# ASSESSMENT ON VARIATION AND SEGREGATION IN $F_2$ POPULATION OF HOT PEPPER (*Capsicum annuum*)

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# ASSESSMENT ON VARIATION AND SEGREGATION IN $F_2$ POPULATION OF HOT PEPPER (*Capsicum annuum*)

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**A thesis submitted to the post-graduate committee of the Yezin Agricultural University as a partial fulfillment of the requirements for the degree of Master of Agricultural Science (Horticulture)**

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The thesis attached hereto, entitled **"Assessment on variation and segregation in $F_2$ population of hot pepper (*Capsicum annuum*)"** was prepared and submitted by Chaw Su Su Htwe under the direction of chairperson of the candidate supervisory committee and has been approved by all members of that committee and board of examiners as a partial fulfillment of the requirements for the degree of **MASTER OF AGRICULTURAL SCIENCE (HORTICULTURE).**

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## DECLARATION OF ORIGINALITY

This thesis represents the original works of the author, except where otherwise stated. It has not been submitted previously for a degree at any other university.

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**DEDICATED TO MY BELOVED PARENTS,**
**(U AUNG NAING) AND DAW HLA AYE**

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# ABSTRACT

A good assessment on the extent of variation of qualitative and quantitative traits in hot pepper is highly important to select desirable traits for varietal improvement. The experiment was carried out to investigate the extent of variations in quantitative traits and to test the segregation pattern for some qualitative traits of selected $F_2$ hot pepper. The seeds were collected from 23 $F_1$ hot pepper plants grown at Yezin Agricultural University Farm. The experiment was laid out in a Randomized Complete Block design (RCBD) with two replications. The traits like leaf length, leaf width, filament length, corolla length, fruit width, thousand seed weight and number of seeds per pod showed important contributors towards genetic variation among the genotypes. No significant difference observed in anther length, fruit length, fruit weight, total fruit weight and fruit pedicel length indicated uniformity in some yield contributing traits in $F_2$ generation. Study on variability highlighted that phenotypic component was the major contributor to total variance for all traits studied. Low and medium heritability values and higher phenotypic coefficient of variation (PCV) suggested that these above phenotypic traits interacted with the environment rather than genetic variation. Segregation pattern 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 absent neck at base of fruit on all genotypes. Segregation distortion 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 to 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 $F_2$ 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 $F_2$ progeny, might be useful for selection of desirable quality in next generation.

**Key words: hot pepper, $F_2$ populations, GCV, PCV, segregation**

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# CHAPTER I
# INTRODUCTION

The genus *Capsicum*, which is commonly known as red chile, hot red pepper, chilli pepper, tabasco, paprika, cayenne, etc., belongs to the nightshade family Solanaceae (Amit, 2004). *Capsicum* forms an important ingredient in people's diet around the world due to the pungency properties of the fruits resulting from the present of capsacinoid alkaloid that may help to fight cancer and inflammation and provide pain relief and due also to the production of high amounts of vitamin C, provitamin A, vitamin E, flavonoids, thiamine ($B_1$), riboflavin ($B_2$) and niacin ($B_3$) (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* consist of over 100 species and even more botanical varieties (Ado, 1999) but their cultivars may vary from the normal description of their species, so intraspecific variation, as well as interspecific variation, must be taken into account under variable study. They are highly heterogeneous plants exhibiting tremendous variability (Walsh and Hoot, 2001, Adetula and Olakojo, 2006; Bozokalfa et al., 2009). Most *Capsicum* species have flowers that are complete and self-compatible and they are generally regenerated through selfing (Bosland and Votava, 2012). However, depending on the species or variety, season and location, *Capsicum* 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 considerable amount of heterozygosity and heterogeneity, eventually resulting in off-type segregants during repeated multiplication cycles. *Capsicum annuum*, which is the most widely cultivated species, differs in many yield and quality characters such as plant height, fruit shape, fruit weight, fruit color, pungency, and maturity. Cultivation in smallholdings by individual farmers under diverse environmental conditions is thought to have contributed to this vast variability. Natural gene introgression is believed to be the prime force responsible for high variability (Grubben and El-Tahir, 2004).

It is well known that the extent of genetic variation present in the crop is the basis for the improvement of that crop and the degree of improvement depends on the 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). The introduction of $F_1$ hybrid cultivars has influenced crop production due to their vigor, uniformity, disease resistance, stress tolerance and good traits including earliness, long shelf-life and giving consistent stable high yield. Elite hybrid varieties or $F_1$ hybrids can be used as gene reservoir for breeding programmes (Van der Have, 1979) and selection has to be made in $F_2$ and the subsequent generations (Somadshaka and Salimath, 2006). $F_2$ generation obtained from selfing of $F_1$ hybrid provides all possible variations (Ghosh et al., 2010).

In Myanmar, however, more than 80% of growers used their farm saved seed in different crops (MOFA, 2015) as an alternative to reduce the production cost although not recorded for *Capsicum*. 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. Information on variability and genetic inheritance of important traits in $F_2$ generation of *Capsicum* is lacking in Myanmar.

Therefore, the experiment was carried out with the following objectives:

(1) To investigate the extent of variations in quantitative traits of $F_2$ population of selected hot pepper

(2) To test segregation pattern for some qualitative traits in $F_2$ hot pepper

# CHAPTER II
# LITERATURE REVIEW

## 2.1 Origin and History of *Capsicum*

*Capsicum* species, members of the Solanaceae, are common throughout the cuisines of the world (Bletter et al., 2010). Prior to the arrival of humans in the Neotropics, the fruit and seeds of the wild ancestors of each domesticated species of pepper would have been dispersed by birds and other animals (Pickersgill, 1984). It consists of over 100 species and even more botanical varieties (Ado, 1999). At least five different species of *Capsicum* were brought into cultivation and were eventually domesticated by the indigenous peoples of the Neotropics. Archaeological data, phytogeography and genetic analyses have led researchers to suggest that *Capsicum annuum* was initially domesticated in Mexico or Northern Central America, *Capsicum frutescens* in the Caribbean, *Capsicum baccatum* in lowland Bolivia, *Capsicum chinense* in northern lowland Amazonia, and *Capsicum pubescens* in the mid-elevation of Southern Andes (Eshbaugh, 1993; Loaiza-Figueroa et al., 1989; Pickersgill, 1971; Pickersgill, 1984).

Among the domesticated species, *Capsicum annuum* was the most successful, probably due to its being the first *Capsicum* that arrived to Europe, rather than to any superior agronomic trait (Andrews, 1993). *Capsicum chinense* and *Capsicum frutescens* became also popular in Africa and Asia, whereas *Capsicum baccatum* and *Capsicum pubescens* mostly remained in South America and Andean regions (Bosland and Votava, 2011). *Capsicum annuum* is the most widely cultivated species, and include many common varieties such as bell peppers, wax, cayenne, jalapeños, and the chiltepin. The species *Capsicum baccatum* contains the South American aji peppers, a chilli that is integral in Peruvian cooking. *Capsicum chinense* varieties include the hottest peppers such as the naga, nabanero, datil and Scotch bonnet. *Capsicum frutescens* is the species that includes the famous tabasco, malagueta and Thai peppers, piri piri, and Malawian Kambuzi, and is considered by some to be wild rather than domesticated. Varieties of peppers like the South American rocoto peppers and manzano that thrive in higher elevations are classified in *Capsicum pubescens* (Asad and Marcos, 2014).

## 2.2 Morphology and Growth of *Capsicum*

*Capsicum* which exhibits considerable morphological variation is a highly

heterogeneous plant species (Walsh and Hoot, 2001). The young stems are angular, usually becoming circular in cross section as they mature. The stem may have anthocyanin along its length, and anthocyanin may or may not be present at the nodes. The stem can be glabrous, pubescent or a gradation between entirely glabrous and entirely pubescent. There are indeterminate types of pepper, which grow like vines, and semi-indeterminate types where the plant slows its growth as it sets fruit. The leaves of pepper show variation in size, shape and color. Most are simple, entire and symmetrical. They can be flat and smooth or wrinkled and glabrous or subglabrous. The leaf blade may be ovate, elliptic or lanceolate. The leaves are usually green but types with purple, variegated, or yellowish color are known. The leaf petiole can be short or long, depending on species and cultivar. Leaves develop in clusters, singularly in a spiral system, or in pairs in opposite position. On the main axis, leaves are, as a rule, in a spiral arrangement. The leaf apex is usually acuminate but can be acute or obtuse. The leaf base either gradually narrows into the petiole or is abruptly acute (Bosland and Votava, 2012).

