Estimation of the Coefficient of Variation and Some Genetic Parameters of Some Landraces of Cowpea

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ABSTRACT

The present investigation was carried out during two successive summer seasons of years 2019 and 2020 at the Faculty of Agriculture (Saba Basha), Alexandria University and the laboratory of the vegetable seed of Sabahya Horticulture Research Station, Alexandria Governorate, Egypt to evaluate six local cultivars and landraces of cowpea for some morphological characters, yield and its components as well as estimate some genetic parameters. Results reflected obvious differences among the six genotypes of cowpea for most of the studied characters. The coefficient of variation (C.V.) was less than 10 % for all the studied traits in all genotypes of cowpea. These results indicate that the six genotypes of cowpea are genetically identical concerning these traits. Analysis of variance showed that variances of genotypes were highly significant in all studied traits. These findings refer to that there were highly variations between genotypes under study. Generally, the data prove that all of the studied traits could be improved through the selection method, but with different degrees of the improving depending upon the amount of variation present in each population. Meanwhile, mean squares of years were significant only in height of the first flower, this can be interpreted as this property being affected by the different environmental conditions in both years of the study. Cluster analysis, based on RAPD plus ISSR analysis, divided the 6 studied genotypes into 3 major groups. The first contained Geza and Kareem7 Cvs. with similarity of (30%), the second consisted of Fowa Lr. and Kaha Cv., and the third one contained the ones of Behira Lr. and Kafr Elshikh Cv.

INTRODUCTION

According to FAOSTAT (2019), cowpea (Vigna unguiculata (L.) Walp.), 2n=22, is one of the most widely grown legume crops. Currently, Africa is considered the main producer of cowpea in the world, with 95.2 of the world's productions. Nigeria is the biggest country in production (3.5 million tons), Egypt produced 7180 tons. By a total area of 1853 hectares (4474 feds).

Cowpea is mainly grown for its seeds, which are high in protein, although the leaves and immature seed pods can also be consumed. The whole plant is used as forage for...
animals, with its use as cattle feed likely responsible for its name (Therese et al., 2019). Four subspecies of cowpeas are recognized, of which three are cultivated. A high level of morphological diversity is found within the species with large variations in the size, shape, and structure of the plant. Cowpeas can be erect, semi-erect (trailing), or climbing.

Cowpea suitable for poor soils (Moroke et al., 2005). It is valued for its ability to tolerate drought, and fix atmospheric nitrogen (rhizobium bacteria) which allows it to grow and improve poor soils, these make it an important component in many cropping systems (Mahalakshmi et al., 2006).

There are several diverse uses of cowpea due to which the varietal requirement in terms of plant type, seed type, maturity, the pattern of use and growth are diverse from region to region. Therefore, the cowpea breeding program becomes more complex and no single variety can be suitable for all the objectives. Thus, there is a need to develop varieties suitable for a specific region and or use. Traditionally, diversity within and between varieties was determined by assessing the difference in morphology. Cowpea is primarily a self-pollinating crop and its genetic base is considered to be narrow (Fana et al., 2004). Genetic diversity plays an important role in the success of any breeding program (Ali et al., 2007). Knowledge of genetic diversity in available varieties and genotypes is very useful for plant improvement all over the world, promoting the efficient use of genetic variations in breeding programs through supporting a proper selection of cross combination among large sets of parental genotypes (Mafakheri et al., 2017).

For any crop improvement program, the evaluation of verities to assess the existing variability is the first step. Greater variability present in the initial material better would be the chances for evolving desired types. A clear understanding of the variability of various characters of the breeding materials is an asset to the plant breeder for selecting superior genotypes on the basis of their phenotypic expression. In this regard, estimates of genotypic and phenotypic variance for various quantitative characters along with heritability and genetic advance expected by selection for yield and its components are useful in designing an effective breeding program (Sarath and Reshma, 2017).

The limited number of cowpea breeding programs in Egypt has contributed to the country’s ineffectiveness in taking advantage of the continent’s high genetic potential. A significant pool of cowpea landraces is thought to be available, but the limited detailed information available about their diversity and agronomic potential makes it difficult for breeding programs to thrive. Thus, the characterization of cowpea genetic resources available in Egypt is of extreme importance for conservation and breeding (Fadia et al., 2019). Unlike commercial varieties, landraces maintained by farmers usually have high levels of genetic variability as they have evolved from years of uncontrolled cross-regional and infield genetic exchange, even between previously released and discontinued open-pollinated varieties, not being subjected to selection over a long period of time. However, knowledge about their variability is usually limited (Ana et al., 2020).

Since the gene theory was put forward, genotypic selection has replaced phenotypic selection gradually. Since then, DNA molecular markers are becoming a research hot spot. The research on AFLP, SSR and RAPD is changing rapidly. Analysis of genetic diversity for cowpea breeding, the genetic diversity information is extremely important, which is the basis of breeding and genetic research. Accurate assessment of genetic variability is important for the preservation and utilization of germplasm resources, and the improvement of cultivars. For this reason, scholars all over the world have made extensive and in-depth research on the genetic diversity of cowpea (Coulibaly et al., 2002; Nkongolo et al., 2003; Malviya et al., 2012)
This investigation was aimed to study the coefficient of variation and genetic differences within and between 6 different genotypes of cowpea as a first step including them in breeding programs to improve and/or establish new cultivars.

**MATERIALS AND METHODS**

The present investigation was carried out during two successive summer seasons of years 2019 and 2020 at the Faculty of Agriculture (Saba Basha), Alexandria University and the laboratory of vegetable seed of Sabahya Horticulture Research Station, Alexandria Government, Egypt to evaluate six local cultivars and landraces of cowpea for morphological characters, yield and its components as well as estimate some genetic parameters i.e. genotypic and phenotypic variation, genotypic and phenotypic coefficient of variation, heritability and correlation coefficient analysis.

**Plant Materials:**

Plant materials for this study consisted of six genotypes of cowpea (Four local cultivars and two landraces). The sources of these genotypes are illustrated in Table (1).

**Table 1. The studied cowpea genotypes and their sources**

| Genotype            | source                                         |
|---------------------|------------------------------------------------|
| Giza 7 (Cv.)        | Registered cultivars at Horticulture Research Institute |
| Karim 7 (Cv.)       | Registered cultivars at Horticulture Research Institute |
| Kafr El-Shikh (Cv.) | Registered cultivars at Horticulture Research Institute |
| Kaha (Cv.)          | Registered cultivars at Horticulture Research Institute |
| Behira              | Landraces collected from Beheira Governorate   |
| Fowa                | Landraces collected from Kafr Al sheik Governorate |

**Field Evaluation:**

Seeds of the studied genotypes were sown on March 15th (during the years 2019 and 2020 summer seasons). The 6 genotypes were, randomly, distributed on a randomized complete blocks design with 3 replicates. Each replicate contained 12 rows, 2 rows for each genotype, rows were 5 m long and 70 cm wide approximately under drip irrigation conditions. The hills were thinned to one plant each 40 cm apart three weeks later. The other normal agricultural practices for cowpea production, i.e., irrigation, fertilization, weeds and pest control were practiced as recommended.

**Recorded Measurements:**

**Morphological Measurements:**

The following measurements were recorded on individual plants in each entry.

**Vegetative Measurements:** i.e., Plant length (cm) (Starting from the surface of the soil to the growing top), Number of branches/plants

**Flowering Measurements:** i.e., Height of the first flower (cm) Starting from the surface of the soil to the first flower appears), Number of days from sowing to the first flower appears (days)

**Yield and Its Components:** i.e., Number of pods/plants, Total pods yield/plant (g), Total seeds yield/plant (g), 100 seeds weight (g).

**Pod measurements:** The following measurements were recorded on randomly 30 pods from each entry; Pod length (cm), Pod width (cm), Pod weight (cm), number of seeds/pods.

**PCR based on RAPD and ISSR Analysis:**

**Genomic DNA Isolation:** Genomic DNA was extracted from the young leaves of the six cow bean genotypes by using DNA extraction kits (Easy Pure Plant Genomic DNA Kit)
DNA samples were stored at -20°C. DNA quality was checked by electrophoresis in a mini gel.

