

# Improving genetic variation for seed vigor and yield components in squash (*Cucurbita pepo* L.) using honey bees as pollinators under shade net houses

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## ABSTRACT

A key advancement has been made in the field of creating high-quality seeds, which are distinguished by great homogeneity at the genetic, environmental, and phenotypic levels. The most crucial seed development approach is the pollination process. The findings revealed persistent issues with Egyptian vegetable seed harvests, including low emergence percentage, poor yields, and seed vigor features. Impaired pollination during seed development is frequently blamed for difficulties in increasing the productivity of crops. Therefore, the goal of this study was to better understand how genetic and phenotypic factors that affect squash seed output and quality are influenced by honey bee pollination. The researcher was forced to become familiar with the scientific methodological procedures to improve the plant population and bring them to a state of homogeneity that is close to stability among their individuals in the so-called pre-breeding programs in response to a new reality regarding the trade balance of payments between countries, in which the seed trade occupies an important place. It was determined that there were definite effects on genetic, environmental, and phenotypic parameters from Honeybee pollination with a high pollen-load population. These results fall under the desired pre-breeding objectives, which are focused on highlighting the variations among the population's plants to undertake an effective selection procedure.

**Keywords:** *Squash, Cucurbita Pepo L., Genetic variability, Pollination, Honey bees, Seed production*

## INTRODUCTION

Squash (*Cucurbita pepo* L.) originates from Mexico, where it was domesticated at least 5000 years ago. Annual Egypt production is about 0.36 million tons of fresh fruits from 17477 hectares; while annual world production is about 28 million tons of fresh fruits from 2.019 million hectares (FAOSTAT, 2020). Squash is one of the most popular vegetables grown in Egypt. Squash fruit contains more than 95% water, is low in calories, sodium, and fat, and is a good source of vitamin C. Its extracts (from different parts of the plant) contain biologically active components which show antidiabetic, antibacterial, antioxidant, anticancer, immunomodulatory, and other miscellaneous effects. In recent years, the phenolic compounds of seeds (as dietary antioxidants) represent potentially health-promoting substances (Krimer-Malešević *et al.*, 2011). Anthesis is a crucial stage in fruit development: ovary tissues stop growing and will resume only with a stimulus like fertilization (Nitsch, 1970). Crane (1964) explained this phenomenon with changes in hormone levels. Several studies have demonstrated that apomictic embryo (Apomixis in flowering plants is defined as the asexual formation of a seed from the maternal tissues of the ovule) development in some species is dependent on pollination (Suessenguth, 1923). An early conclusion that pollen-borne chemical compounds boosted ovary expansion and later indirectly supported embryo development even in the absence of fertilization was reached because of a series of these and related studies (Gustafsson, 1946). After pollination, auxin is required for ovary development, which is typically provided by growing ovules and seeds (Gustafson, 1939). The possibility that pollens extracts mimic auxin's effects led to the theory that auxin present in pollen caused the commencement of ovarian growth (Laibach 1932; Laibach 1933; Gustafson 1937). The formation of hair cells that widen the central ovary cavity is connected to pollination-induced cell division regulation in the placental ridge (Zhang and Neill, 1993). Exogenous inhibitors of auxin and ethylene production revealed that the initial morphological change, the development of ovary wall hair cells, required both auxin and ethylene (Zhang and Neill, 1993). The finding that ethylene treatment increased ovarian growth while also inducing perianth senescence (Strauss and Arditti, 1982) raises the possibility that pollen-borne auxin can be translocated to the ovary (Han *et al.*, 1991; Nichols, 1971; Nichols, 1976; Nichols and Ho 1975a, b).

Ovarian growth responses were probably only indirectly related to pollination since they were most likely brought on by the mobilization of carbohydrates from senescent petals to the ovary. For fruit and seed set,

many crops depend on pollination or by insects, most notably honeybees and wild bees, and crop species that benefit from animal pollination account for about 35% of global agricultural production (Klein *et al.*, 2007). Crop productivity and seed set are both lowered by insufficient insect pollination. Most yield losses are caused by non-developing fruits and fruit deformations (Svensson, 1991; ebrowska, 1998). It was suggested that insufficient pollination was to blame for the asymmetrical fruits seen on trees with few fruit sets. Soon after germination, endogenous gibberellin (GA3 and GA4) concentrations increased in pollen tubes and were positively correlated with fruit growth (Zhang *et al.*, 2010). Researchers Davis *et al.* (1987), Schlichting *et al.* (1990), and Quesada *et al.* (1996) found that variations in pollen load (the number of pollen grains deposited on each stigma) had an impact on not only the number of seeds per fruit but also how quickly the progeny produced by a high pollen-load germinated and developed.

They also showed that fruits with high seed counts on the same plant were more likely to mature than fruits with low seed counts, and they concluded that populations of zucchini squash could increase the average quality of their seeds by selectively aborting fruit depending on seed count. Insects and honey bees are the primary natural pollinators of cucurbit crops in the Cucurbitaceae family (Tepedino, 1981; Stanghellini *et al.*, 1997). The production of cucurbit crops year-round has become more prevalent during the past three decades, prolonging the typical summer season. Because natural pollinators are less active on cool or overcast days, this pattern commonly results in poor fruit set and pollination problems. The seed's three main organs—the seedcoat, endosperm, and embryo—have different morphologies and functions, yet for the seed to germinate, their growth needs to be synchronized (Figueiredo and Köhler, 2018). Consequently, phytohormones (such as auxin, cytokinins-CKs, and GAs) play crucial roles in the execution and upkeep of the strict regulation of the developmental program (Robert, 2019). Nowadays, it is widely acknowledged that auxin is crucial for ovule fertilization, subsequent embryogenesis, and the control of young embryo polarity, among other processes (Lau *et al.*, 2012; Smit and Weijers, 2015; Robert *et al.*, 2018; Matthes *et al.*, 2019). Fundamental plant growth and development processes like flowering, climacteric fruit ripening, aging, dehiscence, seed dormancy release, and germination are regulated by the plant hormone ethylene (ET) (Matilla, 2000; Klee and Clark, 2004; Nath *et al.*, 2006; Matilla, 2007). Similar to this, the plant hormone ET participates in the processes associated with abiotic stress and controls the actions of other hormones by modifying their synthesis, distribution, or signal transduction (Drudge, 2006); Vandendussche and Straeten 2007). Auxins regulate several genes via auxin response factors (ARFs). Numerous ARFs have specialized roles in plant development and have persisted throughout the evolution of plants (Chapman and Estelle, 2009). Additionally, pollen quality and quantity as well as its ability to be released from anthers are declining in the Mediterranean, and there is a lack of synchronization between the time of bee activity and flower opening each day (Nelson, 2009).

For hybridization and selection programs to succeed on two levels—the first being the absence of differences in the genetic, phenotypical, and environmental levels of the varieties or hybrids produced by breeding programs that are commercially marketed, and the second being to achieve the best genetic and phenotypic expression among the individuals of the plant population—necessary processes like pollination and plant nutrition must be studied.