Pepper produces bisexual flowers which are borne at the intersection between the stem and leaves at points where the stem splits into a fork. The inflorescences may vary from solitary to seven flowers at one node. The calyx may range from long, green sepals to truncate sepals to spine-like projections. The pedicel length varies among cultivars, ranging from 3 to 8 cm (Berke, 2000). In the species *C. annuum* the petals are usually white with five to seven individual stamens which vary in color from pale blue to purple anthers. In *C. frutescens* species the corolla is usually greenish-white color. The pistil contains two to four carpels or locules, and a stigma borne at the tip of a slender style. The length of the style and relative position of the stigma and the anthers vary among genotypes, and it is an important factor determining the level of natural cross pollinations of the flowers. The flower color, shape and length also vary with different species and cultivars. The fruits are, botanically, classified as berries with different varieties of shapes, colors and sizes that vary among cultivars. Seeds are cream colored, except for *C. pubescens* which has black seeds (Berke, 2000).

## 2.3 Worldwide *Capsicum* Production

Global production of dry chillies and peppers in 2012 was around 3.2 million tones. Between 1992 and 2012, production grew by 2.6% per year on average. Production and trade of dry chillies and peppers by most important producers in 2011 was shown in Figure 2.1. India is the largest producer (38%), followed by China (8.7%) and Peru

(5.2%). Together they account for about 75% of global exports of dry chillies. Other countries grow specifically for their domestic market and production. The fact that chillies are rain fed crops in many parts of the world, chillies make them depend strongly on weather conditions. Production in China, Peru and Ghana have fluctuated less and has grown steadily over the years. Growth of production in China was mainly due to an increase in the area under cultivation. In Peru it was a combination of a growing area under cultivation and increasing yields. In Ghana growth of production has only been accomplished by increasing yields (Crem and Koekoek, 2015). The top-10 dried-pepper producing countries in 2012 as shown in Figure 2.2 were India, China, Peru, Bangladesh, Pakistan, Thailand, Myanmar, Ghana and Ethiopia (FAOSTAT, 2012).

## 2.4 *Capsicum* Consumption and Production in Myanmar

*Capsicum* is one of the most important crops in Myanmar. Consumers evaluated on fruit firmness, size and shininess during purchase of chillies and chillies purchase is very much linked to the recipe requirement where regular purchase of about 36%, occasional purchase of about 61% while never purchase of only 3% of total purchasing chillies in Myanmar according to Mercy-Corps (2015). Sown acreage and yield of dry chillies by region and state in Myanmar are shown in Table 2.1 and dry chillies and peppers production increased from 121,400 tons to 157,193 tons between 2014 and 2016 (DOA, 2016), however, exports from Myanmar to other countries are mainly restricted to dried chillies and the competitiveness of the Myanmar horticultural sector is declining (Joosten and Koesveld, 2015). Figure 2.3 shows export chain of dried chillies in Myanmar and there will require to focus on export promotion initially on enhancing the competiveness of the existing export crops such as dried chillies.

## 2.5 Nutritional Value and Chemical Composition

Consumption of Capsicum is increasing and may represent an important source of vitamins for world population. Peppers are richer in vitamins C and A than the usually recommended food sources. Capsicum fruits vary in size, shape, color, flavor and pungency and this variation is also reflected in their nutritional composition (Bosland and Votava, 2012). Provitamin A activity, ascorbic acid content, carotenoids, flavonoids, total soluble reducing equivalents, phenolic acids, and antioxidant activity all generally increase with maturity in all cultivars and species of *Capsicum* (Howard et al., 1994; Howard et al., 2000). A great deal of attention has been paid to peppers because of an

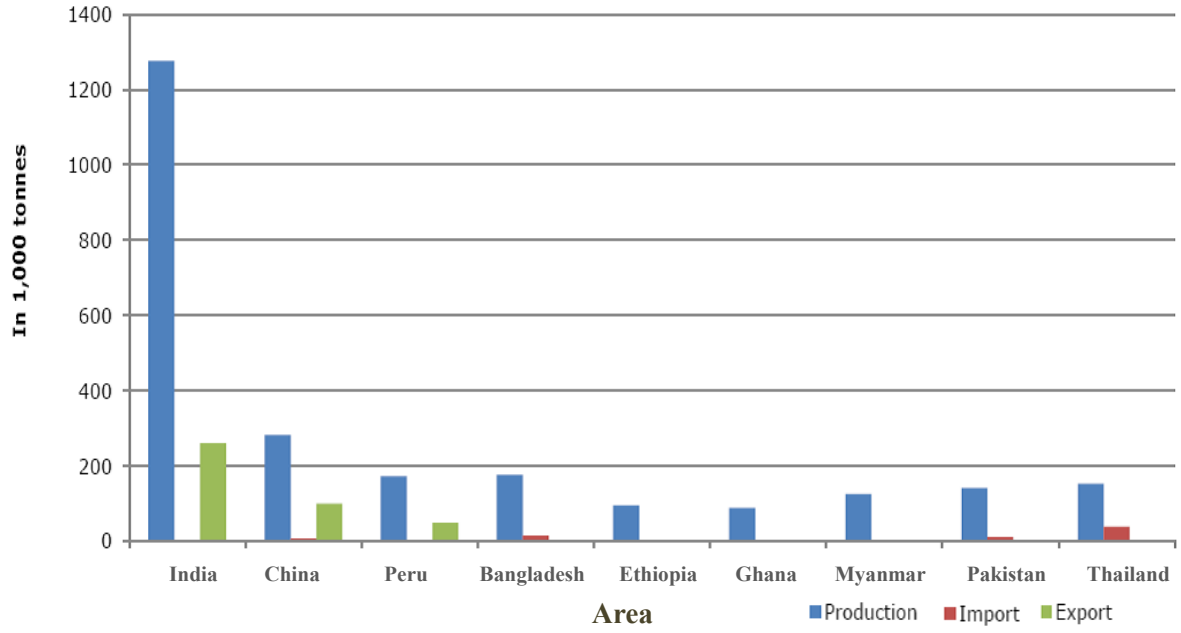



**Figure 2.1 Production and trade of dry chillies and peppers by most important producers, 2011 (*Source*: Crem and Koekoek, 2015)**

