CHARACTERIZATION OF LANDRACES OF ASHGOURD
(*Benincasa hispida* (Thunb.) Cogn.)

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Thesis submitted in partial fulfilment of the requirement
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Dedicated
to
my beloved mother

DECLARATION

I hereby declare that this thesis entitled "**Characterization of landraces of ashgourd (*Benincasa hispida* (Thunb.) Cogn.)**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled "**Characterization of landraces of ashgourd (*Benincasa hispida* (Thunb.) Cogn.)**" is a record of research work done independently by Ms. Resmi. J. (2002-12-20) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Resmi

Resmi. J.

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LIST OF ABBREVIATIONS

%	–	Per cent
µ	–	Microlitre
°C	–	Degree Celsius
°E	–	Degree East
µg	–	Micro gram
µl	–	Micro litre
µM	–	Micromolar
°N	–	Degree North
AFLP	–	Amplified fragment length polymorphic DNA
bp	–	Base pair
CD	–	Critical difference
cm	–	Centimetre
d.f.	–	Degrees of freedom
DNA	–	Deoxy ribonucleic acid
dNTPs	–	Deoxy nucleotides
EDTA	–	Ethylene diamino tetra acetic acid disodium salt
et al.	–	And others
Fig.	–	Figure
g	–	Gram
GA	–	Genetic advance
GCV	–	Genotypic coefficient of variation
i.e.	–	That is
kg	–	kilogram
mM	–	Millimolar
N	–	Normality
ng	–	Nanogram
nm	–	Nanometer
No.	–	Number
NS	–	Not significant
PCA	–	Principal co-ordinate analysis
PCR	–	Polymerase chain reaction
PCV	–	Phenotypic coefficient of variation
PIs	–	Plant introductions
pM	–	Pico mole
RAPD	–	Random amplified polymorphic DNA
SE	–	Standard error
spp.	–	Species
SSR	–	Simple sequence repeats
Tris HCl	–	Tris (hydroxy methyl) aminomethane hydrochloride
UPGMA	–	Unweighted pair group method for arithmetic average
viz.	–	Namely

INTRODUCTION

1. INTRODUCTION

Ashgourd is a monotypic genus with only one cultivated species *Benincasa hispida* (Thunb.) Cogn. It is known by several names: waxgourd, winter melon, hairy melon, ash pumpkin, white pumpkin, whitegourd and Chinese preserving melon. The name waxgourd refers to the thick, waxy cuticle that typically develops on mature fruits. The specific epithet '*hispida*' refers to the hirsute pubescence on the foliage and immature fruits (Robinson and Decker, 1997).

Benincasa is unknown in the wild. It is probably a native of Indo-Malaysia and the cultivated forms may have originated in Southeast Asia. Ashgourd is reported as introduced to India from Japan and Java by foreign navigators and missionaries (Peter, 1998).

The fruit has very high moisture content and is low in calories and carbohydrates (Morton, 1971). It contains 0.4 per cent protein, 1.9 per cent carbohydrates, 0.3 per cent minerals and traces of vitamins A, B and C per 100 g edible portion (Saimbhi, 1993). In addition, the fruit also contains Ca, P, Na, Mg, Fe, K, S and starch in minute quantities.

Ashgourd is used in confectionary and ayurvedic medicinal preparations. The ripe fruits are peeled and cut into pieces and candied to make the well-known *petha sweet*. Young fruits are used as vegetable and are ingredients in curries. The pulp is used as adulterant or as substitute in tomato ketchup in place of tomatoes. Young leaves, vine tips and flower buds are boiled and eaten as greens. Seeds are fried and consumed. Ashgourd is considered good for people suffering from nervousness and debility (Nadkarni, 1927). The fruit is considered as tonic, nutritive and diuretic. Kushmanda lehya, an ayurvedic medicine prepared from pericarp is used for diabetes. Tender stems are found good in liver troubles and muscle pain. Seed powder is used for appendicitis and is antihelmintic. The dry fruit rind serve as containers and an elegant serving bowl for soup. The fruit wax is used to make candles and as a vehicle for carrying

poison for homicide. The petha waste in the form of pith, pulp, seeds and peeling is subjected to vermicomposting (Kumari and Bhadauria, 2002).

In India, a wide range of variability in vegetative and fruit characters is available in ashgourd (Sundararajan and Muthukrishnan, 1982; Peter *et al.*, 1991; Mandal and Sirohi, 2003). Surprisingly, this crop has not been much exploited on commercial basis in the past. Although ashgourd is becoming a crop of industrial importance, relatively less attention has been paid towards the varietal improvement of existing strains available in different parts of the country.

Characterization of varieties is generally being done based on morphological and agronomical characters. The variability that exists in the genome is likely to be detected by use of DNA based molecular markers. Random amplified polymorphic DNA (RAPD) markers based on polymerase chain reaction (PCR) is a powerful technique for determining inter and intra-specific DNA variability. The information generated through DNA profiling using RAPD not only gives a comprehensive picture on diversity and relatedness but also determines the efficacy of each marker to be used in diversity studies.

Taking into consideration of all these aspects, the present study was undertaken with the following objectives:

1. To genetically catalogue the available landraces in *B. hispida.*
2. To identify superior landraces based on yield, quality, pest and disease resistance.
3. To estimate the characters in terms of the extent of available variability, degree and pattern of association, genetic contribution in expression of each character.
4. To characterize the landraces of ashgourd through morphological traits by Mahalanobis's D^2 analysis.
5. To characterize the landraces of ashgourd by RAPD analysis.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Ashgourd is an important warm-season cucurbit vegetable, grown for its succulent hairy fruits, used in confectionary and in ayurvedic medicinal preparations (Indira and Peter, 1987). Crop improvement in ashgourd has been much less compared to other cucurbits. Hence an attempt has been made to review the available literature on various aspects in some important cucurbitaceous crops and presented under the following subheads.

2.1 Morphological characterization

2.2 Molecular characterization

2.1 MORPHOLOGICAL CHARACTERIZATION

2.1.1 Genetic Cataloguing

Genetic cataloguing based on standard descriptors helps to easily describe the morphological features of a genotype and thus helps exchange of information about new accessions.

Morton (1971) described three cultivar types in waxgourd group on the basis of fruit characters namely, fruits nearly round and essentially hairless, fruits nearly round and hairy, fruits oblong and hairy. Walters and Walters (1989) proposed four major categories as cultivar groups in *Benincasa hispida viz.*, unridged winter melon group, ridged winter melon group, fuzzy winter melon group and waxgourd group.

Singh (1989) assigned the pointedgourd plants to four groups based on morphological variations (shape, size and striations) of the fruit. Hazra *et al.* (1998) conducted grouping and characterization of 68 female clones of pointedgourd based on fruit shape and size and their clones fell under four groups.

Mathew (1996) catalogued 28 accessions of bottlegourd based on the descriptor of IBPGR and significant differences were noticed for all the vegetative and fruit characters.

In ridgegourd, Anitha (1998) catalogued 57 accessions collected from different parts of the country and significant difference was observed among them for almost all the characters studied.

2.1.2 Variability

Variability either naturally existing or created artificially forms the basis for any crop improvement programme. Many workers have reported considerable variability in different cucurbitaceous vegetables.

2.1.2.1 Plant Characters

In muskmelon, Deol *et al.* (1981) reported that vine length ranged from 76.9 to 209.3 cm, with a mean of 130.2 cm, while Swamy *et al.* (1985) reported a range between 50.0 and 279.0 cm with a mean of 168.0 cm. High phenotypic and genotypic variance was reported for vine length in pumpkin (Rana *et al.*, 1986; Borthakur and Shadeque, 1990). Low variance for vine length was reported in pointedgourd by Prasad and Singh (1991) and in watermelon by Hegde *et al.* (1994).

Considerable variation among the landraces for internodal length was observed in cucumber (Solanki and Seth, 1980) and pointedgourd (Ram *et al.*, 2001).

In muskmelon, Swamy *et al.* (1985) reported that number of primary branches ranged from 2.3 to 8.3 with a mean of 5.7, while in pumpkin, Mohanty and Mishra (1999) observed low range of variation ranging from 4.1 to 6.0 with a mean 5.13.

Joseph (1999) in ivygourd noticed high PCV and GCV for number of primary and secondary branches per plant, whereas Rakhi and Rajamony (2003) observed low values in melon.

For root-shoot ratio, high degree of phenotypic and genotypic variance was observed in snakegourd (Varghese and Rajan, 1993) and bittergourd (Thakur *et al.*, 1994).

2.1.2.2 Flowering Characters

Deol *et al.* (1981) observed wide varietal variation for days to first male and female flower production in muskmelon. Limited variability was reported in ashgourd (George, 1981) and bittergourd (Mangal *et al.*, 1981).

Priya (2001) noticed wide range of variability for node to first male and female flower in watermelon. High genotypic coefficient of variation was recorded for the character in snapmelon (Jeeva and Pappiah, 2002) and pointedgourd (Dora *et al.*, 2003).

In case of sex ratio, high variability was noticed in watermelon (Thakur and Nandpuri, 1974). High PCV and GCV were recorded for sex ratio in spongegourd (Arora *et al.*, 1983) and ridgegourd (Varalakshmi *et al.*, 1995).

2.1.2.3 Fruit Characters

Wide genetic diversity among the cultivars for yield and yield related characters were reported in bittergourd (Lawande and Patil, 1991) and cucumber (Prasad and Singh, 1994).

Days to first fruit harvest was observed to have a range of 75.0 to 96.6 days with a mean of 84.6 days in muskmelon (Swamy *et al.*, 1985). In cucumber, Rastogi and Deep (1990a) observed low phenotypic and

genotypic coefficient for the character. A high genetic variation for days to first fruit harvest was observed by Chacko (1992) in muskmelon.

Wehner and Cramer (1996) observed genetic variance for fruit shape in three slicing cucumber populations.

Great variability in fruit size was reported by several workers in ashgourd (George, 1981; Randhawa *et al.*, 1983; Hamid *et al.*, 1989; Menon, 1998; Mandal *et al.*, 2002).

Changlin (1998) obtained a range of 12.15 to 33.21 cm for fruit length in ashgourd. High GCV and PCV were observed for fruit length in snakegourd (Mathew and Khader, 1999).

For fruit girth, low genetic variation was observed by Prasad and Singh (1989) in ridgegourd, whereas Iswaraprasad (2000) recorded high genetic variation in bittergourd.

In muskmelon, Nandpuri *et al.* (1975) reported that the number of fruits ranged from 3.6 to 11.69, with a mean of 7.3, while Deol *et al.* (1981) observed between 1.3 and 4.2 with a mean of 2. Low genetic variance was observed for fruits per plant by Babu *et al.* (1986) in bittergourd, whereas Vahab (1989) in bittergourd, Miniraj *et al.* (1993) in ashgourd and Shibukumar (1995) in watermelon observed high values.

Wide variability in fruit weight was noticed in cucumber (Owens *et al.*, 1985), bittergourd (Jaiswal *et al.*, 1990) and ashgourd (Lovely, 2001).

Nandpuri *et al.* (1975) reported that yield per plant ranged from 672 to 4811 g with a general mean of 2821 g in muskmelon. A high genetic variation for fruit yield was observed in bittergourd (Katiyar *et al.*, 1996), while Babu *et al.* (1996) obtained low values in pumpkin. High PCV and GCV were reported for the character by Lovely (2001) in ashgourd.

2.1.2.4 Seed Characters

Ashok (2000) found wide variation in seed characters in snakegourd.

In snakegourd, Pynadath (1978) noticed high genetic variability for seeds per fruit. High PCV and GCV were reported for the character in watermelon by Prasad *et al.* (1988).

Gayathri (1997) reported wide range of variation for 100-seed weight in cucumber. High phenotypic and genotypic coefficients of variation were observed for the character by Priya *et al.* (2004) in watermelon.

2.1.2.5 Pest and Disease Incidence

Wide range of variation was reported for disease resistance in cucumber (Korneev, 1980). Similarly, Chacko (1992) obtained significant differences for reaction towards pest and diseases in muskmelon.

2.1.3 Heritability and Genetic Advance

Effectiveness of selection depends upon the heritability and genetic advance of the character studied. Varalakshmi *et al.* (1995) in ridgegourd and Sriramamurthy (2000) in cucumber reported high values of heritability and genetic advance for most of the characters.

In ashgourd, Parkash *et al.* (2000) reported low heritability and genetic advance for vine length. High heritability coupled with high genetic advance was observed for vine length in watermelon (Priya *et al.*, 2004).

Rastogi and Deep (1990a) recorded high heritability and moderate to low genetic advance for number of primary branches per plant in

cucumber. Menon (1998) noticed high values of heritability and genetic advance for primary branches in ashgourd.

High heritability and low genetic advance was noted for days to first male and female flower in cucumber (Choudhary and Mandal, 1987), ashgourd (Lovely, 2001) and ivygourd (Varghese, 2003).

High values of heritability and genetic advance was noticed for node to first male and female flower in snapmelon (Jeeva and Pappiah, 2002) and watermelon (Priya *et al.*, 2004).

High heritability for sex ratio was found in cucumber (Solanki and Seth, 1980) and muskmelon (Deol *et al.*, 1981).

High heritability along with moderate to low genetic advance was reported for days to first fruit harvest in cucumber (Gayathri, 1997), while low values for days to fruit harvest was observed in snakegourd (Radhika, 1999).

Bisognin and Storck (2000) observed moderate heritability for fruit shape in bottlegourd.

High heritability coupled with high genetic advance for fruit girth was noticed in pumpkin (Singh *et al.*, 1988) and cucumber (Mariappan and Pappiah, 1990). Similar estimates for fruit length were reported in cucumber (Abusaleha and Dutta, 1990; Prasad and Singh, 1992) and snapmelon (Pandey *et al.*, 2003).

In ashgourd, George (1981) found high heritability for fruits per plant. High heritability and genetic advance for fruits per plant was also reported in pumpkin (Rana *et al.*, 1986) and bittergourd (Choudhary *et al.*, 1991).

Reddy and Rao (1984) recorded high heritability for average fruit weight in ridgegourd, whereas Owens *et al.* (1985) recorded intermediate heritability in cucumber. High heritability and genetic advance for average fruit weight was also noticed in muskmelon (Vijay, 1987), ashgourd (Menon, 1998) and snapmelon (Pandey *et al.*, 2003).

Fruit yield per plant was observed to have high heritability along with high genetic advance in ashgourd (Parkash *et al.*, 2000) and melon (Kandasamy, 2004).

Sureshbabu (1989) reported high genetic gain for seeds per fruit (73.05 per cent) in pumpkin. High values of heritability and genetic advance was also reported for the character in watermelon (Rajendran and Thamburaj, 1994) and ashgourd (Lovely, 2001).

In the case of 1000-seed weight, high heritability coupled with high genetic advance was noted in ashgourd (Lovely, 2001) and melon (Rakhi, 2001).

2.1.4 Correlation Studies

Measurement of phenotypic, genotypic and environmental correlations between yield and other characters have been a matter of great importance. Singh *et al.* (1989) observed significant positive association in respect of all the traits in muskmelon.

A high positive correlation was observed between vine length and fruit yield in ashgourd (George, 1981) and cucumber (Satyanarayana, 1991), whereas Shibukumar (1995) reported that in watermelon yield was negatively correlated with vine length.

Fruit yield was observed to have a positive correlation with internodal length in ashgourd (Menon, 1998).

A positive correlation was observed between yield and number of primary branches in watermelon (Sidhu and Brar, 1981). Saikia *et al.* (1995) in cucumber and Kandasamy (2004) in melon reported that number of secondary branches was positively correlated with yield.

In watermelon, Sidhu and Brar (1981) recorded positive correlation of vine length with number of primary and secondary branches.

Varghese (2003) reported that in ivygourd root-shoot ratio had positive correlation with fruit yield.

According to Kuo *et al.* (1988), there exists some correlation between flower type and fruit shape in melon.

Days to first female flower was found to have a positive correlation with yield in pumpkin (Kumaran *et al.*, 1998), whereas a negative correlation was observed in bottlegourd (Badade *et al.*, 2001).

Lovely (2001) reported a negative correlation for days to first female flower and fruits per plant in ashgourd.

A negative association of fruit yield with node to first flower was reported in ridgegourd (Rao *et al.*, 2000) and pumpkin (Lakshmi *et al.*, 2002), whereas a positive correlation was found in snapmelon (Pandey *et al.*, 2003). Kandasamy (2004) reported positive correlation of node to first male flower with node to first female flower in melon.

Murali *et al.* (1986) reported negative correlation of sex ratio with fruits per plant in bottlegourd. In cucumber, Prasunna and Rao (1989) observed that sex ratio was positively correlated with fruit yield.

Fruit yield was observed to have a positive correlation with days to first fruit harvest in bittergourd (Parhi *et al.*, 1995), while a negative correlation was noticed by Kumar and Singh (1998) in bottlegourd.

A positive correlation between yield and fruit length was found in parwal (Singh *et al.*, 1987) and cucumber (Rastogi and Deep, 1990b; Prasad and Singh, 1992), whereas positive association between yield and fruit girth was reported in ashgourd (Parkash *et al.*, 2000) and bittergourd (Bhave *et al.*, 2003).

Salk (1982) recorded positive correlation of fruit girth with average fruit weight in melon. In parwal, Singh *et al.* (1987) reported positive correlation of fruit length with fruit girth and average fruit weight.

Number of fruits produced was found to have a positive association with yield in bittergourd (Lawande and Patil, 1989) and cucumber (Rajput *et al.*, 1991). A high negative correlation between these two characters were reported by Priya (2001) in watermelon.

Salk (1982) reported negative correlation of fruits per plant with average fruit weight and 1000-seed weight in melon.

Fruit yield showed a positive correlation with average fruit weight in muskmelon (Kalloo *et al.*, 1983) and watermelon (Prasad *et al.*, 1988), whereas a negative association was reported in watermelon by Singh and Singh (1988). Devadas *et al.* (1999) reported that fruit weight was correlated with number of seeds per fruit and 100-seed weight in pumpkin.

Sidhu and Brar (1981) in watermelon noticed a negative correlation between number of seeds per kilogram of flesh and fruit yield, while positive association was reported in pointedgourd (Prasad and Singh, 1990), ashgourd (Menon, 1998) and pumpkin (Kumaran *et al.*, 1998).

In watermelon, Priya (2001) obtained a positive correlation between yield and 100-seed weight.

Rakhi (2001) reported that virus disease incidence was negatively correlated with vine length, sex ratio and average fruit weight.

2.1.5 Path Coefficient Analysis

Path analysis facilitates the partitioning of correlation coefficients into direct and indirect effects of various yield attributes.

Average fruit weight and vine length exhibited maximum direct effect on fruit yield per vine in ashgourd (George, 1981).

Sidhu and Brar (1981) found that node to first female flower had high direct as well as indirect effect on yield in watermelon.

A negative direct effect of days to first female flower was noticed in cucumber (Abusaleha and Dutta, 1988) and watermelon (Rajendran and Thamburaj, 1989), while a positive direct effect on fruit yield was obtained in pumpkin (Gopalakrishnan *et al.*, 1989).

Internodal length and days to fruit maturity have positive direct effect on fruit yield in cucumber (Solanki and Shah, 1992). Rajput *et al.* (1995) found that days to fruit harvest exhibited direct negative effect on yield in bittergourd.

In bittergourd, fruit breadth, days to opening of first male and female flower, vine length and number of seeds per fruit had maximum positive direct effect on yield, whereas number of primary branches and fruit length had weak positive direct effect on yield (Parhi *et al.*, 1995). Paranjape and Rajput (1995) found that vine length, number of branches, fruits per plant and seed number indirectly contributed to yield in bittergourd.

Menon (1998) observed that in ashgourd average fruit weight exhibited the highest positive direct effect on fruit yield followed by fruits per plant, female flowers per plant, vine length, internodal length and number of seeds per fruit.

Parkash *et al.* (2000) in ashgourd reported that fruits per plant and average fruit weight had high direct effect on yield, while number of days for flowering had negative direct effect on yield.

Lovely (2001) found that in ashgourd days to first female flower, fruits per plant, fruit length and girth had positive direct effects, while vine length and seeds per fruit had negative direct effects.

Priya (2001) reported that mean fruit weight, vine length, number of primary branches and fruits per plant were the major factors determining the yield per vine by studying their direct and indirect effects in watermelon.

The principal direct or indirect contributors to fruit yield were fruit length, fruits per plant and fruit weight as reported in melon (Rakhi, 2001), bittergourd (Bhave *et al.*, 2003), pointedgourd (Hazra *et al.*, 2003), snapmelon (Pandey *et al.*, 2003) and muskmelon (Choudhary *et al.*, 2004).

2.1.6 Selection Index

Shibukumar (1995) prepared a selection index for a collection of 20 watermelon genotypes based on major components of yield namely, number of fruits per plant, weight of individual fruit and yield per plant. With 20 per cent selection, the varieties Sugar Baby, Asalin Yamato, HW 1 and Fuken were identified superior and suitable for cultivation.

Gayathri (1997) formulated selection index for 22 cucumber genotypes using the characters, node to first female flower, days to first fruit harvest, fruits per plant, average fruit weight, fruit length and girth and yield per plant. The highest index score was recorded by CS 12 followed by CS 11, CS 9 and Punerikhira.

Selection indices with various character combinations *viz.*, number of fruits per vine and vine length were constructed for 51 genotypes of

melon (Lal and Singh, 1997). The relative efficiency was found to be highest in the combination of total yield per vine with weight per fruit.

Fruit length and fruit girth are the important characters that should be taken for selection in improvement programme in ashgourd (Lovely, 2001).

Selection index involving mean weight of fruit, vine length, number of primary branches and number of fruits was suitable to improve yield and quality in watermelon (Priya, 2001).

Rakhi (2001) formulated a selection index for 42 genotypes of *Cucumis melo*, based on vine length, sex ratio, fruits per plant, fruit weight, length of fruit and girth of fruit together with yield per plant. The landraces CM 5, CM 48, CM 6, CM 3, CM 36, CM 46, CM 17, CM 17, CM 35, CM 50 and CM 7 were identified as elite in terms of yield and resistance against mosaic virus.

2.1.7 Genetic Divergence

Mahalanobis's D^2 statistic is one of the potent techniques for measuring genetic divergence at both intra and intercluster levels. Anand and Murthy (1968) have emphasized the merit of D^2 statistics for genetic grouping of germplasm.

Genetic divergence was studied for eight quantitative characters in a collection of 25 cultivars of bittergourd by Ramachandran *et al.* (1981). They grouped into ten clusters and yield per plant, fruits per plant and fruit size were the important factors contributing towards divergence.

Genetic distance among five botanical varieties of *Cucumis melo* was estimated by Mathew *et al.* (1986). Of the four genetic characters studied, seeds per fruit did not contribute to total divergence, while fruits per plant contributed the maximum.

Varghese (1991) studied genetic divergence in snakegourd and 48 genotypes were grouped into ten clusters. The characters fruit weight, fruit number and yield per plant contributed maximum to divergence. Maximum number of genotypes was present in the cluster I (13) followed by III and IV.

Parhi *et al.* (1993) grouped 13 genotypes of bittergourd into six clusters considering 14 quantitative characters in which fruit yield, number of seeds per fruit and 100-seed weight made maximum contribution to total divergence.

Mathew (1999) assessed genetic diversity in a collection of 34 genotypes of snakegourd. Maximum contribution to total divergence was recorded by days to first female flower and number of seeds per fruit.