## MATERIALS AND METHODS

Between the years 2018 and 2020, this research was carried out at the Qaha Vegetable Research Farm in the Qalubia Governorate of Egypt. Clay soil is the description given to the ground at the location of the experiment. We only used one genotype, which was a local cultivar of squash called Eskandarani. The Vegetable Seed Production Unit of the Vegetable Research Departments in Dokki, Giza, Egypt, provided the researchers with the seeds they needed. A comparison was made between the obtained yield and the same field conditions.

This study is carried out in two stages: the first is the effect of pollination intensity on the characteristics of seeds and the second is the effect of the seeds obtained from the first stage on the characteristics of the yield components. The first stage: Their seeds were taken from the same lot and divided into two groups for use in the two populations. The experiment consisted of the two pollen-load treatments used, the treatments were as follows: (I) Hand-pollination with a normal pollen-load, equivalent to one male flower per female. (II) Honeybee pollination with a high pollen load. Honey bees (*Apis mellifera*) were reared in Langstrothbee hives of size 50x40x30 cm at the experimental farm. Healthy honey bee colonies were maintained with regular monitoring and necessary treatments. Squash was grown for seed production in the net house of 360 m<sup>2</sup> area on the experimental farm. Seeds were sown on 15<sup>th</sup> February 2018 and 2019; the population is contiguous in one area (as one net house). Each ridge was 90 cm wide and 50 cm for plant spacing; the seeds were grown in nursery trays, with one seedling per hill. Each net house contains 400 plants. During the anthesis, four frame honeybee colony of *A. mellifera* having approximately 4000 bees in a bee box was kept inside the net house to aid in pollination (Figure 1). Pollination behavior was noticed at Noon when the bright sun shines and more bee activity.

The second stage: Seeds obtained from previous treatments (Each plant contributed ten seeds) were sown on 15<sup>th</sup> February 2019 and 2020 in nursery bags (12×10 cm) arranged in a completely randomized design with four replications. 100 seeds were sown from each plant in 4 bags; the bags were separated from one another by 20 cm spacing, whereas the replications were separated by 50 cm spacing. After recording the data on the viability of the seeds, 120 plants were obtained from them representing each plant in the treatment (population) were transferred to the open field; in all replicates; making an area of 30 m<sup>2</sup> per plot. Other agricultural practices were carried out as recommended for conventional squash planting.



Fig. 1. A bee box was kept inside the net house to aid in pollination

**Experimental design and Statistical analysis**

The statistical analysis is based on the differences between the individual plants. The experiment consisted of a two-factor experiment (two populations; a population affected by hand pollination and a population affected by honeybee pollination).

The acquired data were statistically evaluated using Fisher's analysis of variance (given as a pairwise comparison procedure called the least significant difference (LSD) test). This test should be employed only if the overall F test rejects the hypothesis that all means are equal. If the overall test is significant, any pair of means is tested using a process similar to a standard Student's t-test. No additional tests are run if the total F ratio is not significant. When it is used, the two treatments are deemed different if the absolute difference between the two-sample means is more than 5% using combined ANOVA across years with one-way randomized blocks analysis (Multiple comparisons and trends among treatment means) (Gomez and Gomez, 1984). The experimental unit consisted of one grid with 19 plants (1 central plant + 18). (Figure 2). Both the Hand-pollination with a regular pollen-load population and the Honeybee pollination with a high pollen-load population have these units repeated and contiguous in one region. This method was carried out by Bos and Caligari (1995). Minitab software was used to do all computations (Minitab, 2010).

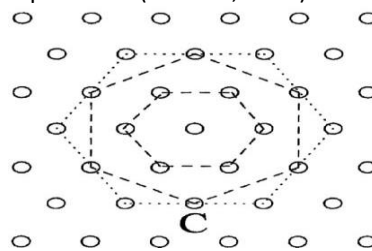


Fig. 2. Within the experimental unit, there is a regular triangle arrangement of plant locations. Each plant is considered a contender in turn and is compared to the plants that occur beside three (grid C) bordering aureoles.

**Data Collection**

Observations were made on several different traits. These are:

**The first stage data:** recorded data of plants affected by pollination intensity on the characteristics of seeds.

**The Weight of seeds per fruit (WSF):** The seed was collected, weighed, and recorded from each fruit in the individual plant and the mean weight was the yield of seeds per fruit expressed in grams (g).

**The number of seeds per fruit (NSF):** The seed was collected, counted, and recorded from each fruit in the individual plant and the mean count was the yield of seeds per plant expressed as a number.

**The Weight of seeds per plant (WSP):** The seed was collected, weighed, and recorded from all individual plants and the mean weight was the yield of seeds per plant expressed in grams (g).

**The number of seeds per plant (NSP):** The seed was collected, counted, and recorded from all individual plants and the mean count was the Yield of seeds per plant expressed as a number.

**Seed Index:** Weight of 100 seeds.

**Emergence index (EI):** Seedling emergence was recorded at 9, 11, 13, 15, 17, and 19 days after planting (DAP) and used to compute EI according to the modified formula of Fakorede and Ojo (1981).

$$EI = \Sigma \frac{\text{(Plants emerged in a day) (Day after planting)}}{\text{Plants emerged by 19 days after planting}}$$

**Emergence percentage (E %):** This was calculated as the percentage of seedlings that emerged 21 DAP relative to the number of seeds sown per plot.

$$E\% = \frac{\text{Seeding emerged by 21 DAP}}{\text{Number of seeds planted}} \times 100$$

**Emergence rate index (ERI) (days):** This was computed by expressing EI as a proportion of E% as follows:

$$ERI = \frac{EI}{E\%}$$

**Seedling vigor index (SVI):** This was computed according to the modified formula of Kharb *et al.* (1994).

$$SVI = \frac{\text{(Vine length + root length)} \times E\%}{100}$$

**The second stage data:** Recorded data from plants grown with seeds obtained from the first stage.

**The number of male flowers per plant (NMF)** was counted at two days intervals from the beginning to the end of the flowering period.

**The number of female flowers per plant (NFF)** was counted at two days intervals from the beginning to the end of the flowering period.

**The number of fruits per plant (NFP).**

**Estimation of phenotypic, genotypic, and environmental variation:**

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

Where, genotypic variance ( $\sigma^2_g$ )

$$\sigma^2_g = \frac{MS_v - MSe}{r \text{ or } n_0}$$

Where, ( $MS_v$ ) and ( $MS_i$ ) are the mean sum of squares due to populations (varieties or treatments) and error, respectively. Environmental variance ( $\sigma^2_e$ ) is equal to the mean sum of squares for error ( $MS_i$ ). Phenotypic variance ( $\sigma^2_p$ ) is comprised of ( $\sigma^2_g$ ) plus ( $\sigma^2_e$ ). In addition,  $r$  = a number of replications (in case of equal sample size) (Singh and Singh, 1994); while  $n_0$  = average sample size (in case of unequal sizes) (Sokal and Rohlf, 1981). The phenotypic and genotypic coefficient of variance was estimated using the formula developed by Burton (1952); Sharma (1988).

$$\text{Coefficient of variation (CV\%)} = \frac{\sqrt{MS_g}}{\bar{x}} \times 100$$

(Where  $MS_g$  = the mean squares of genotypes)

$$\text{Phenotypic Coefficient of variation (PCV)} = \frac{\sqrt{\sigma^2_p}}{\bar{x}} \times 100$$

$$\text{Genotypic Coefficient of variation (GCV)} = \frac{\sqrt{\sigma^2_g}}{\bar{x}} \times 100$$

$$\text{Environmental Coefficient of variation (CVE)} = \frac{\sqrt{\sigma^2_e}}{\bar{x}} \times 100$$

Whereas  $\sqrt{\sigma^2_p}$  = Phenotypic standard deviation.

