MOLECULAR CHARACTERIZATION OF IVYGOURD

[*Coccinia grandis* (L.) Voigt]

S. SURESH

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DECLARATION

I hereby declare that this thesis entitled "**Molecular characterization of ivygourd [*Coccinia grandis* (L.) Voigt]**" 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 "**Molecular characterization of ivygourd [*Coccinia grandis* (L.) Voigt]**" is a record of research work done independently by Mr. S. Suresh (2002-12-17) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Dedicated to

My Dear Ones

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

µ	–	Microlitre
µM	–	Micromolar
AFLP	–	Amplified fragment length polymorphic DNA
bp	–	Base pair
CG	–	*Coccinia grandis*
DNA	–	Deoxy ribonucleic acid
dNTPs	–	Deoxy nucleotides
EDTA	–	Ethylene diamino tetra acetic acid disodium salt
EV	–	Environmental variance
GA	–	Genetic advance
GCV	–	Genotypic coefficient of variation
GV	–	Genotypic variance
h^2	–	Heritability
ISSR	–	Inter simple sequence repeats
mM	–	Millimolar
ng	–	Nanogram
PCR	–	Polymerase chain reaction
PCV	–	Phenotypic coefficient of variation
pM	–	Picomolar
PV	–	Phenotypic variance
RAPD	–	Random amplified polymorphic DNA
RFLP	–	Restriction fragment length polymorphism
SCAR	–	Sequence characterized amplified region
SDS	–	Sodium dodecyl sulphate
SSR	–	Simple sequence repeats
SSRLP	–	Single sequence repeat length polymorphism
STMS	–	Sequence tagged microsatellite sites
Tris_HCl	–	Tris (hydroxy methyl) aminomethane hydrochloride
TSS	–	Total soluble solids
UPGMA	–	Un weighted pair group method for arithmetic average
VNTR	–	Variable number of tandem repeats

Introduction

1. INTRODUCTION

Ivygourd (*Coccinia grandis* (L.) Voigt) also known as Scarletgourd, Kundsru and Tondli is a semiperennial, dioecious creeper widely cultivated in South East Asian countries. In India 'Kowai fruits' as it is popularly known is a common vegetable grown extensively in West Bengal, Karnataka, Andhra Pradesh, Tamil Nadu, Maharashtra and Gujarat. Though its cultivation in Kerala is mainly confined to kitchen gardens, coccinia is attaining the status of a commercial crops in certain parts of Thiruvananthapuram, Pathanamthitta, Kottayam, Malappuram and Kasaragod districts where, farmers find it much profitable (Premnath and Subramonian, 1971).

Ivygourd belongs to the family Cucurbitaceae and consists of male and female plants (Veeraragavathatham *et al.*, 1998). Distinct sex forms in coccinia are due to the presence of characteristically distinct sex chromosomes. Gynodioecious forms consisting of female and hermophrodite flowers have also been observed. Maleness is expressed by the influence of 'y' chromosome. The crop is highly cross pollinated. Seshadri (1986) reported that ivygourd gives yield for three to four years.

Ivygourd is profusely mentioned in the books of Ayurveda. Almost all parts of the plant are said to be of immense medicinal value. It attained historic acclaim as it is mentioned in Vedas and Ithihasas. The nutritive value is assessed as high as the goat's milk and mutton. Tender fruits of ivygourd contain 1.2 per cent protein (Sachan and Chaundawat, 1985). Veeraragavathatham *et al.* (1998) found that tender fruits and shoots of ivygourd are eaten raw or cooked while roots, stem and leaves are used as ingredients of medicines for the treatment of skin diseases, bronchitis and diabetes. Though the main marketed vegetables in Kerala constitute only 20 species, about 60 species are grown in smaller areas strictly for household uses. Ivygourd is one among them (Indira and Peter, 1988).

Breeding attempts on ivygourd have been scanty. This is mainly attributed to the constraints such as the dioecious and semiperennial nature of plants and occurrence of parthenocarpy. No high yielding variety has been identified so far. However local type known as 'Padappai' cultivated in Chengalpattu district in Tamil Nadu is reported to be a prolific bearer of sweet, soft fruits (Veeraragavathatham *et. al.*, 1998).

Development of high yielding varieties of ivygourd is necessary to inspire the interest of farmers and enthuse them to promote large-scale cultivation. In any breeding programme success depends on the availability of genetic variation in a population. Information on variability, genetic advance and components of phenotypic and genotypic variation are basic for crop improvement. The effectiveness of selection of a genotype depends on estimates of heritability based on phenotypic performance. In selection of proper breeding method estimates of heritability coupled with genetic advance are more useful than heritability or genetic advance alone. Similarly, knowledge of genetic divergence with D^2 statistic permit precise comparison among genotypes. Information on direct and indirect effects of yield components on yield would be more useful in making the selection more effective.

A precise system of characterization is essential in varietal identification. Genotypes are usually characterized by morphological and agronomic traits.

However, these approaches are subject to environmental influences and their effectiveness is debatable. DNA-based molecular markers have been developed and accepted over the past few decades for the assessment of genetic diversity in crop plants. Since the marker systems reveal differences at the DNA level, they are not affected by environmental conditions. A major advantage of the DNA-based markers is the abundance of polymorphic loci, which enables estimation of genetic distance among the germplasms with high precision. Several molecular markers, namely, RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), etc. are being utilized for this purpose. Among

these, RAPD marker technique is a powerful technique for determining the genome variation. The variability that exist in the genome is likely to be detected by RAPD due to the random nature of binding of the primers, thereby screening a much larger portion of the genome leading to a better characterization of the organelle genome diversity. Such studies have not been so far conducted in coccinia. In this context the present study was conducted with the following objective.

To study the genetic divergence in the germplasm of coccinia through Mahalanobis D^2 analysis and to characterize them by morphological methods; and

To characterize the coccinia genotypes through molecular markers by RAPD technique and to compare these two methods of characterization.

Review of Literature

2. REVIEW OF LITERATURE

2.1 MORPHOLOGICAL CHARACTERIZATION

Ivygourd is a semiperennial vegetable, popularly grown in West Bengal, Karnataka, Andhra Pradesh, Tamil Nadu, Maharashtra and Gujarat. In Kerala, though it is grown in northern region, still it remains as an under exploited vegetable crop. No high yielding variety is reported in this crop so far. Breeding work in ivygourd is very much limited. It is due to the constraints like dioecious nature of plants, semiperennial habit and occurrence of parthenocarpy.

For any crop improvement programme, it is necessary to collect information on genetic variability, correlation of variables, heritability, genetic advance, path coefficient, genetic diversity etc. The information on these aspects in ivygourd is scarce and scanty. Literature available on other cucurbitaceous vegetables is relevant for an in-depth study of ivygourd as well and hence reviewed under different heads.

2.1.1 Genetic Parameters

Important methods used for improvement of any crop depend to a great extent on selection. An insight into the magnitude of variability present in a crop species is of utmost importance as it provides the basis for effective selection.

The selection is effective only when major part of the variability is genetic. Unraveling the hitherto unknown variability present in any crop species is very important to make selection apt and effective. Variability in a population could be partitioned into heritable and non-heritable components with the aid of genetic parameters such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance (GA), which serves as a basis for selection (Johnson *et al.*, 1955).

2.1.1.1 Variability and Coefficient of Variation

In ivygourd, Raju and Peter (1995) reported considerable variability in fruit length, fruit weight and fruit size. Joseph (1999) observed a wide range of variation for days for first flower opening, fruit length, fruit weight, fruits per plant and yield per plant. The GCV and PCV were high for primary branches and fruit yield.

Sarnaik *et al.* (1999) evaluated 35 genotypes of ivygourd and recorded wide ranges of variation for internode length, fruit length, fruit diameter, fruits and yield per plant. Verghese (2003) recorded maximum variability for yield per plant followed by flowers per plant in ivygourd. Characters *viz.*, leaves per plant, days for first flowering, fruit weight, fruit size, protein content, vitamin A and vitamin C registered a variability of less than 20 percent both at genotypic and phenotypic levels.

Rastogi and Deep (1990) recorded higher PCV and GCV for fruit yield per plant and fruit weight in cucumber. PCV and GCV were minimum for days to first flower opening. Wehner and Cramer (1996) reported genetic variance for total, early and marketable fruits per plot, fruit shape, fruit size and fruit weight in three slicing cucumber populations. Gayathri (1997) reported highest PCV (95.8 per cent) and GCV (92.9 per cent) for yield per plant and lowest PCV (13.6 per cent) for days to first fruit harvest and lowest GCV (11.2 per cent) for days to first flower opening in cucumber.

Chacko (1992) noticed moderate to high GCV for yield per plant in a study for evaluation of dessert type muskmelon. Genotypic differences among the cultivars were the primary source of variation. In culinary melon, Kandaswamy (2004) observed highest PCV and GCV for yield per plant followed by average fruit weight and fruits per plant.

Mangal *et al.* (1981) conducted studies on 21 varieties of bittergourd and observed significant variation for all the characters. The highest GCV was shown by yield per plant and the lowest by days to first female flower opening. Indiresh

(1982) studied 24 lines of bittergourd and observed high GCV for fresh fruit weight, yield per plant and fruit size. Suribabu *et al.* (1986) analysed six lines of bittergourd and found GCV was moderate to high for all the characters except fruits per plant and percentage of fruit set. Choudhary (1987) reported significant variability in respect of various vegetative and yield characters of different bittergourd varieties. The highest PCV and GCV were noticed for yield per plant, fruits per plant, vine length and fruit weight. However, PCV and GCV were low for early female flower formation and early harvest. Vahab (1989) while studying genetic variability in 50 genotypes of bittergourd observed significant differences for 18 characters. The highest PCV was observed for fruit weight, yield and fruits per plant, while earliness exhibited low PCV. The GCV was of high magnitude for majority of characters. Jaiswal *et al.* (1990) found that fruits of various cultivars varied in colour, size, weight, contents of protein, carbohydrates, vitamin A, Vitamin C, acidity and total phenol. They reported considerable variation for these traits. Thakur *et al.* (1994) studied mean square estimate for genotypes of bittergourd and reported significant variability for vine length, branches per plant, root : shoot ratio and flowers per plant.

Mathew *et al.* (2001) evaluated 28 accessions of bottlegourd and recorded high coefficient of variation for the traits fruits per plant (32.50 per cent), fruit length (24.8 per cent), fruit weight (22.40 per cent), vine length (21.45 per cent) and fruit set (21.4 per cent). Rahman *et al.* (1994) conducted biochemical studies and observed considerable variability for nitrogen content, phosphorous, potassium, chlorophyll a and b, carotenoids, sucrose and maltose in the leaves of different type of bottlegourd. The protein, vitamin A and C and starch content of fruits were also varied. In bottlegourd, Kumar *et al.* (1999) noticed maximum GCV for fruits per plant followed by yield per plant. Mathew (1999) found in bottlegourd significant differences for vine length, primary branches, days to first primary branches, days to first female flower opening, root : shoot ratio, sex ratio, fruits per plant, fruit yield per plant, fruit size, 100 seed weight, seeds per fruit and crude fibre content. Bisognin and Storck (2000) reported significant estimates for genetic variance for large fruit diameter and neck diameter in bottlegourd.

In ridgegourd, Varalakshmi *et al.* (1995) reported high PCV and GCV for fruits per plant, fruit weight, seeds per fruit and yield per plant. Variability studies in ridgegourd revealed significant female flower, nodes to first female flower, root : shoot ratio, days to first harvest, fruits per plant, yield per plant, average fruit weight, fruit size, seeds per fruit and crop duration (Anitha, 1998). Choudhury and Sarma (2002) evaluated 12 ridgegourd cultivars and recorded high phenotypic and genotypic coefficients of variation for vine length, fruit weight and yield per hectare. Phenotypic coefficient of variation was greater than genotypic coefficient of variation for all characters. Singh *et al.* (2002) experimented with 80 genetically diverse genotypes of ridgegourd and noticed that high phenotypic and genotypic coefficient of variation for node of first male flower, main axis and branches, fruits per plant, fruit weight and yield per plant.

Varghese (1991) found high variation among 48 genotypes of snakegourd for days to first female flower, fruit harvest, yield per plant, number of flowers per plant and lowest PCV and GCV were recorded for number of days for first flowering. Varghese and Rajan (1993) found GCV was high for fruiting nodes on main vine, flowers per plant, root : shoot ratio, fruits per plant and crude fibre content in snakegourd. Mathew (1999) reported the highest PCV and GCV for mean fruit weight and the lowest PCV and GCV for flesh thickness. Radhika (1999) found that in snakegourd the PCV values ranged from 5.63 to 21.83 per cent. PCV for flesh thickness was the highest. It was followed by yield per plant and fruits per plant. The GCV values ranged from 4.22 per cent (days to first fruit harvest) to 21.54 per cent (flesh thickness). Fruit yield per plant and fruits per plant also had high values of GCV. Ashok (2000) found wide variation in seed, growth and yield characters in a study of character association of seeds with plant morphology in snakegourd.

In pointedgourd Singh *et al.* (1986) reported high GCV for yield per plant, fruit size and fruits per plant. Sarkar *et al.* (1990) observed high genotypic and phenotypic variances for fruits per plant, fruit volume and number of seeds per fruit. Singh *et al.* (1992) reported significant differences for leaf area, leaves per

plant, flowers per plant, fruits per plant and yield in pointedgourd. High GCV was observed for yield and number of fruits.

In ashgourd, George (1981) conducted biochemical studies and reported significant difference for protein content in fruits, vitamin C in fruits, fruit yield, length of main vine, female flowers per plant, fruit weight, weight of first mature fruit, fruits per plant, leaves per plant, fruit size, flesh thickness, seeds per fruit and 100 seed weight. Variability was limited for days to first female flower anthesis.

Mohanty and Mishra (1999) observed high GCV and PCV for yield and fruits per plant in pumpkin and PCV was greater than GCV for all the traits.

Mohanty (2000) studied 19 pumpkin genotypes and observed high phenotypic and genotypic coefficients of variation for yield per plant, number of fruits per plant and average fruit weight. High phenotypic coefficient of variation alone for vine length, number of flowers per plant and primary braches per plant. Chaturvedi (2001) observed that total soluble solids content varied from 3.15 to 5.35 per cent in edible portion and 3.49 to 4.82 per cent in peel portion, crude fibre from 0.44 to 0.76 per cent in both edible portion and peel portion of different pumpkin varieties. Sirohi and Yayasani (2001) revealed distinct variation in the total soluble solids and carotenoid content of eight genetically diverse lines and varieties and their F1 hybrids of pumpkin. The range of mean values of total soluble solids varied from 4.20 to 8.67^0 Brix for F1 hybrids and total carotenoid from 0.32 to 0.82 mg/100g for parents and hybrids. Lakshmi *et al.* (2002) were recorded the maximum genotypic and phenotypic variances for number of seeds per fruit 1000 seed weight and number days to first female flowering in pumpkin.

Hegde *et al.* (1994) found that among the varieties of watermelon cultivated in paddy fallows of Malanad, Kerala considerable variation was found for the yield and yield related characters. Shibukumar (1995) noted significant differences for all the characters in the variability studies in 20 genotypes of watermelon. High PCV and GCV were reported for number of seeds per fruit, number of fruits per plant and node to first female flower.

2.1.1.2 Heritability and Genetic Advance

In the process of crop improvement only the genetic component is transmitted to the subsequent generations. The extent of improvement further depends on the intensity of selection and genetic advance obtained from the population. High habitability is not always an indication of high genetic advance (Johnson *et al.*, 1955).

In ivygourd, Joseph (1999) observed high heritability for vine length primary branches per plant, fruit yield per plant and fruits per vine. In ivygourd, high habitability along with a good genetic advance was found for all the characters studied, except for number of days for first flower opening which exhibited high habitability and low genetic advance (Varghese, 2003).

Prasad and Singh (1992) studied 23 genotypes of cucumber and observed that the heritability estimates ranged from 0.02 per cent for fruits per plant to 48 per cent for fruit length. High heritability coupled with high genetic advance was also observed for fruit length, fruit breadth and fruit weight indicating the action of additive genes for the expression of these characters. Prasad and Singh (1994) reported high heritability and genetic advance for more than 12 growth and yield attributes in a collection of cucumber. Wehner and Cramer (1996) reported low and moderate heritability for fruit yield and number of days to first flower. Gayathri (1997) reported high heritability along with high genetic advance for yield per plant, fruits per plant, fruit weight and days to first female flower.

Vahab (1989) observed high heritability coupled with high genetic gain for fruit weight, yield and fruits per plant in bittergourd. Choudhary *et al.* (1991) also reported high estimates of heritability and genetic advance for yield per plant and fruits per plant. Rajput *et al.* (1995) reported high heritability in bittergourd for almost all the yield and related characters such as fruits per plant, fruit weight and fruit size. Iswaraprasad (2000) recorded high heritability for days to first male flower, days to first female flower, days to first fruit harvest, female flowers per plant, fruits per plant, mean weight of fruit, fruit yield per plant, fruit size, flesh thickness, seeds per fruit, 100 seed weight and crop duration in a study using 7

parents and 21 hybrids of bittergourd. All characters excluding days to first fruit harvest and crop duration showed high estimates of genetic advance.

Anitha (1998) recorded in high heritability along with high genetic advance for vine length, sex ratio, fruits per plant, yield per plant, fruit size and seeds per fruit ridgegourd. Days to first female flower opening and days to harvest had highest heritability but low genetic advance. High heritability and high genetic advance were recorded for the characters vine length, fruit weight and yield per hectare by Choudhury and Sarma (2002) in 12 cultivars of ridgegourd. Singh *et al.* (2002) experimented with 80 genotypes of ridgegourd and found high heritability coupled with high genetic advance for node number of appearance of first male and female flower, length of main axis, number of primary branches, male and female flowers per plant, sex ratio on whole plant, main axis, branches, fruits per plant, fruit set, fruit length, fruit weight, seeds per fruit and yield per plant. In ridgegourd, Varalakshmi *et al.* (1995) observed high heritability values for seeds per fruit, fruit weight, days to first female and male flower, fruit length, 100 seed weight and fruits per plant and low heritability for branches per plant and fruit diameter. Seeds per fruit and 100 seed weight showed high estimates of heritability and genetic advance.

Varghese (1991) noticed in snakegourd high heritability coupled with high genetic gain for flowers per plant, sex ratio and number of branches on the main vine. Varghese and Rajan (1993) observed high magnitude of both heritability and genetic advance for fruits per plant, while yield per plant, fruit length, crop duration, days to first harvest and days to first male flower opening showed high heritability coupled with low genetic gain. Mathew and Khader (1999) reported mean fruit weight, seeds per fruit, fruit length and fruit yield per plant had high heritability coupled with high genetic advance. In snakegourd, Radhika (1999) reported the highest and lowest values of heritability for days to first female flower and vine length respectively. High heritability along with high genetic advance was noticed for days to first male flower opening, days to first female

flower opening, fruit yield per plant, flesh thickness, fruits per plant and 100 seed weight.

Kumar *et al.* (1999) observed high heritability for all the characters studied and high genetic advance was recorded for fruits yield per plant in bottlegourd. It was followed by number of fruits per plant and number of branches per plant. Mathew (1999) reported high heritability and genetic advance for vine length, primary branches, node to first female flower, fruit length, fruit girth and seeds per fruit in bottlegourd. Bisognin and Storck (2000) observed moderate heritability for fruit shape in bottlegourd.

Sarkar *et al.* (1990) in a study with 16 divergent genotypes of pointedgourd found that fruit diameter had high heritability and low genetic advance. In pointedgourd, Singh *et al.* (1992) found that all characters were highly heritable. High heritability with high expected genetic advance was seen for fruits and yield per plant.

Kumaran *et al.* (1997) observed high heritability coupled with high genetic advance for vine length, mean fruit weight, fruits per plant, seeds per fruit and fruit yield per plant in Pumpkin. Mohanty and Mishra (1999) observed moderate heritability with moderately high genetic advance for yield per plant in pumpkin. Days to first anthesis, first female flowering node, flesh thickness, vine length and flowers per plant showed moderate to high heritability accompanied by low genetic advance. High heritability and high genetic advance were recorded for vine length, male and female flowers per plant, length and weight of fruit, fruits per plant and yield per hectare by Bindu *et al.* (2000) through evaluation of 24 genotypes of pumpkin. Mohanty (2000) evaluated 19 pumpkin genotypes and found high heritability and high genetic advance as per cent of mean for the characters fruit weight, fruits per plant and yield per plant and high heritability alone for days to anthesis of first female flower, female flowers per plant and primary branches per plant.

In ashgourd, Menon (1998) observed high heritability for seeds per fruit and size of fruits in a study for cataloguing and identification of promising

ecotypes. Primary branches per plant had the highest genetic advance. High heritability and high genetic advance was seen for primary branches per plant, yield per plant, seeds per fruit and fruit weight.

Chacko (1992) evaluated desert type muskmelon for southern region of Kerala. He observed association of high heritability with high genetic advance for yield per vine

Gopal *et al.* (1996) reported high heritability accompanied by high genetic advance for length of vine and number of branches in watermelon. Deepthy (2000) observed high heritability and genetic advance for keeping quality, yield per plant, seeds per fruit, 100 seed weight, mean fruit weight, flowers per plant, fruits per plant, productive branches per plant and sex ratio in melon. High heritability and genetic advance were send for days to first diameter, flesh thickness, fruiting period and crop duration.

2.1.2 Correlation Studies

Yield is the most important criterion of selection. Complexity of this character made up of several other component characters essentially make it the subject of a distinct study. Correlation studies would facilitate effective selection for improvement of one or many yield contributing components. An estimate of inter-relationship of yield with other traits is of immense help in any crop improvements programme.

In ivygourd, Joseph (1999) observed that fruits per plant, average fruit weight, fruit girth and fruit length, showed significant positive correlation with yield. Fruits per plant had the highest correlation with yield. Sarnaik *et al.* (1999) reported that yield per plant showed positive and significant correlation with fruits per plant and size of fruits and genotypic and phenotypic levels in ivygourd. The yield was also significantly and positively correlated with vine length and number of branches at genotypic level. In ivygourd, Yield per plant exhibited positive association with all the characters except number of days for flowering, which had significant negative correlation with yield (Varghese, 2003).

Chen *et al.* (1994) found that there was a significant positive genotypic correlation between number of flowers, number of parthenocarpic fruits, yield, number of fruits and fruit weight in cucumber. Ma *et al.* (1995) reported that in cucumber total yield had a significant positive correlation with total fruit number, fruit size and fruit weight. Neikov *et al.* (1995) carried out correlation with number of flowers per plant, fruits per plant and fruit weight.

Damarany *et al.* (1995) observed a negative relationship between total yield and number of days for first flowering in summersquash. The total yield had significant positive correlation with total fruit number.

Saika *et al.* (1995) showed that yield per plant had strong positive correlation with main vine length, number of branches, single leaf area, number of leaves, fruiting percentage, fruits per plant, fruit weight and fruit size both at genotypic and phenotypic levels. Zhang *et al.* (1999) reported that the three traits with the largest direct positive action on early yield were fruit weight, harvested fruits per plant and fruit length in cucumber.

Rajput *et al.* (1995) in correlation and path analysis studies on 21 genotypes of bittergourd indicated that fruit yield per vine was positively correlated with number of flowers, fruits per vine, fruit weight, fruit length, percentage of fruit set, vine length, number of leaves and leaf area. Fruit yield per vine was negatively correlated with days to first flower opening both at genotypic and phenotypic levels. Badade *et al.* (2001) reported that in bittergourd, yield was found significantly and positively correlated with number of branches per vine, percentage of female flowers and number of fruits per vine. Days to first male and female flowers appearance and weight of deformed fruits per vine are significantly and negatively correlated with yield at both phenotypic and genotypic levels. In bittergourd, at phenotypic and genotypic levels, fruit yield per vine was positively correlated with flowering duration, fruit length, fruit breadth, fruit rind thickness, number of fruits per vine, fruit weight, biological yield, dry matter and harvest index and negatively correlated with days to first flowering (Bhave *et al.*, 2003).

Singh *et al.* (1993) reported that high yield was positively correlated with number of fruits per plant in pointedgourd. Fruits per plant, days to first flowering and average fruit weight were responsible for yield increase. Sarker *et al.* (1999) conducted correlation studies in pointedgourd and reported that fruit weight, fruit size and primary branches per plant were positively and significantly correlated with yield per plant at genotypic and phenotypic levels.

Kumar *et al.* (1999) reported positive and significant correlations for days to first female flower, vine length, fruit weight, fruits per plant and seeds per fruit with fruit yield per plant in pumpkin. Devadas *et al.* (1999) observed that total number of seeds per fruit and 100 seed weight were the greatest in big fruits. Fruit weight was significantly correlated with polar and equatorial diameter, total number and number of seeds per fruit and 100 seed weight.

Menon (1998) observed that the fruits per plant had the highest positive and significant phenotypic correlation with yield in ashgourd. Highest genotypic correlation of yield was found with female flowers per plant. Yield was positively and significantly correlated with length of main vine, primary branches per plant, nodes on main vine, internode length, leaves per plant, female flowers per plant percentage of female flowers, fruit weight, fruits per plant, percentage of fruit set, circumference of fruit, fruit size and seeds per fruit.

Dhaliwal *et al.* (1996) observed that fruit yield was positively correlated with fruit weight, fruits per vine and flesh thickness in muskmelon. Fruit weight and fruits per vine were negatively correlated. Gopal *et al.* (1996) recorded positive and significant correlations of branches per vine and female flowers per vine with yield in watermelon.