In the present study, two different markers RAPD and ISSR were employed to evaluate the efficiency of these markers in the diversity analysis of cow bean genotypes. The sequences of the used primers are shown in Table 2. PCR reactions were performed in 20µl total volume, using 1µl from diluted DNA, 1µl of each primer for the amplification reaction, 10µl master mix (Taq Ready Mix PCR Kit from the fast gene) and 8µl ddH2O (sterile water) for all reactions. The tubes were capped and placed in a thermocycler and the cycling was started immediately. Amplification protocol was carried out using PCR cycler 600 programmed for initial denaturation step at 94°C for 5 min, followed by 40 cycles each at 94°C for 30 sec, annealing at the recommended temperature for each primer as shown in Table 2 and extension at 72°C for 1min.

Table 2: sequences and annealing temperature of the RAPD and ISSR primers used in the study.

| Molecular marker | Primers | Sequence (5´-3´) | Annealing temperature(C) |
|------------------|---------|------------------|--------------------------|
| RAPD             | OPA2    | GTG ATC GCAG     | 37                       |
|                  | OPA07   | GAAAGGGGTG       |                          |
|                  | OP-B7   | CAG CAC CCA C    |                          |
|                  | Op-B1   | GTAGACC CGT      |                          |
|                  | OP-C9   | CTCACCGTCC       |                          |
| ISSR             | 14A     | (CT)8TG          | 57                       |
|                  | 49A     | (CA)8AG          |                          |
|                  | HB-9    | (CTC)6(TCT)3TGC  |                          |
|                  | HB-12   | (CAC)4GC         |                          |
|                  | HB-15   | (GTG)3GC         |                          |
|                  | HB-10   | (GAG)2(AGA)2TGCCC|                          |

The products of both RAPD and ISSR- based PCR analyses were detected using agarose gel electrophoresis (1.5% in 1X TBE buffer) stained with ethidium bromide (0.3µl). PCR products were visualized on U.V. light; photographed and analyzed using Total Lab Quant soft wear program.

Statistical Procedures:

Data of the studied characters were, statistically, analyzed using a combined analysis of variance for the two evaluated seasons, according to Herbert et al. (1955) and as illustrated in Table (3). The differences among the various means were tested, using Duncan's multiple range tests. The program used in the analysis COSTAT version 3. 303, 2004.

Table 3. The combined analyses of variance

| S.O.V          | DF     | MS      | EMS                              |
|----------------|--------|---------|----------------------------------|
| Blocks         | (r-1)  | MB      |                                 |
| Treatments     | (gs-1) | MT      |                                 |
| Genotypes      | (g-1)  | MG(M1)  | $\delta^2e + r\delta^2gs + rs\delta^2g$ |
| Seasons        | (s-1)  | MS(M2)  | $\delta^2e + r\delta^2gs + rg\delta^2s$ |
| Genotypes*Seasons | (g-1)(s-1) | M G*S(M3) | $\delta^2e + r\delta^2gs$ |
| Error          | (gs-1)(s-1) | ME(M4)  | $\delta^2e$                      |
| Total          | rgs-1  | ME      |                                 |

r = Number of replications, g = Number of genotypes, s = Number of seasons
Estimation of Genetic Parameters:

Components of Variance: Genotypic and phenotypic variances were computed from ANOVA table based on the expected mean sum of squares as follows:
- Genotypic variance (VG) = (M1-M3) / rs
- Seasons variance (VS) = (M2-M3) / rg
- Interaction variance (VGS) = (M3-M4) / r
- Phenotypic variance (VP) = VS + VG + V(GS) + VE

Heritability in broad sense was calculated as illustrated by Falconer (1989) using the following formula:

\[ H_{bs}^2 = \frac{\sigma_g^2}{\sigma_{ph}^2} \times 100 \]

Where, \( \sigma_g^2 \) = Genotypic variance and \( \sigma_{ph}^2 \) = Phenotypic variance

For molecular data and cluster analysis, data were scored for computer analysis on the basis of the presence of the amplified products for each primer. If a product was present in a genotype, it was designated as “1”, if absent, it was designated as “0”, after excluding the unreproducible bands. Pair-wise comparisons of genotype, based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients, according to Jaccard (1908). DNA fragment size was estimated by comparison with a 1-kbp DNA ladder Ready to use from Gene Direx. The similarity coefficients were then used to construct dendrograms, using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical, and Nested clustering) from Past program version 4.03.

RESULTS AND DISCUSSION

Pictures in Figure (1) and results in Table (4) reflected obvious differences among the six genotypes of cowpea for most of the studied characters. The longest plant was obtained by Giza 7 Cv. (73.14 cm), whereas the shortest plant was obtained by Kaha Cv. (43.4 cm). Kafr El-Shiekh Cv. gave the highest No. of branches/plant (25.1), meanwhile, Fowa landraces gave the lowest No. of branches/plant (19.6 branches). Concerning the height of the first flower (cm.), the highest mean value was obtained by Kafr El-Shiekh Cv. (25.4 cm). Meanwhile, the lowest mean value was obtained by Kaha Cv. (11.1 cm). Regarding the number of days to which the first flower appears, Behira landraces were the latest flowering (39.2 days), whereas Giza 7 Cv. was the earliest flowering (26.3 days). Regarding pov. (0.79 cm) and Fowa landraces (0.80 cm). Concerning Pod weight, El-Behira landraces and Giza 7 Cv. scored the highest mean values for pod weight (3.08 and 2.98 g respectively). With respect to the number of seeds/pods, El-Behira and Kafr El-Shiekh Cv. gave the highest number of seeds/pod (8.1 seeds/pod for both). Kafr El-shikh cultivar surpassed the other genotypes of cowpea in all traits of yield and its components. This cultivar gave 75.3 pods/plant, 211.8 g pods yield/plant, 274.4 g seeds yield/plant and 42.6 g weight of 100 seeds, whereas, Fowa landraces gave the lowest mean values for all traits of yield and its components.

The coefficient of variation (C.V.) was less than 10% for all the studied traits in all studied genotypes of cowpea. These results indicated that the six genotypes of cowpea are genetically identical concerning these traits.
Fig. 1. Pictures of the vegetative growth, pods and seeds of the six genotypes of cowpea.

Analysis of variance in Table (5) showed that the mean square of genotypes was highly significant in all studied traits. These findings refer to that there were highly variations between genotypes under study. Generally, the data prove that all of the studied traits could be improved through the selection method, but with different degrees of the improving depending upon the amount of variation present in each population. Similar results were reported by Fana et al., (2004), Gerrano et al., (2015) and Inuwa et al., (2018). They reported that significant and high significant differences between genotypes mean that these genotypes have high expected genetic advance and beginning breeding programs by self-pollination and selection may be very effective generation by generation.

Meanwhile, mean squares of years were significant only in height of the first flower, this can be interpreted as this property being affected by the different environmental conditions in both years of the study. In this regard, Khan et al. (2015) and Mafakheri et al. (2017) reported that the flowering measurements were affected by the change in environmental conditions. However, mean squares of interaction between genotypes ×years were not significant in all studied traits.

All variance components values presented in Table (6) revealed that the large portion of genotypic variance for the following characters: plant height, height of the first flower, number of pods/plants, total pods yield/plant, total seeds yield/plant and 100 seeds weight.
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Moderate values were in remain traits understudied similar results were found by Omoigui 2006 and Patel et al., 2016. They reported that the genotypic and phenotypic variability was a reference point for any breeding program to study the genotypic difference of the most important economic characters. It makes the breeding program by selection more effective.

Genotypic and phenotypic coefficient of variance values (GCV and PCV) showed that there was a narrow range between the genotypic and phenotypic coefficient of variance in characters; Plant height, Height of the first flower, Number of days for the first flower, Number of branches/plants, Pod length, number of Seed/pods, Number of pods/plants, Total pods yield/plant, Total seeds yield/plant and weight of 100 seeds (Table 6). Meanwhile, the wider range was in traits Pod width and Pod weight. Similar results were found by (Pathak et al., 2016) and motioned that the traits which have a wider range between values of (GCV) and (PCV). These results indicating that these characters are more affected by environmental conditions.