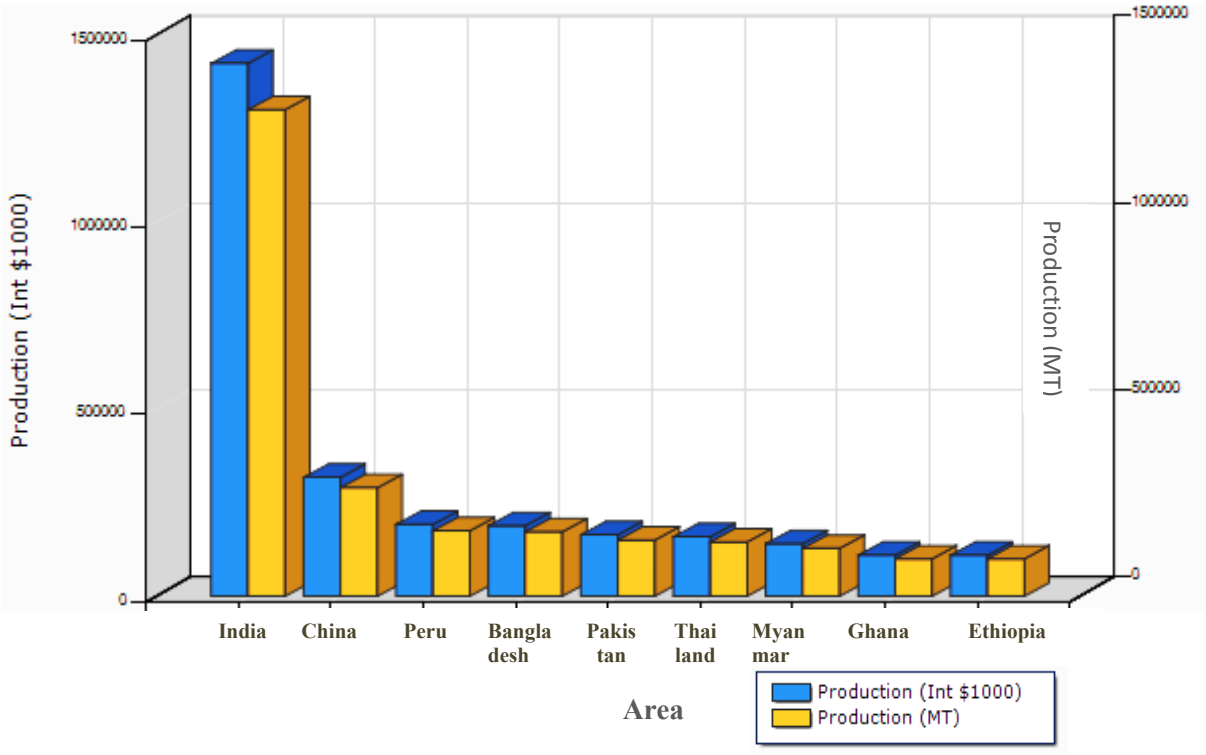



**Figure 2.2 The top-10 dried-pepper producing countries in 2012 (*Source:* FAOSTAT, 2012)**

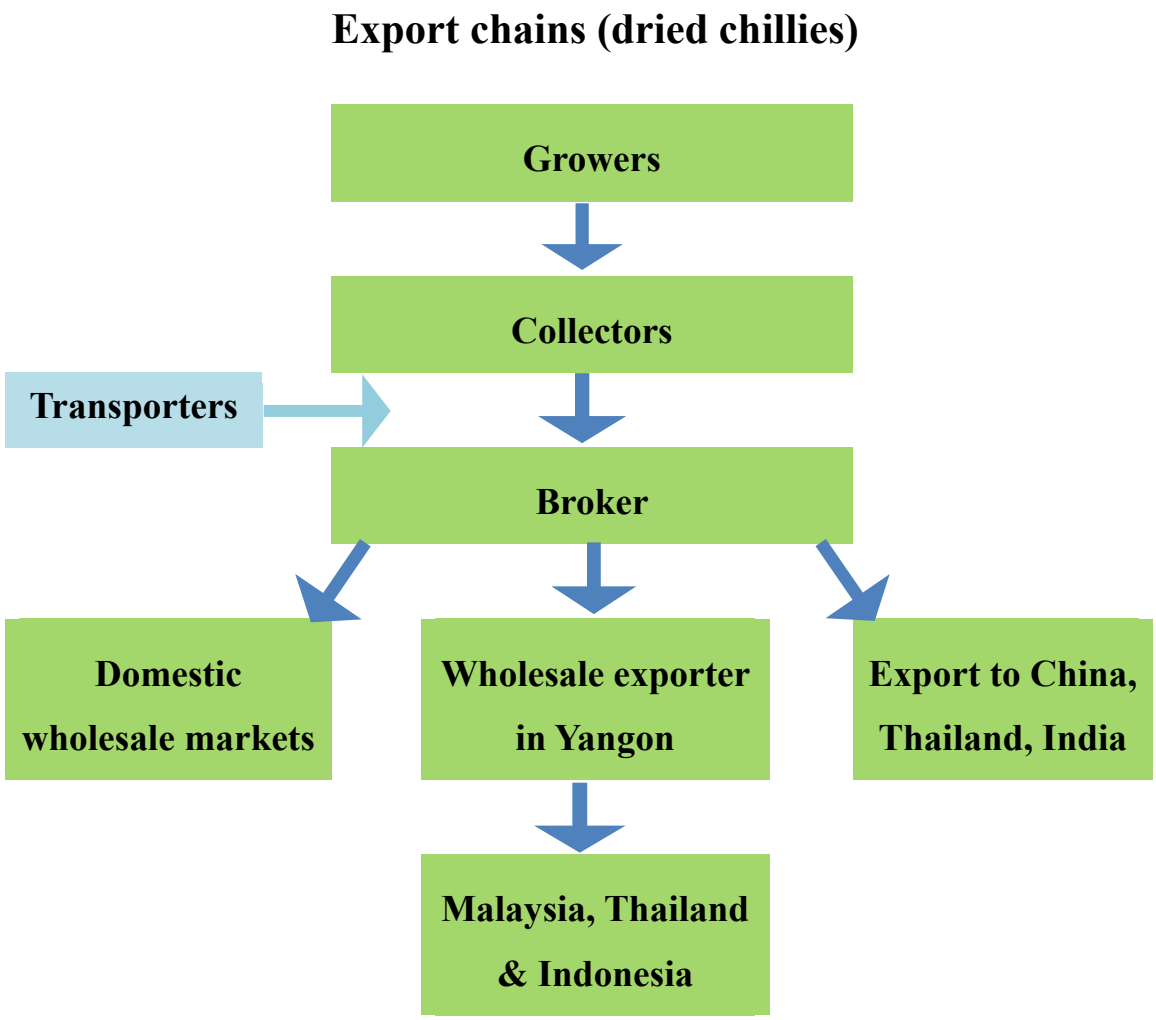



**Figure 2.3 Export chain of dried chillies in Myanmar (*Source*: Joosten and Koesveld, 2014)**

**Table 2.1 Sown acreage and yield of dry chillies by region and state in Myanmar, 2015-16 (*Source*: DOA, 2016)**

| Sr. | Region and State | Sown/Harvesting area (acre) | Yield per acre (kg) |
|---|---|---|---|
| 1 | Naypyitaw | 5211 | 365.3 |
| 2 | Kayah | 5342 | 247.1 |
| 3 | Kayin | 2800 | 326.1 |
| 4 | Chin | 3018 | 169.2 |
| 5 | Sagaing | 4750 | 642.0 |
| 6 | Bago | 4932 | 551.8 |
| 7 | Magway | 25810 | 533.1 |
| 8 | Mandalay | 121534 | 291.0 |
| 9 | Mon | 3377 | 898.2 |
| 10 | Rakhine | 36500 | 468.7 |
| 11 | Yangon | 1131 | 801.8 |
| 12 | Shan | 7974 | 401.9 |
| 13 | Ayeyarwady | 89500 | 657.1 |

excellent source of antioxidants which counter the oxidation of lipids via scavenging oxygen free radicals (Bosland and Votava, 2012). Red peppers are substantially higher in vitamin B (folate) than green peppers (Phillips et al., 2006), while maturity to the red-ripe stage affect carotenoid content, and that immature green peppers has higher concentrations of polyphenols although red ripe fruit had the highest vitamin C and provitamin A contents (Marin et al., 2004).

Peppers contain thousands of chemicals including water, fixed (fatty) oils, steam-volatile oil, carotenoids, resin, protein, fiber, mineral elements and many other chemicals. The numerous chemicals have importance for nutritional value, taste, color and aroma. The two most important groups of chemicals found in peppers may be the carotenoids and the capsaicinoids. The carotenoids contribute to a pepper's color and its nutritional value while the capsaicinoids are the alkaloids that give hot peppers their characteristic heat (Bosland and Votava, 2012). Mature fruit color is the result of reduction in chlorophyll and anthocyanin pigmentation and an accumulation of carotenoid pigments. Capsanthin and capsorubin are the major pigments in red fruit; whereas violaxanthin and ß-carotene are the major pigments in orange fruit (Gross, 1991).

## 2.6 Genetics and Breeding

Most species of chilli pepper are diploid, with 24 chromosomes (2n=2x=24), and have one or two pairs of acrocentric chromosomes with 10 or 11 pairs of metacentric or submetacentric chromosomes (Lanteri and Pickersgill, 1993). A list of known genes can be very useful to pepper breeders. In 1965, a gene list for pepper was produced that included 50 genes (Lippert et al., 1965) and a standardization of rules for naming and symbolizing. An updated gene list was produced by Daskalov and Poulos (1994), and a further update, adding 92 previously unreported genes to bring the total to 292 (Wang and Bosland, 2006).

For selection of desirable characters under a breeding programme, yield and yield contributing traits in chilli pepper including days to 50% flowering, fruit weight, fruit length, fruit width, number of sees per fruit, days to fruit maturity, plant height, plant canopy width, yield per plant play an important role. *Capsicum* breeding, depending on the objectives, involves selection for traits such as high yield, pungency, fruit color, fruit size and shape as well as disease resistance (Liu et al., 2009). These traits require simple traditional breeding methods with few cases of incompatibility. It involves intraspecific hybridization between different cultivars to transfer simple phenotypic characters.

However, limited genetic resources for breeding and increasing demand for better pepper varieties require for pepper breeding. Conventional interspecific hybridization between two species can sometimes result in embryo abscission due to post fertilization genetic barrier. The endosperm degenerates resulting in total or partial sterility of hybrid plants. These barriers have prevented the use of wild species which carry important genes that may be absent in the cultivated species (Monteiro et al., 2011). Pollen viability evaluation was done by visual inspection (Kumar et al., 2004) and by staining method in sweet pepper (Gniffke et al., 2009). However, Kumar et al. (2002) suggested that normal amount of fruits and seeds can be expected on male sterile plants of chilli according to the considerable amount of natural cross pollination in single visit by honeybees.

All pepper species are protogynous and can cross-pollinate. The stigma is positioned slightly below, level with, or exerted beyond the anthers, the latter arrangement increasing the chances of cross pollination. Studies have shown that the frequency of cross pollination in the field can range from just 2% to as high as 90% (Pickersgill, 1997). Pepper breeders and seed producers must try to prevent uncontrolled crosspollination (Bosland, 1993).

## 2.7 Phenotypic and Genotypic Variability and Heritability

To study the extent of variation of traits of pepper germplasm, a good assessment of the qualitative and quantitative traits is highly important. Qualitative characters are those whose variation is discontinuous and in which the individuals comprising the material under consideration are distinguished by some quality or attribute. In quantitative characters the variation may be continuous or discrete and the individuals are distinguished by a measurement or count (Panse and Sukhatme, 1957). Although qualitative and quantitative characters are expected to jointly establish the phenotype, the latter is more important to the plant breeder than the former given its importance in crop improvement (Maga, 2012).

