# STABILITY ANALYSIS AND MOLECULAR EVALUATION NEW GARDEN PEA GENOTYPES IN EGYPT

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## ABSTRACT

Thirteen new promising lines in addition to two commercial cultivars of garden pea (*Pisum sativum* L.) were evaluated under six environments in Lower Egypt (two seasons of 2013/2014 and 2014/2015, and three locations). Data were recorded for plant length, no. of days to flowering, pod length, pod weight, no. of seeds/pod, 100-seeds weight, shelling percentage and total green yield. The linear response of genotypes to environments was highly significant for all studied traits. The mean squares due to Environment + (Genotypes × Environment) was significant for all studied traits. The results of stability analysis indicated that the genotypes $G_1$, $G_5$, $G_6$ and $G_{13,}$ most stable genotypes, gave the maximum total green yield overall the six studied environments and were adapted to environments for total green yield and most studied traits. Also, the genotype $G_{10}$ can be considered promising line as early and short stem length cultivar due to its performance and stability for total green yield and most studied traits. The genetic similarity coefficients among garden pea genotypes evaluated by SCOT markers varied from 68.4% to 99.6%, indicating high level of genetic diversity existing among the pea genotypes which could be valuable for pea breeding in the future. The dendrogram generated with hierarchical UPGMA (Un-weighted Pair Group Method with Arithmetic Averages) cluster analysis of the Jaccard's similarity coefficient matrices revealed two major clusters.

**Key words:** Garden peas, Stability, Regression coefficient, Genotype × Environment, SCOT markers, Genetic similarity

## INTRODUCTION

Garden pea (*Pisum sativum* L.) is a cool season legume crop belongs to family Leguminosae. Recently, the Food and Agriculture Organization (FAO) designated Ethiopia and western Asia as centers of diversity, with secondary centers in southern Asia and the Mediterranean region (DAFF, 2011). In Egypt, this crop is mainly grown for human consumption, and could be used in livestock feed. Also, as a legume crop, it complies well into cereal rotations to provide nitrogen to the soil and reduce the intensity of diseases in non-legume crops if it is managed properly (Ceyhan *et al*., 2012).

One of the main issues to be considered in plant breeding programs is the evaluation of changes in yield and quality of candidate or new cultivars under different environments or seasons (Zayed *et al*., 1999). Genotype-environment (G × E) interaction has been important and challenging issue for plant breeders in developing improved varieties. The development of cultivars adapted to a wide range of divers environments is the ultimate aim of plant breeders in a crop improvement programs (Fikere *et al*., 2009). The adaptability of a genotype is usually tested by the degree of its interactions with diverse environments. A variety is considered more adaptive or stable if it has a high mean of yield with low degree of fluctuation in yield ability to grow across different locations or seasons (Amin *et al*., 2005 and Zayed *et al*., 2005). According to Eberhart and Russell (1966), a stable genotype is one with a high mean, regression coefficient equal to one ($b_i$=1) and mean squares of deviation from regression equal zero ($S^2d_i$=0). A genotype with a high value of $b_i$ and $S^2d_i$ reacts easily to change in the environment and possesses considerable variability, whereas cultivars with a $b_i$<1.0 and $S^2d_i$ near to 0.0 react weakly to changes in growing conditions and are considered to be stable in yield. Fikere *et al*. (2014) indicated that the

deviation from the regression mean square was more efficient than regression coefficient to describe yield stability in field pea.

Pooled analysis of variance for peas grain yield showed significant differences among genotypes, environments and G x E interaction, meanwhile, the magnitude of the environmental effect was by far higher than the genotype effect (Rezene *et al*., 2014). Also, Fikere *et al*. (2010) reported that the environmental factor was highly attributed to the variation in the traits days to flowering, seeds per pod and plant height. Furthermore, the combined analysis of variance for grain yield of different field pea genotypes tested across diverse environments indicated that the large differences among environmental means causing most of the variation in grain yield and the magnitude of the G x E interaction sum of squares was larger than that of genotypes. This indicated that there were differences in genotypic response across environments. Ceyhan *et al*. (2012) demonstrated that environment has a great impact on the performance of studied pea genotypes. Most of these pea genotypes were particularly elevated for plant height, number of pods per plant, seeds per pod and thousand seed weight. Probably they could be grown in different environments without significantly compromising their yield. By contrast, the yield of genotypes exhibited sensitivity to the environment. El-Dakkak (2015a & b) found significant genotype x environment interactions for each of flowering date, pod length, pod diameter, number of seeds/pod, number of pods/plant and pod yield/plant traits. The data indicated that pea genotypes responded differently to various environments; some studied genotypes were not consistent in performance across all environments for pod yield. However, some other genotypes exhibited consistency of their yielding ability under tested environmental conditions. Regression coefficient was less than 1 ($bi<1$) for 10 out of eleven genotypes at least in one to four studied traits. In

addition, Fikere *et al*. (2009) indicated that the majority of the tested genotypes were non-significantly different from a unit regression coefficient ($b_i$=1) and had small deviation from the regression ($S^2d_i$) and thus possessed average stability.

The association between molecular markers and phenotypes is one of the most significant developments in the field of molecular genetics and molecular breeding and provides substantial landmarks for elucidation of genetic variation and detection of genomic regions responsible for the trait, which in turn plays an essential role in the strategy. Improvement of garden pea using marker-assisted selection were reported by Chelkowski *et al*. (2003), Semagn *et al*. (2006), Abu Qamar *et al*. (2008), Adawy *et al*. (2008) and Ellis (2011).

In recent years, a novel marker system namely, Start Codon Targeted Polymorphism (SCoT) was described by Collard and Mackill (2009) based on the observation that the short conserved regions of plant genes are surrounded by the ATG translation start codon (Sawant *et al*., 1999).

SCoT markers are generally reproducible, and it is suggested that primer length and annealing temperature are not the sole factors determining reproducibility ,**Collard and Mackill (2009)**. They are dominant markers, however, number of co-dominant markers were also generated during amplification (Gorji *et al*., 2011). SCoT markers have been successfully used to assess genetic diversity and structure, identify cultivars and for quantitative trait loci (QTL) mapping and DNA fingerprinting in different species, including tritordeums, sugarcane, grape, potato, rice, Jojoba, mango, myricarubra and peanut (Xiong *et al*., 2011, Amirmoradi *et al*., 2012, Cabo *et al*., 2014 and Heikrujam *et al*., 2015).