$\sqrt{\sigma^2_g}$  = Genotypic standard deviation.

$\sqrt{\sigma^2_e}$  = Environmental standard deviation

$\bar{x}$  = the grand mean for each measured trait.

#### Estimation of broad-sense heritability

The formula used for estimating broad-sense heritability was

$$h^2 = \sigma^2_g / \sigma^2_p$$

Where  $\sigma^2_g$  is genetic and  $\sigma^2_p$  is the phenotypic variance (Allard, 1999).

#### Estimation of released genetic gain (observed selection response)

Genetic gain (GG) was defined as the proportional increment in the phenotypic values achieved by selection. GG was calculated following Zheng *et al.* (2006):

$$GC = \frac{X_s - X_c}{X_c} \times 100$$

Where  $X_s$  and  $X_c$  are the mean phenotypic value of progeny in selected and control populations, respectively.

#### Determination of the protein concentration and the identified amino acids in squash seeds samples

This procedure is described by Okoronkwo *et al.* (2017). To obtain the percent concentration of protein contents, a percent solution extinction coefficient ( $\epsilon_{\text{percent}}$ ) was used. In most proteins, the extinction coefficients ( $\epsilon_{\text{percent}}$ ) range from 4.0 to 24.0. Therefore, although any given protein can vary significantly from  $\epsilon_{\text{percent}} = 10$ , the average for a mixture of many different proteins will likely be approximately 10 (Thermo Scientific, 2013). Given that 1% solution equals 1g/100ml measure in a one cm cuvette.

Then, to correct and report in mg/ml, an adjustment factor must be made when using the percent solution extinction coefficients. i.e. for 1g/100 ml (1% solution)

$$\text{Then: } \frac{1 \text{ g}}{100 \text{ ml}} \times \frac{1000 \text{ mg}}{1 \text{ g}} = 10 \text{ mg/ml}$$

$$\text{The percentage concentration} = \frac{\text{Absorbance}}{\epsilon_{\text{percent}}}$$

For 5g/100ml (5% solution) which was the solution used

$$\text{Then: } \frac{5 \text{ g}}{100 \text{ ml}} \times \frac{5 \times 1000 \text{ mg}}{1 \text{ g}} = 50 \text{ mg/ml}$$

$$\therefore \text{Concentration in mg/ml} = \left( \frac{\text{Absorbance}}{\epsilon_{\text{percent}}} \right) \times 50$$

Or concentration in mg/ml = % Concentration  $\times$  5

Absorbance measured at 280 nm (A280), 216 nm (A216), and 298 nm (A298) are used to calculate the protein (g/100g dry seeds), Cysteine (g/100g dry seeds), and Tryptophan (g/100g dry seeds) (amino acids) concentration using the Evolution 300 UV-Vis Spectrophotometer, respectively.

## RESULTS

Genetic parameters and descriptive statistics of seed vigor and yield components characters in two populations (Hand-pollination with a normal pollen-load population and Honeybee pollination with a high pollen-load population) of squash.

Table 1 displays the Mean square (MS) results from the combined analysis of variance components for seed vigor and yield component features in squash employing honeybees and hand pollination under shade net homes. For all the traits, the variance between pollen-load populations was considerably greater than the variance within pollen-load populations (Error), indicating that genetic alterations influenced the performance of the listed squash traits.

Results in Table (2) and Fig. (3) revealed that mean values of the Honeybee pollination with a high pollen-load population concerning the traits of the weight of seeds per fruit (24.474 g), the number of seeds per fruit (204.56), the Weight of seeds per plant (46.34 g), the number of seeds per plant (357.6), seed Index (11.964 g), emergence index (13.504 days), emergence percentage (97.662 %), seedling vigor index (31.125), the number of female flowers per plant (15.850), the number of fruits per plant (14.000), total protein (17.323 g), Cysteine (0.1578 g) and Tryptophan (0.348 g) were significantly higher than those of the Hand-pollination with a normal pollen-load population for the same traits (11.890 g, 137.77, 15.92 g, 181.48, 8.206 g, 8.158 days, 76.100, 18.325, 8.0708, 5.0542, 10.659 g, 0.0596 g, and 0.159 g, respectively). While the mean values of Hand-pollination with a normal pollen-load population of the emergence rate index (17.432 days) and the number of male flowers per plant (8.2250a) were significantly higher than those of the Honeybee pollination with a high pollen-load population for the same traits (11.498 days and 5.6750, respectively). All the previously mentioned results are consistent with improving the phenotypic behavior of seed objectives.

Data of genetic coefficient of variance values for the Honeybee pollination with a high pollen-load population of The Weight of seeds per fruit (7.117), the number of seeds per fruit (6.231), the weight of seeds per plant (31.420), the number of seeds per plant (16.322), seed Index (2.550), emergence index (2.846), emergence percentage (1.199), the Seedling vigor index (1.256), the number of female flowers per plant (4.166), the number of fruits per plant (6.399) and Cysteine (12.582) were decreased in comparison to the Hand-pollination with a normal pollen-load population for the same traits (101.177, 75.152, 107.005, 59.047, 8.116, 8.598, 4.142, 2.800, 4.892, 14.825 and 18.617, respectively). While genetic coefficient of variance values for Hand-pollination with a normal pollen-load population of the emergence rate index (5.216), the number of male flowers per plant (5.690), total protein (6.080) and Tryptophan (6.026) were decreased in comparison to the Honeybee pollination with high pollen-load population for the same traits (7.386, 14.699, 8.715 and 17.127, respectively). High genetic standard deviation indicated that the data are spread out across a large range of values (expressing the variability of a population). On the other hand, a low standard deviation indicates that the data point is close to the mean (expressing the homogeneity of a population).

Environmental coefficient of variance values for the Honeybee pollination with a high pollen-load population of the Weight of seeds per fruit (11.574), the number of seeds per fruit (10.978), the weight of seeds per plant (75.528), the number of seeds per plant (54.634), seed Index (3.093), emergence index (5.742), emergence percentage (1.706), the number of female flowers per plant (5.561), the number of fruits per plant (7.234), total protein (9.145) and Cysteine (18.097) were decreased in comparison to the Hand-pollination with a normal pollen-load population for the same traits (124.411, 92.005, 146.842, 87.967, 11.196, 9.976, 5.975, 10.129, 18.008, 12.375 and 38.610, respectively). While environmental coefficient of variance values for Hand-pollination with a normal pollen-load population of the emergence rate index (8.163), the seedling vigor index (4.025), the number of male flowers per plant (9.574) and Tryptophan (13.103) were decreased in comparison to the Honeybee pollination with high pollen-load population for the same traits (9.771, 4.377, 21.061 and 19.049, respectively).

