



Inheritance of fruit attributes in chilli pepper

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ABSTRACT

Capsicum annuum has been known for the amount of variability present in the genus for fruit traits and also for other morphological traits. The present study used F_1 and F_2 generations developed from diverse parental stocks to report the inheritance pattern for different fruits traits. Our results demonstrate that pepper fruit bearing habit, its colour at unripe stage and fruit apex are simply inherited traits with or without epistatic interactions while fruit length is a quantitative character. Fruit orientation (drooping or erect) and fruit habit (solitary or cluster) showed segregation ratio of 3:1 indicating monogenic inheritance of the trait where drooping character was dominant in nature over erect type and solitary fruit type was dominant over cluster type, fruit bearing habit (single pendant, single erect, cluster pendant and cluster erect) exhibited typical dihybrid ratio of 9:3:3:1, fruit colour at unripe stage (green, purple and mixture of green & purple) segregated in the ratio of 9:3:4 exhibiting recessive gene epistasis and fruit apex (acute or blunt) showed a ratio of 15:1 indicating duplicate dominant gene epistasis. Fruit length exhibited quantitative inheritance with plants showing fruit length greater than the positive parent (parent showing higher fruit length among the two parents) and lesser than the negative parent (parent showing lesser fruit length among the two parents) from different crosses. These results provide new data to clarify and further add on to the information available on the inheritance of chilli pepper fruit attributes.

Keywords: *Capsicum annuum*, fruit traits, genetics, Mendelian ratios.

INTRODUCTION

Capsicum or chilli pepper comprises of more than 30 species which exhibit a lot of variability for yield and quality traits like fruit shape, fruit weight, fruit colour, pungency, plant height, and maturity. Among the different species, five major cultivated species are *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens*. *C. annuum* is the most widely cultivated species which includes both sweet and hot peppers. In *Capsicum*, more than 290 genes have been identified for various genetic characteristics of horticultural importance (Wang and Bosland, 16). Inheritance of different traits in chilli pepper have been studied and elaborately cited in the work of Boswell, 3. The genetics of 12 characters in chilli pepper studied till 1929 was summarised by Matsuura, 11. Inheritance of 16 traits of pepper reviewed by Boswell, 2 included purple foliage, stem colour, red mature fruit colour, blunt fruit apex, bulged fruit base, pendent fruit position and non clasping fruit calyx. The study of other different characters of peppers gained momentum after this. From 1980 onward, efforts were made to tag different genes controlling pepper traits using molecular markers, followed by their cloning and characterization.

Within the genus *Capsicum*, there is an abundance of genetic diversity for fruit and floral characteristics that can be utilized in chilli pepper breeding programmes. Many of these attributes are conditioned by one or several genes but their inheritance pattern has not been completely determined which thereby limit their usefulness for crop improvement. To cite an example, the anthocyanin pigment found in *Capsicum* leaves, flowers, and immature fruit is delphinidin-3-p-coumaroyl-rutinoside-5-glu-coside (Lightbourn, 4). Anthocyanin production was reported to be influenced by an incompletely dominant gene (*A*) called *Anthocyanin* and a second modifying gene (*MoA*) called *Modifier of A* (Deshpande, 4).

From *Capsicum annuum* accessions, pepper breeders have developed new breeding lines and cultivars that combine unique foliar attributes with diverse fruit and plant habit attributes (Stommel and Griesbach, 15). To develop a breeding programme, knowledge of genetics and gene action and interaction is essential for success. Selection of plants from pre-existing populations followed by hybridization can be used to develop breeding lines in *Capsicum*. The development of a new variety that is attractive to the consumer is one of the main goals in any breeding programme. The first step to a successful genetic breeding programme is the selection of the

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parents harboring desirable traits. Various studies have showed that genetically related parents present low heterosis, while hybrids between diverse parents tend to show higher heterosis for fruit and plant size characters. The knowledge of nature and magnitude of gene action is of the utmost importance in the process of selection and predicting the behaviour of the hybrid and segregating generations.

Looking into the importance of information on genetics and inheritance of any trait for its improvement, the present study was undertaken to study the genetics and inheritance of fruit attributes like fruit bearing nature (fruit orientation and fruit habit), colour at unripe stage, fruit apex and fruit length.

