

**Studies on Genetic Diversity among  
Indigenous Landraces of Pumpkin (*Cucurbita  
moschata* Duch. ex Poir)**

**Thesis**

Submitted to the

**Central Agricultural University, Imphal**

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For the award of the degree of

**Master of Science (Horticulture)**

In

**Vegetable Science**

by

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**Pasighat, Pin: 791102, Arunachal Pradesh, India**

**June, 2018**

AFFECTIONATELY

DEDICATED

TO MY BELOVED

PARENTS,

MY BROTHERS

AND

MY SISTERS



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## CERTIFICATE – I

Certified that **Mr. Md. Ramjan** [Registration No. CAU/46-H/16(M)] has satisfactorily prosecuted his course of research for a period of not less than two semesters and that the thesis entitled “**Studies on Genetic Diversity among Indigenous Landraces of Pumpkin (*Cucurbita moschata* Duch. ex Poir )**” submitted by him to the Central Agricultural University, Imphal-795004 (Manipur) in partial fulfilment of the requirements for the award of the degree of **Master of Science (Horticulture)** in the subject of **Vegetable Science** is the result of original research work conducted by him under my supervision and is sufficiently of high standard to warrant its presentation to the examination.

I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

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## **CERTIFICATE – II**

This is to certify that the thesis entitled “**Studies on Genetic Diversity among Indigenous Landraces of Pumpkin (*Cucurbita moschata* Duch. ex Poir )**” Submitted by **Mr. Md. Ramjan [Registration No. CAU/46-H/16(M)]** submitted to the Central Agricultural University, Imphal-795004 (Manipur) in partial fulfilment of the requirements for the award of the degree of **Master of Science (Horticulture)** in the subject of **Vegetable Science** has been approved by Student’s Advisory Committee after oral examination jointly with Dean’s Nominee.

  
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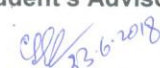
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# DECLARATION

I hereby declare that the thesis entitled “**STUDIES ON GENETIC DIVERSITY AMONG INDIGENOUS LANDRACES OF PUMPKIN (*Cucurbita moschata* Duch. ex Poir)**” is an authentic record of the work done by me and that no part thereof has been presented for the award of any other degree, diploma, associateship, fellowship or any other similar title.

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Place: Pasighat

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**Pasighat**

**(Md. Ramjan)**

**June, 2018**

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# LIST OF SYMBOLS/ABBREVIATIONS

@	at the rate
°B	°brix
°C	°celcius
µg	microgram
µl	microliter
/	per
%	percent
CHFPUM	college of horticulture and forestry pumpkin
cm	centimeter (s)
DUS	distinctiveness, uniformity, stability
EDTA	ethylene diamine tetra acetic acid
g	gram (s)
ha	hectare
K <sub>2</sub> O	potassium oxide
kg	kilogram
m	metre (s)
M	molar
ml	milliliter
MSL	mean sea level
MT	metric tonne (s)
N	nitrogen
NaOH	sodium hydroxide
nm	nanometer
P <sub>2</sub> O <sub>5</sub>	phosphorus pentoxide
q	quintal (s)
RCBD	randomized complete block design
S.N.	serial number

SDS	sodium dodecyl sulphate
t	tonne (s)
TEMED	tetramethylethylenediamine
TSS	total soluble solids
<i>Viz.</i> ,	namely

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#### **ABSTRACT**

The present investigation was conducted during 2017 at Vegetable Research Farm, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh in 25 diverse genotypes of pumpkin including 2 check cultivars viz., CHFPUM-7 (Kashi Harit) and CHFPUM-23 (Narendra Agrim). The experiment was laid out in Randomized Block Design with three replications. Genetic diversity was studied for twenty quantitative traits viz., vine length, number of branches per plant, days to first satminate flower anthesis, days to first pistillate flower anthesis, node bearing first male flower, node bearing first female flower, days to first fruit harvest, number of fruits per plant, polar circumference, equatorial circumference, flesh thickness, cavity length, number of seeds per fruit, 100 seed weight, average fruit weight, fruit yield per plant, total fruit yield q/hac, total soluble solids (TSS), carbohydrates and vitamin A. Data were analyzed statistically for phenotypic and genotypic variance, coefficient of variation, heritability, genetic advance, genetic gain, correlation coefficient, path coefficient, genetic divergence and seed protein banding pattern.

Analysis of variance revealed significant differences among the genotypes for all the characters studied. High PCV and GCV, heritability and genetic gain were observed for vitamin A, cavity length, 100 seed weight, number of seed per fruit, node bearing first male flower, total fruit yield q/ha, average fruit weight, fruit yield per plant, number of fruits per plant, flesh thickness, and carbohydrate. Correlation studies indicated that fruit yield per plant was positively and significantly correlated with average fruit weight, fruit length, fruit diameter and number of seeds per fruit which indicated the importance of these traits in selection for yield. Path analysis revealed that maximum positive direct effect on fruit yield per plant was imposed by average fruit weight followed by number of fruits per plant, node bearing first pistillate flower, days to first pistillate flower anthesis, 100 seed weight and total soluble solids at genotypic level. This indicated that these are the real independent characters and have maximum contribution towards increase in fruit yield per plant. Divergence study revealed that vitamin A contributed maximum per cent 66.45 to the diversity followed by, seed weight, number of branches per plant, fruit yield per plant, fruit diameter and days to first harvest. Maximum inter cluster distance was observed between cluster IV and VI which indicated that the genotypes within these clusters were highly divergent.

SDS-PAGE analysis showed considerable variation in band number of protein which ranged from 13-26. Protein banding profile showed that the genotype CHFPUM-1 was most distantly related to CHFPUM-21, CHFPUM-3 with CHFPUM-13 and CHFPUM-20. Hence, it was recommended that these two genotypes could be utilized for crossing programme to create more genetic diversity. SDS-PAGE marker data provided more sub groupings and revealed higher amount of diversity as compared to morphological data in present study.

**Key words:** Pumpkin, correlation, divergence, genotypes, heritability, SDS-PAGE.

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Chairperson

**(Md. Ramjan)**  
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# CHAPTER-1

## INTRODUCTION

# Chapter-1

## Introduction

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India is a developing country which has achieved self-sufficiency and a good degree of sustainability in food production. This has created an urgent need for providing health security to our population by supplying nutrition through balanced diet, where problem of malnutrition in human diet is severe. Vegetable crops play an important role in the balanced diet of human beings as they are rich source of nutrients like proteins, vitamins, minerals and have medicinal values.

India has emerged as the second largest producer of vegetables after China and contributes 14.45 percent share in the world vegetable production. Vegetable production in India during 2016–17 was 176.17 MT from an area of 10.295 million hectares. However, our per capita consumption is quite low (about 210 g). Pumpkin and squashes occupy 15.5 lakh hectares area having an annual production of 21.0 million tones with a productivity 13.48 of t/ha (Anonymous, 2016) and In India it occupies 7.2 lakh hectares area having annual production of 15.82 million tons with a productivity of 22 tons/ha in India (Anonymous, 2016).

Term “Cucurbit” was coined by Liberty Hyde Bailey for cultivated species of the family Cucurbitaceae. The family Cucurbitaceae comprises largest group of summer vegetables. All together there are 2 well defined sub-families, 8 tribes, about 118 genera and 825 species. Out of these, approximately 20 species belonging to 9 genera are under cultivation (Jeffery, 1990). The genus *Cucurbita* includes pumpkin, (*Cucurbita. moschata* Duch), summer squash (*Cucurbita pepo* L), winter squash (*Cucurbita. maxima*), winter squash pumpkin (*Cucurbita mixta* Pang) and Malabar gourd or fig leaf gourd (*Cucurbita ficifolia* Boucha). Among these five cultivated species, pumpkin (*Cucurbita. moschata*), summer squash (*Cucurbita pepo* L), winter squash (*Cucurbita. maxima*) are of great economic importance (Rana, 2014) cucurbita is the most economically valuable genus of this family with 27 species, five of which *Cucurbita pepo*, *Cucurbita mixta*, are cultivated all over the tropical and sub-tropical in the world. *Cucurbita moschata* Duch. ex Poir, known as pumpkin, is the most important and extensively cultivated species in India, Africa, Latin America, Southern Asia and United States. However, the English name for the species, “tropical pumpkin” is an appropriate one because the greatest diversity lies in the neotropics where the vines are grown under a wide range of ecological conditions (Andres, 2004).

Among the cucurbits, pumpkin is one of the most popular warm season vegetable crops commercially grown all over India. Pumpkin is also known as *Kashiphal* or *Sitaphal* or *Lal Kaddu*. It is extensively grown in different parts of the country for its immature and mature fruits. Its great significance is due to its high carotene content, long shelf life, excellent response to vegetable forcing, high nutritive estimates and better transport qualities.

Pumpkin (*Cucurbita moschata* Duch. ex Poir.) is one of the most important Cucurbitaceous vegetable crops grown extensively in tropical and sub-tropical parts of the country. Pumpkin belongs to the family Cucurbitaceae with chromosome number  $2n=40$ . It is an annual, robust, tendril-bearing climber, usually creeping over the ground. Tendrils are inserted besides the leaves, on robust stalk, 3-4 fid clothed with patent unequal, with hairs, light or yellowish green, 10-40 cm long and inclusive. Stalks are 1.5-7 cm long. Leaves are distichous, long stalked, simple, in outline orbicular-reniform or broadly ovate, base deeply and widely cordate, apex shortly acuminate, obtuse or rounded; 5-7 lobed, densely toothed; teeth obtuse glandular; at the base coarsely serrate or lobed, hispid along the margins, light or dark green and feebly shining above, light green and dull beneath, on both surfaces with white transparent veins, 7-35 cm long and 10-35 cm wide. Petiole is very robust, fistular, with numerous longitudinal faint grooves, densely clothed with patent, very rough hairs, light or dark green, 4-30 cm long and 0.5-1 cm thick. Stipule is absent or replaced by a gland. Flowers are peduncled, large, unisexual-monoecious, and solitary in the leaf-axils, for the greater part male, very hairy, dotted with many glands and 6-15 cm in diameter. Stalk of male flowers are longer and thinner than female, often subteret, 2-25 cm long and 0.5-0.7 mm thick. Stalk of female flowers is distinctly 5-angular and longitudinally furrowed, 2-7 cm long, 0.75-1.00 cm thick, much lengthened after the anthesis. Calyx-segments are 5, in the male flowers 2-5 cm long, 0.25-0.3 cm wide; in the female flowers 3-6 cm long, at the top 0.5-3.0 cm wide. Corolla is campanulate, on the outside light yellow with very prominent, yellowish green longitudinal ribs or veins. The female corolla is 7-15 cm long and 10-15 cm in diameter. Lobes are broadly ovate or broadly rhomboid, acutely acuminate with light green tops, widely patent, 2.5-5.0 cm or in the female flower 4-7.5 cm long and wide. Male flowers are with entirely connate stamens of 2-3 cm long; staminal column robust, with a broad base, sulpherious, 0.5-1.25 cm long; anthers oblong, dorsally connate, ochreous and 1.5-2.5 cm long. Ovary in the female flower is large, globose ovoid or oblong, light green or grayish green, densely irregularly gibbose or warty, with white spots; style columnar, angular, at the apex 2-3 partite, yellowish white, glabrous and 1.5-2.0 cm long. Stigmas are thick, deeply two lobed, ovoid or oblong, dark orange-colored, densely papillose, and 1.25-2.00 cm long. Fruits are long robust stalks, pendulous, very variable in shape, colour and dimensions,

rotate, ovoid or oblong lanceolate-ellipsoide, more or less deeply longitudinally furrowed or lobed, blotched or marbled, sordidly orange-coloured, when quite ripe, and flesh orange-coloured. Seeds are numerous and compressed. (Rai and Yadav, 2005).

Although “Butternut squash” popularly called as pumpkin in Western countries, (Bailey, 1929) described pumpkins as the marvels of the vegetable world because of their many unusual and extravagant characters. The genus *Cucurbita* is indigenous to new world (America). Archaeological remains trace the association of pumpkins and squashes with man in America for at least 10,000 years. The American Indians selected the cultivated species long before 1492 and they were staple items of their diet. Archaeological evidences showed that pumpkin, *Cucurbita moschata* Duch. ex Poir, was widely distributed in both North and South America with the primary centre of origin being Mexico and Peru. Considering its seed colour, pumpkin is also believed to be the native of Guatemala and Columbia. However, (Andres, 2004) have put forward the latest as well as concrete evidence as North Western part of South America being the centre of origin of pumpkin. Wild forms of the species have not been described, but primitive-appearing landraces are known from Central America to Northern Peru. This, coupled with the archaeological evidence, suggests that the centre of origin is in North-Western South America (Andres, 2004). Ferriol *et al.*, 2004 suggested the existence of two independent domesticating regions of pumpkin in Central and South America.

Pumpkin is a native to Central or South America. Archeological evidences suggest that *Cucurbita moschata* was widely distributed in America, Mexico and Peru being the primary center of origin. The pumpkin appeared in Europe in mid-16<sup>th</sup> century. It was introduced in India from South America by foreign navigators and emissaries. The name pumpkin originated from Greek word ‘*Pepon*’ used for long melon (Bahadur and Singh, 2014). Pumpkin after its introduction from South America by foreign navigators and emissaries, has well been accepted in India and its wide spread cultivation has caused the development of wide genetic diversity in different areas of India. Pumpkin now occupies a prominent place among the vegetables owing to its high productivity, nutritive value, good storability, long period of availability and better transportability. It is one of the important and popular vegetables extensively grown during rainy and summer months in all parts of the country particularly in Assam, West Bengal, Tamil Nadu, Karnataka, Madhya Pradesh, Uttar Pradesh, Odisha and Bihar.

Pumpkin is used as fresh vegetable, processed food and livestock feed. Both mature and immature fruits are consumed raw, cooked, stewed, boiled, or baked, and also used for preparation of jam, puree and candy. Its young leaves, tender flowers are also cooked and consumed. The flowers are used for excellent preparation so called “pakora”.

The flowers of pumpkin are more nutritive than fruits (Rana, 2014). The fruits are sweetish when fully mature and can be used in preparing sweets, candy or fermented into beverages. Fruits with orange or yellow flesh generally have high concentration of carotenoid (Pandey *et al.*, 2003). Delicate sweet items like “halwa”, other sweets and jams are prepared from meshed flesh of fully matured fruit. Pulp is also mixed with tomato in the preparation of GTE (glucose tolerance factor) pumpkin milk powder (Xingsheng *et al.*, 1998) which can be used as a diabetic food and pumpkin ice cream. The “Yerusseri” prepared from immature fruit is very popular in Kerala (Rajan and Markose, 2001). Pumpkin fruits are rich in vitamins, particular in precursor of vitamin A, and minerals. The edible portion of pumpkin constitutes around 80 %. Pumpkin seeds contain 40.27 % protein, 34.59 % crude fibre, and 4.4.5 % ash. Oleic, linoleic and palmitic acids are the predominant fatty acids in seed oil. Glutamic acid, arginine, glycine and aspartic acid are the most abundant amino acids (Aboul-Nasr *et al.* 1997).

Pumpkin has some medicinal properties also because of its curative and medicinal properties has been known since ancient time, and thus, associated with man for at least 10 thousand years. The fruit is used as cure of stomach catarrh, hyperacidity, and stomach ulcers, especially peptic ulcers. Raw pulp of pumpkin has purgative effect, and it is recommended in case of obesity and deterioration in the function of gall bladder. Pumpkin juice as well as raw and boiled fruits has diuretic and cholagogue properties. Its juice and decoction with honey is also advisable for people suffering from insomnia or from uneasy and troubled sleep (Rana, 2014). Its combination with sugar and honey has soothing effect to ulcers and blisters and dried pulps are also used in cases of lungs bleeding and some other bleeding problems (Chaturvedi and Chaturvedi, 2001). It is used for the treatment of diabetes, rheumatism, eczema and burns, and against worms and other parasites (Vucetic *et al.*, 1989). The seeds with sugar and castor oil are given to the patients suffering from tapeworm (Chaturvedi and Chaturvedi, 2001). The seeds after removing seed coats are used in confectionary. Seed oil (50% of the seed) is a common salad oil in the southern parts of Austria, Slovenia and Hungary. It is dark green and has a high content of free fatty acids and vitamin E, especially gamma-tocopherol. However, due to its colour, the oil cannot be used for cooking.

Genetic resources are basic foundation block in any crop improvement programme. These include wide range of available genetic variability in the form of landraces, traditional cultivars, putative ancestor form, primitive cultivars, wild relatives' forms and related non-edible wild weedy species (Swaroop, 2014). Genetic variability is the basic need for a plant breeder to initiate any breeding programme. The role of genetic variability in pumpkin is one of the paramount importance in selecting the best genotype for making rapid

improvement in yield and related characters as well as to select the most potential parents for making the hybridization programme successful.

The success of any crop improvement program depends, to a large extent, on the amount of genetic variability present in the population. Intensive research efforts are needed in several areas particularly in selection of superior pumpkin genotypes. There is a wide genetic variability among the existing genotypes (Aliu *et al.*, 2011) and thus, the utilization of such variability in the breeding programs of this crop is possible. Heritability and genetic advance help in determining the influence of environment in expression of the characters and the extent to which improvement is possible after selection (Srikanth *et al.*, 2017).

Co-efficient of variance is useful in the assessment of genetic variability for the particular characters. Heritability denotes the proportion of phenotypic variation due to genotypes thus help the breeder to select the elite variety for a character. Heritability is the ratio of genotypic variance to the total variance in a non-segregating population. The estimation of an amount of heritability indicates the extent to which improvement is possible through selection and relative degree to which a character is transmitted from parent to offspring. Most of the quantitative characters, which are of economic value, are highly influenced by environment (Punetha, 2014).

Genetic advance denotes the improvement in the mean genotypic values of selected families over base population and thus helps the breeder to select the progenies in the earlier generation itself (Singh and Narayan, 1993). Yield being a complex a quantitative character, direct selection for yield may not result in successful improvement. Information on character association and direct and indirect effects of component traits on yield would greatly help in formulating the selection criteria and using them effectively in crop improvement programme.

Correlation coefficient analysis measures the mutual association between two variables. The result of correlation is of great value in the determination of the most effective procedures for selection of superior genotypes (Shivananda and Madalageri 2013). Path coefficient analysis is a statistical tool that helps the plant breeder to better understand what types of relationships exists between two variables. The relationship between two variables can be such that one variable directly causes an effect on the other variable (Camacho *et al.*, 2006). Genetically divergent parents are generally considered to be useful. In such crosses more variability could be expected in the resulting segregating progenies.

Genetic divergence can be estimated by  $D^2$  Statistic suggested by Mahalanobis and in turn is based on multivariate analysis of quantitative characters (Sultana

*et al.*, 2015).  $D^2$  analysis is very useful tool in studying the nature and cause of diversity prevalent into the available genotypes. Taking into account of the more reliability and adequacy, Hybrid breeding is prominent among the methods used in the improvement of vegetable crops, especially in cross-pollinated crop like pumpkin. Hybrids offer opportunity for improvement in productivity, earliness, uniformity and quality and for the rapid deployment of dominant genes for resistance to disease and pests (Punetha, 2014).

Varietal characterization based on morphological data is becoming difficult because these morphological traits are highly influenced by environment. Morphologies reflect not only genetic constitution of cultivars, but also interaction of the genotype with the environment. Due to the GE effects, it is appropriate to discriminate ambiguity among similar morphological expressions. Descriptions based on morphologies are fundamentally flawed in their ability to provide reliable information for calculation of genetic distance or validation of pedigrees. Therefore, *in vitro* identification/selection of cultivars/genotypes for various qualitative and quantitative traits is of great significance as it helps in obtaining desirable result and hence proved a boon to the researchers as well as farmers (Yadav, 2008).

The introduction of biochemical techniques has made possible and a more accurate evaluation of genetic variation, bringing greater precision to measures of genetic diversity. Among the biochemical techniques, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is an economical, simple and extensively used biochemical technique for describing the seed protein diversity of crop germplasm (Cook, 1995; Fufa *et al.*, 2005., Iqbal *et al.*, 2005). Seed proteins are used as genetic markers in the study of genetic variation because they are the primary products of structural genes; any change in the coding sequence of a gene generally reflects the corresponding change in the primary structure of protein (Srivalli *et al.*, 1999). Keeping these points in view, the present investigation was planned with the following objectives:

- (i) To estimate the magnitude of genetic variability, heritability and genetic advance for yield and its component traits
- (ii) To determine correlation coefficient between different pairs of characters
- (iii) To estimate direct and indirect effects of yield attributes on fruit yield per plant
- (iv) To study genetic divergence using Mahalanobis technique
- (v) To study genetic diversity and seed protein banding pattern amongst germplasm

**CHAPTER-2**  
**REVIEW**  
**OF**  
**LITERATURE**

# Chapter-2

## Review of Literature

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Pumpkin is a cross pollinated crop and therefore, the selection breeding is most important technique for its improvement. The relevant information on pumpkin was reviewed under the following heads. A brief review of literature embracing the relevant references related to the different aspects of the present investigation has been presented below:

### **2.1 Genetic variability**

### **2.2 Heritability and genetic advance**

### **2.3 Correlation studies**

### **2.4 Path coefficient analysis**

### **2.5 Genetic divergence**

### **2.6 Quality attributes**

### **2.7 Protein banding pattern**

### **2.1 Genetic Variability**

Genetic variability is the basic need for a plant breeder to initiate any breeding programme. The role of genetic variability in pumpkin is one of the paramount importances in selecting the best genotypes for making rapid improvement in yield and related characters as well as to select the most potential parents for making the hybridization programme successful. The success of any crop improvement program depends, to a large extent, on the amount of genetic variability present in the population. Intensive research efforts are needed in several areas particularly in selection of superior pumpkin genotypes. There is a wide genetic variability among the existing genotypes (Aliu *et al.*, 2011) and thus,

the utilization of such variability in the breeding programs of this crop is possible. In a crop selection program, knowledge of the interrelationships among yield and yield contributing characters are necessary. Genotype and environment interaction help to optimize yield and quality of crops. Thus, partitioning of phenotypic and genotypic variation of accessions requires evaluating performance in many sites (El-Hamed and Elwan, 2011). The fact that the genetics of the crop has not been fully understood makes its improvement of the crop difficult. Hence, there is need to exploit the genetic variability present in the base population to breed superior and high yielding genotypes for commercial cultivation in order to meet increasing demand for the crop. The level of success in breeding depends on the quantity of heritable constituents expressed in a phenotype (Kehinde and Ayo-Vaughan, 1999). Variation precedes and guides selection; hence the clear understanding of the within species variation is a fundamental track to improvement (Chedda and Fatokun, 1982). The magnitude of variability and its genetic components are the most important aspects of breeding material. Hence, basic understanding of the genetic variability is a prerequisite for the planning of breeding programme. A great deal of information has been generated on genetic variability of various components of pumpkin (Johannsen, 1909). There are number of local cultivars with wide range of variability in size, shape and color of fruits available in India that can be exploited for its improvement and by this we can easily fulfill the gap by developing high yielding hybrid variety. Besides, information on quality aspects of fruits is very scanty in India. So it is the demand to a breeder to develop high yielding with high quality varieties through selection, either from the existing genotypes or from the segregates of a cross. Information on variability, character association and path coefficient analysis in respect of yield, its contributing characters and quality aspects required to be properly assessed for its improvement (Akter *et al.*, 2013). Phenotypic variability being highly influenced by the environment, does not give a real picture of the potential genotypic variability. Hence, knowledge about heritability and genetic advance on yield and yield contributing characters are necessary for the crop improvement through selection. Further, yield is a complex quantitative trait governed by large number of genes and is also greatly influenced by environmental factors. Hence, selection of superior accessions based on yield as such is not effective (Sampath and Krishnamoorthy, 2017).

Kumar *et al.* (2017) investigated twenty one genotypes of pumpkin for morphological, yield and biochemical traits *viz.*, days to first male flower anthesis, days to first female flower anthesis, node number to first male flower, node number to first female flower, days to first fruit harvest, number of primary branches per plant, equatorial circumference of fruit, polar circumference of fruit, flesh thickness, internodal length (cm), vine length (m), average fruit weight (kg), number of fruits per plant, fruit yield per plant (kg),

dry matter content, total soluble solids, total sugars, reducing sugars, non-reducing sugars, ascorbic acid content and  $\beta$ -carotene. During the investigations high phenotypic and genotypic coefficient of variation were observed for vine length, number of fruits per plant, fruit yield per plant, reducing sugars and  $\beta$ -carotene.

Srikanth *et al.* (2017) studied the genetic variability among the twenty three genotypes of pumpkin. Analysis of variance showed that there was a high significant variation for all of the studied traits between genotypes. In the present investigation PCV was higher than the GCV for all the characters indicating the substantial modifying effect of environment in the expression of all traits studied. The highest GCV as well as PCV was observed for vine length at 90 days after sowing, fruit cavity, number of seeds per fruit, rind thickness and number of fruits per vine.

The genotypes were evaluated for variability estimates, heritability, genetic advance as percent of mean, magnitude of association between characters, their interdependence, direct and indirect effects of yield attributes on yield per plant. High estimates of genotypic and phenotypic coefficient of variation were observed for yield per plant, vine length, number of fruits per plant, fruit weight and 100 seed weight (Sampath and Krishnamoorthy, 2017).

Tamilselvi *et al.* (2017) suggested that pumpkin has got industrial importance with the development of pulp powder as a nutraceutical supplement to Vitamin A requirement. Pumpkin is cheaper source of Vitamin A when compared to carrot which necessitates specific climatic requirement for its production and high productivity per unit area. About 15 pumpkin genotypes were collected from various places and evaluated for different quantitative characters *viz.*, vine length, days to first female flower appearance, node number for first female flower appearance, sex ratio, days to first harvest, fruit number per vine, fruit weight (kg), flesh thickness (cm) and fruit yield per vine (kg/vine).

Sultana *et al.* (2015) evaluated twenty one genotypes of pumpkin (*Cucurbita moschata* L.) to measure the variability among the genotypes for several characters, estimate genetic parameters, association among the characters and their contribution to yield. There was a great deal of significant variation for all the characters among the genotypes. High variability was observed in number of female flowers/plant, number of male flowers/plant, single fruit weight and fruit yield/plant. All the characters except days to first male flowering and days to first female flowering showed.

Akter *et al.* (2013) conducted traits of 30 pumpkin genotypes. Significant variations were found among the genotypes for most of the characters studied. High genotypic coefficient of variation (GCV) and high heritability coupled with high genetic

advance in percent of mean were observed for beta-carotene followed by non-reducing sugar, number of seeds per fruit, yield per plant, dry weight, flesh thickness, number of male flowers per plant and number of female flowers per plant which indicated that these characters were under additive gene control and selection for genetic improvement for these traits might be effective.

Kulkarni and Joshi (2013) evaluated the physical properties of pumpkin fruit pulp, peel and seed content. A large variation was found in the mass of pumpkin due to the irregular size and shape. Pumpkin fruit having more fruit mass and pulp content had very high edible index and low waste index. Pumpkin pulp had very high moisture content of 95.06 % (wb). Carbohydrate and crude fiber were found to be moderately high in pumpkin pulp. The micronutrient in the pumpkin pulp such as carotene (3 mg /100 g) was found to be very high while vitamin C and minerals such as Ca, P, Fe and K were also present in considerable amounts.

Onyishi *et al.* (2013) conducted an experiment to determine the extent of variability that exists in fruit, seed and leaf yield of the crop and to constitute a germplasm collection. Results of the study showed that there exists a sufficient genetic variability among the South Eastern Nigerian accessions that could be utilized as a germplasm pool for further improvement of the crop in Nigeria.

Sharma and Rao (2013) studied the nutritional potential of an important underutilized fruit of pumpkin (*Cucurbita maxima* D.) at its sequential stages of development. Changes in various physico-biochemical properties such as pH, titratable acidity (TA), carbohydrates, free amino acids, total proteins, total phenols, carotenoids, ascorbic acid and the specific activities of softening enzymes ( $\beta$ -galactosidase, cellulase, pectin methylesterase and polygalacturonase), were studied that pumpkin fruits after ripening accumulated considerable amount of carotenoids, vitamin C and proteins along with carbohydrates.

Fayeun *et al.* (2012) while studying the genetic variability and correlation studies in fluted pumpkin observed considerable variation among the genotypes for all characters studied. Further they recorded low level of environmental influence was observed in the expression of all the characters studied except number of branches per plant and internode length.

Gohari *et al.* (2011) studied the chemical composition and physico-chemical properties of pumpkin seeds and fatty acids content of oil. They found that the seeds contained 41.59% oil and 25.4% protein. Moisture, crude fiber, total ash, and carbohydrate contents were 5.2%, 5.34%, 2.49%, and 25.19%, respectively.

Hossain *et al.* (2010) reported wide variability for the plant characteristics of days to seed germination, vine length harvest, petiole length and yield contributing characters namely, days to first male and female flowering, number of fruits per plant, average fruit weight, fruit length and fruit diameter. The highest GVC was recorded in yield per plant (42.75%) where number of fruits per plant (33.41%), fruit length (27.57%), number of lateral shoots (24.19%), average fruit weight (22.14%), petiole length (16.10%), node order at which male and female flower opened (13.28% and 12.62%) were recorded. Among the long type cucumber accessions, CSL51 gave the highest yield per plant (2.69kg).

Kumar *et al.* (2011) studied the genetic variability for different characters in 21 pumpkin genotypes. The study indicated existence of considerable amount of genetic variability for all the traits. Based on mean performance of yield and yield components PPU-60 was identified as most promising genotype. The maximum phenotypic and genotypic coefficients of variation were observed for fruit yield per plant.

## **2.2 Heritability and Genetic advance**

Heritability and genetic advance help in determining the influence of environment in expression of the characters and the extent to which improvement is possible after selection (Srikanth *et al.*, 2017). Heritability is the ratio of genotypic variance to the total variance in a non-segregating population. Since, the estimate of heritability gives indication of the amount of progress expected from selection, as they are most meaningful when accompanied by estimate of genetic advance (Hanson *et al.*, 1956). Genetic advance is the measure of improvement that can be achieved by practicing selection in a population. The literature on PCV, GCV, heritability and genetic advance as per cent mean are presented. Genetic advance is a product of heritability and infers the potentiality of selection intensity. Genetic advance, when considered along with heritability gives reasonable assessment of the resultant effects of selection in breeding populations (Johnson *et al.*, 1955). The information on heritability alone may not help in identifying characters for enforcing selection; therefore, heritability estimates in conjunction with predicted genetic advance is more reliable. Heritability provides information on the magnitude of the inheritance of characters from parent to off spring, while genetic advance is helpful in finding the actual gain expected under selection (Khan *et al.*, 2017).

Kumar *et al.* (2017) observed high heritability coupled with high genetic advance for number of fruits per plant, fruit yield per plant, reducing sugars, ascorbic acid and  $\beta$ -carotene and suggested for improvement of these traits through selection.

Mutter *et al.* (2017) observed high heritability and genetic advance among genotypes for all the traits studied except for the number of ridges per fruit in pumpkin. High

estimates of heritability coupled with high values of genetic advance over mean was observed for average fruit weight,  $\beta$ -carotene content of fruit, seed cavity length, sex ratio, number of seeds per fruit, fruit yield per vine, vine length at 45 and 75 DAS, number of primary branches per vine at 45 and 75 days after sowing (DAS), number of fruits per vine and fruit length.

Heritability estimates in broad sense were higher for almost all the characters. The characters namely, fruit length, single fruit weight, yield per plant and brix% had high genotypic coefficient of variation coupled with heritability gave high genetic advance expressed as percentage of mean ranged from 76.84 to 96.06 which indicated that these characters were less influenced by environment confirming additive gene action, and therefore, selection of these characters would be more effective for yield improvement of pumpkins. Total six traits likely fruit length, fruit diameter; flesh thickness, single fruit weight and number of fruits per plant were positively and significantly associated with yield per plant Mohsin *et al.* (2017).

Nagar *et al.* (2017) estimated high heritability coupled with high genetic gain for all the seed traits under study, which indicated that these traits are under additive gene effects and are more reliable for effective selection. The maximum PCV and GCV were observed for seed yield/plant (g) followed by 100 seed weight (g), seed area (mm<sup>2</sup>) reflecting presence of high genetic variability among all the genotypes for studied characters. Further, the experimental results revealed that seed area followed by seed yield per plant and 100 seed weight had maximum positive direct effect on fruit yield per plant. The maximum positive indirect effect of seed breadth, seed length, 100 seed weight and seed yield/plant via seed area was observed on fruit yield per plant.

Srikanth *et al.* (2017) studied the heritability and genetic advance among the twenty three genotypes of pumpkin. The highest estimated of heritability (in broad sense) was recorded for fruit cavity (84.80 %) followed by inter nodal length (76.90 %), and fruit yield per hectare (73.60 %). Highest genetic advance (as per cent of mean) was observed for the character of fruit cavity.

Sampath and Krishnamoorthy (2017) observed high heritability with high genetic advance for number of primary branches, vine length, petiole length, inter node length, first male flower node, sex ratio, number of fruits per plant, fruit length, fruit diameter, flesh thickness, fruit weight, 100 seed weight and yield per plant.

Shrikant *et al.* (2017) estimate high heritability coupled with high values of genetic advance for average fruit weight,  $\beta$ -carotene content of fruit, seed cavity length, sex ratio, number of seeds per fruit, fruit yield per vine, vine length at 45 and 75 DAS, number of

primary branches per vine at 45 and 75 days after sowing (DAS), number of fruits per vine and fruit length.

Kiramana *et al.* (2016) observed high heritability and high genetic advance for seed number and 100-seed weight indicating that effective selection can be based on these characters. The high GCV, PCV, heritability and genetic advance values can be applied to select and isolate high yielding accessions for improvement into commercial cultivars.

Sultana *et al.* (2015) evaluated high heritability along with high genetic advance in percent of mean. The positive and strong association of number of female flowers/plant ( $r_g=0.918$ ,  $r_p=0.839$ ), number of male flowers/plant ( $r_g=0.687$ ,  $r_p=0.638$ ), fruit length ( $r_g=0.691$ ,  $r_p=0.520$ ), fruit breadth ( $r_g=0.518$ ,  $r_p=0.420$ ) and single fruit weight ( $r_g=0.492$ ,  $r_p=0.431$ ) with fruit yield/plant revealed the importance of these characters in determining fruit yield/plant. On the other hand, days to first male flowering ( $r_g = -0.623$ ,  $r_p = -0.550$ ) and days to first female flowering ( $r_g = -0.689$ ,  $r_p = -0.543$ ) correlated significantly and negatively with fruit yield/plant.

Fayeun *et al.* (2012) estimated high heritability accompanied by high expected genetic gain in vine length, fresh leaf weight, vine weight, number of leaves per plant and marketable leaf yield. Vine length had the highest broad sense heritability and number of branches had the least value (75.00 and 29.28%, respectively).

Rana *et al.* (2011) observed high to moderate heritability as well as genetic advances for days to seedling emergence, number of seeds per fruit, days to 50% male flower opening, days to first fruit setting, days to first female flower opening, days to 50% fruit setting and total number of male flowers.

Kumar *et al.* (2011) studied genetic heritability and genetic advance for different characters in 21 pumpkin genotypes. The studies indicated existence of considerable amount of genetic variability for all the traits. Based on mean performance of yield and yield components, PPU-60 was identified as the most promising genotype. The maximum phenotypic and genotypic coefficients of variation were observed for fruit yield per plant. High estimates of heritability (broad sense) along with high genetic advance as percent of mean were recorded for most of the traits except days to first staminate flower anthesis.

## **2.3 Correlation studies**

A very little attention has been given for the genetic improvement of pumpkin by collecting diverse germplasm, their morphological characterization and assessing the variability parameters like coefficient of variation, coefficient of correlation and path analysis. The appropriate breeding methodologies may be adopted for genetic improvement of this

crop for simultaneously improvement of different characters. Information regarding mutual relationship among the characters and direction of correlation analysis provides an effective means of finding out direct and indirect causes of association among causal variables.

(Yadav *et al.* 2017) reported that correlation measures the mutual association between two variables. The result of correlation is of great value in the determination of the most effective procedures for selection of superior genotypes. When there is a positive association of major yield characters, component breeding would be very effective but when these characters are negatively associated, it would be difficult to exercise simultaneous selection for them in developing a cultivar. In this regard, the highest significant positive association of fruit yield per vine with average fruit weight followed by vine length, leaf size, fruit flesh thickness and number of fruits per vine was detected (Shivananda *et al.* 2013). Correlation studies along with the path coefficient analysis provide a better understanding of the association of different characters with yield. Path coefficient analysis separates the direct effects from the indirect effects through other related characters by partitioning the correlation coefficients in pumpkin genotypes especially in preliminary generation of breeding and selection programs (Yadegari *et al.* 2012). Especially in correlation and path coefficient analysis provide information about the association between two traits and the partitioning of the relationship into direct and indirect effects showing the relative importance of each of the causal factors. Characters having a high genotypic coefficient of variation indicate high potential for effective selection (Khan *et al.* 2016). A correlation coefficient measures the total linear association between two traits, say Y and X<sub>1</sub>; and includes both the direct effect of X<sub>j</sub> on Y plus the indirect effects of other traits (X<sub>2</sub>,...,X<sub>n</sub>) related to X<sub>j</sub>. For example, the correlation between Y and X<sub>x</sub> can be partitioned into the direct effect of trait X: (quantified by the path coefficient, P: which measures the direct influence independent from other variables) and the indirect effects of X: via its correlation with other traits, X<sub>2</sub> to X<sup>^</sup>. Path analysis can use phenotypic, genetic or environmental correlation coefficients. Genetic correlations are particularly important because they provide the plant breeder with information about heritable associations between traits. This information is useful in the development of appropriate strategies for breeding for multiple traits in a plant improvement program or for employing indirect selection for a particular trait such as yield. Selection for one trait will result in a response in genetically correlated traits (Camacho *et al.*, 2006). Literature pertaining to Correlation studies and its components in pumpkin has been cited.

Highest genotypic coefficient of variation was recorded for fruit length (cm), single fruit weight (kg), Brix (%) and yield per plant (kg) in pumpkin Mohsin *et al.* (2017).

Correlation co-efficient between yields per plant with number of primary branches, number of fruits per plant and single fruit weight were positive and highly

significant in pumpkin. On the other hand, days to first female flower, number of male flowers per plant,  $\beta$ - carotene and total sugar showed positive and insignificant correlation with yield per plant. But correlation between brix content and yield per plant were significantly negative. Akter *et al.* (2013)

Fayeun *et al.* (2012) reported significant and positive association among all the ten characters. Direct selection for improvement of these traits may be carried out on these characters, as their phenotypic expression would be good indicators of their genotypic potentials.