Phenotypic coefficient of variance values for the Honeybee pollination with a high pollen-load population of the weight of seeds per fruit (13.587), the number of seeds per fruit (12.623), the weight of seeds per plant (81.803), the number of seeds per plant (57.020), seed Index (4.009), emergence index (6.409), emergence percentage (2.086), the seedling vigor index (4.553), the number of female flowers per plant (6.948), the number of fruits per plant (9.658), total protein (12.633), and Cysteine (22.041) were decreased in comparison to the Hand-pollination with a normal pollen-load population for the same traits (160.359, 118.79, 181.694, 105.947, 13.829, 13.170, 7.271, 4.903, 11.249, 23.325, 13.788 and 42.864, respectively). While the Phenotypic coefficient of variance values for Hand-pollination with a normal pollen-load population of the emergence rate index (9.687), the number of male flowers per plant (11.137) and Tryptophan (14.422) were decreased in comparison to the Honeybee pollination with high pollen-load population for the same traits (12.249, 25.683 and 25.617, respectively).

Heritability is a proportion its numerical value will range from 0.0 (Genes do not contribute at all to phenotypic individual differences) to 1.0 (Genes are the only reason for individual differences, as explained by Colorado.edu (<http://psych.Colorado.edu/~carey/hgss/hgssapplets/heritability/heritability.intro.html>)). Accordingly, the results showed remarkable changes in the values of heritability for all traits affected by pollen-load treatments. The heritability values of the Honeybee pollination with a high pollen-load population in respect to the traits of the seed index (0.40), emergence percentage (0.33), the emergence rate index (0.36), the number of fruits per plant (0.44), Total protein (0.48), Cysteine (0.33) and Tryptophan (0.45) were higher than those of the Hand-pollination with a normal pollen-load population for the same traits (0.34, 0.32, 0.29, 0.26, 0.19, 0.40, 0.19, 0.19 and 0.17, respectively). While the heritability values of Hand-pollination with a normal pollen-load population of the weight of seeds per fruit (0.34), the number of seeds per fruit (0.40), the weight of seeds per plant (0.35), the number of seeds per plant (0.31), emergence index (0.43) and the seedling vigor index (0.33) were higher than those of the Honeybee pollination with high pollen-load population for the same trait (0.27, 0.24, 0.15, 0.08, 0.20 and 0.08, respectively).

Genetic standard deviation values for the Honeybee pollination with a high pollen-load population of The weight of seeds per fruit (1.741), the number of seeds per fruit (12.747), the weight of seeds per plant (14.56), the number of seeds per plant (58.369), seed index (0.305), emergence index (0.384), emergence percentage (1.171), the emergence rate index (0.849), the seedling vigor index (0.391) were decreased in comparison to the Hand-pollination with a normal pollen-load population for the same traits (12.029, 103.53, 17.035, 107.158, 0.666, 0.701, 3.152, 0.909 and 0.513, respectively). The number of male flowers per plant (0.468), the number of female flowers per plant (0.394), the number of fruits per plant (0.749), total protein (0.648), cysteine (0.011), and tryptophan (0.01) had genetic standard deviation values that were lower for hand-pollination with a normal pollen-load population than for honeybee pollination with a high pollen-load population for the same traits (0.834, 0.66, 0.895, 1.509, 0.019 and 0.059, respectively). Given the high genetic standard deviation, the data are dispersed throughout a wide range of values (expressing the variability of a population). A low standard deviation, on the other hand, denotes that the data point is close to the mean (expressing the homogeneity of a population).

Minimum values of Environmental standard deviation (i.e., they were more homogeneous) for the Honeybee pollination with a high pollen-load population of the weight of seeds per fruit (2.832), the number of seeds per fruit (22.456), seed Index (0.37), emergence index (0.775), emergence percentage (1.667) and the emergence rate index (1.123) were decreased in comparison to the Hand-pollination with a normal pollen-load population for the same traits (14.792, 126.755, 0.918, 0.813, 4.547 and 1.423, respectively). While environmental standard deviation values for Hand-pollination with a normal pollen-load population of the weight of seeds per plant (23.377), the number of seeds per plant (159.643), the seedling vigor index (0.737), the number of male flowers per plant (0.787), the number of female flowers per plant (0.817), the number of fruits per plant (0.91), total protein (1.319), Cysteine (0.023) and Tryptophan (0.02) were decreased in comparison to the Honeybee pollination with high pollen-load population for the same traits (35, 195.374, 1.362, 1.195, 0.881, 1.012, 1.584, 0.028 and 0.066, respectively).

Minimum values of phenotypic standard deviation (i.e., they were more homogeneous) for the Honeybee pollination with a high pollen-load population of the weight of seeds per fruit (3.325), the number of seeds per fruit (25.822), seed index (0.479), emergence index (0.865), emergence percentage (2.037), the emergence rate index (1.408) were decreased in comparison to the Hand-pollination with a normal pollen-load population for the same traits (19.066, 163.667, 1.134, 1.074, 5.533 and 1.688, respectively). While, the phenotypic standard deviation value for Hand-pollination with a normal pollen-load population of the weight of seeds per plant (28.925), the number of seeds per plant (192.273), the seedling vigor index (0.898), the number of male flowers per plant (0.916), the number of female flowers per plant (0.907), the number of fruits per plant (1.178), total protein (1.469), Cysteine (0.025) and Tryptophan (0.022) were decreased in comparison to the Honeybee pollination with high pollen-load population for the same traits (37.907, 203.906, 1.417, 1.457, 1.101, 1.352, 2.188, 0.034 and 0.089, respectively).

The percentage of genetic gain the Honeybee pollination with a high pollen-load population of all traits; the Weight of seeds per fruit, the number of seeds per fruit, the weight of seeds per plant, the number of seeds per plant, seed Index, emergence index, emergence percentage, emergence rate index, seedling vigor index, the number of male flowers per plant, the number of female flowers per plant, the number of fruits per plant, total protein, Cysteine, and Tryptophan.(105.836, 48.479, 191.08, 97.046, 45.807, 65.524, 28.333, 34.04, 69.849, 31.003, 96.386, 176.997, 62.519, 164.814 and 118.916, respectively). Noting that some negative results for the genetic gain are consistent with breeder objectives for improving the genetic behavior.

