

Variation and Segregation in F₂ Population of Hot Pepper (*Capsicum annuum*)

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Abstract

The experiment was carried out to investigate variations in quantitative traits and segregation pattern for some qualitative traits of selected F₂ hot pepper. The seeds were collected from 23 F₁ hot pepper plants grown at Yezin Agricultural University Farm. The experiment was laid out in a Randomized Complete Block design (RCBD) with two replications. Leaf length, leaf width, filament length, corolla length, fruit width, thousand seed weight and number of seeds per pod showed important contributors towards total variation among the genotypes. Variability study on these seven traits highlighted that phenotypic component was the major contributor to total variance. Low and medium heritability values and higher phenotypic coefficient of variation (PCV) suggested that all the above phenotypic traits interacted with the environment rather than genetic variation. Segregation patterns of 21 qualitative traits indicated similar characters for angled stem, erect flower, white corolla and corolla spot, rotate corolla, white filament, elongate fruit, obtuseness of fruit at pedicel attachment and absence of neck at the base of fruit. Segregation distortions observed in some traits: nodal anthocyanin, branching habit, leaf shape, mature fruit color suggested that they are polygenic traits. Calyx margin and fruit bearing characters followed the Mendelian ratio (3:1), highlighting the monogenic recessive nature of the gene. Anther color expressed independent assortment with complete dominance (9:3:3:1). Plant growth habit, stigma exsertion, fruit surface, leaf color and male sterility resulted as modified F₂ dihybrid ratios indicating the involvement of two genes controlling each trait. From the breeding point of view, variation in quantitative traits, on which environmental factors have a profound effect, may hinder the progress in selection for progeny containing favorable genes. However, variation in qualitative traits as a result of segregation in F₂ progeny might be useful for selection of desirable quality in next generation.

Key words: hot pepper, F₂ populations, GCV, PCV, segregation

Introduction

The genus *Capsicum*, which is commonly known as red chilli, hot red pepper, chilli pepper, tabasco, paprika, cayenne, etc., belongs to the nightshade family Solanaceae. *Capsicum* forms an important ingredient in people's diet around the world due to pungency properties of the fruits resulting from the presence of capsaicinoid alkaloid that may help fight cancer and inflammation and provide pain relief (Bosland and Votava, 2012).

Capsicum is one of the most important crops in Myanmar: the production of dry chilli and pepper increased from 121,400 tons to 157,193 tons between 2014 and 2016 (DOA, 2016).

Capsicums are highly heterogeneous plants exhibiting tremendous variability (Walsh and Hoot, 2001; Adetula and Olakojo, 2006; Bozokalfa et al., 2009). It is recorded as a facultative cross-pollinating species with insect-mediated out-crossing rates of 2% to 90% (Pickersgill, 1997). Such a level of out-crossing will maintain a

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considerable amount of heterozygosity and heterogeneity, eventually resulting in off-type segregants during repeated multiplication cycles. From the genetic improvement point of view, the genetic variability available in the segregating populations can be exploited to produce new combinations through the selection of new hybrid lines.

It is well known that the extent of genetic variation present in a crop is the basis for the improvement of that crop, and the degree of improvement depends on its magnitude of available, beneficial genetic variability. Therefore, in-depth understanding of the extent and magnitude of genetic variation within and between a breeding population is required to develop mechanisms for detecting purity and authenticity of parents and hybrids in commercial plant breeding programmes (Se-Jong et al., 2012). Elite hybrid varieties or F₁ hybrids can be used as gene reservoir for breeding programmes (Van der Have, 1979) and selection has to be made in F₂ and the subsequent generations (Somadshaka and Salimath, 2006). However, information on variability study in F₂ generation of *Capsicum* is lacking in Myanmar and therefore, this study was conducted to investigate the extent of variations in quantitative traits of F₂ population of selected hot pepper and to test segregation pattern for some qualitative traits in them.