This study aimed to estimate stability of fifteen garden pea genotypes for yield, yield components and some economic characters and evaluate the performance of these characters across six environments in order to select the best genotypes for developing new garden pea cultivars of high yield and desirable traits. In addition, the study aimed to characterize and assess the level of genetic diversity among and within studied genotypes using morphological traits and molecular markers to aid in the selection and more efficient use of this germplasm in breeding programs.

## MATERIALS AND METHODS

Thirteen new promising lines and two check cultivars of garden pea were evaluated under six environments. Advanced lines were derived from the crosses Master × Sugar daddy, Master × Snow wind and Master × Victory freezer through a breeding program of garden pea, Horticulture Research Institute, ARC, Egypt (Hamed, 2005 and Hamed, 2012). Also, two parents (Sugar daddy and Snow wind) were used only in the genetic diversity study because they are sugar peas cultivars and can not be evaluated with the other garden pea cultivars as shown in Table (1). The six environments were three locations in the first season (2013/2014) in Lower Egypt, *i.e.*, Kalubia Governorate (Kaha), Alex desert road (Abo Ghaly) and Sharkea (Belbais). In the second season (2014/2015), they were three locations in Kalubia (Kaha), Alex desert road (Wadi El-netroon) and Sharkea (Salehya). The drip irrigation system was used in all environments. The experimental layout was a randomized complete blocks design (RCBD) with three replications for each experiment. The experimental plot consisted of one row for each genotype. Rows were 6 m long and 75 cm apart. Spacing within row was 5 cm. Planting date was

first week of November at all locations in both seasons. Data were recorded for the traits plant length (cm), no. of days to flowering, pod length (cm), pod weight (g), no. of seeds/pod, 100-green seeds weight (g), shelling percentage (%) and total green yield (ton/fed). Combined analysis of variance was performed across the six environments (two years and three locations) to detect the Genotype × Environment interaction effects as described by Snedecor and Cochran (1967). The data of each trait were statistically analyzed for stability according to Eberhart and Russell (1966).

**Table 1:** Pedigree of the studied garden pea genotypes.

| No. | Genotypes | From | Origin |
|---|---|---|---|
| **$G_1$** | $F_7$ 7-37-5-7/13 | Master × (Master × Sugar daddy) | Egypt |
| **$G_2$** | $F_8$ 7-37-3-4/13 | Master × Sugar daddy | Egypt |
| **$G_3$** | $F_8$ 4-31-5-8/13 | Master × Sugar daddy | Egypt |
| **$G_4$** | $F_8$ 7-37-15-6/13 | Master × Sugar daddy | Egypt |
| **$G_5$** | $F_8$ 4-32-5-2/13 | Master × Sugar daddy | Egypt |
| **$G_6$** | $F_8$ 4-32-7-4/13 | Master × Sugar daddy | Egypt |
| **$G_7$** | $F_8$ 4-33-2-3/13 | Master × Sugar daddy | Egypt |
| **$G_8$** | $F_8$ 4-33-2-7/13 | Master × Sugar daddy | Egypt |
| **$G_9$** | $F_6$ 5-1-1/13 | Master × Snow wind | Egypt |
| **$G_{10}$** | $F_6$ 33-2-1/13 | Master × Snow wind | Egypt |
| **$G_{11}$** | $F_7$ 4-1-1-8/13 | Master × (Master × Sugar daddy) | Egypt |
| **$G_{12}$** | $F_6$ 33-1-1/13 | Master × Snow wind | Egypt |
| **$G_{13}$** | $F_8$ 9-15-3-2/13 | Master × Victory freezer | Egypt |
| **$G_{14}$** | Victory freezer (Check) | Pop Vrient Co. | U.S.A. |
| **$G_{15}$** | Master (Check) | Hort. Res. Inst., Egypt | Egypt |
| **$G_{16}$** | Sugar daddy | Territoral Seeds Co. | U.K. |
| **$G_{17}$** | Snow wind | Syngenta Co. | U.S.A. |

**SCoT-PCR Reactions:** Ten SCOT primers were used as described by **Collard and Mackill (2009)**. Primer sequences employed in the present study were designed based on the consensus sequences of translation initiation codon region in higher plants (Table 2). PCR reactions were performed in a total volume of 25 ul, containing 1X

reaction buffer (10 mM Tris-HCl, pH 8.3 and 50 mM KCl), 1.5 mM $MgCl_2$, 1U Taq DNA polymerase (promega), 2.5 mM dNTPs, 25 pmol of primer and 30 ng genomic DNA. SCoT- thermo cycling profile and detection of PCR amplification products was carried out in a Perkin-Elmer/GeneAmp®PCR System 9700 (PE Applied Biosystems) thermo cycler. The SCoT amplification conditions were as follows: an initial extended step of denaturation at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min and elongation at 72°C for 2 min. The primer extension segment was extended to 10 min at 72°C in the final cycle. The amplification products were resolved by electrophoresis on 2% agarose gel containing ethidium bromide (0.5µg/ml) in 1X TBE buffer. A 100 bp DNA plus ladder was used as a molecular weight standard. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD).

**Table 2:** Sequence of ten decamer arbitrary (18-mer) primers assayed in SCOT-PCR marker.

| Primer | Sequence (5′ - 3′) | Primer | Sequence (5′ - 3′) |
|---|---|---|---|
| **SCoT- 1** | ACCATGGCTACCAGCGCG | **SCoT- 6** | CAATGGCTACCACTACAG |
| **SCoT- 2** | ACCATGGCTACCACCGGC | **SCoT- 7** | ACAATGGCTACCACTGAC |
| **SCoT- 3** | CGACATGGCGACCCACA | **SCoT- 8** | ACAATGGCTACCACTGAG |
| **SCoT- 4** | ACCATGGCTACCACCGCA | **SCoT- 9** | ACAATGGCTACCACTGCC |
| **SCoT- 5** | CAATGGCTACCACTAGCG | **SCoT- 10** | ACAATGGCTACCACCAGC |

# RESULTS AND DISCUSSION

## Analysis of variance

Combined analysis of data showed that the genotype (G) and environment (E) variances were highly significant for all studied traits, indicating the presence of considerable genotypic variation in the germplasm material and environments for these traits (Table 3). Genotype

× Environment (G × E) interaction variance was also highly significant for plant length, no. of days to flowering, pod length and pod weight traits, indicating the impact of environments on the expression of these traits in pea genotypes (Table 3). However, it was non-significant for no. seeds/pod, 100-green seeds weight, shelling percentage and total yield traits. The magnitude of the environmental effect was higher than the genotype effect for the traits plant length, no. of seeds/pod, shelling percentage and total green yield. However, the magnitude of the genotype effect was higher than the environmental effect for the traits no. of days to flowering, pod length, pod weight and 100-green seeds weight. These results are in agreement with Rezene *et al*. (2014) for peas grain yield. Also, results partially agree with those reported by Fikere *et al*. (2010), who indicated that the environmental factor highly attributed for the variation in the traits seeds per pod and plant height.