Tamilselvi *et al.* (2012) observed in correlation analysis that fruit yield per vine was significantly and positively correlated with fruit number per vine, flesh thickness and total carotenoids content. However negative association was observed with days to first female flower appearance, node number for first female flower appearance, sex ratio, days to first harvest, fruit weight, fruit equatorial diameter, fruit polar diameter and crude fiber content. Hence selecting pumpkin genotypes with narrow sex ratio, more number of fruits per vine, fruits with high flesh thickness and total carotenoids content will help to improve yield per vine and quality of pumpkin fruits.

Rana and Pandit (2011) observed in correlation studies that fruit yield had significant positive correlation with total number of fruits, total number of primary branches and total number of nodes. The highest direct positive effects were recorded for days to first female flower opening. For selecting high yielding genotypes, along with the above traits special emphasis should be given on days to seedling emergence, days to 50% female flower opening, days to first fruit setting and days to first female flower opening.

The correlation analyses revealed that the weight of harvested fruits had significant and positive relationships with days to 50% flowering, number of male flowers, number of female flowers, number of fruits per plant, number of healthy fruits, fruit diameter, fruit length, number of seeds per fruit, 100-seed weight and seed weight. The days to 50% emergence showed negative but significant correlations with days to 50% flowering, number of female flowers, fruit length, seed length and 100-seed weight, suggesting that early crop emergence would imply prolonged vegetative growth phase and the production of higher number of female flowers that would translate to higher seed yield Aruah *et al.* (2010).

Hossain *et al.* (2010) reported that correlation co-efficient analysis yield per plant had highly positive and significant association with fruit length and diameter, average fruit weight and number of fruits per plant. Path analysis showed that the fruit length and diameter, average fruit weight and number of fruits per plant directly contributed towards the

yield per plant in the long type cucumber. Through clustering analysis, 58 long types grouped into eight and nine clusters, respectively.

Shivananda *et al.* (2013) carried out correlation and path analysis on 19 growth parameters, *viz.*, earliness, yield and quality traits in 57 genotypes of pumpkin (*Cucurbita* spp.). There was highest significant positive association of fruit yield per vine with average fruit weight followed by vine length, number of leaves per vine, number of seeds per fruit, length of fruit, fruit cavity size, leaf size, 100 seed weight, fruit flesh thickness, number of primary branches per vine, total soluble solids, number of fruits per vine and circumference of fruit.

Naik *et al.* (2015) suggested that Correlation co-efficient between yields per plant had significant positive correlation with days to first female flower, average fruit weight, fruit length, seed cavity length, placenta weight, number of fruits per vine. But correlation between fruit yield and days to first fruit harvest were significantly negative.

Correlation analysis of growth parameters showed that leaf length and days to first female flowering had positive significant association with fruit yield. The yield traits revealed that, the traits days to fruit maturity, fruit length, fruit diameter, fruit weight and 100 seed weight showed high positive significant association with fruit yield per plant Sampath and Krishnamoorthy (2017).

Sultana *et al.* (2015) reported significant positive correlation with fruit yield/plant was observed because of the combination of the direct and indirect effects of fruit length and single fruit weight to fruit yield/plant. Overall, the results indicated that days to first female flowering, number of female flowers, fruit length, fruit breadth and single fruit weight can be used as useful selection criteria to increase fruit yield/plant in pumpkin..

## **2.4 Path coefficient analysis**

Path coefficient analysis is a statistical tool that helps the plant breeder to better understand what types of relationships exists between two variables. The relationship between two variables can be such that one variable directly causes an effect on the other variable. Alternatively, a variable can cause an indirect effect by way of (via the "path" of) a third, or more variables. In general, the path coefficient analysis consists of partitioning the correlation coefficient into the direct and indirect effects that trait  $X_j$  has on trait  $Y$  ( $X$  is assumed to be the independent, and  $Y$  the dependent, variable) (Camacho *et al.*, 2006). Path analysis would help in partitioning the correlation coefficient into direct and indirect effects of various traits on the genetic variability, character association and the direct and indirect contributions of some yield characters towards fruit yield (Sultana *et al.*, 2015). The

studies of simple correlation do not provide an exact picture of relative importance of direct and indirect influence of each of the component character towards the desired character. So, this can be overcome by following path coefficient analysis technique by further partitioning the correlation coefficient into direct and indirect effects. Path coefficient analysis measures direct influence of one variable upon another and permits separation of correlation coefficient in component direct and indirect effect, which can be used for crop improvement through selection of component traits (Nagar *et al.*, 2017). In path co-efficient analysis, the correlation co-efficient of the component character are partitioned into direct and indirect effects. Hence, it has greater significance and could be effectively utilized in formulating an effective selection scheme. Hence, knowledge of association between the traits can greatly help in avoiding inversely related compensation effects during selection (Shivananda *et al.*, 2013). Literature pertaining to Path coefficient analysis and its components in pumpkin has been cited as under.

Path analysis revealed that vine length, leaf length, inter node length, first male flower node, days to first male flowering, days to first female flowering, sex ratio, days to fruit maturity, number of fruits per plant, fruit length, fruit weight registered positive direct effect on yield (Sampath *et al.*, 2017).

Mohsin *et al.* (2017) reported that path coefficient analysis revealed maximum contribution of single fruit weight (0.869) to yield and this was followed by the contribution of number of fruit per plant (0.527) at genotypic level.

Sultana *et al.* (2015) observed that path co-efficient analysis expressed the highest positive direct effect in number of female flowers (0.887) to fruit yield and high direct effect was found in case of days to first female flowering (0.798). Fruit breadth was observed to have the highest positive indirect effect (0.899). In case of fruit length (0.381) and single fruit weight (0.398), the significant positive correlation with fruit yield/plant was observed because of the combination of the direct and indirect effects of fruit length and single fruit weight to fruit yield/plant. Overall, the results indicated that days to first female flowering, number of female flowers, fruit length, fruit breadth and single fruit weight can be used as useful selection criteria to increase fruit yield/plant in pumpkin.

Akter *et al.* (2013) reported that path coefficient analysis revealed the maximum direct contribution towards yield which was obtained through number of fruits per plant followed by days to first female flower and single fruit weight. Indicated that these traits should be considered as primary components of yield. Negative direct effect on yield was exerted by total sugar followed by number of female flowers per plant.

Path coefficient analysis in tropical pumpkin (*Cucurbita moschata* Duchesne) for yield per plant as a function of number of fruit per plant and weight per fruit, using phenotypic and genetic correlations from two diallels: one using five varieties (variety diallel) and a second using five S1 lines derived from the varieties (line diallel). A randomized complete block design with five replications was used to evaluate 15 genotypes from each diallel, not including reciprocals. Direct effects were 300 to 500% greater than indirect effects in the line diallel. In contrast, direct effects were only 17 to 68% greater than indirect effects in the variety diallel. Effects (direct and indirect) were greater (17 to 500%) when genetic correlations rather than phenotype correlations (42 to 440%) were used in the path analysis. Fruit weight showed a greater effect on yield than did number of fruit, with values between 3.2 to 4.9 times the residual effects. Fruit weight and number of fruit can be used as selection criteria to increase yield in tropical pumpkin Miguel *et al.* (2006).

Naik *et al.* (2015) path coefficient analysis studied and observed that the maximum direct contribution towards yield was through fruit weight, fruit length, days to first female flower, no of fruits per vine showed high direct effect on fruit yield. Negative direct effect on yield was days to first male flower appearance, fruit diameter.

Gwanama *et al.* (1998) investigated traits contributing to *Cucurbita moschata* fruit yield to identify selection aids. The contribution of traits to fruit yield were partitioned by path analysis. Mid-season traits (internode with first female flowers, length of internode with first female flowers, length of primary axis, number of primary branches and number of leaves per plant) exhibited insignificant phenotypic (P) and genotypic (G) direct effects ( $P, G < 0.17$ ) on fruit yield. The length of the primary axis, number of primary branches and number of leaves per plant had genotypic indirect effects of intermediate magnitude on the fruit yield through the number of fruits per plant (-0.27, 0.33, 0.42; respectively). Late season traits (weight of first mature fruit and number of fruits per plant) had significant genotypic direct effects on fruit yield ( $G=0.67$  for both characters). Therefore, in selecting for pumpkin yield, special attention should be given to number of fruits per plant and weight of first mature fruit of each plant.

## 2.5 Genetic divergence

Genetically divergent parents are generally considered to be useful. In such crosses more variability could be expected in the resulting segregating progenies. Genetic divergence can be estimated by  $D^2$  Statistic suggested by Mahalanobis and in turn is based on multivariate analysis of quantitative characters (Sultana *et al.*, 2015). To arrange the diversity in the germplasm the  $D^2$  statistics is the useful tool which measures the degree of diversification of genotypes and also determines the relative contribution of each component

character to the total divergence (Shivananda *et al.*, 2013). Multivariate analysis is a useful tool in quantifying the degree of genotypic divergence in a biological population like an inbred population of pumpkin. It helps to assess the relative contribution of different components to the total divergence both at inter and intra-cluster levels (Das and Gupta, 1984). Efficient use of such genetically diverse parents has a bright scope for the development of high yielding open pollinated and hybrid pumpkin variety. From the plant breeding point of view the degree or extent of genetic diversity between two parents is an index of determining the hybridity over parents or nature of segregates in the follow-up generations (Ahmed *et al.*, 2016). The genetic divergence of parents is the basis of cross breeding for most crops. Therefore efforts had made to determine the genetic divergence of inbred lines in *C. moschata* using Multivariate statistical methods to reveal the level of genetic diversity of the species and to provide a reference for hybrid breeding because of heterosis in pumpkin and squash (Xiaohua *et al.*, 2011). Literature pertaining to in pumpkin has been cited as under.

Chaudhari *et al.* (2017) carried out genetic divergence study on 40 diverse genotypes of pumpkin. These forty genotypes were grouped into fourteen clusters. Total sugar content contributed maximum to total genetic divergence followed by fruit weight, fruit yield per plant,  $\beta$ -carotene, flesh thickness, first male flowering node, test weight of 100 seeds, equatorial circumferences of fruit and number of seeds per fruit. Intra cluster distance was maximum along cluster VI followed by V, I, III and II. The maximum inter-cluster divergence was observed between cluster IX and XIV followed by cluster X and XIII, cluster X and cluster XIV and cluster X and XII. Cluster IX recorded the highest mean values for fruit yield per plant, least mean values for days to harvest and flesh thickness. Cluster X recorded the highest mean values for equatorial circumferences of fruit and flesh thickness. Cluster XIII recorded least mean values for days to opening first female flower and highest mean values for polar circumferences of fruit and cluster IV recorded highest mean values for test weight of 100 seeds and total sugar content.

Ahmed *et al.* (2016) estimated principal component analysis and Mohalanobis's  $D^2$  analysis. Inbred lines were grouped into five clusters. Cluster I, II, III, IV and V were composed of five, three, four, six and one inbred lines respectively. Maximum inter cluster distance was observed between the cluster III and V (72.69) followed by the cluster I and V (63.33). Minimum inter cluster distance was observed between cluster II and IV (10.64). The mean value of cluster III indicated the importance of days to first male and female flower open, node order to open 1st female flower and TSS. Cluster IV showed the importance of days to first female flower open, fruit diameter, cavity diameter, flesh thickness and average fruit weight. Similarly, cluster V indicated the importance of yield per plant, fruits per plant and fruit length. Days to first male and female flower open, fruit length and cavity

length had the highest contribution towards divergence among the inbred lines. Considering the magnitude of cluster distance, cluster means for different characters and contribution of characters towards divergence, the inbred lines from clusters I, III and IV could be selected as parent for hybridization program.

Sultana *et al.* (2015) studied the genetic diversity using Mahalanobis's  $D^2$  technique was studied for yield and its components on twenty one genotypes of pumpkin (*Cucurbita moschata* L.). Quantification of variability for each character was done using the Shannon Weaver Diversity Index. High degree of variation was exhibited within the collection, as reflected by mean diversity index value of 0.80. Data were subjected to principal component analysis (PCA), principal coordinate analysis (PCO), canonical variate analysis (CVA) and non-hierarchical clustering to identify suitable parents having distant relationship for hybridization program. The genotypes were grouped into five different clusters. Cluster IV contained the maximum number of seven genotypes whereas cluster I contained least number having only one genotype. The lowest inter-genotypic distance (0.75) was found between BD-2174 and BD-9489 where the highest (47.46) was between BARI Mistikumra-1 and BD-2150. The maximum inter cluster distance was observed between cluster II and III (17.922) and the minimum inter cluster distance was observed between cluster II and IV (6.825). The maximum intra cluster distance was noticed for the cluster V (0.261) and the minimum intra cluster distance was found in cluster I (0.00). Cluster I contained the highest mean values for pedicel length of male flower, number of male flowers/plant, fruit length, fruit breadth, single fruit weight and fruits/plant. Cluster II contained the highest mean values for days to first male and female flowering. Cluster III contained the highest mean values for leaf breadth, pedicel length of female flower and number of female flowers/plant. Leaf breadth, pedicel length of male flower, number of male flowers/plant and fruits/plant were the important components of genetic divergence in the studied materials. Based on inter cluster distance, inter genotypic distance and consideration of desirable characters for high yield potential, the genotypes G19 (BARI mistikumra-1) and G20 (BARI mistikumra-2) from cluster II; G21 (BD-2150) from cluster I and G1 (BD-2151) and G13 (BD-266) from cluster III can be selected as better parents for future hybridization programme.

Shivananda *et al.* (2013) studied on genetic divergence on growth, earliness, yield and quality traits in fifty seven genotypes of pumpkin (*Cucurbita* spp.) These fifty seven genotypes were grouped into fifteen clusters. Yield per vine contributed maximum to total genetic diversity followed by leaf size, cavity size, carotene content, number of seeds per fruit and hundred seed weight. Intracluster distance was maximum among cluster XIV, I, XIII and XII. Maximum Inter-cluster divergence was observed between clusters X and XIV followed by cluster XI and XIV, cluster XIV and XV and cluster IV and XIV. Cluster XV

recorded highest mean values for yield per vine, number of seeds per fruit, fruit length, average fruit weight and least mean value for days to first female flower, cluster XIV recorded highest mean value for leaf size, cluster XIII recorded highest mean values for fruit number, carotene content, TSS and least mean value for cavity size, cluster VIII recorded maximum mean values for hundred seed weight, flesh thickness and fruit circumference, cluster IV noticed least mean values for days to first harvest and nodes up to first female flower.

Lakshman and Prasad (2015) studied 25 cultivars of Pumpkin using Mahalanobis  $D^2$  statistic. Based on  $D^2$  analysis, the genotypes were grouped into 5 different clusters, where the cluster V possessed higher number (8) of genotypes followed by the cluster I (5) cluster IV (5), III (3), II (2) and VI (2). Clustering pattern revealed that geographical diversity was not associated with genetic diversity. The maximum inter-cluster distance was observed between the clusters III, VI and cluster II, III that of minimum in between the Clusters V and Cluster VI. The wider genetic diversity was observed in cluster II, III and VI which indicate the potentiality of this diverse genotype collection for providing basic material for future breeding programmes.

Srikanth *et al.* (2017) observed highest genetic advance (as per cent of mean) for the character of fruit cavity. However, it ranged from (72.32 to 3.44%) for all the characters. Therefore, selection of superior genotypes in view point of desirable morphologic traits, with high genetic distance could be selected for hybridization programme and recognition of best genotypes for different traits to produce new elite hybrids in pumpkin.

Mohsin *et al.* (2016) estimated the genetic divergence among the 21 genotypes of pumpkin using  $D^2$  analyses. The genotypes under study grouped in to six clusters. The inter-cluster distance was larger than the intra-cluster distance suggesting wider genetic diversity among the genotypes of different groups. The intra-cluster value was maximum in cluster VI and minimum in cluster III. The maximum inter cluster distance was observed between cluster I and cluster III (25.75) indicating wide range of genetic diversity between these two clusters. Thus the genetically diverged genotypes could be used as parent in hybridization program for getting desirable segregate. The lowest inter cluster divergence was observed between cluster III and IV (7.14). Cluster I showed the lowest mean values for days to first male and female flowering and highest mean values for flesh thickness, number of seeds per fruit and brix (%), while cluster VI revealed the highest mean vales for fruit diameter, single fruit weight and yield per plant. Germplasms much in use of the above mentioned characters both in cluster I and VI would offer a good scope of improvement of the crop through rational selection for hybrid breeding program.

Nagar *et al.* (2017) studied genetic divergence among seventy six diverse genotypes of pumpkin and observed considerable diversity for seed traits. The cluster I and cluster IV were found more divergent (9.864) and have chances of getting transgressive segregates in F<sub>2</sub> from crossing of genotypes from cluster I and cluster IV. Therefore, hybridization between these groups can be very effective for improvement of seed yield in pumpkin.

Lakshman *et al.* (2015) observed significant differences among all the genotypes for all the characters under study in pumpkin. Majority of the characters like fruit diameter, seed cavity length, flesh thickness, placenta weight exhibited moderate PCV, GCV values and fruit weight exhibited higher GCV and PCV values. All the characters exhibited high broad sense heritability. High heritability coupled with high GCA for the characters fruit weight, fruit diameter, seed cavity length suggesting that they can be improved through direct selection due to predominant additive variation. Based on D<sup>2</sup> analysis, the genotypes were grouped into 5 different clusters, where the cluster V possessed higher number (8) of genotypes followed by the cluster I (5) cluster IV (5), III (3), II(2) and VI (2). The maximum inter-cluster distance was observed between the clusters III, VI and cluster II, III that of minimum in between the Clusters V and Cluster VI.

## 2.6 Quality attributes

A large number of pumpkin varieties, each of which containing different amounts of carotenoids, are cultivated in the world wide. *C. moschata* cultivars are known to contain high amount of  $\alpha$ - and  $\beta$ -carotene. Carotenoids have antioxidant activity, but few are converted by the body into retinol, the active form of vitamin A. Among the 600 carotenoids with pro-vitamin A activity, the most common are  $\alpha$ - and  $\beta$ -carotene. These carotenoids are susceptible to degradation (e.g., isomerization and oxidation) during cooking (Carvalho *et al.*, 2014). The pulp of round and cylindrical pumpkin fruits containing white seeds were analyzed for some physicochemical properties such as protein, ash, titratable acidity (TA), pH and minerals (Ca, Na, Zn, K, Mg and Fe). The pulps of the fruit were peeled, cut, grated, fermented and the juices extracted were blended with MD2-pineapple juice in different formulation ratios. Sensory evaluation of the juice blend was carried out using the nine-point hedonic scale. Preference ranking test was also carried out on the formulated juices (Adubofuor *et al.*, 2016).

Adubofuor *et al.* (2016) reported moisture content of (95.03 and 95.66%), ash (0.66 and 0.83%), crude protein (2.58 and 2.42), pH (4.39 and 4.13), titratable acidity (0.38 and 0.34% as citric acid), for round and cylindrical pulp, respectively. Significant differences ( $p < 0.05$ ) existed in TA, ash, pH and moisture contents of the pulps from the round and

cylindrical fruits. The predominant minerals in the pulps were potassium and calcium which were 266.30 and 13.18 mg/100g for the round and 363.05 and 9.63mg/ 100g for the cylindrical, respectively.

Sharma and Rao (2013) studied the various physico-biochemical properties viz pH, titratable acidity (TA), carbohydrates, free amino acids, total proteins, total phenols, carotenoids, ascorbic acid and the specific activities of softening enzymes ( $\beta$ -galactosidase, cellulase, pectin methylesterase and polygalacturonase), hydrolytic enzymes (amylase and invertase) and antioxidant enzymes (peroxidase, polyphenoloxidase, superoxide dismutase, catalase and ascorbic acid oxidase) were investigated. The results revealed that pumpkin fruits on ripening accumulated considerable amount of carotenoids, vitamin C and proteins along with carbohydrates.

Karanja *et al.* (2013) observed significant differences ( $p < 0.05$ ) for crude fiber (11.69-24.85%), crude fat (31.9-41.37%), crude protein (14.05-33.29%) and carbohydrates (8.66-27.35%). Fatty acid profile showed a high content of unsaturated fatty acids and the dominant fatty acids were palmitic (1.16-20.81%), stearic (0.16-5.56%), oleic (15.56-30.79%), and linoleic acids (26.18-81.21%). The highest elemental minerals were potassium and sodium (124-335 and 70-148 mg/100 g) respectively.

Carvalho *et al.* (2014) reported that raw pumpkin samples contained 236.10, 172.20, 39.95, 3.64 and 0.8610  $\mu\text{g}\cdot\text{g}^{-1}$  of total carotenoids,  $\beta$ -carotene,  $\alpha$ -carotene, 13-*cis*- $\beta$ -carotene, and 9-*Z*- $\beta$ -carotene, respectively. The samples cooked in boiling water contained 258.50, 184.80, 43.97, 6.80, and 0.77  $\mu\text{g}\cdot\text{g}^{-1}$  of total carotenoids,  $\beta$ -carotene,  $\alpha$ -carotene, 13-*Z*- $\beta$ -carotene, and 9-*Z*- $\beta$ -carotene, respectively. The steamed samples contained 280.77, 202.00, 47.09, 8.23, and 1.247  $\mu\text{g}\cdot\text{g}^{-1}$  of total carotenoids,  $\beta$ -carotene,  $\alpha$ -carotene, 13-*Z*- $\beta$ -carotene, and 9-*Z*- $\beta$ -carotene, respectively. The samples cooked with added sugar contained 259.90, 168.80, 45.68, 8.31, and 2.03  $\mu\text{g}\cdot\text{g}^{-1}$  of total carotenoid,  $\beta$ -carotene,  $\alpha$ -carotene, 13-*Z*- $\beta$ -carotene, and 9-*Z*- $\beta$ -carotene, respectively.

Nwofia *et al.* (2012) studied nineteen accessions of *Cucurbita* for their physical attributes, proximate values, minerals, vitamins and ant nutritional factors. The result indicated that there was significant differences in the accessions for moisture content, crude fiber, ash, crude protein, fat and carbohydrate in the fruits ( $p < 0.01$ ) while crude fiber and carbohydrate was not significant in the seeds ( $p > 0.05$ ). The moisture content ranged from 78.46 to 91.97% in the fruits while in the seeds, it ranged from 44.73 to 53.04%. The ash, crude fat and crude protein content was generally higher in the seeds than the fruit pulp indicating that the seeds are high in essential nutrients.

## 2.7 Protein banding pattern

Varietal characterization based on morphological data is becoming difficult because these morphological traits are highly influenced by environment. Morphologies reflect not only genetic constitution of cultivars, but also interaction of the genotype with the environment. Due to the GE effects, it is inappropriate to discriminate ambiguity among similar morphological expressions. Descriptions based on morphologies are fundamentally flawed in their ability to provide reliable information for calculation of genetic distance or validation of pedigrees. Therefore, *in vitro* identification/selection of cultivars/genotypes for various qualitative and quantitative traits is of great significance as it helps in obtaining desirable result and hence proved a boon to the researchers as well as farmers (Yadav, 2008). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a technique for separating proteins based on their ability to move within an electrical current, which is a function of the length of their polypeptide chains or of their molecular weight. This is achieved by adding SDS detergent to remove secondary and tertiary protein structures and to maintain the proteins as polypeptide chains. The SDS coats the proteins, mostly proportional to their molecular weight, and confers the same negative electrical charge across all proteins in the sample. Glycosylated proteins may not migrate at their expected molecular weight since their migration is based more on the mass of their polypeptide chains, not the sugars that are attached. The most widely used gel system for separating a broad range of proteins by SDS-PAGE is the Laemmli system which uses tris-glycine gels comprised of a stacking gel component (which is used to help focus the proteins into sharp bands at the beginning of the electrophoretic run) and the resolving gel where varying acrylamide gel percentages are used to separate the proteins based on their mass weight (Roy and Kumar, 2012). Polyacrylamide gel electrophoresis (PAGE), describes a technique widely used in biochemistry, forensics, genetics, molecular biology and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility. Mobility is a function of the length, conformation and charge of the molecule. As with all forms of gel electrophoresis, molecules may be run in their native state, preserving the molecules' higher-order structure or a chemical denaturant may be added to remove this structure and turn the molecule into an unstructured linear chain whose mobility depends only on its length and mass-to-charge ratio. For nucleic acids, urea is the most commonly used denaturant. For proteins, sodium dodecyl sulfate (SDS) is an anionic detergent applied to protein sample to linearize proteins and to impart a negative charge to linearized proteins. This procedure is called SDS-PAGE. In most proteins, the binding of SDS to the polypeptide chain imparts an even distribution of charge per unit mass, thereby resulting in a fractionation by approximate size during electrophoresis. Proteins that have a greater hydrophobic content, for instance many membrane proteins, and those that interact

with surfactants in their native environment, are intrinsically harder to treat accurately using this method, due to the greater variability in the ratio of bound SDS (Roy and Kumar, 2012).

Seed proteins are used as genetic markers in the study of genetic variation because they are the primary products of structural genes. Any change in the coding sequence of a gene generally reflects the corresponding change in the primary structure of protein (Srivalli *et al.*, 1999). Electrophoresis of seed or seedling extracts followed by appropriate protein or activity stains are all based on the concept that each cultivar is distinct and relatively homogeneous at the genetic level. Thus by screening enough loci one should be able to uniquely define each cultivar. Electrophoresis is basically a process of forced diffusion within an electric field. Protein molecules of the sample are moved through a medium that is gel, paper, or cellulose by applying an electrical gradient. The protein molecules are separated on the basis of their molecular weight or electrical charge. During electrophoresis the lighter molecules move faster and travel more distance in the gel medium and *vice-versa*. Therefore, the protein molecules with low molecular weight will be stacked at the bottom of the gel. Brief reviews regarding these parameters have been given as under.

Singh *et al.* (2017) studied seed protein profiling in twelve genotypes of Pea (*Pisum sativum* L.) cultivars for protein profiling of seed protein through SDS-PAGE. On the basis of banding patterns through SDS-PAGE, indicated that the number of bands found in cultivars ranged from 12 to 19 with Rm value 0.12 to 0.9. Among all the cultivars, the cultivar KPMR-400 had recorded highest number of bands (19) whereas, the minimum number of bands (12) observed in three cultivars viz., KPMR-921, KPMR-902 and KPMR-913. The total seed protein variation were also analyzed using Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) and resultant cluster analysis based on the data of protein profiling,

Khan *et al.* (2016) extracted seed storage proteins from the seeds of each line and run through Slab-vertical gel Electrophoresis. After staining and de-staining the Electrophoregram was divided into two zones (R-I and R-II). The R-1 was consists of five bands (B-1, 2, 3, 4 & 5) ranging from 180KDa to 34KDa, which calculated < 1% genetic diversity (GD). Out of which B-1, 2 and 3 were polymorphic, while B-4 and B-5 were monomorphic. Due to strong genetic association and low level of GD, the R-I divided the total germplasm into two clusters. Similarly, R-II was comprised of nine bands ranging from 33KDa -10KDa. All the loci were polymorphic and showed >39% genetic diversity. The R-II divided the total lines into four clusters. In cluster plating, 43.74% lines were grouped in C-03, 30.43% in C-01, 13.39% in C-04 and 8.69% in C-02. Variation in bands profile and multiple clustering authenticates that conventional plant breeding enhances the level of

genetic variation in seed storage protein profile in pea germplasm detected through SDS-PAGE.

Khan *et al.* (2016) studied thirty three (33) genotype of *Vigna mungo* based on seeds storage protein profiles. Dendrogram based on SDS –PAGE analysis was divided into linkages group (A and B) at 25% genetic distance. Linkage A consisted of two clusters viz; C1 and C2 while linkage B had clusters C3 and C4. Maximum genetic diversity (63%) was found in the genotype of Berari, Maskari, Nasarabad, Nehag, Chaper, Usheri, Shahibagh, Gogyal, Darora, and Jatgram. While minimum genetic diversity (37%) was found in the genotypes of Wari and Dir.

Singh *et al.* (2016) studied seed protein profiling in 12 genotypes of peas (*Pisum sativum* L.) through SDS-PAGE, and observed that the number of bands found in cultivars ranged from 12 to 19 with Rm value 0.12 to 0.9. The results found that, the cultivar KPMR-400 had recorded highest number of bands (19) whereas, the minimum number of bands (12) was observed in three cultivars viz., KPMR-921, KPMR-902 and KPMR-913. The total seed protein variation were also analyzed using Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) Among all the cultivars, the KPMR-906 in cluster IV having wider genetic diversity and suggested to utilize in future crop improvement program.

Dudwadkar *et al.* (2015) studied diversity analysis among few cucurbitaceae using seed protein profile. The study endeavours to differentiate the members of cucurbitaceae with eco-agronomical essence at intra and inter genus level by profiling seed storage proteins. About 20-25 unique bands were scored in *C. grandis* and other cucurbitaceae members respectively. Cluster analysis performed based on Jaccard's similarity coefficient and SPSS software (Version 14.0) showed 3 clusters. Similarity matrix showed that the greatest similarity and minimum genetic distance belonged to populations with the similarity coefficient 0.28 and 0.13 respectively.

Choudhary *et al.* (2015) studied 7 genotypes of *Brassica napus* for total seed protein content using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Ten reproducible bands were used for cluster analysis and to estimate the genetic diversity. Out of these bands, five bands were observed polymorphic. A dendrogram was constructed and the genotypes were divided into two main groups comprising 4 clusters. The results obtained from these clusters showed genetic diversity in these accessions on SDS-PAGE level. The results showed that the technique of SDS-PAGE was feasible to distinguish the different species of *Brassica napus* genotypes.

Kandasamy (2014) studied electrophoretic characterization of 20 lines of cucumber by sodium dodecyl sulphate polyacrylamide vertical slab gel electrophoresis (SDS-

PAGE). The seed protein could be resolved into total 11 bands distributed in 3 zones *i.e.* A, B and C. Zone A has 5 bands, zone B has 3 bands and zone C included 3 bands. Based on electrophoregrams, genotypes were classified into 6 dissimilar groups. Hence, seed protein profiles proved useful in identifying cucumber genotypes.

Yatung *et al.* (2014) studied seed protein profiling in 30 chilli (*Capsicum annuum* L) genotypes of Northeast India by SDS- PAGE analysis. On the basis of presence (+) or absence (-) of bands, similarity index was calculated and genotypes were grouped in three major clusters which were further sub divided in 9 sub-clusters. The genotype CHFC9 was most distantly related to CHFC18. Hence, it was recommended that genotype CHFC9 and CHFC18 could be utilised for crossing programme to create more genetic diversity or segregants of desired characteristics through chilli breeding programmes.

The dendrogram from SDS-PAGE analysis in nine taxa in *Vicia* that all studied taxa constituted two clusters. The first one consisted of *V. noeana* var. *noeana*, *V. noeana* var. *megalodonta*, *V. truncatula*, *V. peregrina*, *V. michauxii* and *V. grandiflora* second one by *V. mollis*, *V. hybrida*, and *V. assyriaca*. Present results showed that all studied species have similar total protein content. But *V. truncatula* (86.837 µg/ml) and *V. hybrida* (83.209 µg/ml) have highest total protein content. Whereas *V. grandiflora* (65.860 µg/ml) has low total protein content Emre (2011).

Akbar *et al.* (2010) studied exotic genotypes of *Capsicum* (chilli) using SDS-PAGE analysis. Total seed proteins were extracted and separated on 12.5% Polyacrylamide gels using standard protocols. Protein fragments of various molecular weights were separated in various *Capsicum* genotypes. Individual protein fragments were considered as allele / loci. Estimates of Genetic Distances (GD) ranged from 0 to 100%. Nineteen *Capsicum* genotypes were clustered in four groups A, B, C and D comprising 7, 5 and 7 genotypes, respectively.

Kumar *et al.* (2010) studied eighteen genotypes of chilli pepper (*Capsicum* L). A total of 21 protein polypeptide bands with molecular weight ranging from 18.6 to 72.0 kD were recorded. Among the genotypes 'CA18', 'CA21' and 'CA27' represented maximum number of protein bands (12). Band no. (11) and (5,12) are exclusive to *C. annuum* L. and *C. frutescens* L. genotypes respectively. Average percent similarity was highest (100%) between 'CA2' and 'CA8' genotypes and the UPGMA dendrogram represented low genetic diversity.

**CHAPTER-3**  
**MATERIALS**  
**AND**  
**METHODS**

## Chapter-3

# Materials and Methods

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The present investigation entitled “Studies on genetic diversity among indigenous landraces of pumpkin (*Cucurbita moschata* Duch. ex Poir) was carried out at Vegetable Research Farm, Department of Vegetable Science, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh during 2017. The details of material and methods used in the present investigation are described below:

### 3.1 Experimental site

The experiment was conducted at Vegetable Research Farm, College of Horticulture and Forestry, Central Agricultural University, Pasighat, East Siang, Arunachal Pradesh, India (Figure 3.1). The geographical location of the research farm is having an altitude of 153 m above mean sea level, latitude of 28°04`N and longitude of 95°22`E. The climate of this area is humid, sub-tropical and maximum rainfall occurs between June-September. The soil is sandy loam with pH 6.7 and organic carbon 2.1%. The agro-meteorological data pertaining to crop periods have been given in (Appendix-I).

### 3.2 Experimental layout and design

The experiment was laid out in Randomized Block Design (RBD) with three replications (Table 3.2). The details of experimental plan are given below:

Date of sowing in nursery	: 01 March, 2017
Number of genotypes	: 25
Number of replications	: 03
Row to row distance	: 3 m
Plant to plant distance	: 1.3 m
Place of collection	: North Eastern Region

### 3.3 Experimental material

The experimental materials for the present study comprised of 23 diverse genotypes of Pumpkin (*Cucurbita moschata* Duch. ex. Poir) Collected from Northeast India along with 2 suitable checks viz., CHFPUM-7 (Kashi Harit) and CHFCPUM-23 (Narendra Agrim) thereby comprising 25 genotypes in total. The list of genotypes along with their sources is given in (Table 3.1).

**Table 3.1 List of pumpkin genotypes with their sources of collection**

S. N.	Genotype	Source
1	CHFPUM-1	A Landrace of Pasighat, Arunachal Pradesh
2	CHFPUM-2	A landrace of Pasighat, Arunachal Pradesh
3	CHFPUM-3	A landrace of Pasighat, Arunachal Pradesh
4	CHFPUM-4	A landrace of Pasighat, Arunachal Pradesh
5	CHFPUM-5	A landrace of Pasighat, Arunachal Pradesh
6	CHFPUM-6	A landrace of Pasighat, Arunachal Pradesh
7	CHFPUM-7	A landrace of IIVR, Varanasi (U.P.)
8	CHFPUM-8	A landrace of Pasighat, Arunachal Pradesh
9	CHFPUM-9	A landrace of Pasighat, Arunachal Pradesh
10	CHFPUM-10	A landrace of Ziro, Arunachal Pradesh
11	CHFPUM-11	A landrace of Aizawl Mizoram
12	CHFPUM-12	A landrace of Aizawl Mizoram
13	CHFPUM-13	A landrace of Aizawl Mizoram
14	CHFPUM-14	A landrace of Aizawl Mizoram
15	CHFPUM-15	A landrace of Imphal, Manipur
16	CHFPUM-16	A landrace of Imphal, Manipur
17	CHFPUM-17	A landrace of Imphal, Manipur
18	CHFPUM-18	A landrace of Gangtok Sikkim
19	CHFPUM-19	A landrace of Gangtok Sikkim
20	CHFPUM-20	A landrace of Kohima Nagaland
21	CHFPUM-21	A landrace of Kohima Nagaland
22	CHFPUM-22	A landrace of Kohima Nagaland
23	CHFPUM-23	A landrace of NDUAT, Faizabad (U.P.)
24	CHFPUM-24	A landrace of Agartala, Tripura
25	CHFPUM-25	A landrace of Agartala, Tripura

**Table 3.2 Allotment of treatments (genotypes) in experimental field under RBD**

<b>Replication-1</b>	<b>Replication-2</b>	<b>Replication-3</b>
CHFPUM-1	CHFPUM-25	CHFPUM-15
CHFPUM-2	CHFPUM-24	CHFPUM-14
CHFPUM-3	CHFPUM-23	CHFPUM-13
CHFPUM-4	CHFPUM-22	CHFPUM-12
CHFPUM-5	CHFPUM-21	CHFPUM-11
CHFPUM-6	CHFPUM-20	CHFPUM-10
CHFPUM-7	CHFPUM-19	CHFPUM-9
CHFPUM-8	CHFPUM-18	CHFPUM-8
CHFPUM-9	CHFPUM-17	CHFPUM-7
CHFPUM-10	CHFPUM-16	CHFPUM-6
CHFPUM-11	CHFPUM-15	CHFPUM-5
CHFPUM-12	CHFPUM-14	CHFPUM-4
CHFPUM-13	CHFPUM-13	CHFPUM-3
CHFPUM-14	CHFPUM-12	CHFPUM-2
CHFPUM-15	CHFPUM-11	CHFPUM-1
CHFPUM-16	CHFPUM-10	CHFPUM-25
CHFPUM-17	CHFPUM-9	CHFPUM-24
CHFPUM-18	CHFPUM-8	CHFPUM-23
CHFPUM-19	CHFPUM-7	CHFPUM-22
CHFPUM-20	CHFPUM-6	CHFPUM-21
CHFPUM-21	CHFPUM-5	CHFPUM-20
CHFPUM-22	CHFPUM-4	CHFPUM-19
CHFPUM-23	CHFPUM-3	CHFPUM-18
CHFPUM-24	CHFPUM-2	CHFPUM-17
CHFPUM-25	CHFPUM-1	CHFPUM-16

### **3.4 Land preparation**

The experimental field was prepared by one time deep ploughing followed by one harrowing and leveling to get good tilth of the soil for seed sowing. All the recommended packages of practice were followed to raise healthy crop.

#### **3.4.1 Seed sowing**

. Seeds were directly sown in the field with five pits of each genotype in each replication on 01 March, 2017. Two seeds of each genotype were sown in each pit in each replication at spacing of 3 x 1.3 m. Each genotype was sown in a Randomized Block Design with three replications. After the emergence of seedlings, only healthy seedlings of each genotype per replication were retained.