MATERIALS AND METHODS

Plant Material

Eight varietal lines popularly cultivated in India with considerable divergence for fruit attributes were selected as parents for producing F_1 and segregating F_2 generations. A detail of the fruit characters of each of these parents is given in Table 1. Five fruit attributes like fruit orientation, fruit habit, fruit bearing habit, fruit colour at unripe stage, fruit shape at apex and fruit length were studied. Fruit orientation was considered as drooping (D) if the fruit apex faced downwards and erect (E) if it faced upwards, Fruiting habit was considered clustered (C) for those plant which bore two or more than two fruits at a node while it was considered solitary (S) if it had single fruit per node. Taking the two traits together fruit bearing habit was further classified as single drooping (SD), single erect (SE), cluster drooping (CD) and cluster erect (CE) (Fig. 1). Two colours of fruit were observed at unripe stage green and purple. The segregating population was observed for appearance of green (G), purple (P) or mixture of green and purple (GP) colour in fruits (Fig. 2). Fruit apex refers to the appearance of fruit tip. Fruit apex was classified

as acute if it was pointed and blunt if it was having a sunken tip. For fruit length, measurement was taken from the base of the fruit to the tip on all the individuals of populations under study.

For each of these traits three populations were studied which were developed using the parents contrasting for the corresponding trait under study. The list of the F_2 populations phenotyped for the traits under study is given in Table 2. The respective populations were developed by crossing green house grown plants using standard emasculatation practices.

For crossing, the freshly borne flower buds on selected pollen (male) and female parents during the early flowering phase were preferred. In the selected female parents, the flower buds, which are likely to open the following day were selected and emasculated between 5:00 to 6:30 PM. The emasculated flowers were immediately bagged with butter paper covers. Similarly, in the selected male

Table 2. F_2 generations studied for fruit traits.

Fruit trait	Crosses studied	Size of the population studied
Fruit bearing habit	AS × WBC	114
	PC × PS	102
	GVC × WBC	63
Fruit colour at unripe stage	PS × WBC	47
	AS × WBC	101
	PJ × WBC	64
Fruit shape at apex	PS × KeJ	65
	PJ × KeJ	73
	PM × KeJ	89
Fruit length (cm)	PM × KeJ	218
	PS × VA	142
	PS × KeJ	113

Table 1. Fruit characters of parents used for generating F_2 populations.

Name of the parent	Fruit bearing habit	Fruit colour at unripe stage	Fruit shape at apex	Fruit length (cm)
Pusa Sadabahar (PS)	Cluster erect	Medium Green	Pointed	5
Phule Mukta (PM)	Solitary, drooping	Medium Green	Pointed	5
Arka Suphal (AS)	Solitary, drooping	Medium Green	Pointed	7
WBC-Sel-5 (WBC)	Cluster erect	Deep Purple	Pointed	5
GVC-101 (GVC)	Solitary, drooping	Light green	Pointed	10
Pusa Jwala (PJ)	Solitary, drooping	Light green	Pointed	10
Ke Jiaon (KeJ)	Solitary, drooping	Medium green	Blunt	13
Vellayani Athulaya (VA)	Solitary, drooping	Light green	Pointed	12

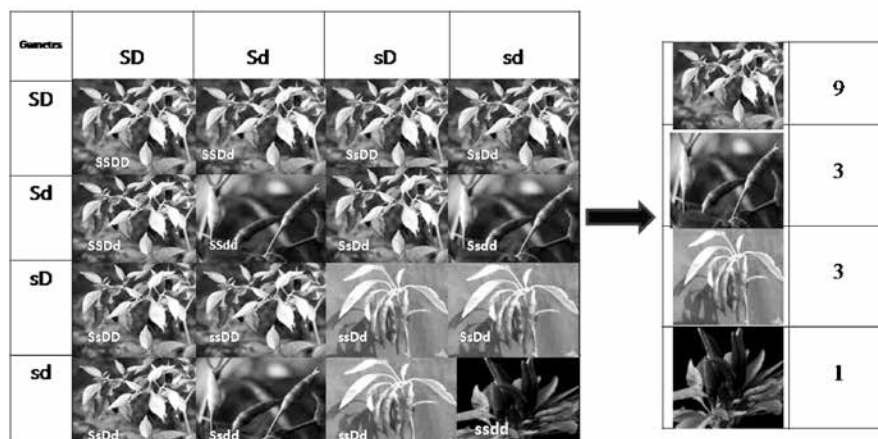


Fig. 1. Phenotypic ratio of 9:3:3:1 observed for fruit bearing habit in a dihybrid cross (SsDd × SsDd).