Gwanama *et al.* (1998) reported mid season traits (length of internodes with first female flower, length of primary axis, primary branches and leaves per plant) exhibited insignificant genotypic and phenotypic correlation with fruit yield in muskmelon. Late season traits (weight of first mature fruit and fruits per plant) had insignificant genotypic correlation on fruit yield.

2.1.3 Path Coefficient Analysis

Different character traits and the causes of their association either direct or indirect are analysed for an indepth understanding of the relative importance of each factor.

In ivygourd, almost all characters showed positive direct effect on fruit yield per plant excluding number of leaves per plant, root : shoot ratio and number of days for first flowering, which showed negative direct effect (Varghese, 2003).

Pandita *et al.* (1990) found that number of fruits and fruit weight had the highest positive direct effect in round melon. Number of days for first flowering had negative direct effect on yield. Rajput *et al.* (1991) reported that in cucumber harvest period also influenced yield but its degree of association was reduced with increasing vine length. Prasad and Singh (1992) in the path analysis of yield and its components in 23 genotypes of cucumber revealed positive direct effect of vine length, days to female flower appearance, fruit weight and fruit length on yield. However, the positive direct effects of these components were partially counter balanced by their negative indirect effects. Solanki and Shah (1992) revealed through path co-efficient analysis of 11 yield components in cucumber that vine length, number of female flowers and days to first female flower and positive and highly significant direct effect on fruit yield. Saika *et al.* (1995) in the path coefficient analysis in 8 genotypes of cucumber revealed that fruits per plant had maximum direct effect on yield followed by fruit weight. These traits were considered as important parameters in all selection programmes for the yield improvement in cucumber. Gayathri (1997) reported that fruit size exerted maximum direct positive effect on yield followed by average fruit weight and fruits per plant in cucumber. Choudhary and Mandal (1987) in path co-efficient analysis in 30 diverse genotypes of cucumber revealed fruit number, female flowers per plant, fruit length, fruit weight and fruit diameter as the important characters determining yield.

In Pumpkin, Kumaran *et al.* (1998) reported that fruits per plant exhibited the highest direct effect on yield. High indirect positive effects were exerted by fruits per plant and mean fruit weight.

Paranjape and Rajput (1995) found that in bittergourd yield was mainly contributed by fruits per vine, fruit weight, fruit size and number of flowers. The fruit weight had maximum direct bearing on yield. However, vine length, number of braches, leaf area, fruits per vine and seed content indirectly contributed towards yield. In bittergourd number leaves per vine, fruit weight, seeds per fruit, fruits per vine, biological yield and keeping quality had direct positive effects on fruit yield, whereas fruit length had positive and indirect effects on fruit yield (Bhave *et al.*, 2003).

In ridgegourd, Rao *et al.* (2000) reported that fruits per vine and weight of fruit had high direct effect on yield. Sidhu and Brar (1981) made path co-efficient analysis in watermelon and found that the number of female flower and fruit weight had high positive direct effect on fruit yield

Positive direct effect of fruits per vine and fruit weight was reported by Vikram *et al.* (1984) in ashgourd. Menon (1998) found that in ashgourd average fruit weight exhibited the highest positive direct effect on fruit yield followed by number of fruits, female flowers per plant, vine length, leaf area and number of seeds per fruit. Leaves per plant exhibited a negative direct effect on fruit yield.

In watermelon, Shibukumar (1995) reported that fruit yield was directly affected by days to first flower followed by fruits per vine, length of vine and fruit weight.

Dhaliwal *et al.* (1996) reported that in muskmelon, fruit yield was directly affected by day to first flower opening followed by fruits per vine, number of flowers and fruit weight. Lal and Singh (1997), showed that number of node on which first female flower appeared, fruits per vine and fruits weight had positive direct effect on yield of muskmelon.

2.1.4 Genetic Divergence and Clustering of Genotypes

Nature and degree of divergence of different groups in a population is a subject of exclusive study and investigation in heterosis breeding. Genetic diversity plays an important role in plant breeding because hybrids between lines of diverse origin generally display a greater heterosis than those between closely related ones. Clustering of genotypes using Mahalanobis D^2 statistic measures the degree of diversification and determines the relative proportion of each component character to the total divergence. The genotypes grouped together in a single cluster are less divergent than the ones which are placed in different clusters.

Varghese (2003) studied genetic divergence of a collection of 50 genotypes of ivygourd. Based on eight morphological characters, the genotypes were grouped into 11 clusters in which flowers per plant and leaves per plant were found to be the two characters that contributed maximum for the divergence of the genotypes.

Vahab (1989) found that the bittergourd genotypes differed for all the 18 characters studied. The genotypes were grouped into 5 clusters. Parhi *et al.* (1993) studied genetic divergence in bittergourd for 14 quantitative characters. The 13 genotypes were grouped into 6 clusters. The characters 100 seed weight, number of seeds per fruit and yield per plant made maximum contribution to divergence. Genetic divergence among 13 genotypes of bittergourd was studied by Parhi *et al.* (1995) and the results revealed that the genotypes were grouped into six clusters and characters like hundred-seed weight, number of seeds per fruit and yield per vine contributed the highest towards divergence. Vahab and Gopalakrishnan (1993) studied genetic divergence among fifty genotypes in bitter gourd and grouped them into five clusters based on D^2 values. This study revealed that the grouping pattern of the genotypes was not always directly associated with the geographical diversity.

Varghese (1991) grouped 48 genotypes of snakegourd into 10 clusters in which fruit weight, fruit number and yield per plant contributed maximum to total

divergence. Maximum number of genotypes was present in the cluster 1(13) followed by III and V. Mathew (1999) assessed genetic diversity in a collection of 34 genotypes of snakegourd. Maximum contribution to total divergence was recorded by days to fist female flower, fruit weight, crop duration and seeds per fruit.

Rios *et al.* (1997) in a multivariate analysis of pumpkin genotypes identified 6 clusters and it was recorded that skin colour and yield contributed most to genotype clustering. Babu *et al.* (1996) classified fifty pumpkin genotypes into 5 clusters based on Mahalanobis D^2 statistics, containing 2, 7, 9, 12 and 20 genotypes respectively.

Twenty diverse cultivars of bottlegourd were studied by Badade *et al.* (2001) and the cultivars were grouped into 10 clusters. Considerable diversity with in and between clusters was observed for vine length, number of branches, percentage of female flowers, fruits per vine, length and diameter of fruit and yield per vine. Twenty eight accessions of bottlegourd were assessed by Mathew *et al.* (2001) and accessions were grouped into eight clusters. The clustering pattern indicated that there was no association between geographical distribution of accessions and genetic divergence. The characters like number of fruits per plant, number of seeds per fruit, length of fruit, average fruit weight, vine length and fruit set percentage contributed the highest to genetic divergence.

The studies on genetic divergence by Dora *et al.* (2001) revealed that 11 genotypes of pointedgourd were found grouped in four clusters. The cluster pattern of genotypes showed that the geographical diversity was not related with genetic diversity. Vine length, number of branches per plant, fruit weight and yield per plant contributed more towards yield.

Singh and Lal (2000) studied 21 muskmelon genotypes and grouped them into 13 clusters. The trait node at which first female flower appeared contributed the highest towards genetic divergence, while the least divergence was provided by total fruit yield per vine.

In D^2 analysis, the forty genotypes of melon were grouped into twenty clusters. Maximum genotypes were in cluster 1 and minimum in cluster XX. The analysis differentiated culinary types of melon from dessert types (Kandaswamy, 2004).

2.2 MOLECULAR CHARACTERIZATION

2.2.1 Molecular Markers

Molecular markers are genotypic markers (Bretting and Widrlechner, 1995). They are used to study the differences among strains at molecular level. Molecular markers constitute biochemical constituents (secondary metabolites in plant) and macro molecules (protein, DNA). Biochemical markers which have been used since long for the characterization of variation in a plant are now considered to be inappropriate as universal markers (Cooke, 1994).

Molecular markers have been shown to be useful for diversity assessment in a number of plant species (Waugh and Powel, 1992). Molecular markers are direct manifestations of genetic content (Weising *et al.*, 1995). They serve as reliable indices of genetic variation. In a past decade, molecular markers have very rapidly complemented the classical strategies.

The genetic markers are used for clonal identification, linkage mapping, population diversity, evolutionary studies, determining the genetic fidelity during micro propagation, germplasm conservation etc. (Bretting and Widrlechner, 1995).

2.2.1.1 DNA Markers

The term DNA fingerprinting was introduced by Jeffrey *et al.* (1985). Presently the term DNA fingerprinting/profiling is used to describe the combined use of several single locus detection systems and are being used as versatile tools for investigating various aspects of plant genomes. These included characterization of genetic variability, genome fingerprinting, genome mapping,

gene localization, analysis of genome evolution, population genetics, taxonomy, plant breeding and diagnostics.

With the advent of molecular biology techniques, DNA based markers have replaced enzyme markers in germplasm identification and characterization as well as in gene mapping because of its plasticity, ubiquity and stability. DNA is the ideal molecule for such analysis (Caetano–Anolles *et al.*, 1991). Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization based markers and polymerase chain reaction (PCR) based markers (Joshi *et al.*, 1999).

2.2.1.1.1 Hybridization based DNA Markers

The hybidization based DNA makers techniques utilize labeled nucleicacid molecules as hybridization probes (Caetano–Anolles *et al.*, 1991). Probe molecules range from synthetic oligonuleotides to cloned DNA. Some of the important hybridization based markers are Restriction Fragment Length Polymorphism (RFLP), Hypervariable sequences and variable Number of Tandom Repeats (VNTRs).

2.2.1.1.1.1 Restriction Fragment Length polymorphism (RFLP)

RFLP analysis involves digesting the genome with restriction enzymes, separating the fragments electrophoretically and then preferentially visualizing the fragments containing particular homologous sequences by hybridizing them with a specific labelled DNA probe (Deverna and Alpert, 1990).

Neuhausen (1992) used RFLP to determine interrelationships among cultivated varieties of *C. melo*. The study indicate that within *C. melo*, the differences among accessions are due to infrequent base subtitutions, where as between the two species, difference are mainly due to genome rearrangements such as inversions and deletions or numerous base substitution. RFLP, in 81 accessions of Japanese local and improved *Cucumis sativus* varieties were determined using 4 genomic clones (PO23, PO51, PO61 and P148) and one DNA clone (C143) (Matsura and Fujita, 1995). Genetic diversity in *Cucumis* species

was documented by using RFLP (Baudracco-Arnas and Pitrat, 1996). Thirty four RFLPs were analysed for linkage in 218 F_2 plants derived from two divergent cultivars. RFLP markers detected similar polymorphism levels. RFLPs were largely due to base substitutions rather than insertions, deletions. Twelve percent of markers showed distorted segregation. Garcia-Mas *et al.* (2000) used three different types of molecular markers, RFLP, RAPD and AFLP to measure genetic diversity among six genotypes of *Cucumis melo* L .

Miller and Tanksley (1990) analysed phylogenetic relationship and genetic variation in tomato using RFLP marker

RFLP analysis was used for the construction of linkage map in disease resistance like root knot nematode in *Solanum bulbocastanum* (Brown *et al.*, 1996) and bacterial canker resistance in *Lycopersicon peruvianun* (Sandbrink *et al.*, 1995).

2.2.1.1.1.2 PCR based DNA Markers

The fingerprinting techniques that use an *in vitro* enzymatic reaction to specifically amplify a multiplicity of target sites in one or more nucleicacid molecules (Michelli and Bova, 1996).

Among the PCR based marker techniques, the important ones are Amplified fragment length polymorphism, Microsatellite, Sequence characterized amplified region and Random amplified polymorphic DNA.

2.2.1.1.1.2.1 Amplified Fragment Length Polymorphism (AFLP)

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

RAPD, microsatellite and AFLP markers were evaluated for linkage analysis in melon (*Cucumis melo* L.) varieties MR-1 (resistant to fuzarium wilt, powdery mildew and downy mildew) and Ananos yokneum (Ay, susceptible to these diseases), to construct a detailed genetic map (Wang *et al.*, 1997). AFLP markers were more efficient in mapping the melon genome than RAPD or microsatellite markers. The map contains 197 AFLP, six RAPDs and one micro

satellite markers assigned 14 major and six minor linkage groups. The map had immediate utility for identifying markers linked to disease resistance genes that are suitable for marker assisted breeding. Garcia–Mas *et al.* (2000) studied the genetic diversity of six genotypes of *Cucumis melo* by using AFLP, RAPD, and RFLPs. Perin *et al.* (2002) reported a reference map of *Cucumis melo* based on two inbred line population by using AFLP method.

Mace *et al.* (1999) evaluated the genetic distance among the cultivated egg plant (*Solanum melongina*) and related species (*Solanum* sub genus *Leptostemonum*, section *Melongina*, series *Incaniformia*) by using AFLP techniques. The result indicated that the AFLP technique is both efficient and effective tool for determining genetic relationships among species of *Solanum*.

Nine virus free clones (Hiroshima 1 go – Hiroshima 9 go) of wakegi onion (*A. Wakegi*) were examined by fluorescent AFLP technique using 16 primer combinations. The results verified that wakegi onion, are interspecific hybrids whose parents are the Japanese bunching onion and shallot. Thus the fluorescent AFLP technique is a useful method for discriminating wakegi onion cultivars (Araki *et al.*, 2003).

Genetic diversity of eight selected Argentinean garlic clones with AFLP produced the dendrogram show 6 arbitrary groups. The garlic clones were clustered according to the physiological group and bulb colour. The potential use of AFLP could allow not only the differentiation among species, but also between botanical varieties and well defined ecotype groups as reported by Garcia-Lampasona *et al.* (2003).

The *gms* gene of Chinese cabbage (*Brassica compestris* spp. *chinensis*) conferring a recessive genetic male sterility was mapped with AFLP markers. Four markers were tightly linked to that gene. The AFLP, were cloned and sequenced. The sequence tagged site (Sts) could be used for marker assisted selection of male sterile plants among segregating populations (Ying *et al.*, 2003).

2.2.1.1.1.2.2 Microsatellite

The term microsatellite was coined by Litt and Luty (1989). DNA sequences with short repeated motifs (2-6 bp) are called simple sequence repeats (SSRs) or microsatellites (Epplen *et al.*, 1991) because microsatellite are highly polymorphic, randomly distributed in the genome and easily analysed as a general and novel source of genetic markers (Thottappilly *et al.*, 2000).

Microsatellite consists of randomly arranged di-tri-tetra nucleotide repeats, which are hypervariable and ubiquitously distributed throughout eukaryotic genomes. Microsatellite markers, which can be directly amplified by PCR, have been developed using the unique sequences that flank microsatellite (Webber and May, 1989).

Katzir *et al.* (1996) carried out the seven SSR, which were used to test a diverse sample of Cucurbitaceae, including 8 melon, 11 cucumber, 5 squash, 1 pumpkin and 3 watermelon genotypes. Five of the seven SSR, detected length polymorphism among the 8 melon genotypes with gene diversity values ranging from 0.53 to 0.75 microsatellite. Four of the seven SSR, detected polymorphism among the 11 cucumber genotypes with gene diversity values ranging between 0.18 and 0.64 primers specific to SSRs of *C. melo* and *C. sativus* also amplified DNA extracted from genotypes belonging to other genera of the Cucurbitaceae family.

Staub *et al.* (2000) have successfully employed seventeen SSR markers that were used to characterize genetic relationship among 46 accession in two *Cucumis melo* L. sub sp. *melo* (Cantalupensis, Inodorus) and sub sp. *agrestis* (Conomon and Flexuosus) groups. Empirical estimations of variances associated with each marker type in the accessions examined indicated that, per band Lower coefficient of variation can be attained in the estimation of genetic diversity when using RAPDs compared to SSRs.

Damin-Poleg *et al.* (2001) expressed that sixty one *Cucumis* SSR markers were developed, most of them (46) from melon (*Cucumis melo* L.) genomic

Libraries. Forty of the markers (30 molons and 10 cucumber SSRs) were evaluated for length polymorphism in a sample of 13 melon genotypes and 11 cucumber (*Cucumis sativus* L.) genotypes. SSR data were applied to phylogenetic analysis among the melon and cucumber genotypes. A clear distinction between the exotic groups and the sweet cultivated groups was demonstrated in melon. In cucumber, seperation between the two sub sp. *C. sativus* var. *sativus* and *C. sativus* var. *hardwickii* was obtained.

Lopez-Sese *et al.* (2002) analysed 15 genotypes of Spanish melon (*C. melo* L.) in allele variation, at 12 microsatellite (SSR) Loci. Many SSR loci suggested that some populations were in genotypic disequilibrium. Moreover, a high level of genetic variation was observed between cassaba market classes than within accessions. Resulted bulk sampling technique coupled with molecular analysis technique that employ a unique array of discriminating markers can provide information leading to effective.

Paris *et al.* (2003) compared forty five accessions of *Cucurbita pepo* for presence or absence of 448 AFLP, 147 ISSR and 20 SSR bands. Clustering was in accordance with the division of *C. pepo* into three sub species, *fraternal*, *texana* and *pepo*. The sub sp. *texana* cluster consisted of six sub-clusters, one each for the representatives of its five cultivar groups (Acorn, Crookneck, Scallop, straightneck and orifera gourd) and wild gourds. The smallest-fruited accession, 'Miniature Ball' appeared to occupy a genetically central position with in *C. pepo*.

2.2.1.1.1.2.3 Sequence Characterized Amplified Region (SCAR)

SCAR DNA analysis was developed to produce reliable PCR based results. Efficiency of RAPD to SCAR marker conversion and comparative PCR sensitivity in cucumber was reported by Horejsi *et al.* (1999) and sex determination in papaya by using SCAR primers was reported by Deputy *et al.* (2002).

SCAR markers were used to detect linkage of fom-2 fusarium wilt resistance in melon (Zheng *et al.*, 1999).

2.2.1.1.1.2.4 Random Amplified Polymorphic DNA (RAPD)

Polymerase chain reaction in conjunction with random primers, was used for fingerprinting genomes (Welsh and Mc Clelland, 1990), population biology studies (Astley, 1992), identification of genome specific markers and other uses (Williams *et al.*, 1990; Erlich *et al.*, 1991). Several authors have applied RAPD technique to investigate genetic variability and found the technique as very efficient and reliable (Brown *et al.*, 1993; Munthali *et al.*, 1996). Analysis of RAPDs offers several advantages compared to RFLP. The most important advantage is that RAPD is not a labour intensive procedure. It is not necessary to construct or maintain genomic library. RAPD requires smaller quantities of genomic DNA than RFLP analysis. Also it is less costly compared to RFLP. Generation of RAPD is quicker than RFLP and can be used to detect even single gene mutation (Williams *et al.*, 1990).

2.2.1.1.1.2.4.1 RAPD and Linkage Maps

RAPD assay has been used by several groups as an efficient tool for indentification of markers linked to agronomically important traits which are introgressed during the development of mere isogenic lines.

Levi *et al.* (2001) employed RAPD markers for constructing genetic linkage map for watermelon (*Citrullus lanatus*) using BC_1 population PI 296341 (Fusarium wilt resistant) X New Hamshire midget (Fusarium susceptiable) X New Hamshire Midget. The Map should be useful for identification of markers linked closely to genes that control fruit quality and fusarium wilt (races 1 & 2) resistance in watermelon.

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

An F_2 population of two celery cultivated types (*Apium graveolens* vars. *rapaceum* and *secalinum*) was used to construct a linkage map consisting of 29 RFLP, 100 RAPD, isozyme, one disease resistance and one growth habit markers.

The map contains 11 major groups and 9 small groups and has a total length of 803 cM with an average distance of 6.4 cM between two adjacent loci (Yang and Quiros, 1995).

2.2.1.1.1.2.4.2 RAPD and Hybrids

RAPD technique has been used for the identification of hybrids and their parents determination as well. Wang *et al.* (1994) proposed RAPD fingerprinting as a convenient tool for the identification, protection and parentage determination of plant hybrids. In their study, DNA from three families of rice plant selected in Northern China (each comprising the male sterile, the restorer, the hybrid F_{15} and maintainer lines) was extracted and amplified by RAPD techniques. The results obtained were useful for identification of each single plant line.

Truksa and Prochazha (1996) reported different banding pattern based on the DNA polymerase used for testing three lines of cucumber for the production of hybrid seeds. Low level of polymorphism was obtained which indicated that RAPD was not suitable for verifying the hybridity of seeds.

Ilbi (2003) evaluated the potential of random amplified polymorphic DNA (RAPD) markers in varietal identification and genetic purity test of 5 Jalepeno hybrid varieties and their corresponding parents. He concluded that RAPD markers may be useful for cultivar identification and hybrid purity test in pepper (*Capsicum annum*) especially for routine seed quality control programme.

Etoh and Jian (2001) used RAPD technique to findout molecular markers associated with the trait of pollen fertility. Twelve pollen fertile and sterile garlic clones were screened using 60 RAPD primers to find the DNA fragments related pollen fertility.

RAPD technique has been used for the determination of genetic purity of F_1 hybrids. Crockett *et al.* (2002) demonstrated the use of RAPD–PCR for evaluating seed purity in a commercial F_1-hybrid broccoli (*Brassica oleraceae* var. *italica*) cultivar. The study clearly demonstrate that RAPD-PCR is a useful tool for determining genetic purity of commercial F_1-hybrid broccoli seeds.

RAPD technique was used to analyse the diversity and uniformity of DH lines derived from cabbage (CVs). Kamienna Glowa, Slawa 2 Enkhuizen and Langendijker (Kaminshi *et al.*, 2003). The result showed the occurrence of the differences at the molecular level among ten plants which indicated that their parental Ro plant probably obtained from somatic cells, not by androgenesis.

RAPD markers were used to identify commercial F_1 hybrid seeds of 2 chinese cabbage and their parents (Hua and Ying, 2003).

2.2.1.1.1.2.4.3 RAPD and Taxonomic studies

RAPD markers have been widely used for taxonomic and related studies. Demeke *et al.* (1992) investigated the potential use of RAPD for taxonomic studies using *Brassica*, *Sinapsis* and *Raphanus* taxa. Analysis of RAPD bands revealed the relationship between diploid and amphidiploid *Brassica* taxa. Results showed that the *Raphanus* sativus and *Sinapsis alba* were distinct from the *Brassica* taxa.

According to Jeon *et al.* (1994) RAPD markers generated by 6 out of 50 arbitary 10-mer primers were effective in discriminating among 9 *Cucurbita moschata* and 6 *C. pepo* cultivars. The 6 primers produced 64 useful RAPD marker of 350 to 6000 bp.

The genetic relationship in cultivated cucumber and its wild relative, wild *Cucumis* sp. (*C. hystrix*), interspecific hybrids and BC_1, S_2s and melon cultivars was studied using RAPD markers. The results from the UPGMA cluster analysis suggested that the 23 cultigens could be classified into four groups. These were the cultivated cucumber and its wild relative, *C. hystrix, C. hytivus* and the melon cultivars (Yun and Feng, 2003).

Friesen and Blatiner (2000) used RAPD analysis to study the phylogenetic relationships between species in *Allium* section *schonoprasum* for the investigation of the intraspecific differenciation. The analysis clearly distinquishes the species of section *schoenoprasum.*

RAPD and PCR-RFLP analysis were conducted to establish the phylogenetic relationships among collected accessions of shallot (*Allium cepa* and *ascalonicum*) and *Allium* X *wakegi*, and to asses the origin of *A. wakegi* (Arifin *et al.* 2000). From the RFLP analysis of amplified *mat* K gene of ctDNA it was demonstrated that *Allium* X *wakegi* originated from shallot as a maternal plant X welsh onion as a parental plant as well as from reciprocal crosses.

2.2.1.1.1.2.4.4 RAPD Detection of Genetic Variability

Staub *at al.* (1997) analysed variation at isozyme and RAPD loci in 8 cucumber and 7 melon cultivars to determine genetic variation among population of each species. Empirical estimate of variances associated with each marker type in the cucumber and melon accessions examined indicated that per band, lower coefficient of variation can be attained in the estimation of genetic difference when using RAPDs compared to isozymes.

RAPD markers and agronomic traits were used to determine the genetic relationships among 32 breeding lines of melon belonging to seven varietal types (Garcia-Mas *et al.*, 1998). The results indicated that RAPDs were more suitable markers than agronomic traits in predicting genetic distance among the breeding lines.

Garcia-Mas *et al.* (2000) used three different types of molecular markers viz. RAPD, RFLP and AFLP to measure genetic diversity among 6 genotypes of *Cucumis melo*. They separated the genotypes into two main groups (1 sweet type, cultivated melon and 2. the exotic type, non cultivated melon) by using the 3 types of markers.

Staub *et al.* (2000) used RAPD and SSR markers to characterize genetic relationship among 46 accessions in two *Cucumis melo* L. sub sp. *melo* (Cantalupensis, Inodorus) and sub sp. *agretis* (conomon and flexuosus) groups and band polymorphisms observed with 21 RAPD primers and 7 SSR primers. According to them the genetic relationships identified using these markers were generally similar. The disparity between the analyses of the two markers made

may be related to the amount of genome coverage which is characteristic of a particular marker system and / or its efficiency in sampling variation in a population and they suggested that 80 RAPD marker bands were adequate for assessing the genetic variation present in the accessions examined.