**Table 3:** Significance of mean squares values of combined analysis of variance for the studied traits of 15 garden pea genotypes over six environments.

| SOV | df | Plant length | No. of days to flowering | Pod length | Pod weight | No. seeds/ pod | 100-green seeds weight | Shelling percentage | Total green yield |
|---|---|---|---|---|---|---|---|---|---|
| **Environments (E)** | 5 | 10415.85** | 460.87** | 6.84** | 10.11** | 32.88** | 284.57** | 325.58** | 80.36** |
| **Replication × E** | 12 | 84.35 | 4.16 | 0.17 | 0.44 | 0.29 | 25.87 | 11.88 | 1.54 |
| **Genotypes (G)** | 14 | 7341.12** | 1079.53** | 17.22** | 31.93** | 8.77** | 1526.51** | 66.93** | 15.96** |
| **E × G** | 70 | 295.22* | 20.73* | 0.75** | 2.18** | 0.85 | 65.18 | 19.20 | 2.05 |
| **Error** | 168 | 192.98 | 13.66 | 0.46 | 1.25 | 0.70 | 65.31 | 17.35 | 1.83 |

* and ** significant at 0.05 and 0.01 levels of probability, respectively.

Data in Table (4) showed that the linear response of environments was highly significant for all studied traits, indicating that genotypes differed in their regression on the environmental index. Therefore, the regression coefficient (b) and deviation from regression ($S^2_d$) was calculated. The mean squares due to E + (G × E) was significant for all studied traits, indicating that genotypes considerably interacted with the six environmental conditions. These results are in agreement with those reported by Fikere *et al*. (2010) and El-Dakkak (2015a).

## Estimates of stability parameters

Stability parameters were calculated across six environments using Eberhart and Russell (1966) model (Table 5). The regression coefficients ($b_i$) were not significantly different from 1.0 in ten genotypes for yield trait, and the $b_i$ values ranged between 0.418 ($G_{14}$) and 1.689 ($G_2$). Residual mean square values ($S^2_d$), which are indicative of deviations from the regression, were close to 0.0 in the genotype $G_6$ ($S^2_d$=0.006), while $G_{11}$ had the highest $S^2_d$ (1.144). The other genotypes $b_i$ and $S^2_d$ values were between these values for yield trait.

**Table 4:** Stability analysis of variance for the studied traits of 15 garden pea genotypes evaluated under six different environmental conditions.

| SOV | df | Mean squares | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Plant length** | **No. of days to flowering** | **Pod length** | **Pod weight** | **No. seeds/ pod** | **100-green seeds weight** | **Shelling percent-age** | **Total green yield** |
| **Genotypes (G)** | 14 | 2447.04** | 359.84** | 5.74** | 10.64** | 2.92** | 508.84** | 22.31** | 5.32** |
| **E+(G×E)** | 75 | 323.31** | 26.69** | 0.884* | 2.904** | 0.994** | 66.60* | 23.21** | 2.42** |
| **E (linear)** | 1 | 17359.75** | 838.99** | 11.41** | 16.85** | 54.81** | 474.29** | 542.64** | 133.93** |
| **G×E (linear)** | 14 | 283.70** | 12.17** | 0.47** | 1.77** | 0.666** | 20.80 | 11.81* | 1.30** |
| **Pooled deviation** | 60 | 48.61 | 4.04 | 0.18 | 0.67 | 0.17 | 22.83 | 5.18 | 0.49 |
| **$G_1$** | 4 | 4.91 | 0.72 | 0.04 | 0.10 | 0.12 | 14.76 | 8.24 | 0.37 |
| **$G_2$** | 4 | 18.57 | 3.27 | 0.11 | 1.06 | 0.07 | 5.37 | 2.14 | 0.61 |
| **$G_3$** | 4 | 84.57 | 0.57 | 0.06 | 0.17 | 0.02 | 4.02 | 8.26 | 0.25 |
| **$G_4$** | 4 | 39.81 | 0.85 | 0.01 | 0.56 | 0.46 | 7.51 | 3.03 | 1.17 |
| **$G_5$** | 4 | 37.40 | 1.38 | 0.11 | 1.93 | 0.07 | 22.49 | 5.55 | 0.44 |
| **$G_6$** | 4 | 47.96 | 0.21 | 0.03 | 0.15 | 0.10 | 15.37 | 2.60 | 0.28 |
| **$G_7$** | 4 | 34.83 | 0.54 | 0.08 | 0.29 | 0.05 | 11.18 | 5.91 | 0.04 |
| **$G_8$** | 4 | 30.19 | 3.33 | 0.02 | 0.08 | 0.15 | 11.86 | 2.27 | 0.26 |
| **$G_9$** | 4 | 104.01 | 1.35 | 0.21 | 0.97 | 0.22 | 5.42 | 4.67 | 0.21 |
| **$G_{10}$** | 4 | 9.86 | 3.26 | 0.39 | 1.74 | 0.15 | 46.16 | 15.19 | 0.21 |
| **$G_{11}$** | 4 | 137.32 | 3.10 | 0.07 | 0.44 | 0.06 | 16.55 | 6.23 | 1.43 |
| **$G_{12}$** | 4 | 51.83 | 20.41 | 0.02 | 1.27 | 0.13 | 127.30 | 8.29 | 0.13 |
| **$G_{13}$** | 4 | 10.49 | 0.55 | 0.03 | 0.17 | 0.27 | 11.24 | 1.52 | 0.53 |
| **$G_{14}$** | 4 | 75.30 | 0.74 | 0.27 | 0.66 | 0.52 | 10.88 | 1.44 | 0.37 |
| **$G_{15}$** | 4 | 42.18 | 20.32 | 1.25 | 0.47 | 0.21 | 32.33 | 2.36 | 1.09 |
| **Pooled Error** | 180 | 61.91 | 4.34 | 0.15 | 0.40 | 0.22 | 20.89 | 5.66 | 0.60 |