Heritability estimates in the broad sense in Table (6) showed that differences between genotypic variance and phenotypic variance were narrow in the same traits which exhibited high heritability values the highest heritability values were in traits Plant height, Height of the first flower, Number of days for the first flower, Number of branches/plant, Pod length, Seeds number/pod, Number of pods/plant, Total pods yield/plant, Total seeds yield/plant and 100 seeds weight (estimates were 90.74, 82.81, 84.54, 85.76, 89.12, 84.67, 90.10 and 90.42% for previous traits respectively). Moderate values were in Pod width and Pod weight (estimates were 73.70 and 66.81 for Pod width and Pod weight, respectively). Similar results were found by Shanko et al., 2014. They found high heritability estimates in a broad sense for plant height, number of pods/plants, seeds yield/plant, 100-seed weight, number of days to flowering. Also (Udensi et al., 2011) found that superior estimates were obtained for pod measurements, the average number of pods/plant and the average number of seeds/plants.

Table 4: Mean performance, range and coefficient of variation (C.V) of vegetative, flowering and pod measurements, yield and its components of the six genotypes from cowpea, calculated from the combined data over both 2019 and 2020 summer seasons.

| Genotypes       | Plant length (cm.) | No. of branches/plant | No. of days from seeing to the first flower appears (days) | Height of the first flower (cm.) |
|-----------------|--------------------|-----------------------|-------------------------------------------------|---------------------------------|
|                 | Mean | Range | C.V | Mean | Range | C.V | Mean | Range | C.V | Mean | Range | C.V |
| Fóvá (landrace) | 62.52 | 59.21-66.80 | 1.00 | 19.57 | 17.99-20.94 | 2.36 | 36.27 | 33.65-38.82 | 2.31 | 23.81 | 22.03-25.61 | 2.58 |
| Behira (landrace) | 54.50 | 50.36-58.15 | 1.51 | 20.75 | 18.91-22.52 | 3.02 | 38.29 | 36.66-40.03 | 2.16 | 33.44 | 29.90-37.55 | 2.06 |
| Kátta (CV) | 43.37 | 40.37-46.06 | 0.91 | 20.73 | 18.15-27.65 | 4.85 | 27.13 | 25.08-36.35 | 2.28 | 11.14 | 9.98-17.81 | 5.58 |
| Kátta El-Shishé (CV) | 68.80 | 63.01-78.00 | 1.08 | 25.00 | 23.17-27.25 | 2.54 | 36.67 | 34.05-39.24 | 2.31 | 25.44 | 22.80-27.85 | 2.06 |
| Gúrý (CV) | 73.14 | 68.85-77.37 | 1.95 | 23.76 | 21.99-25.85 | 3.54 | 36.76 | 34.28-39.65 | 2.60 | 18.87 | 17.32-20.93 | 5.58 |
| Kárým (CV) | 65.93 | 61.78-69.80 | 1.13 | 24.20 | 22.46-25.98 | 2.27 | 28.99 | 26.61-31.52 | 2.60 | 22.61 | 20.90-24.59 | 4.10 |

Means with the same alphabetical letter in the column are not significantly different from each other using Duncan’s Multiple Range Test at 5% probability.
Table 5. Mean squares of plant length and flowering and pod measurements, yield and its components for all genotypes under study, over two years of the study (2019 and 2020 summer seasons).

| S.O.V. | D. F. | Vegetative measurements | Flowering measurements |
|--------|-------|-------------------------|------------------------|
|        |       | Plant length | Height of the first flower | The number of days to the first flower appears | Number of branches of flower holder |
| Blocks | 2     | 0.05** | 0.74** | 0.52** | 0.28** |
| Years(Y) | 1    | 0.01** | 1.64** | 0.22** | 1.05** |
| Genotypes(G) | 5     | 14.12** | 20.19** | 12.01** | 10.83** |
| G × Y | 5     | 0.17** | 0.38** | 0.30** | 0.40** |
| Error | 22    | 0.28 | 0.36 | 0.42 | 0.32 |

| S.O.V. | D. F. | Yield and its components | Pod measurements |
|--------|-------|-------------------------|-----------------|
|        |       | Number of pods/plants | Total pods yield/plant | Total seeds yield/plant | 100 seeds weight |
| Blocks | 2     | 0.60** | 2.06** | 0.13** | 1.64** |
| Years(Y) | 1    | 0.0005** | 0.66** | 0.41** | 0.27** |
| Genotypes(G) | 5     | 27.37** | 58.66** | 94.14** | 28.33** |
| G × Y | 5     | 0.43** | 2.07** | 0.60** | 0.18** |
| Error | 22    | 0.64 | 1.64 | 2.24 | 0.65 |

** Highly significant differences at 1% level of probability.
Ns: not significant differences.

Table 6. Variance components values ($\sigma^2_G$, $\sigma^2_E$ and $\sigma^2_{PH}$) genotypic and phenotypic coefficient of variability (GCV, PCV) and heritability (over mean of 12 traits understudied).

| Traits | Variance | Coefficient of variability | Heritability in broad sense %a |
|--------|----------|-----------------------------|------------------------------|
| σ2_Y: Years variance | σ2_G: Genotypic variance | σ2_E: Error variance | σ2_PH: Phenotypic variance | GCV | PCV |
| Plant height | -0.09067 | 2.32559 | -0.03653 | 0.28329 | 2.56292 | 1.50803 | 4.146604 | 90.7461 |
| Height of the first flower | 0.06916 | 3.101249 | 0.004107 | 0.364856 | 1.374032 | 15.8048 | 17.09642 | 88.269003 |
| Number of days for first flower | -0.00077 | 1.977963 | -0.00153 | 0.422873 | 2.399971 | 5.976559 | 7.216556 | 82.81736 |
| Number of branches of flower holder | 0.098137 | 1.395434 | 0.022348 | 0.324888 | 1.310128 | 1.854457 | 9.614242 | 82.4718 |
| Pod length | 0.004943 | 1.342556 | -0.006267 | 0.299135 | 1.588059 | 10.12655 | 11.973852 | 84.540675 |
| Pod width | -2.38-35 | 0.004575 | -0.00097 | 0.002004 | 0.010504 | 0.524252 | 0.711306 | 73.70369 |
| Pod weight | -0.00121 | 0.037816 | 0.00993 | 0.019059 | 0.050597 | 1.363517 | 2.046868 | 66.81667 |
| Seeds number / pod | -0.00126 | 0.080556 | 0.006242 | 0.008384 | 0.009392 | 1.179821 | 1.375595 | 85.780603 |
| Number of pods / plant | -0.02374 | 4.491116 | -0.02757 | 0.445948 | 5.093926 | 6.924585 | 7.797803 | 89.128189 |
| Total pods yield / plant | -0.07826 | 8.125141 | 0.180861 | 1.644085 | 11.13942 | 14.99154 | 5.895624 | 84.9794 |
| Total seeds yield / plant | -0.09295 | 15.774098 | 0.01423 | 2.248075 | 12.2862 | 8.061350 | 0.045499 | 90.103132 |
| 100 seeds weight | 0.005139 | 4.691259 | -0.15685 | 0.481237 | 5.188036 | 15.41656 | 17.04921 | 90.42588 |

$\sigma^2_Y$: Years variance, $\sigma^2_G$: Genotypic variance, $\sigma^2_E$: Error variance, $\sigma^2_PH$: Phenotypic variance, PCV: Phenotypic coefficient of variability and GCV: Genotypic coefficient of variance.

Cluster analysis based on morphological traits provides two major groups the first one includes Kaha Cv. and the second includes the rest of the genotypes. Meanwhile, the second cluster is divided into 3 sup groups the first include Kafr Elshikh Cv., the second includes Geza7 and Kareem7 Cvs. and the third contains Fowa and Behira Lrs (Fig. 2).