Genetic divergence studies by Lovely (2001) resulted in clustering of 25 genotypes of ashgourd into 8 group constellations. The maximum number of genotypes (8) were included in Cluster III, followed by cluster I (7), cluster II (4) and cluster IV (2). The clusters V, VI, VII and VIII had only one genotype in them. The genetic distance was maximum between II and IV and minimum between VII and VIII. The character seeds per fruit contributed maximum to the total divergence.

Kale *et al.* (2002) assessed genetic diversity in 24 pumpkin genotypes considering fourteen quantitative characters and grouped them into eight clusters. It was observed that fruit weight, seeds per fruit and yield per cluster had the greatest contribution to genetic divergence.

Prasad *et al.* (2002) grouped 48 inbreds of watermelon based on thirteen characters into ten different clusters of different sizes under D^2 canonical analysis.

Genetic divergence for fifteen quantitative traits was studied in 31 cucumber cultivars by Rao *et al.* (2003). Based on D^2 values, the cultivars were grouped into 16 highly divergent clusters.

Varghese (2003) studied genetic divergence in ivygourd using Mahalanobis's D^2 statistic and grouped 50 local cultivars into eleven clusters. Number of flowers per plant contributed maximum to the total divergence.

Lakshmi *et al.* (2003) grouped 21 diverse pumpkin genotypes into ten clusters and observed that days to first female flower, vine length, node to first female flower, fruit weight, number of fruits per vine, number of seeds per fruit and 1000-seed weight contributed maximum to genetic divergence.

Kandasamy (2004) classified forty genotypes of *C. melo* into 20 clusters based on D^2 analysis. Maximum genotypes were in cluster I and minimum in XX. D^2 analysis differentiated culinary types of melon from dessert types.

2.1.8 Reaction Towards Pests and Diseases

2.1.8.1 Reaction Towards Mosaic Virus

In India, mosaic diseases are common on almost all the cucurbit vegetables. There are different strains like cucumber mosaic virus, watermelon mosaic virus, pumpkin yellow vein mosaic virus and kakri mosaic virus.

A variety of mosaic symptoms occur on different members of the Cucurbitaceae (Singh, 1992). The mosaic disease symptoms consisted of distinct pattern of irregular dark green and light green patches on the leaf lamina, raised blisters on the leaf lamina, reduced leaf size, shortened and retarded growth.

The yield loss due to virus infection in pumpkin was 100 per cent when the plants were inoculated at seedling stage (Jayasree, 1984). Nandakumar (1999) reported that the incidence of mosaic in bittergourd adversely affected not only the yield but also the quality of the bittergourd fruits.

2.1.8.1.1 Benincasa-Mosaic Virus Relationship

Shankar *et al.* (1972) reported that pumpkin mosaic virus produced systemic mosaic symptoms on *Benincasa hispida*. Wu and Su (1977) observed that watermelon mosaic virus could infect 16 plants coming under the family Cucurbitaceae, but waxgourd (*Benincasa cerifera*) was hypersensitive.

Ghosh and Mukhopadhay (1979) isolated A3 strain (vein yellowing virus) from *Cucurbita moschata* from West Bengal and recorded that *Benincasa cerifera* is a host plant. They have also reported that *Benincasa campestris* var. *sarson* was found to act as symptomless carrier.

In the host range studies, *Benincasa hispida* was found to be immune to pumpkin yellow vein mosaic (Jayasree, 1984) and bittergourd mosaic virus (Purushothaman, 1994).

2.1.8.1.2 Source of Resistance

PKM-1, a new snakegourd was found to be moderately tolerant to virus diseases in a study conducted at Horticultural Research Station, Periyakulam (Pillai *et al.*, 1979).

In India, CGMMV resistance has been located both in wild species of *Cucumis viz., C. africanus*, *C. figarei*, *C. ficifolius*, *C. meeusii*, *C. zeyheri* (Rajamony *et al.*, 1990a) and in culinary melon like 'Phoot' and 'Kachri' (Rajamony *et al.*, 1990b).

Rakhi (2001) evaluated 42 collections of *C. melo* and CM 5, CM 48, CM 6, CM 31, CM 36, CM 46, CM 17, CM 35 and CM 50 were identified as elite in terms of resistance against mosaic virus.

Out of 86 genotypes of bittergourd screened against Bittergourd distortion mosaic virus (BDMV), Arunachalam (2002) observed that nine genotypes from northern and central parts of Kerala were found to be resistant.

In a CYT with eight genotypes of ashgourd, there was no mosaic incidence in AG 1, AG 22, AG 50 and AG 53. Mild incidence at the fag end of the crop was noticed in AG 23, AG 25 and AG 54 without affecting the yield (Gopalakrishnan, 2004).

2.1.8.2 Reaction Towards Root-knot Nematode

Root-knot nematode, *Meloidogyne* spp. are minute eelworms, causing swellings or galls on the roots of host plants. *Meloidogyne* spp. are unique in their capability to induce giant cells as well as causing extensive pericycle hyperplasia and cortical hypertrophy resulting in galls (Dropkin, 1969).

The cucurbits are highly susceptible to nematode infections. Winstead and Sasser (1956) tested 50 varieties of cucumber and all were highly susceptible to *M. incognita*, *M. incognita acrita*, *M. javanica javanica* and *M. arenaria arenaria*. Thomason and McKinney (1959) found that cultivars of watermelon, cucumber, muskmelon, pumpkin and squash are susceptible to three widely distributed species of root-knot nematodes: *Meloidogyne incognita acrita* Chitwood, *M. javanica* (Treub) Chitwood and *M. hapla* Chitwood.

Nair (1968) reported that *Meloidogyne* do not infest the gourds, bittergourd and snakegourd.

Mukherji and Sharma (1973) studied the symptoms of young and older plants of *Trichosanthes dioica* caused by *M. incognita*. Infection on young plants resulted in stunting, occasional chlorosis and reduced stands whereas on older plants made the stem thin, weak and pale coloured. Root system was reduced, knots on the taproots were large and confluent.

Darekar and Mahse (1988) reported that root-knot nematode cause a yield loss of 36.72 to 47.29 per cent in bittergourd.

2.1.8.2.1 Source of Resistance

Resistance to *Meloidogyne incognita* has been observed in wild species of *Cucumis dipsaceus* and *C. anguria* and in *C. sativus* cultivars, Fem Cap, Rozental Tsepellin, Superator and Kue-Vo-Kha-bakh (Udalova and Prikhod'Ko, 1985).

Some work has been initiated to locate resistant sources for transfer into cultivated varieties. *Cucumis metuliferus* is one, which has high resistance to nematode and some attempts are successful in interspecific hybridization with *Cucumis melo* (Seshadri, 1993).

2.2 MOLECULAR CHARACTERIZATION

Molecular markers are genotypic markers. Unlike morphological characters, molecular markers characterize diversity at the molecular level, and therefore are environmentally independent. The use of these markers provide a potential effective selection technique for crop improvement and has advantage over selection based on phenotype alone.

Molecular markers have been widely used in genetic analysis and diversity assessment in a number of plant species (Waugh and Powell, 1992; Bretting and Widerlechner, 1995; Staub *et al.*, 2004).

Molecular markers that reveal polymorphism at the DNA level are known as DNA markers. They provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann, 1983). Various types of molecular markers are utilized to evaluate DNA polymorphism and among them, the most important is polymerase chain reaction (PCR) based markers.

2.2.1 Polymerase Chain Reaction Based DNA Markers

PCR based DNA marker techniques are fingerprinting techniques that use an *in vitro* enzymatic reaction to specifically amplify a multiplicity of target sites in one or more nucleic acid molecules. Among the PCR based marker techniques, the important ones are Random Amplified Polymorphic DNA, Amplified Fragment Length Polymorphism and Microsatellite.

2.2.1.1 Random Amplified Polymorphic DNA (RAPD)

RAPD is a multiplex marker system that conventionally uses single-primer PCR to amplify random DNA fragments.

RAPD technique is particularly well suited to high through-put systems required for germplasm assessment because of their simplicity, speed and relatively low cost (Hadrys *et al.*, 1992). RAPD markers are commonly used for molecular characterization studies despite disadvantages in reliability (Peteira *et al.*, 1999).

RAPD is now being applied to a wide range of research activities including genome fingerprinting (Welsh and McClelland, 1990), identification of genome specific markers (Williams *et al.*, 1990; Erlich *et al.*, 1991), population biology studies (Astley, 1992), discrimination among specific genotypes, estimation of genetic variation and systematics (Lee *et al.*, 1996; Youn and Chung, 1998; Lopez-Sese and Staub, 2001).

i. RAPD and linkage maps

A high-density genetic linkage map or molecular map allows the location of all major genes regulating the expression of a particular trait to be determined. Random amplified polymorphic DNA analysis has been considered as the most rapid method for constructing genetic map of any crop (Martin *et al.*, 1991; Paran *et al.*, 1991).

The genetic linkage maps have been created in cucumber (Staub and Serquen, 2000; Bradeen *et al.*, 2001), melon (Oliver *et al.*, 2001; Silberstein *et al.*, 2003) and watermelon (Hashizumi *et al.*, 2003; Zhang *et al.*, 2004) using RAPD.

A saturated genetic map of Spanish melon with 385 molecular markers including RAPDs, one morphological trait and one disease resistance gene has been constructed (Oliver *et al.*, 2000). All markers were distributed in 12 linkage groups covering a total genetic distance of 1185 cM with an average map density of 3.1 cM/marker.

Perin *et al.* (2000) constructed a reference genetic map in melon using RAPD where thirteen genes involved in the genetic control of disease resistance or fruit and seed characteristics have been localized.

A genetic linkage (RAPD based) map constructed by Levi *et al.* (2001a) was useful for identification of marker linked closely to genes that control fruit quality and Fusarium wilt resistance in watermelon.

In watermelon, Hawkins *et al.* (2001) reported that several RAPD loci were identified to be loosely linked to morphological characteristics.

RAPD markers were used to construct a partial map of the *Cucurbita* genome (Brown and Myers, 2002). The map covers 1954 cM, which is estimated to be 75 per cent of the *Cucurbita* genome.

ii. RAPD and taxonomic studies

RAPD markers have been widely used for taxonomic and related studies.

Jeon *et al.* (1994) reported that RAPD markers generated by six out of fifty arbitary 10-mer primers were effective in discriminating among nine *C. moschata* and six *C. pepo* cultivars. The average dissimilarity coefficient matrix of markers was 5.84 between *C. moschata* and *C. pepo*, 3.41 between *C. moschata* cultivars and 2.90 between *C. pepo* cultivars.

RAPD analysis was done by Levi *et al.* (2000) to estimate the genetic relatedness among 34 PIs of the genus *Citrullus* and five watermelon cultivars. The analysis delineated three major clusters and the results indicated higher genetic variation with *C. colocynthis* and *C. lanatus* var. *citroides* as compared to *C. lanatus* var. *lanatus*.

Levi *et al.* (2001b) proposed the use of RAPD for scoring *Citrullus* PIs into phylogenetic groups prior to their evaluation for disease and pest resistance. Decker-Walters *et al.* (2001) proposed the use of RAPD to clarify the evolutionary history of bottlegourd landraces and cultivars.

Results of studies by Ferriol *et al.* (2003a) showed that RAPDs didn't group the nineteen accessions of *C. maxima* and eight related *Cucurbita* accessions according either to fruit morphological criteria or to passport data (origin and agro-climatic conditions).

Yun and Feng (2003) investigated the use of RAPD markers for estimating genetic relationship in 23 cultigens including cucumber and its wild relatives. The results from the UPGMA and cluster analysis suggested that the 23 cultigens could be classified into four groups.

iii. RAPD for detection of genetic variability

Random amplified polymorphic DNA analysis provides a quick and efficient method for resolving genetic relationship.

Garcia *et al.* (1998) revealed the use of 115 RAPD loci and 24 agronomic traits to estimate genetic distance among 32 elite melon-breeding lines to evaluate their potential as tools for germplasm management. The results indicated that RAPDs were suitable markers than agronomic traits in predicting genetic distance among the breeding lines.

Horejsi and Staub (1999) examined the genetic relationships in diverse germplasm of 168 *C. sativus* accessions using variation at 71 RAPD loci. Each accession had a unique marker profile, indicating that RAPD analysis was useful in genotypic differentiation.

Gwanama *et al.* (2000) reported that cluster analysis based on 39 polymorphic and 105 monomorphic DNA fragments amplified by sixteen RAPD primers, was used to show relationships among 31 genotypes of pumpkin obtained from Zambia and Malawi. The analysis revealed four clusters, with genotypes from Malawi mainly clustering in three clusters while all genotypes from Zambia and three from Malawi clustered in one cluster. The pairwise mean genetic distance was 0.32 ± 0.04 for samples from Malawi and 0.26 ± 0.04 for samples from Zambia.

Mliki *et al.* (2001) carried out genetic diversity studies in 126 melon accessions using RAPD. Although differences in grouping occurred after multidimensional scaling and cluster analysis, both analysis placed African accessions in two groups, which were separate from Reference Array groupings.

Ferriol *et al.* (2001) analysed polymorphism within and among eight different *C. maxima* accessions by RAPD. The average genetic distance between accessions using bulks was 0.4328 $\pm$ 0.078, which was greater than that obtained within accessions. A dendrogram constructed from the bulks revealed three clusters that correspond partially within the grouping based on fruit morphological characters.

Levi *et al.* (2001c) used RAPD markers to investigate genetic diversity and relatedness among 46 watermelon cultivars. The study revealed low genetic diversity among watermelon cultivars. Decker-Walters *et al.* (2002) used RAPD technique for confirming the relationship among intraspecific taxa of *C. pepo*.

Random amplified polymorphic DNA markers were successfully employed to analyse genetic diversity in cucumber (Ping *et al.*, 2002; Mliki *et al.*, 2003) and reported that UPGMA analysis distinguished 4 and 3 distinct groups respectively in their studies. Woo and Hyeon (2003) also used RAPD to investigate genetic relationship in fifty melon accessions and were separated into two main groups.

Kandasamy (2004) reported that RAPD marker analysis using four decamer primers gave a perfect differentiation of dessert melon from culinary melon which was agreeable with morphological characterization.

iv. RAPD and other uses

Singh *et al.* (2002) proposed the use of RAPD to study genetic and molecular basis of dioecism in *T. dioica*.

Multilateral branching in cucumber was identified with two RAPD markers W7-2 and BC-551 (Fazio *et al.*, 2003). Statistical analysis showed significant association of multilateral branching with these markers.

2.2.1.2 Amplified Fragment Length Polymorphism (AFLP)

AFLP is based on PCR amplification of restriction fragments generated by specific restriction enzymes and oligonucleotide adapters of few nucleotide bases (Vos *et al.*, 1995).

Ferriol *et al.* (2003b) employed AFLP for molecular variability studies in *Cucurbita pepo* and reported that PCA and cluster analysis using the UPGMA method clearly separate the accessions into two subspecies through the use of marker.

Riccardi *et al.* (2003) used AFLP markers to describe genetic variation in *C. melo* and the results pointed out a large variation in the material analysed. Paris *et al.* (2003) also used AFLP to compare 45 accessions of *C. pepo*.

Peng *et al.* (2003) assessed genetic diversity among 30 genotypes of watermelon using AFLP and reported that each genotype could be successfully distinguished based on AFLP scoring. Cluster grouping of accessions based on the AFLP analysis was consistent with that from classification by pedigrees and ecotypes.

AFLP markers were used for molecular analysis of 47 accessions of *Cucurbita maxima* (Ferriol *et al.*, 2004). The accessions clustered according to geographical origin.

2.2.1.3 Microsatellite

Microsatellite markers, also known as simple sequence repeats or SSRs are cluster of short (usually 2 to 6) tandemly repeated nucleotide bases distributed through out the genome (Litt and Luty, 1989).

Higher levels of polymorphism (71%) associated with SSR loci have been demonstrated in *C. melo* (Katzir *et al.*, 1996). Gene diversity values obtained with SSRs in melon were high (0.42 – 0.75) with two to six alleles for each SSR in a sample of eight varieties belonging to four melon groups.

Lopez-Sese *et al.* (2002) analysed 15 genotypes of Spanish melon in allele variation at 12 SSR loci and reported a high level of genetic variation between Cassaba market classes than within the genotypes.

Chiba *et al.* (2003) proposed the use of melon microsatellite loci as anchor markers in studies on synteny in Cucurbitaceae. The species to which the melon microsatellite markers would be most applicable was bittergourd (24 markers), followed by cucumber (20 markers) and pumpkin (18 markers).

Microsatellite was used to study genetic diversity in melon (Manforte *et al.*, 2003; Ritchel *et al.*, 2004). Cluster analysis suggests the division of the melon accessions into two major groups in both studies.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study entitled "Characterization of landraces of ashgourd [*Benincasa hispida* (Thunb.) Cogn.]" was carried out at the Department of Olericulture and the Department of Plant Biotechnology, College of Agriculture, Vellayani during 2003-2004. For morphological characterization, the experimental field was laid at Instructional Farm, College of Agriculture, Vellayani (Plate 1). It is situated at 8.5°N latitude, 76.9°E longitude at an altitude of 29.0 m above MSL. The site has a lateritic red loam soil. The area enjoys a humid tropical climate.

The study consisted of the following experiments.

3.1 Morphological characterization

3.2 Molecular characterization

3.1 MORPHOLOGICAL CHARACTERIZATION

3.1.1 Genetic Cataloguing of *B. hispida*

The basic material for the study included 25 landraces of ashgourd collected from different agroclimatic regions of Kerala, Tamil Nadu and Karnataka. The details of the landraces and their sources are presented in Table 1. The descriptor developed by IBPGR (1983) for cucurbits was used for cataloguing (Table 2).

3.1.2 Variability in *B. hispida*

Twenty-five landraces of ashgourd were grown during October 2003 to February 2004, to identify superior landraces with yield, quality and reaction towards the incidence of pests and diseases.

Table 1. Particulars of landraces of *Benincasa hispida* used in the study and their sources

Sl. No.	Landrace number	Source
1	BH1	Thakkala, Nagercoil, Tamil Nadu
2	BH 2	Cherthala, Alappuzha
3	BH 3	Vadakkancheri, Thrissur
4	BH 4	Balaramapuram, Thiruvananthapuram
5	BH 5	CO-1, TNAU
6	BH 6	Thiruvalla, Pathanamthitta
7	BH 7	Kattakada, Thiruvananthapuram
8	BH 8	Cheruplasseri, Palakkad
9	BH 9	Indu, KAU
10	BH 10	Ambalathara, Thiruvananthapuram
11	BH 11	Vadakara, Kozhikode
12	BH 12	Periya, Wayanad
13	BH 13	Bangalore
14	BH 14	Aryanad, Thiruvananthapuram
15	BH 15	Neyattinkara, Thiruvananthapuram
16	BH 16	Madhurai, Tamil Nadu
17	BH 17	Kalpetta, Wayanad
18	BH 18	Ettumannoor, Kottayam
19	BH 19	Edathua, Alappuzha
20	BH 20	Nagarcoil, Tamil Nadu
21	BH 21	Thodupuzha, Idukki
22	BH 22	Pala, Kottayam
23	BH 23	KAU local, KAU
24	BH 24	Kottarakara, Kollam
25	BH 25	Nemom, Thiruvananthapuram

Table 2. Genetic Cataloguing of *B. hispida*

1. Vegetative characters
1.1 Growth habit – Less viny/Moderately viny/Highly viny
1.2 Internodal length – Short/Medium/Long
1.3 Density of foliage hairs per branch – Few/Moderate/Dense
1.4 Leaf size – Small/Medium/Large
1.5 Leaf shape – Ovate/Pedate/Reniform
1.6 Leaf lobes – Somewhat lobeless/Shallowly lobed/Deeply lobed
1.7 Leaf dorsal surface pubescence – Soft hairy/Bristle like
2. Flowers and fruits
2.1 Flower size – Very small/Small/Medium/Large/Very large
2.2 Fruit size – Very small/Small/Medium/Large/Extra large
2.3 Fruit form – Round/Oval/Globular/Elliptical/Elongate/Elongate flattened/Elongate bottlelike
2.4 Skin texture – Waxy/Smooth
2.5 Skin colour – White/Green
2.6 Fruitshape at stemend –Deeprounded/ Rounded/ Flattened/ Taperpoint
2.7 Fruitshape at blossomend-Deeprounded/ Rounded/ Flattened/ Taperpoint
2.8 Peduncle length – Short/Medium/Long
2.9 Peduncle detachment from fruit – Easily/Difficult
2.10 Earliness of harvest – Early/Medium/Late
2.11 Fruit storage ability – >6 weeks/ <4 weeks/ <2 weeks
3. Seeds
3.1 Seed quantity per fruit – Very few/Few/Intermediate/Many/Very many
3.2 Seed size – Small/Medium/Large/Very large
3.3 Seed coat colour – Whitish yellow/Yellow/Brown
3.4 Seed surface lushe – Dull/Glossy
3.5 Seed separation from placenta – Easily/Medium/Difficult

Statistical details were as furnished below:

Design : RBD

Replications : 2

Treatments : 25 landraces

Spacing : 4.5 × 2.0 m

Plot size : 4 plants per plot

The cultural and management practices were adopted according to package of practices recommendations of Kerala Agricultural University (KAU, 2002).

3.1.2.1 Observations

Four plants per landrace per replication were selected for taking observations and the mean worked out for each replication as per standard procedures. Four fruits per landrace per replication were selected for taking observations of fruit characters.

1. Vine length (cm)

Measured from the collar region to the tip of the main vine using the measuring tape after pulling out the vine at the time of harvest.

2. Internodal length (cm)

Distance between 10^{th} and 11^{th} nodes of the vine.

3. Number of primary branches

The number of primary branches per plant counted at the full maturity of the plant.

4. Number of secondary branches

The number of secondary branches per plant counted at the full maturity of the plant.

5. Root-Shoot length ratio

Ratio between length of the root to the length of the shoot.

6. Days to first male flower

The number of days from sowing of seeds to the opening of the first male flower.

7. Node to first male flower

Node of the first male flower counting from the first true leaf.

8. Days to first female flower

The number of days taken from sowing to the bloom of the first female flower.

9. Node to first female flower

Number of nodes from the base of the plant to the node where the first female flower appeared.

10. Sex ratio

Number of male and female flowers were counted starting from the commencement of flowering till its completion and expressed as male to female ratio.

$$\text{Sex ratio} = \frac{\text{Number of male flowers}}{\text{Number of female flowers}}$$

11. Days to first fruit harvest

Number of days taken from sowing to the harvest of the first formed fruit.

12. Fruit length (cm)

The length of the fruit measured from the stalk end to the blossom end.

13. Fruit girth (cm)

The girth at the middle of the same fruit used for the length measurement.

14. Fruits per plant

The total number of fruits produced on a single plant observed.

15. Average fruit weight (kg)

Weight of four fruits from each replication were taken and average worked out.

16. Yield per plant (kg)

Weight of whole fruits from each plant of the landrace.

17. Seeds per fruit

One well-ripened fruit from each plant was selected at random and seeds with the mucilage were extracted carefully. It was washed, cleaned and dried under shade for three to four days and number of seeds were counted.

18. 1000 Seed weight (g)

The dry weight of randomly selected 1000 seeds were taken.

19. Scoring for pests and diseases

Though pest incidence of American serpentine leafminer and fruitfly were noted in initial growth stages of the crop, no scoring has to be performed further due to effective control measures. The incidence of disease like mosaic was recorded under natural field conditions. Root-knot nematode incidence was noted at harvest stage and scoring was done for its incidence.

a. Scoring for mosaic virus incidence

The rating scale given by Rajamony *et al.* (1990b) in melon was used for scoring with minor modifications. This was done according to the characteristic symptoms of the individual plant (Table 3 and Plates 2 to 5). The scoring was done 60-75 days after sowing.