Materials and Methods

Field experiment was carried out at the Department of Horticulture and Agricultural Biotechnology, Yezin Agricultural University, from November 17, 2013 to May 31, 2014. Hot pepper fruits were collected from 23 F₁ plants of Known-You variety grown at Yezin Agricultural University Farm based on their physical characteristics. The seeds of 23 plants were extracted separately by hand, air-dried and packed. Seeds were sown directly into the seed trays which were filled with a well-prepared compost, well decomposed cow-dung manure and burnt paddy husk at the ratio of 3:1:1 by volume.

The experiment was laid out in a Randomized Complete Block design (RCBD) with two replications. As 7 plants per genotype were selected

and grown one seedling per hole, there were a total of 161 plants per block. Thirty-two days after seeding (32 DAS), vigorous and healthy seedlings were selected and transplanted in the well-prepared field. Inter-cultivation, weeding and watering were done as necessary during the experiment. To prevent cross pollination, each plant was covered with a nylon cage. All parameters (12 quantitative and 21 qualitative values) were recorded according to the descriptor for *Capsicum* (IPGRI, 1995).

Statistical Analysis

Phenotypic and genotypic variability and heritability for quantitative traits

Twelve quantitative data were analysed using SAS (version 9.1) software. The genotype variability was estimated by simple measure, namely mean, phenotypic and genotypic variance and coefficient of variation and heritability according to the methods suggested by Singh and Chaudhary (1985):

$$\text{Genotypic variance, } \theta^2 g = (Mg - Me) / r$$

Where, Mg = Mean square of genotypes
Me = Mean square of error
r = Number of replication

$$\text{Phenotypic variance, } \theta^2 p = \theta^2 g + Me$$

$$\text{Phenotypic coefficient of variation, } PCV\% = \frac{\sqrt{\sigma^2 p}}{\bar{x}} \times 100$$

$$\text{Genotypic coefficient of variation, } GCV\% = \frac{\sqrt{\sigma^2 g}}{\bar{x}} \times 100$$

Where, \bar{x} = Population mean

$$\text{Heritability in broad sense, } h^2 b = \frac{\theta^2 g}{\theta^2 p} \times 100$$

Where, h^2_b = Heritability in broad sense

$\theta^2 g$ = Genotypic variance

$\theta^2 p$ = Phenotypic variance

Estimation of segregating pattern for qualitative traits

Frequency distribution was used to predict the expected frequencies based on 21 qualitative traits. The segregated ratios in F₂ progenies were

subjected to Mendelian genetic models using a chi-square (multinomial) test (Panse and Sukhatme, 1957) using STATISTIX 8 programme.

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

Where, O = Observed value, E = Expected value

Results and Discussion

Analysis of Variance (ANOVA) for Quantitative Traits

Out of 12 quantitative traits for 23 F_2 hot pepper genotypes, seven traits (leaf width, corolla length, fruit width, seeds per pod, leaf length, filament length and thousand-seed weight) showed significant difference at 5% probability level (Table 1). This indicated the presence of appreciable level of variation for those traits among the tested F_2 genotypes. This could be due to variation of genotypes in F_2 generation of *Capsicum*. Barroso et al. (2015) also observed significant differences in corolla length and anther length in F_2 genotypes of ornamental pepper. Similar findings of significant

differences in leaf width in F_2 generation of ornamental peppers were also observed by Pessoa et al. (2015). However, there was no significant difference for anther length, fruit length, fruit weight, total fruit weight and fruit pedicel length indicating uniformity in some yield-contributing traits in F_2 generation of hot pepper.

Mean Performance, Phenotypic and Genotypic Variance, Heritability in Broad Sense, Genotypic Coefficient of Variation and Phenotypic Coefficient of Variation for 7 Quantitative Traits in 23 F_2 Population of Hot Pepper

The extent of variability in respect to mean, phenotypic and genotypic variances, heritability, phenotypic and genotypic coefficients of variation of the seven traits was given in Table 2. There was a wide range of phenotypic and genotypic variances. Genotypic variance values ranged from 0.001 to 34.79 while phenotypic variance values ranged from 0.004 to 94.845 for filament length and number of seeds per pod, respectively. The magnitude of the phenotypic variance for all traits was higher than the genotypic variance, indicating that phenotypic

