6.30	7.13	0.30	61.10
34	BC-SP19	100.87	4.97	2.87	11.77	21.40	5.53	6.07	0.11	50.00
35	BC-SP20	97.07	5.03	2.97	8.30	19.50	5.17	7.10	0.09	44.63
36	BC-SP21	95.17	7.17	2.40	6.53	16.00	5.17	7.10	0.14	59.07
37	BC-SP22	105.27	3.80	4.00	12.37	29.03	6.50	6.23	0.37	42.60
38	BC-SP23	295.60	3.43	3.47	19.30	59.50	5.47	5.70	0.31	30.40
39	BC-O-1	91.00	2.97	3.30	12.23	28.40	5.10	5.30	0.31	46.27
40	BC-O-2	160.90	6.13	3.87	9.17	31.27	6.23	5.37	0.15	43.57

41	BC-O-3	220.63	6.07	3.90	16.90	35.13	5.83	5.37	0.22	34.80
42	BC-O-4	162.77	8.07	6.17	19.00	32.93	5.50	6.33	0.14	45.13
43	BC-O-5	115.53	5.13	4.07	18.07	30.77	6.00	6.93	0.20	49.53
44	BC-O-6	160.37	6.27	5.23	8.13	34.90	6.07	7.10	0.12	52.90
45	BC-O-7	148.57	5.17	4.70	12.00	27.45	4.77	4.90	0.30	28.47
46	BC-O-8	314.03	7.07	3.17	13.07	55.70	5.27	5.63	0.11	36.00
47	BC-O-9	135.37	4.90	4.03	8.97	24.97	6.33	7.30	0.18	54.83
48	BC-O-10	130.47	5.20	3.93	16.07	27.63	7.23	7.67	0.28	68.43
49	BC-O-11	120.47	3.90	3.17	9.13	30.90	7.47	6.80	0.17	61.37
50	BC-O-12	293.40	5.80	2.80	17.13	47.97	5.50	5.97	0.18	38.17
51	BC-O-13	140.67	6.00	5.93	14.00	25.27	7.0	7.13	0.31	59.30
52	BC-NC-1	115.13	5.47	8.07	3.67	31.37	5.73	6.20	0.26	41.90
53	BC-NC-2	203.73	5.93	5.93	9.10	35.30	6.30	7.17	0.30	47.80
54	BC-NC-3	136.43	4.77	4.53	20.53	31.63	6.20	6.47	0.30	44.97
55	BC-NC-4	141.77	5.20	4.90	9.23	23.40	8.70	8.93	0.39	60.97
56	BC-NC-5	208.47	9.00	3.53	9.33	28.30	5.50	5.37	0.19	35.27
57	BC-NC-6	138.73	4.97	3.97	9.73	28.97	5.93	6.03	0.25	32.83
58	BC-NC-7	150.10	5.43	5.00	10.27	30.13	7.13	6.23	0.24	42.70
59	BC-NC-8	107.10	4.80	4.70	8.90	19.43	6.10	6.37	0.25	40.33
60	BC-NC-9	102.57	5.00	5.97	12.97	21.30	7.10	6.37	0.10	47.07
61	BC-NC-10	203.23	5.97	5.03	9.07	29.10	6.17	7.33	0.39	49.37
62	BC-NC-11	106.83	4.80	5.00	9.00	15.73	4.87	5.23	0.39	51.33
63	BC-NC-12	210.33	6.63	3.73	9.97	28.70	5.23	6.17	0.12	39.13
64	BC-NC-13	110.20	7.00	4.00	5.00	28.77	5.73	6.10	0.16	46.40
65	BC-NC-14	90.60	3.87	3.00	9.17	15.97	7.10	7.17	0.17	49.87
66	BC-NC-15	140.23	3.97	3.17	8.10	30.70	6.27	5.23	0.22	42.47
67	BC-NC-16	135.33	5.00	6.00	10.00	21.00	5.27	6.20	0.26	39.70
68	BC-NC-17	190.23	6.97	4.00	9.00	35.70	4.93	4.93	0.15	25.87

Continued.....