**Table 5:** Stability parameters for some economic characters of 15 garden pea genotypes grown under six different environments.

| Genotypes | Plant length (cm) | | | No. days to flowering | | | Pod length (cm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | × | $b_i$ | $S^2_d$ | × | $b_i$ | $S^2_d$ | × | $b_i$ | $S^2_d$ |
| **$G_1$** | 79.43 | 0.848 | -16.400 | 54.44 | 1.478** | -0.892 | 11.32 | 1.249 | -0.001 |
| **$G_2$** | 93.14 | 0.694* | -2.74 | 56.39 | 1.420* | 1.661 | 10.85 | 1.821** | 0.067** |
| **$G_3$** | 87.29 | 1.332* | 63.250** | 52.94 | 1.657** | -1.044 | 10.98 | 0.980 | 0.012 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **$G_4$** | 91.24 | 1.530** | 18.496 | 59.33 | 1.016 | -0.762 | 10.92 | 1.017 | -0.035 |
| **$G_5$** | 94.75 | 1.300* | 16.083 | 55.28 | 0.737 | -0.234 | 10.95 | 0.013** | 0.069** |
| **$G_6$** | 103.43 | 1.783* | 26.640 | 57.44 | 1.159 | -1.399 | 11.09 | 0.144** | -0.017 |
| **$G_7$** | 92.24 | 0.773 | 13.512 | 56.22 | 0.976 | -1.074 | 11.01 | 0.001** | 0.030 |
| **$G_8$** | 102.01 | 1.090 | 8.873 | 56.22 | 0.998 | 1.719 | 11.25 | 1.007 | -0.025 |
| **$G_9$** | 92.88 | 1.642** | 82.692** | 56.17 | 1.121 | -0.264 | 11.04 | 2.000** | 0.164** |
| **$G_{10}$** | 39.58 | 0.450** | -11.459 | 37.28 | 0.103** | 1.651 | 11.30 | 2.663** | 0.345** |
| **$G_{11}$** | 82.52 | 0.707* | 116.005** | 56.33 | 1.253 | 1.486 | 11.20 | 1.297 | 0.026 |
| **$G_{12}$** | 73.76 | 0.422** | 30.514 | 43.89 | 1.081 | 18.800** | 11.24 | 0.407* | -0.023 |
| **$G_{13}$** | 95.25 | 0.916 | -10.829 | 55.33 | 1.367* | -1.065 | 10.04 | 1.028 | -0.015 |
| **$G1_4$** | 81.27 | 1.429* | 53.984** | 62.72 | 0.598* | -0.869 | 7.541 | 0.050** | 0.225** |
| **$G_{15}$** | 35.20 | 0.085** | 20.867 | 36.00 | 0.036** | 18.703** | 9.80 | 1.324 | 1.208** |
| **Mean** | 82.93 | | | 53.07 | | | 10.70 | | |
| **LSD 0.05** | 5.39 | | | 1.48 | | | 0.25 | | |
| **LSD**0.01 | 7.21 | | | 1.98 | | | 0.33 | | |

* and ** indicate significant at 0.05 and 0.01probability levels, respectively.

**Table 5:** Cont.

| **Genotypes** | **Pod weight (g)** | | | **No. seeds/pod** | | | **100-green seeds weight (g)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **×** | **$b_i$** | **$S^2_d$** | **×** | **$b_i$** | **$S^2_d$** | **×** | **$b_i$** | **$S^2_d$** |
| **$G_1$** | 7.06 | 0.589 | -0.005 | 8.17 | 1.339 | 0.014 | 42.13 | 0.951 | 2.655 |
| **$G_2$** | 8.75 | 1.526 | 0.953** | 7.43 | 0.925 | -0.040 | 54.58 | 1.561 | -6.733 |
| **$G_3$** | 6.68 | 0.344* | 0.063 | 8.53 | 1.108 | -0.092 | 41.39 | 0.825 | -8.082 |
| **$G_4$** | 6.67 | 1.030 | 0.459** | 8.38 | 0.281** | 0.354** | 39.27 | 0.655 | -4.594 |
| **$G_5$** | 6.71 | 0.679 | 1.824** | 8.52 | 1.161 | -0.035 | 39.01 | 0.353 | 10.384 |
| **$G_6$** | 6.79 | 0.731 | 0.052 | 8.47 | 0.802 | -0.004 | 37.98 | 0.902 | 3.268 |
| **$G_7$** | 6.57 | 0.201* | 0.184** | 8.09 | 0.548* | -0.059 | 37.94 | 0.724 | -0.925 |
| **$G_8$** | 6.88 | 0.332* | -0.023 | 8.71 | 1.420* | 0.044 | 36.31 | 1.086 | -0.241 |
| **$G_9$** | 8.52 | 2.810** | 0.871** | 7.45 | 0.862 | 0.111 | 52.55 | 0.928 | -6.681 |
| **$G_{10}$** | 10.19 | 2.247** | 1.635** | 7.91 | 1.508** | 0.041 | 62.16 | 2.540* | 34.056** |
| **$G_{11}$** | 6.46 | 0.523 | 0.338** | 8.34 | 1.386* | -0.044 | 39.85 | 0.842 | 4.451 |
| **$G_{12}$** | 8.49 | 0.345* | 1.165** | 7.39 | 0.203** | 0.024 | 63.40 | 1.907 | 115.200** |
| **$G_{13}$** | 5.99 | 0.641 | 0.069 | 7.73 | 0.781 | 0.159** | 42.54 | 0.736 | -0.856 |
| **$G_{14}$** | 4.65 | 0.677 | 0.559** | 6.04 | 1.089 | 0.408** | 34.46 | 0.536 | -1.216 |
| **$G_{15}$** | 6.83 | 2.326** | 0.369** | 7.29 | 1.585** | 0.107 | 44.29 | 0.455 | 20.233** |
| **Mean** | 7.15 | | | 7.90 | | | 44.52 | | |
| **LSD 0.05** | 0.37 | | | 0.38 | | | 4.06 | | |
| **LSD** 0.01 | 0.50 | | | 0.51 | | | 5.43 | | |

* and ** indicate significant at 0.05 and 0.01probability levels, respectively.