Five primers for RAPD and six for ISSR techniques were screened for their ability to amplify the genomic DNA of the six studied cowpea genotypes. Data were analyzed based on the comparison of the amplified fragments using gel documentation for each primer. If a fragment was present in a sample, it was designated as "1", if absent, it was designated as "0". If a fragment was present or absent in the genotype then absent or present in the others, it was called a unique species-specific marker, but if a fragment was absent and present in more than one genotype, it was called polymorphic finally if the fragments were present in all genotypes, it was called monomorphic.
A total of 98 RAPD fragments were amplified with the five used primers ranged from 16 (primer 3) to 27 (primer 4), zero of them were common fragments (monomorphic), 24 of them showed to be polymorphic and other 74 showed to be unique fragments (Tables 7 - 11 and Plate 1).

**Table 7:** Amplified DNA fragments (AF) obtained for the six genotypes using first RAPD primers.

| Fragments | RF   | Sizebp | Genotypes | Polymorphism |
|-----------|------|--------|-----------|--------------|
|           |      |        | Geza7     | Kareem7    | Fowa       | Kaha      | Behira    | Kaf Elshek |          |
| 1         | 0.198| 810.532| 0        | 0          | 0          | 0         | 0         | 1          | Unique     |
| 2         | 0.225| 698.290| 0        | 0          | 1          | 0         | 0         | 0          | Unique     |
| 3         | 0.236| 657.147| 0        | 0          | 0          | 1         | 0         | 0          | Unique     |
| 4         | 0.247| 618.428| 0        | 1          | 0          | 0         | 0         | 0          | Unique     |
| 5         | 0.264| 563.029| 1        | 0          | 0          | 0         | 0         | 0          | Unique     |
| 6         | 0.269| 547.700| 0        | 0          | 1          | 1         | 0         | 0          | Polymorphic |
| 7         | 0.286| 498.637| 0        | 1          | 0          | 0         | 0         | 0          | Unique     |
| 8         | 0.297| 469.257| 0        | 0          | 0          | 0         | 0         | 1          | Unique     |
| 9         | 0.313| 429.586| 1        | 0          | 0          | 0         | 0         | 0          | Unique     |
| 10        | 0.335| 380.455| 0        | 0          | 0          | 0         | 1         | 0          | Unique     |
| 11        | 0.341| 368.089| 1        | 0          | 0          | 0         | 0         | 0          | Unique     |
| 12        | 0.352| 346.374| 0        | 0          | 0          | 0         | 0         | 1          | Unique     |
| 13        | 0.357| 336.943| 0        | 0          | 0          | 1         | 0         | 0          | Unique     |
| 14        | 0.385| 288.685| 1        | 1          | 0          | 0         | 0         | 0          | Polymorphic |
| 15        | 0.390| 280.826| 0        | 0          | 0          | 1         | 1         | 1          | Polymorphic |
| 16        | 0.407| 255.669| 1        | 1          | 0          | 0         | 0         | 0          | Polymorphic |
| 17        | 0.409| 252.862| 0        | 0          | 0          | 1         | 0         | 1          | Polymorphic |
| 18        | 0.429| 226.429| 0        | 0          | 0          | 0         | 1         | 0          | Unique     |
| 19        | 0.434| 220.264| 1        | 0          | 1          | 1         | 0         | 0          | Polymorphic |
| 20        | 0.445| 207.286| 0        | 1          | 0          | 0         | 0         | 0          | Unique     |
| 21        | 0.462| 188.718| 1        | 0          | 0          | 0         | 0         | 0          | Unique     |

Cluster analysis, according to DNA- RAPD analysis, divided the 6 studied genotypes into 3 main clusters. The first cluster includes Kaf Elshik Cv., the second cluster includes Behira Lr., meanwhile, the third cluster includes Geza7, Kareem7, Kaha Cvs. and Fowa Lr.; which contain two sup order the first one contain Geza7 and Kareem7 Cvs. with similarity (15%), The second contains Fowa Lr. and Kaha Cv. with similarity (30%) (Fig.2).

**Table 8:** Amplified DNA fragments (AF) obtained for the six genotypes using second RAPD primers.

| Fragments | RF   | Sizebp | Genotypes | Polymorphism |
|-----------|------|--------|-----------|--------------|
|           |      |        | Geza7     | Kareem7    | Fowa       | Kaha      | Behira    | Kaf Elshek |          |
| 1         | 0.156| 1045.858| 0        | 1          | 0          | 0         | 0         | 0          | Unique     |
| 2         | 0.171| 950.716 | 1        | 0          | 0          | 0         | 1         | 0          | Polymorphic |
| 3         | 0.180| 897.838 | 0        | 1          | 0          | 0         | 0         | 0          | Unique     |
| 4         | 0.190| 842.526 | 1        | 0          | 0          | 0         | 0         | 0          | Unique     |
| 5         | 0.220| 696.210 | 0        | 1          | 0          | 0         | 0         | 0          | Unique     |
| 6         | 0.229| 657.487 | 0        | 0          | 0          | 0         | 0         | 1          | Unique     |
| 7         | 0.249| 578.973 | 0        | 0          | 0          | 0         | 0         | 1          | Unique     |
| 8         | 0.254| 560.855 | 0        | 0          | 0          | 0         | 0         | 1          | Unique     |
| 9         | 0.263| 529.661 | 0        | 1          | 0          | 0         | 0         | 0          | Unique     |
| 10        | 0.283| 466.411 | 0        | 0          | 1          | 0         | 0         | 0          | Unique     |
| 11        | 0.288| 451.816 | 0        | 0          | 0          | 0         | 1         | 0          | Unique     |
| 12        | 0.322| 363.976 | 1        | 1          | 0          | 0         | 0         | 0          | Polymorphic |
| 13        | 0.327| 352.586 | 0        | 0          | 0          | 0         | 1         | 0          | Unique     |
| 14        | 0.341| 322.556 | 0        | 0          | 0          | 1         | 0         | 0          | Unique     |
| 15        | 0.361| 284.038 | 0        | 1          | 0          | 0         | 0         | 0          | Unique     |
| 16        | 0.366| 275.150 | 0        | 0          | 0          | 1         | 0         | 0          | Unique     |
| 17        | 0.390| 236.208 | 0        | 0          | 0          | 1         | 0         | 0          | Unique     |

Detectable fragments | 3 | 6 | 1 | 3 | 4 | 2 |
### Table 9: Amplified DNA fragments (AF) obtained for the six genotypes using third RAPD primers.

| Fragments | RF    | Sizebp | Genotypes | Polymorphism |
|-----------|-------|--------|------------|--------------|
| Geza7     | Kareem7 | Fowa | Kaha | Behira | Kafr Elshek | |
| 1         | 0.222 | 811.211 | 0 | 0 | 0 | 0 | 1 | Unique |
| 2         | 0.236 | 742.264 | 0 | 0 | 1 | 0 | 0 | 0 | Unique |
| 3         | 0.253 | 666.373 | 0 | 0 | 0 | 0 | 0 | 1 | Unique |
| 4         | 0.258 | 645.565 | 0 | 0 | 1 | 0 | 0 | 0 | Unique |
| 5         | 0.264 | 621.452 | 1 | 0 | 0 | 0 | 0 | 0 | Unique |
| 6         | 0.283 | 550.878 | 0 | 1 | 0 | 0 | 0 | 0 | Unique |
| 7         | 0.286 | 540.492 | 1 | 0 | 0 | 0 | 0 | 0 | Unique |
| 8         | 0.306 | 476.082 | 0 | 0 | 0 | 0 | 1 | 0 | Unique |
| 9         | 0.308 | 470.079 | 0 | 0 | 0 | 0 | 0 | 1 | Unique |
| 10        | 0.350 | 360.118 | 0 | 0 | 1 | 0 | 0 | 0 | Unique |
| 11        | 0.353 | 325.332 | 1 | 0 | 0 | 0 | 0 | 0 | Unique |
| 12        | 0.372 | 313.203 | 0 | 0 | 0 | 0 | 0 | 1 | Unique |
| 13        | 0.386 | 286.583 | 0 | 0 | 0 | 1 | 0 | 0 | Unique |
| 14        | 0.411 | 244.549 | 0 | 0 | 0 | 1 | 0 | 0 | Unique |
| 15        | 0.433 | 212.690 | 0 | 0 | 1 | 0 | 0 | 1 | Polymorphic |
| 16        | 0.453 | 187.344 | 0 | 0 | 0 | 1 | 0 | 0 | Unique |
| Detectable fragments | 3 | 1 | 4 | 3 | 1 | 5 |