### **3.5 Cultural practices**

The standard cultural practices recommended in the Package of Practices for Vegetable Crops were followed to produce a healthy crop stand (Anon, 2016 b). Were applied 5kg FYM along with 200 g N, 150 g P<sub>2</sub>O<sub>5</sub> and 150 g K<sub>2</sub>O in five pits of each genotype in each replication at the time of pits preparation. Besides the application of Farm Yard Manure @ 25 t/ha, chemical fertilizers were applied as per the recommendation of package of practices i.e. 107 kg N, 110 kg P<sub>2</sub>O<sub>5</sub> and 40kg K<sub>2</sub>O/ha. Half dose of N along with full doses of P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O and FYM were applied as basal dressing. Remaining dose of N was top dressed in 2 split doses i.e. 25-30 days after sowing and 50-55 days after sowing. Although pumpkin are deep rooted but most of the feeding roots remain confined near the soil surface, thus deep inter-cultivation is very harmful, destroying many of the fine roots near the soil surface. Weeds pose very problem during early growth period, therefore, shallow hand hoeing in early crop stages is usually recommended in pumpkin crop to avoid damage to the roots. First manual weeding was done at 20-25 days and second at 40-45 days after sowing. Mulching is also found for controlling weeds, mulching was done before sowing of seed in pits. The irrigation was applied in early growth stage with water cane, sufficient moisture in the root zone were maintained to promote rapid taproot development so light and frequent irrigation is very essential. The remaining intercultural operations were carried out in accordance with the recommended package of practices.

### **3.6 Observations recorded**

Observations were recorded on three randomly selected plants from each genotype in each replication. The observations recorded were averaged to get mean values. Characters studied and procedures adapted to record the observation have been described below:

### **3.6.1 Morphological characters**

The characterization of genotypes with following morphological traits.

#### **3.6.1.1 Fruit skin colour**

The skin colour of fruits was observed visually after harvesting with the help of visuality test by a group of 5 members.

#### **3.6.1.2 Fruit shape**

The fruit shape was observed visually after harvesting with the help of visuality taste by a group of 5 members

#### **3.6.1.3 Fruit ribs**

The fruit ribs present/absent was observed visually after harvesting with the help of visuality taste by a group of 5 members

#### **3.6.1.4 Fruit flesh colour**

The flesh colour observed visually after harvesting with the help of visuality taste by a group of 5 members

### **3.6.2 Quantitative characters**

The observations were recorded on three randomly selected plants of each genotype from each replication and the average was worked out in each replication for different traits. The observations were recorded on the following traits:

#### **3.6.2.1 Vine length (m)**

The length of vine from base of plant up to tip was measured at the maturity

#### **3.6.2.2 Number of branches per plant**

Number of branches of each genotypes was counted manually in each replication and mean value was taken at the time of last picking of fruits.

#### **3.6.2.3 Days to first staminate flower anthesis**

Number of days taken for the anthesis of first staminate flower was noted down.

#### **3.6.2.4 Days to first pistillate flower anthesis**

Number of days taken for the anthesis of first pistillate flower was noted down.

### **3.6.2.5 Node bearing first staminate flower**

Node number of each genotype, at which the first staminate flower appeared, was counted in each replication and mean value was worked out to estimate the earliness of the variety.

### **3.6.2.6 Node bearing first pistillate flower**

Node number of each genotype, at which the first pistillate flower appeared, was counted in each replication and mean value was worked out to estimate the earliness of the variety.

### **3.6.2.7 Days to first harvest**

The number of days to first harvest was counted from date of sowing of seeds to the date of first harvesting

### **3.6.2.8 Number of fruits per plant**

Total number of fruits harvested from all pickings of individual plant was counted and summed up to get total number of fruits per plant.

### **3.6.2.9 Polar circumference of fruit (cm)**

The polar circumference of fruit are taken from three fruit

### **3.6.2.10 Equatorial circumference of fruit (cm)**

The Equatorial circumference of fruit are taken from three fruit

### **3.6.2.11 Fruit flesh thickness (cm)**

The flesh thickness of three fruits was measured and average was calculated.

### **3.6.2.12 Cavity length (cm)**

The cavity length of three fruits was measured and average was calculated

### **3.6.2.13 Number of seeds per fruit**

The total number of seeds was counted manually from three selected fruits of each genotype and mean value was worked out to find the number of seeds per fruit.

### **3.6.2.14 100 seed weight (g)**

Weight of 100 seeds from three selected fruits of each genotype was taken by using weighing balance and the average was worked out

### **3.6.2.15 Average fruit weight (kg)**

The total number of fruits harvested from all pickings of individual plant weighed and the average fruit weight worked out by dividing total weight of fruits from all the pickings with total number of fruits from all pickings and expressed in gram (kg).

#### **3.6.2.16 Fruit yield per plant (kg)**

The total weight of harvested fruits was weighed from 5 selected plants of each genotype using weighing balance and the average was worked out to get the fruit yield per plant and expressed in kg.

#### **3.6.2.17 Fruit yield per hectare (q)**

Total weight of all fruits harvested from each plot was recorded and expressed as yield per plot. From this value, yield per hectare was calculated and expressed in quintals per hectare.

### **3.6.3 Qualitative characters**

#### **3.6.3.1 Total Soluble Solids ( $^{\circ}$ B)**

Three fruits were selected randomly after harvesting from each plot and Total Soluble Solids (TSS) was observed with the help of Hand Refractometer.

#### **3.6.3.2 Total carbohydrate (mg/100 g)**

Estimation of total carbohydrate content was determined by Anthrone method as described by Hedge and Hofreiter (1962).

#### **Reagents:**

- a) Anthrone reagent:** 200 mg of anthrone in 100 ml of ice-cold 95 % sulphuric acid were prepared fresh before used.
- b) Standard glucose solution: Stock:** 100 mg in 100 ml distilled water.
- c) Working Standard:** 10 ml of stock diluted to 100 ml with distilled water stored in refrigerator adding a few drops toluene.
- d) 2.5 N HCl:** 14 ml HCl in 100 ml distilled water

#### **Procedures:**

Sample (100 mg) 1 g was taken in a boiling tube and hydrolysed by keeping it in a water bath for three hours with 5 ml of 2.5 N HCl. The sample was cooled to room temperature and was neutralized until the effervescence ceased. The volume was made up to 100 ml and the content was centrifuged and supernatant was collected. An aliquot of 0.5 ml was taken and 4 ml of anthrone reagent was added to it. The content was heated for eight minutes in boiling water bath and was cooled rapidly. Dark green colour of the sample was

read against the blank solution at 630 nm using a U V-visible spectrophotometer. The concentration of total carbohydrate in the sample was calculated from the slope of the total carbohydrate standard curve. The total carbohydrate (mg) was calculated by using the formulae:

Amount of carbohydrate present in 100 mg of the sample

$$= \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

### 3.6.3.3 Vitamin A (mg/100 g)

Estimation of Vitamin A content was described Bayfield and Cole (1980).

#### Reagents:

a) Potassium hydroxide in 90% alcohol, 2 N

b) Petroleum ether

c) Sodium sulphate

d) **Trichloroacetic acid:** Dissolve 15 g trichloroacetic acid (clear) in 25 ml of chloroform (alcohol-free) and store in dark.

e) **Standard vitamin A:** 1.5 mg vitamin A palmitate dissolved in 10 ml chloroform

#### Procedures:

Sample (100 mg) 1 g was grind to a fine paste and added 10 ml of saponification mixture (2 N KOH in 90% alcohols). Reflex the tubes gently for 20 min at 60°C. Cool the tubes at room temperature; added 20 ml water and mix well. Extract vitamin A with 10 ml portions of petroleum ether in a separating funnel, twice. Pool the extract (organic layer) and added sodium sulphate (anhydrous) to remove the moisture for 30-40 min. Evaporated the 5 ml aliquot of the ether extract to dryness at 60°C. Dissolve the dried the residue in 1.0 ml of chloroform. Added 2.0 ml of TCA from a fast delivery was pipette, rapidly mixing the contents of the tube. Absorbance was recorded at 620 nm in a spectrophotometer. Standard graph was constructed plotting the  $A_{620}$  in the Y-axis and vitamin A concentration in the X-axis. Calculate the amount of vitamin A (mg) per 100 g of sample.

## 3.6.4 Protein banding pattern

### 3.6.4 (a) Protein

Estimation of protein was done as per Lowry's method (Lowry *et al.* 1951). Seeds of 25 germplasm lines were collected and subjected to gel electrophoresis. 1 g of seed sample were macerated in mortar and pestle with 10 ml of buffer (0.06 M Tris-HCl, 2.5% Glycerol, 0.5% SDS, 1.25%  $\beta$ -mercaptoethanol, 0.1% TCA, 10 mM urea, 1 mM EDTA)

and transferred to centrifuge tubes. The materials were then centrifuged at 8000 rpm for 20 min. The supernatants were collected and the procedure was repeated 4-5 times. The supernatants were mixed and volume made up to 50 ml with phosphate buffer. 1 ml of 20% TCA was added to 1 ml of the extract and the mixture was kept for 30 min. The mixture was then centrifuged at 8000 rpm for 20 min. The resultant pellets were washed twice with acetone and again centrifuged. The supernatant was then discarded. The pellet was collected and dissolved in 5 ml of 0.1N NaOH till it had dissolved. 1 ml of the aliquot was taken in which 5 ml of freshly prepared alkaline copper sulphate reagent were added and mixed properly. After 10 min, 0.5 ml of Folin's reagent was added and mixed instantaneously and allowed to develop colour for 30 min. Absorbance at 660 nm was recorded after setting the instrument with reagent blank which contained 1 ml of 0.1 N NaOH instead of the sample aliquot.

In another set of tubes, suitable aliquots of BSA solution (in the range of 0-100  $\mu$ l) were taken and volume made up to 1 ml with 0.1 N NaOH and allowed to develop colour as described above. A standard curve of absorbance at 660 nm versus  $\mu$ g of BSA was drawn and from this standard curve, the amount of protein in the sample tube was determined as protein per gram of the sample.

### **3.6.3 (b) Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE)**

Polyacrylamide gel electrophoresis in presence of denaturing agent (SDS) was carried out as per procedure described by Laemmli (1970) with some modifications.

#### **Reagents**

**a) Acrylamide Solution:** 29.2 g acrylamide and 0.8 g bisacrylamide were dissolved in water and the final volume was made up to 100 ml and stored at 4°C.

**b) Separating Gel Buffer:** 1.5M Tris-HCl of pH 8.8 was prepared and stored at 4°C.

**c) Stacking Gel Buffer:** 1M Tris-HCl of pH 6.8 was prepared and stored at 4°C.

**d) Sodium Dodecyl Sulphate solution:** 2% aqueous solution of SDS was prepared.

**e) Ammonium per sulphate solution:** 10% aqueous solution of ammonium persulphate was prepared.

**F) Bromophenol blue solution:** 0.1% aqueous solution of bromophenol blue was prepared.

**g) Electrophoresis buffer:** 3.0 g of Tris base and 14.4 g of glycine was dissolved in water and the final volume was made up to 1liter. The final pH was adjusted to 8.3 with glycine solution.

**h) Staining Solution:** Staining solution comprised of fixing solution, sensitizing solution, staining solution, developing solution and terminating solution.

- ❖ **Fixing Solution:** 50% ethanol, 12% glacial acetic acid, 0.05ml formaldehyde was prepared in double distilled water.
- ❖ **Sensitizing Solution:** 0.02% aqueous solution of sodium thiosulphate was prepared.
- ❖ **Silver stain solution:** 0.2% Silver nitrate and 0.076% formaldehyde was prepared in double distilled water.
- ❖ **Developing Solution:** 6% Sodium carbonate, 0.004% sodium thiosulphate and 0.05% formaldehyde solution was prepared in double distilled water.
- ❖ **Terminating/Stopping Solution:** 12% acetic acid solution was prepared.

#### Formulation for 15% Acrylamide Separating Gel

Water	6.9 ml
30% Acrylamide mixture	15 ml
Separating gel buffer (1.5M Tris-HCl, pH 8.8)	7.5ml
2% SDS	0.3 ml
10 % Ammonium persulphate	0.3 ml
TEMED	0.012 ml

The mixture was transferred to gel cassette by running the solution carefully down one edge between the glass plates till it reaches 1 cm from the bottom of sample loading comb. To ensure that the gel sets with a smooth surface distilled water was run down one edge in the cassette. It was allowed to polymerize.

#### Formulation for 5% Acrylamide Stacking Gel

Water	5.5 ml
30% Acrylamide mixture	1.3 ml
Separating gel buffer (1.5M Tris-HCl, pH 6.8)	1 ml
2% SDS	0.06ml
10 % Ammonium persulphate	0.06 ml
TEMED	0.008 ml

#### Preparation of Sample

50mM Tris-HCl (pH 6.8)	100µl
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2% SDS	50 µl
0.1% Bromophenol blue solution	30µl
10% Glycerol	120µl
Protein	200µl

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The sample was kept in water bath at 90<sup>0</sup>C for 5 minutes for denaturation of enzyme. Since, the gel run is very slow, SDS-PAGE is performed in refrigerator at 4<sup>0</sup>C to prevent overheating of plate.

### **Procedure**

The above polymerized gel was clamped in the electrophoresis assembly and both the tanks were filled with electrode buffer. Desired volume of the purified enzyme and known proteins were placed in previously prepared wells of the stacking gel. Cathode and anode terminals were connected to the electrophoretic power supply and the SDS-PAGE was carried out at a constant current of 25 mA. The gel was run until the bromophenol blue reaches the bottom of separating gel. The power supply was switched off when the tracking dye approached the bottom of the gel. The system was disconnected and the gel was taken out from the slab. The gel was then subjected to silver staining and the protein bands on the gel were visualized by silver staining.

### **Silver staining**

Silver staining was performed as method described by Mortz *et al.* (2001).

### **Method**

The gel was first sensitized by 0.02% sodium thiosulphate solution for 5 minutes. The gel was then washed twice with double distilled water for 1 minute. It was then transferred to staining solution and kept on gel rocker for 20 minutes in dark. The gel was then washed twice with distilled water for 45 seconds. The gel was then transferred to developing solution and finally the reaction was stopped with 12% acetic acid solution. Gel was washed with double distilled water before visualizing the dark brown band.

## **3.7 Statistical analysis**

Statistical analysis of the data was carried out by using Statistical Package for Agricultural Research (SPAR 2.0) and Windostat Version 9.2 from Indostat services, Hyderabad, licensed to Plant Breeding Division, Sugarcane Breeding Institute, Coimbatore.

### **3.7.1 Mean**

On the basis of individual plant observation, the mean for each character in all population was calculated as follows:

$$\bar{Y} = \frac{1}{n} (\sum_{i=1}^n Y_i)$$

Where,

$\bar{Y}$  = Population mean

$Y_i$  = Individual value

n = Number of observations

### 3.7.2 Range

The minimum and maximum value on the basis of individual plant observation was used to indicate the range for a given character.

### 3.7.3 Variance and standard deviation

In all the populations, variance and standard deviation was calculated for all the characters as follows:

$$\text{Variance} = \frac{1}{n-1} \sum (Y_i - \bar{Y})^2$$

Where,

$Y_i$  = Individual value

$\bar{Y}$  = Population value

n = Number of observations

Standard deviation (SD) =  $\sqrt{\text{variance}}$

### 3.7.4 Coefficient of variation

The ratio of standard deviation of a sample to its mean expressed in percentage is called coefficient of variation.

$$\text{Coefficient of variation (CV)} = \frac{SD}{\bar{X}} \times 100$$

Where,

$$\text{Standard deviation (SD)} = \sqrt{\text{variance}}$$

### 3.7.5 Analysis of variance

Analysis of variance (ANOVA) and components of variance for individual character was carried out as per procedure adopted by Gomez and Gomez (1984).

#### Structure of ANOVA table

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Expected value of MS	Calculated F

Replications(r)	(r-1)	SSr	$MSr = \frac{RSS}{r-1}$		
Genotypes (g)	(g-1)	SSg	$MSg = \frac{GSS}{g-1}$	$\sigma_e^2 + r \sigma_g^2$	MSg/MSe
Errors (e)	(r-1)(g-1)	Sse	$MSe = \frac{ESS}{(r-1)(g-1)}$	$\sigma_e^2$	
Total	(rg - 1)				

Where,

r = Number of replications

g = Number of treatments

Sr = Sum of squares due to replications

Sg = Sum of squares due to treatments

S<sub>e</sub> = Sum of squares due to errors

MS r = Mean sum of squares due to replications

MSg = Mean sum of squares due to genotypes

MSe = Mean sum of squares due to errors

$\sigma_g^2$  = Genotypic variance

$\sigma_e^2$  = Error variance

The standard error of mean ( $SE_{m\pm}$ ), Standard error ( $SE_{d\pm}$ ) for genotypes and critical difference (CD) for comparing the means of genotypes were computed as follows:

$$SE_{m\pm} = \sqrt{MSe/r}$$

$$SE_{d\pm} = \sqrt{2MSe/r}$$

CD at 5% =  $SE_{(d)}$  x t value at error degree of freedom at 5% level of significance.

The calculated F values were compared with the tabulated F values at P=0.05 and P=0.01, If the calculated F value was found higher than the tabulated, it was considered to be significant. All the characters which showed significant differences among the genotypes were subjected further to the analysis of the following parameters:

1. Coefficient of variability (Phenotypic and genotypic)
2. Heritability (%)
3. Genetic advance(GA)
4. Genetic gain (GG)
5. Correlation studies
6. Path coefficient analysis

### 3.7.6 Coefficients of variability

The phenotypic and genotypic coefficient of variability was calculated as per formula given by Burton and de Vane (1953).

### 3.7.6.1 Phenotypic coefficient of variability (PCV)

$$\text{PCV (\%)} = \frac{\sqrt{\text{Phenotypic variance } (\sigma_p^2)}}{\text{General mean of the character } (\bar{Y})} \times 100$$

### 3.7.6.2 Genotypic coefficient of variability (GCV)

$$\text{GCV (\%)} = \frac{\sqrt{\text{Genotypic variance } (\sigma_g^2)}}{\text{General mean of the character } (\bar{Y})} \times 100$$

PCV and GCV were classified as suggested by Shivasubramanian and Menon (1973) as follows:

0-10% = low

10-20% = Moderate

> 20% = High

### 3.7.7 Heritability ( $h^2$ )

Heritability ( $h^2$ , broad sense) was calculated as per formula given by Burton and de Vane (1953).

$$\text{Heritability } (h^2, \text{ broad sense}) \% = \frac{\text{Genotypic variance } (\sigma_g^2)}{\text{Phenotypic variance } (\sigma_p^2)} \times 100$$

Where,

$h^2$  = Heritability (%)

$\sigma_g^2$  = Genotypic variance [ $\sigma_g^2 = (Mg - Me) / r$ ]

$\sigma_p^2$  = Phenotypic variance ( $\sigma_g^2 + \sigma_e^2$ )

Heritability percentage was categorised as demonstrated by Robinson *et al.* (1949).

0 – 30% = Low

30 – 60% = Moderate

> 60% = High

### 3.7.8 Genetic advance

The expected genetic advance resulted from selection of 5 percent superior individuals were worked out as suggested by Johnson *et al.* (1955).

$$\text{Genetic advance (GA)} = h^2 (\text{bs}) \times \sigma_p \times k$$

Where,

$h^2$  = Heritability

$\sigma_p$  = Phenotypic standard deviation of the character

k = Selection differential at 5 per cent selection intensity *i.e.*, 2.06

### 3.7.9 Genetic gain

Genetic gain is the percentage ratio of genetic advance and population mean as suggested by Johnson *et al.* (1955).

$$\text{Genetic gain (\%)} = \frac{\text{Genetic advance (GA)}}{\text{General mean of the character}} \times 100$$

The genetic advance as percentage of mean was categorised into low, moderate and high as suggested by Johnson *et al.* (1955).

0 – 10 = Low

10 – 20 = Moderate

>20 = High

### 3.7.10 Analysis of covariance

The analysis of covariance and components of covariance (genotypic and phenotypic) were analyzed between character as per procedure adopted by Singh and Chaudhary (1985).

Source of variation	Degree of freedom	Sum of products between X and Y	Mean sum of products between X and Y	Expected value of mean sum of products between X and Y	Calculated F. value
Replications (r)	r-1	RSP <sub>xy</sub>	RSP <sub>xy</sub> /r-1=MSP <sub>r</sub>		
Genotypes (g)	g-1	GSP <sub>xy</sub>	GSP <sub>xy</sub> / g-1=MSP <sub>g</sub>	$\sigma_{e1e2}^+$ $r\sigma_{g1g2}$	MSP <sub>g</sub> /MSP <sub>e</sub>
Error (e)	(r-1)(g-1)	ESP <sub>xy</sub>	ESP <sub>xy</sub> /(r-1) (g-1)=ESP <sub>e</sub>	$\sigma_{e1e2}$	
Total	(rg-1)				

Where,

r = Number of replications

$g$  = Number of genotypes

$MSP_r$  = Mean sum of products due to replications

$MSP_g$  = Mean sum of products due to genotypes

$MSP_e$  = Mean sum of products due to error

$\sigma_{g_1 g_2}$  = Genotypic covariance between character X and Y

$\sigma_{e_1 e_2}$  = Error covariance between character X and Y

### 3.7.11 Coefficients of covariance

Genotypic and phenotypic covariance was computed based on expected sum of products from ANOVA table as follows:

$$MSP_g = \sigma_{e_1 e_2} + r\sigma_{g_1 g_2}$$

$$MSP_e = \sigma_{e_1 e_2}$$

$$\text{Hence, } MSP_g = MSP_e + r\sigma_{g_1 g_2}$$

$$\text{Genotypic covariance } (\sigma_{g_1 g_2}) = \frac{MSP_g - MSP_e}{r}$$

$$\text{Phenotypic covariance } (\sigma_{p_1 p_2}) = \sigma_{g_1 g_2} + \sigma_{e_1 e_2}$$

### 3.7.12 Correlation coefficient

Genotypic ( $v_g$ ) and phenotypic ( $v_p$ ) correlation coefficients were estimated according to the formulae given by Al-Jibouri *et al.* (1958).

$$\text{Genotypic correlation } r_{xy}(g) = \frac{Cov_{xy}(g)}{\sqrt{V_x(g) \times V_y(g)}}$$

$$\text{Phenotypic correlation } r_{xy}(p) = \frac{Cov_{xy}(p)}{\sqrt{V_x(p) \times V_y(p)}}$$

Where,

$Cov_{xy}(p)$  = Phenotypic covariance between X and Y character

$Cov_{xy}(g)$  = Genotypic covariance between X and Y character

$V_x(g)$  = Genotypic variance of character X

$V_y(g)$  = Genotypic variance of character Y

$V_x(p)$  = Phenotypic variance of character X

$V_y(p)$  = Phenotypic variance of character Y

The significance of phenotypic and genotypic correlation coefficient was compared with table r values, as given by Fisher and Yates (1963) at n-2 degree of freedom where 'n' denotes number of observations.

### 3.7.13 Path coefficients analysis

The path coefficient analysis was done to calculate direct and indirect contribution of different characters towards yield. The direct and indirect effects were calculated by solving the following set of simultaneous equations proposed by Dewey and Lu (1959).

$$\begin{array}{rcccccc}
 Py_1 & + & Py_2 r_{12} & + & Py_3 r_{13} & + \dots + & Py_n r_{1n} & = & ry_1 \\
 Py_1 r_{12} & + & Py_2 & + & Py_3 r_{23} & + \dots + & Py_n r_{2n} & = & ry_2 \\
 & - & & - & & - & & - & \\
 & - & & - & & - & & - & \\
 Py_1 r_{1n} & + & Py_2 r_{2n} & + & Py_3 r_{3n} & + \dots + & Py_n & = & ry_n
 \end{array}$$

Where,

$Py_1, Py_2, Py_3, \dots, Py_n$  are the direct path effects of 1, 2, 3, .....n variables on dependent variable 'y'

$r_{12}, r_{13}, r_{19}, \dots, r_{(r-1)_n}$  are the genotypic coefficients of correlation between various independent variables

$ry_1, ry_2, \dots, ry_n$  are the genotypic correlation coefficients of independents variables with dependent variables 'y'

$Py_1 r_{12}, Py_3 r_{23}, \dots, Py_n r_{2n}$  are the indirect effects.

The variation in the independent variable which remained undetermined by including all variables was assumed to be due to variable (s) not included in the present investigation. The degree of determination ( $P^2_{yx}$ ) of such variable (s) on dependent variable was calculated as follows:

$$P^2_{yx} = 1 - (P^2_{y_1} + 2Py_1 Py_2 r_{12} + 2Py_1 Py_3 r_{13} \dots + P^2_{y_2} + 2Py_2 Py_3 r_{23} + P^2_{y_3} + 2Py_3 Py_4 r_{34} \dots + P^2_{y_n})$$

And

$$\text{Residual effect } (R^2) = \sqrt{P^2_{yx}}.$$

### 3.7.14 Genetic divergence

#### 3.7.14.1 Mahalanobis D<sup>2</sup> analysis

Mahalanobis (1936) D<sup>2</sup> statistical analysis was used for assessing the genetic divergence among the test entries. The generalized distance between any two populations is given by formula.

$$D^2 = \sum \sum \lambda_{ij} S_i S_j$$

Where,

$D^2$  = Square of generalized distance

$\lambda_{ij}$  = Reciprocal of the common dispersal matrix

$S^{ai} = (\mu_i - \mu)^2$

$S^{aj} = (\mu_j - \mu)^2$

$\mu$  = General mean

Since the formula for computation requires inversion of higher order determinant, transformation of the original correlated unstandardized character means (Xs) to standardized uncorrelated variable (Ys) was done to simplify the computational procedure. The  $D^2$  values were obtained as the corresponding uncorrelated (Ys) values of any two genotypes as per procedure illustrated by Rao (1952).

### 3.7.14.2 Clustering of $D^2$ values

All then  $(n(n-1)/2)$   $D^2$  values were clustered using Tocher's method as described by Rao (1952).

### 3.7.14.3 Intra cluster distance

The intra cluster distances were calculated by the formula given by Singh and Chaudhary (1977).

$$\text{Square of intra cluster distance} = \frac{\sum D_i^2}{n}$$

Where,

$\sum D_i^2$  = The sum of distance between all possible combinations

$N$  = Number of all possible combinations.

### 3.7.14.4 Inter cluster distance

The inter cluster distance were calculated by the formulae described by Singh and Chaudhary (1977).

$$\text{Square of inter cluster distance} = \frac{\sum D_i^2}{n_i \times n_j}$$

Where,

$\sum D_i^2$  = The sum of distances between all possible combinations ( $n_i n_j$ ) of the entries included in the cluster study.

$n_i$  = Number of entries in cluster  $i$

$n_j$  = Number of entries in cluster  $j$

### 3.7.15 Data analysis for protein banding pattern

The gels were scored as presence (+) or absence (-) of protein polypeptide bands. Depending upon the presence or absence of polypeptide bands, similarity index (SI) (Nei and Li, 1979) between the genotypes was calculated by the following formula:

$$SI = \left( \frac{2Z}{X+Y} \right) \times 100$$

Where,

$Z$  = Number of similar bands between the genotypes, and

$X+Y$  = Total number of bands in the two genotypes compared. Cluster analysis UPGMA (Unweighted pair group method with arithmetic mean analysis) by using statistical software SPSS for windows package (Version 16).



**Fig 3.1 View of the Experimental Farm**

**CHAPTER-4**  
**EXPERIMENTAL**  
**RESULTS**

# Chapter-4

## Experimental Results

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The present work entitled “Studies on genetic diversity among indigenous landraces of pumpkin (*Cucurbita moschata* Duch ex Poir.)” in North eastern India through morphological traits and seed protein profiling was carried out in 25 genotypes of pumpkin for various horticultural traits. The experimental results are presented under the following sub heads:

### 4.1 Morphological characters

4.1.1 Fruit colour

4.1.2 Fruit shape

4.1.3 Fruit ribs

4.1.4 Flesh colour

### 4.2 Quantitative characters

4.2.1 Mean performance of genotypes

4.2.2 Parameter of variability

4.2.3 Correlation coefficient

4.2.4 Path coefficient analysis

4.2.5 Genetic diversity

4.2.5.1 Mahalanobis  $D^2$  analysis

4.2.5.2 Inter cluster distance

4.2.5.3 Intra cluster distance

4.2.5.4 Cluster mean analysis

### 4.3 Seed protein banding pattern through SDS-PAGE

#### 4.1 Morphological characters

The morphological characters for pumpkin plant under studies viz. fruit colour, the fruit colour at mature stage different depending on genotypes. Commonly observed fruit colours were: yellow, orange, green, brown. The shapes of fruit were found: round, elongated, oblong, flattened, oval and pyriform. The fruit ribs presence or absence was also observed among the genotypes. The flesh colour of fruits was observed yellow; orange and cream. Mostly three numbers of locules were also observed among the genotypes (Table 4.1 and Figure 4.1).

#### **4.1.1 Fruit colour**

Yellow colour surface was observed in six genotypes viz., CHFPUM-1, CHFPUM-3, CHFPUM-5, CHFPUM-11, CHFPUM-20 and CHFPUM-22; Green colour in 11 genotypes viz., CHFPUM-4, CHFPUM-6, CHFPUM-7, CHFPUM-8, CHFPUM-9, CHFPUM-10, CHFPUM-14, CHFPUM-17, CHFPUM-19, CHFPUM-23 and CHFPUM-25; Brown colour in 4 genotypes viz., CHFPUM-2, CHFPUM-13, CHFPUM-15 and CHFPUM-16; Cream colour in 2 genotypes viz., CHFPUM-12 and CHFPUM-24; Grey colour in CHFPUM-18.

#### **4.1.2 Fruit shape**

Shape of fruits was found as elongated in CHFPUM-1, CHFPUM-6, CHFPUM-16 CHFPUM-20; Round in CHFPUM-2, CHFPUM-3, CHFPUM-4, CHFPUM-5, CHFPUM-8, CHFPUM-9, CHFPUM-13, CHFPUM-15, CHFPUM-18, CHFPUM-19, CHFPUM-21 and CHFPUM-23; Flattened in CHFPUM-7, CHFPUM-14, CHFPUM-22 and CHFPUM-25; Pyriform CHFPUM-10; Oblong in CHFPUM-11 and CHFPUM-17; Oval in CHFPUM-12 and CHFPUM-24.

#### **4.1.3 Fruit ribs**

The types of fruit ribs present or absent were also observed in genotypes. Intermediate rib present was found in CHFPUM-1, CHFPUM-3, CHFPUM-7, CHFPUM-8, CHFPUM-13, CHFPUM-15 and CHFPUM-18; Superficial in CHFPUM-2, CHFPUM-4, CHFPUM-5, CHFPUM-11, CHFPUM-17, CHFPUM-22 and CHFPUM-23; Deep in CHFPUM-6, CHFPUM-14, CHFPUM-19, CHFPUM-21 and CHFPUM-25. Fruit without rib were also observed in CHFPUM-9, CHFPUM-10, CHFPUM-12, CHFPUM-16 and CHFPUM-24.

#### **4.1.4 Flesh colour**

Creamish white flesh colour was observed in two genotypes viz., CHFPUM-6 and CHFPUM-24; Orange in CHFPUM-15, CHFPUM-16, CHFPUM-17 CHPUM-22 and CHFPUM-25. The remaining 18 genotypes showed yellow flesh colour.

**Table 4.1 Morphological characters of pumpkin genotypes**

<b>S. N.</b>	<b>Genotype</b>	<b>Fruit colour</b>	<b>Fruit shape</b>	<b>Fruit ribs</b>	<b>Flesh colour</b>
1.	<b>CHFPUM-1</b>	Yellow	Elongated	Intermediate	Yellow
2.	<b>CHFPUM-2</b>	Brown	Round	Superficial	Yellow
3.	<b>CHFPUM-3</b>	Yellow	Round	Intermediate	Yellow
4.	<b>CHFPUM-4</b>	Green	Round	Superficial	Yellow
5.	<b>CHFPUM-5</b>	Yellow	Round	Superficial	Yellow
6.	<b>CHFPUM-6</b>	Green	Elongated	Deep	Cream
7.	<b>CHFPUM-7</b>	Green	Flattened	Intermediate	Yellow
8.	<b>CHFPUM-8</b>	Green	Round	Intermediate	Yellow
9.	<b>CHFPUM-9</b>	Green	Round	Absent	Yellow
10.	<b>CHFPUM-10</b>	Green	Pyriform	Absent	Yellow
11.	<b>CHFPUM-11</b>	Yellow	Oblong	Superficial	Yellow
12.	<b>CHFPUM-12</b>	Cream	Oval	Absent	Yellow
13.	<b>CHFPUM-13</b>	Green	Round	Intermediate	Yellow
14.	<b>CHFPUM-14</b>	Green	Flattened	Deep	Yellow
15.	<b>CHFPUM-15</b>	Brown	Round	Intermediate	Orange
16.	<b>CHFPUM-16</b>	Brown	Elongated	Absent	Orange
17.	<b>CHFPUM-17</b>	Green	Oblong	Superficial	Orange
18.	<b>CHFPUM-18</b>	Grey	Round	Intermediate	Yellow
19.	<b>CHFPUM-19</b>	Green	Round	Deep	Yellow
20.	<b>CHFPUM-20</b>	Yellow	Elongated	Absent	Yellow
21.	<b>CHFPUM-21</b>	Green	Round	Deep	Yellow
22.	<b>CHFPUM-22</b>	Yellow	Flattened	Superficial	Orange
23.	<b>CHFPUM-23</b>	Green	Round	Superficial	Yellow
24.	<b>CHFPUM-24</b>	Cream	Oval	Absent	Cream
25.	<b>CHFPUM-25</b>	Green	Flattened	Deep	Orange



**CHFPUM-1**



**CHFPUM-2**



**CHFPUM-3**



**CHFPUM-7**



**CHFPUM-12**



**CHFPUM-15**



**CHFPUM-16**

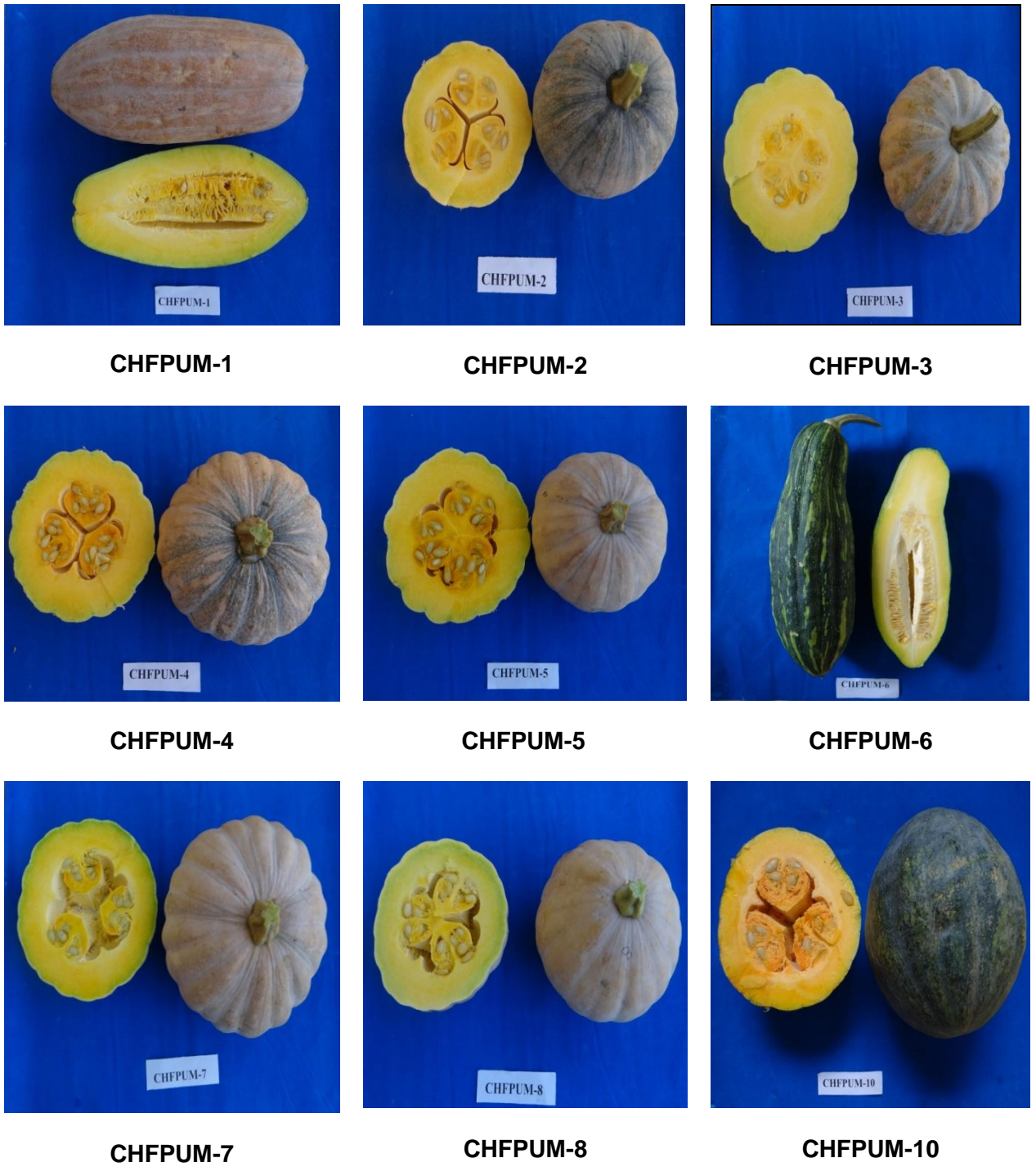


**CHFPUM-17**



**CHFPUM-24**

**Fig 4.1 (a) Glimpse of pumpkin genotypes under study**



**Fig 4.1 (b) Glimpse of pumpkin genotypes under study**



**CHFUM-11**



**CHFUM-12**



**CHFUM-13**



**CHFUM-14**



**CHFUM-15**



**CHFUM-16**



**CHFUM-17**



**CHFUM-18**



**CHFUM-19**

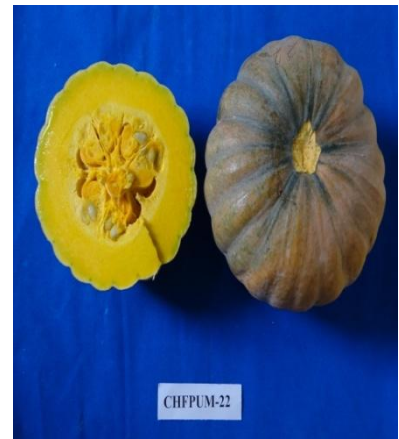
**Fig 4.1 (c) Glimpse of pumpkin genotypes under study**



**CHFUM-20**



**CHFUM-21**



**CHFUM-22**



**CHFUM-23**



**CHFUM-24**



**CHFUM-25**

**Fig 4.1 (d) Glimpse of pumpkin genotypes under study**

## **4.2 Quantitative characters**

### **4.2.1 Mean performance of genotypes**

The analysis of variance for all the traits indicated highly significant variation among the genotypes (Appendix-II) which revealed the existence of sufficient variability in the germplasm. The mean values obtained for various traits under present study are presented in (Table 4.2.). The character *viz* mean performance of different genotypes is presented here as under.