Genetic variability is a measure of the tendency of an individual genotype in a population to vary from one another and it determines the success of any breeding program. Several methods have been used for the assessment of genetic variation in plant populations including morphological, molecular, cytogenetic and DNA based marker selection techniques. Morphological characterisation is the first and simplest approach in the description and classification of germplasm and is the only means by which plants can be differentiated based on their physical appearance. Morphological markers are readily

available and very easy to identify and in most cases do not require special skills. Even though morphological characterization is important in variety identification, its application is influenced by prevailing environmental factors (Smith and Smith, 1989).

The study in *Capsicum annuum* has shown that both morphological traits and amplified length fragment polymorphism (ALFP) markers generally separate pepper genotypes according to fruit traits and a significant positive correlation between the morphological data and ALFP marker-based matrices indicate that ALFP distances tend to reflect morphological distances (Geleta et al., 2005), hence phenotypic distances can sufficiently be used in discriminating a genotype.

Phenotypic variance of individuals is the result of the genotypic constitution of an individual and its expression in the environment (Falconer, 1960). The comparison of characters could be better judged by the estimation of genotypic coefficient of variation (GCV) in relation to their respective phenotypic coefficient of variation (PCV) as regards to the extent of genetic variation (Solomon et al., 2013). PCV) and GCV values were categorized as low (<10%), moderate (10-20%) and high (>20%) (Deshmukh et al., 1986).

Heritability is defined as the ratio of additive genetic component of variance to the total phenotypic variance (Wright, 1934). Heritability in broad sense refers to the genetic variation in the population in relation to the total observed variation. For improvement of desirable characters, heritability estimate is very essential to assess the relative effect of genotype and environment on a character in order to predict the extent of possible improvement. Therefore, heritability is one of the major indicators of response to selection for a successful breeding program. High heritability coupled with high genetic advance indicates that improvement could be made for a character by simple selection on phenotypic performance (Kumar, 1990). The heritability estimates above 60% are considered as high, 30% to 60% are moderate and below 30% as poor according to Robinson et al. (1949), Usman et al. (2014) and Laboni et al. (2015). For selecting the desirable genotypes, higher variability has better chance (Laboni et al., 2015).

## 2.8 Mendelian Genetics and Gene Interaction

The discovery of the laws of inheritance of nuclear genetic material was made in the mid-1800s by the Austrian monk Gregor Mendel, who performed a series of crossing experiments in garden peas. Mendel's monohybrid crosses between varieties of peas differing in phenotype for a single character established his first three laws: (1) Unit

factors come in pairs; (2) Unit factors are either dominant or recessive to one another; and (3) Unit factors segregate randomly. Mendel also studied the inheritance of pairs of factors that led to his fourth law of independent assortment. Mendel's laws and Charles Darwin's theory of evolution provide the fundamental basis of the science of genetics (White et al., 2007).

In genetic studies, the organisms produced by a mating constitute the $F_1$ generation. Crossing between members of the $F_1$ generation produces the $F_2$ generation. Although the recessive trait is not expressed in the hybrid progeny of a monohybrid cross, it reappears in the next generation when the hybrid progeny is allowed to undergo self-fertilization and Mendel counted the $F_2$ pea seeds of the hybrid with the ratio of approximately 3:1. Dihybrid crosses differ in two genes. In the dihybrid $F_2$, the phenotypic ratios are 9:3:3:1 and provided that both the *A* and the *B* alleles are dominant and that the genes undergo independent assortment (Daniel and Elizabeth, 1998).

In the years following the rediscovery of Mendel's four basic laws of inheritance, several exceptions were discovered. The alleles controlling characters in peas that Mendel studied acted dominantly or recessively to one another. Incomplete or partial dominance is an exception to Mendel's second rule, where the effects of each of the alleles are blended to produce an intermediate phenotype. Another exception to the second law is codominance, where the phenotype reveals expression of both alleles, such as with allozymes. Two characters (or loci) located on the same chromosome do not always undergo independent assortment, which is a deviation from Mendel's fourth law. This can be due to genetic linkage. The genetic distance that two loci are from each other on chromosomes is estimated by counting the number of gametes that have undergone crossing over. The proportion of recombinant gametes to the total is called the recombination fraction and is the standard estimator for the degree (*i.e.* tightness) of genetic linkage (White et al., 2007).

In genetic crosses in which two mutations that affect different steps in a single pathway are both segregating, the typical $F_2$ dihybrid ratio of 9:3:3:1 is not observed. Any type of gene interaction that results in the $F_2$ dihybrid ratio of 9:3:3:1 being modified into some other ratio is called *epistasis.* If there is epistasis that renders two or more of the phenotypes indistinguishable, then the $F_2$ ratio is modified. Unmodified and modified $F_2$ dihybrid ratios are shown in Figure 2.3. Taking all the possible modified ratios together, there are nine possible dihybrid ratios when both genes show complete dominance (Daniel and Elizabeth, 1998).

**Duplicate recessive epistasis/ Complementary Genes (9:7) -** This ratio is observed when a homozygous recessive mutation in either or both of two different genes results in the same mutant phenotype. It is exemplified by the segregation of purple and white flowers. Genotypes that are *C*— for the *C* gene and *P*— for the *P* gene have purple flowers; all other genotypes have white flowers. In this notation, the dash in *C*— means that the unspecified allele could be either *C* or *c*, and so *C*— refers collectively to *CC* and *Cc.* Similarly, the dash in *P*— means that the unspecified allele could be either *P* or *p* (House, 1985; Daniel and Elizabeth, 1998).

**Dominant epistasis/ Masking Genes (12:3:1) -** A modified dihybrid ratio of the 12:3:1 variety results when the presence of a dominant allele of one gene masks the genotype of a different gene. For example, if the *A*— genotype renders the *B*— and *bb* genotypes indistinguishable, then the dihybrid ratio is 12:3:1 because the *A*— *B*— and *A*— *bb* genotypes are expressed as the same phenotype (House, 1985; Daniel and Elizabeth, 1998).

**Dominant and recessive epistasis/ Inhibiting Genes (13:3) -** One gene may inhibit the expression of another gene, e.g., if *A*— produces a red flower color and *aa* white, the presence of a dominant allele *B* at another locus may suppress the action of *A*. Thus *A*—*B*, *aaB*—, and *aabb* produce white flowers, and only *A*—*bb* produces red flowers. The *A* allele is necessary for red colored flowers, but the *B* allele is a dominant inhibitor of red coloration and the phenotypic ratio in this situation is 13 (white): 3 (red) (House, 1985).

**Recessive epistasis/ Modifying Genes (9:4:3) -** This dihybrid ratio (often stated as 9:3:4) is observed when homozygosity for a recessive allele with respect to one gene masks the expression of the genotype of a different gene. For example, if the *aa* genotype has the same phenotype regardless of whether the genotype is *B*— or *bb*, then the 9:4:3 ratio results (House, 1985; Daniel and Elizabeth, 1998).

**Duplicate interaction/ Additive Genes (9:6:1) -** This dihybrid ratio is observed when homozygosity for a recessive allele of either of two genes results in the same phenotype, but the phenotype of the double homozygote is distinct. The 9:6:1 ratio results from the fact that both single recessives have the same phenotype (House, 1985; Daniel and Elizabeth, 1998).

**Duplicate dominant epistasis/ Duplicate Genes (15:1) -** If a dominant allele is present at either locus or at both loci, the phenotype is the same. Thus *A*—*B*—, *A*—*bb* and

*aaB*— all result in the same phenotype, and only the homozygous resessive (aabb) is different. This type of gene action results in a 15:1 phenotype ratio (House, 1985).

## 2.9 Chi-square Test

Chi-square is defined as the sum of squares of independent, normally distributed variables with zero mean and unit variance. The chi-square test criterion is most commonly associated with enumeration data which generally involved a discrete variable, that is, a qualitative rather than a quantitative characteristic. The $X^2$ distribution, when associated with discrete data, is usually in conjunction with a test of goodness of fit (Steel and Torrie, 1980).

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

Where, $O$ = Observed value, $E$ = Expected value

The $X^2$ test is a statistical test commonly used perhaps most widely in connection with genetic experiments. It can be employed to test the significance of deviation of an observed segregation from a theoretical one and can also be adapted to the simultaneous testing of a number of questions, such as, single factor ratios, linkage and heterogeneity. The observed segregation may agree with more than one ratio and in consequence no decisive conclusion can be reached unless the size of the experiment is adequate (Panse and Sukhatme, 1957). The outcome of the chi-square test is heavily dependent on sample size. The larger the sample size, the more reliable is the test (Griffiths et al., 2000).