Lopez-Sese *et al.* (2001) revealed that the population structure of 5 spanish melon (*Cucumis melo* L.) accessions, mostly of group Indodorus, was assessed by using 100 random amplified polymorphic DNA (RAPD) bands produced by 36 primers. A relatively high level of polymorphism was detected using RAPD markers.. Moreover, a higher level of genetic variation was observed between Cassaba market classes than within accessions. Result of bulk sampling technique coupled with molecular analysis technique that employ a unique array of discriminating markers can provide information leading to effective.

Genetic diversity of 50 cucumber (*Cucumis sativus*) accessions from America, Holland, Japan and China was detected and evaluated using RAPD markers (Ping *et al.*, 2002). Thus 25 selected primers produced 215 scorable polymorphic RAPD bands and the ratio of polymorphism was 86.98%. The result confirmed that cucumber is a narrow – based germplasm, nevertheless, RAPD analysis was still useful in cucumber genotypic differentiation.

RAPD markers were used to investigate the genetic relationship of 50 melon accessions. Thirty five random primers were tested, and 20 primers showing polymorphism were selected. The amplified fragment ranged from 0.25 to 3 kb in size. The results showed that it was difficult to differentiate the varieties reticulatus, cantalupensis, inodorus and flexuosus belonging to the western groups from each other (Woo and Hyeon, 2003).

Kandaswamy (2004) used RAPD markers to characterize forty genotypes of *Cucumis melo*. The study clearly differentiate the dessert melons from culinary melons of South India.

According to Hawkins *et al.* (2001) several RAPD loci were identified to be loosely linked to morphological traits of an F_3 generation derived from a cross

between *Citrulus lanatus* var. *lanatus* cv. New Hamshire midget and *C. lanatus* var. *citroides* cv. PI 296341.

According to Kochieva *et al.* (2002) genome divergence among cross pollinating tomato species was higher than in self pollinating species. They analysed the genetic polymorphism in 53 species and cultivars of *Lycopersicon* by using RAPD markers.

Shekara *et al.* (2003) evaluated the genetic distance of 4 species of tomato, local selection (*L. esculentum*), LA 1478 (*L. Pimpinellifolium*), PI 126443 (*L. glandulosum*) and LA 1777 (*L. hirsutum*) by using RAPD markers, twelve random primers (OPC 6, OPD19, OPF 1, OPF3, OPF10, OPF11, OPF13, OPF 16, OPF 19, OPF 20, OPH 12, and OPK 8) generated 61 polymorphic bands. Finally they suggested the need for more number of random primers for discerning the genetic relationship effectively among the genotypes.

The RAPD technique was used for cultivar identification of 11 aubergine cultivars (*Solanum melongena* L.). Twelve 10-mer primers were used, 9 of which revealed polymorphism in cultivars (Kochieva *et al.* 1999)

RAPD markers were used to measure the genetic diversity within and among 13 accessions of *Capsicum pubescens* from Boliva, Ecuador, peru, Guatemala, Mexico or Costa Rica (Votava and Bosland, 2001).

Baral and Bosland (2002) was investigated the genetic diversity in *Capsicum annum* var. *annum* landraces from Nepal using RAPD markers and compared with that of *C. annum* var. *annum* landraces from centre of diversity, Mexico. RAPD marker based cluster analysis of *C. annum* var. *annum* clearly separated each accession. The analysis indicated that the Nepalese chilli population went through an additional evolutionary bottleneck or founder effect probably due to intercontinental migration.

Lanteri *et al.* (2003) used RAPD and AFLP markers to assesses genetic diversity within and between 5 populations of a land race of *Capsicum annum* L. genome in a Limited area in north-west Italy and locally known as 'cuneo pepper'.

RAPD markers were utilized to estimate the diversity among 42 *Abelmoschus* and one *Hibiscus* accessions (Martinello *et al.*, 2003). Thirty one random decamer primers were used to amplify the DNA by PCR and 103 RAPD fragments were generated.

Yung *et al.* (2001) investigated the genetic variation and relationships of onion germplasm by RAPD PCR analysis. In the test of 120 primers with 58 onion lines and cultivars collected from local and abroad, eight primers turned out to be useful for further RAPD analysis.

Peng *et al.* (2001) analysed 14 *Allium* cultivars by RAPD technique to determine their genetic relations. Finally they were classified the welsh onion, chive and bunching onion into *A. fistulosum*, bulb onion and shallot in to *A. cepa* and the remaining under *A. porrum* and *A. rhiziridium*.

Shigyo *et al.* (2002) identified two cultivated and related species of sections *cepa* and *phyllodolon* in *Allium* by using 60 RAPD markers. Some of the RAPDs were effective for identifying interspecific hybrids. A total of 393 RAPDs were detected between the cultivated species, *A. fistulosum* and shallot. These RAPDs will be useful as genetic markers in the two sections.

Tanikawa *et al.* (2002) found that the genetic diversity is low among 22 onion (*A. cepa*) cultivars by using RAPD markers. Seventeen of the 100 primers screened produced clear, reproducible, polymorphic banding profiles. A total of 88 fragments were produced by 17 primers, of which 35 were polymorphic among the cultivars.

With the aim of identifying RAPD markers for 14 broccoli and 12 cauliflower cultivars, Hu and Quiros (1991) used four 10-mer Operon primers to amplify DNA from broccoli and cauliflower. The markers generated by 2 and 3 primers were sufficient to distinguish each of the broccoli and cauliflower cultivars, respectively. The result showed that RAPD makers provided a quick and reliable alternative to identification of broccoli and cauliflower cultivars.

RAPD markers were used to discriminate among 16 commercial cultivars of cabbage (*Brassica oleraceae capitata* groups). Out of 100 primers screened,

18 decamer primers generated 105 polymorphic bands. Thus RAPD provides a quick and reliable alternative for the identification of cabbage cultivars (Cansian and Echeverigaray, 2000).

RAPD markers were used to investigate the genetic polymorphism present among 33 cultivars of vegetable *Brassica compestris*, including turnips (Subsp. *rapifera*), non-heading Chinese cabbages [sub sp. *chinensis* (*B. chinensis*)] and heading Chinese cabbages [sub sp. *Pekinensis* (B. pekinensis)] (Peng *et al.*, 2000).

Briad *et al.* 2002 used RAPD technique for analysis of the variability of wild seakale (*Crambe maritime* L.) for commercialization of that species.

The genetic relationship among 20 chinese kale cultivars (*Brassica oleracea* var. *alboglabra*) was examined by RAPD analysis using 12 decamer oligonucleotide primers and the cultivars were classified into 3 groups based on the numerous polymorphisms of DNA fingerprints. The result suggested that the Chinese kale cultivars with yellow petals diverged from those with white petals when they were introduced into Taiwan from China (Matsui *et al.*, 2002).

RAPD analysis was used to evaluate polymorphism and genetic relationships of 11 local germplasm of Chinese cabbage. The result indicated that, it was possible to study genetic relationship and polymorphism by RAPD with specific primer within subspecies (Yong *et al.*, 2003).

Evaluation of the genetic diversity among 26 carrot accessions from the collection of *Daucus* was done by Grzebelus *et al.* (2002) using RAPD technique. Out of the 33 primers screened, 6 primers generated the most polymorphic and reproducible bands. Six groups could be seen on the dendrogram showing genetic distances within the tested collection of carrots.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled 'Molecular Characterization of ivygourd [*Coccinia grandis* (L.) Voigt]' 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 garden land of Department of Olericulture, College of Agriculture, Vellayani. 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 warm humid tropical climate.

The study consisted of the following two experiments.

3.1 Morphological characterization

3.2 Molecular characterization

3.1 MORPHOLOGICAL CHARACTERIZATION

3.1.1 Materials

The basic material for the study included twenty five landraces of ivygourd collected from different agroclimatic regions of Kerala and Tamil Nadu. The details of the accessions and their sources are presented in Table 1 and Plate 1.

3.1.2 Methods

3.1.2.1 Design and Layout

The vine cuttings of pencil thickness from twenty-five landraces of ivygourd were planted in randomized block design with two replications. In each replication four plants were maintained. The cultural and management practices were adopted according to package of practices recommendations of Kerala Agricultural University (KAU, 2002).

3.1.2.2 Biometric Observations

Two plants per genotype per replication were randomly selected for taking observations and the mean value was worked out for each trait as

Table 1. Particulars of accessions of *Cccinia grandis* used in the study and their sources

Sl. No.	Accession number	Source
1	CG 21	Pala, Kottayam
2	CG 3	TNAU, Coimbatore
3	CG 13	Perinthalmanna, Malappuram
4	CG 17	Neyyattinkara, Thiruvananthapuram
5	CG 11	AC & RA, Madurai
6	CG 24	Kalliyoor, Thiruvananthapuram
7	CG 25	Vellayani-1, Thiruvananthapuram
8	CG 2	Vellanikara, Thrissur
9	CG 9	Aanad, Thiruvananthapuram
10	CG 22	Vellayani-2, Thiruvananthapuram
11	CG 6	Edakkade, Kannoor
12	CG 10	Perigamala, Thiruvananthapuram
13	CG 1	Vadakkanchery, Thrissur
14	CG 4	Pattambi, Palakkadu
15	CG 23	Kottiyam, Kollam
16	CG 7	Parasalai, Thiruvananthapuram
17	CG 5	Taliparamba, Kannoor
18	CG 8	Palapoor, Thiruvananthapuram
19	CG 20	Kazhakuttam, Thiruvananthapuram
20	CG 18	Coimbatore, Tamil Nadu
21	CG 14	Pothenkode, Thiruvananthapuram
22	CG 15	Sreekaryam, Thiruvananthapuram
23	CG 19	Balaramapdram, Thiruvananthapuram
24	CG 16	Saathacoil, Thiruvananthapuram
25	CG 12	Punkulam, Thiruvananthapuram

Plate 1. A comprehensive representation of 25 accessions of ivygourd used in the study

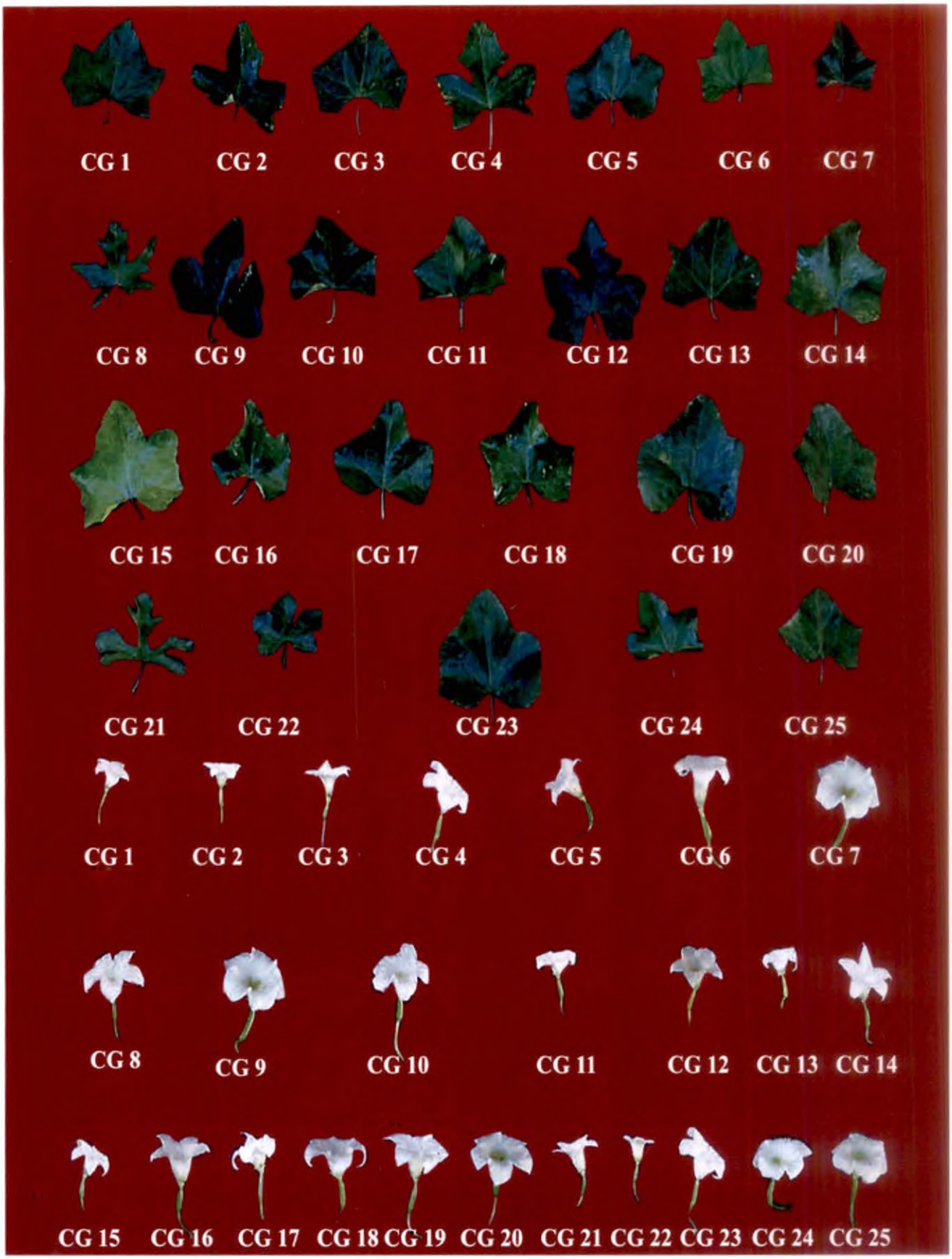


Plate 1. Continued

per standard procedures. Five fruits per genotype per replication were selected for taking observations of fruit characters.

1. Primary branch length (m)

Measured from the origin at main vine to the tip of the primary branches using the measuring tape after pulling out the vine at the time of last harvest.

2. Internodal length (cm)

Distance between two adjacent nodes were taken from the bottom portion, middle and top of the vine and average was calculated and expressed in centimeters.

3. Days to first flower

The number of days taken from planting to the bloom of the first flower was counted.

4. Node to first flower

The position of the node from the base of the plant to the one where the first flower appeared was counted.

5. Fruit length (cm)

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

6. Fruit girth (cm)

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

7. Fruits per plant

The total number of fruits from all harvest was counted as expressed.

8. Average fruit weight (g)

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

9. Seeds per fruit

Two 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 days and number of seeds were counted.

10. Yield per plant (Kg)

Weight of whole fruits from all harvests in each plants taken and average worked.

11. Total number of harvest

The total number of harvest from each accessions was noted and recorded.

12. Protein (mg)

Protein content of fresh fruits was estimated by used Lowery's method (Sadasivam and Manickam, 1996).

Procedure :

Fresh rhizome sample of 500 mg each were weighed and ground well with a mortar pestle in 5-10 ml of phosphate butter and centrifuged at 5000 rpm for 15 minutes. From the supernatant, 1 ml of aliquot was taken and 5 ml of alkaline copper solution was added to each of the samples including the blank. After 10 minutes, 0.5 ml of Folin-ciocalteaus reagent was added and incubated at room temperature in dark for 30 minutes. The blue colour developed was measured at 660 nm in a spectrophotometer (Systonics UV-VIS spectrophotometer 118). The amount of protein present in the sample was found out by using standard graph prepared by using bovine serum albumin (fraction V). The amount of protein was expressed as milligram albumin equivalent of soluble protein per 100 gram on fresh weight basis.

13. Organoleptic quality

The Organoleptic qualities and acceptability traits were done using the scoring method proposed by Jijimma (1986). The following major quality attributes were included in the score.

1. Appearance / colour

2. Doneness
3. Bitterness
4. Odour
5. Taste

Each of the above mentioned quality was assessed by a five point rating scale starting from 1 to 5 as furnished in Table 2.

14. Scoring for pests and diseases

No significant incidence of pests and diseases were noticed in the crop in any of the growth stages and hence no scoring for pests and diseases incidence was done.

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 as per Panse and Sukhatme 1967.

2. Mean : The mean of the i^{th} character Xi ($\overline{xi}$) was worked out.

3. Variability components at phenotypic and genotypic levels for different characters were 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 respectively from ANOVA and r, the number of replications.

Table 2 Score card for the organoleptic evaluation of ivygourd

Quality attributes	**Subdivisions of attributes**	**Score**
Appearance / colour	Natural color	5
	Colour fairly preserved	4
	Slightly discoloured	3
	Moderately discoloured	2
	Highly discoloured	1
Doneness	Highly acceptable	5
	Fairly acceptable	4
	Moderately acceptable	3
	Slightly acceptable	2
	Least acceptable	1
Bitterness	No bitterness	5
	Slight bitterness	4
	Moderate bitterness	3
	High bitterness	2
	Very high bitterness	1
Odour	Highly acceptable	5
	Fairly acceptable	4
	Moderately acceptable	3
	Slightly acceptable	2
	Least acceptable	1
Taste	Highly acceptable	5
	Fairly acceptable	4
	Moderately acceptable	3
	Slightly acceptable	2
	Least acceptable	1

For two characters X_i and X_j,

Environmental covariance, σ_{eij} = MSPE

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

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 genotype 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 Chaudhary, 1979).

For the character X_i,

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

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

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 :

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).

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 (1979).

$$\text{Genotypic correlation coefficient } r_g(ij) = \frac{\sigma_g ij}{\sigma_g i \times \sigma_g j}$$

$$\text{Phenotypic correlation coefficient } r_p(ij) = \frac{\sigma_p ij}{\sigma_p i \times \sigma_p j}$$

Environmental correlation coefficient $r_e(ij) = \frac{\sigma_e ij}{\sigma_e i \times \sigma_e j}$

7. Path analysis

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

8. Mahalanobi's D^2 analysis

Mahalanobi's D^2 analysis (1936) was applied for classificatory studies by Murthy and Arunachalam (1966) in crop plants. The same methodology was applied to cluster the genotypes of ivygourd in the experiment.

For i^{th} and j^{th} genotypes, the D^2 value is computed as

$$D^2 = \sum_{l=1}^{k} (X_{il} - X_{jl})^2$$

where k is the number of characters.

The genotypes were grouped into several clusters based on the D2 values by Tochers method of clustering (Rao, 1952).

3.2 MOLECULAR CHARACTERIZATION

3.2.1. Materials

The twenty five accessions of ivygourd used in the experiment I were studied for molecular characterization.

3.2.2 Methods

Isolation of genomic DNA

For the isolation of genomic DNA, leaf samples were collected from young new leaves of ivygourd plants. The method of isolation followed was the modified procedure of Murray and Thompson (1980). Briefly 1g of leaf material was first washed in running tap water and later in distilled water two 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 2.0ml centrifuge tube and enough extraction buffer (0.7N Nacl, 1% CTAB, 50mM Tris-HCl (pH 8.0), 10mM EDTA) was added to it so that clumps could easily be dispersed but the solution remains somewhat viscous. One millilitre of buffer was used for 100 mg of powder. 200-300µl PVP and 50-100 µl β-mercaptoethanol was also added to the centrifuge tube and was incubated in water bath at 60^0C for 45 minutes with occasional gentle shaking and centrifuged at 15000 rpm for 10 minutes at 4^0c and collected the aqueous phase, then added $^1/_3{}^{rd}$ volume of phenol : chloroform : Isoamyl alcohol (25:24:1) solution to the centrifuge tube, the two phases were mixed gently and centrifuged at 15000 pm for 10 minutes at 4^0C and collected the aqueous phase. To the aqueous phase, added the chloroform : Isoamyl alcohol (24:1) mixture the two phases were mixed gently and centrifuged at 15000 pm for 10 minutes at 4^0C and collecting aqueous phase and repeated the same until the interphase disappeared. After that $^1/_{10}{}^{th}$ volume of 3.0M sodium acetate was added followed by double volume of chilled absolute isopropyl alcohol. It was kept in refrigerator at 4^0C for 30 minutes and was centrifuged at 10000 rpm for 10 minutes at 4^0C to pellet the DNA. The supernatent was discarded and the pellet was washed in 70% ethanol. Then it was centrifuged at 5000 rpm for 5 minutes at 4^0C

The supernatant was discarded and the pellet was air dried for 20 minutes. Then the pellet was dissolved in 0.5ml of 1xTris EDTA buffer (10mM Tris Hcl, 1mm EDTA, P^H 8) and stored at 4^0C

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.

3.2.2. 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 dissolved was taken in a cuvettte to calibrate the spectrophotometer at 260 and 280nm wavelength. The optical density (OD) of the DNA samples dissolved in the buffer was recorded at both 260 and 280nm.

The quantity of DNA in the sample was estimated by employing the following formula: Amount of the DNA (ng/µl) = A_{260} × 50x dilution factor/1000. where, A_{260} – absorbance at 260nm.

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

3.2.3 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 (0.04M Tris acetate, 0.001M EDTA (pH 8.0) by boiling. After cooling to about 50°C, ethidium bromide was added to a final concentration of 0.5µg 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 by the buffer. The DNA sample was mixed with the required volume of gel loading buffer (60 x loading dye *viz.*, 40 per cent sucrose, 0.25 per cent bromophenol blue). Each well was loaded with 20µl of sample, for visualizing the genomic DNA added 5µl isolated sample. One of the wells was loaded with 5.0 µl of molecular weight marker along with required volume of gel loading buffer. Electrophoresis was performed at 75volts until the loading dye reached 3/4th of the length of the gel. The gel was visualized using an ultraviolet visible (UV-vis) transilluminator.

Random Amplified Polymorphic DNA (RAPD) analysis

DNA amplification was done using arbitarily designed decamer primers (Operon Inc., CA, USA) adopting the procedure of Williams et al. (1990) with required modifications. Polymerase chain reactions of genomic DNA were performed in 25 µl of reaction mixture containing 2.5 µl 10X PCR buffer (10mM Tris (pH 9.0), 1.50 mM $MgCl_2$, 50mM KCl and 0.01 per cent gelatin). 10 picomoles primer, 200µM each of dNTPs, 0.8 units of Taq DNA polymerase and 40ng genomic DNA. Amplification was performed in a Programmable Thermal Controller (MJ Research, Inc.) with an initial denaturation at 94^0C for 5 minute followed by 45 cycles of denaturation at 94^0C for 15seconds, annealing at 35^0C for 15 seconds and extension at 72^0C for 1.5 minutes. A step of extension at 72^0C for 7 minutes was included after the last cycle. Finally the products of amplification were cooled to 4 ^{0}C. A negative control containing sterile water instead of template was included in each reaction set. The DNA fragments produced and the PCR molecular weight markers were visualized in 1.2 percent agarose gel electrophoresis, stained with ethidium bromide and photographed with the help of gel doc system. The RAPD bands were represented as '1' for presence and '0' for absence and recorded. The PCR was repeated twice in order to confirm the reproducibility. The amplified products of four primers alone which could produce amplification for most of the clones were used for further analysis.

3.2.5 Data analysis

The reproducible bands were scored for their presence (1) or absence (0) for all the ivygourd genotypes studied. A genetic similarity matrix was constructed using Jaccard's similarity coefficient method (Jaccard, 1908).

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

Where,

a : number of bands present in both the varieties

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

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

Based on the similarity coefficient, the distance between the genotypes was computed with the help of the software package NTSYS (version 2.02). Using these values of distances, between genotypes, a dendrogram was constructed by following the UPGMA (Un weighted pair group method for arithmetic average) methods. Association between the various genotypes was found out from the dendrogram.

Results

4. RESULTS

Twenty five diverse accessions of ivygourd (*Coccinia grandis* (L.) Voigt) were evaluated in the field during 2003-2004 for biometric characters and quality attributes. The accessions were characterized on morphological and molecular basis. The data were statistically analysed. The accessions were also grouped into different clusters bases on the D^2 values and RAPD analysis. The result are presented under the following heads.

4.1. Genetic variability and genetic divergence

4.2. Molecular characterization (RAPD)

4.1. GENETIC VARIABILITY AND GENETIC DIVERGENCE

4.1.1 Analysis of Variance

General analysis of variance revealed significant differences among the 25 accessions for all the twelve characters.

4.1.2 Mean Performance of the Accessions

Performance of the accessions for twelve characters viz., primary branch length, internodal length, days to first flowering, node to first flower, fruit length, fruit girth, average fruit weight, seeds per fruit, fruits per plant, protein content, total number of harvest and yield per plant are presented in Table 3.

4.1.2.1 Primary Branch Length

The accession CG 19 possessed longest primary branch (8.03 m) followed by CG 4 (6.03 m) and CG 14 (3.93 m). The accessions CG 4, CG 5, CG 8, CG 9, CG 11, CG 13, CG 14, CG 17, CG 19, CG 21, CG 22, and CG 25exceeded the general mean (2.95m) for this trait. Whereas the line CG 20 (1.1m) had shortest primary branch.