### Table 10: Amplified DNA fragments (AF) obtained for the six genotypes using forth RAPD primers.

| Fragments | RF    | Sizebp | Genotypes | Polymorphism |
|-----------|-------|--------|------------|--------------|
| Geza7     | Kareem7 | Fowa | Kaha | Behira | Kafr Elshek | |
| 1         | 0.161 | 1240.209 | 0 | 0 | 1 | 0 | 0 | 0 | Unique |
| 2         | 0.185 | 1070.896 | 0 | 0 | 1 | 0 | 0 | 0 | Unique |
| 3         | 0.211 | 913.456 | 0 | 0 | 1 | 0 | 0 | 0 | Unique |
| 4         | 0.252 | 710.862 | 0 | 0 | 1 | 1 | 0 | 0 | Polyomorphic |
| 5         | 0.276 | 613.816 | 0 | 0 | 0 | 1 | 0 | 0 | Unique |
| 6         | 0.293 | 553.202 | 0 | 0 | 0 | 1 | 0 | 0 | Unique |
| 7         | 0.299 | 533.269 | 0 | 0 | 0 | 0 | 0 | 1 | Unique |
| 8         | 0.323 | 460.468 | 0 | 0 | 0 | 1 | 0 | 0 | Polyorphic |
| 9         | 0.328 | 446.600 | 0 | 0 | 0 | 0 | 0 | 0 | Unique |
| 10        | 0.355 | 378.619 | 0 | 1 | 0 | 1 | 0 | 0 | Polyorphic |
| 11        | 0.358 | 371.736 | 0 | 0 | 1 | 0 | 0 | 0 | Unique |
| 12        | 0.361 | 364.977 | 0 | 0 | 0 | 0 | 1 | 0 | Unique |
| 13        | 0.372 | 341.231 | 1 | 0 | 0 | 0 | 0 | 0 | Unique |
| 14        | 0.378 | 329.936 | 0 | 0 | 0 | 1 | 0 | 0 | Unique |
| 15        | 0.381 | 322.956 | 0 | 0 | 0 | 1 | 0 | 0 | Unique |
| 16        | 0.393 | 300.102 | 0 | 0 | 0 | 0 | 0 | 1 | Unique |
| 17        | 0.399 | 289.289 | 0 | 0 | 0 | 0 | 1 | 0 | Unique |
| 18        | 0.405 | 278.866 | 0 | 1 | 0 | 1 | 0 | 0 | Polyorphic |
| 19        | 0.416 | 260.722 | 0 | 0 | 0 | 0 | 1 | 0 | Unique |
| 20        | 0.419 | 255.982 | 1 | 0 | 0 | 1 | 0 | 0 | Polyorphic |
| 21        | 0.434 | 233.543 | 0 | 1 | 0 | 0 | 1 | 0 | Polyorphic |
| 22        | 0.437 | 229.297 | 0 | 0 | 0 | 0 | 0 | 1 | Unique |
| 23        | 0.449 | 213.071 | 0 | 0 | 0 | 1 | 1 | 0 | Polyorphic |
| 24        | 0.455 | 205.394 | 0 | 1 | 0 | 0 | 0 | 0 | Unique |
| 25        | 0.472 | 185.112 | 1 | 0 | 0 | 0 | 0 | 0 | Unique |
| 26        | 0.525 | 133.862 | 1 | 0 | 0 | 0 | 0 | 0 | Unique |
| 27        | 0.554 | 112.106 | 1 | 0 | 0 | 0 | 0 | 0 | Unique |
| Detectable fragments | 6 | 4 | 5 | 9 | 6 | 4 |
**Table 11:** Amplified DNA fragments (AF) obtained for the six genotypes using fifth RAPD primers.

| Fragments | RF    | Sizebp | Geza7 | Kareem7 | Fowa | Kaha | Behira | Kafr Elshek | Polymorphism |
|-----------|-------|--------|-------|----------|------|------|--------|--------------|--------------|
| 1         | 0.163 | 1334.914 | 0     | 0       | 1    | 1    | 0      | 1            | Polymorphic  |
| 2         | 0.191 | 1169.424 | 0     | 0       | 1    | 1    | 0      | 1            | Polymorphic  |
| 3         | 0.224 | 1000.522 | 0     | 0       | 0    | 1    | 0      | 0            | Unique       |
| 4         | 0.227 | 986.434  | 0     | 0       | 1    | 0    | 0      | 0            | Unique       |
| 5         | 0.247 | 897.449  | 0     | 0       | 1    | 1    | 0      | 0            | Polymorphic  |
| 6         | 0.305 | 682.247  | 1     | 0       | 0    | 0    | 0      | 0            | Unique       |
| 7         | 0.320 | 635.548  | 0     | 0       | 0    | 1    | 0      | 0            | Unique       |
| 8         | 0.343 | 570.075  | 1     | 1       | 0    | 0    | 0      | 0            | Polymorphic  |
| 9         | 0.355 | 538.638  | 0     | 0       | 1    | 0    | 0      | 0            | Unique       |
| 10        | 0.383 | 471.863  | 1     | 0       | 1    | 1    | 0      | 0            | Polymorphic  |
| 11        | 0.432 | 374.303  | 0     | 0       | 1    | 1    | 0      | 1            | Polymorphic  |
| 12        | 0.461 | 326.355  | 1     | 0       | 1    | 1    | 0      | 1            | Polymorphic  |
| 13        | 0.489 | 285.896  | 1     | 0       | 1    | 1    | 1      | 0            | Polymorphic  |
| 14        | 0.526 | 240.022  | 0     | 0       | 0    | 0    | 1      | 0            | Unique       |
| 15        | 0.547 | 217.340  | 1     | 0       | 0    | 0    | 0      | 0            | Unique       |
| 16        | 0.555 | 209.275  | 0     | 0       | 0    | 0    | 0      | 1            | Unique       |
| 17        | 0.610 | 161.365  | 1     | 0       | 0    | 0    | 0      | 0            | Unique       |

| Detectable fragments | 7 | 1 | 9 | 9 | 2 | 5 |

**Plate 1:** RAPD banding patterns in the six genotypes accessions generated using 5 primers. (1, 2, 3, 4, 5 and 6 for Geza7, Kareem7, Fowa, Kaha, Behira and Kafr El-Shikh, respectively).

A total of 99 ISSR fragments were amplified with the six used primers ranged from 10 to 22, 8 of them were common fragments (monomorphic), 33 of them showed to be polymorphic and 58 showed to be unique fragments (Tables 12 - 17 and Plate 2).

Cluster analysis, according to DNA- ISSR analysis, divided the 6 studied genotypes into 2 major groups. The first main group contained Kafr Elshikh. The second main group
contains the rest genotypes, which contain two sups order the first one contains Geza7 Cv. Meanwhile, the includes Kareem7 and Kaha Cvs. and Behira and Fowa Lrs. (Fig. 2).

Of the total 347 reproducible amplicons generated by the 11 RAPD and ISSR primers in sum, showing 66 fragments for Geza7, 50 for Kareem7, 58 for Fowa, 71 for Kaha, 46 for Behira and 56 for Kafr El-Shikh. 132 fragments were unique fragments 29 of them detected in Geza7, 17 in Kareem7 and Behira,18 in Fowa,24 in Kaha and 27 for Kafr El-Shikh genotypes (Tables 18 to 20).

Cluster analysis, based on RAPD plus ISSR analysis, divided the 6 studied genotypes into 3 major groups. The first contained Geza and Kareem7 Cvs. with similarity of (30%), the second consisted of Fowa Lr. and Kaha Cv., and the third one contained Behira Lr. and Kafr Elshikh Cv. (Fig. 3).