If the p-value for the calculated chi-square is $p > 0.05$, accept the null hypothesis. 'The deviation is small enough that chance alone accounts for it. A p-value of 0.6, for example, means that there is a 60% probability that any deviation from expected is due to chance only. This is within the range of acceptable deviation. If the p-value for the calculated chi-square is $p < 0.05$, reject your hypothesis, and conclude that some factor other than chance is operating for the deviation to be so great (Fisher and Yates, 1963).

The p-value can be looked upon as a statistical gauge of internal validity: studies with large p-values are more likely to falsely connect cause and effects, and thus more likely to be designed in a way that does not isolate and exclude the mechanism by which chance may lead to seemingly significant results. The lower the p-value, the greater is the likelihood that the study has good internal validity (Alberto and Mark, 2011).

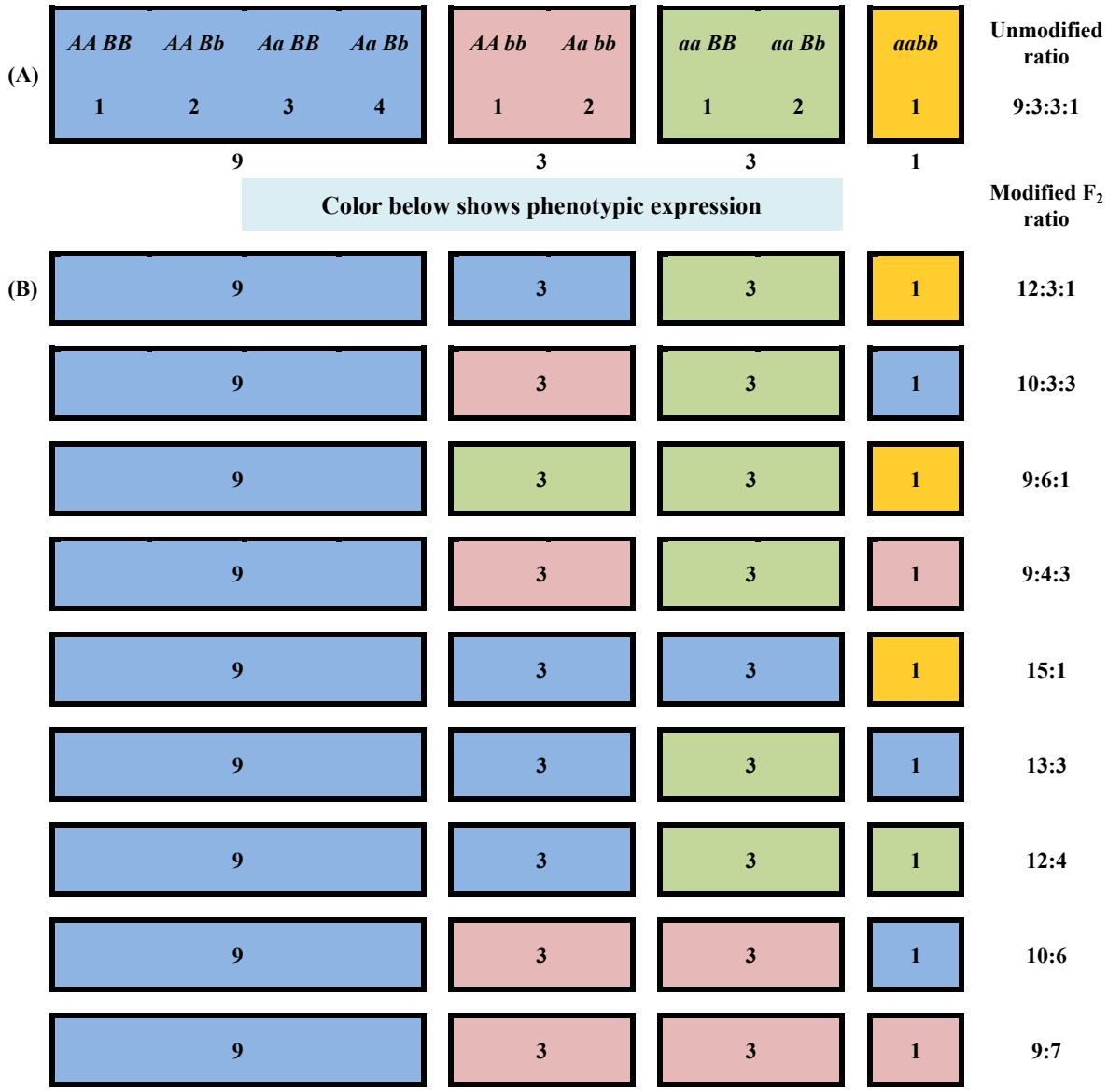



**Figure 2.4** **Modified $F_2$ dihybrid ratios. In each row, different colors indicate different phenotypes. (A) The $F_2$ genotypes of two independently assorting genes with complete dominance result in a 9:3:3:1 ratio of phenotypes if there is no interaction between the genes (epistasis). (B) If there is epistasis that renders two or more of the phenotypes indistinguishable, indicated by the colors, then the $F_2$ ratio is modified.**

# CHAPTER III
# MATERIALS AND METHODS

## 3.1 Experimental Site and Period

Field experiment was carried out at the Department of Horticulture and Agricultural Biotechnology, Yezin Agricultural University, Latitude 19°91' N and Longitude 96°07' E. The experiment was conducted from November 17, 2013 to May 31, 2014 (dry season) with the plot size of 26.5 meter in length and 23 meter in width. The soil of experimental site was loamy sand and the soil pH was 6.8. Available N was accounted for 241 kg/ha, available P was 36 kg/ha, available K was 396 kg/ha and organic matter percent was 1.43% (Tyurin's method).

## 3.2 Materials

Hot pepper fruits from 23 $F_1$ plants of Known-You variety grown at Yezin Agricultural University Farm were collected based on their physical characteristics.

## 3.3 Methods

Seeds of 23 plants were extracted separately by hand, air-dried and packed separately. Before sowing, the seeds were treated with 3% sodium hypochloride (NaOCl) for about 20 minutes for surface sterilization. Seeds were sown directly into the seed tray which were filled with well-prepared potting mixture (compost, well decomposed cow dung manure and burnt paddy husk) at the ratio of 3:1:1 by volume. The seedlings were carefully protected from pests and diseases and watered daily.

Land clearing, plowing, harrowing, cleaning weeds, making planting holes spaced by 90cm × 90cm were done before planting. Each planting hole was filled with mixture of top soil, farm yard manure, compound fertilizer, gypsum and furadan. Details of crop husbandry during the experiment were shown in Appendix 1.

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 experiment period. To control cross pollination, each plant was covered with isolation cages of nylon fabric.

### 3.4 Data Collection

All parameters (12 quantitative and 21 qualitative values) were recorded according to the descriptor for *Capsicum* (IPGRI, 1995).

#### 3.4.1 Quantitative traits

**Leaf Length (cm):** Average 10 mature leaves from the main branch of the plant were recorded when the first fruit has begun ripen in 50% of plants.

**Leaf Width (cm):** It was the widest part of leaf. Average 10 mature leaves from the main branch of the plant were measured when the first fruit has begun ripen in 50% of plants.

**Anther Length (cm):** Average anther length of 10 representative flowers was recorded just after anthesis.

**Filament Length (cm):** Average filament length of 10 representative flowers was measured just after anthesis.

**Corolla Length (cm):** Average 10 petals of dissected corolla were recorded just after anthesis.

**Fruit Length (cm):** Average 10 ripe fruits of second harvest were measured.

**Fruit Width (cm):** It was the widest portion of the fruit. Average 10 ripe fruits of second harvest were recorded.

**Fruit Weight (g):** Average of 10 ripe fruits of second harvest were measured.

**Fruit Pedicel Length (cm):** Average 10 pedicels of second harvest were measured.

**Total Fruit Weight (g):** Total fruits of three times harvests were weighed.

**Number of Seeds per Fruit:** The average number of seeds counted from 3 selected fruits per plant.

**1000 Seed Weight (g):** Two hundred seeds were weighed and multiplied by five times.

#### 3.4.2 Qualitative traits

Twenty one qualitative traits were recorded as shown in Table 3.1.

### 3.5 Statistical Analysis

#### 3.5.1 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):

**Genotypic variance,** $\sigma^2_g = (Mg-Me)/r$

Where, Mg = Mean square of genotypes

Me = Mean square of error

r = Number of replication

**Phenotypic variance,** $\sigma^2_p = \sigma^2_g + Me$

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

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

Where, $\bar{x}$ = Population mean

**Heritability in broad sense,** $h^2_b\ (\%) = \sigma^2_g / \sigma^2_p \times 100$

Where, $h^2_b$ = Heritability in broad sense

$\sigma^2_g$ = Genotypic variance

$\sigma^2_p$ = Phenotypic variance

### 3.5.2 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 $F_2$ progenies were subjected to Mendelian genetic models using a chi-square (multinomial) test (Panse and Sukhatme, 1957) using STATISTIX 8 programme.