Table 3. Mean performance of 25 accessions of ivygourd in 12 characters

Accession	1	2	3	4	5	6	7	8	9	10	11	12
CG 1	1.93	5.84	39.75	27.50	5.34	6.57	13.00	67.25	257.00	55.50	6.00	3.33
CG 2	1.58	5.34	56.75	23.00	5.34	6.03	9.70	64.75	287.50	46.50	5.00	2.70
CG 3	1.68	5.49	56.85	22.25	5.40	5.98	9.50	67.75	274.75	43.50	5.00	2.62
CG 4	6.03	6.31	55.25	22.25	5.17	7.54	14.05	63.00	504.00	68.00	13.50	7.11
CG 5	3.13	6.71	65.75	30.25	5.06	5.98	12.12	77.00	124.75	60.00	6.50	1.51
CG 6	1.98	5.83	52.75	22.00	6.84	7.04	14.55	71.75	106.25	61.50	5.50	1.55
CG 7	2.73	6.28	65.25	20.75	8.18	6.15	12.85	65.75	270.50	47.00	7.50	3.49
CG 8	3.18	7.38	67.50	32.75	4.79	5.69	9.95	73.50	130.50	57.50	8.50	1.30
CG 9	3.08	8.42	43.00	18.25	8.82	7.43	18.95	92.50	492.50	68.00	10.50	9.36
CG 10	2.45	8.16	40.75	23.75	8.22	7.15	18.90	92.75	393.75	74.00	9.00	7.43
CG 11	3.58	5.64	35.75	21.75	5.58	6.72	10.10	66.50	1072.25	62.00	10.50	10.88
CG 12	1.63	5.64	40.50	23.50	6.03	7.39	13.10	82.75	413.75	64.50	6.50	5.44
CG 13	3.48	8.50	43.25	19.25	9.60	7.58	19.60	93.00	666.25	51.00	12.50	13.07
CG 14	3.93	8.07	64.00	27.50	7.63	7.30	12.35	84.75	208.25	37.50	9.00	2.58
CG 15	2.83	7.11	44.00	21.25	8.04	7.38	14.60	72.25	321.00	56.00	8.00	4.70
CG 16	1.05	5.27	59.50	16.50	6.17	5.80	10.65	60.75	164.75	56.00	4.50	1.76
CG 17	3.30	8.23	41.75	20.25	8.09	7.11	17.40	88.75	527.00	54.00	9.00	9.21
CG 18	2.48	7.92	44.75	25.75	8.18	6.67	17.75	85.25	278.50	81.00	8.00	4.96
CG 19	8.03	8.94	44.75	30.00	6.08	8.26	19.05	112.50	963.25	60.00	16.00	18.42
CG 20	1.10	6.42	50.75	21.00	6.57	7.60	13.30	82.25	200.25	82.00	7.00	2.66
CG 21	3.43	7.96	62.50	28.50	5.59	5.23	10.85	71.25	150.50	64.50	9.50	1.62
CG 22	3.85	7.27	66.25	32.25	5.50	6.62	9.45	72.25	127.50	57.00	8.00	1.21
CG 23	2.18	6.92	41.00	20.00	6.45	7.17	16.85	81.75	315.75	92.00	7.00	5.84
CG 24	2.10	7.73	41.75	21.75	7.17	5.99	15.20	80.75	368.25	68.00	8.50	5.60
CG 25	3.18	7.36	38.00	18.50	6.04	8.14	17.65	97.25	278.00	60.50	12.00	5.24
Mean	2.95	6.99	50.48	23.62	6.64	6.82	14.06	78.72	355.87	61.10	8.52	5.34
CD	0.41	0.29	4.80	3.94	0.34	0.37	1.48	5.29	61.98	17.19	1.92	0.95

1 Primary branch length (m)
2 Internodal length(cm)
3 Days to first flower
4 Node to first flower
5 Fruit length (cm)
6 Fruit girth (cm)
7 Average fruit weight (g)
8 Seeds per fruit
9 Fruits per plant
10 Protein (mg)
11 Total number of harvest
12 Yield per plant (kg)

4.1.2.2 Internodal Length

The accession CG 19 (8.94 cm) had longest internode followed by CG 13 (8.5 cm) and CG 9 (8.42 cm) where as CG 16 (5.27 cm) was the shortest. The accessions CG 1,CG 2, CG 3, CG 4, CG 5, CG 6, CG 7, CG 11, CG 12, CG 16, CG 20, and CG 23 registered value lesser than the grand mean (6.99 cm).

4.1.2.3 Days to First Flowering

The accession CG 11 (35.75 days) was the earliest to flower while CG 8 (67.50 days) was the latest to flower. Accessions CG 1, CG 5, CG 9, CG 10, CG 11, CG12, CG 13, CG 15, CG 17, CG 18, CG 19, CG 23, CG 24 and CG 25 flowered earlier then the average (50.48 days)

4.1.2.4 Node to First Flower

The landrace CG 16 produced the first flower at the lowest node (16.5) and CG 8 at the highest (32.75). Fifteen accessions viz., CG 2, CG 3, CG 4, CG 7, CG 9, CG 11, CG 12, CG 13, CG 15, CG 16, CG 17, CG 20, CG 23, CG 24, and CG 25 exhibited lower values for this trait when compared to the general mean (23.62).

4.1.2.5 Fruit Length

The longest fruit was observed in CG 13 (9.6 cm) followed by CG 9 (8.82cm) and CG 10 (8.22 cm) whereas the accessions CG 8 had the shortest fruit (4.79cm). Fruits longer than the general mean (6.64 cm) were observed in CG 6, CG 7, CG 9, CG 10, CG 13, CG 14, CG 15,CG 17, CG 18, and CG 24.

4.1.2.6 Fruit Girth

The fruit girth was maximum in CG 19 (8.26 cm) followed by CG 25 (8.14 cm) and CG 13 (7.58cm). Greater than the average (6.82 cm) fruit girth was observed in accessions CG 4, CG 6, CG 9, CG 10, CG 12, CG 13, CG 14, CG 15, CG 17, CG 19, CG 20, CG 23, and CG 25. Lowest fruit girth in CG 21 (5.23cm).

4.1.2.7 Average Fruit Weight

CG 13 (19.6 g) recorded highest average fruit weight followed by CG 19 (19.05 g) and CG 9 (18.95 g) and the lowest was recorded in CG 3 (9.5 g). The accessions CG 6, CG 9, CG 10, CG 13, CG 15, CG 17, CG 18, CG 23, CG 24, and CG 25 recorded average fruit weight greater than average (14.05 g).

4.1.2.8 Seeds per Fruit

The accession CG 19 (112.5) had maximum seeds per fruit followed by CG 25 (97.25) and CG 13 (93.00) and minimum seeds were in CG 16 (60.75). The accessions CG 9, CG 10, CG 12, CG 13, CG 14, CG 17, CG 18, CG 19, CG 20, CG 23, CG 24, and CG 25 exceeded the general mean (78.72).

4.1.2.9 Fruits per Plant

Maximum fruits per plant was in CG 11 (1072.25) followed by CG 19 (963.25) and CG 13 (666.25) whereas the minimum was in CG 6 (106.25). The accessions which recorded above the mean (355.87) for this trait were CG 4, CG 9, CG 10, CG 11, CG 12, CG 13, CG 17, CG 19, and CG 24.

4.1.2.10 Protein

The protein content was maximum in CG 23 (92 mg) followed by CG 20 (82 mg) and CG 18 (81mg). The minimum protein content was in CG 14 (37.5 mg).

4.1.2.11 Total Number of Harvest

The accession CG 19 (16.00) obtained highest harvest followed by CG 4 (13.5) and CG 13 (12.5) whereas minimum was in CG 16 (4.5). The accessions which showed above the grand mean (8.52) were CG 4, CG 9, CG 11, CG 13, CG 19, and CG 21.

4.1.2.12 Yield per Plant

The highest yield per plant was obtained from accession CG 19 (18.41 kg) followed by CG 13 (13.07 kg) and CG 11 (10.88 kg) where as the lowest was

recorded in CG 8 (1.3 kg) (Plate 2). The accessions CG 4, CG 9, CG 10, CG 11, CG 12, CG 13, CG 17, CG 19, and CG 24 exceeded the grand mean (5.34 kg).

4.1.2.13 Organoleptic Quality

The quality attributes considered under organoleptic evaluation were colour, doneness, bitterness, odour and taste. The accessions did not differ significantly for all these attributes. The accessions CG 14 (20.14) followed by CG 23 (18.85) and CG 17 (18.4) were organoleptically superior than others based on the score. The accessions CG 8, CG 5, CG 21 and CG 22 which were highly bitter did not have any score (Fig. 1).

4.1.2.14 Incidence of Pest and Diseases

There was no severe incidence of pest and diseases. However, a minor attack of fruit fly was observed. The incidence of pest and diseases were not serious and no scoring was done (Plate 3).

4.1.3 Variability Studies

The phenotypic variance, genotypic variance, environmental variance and coefficient of variation for the biometric characters are presented in Table 4 and Fig. 2.

Number of fruits per plant showed the highest genotypic variance (59543.1) followed by seeds per fruit (157.117) and protein content (118.165). Least value was recorded for fruit girth (0.621) and internodal length (1.264).

The phenotypic variance was also maximum for fruits per plant (60445.03) followed by protein content (187.502) and seeds per fruit (163.689). Lower phenotypic variance was noticed for girth of fruit (0.653) and internodal length (1.283).

Maximum GCV in per cent was observed for yield per plant (78.695) followed by fruits per plant (68.569), primary branches length (50.702), total number of harvest (32.043) and average fruit weight (23.873). Lowest GCV in

Table 4. Components of variance for 12 characters in ivygourd

Characters	Variance			Coefficient of variation	
	GV	PV	EV	GCV (%)	PCV (%)
Primary branch length (m)	2.24	2.28	0.04	50.70	51.15
Internodal length (cm)	1.26	1.28	0.02	16.09	16.21
Days to first flower	107.57	112.97	5.40	20.54	21.05
Node to first flower	18.38	22.02	3.64	18.15	19.87
Fruit length (cm)	1.82	1.84	0.03	20.32	20.47
Fruit girth (cm)	0.62	0.65	0.03	11.55	11.85
Average fruit weight (g)	11.26	11.78	0.52	23.87	24.41
Seeds per fruit	157.12	163.69	6.57	15.92	16.25
Fruits per plant	59543.10	60445.03	901.93	68.57	69.09
Protein (mg)	118.17	187.50	69.34	17.79	22.41
Total number of harvest	7.45	8.32	0.86	32.04	33.85
Yield per plant (kg)	17.67	17.88	0.21	78.70	79.16

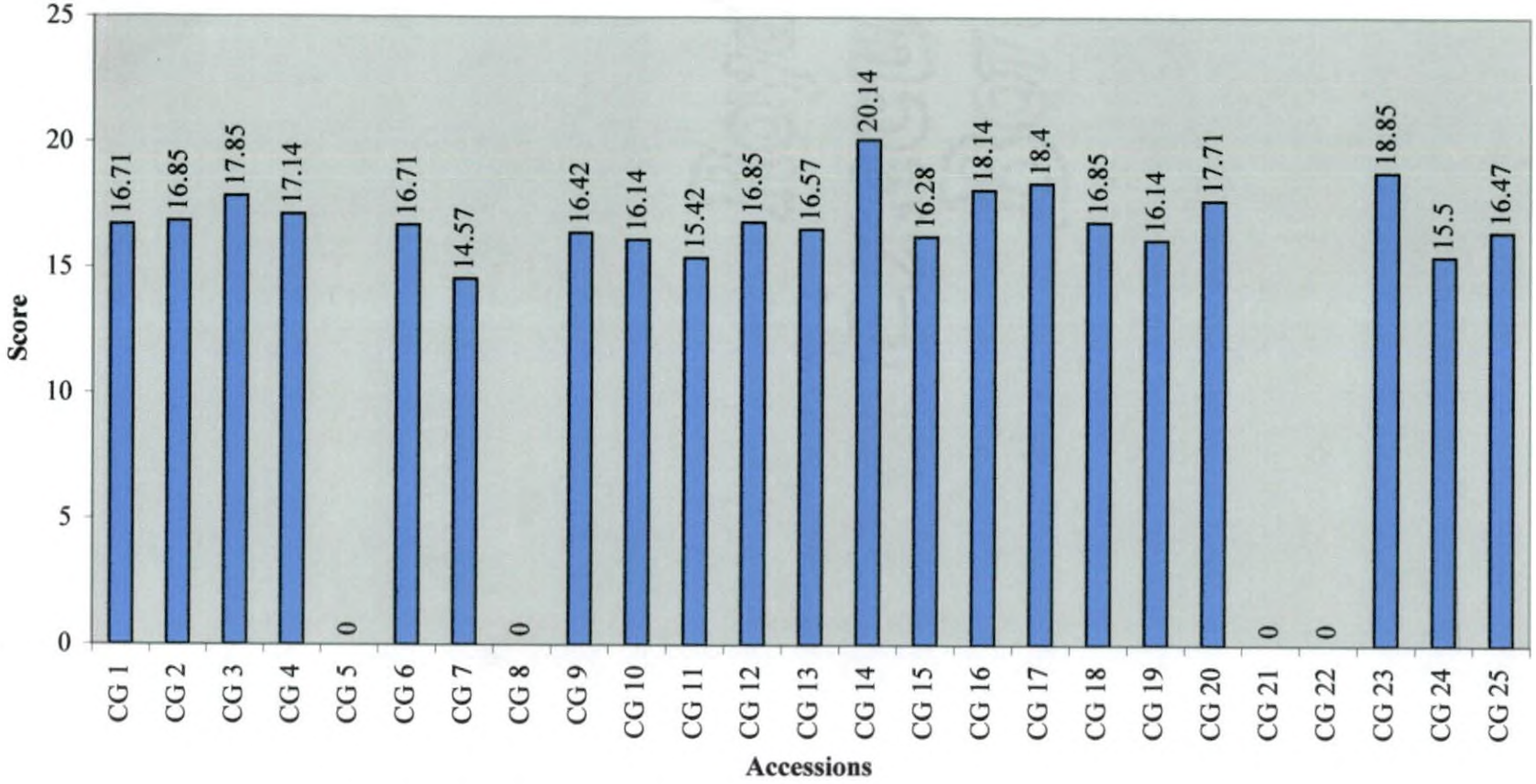
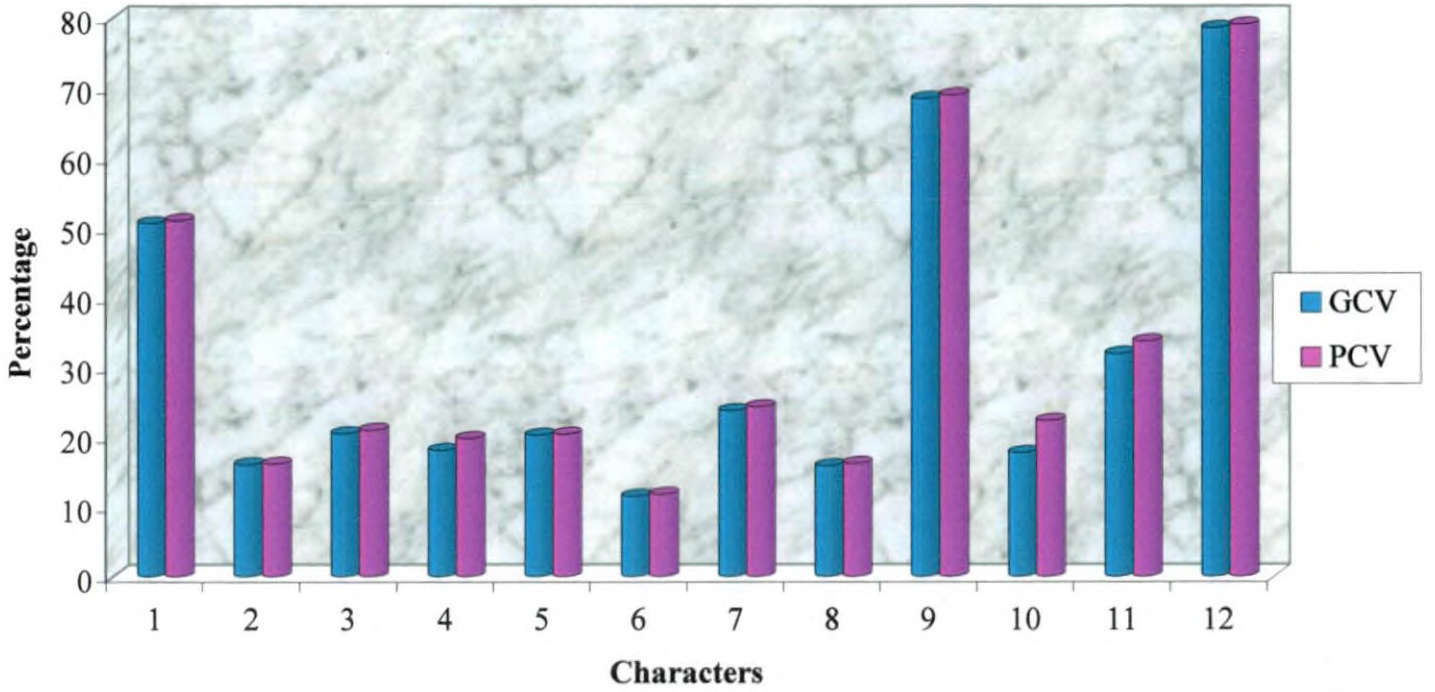


Fig. 1. Score obtained in the organoleptic evaluation of *Coccinia grandis* accessions



1	Primary branch length (m)	7	Average fruit weight (g)
2	Internodal length (cm)	8	Seeds per fruit
3	Days to first flower	9	Fruits per plant
4	Node to first flower	10	Protein (g)
5	Fruit length (cm)	11	Total number of harvest
6	Fruit girth (cm)	12	Yield per plant (Kg)

Fig. 2. Coefficient of variation for 12 characters in 25 accessions of ivygourd

Plate 2. CG 19 and CG 13 - high yielding superior accessions of ivygourd

Plate 3. Fruit fly infected fruit

per cent was noted for fruit girth (11.550) followed by seeds per fruit (15.923) and internodal length (16.091).

The highest PCV in percentage observed for yield per plant (79.160) followed by number of fruits per plant (69.086), primary branche length (51.154), total number of harvest (33.848) and average fruit weight (24.414). Lowest PCV was observed for fruit girth (11.848) followed by intenodal length (16.213) and seeds per fruit (16.253).

4.1.4 Heritability and Genetic Advance

The estimates of heritability and genetic advance are presented in Table 5 and Fig. 3.

High values of heritability were recorded for all the traits studied, among these yield per plant had highest value (98.83) followed by fruit length (98.56), fruits per plant (98.51), internodal length (98.50), primary branch length (98.24), seeds per fruit (95.98), average fruit weight (95.62) days to first flowering (95.22), fruit girth (95.05), total number of harvest per plant (89.62), node to first flower (83.49) and protein content (63.02).

Expected genetic advance as percentage of mean were highest for yield per plant (161.15) followed by fruits per plant (140.19), primary branch length (103.5244), total number of harvest (62.489), average fruit weight (48.08), fruit length (41.57) and days to first flowering (41.29). These traits also possessed high heritability values.

Medium genetic advance as percentage of mean was for node to first flower (34.16) followed by internodal length (32.89), seeds per fruit (32.13), protein content (29.09) and fruit girth (23.19).

4.1.5 Correlation Studies

The Phenotypic, genotypic and environmental correlation among 12 characters were worked out and presented in Table 6, 7 and 8 respectively.

Table 5. Heritability and genetic advance for 12 characters in ivygourd

Characters	Heritability (%)	GA as % of mean
Primary branch length (m)	98.24	103.52
Internodal length (cm)	98.50	32.90
Days to first flower	95.22	41.30
Node to first flower	83.49	34.17
Fruit length (cm)	98.56	41.56
Fruit girth (cm)	95.05	23.20
Average fruit weight (g)	95.62	48.09
Seeds per fruit	95.98	32.14
Fruits per plant	98.51	140.19
Protein (mg)	63.02	29.09
Total number of harvest	89.62	62.49
Yield per plant (kg)	98.83	161.16

Table 6. Phenotypic correlation coefficient among 12 characters in ivygourd

Characters	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	0.519	1										
3	0.033	-0.153	1									
4	0.365	0.211	0.457	1								
5	-0.064	0.535	-0.337	-0.445	1							
6	0.407	0.331	-0.544	-0.251	0.355	1						
7	0.274	0.662	-0.618	-0.309	0.682	0.664	1					
8	0.409	0.769	-0.446	0.039	0.435	0.648	0.782	1				
9	0.544	0.227	-0.574	-0.184	0.177	0.452	0.366	0.379	1			
10	-0.093	0.126	-0.4	-0.127	0.031	0.189	0.35	0.192	0.022	1		
11	0.833	0.635	-0.274	0.055	0.176	0.562	0.522	0.575	0.643	0.081	1	
12	0.619	0.496	-0.595	-0.177	0.368	0.601	0.658	0.649	0.912	0.086	0.744	1

1 Primary branch length (m)
2 Internodal length(cm)
3 Days to first flower
4 Node to first flower
5 Fruit length (cm)
6 Fruit girth (cm)
7 Average fruit weight (g)
8 Seeds per fruit
9 Fruits per plant
10 Protein (mg)
11 Total number of harvest
12 Yield per plant (kg)

Table 7. Genotypic correlation coefficient among 12 characters in ivygourd

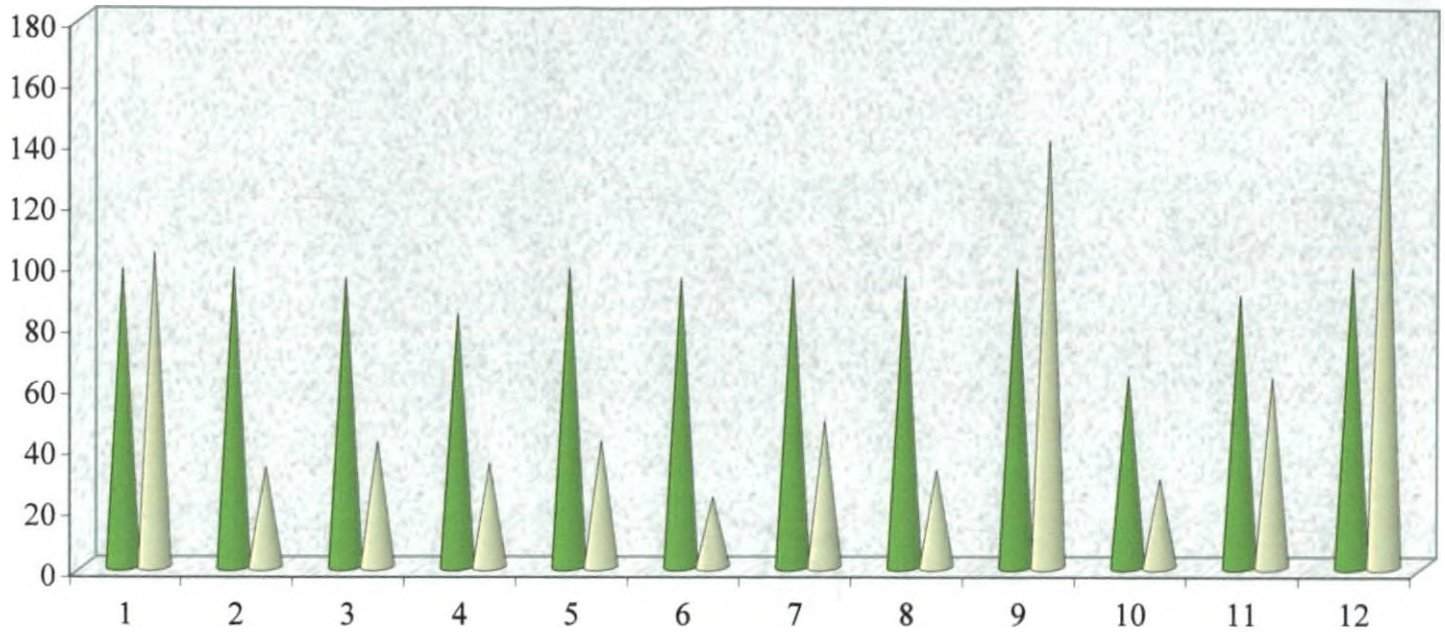
Characters	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	0.532	1										
3	0.035	-0.162	1									
4	0.399	0.212	0.503	1								
5	-0.062	0.54	-0.35	-0.494	1							
6	0.414	0.348	-0.592	-0.295	0.361	1						
7	0.284	0.678	-0.659	-0.374	0.699	0.694	1					
8	0.426	0.782	-0.47	0.001	0.447	0.686	0.81	1				
9	0.552	0.236	-0.593	-0.183	0.178	0.47	0.382	0.396	1			
10	-0.11	0.166	-0.437	-0.131	0.062	0.246	0.464	0.281	0.048	1		
11	0.894	0.681	-0.293	0.093	0.202	0.601	0.566	0.641	0.688	0.079	1	
12	0.628	0.502	-0.618	-0.193	0.369	0.621	0.674	0.667	0.913	0.143	0.8	1

1 Primary branch length (m)
2 Internodal length(cm)
3 Days to first flower
4 Node to first flower
5 Fruit length (cm)
6 Fruit girth (cm)
7 Average fruit weight (g)
8 Seeds per fruit
9 Fruits per plant
10 Protein (mg)
11 Total number of harvest
12 Yield per plant (kg)

Table 8. Environmental correlation coefficient among 12 characters in ivygourd

Characters	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	-0.247	1										
3	-0.014	0.135	1									
4	0.064	0.386	0.103	1								
5	-0.159	0.205	0.078	0.073	1							
6	0.253	-0.193	0.383	0.132	0.216	1						
7	-0.064	0.142	0.237	0.296	0.104	0.054	1					
8	-0.163	0.375	0.065	0.469	0.003	-0.151	0.149	1				
9	0.063	-0.358	0.014	-0.353	0.116	-0.096	-0.21	-0.236	1			
10	-0.074	-0.075	-0.462	-0.129	-0.243	-0.014	-0.081	-0.215	-0.211	1		
11	-0.132	-0.131	-0.052	-0.196	-0.369	0.098	-0.027	-0.313	-0.09	0.109	1	
12	0.046	-0.018	0.213	-0.049	0.32	-0.044	0.119	-0.039	0.833	-0.406	-0.237	1

1 Primary branch length (m)
2 Internodal length(cm)
3 Days to first flower
4 Node to first flower
5 Fruit length (cm)
6 Fruit girth (cm)
7 Average fruit weight (g)
8 Seeds per fruit
9 Fruits per plant
10 Protein (mg)
11 Total number of harvest
12 Yield per plant (kg)



1	Primary branch length (m)	7	Average fruit weight (g)
2	Internodal length (cm)	8	Seeds per fruit
3	Days to first flower	9	Fruits per plant
4	Node to first flower	10	Protein (g)
5	Fruit length (cm)	11	Total number of harvest
6	Fruit girth (cm)	12	Yield per plant (Kg)

Fig. 3. Heritability and genetic advance as percentage of mean for 12 characters in 25 accessions of ivygourd

4.1.5.1 Phenotypic Correlation Coefficients

High positive correlation was observed between yield per plant and fruits per plant (0.912) followed by total number of harvest (0.744) and average fruit weight (0.658). Positive association was also noted for characters viz., seeds per fruit (0.649), primary branch length (0.619), fruit girth (0.601), internodal length (0.496) and fruit length (0.368).