**Table 5:** Cont.

| Genotypes | Shelling percentage (%) | | | Total green yield (ton/fed) | | |
|---|---|---|---|---|---|---|
| | × | $b_i$ | $S^2_d$ | × | $b_i$ | $S^2_d$ |
| $G_1$ | 44.89 | 0.822 | 5.404** | 4.943 | 1.208 | 0.085 |
| $G_2$ | 46.72 | 0.796 | -0.694 | 6.794 | 1.689** | 0.324 |
| $G_3$ | 47.78 | 1.338 | 5.423** | 4.744 | 0.728 | -0.036 |
| $G_4$ | 49.33 | 0.463 | 0.195 | 4.759 | 1.608** | 0.885** |
| $G_5$ | 50.18 | 1.132 | 2.718 | 5.007 | 1.273 | 0.149 |
| $G_6$ | 45.77 | 1.317 | -0.237 | 5.795 | 0.964 | -0.006 |
| $G_7$ | 48.49 | 1.243 | 3.080 | 4.805 | 0.485** | -0.251 |
| $G_8$ | 45.62 | 1.002 | -0.567 | 4.665 | 0.821 | -0.027 |
| $G_9$ | 48.08 | 0.222** | 1.834 | 4.080 | 1.060 | -0.079 |
| $G_{10}$ | 45.76 | 2.347** | 12.361** | 4.114 | 0.914 | -0.074 |
| $G_{11}$ | 48.98 | 1.061 | 3.396 | 5.432 | 1.327 | 1.144** |
| $G_{12}$ | 43.81 | 1.379 | 5.454** | 4.846 | 0.790 | -0.163 |
| $G_{13}$ | 47.46 | 0.878 | -1.316 | 4.923 | 1.146 | 0.239 |
| $G_{14}$ | 44.02 | 0.325* | -1.398 | 2.790 | 0.418** | 0.086 |
| $G_{15}$ | 46.89 | 0.676 | -0.474 | 3.397 | 0.570* | 0.797** |
| **Mean** | 46.92 | | | 4.740 | | |
| **LSD 0.05** | 1.96 | | | 0.627 | | |
| **LSD** 0.01 | 2.63 | | | 0.838 | | |

* and ** indicate significant at 0.05 and 0.01probability levels, respectively.

The results in Table (5) indicates that values of deviation from regression ($S^2_d$) were significant in some genotypes for specific traits, indicating the instability of these genotypes regarding these traits. It should be mentioned that the performance of a genotype which had non-significant regression coefficients (b=1) may be predicted and stable (Eberhart and Russell, 1966). The genotypes with least insignificant deviation from regression are most phenotypically stable and *vice versa*. Accordingly, again, it is evident that stability analysis showed a wide variation among genotypes; some genotypes exhibited wide adaptation, while others showed specific adaptation either to favorable or unfavorable environments.

In general, preferred genotypes show low G×E interaction variance, high mean yield potential across environments and below deviation from the expected response within a target environment (Lin and Binns, 1988). The results in Table (4) indicated that the high yielding genotype $G_6$ (medium stem length and late genotype) and $G_{10}$ (short stem

length and early genotype) produced high mean yields (5.798 and 4.114 tons/fed, respectively) across all environments, had regression coefficient (b) close to unity (0.964 and 0.914, respectively) and deviation from regression ($S^2_d$) not significantly from zero. These results indicated that their high yielding performance based on wide adaptation and stability of performance across all environments.

The genotypes $G_1$, $G_5$ and $G_{13}$ produced high yield across a range of environments, showed high regression coefficient ($b_i>1$) and non-significant deviation from regression ($S^2_d$), indicating specific adaptability of these genotypes to favorable or high yielding environments. Results indicated that these genotypes could produce high yield at favorable environments with fertile soil, adequate water and other inputs. On the contrary, the genotypes $G_{3,}$ $G_6$, $G_7$ and $G_{12}$ as well as the short and early genotype $G_{10}$ showed low regression coefficient ($b_i<1$) and non-significant deviation from regression ($S^2_d$), indicating specific adaptability of these genotypes to harsh (unfavorable) environments. It is evident that these genotypes could be used as stress tolerant genotypes under stressed environments (poor yielding or unfavorable environments). Again, according to Eberhart and Russell (1966), genotypes with “b” value less than 1.0 and higher $S^2_d$ than zero are said to be specifically adapted to poor or unfavorable environments, while, genotypes having high “b” value are specifically adapted to favorable or high yielding environments. Genotypes $G_1$, $G_2$, $G_4$, $G_5$, $G_{11}$ and $G_{13}$ with above average regression coefficient (b>1) for total yield, could produce higher yield at favorable environments with fertile soil, adequate water and other inputs.

**Molecular analysis**

A total of 10 primers were tested for selective amplification of DNA fragments. The Primer name, number of total bands, polymorphic bands and percentage of polymorphism as detected by SCoT are listed in Table (6). The ten SCoT primers produced reliable PCR products. However, only four SCoT primers (40%) showed discernible polymorphism between genotypes. Thus, analysis of segregation among the genotypes was performed using these four SCoT polymorphic primers (Table 6 and Fig. 1).

A total of 108 major SCoT bands (with average 10.8) were observed, 15 of which (13.8%) were polymorphic among the genotypes. The number of amplicons/primer ranged from 6 to 16 (SCoT-4, SCoT-9, respectively) , the number of polymorphic amplicons varied from (2) to (6). The primer (SCoT-5) produced the least number of polymorphic products (2), while, the primer (SCoT-9) produced the highest number of polymorphic products (6). The primers (SCoT-2, SCoT-3, SCoT-4, SCoT-6, SCoT-7 and SCoT-8) failed to produce polymorphic bands.

In addition, a number of unique bands were recorded for particular genotypes at different loci. For example, genotypes $G_{16}$ and $G_{17}$ recorded unique bands at molecular weight 180bp (Fig 1b). Such exclusive alleles could be important from a breeding point of view.

Overall, a high level of genetic diversity was revealed among genotypes through the use of these SCOT markers, which is in line with previous studies that reported a great extent of diversity in the pea gene pool (Cabo *et al*., 2014 and Heikrujam *et al*., 2015).