Table 1. Analysis of variance for 12 quantitative traits of 23 F_2 population of hot pepper

	Source	Rep.	Geno.	Error	CV%
	d.f	1	22	160	
Mean square	Leaf length (cm)	0.476	0.656*	0.4	15.65
	Leaf width (cm)	0.023	0.195**	0.091	17.677
	Anther length (cm)	0.469×10^{-3}	3.143×10^{-3}	0.003	20.932
	Filament length (cm)	0.134×10^{-2}	0.547×10^{-2}*	0.003	20.699
	Corolla length (cm)	0.026	0.043**	0.019	10.699
	Fruit length (cm)	0.019	0.527	0.369	11.57
	Fruit width (cm)	0.052	0.035**	0.016	14.646
	Fruit weight (g)	0.043	0.082	0.09	21.594
	Total fruit weight (g)	352.313	749.338	648.987	19.095
	Fruit pedicel length (cm)	0.137	0.361	0.261	15.887
	Thousand-seed weight (g)	0.501	2.525*	1.516	22.712
	Seeds per pod (no.)	141.043	129.625**	60.062	14.098

** , * = significant at 1% and 5% level, respectively, d.f= degree of freedom

component was the major contributor to total variance. Leaf width, corolla length, fruit width and seeds per pod have medium heritability values while leaf length, filament length and thousand seed weight have low heritability values showing a relatively larger contribution of environment to the phenotype (Table 2). These data revealed that the magnitude of phenotypic coefficient of variation (PCV) was higher than that of genotypic coefficient of variation (GCV). These data indicated that all these traits interacted with the environment to some extent. Sahoo et al. (1990) described medium heritability values for fruit diameter in F₂ generation of chilli pepper. Other studies also found that genetic and environmental variation coefficients ratio was greater than 1.0 for corolla length and anther length (Barroso et al., 2015) and medium GCV and PCV values were recorded for fruit length, fruit diameter, fruit weight and 100 seeds weight in F₂ generation of peppers (Sahoo et al., 1990). Comstock and Moll (1963) reported that the more diverse the environment, the smaller the estimates of genetic variance, which supports the present results of low estimates of genetic variance.

Segregation Pattern of Qualitative Traits

Table 3 showed the frequency distribution of 21

qualitative morphological traits of 23 hot pepper genotypes. Similar traits (angled stem, erect flower, white corolla and corolla spot, rotate corolla, white filament, elongated fruit, obtuseness of fruit at pedicel attachment and absence of neck at the base of the fruit) were observed on all genotypes. Shaw and Khan (1928) stated that corolla color is one of the most consistent features of distinguishing *Capsicum* species and Bosland and Votava (2012) mentioned that the petals are usually white for the species *C. annuum*. Hence, the variety of hot pepper in this study is confirmed to be a variety of *Capsicum annuum*.

Segregation pattern of plant growth habit

Two types of plant growth habit were observed: intermediate (compact) and erect. A large number of plants exhibited intermediate growth habit in F₂ generation. The computed chi-square value (2.89) was lower than the chi-square table value (3.84) at 1 degree of freedom and thus the p-value (0.089) was greater than 0.05 (Table 4). Segregation of F₂ hot pepper based on plant growth habit supported the expected ratio of 9:7. In this study, compact plant growth habit was dominant over erect growth habit and can be determined by the complementary gene action. Erect plant type can be produced when the

Table 2. Mean, genotypic variance, environmental variance, phenotypic variance, genotypic coefficient of variation and phenotypic coefficient of variation for the 7 quantitative traits in 23 F₂ population of hot pepper

Characters	Range	Mean	σ^2_g	σ^2_p	$h^2_b(\%)$	GCV(%)	PCV(%)
Leal length (cm)	3-5	4.040	0.128	0.528	24.242	8.856	17.986
Leaf width (cm)	1-2.3	1.703	0.052	0.143	36.364	13.390	22.205
Filament length (cm)	0.2-0.4	0.260	0.001	0.004	25.000	13.652	24.956
Corolla length (cm)	0.9-1.6	1.290	0.012	0.031	38.710	8.492	13.649
Fruit width (cm)	0.6-1.2	0.860	0.010	0.026	38.462	11.333	18.568
Thousand-seed weight (g)	3-8	5.420	0.505	2.025	24.938	13.111	26.255
Seeds per pod (no.)	40-70	54.970	34.785	94.845	36.676	10.729	17.717