#### **4.2.1.1 Vine length (m)**

Observation recorded on vine length (Table 4.2.a) ranged from 6.36 to 11.36 m with a mean of 7.63 m. maximum vine length was recorded in CHFPUM-12 (11.37) which was at par with CHFPUM-1 (11.00), whereas minimum vine length was recorded in CHFPUM-22 (6.37) followed by CHFPUM-19 (6.57) and CHFPUM-24 (6.6) Out of 25 genotypes, nine genotypes had significantly longer vines when compared to grand mean.

#### **4.2.1.2 Number of primary branches per plant**

Significant variations were obtained among all the genotypes for number of primary branches per plant (Table 4.2.a) which ranged from 8.06 to 13.63 with a mean of 10.95. The highest number of branches was recorded in CHFPUM-2 (13.63) which was at par with CHFPUM-12 (11.97), CHFPUM-3 (11.97), CHFPUM-4 (12.33), CHFPUM-22 (12.73) and CHFPUM-25 (12.87) While CHFPUM-8 (8.06) followed by CHFPUM-13 (8.10) and CHFPUM-19 (9.00). Lowest number of branches per plant had recorded. Out of 25 genotypes, sixteen genotypes had more number of primary branches per plant than the general mean.

#### **4.2.1.3 Days to first stminate flower anthesis**

Observation recorded on days to first staminate flower anthesis (Table 4.2.a) showed ranged from 58.53 to 63.50 days. General mean for the character was (61.27). Among all genotypes, CHFPUM-15 produced its first days to first staminate flower anthesis (58.53 days), it was found statistically at par with CHFPUM-14 (58.83), CHFPUM-4 (58.87), CHFPUM-3 (59.10), CHFPUM-19 (59.40), CHFPUM-16 (59.73), CHFPUM-13 (59.73), CHFPUM-5 (60.53) and CHFPUM-25 (60.63) whereas CHFPUM-2 (63.50) and CHFPUM-18 (63.43) took more days to produced their first satminate flower anthesis. Out of 25 genotypes, thirteen genotypes were early in flowering when compared to average mean of value of (61.27) days to first staminate flower anthesis.

#### **4.2.1.4 Days to first pistillate flower anthesis**

Significant variations were obtained among all the genotypes for days to first pistillate flower anthesis (Table 4.2.a) which ranged from 62.00 to 70.73 days. General mean

for the character was 65.22. Among all genotypes, CHFPUM-5 (62.00) took minimum days to first pistillate flower anthesis. It was found statistically at par with CHFPUM-23 (62.30), CHFPUM-19 (62.53), CHFPUM-14 (62.73), CHFPUM-25 (63.17), CHFPUM-3 (63.40), CHFPUM-21 (63.53), CHFPUM-15 (63.53), CHFPUM-9 (63.53), CHFPUM-20 (63.73), CHFPUM-8 (63.87) and CHFPUM-13 (63.97) whereas CHFPUM-2 (70.73) and CHFPUM-10 (70.20) took more days to produce their first pistillate flower anthesis. Out of 25 genotypes, sixteen genotypes produce their first pistillate flower anthesis earlier when compared to grand mean (65.22).

#### 4.2.1.5 Node bearing first staminate flower

Significant difference was noticed among genotypes for observation recorded on node bearing to first staminate flower which ranged from 2.73 to 6.33 nodes (Table 4.2.a). General mean for the character was 4.04. Among all genotypes, CHFPUM-24 (2.73) produced their first staminate flower at lowest nodes. It was found statistically at par with CHFPUM-20 (3.00), CHFPUM-21 (3.20), CHFPUM-18 (3.30), CHFPUM-13 (3.30), CHFPUM-17 (3.33), CHFPUM-12 (3.53), CHFPUM-8 (3.53), CHFPUM-14 (3.63), CHFPUM-22 (3.73), CHFPUM-9 (3.73), CHFPUM-3 (3.73) and CHFPUM-7 (3.77) whereas highest number of nodes were recorded in CHFPUM-1 (6.33) and CHFPUM-2 (5.63). Out of 25 genotypes, sixteen genotypes were found to be having less node number to first staminate flower appearance than their general mean (4.04).

**Table 4.2 (a) Mean performances of pumpkin genotypes for yield and yield attributing characters**

S.N ...	Genotypes	Vine Length (m)	Number of branches per plant	Days to first staminate flower anthesis	Days to first pistillate flower anthesis	Node bearing first staminate flower
1	<b>CHFPUM-1</b>	11.00	10.53	62.97	66.17	6.33
2	<b>CHFPUM-2</b>	7.10	13.63	63.50	70.73	5.63
3	<b>CHFPUM-3</b>	8.10	11.97	59.10	63.40	3.73
4	<b>CHFPUM-4</b>	7.07	12.33	58.87	66.33	4.40
5	<b>CHFPUM-5</b>	6.70	9.53	60.53	62.00	5.07
6	<b>CHFPUM-6</b>	8.50	11.10	62.07	67.73	5.00

7	<b>CHFPUM-7</b>	7.33	11.53	61.73	64.63	3.77
8	<b>CHFPUM-8</b>	7.77	8.07	61.10	63.87	3.53
9	<b>CHFPUM-9</b>	7.40	10.97	60.73	63.53	3.73
10	<b>CHFPUM-10</b>	7.88	9.67	63.40	70.20	4.73
11	<b>CHFPUM-11</b>	7.20	11.33	63.07	69.07	4.73
12	<b>CHFPUM-12</b>	11.37	11.97	62.10	66.53	3.53
13	<b>CHFPUM-13</b>	7.90	8.10	59.73	63.97	3.30
14	<b>CHFPUM-14</b>	6.70	9.53	58.83	62.73	3.63
15	<b>CHFPUM-15</b>	7.67	10.07	58.53	63.53	4.43
16	<b>CHFPUM-16</b>	7.90	11.73	59.73	65.00	4.50
17	<b>CHFPUM-17</b>	8.57	10.83	61.20	64.87	3.33
18	<b>CHFPUM-18</b>	6.83	10.97	63.43	70.10	3.30
19	<b>CHFPUM-19</b>	6.57	9.00	59.40	62.53	3.93
20	<b>CHFPUM-20</b>	6.90	11.10	62.77	63.73	3.00
21	<b>CHFPUM-21</b>	7.07	11.53	62.07	63.53	3.20
22	<b>CHFPUM-22</b>	6.37	12.73	62.43	66.20	3.73
23	<b>CHFPUM-23</b>	7.03	11.17	60.97	62.30	3.97
24	<b>CHFPUM-24</b>	6.60	11.67	62.87	64.63	2.73
25	<b>CHFPUM-25</b>	7.27	12.87	60.63	63.17	3.97
	<b>Mean</b>	<b>7.63</b>	<b>10.96</b>	<b>61.27</b>	<b>65.22</b>	<b>4.04</b>
	<b>CV %</b>	<b>8.74</b>	<b>10.15</b>	<b>2.09</b>	<b>2.11</b>	<b>17.93</b>
	<b>SEm±</b>	<b>0.38</b>	<b>0.64</b>	<b>0.74</b>	<b>0.79</b>	<b>0.41</b>
	<b>LSD (5%)</b>	<b>1.09</b>	<b>1.82</b>	<b>2.10</b>	<b>2.26</b>	<b>1.19</b>

#### **4.2.1.6 Node bearing first pistillate flower**

Observation recorded on node bearing to first pistillate flower (Table 4.2.b) showed ranged from 10.43 to 14.33. General mean for the character was 12.32. Among all genotypes, CHFUM-24 (10.43) produced the first pistillate flower at lowest nod. It was found statistically at par with the CHFUM-14 (10.77), CHFUM-20 (11.20), CHFUM-1 (11.33), CHFUM-16 (11.37), CHFUM-12 (11.77), CHFUM-21 (11.83), CHFUM-18 (11.87), CHFUM-5 (12.00), CHFUM-15 (12.07), CHFUM-25 (12.10) ,CHFUM-9 (12.10), CHFUM-3 (12.10), CHFUM-13 (12.13), CHFUM-7 (12.20), CHFUM-11 (12.27) and CHFUM-2 (12.30) whereas highest number of nodes were recorded in CHFUM-23 (14.33) and CHFUM-19 (14.13). Out of 25 genotypes, seventeen genotypes were found to have less node number to first pistillate flower appearance when compared to as average mean (12.32).

#### **4.2.1.7 Days to first fruit harvest**

Data recorded on days to first fruit harvest showed significant variations among the genotypes (Table 4.2.b). Days to first fruit harvest ranged from 79.20 to 103.33 days. General mean for the character was (86.90). Among all genotypes, the minimum days to first fruit harvest was recorded in CHFUM-21 (79.20) which was statistically at par with CHFUM-23 (81.10), CHFUM-24 (81.30), CHFUM-14 (81.40), CHFUM-13 (82.00), CHFUM-22 (82.20), CHFUM-15 (82.37) and CHFUM-20 (82.70) while CHFUM-2 (103.33) and CHFUM-11 (93.43) took more days to produce first fruit harvest. Out of 25 genotypes, thirteen genotypes recorded earlier harvest when compared to grand mean (86.90).

#### **4.2.1.8 Number of fruits per plant**

Significant differences were noticed among genotypes for number of fruits per plant (Table 4.2.b). The number of fruits per plant ranged from 1.43 to 3.73. General mean for the character was (2.66). Out of 25 genotypes, maximum number of fruits per plant was recorded in CHFUM-2 (3.73) and it was found statistically at par with CHFUM-16 (3.57), CHFUM-19 (3.57) and CHFUM-13 (3.40). While minimum number of fruits was noted in CHFUM-12 (1.43) and CHFUM-7 (1.63). Among 25 genotypes, thirteen genotypes had more number of fruits as compared to average number of fruits per plant (2.66).

#### **4.2.1.9 Polar circumference (cm)**

All the genotypes studied indicated significant variations for polar circumference (Table 4.2.b). The range of polar circumference was observed from 41.93 to 68.93. General mean for the character was (58.83). Among all genotypes, the maximum polar circumference was recorded in CHFUM-20 (68.93) which was statistically at par with CHFUM-10 (67.63), CHFUM-23 (66.80), CHFUM-6 (66.10) and CHFUM-19 (64.93)

while the lowest polar circumference was noted in CHFPUM-8 (41.93) and CHFPUM-5 (50.00). Out of 25 genotypes, twelve genotypes had maximum polar circumference when compared to grand mean (58.83).

#### 4.2.1.10 Equatorial circumference (cm)

Significant differences were noticed among genotypes for equatorial circumference (Table 4.2.b). Equatorial circumference ranged from 33.93 to 73.8 cm.

General mean for the character was (56.57). The highest equatorial circumference was noted in CHFPUM- 23 (73.80) followed by CHFPUM-10 (67.93) and CHFPUM-21 (64.13) while the minimum equatorial circumference was observed in CHFPUM-16 (33.93) followed by CHFPUM-1 (37.80) and CHFPUM-20 (40.30). Out of 25 genotypes, sixteen genotypes had highest equatorial circumference as compared to grand mean (56.57).

**Table 4.2 (b) Mean performances of pumpkin genotypes for yield and yield attributing characters**

S. N.	Genotypes	Node bearing first staminate flower	Days to first harvest	Number of fruits per plant	Polar circumference (cm)	Equatorial circumference (cm)
1	CHFPUM-1	11.33	88.87	3.37	62.67	37.80
2	CHFPUM-2	12.30	103.33	3.73	52.90	51.50
3	CHFPUM-3	12.10	87.60	2.90	55.10	60.00
4	CHFPUM-4	14.00	89.67	2.33	60.13	63.50
5	CHFPUM-5	12.00	86.67	2.07	50.00	51.63
6	CHFPUM-6	13.60	91.43	2.87	66.10	46.80
7	CHFPUM-7	12.20	87.03	1.63	52.30	61.00
8	CHFPUM-8	13.33	87.63	2.77	41.93	48.10
9	CHFPUM-9	12.10	89.10	3.13	51.00	52.43
10	CHFPUM-10	12.83	93.40	2.90	67.63	67.93
11	CHFPUM-11	12.27	93.43	2.80	57.30	58.81
12	CHFPUM-12	11.77	90.20	1.43	63.80	58.30
13	CHFPUM-13	12.13	82.00	3.40	63.60	63.30
14	CHFPUM-14	10.77	81.40	1.87	56.80	63.13

15	<b>CHFPUM-15</b>	12.07	82.37	2.60	55.63	59.30
16	<b>CHFPUM-16</b>	11.37	85.00	3.57	64.13	33.93
17	<b>CHFPUM-17</b>	13.33	85.20	2.50	58.73	61.93
18	<b>CHFPUM-18</b>	11.87	91.10	2.63	58.00	56.10
19	<b>CHFPUM-19</b>	14.13	86.27	3.57	64.93	61.30
20	<b>CHFPUM-20</b>	11.20	82.70	2.00	68.93	40.30
21	<b>CHFPUM-21</b>	11.83	79.20	2.10	59.00	64.13
22	<b>CHFPUM-22</b>	12.73	82.20	2.40	56.93	61.30
23	<b>CHFPUM-23</b>	14.33	81.10	2.70	66.80	73.80
24	<b>CHFPUM-24</b>	10.43	81.30	2.13	63.63	58.50
25	<b>CHFPUM-25</b>	12.10	84.50	3.13	52.80	59.43
	<b>Mean</b>	<b>12.33</b>	<b>86.91</b>	<b>2.66</b>	<b>58.83</b>	<b>56.57</b>
	<b>CV %</b>	<b>10.11</b>	<b>2.76</b>	<b>9.21</b>	<b>4.29</b>	<b>3.23</b>
	<b>SEm±</b>	<b>0.71</b>	<b>1.38</b>	<b>0.14</b>	<b>1.45</b>	<b>1.05</b>
	<b>LSD (5%)</b>	<b>2.04</b>	<b>3.94</b>	<b>0.40</b>	<b>4.14</b>	<b>3.00</b>

#### 4.2.1.11 Flesh thickness (cm)

All the genotypes studied indicated significant variations for flesh thickness (Table 4.2.c) ranged from 2.33 to 5.16. General mean for the character was (3.40). Out of 25 genotypes, the data pertaining to flesh thickness was recorded maximum in CHFPUM-12 (5.17) which was statistically at par with CHFPUM-17(4.76) whereas minimum flesh thickness was found in CHFPUM-20 (2.33) followed by CHFPUM-6 (2.43) and CHFPUM-16 (2.60). Out of 25 genotypes, ten genotypes had more flesh thickness when compared to average mean flesh thickness (3.40).

#### 4.2.1.12 Cavity length (cm)

Significant variations among genotypes for cavity length (Table 4.2.c) the cavity length of fruits was observed in range from 5.93 to 23.56. General mean for the character was (11.76). Out of 25 genotypes, minimum cavity length was noted in CHFPUM-5 (5.93) followed by CHFPUM-9 (7.63) and CHFPUM-2 (7.76) while the maximum cavity length was found in CHFPUM-20 (23.57) followed by CHFPUM-1 (18.57) and CHFPUM-6 (18.17). Among the 25 genotypes, eighteen genotypes had minimum cavity length when compared to grand mean (11.76).

#### 4.2.1.13 Number of seed per fruit

Data recorded on number of seed per fruit showed significant variations among the genotypes (Table 4.2.c). The number of seeds per fruit ranged from 134.33 to 401.10 seed. General mean for the character was 237.12. Among all the genotypes, minimum number of seed per fruit was recorded in CHFPUM-23 (134.33) followed by CHFPUM-6 (183.33) and CHFPUM-8 (186.07) while the maximum number of seed per fruit was recorded in CHFPUM-5 (401.10) followed by CHFPUM-2 (318.33) and CHFPUM-14 (314.00). Out of 25 genotypes, seventeen genotypes had less number of seed per fruit when compared to average seed per fruit (237.12).

#### 4.2.1.14 100 seed weight (g)

All the genotypes studied resulted in significant differences for 100 seed weight (Table 4.2.c). The 100 seed weight per fruit range was noted from 4.76 to 14.56 g. General mean for the character was 8.94. Out of 25 genotypes, maximum seed weight was noted in CHFPUM-10 (15.57) which was statically at par with CHFPUM-23 (14.37) while the minimum 100 seed weight was recorded in CHFPUM-8 (4.77) followed by CHFPUM-6 (5.06) and CHFPUM-9 (5.83). Out of 25 genotypes, thirteen genotypes had recorded higher weight of 100 seed when compared to grand mean (8.94).

#### 4.2.1.15 Average fruit weight (kg)

Significant differences were noticed among genotypes for average fruit weight (Table 4.2.c). The average fruit weight among all the genotypes range from 1.36 to 3.00 kg. General mean for the character was 2.18 kg. Out of 25 genotypes, maximum average fruit weight was found in CHFPUM-23 (3.00) it was found statistically at par with CHFPUM-14 (2.90), CHFPUM-19 (2.90), CHFPUM-12 (2.87), CHFPUM-15 (2.75), CHFPUM-11 (2.70) and CHFPUM-24 (2.63) while the minimum average fruit weight was observed in CHFPUM-2 (1.36) and CHFPUM-1 (1.57). Out of 25 genotypes, twelve genotypes had noted higher average fruit weight when compared to mean (2.18).

**Table 4.2 (c) Mean performances of pumpkin genotypes for yield and yield attributing characters**

S.N.	Genotypes	Flesh thickness (cm)	Cavity length (cm)	Number of seed per fruit	100 seed weight (gm)	Average fruit weight (kg)
1	CHFPUM-1	2.77	18.57	209.67	9.67	1.57
2	CHFPUM-2	3.23	7.77	318.33	8.20	1.37
3	CHFPUM-3	4.50	10.17	213.33	7.57	2.00
4	CHFPUM-4	4.50	11.90	205.00	9.80	1.82

5	<b>CHFPUM-5</b>	2.77	5.93	401.00	8.37	1.60
6	<b>CHFPUM-6</b>	2.43	18.17	183.33	5.07	2.00
7	<b>CHFPUM-7</b>	3.67	10.23	301.67	9.23	1.70
8	<b>CHFPUM-8</b>	2.90	8.67	186.67	4.77	1.65
9	<b>CHFPUM-9</b>	3.87	7.63	290.00	5.83	2.15
10	<b>CHFPUM-10</b>	3.77	11.27	263.33	14.57	2.37
11	<b>CHFPUM-11</b>	2.80	11.53	226.67	6.97	2.70
12	<b>CHFPUM-12</b>	5.17	10.60	198.33	6.03	2.87
13	<b>CHFPUM-13</b>	2.83	11.57	229.33	10.30	2.07
14	<b>CHFPUM-14</b>	4.30	13.27	314.00	11.23	2.90
15	<b>CHFPUM-15</b>	3.23	10.53	236.67	10.37	2.75
16	<b>CHFPUM-16</b>	2.60	18.17	209.33	8.97	1.70
17	<b>CHFPUM-17</b>	4.77	8.07	206.00	12.03	1.77
18	<b>CHFPUM-18</b>	3.23	9.50	296.67	6.47	2.47
19	<b>CHFPUM-19</b>	2.83	10.50	218.33	6.23	2.90
20	<b>CHFPUM-20</b>	2.33	23.57	206.67	11.50	1.67
21	<b>CHFPUM-21</b>	2.93	10.50	188.67	7.93	2.50
22	<b>CHFPUM-22</b>	3.33	9.77	189.67	9.90	2.40
23	<b>CHFPUM-23</b>	3.50	10.77	134.33	14.37	3.00
24	<b>CHFPUM-24</b>	3.63	14.73	204.33	7.10	2.63
25	<b>CHFPUM-25</b>	3.17	10.83	296.67	11.03	2.20
	<b>Mean</b>	<b>3.40</b>	<b>11.76</b>	<b>237.12</b>	<b>8.94</b>	<b>2.18</b>
	<b>CV %</b>	<b>9.69</b>	<b>4.48</b>	<b>10.01</b>	<b>6.42</b>	<b>12.98</b>
	<b>SEm±</b>	<b>0.19</b>	<b>0.30</b>	<b>13.71</b>	<b>0.33</b>	<b>0.16</b>
	<b>LSD (5%)</b>	<b>0.54</b>	<b>0.86</b>	<b>39.00</b>	<b>0.94</b>	<b>0.46</b>

#### **4.2.1.16 Fruit yield per plant (kg)**

Observation recorded on fruit yield per plant showed significant variation among the genotypes (Table 4.2.d). The fruit yield per plant ranged from 2.83 to 7.36 kg. General mean for the character was 5.13 kg. Out of 25 genotypes, the maximum fruit yield per plant was recorded in CHFPUM-16 (7.36) and it was found statistically at par with and CHFPUM-19 (7.07) while the minimum fruit yield per plant was found in CHFPUM-5 (2.83)

followed by CHFUM-12 (3.03) and CHFUM-8 (3.40). Out of 25 genotypes, fourteen genotypes had noted more fruit yield per plant when compared with grand mean (5.13).

#### **4.2.1.17 Total fruits yield (q/ha)**

All the genotypes studied resulted in significant differences for fruit yield per hectare (Table 4.2.d). The Total fruits yield per hectare was recorded in ranged from 121.06 to 314.76 quintal per hectare. General mean for this attribute was 219.16. Among all the genotypes, maximum fruit yield per hectare was recorded in CHFUM-16 (314.76) which was found statically at par with CHFUM-19 (301.93) while the minimum fruit yield per hectare was found in CHFUM-5 (121.07) followed by CHFUM-12 (129.60) and CHFUM-8 (145.23). Out of 25 genotypes, thirteen genotypes had maximum fruit yield per hectare as compared to average mean (219.16).

#### **4.2.1.18 Total soluble solids (°Brix)**

Observation recorded on Total soluble solids (TSS) showed significant variation among the genotypes (Table 4.2.d). Total soluble solids (TSS) ranged from 5.60 to 8.66<sup>°B</sup>. General mean for the character was 7.01. Out of 25 genotypes, maximum TSS was observed in CHFUM-17 (8.66) followed by CHFUM-2 (8.57) and CHFUM-5 (8.23), CHFUM-14 (7.93), CHFUM-9 (7.83), CHFUM-25 (7.77), CHFUM-1 (7.63), CHFUM-20 (7.50), CHFUM-19 (7.30), CHFUM-3 (7.27), CHFUM-4 (7.10), CHFUM-7 (7.10), CHFUM-16 (6.90) and CHFUM-21 (6.90) while the minimum TSS was recorded in CHFUM-11 (5.60) and CHFUM-10 (5.66). Out of 25 genotypes, twelve genotypes gave higher total soluble solids (TSS) than population mean (7.01).

#### **4.2.1.19 Total carbohydrates (mg/100g)**

All the genotypes studied resulted in significant differences for total carbohydrate content (Table 4.2.d) which ranged from 126 to 559.50 mg. General mean for the character was 481.14. Among all genotypes, maximum carbohydrate was estimated in CHFUM-22 (559.50) it was found statically at par with CHFUM-21 (550.50), CHFUM-20 (547.50), CHFUM-19 (541.50) and CHFUM-17 (540.00) while the minimum carbohydrates was estimated in CHFUM-24 (126.00) and Out of 25 genotypes, nineteen genotypes expressed significantly higher content of carbohydrates when compared to as average mean (481.14).

#### **4.2.1.20 Vitamin A (mg/100g)**

Observation on vitamin A showed significant variation among the genotypes (Table 4.2.d) which ranged from 0.52 to 14.57 mg. General mean for the character was 6.68. Out of 25 genotypes; maximum vitamin A content was estimated in CHFUM-1 (14.57) followed by CHFUM-7 (13.85) and CHFUM-4 (13.79) while the lowest content of vitamin A was found in CHFUM-19 (0.52) followed by CHFUM-22 (0.53) and CHFUM-14 (0.77). Among all

genotypes, eleven genotypes were found to have maximum vitamin A content when compared to grand mean (6.68).

**Table 4.2 (d) Mean performances of pumpkin genotypes for yield and yield attributing characters**

S. N.	Genotypes	Fruits yield per plant (kg)	Total fruits yield (q/ha)	Total Soluble Solids ( <sup>o</sup> Brix)	Carbohydrates (mg/100g)	Vitamin A (mg/100)
1	CHFPUM-1	4.97	212.20	7.63	480.00	14.57
2	CHFPUM-2	5.83	249.03	8.57	498.00	13.69
3	CHFPUM-3	5.70	210.20	7.27	529.50	13.61
4	CHFPUM-4	4.66	199.10	7.10	502.50	13.79
5	CHFPUM-5	2.83	121.07	8.23	507.00	12.65
6	CHFPUM-6	5.20	222.20	6.67	355.50	12.84
7	CHFPUM-7	4.23	180.87	7.10	510.00	13.85
8	CHFPUM-8	3.40	145.23	6.30	438.00	12.32
9	CHFPUM-9	5.83	249.23	7.83	508.50	12.99
10	CHFPUM-10	6.27	267.77	5.67	375.00	12.82
11	CHFPUM-11	6.03	257.80	4.67	522.00	1.42
12	CHFPUM-12	3.03	129.60	5.83	514.50	1.21
13	CHFPUM-13	5.17	220.73	6.80	526.50	3.93
14	CHFPUM-14	4.83	206.50	7.93	510.00	0.77
15	CHFPUM-15	6.43	274.67	6.50	534.00	1.43
16	CHFPUM-16	7.37	314.77	6.90	531.00	6.53
17	CHFPUM-17	4.63	197.97	8.67	540.00	7.36
18	CHFPUM-18	5.97	254.97	5.97	511.50	1.57
19	CHFPUM-19	7.07	301.93	7.37	541.50	0.52
20	CHFPUM-20	3.60	153.80	7.50	547.50	2.09
21	CHFPUM-21	3.80	162.33	6.90	550.50	2.50

22	<b>CHFPUM-22</b>	6.00	256.17	6.00	559.50	0.53
23	<b>CHFPUM-23</b>	5.77	246.17	6.53	273.00	1.74
24	<b>CHFPUM-24</b>	3.90	166.60	6.37	126.00	1.25
25	<b>CHFPUM-25</b>	5.73	244.97	7.77	537.00	1.22
	<b>Mean</b>	<b>5.13</b>	<b>219.16</b>	<b>7.01</b>	<b>481.14</b>	<b>6.68</b>
	<b>CV %</b>	<b>5.92</b>	<b>5.93</b>	<b>10.55</b>	<b>2.84</b>	<b>3.54</b>
	<b>SEm±</b>	<b>0.17</b>	<b>7.50</b>	<b>0.42</b>	<b>7.90</b>	<b>0.13</b>
	<b>LSD (5%)</b>	<b>0.49</b>	<b>28.48</b>	<b>1.21</b>	<b>22.46</b>	<b>0.38</b>

## 4.2.2 Variability of parameters

In order to determine the scope and response of selection for various traits, the estimates of coefficients of variability at phenotypic (PCV) and genotypic (GCV) level, heritability (broad sense), genetic advance and genetic advance as percentage of the mean (genetic gain) were presented in (Table 4.3 and Figure 4.2, Figure 4.3).

### 4.2.2.1 Coefficients of variability

The perusal of the data presented in the (Table 4.3 and figure 4.3) indicated that phenotypic coefficients of variability were higher in magnitude than their corresponding genotypic coefficients of variability for all the characters.

Phenotypic and genotypic variance were highest (10172.68 and 9985.36) for carbohydrates mg/100g and the lowest phenotypic and genotypic variance was recorded in (0.28 and 0.22) for average fruit weight.

The highest estimates of phenotypic (PCV) and genotypic (GCV) coefficient of variation were observed for vitamin A (PCV=86.19%, GCV=86.12%) followed by cavity length (PCV=35.52%, GCV=34.24%), 100 seed weight (PCV=30.04%, GCV=29.37%), number of seeds per fruit (PCV=26.09%, GCV=24.09%), node bearing first staminate flower (PCV=25.60%, GCV=18.36%), total fruit yield q/ha (PCV=24.74%, GCV=23.38%), average fruit weight (PCV=24.42%, GCV=22.16%), fruit yield per plant (PCV=24.22%, GCV=23.58%), number of fruits per plant (PCV=24.07%, GCV=22.87%), flesh thickness (PCV=23.53%, GCV=20.16%) and Carbohydrate (PCV=20.97%, GCV=20.77%) However, the moderate estimates of PCV and GCV were recorded in case of total soluble solids (PCV=18.84%, GCV= 10.26%) followed by vine length (PCV=17.50%, GCV= 15.19%), equatorial circumference (PCV=16.89%, GCV=16.49%), number of primary branches (PCV=15.25%, GCV=11.39%), node bearing first staminate flower (PCV=11.63%, GCV=5.76%) and polar circumference (PCV=11.63%, GCV=10.81%). The lowest estimates of PCV and GCV were recorded in case of days to first fruit harvest (PCV=6.580%, GCV= 5.88%), days to first staminate flower anthesis (PCV=4.32%, GCV=3.76%) and days to first staminate flower anthesis (PCV=3.15%, GCV= 2.30%).

### 4.2.2.2 Heritability (%)

Heritability (bs) estimates ranged from 100% to 25%. In the present investigation, all of the characters studied recorded high heritability estimates (>60%). Higher heritability estimates was recorded for vitamin A (100%) followed by cavity length (98.00%), carbohydrates (98%), 100 seed weight (96%), fruit yield per plant (95%), number of fruit per plant (90%), total fruit yield q per hectare (89%), polar circumference (86%), equatorial

circumference (68%), number of seed per fruit (85%), flesh thickness (84%), days to first fruit harvest (82%), average fruit weight (82%), days to first staminate flower anthesis (76%) and vine length (75%).

#### **4.2.2.3 Genetic advance**

In the present investigation, genetic advance was high for carbohydrate (203.95) followed by number of seed per fruit (108.65) and total fruit yield q/ha (99.12). However, equatorial circumference (18.86), polar circumference (12.18) and vitamin A (11.86) showed moderate genetic gain.

#### **4.2.2.4 Genetics advance as percentage of mean**

Genetic gain is the genetic advance expressed as percent of population mean. In the present investigation, genetic gain was high for vitamin A (177.27%) followed by cavity length (69.98%), 100 seed weight (59.17%), fruit yield per plant (47.36%), number of seed per fruit (45.82%), total yield q per hectare (45.50%), number of fruit per plant (44.73%), carbohydrate (42.38%), average fruit weight (41.28%), flesh thickness (40.88%), equatorial circumference (33.33%), vine length (27.12%) and polar circumference (20.70%). However, number of primary branches (17.53%), node bearing first staminate flower (14.07%), total soluble solid (12.06%) and days to first harvest (10.95%) showed moderate genetic gain.

**Table 4.3 Genetic parameters of yield and its component characters in pumpkin**

S.N	Characters	Mean ± SE(m)	Range		Variance		Coefficient of variability (%)		Heritability %	Genetic advance	Genetic advance as % of mean (Genetic gain)
			Min	Max	Phenotypic	Genotypic	PCV	GCV			
1	Vine length (m)	7.63±0.38	6.36	11.36	1.78	1.34	17.50	15.19	75	2.07	27.13
2	Number of primary branches	10.95±0.64	8.06	13.63	2.77	1.54	15.25	11.39	56	1.92	17.53
3	Days to first staminate flower anthesis	61.27±0.74	58.53	63.50	3.68	2.04	3.15	2.30	54	2.17	3.54
4	Days to first pistillate flower anthesis	65.22±0.79	62.00	70.73	7.93	6.02	4.32	3.76	76	4.40	6.75
5	Node bearing first staminate flower	4.04±0.41	2.73	6.33	1.07	0.55	25.60	18.36	51	0.57	14.06
6	Node bearing first staminate flower	12.32±0.71	10.43	14.33	2.05	0.50	11.63	5.76	25	0.72	5.84
7	Days to first fruit harvest	86.90±1.38	70.20	103.33	31.88	26.12	6.50	5.88	82	9.53	10.97
8	Number of fruits per plant	2.66±0.12	1.43	3.73	0.41	0.37	24.07	22.87	90	1.19	44.74
9	Polar circumference (cm)	58.83±1.45	41.93	68.93	46.83	40.46	11.63	10.81	86	12.18	20.70
10	Equatorial circumference (cm)	56.57±1.05	33.93	78.30	90.38	87.04	16.89	16.49	86	18.86	33.34
11	Flesh thickness (cm)	3.40±0.19	2.33	5.16	0.64	0.54	23.53	20.16	84	1.39	40.88
12	Cavity length (cm)	11.76±0.30	5.93	23.56	16.48	16.28	35.52	34.24	98	8.23	69.98
13	Number of seed per fruit	237.12±13.1	134.33	401.10	3827.44	3263.08	26.09	24.09	85	108.65	45.82
14	100 seed weight (g)	8.94±0.33	4.77	14.57	7.21	6.89	30.04	29.37	96	5.29	59.17
15	Average fruit weight (kg)	2.18±0.13	1.37	3.00	0.28	0.23	24.42	22.16	82	0.90	41.28
16	Fruit yield per plant (kg)	5.13±0.16	2.83	7.37	1.54	1.46	24.22	23.58	95	2.43	47.37
17	Total fruit yield per hectare (q)	219.16±10.18	121.7	314.77	2904.11	2592.90	24.74	23.38	89	99.12	45.50
18	Total soluble solids ( <sup>o</sup> Brix)	6.96±0.63	4.67	8.67	1.72	0.51	18.84	10.26	30	0.84	12.07
19	Carbohydrates (mg/100g)	481.14±7.90	126.00	559.50	10172.6 2	9985.36	20.97	20.77	98	203.95	42.39
20	Vitamin A (mg/100g)	6.63±0.11	0.52	14.57	33.21	33.18	86.27	86.23	100	11.86	177.28

### Genetic Parameters

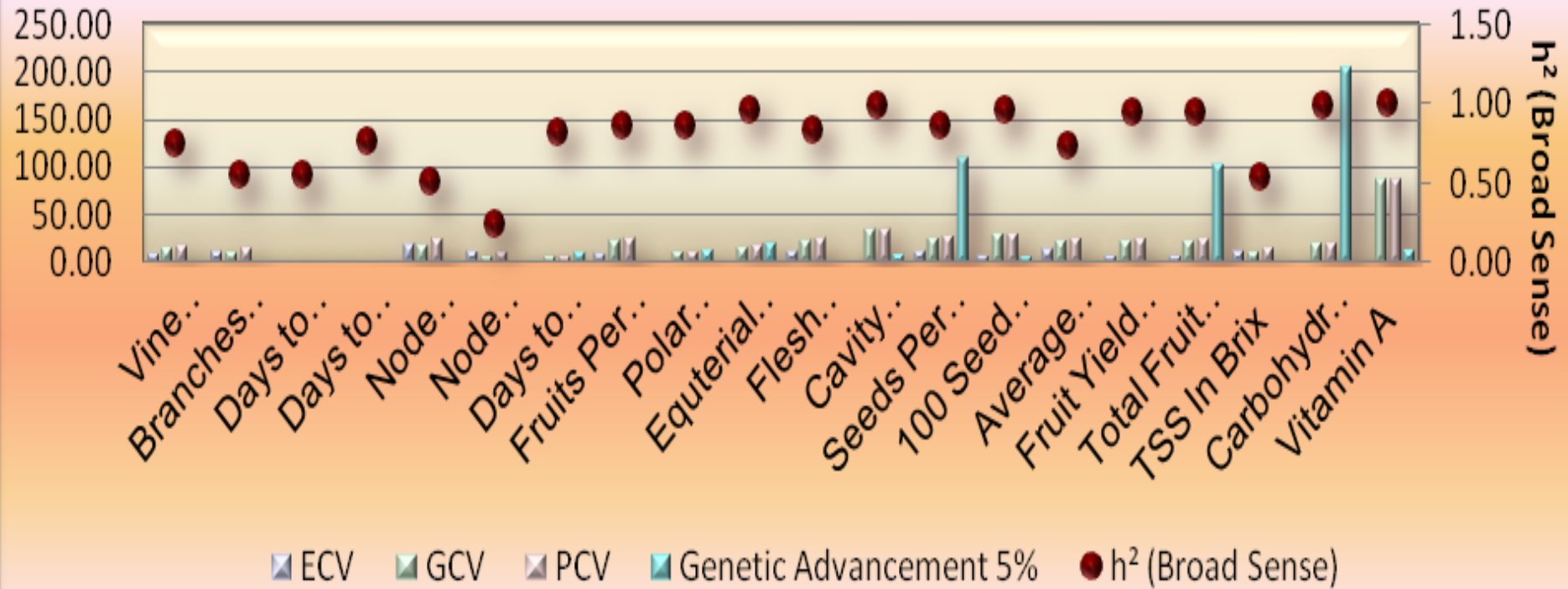


Fig 4.2 Genetic parameters for yield and yield attributing characters in pumpkin



**Fig 4.3 Glimpse of 25 variables in pumpkin**

### **4.2.3 Correlation studies**

The phenotypic ( $r_p$ ) and genotypic ( $r_g$ ) correlation coefficients among different characters were estimated for all possible combinations (Table 4.4 and 4.5). In general, it was observed that that genotypic correlation coefficient ( $r_g$ ) values were higher in magnitude than that of phenotypic correlation coefficient ( $r_p$ ) values.

#### **4.2.3.1 Vine length (m)**

The vine length had positive and significant phenotypic correlation ( $r_p$ ) with node bearing first staminate flower (0.284) and vitamin A content (0.229) where as it had negative significant phenotypic correlation with equatorial circumference of fruit (-0.279). However, vine length did not show either positive or negative and significant correlation at genotypic correlation ( $r_g$ ).

#### **4.2.3.2 Number of primary branches per plant**

Numbers of primary branches per plant had positive and significant phenotypic correlation ( $r_p$ ) with days to first pistillate flower anthesis (0.233) and none of the traits established significant and negative associated with number of branches per plant while number of primary branches had positive and significant genotypic correlation with days to first pistillate flower anthesis (0.400) while negative and significant correlation did not showed for number of primary branches at genotypic correlation ( $r_g$ ).