Characters like days to first flowering (-0.595) and node to first flowering (-0.177) had negative correlation with yield.

Primary branch length was positively correlated with total number of harvest (0.833), fruits per plant (0.544), seeds per fruit (0.409), average fruit weight (0.274), fruit girth (0.407), node to first flower (0.365), internodal length (0.519) and days to first flowering (0.033) but it was negatively associated with fruit length (-0.064).

Total number of harvest had high positive correlation with internodal length (0.635) fruits per plant (0.643) seeds per fruit (0.575), fruit girth (0.562), average fruit weight (0.522) and fruit length (0.176).

Almost all characters had negative correlation with days to first flowering. But node to first flower (0.457) had positive association.

Fruits per plant (-0.184), average fruit weight (-0.309), fruit girth (-0.251) and fruit length (-0.445) are negatively associated with node to first flower. But seeds per fruit (0.039) had positive correlation.

Seeds per fruit had positive association with internodal length (0.709), fruit girth (0.648), fruit length (0.435) and average fruit weight (0.782).

Average fruit weight was positively correlated with internodal length (0.662) fruit length (0.682) and fruit girth (0.664).

Fruit girth showed positive association with internodal length (0.331) and fruit length (0.355). Similarly fruit length was positively correlated with internodal Length (0.535).

4.1.5.2 Genotypic Correlation Coefficients

Total yield per plant was positively and highly correlated with fruits per plant (0.913), total number of harvest (0.800), average fruit weight (0.674). It was also positively correlated with seeds per fruit (0.667), primary branch length (0.628), fruit length (0.369), fruit girth (0.621), internodal length (0.502), and protein content (0.143). However a negative association was found for characters like node to first flower (-0.193) and days to first flower (-0.618) with yield.

Total number of harvest showed a high positive correlation with primary branch length (0.894), internodal length (0.681), node to first flower (0.093), fruit length (0.202), fruit girth (0.601), average fruit weight (0.566), seeds per fruit (0.641), fruits per plant (0.688) and protein content (0.079), whereas it had negative correlation with days to first flower (-0.293).

Protein content had positive association with internodal length (0.166), fruit length (0.062), fruit girth (0.246), average fruit weight (0.464), fruits per plant (0.048) and seeds per fruits (0.281).

Fruits per plant showed a high positive correlation with primary branch length (0.552), internodal length (0.236), fruit length (0.178), fruit girth (0.470), average fruit weight (0.382) and seeds per fruit.

Seeds per fruit was positively correlated with primary branch length (0.426), internodal length (0.782), fruit length (0.447) fruit girth (0.686), average fruit weight (0.81) and node to first flower (0.001).

Average fruit weight showed a positive correlation with primary branch length (0.284), internodal length (0.678), fruit length (0.699) and fruit girth (0.694).

Fruit girth showed positive correlation with primary branch length (0.414), internodal length (0.348) and fruit length (0.361) and it was negatively correlated with days to first flower (-0.592) and node to first flower (-0.295). Fruit length had positive correlation with internodal length (0.540) and it was negatively

correlated with primary branch length (-0.062), days to first flower (-0.35) and nodes to first flower (-0.494).

Nodes to first flower showed positive correlation with primary branch length (0.399), internodal length (0.212) and days to first flower (0.503).

Days to first flower was positively correlated with primary branch length (0.035) and it was negatively correlated with internodal length (-0.162). Internodal length had positive association with primary branch length (0.532).

4.1.5.3 Error Correlation Coefficient

Most of the error correlation coefficients were very low indicating that the effect of environment on expression of the association between the character was not so strong as to alter it markedly. However the error correlation between yield and fruits per plant (0.833), days to first flower (0.213) and fruit length (0.32) had high positive correlation. Primary branch length (0.046) and average fruit weight (0.119) were also positive correlated with yield per plant.

4.1.6 Path Coefficient Analysis

The genotypic correlation among yield and its component character were partitioned into different components to find out the direct and indirect contribution of each character on yield. Primary branch length, internodal length, fruit length, fruit girth, average fruit weight, seeds per fruit, fruits per plant and total number of harvest were the characters selected for path coefficient analysis.

Direct effect and correlation of the yield components are presented in Table 9 and Fig. 4.

The primary branch length had genotypic correlation of 0.628 with yield. The major portion of this was contributed by its direct effect on yield (0.6771). The indirect effect of primary branch length on yield, through total number of harvest, seeds per fruits and average weight of fruit were 0.3174, 0.1775 and 0.0142 respectively. The indirect effect through internodal length (-0.0489), fruit

length (-0.0081), fruit girth (-0.0836), and total number of harvest (-0.3277) were negative.

The direct effect of internodal length on yield was negative (-0.0919). The internode length had indirect effect on yield mainly through primary branch length (0.3601) followed by seeds per fruit (0.3256), fruits per plant (0.1358), fruit length (0.0717) and average weight fruit (0.0338). The indirect effect through fruit girth (-0.0702) and total number of harvest (-0.2498) were negative. The genotypic correlation of internodal length on yield was 0.502.

The genotypic correlation of fruit length on yield was 0.369 and its direct effect on yield was 0.1300. The major contribution of its total correlation was through indirect effect of seeds per fruits (0.1861) followed by fruits per plant (0.1025) and average fruit weight (0.0349). The effects through primary branch length (-0.0422) internodal length (-0.0496), fruit girth (-0.0729) and total number of harvest per plant were low and negative.

The genotypic correlation of fruit girth on yield was higher than that of fruit length and was 0.621. But its direct effect was negative (-0.2019). The indirect effect on yield through internodal length (-0.0320) and total number of harvest was negative. But the effects through primary branch length (0.2802), seed per fruit (0.2858), fruits per plant (0.2703), fruit length (0.0469) and average fruit weight (0.0346) were positive.

The total genotypic correlation of average fruit weight on yield was high (0.674). The direct effect was low (0.0499) and positive. The rest of its effect on yield was contributed by indirect effect through seeds per fruit (0.3375), fruits per plant (0.2197), primary branch length (0.1924) and fruit length (0.0909). Whereas through characters like internodal length (-0.0623), fruit girth (-0.1402) and total number of harvest (-0.2074) were negative.

Both genotypic correlation (0.667) and direct effect (0.4166) of seeds per fruit on yield was high. The indirect effect of seeds per fruit on yield mainly through primary branch length (0.2884), fruits per plant (0.2273), fruit length

Table 9. Direct and indirect effects of component characters on fruit yield per plant

Characters	1	2	3	4	5	6	7	8
1	0.6771	-0.0489	-0.0081	-0.0836	0.0142	0.1775	0.3174	-0.3277
2	0.3601	-0.0919	0.0702	-0.0702	0.0338	0.3256	0.1358	-0.2498
3	-0.0422	-0.0496	0.1300	-0.0729	0.0349	0.1861	0.1025	-0.0741
4	0.2802	-0.0320	0.0470	-0.2019	0.0346	0.2858	0.2703	-0.2204
5	0.1924	-0.0623	0.0910	-0.1402	0.0499	0.3375	0.2197	-0.2074
6	0.2884	-0.0718	0.0581	-0.1385	0.0404	0.4166	0.2273	-0.2352
7	0.3740	-0.0217	0.0232	-0.0950	0.0191	0.1648	0.5747	-0.2524
8	0.6050	-0.0626	0.0263	-0.1214	0.0282	0.2672	0.3956	-0.3667

Diagonal value = direct effect Off diagonal value = indirect effect
Residual effect = 0.19121

1 Primary branch length (m)
2 Internodal length(cm)
3 Fruit length (cm)
4 Fruit girth (cm)
5 Average fruit weight (g)
6 Seeds per fruit
7 Total number of harvest
8 Fruits per plant

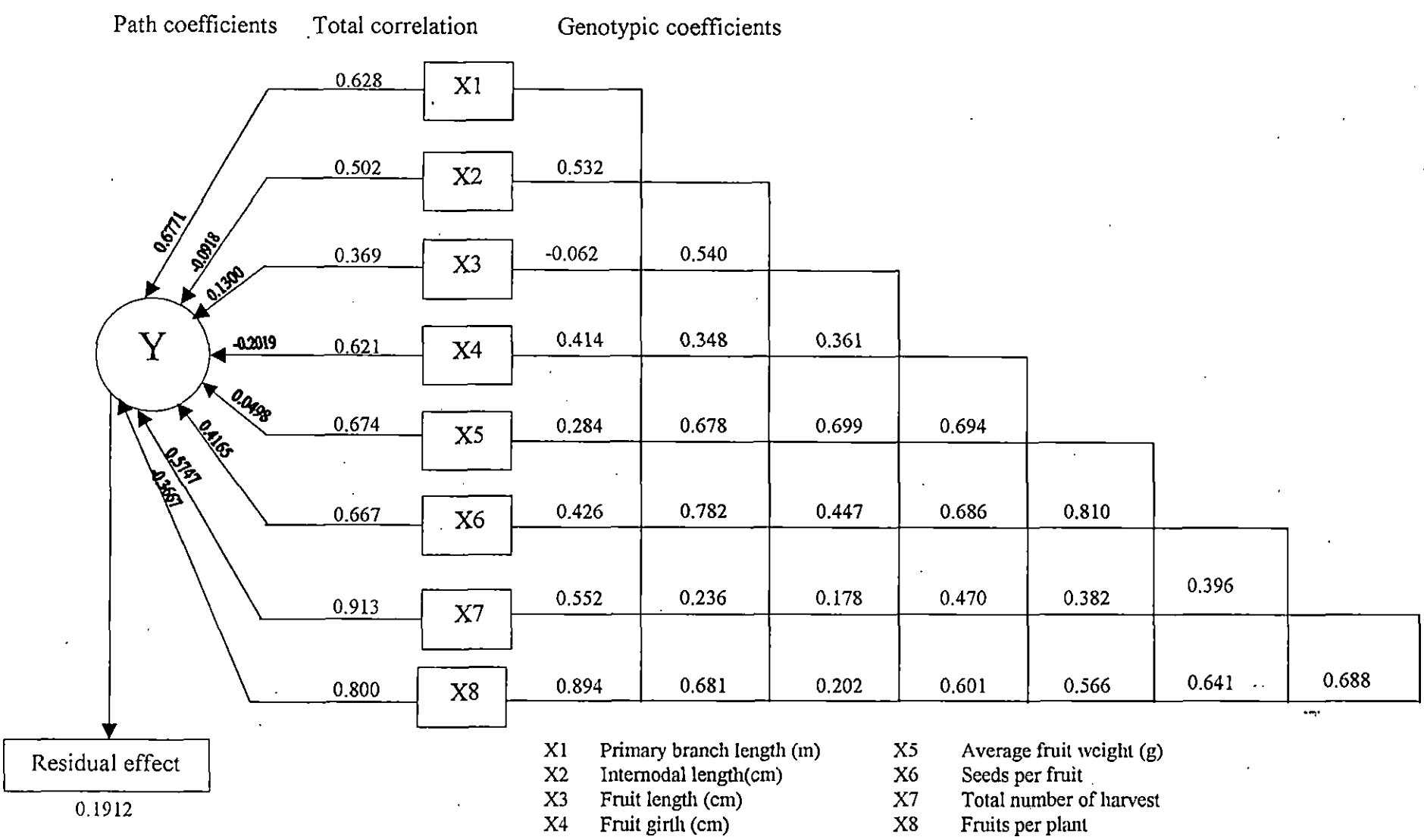


Fig. 4. Path diagram showing direct effects and interrelationship in accessions of ivygourd

(0.0581) and average fruit weight, whereas through internodal length (-0.0718), fruit girth (01385) and total number of harvest (-0.2352) had negative indirect effect.

The direct effect of fruits per plant (0.5747) on yield was high and total genotypic correlation was also high (0.913). The major potion of its indirect effects on yield through primary branch length (0.3740) followed by seeds per fruit (0.1648), fruit length (0.0232) and average fruit weight (0.0191). The effects through internodal length (-0.0217), fruit girth (-0.0949) and total number of harvest (-0.2524) were negative.

The direct effect of total number of harvest on yield was low (-0.3667) and negative but its genotypic correlation with yield was high and positive (0.800). It is indirect on yield mainly through primary branch length (0.605) followed by fruits per plant (0.3956), seed per fruit (0.2672), average fruit weight (0.0282) and fruit length (0.0263). The indirect effect through of internodal length (-0.0626), fruit girth (-0.1214) and total number of harvest (-0.3667) was negative.

Among the characters studied the primary branch length had highest direct effect on yield followed by fruits per plant, seeds per fruit, fruit length and average weight of fruit.

The residue was 0.19121 indicating that the selected eight characters contributed the reaming 81 percent.

4.1.7 Genetic Divergence

The 25 accessions of ivygourd were divided into two groups based on the extent of bitterness. The first group consisted 21 accessions which were all bitterless and second group consisted four accessions which were highly bitter. The accessions CG 8, CG 5, CG 21 and CG 22 belonged to this group.

The first 21 accessions are subjected to Mahalanobis D^2 analysis. Observations on eleven characters namely, primary branch length, internodal length, days to first flowering, node to first flower, fruit length, fruit girth, average fruit weight, seeds per fruit, fruit per plant, total number of harvest and yield per

plant were subjected Mahalanobis D^2 analysis using Tochers method. Based on this D^2 values and as such six clusters are presented in Table 10.

The cluster III had the highest number of accessions (9) which included accessions CG 4, CG 12, CG 15, CG 18, CG 20, CG 23, CG 24, CG 25 and CG 16 followed by cluster II with 5 accessions viz., CG 13, CG 14, CG 17, CG 11 and CG 18. The other clusters, cluster I (CG 2 and CG 3), cluster IV (CG 1 and CG 6) and cluster V (CG 10 and CG 7) had two accessions each. The accessions CG 19 remained as divergent accessions that could not be accommodated in any of the other clusters which remained as a separate cluster.

Based on the total D^2 values the average inter and intra cluster distance were estimated and presented in Tables 11, 12 and Fig. 5, the average intra cluster distance varied from zero (cluster VI) to 33.896 (cluster I). The inter cluster distance varied from 14.866 (cluster I & IV) to 94.027 (cluster I and VI).

The maximum and minimum divergence between the clusters, the cluster I had the maximum distance (94.027) from cluster VI followed by II, V, III and IV. The cluster II had the maximum distance (58.834) from cluster I followed by cluster VI, IV, III and V. Cluster III had greatest distance (73.44) from cluster VI followed by II, I, V and IV. The cluster IV had maximum distance (88.741) from cluster VI followed by II, V, III and I. The cluster V had maximum distance (69.749) from cluster VI followed by I, IV, II and III. The cluster VI had greatest distance from (94.027) cluster I followed by IV, III, V and II.

Among all clusters, VI exhibited very high distance from four of the six clusters. The cluster VI had only one accession CG 19 which is high yielder among all accessions.

Among the 11 characters subjected to D^2 analysis the trait yield per plant (44.2857) had highest contribution to cluster formation followed by total number of harvest (23.8095) and fruits per plant (17.619). The traits node to first flower (0.4762) and primary branch length (0.952) had least contribution. The characters average fruit weight not involved in cluster formation (Table 13).

Table 10. Group constellation in 21 accessions of ivygourd

Cluster number	Number of accessions	Members
I	2	2 3
II	5	11 13 14 17 8
III	9	4 12 15 16 18 20 23 24 25
IV	2	1 6
V	2	10 7
VI	1	19

Table 11. Average intra and inter cluster distance (D^2)

Characters	I	II	III	IV	V	VI
I	6.429	3461.477	1202.859	220.993	1785.249	8841.08
II		1019.387	1711.498	2855.746	1154.512	2877.913
III			970.753	889.511	996.864	5393.45
IV				254.264	1397.256	7875.016
V					1148.968	4864.944
VI						0

Diagonal value = intra cluster distance
Off diagonal value = inter cluster distance

Table 12. Average intra and inter cluster distance (D)

Characters	I	II	III	IV	V	VI
I	2.536	58.834	34.682	14.866	42.252	94.027
II		31.928	41.37	53.439	33.978	53.646
III			31.157	29.825	31.573	73.44
IV				15.946	37.38	88.741
V					33.896	69.749
VI						0

Diagonal value = intra cluster distance
Off diagonal value = inter cluster distance

Table13. Contribution of each characters to divergence

Characters	Percentage of contribution
Primary branch length (m)	0.9524
Internodal length (cm)	2.381
Days to first flower	2.8571
Node to first flower	0.4762
Fruit length (cm)	3.8095
Fruit girth (cm)	2.381
Average fruit weight (g)	0
Seeds per fruit	1.4286
Fruits per plant	17.619
Total number of harvest	23.8095
Yield per plant (kg)	44.2857

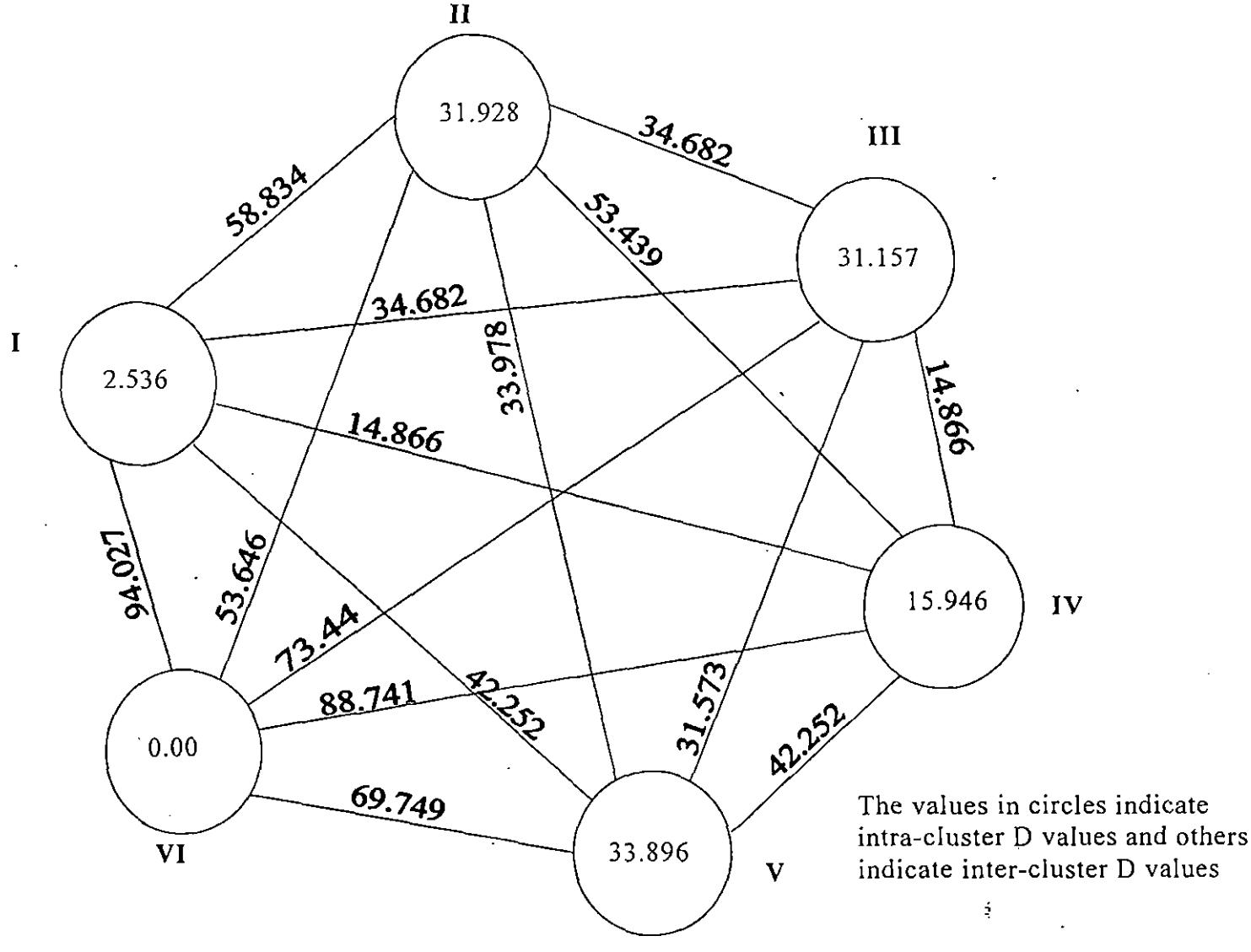


Fig. 5 Diagrammatic representation of clustering of 21 accessions of ivygourd

4.2 MOLECULAR CHARACTERIZATION

The results of the investigations carried out for characterization of the ivygourd accessions using RAPD markers are presented in this chapter.

4.2.1 DNA Isolation

DNA Pellets were brown in colour. Extraction with Phenol: Cholroform: Isoamyl alcohol (25:24:1) reprecipitation with ethyl alcohol and washing with 70 per cent ethanol reduced the browning of the pellet. Then the pellete was dissolved with TE buffer.

The DNA yield of 25 accessions estimated using UV-vis spectrophotometer ranged from 1.44 ng/ µl (CG 4) to 9.24 ng/ µl (CG 23). The purity of DNA (A_{260}/A_{280}) ranged from 1.29 (CG 15) to 2.28 (CG 19) as shown in Table 14.

4.2.2 Gel Electrophoresis

The quality of DNA was assessed by gel electrophoresis. In some of the samples smearing was observed indicating shearing of DNA. The rest of the DNA was observed as a crisp single band. For those samples showing sheared DNA, the isolation process was repeated and electrophoresed. The bands indicated unsheared good quality DNA. RNA was observed as a thick band below the genomic DNA.

4.2.3 Polymerase Chain Reaction

Polymerase chain reaction was standardized for the amplification of the DNA from *Cucumis melo* (Staub *et al.*, 2000) was used for the amplification of 25 accessions of ivygourd with slight modifications. Fourty nanogram of DNA, 200 µM each of the four dNTP, one unit of Taq DNA polymerase and 5 pico moles of primer in presence of 2.5 µl of 10x Taq buffer (10 mM Tris-HCL, pH 9.0, 1.5 mM KCL and 0.01 % gelatin) and 3 mM $MgCl_2$ gave good amplification.

Twenty eight decamer primers were screened for their efficiency using the DNA isolated from CG 1. Out of the 28 decamer primers screened, 17 yielded

Table 14. Quality and quantity of DNA isolated from ivygourd genotypes

Accessions	260nm	280nm	260 nm/ 280 nm	DNA yield ng/ul
CG1	0.171	0.102	1.69	5.13
CG2	0.090	0.057	1.57	2.70
CG3	0.095	0.048	1.66	2.85
CG4	0.048	0.026	1.84	1.44
CG5	0.110	0.058	1.89	3.30
CG6	0.147	0.077	1.90	4.41
CG7	0.072	0.034	2.11	2.16
CG8	0.166	0.087	1.90	4.98
CG9	0.145	0.076	1.90	4.35
CG10	0.165	0.098	1.68	4.95
CG11	0.136	0.065	2.09	4.08
CG12	0.081	0.051	1.58	2.43
CG13	0.125	0.069	1.81	3.75
CG14	0.193	0.112	1.72	5.79
CG15	0.204	0.158	1.29	6.18
CG16	0.111	0.054	2.05	3.33
CG17	0.159	0.081	1.96	4.77
CG18	0.075	0.042	1.78	2.25
CG19	0.048	0.021	2.28	1.44
CG20	0.116	0.054	2.14	3.48
CG21	0.145	0.078	1.85	4.35
CG22	0.149	0.082	1.81	4.47
CG23	0.308	0.172	1.79	9.24
CG24	0.269	0.155	1.73	8.07
CG25	0.291	0.170	1.71	8.73

amplification products. There was no amplification with the primers like OPA-02, OPA-04, OPA-06, OPA-07, OPA-08, OPA-16, OPA-19, OPA-20, OPB-17 and OPB-20. The total number of bands, number of faint bands and number of intense bands produced by the primers are given in Table 15, Fig. 6 and 7.

A total of 41 RAPDs (average of 1.46 bands per primer) were generated of which 39 bands were polymorphic.

The highest number of RAPDs were produced by the primer OPA-18 (6 bands) followed by OPB-11 (5 bands) and OPB-10 (4 bands). Of these primers the highest number of intense bands (4 bands) were produced by OPA-18. OPB-11 produced 3 intense and 2 faint bands and OPB-04 produced two each of faint and intense bands.