**Table 1.** Combined Analysis of Variance over seasons (Pooled ANOVA) for seed vigor and yield components characters in squash using honeybees and hand pollination under shade net houses as two pollen-load treatments (Hand-pollination with a normal pollen-load population and Honeybee pollination with a high pollen-load population).

| Traits <sup>1</sup> | Source of variation             | DF  | Adj SS   | Adj MS  | F-Value  | P-Value |
|---------------------|---------------------------------|-----|----------|---------|----------|---------|
| WSF                 | Between pollen-load populations | 1   | 19004    | 19004.0 | 435.69   | <0.05   |
|                     | Within pollen-load populations  | 478 | 20850    | 43.6    |          |         |
| NSF                 | Between pollen-load populations | 1   | 535402   | 535402  | 178.80   | <0.05   |
|                     | Within pollen-load populations  | 478 | 1431356  | 2994    |          |         |
| WSP                 | Between pollen-load populations | 1   | 111042   | 111042  | 172.29   | <0.05   |
|                     | Within pollen-load populations  | 478 | 308067   | 644     |          |         |
| NSP                 | Between pollen-load populations | 1   | 3720817  | 3720817 | 139.56   | <0.05   |
|                     | Within pollen-load populations  | 478 | 12744317 | 26662   |          |         |
| SI                  | Between pollen-load populations | 1   | 1694.9   | 1694.93 | 7538.64  | <0.05   |
|                     | Within pollen-load populations  | 478 | 107.5    | 0.22    |          |         |
| EI                  | Between pollen-load populations | 1   | 3429.4   | 3429.35 | 5357.33  | <0.05   |
|                     | Within pollen-load populations  | 478 | 306.0    | 0.64    |          |         |
| E%                  | Between pollen-load populations | 1   | 55793    | 55793.0 | 4861.94  | <0.05   |
|                     | Within pollen-load populations  | 478 | 5485     | 11.5    |          |         |
| ERI                 | Between pollen-load populations | 1   | 4224.8   | 4224.77 | 4831.85  | <0.05   |
|                     | Within pollen-load populations  | 478 | 417.9    | 0.87    |          |         |
| SVI                 | Between pollen-load populations | 1   | 19660.5  | 19660.5 | 16649.36 | <0.05   |
|                     | Within pollen-load populations  | 478 | 564.5    | 1.2     |          |         |
| NMF                 | Between pollen-load populations | 1   | 780.3    | 780.300 | 1156.54  | <0.05   |
|                     | Within pollen-load populations  | 478 | 322.5    | 0.675   |          |         |
| NFF                 | Between pollen-load populations | 1   | 7261.9   | 7261.85 | 10137.87 | <0.05   |
|                     | Within pollen-load populations  | 478 | 342.4    | 0.72    |          |         |
| NFP                 | Between pollen-load populations | 1   | 9603.4   | 9603.35 | 10378.58 | <0.05   |
|                     | Within pollen-load populations  | 478 | 442.3    | 0.93    |          |         |
| TPR                 | Between pollen-load populations | 1   | 5329.9   | 5329.95 | 2826.04  | <0.05   |
|                     | Within pollen-load populations  | 478 | 901.5    | 1.89    |          |         |
| CYS                 | Between pollen-load populations | 1   | 1.1567   | 1.15670 | 2468.71  | <0.05   |
|                     | Within pollen-load populations  | 478 | 0.2240   | 0.00047 |          |         |
| TRY                 | Between pollen-load populations | 1   | 4.2992   | 4.29919 | 6130.92  | <0.05   |
|                     | Within pollen-load populations  | 478 | 0.3352   | 0.00070 |          |         |

<sup>1</sup>: WSF= The Weight of seeds per fruit; NSF= The number of seeds per fruit; WSP = The Weight of seeds per plant ; NSP= The number of seeds per plant; SI= Seed Index; EI= Emergence index ; E%= Emergence percentage ; ERI= Emergence rate index; SVI = Seedling vigor index; NMF= The number of male flowers per plant; NFF= The number of female flowers per plant ; NFP=The number of fruits per plant ; TPR= Total protein; CYS= Cysteine; TRY= Tryptophan. Between pollen-load populations = Hand-pollination with a normal pollen-load population and honeybee pollination with high pollen-load population



**Table 2.** Genetic parameters and Descriptive statistics of seed vigour and yield components characters in squash using honeybees and hand pollination under shade net houses as two pollen-load treatments (Hand-pollination with a normal pollen-load population and Honeybee pollination with high pollen- load population).