#### **4.2.3.3 Days to first staminate flower anthesis**

Days to first staminate flower anthesis had positive and significant phenotypic correlation ( $r_p$ ) with days to first pistillate flower anthesis (0.551), days to first fruit harvest (0.327) while none of the traits showed significant and negative associated with days to first staminate flower anthesis.

However, At genotypic correlation ( $r_g$ ) days to first staminate flower anthesis showed positive and significant correlation with days to first pistillate flower anthesis (0.758) and days to first fruit harvest (0.491) while negative and significant correlation did not showed for days to first staminate flower anthesis.

#### **4.2.3.4 Days to first pistillate flower anthesis**

Days to first pistillate flower anthesis showed positive and significant correlation at phenotypic level ( $r_p$ ) with node bearing first staminate flower (0.296) and days to first fruit harvest (0.709) whereas no any traits showed significant and negative associated with days to first pistillate flower anthesis.

However, Days to first pistillate flower anthesis at genotypic level ( $r_g$ ) had positive and significant correlation was observed with node bearing first staminate flower (0.440) and days to first fruit harvest (0.815) while negative and significant genotypic correlation ( $r_g$ ) did not show for the days to first pistillate flower anthesis.

#### **4.2.3.5 Node bearing first staminate flower**

Node bearing first staminate flower showed positive and significant phenotypic correlation ( $r_p$ ) with days to first fruit harvest (0.436), number of fruits per plant (0.353) and vitamin A (0.442) while negative and significant phenotypic correlation ( $r_p$ ) with equatorial circumference (-0.277), flesh thickness (-0.245) and average fruit weight (-0.262).

The positive and significant correlation was observed with days to first fruit harvest (0.709), number of fruits per plant (0.540) and vitamin A content (0.608) but negative and significant correlation with fruit equatorial circumference (-0.408), average fruit weight (-0.438) at genotypic level ( $r_g$ ).

#### **4.2.3.6 Node bearing first pistillate flower**

Node bearing first pistillate flower recorded positive and significant correlation with equatorial circumference (0.235) while it was negatively correlated with cavity length (-0.233) at phenotypic level ( $r_p$ ).

Node bearing first pistillate flower was recorded positive and significant correlation with number of fruits per plant (0.382), equatorial circumference (0.567) while it was negatively significant correlated with cavity length (-0.452), number of seeds per fruit (-0.504) at genotypic level ( $r_g$ ).

#### **4.2.3.7 Days to first fruit harvest**

Days to first fruit harvest showed positive and significant correlation with number of fruits per plant (0.292), number of seeds per fruit (0.267) and vitamin A (0.500) while negative and significant correlation was observed with 100 seed weight (-0.250) and average fruit weight (-0.332) at phenotypic level ( $r_p$ ).

However, at genotypic level days to first fruit harvest showed positive and significant correlation with number of fruits per plant (0.382) and vitamin A (0.555) while negative and significant correlation was not observed with days to first fruit harvest.

#### **4.2.3.8 Number of fruits per plant**

Number of fruits per plant exhibited positive and significant correlation with fruits yield per plant (0.653) and total fruit yield/ha (0.657) but negative and significant

correlation was observed with equatorial circumference (-0.251) and flesh thickness (-0.365) at phenotypic level ( $r_p$ ).

At genotypic level ( $r_g$ ), number of fruits per plant exhibited positive and significant correlation with fruits yield per plant (0.736) and total fruits yield/ha (0.734) while negative and significant correlation with flesh thickness (-0.436).

#### **4.2.3.9 Polar circumference**

At phenotypic level ( $r_p$ ), polar circumference showed positive and significant correlation with cavity length (0.608), 100 seeds weight (0.330) and average fruits weight (0.293) while significant and negative correlation with number of seeds per fruit (-0.448), carbohydrates (-0.274) and vitamin A (-0.293).

Polar circumference exhibited positive and significant correlation with cavity length (0.650) while significant and negative correlation with number of seeds per fruit (-0.489) at genotypic level ( $r_g$ ).

#### **4.2.3.10 Equatorial circumference**

Equatorial circumference showed positive and significant correlation with flesh thickness (0.462), 100 seeds weight (0.359) and average fruit weight (0.542) whereas significant and negative correlation with cavity length (-0.549), total soluble solids (-0.242) and vitamin A (-0.291) at phenotypic level ( $r_p$ ).

However, at genotypic level ( $r_g$ ) equatorial circumference showed positive and significant correlation with flesh thickness (0.521), average fruit weight (0.646) while significant and negative correlation with cavity length (-0.561).

#### **4.2.3.11 Flesh thickness**

Flesh thickness had negative and significant correlation with cavity length (-0.372). There was no significant correlation. At genotypic level ( $r_g$ ) flesh thickness had negative and significant correlation with cavity length (-0.408) while no positive and significant correlation showed for flesh thickness at genotypic level ( $r_g$ ).

#### **4.2.3.12 Cavity length**

Cavity length exhibited negative and significant correlation with number of seeds per fruit (-0.365) while no positive significant showed at phenotypic level ( $r_p$ ) for the cavity length.

However, at genotypic level ( $r_g$ ) cavity length exhibited negative and significant correlation with number of seeds per fruit (-0.402) while no positive and significant correlation showed at genotypic level ( $r_g$ ) for the cavity length.

#### **4.2.3.13 Number of seed per fruit**

At phenotypic level ( $r_p$ ) number of seed per fruit showed positive and significant correlation (0.338) with total soluble solids carbohydrate (0.258). However, at genotypic level ( $r_g$ ) number of seed per fruit exhibited positive and significant correlation with total soluble solids (0.511).

#### **4.2.3.14 100 seed weight**

100 seeds weight did not showed either positive or negative significant correlation with others traits at phenotypic level ( $r_p$ ) while at genotypic level ( $r_g$ ) 100 seeds weight also did not showed any correlation with others traits.

#### **4.2.3.15 Average fruit weight**

Average fruit weight recorded negative and significant correlation with total soluble solids (-0.442) and vitamin A (-0.668). However, at genotypic level ( $r_g$ ) average fruit weight also showed negative and significant correlation with total soluble solids (-0.681) and vitamin A (-0.778).

#### **4.2.3.16 Fruit yield per plant**

Fruit yield per plant showed positive and significant correlation with total fruit yield (0.966). However, at genotypic level ( $r_g$ ) fruit yield per plant showed positive and significant correlation with total fruit yield (1.001).

#### **4.2.3.17 Total fruit yield**

Total fruit yield did not showed correlation with any traits at phenotypic level ( $r_p$ ) and at genotypic level ( $r_g$ ) respectively.

#### **4.2.3.18 Total soluble solid**

Total soluble solids exhibited positive and significant correlation with carbohydrate (0.387) and vitamin A (0.250). However, total soluble solids positive and significant correlation was observed with carbohydrate (0.525) while none any traits showed significant and negative correlation at genotypic level ( $r_g$ ) for the total soluble solids.

#### **4.2.3.19 Carbohydrate**

Carbohydrates did not show correlation at phenotypic level ( $r_p$ ) and at genotypic level ( $r_g$ ) respectively for the carbohydrates.

#### **4.2.3.20 Vitamin A**

On over all basis, it exhibited positive and significant correlation with vine length (0.229), node bearing first staminate flower (0.442), days to first harvest (0.500) and

total soluble solids (0.250) while significant and negatively correlated with polar circumference (-0.293), equatorial circumference (-0.292) and average fruit weight (-0.668) at phenotypic level ( $r_p$ ).

However, positive and significant correlation with node bearing first staminate flower (0.608), days to first harvest (0.555) while significant and negatively correlated with average fruit weight (-0.778) at genotypic level ( $r_g$ ).

**Table 4.4 Phenotypic correlation coefficients ( $r_p$ ) between twenty morphological traits in pumpkin**

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Vine length (m)	1.000	-0.003	0.046	0.137	0.284*	-0.040	0.189	-0.006	0.184	-0.279*	0.219	0.195	-0.197	-0.086	-0.101	-0.151	-0.159	0.001	0.057	0.229*
Number of primary branches		1.000	0.225	0.234*	0.022	-0.156	0.209	-0.034	0.015	-0.032	0.204	0.034	-0.016	0.020	-0.041	0.076	0.074	0.115	0.018	0.007
Days to first staminate flower anthesis			1.000	0.551**	0.121	-0.121	0.328**	-0.079	0.120	-0.141	-0.157	0.118	-0.044	-0.076	-0.089	-0.140	-0.132	-0.093	-0.222	0.034
Days to first pistillate flower anthesis				1.000	0.296**	0.091	0.709**	0.167	0.125	-0.085	0.021	0.019	0.026	-0.101	-0.145	0.213	0.216	-0.179	-0.061	0.190
Node bearing first staminate flower					1.000	0.171	0.436**	0.353**	-0.011	-0.277*	-0.245*	0.058	0.165	0.031	-0.263*	0.211	0.200	0.193	0.067	0.442**
Node bearing first staminate flower						1.000	0.116	0.139	-0.003	0.235*	0.010	-0.233*	-0.220	0.044	0.020	0.165	0.168	-0.138	0.000	0.141
Days to first fruit harvest							1.000	0.292*	-0.132	-0.187	0.043	-0.174	0.267*	-0.251*	-0.332**	0.153	0.151	0.047	0.038	0.501**
Number of fruits per plant								1.000	0.041	-0.251*	-0.365*	0.020	-0.037	-0.041	-0.189	0.654**	0.657**	0.087	0.058	0.203
Polar circumference (cm)									1.000	0.036	-0.080	0.608**	-0.448**	0.330**	0.294*	0.203	0.203	-0.210	-0.274*	-0.294*
Equatorial circumference (cm)										1.000	0.462**	-0.549**	-0.054	0.359**	0.542**	0.052	0.050	-0.242*	-0.154	-0.293*
Flesh thickness (cm)											1.000	-0.372**	0.011	0.133	0.202	-0.165	-0.153	-0.005	-0.006	0.057
Cavity length (cm)												1.000	-0.365**	0.118	-0.106	0.013	0.013	-0.006	-0.160	-0.104
Number of seed per fruit													1.000	0.016	-0.197	-0.065	-0.063	0.339*	0.259*	0.218
100 seed weight (g)														1.000	0.060	0.149	0.149	0.132	-0.047	-0.125
Average fruit weight (kg)															1.000	0.219	0.222	-0.443*	-0.182	-0.668**
Fruit yield per plant (kg)																1.000	0.997	-0.100	0.127	-0.126
Total fruit yield per hectare (q)																	1.000	-0.099	0.128	-0.127
Total soluble solids (%Brix)																		1.000	0.388*	0.250*
Carbohydrates (mg/100 g)																			1.000	-0.031
Vitamin A (mg/100 g)																				1.000

\*, \*\* indicate at 5.0% and 1.0% level of probability, respectively

**Table 4.5 Genotypic correlation coefficients ( $r_g$ ) among twenty characters in pumpkin**

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Vine length (m)	<b>1.000</b>	-0.020	0.204	0.200	0.344	-0.162	0.236	0.006	0.199	-0.306	0.302	0.210	-0.305	-0.118	-0.149	-0.225	-0.221	-0.115	0.061	0.263
Number of primary branches		<b>1.000</b>	0.354	0.400*	0.185	-0.046	0.305	-0.098	0.054	-0.030	0.253	0.058	-0.065	0.002	-0.159	0.136	0.137	0.193	0.034	-0.000
Days to first staminate flower anthesis			<b>1.000</b>	0.758**	0.126	-0.259	0.491**	-0.059	0.201	-0.194	-0.255	0.165	-0.005	-0.073	-0.153	-0.201	-0.207	-0.224	-0.315	0.038
Days to first pistillate flower anthesis				<b>1.000</b>	0.440**	-0.032	0.815**	0.205	0.171	-0.092	0.000	0.027	0.073	-0.099	-0.118	0.250	0.248	-0.266	-0.067	0.215
Node bearing first staminate flower					<b>1.000</b>	0.102	0.709**	0.540**	-0.042	-0.408*	-0.293	0.071	0.281	0.049	-0.438*	0.309	0.317	0.281	0.052	0.608**
Node bearing first pistillate flower						<b>1.000</b>	0.267	0.382*	0.062	0.567**	0.103	-0.452*	-0.504**	0.102	0.068	0.321	0.317	-0.085	-0.059	0.278
Days to first fruit harvest							<b>1.000</b>	0.382*	-0.170	-0.214	0.058	-0.201	0.332	-0.305	-0.363	0.180	0.182	0.155	0.060	0.555**
Number of fruits per plant								<b>1.000</b>	0.026	-0.270	-0.436*	0.011	-0.040	-0.051	-0.260	0.736**	0.734**	0.143	0.069	0.216
Polar circumference (cm)									<b>1.000</b>	0.037	-0.068	0.650**	-0.489*	0.378	0.312	0.203	0.204	-0.344	-0.291	-0.317
Equatorial circumference (cm)										<b>1.000</b>	0.521**	-0.561**	-0.057	0.373	0.646**	0.053	0.053	-0.339	-0.157	-0.295
Flesh thickness (cm)											<b>1.000</b>	-0.408	-0.028	0.137	0.250	-0.172	-0.178	-0.029	-0.009	0.066
Cavity length (cm)												<b>1.000</b>	-0.402*	0.122	-0.136	0.008	0.009	-0.013	-0.160	-0.105
Number of seed per fruit													<b>1.000</b>	-0.046	-0.278	-0.077	-0.077	0.511**	0.275	0.243
100 seed weight (g)														<b>1.000</b>	0.059	0.165	0.164	0.210	-0.046	-0.124
Average fruit weight (kg)															<b>1.000</b>	0.229	0.227	-0.681**	-0.243	-0.778**
Fruit yield per plant (kg)																<b>1.000</b>	1.001**	-0.155	0.128	-0.129
Total fruit yield per hectare (q)																	<b>1.000</b>	-0.155	0.127	-0.128
Total soluble solids ( <sup>o</sup> Brix)																		<b>1.000</b>	0.525**	0.345
Carbohydrates (mg/100 g)																			<b>1.000</b>	-0.031
Vitamin A (mg/100 g)																				<b>1.000</b>

\*, \*\* indicate at 5.0% and 1.0% level of probability, respectively

## 4.2.4 Path coefficient analysis

Path coefficient analysis devised by Dewey and Lu. However, provides a realistic basis for allocation of appropriate weightage to various attributes, while designing a pragmatic programme for the improvement of yield. The data on path coefficient analysis at both phenotypic and genotypic level showing the direct and indirect effects of significant traits on fruit yield per plant. The results have been presented in (Table 4.6 and Figure 4.4, Table 4.7 and Figure 4.5).

### 4.2.4.1 Path coefficient analysis at phenotypic level

A perusal of phenotypic path coefficient analysis showed that maximum direct positive effect on fruit yield per plant was imposed by number of fruits per plant (0.664) followed by days to first pistillate flower anthesis (0.176), carbohydrates (0.171), node bearing first staminate flower (0.144), polar circumference (0.113), flesh thickness (0.104), number of primary branches per plant (0.091), equatorial circumference (0.80), 100 seeds weight (0.71), cavity length (0.66), number of seeds per fruit (0.32) and node bearing first pistillate flower (0.025). While, maximum negative direct effects on fruit yield per plant were recorded for vitamin A (-0.199), vine length (-0.186), total soluble solids (-0.167), days to first staminate flower anthesis (-0.164), days to first fruit harvest (-0.015).

The maximum and positive indirect effect on fruit yield per plant was imposed by number of fruits per plant through node bearing first staminate flower (0.235), number of fruits per plant through days to first fruit harvest (0.194), number of fruits per plant through vitamin A (0.135) and days to first pistillate flower anthesis through days to first fruit harvest (0.125), number of fruits per plant through days to first pistillate flower anthesis (0.110).

The characters like number of fruits per plant showed maximum negative indirect effect on fruit yield per plant through flesh thickness (-0.242) and equatorial circumference (-0.167), vitamin A through days to first fruit harvest (-0.100).

Residual effect at phenotypic level was observed to be 0.595.

### 4.2.4.2 Path coefficient analysis at genotypic level

Days to first pistillate flower anthesis (1.185) had maximum positive direct effect on fruit yield per plant followed by number of fruits per plant (0.681), node bearing first staminate flower (0.293), polar circumference (0.259), total soluble solids (0.133), number of primary branches per plant (0.103) and flesh thickness (0.043). However, maximum negative direct effect on fruit yield per plant was imposed by days to first fruit harvest (-0.763), days to

first staminate flower anthesis (-0.719), cavity length (-0.372), vine length (-0.305), number of seeds per fruit (-0.232), node bearing first staminate flower (-0.220), 100 seeds weight (-0.103), vitamin A (-0.081) carbohydrates (-0.031) and equatorial circumference (-0.002).

The maximum and positive indirect effect on fruit yield per plant was imposed by days to first pistillate flower anthesis through days to first fruit harvest (0.996) and days to staminate flower anthesis (0.899), node bearing first staminate flower (0.522), number of primary branches per plant (0.474). Number of fruits per plant through node bearing first staminate flower (0.368) and node bearing first pistillate flower (0.261), days to first fruit harvest (0.260).

The characters like days to first fruit harvest imposed high negative indirect effect through days to first pistillate flower anthesis (-0.623), node bearing first staminate flower (-0.542), vitamin A (-0.424), days to first staminate flower anthesis (-0.375), number of fruits per plant (-0.292), number of seeds per fruit (-0.254), number of branches per plant (-0.233) and node bearing first pistillate flower (-0.204).

Residual effect at genotypic level was observed to be 0.250

**Table 4.6 Direct (diagonal) and indirect effects of fruit yield components on fruit yield per plant at phenotypic level in pumpkin**

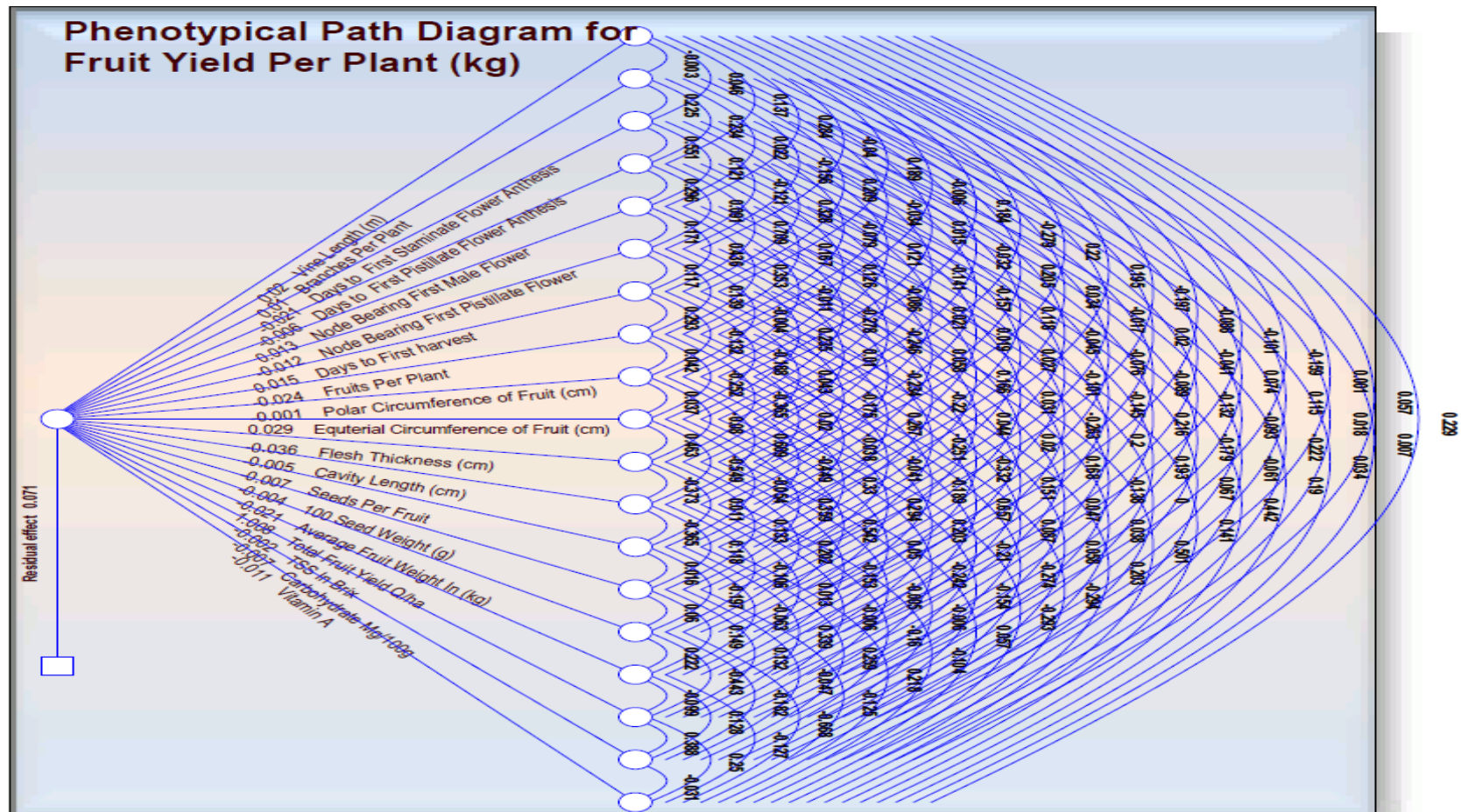
Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Vine length (m)	<b>-0.186</b>	0.001	-0.009	-0.026	-0.053	0.007	-0.035	0.001	-0.034	0.052	-0.041	-0.036	0.037	0.016	0.000	-0.011	-0.043
Number of primary branches	0.000	<b>0.091</b>	0.021	0.021	0.002	-0.014	0.019	-0.003	0.001	-0.003	0.019	0.003	-0.002	0.002	0.011	0.002	0.001
Days to first staminate flower anthesis	-0.008	-0.037	<b>-0.164</b>	-0.091	-0.020	0.020	-0.054	0.013	-0.020	0.023	0.026	-0.019	0.007	0.012	0.015	0.036	-0.006
Days to first pistillate flower anthesis	0.024	0.041	0.097	<b>0.177</b>	0.052	0.016	0.125	0.029	0.022	-0.015	0.004	0.003	0.005	-0.018	-0.032	-0.011	0.033
Node bearing first staminate flower	0.041	0.003	0.018	0.043	<b>0.144</b>	0.025	0.063	0.051	-0.002	-0.040	-0.035	0.008	0.024	0.004	0.028	0.010	0.064
Node bearing first pistillate flower	-0.001	-0.004	-0.003	0.002	0.004	<b>0.025</b>	0.003	0.003	0.000	0.006	0.000	-0.006	-0.006	0.001	-0.003	0.000	0.004
Days to first fruit harvest	-0.003	-0.003	-0.005	-0.011	-0.007	-0.002	<b>-0.015</b>	-0.004	0.002	0.003	-0.001	0.003	-0.004	0.004	-0.001	-0.001	-0.008
Number of fruits per plant	-0.004	-0.023	-0.053	0.111	0.235	0.093	0.195	<b>0.665</b>	0.028	-0.167	-0.243	0.014	-0.025	-0.027	0.058	0.039	0.135
Polar circumference (cm)	0.021	0.002	0.014	0.014	-0.001	0.000	-0.015	0.005	<b>0.114</b>	0.004	-0.009	0.069	-0.051	0.038	-0.024	-0.031	-0.033
Equatorial circumference (cm)	-0.023	-0.003	-0.011	-0.007	-0.022	0.019	-0.015	-0.020	0.003	<b>0.081</b>	0.037	-0.044	-0.004	0.029	-0.020	-0.012	-0.024
Flesh thickness (cm)	0.022	0.021	-0.016	0.002	-0.025	0.001	0.004	-0.038	-0.008	0.048	<b>0.104</b>	-0.038	0.001	0.013	-0.000	-0.000	0.005
Cavity length (cm)	0.013	0.002	0.007	0.001	0.003	-0.015	-0.011	0.001	0.040	-0.036	-0.024	<b>0.066</b>	-0.024	0.007	-0.000	-0.010	-0.006
Number of seed per fruit	-0.006	-0.000	-0.001	0.000	0.005	-0.007	0.008	-0.001	-0.014	-0.001	0.000	-0.011	<b>0.032</b>	0.000	0.011	0.008	0.007
100 seed weight (g)	-0.006	0.001	-0.005	-0.007	0.002	0.003	-0.017	-0.002	0.023	0.025	0.009	0.008	0.001	<b>0.071</b>	0.009	-0.003	-0.008
Total soluble solids	-0.000	-0.019	0.015	0.030	-0.032	0.023	-0.007	-0.014	0.035	0.040	0.000	0.001	-0.056	-0.022	<b>-0.167</b>	-0.065	-0.041
Carbohydrates mg/100 g	0.009	0.003	-0.038	-0.010	0.011	0.000	0.006	0.009	-0.046	-0.026	-0.001	-0.027	0.044	-0.008	0.066	<b>0.171</b>	-0.005
Vitamin A mg/100 g	-0.045	-0.001	-0.006	-0.037	-0.088	-0.028	-0.100	-0.040	0.058	0.058	-0.011	0.020	-0.043	0.024	-0.050	0.006	<b>-0.199</b>
<b>Fruit yield per plant (kg)</b>	<b>-0.151</b>	<b>0.076</b>	<b>-0.140</b>	<b>0.213</b>	<b>0.211</b>	<b>0.164</b>	<b>0.152</b>	<b>0.653</b>	<b>0.202</b>	<b>0.051</b>	<b>-0.165</b>	<b>0.013</b>	<b>-0.064</b>	<b>0.149</b>	<b>-0.099</b>	<b>0.126</b>	<b>-0.126</b>
Partial R <sup>2</sup>	0.028	0.006	0.023	0.037	0.030	0.004	-0.002	0.434	0.023	0.004	-0.017	0.000	-0.002	0.010	0.016	0.021	0.025

R square = 0.6459

Residual effect = 0.595

Where,

1. Vine length (m), 2. Number of branches per plant, 3. Days to first staminate flower anthesis, 4. Days to first pistillate flower anthesis, 5. Node bearing first staminate flower, 6. Node bearing first pistillate flower, 7. Days to first fruit harvest, 8. Number of fruits per plant, 9. Polar circumference (cm), 10. Equatorial circumference (cm), 11. Flesh thickness (cm), 12. Cavity length (cm), 13. Number of seeds per fruit, 14. 100 seeds weight (g), 15. Total soluble solids (<sup>0</sup>Brix), 16. Carbohydrate mg/100 g, 17. Vitamin A mg/100 g.



**Fig 4.4 Direct (diagonal) and indirect effects of fruit yield components on fruit yield per plant at phenotypic level in pumpkin**

**Table 4.7 Direct (diagonal) and indirect effects of fruit yield components on fruit yield per plant at genotypic level in pumpkin**

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Vine length (m)	<b>-0.305</b>	0.006	-0.062	-0.061	-0.105	0.049	-0.072	-0.002	-0.060	0.093	-0.092	-0.064	0.093	0.036	0.035	-0.018	-0.080
Number of primary branches	-0.002	<b>0.103</b>	0.036	0.041	0.019	-0.004	0.031	-0.010	0.005	-0.003	0.026	0.006	-0.006	0.000	0.020	0.003	0.000
Days to first staminate flower anthesis	-0.147	-0.254	<b>-0.719</b>	-0.545	-0.090	0.186	-0.353	0.042	-0.144	0.139	0.184	-0.119	0.003	0.052	0.161	0.227	-0.027
Days to first pistillate flower anthesis	0.237	0.474	0.899	<b>1.185</b>	0.522	-0.039	0.966	0.243	0.203	-0.109	0.000	0.031	0.087	-0.118	-0.316	-0.080	0.255
Node bearing first staminate flower	0.100	0.054	0.037	0.129	<b>0.293</b>	0.030	0.208	0.158	-0.012	-0.119	-0.085	0.020	0.082	0.014	0.082	0.015	0.178
Node bearing first pistillate flower	0.035	0.010	0.057	0.007	-0.022	<b>-0.220</b>	-0.059	-0.084	-0.013	-0.125	-0.022	0.099	0.111	-0.022	0.018	0.013	-0.061
Days to first fruit harvest	-0.180	-0.233	-0.375	-0.623	-0.542	-0.204	<b>-0.763</b>	-0.292	0.129	0.164	-0.044	0.153	-0.254	0.233	-0.118	-0.046	-0.424
Number of fruits per plant	0.004	-0.066	-0.040	0.140	0.368	0.261	0.260	<b>0.681</b>	0.018	-0.184	-0.297	0.007	-0.027	-0.035	0.098	0.047	0.147
Polar circumference (cm)	0.051	0.014	0.052	0.044	-0.011	0.016	-0.044	0.007	<b>0.259</b>	0.009	-0.017	0.169	-0.127	0.098	-0.089	-0.075	-0.082
Equatorial circumference (cm)	0.000	0.000	0.000	0.000	0.001	-0.001	0.000	0.000	-0.000	<b>-0.002</b>	-0.001	0.001	0.000	-0.001	0.000	0.000	0.000
Flesh thickness (cm)	0.013	0.011	-0.011	0.000	-0.012	0.004	0.002	-0.019	-0.003	0.022	<b>0.043</b>	-0.017	-0.001	0.006	-0.001	-0.000	0.002
Cavity length (cm)	-0.078	-0.021	-0.061	-0.010	-0.026	0.168	0.074	-0.004	-0.242	0.209	0.152	<b>-0.372</b>	0.150	-0.045	0.004	0.059	0.039
Number of seed per fruit	0.071	0.015	0.001	-0.017	-0.065	0.117	-0.077	0.009	0.114	0.013	0.006	0.093	<b>-0.232</b>	0.010	-0.119	-0.064	-0.056
100 seed weight (g)	0.012	-0.000	0.007	0.010	-0.005	-0.010	0.031	0.005	-0.039	-0.038	-0.014	-0.012	0.004	<b>-0.103</b>	-0.021	0.004	0.012
Total soluble solids	-0.015	0.025	-0.030	-0.035	0.037	-0.011	0.020	0.019	-0.046	-0.045	-0.003	-0.001	0.068	0.028	<b>0.133</b>	0.070	0.046
Carbohydrates mg/100 g	-0.001	-0.001	0.009	0.002	-0.001	0.001	-0.001	-0.002	0.009	0.004	0.000	0.005	-0.008	0.001	-0.016	<b>-0.031</b>	0.001
Vitamin A mg/100 g	-0.021	0.000	-0.003	-0.017	-0.049	-0.022	-0.045	-0.017	0.025	0.024	-0.005	0.008	-0.019	0.010	-0.028	0.002	<b>-0.081</b>
<b>Fruit yield per plant (kg)</b>	<b>-0.225</b>	<b>0.136</b>	<b>-0.201</b>	<b>0.250</b>	<b>0.309</b>	<b>0.321</b>	<b>0.180</b>	<b>0.736</b>	<b>0.203</b>	<b>0.053</b>	<b>-0.172</b>	<b>0.008</b>	<b>-0.077</b>	<b>0.165</b>	<b>-0.155</b>	<b>0.128</b>	<b>-0.129</b>
Partial R <sup>2</sup>	0.069	0.014	0.145	0.296	0.090	-0.070	-0.138	0.502	0.053	-0.000	-0.007	-0.003	0.017	-0.017	-0.020	-0.004	0.010

R Square = 0.9372

Residual Effect = 0.250

Where,

1. Vine length (m),
2. Number of branches per plant,
3. Days to first staminate flower anthesis,
4. Days to first pistillate flower anthesis,
5. Node bearing first staminate flower,
6. Node bearing first pistillate flower,
7. Days to first fruit harvest,
8. Number of fruits per plant,
9. Polar circumference (cm),
10. Equatorial circumference (cm),
11. Flesh

thickness (cm), **12.** Cavity length (cm), **13.** Number of seeds per fruit, **14.** 100 seeds weight (g), **15.** Total soluble solids (<sup>o</sup>Brix), **16.** Carbohydrate mg/100 g, **17.** Vitamin A mg/100 g.



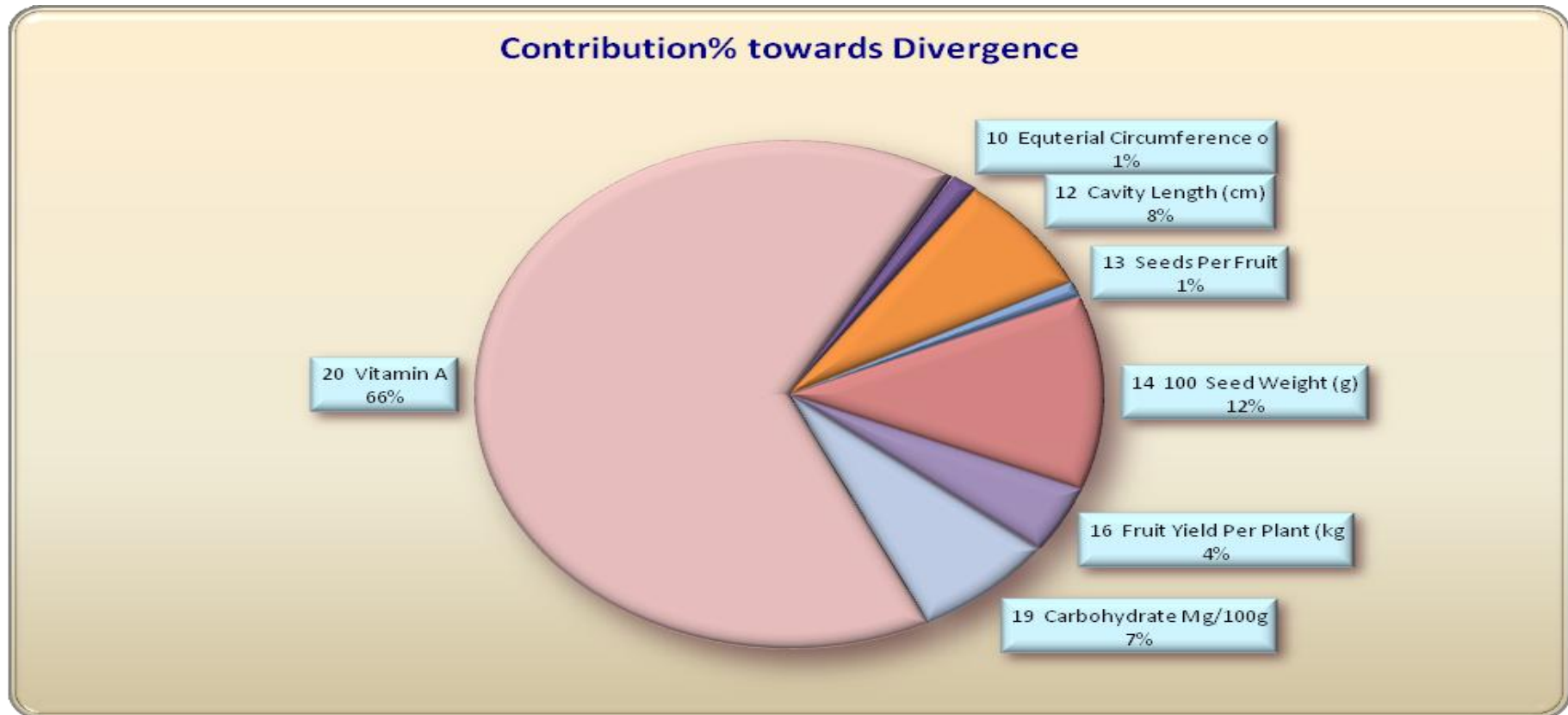
## 4.2.5 Genetic divergence

### 4.2.5.1 Mahalanobis generalized distance ( $D^2$ )

The genetic diversity among 25 genotypes was measured by employing  $D^2$  statistics (Mahalanobis). Out of 20 characters studied; vitamin A content (mg/100 g) contributed maximum percent towards genetic divergence (65.67%) followed by 100 seed weight (12.33%), cavity length (7.67%), carbohydrate (7.33%), fruit yield per plant (4.33%), equatorial circumference (1.33%), number of seed per fruit (1.00%) and days to first pistillate flower anthesis (0.33%) (Table 4.8 and figure 4.6).