Sl. No	Clones	Fruit length (cm)	Fruit girth (cm)	Fruit volume (cc)	Fruit weight (g)	Pulp content (g)	Pericarp Thickness (cm)	Seeds / fruit	Protein content (g)	Fruits per plant	Fruit yield /plant (g)
1	BC-S-1	5.77	9.20	29.47	22.93	8.60	0.38	9.57	1.80	17.00	389.77
2	BC-S-2	4.33	8.13	29.90	20.77	9.57	0.30	10.17	1.83	26.57	551.67
3	BC-S-3	5.50	8.30	21.37	17.07	6.80	0.39	7.00	1.60	32.73	558.80
4	BC-S-4	4.97	8.63	35.43	32.33	9.57	0.49	8.10	1.83	26.47	856.13
5	BC-S-5	6.13	8.27	29.60	24.97	9.45	0.40	9.43	1..73	25.47	635.80
6	BC-S-6	6.17	8.37	30.93	29.87	9.77	0.41	11.33	1.83	37.27	1146.60
7	BC-S-7	5.17	7.93	20.40	14.73	6.10	0.40	7.07	1.87	42.17	621.70
8	BC-S-8	5.37	8.10	20.87	18.73	5.73	0.30	8.47	1.77	30.37	568.00
9	BC-S-9	5.27	9.27	20.37	14.63	7.57	0.39	10.27	1.77	41.90	613.47
10	BC-S-10	5.67	9.43	30.10	28.70	10.10	0.38	8.53	1.83	27.27	783.33
11	BC-S-11	6.10	7.27	20.60	14.53	4.93	0.29	8.37	1.83	21.47	312.87
12	BC-S-12	5.07	8.30	25.43	19.00	5.97	0.40	9.07	1.73	24.90	507.37
13	BC-S-13	6.03	8.93	24.33	25.60	7.73	0.49	9.13	1.80	56.63	1450.43
14	BC-S-14	6.10	8.30	25.40	25.57	7.67	0.29	8.87	1.77	51.57	1319.90
15	BC-S-15	5.40	8.27	25.57	20.43	7.07	0.30	9.33	1.83	46.87	958.63
16	BC-SP1	5.03	9.87	36.87	29.60	9.97	0.30	15.30	1.97	29.03	859.73
17	BC-SP2	8.90	10.20	35.67	30.30	8.53	0.39	9.50	1.87	32.07	1034.30
18	BC-SP3	8.70	10.57	25.87	29.57	7.23	0.29	13.17	1.83	34.33	715.47
19	BC-SP4	9.40	9.93	36.10	24.40	9.30	0.30	12.40	1.83	24.17	814.37
20	BC-SP5	8.83	9.77	35.00	24.50	12.33	0.40	12.37	1.83	32.80	925.03
21	BC-SP6	8.70	9.87	31.87	30.33	9.43	0.29	16.40	1.87	37.70	1045.47
22	BC-SP7	8.83	10.23	38.83	31.40	8.23	0.40	12.27	1.77	34.13	1801.63
23	BC-SP8	8.23	9.97	30.90	25.47	11.60	0.21	13.17	1.87	57.30	1067.50
24	BC-SP9	10.70	10.47	60.50	39.23	13.30	0.42	20.43	1.83	41.87	1645..03
25	BC-SP10	9.13	8.67	44.90	37.27	13.13	0.39	12.93	1.77	33.47	1441.91
26	BC-SP11	7.67	9.53	27.67	18.43	7.50	0.40	10.83	1.83	37.63	672.63
27	BC-SP12	7.73	9.03	31.07	24.30	8.30	0.40	19.17	1.83	36.43	521.67
28	BC-SP13	10.27	11.13	40.27	28.40	9.20	0.40	18.17	1.93	21.40	952.90
29	BC-SP14	8.43	10.30	41.83	33.30	10.20	0.39	18.47	1.87	19.47	615.77
30	BC-SP15	8.13	10.43	31.67	24.23	9.30	0.37	14.10	1.85	20.10	551.93
31	BC-SP16	8.40	10.33	48.93	35.63	11.07	0.31	16.93	1.83	25.60	914.00
32	BC-SP17	7.73	10.57	36.53	24.90	9.03	0.39	16.23	1.77	26.97	673.13
33	BC-SP18	8.47	10.70	39.73	24.90	11.33	0.49	8.30	1.77	16.53	467.93
34	BC-SP19	8.53	10.43	25.33	24.40	10.53	0.39	9.60	1.73	18.17	408.33
35	BC-SP20	9.30	10.60	28.27	24.53	10.27	0.39	9.47	1.77	33.70	827.70
36	BC-SP21	7.80	10.00	30.93	21.50	10.10	0.37	9.17	1.80	19.43	418.03
37	BC-SP22	8.20	10.97	38.97	33.23	11.20	0.80	14.83	1.77	18.80	625.67
38	BC-SP23	8.33	11.20	41.23	31.20	11.20	0.46	14.53	1.77	20.13	628.4
39	BC-O-1	7.03	9.37	27.07	25.33	10.10	0.40	18.67	1.93	39.92	1013.40
40	BC-O-2	8.00	10.17	31.20	29.27	10.17	0.40	14.93	1.77	11.53	336.93
41	BC-O-3	7.67	10.43	36.07	31.30	11.17	0.51	9.43	1.90	24.47	765.93
42	BC-O-4	7.30	10.73	29.47	24.10	7.10	0.40	9.60	1.77	34.63	835.97
43	BC-O-5	6.80	10.33	25.37	23.27	10.07	0.30	13.60	1.73	18.03	419.93
44	BC-O-6	7.17	10.27	26.30	23.17	10.30	0.40	9.50	1.77	21.73	504.03
45	BC-O-7	8.83	10.45	27.27	25.00	11.00	0.39	11.17	1.97	26.37	659.90
46	BC-O-8	7.30	10.40	37.27	31.43	13.10	0.49	18.20	1.90	32.07	1008.97
47	BC-O-9	7.00	10.83	50.40	38.00	12.10	0.59	13.23	1.97	13.20	502.33