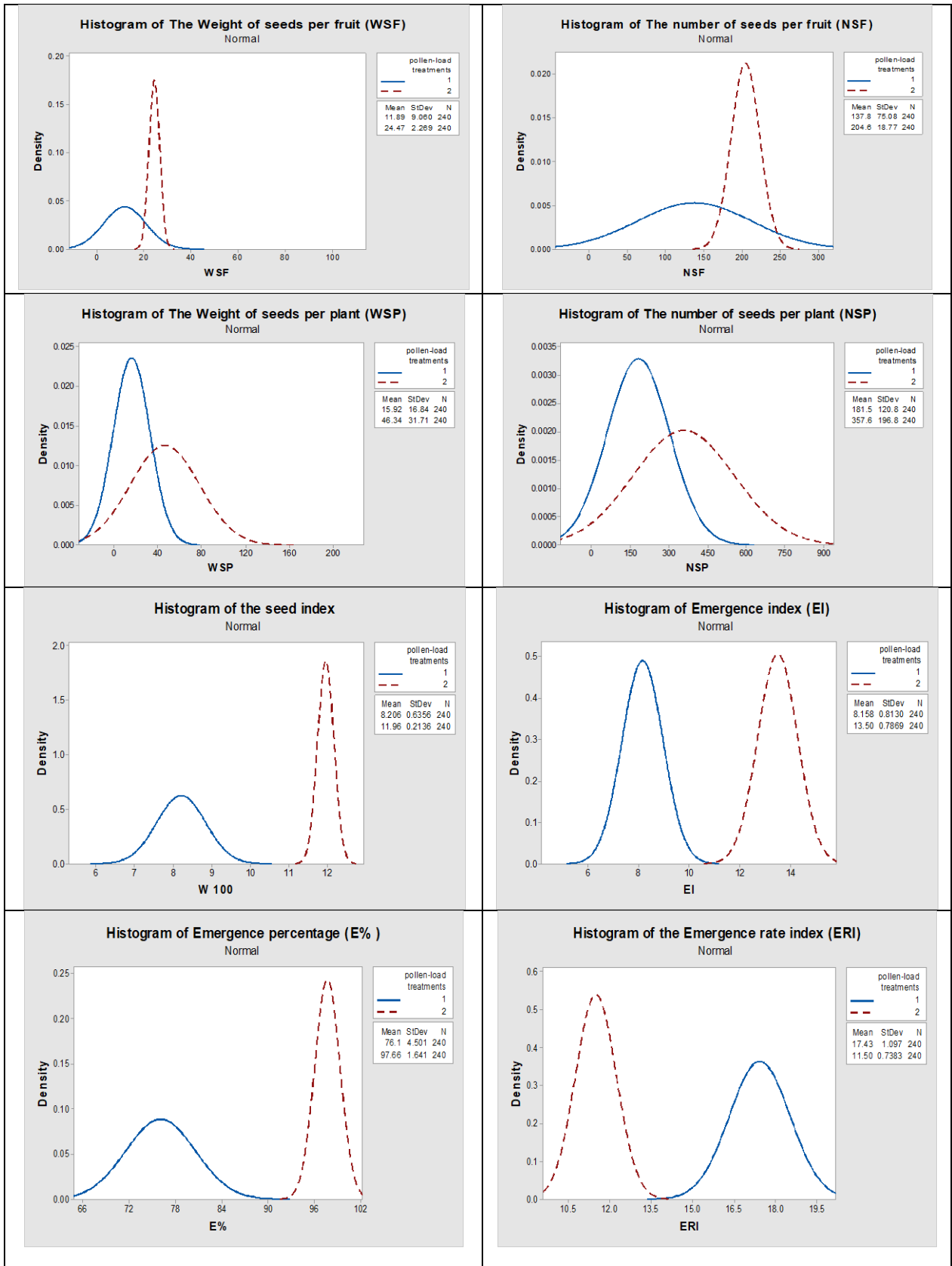
| Genetic parameters and Descriptive statistics 1        | Traits2 |         |         |         |         |         |         |         |
|--|---------|---------|---------|---------|---------|---------|---------|---------|
|  | WSF     | NSF     | WSP     | NSP     | SI      | EI      | E%      | ERI     |
| Hand-pollination with a normal pollen-load population  |         |         |         |         |         |         |         |         |
| Mean   | 11.890b | 137.77b | 15.92b  | 181.48b | 8.206b  | 8.158b  | 76.100b | 17.432a |
| CoefVar  | 76.2    | 54.5    | 85.81   | 66.55   | 7.75    | 9.97    | 5.91    | 6.29    |
| Genetic variance                                       | 144.72  | 10720   | 290.2   | 11483   | 0.4436  | 0.4921  | 9.94    | 0.827   |
| environmental variance                                 | 218.82  | 16067   | 546.5   | 25486   | 0.8442  | 0.6625  | 20.68   | 2.025   |
| Phenotypic variance                                    | 363.54  | 26787   | 836.7   | 36969   | 1.2878  | 1.1546  | 30.62   | 2.852   |
| (GCV)%   | 101.177 | 75.152  | 107.005 | 59.047  | 8.116   | 8.598   | 4.142   | 5.216   |
| (ECV)%   | 124.411 | 92.005  | 146.842 | 87.967  | 11.196  | 9.976   | 5.975   | 8.163   |
| (PCV)%   | 160.359 | 118.79  | 181.694 | 105.947 | 13.829  | 13.170  | 7.271   | 9.687   |
| Heritability   | 0.34    | 0.40    | 0.35    | 0.31    | 0.34    | 0.43    | 0.32    | 0.29    |
| GSD  | 12.029  | 103.53  | 17.035  | 107.158 | 0.666   | 0.701   | 3.152   | 0.909   |
| ESD  | 14.792  | 126.755 | 23.377  | 159.643 | 0.918   | 0.813   | 4.547   | 1.423   |
| PSD  | 19.066  | 163.667 | 28.925  | 192.273 | 1.134   | 1.074   | 5.533   | 1.688   |
| Honeybee pollination with high pollen- load population |         |         |         |         |         |         |         |         |
| Mean   | 24.474a | 204.56a | 46.34a  | 357.6a  | 11.964a | 13.504a | 97.662a | 11.498b |
| CoefVar  | 9.27    | 9.18    | 68.42   | 55.04   | 1.79    | 5.83    | 1.68    | 6.42    |
| Genetic variance                                       | 3.034   | 162.5   | 212     | 3407    | 0.09323 | 0.1478  | 1.372   | 0.7214  |
| environmental variance                                 | 8.024   | 504.3   | 1225    | 38171   | 0.13702 | 0.6014  | 2.779   | 1.2622  |
| phenotypic variance                                    | 11.058  | 666.8   | 1437    | 41578   | 0.23015 | 0.7492  | 4.151   | 1.9836  |
| (GCV)%   | 7.117   | 6.231   | 31.420  | 16.322  | 2.550   | 2.846   | 1.199   | 7.386   |
| (ECV)%   | 11.574  | 10.978  | 75.528  | 54.634  | 3.093   | 5.742   | 1.706   | 9.771   |
| (PCV)%   | 13.587  | 12.623  | 81.803  | 57.020  | 4.009   | 6.409   | 2.086   | 12.249  |
| Heritability   | 0.27    | 0.24    | 0.15    | 0.08    | 0.40    | 0.20    | 0.33    | 0.36    |
| GSD  | 1.741   | 12.747  | 14.56   | 58.369  | 0.305   | 0.384   | 1.171   | 0.849   |
| ESD  | 2.832   | 22.456  | 35      | 195.374 | 0.37    | 0.775   | 1.667   | 1.123   |
| PSD  | 3.325   | 25.822  | 37.907  | 203.906 | 0.479   | 0.865   | 2.037   | 1.408   |
| Genetic gain (R)                                       | 1.058   | 0.484   | 1.91    | 0.97    | 0.458   | 0.655   | 0.283   | -0.3404 |
| Genetic gain% (R%)                                     | 105.836 | 48.479  | 191.08  | 97.046  | 45.807  | 65.524  | 28.333  | -34.04  |

<sup>1</sup>: coefvar = coefficient variance; GCV% = Genetic coefficient of variability; (ECV)%= Environmental coefficient of variation; PCV% = Phenotypic coefficient of variability; GSD= Genetic Standard deviation; ESD= Environmental standard deviation; PSD= Phenotypic Standard deviation. <sup>2</sup>: WSF= The Weight of seeds per fruit; NSF= The number of seeds per fruit; WSP = The Weight of seeds per plant ; NSP= The number of seeds per plant; SI= Seed Index; EI= Emergence index ; E%= Emergence percentage ; ERI= Emergence rate index; SVI = Seedling vigor index; NMF= The number of male flowers per plant; NFF= The number of female flowers per plant ; NFP= The number of fruits per plant ; TPR= Total protein; CYS= Cysteine; TRY= Tryptophan. Means within columns followed by the same letter are not statistically different at the 5% level (Unpaired two-tailed Student's t-test).

**Table 2.** Cont.: Genetic parameters and descriptive statistics of seed vigor and yield components characters in squash using honeybees and hand pollination under shade net houses as two pollen-load treatments (Hand-pollination with a normal pollen-load population and Honeybee pollination with a high pollen-load population).