**Table 4.8 Percentage contribution of twenty characters towards diversity in pumpkin genotypes**

S.N.	Character	Contribution (%)	Times Ranked 1st
1.	Vine length (m)	0.01	0
2.	Number of primary branches	0.01	0
3.	Days to first staminate flower anthesis	0.01	0
4.	Days to first pistillate flower anthesis	0.33	1
5.	Node bearing first staminate flower	0.01	0
6.	Node bearing first staminate flower	0.01	0
7.	Days to first fruit harvest	0.01	0
8.	Number of fruits per plant	0.01	0
9.	Polar circumference (cm)	0.01	0
10.	Equatorial circumference (cm)	1.33	4
11.	Flesh thickness (cm)	0.01	0
12.	Cavity length (cm)	7.67	23
13.	Number of seed per fruit	1.00	3
14.	100 seed weight (g)	12.33	37
15.	Average fruit weight (kg)	0.01	0
16.	Fruit yield per plant (kg)	4.33	13
17.	Total fruit yield per hectare (q)	0.01	0
18.	Total soluble solids	0.01	0
19.	Carbohydrates mg/100 g	7.33	22
20.	Vitamin A mg/100 g	65.67	197



**Fig 4.6 Contribution % towards genetic divergence**

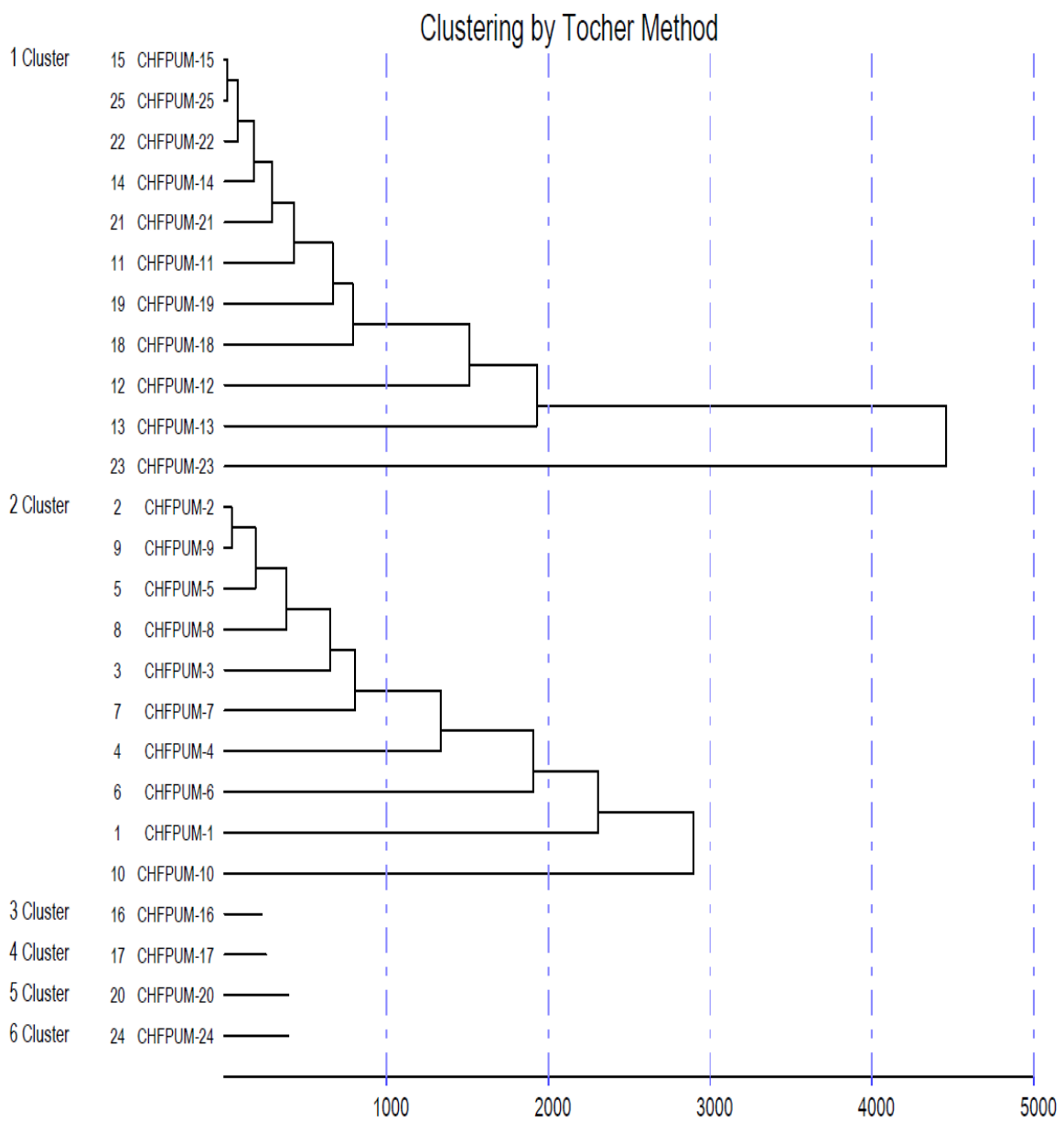
As per Tocher's method (Rao, 1952) were groups into six clusters based on  $D^2$  values (Figure 4.7). The  $D^2$  statics which being the sum of square for each Y values was calculated for all combinations. Cluster I was largest group comprising of 11 genotypes, followed by cluster II with 10 genotypes, cluster III, cluster IV, cluster V and cluster VI were containing single genotype each (Table 4.9).  $D^2$  value ranged from 409.051 to 3064.727 among genotypes (Table 4.10).

**Table 4.9 Clustering pattern of 25 pumpkin genotypes by Tocher's method**

<b>Cluster</b>	<b>Number of genotypes</b>	<b>Genotypes</b>
<b>Cluster I</b>	11	CHFPUM-15, CHFPUM- 25,CHFPUM-22, CHFPUM-14, CHFPUM-21, CHFPUM-11, CHFPUM-19, CHFPUM-18, CHFPUM-12, CHFPUM-13, CHFPUM-23
<b>Cluster II</b>	10	CHFPUM-2, CHFPUM-9, CHFPUM-5, CHFPUM-8, CHFPUM-3, CHFPUM-7, CHFPUM-4, CHFPUM-6, CHFPUM-1, CHFPUM-1
<b>Cluster III</b>	1	CHFPUM-16
<b>Cluster IV</b>	1	CHFPUM-17
<b>Cluster V</b>	1	CHFPUM-20
<b>Cluster VI</b>	1	CHFPUM-24

**Table 4.10 nearest and farthest cluster from each cluster based on D<sup>2</sup> value in pumpkin**

<b>Cluster</b>	<b>Nearest cluster with D<sup>2</sup> value</b>	<b>Farthest cluster with D<sup>2</sup> value</b>
<b>Cluster I</b>	III (514.535)	II (2301.126)
<b>Cluster II</b>	IV (692.149)	V (3064.727)
<b>Cluster III</b>	IV (409.051)	II (1333.509)
<b>Cluster IV</b>	III (409.051)	VI (1330.181)
<b>Cluster V</b>	III (534.334)	II (3064.727)
<b>Cluster VI</b>	I (526.230)	II (2744.324)



**Fig 4.7 Dendrogram based on morphometric character of 25 genotypes of pumpkin for twenty traits using hierarchical cluster analysis (Tocher's method)**

#### 4.2.5.2 Inter cluster distance

Inter cluster  $D^2$  values are given in the (Table 4.12 and Figure 4.6). The inter cluster distance was maximum (3064.727) between cluster II and V. The minimum (409.051) was observed between cluster III and IV. This indicates close relationship among the genotypes included in these clusters.

#### 4.2.5.3 Intra cluster distance

Intra cluster distance was observed between in cluster I and II and as the remaining clusters contained only one constituent genotype each. Intra cluster distance was maximum in the cluster II (233.901) followed by cluster I (188.503) distance in (Table 4.11 and Figure 4.8).

**Table 4.11 Average inter and intra cluster distances ( $D^2$ ) for 25 genotypes**

Cluster number	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	<u>188.503</u>	2301.126	514.535	738.328	611.374	526.230
Cluster II		<u>233.901</u>	1333.509	692.149	3064.727	2744.324
Cluster III			<u>0.000</u>	409.051	534.334	1072.981
Cluster IV				<u>0.000</u>	1268.010	1330.181
Cluster V					<u>0.000</u>	1123.036
Cluster VI						<u>0.000</u>

# Tocher Method

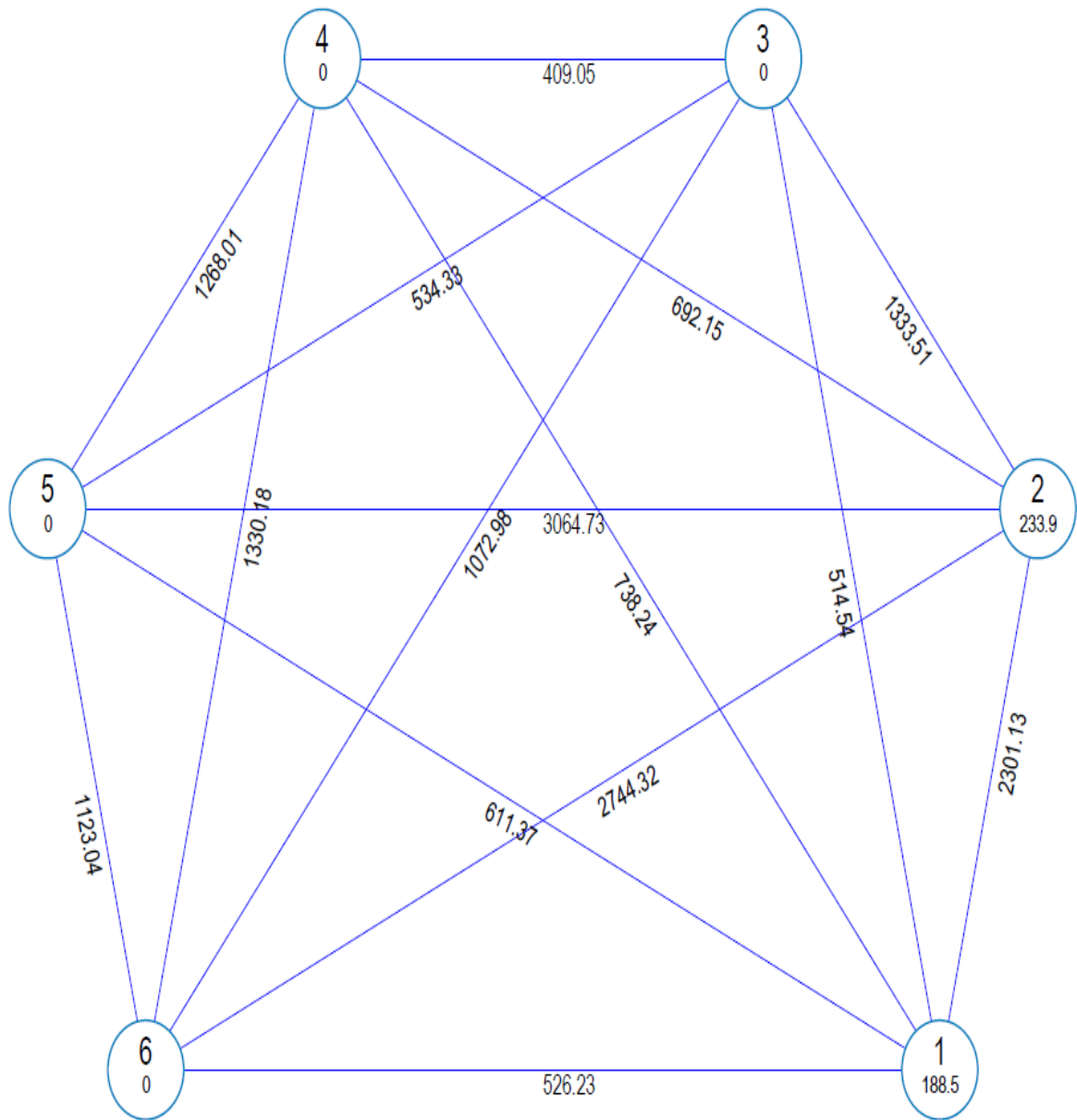


Fig 4.8 Cluster diagram with inter and intra cluster distance

#### 4.2.5.4 Cluster mean analysis

Cluster means were computed in clusters for twenty characters studied (Table 4.12), it is evident that mean value of vine length was maximum in cluster IV (8.57) and minimum in cluster VI (6.60). The number of branches per plant was found maximum in cluster III (11.73) and minimum in cluster IV (10.83). Minimum days to first staminate flower anthesis was recorded in cluster III (59.73 days) while maximum days to first satminate flower anthesis was recorded in cluster VI (62.86 days). The genotypes in cluster V exhibited minimum days to first pistillate flower anthesis (63.73 days) while those in cluster II exhibited maximum days to first pistillate flower anthesis (65.86 days). Further the node bearing first staminate flower was recorded lowest in cluster VI (2.73) while the maximum was recorded in cluster I (3.79.). With regards to node baring first pistillate flowers cluster VI showed the minimum mean value (10.43), while cluster IV showed the maximum (13.33).

Days to first fruit harvest was minimum in cluster VI (81.30 days) and maximum in cluster II (90.47 days). Cluster III exhibited maximum number of fruits per plant (3.56) while cluster V exhibited minimum number of fruits per plant (2.00). Polar circumference of fruits was maximum in cluster V (68.93) while it was minimum in cluster II (55.97). Equatorial circumference was observed highest in cluster IV (61.93) while it was observed lowest in cluster V (40.30). The genotypes in cluster IV exhibited maximum flesh thickness (4.76) while genotypes in cluster V exhibited minimum flesh thickness (2.33). The genotypes of cluster V had maximum cavity length (23.56 cm) while genotypes of cluster IV had minimum cavity length (8.06 cm). Number of seeds per fruit was recorded minimum in cluster VI (204.33) and maximum in cluster II (257.23). 100 seed weight was found maximum in cluster IV (12.03 g) and minimum in cluster VI (7.10 g). The genotypes in cluster VI exhibited maximum average fruit weight (2.63 kg) while that in cluster V exhibited minimum average fruit weight (1.66 kg).

Fruits yield per plant was recorded maximum in cluster III (7.36 kg) and minimum in cluster V (3.60 kg). Highest total fruits yield q/ha was found in cluster III (314.76 q) while the minimum total fruits yield was found in cluster V (153.80). The highest total soluble solids (TSS) content was found in cluster IV (8.66°B) while it was lowest in cluster VI (5.60°B). Carbohydrates were recorded highest in cluster V (547.50 mg/100 g) and lowest in cluster VI (126.00 mg/100 g). The highest vitamin A was recorded in cluster II (13.31mg/100 g) and lowest in cluster VI (1.24mg/100g).

**Table 4.12 Mean values of clusters for twenty characters studied in pumpkin**

<b>Cluster Number</b>	<b>Vine length</b>	<b>Number of primary branches per plant</b>	<b>Days to first staminate flower anthesis</b>	<b>Days to first staminate flower anthesis</b>	<b>Node bearing first staminate flower</b>	<b>Node bearing first staminate flower</b>	<b>Days to first fruits harvest</b>	<b>Number of fruits per plant</b>	<b>Polar circumferences</b>	<b>Equatorial circumferences</b>
<b>Cluster I</b>	7.45	10.84	61.02	64.88	3.79	12.36	84.89	2.60	59.60	61.72
<b>Cluster II</b>	7.88	10.93	61.40	65.86	4.59	12.58	90.47	2.77	55.98	54.07
<b>Cluster III</b>	7.90	11.73	59.73	65.00	4.50	11.37	85.00	3.57	64.13	33.93
<b>Cluster IV</b>	8.57	10.83	61.20	64.87	3.33	13.33	85.20	2.50	58.73	61.93
<b>Cluster V</b>	6.90	11.10	62.77	63.73	3.00	11.20	82.70	2.00	68.93	40.30
<b>Cluster VI</b>	6.60	11.67	62.87	64.63	2.73	10.43	81.30	2.13	63.63	58.50

<b>Cluster Number</b>	<b>Flesh thickness</b>	<b>Cavity length</b>	<b>Number of seeds per fruit</b>	<b>100 seed weight</b>	<b>Average fruit weight</b>	<b>Fruit yield per plant</b>	<b>Fruit yield per hectare</b>	<b>Total Soluble Solids</b>	<b>Carbohydrate</b>	<b>Vitamin A</b>
<b>Cluster I</b>	3.39	10.85	229.4	9.17	2.61	5.44	232.5	6.70	507.7	1.531
<b>Cluster II</b>	3.44	11.03	257.3	8.31	1.82	4.89	209.2	7.24	470.0	13.313
<b>Cluster III</b>	2.60	18.17	209.3	8.97	1.70	7.37	314.7	6.90	531.0	6.527
<b>Cluster IV</b>	4.77	8.07	206.0	12.03	1.77	4.63	197.7	8.67	540.0	7.360
<b>Cluster V</b>	2.33	23.57	206.7	11.50	1.67	3.60	153.0	8.27	547.0	2.093
<b>Cluster VI</b>	3.63	14.73	204.3	7.10	2.63	3.90	166.0	5.60	126.0	1.247

## 4.2.6 Genotype characterization through seed protein profiles

Protein banding pattern of 25 pumpkin genotypes was generated by SDS-PAGE (Figure 4.10). Protein distribution patterns in 25 genotypes of pumpkin were studied and subjected for cluster analysis (Table 4.13). Cluster analysis of banding pattern of 25 genotypes based on similarity and UPGMA resulted in distinct clusters (Table 4.14 and Figure 4.9). A total of 89 protein bands as per Rm values were identified by silver staining. The genotypes exhibited considerable variation in protein band number ranging from 13-26. Among the genotypes CHFUM-6, CHFUM-18 and CHFUM-25 showed maximum numbers (26) of protein bands while the minimum numbers (13) of bands were present in genotypes CHFUM-13.

Band number 2 (Rm=0.11) was recorded in genotypes CHFUM-5 only similarly, band number 5 (Rm=0.14), 7 (Rm=0.16) and 8 (Rm=0.17) was present only one genotypes CHFUM-5, CHFUM-3 and CHFUM-6 respectively whereas it was absent in all other genotypes.

Band number 1 (Rm=0.10) was present in genotype CHFUM-3 and CHFUM-6 only whereas it was absent in all the other genotypes. Similarly, band number 6 (Rm=0.15) was to present in CHFUM-2 and CHFUM-6. 19 (Rm=0.28) present in CHFUM-5 and CHFUM-10 only. 38 (Rm=0.47) present in CHFUM-6 and CHFUM-19. 60 (Rm=0.69) present in CHFUM-2 and CHFUM-12 and band number 82 (Rm=0.91) was found to be present only in genotype CHFUM-11 and CHFUM-19 whereas it was absent in all other genotypes. Band number 3 (Rm=0.12) and 4 (Rm=0.13) was to absent all the genotypes.

Band number 15 (Rm=0.24) was found to be present in CHFUM-2, CHFUM-4 and CHFUM-5 only. Similarly, band number 65 (Rm=0.74) present in CHFUM-2, CHFUM-8 and CHFUM-25 only. Band number 70 (Rm=0.79) present in CHFUM-7, CHFUM-9 and CHFUM-25. Band number 72 (Rm=0.81) present in CHFUM-4, CHFUM-6 and CHFUM-7. Band number 77 (Rm=0.86) present in CHFUM-6, CHFUM-16 and CHFUM-19. Band number 88 (Rm=0.97) present in CHFUM-1, CHFUM-14 and CHFUM-17.

Band number 23 (Rm=0.32), band number 39 (Rm=0.48) and band number 81(Rm=0.91) was found to be present in maximum (13) number of genotypes. CHFUM-4, CHFUM-7, CHFUM-8, CHFUM-17 and CHFUM-22 were found to possess of three bands. CHFUM-1, CHFUM-3 CHFUM-14 were to found absent of three bands. Band number 39 (Rm=0.48) and band number 81 (Rm=0.90) possess in CHFUM-2, CHFUM-9, CHFUM-20, CHFUM-21and CHFUM-23 but lack in band number 23 (0.32) while CHFUM-5,

CHFPUM-10, CHFPUM-11, CHFPUM-12 and CHFPUM-19 were to found lack in band number 39 (Rm=0.48) and band number 81 (Rm=0.90).

**Table 4.13 Major cluster produced by SDS-PAGE analysis in thirty genotypes of pumpkin**

<b>Cluster</b>	<b>Sub cluster</b>	<b>Sub-sub cluster</b>	<b>Genotypes</b>
<b>I</b>	<b>IA</b>	<b>IA1</b>	CHFPUM-5, CHFPUM-12, CHFPUM-9, CHFPUM-6 CHFPUM-2, CHFPUM-7 CHFPUM-14, CHFPUM-10 CHFPUM-4, CHFPUM-3 CHFPUM-13, CHFPUM-24 CHFPUM-25, CHFPUM-8 CHFPUM-19, CHFPUM-21 CHFPUM-15 CHFPUM-20 CHFPUM-23, CHFPUM-16 and CHFPUM-18
		<b>IA2</b>	CHFPUM-11, CHFPUM-17
	<b>IB</b>	CHFPUM-22	
<b>II</b>	CHFPUM-1		

H I E R A R C H I C A L C L U S T E R A N A L Y S I S

Dendrogram using Average Linkage (Between Groups)

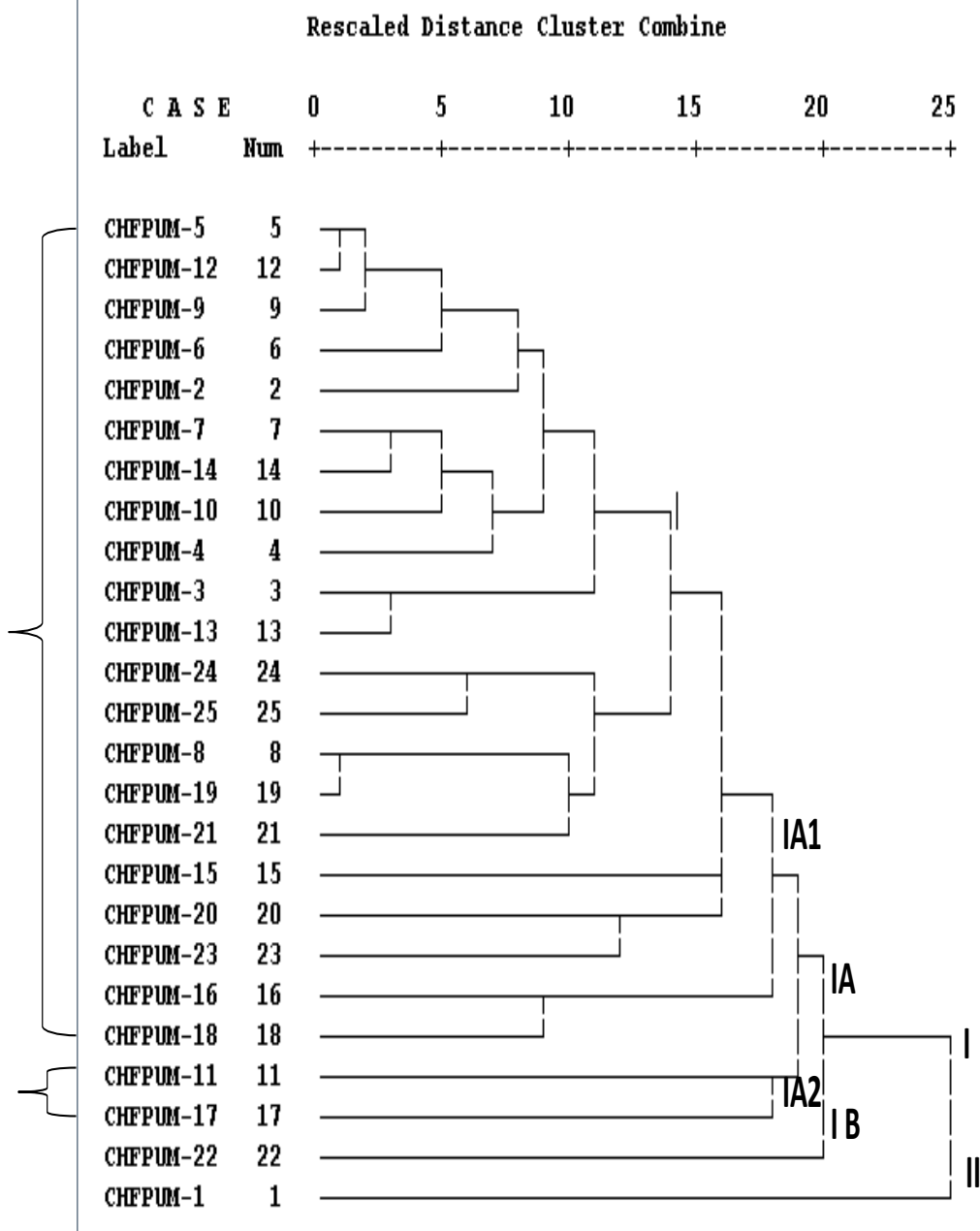
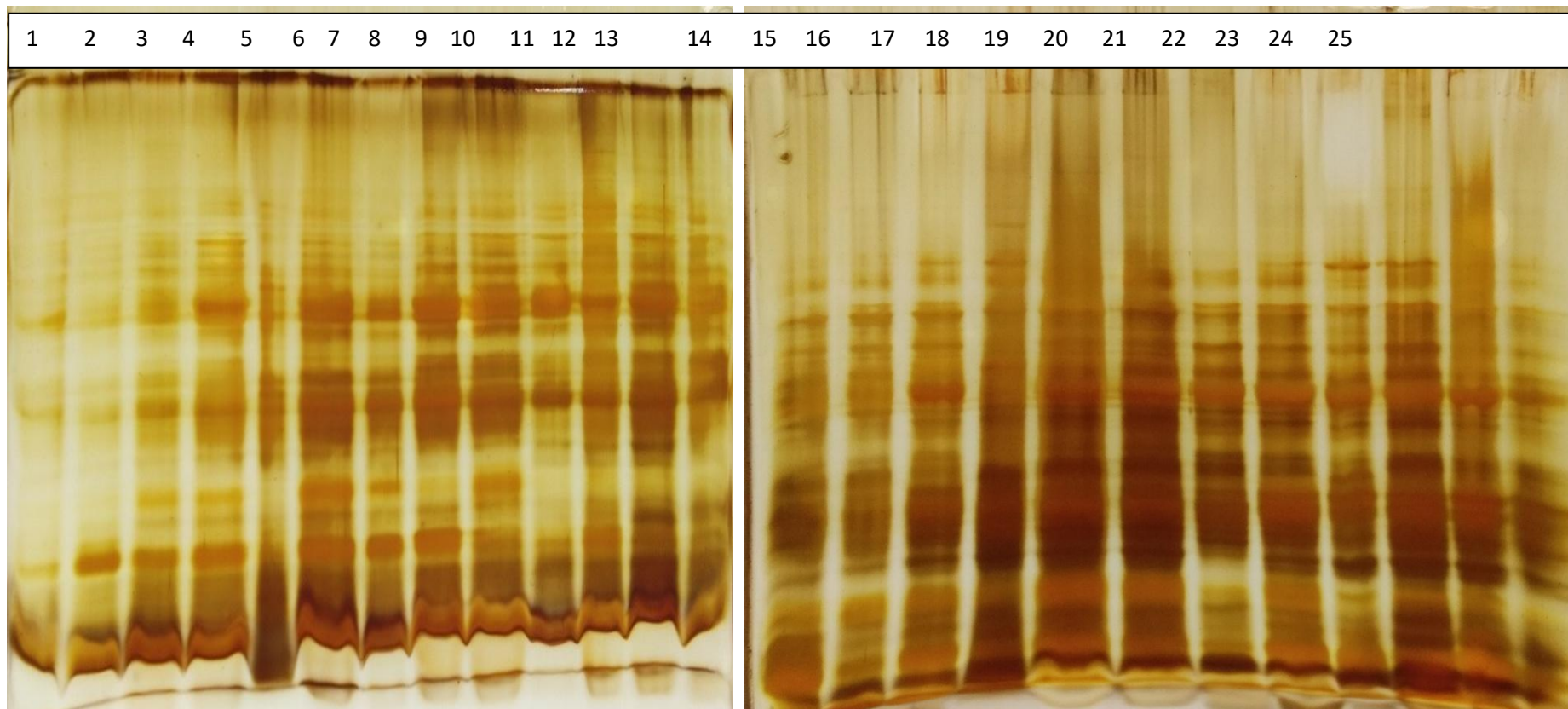


Fig 4.10 UPGMA of 25 pumpkin genotypes based on total seed protein profiles obtained by SDS-PAGE



**Fig. 4.9 Seed protein banding pattern in 25 pumpkin genotypes**

#### 4.2.7 Genetic relationship

The similarity index coefficient matrix between data analysis for the twenty five genotypes of pumpkin under described in (Table 4.15) and the dendrogram generated with matrix value has been presented in (Figure 4.10). Percentage similarity index ranged from 0 to 62.86. Values of similarity index coefficient matrix suggested least genetic distance of genotypes was observed between CHFUM-3 with CHFUM-13 and CHFUM-20 (0.00%) while maximum similarity index was observed between the genotypes CHFUM-1 and genotypes CHFUM-11 as evident by coefficient value of (62.86%), (Table 4.14).

Based on the dendrogram, all the genotypes were grouped into 2 major clusters. Cluster I was further sub-divided into 2 sub clusters, which were again sub-divided into 2 sub-sub clusters each. Cluster I was contain 21 genotypes while cluster II was contain only 1 genotype (CHFUM-1). The first sub-sub cluster (IA1) was found to be incorporating 21 genotypes viz., CHFUM-5, CHFUM-12, CHFUM-9, CHFUM-6, CHFUM-2, CHFUM-7, CHFUM-14, CHFUM-10, CHFUM-4, CHFUM-3, CHFUM-13, CHFUM-24, CHFUM-25, CHFUM-8, CHFUM-19, CHFUM-21, CHFUM-15, CHFUM-20, CHFUM-23, CHFUM-16 and CHFUM-18. Second sub-sub cluster (IA2) was incorporating with 2 genotypes viz., CHFUM-11 and CHFUM-17.

The dendrogram generated by Tocher's method analysis grouped 25 genotypes into 6 major clusters while SDS-PAGE analysis grouped into 2 major clusters and cluster I into 2 sub clusters which were again sub-divided into 2 sub-sub clusters each. Genotypes CHFUM-16, CHFUM-17, CHFUM-20 and CHFUM-24 these were grouped in individual cluster (cluster 3), (cluster 4), (cluster 5) (cluster 6) respectively. Dendrogram generated by Tocher's method analysis but the dendrogram based on SDS-PAGE banding pattern grouped CHFUM-16, CHFUM-17, CHFUM-20 and CHFUM-24 separated in first sub-sub cluster (IA1) while CHFUM-11 and CHFUM-17 in the Second sub-sub cluster (IA2).

The genotype CHFUM-1 was grouped in cluster II, while it was grouped with CHFUM-2, CHFUM-9, CHFUM-6, CHFUM-8, CHFUM-3, CHFUM-7, CHFUM-4, CHFUM-6 and CHFUM-10 in cluster II in dendrogram generated by Tocher's method analysis.

CHFUM-22 was grouped in first cluster (Cluster I) in dendrogram based on  $D^2$  value but in dendrogram based on SDS-PAGE banding pattern, CHFUM-22 was grouped in first sub cluster (IB) only.

Although, some similarities were found in the clustering pattern of both the dendrograms generated by Tocher's method analysis and SDS-PAGE but more sub groupings was observed in SDS-PAGE banding pattern and thus more variation could be worked out by the dendrogram generated by SDS-PAGE banding pattern.

**Table 4.14 Percentage similarity index estimates among 25 genotypes of pumpkin using SDS-PAGE analysis**

Genotypes	CHF PU M-1	CHF PU M-2	CHF PU M-3	CHF PU M-4	CHF PU M-5	CHF PU M-6	CHF PU M-7	CHF PU M-8	CHF PU M-9	CHF PU M-10	CHF PU M-11	CHF PU M-12	CHF PU M-13	CHF PU M-14	CHF PU M-15	CHF PU M-16	CHF PU M-17	CHF PU M-18	CHF PU M-19	CHF PU M-20	CHF PU M-21	CHF PU M-22	CHF PU M-23	CHF PU M-24	CHF PU M-25
CHFPUM-1	0.00																								
CHFPUM-2	15.78	0.00																							
CHFPUM-3	21.62	9.30	0.00																						
CHFPUM-4	15.00	39.13	17.77	0.00																					
CHFPUM-5	30.00	4.34	35.55	25.00	0.00																				
CHFPUM-6	14.28	12.50	25.53	28.00	20.00	0.00																			
CHFPUM-7	10.00	21.70	13.33	29.16	25.00	36.00	0.00																		
CHFPUM-8	15.38	22.22	27.27	29.78	34.04	28.57	34.04	0.00																	
CHFPUM-9	25.00	17.39	31.11	25.00	12.50	24.00	20.83	25.53	0.00																
CHFPUM-10	16.21	27.90	9.52	26.66	22.22	34.04	31.11	27.27	26.66	0.00															
CHFPUM-11	62.86	14.63	20.00	37.20	27.90	26.66	27.90	28.57	18.60	30.00	0.00														
CHFPUM-12	18.18	5.12	26.31	19.51	19.51	13.95	29.26	20.00	14.63	15.78	27.77	0.00													
CHFPUM-13	13.79	17.14	0.00	17.02	12.76	10.25	21.27	38.88	32.43	23.52	12.50	20.00	0.00												
CHFPUM-14	6.00	15.38	10.00	19.51	14.63	18.60	4.87	30.00	9.75	10.52	33.33	17.64	26.66	0.00											
CHFPUM-15	14.63	29.70	17.39	12.24	24.48	31.37	20.40	41.66	20.40	17.39	9.90	14.28	26.31	33.33	0.00										
CHFPUM-16	21.05	18.18	32.55	21.73	17.39	25.00	17.39	35.55	13.04	37.20	43.90	25.64	17.14	30.76	38.29	0.00									
CHFPUM-17	25.00	30.43	13.33	29.16	25.00	24.00	37.50	38.29	25.00	40.00	27.90	14.63	32.43	29.26	44.89	52.17	0.00								
CHFPUM-18	9.52	0.83	25.53	24.00	28.00	34.61	20.00	36.65	24.00	29.78	22.22	27.90	15.38	41.86	58.82	20.83	44.00	0.00							
CHFPUM-19	10.00	17.39	26.66	29.16	20.83	44.00	20.80	17.02	20.83	22.22	27.90	24.39	21.62	29.26	44.89	34.78	41.66	48.00	0.00						
CHFPUM-20	9.52	41.60	0.00	44.00	16.00	26.92	36.00	40.81	32.00	34.04	26.66	23.25	5.64	27.90	31.37	37.50	44.00	38.46	36.00	0.00					
CHFPUM-21	0.00	35.89	10.52	39.02	9.75	23.25	34.14	25.00	19.51	15.78	27.77	17.64	13.33	35.29	18.60	15.38	24.39	46.51	14.63	51.16	0.00				
CHFPUM-22	10.25	13.33	31.80	34.04	29.78	20.40	25.50	39.13	34.04	40.90	52.38	35.00	27.77	35.00	33.33	48.88	55.31	48.97	34.04	32.65	25.00	0.00			
CHFPUM-23	10.15	35.55	13.63	42.55	17.02	24.48	29.78	34.78	34.04	22.72	14.28	15.00	16.66	15.00	41.66	35.55	38.29	28.57	25.53	44.89	40.00	26.08	0.00		
CHFPUM-24	5.10	31.11	22.72	25.53	12.76	28.57	29.78	26.08	12.76	31.81	28.57	20.00	11.11	40.00	29.16	44.44	42.55	28.57	25.53	36.73	35.00	34.78	43.47	0.00	
CHFPUM-25	14.28	37.50	34.04	28.00	28.00	42.30	28.00	32.65	40.00	25.53	26.66	18.60	20.51	23.25	47.05	25.00	24.00	46.15	40.00	36.73	27.90	32.65	40.81	8.16	0.00

**Table 4.15 Comparison of scorable protein bands among 25 pumpkin genotypes**

Band number	Rm value	P-1	P-2	P-3	P-4	P-5	P-6	P-7	P-8	P-9	P-10	P-11	P-12	P-13	P-14	P-15	P-16	P-17	P-18	P-19	P-20	P-21	P-22	P-23	P-24	P-25
1	0.1	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	0.11	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	0.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	0.14	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	0.15	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	0.16	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	0.17	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	0.18	+	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
10	0.19	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-
11	0.2	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	0.21	-	-	+	+	+	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
13	0.22	-	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
14	0.23	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	0.24	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	0.25	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	0.26	-	-	-	-	-	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
18	0.27	-	-	-	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-
19	0.28	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	0.29	-	-	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
21	0.3	-	+	-	+	+	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	+
22	0.31	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-
23	0.32	-	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-
24	0.33	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	+	-	+	+	-
25	0.34	-	-	-	-	-	+	+	-	+	+	+	-	-	+	+	-	-	-	+	+	-	-	-	-	+
26	0.35	-	-	+	-	+	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-
27	0.36	-	+	-	+	-	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
28	0.37	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	+	+	-	+	-	-	-	-	+	-
29	0.38	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	+	-	+
30	0.39	+	-	+	+	-	-	+	-	-	+	-	+	-	-	+	-	-	+	-	-	-	-	+	-	+
31	0.4	-	+	-	-	-	+	+	+	-	+	-	-	-	-	+	+	+	+	-	+	-	-	+	+	-
32	0.41	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
33	0.42	-	-	-	+	-	-	-	+	-	+	+	-	-	-	+	-	+	+	-	-	-	+	-	-	-
34	0.43	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	+	+	-	-	+	-
35	0.44	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	+

36	0.45	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	-	+	+	+	+	
37	0.46	+	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	
38	0.47	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
39	0.48	-	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-	+	+	-	+	+	+	+	-	+	
40	0.49	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	
41	0.5	-	-	-	-	-	+	-	+	-	+	-	+	-	+	+	+	+	+	-	-	-	+	-	+	+	
42	0.51	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	
43	0.52	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	
44	0.53	-	-	+	+	-	+	-	-	-	-	+	-	-	+	-	+	-	+	+	-	-	+	-	-	+	
45	0.54	-	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-	+	-	-	+	-	-	+	+	-	
46	0.55	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	+	+	-	+	-	-	-	
47	0.56	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
48	0.57	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	+	+	-	+	-	-	-	
49	0.58	-	-	+	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	+	
50	0.59	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	
51	0.6	-	-	-	-	-	+	-	-	+	-	-	-	-	+	+	-	-	+	-	+	-	-	+	-	+	
52	0.61	-	-	+	-	+	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	
53	0.62	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	+	-	-	+	-	+	
54	0.63	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+	-	+	-	
55	0.64	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	
56	0.65	-	+	+	-	+	+	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	-	+	+	
57	0.66	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	
58	0.67	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	
59	0.68	-	-	-	-	-	+	-	+	-	+	-	-	-	+	-	-	-	+	-	+	+	-	-	+	+	
60	0.69	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	
61	0.7	-	-	-	-	+	-	-	+	-	-	-	-	+	+	+	+	-	-	-	+	-	+	+	-	+	
62	0.71	+	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	
63	0.72	-	+	-	+	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	
64	0.73	-	-	-	-	-	+	+	-	+	-	-	-	+	-	+	-	+	+	+	-	+	-	-	-	+	
65	0.74	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
66	0.75	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	+	+	-	-	+	-	-	+	+	-	
67	0.76	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	
68	0.77	-	+	-	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	
69	0.78	-	-	-	+	+	-	-	+	-	-	+	+	-	+	-	-	-	+	-	+	+	+	+	+	-	
70	0.79	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
71	0.8	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	-	+	+	+	-	
72	0.81	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
73	0.82	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
74	0.83	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+	-	+	+	-	+	+	-	-	+	
75	0.84	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	

76	0.85	-	+	-	-	-	-	-	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	-	-	+
77	0.86	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
78	0.87	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-
79	0.88	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	+	-
80	0.89	+	-	+	-	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-	-	-	-	+	-	+
81	0.9	-	+	-	+	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+	-
82	0.91	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
83	0.92	-	-	-	-	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-
84	0.93	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-
85	0.94	-	-	-	-	-	-	+	+	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-	+
86	0.95	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	+	+	-	-	-	-	+	-	+	-
87	0.96	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	+	+	-	+	-	-
88	0.97	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
89	0.98	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+
	<b>Total bands</b>	<b>16</b>	<b>22</b>	<b>21</b>	<b>24</b>	<b>24</b>	<b>26</b>	<b>24</b>	<b>23</b>	<b>24</b>	<b>21</b>	<b>19</b>	<b>17</b>	<b>13</b>	<b>17</b>	<b>25</b>	<b>22</b>	<b>24</b>	<b>26</b>	<b>24</b>	<b>25</b>	<b>17</b>	<b>23</b>	<b>23</b>	<b>23</b>	<b>26</b>

# CHAPTER-5

# DISCUSSION

## Chapter-5

# Discussion

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The result obtained during the course of the experiment as the reported in the chapter-4 have been discussed in the present section. Pumpkin (*Cucurbita moschata* Duch. ex Poir) is the one of the important Cucurbitaceous crops. Vegetables are primary dietary source of essential nutrients *viz.*, vitamins and minerals and they provide many nutritionally less defined, yet important components of our diet e.g. fibres and anti-oxidants. The world's rapid population growth is demanding increased production and greater diversification of crops. So, there is a need to diversify our food basket by crop having potential of good yield.