. This diversity could be a resource of genes for various desirable traits in pea breeding.

**Table 6:** Levels of polymorphism, total number of bands, monomorphic bands, polymorphic bands and percentage of polymorphism as revealed by SCOT markers within and among seventeen garden pea genotypes.

| No. | Primers | Total number of bands | Mono morphic bands | Poly morphic bands | % of polymorphism |
|---|---|---|---|---|---|
| 1 | SCoT- 1 | 12 | 8 | 4 | 33 |
| 2 | SCoT- 2 | 9 | 9 | 0 | 0 |
| 3 | SCoT- 3 | 11 | 11 | 0 | 0 |
| 4 | SCoT- 4 | 6 | 6 | 0 | 0 |
| 5 | SCoT- 5 | 10 | 8 | 2 | 20 |
| 6 | SCoT- 6 | 10 | 10 | 0 | 0 |
| 7 | SCoT- 7 | 13 | 13 | 0 | 0 |
| 8 | SCoT- 8 | 8 | 8 | 0 | 0 |
| 9 | SCoT- 9 | 16 | 10 | 6 | 37 |
| 10 | SCoT- 10 | 13 | 10 | 3 | 23 |
| Total | | 108 | 93 | 15 | 13.8 |

The genetic similarity among seventeen garden pea genotypes was estimated in terms of using Dice's similarity coefficients (DSC's) to compute the similarity matrix based on the scored SCOT data matrix.

This similarity matrix was used to generate a dendrogram using the UPGMA method. SCOT data analysis showed that the genetic similarity among the seventeen garden pea genotypes ranged from 68.4% to 99.6%, with an average value of 84% as illustrated in Table (7). In addition to SCOT analysis, the highest similarity level (99.6%) was detected between $G_2$ and $G_7$ genotypes which are closely related accessions. While, the least genetic similarity (68.4%) was detected between $G_7$ and $G_{17}$ genotypes.

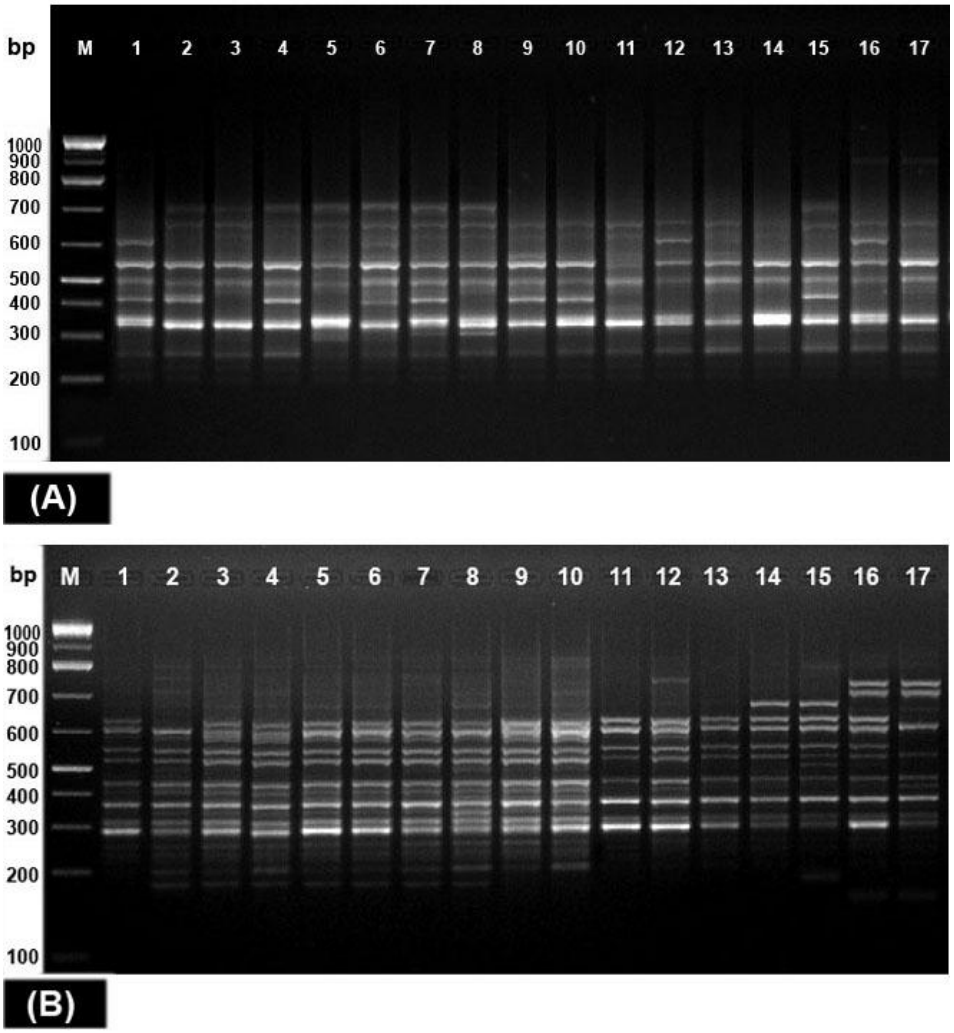


**Fig. 1.** SCOT profiles of seventeen garden pea genotypes (1-17) as detected with primers **(A)** SCoT- 1 and **(B)** SCoT- 9. DNA molecular weight standards (**M)** 100 bp DNA ladder.

The results showed presence of similarity among seven pea genotypes $G_2$, $G_3$, $G_4$, $G_5$, $G_6$, $G_7$ and $G_8$ which came from intercrosses between (Master × Sugar daddy) genotypes ranged from 98.8% to 99.5%, while these genotypes produced 97.4% similarity percentage with $G_1$ Master × (Master × Sugar daddy) and 82.8% similarity percentage with $G_{15}$ (Master), on another side these genotypes produced 81.3% and 80.7% similarity percentage with $G_{13}$ and $G_{16}$ (Sugar daddy), respectively, indicating that these genotypes were more uniform showing low level of genetic diversity. Uniformity of pea accession could be ascribed to their possible inclusion in modern breeding programs that

usually result in low level of genetic diversity. The results indicated that the genotypes $G_1$, $G_5$, $G_6$ and $G_{13}$ were stable genotypes, thus the superiority of these genotypes under the six studied environments indicat the impact of environments in the expression of these traits in pea genotypes.