Studies on genetic diversity and relatedness at its molecular level have been surprisingly scarce. Hossain et al. (2003) characterized cold-tolerant and cold-sensitive Jew's mallow germplasms. Qi et al. (2003a, b) classified wild Jew's mallow species using Inter Simple Sequence Repeat (ISSR) marker. Recently Akter et al. (2008) and Mir et al. (2008) reported the utility of studying genetic variability for different traits in Jew's mallow genotypes using Jew's mallow-specific SSR markers. ISSRs will have an important role in securing plant variety rights by virtue of its unique efficiency in distinguishing even closely related germplasm. To date, more polymorphism has been detected with the use of ISSRs than that with any other assay procedure (Gupta et al., 1994).

Table 12: Amplified DNA fragments (AF) obtained for the six genotypes using first ISSR primers.

| Fragments | RF  | Sizebp | Geza7 | Kareem7 | Fowa | Kaha | Behira | Kafr Elshek | Polymorphism |
|-----------|-----|--------|-------|---------|------|------|--------|-------------|--------------|
| 1         | 0.253 | 791,535 | 0     | 0       | 0    | 1    | 0      | 0           | Unique       |
| 2         | 0.259 | 769,035 | 0     | 0       | 0    | 0    | 0      | 1           | Unique       |
| 3         | 0.290 | 662,580 | 1     | 0       | 0    | 0    | 0      | 0           | Unique       |
| 4         | 0.293 | 653,094 | 0     | 0       | 0    | 0    | 0      | 1           | Unique       |
| 5         | 0.338 | 526,072 | 0     | 1       | 0    | 0    | 0      | 0           | Unique       |
| 6         | 0.343 | 513,581 | 0     | 0       | 1    | 0    | 0      | 0           | Unique       |
| 7         | 0.355 | 484,798 | 0     | 0       | 0    | 1    | 1      | 0           | Polymorphic  |
| 8         | 0.377 | 436,153 | 0     | 1       | 0    | 0    | 0      | 0           | Unique       |
| 9         | 0.389 | 411,709 | 1     | 0       | 0    | 0    | 0      | 0           | Unique       |
| 10        | 0.421 | 353,017 | 0     | 1       | 0    | 1    | 1      | 0           | Polymorphic  |
| 11        | 0.426 | 344,634 | 1     | 0       | 0    | 0    | 0      | 0           | Unique       |
| 12        | 0.438 | 325,320 | 0     | 0       | 0    | 1    | 0      | 0           | Unique       |
| 13        | 0.449 | 308,567 | 1     | 1       | 0    | 0    | 1      | 0           | Polymorphic  |
| 14        | 0.466 | 284,357 | 1     | 0       | 1    | 0    | 0      | 0           | Polymorphic  |
| 15        | 0.491 | 252,163 | 1     | 0       | 1    | 0    | 0      | 0           | Polymorphic  |
| 16        | 0.503 | 238,031 | 0     | 0       | 0    | 1    | 0      | 0           | Unique       |
| 17        | 0.512 | 227,954 | 0     | 0       | 0    | 0    | 1      | 0           | Unique       |
| 18        | 0.515 | 224,690 | 0     | 0       | 1    | 0    | 0      | 0           | Unique       |
| 19        | 0.522 | 217,257 | 1     | 0       | 0    | 0    | 0      | 0           | Unique       |
| 20        | 0.531 | 208,059 | 0     | 0       | 0    | 0    | 0      | 1           | Unique       |
| 21        | 0.546 | 193,587 | 0     | 0       | 0    | 1    | 0      | 0           | Unique       |

Detectable fragments  7  4  3  6  5  4
Table 13: Amplified DNA fragments (AF) obtained for the six genotypes using second ISSR primers.

| Fragments | RF     | Sizebp | Genotypes      | Polymorphism |
|-----------|--------|--------|----------------|--------------|
|           |        |        | Geza7 Kareem7 Fowa Kaha Behira Kafr Elshek |              |
| 1         | 0.206  | 962.158| 0 0 1 0 0 0     | Unique       |
| 2         | 0.228  | 848.128| 1 0 0 0 0 0     | Unique       |
| 3         | 0.233  | 824.138| 0 0 0 0 0 1     | Unique       |
| 4         | 0.236  | 810.102| 0 0 1 0 0 0     | Unique       |
| 5         | 0.253  | 734.862| 1 0 1 1 0 1     | Polymorphic  |
| 6         | 0.267  | 678.176| 0 0 0 1 0 0     | Unique       |
| 7         | 0.268  | 674.299| 0 1 1 1 0 0     | Polymorphic  |
| 8         | 0.286  | 608.175| 1 0 0 0 0 0     | Unique       |
| 9         | 0.311  | 526.954| 0 0 1 0 0 0     | Unique       |
| 10        | 0.314  | 517.967| 0 0 0 1 0 0     | Unique       |
| 11        | 0.325  | 486.306| 1 0 0 0 0 0     | Unique       |
| 12        | 0.364  | 388.858| 0 0 1 0 0 0     | Unique       |
| 13        | 0.372  | 371.423| 1 0 0 0 0 0     | Unique       |
| 14        | 0.410  | 298.703| 0 0 1 1 1 1     | Polymorphic  |
| 15        | 0.433  | 261.797| 0 1 1 0 0 0     | Polymorphic  |
| 16        | 0.439  | 252.944| 0 0 0 0 0 1     | Unique       |
| 17        | 0.481  | 198.808| 1 0 0 0 0 0     | Unique       |
| Detectable fragments | 6 2 8 3 2 3 |

Table 14: Amplified DNA fragments (AF) obtained for the six genotypes using third ISSR primers.

| Fragments | RF     | Sizebp | Genotypes      | Polymorphism |
|-----------|--------|--------|----------------|--------------|
|           |        |        | Geza7 Kareem7 Fowa Kaha Behira Kafr Elshek |              |
| 1         | 0.216  | 874.623| 0 0 0 1 0 0     | Polymorphic  |
| 2         | 0.236  | 781.263| 1 1 0 1 0 0     | Polymorphic  |
| 3         | 0.241  | 759.524| 0 0 0 0 0 1     | Unique       |
| 4         | 0.268  | 652.168| 0 1 0 1 0 1     | Polymorphic  |
| 5         | 0.282  | 602.619| 0 0 0 0 1 0     | Unique       |
| 6         | 0.301  | 541.340| 0 0 0 1 0 0     | Unique       |
| 7         | 0.307  | 523.315| 1 1 0 0 0 1     | Polymorphic  |
| 8         | 0.323  | 478.128| 0 0 0 1 0 1     | Polymorphic  |
| 9         | 0.329  | 462.208| 0 1 0 0 0 0     | Unique       |
| 10        | 0.349  | 412.870| 0 1 1 1 0 0     | Polishorphic |
| 11        | 0.359  | 390.213| 0 0 0 0 1 0     | Unique       |
| 12        | 0.403  | 304.404| 0 0 0 0 0 1     | Unique       |
| 13        | 0.416  | 282.868| 0 0 0 1 0 1     | Unique       |
| 14        | 0.433  | 256.989| 0 0 0 1 0 0     | Unique       |
| 15        | 0.441  | 245.643| 0 0 1 0 0 0     | Unique       |
| 16        | 0.477  | 200.476| 0 0 0 0 1 0     | Unique       |
| 17        | 0.485  | 191.625| 0 0 1 0 0 0     | Unique       |
| Detectable fragments | 2 5 3 6 3 7 |

Table 15: Amplified DNA fragments (AF) obtained for the six genotypes using fourth ISSR primers.