Pumpkin (*Cucurbita moschata* Duch. ex Poir) being monoecious in nature, its high cross-pollinated and posses with wide variability provide immense scop for germplasm collection, maintenance and evolution for economically important traits prerequisite for genetic important of any crop. There is a constant need to evaluate to germplasm to identity the genetically diverse lines with desirable character combination to develop an idio type.

Vegetable crops grown extensively in tropical and sub-tropical parts of the country. Pumpkin is a thermophilic and frost susceptible species growing best at a temperature above 20°C and cultivated both in summer and rainy seasons in India. It is grown for its tender fruits, which are consumed as fresh vegetable, processed food and livestock feed. Both mature and immature fruits are consumed raw, cooked, stewed, boiled, or baked, and also used for preparation of jam, puree and candy. Its young leaves, tender flowers are also cooked and consumed. The flowers are used for excellent preparation so called "pakora". The flowers of pumpkin are more nutritive than fruits (Rana 2014). The fruits are sweetish when fully mature and can be used in preparing sweets, halwa, candy or fermented into beverages. Fruits with orange or yellow flesh generally have high concentration of carotenoid (Pandey *et al.*, 2003). Pumpkin has some medicinal properties also because of its curative and medicinal properties has been known since ancient time, and thus, associated with man for at least 10 thousand years. The fruit is used as cure of stomach catarrh, hyperacidity, and stomach ulcers, especially peptic ulcers. Raw pulp of pumpkin has purgative effect, and it is recommended in case of obesity and deterioration in the function of gall bladder. Pumpkin juice as well as raw and boiled fruits has diuretic and cholagogue properties.

Although pumpkin is cultivated in all the states of North Eastern Region of India but there is no improved variety of pumpkin that can be recommended to the farmers for its commercial cultivation in the region. Therefore, a systematic breeding approach for crop improvement of pumpkin is initiated.

The immediate and long term objective of crop breeding remains the increase of productivity or yield. For improving yield through breeding, it is necessary to study the components, their inter relationship with yield and their direct and indirect contributions. Therefore, identification of superior genotypes becomes imperative for promoting its production, high productivity and better quality of the produce. To achieve these goals, crop breeder need to know the extent of genetic variability/diversity present in a population. Larger variability ensures better chance of producing new derived forms. Variation at phenotypic level is a combination of genotypic as well as environmental variability. It is the genotypic variability in which a breeder is primarily interested because it decides response to selection. Statistical tools like range, mean coefficient of variability, heritability and genetic advance are useful tools to estimate the total genetic variation to calculate degree of genetic control for a particular trait. Moreover, it provides basic information for successful breeding programme.

Yield is a complex character as it is under polygenic control and is more responsive to varying environmental conditions. An efficiency of selection in breeding programme mainly depends on the knowledge of the role of environment and degree of association of the component characters. Correlation coefficient analysis is useful in developing an effective basis of phenotypic selection in plant population. Study of character may be bring about simultaneous effect on other, depending as the intensity of association between the two traits under consideration.

The phenotypic and genotypic correlation reveals the extent of association between different characters. Thus, it is helpful to base selection procedure to a required balance, when two opposite desirable characters affecting the principal characters are being selected. Path analysis also gives a cause and effect relationship. It critically breaks up different direct and indirect effects which finally makeup correlation coefficient. In present study, genotypic and phenotypic path analysis for yield per plant was analysed by selecting most influencing independent variables. In crop improvement programme, genetic diversity is one of the important tools to quantify genetic variability in both cross and self-pollinated crops. The  $D^2$  statistics enables to discriminate among different cultivars according to the diversity present (Mahalanobis, 1936). It gives clear idea about the diverse nature of the populations. The clusters formed according to Tocher's method (Rao, 1952) were used to

know the distance between and within the cluster. The results could give an insight about diverse nature of the genotypes in a cluster.

Varietal identification based on morphological traits is not accurate in pumpkin because these traits are highly influenced by environment. Considering the difficulties, the introduction of biochemical techniques has made possible and a more accurate evaluation of genetic variation, bringing greater precision to measures of genetic diversity. Electrophoresis of seed proteins is based on the concept that each genotype/cultivar is distinct and relatively homogenous at the genetic level. Seed protein electrophoresis is frequently employed as an additional approach for species identification and as a useful tool for back-tracking the evolution of various groups of plants (Ladizinski and Hymowitz, 1979). Among the biochemical techniques, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is an economical, simple and extensively used biochemical technique for describing the seed protein diversity of crop germplasm (Cook, 1995; Das and Mukarjee, 1995; Fufa *et al.*, 2005 and Iqbal *et al.*, 2005). Furthermore, seed proteins used as genetic marker in the study of genetic variation because they are the primary products of structural genes, any change in the coding sequence of a gene generally reflects the corresponding change in the primary structure of protein (Srivalli *et al.*, 1999).

In the present investigation 25 genotypes of pumpkin were used to study the variability. It was considered imperative to carry out a study to obtain information on genetic variability, correlation, path analysis, genetic divergence and seed protein electrophoresis in order to device breeding strategies for the improvement of pumpkin using indigenous material. The choice of plant breeding methodology for increasing yield potential largely depends on the amount of variability. Owing to the existence of large amount of variability in pumpkin, it was considered worthwhile to evaluate and characterize germplasm lines and diverse collections from different part of NER maintained at College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh both morphologically and electrophoretically. The salient findings of the present investigation are being discussed in the following sub headings:

- 5.1 Analysis of variance
- 5.2 Mean performance of the genotypes
- 5.3 Genetic variability
- 5.4 Heritability and genetic advance
- 5.5 Correlation coefficient analysis
- 5.6 Path coefficient analysis

- 5.7 Genetic divergence
- 5.8 Genotype characterization through seed protein profiles
- 5.9 Potential genotype(s) for various traits in pumpkin

## 5.1 Analysis of variance

The analysis of variance in the present investigation revealed significant differences among 25 pumpkin genotypes for all the traits studied and a wide range of variability was observed for vine length (m), number of branches per plant, days to first staminate, flower anthesis, days to first pistillate flower anthesis, node bearing first staminate flower, node bearing first pistillate flower, days to first fruit harvest, number of fruits per plant, polar circumference (cm), equatorial circumference (cm), flesh thickness (cm), cavity length (cm), number of seeds per fruit, 100 seed weight (g), average fruit weight (kg), fruit yield per plant (kg), fruit yield per ha (q), total soluble solid (TSS °Brix), carbohydrates content (mg/100g) and vitamin A content (mg/100g). Similar results have also been reported by under environmental conditions (Shrikant *et al*, 2017, Kumar *et al*, 2017, Kumar *et al*, 2011, Tamilselvi and Jansirani, 2017) which indicated the scope for selection of suitable initial breeding material for further crop improvement.

## 5.2 Mean performance of genotypes

Significant differences were observed among all the genotypes for all the characters under study. The highest vine length for getting higher yield was recorded in CHFPUM-12 (11.36) followed by CHFPUM-1 (11.00), CHFPUM-17 (8.56), CHFPUM-6 (8.5) and CHFPUM-13, CHFPUM-116 (7.9). The maximum number of primary branches per plant was observed in CHFPUM-2 (13.63) followed by CHFPUM-12 (11.97), CHFPUM-3 (11.97), CHFPUM-4 (12.33), CHFPUM-22 (12.73) and CHFPUM-25 (12.87). Variability in vine length and number of primary branches per plant was also reported by Shrikanth *et al*. (2017), Kumar *et al*. (2011). The selection of genotypes with high vine length and number of primary branches per plant will be an important criterion for getting higher yield.

Among the horticultural characters, days to first staminate flower anthesis, days to first pistillate flower anthesis, node bearing first staminate flower, node bearing first festaminate flower, days to first harvest are the traits which determine the earliness of a variety. Among the earliness traits, in case of days to first staminate flower anthesis, the lowest duration for days to first staminate flower anthesis was recorded in CHFPUM-15 (58.53) followed by CHFPUM-14 (58.83), CHFPUM-4 (58.87), CHFPUM-3 (59.10), CHFPUM-19 (59.40), CHFPUM-16 (59.73), CHFPUM-13 (59.73), CHFPUM-5 (60.53). The

lowest duration for days to first pistillate anthesis was recorded in CHFUM-5 (62.00) followed by CHFUM-23 (62.30), CHFUM-19 (62.53), CHFUM-14 (62.73), CHFUM-25 (63.17), CHFUM-3 (63.40), CHFUM-21 (63.53), CHFUM-15 (63.53), CHFUM-9 (63.53) and CHFUM-20 (63.73). Among all genotypes, CHFUM-24 (2.73) produced their first staminate flower at lowest nodes followed by CHFUM-20 (3.00), CHFUM-21 (3.20), CHFUM-18 (3.30), CHFUM-13 (3.30), CHFUM-17 (3.33), CHFUM-12 (3.53), CHFUM-8 (3.53) and CHFUM-14 (3.63). The genotype CHFUM-24 (10.43) produced the first pistillate flower at lowermost node followed by CHFUM-14 (10.77), CHFUM-20 (11.20), CHFUM-1 (11.33), CHFUM-16 (11.37), CHFUM-12 (11.77) and CHFUM-21 (11.83). Among all genotypes, the minimum days to first fruit harvest was recorded in CHFUM-21 (79.20) followed by CHFUM-23 (81.10), CHFUM-24 (81.30), CHFUM-14 (81.40), CHFUM-13 (82.00), CHFUM-22 (82.20), CHFUM-15 (82.37). Srikanth *et al.* (2017), Kumar *et al.* (2017) also reported varying mean performance of pumpkin genotypes in their experiments for earliness traits. These genotypes may be used in crossing programmes for imparting earliness in the hybrids.

Among the horticultural characters, number of fruits per plant, polar circumference, equatorial circumference, flesh thickness, cavity length, average fruit weight and fruits yield per plant are the major yield contributing traits.

Out of 25 genotypes, maximum number of fruits per plant was recorded in CHFUM-2 (3.73) followed by CHFUM-16 (3.57), CHFUM-19 (3.57), CHFUM-13 (3.40) and CHFUM-1 (3.36). The maximum polar circumference was recorded in genotype CHFUM-20 (68.93) followed by CHFUM-10 (67.63), CHFUM-23 (66.80), CHFUM-6 (66.10) and CHFUM-19 (64.93). The highest equatorial circumference was noted in CHFUM-23 (73.80) followed by CHFUM-10 (67.93), CHFUM-21 (64.13) and CHFUM-14 (63.13). The flesh thickness was recorded maximum in CHFUM-12 (5.16) followed by CHFUM-17 (4.76) and CHFUM-3, CHFUM-4 (4.50). The minimum cavity length was noted in CHFUM-5 (5.93) followed by CHFUM-9 (7.63), CHFUM-2 (7.76) and CHFUM-17 (8.06). These findings are in conformity with reported of Nagar *et al.* (2017), Kumar *et al.* (2017), Srikanth *et al.* (2017) and Martins *et al.* (2015a).

The maximum number of seed per fruit was recorded in CHFUM-5 (401.10) followed by CHFUM-2 (318.33), CHFUM-14 (314.00) and CHFUM-7 (301.66) while maximum seed weight was noted in CHFUM-10 (15.57) followed by CHFUM-23 (14.37), CHFUM-10 (14.56) and CHFUM-23 (14.36). Similar findings were also reported by Martins *et al.* (2015) and Srikanth *et al.* (2017).

The maximum fruit weight was found in CHFUM-23 (3.00 kg) followed by CHFUM-14 (2.90 kg), CHFUM-19 (2.90 kg), CHFUM-12 (2.87 kg), CHFUM-15 (2.75 kg), CHFUM-11 (2.70 kg) and CHFUM-24 (2.63 kg). Thus genotypes having more fruit weight and number of fruits per plant and total fruit yield per hectare could be exploited as promising variety for improved fruit yield. Moreover, these genotypes may be used in future breeding programmes for improvement of fruit traits and yield. Variability in major yield contributing traits has also been by Kumar *et al.* (2017), Martins *et al.* (2015).

The maximum fruit yield per plant was recorded in CHFUM-16 (7.36 kg) followed by CHFUM-19 (7.07 kg), CHFUM-22 (6.00 kg), CHFUM-2, CHFUM-9 (5.83 kg) and CHFUM-18 (5.96 kg) while maximum fruit yield per hectare was recorded in CHFUM-16 (314.76 q/ha) followed by CHFUM-19 (301.93 q/ha), CHFUM-15 (274.66 q/ha), CHFUM-10 (267.76 q/ha) and CHFUM-22 (256.166 q/ha) Similar results have also been reported by Nagar *et al.* (2017), Kumar *et al.* (2017), Srikanth *et al.* (2017).

The maximum TSS was observed in CHFUM-17 (8.66 °B) followed by CHFUM-2 (8.57 °B) and CHFUM-5 (8.23 °B), CHFUM-14 (7.93 °B), CHFUM-9 (7.83 °B), CHFUM-25 (7.77 °B), CHFUM-1 (7.63 °B) and CHFUM-20 (7.50 °B) similar results have also been reported by Kumar *et al.* (2017).

The maximum carbohydrate was recorded in CHFUM-22 (559.50 mg/100 g) and it was found statically at par with CHFUM-21 (550.50 mg/100 g), CHFUM-20 (547.50 mg/100 g), CHFUM-19 (541.50 mg/100 g) and CHFUM-17 (540.00 mg/100 g). Highest vitamin A was estimated in CHFUM-1 (14.57 mg/100 g) followed by CHFUM-7 (13.85 mg/100 g) and CHFUM-4 (13.79 mg/100 g).

As the *per se* performance cannot be the sole criteria for selection programme as the value of the parent selected may not be observed in progeny. Hence, it is worthwhile to study variability parameters.

### **5.3 Genetic variability**

The success of any breeding programme depends on the availability of genetic variability present in the population, which is however, not directly measurable by itself, but has to be inferred with the phenotypic expression. The phenotypic may be therefore defined as a linear function of genotypes (G), environment (E) and genotype × environment (G × E) interaction effect. A wide spectrum of variability will provide an insight to be local condition.

A wide range of variability was observed in the genotypes. However, the information based on the range provides rough estimates of the degree of the variation

present in the materials and is more reliable if based on coefficients of variability. For all the characters studied, phenotypic coefficients of variability were higher in magnitude than genotypic coefficients of variability, though difference was very less in majority of the cases. Thus, showing that these traits are less influenced by environmental factors. Coefficients of variability varied in magnitude from character to character (either low or moderate or high). Therefore, it indicated that there was a great diversity in the experimental material used.

The extent of genetic variability observed in the present study for the most of the attributes in pumpkin was quite high and the same can be exploited by the breeders for increasing the productivity. In the present investigation, the high genotypic coefficient of variation (GCV) were observed for vitamin A, cavity length, 100 seed weight, number of seeds per fruit, node bearing first staminate flower, total fruit yield, average fruit weight, fruit yield per plant number of fruit per plant, flesh thickness and carbohydrates. This reflects greater genetic variability among the genotypes for these characters for making further improvement by selection. These results were in conformity with Srikanth *et al.* (2017); Shrikant *et al.* (2017), Kumar *et al.* (2017), Sulatana *et al.* (2015), and Kumar *et al.* (2011). However, the moderate GCV were recorded in case of total soluble solids, vine length equatorial circumference, node bearing first festaminate flower and polar circumference. The estimates of these parameters are in line with the findings of Shrikant *et al.* (2017), Kumar *et al.* (2017), and Kumar *et al.* (2011). The lowest estimates of GCV were recorded in case of days to first harvest, days to first pistillate flower anthesis and days to first staminate flower anthesis. The estimates of these parameters are in line with the findings of Shrikant *et al.* (2017), Kumar *et al.* (2017), and Kumar *et al.* (2011). Wide variability present in experimental materials suggested that there are ample scopes for bringing out improvement in these characters.

#### **5.4 Heritability and genetic advance**

The genotypic coefficient of variation does not offer full scope to estimate the variations that are heritable and therefore, estimation of heritability becomes necessary. The effectiveness of selection depends upon genetic advance of the character selected along with heritability. In the present investigation the estimates of heritability (broad sense) was high for all of the characters under study. This indicates that selection for these characters will be effective as phenotype is a true representative of genotype. Heritability estimates in broad sense will be reliable if accompanied by high genetic advance (Burton and de Vane, 1953).

In the present investigation, high heritability estimates (broad sense) was recorded for all the characters under study *viz.*, vitamin A, cavity length, carbohydrates, 100

seed weight, fruit yield/plant, number of fruits/plant, total fruit yield, polar circumference, equatorial circumference, number of seeds/fruit, flesh thickness, average fruit weight days to first staminate flower anthesis, and vine length. High heritability for different traits indicated that large proportion of phenotypic variance has been attributed to genotypic variance and suggested that selection could be made for these traits on the basis of phenotypic expression. Burton (1952) also suggested that a genotypic coefficient of variation along with high heritability gives clear picture of gain to be expected from selection. Mahmud *et al.* (2016) have also suggested that the character that shows high heritability with high genetic advance is controlled by additive gene action and can be improved through simple or progeny selection methods. Selection for the traits having high heritability coupled with high genetic advance is likely to accumulate more additive genes leading to further improvement of their performance. The characters showing high heritability along with moderate or low genetic advance can be improved by intermating superior genotypes of segregating population developed from combination breeding. The high heritability values for yield and component characters are in line with the findings of Kumar *et al.* (2017), Srikanth *et al.* (2017), Mahmud *et al.* (2016), and Kumar *et al.* (2011).

Further, Johnson *et al.* (1955) reported that high heritability estimates along with high genetic gain were useful than heritability alone for effective selection. Similarly, in the present study the characters like vitamin A, cavity length, carbohydrates, 100 seed weight, fruit yield per plant, number of fruit per plant, total fruit yield, polar circumference, equatorial circumference, number of seed per fruit, flesh thickness, average fruit weight, days to first staminate flower anthesis, and vine length, high heritability with high genetic advance indicated that these characters are under additive gene effects and hence these characters are more reliable for effective selection (Panse, 1957). These findings were in agreement with Kumar *et al.* (2017), Srikanth *et al.* (2017), Mahmud *et al.* (2016), and Kumar *et al.* (2011).

High heritability coupled with moderate genetic gain was observed for number of primary branches per plant, node bearing first staminate flower, total soluble solids, and days to first fruit harvest. This indicated that these characters are under non-additive gene effects and selection for these characters will be less effective. Such traits are more under the influence of environment and do not respond to selection. These findings contradicted the findings of Kumar *et al.* (2017).

However, the breeder should adopt suitable breeding methodology to utilize both additive and non-additive gene effects simultaneously, since varietal and hybrid development will go a long way in the breeding programmes in case of pumpkin.

## 5.5 Correlation studies

Yield being a complex character, is influenced by many yield components. Knowledge on the impact of various components on yield is essential before selection of desirable genotypes. In this context, correlation analysis will indicate possible association between the yield and yield attributes of pumpkin genotypes. Estimation of correlation is a simple tool to select pumpkin genotypes suitable for further crop improvement programme. The genotypic correlation coefficients among the yield and yield attributes in pumpkin suggested that inter correlation among the yield components need to be estimated because one component influences the other related components (Tamil selvi *et al.*, 2012).

Correlation studies have been done at both genotypic and phenotypic level. The perusal of the data revealed that the correlation at the genotypic as well as phenotypic levels has the same trend for most of the traits studied. The magnitude of genotypic correlation coefficients was relatively higher than the corresponding phenotypic correlation coefficients in almost all the characters paired indicated the inherent association between various characters. Similar result was obtained by Chaudhari *et al.* (2017). Lower phenotypic correlation coefficients than genotypic correlation coefficients indicate that both genotypic and environment correlation in those study act in same direction, and finally maximize their expression at phenotypic level. Correlation measures only mutual relationship without concern cause and effects of relationship Yadav *et al.* (2017).

Path coefficient analysis provides a method for separating out direct and indirect effects and measures the relative importance of casual factor which ultimately affect the yield.

The study on correlation coefficients indicates the nature of association and this alone does not provide an exact insight into relative influence of each component character towards yield because a character may not be directly correlated with yield but may influence it through other characters. These component characters are not independent in their action but are interlinked and in this interlinked complex genetic system, selection practiced for one individual character might subsequently bring about a simultaneous change in other. The association between two traits that can be directly observed is the phenotypic correlation. A phenotypic correlation is usually estimated by the product moment correlation (simple correlation). The genotypic correlation in its true sense may be interpreted as the correlation of breeding values (additive genetic). Therefore selection made for one trait influenced the other linkage or pleiotropically affected traits.

In the present investigation, the genotypic correlation coefficients were higher in magnitude than phenotypic correlation coefficients for all the characters; indicated strong association between the two characters genetically. Correlation studies revealed that characters like number of fruit per plant had significant positive correlation with fruit yield per plant both at genotypic and phenotypic level which indicated the importance of these traits in selection for yield. Direct selection based on these traits would result in simultaneous improvement of aforesaid traits and fruit yield *per se* in pumpkin. Similar results were also reported by Chaudhari *et al.* (2017), Shrikant *et al.* (2017); Yadav *et al.* (2017) in their experiments. Positive and significant correlation could possibly be used in selecting better yield.

None of the characters showed a negative correlation with the fruit yield. The similar result reported by the Khan *et al.* (2016).

Days to first staminate flower anthesis and days to first pistillate flower anthesis had highly positive correlation and significant to node bearing first staminate flower, node bearing first festaminate flower respectively at both genotypic and phenotypic level to be considered for simultaneous selection. Thus, emphasis should be given to selection of these characters for the yield improvement in pumpkin. Similar results were also reported by Chaudhari *et al.* (2017) and Khan *et al.* (2016). In general, earliness in cucurbits is measured as the days taken for first staminate flower appearance and node number for first staminate flower appearance and are considered as desirable traits in any hybrid development programme (Tamil selvi *et al.*, 2012).

On the basis of correlation association analysis, it could be concluded that the selection criteria based on number of fruits per plant can provide better results for the improvement of fruit yield in pumpkin.

## **5.6 Path coefficient analysis**

Correlation study only provides information on relationship and does not give any idea about their direct and indirect contribution. Consequently, this information is sometimes misleading with respect to identification of yield components. Path coefficient analysis is one such method which partitions correlation into direct and indirect effects (Wright, 1921; Dewey and Lu, 1959). Path analysis breaks correlation between traits into their direct and indirect effects on the economic product, permitting a critical examination of specific trait contributing individually and collectively to produce the total effect. It also helps to measure the relative importance of each trait Chaudhari *et al.* (2017). The path analysis at phenotypic level may not provide a true picture of direct and indirect causes, and it is

advisable to understand the contribution of different traits towards the fruit yield per plant at the genotypic level. The residual effect in path coefficient analysis usually indicates that there are traits other than those included in pathways that contribute to the dependent variable. Path coefficient analyses did not account for all variation in fruit yield as indicated by the magnitude of residual effect indicating that there are other traits also that contributed to fruit yield. The low residual effect (0.02352) at genotypic level indicates that all the important characters correlated with fruit yield in bitter melon (Singh *et al.*, 2014). In the present investigation at genotypic level, fruit yield per plant was taken as dependent variable and other 17 traits were considered as causal variables.

Days to first pistillate flower anthesis (1.185) had maximum positive direct effect on fruit yield per plant followed by number of fruits per plant (0.681), node bearing first staminate flower (0.293), polar circumference (0.259), total soluble solids (0.133), number of primary branches per plant (0.103) and flesh thickness (0.043) at genotypic level; indicated that these are the real independent characters and have maximum contribution towards increase in fruit yield per plant. These observations were in conformity with Chaudhari *et al.* (2017), Sampath and Krishnamoorthy (2017), Khan *et al.* (2016), Srikanth *et al.* (2015), Naik *et al.* (2015), Sultana *et al.* (2015) and Shivanada *et al.* (2013).

The high positive direct effect of days to first pistillate flower anthesis on fruit yield per plant was counter balanced by its positive indirect effect *via* days to first fruit harvest and days to staminate flower anthesis, node bearing first staminate flower, number of primary branches per plant, vitamin A, vine length, number of fruits per plant, polar circumference, at genotypic level. However, high positive direct effect of number of fruits per plant on fruit yield per plant was counter balanced by its positive indirect effect *via* node bearing first staminate flower, node bearing first pistillate flower, days to first fruit harvest, vitamin A and days to first pistillate flower at genotypic level.

However, maximum negative direct effect on fruit yield per plant was imposed by vitamin A, vine length, days to first fruit harvest and days to first staminate flower anthesis (both at phenotypic and genotypic level), total soluble solids (only at phenotypic level), cavity length, number of seeds per fruit, node bearing first staminate flower, 100 seeds weight, carbohydrates and equatorial circumference (only at genotypic level),

Days to first fruits harvest had negative direct effect on fruit yield per plant at genotypic level; however its positive significant association with fruit yield was achieved due to indirect positive contribution through 100 seeds weight, equatorial circumference, cavity length and polar circumference. These observations were in conformity with Srikanth *et al.*

(2015), Shivanada *et al.* (2013), Sampath and Krishnamoorthy (2017) and Nagar *et al.* (2017).

Path coefficient analysis revealed that days to first pistillate flower anthesis, number of fruits per plant, node bearing first staminate flower, polar circumference, total soluble solids, number of primary branches per plant and flesh thickness cannot be ignored when selection is practiced for improving the fruit yield per plant in pumpkin.

Low value of residual effect at genotypic level (0.250) indicated that 75.00 % of variability could be explained through the component characters.

## **5.7 Genetic divergence**

### **5.7.1 Mahalanobis generalized distance**

Mahalanobis  $D^2$  statistics of multivariate analysis is recognized as a powerful tool in quantifying the degree of genetic divergence among the populations.

More diverse the parents within a reasonable range, better are the chances of improving economic characters under consideration in the offspring. Being a numerical estimate, it has added advantage over other criteria permitting precise comparison among all possible pairs of population in any group. When considering data on genotype divergence derived with use of Mahalanobis  $D^2$  statistics revealed that 25 genotypes included in the present study, varied significantly for all the characters under evaluation. The results of the present study pointed out a positive contribution of genetic divergence and yield components; this can be of considerable help in selection of yield and other economic traits.

Based on  $D^2$  value, 25 genotypes were grouped into 6 clusters which indicated a large genetic diversity. Out of the 6 clusters, maximum number of genotypes were accommodated in cluster I with 11 genotypes, cluster II with 10 genotypes, cluster III, cluster IV, cluster V and cluster VI were solitary containing single genotype each namely CHFFUM-16, CHFPUM-17, CHFPUM-20 and CHFUM-24 respectively. Different genotypes with their respective clusters are presented in Table 4.9. In many cases, the same cluster included the geographic distribution and genetic divergence did not follow the same trend. These results were in concurrence with Sultana *et al.* (2015). The absence of relationship between genetic diversity and geographical distance indicated that forces other than geographical origin, such as exchange of genetic stocks, genetic drift, variation, natural and artificial selection were responsible for genetic diversity.

In the present study, vitamin A contributed maximum percent to the diversity followed by cavity length, carbohydrates, fruit yield per plant, equatorial circumference, number of seeds per fruit and days to first pistillate flower anthesis. Similar contributions

towards diversity have also been reported by Sivananda *et al.* (2013). 25 genotypes were grouped into 6 clusters which showed inter cluster  $D^2$  value ranged from 409.051 to 3064.727 among genotypes. The inter cluster distance between cluster III and IV (409.051) indicated that genotypes (CHFPUM-16 and CHFPUM-17) were genetically close to each other.

Maximum inter cluster distance was observed between cluster II and V (3064.727) and indicated that genotypes (CHFPUM-2, CHFPUM-9, CHFPUM-5, CHFPUM-8, CHFPUM-3, CHFPUM-7, CHFPUM-4, CHFPUM-6, CHFPUM-1 and CHFPUM-10) and (CHFPUM-20) are highly divergent. These two clusters revealed highly divergent parents with desirable traits and may be recommended for future breeding programmes. Inter crossing the genotypes from these two clusters may generate wider variability and is expected to throw high yielding transgressive segregants in a population improvement programme.

### **5.7.2 Cluster mean analysis**

The mean values obtained for varying number of genotypes in each cluster, although, cannot be compared statistically, but to get a relative idea of diversity among the clusters they are compared. Based on the range of means for each character, it became possible to know the characters influencing the divergence. It also helps to categorize the cluster under high fruit yield per plant bearing groups or according to their average performance for a particular character *viz.*, clusters III, I, II, IV, VI and V. Cluster III (CHFPUM-16) recorded highest fruit yield per plant because it has higher side of values for vine length, number of branches per plant, and number of fruits per plant. While cluster V (CHFPUM-5) formed the lowest performing group for fruit yield per plant.

Genetic divergence among 25 genotypes revealed that cluster II with genotypes CHFPUM-2, CHFPUM-9, CHFPUM-5, CHFPUM-8, CHFPUM-3, CHFPUM-7, CHFPUM-4, CHFPUM-6, CHFPUM-1 and CHFPUM-10 were the more divergent for improving for days to first pistillate flower anthesis, node bearing first staminate flower, days to first fruit harvest, number of seeds per fruit and vitamin A. Cluster V with single genotype (CHFPUM-20) is found to be promising for improving polar circumference, cavity length and carbohydrates. Cluster VI with single genotype (CHFPUM-24) was found to be promising for improving days to first staminate flower anthesis, and average fruit weight. Cluster IV with single genotypes (CHFPUM-17) was found to be promising for improving vine length, node bearing first pistillate flower, equatorial circumference flesh thickness, 100 seeds weight and total soluble solids. Cluster III with single genotypes (CHFPUM-16) was found to be promising for improving number of branches per plant, number of fruits per plant, fruit yield

per plant and total fruit yield q per hectare. Hence, genotypes in these clusters can be utilized in pumpkin improvement programme as donor parents for improving all these characters.

Hence, apart from selecting genotypes from these clusters which have high inter-cluster distance for hybridization, one can also think of selecting parents based on extent of genetic divergence in respect to a particular character of interest. This is to mean that, if breeder's intention is to improve fruit yield, they can select parents which are highly divergent with respect to these characters.

## **5.8 Characterization of genotypes through seed protein profiles**

The introduction of biochemical techniques like Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), isozyme markers has been particularly helpful in deducing systematic relationships between groups where morphological and cytological data were not corollary. SDS-PAGE is an economical, simple and extensively used technique for describing the seed protein diversity of crop germplasm (Fufa *et al.*, 2005; Iqbal *et al.*, 2005). Seed storage protein analysis helps in protein identification and characterization of diversity in crop varieties and their wild relatives. Variation in protein band provides information on the relationship among the seeds collected from various geographic regions. Storage proteins play main role that includes nitrogen and energy source and defense against insect and pathogens such as bacteria and fungi (Singh *et al.*, 2015).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is an economical, simple and extensively used biochemical technique for describing the seed protein diversity of crop germplasm (Cook, 1995; Das and Mukarjee, 1995). Seed protein variants have been observed to be the most widely used biochemical genetic markers during the last quarter century. Its success depends upon the polymorphism of seed and seedling proteins and the fact that these proteins represent primary gene products and are largely unaffected by the environmental interactions (Smith and Smith, 1992). Caution should be taken in interpreting darkness and thickness as the kind of variation may be due to the lack of separation on the gels of several proteins having similar migration rates and studies are required to estimate the number of genes causing quantitative variation in seed protein bands (Ladizinsky and Hymowitz, 1979).

The seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary relationships among crops and their wild relatives (Das and Mukarjee, 1995). The SDS-PAGE is the most commonly methods used for studying protein differences between species. However previously varieties were described on the basis of their morphological characters. But now a day's SDS is an anionic

detergent which binds strongly and denatures proteins. Hence, the number of SDS molecule bound to a polypeptide chain is approximately half the number of amino acid residues in that chain. There after the protein SDS complex carries net negative charges. And move towards the anode and the finally separation is based on the size of the protein. Therefore, modification of PAGE called SDS-PAGE, an oligomer protein may be dissociated into its sub-units and the molecular weights of sub-units are determined (Singh *et al.*, 2016).

The Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was carried out to determine the protein banding patterns of 25 pumpkin genotypes. The genotypes showed considerable variation in band number of protein in present investigation which ranged from 13-26. Among the genotypes CHFUM-6, CHFUM-18 and CHFUM-25 showed maximum numbers (26) of protein bands while the minimum numbers (13) of bands were present in genotypes CHFUM-13.

Cluster analysis utilizing SDS-PAGE banding patterns produced a dendrogram depicting clear separation of genotypes. The genotype CHFUM-20, CHFUM-24, CHFUM-17 and CHFUM-16 formed an individual cluster in dendrogram generated by Tocher's method analysis but the dendrogram based on SDS-PAGE banding pattern grouped genotype CHFUM-20, CHFUM-24 and CHFUM-16 with CHFUM-5, CHFUM-12, CHFUM-9, CHFUM-6, CHFUM-2, CHFUM-7, CHFUM-14, CHFUM-10, CHFUM-4, CHFUM-3, CHFUM-13, CHFUM-25, CHFUM-8, CHFUM-19, CHFUM-21, CHFUM-15, CHFUM-23, CHFUM-16, and CHFUM-18 in first sub-sub cluster (IA1), while genotype CHFUM-17 with CHFUM-11 in second sub-sub cluster (IA2). This shows that CHFUM-20, CHFUM-24 and CHFUM-16 is more closely related to CHFUM-5, CHFUM-12, CHFUM-9, CHFUM-6, CHFUM-2, CHFUM-7, CHFUM-14, CHFUM-10, CHFUM-4, CHFUM-3, CHFUM-13, CHFUM-25, CHFUM-8, CHFUM-19, CHFUM-21, CHFUM-15, CHFUM-23, CHFUM-16, and CHFUM-18. Also, CHFUM-17 is more closely related to CHFUM-11 as depicted in dendrogram generated by SDS-PAGE banding pattern.

Similarly, the genotype CHFUM-1 was solitary grouped in cluster II in dendrogram generated by SDS-PAGE banding pattern while it was grouped with CHFUM-, CHFUM-2, CHFUM-9, CHFUM-5, CHFUM-8, CHFUM-3, CHFUM-7, CHFUM-4, CHFUM-6 and CHFUM-10 in cluster II in dendrogram generated by Tocher's method analysis. This shows that the genotype CHFUM-1 is not closely related to other genotypes at genetic level.

It was also noticed that both the dendrogram placed the pumpkin genotypes CHFUM-15, CHFUM-25, CHFUM-16, CHFUM-21, CHFUM-11, CHFUM-19,

CHFPUM-18, CHFPUM-12, CHFPUM-13, and CHFPUM-23 in one major cluster based on both the dendrogram.

The similarity index among 25 genotypes of pumpkin using SDS-PAGE analysis showed a maximum degree of similarity index percentage exhibiting a value of 62.86 between genotypes CHFPUM-1 with CHFPUM-11. Further, the genotype CHFUPM-1 with CHFPUM-21, CHFUM-3 with CHFUM-13 and CHFPUM-20 showed minimum percentage of similarity index between each other as evident by lowest value of similarity index (0.00%). Genetic diversity analysis, based on seed protein profile using SDS-PAGE was also reported by Singh *et al.* (2016) in garden pea, Singh *et al.* (2015) in chick pea and Kandasamy (2014) in pumpkin, Yatung *et al.* (2014) in chilli, Dubey and Ram (2008) in bottle gourd and by Singh and Ram (2001) in pumpkin.

Thus, SDS-PAGE marker data provided more sub groupings and revealed higher amount of diversity as compared to morphological data analysis. It is evident from the present study that genetic relationship estimated from protein banding pattern enhanced the resolution of diversity and thus provided a better picture of variability as compared to morphological markers. Although SDS-PAGE analysis could show discrete variation among few genotypes of pumpkin under study, this protein marker should be applied in future to more number of genotypes to arrive at a reasonable conclusion. Therefore, seed protein electrophoresis could be proved to be a successful technique in certain cases to distinguish morphologically indistinguishable genotypes. SDS-PAGE is a predominant tool to study molecular systematic for identification of genotypes based on proteins and a promising method to discriminate between morphologically similar pumpkin genotypes in a limited way.

The method used for protein extraction has proved to be efficient for extraction of soluble seed storage proteins. The divergence observed in pumpkin should be further confirmed using molecular tools. Protein profiling is an efficient method for studying genetic diversity, coupling biochemical method with molecular approaches would help plant taxonomist to understand the variance among plants in further depth. The divergence observed in pumpkin should be further confirmed using both proteomic and molecular tools.

## **5.9 Potential genotypes for various traits in pumpkin**

The variable genotypes offered sufficient opportunity to identify desirable types. Some genotypes with good horticultural traits and yield were sorted out and presented in (Table 5.1).

