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# Molecular Characterization of some Mung Bean (*Vigna rediata* L.) Genotypes by Using ISSR Technique

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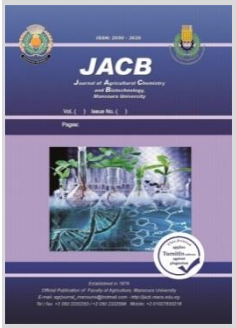


## ABSTRACT

The present study was carried out to evaluate ten genotypes of mung bean for ten important agricultural characteristics. Ten mung bean genotypes were obtained from Bahteem Research Station of the Field Crop Research Institute in Giza in 2019. The complete randomized block design was performed with four replicates, the best genotype selection was made on the basis of ten important agricultural characteristic. Data were recorded for, plant height (cm), branches' number per plant, number of fertile nods per plant, number of pods per nods, pods length (cm), number of seeds per pod, number of pods per plant, number of seeds per plant, seed weight per plant (g) and 100-seed weight (g). The results indicated that there were slightly important differences between the genotypes and genetic diversity was observed for the studied traits and thus, there is an opportunity for genetic improvement. Molecular markers analysis evaluated using Inter Simple Sequence Repeat (ISSR) with ten primers were used on ten mung bean genotypes. A high level of polymorphism was found, where 65 polymorphic bands and 52 monomorphic bands were detected out of total 117 bands. The results showed that there is a high degree of genetic similarity ranged between 0.88 and 0.76 where the highest was 0.88 between genotypes 1, 2 and the least was 0.76 between genotypes 4, 5 and 2, 9.

***Keywords:*** Mung bean (*Vigna rediata* L.) - Genetic variability – ISSR marker – Dendrogram.

## INTRODUCTION

Mung bean (*Vigna rediata* L.) is one of the famous plants in China and India, belongs to family Leguminosae and cultivated mostly in South, East and Southeast Asia with chromosome number of 2n=2x=22 (Lixia *et al*., 2016). It is a good source of nutritional balance and contains high levels of both minerals such as calcium, potassium, iron, manganese, copper, magnesium and vitamins such as A, B, C, E and K. In addition, it is easy in digestion, it has low calories, high fiber content, low fat content, and it is an excellent source of antioxidants and phytonutrients. Also, in some early research suggests that bean has a variety of health benefits. It reduces the formation and accumulation of cholesterol in the walls of arteries and capillaries, which reduces the risk of cardiovascular disease. Moreover, it reduces blood sugar and protects against cancer (Dianzhi *et al*., 2019). Previous studies were used to estimate the morphological characteristics and showed that the germplasm restricted a low level of diversity in the working genes in Mung bean (Ramanujam, 1981). In general, the most responsive to genetic improvement are parents who have the most genetic diversity (Arunachalam, 1981). One of the typical techniques that give a quickly and stable results even if they are conducted in multiple places is Inter Simple Sequence Repeat (ISSR) technique, which depends on the Polymerization Chain Reaction (PCR) that requires a small amount of DNA. This technique was used to study the genetic variability in rice (Joshi *et al*., 2000) and potatoes (Bornet *et al*., 2002). ISSR have been utilized successfully in population genetic studies for a variety of organisms including clonal plants (King *et al*., 2002 and Wang *et al*., 2004). However, from the foundation to be examined and characterization of the genetic structure of the nature and extent of genetic diversity content by using the parental genotypes containing a large amount of genetic diversity. Several other researchers have studied genetic divergence in mung bean (Thulasidas 1984; Sharma *et al*., 1996 and Bish *et al*., 1998), sorghum (Mehdi & Asghar 1999), cotton (Ponitha & Raveendram 2000), rice (Mahapatra *et al*., 1995; Bharawadraj *et al*., 2001 and Cheema *et al*., 2004) and wheat (Peleg *et al*., 2005 and Martynov *et al*., 2006). Currently, ISSR is widely being used for line identification and genetic variability between various genotypes (Zhang and Dai, 2010; Xavier *et al*., 2011 and Abdel Khalik *et al*., 2012).

The aim of this study is to examine the genetic variability of ten mung bean genotypes by using ISSR technique and to select the best studied mung bean genotypes that show unique parameters and that is recommended to use them in breeding programs to produce high yield and good quality varieties.