Table 3. Scoring for mosaic virus incidence

Rating scale	Symptom	Category
0	No symptom	Highly resistant
1	Very light mottling of green colour	Resistant
2	Mottling of leaves with light and dark green colour	Moderately resistant
3	Blisters and raised surface on the leaves	Moderately susceptible
4	Distortion of leaves	Susceptible
5	Stunting of the plants with negligible or no flowering	Highly susceptible

The individual plant score was utilized to work out the 'Severity Index' or 'Vulnerability Index' (V.I.), so as to measure the resistance. The vulnerability index was calculated using an equation adopted by

Silbernagel and Jafri (1974) for measuring resistance in snap bean (*Phaseolus vulgaris*) to beet curly top virus and modified later by Bos (1982).

$$\text{Vulnerability Index (V.I.)} = \frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5)}{n_t\,(n_c-1)} \times 100$$

where,

$n_0, n_1, \ldots, n_5$ = number of plants in category 0,1,...,5 respectively

n_t = total number of plants

n_c = total number of categories = 6

The Vulnerability Index was used to classify the landraces into different categories.

Sl. No.	Vulnerability Index	Category
1	0-20	Highly resistant
2	21-40	Resistant
3	41-60	Moderately resistant
4	61-80	Susceptible
5	81-100	Highly susceptible

b. Scoring for root-knot nematode incidence

Gall formation on roots

Gall counts were taken in the uprooted plants. All the roots of a plant were carefully cut out and the galls on each rootlet were counted and recorded. The gall population or gall index was expressed as number of galls per 10 cm of root (Rajitha, 2003). Root-knot indexing in varietal screening was done as follows (Table 4 and Plate 6).

Plate 2. Scoring of mosaic intensity on the leaves of *B. hispida*

Plate 3. BH 10 - a landrace found highly resistant in the field (Score 0)

Plate 4. BH 16 - a landrace found moderately resistant in the field (Score 2)

Plate 5. BH 22 - a landrace found highly susceptible in the field (Score 5)

Table 4. Root-knot indexing in varietal screening

Number of galls per plant	Root-knot index	Reaction
0	1	Highly resistant
1-10	2	Resistant
11-30	3	Moderately resistant
31-100	4	Susceptible
>100	5	Highly susceptible

3.1.2.2 Statistical Analysis

1. Analysis of variance (ANOVA) and covariance (ANCOVA) for Randomized Block Design (RBD) in respect of the various characters was done (Panse and Sukhatme, 1967).

2. Mean : The mean of the character X_i ($\overline{X_i}$) was worked out.

3. Variability components (phenotypic and genotypic) for different characters was estimated as suggested by Kempthorne (1977).

(a) The variance and covariance components were calculated as per the following formulae :

For the character X_i,

Environmental variance, $\sigma_{ei}^2 = MSE$

Genotypic variance, $\sigma_{gi}^2 = \frac{MST - MSE}{r}$

Phenotypic variance, $\sigma_{pi}^2 = \sigma_{gi}^2 + \sigma_{ei}^2$

where, MST and MSE are respectively, the mean sum of squares for treatment and error from ANOVA and 'r', the number of replications.

For two characters X_i and X_j,

$$\text{Environmental covariance, } \sigma_{eij} = \text{MSPE}$$

$$\text{Genotypic covariance, } \sigma_{gij} = \frac{\text{MSPT - MSPE}}{r}$$

$$\text{Phenotypic covariance, } \sigma_{pij} = \sigma_{gij} + \sigma_{eij}$$

where, MSPT and MSPE are respectively, the mean sum of products between the i^{th} and j^{th} characters for landrace and environment respectively from Analysis of Covariance (ANCOVA).

(b) Coefficient of variation

Variability that existed in the population for various characters were apportioned using the estimates of coefficient of variation (Singh and Choudhary, 1985).

For the character X_i,

$$\text{Phenotypic coefficient of variation, PCV} = \frac{\sigma_{pi}}{\overline{X}_i} \times 100$$

$$\text{Genotypic coefficient of variation, GCV} = \frac{\sigma_{gi}}{\overline{X}_i} \times 100$$

$$\text{Environmental coefficient of variation, ECV} = \frac{\sigma_{ei}}{\overline{X}_i} \times 100$$

where, σ_{pi}, σ_{gi} and σ_{ei} are respectively the phenotypic, genotypic and environmental standard deviations with respect to each character.

4. Heritability

Hanson *et al.* (1956) proposed the mathematical relationship of variance estimates on computation of heritability, which is usually expressed as a percentage.

$$\text{Heritability (broad sense), } H^2 = \frac{\sigma_{gi}^2}{\sigma_{pi}^2} \times 100$$

The range of heritability was categorized as suggested by Robinson *et al.* (1949) namely, low (0 – 30 per cent), moderate (31 – 60 per cent) and high (61 per cent and above).

5. Genetic Advance

Genetic advance as percentage over mean was calculated as per the formula given by Lush (1949) and Johnson *et al.* (1955).

$$\text{Genetic advance, } GA = \frac{kH^2 \sigma_{pi}}{\overline{X}_i} \times 100$$

where, H^2 - heritability in broad sense.

σ_{pi} - phenotypic standard deviation

k - selection differential which is 2.06 in case of 5 % selection in large samples (Miller *et al.*, 1958; Allard, 1960).

Genetic advance was categorized according to Robinson *et al.* (1949) as follows :

Definition		**Category**
Less than 20 per cent	:	Low
Greater than 20 per cent	:	High

6. Correlation Analysis

Phenotypic, genotypic and environmental correlation coefficients were worked out according to the procedure suggested by Singh and Choudhary (1985).

7. Path Analysis

The direct and indirect effects of yield contributing factors were estimated through path analysis technique (Wright, 1954; Dewey and Lu, 1959).

8. Mahalanobis's D^2 Analysis

Genetic divergence was studied based on eleven characters taken together using Mahalanobis's D^2 statistic as described by Rao (1952). The landraces were clustered by Tochers method.

9. Selection Index

The selection index developed by Smith (1936) using the discriminant function of Fisher (1936) was used to discriminate the landraces based on eleven characters. The selection index is described by the function

$$I = b_1 X_1 + b_2 X_2 + \ldots b_k X_k$$

The function $H = a_1G_1 + a_2G_2 + \ldots a_kG_k$ describes the merit of a plant, where $X_1, X_2, \ldots, X_k$ are the phenotypic values and $G_1, G_2, \ldots, G_k$ are the genotypic values of the plant with respect to the characters $X_1, X_2, \ldots, X_k$. H denotes the genetic worth of the plant. The economic worth assigned to each character is assumed to be equal to unity i.e., $a_1, a_2, \ldots, a_k = 1$. The regression coefficients $b_1, b_2, \ldots, b_k$ are estimated in such a way that the correlation between H and I is maximum. The procedure will reduce to an equation of the form $b = P^{-1}Ga$, where P and G are the phenotypic and genotypic variance-covariance matrices respectively. Based on the

'b' estimates and the mean values for the eleven characters with respect to each landrace, scores were calculated and the landraces were ranked.

3.2 MOLECULAR CHARACTERIZATION

3.2.1 Materials

The twenty five landraces of ashgourd used in the first experiment were studied for molecular characterization.

3.2.2 Methods

1. Isolation of genomic DNA

For the isolation of genomic DNA, leaf samples were collected from young new leaves of ashgourd plants. The method of isolation followed was modified from that of Murray and Thompson (1980). Briefly 0.5 g of leaf material was first washed in running tap water and later in distilled water two or three times after chopping the leaves coarsely. After wiping off the water using tissue paper, the chopped leaves were pulverized in liquid nitrogen in a pre-cooled mortar by rapid grinding to a fine powder. Dry powder of plant material was transferred to a 2.0 ml centrifuge tube and enough extraction buffer (0.7 N NaCl, 1% CTAB, 50 mM Tris HCl (pH 8.0), 10 mM EDTA) was added to it so that clumps can easily be dispersed but the solution remains somewhat viscous. For this, 1.0 ml per 30-100 mg dry weight of powder was required. 200-300 µl PVP and 50-100 µl β-mercaptoethanol was also added to the centrifuge tube and was incubated in waterbath at 60°C for 45 minutes with occasional gentle shaking. The mixture was then subjected to centrifugation at 15000 rpm for 10 minutes. The clear supernatant was taken and the remaining extraneous matter was discarded. After that one-third volume of Phenol : Chloroform : Isoamyl alcohol (25:24:1) solution was added to the centrifuge tube, the two phases were

mixed gently and centrifuged at 12000 rpm for 10 minutes at 4.0°C. Then the supernatant was collected and to this one-third volume of Chloroform : Isoamyl alcohol (24:1) solution was added and centrifuged as in the previous step after thorough mixing. After collecting the upper phase, again the Chloroform : Isoamyl alcohol (24:1) extraction was repeated until the interphase disappeared. After that to the supernatant, one-tenth volume of 3.0 M Sodium acetate followed by double volume of chilled absolute isopropyl alcohol were added. It was kept in refrigerator at 4°C for 30 minutes. It was then centrifuged at 10000 rpm for 10 minutes at 4°C to pellet the DNA. The supernatant was discarded and the pellet was washed in 50 per cent ethanol. Then it was centrifuged at 10000 rpm for 5 minutes at 4°C. The supernatant was again discarded and the pellet was air dried for 20 minutes. Then the pellet was dissolved in 0.5 ml of 1x Tris EDTA buffer (10 mM Tris HCl, 1 mM EDTA, pH 8) and stored at 4°C.

All the materials used in the preparation and storage of reagents including reagent bottles, conical flasks, centrifuge tubes, spatula, glassrodes and tips of micropipettes were washed with Labolin solution and rinsed with distilled water and autoclaved.

2. Quantification of DNA

The quantification of DNA is necessary before it is subjected to amplification by PCR. DNA quantification was carried out with the help of UV-Vis Spectrophotometer (Spectronic Genesys 5).

The buffer in which the DNA was already dissolved was taken in a cuvette to calibrate the Spectrophotometer at 260 and 280 nm wavelength. The optical density (O.D.) of the samples dissolved in the buffer was recorded at both 260 and 280 nm.

The quantity of DNA in the sample was estimated by employing the following formula :

$$\text{Amount of DNA } (\mu g\ \mu l^{-1}) = \frac{A_{260} \times 50 \times \text{dilution factor}}{1000}$$

where, A_{260} – absorbance at 260 nm

The quality of DNA could be judged from the ratio of the O.D. values recorded at 260 and 280 nm. The A_{260} / A_{280} ratio between 1.8 and 2.0 indicates good quality of DNA, where A_{280} is the absorbance at 280 nm.

3. Agarose gel electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit supplied by the Bangalore Genei. The required amount of agarose was weighed out (0.7 per cent for visualizing the genomic DNA and 1.2 per cent for visualizing the amplified products) and melted in 1x TAE buffer (0.04 mM Tris acetate, 0.001 mM EDTA, pH 8) by boiling. After cooling to about 50°C, ethidium bromide was added to a final concentration of 0.5 μg ml^{-1}. The mixture was then poured to a preset template with appropriate comb. After solidification, the comb and the sealing tapes were removed and the gel was mounted in an electrophoresis tank filled with 1x TAE buffer. The gel was completely covered on the surface by the buffer. The DNA sample was mixed with the required volume of gel loading buffer (6x loading dye *viz.* 40 per cent sucrose, 0.25 per cent bromophenol blue). Each well was loaded with 20 μl of sample. One of the wells was loaded with 5.0 μl of molecular weight marker along the required volume of gel loading buffer. Electrophoresis was performed at 75 volts until the loading dye

reached 3/4th of the length of the gel. The gel was visualized using an ultraviolet visible (UV-Vis) transilluminator.

4. Random Amplified Polymorphic DNA (RAPD) analysis

DNA amplification was done using 40 arbitrarily designed decamer primers (Operon Inc., CA, USA) adopting the procedure of Staub *et al.* (2000) with required modifications.

Polymerase chain reactions of genomic DNA were performed in 25 µl containing 2.5 µl 10x PCR buffer, 1 µl $MgCl_2$, 2 µl each of dNTPs, 10 pM primer, 1 unit of Taq DNA polymerase (Invitrogen, USA) and 40 ng genomic DNA. Amplification was performed in a Programmable Thermal Controller (PTC-100, MJ Research Inc.) for an initial denaturation at 94°C for 5 minutes, followed by 44 cycles of denaturation at 94°C for 15 seconds and annealing at 35°C for 15 seconds. An extension at 72°C for 75 seconds was included after the last cycle. Finally the products of amplification were cooled to 4°C. A negative control containing sterile water instead of template was included in each reaction set.

The PCR product was size fractioned on a 1.2 per cent agarose gel prepared in 1x TAE buffer and stained with ethidium bromide. DNA fragments were visualized under UV transilluminator and photographed using a gel documentation system (BIO RAD, USA). The RAPD bands were represented as '+' for presence and '-' for absence and recorded. The PCR was repeated twice in order to confirm the reproducibility. The amplified products of three primers alone which could produce amplification for most of the clones were used for further analysis.

5. Data analysis

The reproducible bands were scored for their presence (+) or absence (-) for all the ashgourd landraces. A genetic similarity matrix was constructed using Jaccard's coefficient method (Jaccard, 1908).

$$Sj = a / (a + b + c)$$

where,

a : number of bands present in both the landraces in a pair

b : number of bands present in the first landrace but not in the second one

c : number of bands present in the second landrace but not in the first

Based on the similarity coefficient, the distance between the landraces was computed with the help of the software package NTSYS PC (Version 2.02i). Using these values of distances between landraces, a dendrogram was constructed by UPGMA (Unweighted pair group method with arithmetic average). Association between the various landraces was found out from the dendrogram.

RESULTS

4. RESULTS

Experimental data recorded during the course of investigation were subjected to statistical analysis and are presented under the following heads.

4.1 Genetic cataloguing

4.2 Genetic variability and divergence

4.3 Molecular characterization (RAPD)

4.1 GENETIC CATALOGUING

Twenty-five landraces of *B. hispida* were genetically catalogued for vegetative, flower, fruit and seed characters as per IBPGR (1983) descriptor list (Tables 5 to 7 and Plates 7 to 9).

Most of the landraces fall in moderate to high viny growth habit and short to long internodal length. Density of foliage hairs per branch ranged from few to dense. Leaf size varied from small to large with ovate, pedate or reniform shape. Most of the landraces had shallowly lobed leaves but exceptions of deeply lobed and lobeless cases were also found. Leaf dorsal surface pubescence was either soft hairy or bristle like.

Variability was more pronounced for flower and fruit characters. Flower and fruit size ranged from small to very large. Fruit form was either round, oval, globular or elongate bottle like. Skin colour was green in smooth textured fruits and white in waxy textured fruits. Fruit shape at stem end and blossom end ranged from deep round to taper point. Peduncle length ranged from short to long. Peduncle detachment from fruit was difficult in most of the landraces. Most of the landraces possess

Table 5. Vegetative characters in *B. hispida* landraces

Landrace Number	Growth habit	Internodal length	Density of foliage hairs/branch	Leaf size	Leaf shape	Leaf lobes	Leaf dorsal surface pubescence
BH 1	Less viny	Long	Moderate	Small	Ovate	Shallowly lobed	Soft, hairy
BH 2	Moderately viny	Medium	Few	Medium	Ovate	Shallowly lobed	Soft, hairy
BH 3	Moderately viny	Medium	Dense	Small	Pedate	Deeply lobed	Bristle like
BH 4	Moderately viny	Medium	Few	Small	Ovate	Shallowly lobed	Soft, hairy
BH 5	Moderately viny	Medium	Moderate	Large	Ovate	Shallowly lobed	Bristle like
BH 6	Highly viny	Long	Dense	Small	Ovate	Shallowly lobed	Bristle like
BH 7	Highly viny	Long	Dense	Large	Ovate	Shallowly lobed	Bristle like
BH 8	Highly viny	Long	Moderate	Small	Ovate	Shallowly lobed	Soft, hairy
BH 9	Moderately viny	Medium	Moderate	Medium	Ovate	Shallowly lobed	Soft, hairy
BH 10	Highly viny	Medium	Moderate	Medium	Pedate	Deeply lobed	Soft, hairy
BH 11	Highly viny	Medium	Dense	Medium	Ovate	Shallowly lobed	Bristle like
BH 12	Less viny	Medium	Few	Medium	Ovate	Shallowly lobed	Soft, hairy
BH 13	Highly viny	Long	Moderate	Medium	Ovate	Shallowly lobed	Bristle like
BH 14	Highly viny	Long	Few	Medium	Pedate	Deeply lobed	Soft, hairy
BH 15	Highly viny	Short	Dense	Large	Ovate	Shallowly lobed	Bristle like
BH 16	Moderately viny	Medium	Few	Medium	Ovate	Shallowly lobed	Soft, hairy
BH 17	Less viny	Medium	Moderate	Medium	Reniform	Somewhat lobeless	Soft, hairy
BH 18	Highly viny	Long	Dense	Large	Ovate	Shallowly lobed	Bristle like
BH 19	Less viny	Medium	Few	Medium	Pedate	Deeply lobed	Soft, hairy
BH 20	Moderately viny	Medium	Moderate	Large	Ovate	Shallowly lobed	Soft, hairy
BH 21	Less viny	Medium	Few	Small	Ovate	Shallowly lobed	Soft, hairy
BH 22	Highly viny	Long	Moderate	Medium	Pedate	Deeply lobed	Bristle like
BH 23	Moderately viny	Long	Moderate	Large	Ovate	Shallowly lobed	Soft, hairy
BH 24	Highly viny	Short	Few	Medium	Ovate	Shallowly lobed	Soft, hairy
BH 25	Moderately viny	Medium	Moderate	Large	Ovate	Shallowly lobed	Soft, hairy

Table 6. Flower and fruit characters in *B. hispida* landraces

Landrace Number	Size		Fruit form	Skin		Fruit shape at		Peduncle		Earliness of harvest	Fruit storage ability
	Flower	Fruit		Texture	Colour	Stem end	Blossom end	Length	Detachment from fruit		
BH 1	Large	Large	Round	Waxy	White	Rounded	Rounded	Short	Easily	Medium	> 6 weeks
BH 2	Large	Large	Oval	Waxy	White	Flattened	Flattened	Long	Difficult	Early	> 6 weeks
BH 3	Very large	Large	Globular	Waxy	White	Rounded	Rounded	Short	Easily	Medium	> 6 weeks
BH 4	Large	Large	Round	Waxy	White	Rounded	Flattened	Medium	Difficult	Medium	< 2 weeks
BH 5	Large	Large	Elliptical	Waxy	White	Taper point	Rounded	Medium	Easily	Medium	> 6 weeks
BH 6	Medium	Large	Oval	Waxy	White	Rounded	Flattened	Long	Difficult	Early	< 4 weeks
BH 7	Small	Extra large	Elongate flattened	Smooth	Green	Rounded	Taper point	Long	Difficult	Late	< 2 weeks
BH 8	Very small	Very small	Oval	Waxy	White	Rounded	Rounded	Short	Easily	Medium	> 6 weeks
BH 9	Medium	Large	Oval	Waxy	White	Rounded	Deep rounded	Short	Difficult	Medium	> 6 weeks
BH 10	Small	Small	Oval	Waxy	White	Rounded	Taper point	Medium	Difficult	Late	> 6 weeks
BH 11	Very small	Very small	Round	Waxy	White	Deep rounded	Rounded	Small	Easily	Medium	> 6 weeks
BH 12	Very large	Medium	Elongate	Waxy	White	Deep rounded	Taper point	Long	Difficult	Late	< 4 weeks
BH 13	Large	Medium	Elongate, bottle like	Waxy	White	Flattened	Taper point	Long	Difficult	Late	< 4 weeks
BH 14	Small	Medium	Globular	Waxy	White	Rounded	Deep rounded	Medium	Difficult	Medium	> 6 weeks
BH 15	Medium	Extra large	Round	Waxy	White	Deep rounded	Rounded	Long	Easily	Early	> 6 weeks
BH 16	Medium	Large	Elliptical	Waxy	White	Rounded	Rounded	Small	Easily	Medium	< 4 weeks
BH 17	Large	Large	Elongate bottle like	Smooth	Green	Taper point	Taper point	Small	Easily	Late	< 2 weeks
BH 18	Large	Large	Elongate	Waxy	White	Rounded	Rounded	Medium	Easily	Medium	< 2 weeks
BH 19	Very large	Large	Round	Waxy	White	Rounded	Flattened	Long	Difficult	Late	< 2 weeks
BH 20	Medium	Medium	Elongate	Smooth	Green	Taper point	Taper point	Medium	Difficult	Medium	< 2 weeks
BH 21	Small	Small	Oval	Waxy	White	Rounded	Deep rounded	Medium	Difficult	Late	> 6 weeks
BH 22	Small	Very small	Oval	Waxy	White	Deep rounded	Rounded	Long	Difficult	Medium	> 6 weeks
BH 23	Large	Large	Elliptical	Waxy	White	Deep rounded	Deep rounded	Long	Difficult	Early	> 6 weeks
BH 24	Medium	Medium	Round	Waxy	White	Flattened	Flattened	Small	Easily	Medium	< 4 weeks
BH 25	Medium	Large	Elongate bottle like	Smooth	Green	Rounded	Taper point	Long	Difficult	Late	< 2 weeks

Table 7. Seed characters in *B. hispida* landraces

Landrace Number	Seed quantity per fruit	Seed size	Seed coat colour	Seed surface lushe	Seed separation from placenta
BH 1	Very many	Large	Yellow	Glossy	Difficult
BH 2	Very many	Large	Yellow	Glossy	Difficult
BH 3	Very many	Large	Brown	Dull	Medium
BH 4	Few	Large	Yellow	Glossy	Medium
BH 5	Many	Large	Yellow	Glossy	Difficult
BH 6	Very many	Large	Whitish yellow	Glossy	Difficult
BH 7	Few	Large	Brown	Dull	Medium
BH 8	Very few	Small	Yellow	Glossy	Easily
BH 9	Many	Large	Brown	Dull	Medium
BH 10	Few	Medium	Whitish yellow	Glossy	Easily
BH 11	Few	Small	Yellow	Glossy	Easily
BH 12	Intermediate	Medium	Yellow	Glossy	Easily
BH 13	Intermediate	Medium	Yellow	Glossy	Easily
BH 14	Few	Medium	Whitish yellow	Glossy	Easily
BH 15	Very many	Very large	Brown	Dull	Difficult
BH 16	Very many	Large	Brown	Dull	Medium
BH 17	Intermediate	Large	Brown	Dull	Difficult
BH 18	Few	Large	Yellow	Glossy	Medium
BH 19	Many	Large	Yellow	Glossy	Medium
BH 20	Few	Medium	Brown	Dull	Easily
BH 21	Few	Small	Whitish yellow	Glossy	Easily
BH 22	Few	Medium	Whitish yellow	Glossy	Easily
BH 23	Very many	Large	Brown	Dull	Medium
BH 24	Few	Medium	Whitish yellow	Glossy	Easily
BH 25	Few	Medium	Yellow	Glossy	Medium