**Table 5.1 Three best potential genotypes for productivity characters in pumpkin**

<b>S.N.</b>	<b>Traits</b>	<b>Genotypes</b>
1.	<b>Vine length (m)</b>	CHFPUM-12 (11.36), CHFPUM-1 (11.00) and CHFPUM-17 (8.56)
2.	<b>Number of primary branches</b>	CHFPUM-2 (13.63), CHFPUM-25 (12.87) and CHFPUM-22 (12.73),
3.	<b>Days to first staminate flower anthesis</b>	CHFPUM-15 (58.53), CHFPUM-14 (58.83) and CHFPUM-4 (58.87),
4.	<b>Days to first pistillate flower anthesis</b>	CHFPUM-5 (62.00), CHFPUM-23 (62.30) and CHFPUM-19 (62.53),
5.	<b>Node bearing first staminate flower</b>	CHFPUM-24 (2.73), CHFPUM- 20 (3.00) and CHFPUM-21 (3.20),
6.	<b>Node bearing first staminate flower</b>	CHFPUM-24 (10.43), CHFPUM-14 (10.77) and CHFPUM-20 (11.20),
7.	<b>Days to first fruit harvest</b>	CHFPUM-21 (79.20), CHFPUM-23 (81.10) and CHFPUM-24 (81.30),
8.	<b>Number of fruits per plant</b>	CHFPUM-2 (3.73), CHFPUM-16 (3.57) and CHFPUM-19 (3.57)
9.	<b>Polar circumference (cm)</b>	CHFPUM-20 (68.93), CHFPUM-10 (67.63) and CHFPUM-23 (66.80),
10.	<b>Equatorial circumference (cm)</b>	CHFPUM- 23 (73.80), CHFPUM-10 (67.93) and CHFPUM-21 (64.13)
11.	<b>Flesh thickness (cm)</b>	CHFPUM-12 (5.16), CHFPUM-17(4.76) and CHFPUM-3, CHFPUM-4 (4.50)
12.	<b>Cavity length (cm)</b>	CHFPUM-5 (5.93), CHFPUM-9 (7.63) and CHFPUM-2 (7.76)
13.	<b>Number of seed per fruit</b>	CHFPUM-23 (134.33), CHFPUM-6 (183.33) and CHFPUM-8 (186.07)
14.	<b>100 seed weight (g)</b>	CHFPUM-10 (14.57), CHFPUM-23 (14.37) and CHFPUM-20 (11.50)
15.	<b>Average fruit weight (kg)</b>	CHFPUM-23 (3.00), CHFPUM-14 (2.90) and CHFPUM-19 (2.90),
16.	<b>Fruit yield per plant (kg)</b>	CHFPUM-16 (7.36), CHFPUM-19 (7.07) and CHFPUM-15 (6.43)
17.	<b>Total fruit yield per hectare (q)</b>	CHFPUM-16 (314.76), CHFPUM-19 (301.93) and CHFPUM-15 (274.66)
18.	<b>Total soluble solids</b>	CHFPUM-17 (8.66), CHFPUM-2 (8.57) and CHFPUM-5 (8.23),
19.	<b>Carbohydrates mg/100g</b>	CHFPUM-22 (559.50), CHFPUM-21 (550.50) and CHFPUM-20 (547.50),
20.	<b>Vitamin A mg/100g</b>	CHFPUM-1 (14.57), CHFPUM-7 (13.85) and CHFPUM-4 (13.79)

**CHAPTER-6**  
**SUMMARY**  
**AND**  
**CONCLUSION**

## Chapter-6

# Summary and Conclusion

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The present investigation entitled “Studies on genetic diversity among indigenous landraces of pumpkin (*Cucurbita moschata* Duch. ex Poir.)” of North Eastern India through morphological traits and seed protein profiling was carried out at Vegetable Research Farm, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh during 2017 with the objective of studying different genetic parameters such as mean, range, genotypic coefficient of variation, phenotypic coefficient of variation, correlations, direct and indirect effect of important characters towards fruit yield, genetic divergence and seed protein profile.

The experimental materials comprised of 25 diverse genotypes of pumpkin. The different genotypes were raised in Randomized Block Design with three replications. Along with four morphological traits, observation were also recorded for 20 quantitative traits viz., vine length, number of branches per plant, days to first staminate flower anthesis, days to first pistillate flower anthesis, node bearing first staminate flower, node bearing first festaminate flower, days to first harvest, number of fruits per plant, polar circumference of fruit, equatorial circumference of fruit, fruit flesh thickness, cavity length, number of seeds per fruit, 100 seed weight, average fruit weight, fruit yield per plant, fruit yield per hectare, total soluble solids, total carbohydrate and vitamin A.

Salient findings of present investigation are summarized below:

1. The analysis of variance in the present investigation revealed significant differences for all the 20 traits among 25 genotypes of pumpkin which indicated the scope for selection of suitable initial breeding material for further crop improvement.
2. On the basis of mean performance of the genotypes for all the traits studied, the genotypes CHFPUM-16, CHFPUM-19, CHFPUM-22, CHFPUM-2, CHFPUM-9 and CHFPUM-18 were found to be superior for the most of the yield components and fruit quality traits. So, these genotypes can be used as parental source in breeding programme.
3. Genotypic and phenotypic coefficients of variation were found high for vitamin A, cavity length, 100 seed weight, number of seeds per fruit, node bearing first staminate flower, total fruit yield, average fruit weight, fruit yield per plant number of fruit per

plant, flesh thickness and carbohydrates. This reflects greater genetic variability among the genotypes for these characters for making further improvement through selection. Wide variation present in experimental materials suggested that there is ample scope for bringing out improvement in these characters.

4. The characters like, vitamin A, cavity length, carbohydrates, 100 seed weight, fruit yield per plant, number of fruits/plant, total fruit yield, polar circumference, equatorial circumference, number of seeds/fruit, flesh thickness, average fruit weight, days to first staminate flower anthesis and vine length recorded high heritability coupled with high genetic advance which indicated that these characters are under additive gene effects and hence these characters are more reliable for effective selection. Hence, selection on the basis of these characters will be more useful for the improvement of this crop towards higher fruit yield and quality production.
5. Correlation studies revealed that characters like number of fruits/plant has positive correlation significant with fruit yield per plant both at genotypic and phenotypic level which indicated the importance of these traits in selection for yield. Direct selection based on these traits would result in simultaneous improvement of aforesaid traits and fruit yield per plant in pumpkin.
6. Days to first pistillate flower anthesis had maximum positive direct effect on fruit yield per plant followed by number of fruits per plant, node bearing first staminate flower, polar circumference, total soluble solids, number of primary branches per plant and flesh thickness at genotypic level. This indicated that these are the real independent characters and have maximum contribution towards increase in fruit yield per plant. Hence, it would be rewarding to lay stress on these characters in selection programme for increasing the fruit yield per plant in pumpkin.
7. Genetic diversity assessed by using Mahalanobis  $D^2$  statistics. 25 genotypes of pumpkin were grouped into 6 clusters. The inter-cluster  $D^2$  value was observed maximum between cluster II and V. Genetic divergence among 25 genotypes revealed that cluster cluster II with genotypes CHFPUM-2, CHFPUM-9, CHFPUM-5, CHFPUM-8, CHFPUM-3, CHFPUM-7, CHFPUM-4, CHFPUM-6, CHFPUM-1 and CHFPUM-10 were the more divergent for improving for days to first pistillate flower anthesis, node bearing first staminate flower, days to first fruit harvest, number of seeds per fruit and vitamin A. Cluster V with single genotype (CHFUM-20) was found to be promising for improving polar circumference, cavity length and carbohydrates. Cluster VI with single genotype (CHFPUM-24) was found to be promising for improving days to first staminate flower anthesis and average fruit weight. Cluster IV

with single genotypes (CHFPUM-17) was found to be promising for improving vine length, node bearing first pistillate flower, equatorial circumference flesh thickness, 100 seeds weight and total soluble solids. Cluster III with single genotypes (CHFPUM-16) was found to be promising for improving number of branches per plant, number of fruits per plant, fruits yield/plant and total fruit yield q/ha. Hence, genotypes in these clusters can be utilized in pumpkin improvement programme as donor parents for improving all these characters. Moreover, out of 20 characters studied vitamin A, cavity length, carbohydrates, fruit yield per plant, equatorial circumference, number of seeds/fruit and days to first pistillate flower anthesis are most rewarding. Hence, the above characters are to be taken into consideration while attempting crop improvement studies in pumpkin.

8. The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) showed considerable variation in protein band numbers in 25 pumpkin genotypes ranging from 13-26. Accessions from different regions seemed to be closely related and accessions from the same region had different genetic background. Therefore, seed protein electrophoresis could be proved to be a successful technique in certain cases to distinguish morphologically indistinguishable genotypes. Molecular studies were useful to confirm the genetic diversity based on morphological characters and to characterize these landraces for more detailed examination. The genotype CHFUPM-1 with CHFPUM-21, CHFUM-3 with CHFUM-13 and CHFPUM-20 showed only (0.00%) percent similarity index. Hence, it can be recommended that these two genotypes could be utilized for crossing programme to create more genetic diversity or segregants of desired characteristics through pumpkin breeding programmes. From the findings of present investigation, it may be concluded that the genotypes CHFPUM-16, CHFPUM-19, CHFPUM-22, CHFPUM-2, CHFPUM-9 and CHFPUM-18 were found to be superior for the most of the yield components and fruit quality traits. So, these genotypes might be used as parental source in any breeding programme. From the correlation studies, it is evident that number of fruits per plant could improve fruit yield per plant. Therefore, recurrent selection programme aimed in this direction may yield fruitful results.
9. Path analysis revealed that days to first pistillate flower anthesis had maximum positive direct effect on fruit yield per plant followed by number of fruits per plant could be regarded as primary yield contributing components in pumpkin and hence it would be rewarding to lay stress on these characters in selection programme for increasing the fruit yield per plant in pumpkin.

10. Genetic diversity study revealed that vitamin A, cavity length, carbohydrates, 100 seed weight, fruit yield per plant, number of fruit per plant, total fruit yield, polar circumference, equatorial circumference, number of seed per fruit, flesh thickness, average fruit weight days to first staminate flower anthesis and vine length may be considered as important parameters in selecting genetically diverse parents for hybridization programme as well as for study of genetic diversity in pumpkin as these traits together accounted for 99.99 % to the total divergence. On the basis of maximum inter-cluster distance and *per se* performance, there is a scope of varietal improvement through hybridization programme involving the genotypes under cluster III (CHFPUM-16) with genotype under cluster V (CHFUM-20), cluster VI (CHFPUM-24) and cluster IV (CHFPUM-17).
11. Seed protein banding pattern through SDS-PAGE showed that the genotypes CHFUM-1 with CHFPUM-21, CHFUM-3 with CHFUM-13 and CHFPUM-20 were more distantly related to each other at genotypic level. Hence, it can be concluded that these two genotypes could be utilized for crossing programme to create more genetic diversity or segregants of desired characteristics through pumpkin breeding programmes.

## **6.1 Future line of work**

1. The wide range of variability was present for the all characters under present study. Hence, attention needs to be given for these characters during selection for improvement of fruit yield per plant.
2. Genotypes Identified for higher fruit yield per plant i.e., CHFPUM-16, CHFPUM-19, CHFPUM-22, CHFPUM-2, CHFPUM-9 and CHFPUM-18., may be further tested over locations and years to know their stability.
3. From the correlation studies, it was evident that number of fruits per plant could improve fruit yield per plant. Therefore, recurrent selection programme aimed in this direction may yield fruitful result.
4. Selected parents with desirable yield per plant with respect to different component traits can be involved in multiple crossing schemes to recombine different productivity components.
5. Genotypes with similar banding patterns should be further characterized by 2-D electrophoresis.
6. Advanced molecular techniques could be employed to identify duplicate genotypes for efficient management of pumpkin germplasm and to tag important gene available in the germplasm through linkage to DNA markers.

# BIBLIOGRAPHY

# Bibliography

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- Aboul-Nasr, M.H., Ramdan, B.R. and El-Dengawy, R.A. (1997). Chemical composition of pumpkin seeds. *Assiut. J. Agric. Sci.*, 28(1): 163-172.
- Adubofuor, J., Amoah, I., and Agyekum, P.B. (2016). Physicochemical properties of pumpkin fruit Pulp and sensory evaluation of pumpkin-pineapple juice blends. *American J. Food Sci. and Tech.*, 4(4): 89-96.
- Ahmed, B., Masud, M.A.T., Zakaria, M. Hossain, M.M. and Mian, M.A.K. (2016). Genetic divergence among inbred lines of pumpkin (*Cucurbita moschata* Duch ex Poir). *Bangladesh H. J. Pl. Breed. Genet.*, 29(2): 25-31.
- Akbar, A., Ahmed, H., Ghafoor, S. and Khan, A.I. (2010). Phylogeny and genetic diversity studies in *Capsicum* using seed storage proteins. *Curr. Res. J. Biol. Sci.*, 2(4): 250-252.
- Akter, R., Rasul, M.G., Islam, A.K.M. and Hossain, M.M. (2013). Genetic variability, correlation and path coefficient analysis of yield and quality traits in pumpkin (*cucurbita moschata* Duch ex Poir.). *Bangladesh J. Pl. Breed. Genet.*, 26(1): 25-33.
- Aliu, S. A., Haziri, S., Fetahu, N., Aliage, I., Rusinove, I., Haziri and Arapi, V. (2011). Morphological and nutritive variation in a collection of *Cucurbita pepo* L. growing in Kosova. *Notulae Scientia Biologicae.*, 3(2): 119-122.
- Al-Jibouri, H.A., Miller, P.A. and Robinson, H.F. (1958). Genotypic and environmental variances and covariances in an upland cotton cross of inter specific origin. *Agron. J.*, 50(10): 633-636.
- Andres, T.C. (2004). Diversity in tropical pumpkin (*Cucurbita moschata*) a review of infraspecific classification. *Progress in cucurbit.* Czech Republic. p.107-112.
- Anonymous, (2016 a). All India Area and Production of Fruits and Vegetables. Indian Horticulture Database, National Horticulture Board, NHB, website: [www.nhb.gov.in](http://www.nhb.gov.in).
- Anonymous. (2016 b). Package of practices for vegetable crops. Directorate of Extension, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, p. 35
- Aruah, C.B., Uguru, M.I. and Oyiga, B.C. (2010). Variations among some Nigerian *Cucurbita* landraces. *African J. Plant Sci.*, 4 (10): 374-386.
- Bahadur, A. and Singh, K.P. (2014). Pumpkin. In: Rana, M.K. (ed.). Scientific cultivation of vegetables. Kalyani Publishers, New Delhi, p. 209-246.
- Bailey, L.H. (1929). The domesticated cucurbits. *J. Genets. Herb.*, 2: 62.
- Bayfield, R.F. and Cole, E.R. (1980). Colorimetric estimation of vitamin A with trichloroacetic acid, *Methods in Enzymology*, 67: 180-195.
- Burton, G.W. (1952). Quantitative inheritance in grasses. In: Proceedings of 6<sup>th</sup> International Grassland Congress. 1(2): 227-285.
- Burton, G.W. and de Vane, E.H. (1953). Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. *Agron. J.*, 45(10): 478-481.
- Camacho, M.E., Franco, A., Cabrera, V., Garcia, D.B. and Beaver, L.W. (2006). Path analysis of yield components in tropical pumpkin. *J. Agric. Univ. P.R.*, 90(3-4): 183-191.
- Carvalho, L.M.J.D., Smiderle, L.D A.S.M., Carvalho, J.L.V.D., Cardoso, F.D.S.N., and Koblitz, M.G.B. (2014). Assessment of carotenoids in pumpkins after different home cooking conditions. *Food Sci. and Tech. (Campinas)*. 34(2): 365-370.
- Chaturvedi, S. and Chatervedi, S. (2001). Qualitative traits of some pumpkin (*Cucurbita moschata*). *Veg. Sci.*, 28(2): 182-183.
- Chaudhari, D.J., Acharya, R.R., Gohil, S.B. and Patel, N.A. (2017 a). Genetic divergence study in pumpkin (*Cucurbita moschata* Duch. Ex. Poir). *J. Pharmacogn. Phytochem.*, 6(4): 744-747.

- Chaudhari, D.J., Acharya, R.R., Gohil, S.B. Patel, N.A. and Bhalala, K.C. (2017 b). Variability, correlation and path analysis in pumpkin (*Cucurbita moschata* Duch. ex. Poir.). *J. Pharmacogn. Phytochem.*, 6(6): 142-145.
- Chheda, H.R., Fatokun, C.A. (1982). Numerical taxonomy of variation pattern in okra (*Abelmoschus esculentus* (L) Moench). *Botanical Gazette.*, 143: 253-261.
- Choudhary, R., Rai, G.K., Rai, S.K., Parveen, A., Rai, P.K. and Salgotra, R.K. (2015). Genetic diversity of brassica napus using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). *SABRAO J. Breed. Genet.*, 47(1): 14-20.
- Cook, R.J. (1995). Gel electrophoresis for the identification of plant varieties. *J. Chrom.*, 698: 281-299.
- Das, P.K. and Gupta, T.D. (1984). Multivariate analysis in black gram. *Indian J. Genet.* 44(2): 243-247.
- Das, S. and Mukarjee, K.K. (1995). Comparative study on seed proteins of *Ipomoea*. *Seed Sci. Technol.*, 23: 501-509.
- Dewey, J.R. and Lu, K.H. (1959). A correlation and path-coefficient analysis of components of crested wheat grass seed production. *Agron. J.*, 5(9): 515-518.
- Dubey R.K. and Ram, H.H. (2008) Characterization of advanced breeding lines and assessment of genetic diversity in bottle gourd (*Lagenaria siceraria* (Mol.) Standl.) through SDS-PAGE. *Int. J. Pl. Breed.*, 2(2): 85-86.
- Dudwadkar, S., Parab, M. and Singh, S. (2015). Diversity analysis among few cucurbitaceae using seed protein profile. *Int. J. Plant Anim. Environ. Sci.*, 5(1): 146-151.
- El-Hamed, K.E.A. and Elwan, M.W.M. (2011). Dependence of pumpkin yield on plant density and variety. *American J. Plant Sci.*, 2: 636-643.
- Emre, I. (2011). Determination of genetic diversity in the *Vicia* L. (section *Vicia*) by using SDS-PAGE. *Pak. J. Bot.*, 43(3): 1429-1432.
- Fayeun, L.S., Odiyi, A.C., Makinde, S.C.O. and Aiyelari, O.P. (2012). Genetic variability and correlation studies in the fluted pumpkin (*Telfairia occidentalis* Hook F.). *J. Pl. Breed. Crop Sci.*, 4(10): 156-160.
- Ferriol, M., Pico, B. Cordova, P.F. de., Nuez, F. and de Cordova, P.F. (2004). Molecular diversity of a germplasm collection of squash (*Cucurbita moschata*) determined by SRAP and AFLP markers. *Crop Sci.*, 44(2): 653-664.
- Fisher, R.A. and Yates, F. (1963). Statistical tables for biological, agricultural and medical research. Oliver and Boyd. Limited. p. 63.
- Fufa, H., Baenziger, P.S., Beecher, B.S., Dweikat, I., Graybosch, R.A. and Eskridge, K.M. (2005). Comparison of phenotypic and molecular marker based classifications of hard red winter wheat cultivars. *Euphytica*, 145:133-146.
- Gohari, A., Ardabili, F.R. and Khodaparast, M.H.H. (2011). Chemical composition and physico-chemical properties of pumpkin seeds. *Agr. Sci. Tech.*, 13: 1053-1063.
- Gomez, A.K. and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research. 2<sup>nd</sup> edition. Wiley India Private Limited, Ansari road, Daryaganj, New Delhi, pp. 134-138.
- Gwanama, C., Mwala, M.S. and Nichterlein, K. (1998). Path analysis of fruit yield components of *cucurbita moschata* Duch. *Tropical Agric. J. Res. Exten.*, 1(1): 19-22.
- Hanson, C.H., Robinson, H.R. and Comstock, R.S. (1956). Biometrical studies of yield in segregating populations of Korean Lespedeza. *Agron. J.*, 48(6): 268-272.
- Hedge, J.E. and Hofreiter, B.T. (1962). In: *Carbohydrate Chemistry*, 17 (Eds. Whistler R.L. and Be Miller, J.N.), Academic Press, New York.
- Hossain, M.F., Rabbani, M.G., Hakim, M.A., Amanullah, A.S.M. and Ahsanullah, A.S.M. (2010). Study on variability character association and yield Performance of cucumber (*Cucumis sativus* L.). *Bangladesh Res. Publications J.*, 4(3): 297-311.
- Iqbal, S.H., Ghafoor, A. and Ayub, N. (2005). Relationship between SDS-PAGE markers and *Ascochyta* blight in chickpea. *Pakistan J. Bot.*, 37: 87-96.

- Jeffery, C. (1990). Systematics of the Cucurbitaceae: an overview. In: D. M. Bates, R. W. Robinson, and C. Jeffery, (eds.) *Biology and utilization of the cucurbitaceae*. Cornell University Press, Ithaca, New York, p. 3-9.
- Johannsen, W. (1909). *Elements der exateten exblich keitslehra Jena: Gustan Fishe*, p. 20
- Johnson, H.W., Robinson, H.F. and Comstock, R.E. (1955). Estimate of genetic and environmental variability in soybeans. *Agron. J.*, 47(7): 314-318.
- Kandasamy, R. (2014). Electrophoretic characterization of genotypes of cucumber (*Cucumis sativus* L.) based on seed protein profiles. *Adv. Res. J. Crop Improv.*, 5(2): 229-231.
- Karanja, J.K., Mugendi, B.J., Khamis, F.M., and Muchugi, A.N. (2013). Nutritional composition of the pumpkin (*Cucurbita spp.*) seed cultivated from selected regions in Kenya. *J. Hortic. Letters*. 3(1): 17.
- Kehinde, OB, Ayo-Vaughan, M.A. (1999). An Inheritance study of impermeable seed in soybeans. *Field Crop Res.*, 1: 65-70.
- Khan, A., Shinwari, Z.K., Iqbal, A., Wadood, S.F., Nausheen, & Nisar, M. (2016). Conventional breeding enhances genetic diversity in *pisum sativum* (L.) detected through SDS-PAGE. *Pak. J. Bot.*, 48(6): 2433-2438.
- Khan, A.S.M.M.R., Eyasmin, R., Rashid, M.H., Ishtiaque, S. and Chaki, A.K. (2017). Variability, heritability, character association, path analysis and morphological diversity in snake gourd. *Agric. and Nat. Resour.*, 50: 483-489.
- Khan, J., Shah, A.H., Nisar, M., Ali, N., Khan, U., Khan, W. and Ahmad, H. (2016). Evaluation of genetic diversity in black gram through SDS-PAGE. *Pl. Cell Biotechnol. Mol. Biol.*, 17(3&4): 87-95.
- Kiramana, J.K., Isutsa, D.K. and Nyende, A.B. (2016). Evaluation of quantitative yield performance of pumpkin accessions in Kenya using mother trials. *Int. J. Advn .Biol. Res.*, 6(4): 439-447.
- Kulkarni, A.S. and Joshi, D.C. (2013) Studied on selected physio-chemical properties of pumpkin. *Asian J. Dairy & Food Res.*, 32(2): 126-129.
- Kumar, J., Rajnish, K.S. and Krishan, P. (2011). Variability and character association in pumpkin (*Cucurbita moschata* Duch. ex. Poir). *Indian J. Agric. Res.*, 45(1): 87-90.
- Kumar, O.A. and Tata, S.S. (2010). SDS-PAGE seed storage protein profiles in chili peppers (*Capsicum* L.). *Not. Sci. Biol.*, 2(3): 86-90.
- Kumar, V., Mishra, D.P. Yadav G.C. and Dwivedi, D.K. (2017). Genetic diversity assessment for morphological, yield and biochemical traits in genotypes of pumpkin. *J. Pharmacogn and Phytochem.*, 6 (3): 14-18.
- Ladizinski, G. and Hymowitz, T. (1979). Seed protein electrophoresis in taxonomic and evolutionary studies. *Theor. Appl. Genet.*, 54: 145-151.
- Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nat.*, 227: 680-685
- Lakshman Naik, M. and Prasad, V.M. (2015). Studies on genetic divergence in pumpkin. *Electron. J. Pl. Breed.*, 6(4): 1088-1095.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Mahalanobis, P.C. (1936). On the generalized distance in statistics. *Proceedings of National Academic Sciences*. 2: 55-79.
- Mahbubur Rahman Khan, A.S.M. Eyasmin, R. Rashid, M.H. Ishtiaque, S. and Chaki, A. (2016). Variability, heritability, character association, path analysis and morphological diversity in snake gourd. *Agric. Nat. Resour.*, 50, 483-489.
- Mahmud, E., Karim, M.R., Talukder, M.M.R., Hasan, G.N. and Islam, M.N. (2016). Phenotypic variability among pumpkin germplasm (*Cucurbita moschata* Duch. ex Poir.) in southern part of Bangladesh. *Int. J. Agric.*, 1(2): 22–26.
- Martins, S., Ribeiro de carvalho, C and Carnide, V. (2015). Assessing phenotypic diversity of *Cucurbita* Portuguese germplasm. *Agric. Forest.*, 61(1): 27-33.

- Mekala Srikanth, S.G., Bharad, L.B. Thulasiram and Potdukhe, N.R. (2017). Studies on genetic variability, heritability and genetic advance in pumpkin (*Cucurbita moschata* Duch ex Poir.). *Int. J. Curr. Microbiol. App. Sci.* 6(6): 1416-1422.
- Miguel, E.C., Franco, A.V.C., Diosdado, B.G. and Linda, W.B. (2006). Path analysis of yield components in tropical pumpkin. *J. Agrie. Univ. P.R.*, 90(3-4): 183-191.
- Mohsin, G.M., Islam, M.S., Rahman, M.S., Ali, L. and Hasanuzzaman, M. (2017). Genetic variability, correlation and path coefficients of yield and its components analysis in pumpkin (*Cucurbita moschata* Duch Ex Poir) *Int. J. Agril. Res. Innov. Tech.*, 7(1): 8-13.
- Mohsin, G.M., Islam, M.S., Rahman, M.S., Hasanuzzaman, M. and Biswas, B.K. (2016). Genetic divergence in Pumpkin (*Cucurbita moschata* Duch Ex Poir). *Advances in Pl. Agri. Res.*, 4 (5).
- Mortz, E., Krogh, T.N., Vorum, H. and Gorg, A. (2001). Improved silver staining protocols for high sensitivity protein identification using matrix-assisted laser desorption/ionization-time of flight analysis. *Proteom.*, 1(11): 1359-1363.
- Mutter, M.S., Ravindara, M., Gasti, V.D., Ananad, M.B., Sumangala, K. and Nagesh, G.C. (2017). Genetic variability studies in F4 generation of pumpkin (*Cucurbita* SPP.). *Int. J. Agric. Sci.*, 9(1): 3603-3605.
- Nagar, A., Sureja, A.K., Kar, A., Bhardwaj, R., Krishnan, S.G. and Munshi, A.D. (2017a). Variability, correlation and path analysis studies in pumpkin (*Cucurbita moschata* Duchesne ex Poir.) *Chem. Sci. Review. Letters.* 6(21): 574-580.
- Nagar, A., Sureja, A.K., Munshi, A.D., Bhardwaj, R., Kumar, S. and Tomar. B.S. (2017 b). Heritability, correlation and genetic divergence for different seed traits in pumpkin (*Cucurbita moschata*) *Indian J. Agric. Sci.*, 87(11): 19–23.
- Naik, L.M. and Prasad, V.M. (2015 a). Studies on genetic divergence in pumpkin. *The Bioscan.*, 10(4): 2085-2088.
- Naik, M.L., Prasad, V.M., and Laxmi, R.P. (2015 b). A study on character association and path analysis in pumpkin (*Cucurbita moschata* Duch. ex Poir.). *Int. J. Adv.Res.*, 3(1): 1030-1034.
- Nei, M. and Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences, USA.* 76(10): 5269-5273.
- Nwofia, G.E., Victoria, N.N., and Blessing, K.N. (2012). Nutritional variation in fruits and seeds of pumpkins (*Cucurbita spp*) accessions from Nigeria. *Pakistan J. Nutri.*, 11(10): 848-858.
- Onyishi, G.C., Ngwuta, A.A., Onwuteaka, C. and Okporie, E.O. (2013). Assessment of genetic variation in twelve accessions of tropical pumpkin (*Cucurbita maxima*) of south Eastern Nigeria. *World App. Sci. J.*, 24(2): 252-255.
- Pandey, S., Singh, J. Upadhaya, A.K., Ram, D. and Rai, M. (2003). Ascorbate and carotenoid content in Indian collection of pumpkin (*Cucurbita moschata* Duch. Ex Poir.) *Cucurbit Genet. Coop. Report.*, 26: 51-53.
- Panse, V.S. (1957). Genetics of quantitative characters in relation to plant breeding. *Indian J. Genet.*, 17: 318-328.
- Punetha, S. (2014). Molecular characterization of parental lines and genetical studies in crosses of cucumber (*Cucumis sativus* L.) M.Sc. Thesis Submitted to the G.B.P.U.A. and T. Pantnagar.
- Rai, N. and Yadav, D.S. (2005). Cucurbitaceous vegetables In: *Advances in vegetable production. Research Book Centre, New Delhi*, p. 369-378.
- Rajan, S. and Markose, B. (2001). Pumpkin. In: *Text book of vegetable, tuber crops and spices* (Ed. S. Thamburaj and N. Singh), ICAR, p. 292-300.
- Rana, M.K. (2014). Pumpkin In: *Scientific cultivation of vegetables*. Kalyani Publishers, New Delhi, p. 209-246.
- Rana, N.P. and Pandit, M.K. (2011). Studies on genetic variability, character association and path analysis in snake gourd (*Trichosanthes anguina* L.) genotypes. *J. Crop Weed.*, 7(2): 91-96.
- Rao, C.R. (1952). *Advanced statistical methods in biometrical research*. John Wiley and Sons, New York, p. 357-369.

- Robinson, H.F., Comstock, R.E. and Harvey, P.H. (1949). Genotypic and phenotypic correlation in corn and their implication in selection. *Agron. J.*, 43: 282-287.
- Roy, S. and Kumar, V. (2012). A practical approach on SDS PAGE for separation of protein. *Int. J. Sci. Res.*, 3, 358.
- Sampath, S. and Krishnamoorthy, V. (2017). Genetic variability, correlation and path analysis in pumpkin (*Cucurbita moschata* Duch. ex. Poir). *Int. J. Curr. Microbiol. App. Sci.*, 6(6): 3027-3035.
- Sharma, S., & Rao, R. (2013). Nutritional quality characteristics of pumpkin fruit as revealed by its biochemical analysis. *Int. Food. Res. J.* 20(5): 2309-2316.
- Sheng, Xing, Dai, Yu, Li., Yan, Hao., Dong Hai., Ma, X.S., Dai, Xy., Li, Y.L. and Hao, D.H. (1998). *China Dairy Industri.* 6: 17-19.
- Shivananda, M.M. and Madalageri, M.B. (2013). Correlation and path co efficient studies in pumpkin (*Cucurbita moschata* Dutch. Ex. Poir.). *Int. J. Agric. Sci.*, 9: 76-79.
- Shivananda, M.M., Madalageri, M.B., Srinivas, C.S., Mohankumar, A.B. and Yathiraj, K. (2013). Genetic divergence studies in pumpkin (*Cucurbita* spp.). *Int. J. Plant. Sci.*, 8(1): 29-34.
- Shivasubramanian, S. and Menon, M. (1973). Heterosis and inbreeding depression in rice. *Madras Agric. J.*, 60: 1139-1144.
- Shrikant, M.M. Ravindra, M. Gasti, V.D. Mastiholi, A.B. Sumangala, K. and Nagesh (2017). Genetic variability studies in 4 generation of pumpkins (*Cucurbita* spp.). *Int. J. Agric. Sci.*, 9(1): 3603-3605.
- Singh, B.K., Hotti, A.H., Singh, P.K. and Mandal, N. (2016). Analysis of seed storage protein diversity among twelve different cultivars of pea (*Pisum sativum* L.) using SDS-PAGE. *Electron. J. Pl. Breed.*, 7(2): 378-385.
- Singh, B.K., Singh, A.K., Hotti, A.H., Kumar, J. & Singh, S.K. (2017). Diversity analysis through SDS-PAGE of seed storage protein of pea genotypes. *Res. Environ. Life Sci.*, 10(5): 449-452.
- Singh, D.K. and Ram, H.H. (2001). Characterization of indigenous germplasm lines of cucumber (*Cucumis sativus* L.) through SDS PAGE. *Veg. Sci.*, 28: 22-23.
- Singh, M.K. Bhardwaj, D.R. and Upadhyay, D.K. (2014). Genetic architecture and association analysis in bitter melon (*Momordica charantia* L.) landraces. *The Biosacr.*, 9(2): 707-711.
- Singh, P. and Narayan, S.S. (1993) Biometrical techniques in plant breeding. Kalyani Publisher, Ludhiana India, p. 318.
- Singh, R.K. and Chaudhary, B.D. (1977). Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, p. 266.
- Singh, R.K. and Chaudhary, B.D. (1985). Biometrical methods in Quantitative genetic analysis. Kalyani Publishers, New Delhi, p. 318.
- Smith, J.S.C. and Smith, O.S. (1992). Fingerprinting in crop varieties. *Adv. Agron.*, 47: 85-140.
- Srikanth, M. Bharad, S.G. Potdukhe, N.R. and Ravinayak, B. (2015). Character association and path analysis in pumpkin (*Cucurbita moschata* Duch ex. Poir). *Res. J. Agric. Sci.*, 6(3): 495-498.
- Srikanth, M., Bharad, S.G., Thulasiram L.B. and Potdukhe, N.R. (2017). Studies on genetic variability, heritability and genetic advance in pumpkin (*Cucurbita moschata* Duch ex Poir.). *Int. J. Curr. Microbiol. App. Sci.*, 6(6): 1416-1422.
- Srivalli, T., Lakshmi, N. and Gupta, C.H.G. (1999). Analysis of seed proteins by 11 polyacrylamide gel electrophoresis (PAGE) in diploids, triploids and tetraploid hybrids of *Capsicum*. *Capsicum and Eggplant Newsletter.* 18: 48-51.
- Sultana, S. Kawochar, M.A., Naznin, S., Siddika. A and Mahmud, F. (2015 b). Genetic divergence in pumpkin (*Cucurbita moschata* l.) genotypes. *Bangladesh J. Agril. Res.*, 40(4): 683-692.
- Sultana, S., Kawochar, M.A., Naznin, S. Siddika. A and Mahmud, F. (2015 a). Variability, correlation and path analysis in pumpkin (*Cucurbita moschata* L.). *Bangladesh J. Agril. Res.*, 40(3): 479-489.

- Swarup V. (2014). Vegetable science and technology in India. Kalyani Publishers, New Delhi, p. 7-29.
- Tamilselvi, N.A. and Jansirani, P. (2017). Evaluation of pumpkin (*Cucurbita moschata* L.) genotypes for earliness, yield and quality. *Int. J. Curr. Microbiol. App. Sci.*, 6(3): 1554-1559.
- Tamilselvi, N.A., Jansirani, P., Pugalendhi, L. and Nirmalakumari, A. (2012). Performance of genotypes and correlation analysis in pumpkin (*Cucurbita moschata* Duch.ex poir) *Electron. J. Pl. Breed.*, 3(4): 987- 994.
- 1) Vucetic, J. Cirovic, M. and Matic, V. (1989). Chemical composition, nutritive value and healing properties of pumpkin (*Cucurbita moschata* Duch.). *Hrana-I-Ishrana*. 30(3-4): 159-161.
- Wright, S. (1921). Correlation and causation. *J. Agric. Res.*, 20(7): 557-585.
- Xiaohua, D., Sun, Y., Li, X., Zhou, J. and Li, X. (2011). Genetic divergence among inbred lines in *Cucurbita moschata* from China. *Scientia Hortic.*, 127: 207–213.
- Sheng Xing, Dai, Yu, Li., Yan, Hao., Dong Hai., Ma, X.S., Dai, Xy., Li, Y. L. and Hao, D. H. (1998). *China Dairy Industri*. No. 6: 17-19.
- Yadav, A.N., Singh, V.B., Yadav, G.C. and Kumar, V. (2017). Determining relationships between different horticultural and yield traits in sponge gourd (*Luffa cylindrica* Roem.) genotypes with path coefficient analysis. *J. Pharmacogn. Phytochem.*, 6(3): 342-345.
- Yadav, R.K. (2008). Seed protien electrophoresis studies in cucurbits –A reivew. *Agric. Rev.*, 29(1): 21-30.
- Yadegari, M., Golparvar, A.R. and Barzegar, R. (2012). Multivariate analysis of quantitative traits in Iranian pumpkin lines (*Cucurbita* spp.). *African J. Agril. Res.*, 7(5): 764-774.
- Yatung, T., Dubey, R.K., Singh, V., Upadhyay, G. and Singh, S. (2014). Studies on seed protein profiling in chilli (*Capsicum annuum* L.) genotypes of Northeast India. *Aust. J. Crop Sci.*, 8(3): 369-377.

## APPENDIX-I

### Meteorological data for the crop period during the experiment from March- August (2017)

Month/ year	Temp. (°C)				RH%		Rainfall (mm)	No. of rainy days	Evaporation (mm)
	FN	AN	FN	AN	FN	AN			
	Max.	Max.	Min.	Min.					
<b>January, 2017</b>	20.8	27.7	11.7	13.5	77.4	60.1	00	00	4.46
<b>February, 2017</b>	21.1	27.3	14.1	14.6	81.1	71.8	75.3	8	4.48
<b>March, 2017</b>	21.1	25.1	14.3	15.2	87.2	81.1	192.4	16	4.83
<b>April, 2017</b>	25.3	28.0	18.1	17.9	92.2	89.6	450.2	17	4.06
<b>May, 2017</b>	24.7	28.7	NA	NA	88.8	82.3	858.2	19	4.95
<b>June, 2017</b>	27.3	31.0	NA	NA	88.6	81.5	749.5	18	6.2
<b>July, 2017</b>	27.8	31.9	NA	NA	94.5	91.9	784.3	22	4.13
<b>August, 2017</b>	27.7	31.1	NA	NA	92.8	89.5	953.4	18	3.98

Source: Meteorological observatory, Department of Natural Resource Department, C.H.F., C.A.U. Pasighat

## APPENDIX-II

### Analysis of variance for different character in pumpkin

S.N.	Source of variation	Mean square		
		Replication	Genotype	Error
	Df	2	24	48
1.	Vine length (m)	0.11	4.47**	0.44
2.	Number of branches per plant	1.14	5.86**	1.23
3.	Days to first staminate flower anthesis	2.61	7.76**	1.64
4.	Days to first pistillate flower anthesis	2.94	19.97**	1.91
5.	Node bearing first staminate flower	0.03	2.17**	0.52
6.	Node bearing first pistillate flower	1.76	3.06*	1.55
7.	Days to first fruit harvest	12.66	84.12**	5.76
8.	Number of fruits per plant	0.46	1.15**	0.04
9.	Polar circumference (cm)	0.35	127.76**	6.37
10.	Equatorial circumference (cm)	9.13	264.48**	3.34
11.	Flesh thickness (cm)	0.06	1.72**	0.1
12.	Cavity length (cm)	0.25	48.9**	0.27
13.	Number of seeds per fruit	1018.92	10353.6**	564.36
14.	100 seed weight (g)	0.35	21.00**	0.32
15.	Average fruit weight (kg)	0.69	0.75**	0.05
16.	Fruit yield per plant (kg)	0.28	4.47**	0.08
17.	Fruit yield per ha (q)	837.49	8089.92**	311.21
18.	Total soluble solid (TSS)	2.37	2.74**	1.21
19.	Carbohydrates mg/100g	57.86	30143.4**	99.57
20.	Vitamin A mg/100g	0.04	99.57**	0.03