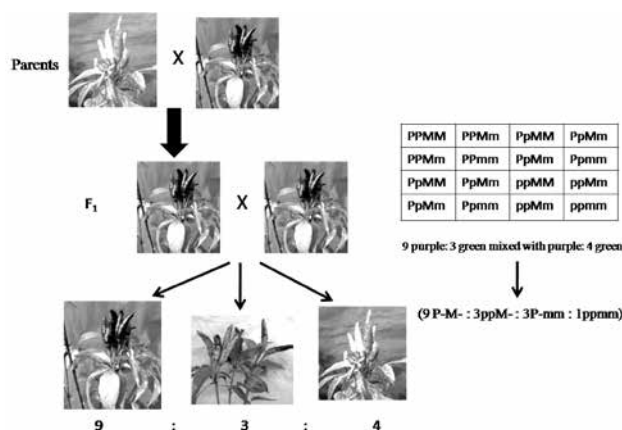


Fig. 2. Recessive epistasis operating for fruit colour expression at unripe stage in chilli.

parents, the flowers likely to be opened in the next day were bagged with butter paper covers to avoid contamination with foreign pollen. On the next day between 7:00 to 9:00 AM, pollen from the bagged flowers of the male parents were collected and dusted on to the stigma of their respective emasculated flowers using a pointed forceps tip and the bags were replaced immediately and labeled. After fruit set, the bags were removed to facilitate uninterrupted development of the fruits. Seeds were extracted from fully ripe fruits, cleaned and dried for raising F₁ generation. F₁ plants were selfed to obtain F₂ seeds for raising F₂ population. For selfing, the flower buds, which are expected to open in the subsequent day, were bagged separately with butter paper envelopes covering both male and female reproductive organs. Progeny of each population were grown in net house. Forty five days old seedlings of each genotype were transplanted in first week of August, 2016 at Indian Agricultural Research Institute, New Delhi.

F₁ and F₂ generation of a cross for a particular fruit trait were observed for their expression and recorded. In case of qualitative traits, to estimate the number of genes conditioning expression of fruit traits in among these populations, a Chi square test was carried out. The Chi square analysis was used to test the goodness of fit of observed segregations to the expected genetic ratios: The agreement of the observed values with the expected was tested by the chi-square test of goodness of fit with degrees of freedom (n-1), where n is the number of classes.

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

If the trait was quantitative, observation on each and every individual of the cross was recorded. Skewness and kurtosis values were estimated for trait exhibiting quantitative inheritance. Skewness was estimated using following formula:

$$\text{Skewness} = \frac{\sum_{i=1}^N (Y_i - Y)^3}{(N-1)s^3}$$

Kurtosis was estimated using following formula:

$$\text{Kurtosis} = \frac{\sum_{i=1}^N (Y_i - Y)^4}{(N-1)s^4}$$

Y_i = value of genotype-i, s = standard deviation, N = Number of data collected

The statistical test of significance of skewness and kurtosis was performed by comparing the skewness and kurtosis with their standard error. The value of skewness, kurtosis and standard error was computed using XLstat base software.

RESULTS AND DISCUSSION

Capsicum annuum offers the most excellent material for genetical studies, as the variations encountered in both the morphological and physiological characters are enormous. Various

efforts have been made to study the genetics of different traits but consistency in results for the traits studied is less. This is mainly attributed to different genetic material employed for these studies. In pepper, there are numerous studies that associate gene names to specific traits (Wang and Bosland, 16). In many of these studies, the inheritance of morphological traits has not been described in detail. In this study, we have focused on fruit traits and have found that two genes with or without epistatic action is involved in expression of three major fruit traits i.e., fruit bearing habit, fruit colour at unripe stage and fruit shape at apex.