| Fragments | RF     | Sizebp | Genotypes      | Polymorphism |
|-----------|--------|--------|----------------|--------------|
|           |        |        | Geza7 Kareem7 Fowa Kaha Behira Kafr Elshek |              |
| 1         | 0.258  | 1111.675| 1 1 1 1 1 1     | Monomorphic  |
| 2         | 0.357  | 406.023 | 1 0 1 1 1 1     | Polymorphic  |
| 3         | 0.333  | 518.315 | 0 0 1 1 1 1     | Polymorphic  |
| 4         | 0.299  | 732.523 | 1 1 1 1 1 1     | Monomorphic  |
| 5         | 0.278  | 907.003 | 0 0 1 1 1 1     | Monomorphic  |
| 6         | 0.238  | 1362.533| 1 1 1 1 1 1     | Monomorphic  |
| 7         | 0.209  | 1830.131| 0 0 0 0 0 1     | Unique       |
| 8         | 0.148  | 3404.144| 0 1 0 1 1 1     | Polymorphic  |
| 9         | 0.205  | 1906.145| 1 1 1 1 1 0     | Polymorphic  |
| Detectable fragments | 6 6 8 9 9 9 |
Table 16: Amplified DNA fragments (AF) obtained for the six genotypes using fifth ISSR primers.

| Fragments | RF  | Sizebp  | Genotypes | Polymorphism |
|-----------|-----|---------|-----------|--------------|
|           |     |         | Geza7     | Kareem7     | Fowa        | Kaha       | Behira     | Kafr Elshek |             |
| 1         | 0.092 | 5432.261 | 0         | 0           | 0           | 0          | 0          | 1           | Unique       |
| 2         | 0.111 | 3822.460 | 1         | 0           | 1           | 1          | 1          | 1           | Monomorphic  |
| 3         | 0.134 | 2497.878 | 1         | 1           | 1           | 1          | 1          | 1           | Monomorphic  |
| 4         | 0.150 | 1857.951 | 1         | 1           | 1           | 1          | 1          | 1           | Monomorphic  |
| 5         | 0.164 | 1434.051 | 1         | 1           | 1           | 1          | 1          | 1           | Monomorphic  |
| 6         | 0.175 | 170.026  | 1         | 0           | 0           | 0          | 0          | 0           | Unique       |
| 7         | 0.196 | 793.397  | 1         | 1           | 0           | 1          | 0          | 1           | Polymorphic  |
| 8         | 0.220 | 508.962  | 1         | 0           | 1           | 0          | 1          | 0           | Polymorphic  |
| 9         | 0.234 | 392.840  | 1         | 1           | 1           | 0          | 0          | 0           | Polymorphic  |
| 10        | 0.253 | 276.425  | 1         | 0           | 0           | 0          | 0          | 0           | Unique       |
| 11        | 0.297 | 122.490  | 1         | 1           | 1           | 1          | 1          | 1           | Polymorphic  |
| 12        | 0.329 | 67.768   | 1         | 1           | 1           | 1          | 1          | 1           | Polymorphic  |

Detectable fragments: 9, 8, 8, 7, 7, 7

Table 17: Amplified DNA fragments (AF) obtained for the six genotypes using sixth ISSR primers.

| Fragments | RF  | Sizebp  | Genotypes | Polymorphism |
|-----------|-----|---------|-----------|--------------|
|           |     |         | Geza7     | Kareem7     | Fowa        | Kaha       | Behira     | Kafr Elshek |             |
| 1         | 0.123 | 5538.394 | 1         | 0           | 0           | 0          | 1          | 0           | Polymorphic  |
| 2         | 0.153 | 3924.725 | 1         | 0           | 0           | 0          | 1          | 1           | Polymorphic  |
| 3         | 0.167 | 3342.010 | 0         | 1           | 0           | 0          | 0          | 0           | Unique       |
| 4         | 0.170 | 3228.868 | 0         | 0           | 1           | 1          | 1          | 1           | Polymorphic  |
| 5         | 0.187 | 2656.386 | 0         | 0           | 0           | 0          | 0          | 1           | Unique       |
| 6         | 0.192 | 2508.200 | 1         | 0           | 1           | 1          | 0          | 0           | Polymorphic  |
| 7         | 0.208 | 2087.320 | 1         | 1           | 1           | 1          | 1          | 1           | Monomorphic  |
| 8         | 0.222 | 1777.410 | 0         | 0           | 0           | 0          | 0          | 1           | Unique       |
| 9         | 0.224 | 1737.064 | 1         | 1           | 1           | 1          | 0          | 0           | Polymorphic  |
| 10        | 0.240 | 1445.582 | 0         | 0           | 0           | 0          | 1          | 0           | Unique       |
| 11        | 0.243 | 1396.643 | 1         | 0           | 0           | 0          | 0          | 0           | Unique       |
| 12        | 0.251 | 1274.086 | 0         | 1           | 0           | 0          | 0          | 0           | Unique       |
| 13        | 0.254 | 1230.952 | 0         | 0           | 0           | 0          | 1          | 0           | Unique       |
| 14        | 0.269 | 1036.225 | 1         | 0           | 0           | 0          | 0          | 0           | Unique       |
| 15        | 0.287 | 842.770  | 1         | 1           | 1           | 1          | 0          | 0           | Polymorphic  |
| 16        | 0.308 | 662.227  | 1         | 0           | 0           | 1           | 0          | 0           | Polymorphic  |
| 17        | 0.310 | 647.195  | 0         | 1           | 0           | 0           | 0          | 0           | Unique       |
| 18        | 0.325 | 344.814  | 0         | 0           | 0           | 1           | 0          | 0           | Unique       |
| 19        | 0.333 | 497.006  | 1         | 0           | 0           | 0           | 0          | 0           | Unique       |
| 20        | 0.351 | 404.219  | 0         | 1           | 0           | 0           | 0          | 0           | Unique       |
| 21        | 0.365 | 344.203  | 0         | 1           | 0           | 0           | 0          | 0           | Polymorphic  |
| 22        | 0.386 | 270.466  | 0         | 0           | 0           | 1           | 0          | 0           | Unique       |

Detectable fragments: 10, 8, 6, 10, 4, 5
Plate 2: ISSR banding patterns in the six genotypes accessions generated using 6 primers. (1, 2,3,4,5 and 6 for Geza7, Kareem7, Fowa, Kaha, Behira and Kafr El-Shikh, respectively).

Cluster analysis based on morphological traits provides two major groups the first one includes Kaha Cv. and the second includes the rest of the genotypes. Meanwhile, the second cluster divided into 3 sup group the first includes Kafr Elshikh Cv., the second includes Geza7 and Karem7 Cvs. and the third contain Fowa and Behira Lrs (Fig. 2 and Tables 18- 20).

Fig.2: Cluster analysis using UPGMA method depicting genetic similarity (correlation) between six genotypes of cowpea derived from sharing data of morphological. (1, 2,3,4,5 and 6 for Geza7, Kareem7, Fowa, Kaha, Behira and Kafr El-Shikh, respectively).
Fig. 3: Cluster analysis using UPGMA method depicting genetic similarity (Jaccards coefficient) between three genotypes of cowpea derived from band sharing data of RAPD, ISSR and pooled RAPD + ISSR data. (1, 2, 3, 4, 5 and 6 for Geza7, Kareem7, Fowa, Kaha, Behira and Kafr El-Shikh, respectively).