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

Where, $O$ = Observed value, $E$ = Expected value

**Table 3.1 List of twenty-one qualitative descriptors and time of data collection**

| No. | Qualitative descriptors | | Time |
|---|---|---|---|
| 1 | **Nodal Anthocyanin** | 1=Green, 3=Light purple, 5=Purple, 7=Dark purple | At maturity |
| 2 | **Stem Shape** | 1=Cylindrical, 2=Angled, 3=Flattened | At maturity |
| 3 | **Branching Habit** | 3=Sparse, 5=Intermediate, 7=Dense | At maturity |
| 4 | **Plant Growth Habit** | 3=Prostrate, 5=Intermediate (compact), 7=Erect, 9=Other | When 50% of the plants bear ripe fruits |
| 5 | **Flower Position** | 3=Pendant, 5=Intermediate, 7=Erect | At anthesis |
| 6 | **Leaf Color** (average 10 mature leaves from the main branches of the plant) | 1=Yellow, 2=Light green, 3=Green, 4=Dark green, 5=Light purple, 6=Purple, 7=Variegated, 8=Other | When the first fruit has begun ripen in 50% of plants |
| 7 | **Leaf Shape** (average 10 mature leaves from the main branches of the plant) | 1=Deltoid, 2=Ovate, 3=Lanceolate | When the first fruit has begun ripen in 50% of plants |
| 8 | **Corolla Color** | 1=White, 2=Light yellow, 3=Yellow, 4=Yellow-green, 5=Purple with white base, 6=White with purple base, 7=White with purple margin, 8=Purple, 9=Other | At flowering time |
| 9 | **Corolla Spot Color** | 1=White, 2=Yellow, 3=Green-yellow, 4=Green, 5=Purple, 6=Other | At flowering time |
| 10 | **Corolla Shape** | 1=Rotate, 2=Campanulate, 3=Other | At flowering time |
| 11 | **Anther Color** | 1=White, 2=Yellow, 3=Pale blue, 4=Blue, 5=Purple, 6=Other | Immediately after blooming |

**Table 3.1 List of twenty-one qualitative descriptors and time of data collection (continued)**

| No. | Qualitative descriptors | | Time |
|---|---|---|---|
| 12 | **Filament Color** | 1=White, 2=Yellow, 3=Green, 4=Blue, 5=Light purple, 6=Purple, 7=Other | Just after anthesis |
| 13 | **Stigma Exsertion**<br>(In relation to anther, average 10 stigmas from representative flowers) | 3=Inserted, 5=Same level, 7=Exserted | At full anthesis |
| 14 | **Male Sterility** | 0=Absent, 1=Present | At full anthesis |
| 15 | **Calyx Margin** | 1=Entire, 2=Intermediate, 3=Dentate, 4=Other | At flowering time |
| 16 | **Mature fruit Color** | 1=White, 2=Lemon-yellow, 3=Pale orange-yellow, 4=Orange-yellow, 5=Pale orange, 6=Orange, 7=Light red, 8=Red, 9=Dark red, 10=Purple, 11=Brown, 12=Black, 13=Other | Just after harvest |
| 17 | **Fruit Shape** | 1=Elongate, 2=Almost round, 3=Triangular, 4=Campanulate, 5=Blocky, 6=Other | Just after harvest |
| 18 | **Fruit Shape at Pedicel Attachment** | 1=Acute, 2=Obtuse, 3=Truncate, 4=Cordate, 5=Lobate | Just after harvest |
| 19 | **Neck at Base of Fruit** | 0=Absent, 1=Present | Just after harvest |
| 20 | **Fruit Surface** | 1=Smooth, 2=Semiwrinkled, 3=Wrinkled | Just after harvest |
| 21 | **Fruit Bearing** | 0=Absent, 1=Present | When 50% of the plants bear ripe fruits |

# CHAPTER IV
# RESULTS AND DISCUSSION

## 4.1 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 4.1). These data 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 the traits of 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 according to 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. The range of twelve quantitative traits in 23 hot pepper genotypes was shown in Appendix 2.

## 4.2 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 is given in Table 4.2.

There was a wide range of phenotypic and genotypic variances. Genotypic variance ranged from 0.001 in filament length and 34.79 in number of seeds per fruit while phenotypic variance ranged from 0.004 in filament length and 94.845 in number of seeds per pod. The magnitude of phenotypic variance of all traits was higher than the genotypic variance, indicating that phenotypic component was the major contributor to total variance. 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. Estimates of heritability in broad sense ranged from 24% for leaf length to 39 % for corolla length. Leaf width, corolla length, fruit width and seeds per pod have medium heritability values while leaf length, filament length and thousand-

**Table 4.1 Analysis of variance for 12 quantitative traits of 23 $F_2$ population of hot pepper**

| Source | d.f | Mean square | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **LFLT** | **LFWD** | **ANLT** | **FMLT** | **CORLT** | **FRLT** | **FRWD** | **FRWT** | **TFRWT** | **FRPLT** | **THOSWT** | **SDPOD** |
| **Rep.** | 1 | 0.476 | 0.023 | $0.469\times10^{-3}$ | $0.134\times10^{-2}$ | 0.026 | 0.019 | 0.052 | 0.043 | 352.313 | 0.137 | 0.501 | 141.043 |
| **Geno.** | 22 | **0.656*** | **0.195**** | $3.143\times10^{-3}$ | **$0.547\times10^{-2}$*** | **0.043**** | 0.527 | **0.035**** | 0.082 | 749.338 | 0.361 | **2.525*** | **129.625**** |
| **Error** | 160 | 0.400 | 0.091 | 0.003 | 0.003 | 0.019 | 0.369 | 0.016 | 0.090 | 648.987 | 0.261 | 1.516 | 60.062 |
| **CV%** | | 15.650 | 17.677 | 20.932 | 20.699 | 10.699 | 11.570 | 14.646 | 21.594 | 19.095 | 15.887 | 22.712 | 14.098 |

**, * = significant at 1% and 5% level, respectively

d.f = degree of freedom

LFLT = leaf length (cm)

LFWD = leaf width (cm)

ANLT = anther length (cm)

FMLT = filament length (cm)

CORLT = corolla length (cm)

FRLT = fruit length (cm)

FRWD = fruit width (cm)

FRWT = fruit weight (g)

TFRWT = total fruit weight (g)

FRPLT = fruit pedicel length (cm)

THOSWT = thousand-seed weight (g)

SDPOD = seeds per pod (no.)

**Table 4.2 Mean performance, genotypic variance, phenotypic 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**

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

$\sigma^2_g$ = genotypic variance

$\sigma^2_e$ = environmental variance

$\sigma^2_p$ = phenotypic variance

**$h^2_b$ (%)** = heritability in broad sense

**GCV (%)** = genotypic coefficient of variation

**PCV (%)** = phenotypic coefficient of variation

seed weight have low heritability values showing a relatively larger contribution of environment to the phenotype (Table 4.2). 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 $F_2$ 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 $F_2$ 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.

## 4.3 Segregation Pattern of Qualitative Traits

Frequency distribution of twenty one qualitative morphological traits of twenty three hot pepper genotypes was shown in Appendix 3. Segregation pattern indicated that similar traits for angled stem shape, erect flower, white corolla and corolla spot, rotate corolla, white filament, elongate fruit, obtuseness of fruit at pedicel attachment and absent neck at the base of the fruit. 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*.

### 4.3.1 Segregation pattern of plant growth habit

Two types of plant growth habit were observed: intermediate (compact) and erect in which a large number of plants exhibited intermediate plant growth habit in $F_2$ 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 4.3). This ratio suggested that the segregation of $F_2$ 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 dominant alleles *Dt* and *Ct* were in the dominant condition (McCammon and Honma, 1984).

### 4.3.2 Segregation pattern of branching habit

Sparse to dense characters were observed for branching habit (Figure 4.1, Plate

**Table 4.3 Chi-square values and probabilities of goodness of fit for segregation pattern of 12 qualitative traits in $F_2$ hot pepper**

| No. | Descriptors | | Hypothesized Proportion | $\chi^2$ | 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 Color | 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 Color | Red:Light red:Orange | 12:3:1 | 7.74 | 0.0208 |

4.1). However, the segregation ratio did not fit the expected 9:3:4 ratio for $F_2$ populations suggesting a segregation distortion at this trait (Table 4.3). It is probably due to multiple genes controlling this trait.

### 4.3.3 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) (Figure 4.2, Plate 4.2). In the species *C. annuum*, each flower has five to seven individual stamens which vary in color from 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 for 5 percent (Table 4.3). Hence, the null hypothesis was accepted and there is no statistically significant difference in the proportion of 9:3:3:1 in segregating population of $F_2$ chilli pepper. Such results are obtained when the two pairs of characters behave independently with complete dominance in $F_2$ genotypes; this is known as independent assortment. Manu et al. (2014) stated that cream and light blue anthers were observed with the segregation ratio of 3:1 in $F_2$ generation of *Capsicum annuum*. The resulted $F_2$ dihybrid ratios indicated the involvement of two genes controlling anther color.