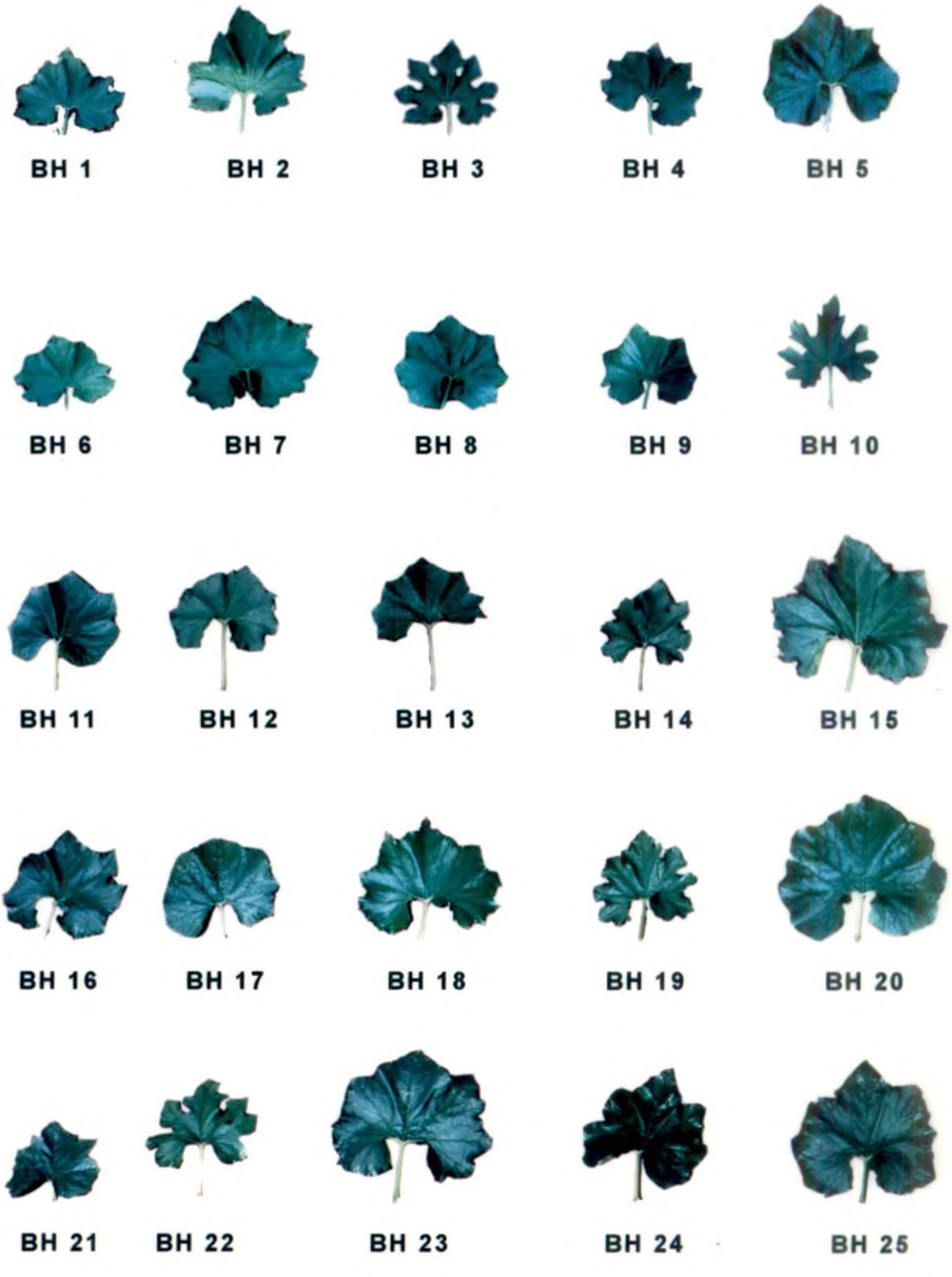
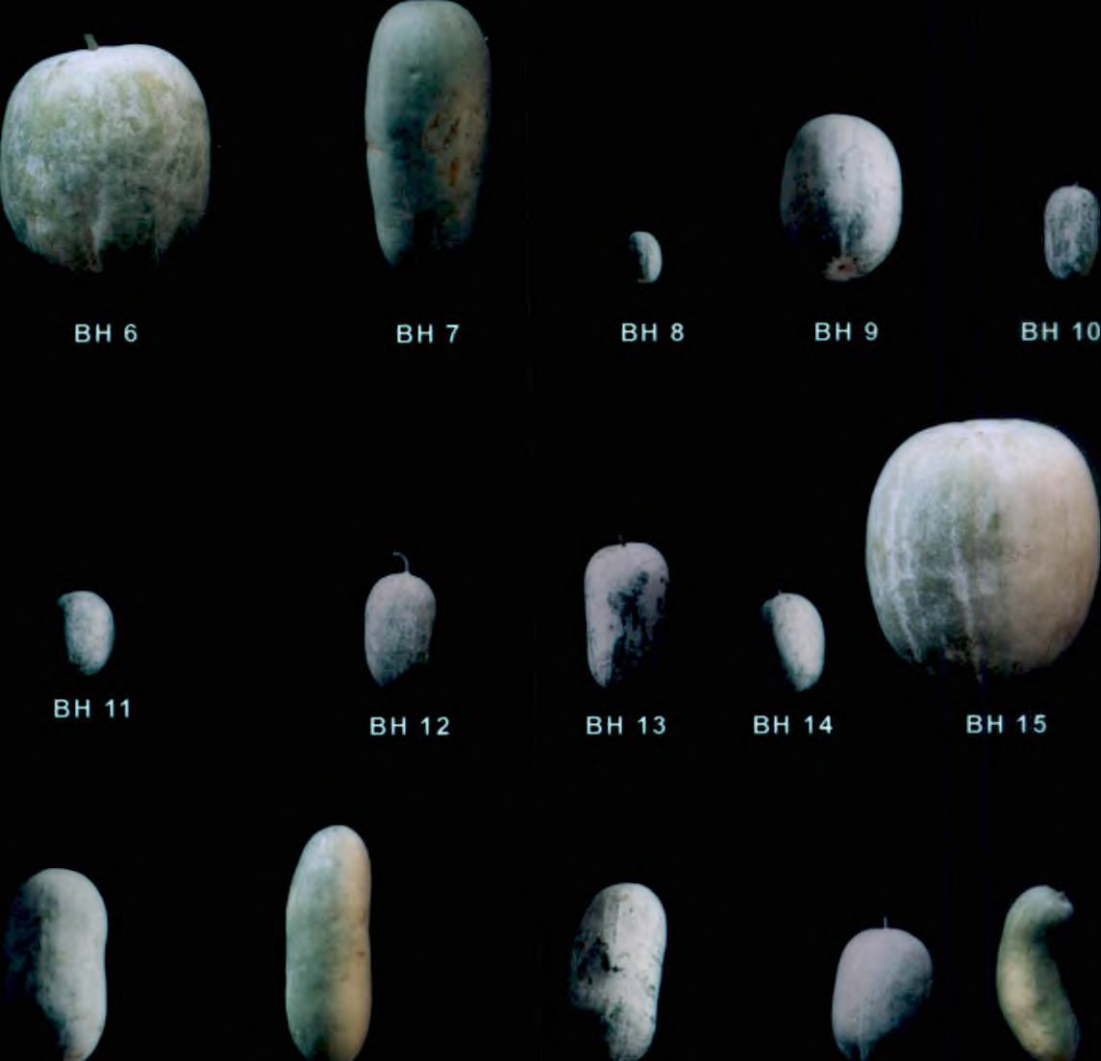


Plate 7. Variability in leaf characteristics of *B. hispida*

Plate 8. Variability in flower characters of *B. hispida*


BH 6
BH 7
BH 8
BH 9
BH 10
BH 11
BH 12
BH 13
BH 14
BH 15

medium earliness of harvest. Fruit storage ability was more than six weeks in most of the landraces.

Seed quantity per fruit ranged from very few to many with small to large seed size. Most of the landraces had yellow seed colour, but exceptions of brown and whitish yellow colour was also found. Glossy to dull seed surface lushe was noticed. Seed separation from placenta was easy in most of the landraces.

4.2 GENETIC VARIABILITY AND DIVERGENCE

4.2.1 Mean Performance

Analysis of variance showed significant differences among the landraces for all the characters studied. The mean values of 25 landraces for different characters are presented in Table 8.

Vine length

There was significant difference among the landraces for vine length. It ranged from 283.00 to 875.00 cm with an overall mean of 541.80 cm. BH 22 was the longest with a length of 875.00 cm. The landrace BH 19 (283.00 cm) was the shortest, which was on par with BH 17 (309.50 cm), BH 5 (334.00 cm) and BH 15 (354.00 cm).

Internodal length

Internodal length was found to vary from 7.20 to 17.55 cm. The landraces on an average had 13.68 cm internodal length. Maximum internodal length was observed in BH 6 (17.55 cm), which was on par with BH 7 (17.25 cm), BH 8 (16.75 cm) and BH 22 (16.30 cm) and minimum in BH 24 (7.20 cm).

Table 8. Mean value of biometric characters in *B. hispida*

Landrace Number	Vine length (cm)	Internodal length (cm)	Number of primary branches	Number of secondary branches	Root shoot length ratio	Days to first male flower	Node to first male flower	Days to first female flower	Node to first female flower	Sex ratio
BH 1	610.00	15.10	2.50	7.00	0.05	50.50	12.12	58.50	21.37	13.50
BH 2	622.50	11.80	2.00	5.00	0.03	47.00	9.62	53.50	19.00	13.52
BH 3	610.50	14.90	2.00	8.00	0.05	50.87	14.75	55.50	21.62	13.15
BH 4	686.50	13.40	3.00	14.00	0.04	49.12	12.50	53.12	18.12	12.92
BH 5	334.00	10.95	3.00	3.50	0.10	53.62	15.87	58.37	22.25	11.52
BH 6	506.50	17.55	1.50	6.00	0.11	56.37	13.50	60.87	22.75	12.29
BH 7	696.00	17.25	3.00	18.50	0.14	56.87	16.87	61.50	22.87	11.85
BH 8	388.50	16.75	2.00	5.50	0.06	50.50	19.12	46.25	27.62	8.59
BH 9	512.50	12.85	3.00	4.50	0.06	50.00	17.37	53.75	26.37	13.90
BH 10	457.00	11.10	3.00	6.50	0.07	64.12	14.25	65.62	21.37	7.88
BH 11	407.00	12.80	2.50	9.00	0.07	49.62	15.37	53.62	21.75	10.88
BH 12	691.50	12.05	3.00	12.50	0.06	52.50	19.62	55.50	26.75	14.65
BH 13	621.00	15.35	3.00	8.00	0.06	59.25	19.00	63.75	26.87	10.26
BH 14	492.50	14.95	3.00	12.50	0.06	48.87	13.25	51.00	19.12	7.21
BH 15	354.00	9.70	3.50	5.50	0.07	49.83	13.00	56.33	22.05	15.73
BH 16	589.00	13.95	3.00	13.50	0.07	48.37	15.00	53.50	22.62	11.60
BH 17	309.50	12.30	1.50	3.00	0.06	53.87	17.00	57.25	24.50	12.91
BH 18	732.00	16.00	2.00	12.50	0.08	49.12	14.71	52.58	19.98	9.20
BH 19	283.00	12.75	2.50	5.50	0.06	58.12	10.62	62.50	20.12	16.40
BH 20	652.00	13.95	3.00	7.50	0.04	51.37	17.50	56.75	28.37	14.40
BH 21	641.50	13.55	2.50	9.00	0.13	51.25	18.50	56.87	26.12	11.42
BH 22	875.00	16.30	4.00	23.00	0.03	56.37	16.00	60.62	24.87	7.89
BH 23	367.00	16.00	2.00	4.00	0.05	48.12	14.75	52.33	21.37	10.56
BH 24	416.00	7.20	2.00	6.00	0.06	48.12	15.62	49.87	21.00	8.40
BH 25	690.00	13.60	3.00	3.50	0.04	48.50	13.87	52.00	18.00	10.47
Mean	541.80	13.68	2.62	8.54	0.06	52.09	15.19	56.06	22.67	11.64
F ratio	33.07**	25.35**	5.25**	23.75**	12.86**	5.34**	9.40**	4.03**	4.10**	1.95*
CD	79.03	1.42	0.78	2.96	0.02	5.36	2.46	6.73	4.34	5.27

* Significant at 5 % ** Significant at 1%

Table 8. Continued

Landrace Number	Days to first fruit harvest	Fruit length (cm)	Fruit girth (cm)	Fruits per plant	Average fruit weight (kg)	Yield per plant (kg)	Seeds per fruit	1000-seed weight (g)	Mosaic incidence (V.I.)
BH 1	98.00	38.79	63.98	2.12	6.60	14.05	1647.00	74.30	47.50
BH 2	93.00	39.40	68.45	2.37	5.95	13.00	1006.00	69.55	55.00
BH 3	95.00	35.00	69.70	2.25	5.50	11.59	1468.00	64.50	40.00
BH 4	93.00	41.05	65.92	2.00	6.20	11.66	276.50	71.10	45.00
BH 5	99.00	50.10	71.95	2.12	8.40	16.55	940.50	76.75	40.00
BH 6	99.50	36.42	63.23	2.50	7.35	15.40	1364.00	70.70	57.50
BH 7	102.00	54.93	62.59	1.87	9.30	15.20	183.50	64.50	75.00
BH 8	88.00	13.65	22.20	8.00	0.27	1.99	73.50	24.90	47.50
BH 9	93.50	33.40	59.85	3.60	4.60	15.71	960.50	63.03	52.50
BH 10	107.50	20.75	27.25	4.87	0.57	2.80	171.50	41.25	27.50
BH 11	93.00	21.00	32.75	4.75	0.59	2.50	174.50	26.30	55.00
BH 12	95.00	33.79	54.70	3.12	2.80	9.71	511.50	33.30	57.50
BH 13	105.50	35.90	51.30	3.87	3.05	10.90	518.00	36.75	40.00
BH 14	90.50	21.90	34.25	5.50	1.02	5.87	220.50	35.00	37.50
BH 15	97.00	44.25	78.05	2.37	9.50	21.20	1411.50	96.95	60.00
BH 16	93.00	48.70	59.25	2.50	6.95	15.30	1282.50	56.25	42.50
BH 17	89.50	43.85	41.75	2.75	2.82	5.75	648.50	65.50	57.50
BH 18	92.00	54.73	57.43	2.87	6.65	16.45	232.00	52.25	55.00
BH 19	104.00	32.90	54.05	2.75	2.60	7.37	914.00	54.35	57.50
BH 20	97.50	28.65	33.90	3.50	1.15	5.05	233.50	46.85	55.00
BH 21	96.50	17.25	25.60	5.62	0.30	1.62	201.00	19.25	35.00
BH 22	101.50	26.50	34.00	8.00	0.85	6.71	241.00	32.70	70.00
BH 23	92.00	45.80	73.10	2.75	8.25	17.44	1555.50	62.35	50.00
BH 24	88.50	25.35	42.35	9.12	1.35	9.89	285.50	30.90	57.50
BH 25	90.50	56.00	22.45	3.00	4.80	13.40	140.50	54.20	70.00
Mean	95.78	36.00	50.80	3.77	4.29	10.68	666.44	52.93	51.50
F ratio	3.85**	39.60**	84.31**	34.67**	171.10**	77.27**	4291.01**	1108.69**	3.44**
CD	7.91	5.62	5.58	1.01	0.69	1.83	23.75	1.71	17.98

* Significant at 5 % ** Significant at 1%

Number of primary branches

Among the landraces, the number of primary branches was found to vary from 1.50 to 4.00 with a general mean of 2.62. Maximum number of 4.00 was found in BH 22 and minimum of 1.50 in BH 6, which was on par with BH 17 (1.50).

Number of secondary branches

Mean number of secondary branches varied from 3.00 in BH 17 to 23.00 in BH 22 with a general mean of 8.54. BH 5 and BH 25 each with 3.50 were on par with BH 17 (3.00).

Root shoot length ratio

Root shoot length ratio observed a range of 0.03 to 0.14. Among the 25 landraces, BH 7 had the maximum ratio (0.14) whereas BH 22 had the minimum (0. 03).

Days to first male flower

Days to first male flower exhibited a range of 47.00 to 64.12. BH 2 was the earliest to flower (47.00). BH 10 (64.12) was the latest, which was on par with BH 13 (59.25).

Node to first male flower

Node to first male flower ranged from 9.62 in BH 2 to 19.62 in BH 12. The landraces BH 8 (19.12) and BH 13 (19.00) were on par with BH 12 while BH 19 (10.62) was on par with BH 2 (9.62).

Days to first female flower

Among the landraces, days to first female flower ranged from 46.25 in BH 8 to 65.62 in BH 10 with an overall mean of 56.06.

Node to first female flower

Range in node to first female flower among the landraces was from 18.00 in BH 25 to 28.37 in BH 20.

Sex ratio

Sex ratio had a range from 7.21 in BH 14 to 16.40 in BH 19 with an overall mean of 11.64.

Days to fruit harvest

Days to fruit harvest exhibited a range of 88.00 to 107.50. BH 8 was the earliest to harvest (88.00) while BH 10 (107.50) was the latest.

Fruit length

A wide range of variation was noticed for fruit length. Maximum fruit length was observed in BH 25 (56.00 cm) and minimum in BH 8 (13.65 cm).

Fruit girth

Girth of fruits varied significantly among the landraces from 22.20 to 78.05 cm. Maximum fruit girth was recorded in BH 15 (78.05 cm), which was on par with BH 23 (73.10 cm). Landrace BH 8 (22.20 cm) had the minimum fruit girth.

Fruits per plant

Fruit number varied considerably from 1.87 in BH 7 to 9.12 in BH 24 with an overall mean of 3.77.

Average fruit weight

Range in average fruit weight among the landraces was from 0.27 to 9.50 kg, highest in BH 15 (9.50 kg) and lowest in BH 8 (0.27 kg).

Yield per plant

A wide range of variation was observed for yield per plant from 1.62 to 21.20 kg. BH 15 had the highest yield (21.20 kg), which was significantly different from all other landraces. The lowest yield was obtained from BH 21 (1.62 kg), which was on par with BH 8 (1.99 kg).

Seeds per fruit

Seeds per fruit observed a range from 73.50 in BH 8 to 1647.00 in BH 1 with an overall mean of 666.44.

1000-seed weight

Among the landraces, 1000-seed weight ranged from 19.25 g in BH 21 to 96.95 g in BH 15.

Mosaic incidence

Mosaic was the only disease observed at fruit maturation stage and hence scoring based on visual observations was done for mosaic incidence. The vulnerability index for mosaic incidence ranged from 27.50 to 75.00. Maximum mosaic incidence was observed in BH 7 (75.00), whereas BH 10 (27.50) was the least affected. The reaction of landraces towards mosaic incidence (Table 9) indicated that six landraces were resistant; sixteen landraces were moderately resistant and remaining three (BH 7, BH 22, BH 25) were susceptible to the disease.

Table 9. Reaction of 25 landraces of *B. hispida* towards mosaic virus under field conditions

Category	Highly resistant	Resistant	Moderately resistant	Susceptible	Highly susceptible
Vulnerability index	0-20	21-40	41-60	61-80	81-100
Landraces	Nil	BH 3, BH 5, BH 10, BH 21, BH 13, BH 14	BH 1, BH 2, BH 4, BH 6, BH 8, BH 9, BH 11, BH 12, BH 15, BH 16, BH 17, BH 18, BH 19, BH 20, BH 23, BH 24	BH 7, BH 22, BH 25	Nil

Root-knot incidence

The root-knot incidence was noticed in three landraces BH 13, BH 15 and BH 23. Among the landraces, the mean number of galls ranged from 14.55 to 52.55. Root- knot index showed that 22 landraces were highly resistant, two (BH 15 and BH 23) were moderately resistant and BH 13 was susceptible to root-knot infestation (Table 10).

4.2.2 Genetic Parameters

The population mean, range, phenotypic, genotypic and environmental variances, phenotypic and genotypic coefficients of variation are given in Table 11.

High phenotypic and genotypic variances were observed for several characters including seeds per fruit and vine length. Wide variation was observed in phenotypic and genotypic variances among the characters. A close association between phenotypic and genotypic variances was noticed for seeds per fruit, 1000-seed weight, yield per plant, average fruit weight and fruits per plant. For most of the characters, genotypic variance makes up the major portion of the phenotypic variance, with very little effect of environment.

Phenotypic and genotypic coefficients of variation (PCV and GCV respectively) observed were high for most of the characters (Fig. 1). Seeds per fruit had the highest PCV (79.98) and GCV (79.96) followed by average fruit weight (72.18 and 71.76), fruits per plant (54.83 and 53.27) and yield per plant (52.06 and 51.39) respectively. The lowest PCV and GCV were exhibited by days to first fruit harvest (6.23 and 4.78 respectively).