**Table 7**: Genetic similarity matrix within and among seventeen garden pea genotypes as computed according to Dice's similarity coefficient from SCOT-markers generated data.

| | $G_1$ | $G_2$ | $G_3$ | $G_4$ | $G_5$ | $G_6$ | $G_7$ | $G_8$ | $G_9$ | $G_{10}$ | $G_{11}$ | $G_{12}$ | $G_{13}$ | $G_{14}$ | $G_{15}$ | $G_{16}$ | $G_{17}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $G_1$ | 100 | | | | | | | | | | | | | | | | |
| $G_2$ | 97.4 | 100 | | | | | | | | | | | | | | | |
| $G_3$ | 97.4 | 99.1 | 100 | | | | | | | | | | | | | | |
| $G_4$ | 97.4 | 98.9 | 99.1 | 100 | | | | | | | | | | | | | |
| $G_5$ | 97.4 | 99.4 | 99.4 | 99.7 | 100 | | | | | | | | | | | | |
| $G_6$ | 97.4 | 98.8 | 99.5 | 99.5 | 99.5 | 100 | | | | | | | | | | | |
| $G_7$ | 97.4 | 99.6 | 99.0 | 99.1 | 99.1 | 99.1 | 100 | | | | | | | | | | |
| $G_8$ | 97.4 | 99.1 | 99.4 | 99.4 | 99.4 | 99.4 | 99.4 | 100 | | | | | | | | | |
| $G_9$ | 79.1 | 74.9 | 74.9 | 74.9 | 74.9 | 74.9 | 74.9 | 74.9 | 100 | | | | | | | | |
| $G_{10}$ | 97.1 | 74.1 | 74.1 | 74.1 | 74.1 | 74.1 | 74.1 | 74.1 | 99.8 | 100 | | | | | | | |
| $G_{11}$ | 98.9 | 98.9 | 98.8 | 98.8 | 98.8 | 98.8 | 98.8 | 98.8 | 84.5 | 84.5 | 100 | | | | | | |
| $G_{12}$ | 79.9 | 75.1 | 75.1 | 75.1 | 75.1 | 75.1 | 75.1 | 75.1 | 99.8 | 99.8 | 82.5 | 100 | | | | | |
| $G_{13}$ | 77.7 | 81.3 | 81.3 | 81.3 | 81.3 | 81.3 | 81.3 | 81.3 | 74.4 | 74.4 | 77.7 | 74.1 | 100 | | | | |
| $G_{14}$ | 71.1 | 79.3 | 79.3 | 79.3 | 79.3 | 79.3 | 79.3 | 79.3 | 71.5 | 71.5 | 71.3 | 71.5 | 83.9 | 100 | | | |
| $G_{15}$ | 83.3 | 82.8 | 82.8 | 82.8 | 82.8 | 82.8 | 82.8 | 82.8 | 89.1 | 89.1 | 82.3 | 89.1 | 89.9 | 91.1 | 100 | | |
| $G_{16}$ | 81.2 | 80.7 | 80.7 | 80.7 | 80.7 | 80.7 | 80.7 | 80.7 | 83.1 | 83.1 | 81.2 | 83.1 | 71.9 | 71.2 | 73.1 | 100 | |
| $G_{17}$ | 69.8 | 68.5 | 68.6 | 68.5 | 68.5 | 68.5 | 68.4 | 68.5 | 84.9 | 84.9 | 69.1 | 84.9 | 69.3 | 70.9 | 72.5 | 91.1 | 100 |

According to Dice's similarity coefficient from SCOT-markers generated data.

The dendrogram (Fig. 2) separated the seventeen garden pea genotypes into two major clusters. The first cluster contained $G_9$, $G_{10}$ and $G_{12}$ accessions which came from crosses between Master and Snow wind**,** while, the second cluster contained the remaining cultivated forms of garden pea, that could be divided into two sub clusters. Garden pea cultivars $G_1$ and $G_{11}$ are grouped together in the first sub cluster, while the rest of garden pea cultivars are grouped together in the second sub cluster as shown in Fig. (2).

These results are in congruence with those obtained by Gixhari *et al*. (2014), who investigated the genetic diversity present in the pea

germplasm stored in the Albanian gene bank, 28 local pea genotypes of Albanian origins were analyzed for 23 quantitative morphological traits, as well as 14 retrotransposon-based insertion polymorphism (RBIP) molecular markers. RBIP marker analysis revealed the genetic similarity in the range from 0.06 to 0.45. ANOVA, principal component analysis (PCA) and cluster analysis were used to visualize the association among different traits. Most of the quantitative morphological traits showed significant differences. PCA and cluster analysis (Ward's method) carried out for morphological traits divided the local pea genotypes into three clusters.

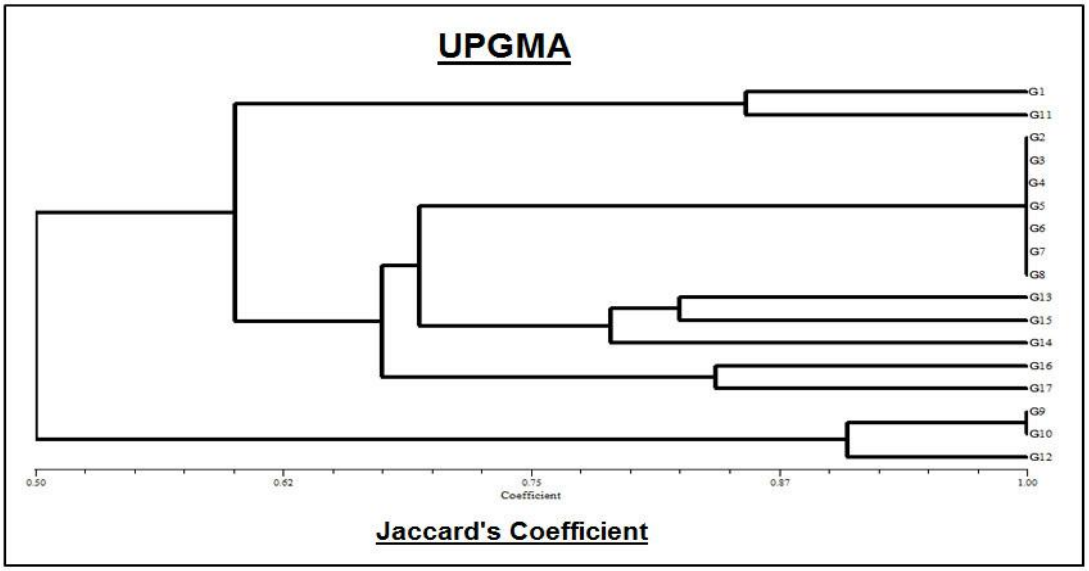


**Figure 2.** Dendrogram for the 17 garden pea genotypes constructed from the SCOT-markers generated data using UPGMA method and similarity matrices computed according to DSC's.