σ^2_g = genotypic variance
 σ^2_e = environmental variance
 σ^2_p = phenotypic variance
 $h^2_b(\%)$ = heritability in broad sense
 GCV(%) = genotypic coefficient of variation
 PCV(%) = phenotypic coefficient of variation

Table 3. Number of populations and relative frequency (%) of qualitative morphological traits for 322 F₂ hot pepper plants

No.	Score Character	Score	No. of Population	Relative Frequency (%)	
1	Plant Growth Habit	Intermediate/compact	5	166	52
		Erect	7	156	48
2	Branching Habit	Sparse	3	70	22
		Intermediate	5	145	45
		Dense	7	107	33
3	Anther Color	Pale blue	3	48	15
		Blue	4	19	6
		Purple	5	182	56
		Other (Yellow)	6	73	23
4	Stigma Exsertion	Same level	5	27	8
		Exserted	7	295	92
5	Calyx Margin	Intermediate	2	246	76
		Dentate	3	76	24
6	Fruit Surface	Non fruited	0	80	25
		Smooth	1	17	5
		Semiwrinkled	2	176	55
		Wrinkled	3	49	15
7	Fruit Bearing	Absent	0	80	25
		Present	1	242	75
8	Leaf Shape	Deltoid	1	9	3
		Ovate	2	209	65
		Lanceolate	3	104	32
9	Nodal Anthocyanin	Purple	5	42	13
		Dark purple	7	280	87
10	Leaf Color	Yellow	1	15	4
		Light green	2	176	55
		Green	3	131	41
11	Male sterility	Absent	0	270	84
		Present	1	52	16
12	Fruit Color	Non fruited	0	80	25
		Orange	6	12	4
		Light red	7	30	9
		Red	8	200	62
13	Fruit Shape	Non fruited	0	80	25
		Elongate	1	242	75
14	Neck at Base of Fruit	Non fruited	0	80	25
		Absent	1	242	75
15	Fruit Shape at Pedicel Attachment	Non fruited	0	80	25
		Obtuse	2	242	75
16	Flower Position	Erect	7	322	100
17	Corolla Color	White	1	322	100
18	Corolla Spot color	White	1	322	100
19	Corolla Shape	Rotate	1	322	100
20	Filament Color	White	1	322	100

dominant alleles *Dt* and *Ct* were in the dominant condition (McCammon and Honma, 1984).

Segregation pattern of branching habit

Sparse to dense characters were observed for branching habit. However, the segregation ratio did not fit the expected 9:3:4 ratio for F₂ populations suggesting a segregation distortion at this trait (Table 4). It is probably due to multiple genes controlling this trait.

Segregation pattern of anther color

In the present experiment, the genotypes could be grouped into four different morphological classes based on anther color: pale blue, blue, purple and other (yellow). In the species *C. annuum*, each flower has five to seven stamens with pale blue to purple anthers (Bosland and Votava, 2012). Chi-square test (p-value > 0.05) indicated that the observed deviation was obtained by chance alone. Hence, the null hypothesis was accepted and there

Table 4. Chi-square values and probabilities of goodness of fit for segregation pattern of 12 qualitative traits in F₂ hot pepper

No.	Descriptors	Hypothesized Proportion	χ^2	Pr.
1	Plant Growth habit (Intermediate/compact:Erect)	9:7	2.89	0.0893
2	Branching Habit (Intermediate: Sparse: Dense)	9:3:4	17.46	0.0002
3	Anther Colour (Purple:Yellow:Pale blue:Blue)	9:3:3:1	5.24	0.1548
4	Stigma Exsertion (Exserted:Same level)	15:1	2.51	0.1135
5	Leaf Shape (Ovate:Lanceolate:Deltoid)	9:6:1	12.76	0.0017
6	Calyx Margin (Intermediate:Dentate)	3:1	0.34	0.5620
7	Fruit Surface (Semiwrinkled:Wrinkled:Smooth)	12:3:1	0.69	0.7087
8	Fruit Bearing (Present:Absent)	3:1	0.00	0.9487
9	Leaf Color (Light green:Green:Yellow)	9:6:1	2.32	0.3135
10	Nodal Anthocyanin (Dark purple:Purple)	13:3	6.88	0.0087
11	Male Sterility (Absent:Present)	13:3	1.43	0.2318
12	Fruit Colour (Red:Light red:Orange)	12:3:1	7.74	0.0208