Table 10. Reaction of 25 landraces of *B. hispida* towards root-knot under field conditions

Root-knot index	Highly resistant (1)	Resistant (2)	Moderately resistant (3)	Susceptible (4)	Highly susceptible (5)
Number of galls	0	1-10	11-30	31-100	>100
Landraces	BH 1, BH 2, BH 3, BH 4, BH 5, BH 6, BH 7, BH 8, BH 9, BH 10, BH 11, BH 12, BH 14, BH 16, BH 17, BH 18, BH 19, BH 20, BH 21, BH 22, BH 24, BH 25	Nil	BH 15, BH 23	BH 13	Nil

Table 11. Range, mean, phenotypic, genotypic and environmental variances, phenotypic and genotypic coefficients of variation for different characters in *B. hispida*

Sl. No.	Character	Range	Mean ± SE_m	σ_p^2	σ_g^2	σ_e^2	PCV (%)	GCV (%)
1	Vine length (cm)	283.00 - 875.00	541.80 ± 27.06	24979.96	23513.70	1466.25	29.17	28.30
2	Internodal length (cm)	7.20 - 17.55	13.68 ± 0.48	6.29	5.81	0.43	18.33	17.62
3	Number of primary branches	1.50 - 4.00	2.62 ± 0.38	0.45	0.30	0.14	25.69	21.19
4	Number of secondary branches	3.00 - 23.00	8.54 ± 1.01	25.61	23.54	2.07	59.26	56.82
5	Root-Shoot length ratio	0.03 - 0.14	0.06 ± 7.82	0.02	0.01	0.00	43.20	39.97
6	Days to first male flower	47.00 - 64.12	52.09 ± 1.83	21.42	14.66	6.75	8.88	7.35
7	Node to first male flower	9.62 - 19.62	15.19 ± 0.84	7.43	6.00	1.43	17.94	16.13
8	Days to first female flower	46.25 - 65.62	56.06 ± 2.30	26.82	16.16	10.65	9.23	7.17
9	Node to first female flower	18.00 - 28.37	22.67 ± 1.49	11.28	6.86	4.42	14.81	11.55
10	Sex ratio	7.21 - 16.40	11.64 ± 1.80	9.63	3.10	6.52	26.65	15.13
11	Days to first fruit harvest	88.00 - 107.50	95.78 ± 2.71	35.66	20.96	14.70	6.23	4.78
12	Fruit length (cm)	13.65 - 56.00	36.00 ± 1.92	150.70	143.28	7.42	34.09	33.24
13	Fruit girth (cm)	22.20 - 78.05	50.80 ± 1.91	312.31	304.99	7.32	34.78	34.37
14	Fruits per plant	1.87 - 9.12	3.77 ± 0.34	4.27	4.03	0.23	54.83	53.27
15	Average fruit weight (kg)	0.27 - 9.50	4.29 ± 0.23	9.62	9.51	0.11	72.18	71.76
16	Yield per plant (kg)	1.62 - 21.20	10.68 ± 0.62	30.95	30.16	0.79	52.06	51.39
17	Seeds per fruit	73.50 - 1647.00	666.44 ± 8.13	284166.90	284034.50	132.41	79.98	79.96
18	1000 Seed weight (g)	19.25 - 96.95	52.93 ± 0.58	382.90	382.21	0.69	36.96	36.92
19	Mosaic virus incidence (Vulnerability Index)	27.50 - 75.00	51.50 ± 6.16	168.70	92.75	75.95	25.22	18.70

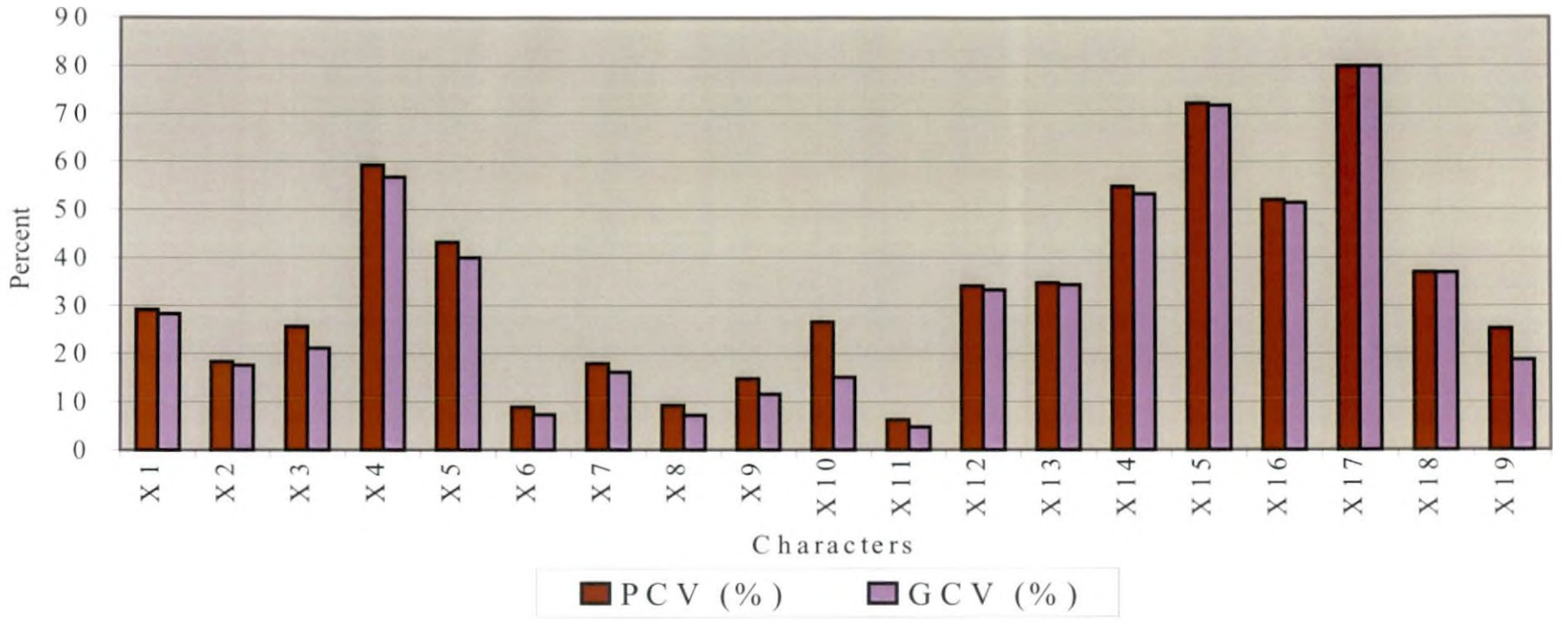


Fig.1 Phenotypic and genotypic coefficient of variation for 19 characters in *B. hispida*

X1 – Vine length
X2 - Internodal length
X3 – Number of primary branches
X4 – Number of secondary branches
X5 – Root – Shoot length ratio
X6 – Days to first male flower
X7 – Node of first male flower
X8 – Days to first female flower
X8 – Days to first female flower
X9 – Node to first female flower
X10 – Sex ratio
X11 – Days to first fruit harvest
X12 – Fruit length
X13 – Fruit girth
X14 – Fruits per plant
X15 – Average fruit weight
X16 – Yield per plant
X17 – Seeds per plant
X 18 – 1000 Seed weight
X 19 – Mosaic incidence

4.2.3 Heritability and Genetic Advance

Heritability and genetic advance for different characters are presented in Table 12 (Fig. 2).

High heritability coupled with high genetic advance was observed for most of the characters, except days to first male and female flower, node to first female flower, sex ratio, days to first fruit harvest and mosaic incidence.

Heritability estimates were high for most of the characters studied *viz.*, seeds per fruit (99.95), 1000-seed weight (99.81), average fruit weight (98.83) and fruit girth (97.65). Sex ratio recorded the lowest but a moderate heritability (32.23).

Genetic advance was highest for seeds per fruit (164.69), followed by average fruit weight (147.08) and lowest for days to first fruit harvest (7.54) and days to first female flower (11.45). High heritability combined with high genetic advance was observed for fruit length, fruit girth, fruits per plant, average fruit weight, yield per plant and seeds per fruit.

4.2.4 Correlation Analysis

The phenotypic, genotypic and environmental correlation coefficients were estimated for 19 characters (Tables 13, 14 and 15).

(A) Phenotypic correlation

(i) Correlation between yield and other characters

Yield per plant recorded high positive significant correlation with fruit length (0.7893), fruit girth (0.8165), average fruit weight (0.9203), seeds per fruit (0.6264) and 1000-seed weight (0.7767). Node to first male and female flower (-0.3074 and -0.2965 respectively) and fruits per plant (-0.5738) was negatively correlated with yield.

Table 12. Heritability and genetic advance for different characters in *B. hispida*

Sl. No.	Characters	Heritability (%)	Genetic advance (%)
1	Vine length	94.13	56.60
2	Internodal length	92.41	34.86
3	Number of primary branches	68.01	35.87
4	Number of secondary branches	91.91	112.17
5	Root-Shoot length ratio	85.57	83.33
6	Days to first male flower	68.46	12.51
7	Node to first male flower	80.77	29.82
8	Days to first female flower	60.25	11.45
9	Node to first female flower	60.85	18.52
10	Sex ratio	32.23	17.69
11	Days to first fruit harvest	58.77	7.54
12	Fruit length	95.07	66.77
13	Fruit girth	97.65	69.98
14	Fruits per plant	94.39	106.36
15	Average fruit weight	98.83	147.08
16	Yield per plant	97.44	104.08
17	Seeds per fruit	99.95	164.69
18	1000 Seed weight	99.81	76.00
19	Mosaic incidence	54.97	28.56

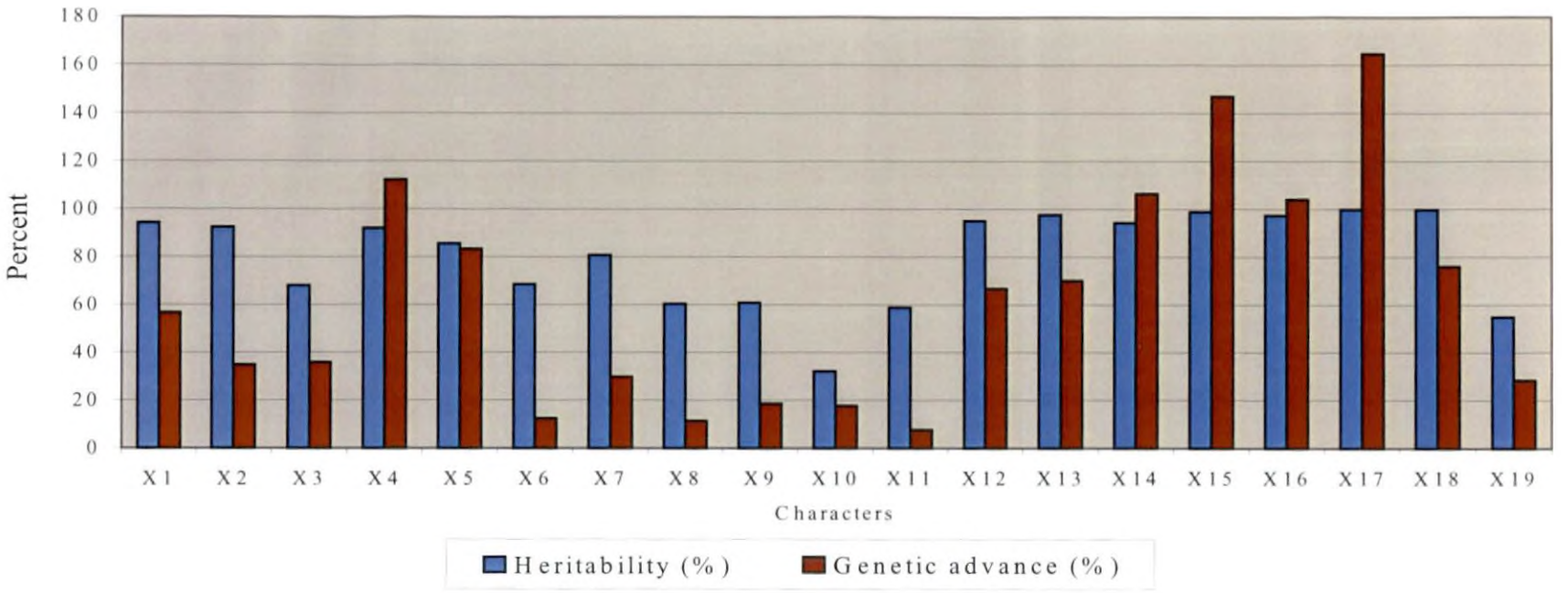


Fig. 2 Heritability and genetic advance for 19 characters in *B. hispida*

X1 – Vine length
X2 - Internodal length
X3 – Number of primary branches
X4 – Number of secondary branches
X5 – Root – Shoot length ratio
X6 – Days to first male flower
X7 – Node of first male flower
X8 – Days to first female flower
X8 – Days to first female flower
X9 – Node to first female flower
X10 – Sex ratio
X11 – Days to first fruit harvest
X12 – Fruit length
X13 – Fruit girth
X14 – Fruits per plant
X15 – Average fruit weight
X16 – Yield per plant
X17 – Seeds per plant
X18 – 1000 Seed weight
X19 – Mosaic incidence

(ii) Correlation among the yield component characters

Vine length was positively correlated with internodal length (0.4181) and number of primary and secondary branches (0.3310 and 0.6852 respectively).

Internodal length had positive correlation with number of secondary branches (0.3965).

A positive correlation was observed between number of primary branches and number of secondary branches (0.4514).

Number of secondary branches observed negative correlation with seeds per fruit (-0.3410).

Days to first male flower recorded high positive correlation with days to first female flower (0.8518), node to first female flower (0.2797) and days to first fruit harvest (0.7945). Days to first female flower also observed high positive correlation with days to first fruit harvest (0.9025).

Node to first male flower exhibited positive correlation with node to first female flower (0.7769) and fruits per plant (0.3101) and negative correlation with fruit girth (-0.3483), average fruit weight (-0.3366), seeds per fruit (-0.3767) and 1000-seed weight (-0.4989).

Node to first female flower had negative correlation with fruit length (-0.3388), average fruit weight (-0.3142) and 1000-seed weight (-0.3241).

Sex ratio was positively correlated with fruit girth (0.4219), average fruit weight (0.3032), seeds per fruit (0.4163) and 1000-seed weight (0.4562), while negatively correlated with fruits per plant (-0.5341).

Table 13. Phenotypic correlation coefficients among yield and its components in *B. hispida*

Characters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19
Vine length (X1)	1.0000																		
Internodal length (X2)	0.4181**	1.0000																	
Number of primary branches (X3)	0.3310*	-0.0792	1.0000																
Number of secondary branches (X4)	0.6852**	0.3965**	0.4514**	1.0000															
Root shoot ratio (X5)	-0.0994	0.1418	-0.0783	0.0910	1.0000														
Days to first male flower (X6)	-0.0332	0.1202	0.2226	0.1159	0.2367	1.0000													
Node to first male flower (X7)	0.1229	0.1170	0.0760	0.1255	0.2432	0.1699	1.0000												
Days to first female flower (X8)	0.0696	0.0690	0.2594	0.1303	0.2216	0.8518**	0.0330	1.0000											
Node to first female flower (X9)	0.0826	0.1576	0.0756	0.0492	0.1289	0.2797*	0.7769**	0.2427	1.0000										
Sex ratio (X10)	-0.0935	-0.1551	-0.0107	-0.2090	-0.0204	-0.0070	-0.1185	0.2236	0.1532	1.0000									
Days to first fruit harvest (X11)	0.1108	0.0844	0.3538*	0.1713	0.2039	0.7945**	-0.0017	0.9025**	0.2188	0.1462	1.0000								
Fruit length (X12)	0.1432	0.0964	-0.0069	-0.006	0.0929	-0.1650	-0.2480	0.0476	-0.3388*	0.1859	-0.0437	1.0000							
Fruit girth (X13)	-0.0905	-0.0196	-0.1017	-0.062	0.0407	-0.1570	-0.3483*	0.0971	-0.2111	0.4219**	0.0853	0.5884**	1.0000						
Fruits per plant (X14)	-0.0053	-0.1292	0.0648	0.1716	-0.1073	0.0112	0.3101*	-0.2714	0.2229	-0.5341**	-0.1770	-0.7103**	-0.6638**	1.0000					
Average fruit weight (X15)	-0.0014	0.1316	-0.0493	-0.053	0.2179	-0.1860	-0.3366*	0.0515	-0.3142*	0.3032*	0.0271	0.8286**	0.8528**	-0.7282**	1.0000				
Yield per plant (X16)	0.0373	0.0172	0.0441	-0.077	0.0637	-0.2580	-0.3074*	-0.0268	-0.2965*	0.2752	-0.0339	0.7893**	0.8165**	-0.5738**	0.9203**	1.0000			
Seeds per fruit (X17)	-0.2767	0.0175	-0.2120	-0.3410*	-0.0722	-0.1430	-0.3767*	0.1031	-0.1176	0.4163**	0.0598	0.3393*	0.7592**	-0.5323**	0.6313**	0.6264**	1.0000		
1000-seed weight (X18)	-0.1782	-0.0707	-0.0345	-0.2620	-0.0169	-0.0990	-0.4989**	0.1437	-0.3241*	0.4562**	0.0718	0.6835**	0.7910**	-0.7483**	0.8535**	0.7767**	0.6887**	1.0000	
Mosaic incidence (X19)	0.1841	0.0892	0.0734	0.2132	-0.0694	-0.0800	-0.0052	-0.0488	-0.0327	0.2185	-0.0809	0.2924*	0.0438	-0.0130	0.1905	0.2327	-0.0848	0.1515	1.0000

* Significant at 5 % ** Significant at 1%

Fruit length observed high positive correlation with fruit girth (0.5884), average fruit weight (0.8286), seeds per fruit (0.3393), 1000-seed weight (0.6835) and mosaic incidence (0.2924), while it showed negative correlation with fruits per plant (-0.7103).

Fruit girth had negative correlation with fruits per plant (-0.6638) and positive correlation with average fruit weight (0.8528), seeds per fruit (0.7592) and 1000-seed weight (0.7910).

Fruits per plant recorded negative correlation with most of the characters, the highest being with 1000-seed weight (-0.7483). Average fruit weight exhibited high positive correlation with seeds per fruits (0.6313) and 1000-seed weight (0.8535).

Seeds per fruit was positively correlated with 1000-seed weight (0.6887).

(B) Genotypic correlation

(i) Correlation between yield and other characters

High positive correlation was observed between yield per plant and sex ratio (0.4433), fruit length (0.8135), fruit girth (0.8354), average fruit weight (0.9322), seeds per fruit (0.6362), 1000-seed weight (0.7900) and mosaic incidence (0.3351), whereas days to first female flower (-0.2812), node to first male and female flower (-0.3199 and -0.3517 respectively) and fruits per plant (-0.6149) exhibited a high negative correlation.

(ii) Correlation among the yield component characters

Vine length had high positive correlation with internodal length (0.3938), number of primary and secondary branches (0.4357 and 0.7005 respectively) and mosaic incidence (0.2947), whereas seeds per fruit (-0.2860) was negatively correlated.

Table 14. Genotypic correlation coefficients among yield and its components in *B. hispida*

Characters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19
Vine length (X1)	1.0000																		
Internodal length (X2)	0.3938	1.0000																	
Number of primary branches (X3)	0.4357	-0.1102	1.0000																
Number of secondary branches (X4)	0.7005	0.3909	0.5774	1.0000															
Root shoot ratio (X5)	-0.1020	0.1683	-0.1870	0.0749	1.0000														
Days to first male flower (X6)	-0.0619	0.1399	0.1560	0.1388	0.3039	1.0000													
Node to first male flower (X7)	0.1478	0.1609	0.1540	0.1531	0.3532	0.1520	1.0000												
Days to first female flower (X8)	0.0802	0.1057	0.2811	0.1550	0.3181	0.9137	-0.1517	1.0000											
Node to first female flower (X9)	0.0517	0.1924	0.1776	0.0426	0.2310	0.2211	0.9236	-0.0263	1.0000										
Sex ratio (X10)	-0.2372	-0.2679	-0.0571	-0.4114	-0.0658	-0.2288	-0.3866	0.1401	-0.0272	1.0000									
Days to first fruit harvest (X11)	0.1356	0.1712	0.4946	0.2441	0.2830	0.9547	-0.0956	1.0059	0.0614	0.1197	1.0000								
Fruit length (X12)	0.1489	0.0967	0.0253	-0.0108	0.0995	-0.1548	-0.2503	0.0890	-0.4472	0.4895	-0.0178	1.0000							
Fruit girth (X13)	-0.1054	-0.0312	-0.0751	-0.0719	0.0574	-0.1714	-0.3961	0.1312	-0.3143	0.7784	0.1252	0.5950	1.0000						
Fruits per plant (X14)	-0.0070	-0.1403	0.0781	0.1732	-0.1050	0.0299	0.4064	-0.2858	0.3562	-0.9420	-0.1992	-0.7398	-0.6867	1.0000					
Average fruit weight (X15)	-0.0060	0.1309	-0.0302	-0.0611	0.2442	-0.2060	-0.3745	0.0717	-0.4165	0.5507	0.0517	0.8398	0.8539	-0.7511	1.0000				
Yield per plant (X16)	0.0418	0.0113	0.0541	-0.0953	0.0711	-0.2812	-0.3199	-0.0118	-0.3517	0.4433	-0.0023	0.8135	0.8354	-0.6149	0.9322	1.0000			
Seeds per fruit (X17)	-0.2860	0.0169	-0.258	-0.3552	-0.0769	-0.1774	-0.4205	0.1261	-0.1613	0.7341	0.0712	0.3484	0.7675	-0.5465	0.6348	0.6362	1.0000		
1000-seed weight (X18)	-0.1861	-0.0780	-0.0484	-0.2731	-0.0146	-0.1293	-0.5575	0.1727	-0.4286	0.8128	0.0821	0.7022	0.7991	-0.7680.	0.8582	0.7900	0.6887	1.0000	
Mosaic incidence (X19)	0.2947	0.1573	0.1021	0.2725	-0.0463	-0.1601	-0.1024	-0.0431	-0.0350	0.2133	-0.1727	0.5351	0.0679	-0.0751	0.2730	0.3351	-0.1178	0.2018	1.0000

Internodal length observed positive correlation with number of secondary branches (0.3909).

Number of primary branches exhibited positive correlation with number of secondary branches (0.5774), days to first female flower (0.2811) and days to first fruit harvest (0.4946). Number of secondary branches had negative correlation with sex ratio (-0.4114) and seeds per fruit (-0.3552).

Root shoot length ratio was positively correlated with days to male and female flower (0.3039 and 0.3181 respectively), node to male flower (0.3532) and days to first fruit harvest (0.2830).

Days to first male flower observed high positive correlation with days to first female flower (0.9137) and days to first fruit harvest (0.9547). Days to first female flower showed negative correlation with fruits per plant (-0.2858).

Node to first male flower had positive correlation with node to first female flower (0.9236) and fruits per plant (0.4064), while negatively correlated with sex ratio (-0.3866), fruit girth (-0.3961), average fruit weight (-0.3745), seeds per fruit (-0.4205) and 1000-seed weight (-0.5575).

Fruit length (-0.4472), fruit girth (-0.3143), average fruit weight (-0.4165) and 1000-seed weight (-0.4286) were negatively correlated with node to first female flower, while fruits per plant (0.3562) was positively correlated with node to first female flower.

Sex ratio observed positive correlation with several characters like fruit length (0.4895), fruit girth (0.7784), average fruit weight (0.5507), seeds per fruit (0.7341) and 1000-seed weight (0.8128) and negative correlation with fruits per plant (-0.9420).

Fruit length had high positive correlation with fruit girth (0.5950), average fruit weight (0.8398), seeds per fruit (0.3484), 1000-seed weight (0.7022) and mosaic incidence (0.5351) and high negative correlation with fruits per plant (-0.7398). Fruit girth had high positive correlation with average fruit weight (0.8539), seeds per fruit (0.7675) and 1000-seed weight (0.7991), while negatively correlated with fruits per plant (-0.6867).

Fruits per plant recorded negative correlation with most of the characters, the highest being with 1000-seed weight (-0.7680), followed by average fruit weight (-0.7511). Average fruit weight exhibited high positive correlation with seeds per fruits (0.6348) and 1000-seed weight (0.8582).

Seeds per fruit was positively correlated with 1000-seed weight (0.6887).

(C) Environmental correlation

Environmental correlation coefficients were found to be negligible among yield and its component characters, except for the correlation between fruits per plant and yield per plant (0.4217).

4.2.5 Path Analysis

In path analysis, the genotypic correlation coefficients among yield and its component characters were partitioned into direct and indirect contribution of each character to fruit yield (Table 16). Vine length, days to first female flower, node to first female flower, sex ratio, fruit length, fruit girth, fruits per plant, average fruit weight, seeds per fruit and mosaic incidence were selected for path coefficient analysis.