For fruit bearing habit, all the three F₁s were solitary drooping in habit which was similar to one of the parent. The F₂ generations came up with expression of two new phenotypes viz., solitary erect and cluster drooping unlike the parents (solitary drooping and cluster erect) (Table 3). The three F₂ populations i.e., AS × WBC, PC × PS and GVC × WBC showed segregation for solitary drooping, solitary erect, cluster drooping and cluster erect fruit bearing habit in the ratio of 9:3:3:1 (Table 4). Fruit orientation and fruit habit both showed a segregation ratio of 3:1 in the F₂ population where drooping fruits (D) were dominant over erect types (d) and solitary fruits (S) were dominant over cluster fruiting habit (s). When we considered both the traits together to obtain information about fruit bearing habit we found that the traits segregated in typical fashion as observed in dihybrid cross. Dihybrid cross is a cross between two different lines that differ in two observed traits. In the Mendelian sense, between the alleles of both these loci there is a relationship of complete dominance – recessive and this is what we observed

here. In the F₂ generation, individuals showed a segregation of 9:3:3:1 for fruit bearing habit i.e., the “9” represents the proportion of individuals displaying both dominant traits, here solitary and drooping fruit type, the first “3” represents the individuals displaying the first dominant trait and the second recessive trait, here solitary and erect fruiting type, the second “3” represents those displaying the first recessive trait and second dominant trait, here cluster and drooping fruit type and “1” represents the homozygous, displaying both recessive traits here cluster erect (Fig.1). This also provides information that genes for fruit orientation and habit show segregation independent of each other thus validating the Mendel’s second law of independent assortment. Therefore the two genes (S- and D-) are either present on two different chromosomes or on one chromosome but far apart so that they do not show linkage at all.

The orientation of fruit segregated in the ratio of 9 Pendant: 3 Intermediate: 4 Erect, indicating the involvement of two complete dominant genes with one gene, when homozygous recessive is epistatic to the others (Basavaraj *et al.*, 2). Here in our study we did not observe any intermediate phenotype. However, Deshpande, 5 found monogenic action with the F₂ segregation ratio of 3:1 similar to our study, whereas, Gopalkrishnan *et al.*, 7 reported 13:3 F₂ segregation ratio where complete dominance operates at both gene pairs, but one gene when dominant is epistatic to the other and the second gene when homozygous recessive is epistatic to the first.

Similar to our studies Ohta and Chung, 9, reported that the cluster habit was controlled by a single recessive gene. Studies of Basavaraj *et al.*, 2 reported fruiting type segregated in the ratio of

Table 3. Phenotypic expression for different fruit trait in F₁ and F₂ generation.

Fruit trait	Generation	Phenotype expressed
Fruit bearing habit	F ₁	Solitary drooping (SD)
	F ₂	Solitary drooping, Solitary erect (SE), cluster drooping (CD), cluster erect (CE)
Fruit colour at unripe stage	F ₁	Purple (P)
	F ₂	Green (G), Purple, Green mixed with purple (GP)
Fruit shape at apex	F ₁	Apex
	F ₂	Apex, Blunt
Fruit length (cm)	F ₁	PM × Kej - 7.89 cm
		PS × VA - 7.78 cm
		PS × Kej - 8.34 cm
	F ₂	PM × Kej - Mean of 6.47 cm
		PS × VA - Mean of 6.04 cm
		PS × Kej - Mean of 9.34 cm

Table 4. Segregation pattern of F₂ generation for different fruit traits.

Fruit trait	Ratio tested	Name of cross	Number of plants in F ₂ generation of each category				χ ² ratio (5%)	Table χ ² value (5%)	
			O	D	E				
Fruit orientation	3:1	AS × WBC	O	85	29		0.011	3.84	
			E						
		PC × PS	O	78	24				0.117
	E								
	GVC × WBC	O	48	15		0.047	3.84		
	E								
Fruit habit	3:1	AS × WBC	O	98	40				1.16
			E						
		PC × PS	O	80	22		0.64	3.84	
	E								
	GVC × WBC	O	40	17		0.71			3.84
	E								
Fruit bearing habit	9:3:3:1	AS × WBC	O	72.00	26.00		13.00	3.00	
			E	64.12	21.37	21.37	7.12		
		PC × PS	O	62.00	18.00	16.00	6.00	0.972	7.815
			E	57.37	19.12	19.12	6.37		
		GVC × WBC	O	32.00	8.00	16.00	7.00	5.430	7.815
			E	35.43	11.81	11.81	3.94		
Fruit colour at unripe stage	9:3:4	PS × WBC	O	26.00	6.00	15.00		1.8	5.991
			E	26.43	8.81	11.75			
		AS × WBC	O	56.00	19.00	26.00		0.034	5.991
			E	56.80	18.93	25.25			
		PJ × WBC	O	34.00	10.00	20.00		1.44	5.991
			E	36.00	12.00	16.00			
Fruit shape at apex	15:1	PS × Kej	O	58.00	7.00			2.265	3.84
			E	60.90	4.00				
		PJ × Kej	O	66.00	7.00			1.389	3.84
			E	68.43	4.56				
		PM × Kej	O	84.00	5.00			0.061	3.84
			E						

45:19 (Solitary: Cluster) indicating the existence of three interacting genes for fruit tip type, namely one basic complementary gene and two complementary duplicate genes.