OPB6 and OPB-14 produced three bands each. Among these two, OPB-6 produced two intense and one faint bands. OPB-14 produced three faint bands.

Two bands each were obtained when OPA-1, OPA-3, OPA-5, OPA-17, OPB-7, OPB-13, OPB-16 and OPB-19 were used for amplification. Of these one band was intense and one faint in OPA-1, OPA-5, OPA-17, OPB-7 and OPB-19. The primers OPB-3, OPB-13 and OPB-16 produced 2 faint bands only.

The primers OPB-08, OPB-09, OPB-05 and OPB-18 produced only one band each. Among these the first three were produced faint bands.

Out of the 28 primers screened four were selected for amplifying DNA from all the ivygourd accessions. PCR reaction was repeated twice for each sample in order to check the reproducibility. The number of bands resolved per amplification was primer dependent and varied from minimum of 8 to maximum 16. The nucleotide sequence of informative RAPD markers given by each primer is shown in Table 16. The GC content of the primers used varied from 60 to 70 per cent.

The highest number of scorable bands (16) was given by the primer OPB-11. The accessions CG 2 and CG 7 gave 13 bands each when OPB-11 was used for amplification. The accessions CG 3, CG 5, CG 6, CG 8, CG 9, CG 13,

Table 15. Primer associated banding patterns with the DNA of CG 1 using 28 primers supplied by the Operon Inc., CA, USA

Sl. No	Primers	Total Number of bands	Number of intense bands	Number of faint bands
1	OPA-1	2	1	1
2	OPA-2	0	0	0
3	OPA-3	2	0	2
4	OPA-4	0	0	0
5	OPA-5	2	1	1
6	OPA-6	0	0	0
7	OPA-7	0	0	0
8	OPA-8	0	0	0
9	OPA-16	0	0	0
10	OPA-17	2	1	1
11	OPA-18	6	4	2
12	OPA-19	0	0	0
13	OPA-20	0	0	0
14	OPB-6	3	2	1
15	OPB-7	2	1	1
16	OPB-8	1	0	1
17	OPB-9	1	0	1
18	OPB-10	4	2	2
19	OPB-11	5	3	2
20	OPB-12	0	0	0
21	OPB-13	2	0	2
22	OPB-14	3	0	3
23	OPB-15	1	0	1
24	OPB-16	2	0	2
25	OPB-17	0	0	0
26	OPB-18	1	1	0
27	OPB-19	2	1	1
28	OPB-20	0	0	0

Table 16. Nucleotide sequences of primers and total number of informative RAPD markers amplified with them in the ivygourd accessions used in this study

Primer	Sequence	Number of informative RAPD markers
OPA-18	AGGTGACCGT	10
OPB-6	TGCTCTGCCC	8
OPB-10	CTGCTGGGAC	14
OPB-11	GTAGACCCGT	16

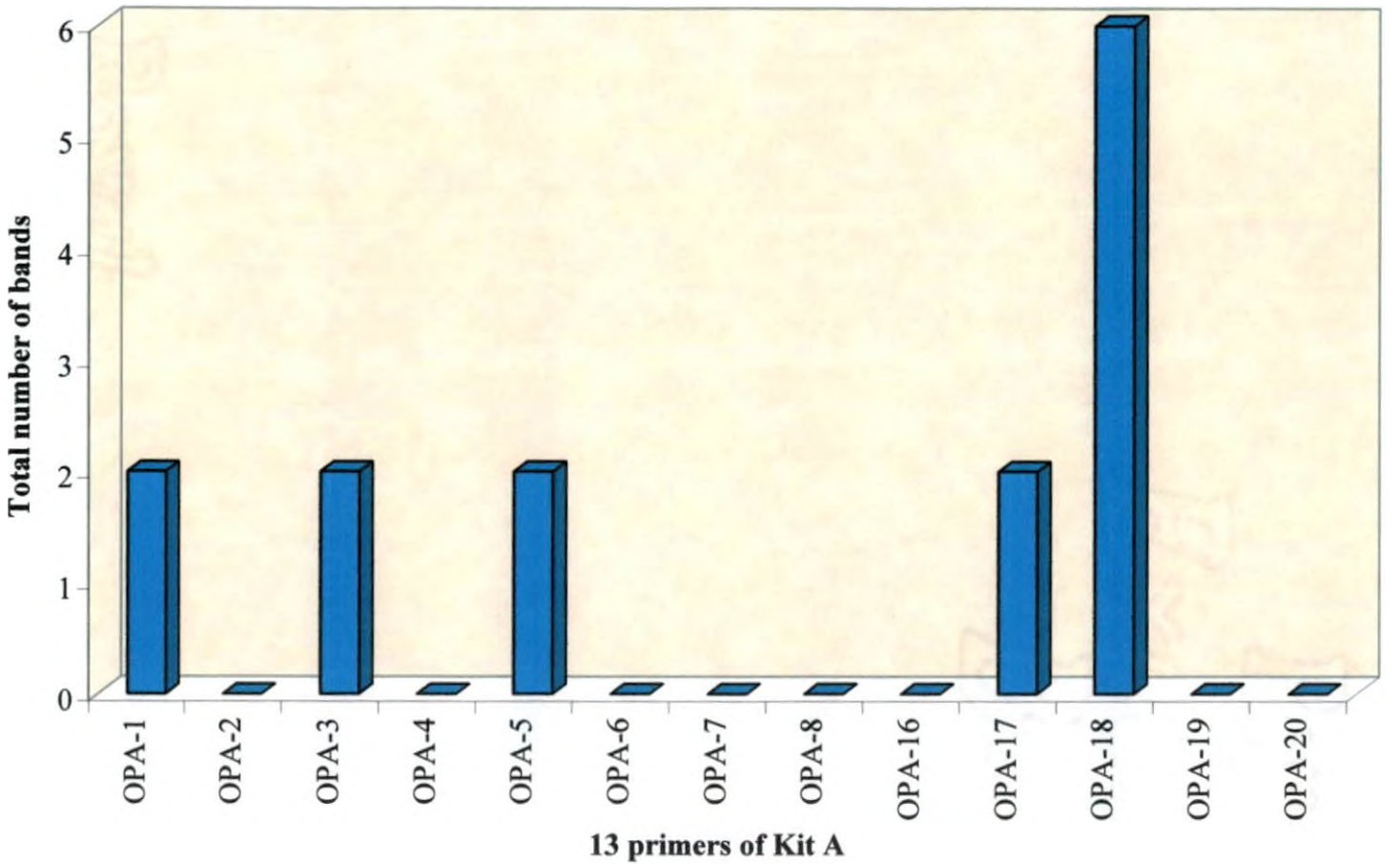


Fig. 6. Amplification profiles (total bands) of the DNA of CG 1 using 13 primers (belongign to Kit A)

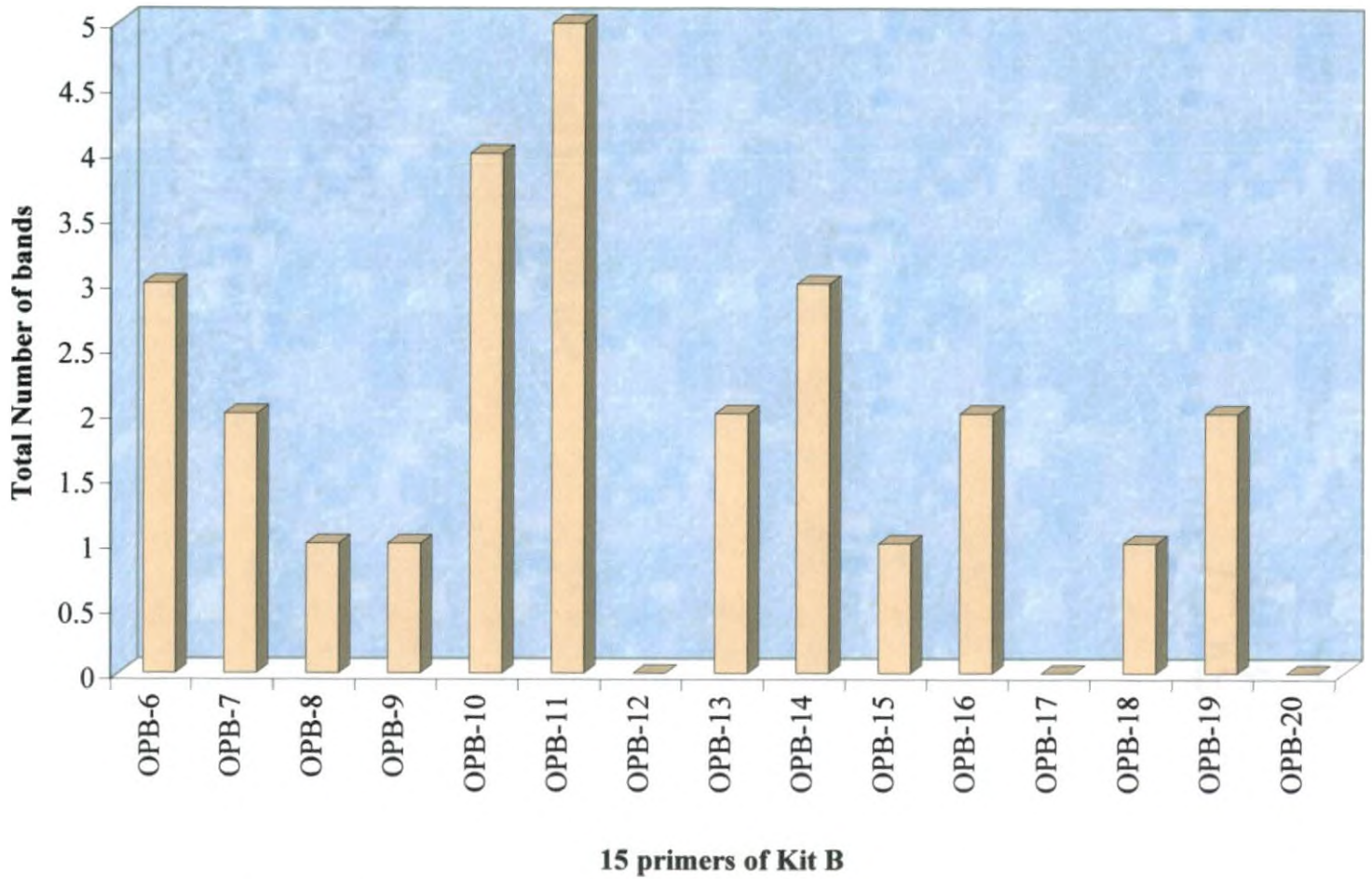


Fig. 7. Amplification profiles (total bands) of the DNA CG 1 using 15 primers (belonging to Kit B)

CG 21, CG 22, CG 23 and CG 24 gave 12 bands each. Eleven bands were produced by CG 1, CG 17 and CG 18. CG 4, CG 10 and CG 25 produced 10 bands. Seven bands were produced by CG 12, CG 15 and CG 17 and four bands were produced by CG 16. With OPB-11 two bands were monomorphic for all the accessions. The accessions CG 9 gave highest intense bands (10 bands), whereas the accessions CG 16 produced only two intense bands. Highest number of faint band were produced by the accession CG 2 (10 bands) (Fig. 8, 9 and Plate 4).

Ten scorable bands were obtained an amplification using the primer OPA-18, Six bands produced by this primer were monomorphic for all accessions. The highest number of bands (9 bands) was given by the accession CG 2, and CG 13. The accessions CG 6, CG 17 and CG 18 gave 7 bands each. All other accessions produce 8 bands each. The accessions CG 1, CG 2, CG 3, CG 5, CG 7, CG 8, CG 16, CG 17, CG 18, CG 21, CG 22, CG 23 and CG 25 were produced intense bands only (Fig. 10, 11 and Plate 5).

When OPB-10 was used for amplification one band was monomorphic in all the accessions. Eleven bands were obtained for CG 10. The samples CG 11 and CG 13 gave nine bands each. The accessions CG 1, CG 7, CG 9, CG 12, CG 18 and CG 24 produced eight bands each. CG 4, CG 16, and CG 17 gave six bands each. CG 4, CG 16, and CG 17 gave six bands each. CG 2, CG 15, CG 19, and CG 20 gave five bands. The accessions CG 5, CG 8, and CG 23 gave four bands and CG 14 produce the least number of bands (3 bands) (Fig. 12, 13 and Plate 6).

Eight scorable bands were obtained on amplification of the samples with the primer OPB-06. One band was monomorphic. Seven bands were produced by the accessions CG 13 and CG 18 (Fig. 14, 15 and Plate 7).

The amplification profiles (intense and faint bands) of the DNA of 25 ivygourd genotypes using the primer OPA 18, OPB 11, OPB 10 and OPB-06 are presented in Table 17.

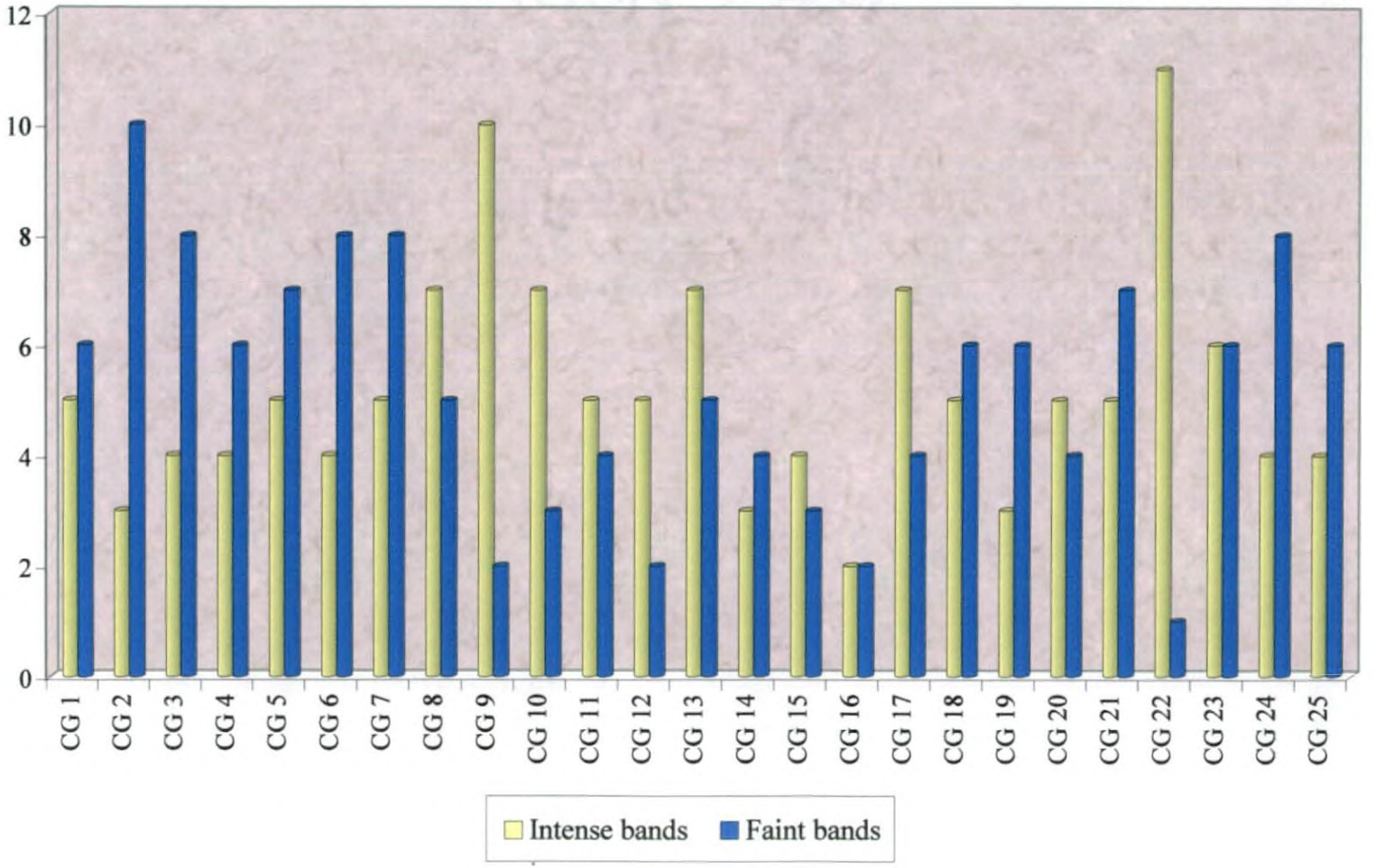


Fig. 8. Representation of the amplification profiles (intense and faint bands) of the DNA of 25 ivygourd accessions using the primer OPB-11

CG 1	CG 2	CG 3	CG 4	CG 5	CG 6	CG 7	CG 8	CG 9	CG 10	CG 11	CG 12	CG 13	CG 14	CG 15	CG 16	CG 17	CG 18	CG 19	CG 20	CG 21	CG 22	CG 23	CG 24	CG 25
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	1	0	0	0	1	1	0
1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	1	1	0	0	1	1	1	1	1
0	0	1	0	0	1	1	1	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
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1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	0	0	0	0	1
1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	0	1	1	1	1	0

1 – Presence of band 0 – Absence of band

Fig. 9. Representation of the amplification profile of the DNA of 25 accessions using the primer OPB-11

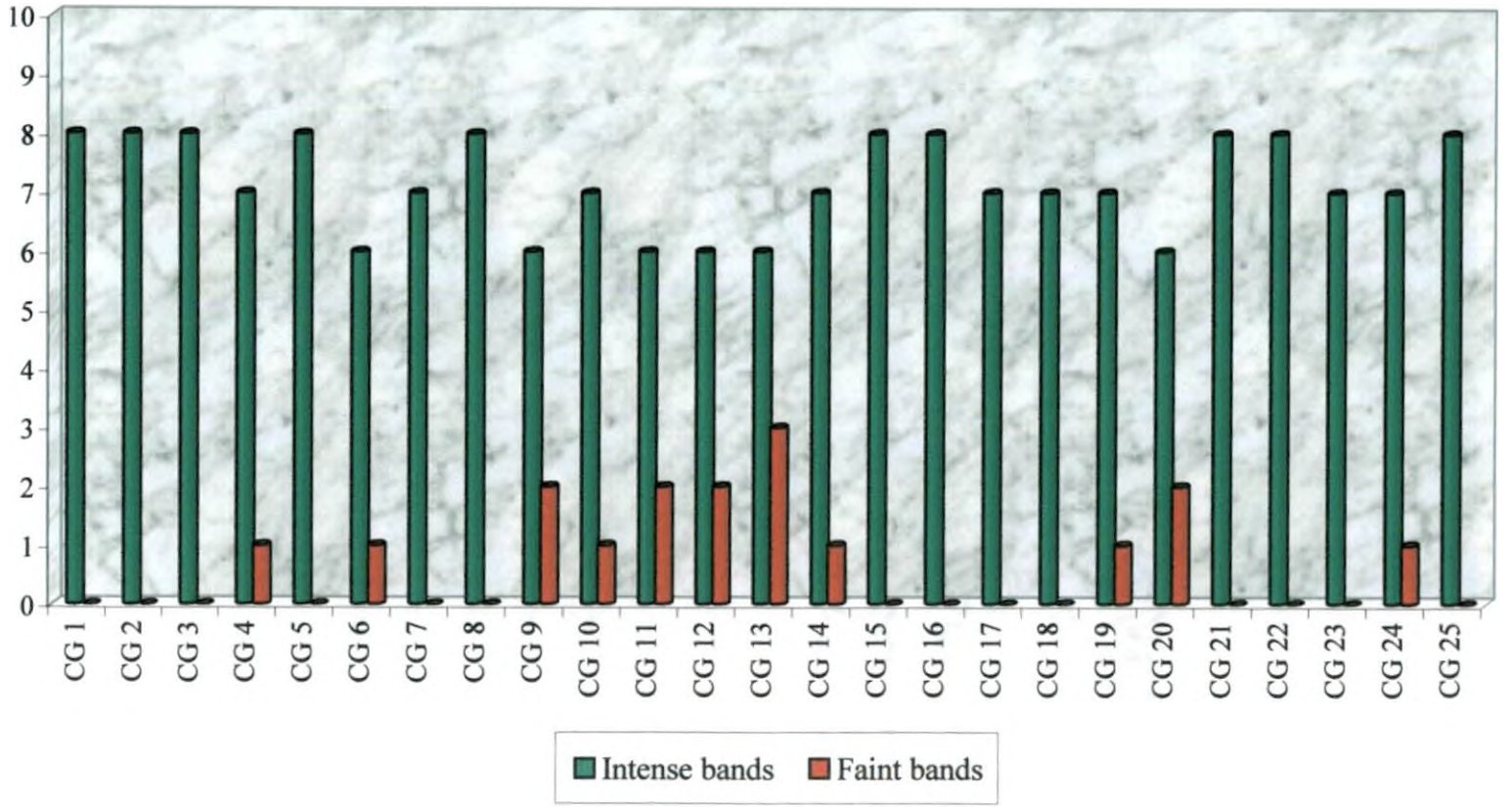

Fig. 10. Representation of the amplification profiles (intense and faint bands) of the DNA of 25 ivygourd accessions using the primer OPA-18

CG 1	CG 2	CG 3	CG 4	CG 5	CG 6	CG 7	CG 8	CG 9	CG 10	CG 11	CG 12	CG 13	CG 14	CG 15	CG 16	CG 17	CG 18	CG 19	CG 20	CG 21	CG 22	CG 23	CG 24	CG 25
0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1
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1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

1 – Presence of band   0 – Absence of band

Fig. 11. Representation of the amplification profile of the DNA of 25 accessions using the primer OPA-18

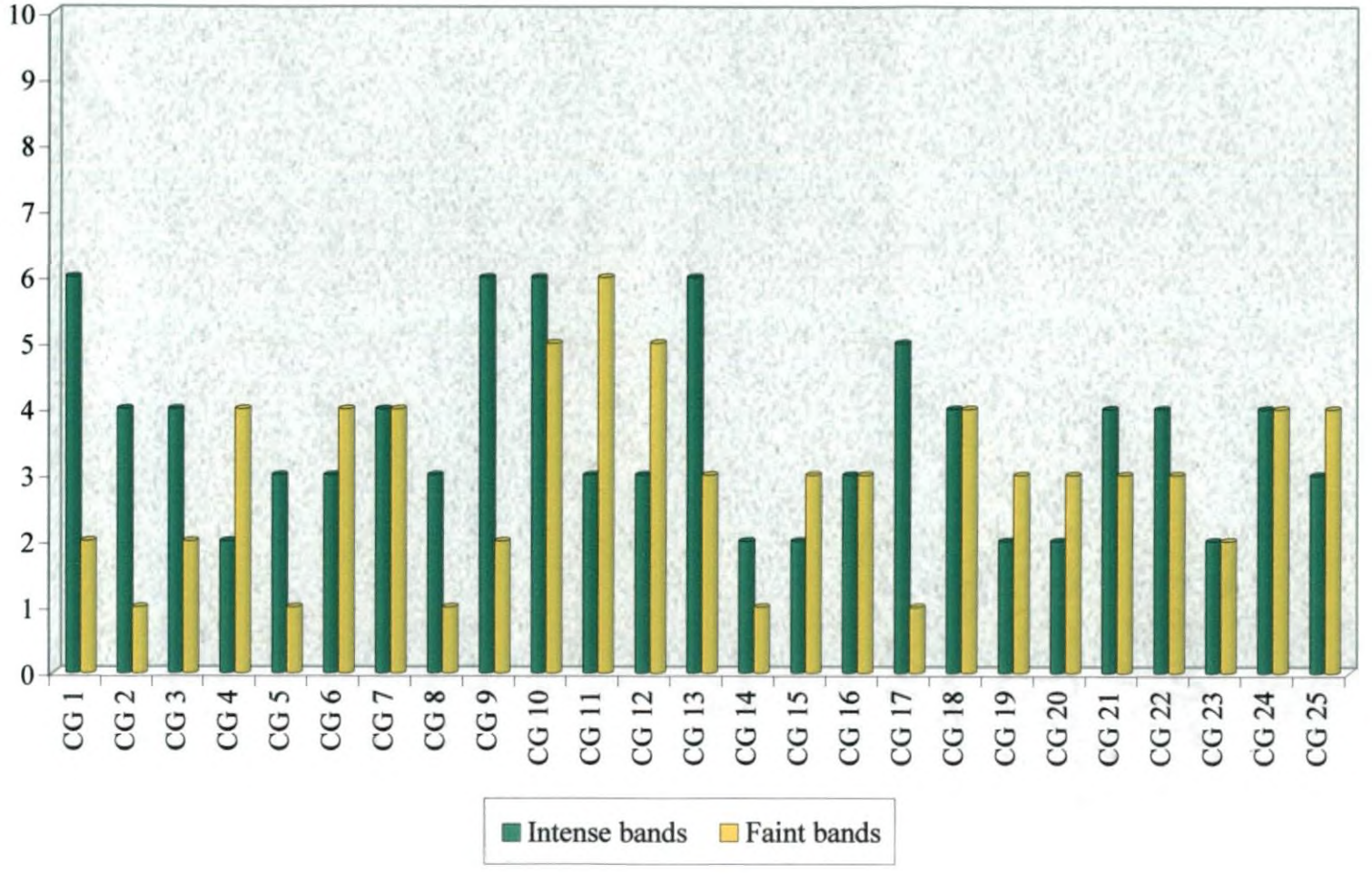

Fig. 12. Representation of the amplification profiles (intense and faint bands) of the DNA of 25 ivygourd accessions using the primer OPB-10