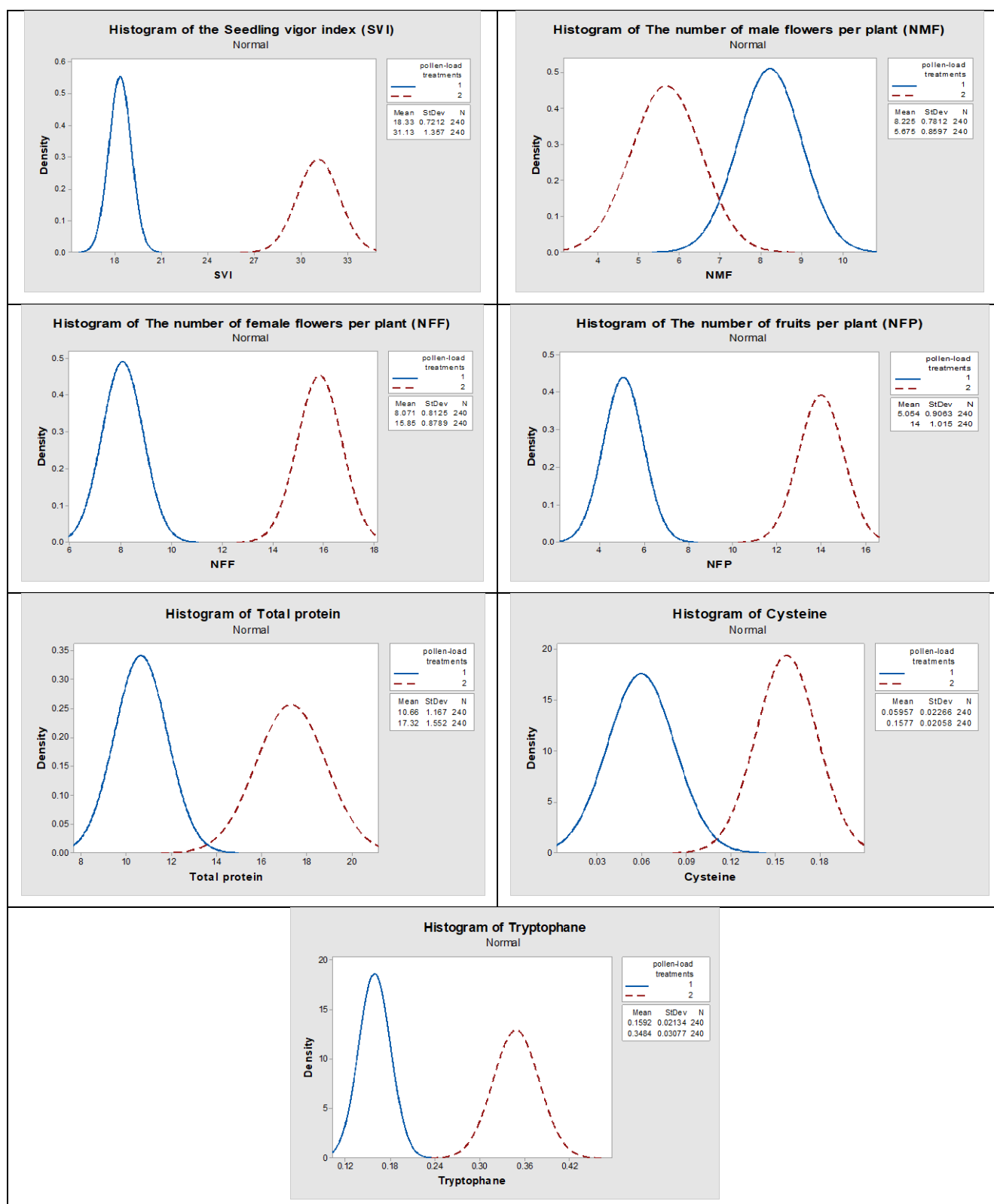
| Genetic parameters and descriptive statistics <sup>1</sup> | Traits <sup>2</sup> |                     |                     |                     |                     |                     |                    |
|--|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|--------------------|
|  | SVI                 | NMF                 | NFF                 | NFP                 | TPR                 | Cys                 | Try                |
| Hand-pollination with a normal pollen-load population      |                     |                     |                     |                     |                     |                     |                    |
| Mean   | 18.325 <sup>b</sup> | 8.2250 <sup>a</sup> | 8.0708 <sup>b</sup> | 5.0542 <sup>b</sup> | 10.659 <sup>b</sup> | 0.0596 <sup>b</sup> | 0.159 <sup>b</sup> |
| CoefVar  | 3.94                | 9.5                 | 10.07               | 17.93               | 10.95               | 38.04               | 13.41              |
| genetic variance   | 0.2633              | 0.2191              | 0.1559              | 0.5615              | 0.42                | 0.000123            | 0.000092           |
| environmental variance                                     | 0.5442              | 0.6201              | 0.6684              | 0.8284              | 1.74                | 0.000529            | 0.000435           |
| phenotypic variance  | 0.8075              | 0.8392              | 0.8243              | 1.3899              | 2.16                | 0.000652            | 0.000527           |
| (GCV)%   | 2.800               | 5.690               | 4.892               | 14.825              | 6.080               | 18.617              | 6.026              |
| (ECV)%   | 4.025               | 9.574               | 10.129              | 18.008              | 12.375              | 38.610              | 13.103             |
| (PCV)%   | 4.903               | 11.137              | 11.249              | 23.325              | 13.788              | 42.864              | 14.422             |
| Heritability   | 0.33                | 0.26                | 0.19                | 0.40                | 0.19                | 0.19                | 0.17               |
| GSD  | 0.513               | 0.468               | 0.394               | 0.749               | 0.648               | 0.011               | 0.01               |
| ESD  | 0.737               | 0.787               | 0.817               | 0.91                | 1.319               | 0.023               | 0.02               |
| PSD  | 0.898               | 0.916               | 0.907               | 1.178               | 1.469               | 0.025               | 0.022              |
| Honeybee pollination with high pollen-load population      |                     |                     |                     |                     |                     |                     |                    |
| Mean   | 31.125 <sup>a</sup> | 5.6750 <sup>b</sup> | 15.850 <sup>a</sup> | 14.000 <sup>a</sup> | 17.323 <sup>a</sup> | 0.1578 <sup>a</sup> | 0.348 <sup>a</sup> |
| CoefVar  | 1.842               | 15.15               | 5.54                | 7.25                | 8.96                | 13.05               | 8.83               |
| genetic variance   | 0.153               | 0.6959              | 0.4361              | 0.8026              | 2.2793              | 0.000394            | 0.003562           |
| environmental variance                                     | 1.856               | 1.4286              | 0.777               | 1.0257              | 2.5099              | 0.000815            | 0.004406           |
| phenotypic variance  | 2.009               | 2.1245              | 1.2131              | 1.8283              | 4.7892              | 0.001209            | 0.007968           |
| (GCV)%   | 1.256               | 14.699              | 4.166               | 6.399               | 8.715               | 12.582              | 17.127             |
| (ECV)%   | 4.377               | 21.061              | 5.561               | 7.234               | 9.145               | 18.097              | 19.049             |
| (PCV)%   | 4.553               | 25.683              | 6.948               | 9.658               | 12.633              | 22.041              | 25.617             |
| Heritability   | 0.08                | 0.33                | 0.36                | 0.44                | 0.48                | 0.33                | 0.45               |
| GSD  | 0.391               | 0.834               | 0.66                | 0.895               | 1.509               | 0.019               | 0.059              |
| ESD  | 1.362               | 1.195               | 0.881               | 1.012               | 1.584               | 0.028               | 0.066              |
| PSD  | 1.417               | 1.457               | 1.101               | 1.352               | 2.188               | 0.034               | 0.089              |
| Genetic gain (R)   | 0.698               | -0.31               | 0.963               | 1.769               | 0.625               | 1.648               | 1.189              |
| Genetic gain% (R%)   | 69.849              | 31.003              | 96.386              | 176.997             | 62.519              | 164.814             | 118.916            |

<sup>1</sup>: coefvar = coefficient variance; GCV% = Genetic coefficient of variability; (ECV)%= Environmental coefficient of variation; PCV% = Phenotypic coefficient of variability; GSD= Genetic Standard deviation; ESD= Environmental standard deviation; PSD= Phenotypic Standard deviation. <sup>2</sup>: WSF= The Weight of seeds per fruit; NSF= The number of seeds per fruit; WSP = The Weight of seeds per plant ; NSP= The number of seeds per plant; SI= Seed Index; EI= Emergence index ; E%= Emergence percentage ; ERI= Emergence rate index; SVI = Seedling vigor index; NMF= The number of male flowers per plant; NFF= The number of female flowers per plant ; NFP= The number of fruits per plant ; TPR= Total protein; CYS= Cysteine; TRY= Tryptophan. Means within columns followed by the same letter are not statistically different at 5% level (Unpaired two-tailed Student's t-test).