We observed that immature black fruit colour was dominant over green fruit colour as observed in F₁ generations of three different crosses involving one parent with purple color immature fruits i.e., PS

× WBC, AS × WBC and PJ × WBC (Table 3). The F₂ generation was observed to have a new phenotype of individuals carrying fruits with a mixture of green and purple colour along with plants with complete green and purple fruits. Across the three populations, maximum number of individuals showed purple fruits followed by green and green mixed with purple. The three category of immature fruits segregated in the

ratio of 9 (purple): 3 (green mixed with purple): 4 (green) (Table 4). With reference to the characters of pigmentation, it is known that presence of pigment in many plant parts is a varietal feature of chilli plant. Inheritance of pigmentation mostly of the presence and the absence of the pigment in various parts of the plant has been studied by many and only a few have considered the intensity of the pigment. Voluminous literature has been accumulated on this aspect which was subsequently summarized by many authors (Deshpande, 5).

The present investigation was taken up to understand the nature of inheritance by studying the segregation of anthocyanin pigmentation in unripened fruits, the presence of which resulted in purple fruits. Pigmentation of fruit segregated in the ratio of 9 purple: 3 mixture of green and purple: 4 green, which is explained by the hypothesis that anthocyanin pigmentation in unripened fruits is governed by two genes. Gene P determines whether the fruit will be purple (PP) or green mixed with purple (pp). Another gene M determines the presence of anthocyanin pigmentation. This gene in recessive form prevents expression of anthocyanin pigments so that the genotype P-M- produces purple fruits, ppM- produces green and purple mixed fruits while P-mm and ppmm produces green fruits. The recessive allele 'm' is epistatic to other gene 'P' or 'p' when homozygous, hence showing it to be "recessive epistasis" in nature (Fig. 2).

Studies of Basavaraj *et al.*, 2 showed segregation of purple and yellow fruits at unripe stage in the ratio of 9 (purple): 6 (purple) and 1 (yellow) which was explained on the hypothesis of two complete dominant genes affecting different expressions and interaction between both dominant gives new phenotype. Ramanujam *et al.*, 13 observed 3:1 F_2 segregation ratio for unripe fruit colour controlled by solitary major gene with some modifiers whereas Jeswani *et al.*, 8 proposed duplicate gene system to account for 15:1 F_2 ratio from the cross between cedar green with lettuce green and similar observations were made by Jeswani *et al.*, 8.

Unlike the previous two traits, fruit shape at apex did not show any new phenotype in the three F_2 populations studied i.e., PS × KeJ, PJ × KeJ and PM × KeJ. The three F_1 s showed acute fruit apex while maximum number of F_2 individuals had acute fruit shape. F_2 plants with acute fruit apex and blunt fruit apex segregated in the ratio of 15:1. (Table 3 & 4). Segregation of fruit apex type in the F_2 showed maximum individuals with pointed or acute fruit apex while very few had blunt tip. This indicated that pointed fruit apex was dominant over blunt

apex (Fig. 3). Further the individuals with acute and blunt fruit tip segregated in the ratio of 15:1 which corresponds to presence of duplicate dominant gene epistasis. The trait is governed by two genes (designated as P_1 & P_2). Whenever both or either of the genes is present in dominant form (P_1-P_2- , $P_1-p_2P_2$ or $p_1P_1P_2-$), it produces acute fruit apex while when both are present in homozygous recessive state ($p_1p_1p_2p_2$) they produce blunt apex. Dominant allele of both the genes is epistatic to expression of blunt fruit tip (Fig. 3).

Basavaraj *et al.*, 2 studied segregation of pointed and lobed fruit tip in chilli and found fruit tip segregated into 45 Pointed: 19 Lobed indicating the existence of three interacting genes for fruit tip type, namely one basic complementary gene and two complementary duplicate genes. Bal *et al.*, 1 reported the F_2 segregation ratio of 1 Pointed: 2 Mixed: 1 Blunt apex suggesting thereby monogenic inheritance of fruit apex. Incomplete dominance was noticed by Deshpande, 4 in chilli for pointed to blunt type of fruit tip.