48	BC-O-10	7.83	9.97	50.17	36.00	11.03	0.49	11.33	1.87	19.90	717.07
49	BC-O-11	7.87	11.80	60.20	46.60	13.00	0.50	13.30	1.87	17.33	807.90
50	BC-O-12	7.20	10.80	36.10	29.07	9.93	0.48	11.40	1.83	51.40	1495.37
51	BC-O-13	9.03	10.70	41.07	41.03	12.33	0.49	15.57	1.97	31.97	1310.50
52	BC-NC-1	6.63	9.37	26.43	24.47	10.47	0.39	8.53	1.87	17.27	423.37
53	BC-NC-2	6.00	9.40	22.30	20.17	8.30	0.49	9.93	1.87	12.73	254.17
54	BC-NC-3	8.37	10.57	31.27	29.53	10.43	0.39	9.20	1.97	21.13	625.33
55	BC-NC-4	7.07	9.37	34.40	31.20	10.47	0.61	12.30	1.90	17.90	557.53
56	BC-NC-5	6.87	9.47	46.33	41.10	10.80	0.61	17.03	1.93	29.10	1199.77
57	BC-NC-6	6.30	8.67	31.33	29.33	8.70	0.48	10.27	1.77	15.87	465.40
58	BC-NC-7	7.17	10.07	31.33	29.23	8.70	0.59	13.03	1.77	20.17	590.37
59	BC-NC-8	6.20	8.40	30.10	27.20	7.80	0.69	11.70	1.80	24.47	667.70
60	BC-NC-9	7.00	8.80	29.67	26.23	8.27	0.67	10.13	1.75	34.40	804.07
61	BC-NC-10	6.03	7.83	29.73	27.33	10.07	0.39	8.27	1.93	18.20	704.50
62	BC-NC-11	6.03	8.73	36.10	35.67	7.07	0.50	10.25	1.93	11.80	341.70
63	BC-NC-12	7.07	8.17	30.90	29.40	7.20	0.49	10.23	1.97	236.53	780.43
64	BC-NC-13	5.83	9.00	31.03	30.43	7.27	0.50	8.43	1.97	20.40	621.37
65	BC-NC-14	5.80	9.17	34.97	30.80	10.00	0.50	12.07	1.87	14.47	446.27
66	BC-NC-15	6.53	9.50	30.07	29.30	8.07	0.49	10.07	1.77	29.27	857.63
67	BC-NC-16	6.83	9.80	29.73	29.17	8.73	0.40	11.50	1.73	27.90	815.47
68	BC-NC-17	6.93	9.07	25.70	24.95	7.93	0.38	35.97	1.77	41.07	1027.43

- **Average performance of 1997-98 and 1998-99 field trials.**

Notations associated with the name of the clones :

S : Clones bearing small sized fruits, SP : Clones bearing spindle shaped fruits, O : Clones bearing oval shaped fruits, NC : Clones bearing near cylindrical fruits.