SD = Standard deviation, coefvar = coefficient of variance and N = number of plants per population. Pollen-load treatments (1= Hand-pollination with a normal pollen-load population; and 2= Honeybee pollination with high pollen-load population).

**Fig. 3.** Histograms of seed vigor and yield components characters in squash using honeybees and hand pollination under shade net houses as two pollen-load treatments (Hand-pollination with a normal pollen-load population and Honeybee pollination with a high pollen-load population).



SD = Standard deviation, coefvar = coefficient of variance and N = number of plants per population. Pollen-load treatments (1= Hand-pollination with a normal pollen-load population; and 2= Honeybee pollination with high pollen-load population).

**Fig. 3. Continued.** Histograms of seed vigor and yield components characters in squash using honeybees and hand pollination under shade net houses as two pollen-load treatments (Hand-pollination with a normal pollen-load population and Honeybee pollination with a high pollen-load population).

## DISCUSSION

In order for hybridization and selection programs to be successful on two levels — the first level being the absence of differences in the genetic, phenotypical, and environmental levels of the varieties or hybrids produced by breeding programs that are commercially marketed, and the second level being to achieve the best genetic and phenotypic expression among the individuals of the plant population — necessary processes like pollination and plant nutrition need to be studied.

Hirsch (1997), cited in Lerner (2002), claimed that heritability can be employed in a confusing and deceptive way and that heredity does not necessarily imply genetic determination. Additionally, when geneticists use the term "heritable," they merely suggest that one can predict the distribution of a characteristic in a group's progeny based on the distribution of that feature in the parent group, particularly the descriptive traits. The heritability value still only describes the extent to which inter-individual differences in a trait distribution measured at one point in time and under one specific set of environmental conditions are associated with inter-individual differences in gene distributions; these statistics do not explain the role of genes. The geneticist does not address the extent to which the trait's expression may change in response to environmental modification.

Because of this, heredity refers to characteristics of a group rather than an individual. Additionally, heritability ( $h^2$ ) may equal one for a population raised under one set of environmental circumstances and zero for the same population raised under a different set of environmental circumstances, according to Ruston (1999), as mentioned in Lerner (2002). Although it can be assumed that negative heredity is zero (Robinson *et al.*, 1955, as quoted in Gusmini and Wehner (2007) and Sabu *et al.* (2009), negative heritability should be recorded in order to contribute to the body of information that can be properly evaluated (Dudley and Moll, 1969, as cited in Gusmini and Wehner, 2007). Rogue practice is only reliable when it has descriptive qualities. These results were in line with those of Nevo *et al.* (1984), who found that when a particular polymorphism is caused by variation at a single locus, the relationship between environmental and phenotypic variation is theoretically best understood and experimentally best investigated. These results were cited in Pamilo (1988). Thoughts have advanced well beyond this straightforward illustration, and currently, multilocus heterozygosity is thought to indicate an adaptive approach connected to the pattern of environmental variation.

## CONCLUSION

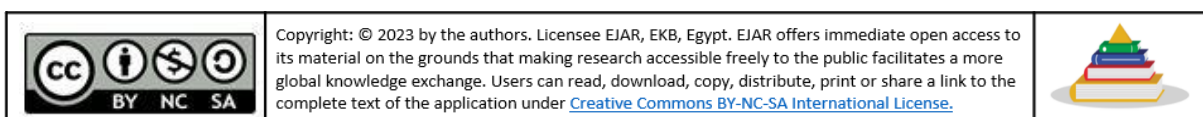
It could be concluded that it was determined that there were definite effects on genetic, environmental, and phenotypic parameters from Honeybee pollination with a high pollen-load population. These results fall under the desired pre-breeding objectives, which are focused on highlighting the variations among the population's plants to undertake an effective selection procedure.

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## تحسين التباين الوراثي لحيوية البذور ومكونات المحصول في الكوسة باستخدام نحل العسل كملقح تحت بيوت شبكية مظلمة

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لقد أحدثت تقنيات تطوير إنتاج البذور والتي من أهمها عملية التلقيح تطوراً جوهرياً في ميدان إنتاج البذور عالية الجودة والتي تتميز بالتجانس العالي على المستوى الوراثي والمظهري والبيئي. وقد فرضت تلك التقنيات واقعا جديدا فيما يتعلق بميزان المدفوعات التجاري بين الدول والتي تحتل فيه تجارة البذور مكاناً هاماً، ما جعل الباحث مطالباً بالإلمام بالإجراءات المنهجية العلمية لتحسين العشائر النباتية والوصول بها لحالة التجانس الذي يقترّب من الثبات بين أفرادها فيما يطلق عليه ببرامج ما قبل التربية. يهدف هذا البحث إلى دراسة الإجراءات الضرورية مثل التلقيح وتغذية النبات التي تجعل برامج التهجين والاختيار تحقق أهدافها على مستويين، الأول هو عدم وجود اختلافات في المستويات الجينية والمظهرية والبيئية للأصناف أو الهجن الناتجة عن برامج التربية والتي يتم تسويقها تجارياً؛ والثاني هو الوصول إلى التعبير الجيني والمظهري الأمثل بين أفراد العشيرة النباتية كهدف رئيسي لمرحلة ما قبل التربية لتنفيذ برامج إنتخاب ناجحة. ناقشت النتائج أن ضعف المحصول وإنخفاض نسبة الإنبات وحيوية البذور يمثلان مشاكل مزمنة في إنتاج بذور محاصيل الخضروات المصرية بصفة عامة ومحصول الكوسة موضع الدراسة بصفة خاصة، غالباً ما تُعزى الصعوبات في تحسين إنتاجية بعض المحاصيل إلى ضعف عملية التلقيح كعملية من أهم عمليات تطوير إنتاج البذور وعلاقة هذه العملية بالتعبير الوراثي والمظهري للنبات. أثبتت الدراسة أن تلقيح نحل العسل مع عدد كبير من حبوب اللقاح كان لها تأثيرات واضحة على العوامل الوراثية والبيئية والمظهرية تتوافق هذه النتائج مع أهداف ما قبل التربية المرغوبة، والتي تهتم بإظهار الاختلافات بين النباتات الفردية للعشيرة النباتية بحيث يمكن تنفيذ برنامج إنتخاب جيد.

**الكلمات المفتاحية:** الكوسة، التباين الوراثي، التباين المظهري، التباين البيئي، التلقيح، نحل العسل، إنتاج البذور