Fruit length appeared to be quantitative in nature with F_1 having intermediate values for fruit length (Table 3). Mean fruit length of 6.47, 6.04 and 9.34 cm was observed in the F_2 populations of PM × KeJ, PS × VA and PS × KeJ crosses respectively. Skewness was positive for all the three populations with values of 0.555, 0.115 and 0.113, respectively for PM × KeJ, PS × VA and PS × KeJ crosses, while kurtosis was negative with values of -0.193, -0.173 and -0.529, respectively (Table 5). Fruit length of F_1 is intermediate between P_1 & P_2 for all the three populations studied, the F_2 individuals distribute multimodally between parental values with some individuals having values greater than parent and lesser than lower parents but the distribution cannot be sharply divided into distinct classes confirming thereby the quantitative nature of the trait. There are many reports stating fruit length to be quantitative in nature (Basavaraj *et al.*, 2). Studies of Khambanonda, 9 indicated that fruit length is influenced by genes governing fruit shape to some extent although there are independent genes for fruit length also.

The skewness analysis of F_2 distribution provides information about nature of gene action (Fisher *et al.*, 6), whereas kurtosis provides information on the number of genes controlling the traits (Robson, 14). Skewness value is zero for normal distribution which indicates the absence of gene interaction. The positive skewness is associated with complementary gene interactions while negative skewness is associated with duplicate gene interactions.

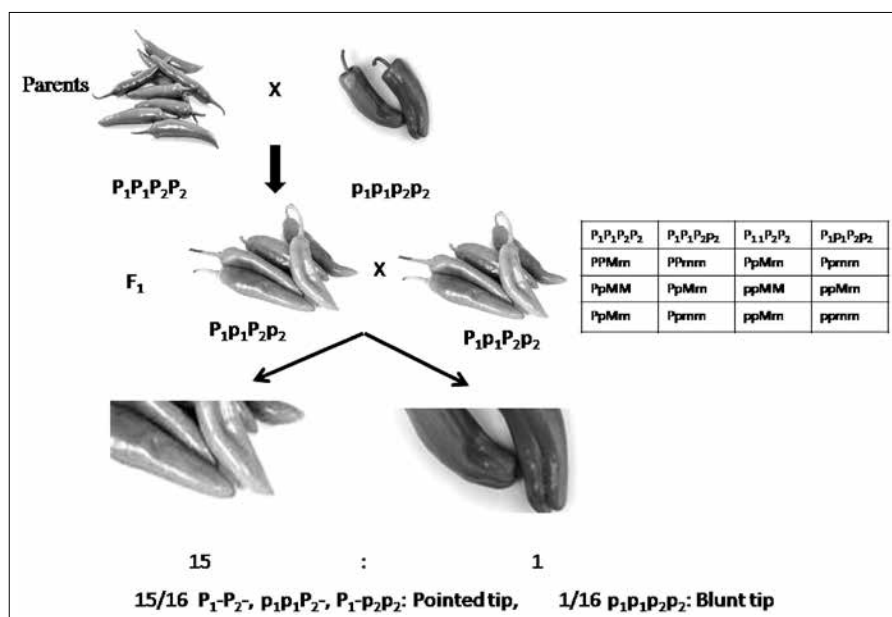


Fig. 3. Duplicate dominant gene epistasis for fruit apex in chilli.

Table 5. Skewness and Kurtosis value for fruit length in three populations studied.

	Mean (cm)	Range for F ₂ individuals (cm)		Skewness	Gene action	Gene	Number of genes
		Min.	Max.				
PM × KeJ	6.47	1	14	0.555**	Complementary	-0.193**	Many genes
PS × VA	6.04	2	11	0.115**	Complementary	-0.173**	Many genes
PS × KeJ	9.34	2	19	0.113**	Complementary	-0.529**	Many genes

** Significance at 1%

Skewness describes the degree of departure of a distribution from symmetry or it is a measure of relative symmetry while kurtosis is a measure of relative peakedness of a distribution. The traits with leptokurtic distribution are usually under the control of few segregating genes and the traits with platykurtic distribution are controlled by many genes. The positive value of kurtosis indicated leptokurtic curve whereas negative kurtosis indicated platykurtic curve.

Skewness values for fruit length were significant for all the three populations screened. Significance of skewness values indicates presence of gene interaction and positive skewness indicates complementary gene interactions. Negative kurtosis values indicate fruit length is governed by many genes.

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