## MATERIALS AND METHODS

### Genotypes and experimental design

Ten genotypes (Table 1) of Mung bean (*Vigna rediata* L.) were obtained from Agriculture Research Centre, Field Crops Research Institute, Genetic Resources Department, Bahteem, Egypt. In season 2018 in a randomized complete block design in four replications. The land was planned by 12 lines in the

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two reeds, and the mung bean was planted on two feathers in the line, and the plants are in the gores the distance between each round is 20 cm, and in each round, two seeds where about thirty three plants per square meter, meaning 140 thousand plants per acre. For each replicate, 15 plants were taken randomly, and data recorded for plant height (cm), branches' number, number of fertile nods per plant, number of pods per nods, pods length (cm), number of seeds per pod, number of pods per plant, number of seeds per plant, seed weight per plant (g) and 100-seed weight (g), the data were analyzed statistically. Then genetic analyzes were performed on the ten genotypes using ISSR technique with ten primers.

**Table 1. Origin of the ten mung bean genotypes and their pedigree were used in the present study.**

| No. | Genotypes | Pedigree | Origin |
|---|---|---|---|
| 1 | L.1 | Line 63 | India |
| 2 | L.2 | Line 73 | India |
| 3 | L.3 | Line74 | India |
| 4 | L.4 | Line 80 | India |
| 5 | L.5 | Line 83 | India |
| 6 | L.6 | Line 111 | India |
| 7 | L.7 | Line 275 | India |
| 8 | L.8 | Line 299 | India |
| 9 | L.9 | Line 301 | India |
| 10 | L.10 | Line 304 | India |

**Then genetic analyzes were performed on the ten genotypes using ISSR technique with ten primers.**

**DNA extraction and purification CTAB**

DNA was extracted according to Porebski *et al*. (1997) with some modifications: 100 mg of frozen leaf tissue were grinded in a mortar with liquid $N_2$. 1 ml of CTAB buffer [100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 2% (w/v) CTAB (Hexa decyl triammonium bromide) and 0.3% β-mercaptoethanol were added immediately before use] was added to the grinded tissue and mixed with pestle. The solution was then transferred to 1.5 ml tube and 2µl of RNase were added. Samples were incubated at 65°C for 30 min. The supernatant was collected in new 1.5 ml tube after centrifugation for 5 min at 10000 rpm. 500µl of 24:1 Chloroform- Isoamyl Alcohol were added and mixed well. The aqueous phase was transferred carefully to a new labeled 1.5 ml tube.

Same volume of cold isopropanol was added and incubatedat-20 ºC for 45 min to an hour. DNA pellet was collected after centrifugation 5 min at maximum speed. 700 µl of cold 70% ethanol was added to wash the pellet. The pellet was dried in a vacuum centrifuge or on a hot plate at 55°C. The dry pellet was allowed to resuspend with 100 µl of water ($dH_2O$) for 1hr at 55°C before using.

**Estimation for the DNA concentration:**

The concentration of genomic DNA was estimated by NanoDrop (machine ID) and the concentration was adjusted, if necessary, with distilled water to (10ng) for PCR amplification.

**Table 2. List of ISSR primers sequences**

| Primers | Sequences |
|---|---|
| ISSR- 1 | 5'-AGAGAGAGAGAGAGAGYC-3' |
| ISSR- 3 | 5'-ACACACACACACACACYT-3' |
| ISSR- 5 | 5'-GTGTGTGTGTGTGTGTYG-3' |
| ISSR- 6 | 5'-CGCGATAGATAGATAGATA-3' |
| ISSR- 7 | 5'-GACGATAGATAGATAGATA-3' |
| ISSR- 9 | 5'-GATAGATAGATAGATAGC-3' |
| ISSR- 10 | 5'-GACAGACAGACAGACAAT-3' |
| ISSR- 14 | 5'-CTCCTCCTCCTCCTCTT-3' |
| ISSR- 19 | 5'-HVHTCCTCCTCCTCCTCC-3' |
| ISSR- 20 | 5'-HVHTGTGTGTGTGTGTGT-3' |

**A: Adenine, T: Thymine, G: Guanine, C: Cytosine, Y: (C or T), H: (A or C or T), V :( A or C or G) and R: (A or G)**

**ISSR-PCR Reactions**

A set of 10 ISSR primers Table 2 was used in the detection of polymorphism. The amplification reaction was carried out in 25 µl reaction volume containing 12.5 µl master mix (sigma), 2.5 µl primer (10 pcmol), 3 µl template DNA (10 ng) and 7 µl $dH_2O$.

**Thermocyling Profile PCR:**

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (*PE* Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 36ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

**Detection of the PCR Products**:

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at (75 mMTris , 90 mM Boric acid , 2 mM Na2-EDTA) 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000) as in Figure 1 and 2.