was no statistically significant difference in the proportion of 9:3:3:1 in segregating population of F₂ chilli pepper. Such results are obtained when the two pairs of characters behave independently with complete dominance in F₂ genotypes; this is known as independent assortment. In F₂ generation of *Capsicum annum*, cream and light blue anthers were observed with the segregation ratio of 3:1 (Manu et al., 2014). The resulted F₂ dihybrid ratios indicated the involvement of two genes controlling anther color.

Segregation pattern of stigma exsertion

Majority of the genotypes had exserted stigma. Lower computed chi-square value (2.51) compared to tabular value (3.84) at 1 degree of freedom and p-value of chi-square (0.114) indicated that the deviation from the expected frequencies was not significant i.e. there is a good fit to a 15:1 ratio (Table 4). Hongsheng et al. (2015) observed that the segregation of low and high stigma exsertion was fitted to a ratio of 3:1 by chi-square test and they suggested that the stigma exsertion was controlled by one pair of recessive genes in wheat. Higher percent of stigma exsertion in this study could be due to high temperature during the flowering time. Similar finding of elongated style under high temperature was also observed in pepper by Wien

(1997) who stated that styles are elongated under excessively high temperatures (32-38°C) and low relative humidity.

Segregation pattern of calyx margin

The computed chi-square value (0.34) was lower than the chi-square table value (3.84) at 1 degree of freedom and also the p-value (0.56) was greater than 0.05 (Table 4). This indicated that the segregation of F₂ chilli pepper based on calyx margin is a better fit to the expected ratio of 3:1 with dentate calyx margin is a character controlled by a single recessive gene. This ratio indicated that this trait is controlled by monogenic dominant gene with typical feature of qualitatively inherited character and of full dominance over recessiveness.

Segregation pattern of fruit surface

Three modes of fruit surface were observed: smooth, semiwrinkled and wrinkled. The computed chi-square value (0.69) was lower than the chi-square table value (5.99) at 2 degrees of freedom indicating that the deviation from the expected frequencies is not significant (Table 4). The segregation of F₂ chilli pepper based on fruit surface is a good fit to a 12:3:1 ratio, highlighting that there were two segregating major genes governing fruit

surface with dominant epistatic gene action.

Segregation pattern of fruit bearing

Fruit bearing plants and plants with no fruit were observed in the studied F_2 population. The ratio of fruit bearing in F_2 generation was very close to 3:1 ratio (Table 4). The p-value of chi-square (0.95) was greater than 0.05 indicating that the deviation from the expected frequencies was not significant i.e. there is a better fit to the expected ratio of 3:1 ratio. According to Panse and Sukhatme (1957), the value of chi-square score will be zero for a complete agreement with the hypothetical distribution. This finding was in agreement with the finding of Bosland (2005), who stated that male sterile plants did not set fruit and when they did, the fruits were too small to be commercially acceptable.

Segregation pattern of leaf shape

Most of the genotypes had ovate and lanceolate leaf shape with a few deltoid. Chi-square analysis for goodness of fit for F_2 population indicated that segregation did not fit to the expected ratio of 9:6:1 (Table 4) as p-value of chi-square (0.002) was less than 0.05. This is probably due to presence of polygenic gene for this trait.

Segregation pattern of nodal anthocyanin

Majority of the genotypes had dark purple nodal anthocyanin. Nodal anthocyanin of F_2 generation showed probability values (0.009) lower than 0.05 and the computed chi-square value (6.88) was larger than the chi-square table value (3.84) at 1 degree of freedom (Table 4). This means that the deviation from predicted segregation ratio of 13:3 may not be due solely to chance. The segregation ratios of the nodal anthocyanin did not fit any hypothesized segregation ratios. Segregation distortion in this study may be probably due to multiple genes controlling this trait.