Table 18: Amplified DNA fragments (AF) obtained for the six genotypes using RAPD and ISSR primers:

| Markers   | Primer | MB | PB | UB | TAF | P%  |
|-----------|--------|----|----|----|-----|-----|
| RAPD      | 1      | 0  | 6  | 15 | 21  | 28.6|
|           | 2      | 0  | 2  | 15 | 17  | 11.8|
|           | 3      | 0  | 1  | 15 | 16  | 6.3 |
|           | 4      | 0  | 7  | 20 | 27  | 25.9|
|           | 5      | 0  | 8  | 9  | 17  | 47.1|
| Total AF  | 0      | 24 | 74 | 98 |     | 24.5|
| %         | 0      | 4.8| 14.8| 19.6|    | 4.9 |
| ISSR      | 1      | 0  | 5  | 16 | 21  | 23.8|
|           | 2      | 0  | 4  | 13 | 17  | 23.5|
|           | 3      | 0  | 5  | 12 | 17  | 29.4|
|           | 4      | 4  | 5  | 1  | 10  | 50.0|
|           | 5      | 3  | 6  | 3  | 12  | 50.0|
|           | 6      | 1  | 8  | 13 | 22  | 36.4|
| Total AF  | 8      | 33 | 58 | 99 |     | 33.3|
| %         | 1.3    | 5.5| 9.7| 16.5|    | 33.3|
| Total(RAPD+ISSR) AF | 8    | 57 | 132| 197|     | 28.9|
Table 19: Amplified specific DNA fragments (AF) obtained for six genotypes using RAPD and ISSR primers.

| Primers | Genotypes | RAPD | Total |
|---------|-----------|------|-------|
|         | Geza7 (Cv.) | Kareem7(Cv.) | Fowa (Lr.) | Kaha (Cv.) | Behira (Lr.) | Kafr Elshek (Cv.) |       |
| 1       | 4          | 3     | 1     | 2     | 2     | 3     | 15    |
| 2       | 1          | 5     | 1     | 3     | 3     | 2     | 15    |
| 3       | 3          | 1     | 3     | 3     | 1     | 4     | 15    |
| 4       | 5          | 1     | 4     | 3     | 4     | 3     | 20    |
| 5       | 3          | 0     | 2     | 2     | 1     | 1     | 9     |
| Total   | 16         | 10    | 11    | 13    | 11    | 13    | 74    |
| ISSR    |            |       |       |       |       |       |       |
| 1       | 4          | 2     | 1     | 4     | 2     | 3     | 16    |
| 2       | 5          | 0     | 4     | 1     | 1     | 2     | 13    |
| 3       | 0          | 1     | 2     | 2     | 3     | 4     | 12    |
| 4       | 0          | 0     | 0     | 0     | 0     | 1     | 1     |
| 5       | 1          | 0     | 0     | 0     | 0     | 2     | 3     |
| 6       | 3          | 4     | 0     | 4     | 0     | 2     | 13    |
| Total   | 13         | 7     | 7     | 11    | 6     | 14    | 58    |
| Total (RAPD+ISSR) | 29 | 17 | 18 | 24 | 17 | 27 | 132 |

Table 20: Amplified DNA fragments (AF) obtained for the six genotypes using RAPD and ISSR primers.

| Primers | Genotypes | RAPD | Total |
|---------|-----------|------|-------|
|         | Geza7 (Cv.) | Kareem7(Cv.) | Fowa (Lr.) | Kaha (Cv.) | Behira (Lr.) | Kafr Elshek (Cv.) |       |
| 1       | 7          | 5     | 3     | 6     | 3     | 5     | 29    |
| 2       | 3          | 6     | 1     | 3     | 4     | 2     | 19    |
| 3       | 3          | 1     | 4     | 3     | 1     | 5     | 17    |
| 4       | 6          | 4     | 5     | 9     | 6     | 4     | 34    |
| 5       | 7          | 1     | 9     | 9     | 2     | 5     | 33    |
| Total   | 26         | 17    | 22    | 30    | 16    | 21    | 132   |
| ISSR    |            |       |       |       |       |       |       |
| 1       | 7          | 4     | 3     | 6     | 5     | 4     | 29    |
| 2       | 6          | 2     | 8     | 3     | 2     | 3     | 24    |
| 3       | 2          | 5     | 3     | 6     | 3     | 7     | 26    |
| 4       | 6          | 6     | 8     | 9     | 9     | 9     | 47    |
| 5       | 9          | 8     | 8     | 7     | 7     | 7     | 46    |
| 6       | 10         | 8     | 6     | 10    | 4     | 5     | 43    |
| Total   | 40         | 33    | 36    | 41    | 30    | 35    | 215   |
| Total(RAPD+ISSR) | 66 | 50 | 58 | 71 | 46 | 56 | 347 |
All three methods assessed a high level of genetic variations. Based on combined results for morphological and molecular genetic diversity estimates, genotype Kafr el-sheikh and fowa were distinct from other genotypes and can be exploited to harness their unique features in breeding programs. Genotypes swapped among different clusters in different methods of clustering (Table 21). Rahman et al. (2011) reported that genotypes also swapped from one cluster to another cluster among different methods and this pattern is somewhat irregular. These differences are not an indicator of the failure or limitation or weakness of the methods (Roldán-Ruiz, et. al., 2001). These results may be due to the diversity at the molecular level, which may not reflect the diversity at the morphological or physiological level, as described by Karhu et al. (1996). Another possible reason for this variation in clustering might be the environmental influence and genotype-environment interaction. Compared to morphological and physiological characteristics, the DNA genome provides a direct comparison of genetic diversity at the DNA level, is phenotypically neutral and is not modified by environment and management practices (Messmer et. al., 1993). Morphological and physiological characters are the ultimate expression of the molecular constitution of a variety where a number of biochemical processes are involved. So where a number of biochemical processes are involved. So different types of clustering in different methods are not unusual (Han-yong et. al., 2004).

Table 21: Grouping of genotypes on the basis of morphological and molecular data by using PAST4.03programe:

| Morphological groups | genotypes | RAPD groups | genotypes | ISSR groups | genotypes | Common genotypes |
|----------------------|-----------|-------------|-----------|-------------|-----------|-----------------|
| A                    | Kaha      | A           | kafrelshik| A           | kafrelshik | kafrelshik      |
| B                    | Geza7, Kareem 7, Fowa, Behira and kafrelshik | B           | Behira    | B           | Geza7, Kareem 7, Fowa, Behira and kaha | Geza7, Kareem 7, Fowa, Behira |
| B1                   | kafrelshik | C           | Geza7, Kareem 7, Fow and kaha | B1         | Kareem7 and kaha   | Kareem7 and kaha |
| B2                   | Geza7 and Kareem 7 | C1          | Geza7 and Kareem 7 | B2         | Fow and behira    | Geza7 and Kareem 7 |
| B3                   | Fow and Behira | C2          | Fow and kaha |            |           | Fowa            |

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تقدير معامل الاختلاف وبعض المقارن الوراثية لبعض الأصناف البلدية من اللوبيا

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تم إجراء هذا البحث خلال موسمين صيفيين متتاليين لعامي 2019 و 2020 في كلية الزراعة (سابة باشا)، جامعة الإسكندرية ومختبر بذور الخضروات بمحطة بحوث البساتين بالصبيحة، الإسكندرية، مصر؛ لتقييم ستة أصناف وسلالات محلية من اللوبيا من ناحية بعض الصفات المورفولوجية والمكونات والمحصول ومكوناتها وتقييم بعض المتغيرات الوراثية.

وقد أظهرت النتائج اختلافات واضحة بين التراكيب الوراثية الستة للوبيا في معظم الصفات المدروسة. كما كان معامل الاختلاف (C.V.) أقل من 10% لجميع الصفات المدروسة في جميع التراكيب الوراثية المدروسة لللوبيا. وتشير هذه النتائج إلى أن التراكيب الوراثية من اللوبيا متطابقة وراثيا فيما يتعلق بهذه الصفات. أظهر تحليل التباين أن تباين التراكيب الوراثية كان ذا دلالة عالية في جميع الصفات المدروسة. تشير النتائج إلى وجود اختلافات كبيرة بين التراكيب الوراثية في جميع الصفات المدروسة بشكل عام، فقد تفيد النتائج أن جميع الصفات المدروسة يمكن تحسينها من خلال طريقة الانتخاب، ولكن بدرجات مختلفة من التحسن اعتمادا على مقدار التباين الموجودة في كل مجموعة. وفي الوقت نفسه، كان متوسط مربعات السنوات معنويًا فقط في ارتفاع الزهرة الأولى، ويمكن تفسير ذلك على أن هذه الخاصية تتأثر بالظروف البيئية المختلفة في كل عام. مثلاً، قد تتميز الظروف الجوية في السنة الأولى إلى مجموعات رئيسيتين. الأول يحتوي على صنف جيزة وأكرم 7 بنسبة تشابه (30%) والثانية تتكون من السلالات فو وصنف جيزة، وقد تجري بطرق التربية المناسبة.

و أبرزت النتائج عن أن صنف جيزة وفوه قد يتم الاستفادة بصفاتهم وتمييزهم بطرق التربية المناسبة.