Primers' data was scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. If a product was present in a genotype, it was designated as "1", if it was absent it was designated as "0" for each primer and was used to count genetic similarity matrix. Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products

Genetic kinship tree was created between the ten genotypes of mung bean depending on the Jaccard coefficient of similarity (Jaccard similarity index) according to Jaccard (1908). It was divided according to the characteristics of genetic and environmental conditions.

## RESULTS AND DISCUSSION

The data in Table 3 showed the mean values for plant height, branches' number per plant, number of fertile nods/plant, number of pods/nods and pods length for ten genotypes of mung bean. It could be noticed that the mean values of plant height ranged from 67.75 to 95 cm and the highest values were recorded in genotypes 5, 1 and 9 with mean values of 95, 93.75 and 92.5cm, respectively. Also, it could be observed that the highest branches' number per plant was recorded in genotype 6 followed by genotype 7 with mean values of 4.75 and 4.5, respectively compared

with other studied genotypes. Furthermore, the results for branches' number per plant showed that no significant differences were found between the studied genotypes No. 1, 3, 8, 9 and 10, respectively. From the obtained data, it's clear that genotype 10 followed by genotype 7 had the highest number of fertile nods per plant with mean values of 39.25 and 36.75, respectively. While, the lowest mean values were recorded in genotype No. 5 and genotype 6 with mean values of 21 and 19.5, respectively. As shown in the same Table, no significant changes were observed in number of pods per nods mean values between genotypes No. 3, 6, 7, 8 and 9, respectively. The results in Table 3 indicated that pods length for different studied genotypes of mung bean ranged from 6.75 to 9.75 cm and the highest mean values recorded in genotypes No. 2, 4 and 7 had the lowest mean values of pods length recorded in genotypes No. 6 and 5, respectively.

From the obtained data in Table 3 continue, it could be noticed that there were slightly significant ($P \leq 0.05$) differences in number of seeds per pods between all studied genotypes and the highest mean value was recorded in genotype No. 10 compared with other studied genotypes. The aforementioned results in the same Table declared that mean values for number of pods per plant which counted in ten studied genotypes ranged from 44.25 and 110 pods and the highest mean values recorded in genotype No. 8 followed by genotype No. 7 with mean values of 110 and 99.5 pods, respectively, while the lowest mean values were recorded in genotypes No. 1 and 6. Referring to data in Table 1 it was clear that the highest mean value of number of seeds per plant was recorded in genotype No. 8 with mean value of 1420 compared with other studied genotypes. The lowest mean value of seed weight per plant was observed in genotype 1with mean value of 19.675 g.

Results given in Table 3 revealed that no significant differences were found in 100-seed weight between the studied genotypes No. 1, 2, 3, 6 and 8. On the other hand, the highest mean value 9.4 g was recorded in genotype No. 5 and the lowest mean value was recorded in genotypes No. 10 and 4, respectively compared with other studied genotypes.

**Table 3. The mean values (mean ± SD, n= 3) within a column with a same superscript were significantly different ($P \leq 0.05$) of ten genotypes for ten studies traits in mung bean.**