Fruit length exhibited the highest positive direct effect on fruit yield (0.549), followed by average fruit weight (0.516) and fruits

Table 15. Environmental correlation coefficients among yield and its components in *B. hispida*

Characters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19
Vine length (X1)	1.0000																		
Internodal length (X2)	0.7615	1.0000																	
Number of primary branches (X3)	-0.1292	0.0523	1.0000																
Number of secondary branches (X4)	0.4885	0.4623	-0.0319	1.0000															
Root shoot ratio (X5)	-0.0845	-0.0745	0.2997	0.2273	1.0000														
Days to first male flower (X6)	0.1216	0.0580	0.3655	0.0364	0.0190	1.0000													
Node to first male flower (X7)	-0.0561	-0.1824	-0.1536	-0.0513	-0.3027	0.2308	1.0000												
Days to first female flower (X8)	0.0602	-0.0568	0.2227	0.0836	-0.0287	0.7482	0.5022	1.0000											
Node to first female flower (X9)	0.2869	0.0773	-0.1092	0.0977	-0.1590	0.3898	0.4721	0.6556	1.0000										
Sex ratio (X10)	0.1862	-0.0392	0.0344	0.0631	0.0454	0.2180	0.2184	0.3119	0.3207	1.0000									
Days to first fruit harvest (X11)	0.0641	-0.2363	0.113	-0.0441	0.013	0.5239	0.2279	0.7509	0.4530	0.1780	1.0000								
Fruit length (X12)	0.0434	0.0932	-0.2165	0.0601	0.0373	-0.3192	-0.2944	-0.1414	0.0091	-0.4657	-0.2132	1.0000							
Fruit girth (X13)	0.2838	0.2378	-0.4674	0.1413	-0.2036	-0.1914	0.0517	-0.0367	0.3242	-0.1180	-0.0975	0.4451	1.0000						
Fruits per plant (X14)	0.0227	0.0277	0.0168	0.1524	-0.1443	-0.0966	-0.4306	0.3741	-0.3174	-0.0743	-0.1882	-0.1795	-0.1222	1.0000					
Average fruit weight (X15)	0.1650	0.2196	-0.4015	0.1609	-0.1649	-0.2662	-0.0427	-0.0555	0.1299	-0.0863	-0.1774	0.6070	0.8384	-0.1079	1.0000				
Yield per plant (X16)	-0.0691	0.1469	0.0004	0.2877	-0.0193	-0.314	-0.3366	-0.1767	-0.2569	0.2031	-0.3134	0.1752	0.0641	0.4217	0.3151	1.0000			
Seeds per fruit (X17)	0.1341	0.2062	0.0584	-0.0329	-0.1384	0.303	0.1212	0.3858	0.6060	-0.0219	0.3753	-0.0738	0.2519	-0.2906	0.1342	-0.4256	1.0000		
1000-seed weight (X18)	0.2086	0.3610	0.2236	0.0119	-0.2091	0.347	0.0919	0.3634	0.3681	-0.1406	0.3268	-0.0595	0.3163	-0.2827	0.2286	-0.3664	0.8440	1.0000	
Mosaic incidence (X19)	-0.1720	-0.1242	0.0289	0.1023	-0.1478	0.0482	0.2145	-0.0567	-0.0297	0.2330	0.0400	-0.6345	-0.0576	0.2576	-0.1486	-0.1174	0.1771	0.0701	1.0000

Table 16. Direct and indirect effect of selected yield components on fruit yield in *B. hispida*

Character	Vine length (cm)	Days to first female flower	Node to first female flower	Sex ratio	Fruit length (cm)	Fruit girth (cm)	Fruits per plant	Average fruit weight (kg)	Seeds per fruit	Mosaic incidence	Correlation with yield
Vine length (cm)	**0.079**	-0.003	0.002	0.012	0.082	-0.027	-0.003	-0.003	-0.044	-0.029	0.0418
Days to first female flower	0.006	**-0.038**	-0.001	0.007	0.049	0.033	-0.129	0.037	0.020	0.004	-0.0118
Node to first female flower	0.004	0.001	**0.046**	-0.001	0.246	-0.080	0.161	-0.215	-0.025	0.003	-0.3517
Sex ratio	-0.019	-0.005	-0.001	**0.050**	0.269	0.198	-0.425	0.284	0.114	-0.021	0.4433
Fruit length (cm)	0.012	-0.003	-0.021	0.024	**0.549**	0.152	-0.334	0.433	0.054	-0.053	0.8135
Fruit girth (cm)	-0.008	-0.005	-0.014	0.039	0.327	**0.255**	-0.310	0.440	0.119	-0.007	0.8354
Fruits per plant	-0.001	0.011	0.016	-0.047	-0.406	-0.175	**0.451**	-0.387	-0.085	0.007	-0.6149
Average fruit weight (kg)	0.000	-0.003	-0.019	0.027	0.461	0.218	-0.339	**0.516**	0.098	-0.027	0.9322
Seeds per fruit	-0.002	-0.005	-0.007	0.036	0.191	0.196	-0.247	0.327	**0.155**	0.012	0.6362
Mosaic incidence	0.023	0.002	-0.002	0.011	0.294	0.017	-0.034	0.141	-0.018	**-0.099**	0.3351

Residue = 0.2483521
Direct effects- diagonal elements
Indirect effects- off diagonal elements

per plant (0.451). The direct effects of vine length, node to first female flower and sex ratio were negligible, whereas days to first female flower and mosaic incidence exerted small and negative direct effect on yield.

Indirect effects through fruit length and average fruit weight were consistently high signifying the importance of these characters. Thus in the case of vine length (0.082), positive correlation with yield was mainly due to their positive indirect effects through fruit length. At the same time sex ratio, fruit girth and seeds per fruit made high positive correlation with yield due to their positive indirect effects through average fruit weight (0.284, 0.440 and 0.327 respectively). High negative correlation of fruits per plant (-0.406) with yield was due to high negative indirect effect through fruit length, while in node to first female flower, high negative correlation was through average fruit weight (-0.215). Days to first female flower (-0.129) exhibited negative correlation with yield due to negative indirect effect through fruits per plant. In the case of mosaic incidence (-0.099), the correlation was mainly built by the direct as well as indirect negative effect.

4.2.6 Selection Index

A discriminant function analysis was carried out for isolating superior landraces. Selection index involving characters *viz.*, vine length (X_1), days to first female flower (X_2), node to first female flower (X_3), sex ratio (X_4), fruit length (X_5), fruit girth (X_6), fruits per plant (X_7), average fruit weight (X_8), yield per plant (X_9), seeds per fruit (X_{10}) and mosaic incidence (X_{11}) were selected for the analysis.

The selection index worked out was as follows :

$$I = 0.922386\ X_1 + 0.572064\ X_2 - 2.20051\ X_3 - 4.56438\ X_4 - 0.239115\ X_5 + 0.581712\ X_6 - 12.0909\ X_7 - 9.37227\ X_8 + 7.41824\ X_9 + 0.994694\ X_{10} + 1.44769\ X_{11}$$

The scores obtained for the landraces based on the selection index were given in Table 17.

Based on selection index, BH 15 (4477.91) ranked first, followed by BH 23 (4089.28) and BH 5 (3886.23) (Plates 10, 11 and 12). The minimum scores were obtained for BH 8 (703.56) and BH 11 (1062.58).

4.2.7 Mahalanobis's D^2 Analysis

Following Mahalanobis's D^2 statistic, the 25 landraces of *B. hispida* were subjected to cluster analysis, based on eleven characters *viz.*, vine length, days to first female flower, node to first female flower, sex ratio, fruit length, fruit girth, fruits per plant, average fruit weight, seeds per fruit and mosaic incidence.

The 25 landraces fell under seven clusters. The clustering pattern is furnished in Table 18. Cluster I was the largest with 8 landraces, followed by cluster II with 4 landraces. Cluster III, IV, V and VI had three landraces each while cluster VII had one landrace.

The cluster means of the eleven characters are presented in Table 19. Cluster VII (BH 8) comprised of landrace with smallest fruits, highest fruits per plant, shorter vine length, earliness in flowering and lowest sex ratio, yield and seeds per fruit. Cluster V consisted of landraces with medium sized fruits with highest mosaic resistance and seeds per fruit. Cluster III had the highest average fruit weight and yield per plant. Cluster VI comprised of large sized fruits with high mosaic incidence.

The average inter and intra cluster distances are presented in Table 20. The cluster diagram is shown in Fig 3.

The intracluster distance was highest for cluster VI (549.72), followed by clusters II and IV (456.32 and 417.05 respectively).

Table 17. Selection indices on the landraces of *B. hispida* arranged in descending order

Rank	Landraces	Selection index
1	BH 15	4477.91
2	BH 23	4089.28
3	BH 5	3886.23
4	BH 6	3757.87
5	BH 16	3698.43
6	BH 1	3606.98
7	BH 2	3247.58
8	BH 9	2939.54
9	BH 3	2562.93
10	BH 19	2381.16
11	BH 12	2335.17
12	BH 13	2206.18
13	BH 22	2101.19
14	BH 18	1949.96
15	BH 4	1875.33
16	BH 17	1860.67
17	BH 7	1780.06
18	BH 25	1675.34
19	BH 20	1629.58
20	BH 21	1434.38
21	BH 14	1328.43
22	BH 24	1327.71
23	BH 10	1107.61
24	BH 11	1062.58
25	BH 8	703.56

Plate 10. BH 15 - a landrace ranked first based on selection index

Plate 11. BH 23 - a landrace ranked second based on selection index

Plate 12. BH 5 - a landrace ranked third based on selection index

Table 18. Clustering pattern of twenty five landraces of *B. hispida*

Cluster No.	Number of landraces	Landraces
I	8	BH 4, BH 10, BH 11, BH 14, BH 20, BH 21, BH 22, BH 24
II	4	BH 2, BH 5, BH 9, BH 19
III	3	BH 6, BH 15, BH 16
IV	3	BH 12, BH 13, BH 17
V	3	BH 1, BH 3, BH 23
VI	3	BH 7, BH 18, BH 25
VII	1	BH 8

Table 19. Cluster means of eleven biometric characters in *B. hispida*

Cluster	Vine length (cm)	Days to first female flower	Node to first female flower	Sex ratio	Fruit length (cm)	Fruit girth (cm)	Fruits per plant	Average fruit weight (kg)	Yield per plant (kg)	Seeds per fruit	Mosaic incidence (V.I.)
I	578.44	55.93	22.59	10.12	24.69	37.07	5.42	1.51	5.77	225.50	47.81
II	438.00	57.03	21.94	13.84	38.95	63.58	2.72	5.39	13.16	955.25	51.25
III	483.17	56.90	22.43	13.21	43.13	66.85	2.46	7.93	17.70	1352.67	53.33
IV	540.67	58.83	26.04	12.61	37.85	49.25	3.25	2.89	8.78	559.33	51.67
V	529.17	55.45	21.46	12.40	39.86	68.93	2.38	6.78	14.36	1556.83	45.83
VI	706.00	55.45	20.28	10.51	55.28	47.49	2.56	6.92	15.02	186.00	57.50
VII	388.50	46.25	27.63	8.59	13.65	22.20	8.00	0.28	1.99	73.50	47.50

Table 20. Average inter and intracluster distances in the landraces of *B. hispida*

Cluster	I	II	III	IV	V	VI	VII
I	397.30	10662.02	23094.50	2580.15	32854.00	1321.92	1322.18
II		456.32	2890.19	3354.86	6621.94	13099.44	17412.36
III			286.55	11357.85	1243.44	25733.91	32266.60
IV				417.05	18283.41	4198.01	6413.52
V					401.25	36516.94	43823.68
VI						549.72	1997.78
VII							0.00

Diagonal elements- intracluster values

Off diagonal elements- intercluster values

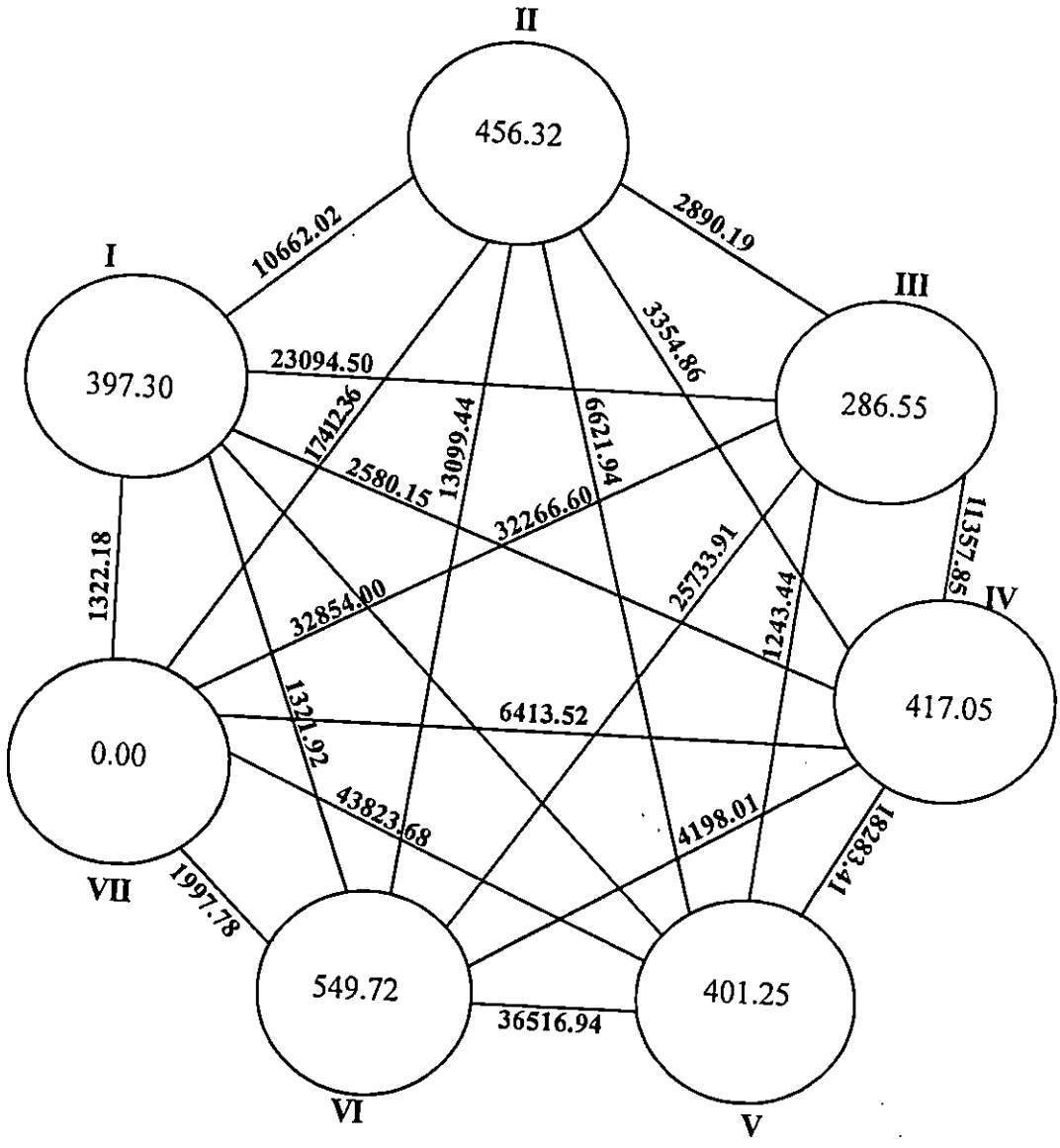


Fig. 3 Cluster diagram of 25 landraces of *B. hispida*

The highest intercluster distance was observed for clusters V and VII (43823.68), followed by clusters V and VI (36516.94) and clusters I and V (32854.00). The genetic distance (D) between clusters I, IV, VI and VII were largest with cluster V. The minimum intercluster distance was observed between clusters III and V (1243.44) indicating a close relationship among the landraces included.

4.3 MOLECULAR CHARACTERIZATION (RAPD)

RAPD (Random amplified polymorphic DNA) analysis was used to characterize genetic variability and relationships among twenty five landraces of *B. hispida* from diverse ecosystems.

4.3.1 Isolation of Genomic DNA

Etiolated 15-20 days old seedlings were used to extract genomic DNA from various landraces of *B. hispida* following the protocol modified from that of Murray and Thompson (1980).

The DNA yield for twenty five landraces of *B. hispida* ranged from 0.21 to 3.90. The purity of DNA (A_{260} / A_{280} ratio) (Table 21) ranged from 1.40 to 2.08 $\mu g\ \mu l^{-1}$.

4.3.2 Testing the Quality of DNA

For RAPD profile analysis, the DNA should be free of RNA and protein. Moreover, it needs intact, unsheared DNA sample of sufficient quantity. To access the quality, all the genomic DNA samples were run on 0.7 per cent agarose gel and the gel was stained with ethidium bromide and bands appeared in the gel were visualized, using ultraviolet transilluminator.

Table 21. Quantitative and qualitative characters of DNA isolated from landraces of *B. hispida* using modified Murray and Thompson method

Sl. No.	Landrace No.	260 nm	280 nm	Ratio $\frac{260}{280}$	DNA yield ($\mu g\ \mu l^{-1}$)
1	BH 1	0.031	0.016	1.94	0.93
2	BH 2	0.010	0.006	1.67	0.30
3	BH 3	0.007	0.004	1.75	0.21
4	BH 4	0.026	0.016	1.63	0.78
5	BH 5	0.080	0.044	1.81	2.40
6	BH 6	0.020	0.013	1.54	0.60
7	BH 7	0.034	0.019	1.79	1.02
8	BH 8	0.028	0.020	1.40	0.84
9	BH 9	0.027	0.014	1.92	0.81
10	BH 10	0.033	0.018	1.83	0.99
11	BH 11	0.050	0.031	1.61	1.50
12	BH 12	0.130	0.089	1.46	3.90
13	BH 13	0.093	0.055	1.70	2.79
14	BH 14	0.030	0.016	1.87	0.90
15	BH 15	0.022	0.013	1.69	0.66
16	BH 16	0.052	0.025	2.08	1.56
17	BH 17	0.104	0.061	1.70	3.12
18	BH 18	0.013	0.008	1.63	0.39
19	BH 19	0.026	0.013	2.00	0.78
20	BH 20	0.046	0.024	1.90	1.38
21	BH 21	0.093	0.047	1.90	2.79
22	BH 22	0.065	0.032	2.03	1.95
23	BH 23	0.032	0.019	1.68	0.96
24	BH 24	0.020	0.011	1.80	0.60
25	BH 25	0.012	0.007	1.71	0.36

4.3.3 Polymerase Chain Reaction (PCR)

Polymerase chain reaction, standardized for the amplification of the DNA from *Cucumis melo* L. (Staub *et al.*, 2000) was used for twenty five landraces of *B. hispida*. Forty decamer primers of series A and B were screened for their efficiency using the DNA isolated from landrace BH 1 as the representative sample. Out of the 40 decamer primers, twenty nine yielded amplification products. The total number of bands, number of intense bands and number of faint bands produced by the primers are given in Table 22.

A total of 83 RAPDs (average 2.08 bands per primer) were generated by the 29 primers, of which 92.77 per cent were polymorphic (77 bands) and six were monomorphic. Five primers showed high level of polymorphism. The maximum number of RAPDs (8 bands) were produced by primer OPA-13, followed by OPA-07 (7 bands), OPA-01 (5 bands), OPA-10 (5 bands) and OPB-10 (4 bands).

For further PCR amplification, three primers were selected (OPA-01, OPA-07 and OPA-13) based on their performance in DNA amplification and production of highest number of bands as well as intense bands (Table 23). Also the selected primers were consistent and heritable when checked for their reproducibility. Hence these were used for DNA amplification of 25 landraces of *B. hispida*. Data obtained from the three primers that give reproducible bands were used for statistical analysis.

The RAPD profile generated by three selected primers *viz.*, OPA-01, OPA-07 and OPA-13 were shown in Plates 13 to 15 and Figures 4 to 6. A total of 20 scorable bands (average of 6.66 bands per primer) were generated of which 2 were monomorphic and rest, 18 were

Table 22. Primer associated banding patterns in DNA sample of landrace BH 1

Sl. No.	Primers	Total number of bands	Number of intense bands	Number of faint bands
1	OPA-01	5	5	0
2	OPA-02	4	2	2
3	OPA-03	0	0	0
4	OPA-04	3	2	1
5	OPA-05	3	1	2
6	OPA-06	0	0	0
7	OPA-07	7	6	1
8	OPA-08	3	2	1
9	OPA-09	0	0	0
10	OPA-10	5	4	1
11	OPA-11	1	1	0
12	OPA-12	0	0	0
13	OPA-13	8	7	1
14	OPA-14	2	1	1
15	OPA-15	2	1	1
16	OPA-16	0	0	0
17	OPA-17	1	0	1
18	OPA-18	0	0	0
19	OPA-19	0	0	0
20	OPA-20	2	2	0
21	OPB-01	3	2	1
22	OPB-02	0	0	0
23	OPB-03	3	2	1
24	OPB-04	3	2	1
25	OPB-05	1	1	0
26	OPB-06	2	1	1
27	OPB-07	3	2	1
28	OPB-08	1	1	0
29	OPB-09	0	0	0
30	OPB-10	4	4	0
31	OPB-11	3	1	2
32	OPB-12	0	0	0
33	OPB-13	3	1	2
34	OPB-14	2	0	2
35	OPB-15	0	0	0
36	OPB-16	3	1	2
37	OPB-17	2	1	1
38	OPB-18	1	1	0
39	OPB-19	1	1	0
40	OPB-20	2	1	1

Table 23. Nucleotide sequences of primers and total number of informative RAPD markers amplified with them in the landraces of *B. hispida* used in this study

Sl. No.	Primer	Sequence (5'-3' direction)	Number of informative RAPD markers
1	0PA-01	CAGGCCCTTC	5
2	0PA-07	GAAACGGGTG	7
3	OPA-13	CAGCACCCAC	8

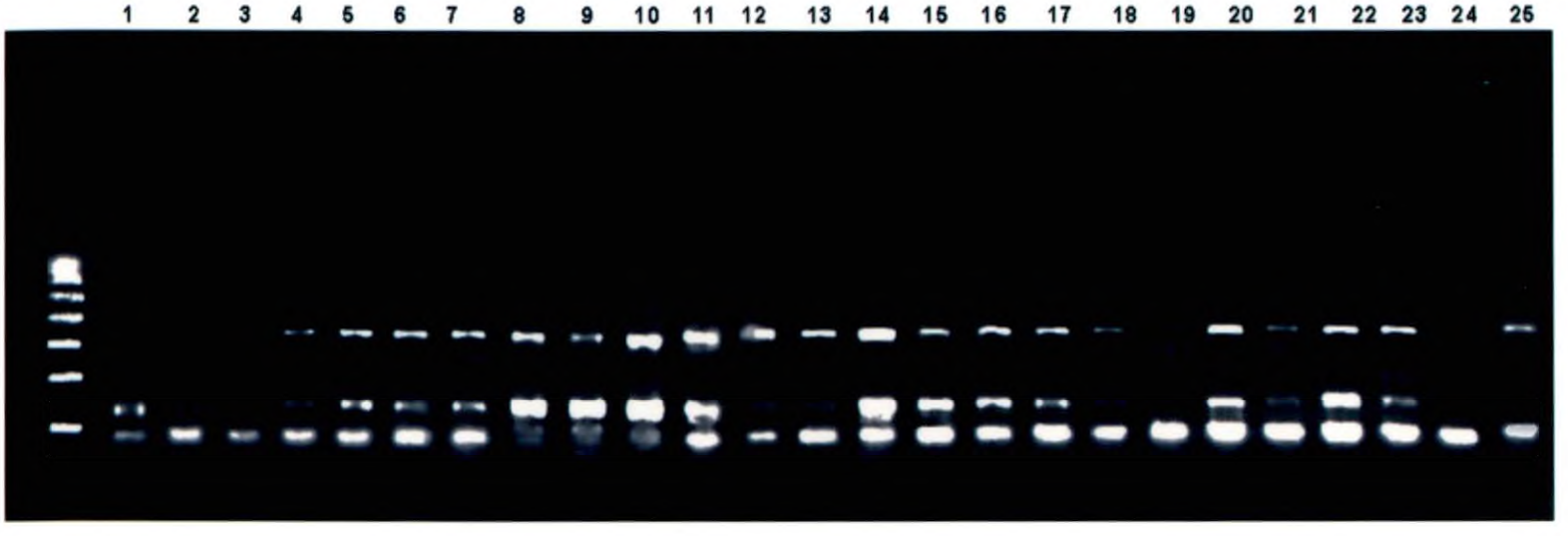


Plate 13. Amplification profiles of the DNA of 25 landraces of *B. hispida* using the primer OPA-01

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Fig. 4. Representation of amplification profile of the DNA of twenty five landraces of *B. hispida* using the primer OPA-01