Segregation pattern of leaf color

Most of the genotypes had leaf color ranging from light green to green and a few had yellow. The computed chi-square value (2.32) was lower than the chi-square table value (5.99) at 2 degrees of freedom and also the p-value of chi-square (0.3135) was indicating that the deviation from the expected

frequencies is not significant (Table 4). The segregation of the leaf color in F_2 generation fall into three classes: light green, green and yellow fitting to the theoretical dihybrid ratio of 9:6:1. Csilléry (1985) studying in *Capsicum baccatum* stated that although the leaf color of the F_1 were normal green, the segregation ratio in F_2 generation was abnormal resulting in 12:3:1 with normal green: pale yellow: yellow and also stated that yellow leaf color was according to the monogenic recessive *lutescens* (*lut-1*) mutant. However, the leaf color may also depend on the concentrations of the chlorophylls and carotenoids and deficiency in any one of several nutrient elements (Bosland and Votava, 2012).

Segregation pattern of male sterility

Relative frequency of male sterility showed that 84% for absence and 16% for presence. Sterile plants were easily distinguishable by visual observation. The computed chi-square value (1.43) was lower than the chi-square table value (3.84) at 1 degree of freedom and also the p-value (0.23) was greater than 0.05 (Table 4). It can be concluded that the segregation of F_2 chilli pepper based on male sterility supported the expected ratio of 13:3. Kumar et al. (2004) also showed frequencies of male fertile and sterile plants as 3:1 in segregation of F_2 pepper plants by selfing. Bosland (2005) also stated that 25% of the F_2 hybrid chilli pepper would be male-sterile theoretically. According to Bosland and Votava (2011), fertility restoration is believed to be controlled by a single dominant gene *Rf* in a sterile cytoplasm, while the recessive *rf* maintains sterility. Peterson (1958) showed that warm temperatures present the most critical environment for sterility expression. Thus, the relative instability of the trait in this study may be attributed to an interaction between temperature and sterility modifier genes.

Segregation pattern of fruit color

This study revealed three fruit colors (red, light red, orange) at mature stage with red as the predominant fruit color in the F_2 generation. The computed chi-square value (7.74) was greater than the tabular chi-square value (5.99) at 2 degrees of freedom (Table 4). P-value in chi-square (0.02) was lower than 0.05 showing deviation from the

predicted 12:3:1 ratio of red: light red: orange in the segregating population of F₂ chilli pepper. The segregation distortion in this study may be due to the multiple genes controlling this trait.

Conclusion

Significant differences among 7 traits (leaf length, leaf width, filament length, corolla length, fruit width, thousand-seed weight and number of seeds per pod) indicated that the presence of appreciable level of variability among the tested 23 hot pepper genotypes. Low and medium heritability values and higher phenotypic coefficient of variation (PCV) than genotypic coefficient of variation (GCV) suggested that all these traits interacted with the environment rather than genetic variation. Similar traits were observed on all genotypes for angled stem, erect flower, white corolla and corolla spot, rotate corolla, white filament, elongated fruit, obtuseness of fruit at pedicel attachment and absence of neck at base of fruit. Segregation distortion observed in nodal anthocyanin, branching habit, leaf shape, mature fruit color suggested that they are polygenic traits. Calyx margin and fruit bearing characters followed the Mendelian ratio (3:1) highlighting the monogenic recessive nature of the gene. Anther color expressed independent assortment with complete dominance (9:3:3:1). Plant growth habit, stigma exertion, fruit surface, leaf color and male sterility resulted as modified F₂ dihybrid ratios indicated the involvement of two genes controlling each trait. Variation in quantitative traits, on which environmental factors have a profound effect, may hinder the progress in selection for progeny containing favorable genes; however, variation in qualitative traits which are due to segregation in F₂ progeny might be useful for selection of desirable quality in next generation.

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