| Traits<br>Genotypes | Plant height (cm) | branches' number | Number of fertile nods per plant | Number of Pods per nods | Pods length (cm) |
|---|---|---|---|---|---|
| 1 | 93.75 ± 2.5 $^{a}$ | 3.25 ± 0.5 $^{c}$ | 24.75 ± 3.304 $^{d}$ | 4.25 ± 0.5 $^{b}$ | 7.75 ± 0.5 $^{d}$ |
| 2 | 67.75 ± 2.63 $^{c}$ | 3.75 ± 0.5 $^{bc}$ | 32.75 ± 2.630 $^{b}$ | 4.5 ± 0.577 $^{ab}$ | 9.75 ± 0.5 $^{a}$ |
| 3 | 82.5 ± 2.887 $^{b}$ | 3.5 ± 0.577 $^{c}$ | 25 ± 1.633 $^{d}$ | 5.5 ± 0.577 $^{a}$ | 8.25 ± 0.5 $^{cd}$ |
| 4 | 72.5 ± 2.887 $^{c}$ | 3.75 ± 0.5 $^{bc}$ | 28.75 ± 1.708 $^{c}$ | 4.5 ± 0.577 $^{ab}$ | 9.5 ± 0.577 $^{a}$ |
| 5 | 95 ± 5.774 $^{a}$ | 3.75 ± 0.5 $^{bc}$ | 19.5 ± 1.291 $^{e}$ | 4.75 ± 0.957 $^{ab}$ | 6.875 ± .250 $^{e}$ |
| 6 | 72.5 ± 2.887 $^{c}$ | 4.75 ± 0.5 $^{a}$ | 21 ± 1.826 $^{e}$ | 5.5 ± 0.577 $^{a}$ | 6.75 ± .645 $^{e}$ |
| 7 | 72.5 ± 2.887 $^{c}$ | 4.5 ± 0.577 $^{ab}$ | 36.75 ± 2.986 $^{a}$ | 5.5 ± 0.577 $^{a}$ | 9.625 ± .479 $^{a}$ |
| 8 | 82.5 ± 2.887 $^{b}$ | 3.5 ± 0.577 $^{c}$ | 24.5 ± 3.109 $^{d}$ | 5.25 ± 0.5 $^{a}$ | 9.125 ± .629 $^{ab}$ |
| 9 | 92.5 ± 2.887 $^{a}$ | 3.5 ± 0.577 $^{c}$ | 31 ± 1.414 $^{bc}$ | 5.5 ± 0.577 $^{a}$ | 7.75 ± .289 $^{d}$ |
| 10 | 82.5 ± 2.887 $^{b}$ | 3.5 ± 0.577 $^{c}$ | 39.25 ± 0.957 $^{a}$ | 4.75 ± 0.5 $^{ab}$ | 8.75 ± .289 $^{bc}$ |

**Table 3. continue**

| Traits<br>Genotypes | Number of seeds per pod | Number of pods per plant | Number of seeds per plant | Seed weight per plant (g) | 100- seed weight (g) |
|---|---|---|---|---|---|
| 1 | 9.25 ± 0.5 $^{de}$ | 51 ± 2.944 $^{e}$ | 477.75 ± 26.588 $^{def}$ | 19.675 ± 1.31 $^{ef}$ | 4.075 ±0.096 $^{d}$ |
| 2 | 10.25 ± 0.5 $^{bcd}$ | 85 ± 5.774 $^{bc}$ | 893.75 ± 30.380 $^{b}$ | 34.65 ± 3.363 $^{c}$ | 4.08 ± 0.340 $^{d}$ |
| 3 | 9.25 ± 0.957 $^{cde}$ | 61 ± 1.826 $^{d}$ | 585.75 ± 17.858 $^{cde}$ | 22.325 ± 1.982 $^{e}$ | 3.775 ±0.359 $^{d}$ |
| 4 | 11.25 ± 0.5 $^{ab}$ | 83.5 ± 2.646 $^{c}$ | 797.25 ± 11.758 $^{bc}$ | 22.7 ± 2.264 $^{e}$ | 2.8 ± 0.283 $^{e}$ |
| 5 | 10.25 ± 0.5 $^{bcd}$ | 33.5 ± 2.646 $^{f}$ | 323.25 ± 5.377 $^{f}$ | 30.525 ±0.793 $^{d}$ | 9.4 ± 0.294 $^{a}$ |
| 6 | 9 ± 0.816 $^{e}$ | 44.25 ± 3.304 $^{e}$ | 397.5 ± 12.583 $^{ef}$ | 17.275 ± 1.742 $^{f}$ | 4.3 ± 0.455 $^{d}$ |
| 7 | 9.75 ± 0.957 $^{cde}$ | 99.5 ± 11.150 $^{a}$ | 1000.2 ± 11.518 $^{b}$ | 50.25 ± 2.167 $^{b}$ | 4.95 ± 0.173 $^{c}$ |
| 8 | 10.5 ± 0.577 $^{abc}$ | 110 ± 7.483 $^{a}$ | 1420 ± 446.393 $^{a}$ | 55.225 ± 1.613 $^{a}$ | 4.275 ±1.024 $^{d}$ |
| 9 | 9.5 ± 0.577 $^{cde}$ | 63.5 ± 2.380 $^{d}$ | 623.75 ± 25.617 $^{cd}$ | 35.575 ± 1.410 $^{c}$ | 5.65 ± 0.289 $^{b}$ |
| 10 | 11.5 ± 0.577 $^{a}$ | 91.75 ± 2.872 $^{b}$ | 869 ± 19.339 $^{b}$ | 22.675 ± 1.846 $^{e}$ | 2.675 ±0.359 $^{e}$ |