CG 1	CG 2	CG 3	CG 4	CG 5	CG 6	CG 7	CG 8	CG 9	CG 10	CG 11	CG 12	CG 13	CG 14	CG 15	CG 16	CG 17	CG 18	CG 19	CG 20	CG 21	CG 22	CG 23	CG 24	CG 25
1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	1	1	0	1	1
0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1
0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	1	0	0	1	1	1	0	1	0	1	1	1	1	0	0	1	1	0	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1
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1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	1	1	0	1	1
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

1 – Presence of band 0 – Absence of band

Fig. 13. Representation of the amplification profile of the DNA of 25 accessions using the primer OPB-10

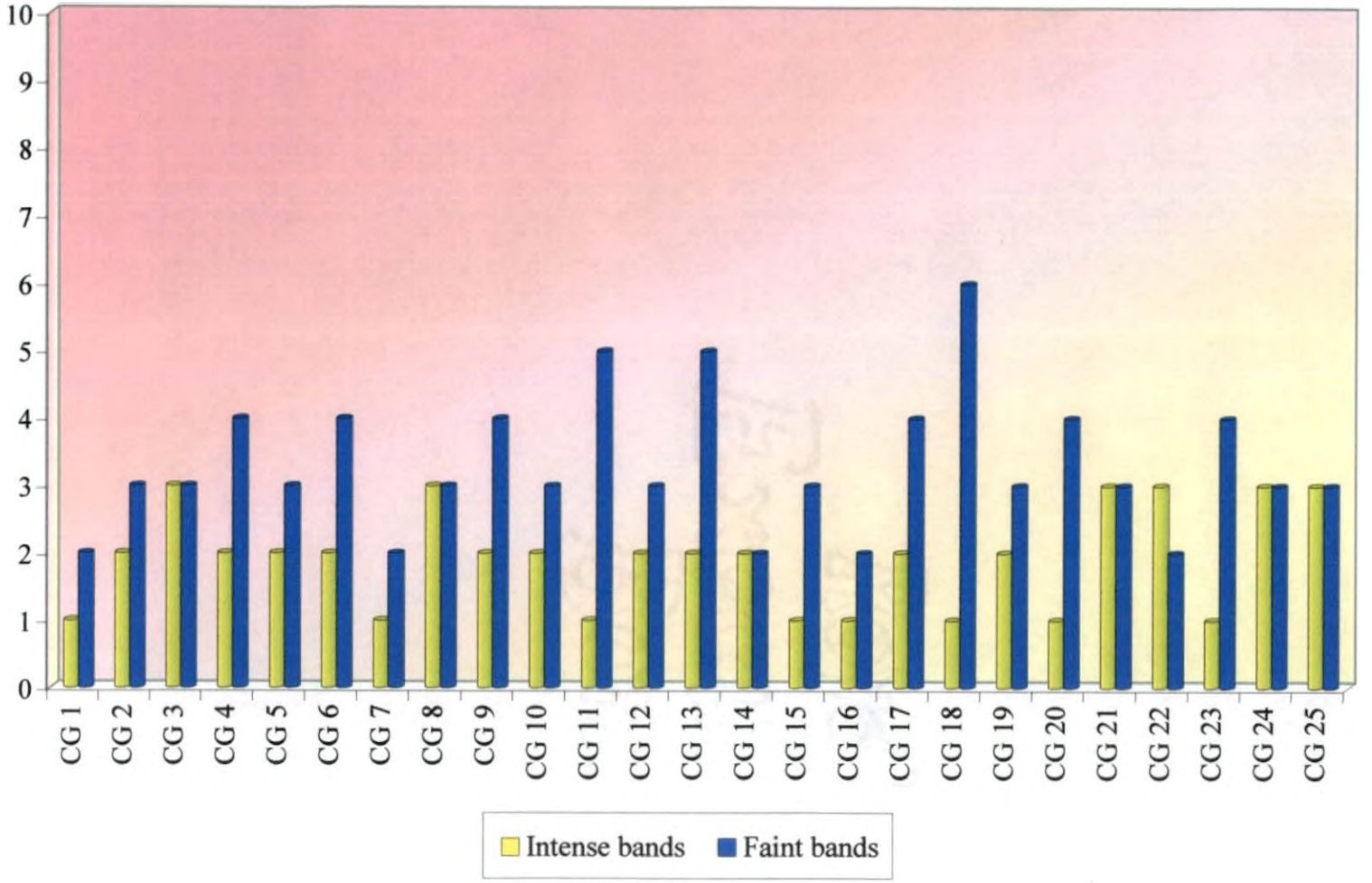

Fig. 14. Representation of the amplification profiles (intense and faint bands) of the DNA of 25 ivygourd accessions using the primer OPB-6

CG 1	CG 2	CG 3	CG 4	CG 5	CG 6	CG 7	CG 8	CG 9	CG 10	CG 11	CG 12	CG 13	CG 14	CG 15	CG 16	CG 17	CG 18	CG 19	CG 20	CG 21	CG 22	CG 23	CG 24	CG 25
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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0	1	1	1	1	1	0	1	0	0	1	1	1	1	0	1	1	1	0	0	0	0	0	1	1
0	1	1	1	0	1	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1

1 – Presence of band 0 – Absence of band

Fig. 15. Representation of the amplification profile of the DNA of 25 accessions using the primer OPB-6

Table 17. Amplification profiles (intense and faint bands) of the DNA of 25 ivygourd genotypes using the primers OPA-18, OPB-11, OPB-10 and OPB-6

Accessions	Primers							
	OPA-18		OPB-11		OPB-10		OPB-6	
	Intense bands	Faint bands	Intense bands	Faint bands	Intense bands	Faint bands	Intense bands	Faint bands
CG 1	8	0	5	6	6	2	1	2
CG 2	8	0	3	10	4	1	2	3
CG 3	8	0	4	8	4	2	3	3
CG 4	7	1	4	6	2	4	2	4
CG 5	8	0	5	7	3	1	2	3
CG 6	6	1	4	8	3	4	2	4
CG 7	7	0	5	8	4	4	1	2
CG 8	8	0	7	5	3	1	3	3
CG 9	6	2	10	2	6	2	2	4
CG 10	7	1	7	3	6	5	2	3
CG 11	6	2	5	4	3	6	1	5
CG 12	6	2	5	2	3	5	2	3
CG 13	6	3	7	5	6	3	2	5
CG 14	7	1	3	4	2	1	2	2
CG 15	8	0	4	3	2	3	1	3
CG 16	8	0	2	2	3	3	1	2
CG 17	7	0	7	4	5	1	2	4
CG 18	7	0	5	6	4	4	1	6
CG 19	7	1	3	6	2	3	2	3
CG 20	6	2	5	4	2	3	1	4
CG 21	8	0	5	7	4	3	3	3
CG 22	8	0	11	1	4	3	3	2
CG 23	7	0	6	6	2	2	1	4
CG 24	7	1	4	8	4	4	3	3
CG 25	8	0	4	6	3	4	3	3

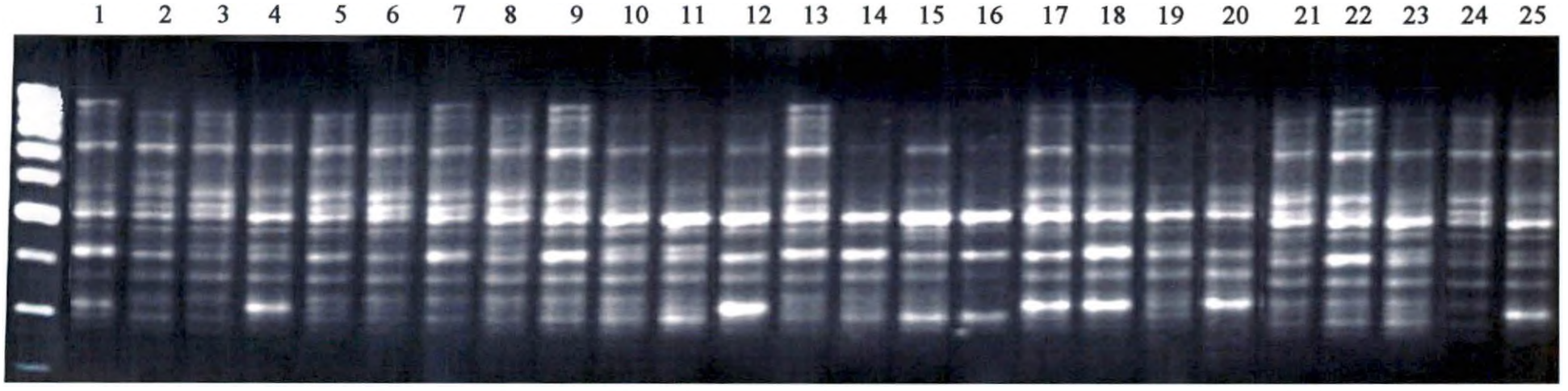

Plate 4. Amplification profiles of the DNA of twenty five *Coccinia grandis* genotypes using the primer OPB-11

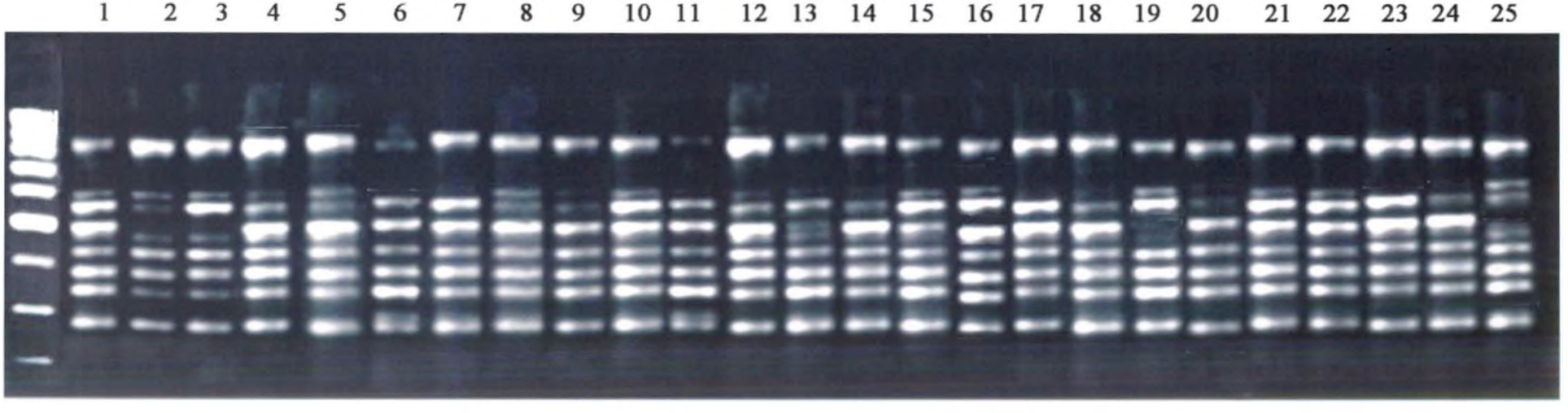

Plate 5. Amplification profiles of the DNA of twenty five *Coccinia grandis* genotypes using the primer OPA-18

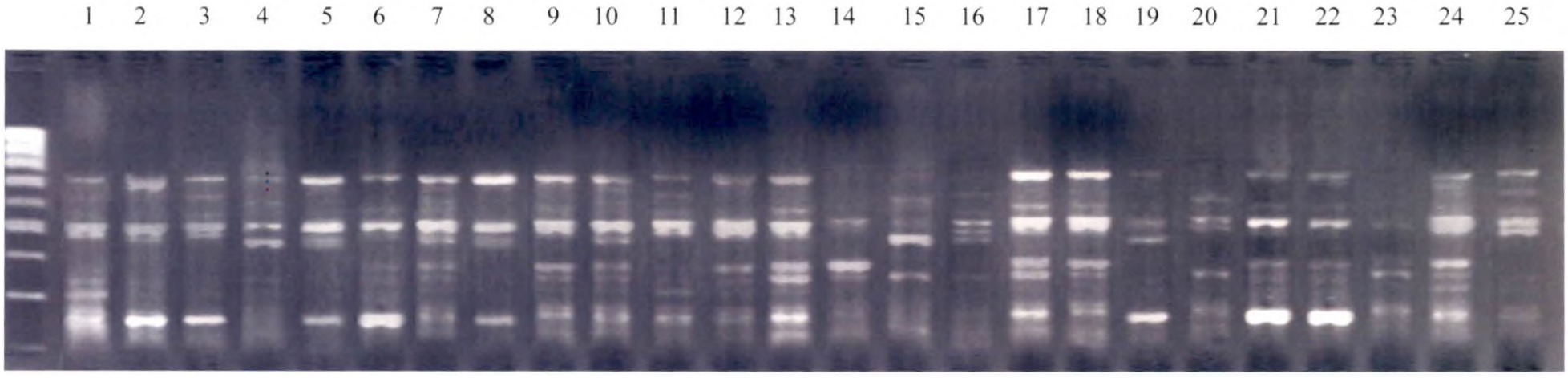

Plate 6. Amplification profiles of the DNA of twenty five *Coccinia granids* genotypes using the primer OPB-10

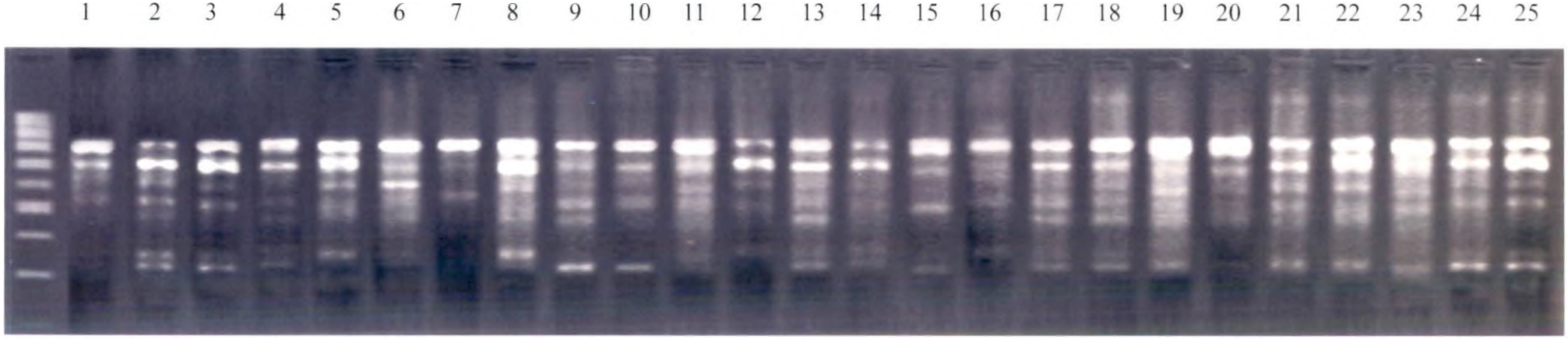

Plate 7. Amplification profiles of the DNA of twenty five *Coccinia granids* genotypes using the primer OPB-6

4.2.4 Statistical Analysis

Reproducible bands were scored for their presence (1) or absence (0) for all the accessions studied. RAPD marker data were analysed by used NTSYS (version 2.02) statistical Package. A genetic similarity matrix was constructed using the Jaccard's similarity coefficient method. The pair wise coefficient values varied between 0.421 and 0.918. The least similarity coefficient values were those of CG 18 and CG 16 (0.421).

The highest value for similarity index was obtained for CG 13 and CG 9 (0.918). Similarity matrix obtained for all the accessions shown in the Table 18.

On drawing a vertical line in the dendrogram (Fig 16), along the point corresponding to a similarity coefficient value of 0.706, all the twenty five accessions got divided into ten clusters. The accessions CG 11, CG 10, CG 18, CG 17, CG 24, CG 13, CG 9, CG 22, CG 21 and CG 6 together formed the largest cluster. Within this cluster CG 22 and CG 21 showed cent percent similarity among them. The accessions in this cluster CG 13 and CG 9 were more close to each other followed by CG 18 and CG 17. The accessions CG 8, CG 5, CG 25, CG 3 and CG2 together formed second largest cluster. The accessions CG 16, CG 19, CG 12, CG 7, CG 4 and CG 1 formed individual separate clusters.

Among the ten clusters two clusters had 2 accessions each, the first cluster had CG 14 and CG 15, whereas the second one had CG 23 and CG 20.

Table 18. Similarity matrix of 25 ivygourd accessions based on the Jaccard's similarity index

Acce-ssions	CG 1	CG 2	CG 3	CG 4	CG 5	CG 6	CG 7	CG 8	CG 9	CG 10	CG 11	CG 12	CG 13	CG 14	CG 15	CG 16	CG 17	CG 18	CG 9	CG 20	CG 21	CG 22	CG 23	CG 24	CG 25
CG 1	1.000																								
CG 2	0.632	1.000																							
CG 3	0.676	0.829	1.000																						
CG 4	0.667	0.632	0.590	1.000																					
CG 5	0.639	0.794	0.743	0.686	1.000																				
CG 6	0.632	0.730	0.730	0.590	0.694	1.000																			
CG 7	0.694	0.615	0.615	0.488	0.622	0.750	1.000																		
CG 8	0.579	0.722	0.771	0.714	0.844	0.771	0.605	1.000																	
CG 9	0.730	0.650	0.692	0.641	0.658	0.784	0.711	0.730	1.000																
CG 10	0.711	0.675	0.675	0.667	0.641	0.675	0.610	0.625	0.769	1.000															
CG 11	0.722	0.641	0.641	0.676	0.605	0.641	0.615	0.590	0.692	0.811	1.000														
CG 12	0.611	0.579	0.538	0.611	0.629	0.579	0.553	0.568	0.676	0.703	0.714	1.000													
CG 13	0.675	0.683	0.725	0.634	0.650	0.769	0.659	0.718	0.919	0.714	0.683	0.667	1.000												
CG 14	0.559	0.618	0.571	0.606	0.625	0.618	0.543	0.606	0.629	0.611	0.667	0.645	0.622	1.000											
CG 15	0.688	0.556	0.600	0.636	0.606	0.556	0.528	0.588	0.611	0.595	0.647	0.576	0.564	0.741	1.000										
CG 16	0.594	0.472	0.514	0.500	0.515	0.472	0.444	0.457	0.447	0.474	0.559	0.485	0.450	0.630	0.731	1.000									
CG 17	0.667	0.590	0.632	0.714	0.595	0.771	0.649	0.667	0.778	0.667	0.722	0.657	0.763	0.606	0.588	0.457	1.000								
CG 18	0.703	0.585	0.625	0.703	0.590	0.757	0.641	0.658	0.861	0.700	0.711	0.694	0.842	0.556	0.541	0.421	0.909	1.000							
CG 19	0.639	0.694	0.649	0.639	0.657	0.525	0.579	0.639	0.615	0.641	0.649	0.500	0.610	0.576	0.606	0.429	0.553	0.550	1.000						
CG 20	0.676	0.639	0.639	0.629	0.600	0.595	0.611	0.583	0.649	0.722	0.639	0.618	0.600	0.667	0.700	0.548	0.629	0.622	0.600	1.000					
CG 21	0.703	0.757	0.806	0.658	0.722	0.857	0.684	0.800	0.861	0.789	0.711	0.605	0.795	0.647	0.629	0.500	0.750	0.737	0.632	0.667	1.000				
CG 22	0.743	0.750	0.800	0.605	0.714	0.853	0.722	0.743	0.857	0.784	0.703	0.639	0.789	0.688	0.667	0.529	0.743	0.730	0.622	0.706	0.939	1.000			
CG 23	0.611	0.667	0.622	0.611	0.676	0.667	0.686	0.657	0.771	0.703	0.622	0.600	0.711	0.700	0.625	0.441	0.657	0.694	0.629	0.719	0.743	0.735	1.000		
CG 24	0.711	0.718	0.763	0.667	0.730	0.763	0.650	0.757	0.865	0.795	0.718	0.703	0.846	0.657	0.595	0.474	0.711	0.789	0.641	0.590	0.838	0.833	0.750	1.000	
CG 25	0.743	0.800	0.853	0.694	0.667	0.658	0.550	0.649	0.625	0.692	0.703	0.595	0.659	0.543	0.618	0.529	0.694	0.684	0.667	0.706	0.730	0.722	0.595	0.692	1.000

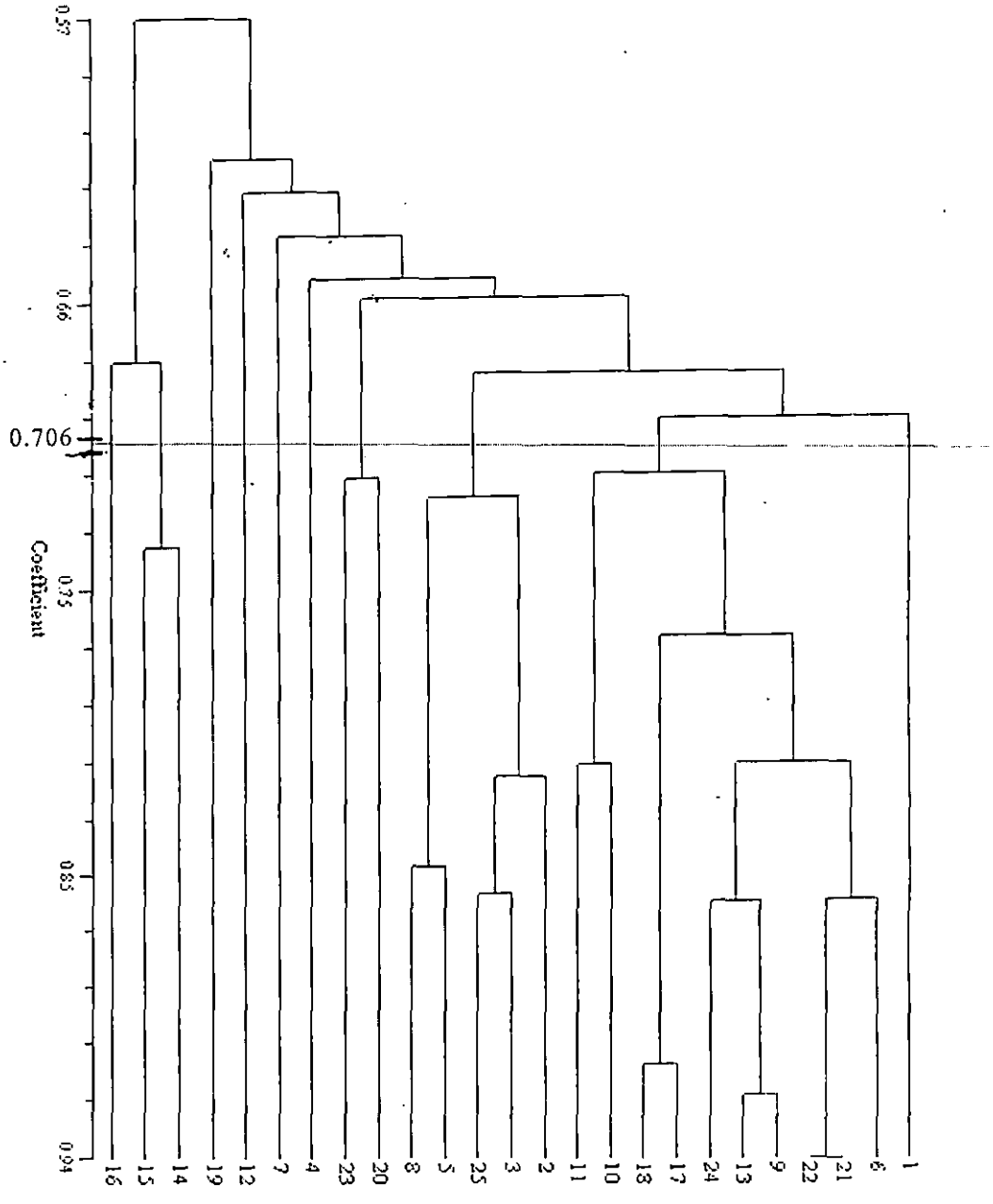

Fig. 16 Dendrogram obtained from RAPD analysis using UPGMA method

Discussion

5. DISCUSSION

Ivygourd (*Coccinia grandis* (L.) Voigt) also known as scarletgourd, kundsra and tondli is an important perennial cucurbitaceous vegetable widely cultivated in South Asian countries. The immature fruits are consumed raw, cooked or candied. The roots, stem and leaves possess medicinal properties for the treatment of skin diseases, bronchitis and diabetes. The fruits are good source of protein, vitamin A and vitamin C. There exists distinct types in coccinia. Wide variation is noticed in respect of size, shape and colour of fruits and leaves. Variation is also observed in respect of extent of bitterness. Despite these high variability and useful traits no authentic work is available on the characterization of the accessions. Though characterization based on morphological attributes was done by few workers the results are not convincing as it is influenced by environment. As it is a vegetatively propagated crop molecular characterization and classification deserves priority in terms of improvement. Information generated through DNA profiling using RAPD markers would give a comprehensive picture on diversity and relatedness of the accessions in a crop like coccinia. Therefore the present investigation was carried out with objective of studying the genetic divergence of the germplasm of coccinia and characterizing them on morphological and molecular basis. Attempts was also made to compare the two methods of characterization.

Twenty five diverse accessions of ivygourd were evaluated for biometric characters and quality attributes. Morphological and molecular characterization of these cultivars were done. The accessions were grouped into clusters based on the above methods. The result obtained are discussed here under.