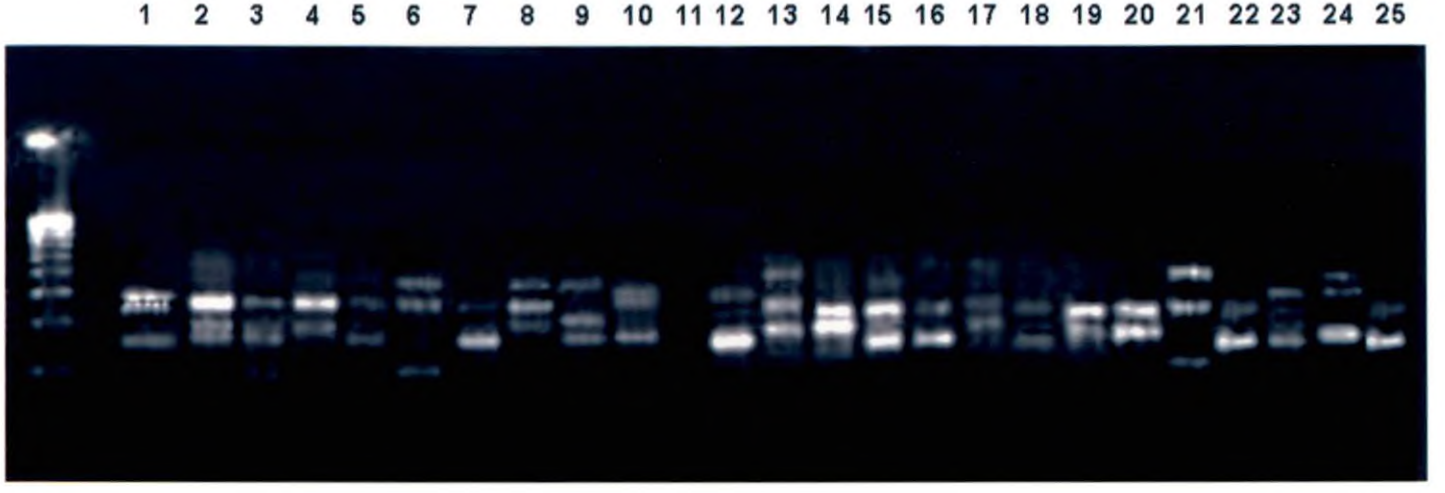


Plate 14. Amplification profiles of the DNA of 25 landraces of *B. hispida* using the primer OPA-07

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
-	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-
+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-
+	+	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	+	+	-	+	+
+	+	+	+	-	-	-	-	-	+	-	+	-	+	-	-	+	+	+	+	-	+	-	-	-
-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-

Fig. 5. Representation of amplification profile of the DNA of twenty five landraces of *B. hispida* using the primer OPA-07

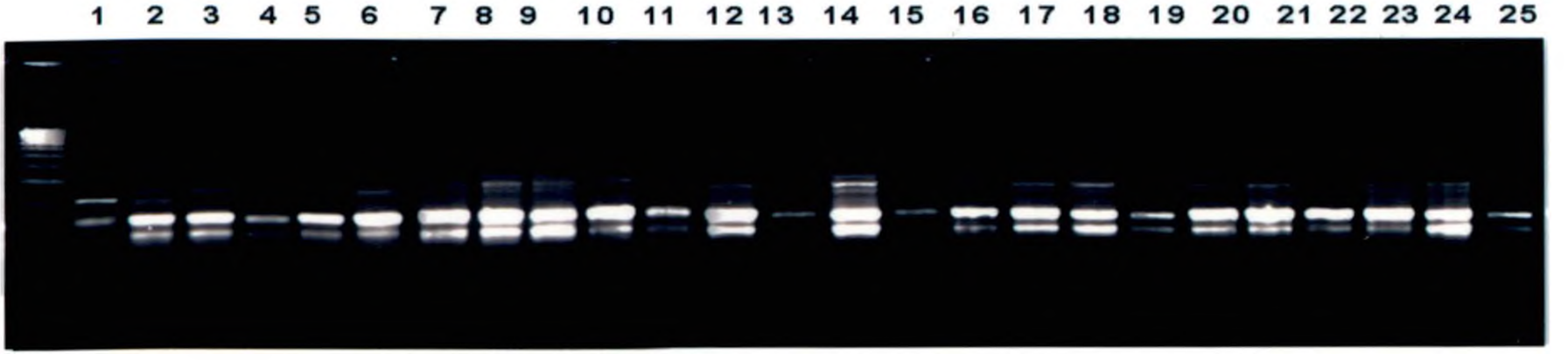


Plate 15. Amplification profiles of the DNA of 25 landraces of *B. hispida* using the primer OPA-13

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
-	+	+	-	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-	+	+	-	+	+	-
+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	+	-	+	+	-
-	-	-	-	-	+	-	+	+	+	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-
-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+
-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+-	+	+	+

Fig. 6. Representation of amplification profile of the DNA of twenty five landraces of *B. hispida* using the primer OPA-13

polymorphic (90.0 %). The number of bands ranged from 1 to 8 with an average of 3 per primer.

The primer OPA-07 was unique as it could distinguish maximum polymorphism among the landraces tested. The highest number of scorable bands was given by OPA-13 of which one of the bands produced was monomorphic. The primer OPA-01 produced five scorable bands of which one band was monomorphic for all the landraces.

4.3.4 Data Analysis

The banding pattern from RAPD analysis for each primer was scored by visual observation. Reproducible bands were scored for their presence (+) or absence (-) for all the landraces of *B. hispida* studied. From this RAPD marker data, Jaccard's similarity coefficient values were calculated for each pair-wise comparison between landraces and a similarity coefficient matrix was constructed (Table 24). This matrix was subjected to UPGMA to generate a dendrogram for the twenty five landraces (Fig.7). All computing were carried out using NTSYS-pc software.

Overall similarity indices ranged from 0.14 to 1.00. Cluster analysis revealed that at about 0.35 similarity coefficient, the twenty five landraces of *B. hispida* grouped into two clusters. Landraces with morphologically distinct smooth and waxy textured fruits grouped into two major clusters with an exception of BH 19 falling in the first cluster.

At 0.51 similarity coefficient, the smooth textured group got differentiated from the exceptional landrace with waxy textured fruits (BH 19). Landraces with smooth textured fruits (BH 7, BH 17, BH 20 and BH 25) again grouped into 2 with two members each at 59 per cent similarity. This grouping was in concordance with their average fruit

Table 24. Similarity matrix for the twenty five landraces of *B. hispida* generated using RAPD primers

X	BH1	BH2	BH3	BH4	BH5	BH6	BH7	BH8	BH9	BH10	BH11	BH12	BH13	BH14	BH15	BH16	BH17	BH18	BH19	BH20	BH21	BH22	BH23	BH24	BH25
BH 1	1.00																								
BH 2	0.40	1.00																							
BH 3	0.33	0.57	1.00																						
BH 4	0.14	0.37	0.71	1.00																					
BH 5	0.28	0.50	0.85	0.62	1.00																				
BH 6	0.33	0.57	0.71	0.50	0.62	1.00																			
BH 7	0.30	0.50	0.42	0.25	0.37	0.42	1.00																		
BH 8	0.22	0.27	0.50	0.36	0.45	0.50	0.44	1.00																	
BH 9	0.20	0.36	1.00	0.45	0.44	0.40	0.40	0.42	1.00																
BH 10	0.22	0.27	0.50	0.36	0.60	0.50	0.44	0.80	0.42	1.00															
BH 11	0.28	0.50	0.85	0.62	0.70	0.62	0.37	0.45	0.44	0.60	1.00														
BH 12	0.25	0.44	0.55	0.40	0.50	0.55	0.33	0.70	0.43	0.54	0.50	1.00													
BH 13	0.25	0.33	0.50	0.50	0.42	0.50	0.16	0.20	0.40	0.20	0.42	0.22	1.00												
BH 14	0.22	0.27	0.50	0.36	0.45	0.50	0.44	0.80	0.42	0.80	1.00	0.54	0.20	1.00											
BH 15	0.25	0.14	0.50	0.50	0.42	0.28	0.16	0.33	0.40	0.33	0.42	0.22	0.50	0.33	1.00										
BH 16	0.20	0.36	0.45	0.33	0.54	0.45	0.40	0.72	0.46	0.72	0.54	0.80	0.18	0.58	0.18	1.00									
BH 17	0.35	0.44	0.55	0.40	0.50	0.55	0.70	0.70	0.40	0.70	0.50	0.77	0.22	0.70	0.22	0.80	1.00								
BH 18	0.22	0.27	0.50	0.36	0.45	0.50	0.44	0.80	0.42	0.80	0.45	0.54	0.20	0.70	0.33	0.58	0.70	0.35							
BH 19	0.30	0.42	0.47	0.37	0.50	0.37	0.50	0.40	0.56	0.40	0.50	0.44	0.14	0.55	0.33	0.36	0.44	0.55	1.00						
BH 20	0.35	0.30	0.55	0.40	0.50	0.55	0.50	0.89	0.40	0.88	0.50	0.60	0.22	0.88	0.37	0.63	0.77	0.88	0.44	1.00					
BH 21	0.25	0.44	0.45	0.40	0.50	0.55	0.50	0.70	0.80	0.70	1.00	0.77	0.22	1.00	0.22	0.80	0.50	0.70	0.44	0.77	1.00				
BH 22	0.50	0.50	0.46	0.42	0.57	0.42	0.60	0.44	0.40	0.44	0.57	0.50	0.16	0.44	0.40	0.40	0.50	0.44	0.80	0.50	0.50	1.00			
BH 23	0.25	1.00	0.55	0.40	0.50	0.55	0.50	0.70	0.80	0.70	0.50	0.77	0.22	0.70	0.22	0.80	0.33	0.70	0.44	0.77	0.46	0.50	1.00		
BH 24	0.22	0.40	0.50	0.36	0.45	0.50	0.44	0.60	0.72	0.63	1.00	0.88	0.20	1.00	0.20	0.90	0.88	0.63	0.40	0.70	0.88	0.44	0.88	1.00	
BH 25	0.36	0.60	0.40	0.48	0.42	0.40	0.45	0.43	0.60	0.43	0.42	0.47	0.40	0.43	0.40	0.40	0.47	0.43	0.50	0.47	0.64	0.64	0.47	0.43	1.00

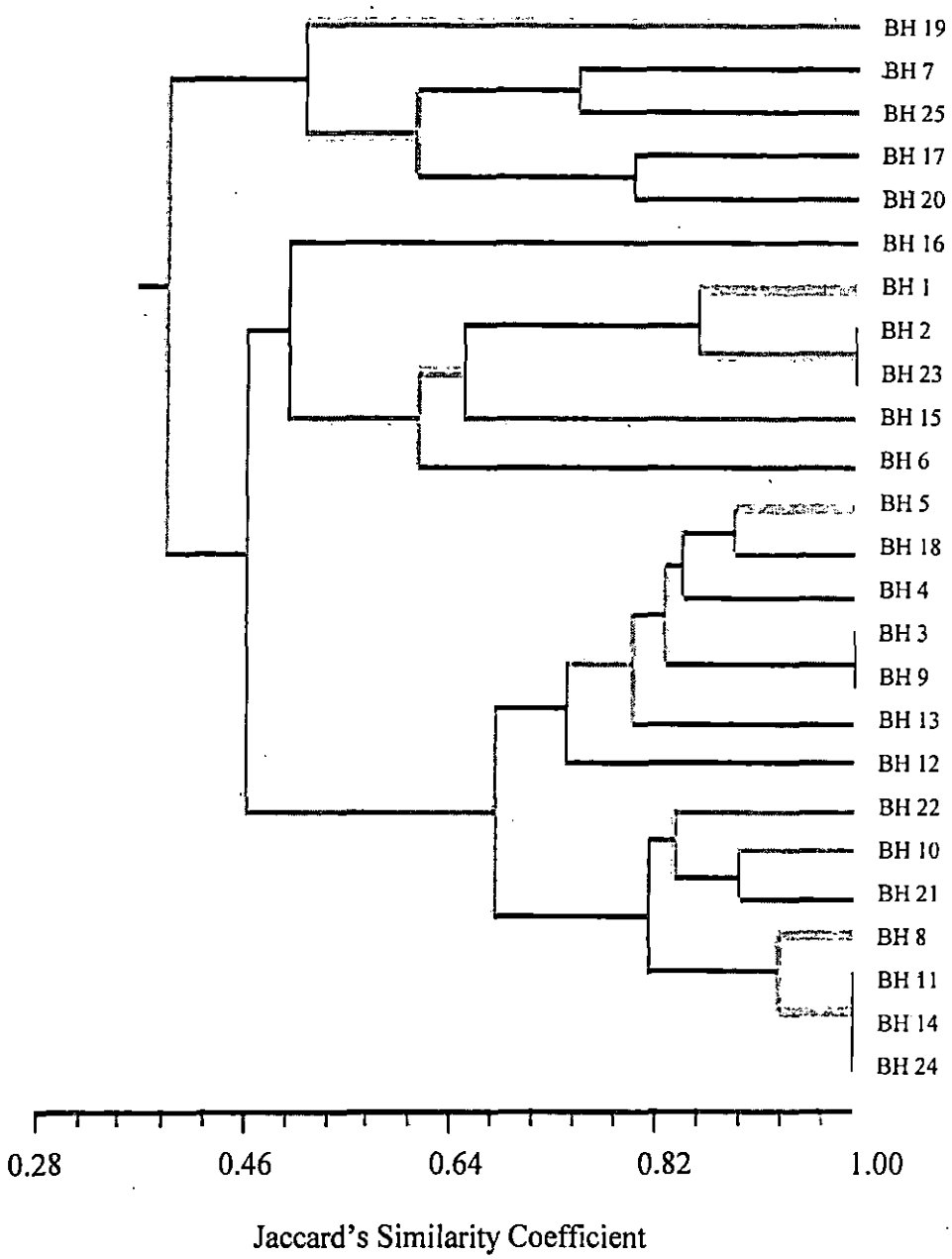


Fig 7. Dendrogram for twenty five landraces of *B. hispida* based on data from RAPD primers

weight. BH 7 showed 74.8 per cent similarity with BH 25 while BH 17 showed 80.2 per cent similarity with BH 20.

Landraces with waxy textured fruits could be split into two subclusters of 6 and 14 members respectively at 0.46 similarity coefficient.

Among waxy textured fruits, landrace BH 16 stood out from the rest of the group at 49.6 per cent similarity.

At 60.7 per cent similarity, first subcluster of landraces with waxy texture was again grouped into two with 4 members (BH 1, BH 3, BH 15 and BH 23) forming one cluster while landrace BH 6 remained distinct. Cluster with four members were further divided into two subgroups at 65.8 per cent similarity. BH 1, BH 2 and BH 23 formed one group while BH 15 remained distinct. At 85.6 per cent similarity, BH 1 got distinct from BH 2 and BH 23. BH 2 showed 100 per cent similarity with BH 23.

At 67.6 per cent similarity, second subcluster comprising of landraces with waxy textured fruits was further grouped into two with seven members each. At 74.2 per cent, the first subgroup was again divided into two with BH 3, BH 4, BH 5, BH 9, BH 12 and BH 18 falling in one subgroup and BH 12 stand singly. Among these, BH 4, BH 5 and BH 18 show genetic similarity at 83.8 per cent while BH 4 become distinct from BH 5 and BH 18 at 84.7 per cent. BH 5 showed 85.2 per cent similarity with BH 18. Similarly, BH 12 and BH 13 stand singly at 0.74 and 0.80 per cent similarity coefficient respectively while BH 3 and BH 9 showed 100 per cent similarity.

Rest of the landraces with waxy textured fruits were grouped together in second subgroup. They showed a similarity ranged from 82 to 100 per cent. Of these landraces, BH 11, BH 14 and BH 24 were most closely related (100 per cent similarity).

In this study, RAPD marker analysis has revealed and grouped the *B. hispida* landraces according to their genetic relationships reliably. The clusters based on RAPD analysis using three primers depict genetic variation among the landraces of *B. hispida*. The 25 landraces of *B. hispida* that were studied formed 8 clusters in the UPGMA cluster analysis. Quite distinct among these were two cluster formed at 0.35 similarity coefficient which clearly separates smooth textured group of landraces from waxy textured group.

DISCUSSION

5. DISCUSSION

Ashgourd is a cucurbitaceous vegetable crop grown under wide agro-climatic conditions both for mature and immature fruits. It is famous among the growers and consumers because of long shelf life under ambient conditions, good portability and appreciably good nutritive value. Ashgourd has wide use in confectionary and ayurvedic medicinal preparations. Although ashgourd is becoming a crop of industrial importance, relatively less attention has been paid towards the varietal improvement of existing strains available in different parts of the country. There is an imperative need to pick up an ideal plant type having maximum desirable traits to meet the growing demand.

Kerala is blessed with diverse climatic and soil conditions, which have helped in the development of different landraces of crops having variability. These landraces, the products of natural selection maintain genetic heterogenity in balance over time. The exploitation of this heterogenity can help in improvement of the crop.

The genetic improvement of any crop aims at increasing the production potential and quality by altering the genetic make up of the existing varieties. For rational approach to improve the yield, it is essential to have the knowledge of variability, heritability, genetic advance and association between characters. The existence of different shape and size of fruits indicates the presence of wide genetic variability in ashgourd germplasm.

Hence a study was undertaken to collect, catalogue and characterize the available landraces of *B. hispida* at morphological and molecular level. Morphological characterization helps to assess the magnitude of genetic variability for identifying superior landraces based on yield, quality, earliness and pest and disease resistance. Molecular characterization

gives a comprehensive picture on diversity and relatedness of available landraces.

5.1 MORPHOLOGICAL CHARACTERIZATION

5.1.1 Genetic Cataloguing

Twenty five landraces of *B. hispida* upon cataloguing showed distinct variations among each other with respect to vegetative, flower, fruit and seed characters. Most of the landraces fall in moderate to high viny growth habit and short to long internodal length. Variability was more pronounced for flower and fruit characters. Flower and fruit size ranged from small to very large. Fruit colour and fruit shape showed wide variations among the landraces. Seed quantity per fruit ranged from very few to many with small to large seed size. There are reports on high variability for morphological characters in pointedgourd (Singh, 1989) and bottlegourd (Mathew, 1996).

5.1.2 Variability

Genetic variability in the base population is a pre-requisite for effective crop improvement. The observed variability in the population is the sum total of the variations that arise due to genotypic and environmental effects. Hence a knowledge on the nature and magnitude of genetic variation contributing to gain under selection is essential.

In the present investigation, analysis of variance revealed high significant differences among the twenty five landraces of *B. hispida* for all the characters studied namely, vine length, internodal length, number of primary branches, number of secondary branches, root shoot length ratio, days to first male flower, node to first male flower, days to first female flower, node to first female flower, sex ratio, days to first fruit harvest, fruit length, fruit girth, fruits per plant, average fruit weight, yield per plant, seeds per fruit, 1000-seed weight and mosaic incidence

indicating sufficient diversity among the landraces. Such variation indicated the scope for improving the population for these characters as reported earlier by Randhawa *et al.* (1983), Madalgeri and Dharmatt (1989) and Parkash *et al.* (2002) in ashgourd.

In respect of vegetative characters, ample variability was observed as evident from the wide range obtained for vine length and internodal length. Among the landraces evaluated, BH 22 was the most vigorous registering the highest values for vine length and BH 6 for internodal length. Considerable variability was reported by Borthakur and Shadeque (1990) for vine length in pumpkin and Ram *et al.* (2001) for internodal length in pointedgourd.

Number of primary branches recorded a low range of variation as reported by Mohanty and Mishra (1999) in pumpkin, while number of secondary branches recorded a wide range of variation as reported by Joseph (1999) in ivygourd. Root shoot length ratio obtained low range of variation compared to other characters.

Days to first male and female flower showed wide range of variation among the landraces. Similar results were also reported by Deol *et al.* (1981) in muskmelon. Node to first male and female flower also recorded wide range of variability as reported by Priya (2001) in watermelon.

Sex ratio in the present study ranged from 7.21 to 16.40. Considerable variation for the character was also reported by Thakur and Nandpuri (1974) in watermelon.

Days to first fruit harvest recorded narrow range of variation. Most of the landraces are harvested in 96 days.

Among the landraces, maximum fruit weight was observed in BH 15. Other landraces with better fruit weight were BH 7 and BH 5. Both fruit length and fruit girth contributed to better fruit weight in high yielders. In the present

study, fruit length ranged from 13.65 to 56.00 cm. Similarly, fruit girth also varied from 22.20 to 78.05 cm, suggesting ample variability and scope for improvement for fruit size in *B. hispida*.

Fruits per plant and yield per plant exhibited high variability as reported by Miniraj *et al.* (1993) in ashgourd and Katiyar *et al.* (1996) in bittergourd respectively. Among the landraces evaluated, fruits per plant was maximum in BH 24 (Kottarakara, Kollam). Other landraces with better fruit number were BH 8 (Cheruplasseri, Palakkad) and BH 22 (Pala, Kottayam). The landraces with high fruit number are small in fruit size while large sized fruits have limited fruits per plant. Among the landraces BH 15 (Neyattinkara, Thiruvananthapuram) is the highest yielder followed by BH 23 (KAU local, KAU) and BH 5 (CO-1, TNAU). The high yield in BH 15 may be attributed to the high fruit length, fruit girth and average fruit weight. BH 23 was characterized by low sex ratio and earliness in male and female flowering and harvest. The landrace BH 5, apart from being better for most of the fruit characters like fruit length, fruit girth, average fruit weight and 1000-seed weight, it also registered high values for resistance against mosaic disease resulting in better yield. This confirms the fact that fruit yield is a complex trait and is the ultimate expression of many component characters.

Seeds per fruit and 1000-seed weight exhibited a wide range of variation from 73.50 to 1647.00 and 19.25 to 96.95 g respectively. Similar results were reported for seeds per fruit by Pynadath (1978) in snake gourd and for 1000-seed weight by Gayathri (1997) in cucumber. Varieties with high fruit seed weight and fruit seed number are preferred not only to increase crop production, but also to meet the needs of the seed industry and farmers.

Mosaic disease is a major constraint in cucurbit cultivation in Kerala. Significant differences were observed among the ashgourd landraces for mosaic incidence, which clearly indicated that the level of

resistance or susceptibility to the disease varied with the landrace. Out of the 25 landraces evaluated, six were resistant; sixteen were moderately resistant and remaining three (BH 7, BH 22, BH 25) were found susceptible to the disease. Screening for mosaic resistance was also done by Rajamony *et al.* (1990a) in wild species of *Cucumis*, Rajamony *et al.* (1990b) in culinary melon, Rakhi (2001) in *Cucumis melo* and Arunachalam (2002) in bittergourd.

Though cucurbits are highly susceptible to nematode infections, no reports are available for root-knot infestations affecting ashgourd. Root-knot index showed that 22 landraces were highly resistant, two (BH 15 and BH 23) were moderately resistant and BH 13 was susceptible to the root-knot incidence.

In the present investigation, the GCV was very near to PCV for most of the characters indicating that these characters are least influenced by the environment and are under the control of genotype itself. High coefficients of variation (phenotypic [PCV] and genotypic [GCV]) were observed for seeds per fruit, average fruit weight, yield per plant and fruits per plant. Similar results were also reported in watermelon (Prasad *et al.*, 1988) and in ashgourd (Miniraj *et al.*, 1993; Lovely, 2001). The high PCV and GCV observed for these characters are evident from their high variability, which in turn offers good scope for selection.

The lowest PCV and GCV were exhibited by days to first fruit harvest, which was in conformity with the findings of Rastogi and Deep (1990a) in cucumber.