**Biodiversity analysis based on ISSR analysis**

**ISSR analysis:**

Ten ISSR primers used in the present investigation with ten examined genotypes Figure 1 produced a total of 117 bands in the profiles Table 15 and Figure 2. The primers ISSR 1, ISSR 3, ISSR 5, ISSR 7, ISSR 9 and ISSR 10 have produced unique bands. In Table 5 there was a unique band with the primer ISSR 1 at the molecular weight 570bp. Also, in Table 6 there was a unique band with the primer ISSR 3 at the molecular weight 1400bp and at the molecular weight 290bp, there was a unique

band with the primer ISSR 5 in Table 7. Likewise, in Table 9 there was a unique band with the primer ISSR 7 at the molecular weight 410bp. As well as, in Table 10 at the molecular weight 810bp there was a unique band with the primer ISSR 9. In addition, at the molecular weight 1050bp there was a unique band with the primer ISSR 10 in Table 11. While, in the rest Tables 8, 12, 13 and 14 there wasn't any unique bands so, it recommends using the primers that were given the unique bands.

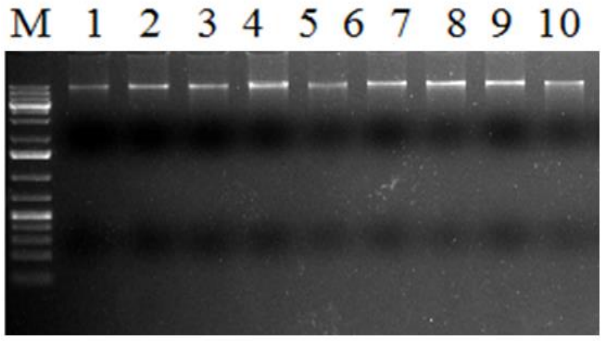


**Fig. 1.Concentration DNA using Agarose Gel**

**Table 4. Concentration DNA using NanoDrop**

| Sample | Concentration |
|---|---|
| 1 ( L.1) | 345.2 |
| 2 ( L.2) | 332.4 |
| 3 ( L.3) | 355.6 |
| 4 ( L.4) | 383.7 |
| 5 ( L.5) | 361.1 |
| 6 ( L.6) | 311.5 |
| 7 ( L.7) | 298.2 |
| 8 ( L.8) | 352.1 |
| 9 ( L.9) | 308.6 |
| 10 ( L.10) | 320.5 |

The highest total number of bands (17) resulted by primer ISSR 3, while the lowest total number of bands (8) revealed by primer ISSR 7. These results were in agreement with that obtained by Khalil *et al.*, (2010). These results indicated the existence of high genetic diversity as a result of the high polymorphism in the mung bean genotypes. The percentage of polymorphism is ranged from 33% (ISSR 6 and ISSR 20) to 67% (ISSR 1), but in (ISSR 5 and ISSR 9) the percentage of polymorphism was 55% and the average of polymorphism of ten mung bean genotypes was (54.3%). Total average of mean band frequency was 0.65 ranged from 0.8 to 0.6, the highest and lowest mean of band frequency values were found for primers ISSR 20 and ISSR (3, 5, 7, 9, 10 and 19), these results were in agreement with that repeated by Das *et al*., (2014) who found that the usage of ten primers amplified a total number of 353 bands under 93 loci through five mung bean genotypes with an average of 9.3 loci/primer showing an overall polymorphism of 52.7%. (Singh *et al*., 2011) also studied ISSR markers that were applied to study the polymorphism of mung bean genotypes. They obtained that the polymorphism percentage had ranged from 25 to 85% with an average of 58.3% among all genotypes. (Tantasawat *et al*., 2010) studied genetic variability and affinity in mung bean and five blackgram (*Vigna mango* L.) genotype by ISSR markers. (Heakel, 2019) suggested that a recent study was carried out on some Egyptian canola genotypes, could utilize ISSR primers for differentiating genotypes, leading to generate ISSR molecular markers, related to high seed yield/plant, and consequently oil percentage.