5.1 MORPHOLOGICAL CHARACTERIZATION

5.1.1 Variability Studies

Genetic variability and divergence were worked out considering 12 morphological and quality characters viz. Primary branch length, internodal

length, days to first flowering, node to first flower, fruit length, fruit girth, average fruit weight, seeds per fruit, fruits per plant, protein content, total number of harvest and yield per plant.

Analysis of variance showed significant difference among the accessions for all the characters studied. The present collection of accessions included accessions from different parts of Kerala and Tamil Nadu in general. Individual accessions confined to particular localities or pockets to satisfy the preference of the people of that area. The individuals maintained the genetic identity as they had been continuously cultivated through vegetative means. As the preference of the crop vary with individual and locality the collected material included diverse accessions of different localities show variation in plant and fruit characters. Therefore the present observed variability is quite rational. Similar variation in plant (or) fruit characters reported by Josepth (1999), Sarnaik (1999) and Varghese (2003) in ivygourd support the present findings.

The average primary branch length was estimated as 2.95 m with a range of 1.1 m to 8.02 m. PCV and GCV are in the high range (>30 percent) and the environmental variance was negligible indicating low influence of environment on this character. These findings were in consonance with the findings of Choudhary (1987) in bittergourd and Mathew (2001) in bottlegourd vine length.

The mean internodal length was 6.98 with a minimum of 5.27 cm and maximum 8.94 cm. The PCV and GCV are in low range and environmental variance also was less. Sarnaik *et al.* (1999) recorded wide range of variation for internodal length in ivygourd.

The average number of days to first flowering was 50.48 days with a minimum of 35.75 days and maximum of 67.2 days. Environmental variance was less and the PCV and GCV were minimum. This findings were in tune with the findings of Varghese (1991) in snakegourd; Ganyathri (1997) in cucumber who reported least GCV and PCV for this trait.

The average node number to first flower was 23.62 with a range of 16.5 to 32.75. Least PCV and GCV was recorded for this trait. These indicates that variation for earliness in terms of days and node to first female flowering comparatively low in the present gerplasm. However, varghese and Rajan (1993) in snakegourd and singh *et al.* (2002) in ridgegourd recorded high PCV and GCV.

The fruit length and fruit girth are the indications of fruit size. The mean of fruit length and girth were 6.63 cm and 6.82 cm respectively. Moderate and Least GCV and PCV were recorded for fruit length and girth respectively indicating more variability for fruit size. However Singh *et al.* (1986) in pointed gourd and Indiresh (1982) in bittergourd recorded high GCV for fruit size. Raju and Peter (1995) reported considerable variation in fruit length of ivygourd. This could be to genotypic characteristics of the present germplasm.

The average fruit weight ranged between 19.69g to 9.3g with an average mean of 14.05g. Moderate PCV and GCV were recorded for this trait and the environmental variability was less. In ridgegourd Varalakshmi *et al.* (1996); Choudhary (1987) and Vahab (1989) in bittergourd reported high GCV and PCV for fruit weight.

Mean fruit the per plant was 355.87 with a range of 1072.25 to 106.25. In this present study very high PCV and GCV were recorded for this character indicating ample variation in the present collection for this character. This findings is in consonance with that of Varalakshmi *et al.* (1996) in ridgegourd; Radhiha (1999) in snakegourd; Joseph (1999) in ivygourd and Kandaswamy (2004) in melon.

Protein content in fruits ranged between 92 mg to 38 mg per 100g fresh weight. Moderate variability was recorded both at Phenotypic and genotypic levels. Similar result were obtained by Jaiswal *et al.* (1990) in bitter gourd and Rahman *et al.* (1994) in bottlegourd.

The total number of harvest is an indication of cropping duration of the crop. Generally the fruits are harvested at 7 days interval. Least number of

harvests indicates short duration and more number of harvests indicates long duration. In the present study the number of harvest ranged from 16 to 4.5 with a mean of 8.52. High PCV and GCV were recorded for these character indicating significant variability in the present collection for cropping period.

The most important economic character is the fruit yield per plant which varied from 1.3 kg to 18.41 kg with an average of 5.34 kg. Fruit yield per plant had higher values of PCV and GCV among all the characters studied. Environmental variance was also very less. This indicates ample variability for yield per plant ensuring better possibility of selection for improvement. These results are in agreement with the findings of Joseph (1999) in ivygourd; Varalakshmi *et al.* (1996) and Mathew (1999) in bottlegourd and Radhika (1999) in snakegourd.

5.1.2 Heritability and Genetic Advance

The success of improvement of the characters through selection depends on the heritability coupled it expected genetic advance. Heritability gives an idea about the relative importance of the genetic and environmental components of variance in the character expression. High values of these coefficient indicate the effectiveness of selection phenotypically superior plants in the next generation. Similarly, the magnitude of improvement in the performance of selected individuals over the population assumes importance. This is estimated through the parameter genetic advance. For easy understanding heritability is classified as high (60-100), moderate (30-60) and low (<30) and genetic advance as high (>20), moderate (10-20) and low (<10).

Heritability along with genetic advance is more useful than heritability alone in predicting the resultant effect of selecting the best individuals (Jhonson *et al.* (1955).

Ivygourd is a vegetatively propagated crop. So all characters recorded high heritability and genetic advance. Among the different characters maximum heritability estimate was for yield per plant followed by fruit length, fruits per

plant, internodal length and primary branch length. All of these characters are yield contributing traits. If five percent selection is to be practiced maximum genetic advance is expected for fruit yield per plant followed by fruits per plant, primary branch length and average fruit weight. According to Panse and Sukhatme (1957), the characters with high heritability and genetic advance were controlled by additive gene action and therefore amenable to improvement through selection. In the present study all the economic characters recorded very high heritability (>95 percent) followed by genetic advance. So selection of phenotypically superior plants with respect to these characters will result in a significant improvement in the next generation.

High heritability along with high genetic advance as percentage of mean for the major characters under study were formerly reported by Joseph (1999), and Varghese (2003) in ivygourd; Varalakshmi *et al.* (1996) and Anitha (1998) in ridge gourd and Radhika (1999) in snake gourd.

5.1.3 Correlation

The degree and direction of the inherent association (genotypic correlation) of characters apart from the observable correlation (Phenotypic correlation) between two characters are important for the simultaneous selection of characters for genetic improvement. Correlation coefficient gives an idea about the mutual relationship between various plant characters and determines the component characters on which selection can be based for genetic improvement in yield. The presence of genotypic correlation may be either due to pleiotropic action of genes or due to linkage or more likely both. If a positive genotypic correlation was observed for pair of characters, certainly the improvement in one character on the correlated response to selection. If the improvement in one characters results a decrease in other character, this will also help the breeder in the selection of characters if necessary.

In the present experiment, primary branch length, internodal length, fruit length, fruit girth, average fruit weight, seeds per fruit, fruits per plant and total number of harvest, showed positive genotypic and phenotypic correlation with

yield. Similar correlation was reported by Joseph (1999), Sarnaik *et al.* (1999) and Varghese (2003) in ivygourd. From this it is evident that, selection based on these characters will result in higher yield. The character also observed to be highly heritable with high genetic gain.

Days to first flowering showed negative genotypic and phenotypic correlation with yield. The same result was obtained by Varghese (2003) in ivygorud.

Among the characters, fruits per plant had the highest correlation with yield. Joseph (1999) in ivygourd reported the same result. So selection based on this trait is more effective.

Fruits per plant depends on primary branch length and internodal length. Longest primary branch length with shortest internode increase the number of nodes per branch, therefore increasing the fruits per plant. Here the primary branch length and internodal length had positive correlation both at phenotypic and genotypic levels. Both traits are also positively correlated with fruits per plant. From this it is evident that selection based on these traits increase the yield. Similar results were reported by Sarnaik *et al.* (1999) on ivygourd and Rajput *et al.* (1995) in bittergourd. But Shibukumar (1995) reported in watermelon negative correlation between vine length and yield per plant.

Average fruit weight is one of the yield contributing factor which is decided by the length and girth of the fruit. Fruit length exhibited positive correlation with fruit girth both phenotypic and genotypic levels. These two character had also positive correlation with average fruits weight at both levels. This is conformity with the findings of Joseph (1999) on ivygourd.

The phenotypic correlation includes both genetic and environmental effects. In the present study, the magnitude of genotypic correlation was higher than the corresponding phenotypic correlation indicating that environment had negligible effect on these characters.

5.1.4 Path Analysis

The technique of path analysis was applied in plant selection by Dewey and Lu (1959). It measures the direct and indirect contribution of independent variables on dependent variable and the residual effects. This technique also provides information about the cause and effect situation and helps in understanding the cause of association between two variables. Direct effect and indirect effects can be classified into very high (>1), high (0.30-0.99), moderate (0.20-0.29), low (0.10-0.19) and negligible (0.00-0.09) (Lenka and Mishra, 1973). Path analysis was done using the characters primary branch length, internodal length, fruits length, fruit girth, average fruit weight, seeds per fruit, fruits per plant and total number of harvest as independent variables which had strong genotypic correlation with fruits yield per plant, the dependent variable.

In the present study majority of the characters recorded positive direct and indirect effect on yield. Among this primary branch length had high positive direct effect on yield per plant. The similar result was obtained by Prasad and Singh (1992) in cucumber. In this case too, it is evident that selection for primary branch length leads to higher yield. Its indirect effect through other selected traits were negligible except fruits per plant.

The genotypic correlation of internodal length was high. Though its indirect effect was negative. But its indirect effect through primary branch length was high.

The direct effect of fruit length was positive. Its indirect effect through other selected characters were moderate. Prasad and Singh (1992) in cucumber and Bhave *et al.* (2003) in bittergourd obtained the same result. Though the direct effect of fruit girth on yield was negative its indirect effect through seeds per fruit, primary branch length and fruits per plant was moderate indicating effective indirect selection through this traits. Chaudhary and Mandal (1987) in cucumber reported similar result.

The direct effect of average fruit weight was least and positive, through its indirect effect through seeds per fruit was high. The genotypic correlation was also high. So indirect selection through seeds per fruits will be more effective than direct selection. Rao *et al.* (2000) and Sidhu and Brar (1981) in ridgegourd reported high positive effect of average fruit weight on yield.

The direct effect of seeds per fruits on yield was high and its indirect effect through primary branch length and fruits per plant was moderate. The same result was obtained by Menon (1998) in ashgourd.

Fruits per plant showed high positive direct effect on yield and its indirect effect through primary branch length was also high. In this case too, its is evident that selection for more fruits per plant and primary branch length leads to higher yield. Similar result was obtained by Saika *et al.* (1995) and Gayathri (1997) in cucumber.

5.1.5 Genetic Divergence

An assessment of genetic diversity is of much importance in plant breeding as for as the selection of parents for hybridization is concerned. The economic value of a plant is determined by several characters which may be correlated. Mahalasnobis D^2 statistics gives quantitative measure of divergence based on multiple characters. In the present study the 21 bitterless accessions of ivygourd were clustered into 6 groups. Fig. 5 shows the genetic diversity at intra and intercluster levels. The greater is the divergence between accessions belonging to the clusters. The accessions within a cluster are less divergent than those in different clusters. In the selection of parents for hybridization the mean points to be considered are the relative contribution of each characters to the total divergence. Choice of the clusters with maximum genetic distance and selection of accessions from such characters. Among the 6 clusters maximum number of accessions were in the cluster II. One accessions (CG 19) did not come under any of the clusters. The maximum intercluster distance was between cluster I and IV and the minimum between cluster II and I. The cluster VI included only one accession (CG 19) which is high yielder and had maximum intercluster distance

with IV of the six clusters. This shows that selection of parents form these divergent clusters will be very effective in hybridization.

Character wise performance at cluster levels the yield per plant had highest contribution to cluster formation, followed by total number of harvest and fruits per plant. These characters can be considered as potential contribution of divergence.

5.2 MOLECULAR CHARACTERIZATION

The present study was undertaken to characterise 25 accessions of ivygourd (*Coccinia grandis* (L.) Voigt) using RAPD makers. The result obtained are discussed in detail in this chapter.

Isolation of genomic DNA of ivygourd was done using modified method of Murray and Thompson (1980). The quantity and quality of the isolated DNA depended on the source as well as efficient disruption of the plant cell wall. The yield of DNA and its purity varied with accessions. The yield ranged from 1.44 ng/ µl (CG 19) 9.25 ng/ µl (CG 23). The purity of the DNA (A^{260}/A^{280}) ranged from 1.29 to (CG 15) 2.28 9cG 19). This could be due to the interference of various compound in the plant tissue during the isolation.

The DNA isolated using modified Murray and Thompson method was slightly brown in colour. This could be due to the presence of phenols. The inclusion of phenol: Chloroform: isoamyl alcohol (25:24:1) and washing with 70 per cent ethanol improved the quality of DNA as observed by the reduction in the colour.

Agarose gel electrophoresis was used for analyzing the genomic DNA isolated from different accessions as well as for the RAPD products. The concentration of gel is an important factor for the separation of DNA fragments. A low concentration of agarose is ideal for the separation of genomic DNA, which are of high molecular weight while small DNA fragments gave better separation in high concentration of agarose gel. Staub *et al.* (2000) in melon used 1.6 percent agarose for RAPD analysis. In the present study 0.9 per cent agarose was

used for genomic DNA and one per cent for RAPD analysis which was found satisfactory for the resolution of bands.

5.2.1 RAPD Analysis

The PCR amplification was carried out using twenty eight decamer primers (Opron Inc., CA, USA) of kit A and Kit B with the DNA of accession CG 1. The procedure standardized by Staub *et al.* (2000) in *Cucumis melo* was used for amplification. Seventeen primers out of twenty-eight screened, yielded amplification products. The total number of bands ranged from 1 to 6.

The primers OPA-2, OPA-4, OPA-6, OPA-7, OPA-8, OPA-16, OPA-19, OPA-20, OPB-17 and OPB-20 did not yield any bands. This indicated that there is no sequence complementary to the sequence of these primers in the DNA of CG 1.

A total of 41 RAPDs (average 1.46 bands per primer) were generated by 17 primers of which 39 were polymorphic.

In the present study four primers were finally selected for the RAPD analysis for all the accessions based on the number of intense bands obtained. They were OPA-18, OPB-11, OPB-06, and OPB-10. These primers were used for amplifying DNA from all of the ivygourd accessions. According to Weising *et al.* (1995) primers with a GC content of at least 50 percent should be used. In this study the GC the content of the primers used varied from 60-70 per cent. The PCR reaction was repeated twice in order to check the reproducibility.

The four primers used in this analysis yielded 48 scorable bands with a average of 12 bands per primer. The number of bands resolved per amplification was primer dependent and varied from 8.16.

The highest number of scorable bands was given by the primer OPB-11 (16 bands) among this, two bands were monomorphic and 14 bands were polymorphic. The highest number of bands (13 bands) were produced by CG 2 and CG 7 whereas, least bands (4 bands) by CG 16.

When using the primer OPA-18 for amplification, 6 bands were monopmorphic. In OPB-10 and OPB-6 the highest number of band was produced by the accessions CG 10 (11 bands) and CG 13 and CG 18 (7 bands) respectively. All the primers together the accessions CG 3 produce highest number of bands (37 bands) whereas CG 16 produced least number of bands (CG21).

It is well known that with polymorphic bands it could be possible to clearly distinguish the various accession rather than monomorphic bands.

Among the four primers used for amplification of the 25 different accessions, the primer OPA-18 produced highest number of monomorphic bands. The primer OPB-11 produce only 2 monomorphic bands and OPB-10 and OPB-6 only one monomorphic bands each and all other bands were polymorphic. Thus these primers serve well in fine differentiation of the 25 genotypes of ivygourd studied

5.2.2 Statistical Analysis

Jaccard's similarity co-efficient values ranged from 0.421 to 0.918. The lowest values was between CG 18 and CG 16 and the highest value between CG 13 and CG9. The estimation of similarity coefficient and construction of dendrogram by using the UPGMA (Un weighted pair group method for arithmetic average) method revealed the extent of genetic similarities among the 25 ivygourd accessions. All the 25 accessions were divided into 10 cluster at 0.706 similarity coefficient. The nine accessions CG 11, CG 10, CG 18, CG 17, CG 24, CG 13, CG 9, CG 22, CG 21 and CG 6 together formed a single large cluster. Among these accessions CG 13, CG 17, CG 18, CG 9, CG 24 and CG 11 were found similar based on morphological observation having longer fruits with prominent yellowish line. The accessions CG 13, CG 5, CG 9, and CG 11 were highest yielders compared to others. The accession 11 produced highest fruits per plant. The accessions CG 21 and CG 22 having equal distance coming under this cluster are bitter types. The dendrogram illustrates a close relationship between the accessions CG 5 and CG 8 which are bitter types. It is also indicated that there is great genetic difference between the different bitter accessions. But these

accessions were found to be morphologically similar and this may be due to the influence of the environment, location and other morphological and physico-chemical attributes.

It is clearly evident from the dendrogram that the highest yielding accession CG 19 is entirely different from the rest of the accessions and it formed a separate cluster. It had longest primary branch, the fruit had wax coating and was a high yielder. The accession CG 12 also formed a separate cluster. It was different from other accessions by duration and it had medium wax coating. The accession CG 16 alone formed a cluster. Among the bitterless accessions, CG 16 was a low yielder and took maximum days for first flowering.

The accessions CG 23 and CG 20 together formed a cluster. The colour, shape and size of the fruits were almost similar. But CG 20 was low yielder. This could be mainly due to the environmental effect. The fruit size, shape, colour and fruit yield per plant were almost similar for the accession CG 3 and CG 2. The dendrogrm for both these came under same cluster but not of equal distance.

In the present study it was evident that the high yielding accession CG 19 possessed unique characteristics both on morphological and molecular bases of characterization. In both methods this accession came under separate cluster. So it was confirmed that the phenotypic variation of this accession is due to genetic makeup of the crop. Though the accession CG 13, which is the second highest yield had longest fruits and good visual appearance, in molecular characterization, it fell into the largest cluster and 50 per cent of other members were similar morphology with only where lower yielders. So it showed that the environment had great influence on yield.

The present investigation of the genetic diversity by using morphological characters revealed, influence of environmental on expression of characters. So DNA based molecular markers were found to be more reliable than any other morphological markers in the characterization of the genotypes. Among the molecular markers, RAPD markers are quicker with less cost. The present results

obtained indicated that RAPDs are more suitable markers than agronomic traits in predicting genetic distance among the breeding lines. Garcia-Mas *et al.* (1998) obtained the similar result in melon supporting the present findings.

Further studies on morphological and agronomic traits and analysis of the accessions with more number of reliable DNA markers may be helpful in confirming the result. Knowledge of the degree of genetic relationships between these accessions will be important for the improvement of the genotypes and to establish a core collection as part of the germplasm maintenance.

Summary

6. SUMMARY

The present study entitled "Molecular characterization of ivygourd [*Coccinia grandis* (L.) Voigt]" was conducted at the Departments of Olericulture and Plant Biotechnology, College of Agriculture, Vellayani during the period 2003-2004. The programme envisaged assessing the variability in the landraces of ivygourd in Kerala and Tamil Nadu on morphology, yield and yield attributes and characterizing them under molecular level using RAPD technique. Twenty five diverse genotypes collected from different parts of Kerala and Tamil Nadu were evaluated for the genetic variability under morphological and molecular level. The salient result of the study are summarized.

For genetic variability studies morphological characters like primary branch length, internodal length, days to first flowering, node to first flower, fruit length, fruit girth, average fruit weight, seeds per fruit, fruits per plant, total number of harvest and yield per plant and quality characters like protein content were considered. Then the genotypes were evaluated organoleptically also.

Morphological studies showed significant differences among the 25 accessions for all the characters. Yield per plant was maximum in CG 19, a genotype collected from Kazhakkuttom, Thiruvananthapuram. High PCV coupled with high GCV was recorded for primary branch length, fruits per plant, total number of harvest and yield per plant, whereas moderate PCV and GCV were recorded for fruit length, average fruit weight and internodal length. Number of days to first flower and fruit girth recorded least PCV and GCV.

All the characters recorded high heritability and genetic advance. Among these yield per plant followed by fruit length and fruits per plant recorded very high heritability. Maximum genetic advance as percentage

of mean was recorded for yield per plant followed by fruits per plant, primary branch length and average fruit weight.

In this experiment primary branch length, internodal length, fruit length, fruit girth, average fruit weight, seeds per fruit, fruits per plant and total number of harvest showed positive genotypic and phenotypic correlation with yield, whereas days to first flowering showed negative correlation both at genotypic and phenotypic level.

Primary branch length, average fruit weight, seeds per fruit and fruits per plant showed high positive direct effect on yield. However, fruit girth had negative effect.

In D^2 analysis the 21 accessions of ivygourd were grouped into six clusters. Maximum number of genotypes was in cluster three. The high yielder (CG 19) stood alone as a separate cluster. Yield per plant contributed maximum for cluster formation.

In molecular characterization the DNA was isolated from young leaves of ivygourd using modified Murray and Thompson method. The yield of DNA ranged from 1.44 ng/µl to 9.24 ng/µl. The purity of DNA ranged from 1.29 to 2.28.

The quality of DNA was assessed by gel electrophoresis. The concentration of agarose was 0.9 per cent and 1.0 per cent for identification of genomic DNA and PCR products respectively.

For amplification of DNA, 40 ng of DNA, 20 µM of each of four dTNP, one unit of Tag DNA polymerase, 10 picomoles primer were used. The programme consisted of an initial denaturation at 94°C for five minutes followed by 43 cycles of denaturation at 94°C for 15 seconds, annealing at 35°C for 15 seconds and extension at 72°C for 75 seconds. The synthesis step of final cycle was extended further by seven minutes. The product of amplification were kept at 4°C until attended.

A total of 41 RAPDs were generated when PCR amplification was carried out using 28 decamer primers (Operon Inc., CA, USA) of Kit A and Kit B. Of these 39 bands were polymorphic. Four primers OPA 18, OPB 11, OPB 10 and OPB 6 produced reproducible banding pattern atleast two runs. These primers yielded 48 scorable bands with an average of 12 bands per primer. The number of bands resolved per amplification was primer depended and varied from 8 – 16.

Similarity coefficient value ranged from 0.421 to 0.918. From the dendrogram the 25 ivygourd genotypes were clustered into 10 groups. The largest group consisted the genotypes CG 11, CG 10, CG 18, CG 17, CG 24, CG 13, CG 9, CG 22, CG 21 and CG 6. The second largest group consisted the genotypes CG 8, CG 5, CG 25, CG 3 and CG 2. The genotypes CG 16, CG 19, CG 12, CG 7, CG 4 and CG 1 formed individual separate clusters.

Attempts to compare the morphological and molecular methods of characterization indicated the similarity in the case of CG 19 the top yielding genotype. Though the genotype CG 13 was second largest yielder with longest fruit and other attributes, in molecular characterisation it fell into the largest cluster with other moderate to low yielder. This shows the impact of environment on the expression of characters like yield. It also reveals the reliability of molecular techniques compared to morphological methods in the characterisation of landraces of ivygourd.

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*Original not seen

MOLECULAR CHARACTERIZATION OF IVYGOURD

[*Coccinia grandis* (L.) Voigt]

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Abstract of the
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ABSTRACT

The experiment entitled "Molecular characterization of ivygourd [*Coccinia grandis* (L.) Voigt]" was conducted at the Departments of Olericulture and Plant Biotechnology, College of Agriculture, Vellayani. The objectives were to assess the genetic divergence of the germplasm both at morphological and molecular level using RAPD markers in 25 ivygourd genotypes.

Analysis of variance of the observations showed significant difference among the accessions for all the characters. The yield obtained in the range of 1.3 kg (CG 2) to 18.41 kg (CG 19) per plant.

High PCV and GCV were observed for primary branch length, fruit per plant, total number of harvest and yield per plant. High heritability along with high genetic gain was observed in all the characters. The range of heritability was 98.83 to 63.02.

All characters except days to first flowering are positively correlated with yield both at phenotypic and genotypic level. Primary branch length, average fruit weight, seeds per fruit and fruits per plant had positive direct effect on yield. In D^2 analysis all the accessions are grouped into six clusters. The cluster III had maximum number of genotypes. The character yield per plant had maximum contribution to cluster formation.

In molecular characterization the yield of DNA ranged from 1.44 ng/µl to 9.24 ng/µl. The purity was 1.29 to 2.28.

A total of 41 RAPDs were generated when PCR amplification was carried out using 28 decamer primers (Operon Inc., CA, USA) of Kit A and Kit B. Of these 39 bands were polymorphic. Four primers, OPA 18, OPB 11, OPB 10 and OPB 6 produced reproducible banding pattern on

atleast two runs. These primers yielded 48 scorable bands with an average of 12 bands per primer.

The similarity coefficient values ranged from 0.421 to 0.918. From the dendrogram the 25 ivygourd genotypes were clustered into 10 groups. The largest group consisted the genotypes CG 11, CG 10, CG 18, CG 17, CG 2, CG 13, CG 9, CG 22, CG 21 and CG 6. The genotypes CG 16, CG 19, CG 12, CG 7, CG 4 and CG 1 formed individual separate clusters.

The result of both morphological and molecular level characterization revealed similarity in the case of genotype CG 19, the highest yield. Though the genotype CG 13 was second in yield with other special attributes, it fell into a cluster along with other moderate to poor yielders. This shows the impact of environment on the expression of the characters and necessity of molecular markers in the characterization of landraces in ivygourd.