Also, the results are in agreement with those obtained by Simioniuc *et al*. (2002), who reported a relatively high similarity range (0.80–0.94) with RAPD markers compared with that obtained using AFLP markers in pea cultivars (0.85–0.94). However, Baranger *et al*. (2004) obtained a very wide range of similarity (0.0-1.0) in 148 *Pisum* genotypes using protein and PCR-based markers. The differences could be attributed to

differences among pea accessions of different origin and software used in this respect. On the other hand, Amirmoradi *et al*. (2012) detected 112 bands among 38 accessions belonging to eight annual *Cicer* species using nine SCoT markers, of which 109 were polymorphic. The number of bands ranged from 7 to 17 with an average of 12.4 per primer. The overall size of amplified products ranged from 220 to 2250 bp. Percent polymorphism ranged from 86.6% to as high as 100% with average polymorphism of 97% across all accessions. While, Luo *et al*. (2010) selected 33 primers for mango cultivars identification and genetic relationship analysis. Among the 50 accessions, 33 SCoT primers yielded a total of 273 clear and bright bands and their sizes ranged between 250 bp and 4000 bp; the number of bands varied from 3 to 15 with an average of 8.27 bands per primer. Out of 273 bands, 208 (76.19%) were found to be polymorphic, the number of polymorphic bands varied from 2 to 14 with an average of 6.3 bands per primer. The detected polymorphism per primer among the tested accessions ranged from 40% to 100%. Also, Xiong *et al*. (2011) used a set of 36 SCoT primers to fingerprint 20 peanut accessions. Eighteen primers generated a total of 157 fragments with a mean of 8.72, ranging from 4 to 17 per primer. Of 157 bands, 97 (61.78%) fragments were present in all the 20 accessions and 60 bands (38.22%) were polymorphic. One to seven polymorphic bands were amplified by each primer, with an average of 3.33 polymorphic bands per primer. Detected polymorphism per primer among the tested accessions ranged from 14.29% to 66.67%, with an average of 36.76%. Polymorphic index (PI) per primer ranged from 0.09 to 1.65, with an average of 0.82.

## CONCLUSION

The results indicated that the genotypes $G_1$, $G_5$, $G_6$ and $G_{13,}$ the most stable genotypes, gave the maximum total green yield overall the six

studied environments and were adapted to environments for most studied traits. Also, the genotype $G_{10}$ was considered promising line as early and of short stem length cultivar for its performance and was found to be suited to low yielding environments and could be used as stress tolerant genotype under stressed environments (poor yielding or unfavorable environments). The molecular analysis explained the differences within and between Master, Victory freezer, Sugar daddy and Snow wind genotypes and intercrosses between them, and suggested the superiority of these genotypes under the six studied environments due to the impact of environments in expression of these traits in pea genotypes. Results indicated that intercrosses between Master (check cultivar) and other pea genotypes as well as inclusion of valuable genotypes into breeding programmers might prevent loss of diversity in the *Pisum* gene pool. In addition, the findings could be used as an input for *in-situ* and *ex-situ* conservation strategies of the *P. sativum* and guide future collection missions.

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# تحليل الثبات والتقويم الجزيئى لتراكيب وراثية جديدة من البسلة الخضراء فى مصر

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أجريت هذه الدراسة بهدف تقييم ثلاثة عشرسلالة جديده مبشره من البسلة الخضراء ومقارنتها بصنفين منزرعين فى مصر وذلك فى ٦ بيئات مختلفة فى دلتا مصر (٣ مناطق خلال الموسمين الزراعيين ٢٠١٤/٢٠١٣ ، و٢٠١٥/٢٠١٤). تم تقييم السلالات الثلاثة عشرالجديده مع الصنفين التجاريين لعدد من الصفات الإقتصاديه وهى طول النبات ، وعدد الأيام حتى التزهير ، وطول القرن ، ووزن القرن ، وعدد البذور بالقرن ، ووزن ١٠٠ بذره ، ونسبة التصافى ، والمحصول الأخضر الكلى. أشارت النتائج إلى أن الإستجابة الخطية لتأثير البيئة كانت عالية المعنوية لكل الصفات المدروسة مؤكدا وجود فروق بين البيئات المختلفة مما يؤثر على هذه الصفات. وكان التفاعل بين التراكيب الوراثية والبيئات معنوياً لجميع الصفات المدروسة مما يدل على أن أداء التركيب الوراثى يختلف اختلافا كبيرا عبر البيئات المختلفة. ويتضح من نتائج تحليل الثبات الوراثى أن السلالات $G_1$، و$G_5$، و$G_6$ ، و$G_{13}$ كانت أكثر السلالات المبشرة ثباتا حيث أعطت أعلى محصول أخضر كلى فى مختلف البيئات ، كما كانت متأقلمة مع البيئات لصفة المحصول الأخضر الكلى ومعظم الصفات المدروسة. وتعتبر السلالة $G_{10}$ مبشرة كسلالة مبكرة وقصيرة الطول وثابتة وراثيا لصفة المحصول الأخضر ومعظم الصفات المدروسة. وكذلك أوضحت نتائج تحليل بيانات SCOT-markers أن نسبة التشابه الوراثى بين أصناف البسلة الخضراء تتراوح ما بين ٦٨.٤ % إلي ٩٩.٦ ٪ مما يدل على مستوى عالى من التباعد الوراثى بينها والقيمة العالية فى برامج تربية البسلة المستقبلية. وقد تم تحليل علاقات درجة القرابة والتشابه الوراثى بناءاً على مصفوفات التشابه المأخوذة من الواسمات الجزيئية بإستخدام طريقة UPGMA لرسم دندروجرامات لتوضيح القرابة الوراثية .