### 4.3.4 Segregation pattern of stigma exsertion

Majority of the genotypes had exserted stigma (Figure 4.3, Plate 4.3). Lower computed chi- square value (2.51) compared to tabular value (3.84) at 1 degree of freedom and p-value of chi-square (0.11) indicated that the deviation from the expected frequencies was not significant i.e. there is a good fit to a 15:1 ratio (Table 4.3). Hongsheng et al. (2015) observed that the segregation of low and high stigma exsertion fitted to a ratio of 3:1 by chi-square test and they suggested that the stigma exertion 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 observed in pepper by Wien (1997) who stated that styles are elongated under excessively high temperatures (32-38°C) and low relative humidity.

### 4.3.5 Segregation pattern of calyx margin

Majority of the genotypes had intermediate calyx margin (Figure 4.4, Plate 4.4). The computed chi- square value (0.34) was lower than the chi-square table value (3.84) at

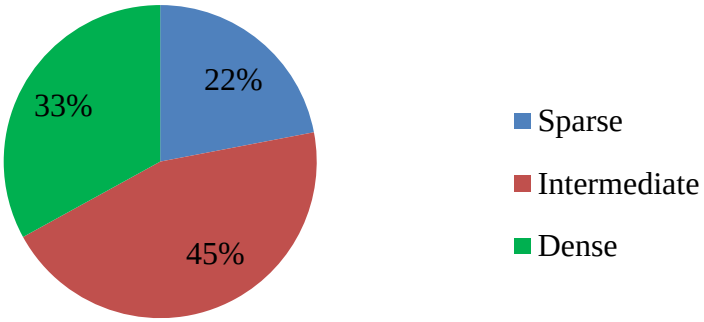



**Figure 4.1 Relative frequency of branching habit among pepper genotypes**

**(a) Sparse** **(b) Intermediate** **(c) Dense**

**Plate 4.1 Different branching habit of hot pepper**

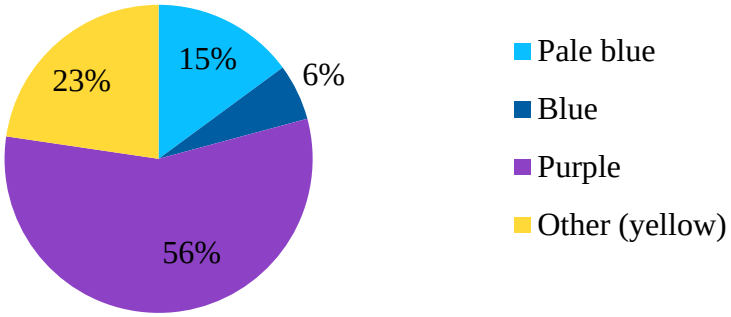



**Figure 4.2 Relative frequency of anther color among pepper genotypes**

**(a) Pale Blue** **(b) Blue** **(c) Purple** **(d) Other (yellow)**

**Plate 4.2 Different anther color**

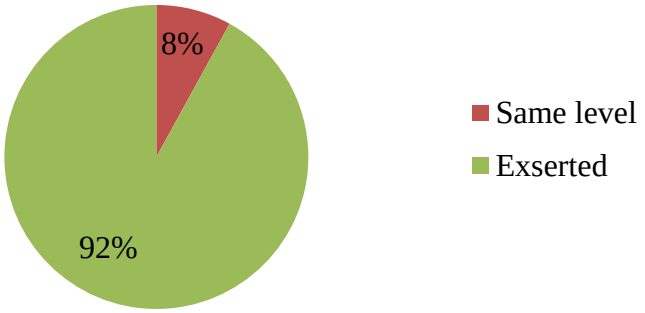



**Figure 4.3 Relative frequency of stigma exsertion among pepper genotypes**

**(a) Same level stigma**

**(b) Exserted stigma**

**Plate 4.3 Stigma exsertion**

1 degree of freedom and also the p-value (0.56) was greater than 0.05 (Table 4.3). These data indicated that the segregation of $F_2$ 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.

### 4.3.6 Segregation pattern of fruit surface

Three modes of fruit surface were observed: smooth, semiwrinkled and wrinkled (Figure 4.5, Plate 4.5). The computed chi- square value (0.69) was lower than the chi-square table value (5.99) at 2 degree of freedom and also the p-value of chi-square indicating that the deviation from the expected frequencies is not significant (Table 4.3). The segregation of $F_2$ 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.

### 4.3.7 Segregation pattern of fruit bearing

Fruit bearing plants and plants with no fruit were observed in the studied $F_2$ population (Figure 4.6, Plate 4.6). 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 expected ratio of 3:1 ratio (Table 4.3). According to Panse and Sukhatme (1957), the value of chi-square score will be zero for a complete agreement with the hypothetical distribution. In this study, fruit bearing character expressed as Mendelian ratio. Bosland (2005) stated that male sterile plants did not set fruit and when they did, the fruits were too small to be commercially acceptable.

### 4.3.8 Segregation pattern of leaf shape

Most of the genotypes had ovate and lanceolate leaf shape with a few have deltoid. Chi-square analysis for $F_2$ population indicated that segregation did not fit to the expected ratio of 9:6:1 (Table 4.3) as p-value of chi-square (0.002) was less than 0.05. This is probably due to presence of polygenic genes for this trait.

### 4.3.9 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-

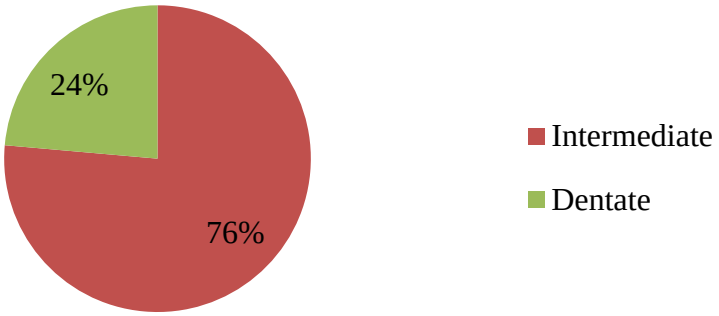



**Figure 4.4 Relative frequency of calyx margin among pepper genotypes**

**(a) Intermediate** **(b) Dentate**

**Plate 4.4 Calyx margin**

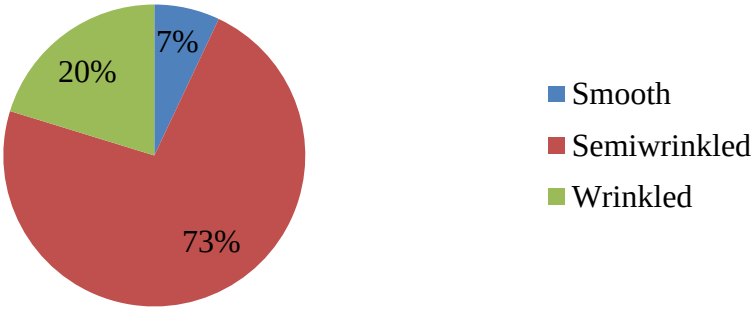
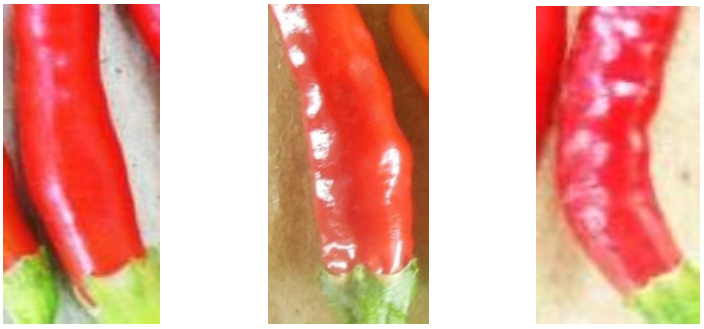



**Figure 4.5 Relative frequency of fruit surface among pepper genotypes**



**(a) Smooth** **(b) Semiwrinkled** **(c) Wrinkled**

**Plate 4.5 Different fruit surface of hot pepper**

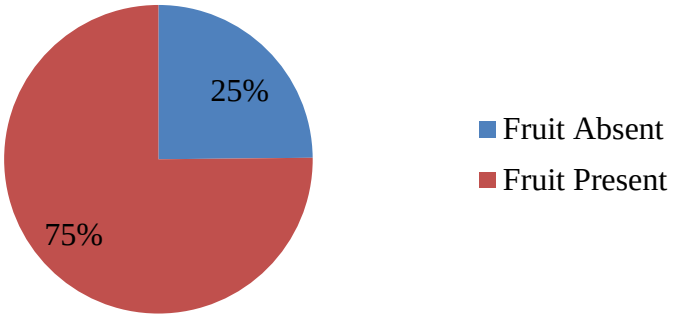



**Figure 4.6 Relative frequency of fruit bearing among pepper genotypes**

**(a) Fruit absent** **(b) Fruit present**

**Plate 4.6 Fruit bearing**

square value (6.88) was larger than the chi-square table value (3.84) at 1 degree of freedom (Table 4.3). 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 for this trait.