5.1.3 Heritability and Genetic Advance

The total variability existing in a population is a sum of heritable and non-heritable components and it is necessary to partition these components, since the magnitude of heritable variability is an important

aspect of genetic constitution of breeding material, which has a close bearing on selection.

High values of heritability were observed for most of the characters studied. Higher magnitude of heritability (>90 %) was recorded for yield per plant, fruits per plant, average fruit weight, fruit length, fruit girth, seeds per fruit, 1000-seed weight, vine length, internodal length and number of secondary branches. Similar findings were also reported in ashgourd by George (1981) for fruits per plant, Menon (1998) for average fruit weight, Parkash *et al.* (2000) for fruit yield and Lovely (2001) for seeds per fruit and 1000-seed weight. Panday *et al.* (2003) in snapmelon and Priya *et al.* (2004) in watermelon also observed high heritability for fruit characters and vine length respectively. High heritability estimates indicate the presence of large number of fixable additive factors and hence these traits can be improved by selection.

High heritability estimates does not necessarily mean a high genetic advance for a particular character. Knowledge of heritability coupled with expected genetic advance of a trait is necessary for assessing the scope of its improvement through selection. The present investigation revealed high heritability coupled with high genetic advance for several biometric characters including average fruit weight, yield per plant, fruits per plant, fruit girth and fruit length. Sriramamurthy (2000) also observed high heritability and genetic advance for several yield characters in cucumber.

High heritability coupled with low genetic advance attributable to non-additive gene action was noticed for days to first male and female flower. Similar results were reported in cucumber (Choudhary and Mandal, 1987), ashgourd (Lovely, 2001) and ivygourd (Varghese, 2003).

Moderate heritability with low genetic advance was observed for sex ratio and days to first fruit harvest. It may be inferred that these

characters were conditioned by non-additive gene action and presence of high genotypic and environmental interaction.

On the basis of the present study, it can be concluded that simultaneous selection based on multiple characters having high estimates of heritability associated with greater genetic advance may be useful for the improvement of this crop.

5.1.4 Correlation Studies

Correlation provides information on the nature and extent of relationship between all pairs of characters. Correlation studies between yield and other characters have been of immense help in selection of suitable plant types.

In the present study, both at phenotypic and genotypic levels, the characters *viz.*, fruit length, fruit girth, average fruit weight, seeds per fruit and 1000-seed weight showed strong positive association with yield per plant. Node to first male and female flower and fruits per plant were negatively correlated with yield.

The very high positive association of average fruit weight with yield indicated that average fruit weight was the primary yield attribute in ashgourd. Similar reports were there in muskmelon (Kalloo *et al.*, 1983) and watermelon (Prasad *et al.*, 1988). Average fruit weight was also positively correlated with number of seeds per fruit and 1000-seed weight. This result was supported by the findings of Devadas *et al.* (1999) in pumpkin.

Positive association of vine length with number of primary and secondary branches was in agreement with the findings of Sidhu and Brar (1981) in watermelon.

Node to first male and female flower was found to be negatively correlated with yield. Similar results were also reported by Lakshmi *et al.* (2002) in pumpkin. Strong positive correlation of node to first male flower with node to first female flower was in conformity with the results of Kandasamy (2004) in melon.

Sex ratio exhibited positive correlation with fruit yield. Similar report was made in cucumber by Prasunna and Rao (1989).

Sex ratio showed high negative correlation with fruits per plant. This suggested that a direct relationship existed between number of female flowers and number of fruits set and total fruit yield. This result was supported by the findings of Murali *et al.* (1986) in bottlegourd.

Positive correlation of fruit length with fruit girth, average fruit weight and yield was in agreement with the findings of Singh *et al.* (1987) in parwal and Prasad and Singh (1992) in cucumber.

The present investigation revealed that fruit girth was positively correlated with average fruit weight and yield. Salk (1982) in melons reported strong positive correlation between fruit girth and average fruit weight. Parkash *et al.* (2000) in ashgourd and Bhave *et al.* (2003) in bittergourd reported the association of fruit girth with yield.

The high negative correlation between fruits per plant with yield was supported by the findings of Priya (2001) in watermelon. Fruits per plant also had high negative correlation with average fruit weight and 1000-seed weight. This makes a clear indication that increase in number of fruits per plant would affect the fruit yield, which is more dependent on fruit weight. Similar results had been reported by Salk (1982) in melons.

5.1.5 Path Coefficient Analysis

Yield is a complex and polygenically controlled character and is highly influenced by environmental factors. Path coefficient analysis furnishes a method for separating out the direct and indirect effects so as to measure the relative importance of each component characters. As evidenced from correlation studies, path coefficient analysis also signifies the importance of characters fruit length and average fruit weight, which exhibited the highest direct and indirect effect. Similar results were also reported by Menon (1998) and Lovely (2001) in ashgourd.

The direct effects of vine length, node to first female flower and sex ratio were negligible, but their indirect effect through fruit length and average fruit weight were consistently high. High negative correlation of fruits per plant with yield was due to high negative indirect effect through fruit length, while in node to first female flower, high negative correlation was through average fruit weight. This was in conformity with the findings of Rakhi (2001) in melon and Pandey *et al.* (2003) in snapmelon.

5.1.6 Selection Index

Selection of landraces based on a suitable index is highly efficient for any crop improvement programme. Selection index involves discriminant function analysis, which is meant for isolating superior landraces based on the phenotypic and genotypic correlations. Identification of superior genotypes of *B. hispida* based on discriminant function analysis was done by Lovely (2001) in ashgourd. A model involving the same set of eleven characters which was used for path coefficient analysis was selected for ranking the landraces. On ranking the scores obtained, the landrace BH 15 (Neyattinkara, Thiruvananthapuram) ranked first, followed by BH 23 (KAU local, KAU) and BH 5 (CO-1, TNAU). These landraces with better yield, fruit quality, earliness in male

and female flowering, narrow sex ratio and mosaic resistance may be recommended as elite types after refinement and multilocational testing.

5.1.7 Mahalanobis's D^2 Analysis

Mahalanobis's D^2 statistic is one of the potent techniques for measuring genetic divergence at both intra and inter cluster levels and thus provides a basis for selection of genetically divergent parents in hybridization programme. Genetic divergence was assessed by Lovely (2001) in ashgourd, Kale *et al.* (2002) and Lakshmi *et al.* (2003) in pumpkin and Kandasamy (2004) in melon.

In the present study, based on Mahalanobis's D^2 statistic, the 25 landraces were grouped into seven gene constellations. The maximum number of landraces (8) were included in Cluster I, followed by cluster II with 4 landraces. Cluster III, IV, V and VI had three landraces each while cluster VII had one landrace. The pattern of clustering almost followed the ranking obtained from selection index.

Considering the cluster means for various characters studied, clusters III and V were superior for most of the biometric characters, whereas clusters I and VI were generally poor. Cluster II and VII was found to be intermediate. For crop improvement programmes, intercrossing among landraces with outstanding mean performance for these characters would be effective.

The present investigation on morphological characterization of 25 *B. hispida* landraces showed wide variation for all the characters. High heritability coupled with high genetic advance was observed for most of the biometric characters, which indicates the scope for effective selection. Correlation and path coefficient analysis revealed that fruit length and average fruit weight are the primary yield component. The landraces BH 15 (Neyattinkara, Thiruvananthapuram), BH 23 (KAU local, KAU)

and BH 5 (CO-1, TNAU) were found to be promising with regard to yield, fruit quality, earliness in male and female flowering, narrow sex ratio and mosaic resistance. The same may be used for further improvement programmes.

5.2 MOLECULAR CHARACTERIZATION

Most of the genetic diversity studies in crop plants have been carried out using morphological markers only. Now-a-days Polymerase chain reaction (PCR) based molecular markers have developed into powerful tools to analyse genetic relationships and genetic diversity. RAPD technique is one among them. The genetic variation as detected by RAPD analysis opens up the avenue for the proper identification and selection of the genotypes that could be used for varietal identification and planning for future crop improvement programme. RAPD analysis has been successfully employed to analyse genetic diversity in melon (Mliki *et al.*, 2001) and in cucumber (Ping *et al.*, 2002; Mliki *et al.*, 2003).

In the present study an attempt was made to determine the extent of genetic diversity in 25 landraces of *B. hispida* based on RAPD markers, making use of arbitrary primers to amplify random DNA sequence in the genome.

Isolation of genomic DNA of ashgourd was done using modified Murray and Thompson (1980) method. Tissues from young tender leaves were found to yield good quality DNA.

The DNA yield for twenty five landraces of *B. hispida* ranged from 0.21 to 3.90. The purity of DNA (A_{260} / A_{280} ratio) ranged from 1.40 to 2.08 $\mu g\ \mu l^{-1}$.

To identify the promising primers for RAPD analysis, forty decamer primers of kit A and B were screened using the DNA of

landrace BH 1. The procedure standardized by Staub *et al.* (2000) in *Cucumis melo* germplasm was tried for amplification. Twenty nine primers, out of the forty decamer primers yielded amplification products indicating presence of sequence complementary to these primer in the DNA of BH 1 landrace. A total of 83 RAPDs (average 2.08 bands per primer) were generated by the 29 primers, of which 92.77 per cent were polymorphic (77 bands) and six were monomorphic. Five primers showed high level of polymorphism. This could be explained by the capability of individual primers to amplify the less conserved and highly repeated regions of the genomic DNA. There is high possibility for the amplified fragments to contain repeated sequences.

For further amplification of DNA from twenty five ashgourd germplasm, three promising primers were identified for RAPD analysis based on performance in DNA amplification, production of highest number of polymorphic bands as well as intense bands and reproducibility. They were OPA-01, OPA-07 and OPA-13. Gwanama *et al.* (2000) identified sixteen RAPD primers to show genetic relationship among pumpkin genotypes while Kandasamy (2004) used four primers in melon for genetic diversity studies. However, Bhat and Jarret (1995) suggested that the number of polymorphisms might be more important than the number of primers for the generation of stable phenogram and it would vary with plant material under investigation and the sequences that are amplified.

A total of 20 scorable bands (average of 6.66 bands per primer) were generated by the selected three primers of which 2 were monomorphic and rest, 18 were polymorphic (90.0 %). The number of bands ranged from 1 to 8 with an average of 3 per primer.

The primer OPA-07 was unique as it could distinguish maximum of the landraces tested. The highest number of scorable bands was given by

OPA-13 of which one of the bands produced was monomorphic. The primer OPA-01 produced five scorable bands of which one band was monomorphic for all the landraces.

The estimation of Jaccard's similarity coefficients and construction of dendrogram by using UPGMA revealed the presence and extent of genetic similarities among the twenty five landraces of *B. hispida* examined. The overall similarity coefficients ranged from 0.14 to 1.00. Cluster analysis revealed that at about 0.35 similarity coefficient, the twenty five landraces of *B. hispida* grouped into two clusters. Landraces with morphologically distinct smooth and waxy textured fruits grouped into two major clusters with an exception of BH 19 falling in the first cluster. This substantiates the moderately broad distribution of genetic variability, which can be attributed to the broad genetic base in their origin.

The waxy textured group formed a more divergent cluster than smooth textured group. With in the group of waxy textured fruits, limited variation was detected among landraces with small sized fruits. Morphologically similar landraces BH 11, BH 14 and BH 24 are grouped together and showed 100 per cent similarity.

Landraces with medium sized fruits also showed limited variability. They formed five subclusters with in the waxy textured group with 100 per cent similarity for BH 2 with BH 23 and BH 3 with BH 9 respectively.

Further, landraces classified as belonging to the same morphotypic group did not always cluster together. This was evidenced from the results of RAPD analysis that morphologically similar landraces with large fruits form distinct clusters with in the major clusters. BH 1, BH 5, BH 6, BH 7, BH 15, BH 18 and BH 25 with high average fruit weight formed distinct clusters under molecular study.

Thus the study revealed that RAPD technique was successful and efficient in discriminating ashgourd germplasm. The clusters based on RAPD analysis using three primers depict wide genetic variation among the landraces of ashgourd. It can easily differentiate *B. hispida* landraces, even the closely related ones. Polymorphism obtained in the present study will be further useful in fingerprinting and in determining genetic diversity among the ashgourd landraces. For future studies on analysis of ashgourd landraces, wider genetic base and greater number of RAPD primers are to be included for accurate results. Finally, the results support the idea that RAPD technique being relatively simpler, quicker, inexpensive and non-radioactive can detect sufficient polymorphisms for germplasm characterization and genetic distance studies.

By characterizing all the twenty five landraces of *B. hispida* using morphological (selection index and D^2 analysis) and molecular (RAPD marker analysis) methods revealed that morphologically distinct and superior lines were genetically differentiable. Also the RAPD analysis gave a perfect differentiation of waxy textured group from smooth textured group, which is in line with morphological characterization.

SUMMARY

6. SUMMARY

The present investigation on "Characterization of landraces of ashgourd [*Benincasa hispida* (Thunb.) Cogn.]" was carried out at the Department of Olericulture and the Department of Plant Biotechnology, College of Agriculture, Vellayani during 2003-2004.

The study envisaged genetic cataloguing of the available germplasm in *Benincasa hispida*, assessment of genetic variability, divergence, association among the characters including direct and indirect effects of various characters on yield, formulation of a selection index for identifying suitable lines based on yield, quality, pest and disease resistance and molecular characterization using RAPD analysis.

The experimental material consisted of 25 landraces of ashgourd collected from different agroclimatic regions of Kerala, Tamil Nadu and Karnataka. The landraces were genetically catalogued based on the descriptor list for cucurbits (IBPGR, 1983). The results revealed distinct variations among the landraces with respect to vegetative, flower, fruit and seed characters.

Significant differences were observed among the twenty five landraces of *B. hispida* for all the characters studied namely, vine length, internodal length, number of primary branches, number of secondary branches, root shoot length ratio, days to first male flower, node to first male flower, days to first female flower, node to first female flower, sex ratio, days to first fruit harvest, fruit length, fruit girth, fruits per plant, average fruit weight, yield per plant, seeds per fruit, 1000-seed weight and mosaic incidence indicating sufficient diversity among the landraces.

The highest yield was observed in BH 15 (Neyattinkara, Thiruvananthapuram, 21.20 kg), which also recorded the maximum

average fruit weight (9.50 kg), fruit girth (78.05 cm) and 1000-seed weight (96.95 g). Among the landraces, maximum fruit length was observed for BH 25 (56.00 cm). BH 22 was the longest in vine length (875.00 cm) and had the highest number of primary and secondary branches (4.00 and 23.00 respectively). BH 24 (7.20 cm) was shortest in internodal length, which was also characterized by maximum fruits per plant (9.12). BH 8 was the earliest to flower (46.25 days) and harvest (88.00 days). The reaction of twenty five landraces of *B. hispida* towards mosaic incidence indicated that six landraces were resistant; sixteen landraces were moderately resistant and remaining three (BH 7, BH 22, BH 25) were susceptible to the disease. BH 10 had the least vulnerability index for mosaic (27.50).

High coefficients of variation (phenotypic [PCV] and genotypic [GCV]) were observed for seeds per fruit, average fruit weight, yield per plant and fruits per plant. The lowest PCV and GCV were exhibited by days to first fruit harvest.

High heritability coupled with high genetic advance was observed for average fruit weight, yield per plant, fruits per plant, fruit girth and fruit length indicating scope for improvement of these characters through selection.

Correlation studies revealed at both phenotypic and genotypic levels, the characters like fruit length, fruit girth, average fruit weight, seeds per fruit and 1000-seed weight were positively correlated with yield per plant. Node to first male and female flower and fruits per plant were negatively correlated with yield.

Path coefficient analysis indicated that fruit length had the maximum positive direct effect on fruit yield (0.5493), followed by average fruit weight (0.5157) and fruits per plant (0.4514). Indirect

effects through fruit length and average fruit weight were consistently high signifying the importance of these characters.

Selection index was worked out using eleven characters *viz.*, vine length, days to first female flower, node to first female flower, sex ratio, fruit length, fruit girth, fruits per plant, average fruit weight, yield per plant, seeds per fruit and mosaic incidence. Based on the index scores obtained, the landrace BH 15 (Neyattinkara, Thiruvananthapuram) ranked first, followed by BH 23 (KAU local, KAU) and BH 5 (CO-1, TNAU).

The 25 landraces of *B. hispida* were grouped into seven gene constellations based on Mahalanobis's D^2 statistic. Cluster I was the largest which contained 8 landraces, followed by cluster II with 4 landraces. Cluster III, IV, V and VI had three landraces each while cluster VII had one landrace. With regard to cluster means, clusters III and V performed better for most of the characters taken. The maximum intercluster distance was observed for clusters V and VII (43823.68), followed by clusters V and VI (36516.94) and clusters I and V (32854.00). The intracluster distance was highest for cluster VI (549.72).

From the morphological characterization of 25 landraces of ashgourd, BH 15, BH 23 and BH 5 were found to be promising based on their superiority in yield, fruit quality, earliness in male and female flowering, narrow sex ratio and mosaic resistance and hence they may be utilized for further improvement.

RAPD (Random amplified polymorphic DNA) analysis was used to characterize genetic variability and relationships among twenty five landraces of *B. hispida* at molecular level. The DNA was isolated from etiolated 15-20 days old seedlings. The DNA yield for twenty five ashgourd landraces ranged from 0.21 to 3.90. The purity of DNA (A_{260} / A_{280} ratio) ranged from 1.40 to 2.08 $\mu g\ \mu l^{-1}$. Each sample was subjected to RAPD analysis. Out of the 40 decamer primers, twenty nine

yielded amplification products. A total of 83 RAPDs (average 2.08 bands per primer) were generated by the 29 primers, of which 92.77 per cent were polymorphic (77 bands) and six were monomorphic. Five primers showed high level of polymorphism. Finally, three promising primers *viz.*, OPA-01, OPA-07 and OPA-13 were identified for RAPD analysis based on their performance in DNA amplification, reproducibility and production of highest number of polymorphic bands as well as intense bands. The primer OPA-07 was unique as it could distinguish maximum polymorphism among the landraces tested while OPA-13 produced maximum number of scorable bands. The selected primers yielded 20 scorable bands (average of 6.66 bands per primer) of which 2 were monomorphic and rest, 18 were polymorphic (90.0 %).

The estimation of Jaccard's similarity coefficients and construction of dendrogram by using UPGMA revealed the presence and extent of genetic similarities among the twenty five landraces of *B. hispida* examined. The overall similarity coefficients ranged from 0.14 to 1.00. Cluster analysis revealed that at about 0.35 similarity coefficient, the twenty five landraces of *B. hispida* grouped into two clusters. Landraces with morphologically distinct smooth and waxy textured fruits grouped into two major clusters. Considering the waxy textured group, it formed a more divergent cluster than smooth textured group. With in the group of waxy textured fruits, limited variation was detected among landraces with small and medium sized fruits. Further, morphologically similar landraces with large fruits form distinct clusters with in the major clusters.

By characterizing all the twenty five landraces of *Benincasa hispida* using morphological (selection index and D^2 analysis) and molecular (RAPD marker analysis) methods revealed that morphologically distinct and superior lines were genetically differentiable. Also the RAPD analysis gave a perfect differentiation of waxy textured group from smooth textured group, which is in line with morphological characterization.

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*Original not seen

CHARACTERIZATION OF LANDRACES OF ASHGOURD
(*Benincasa hispida* (Thunb.) Cogn.)

RESMI J.

Abstract of the
thesis submitted in partial fulfilment of the requirement
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ABSTRACT

The research project "Characterization of landraces of ashgourd [*Benincasa hispida* (Thunb.) Cogn.]" was carried out at the Department of Olericulture and the Department of Plant Biotechnology, College of Agriculture, Vellayani during 2003-2004. The objective of the study was to catalogue the landraces based on the IBPGR descriptor for cucurbits, to estimate the genetic parameters for different traits in the germplasm for identifying superior lines based on yield, quality, pest and disease resistance and to characterize the landraces using molecular techniques (RAPD analysis).

Twenty five landraces of *Benincasa hispida* collected from various sources upon cataloguing pointed out wide variation for several morphological characters. Analysis of variance revealed significant differences among the landraces for all the characters studied namely, vine length, internodal length, number of primary branches, number of secondary branches, root shoot length ratio, days to first male flower, node to first male flower, days to first female flower, node to first female flower, sex ratio, days to first fruit harvest, fruit length, fruit girth, fruits per plant, average fruit weight, yield per plant, seeds per fruit, 1000-seed weight and mosaic incidence.

Among the landraces, BH 15 (Neyattinkara, Thiruvananthapuram) recorded the maximum yield (21.20 kg), average fruit weight (9.50 kg), fruit girth (78.05 cm) and 1000-seed weight (96.95 g). Among the landraces, maximum fruit length was observed for BH 25 (56.00 cm). BH 22 was the longest in vine length (875.00 cm) and had the highest number of primary and secondary branches (4.00 and 23.00 respectively). BH 24 (7.20 cm) was shortest in internodal length, which was also characterized by maximum fruits per plant (9.12). BH 8 was the earliest to flower (46.25 days) and harvest (88.00 days). BH 10 had the least

vulnerability index for mosaic (27.50). High phenotypic and genotypic coefficients of variation were observed for seeds per fruit, average fruit weight, yield per plant and fruits per plant.

High heritability coupled with high genetic advance was observed for average fruit weight, yield per plant, fruits per plant, fruit girth and fruit length.

Correlation studies and path coefficient analysis revealed that fruit length and average fruit weight are the primary yield components as evidenced from its high positive correlation as well as direct and indirect effects on yield.

In the discriminant function analysis, the landrace BH 15 (Neyattinkara, Thiruvananthapuram) ranked first, followed by BH 23 (KAU local, KAU) and BH 5 (CO-1, TNAU). They were found to be promising based on their superiority in yield, fruit quality, earliness in male and female flowering, narrow sex ratio and mosaic resistance and hence they may be utilized for further crop improvement.

Based on the analysis for genetic divergence, the 25 landraces of *B. hispida* were grouped into seven clusters, with the highest intercluster distance observed between clusters V and VII.

DNA isolated from the 25 landraces of *B. hispida* were subjected to RAPD analysis. Out of the 40 decamer primers, twenty nine yielded amplification products. A total of 83 RAPDs (average 2.08 bands per primer) were generated by the 29 primers, of which 92.77 per cent were polymorphic (77 bands) and six were monomorphic. Out of five primers showing high level of polymorphism, three promising primers *viz*., OPA-01, OPA-07 and OPA-13 were selected. The primer OPA-07 was unique as it could distinguish maximum polymorphism among the landraces tested while OPA-13 produced maximum number of scorable bands. The selected three primers yielded 20 scorable bands (average of

6.66 bands per primer) of which 2 were monomorphic and rest, 18 were polymorphic (90.0 %).

The overall Jaccard's similarity coefficients ranged from 0.14 to 1.00. Cluster analysis revealed that at about 0.35 similarity coefficient, the twenty five landraces of *B. hispida* grouped into two clusters. Landraces with morphologically distinct smooth and waxy textured fruits grouped into two major clusters. Considering the waxy textured group, it formed a more divergent cluster than smooth textured group. With in the group of waxy textured fruits, limited variation was detected among landraces with small and medium sized fruits. Further, morphologically similar landraces with large fruits form distinct clusters with in the major clusters.

By characterizing all the twenty five landraces of *Benincasa hispida* using morphological (selection index and D^2 analysis) and molecular (RAPD marker analysis) methods revealed that morphologically distinct and superior lines were genetically differentiable. Also the RAPD analysis gave a perfect differentiation of waxy textured group from smooth textured group, which is in line with morphological characterization.