### 4.3.10 Segregation pattern of leaf color

Most of the genotypes had leaf color ranged from light green to green and a few had yellow. The computed chi- square value (2.32) lower than the chi-square table value (5.99) at 2 degree of freedom and the p-value (0.31) indicated that the deviation from the expected frequencies is not significant (Table 4.3). The segregation of the leaf color in $F_2$ generation fall into three classes: light green, green and yellow fitting to the dihybrid ratio 9:6:1. Csilléry (1985) stydying 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 resulted in 12:3:1 with normal green: pale yellow: yellow and 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).

### 4.3.11 Segregation pattern of male sterility

Relative frequency of male sterility showed that 84% for absence and 16% for presence (Figure 4.7). Sterile plants were easily distinguishable by visual observation (Plate 4.7). 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 4.3). 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) 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.

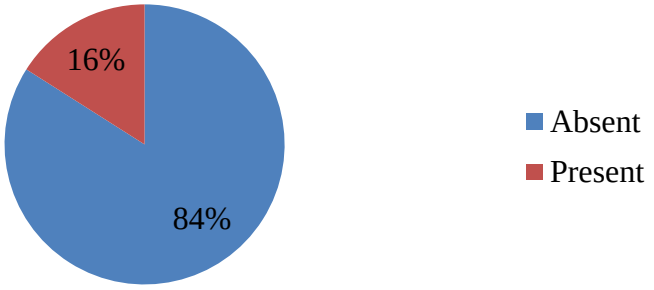
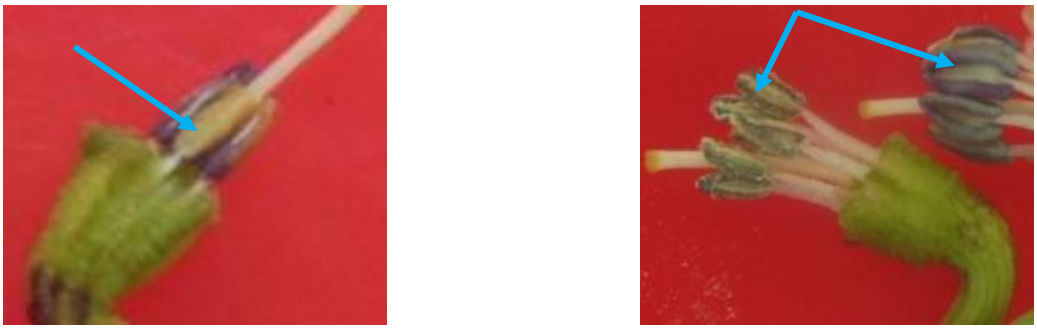



**Figure 4.7 Relative frequency of male sterility among pepper genotypes**



**(a) Sterile anthers**
**(thin, shriveled with not visible pollen)**

**(b) Fertile anthers**
**(full, densely covered with pollen)**

**Plate 4.7 Male sterility (visual observation)**

### 4.3.12 Segregation pattern of fruit color

This study revealed three fruit colors (red, light red, orange) at mature stage (Figure 4.8, Plate 4.8) 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 degree of freedom (Table 4.3). 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 $F_2$ chilli pepper. According to Hurtado-Hernandez and Smith (1985), ripe fruit color in *Capsicum* was controlled by three independent pairs of genes, encoded by the *y*, *c1*, and *c2* loci. However, Bosland and Votava (2011) stated that capsanthin capsorubin synthase (*Ccs*) gene which determined the major pigments in red fruit was a single dominant gene and the absence of which shown to result orange fruit color in *Capsicum* species. The segregation distortion in this study may also be due to the multiple genes controlling on this trait.

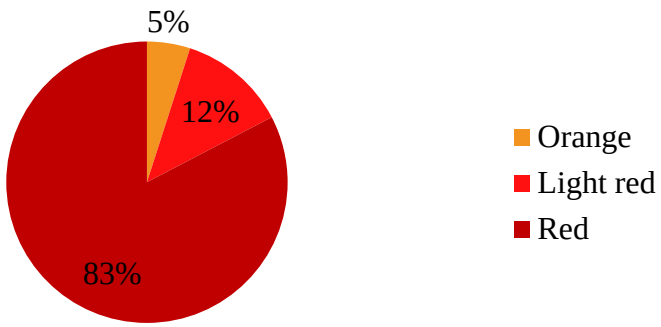



**Figure 4.8 Relative frequency of fruit color among pepper genotypes**

**(a) Orange** **(b) Light red** **(c) Red**

**Plate 4.8 Fruit color**

# CHAPTER V
# CONCLUSION

Quantitative traits showed significant differences among 7 characters (leaf length, leaf width, filament length, corolla length, fruit width, thousand-seed weight and number of seeds per pod) indicating the presence of appreciable level of variability among the tested 23 hot pepper genotypes. 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. In this study, the magnitude of the phenotypic variance of all traits was higher than the genotypic variance, indicating that the phenotypic component was the major contributor to total variance for all the traits studied. 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 these traits. Similarly, phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits and this reflect a marked influence of environmental factors for the expression of these traits. Therefore selection based on these traits may not be significantly effective for genetic improvement.

Study on segregation pattern 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 absent neck at base of fruit. Segregation distortion 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 to Mendelian ratio (3:1) highlighting the monogenic recessive nature of the gene. Anther color expressed independent assortment with complete dominance (9:3:3:1) and plant growth habit, stigma exsertion, fruit surface, leaf color and male sterility resulted as modified $F_2$ dihybrid ratios. These data indicated 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 $F_2$ progeny might be useful for selection of desirable quality in next generation. It is expected that the information generated in this study will provide a basis for breeding purposes and more practical approach such as molecular markers is required

to enhance precision of study. Further assessment studies on yield in $F_2$ hot pepper should be done by open pollination.

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# APPENDICES

**Appendix 1 Details of crop husbandry during the experiment**

| Operation | Date | Remarks |
|---|---|---|
| Seed sowing | 13 November 2013 | |
| Transplanting | 15 December 2013 | |
| Basal fertilizer application | 14 December 2013 | Cowdung manure (8 ton/ha), Compound fertilizer (251 kg/ha), Triple super phosphate (120 kg/ha) |
| 1st side dressing | 30 December 2013 | Urea (52 kg/ha) |
| 2nd side dressing | 14 January 2014 | Urea (52 kg/ha) |
| 3rd side dressing | 29 January 2014 | Urea (52 kg/ha) |
| Bagging the plant | 23 January 2014 | For pollination control |
| 1st weeding | 5 January 2014 | |
| Times of weeding | 5 January 2014 to 12 May 2014 | 10 times with 15 day interval |
| Insecticide and fungicide spraying | 16 January 2014 to 1 May 2014 | 7 times with 15 day interval |
| 1st time fruit picking | 20 April 2014 | |
| 2nd time fruit picking | 10 May 2014 | |
| 3rd time fruit picking | 26 May 2014 | |

**Appendix 2 Range of twelve quantitative traits in 23 $F_2$ hot pepper genotypes**

| **Characters** | **Range** | | |
|---|---|---|---|
| Leaf length (cm) | 3 | - | 5 |
| Leaf width (cm) | 1 | - | 2.3 |
| Anther length (cm) | 0.2 | - | 0.4 |
| Filament length (cm) | 0.2 | - | 0.4 |
| Corolla length (cm) | 0.9 | - | 1.6 |
| Fruit length (cm) | 4.2 | - | 6.3 |
| Fruit width (cm) | 0.6 | - | 1.2 |
| Fruit weight (g) | 0.9 | - | 1.9 |
| Total fruit weight (g) | 90 | - | 179 |
| Fruit pedicel length (cm) | 2.2 | - | 4.2 |
| Thousand-seed weight (g) | 3 | - | 8 |
| Seeds per pod (no.) | 40 | - | 70 |

**Appendix 3 Number of populations and relative frequency (%) of qualitative morphological traits for 322 $F_2$ hot pepper plants**

| No. | Score Character | | Score | No. of Populations | 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 |

**Appendix 3 Number of populations and relative frequency (%) of qualitative morphological traits for 322 $F_2$ hot pepper plants**

| No. | Score Character | | Score | No. of Populations | Relative Frequency (%) |
|---|---|---|---|---|---|
| 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** | Non fruited | 0 | 80 | 25 |
| | **Attachment** | 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 |
| 21 | **Stem Shape** | Angled | 2 | 322 | 100 |