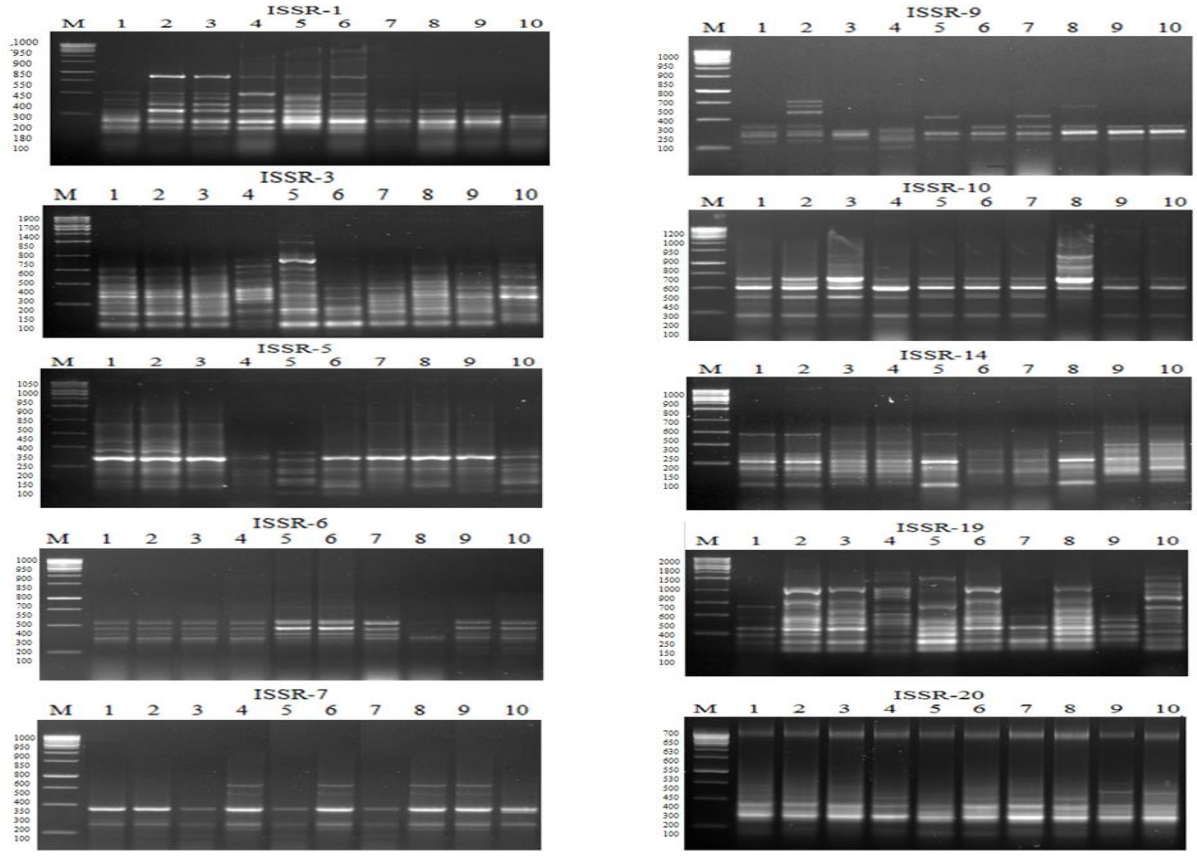


**Fig. 2. DNA polymorphism using ISSR with ten primers in ten genotypes of mung bean (*Vigna rediata* L.).**

**Table 5. Primer ISSR-1, molecular weight (bp) and presence or absence bands for ten of mung bean (*Vigna rediata* L.) genotypes.**

| ISSR-1 | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MW | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
| 850 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0.7 |
| 570 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.1 |
| 480 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0.6 |
| 430 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.3 |
| 380 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0.2 |
| 330 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0.9 |
| 260 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 220 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0.5 |
| 200 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 180 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 160 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 133 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0.7 |

**Table 6. Primer ISSR-3, molecular weight (bp) and presence or absence bands for ten of mung bean (*Vigna rediata* L.) genotypes.**

| ISSR-3 | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MW | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
| 1400 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.1 |
| 850 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0.2 |
| 760 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 710 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0.8 |
| 560 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0.7 |
| 500 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0.2 |
| 470 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0.3 |
| 430 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 380 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0.3 |
| 350 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 310 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 280 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 260 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0.6 |
| 230 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0.6 |
| 220 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0.3 |
| 200 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0.7 |
| 155 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |

**Table 7. Primer ISSR-5, molecular weight (bp) and presence or absence bands for ten of mung bean (*Vigna rediata* L.) genotypes.**

| ISSR-5 | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MW | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
| 850 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0.5 |
| 540 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0.4 |
| 440 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0.3 |
| 250 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 350 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 400 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 220 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.3 |
| 290 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| 180 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 150 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 129 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0.4 |

**Table 8. Primer ISSR-6, molecular weight (bp) and presence or absence bands for ten of mung bean (*Vigna rediata* L.) genotypes.**

| ISSR-6 | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MW | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
| 770 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0.2 |
| 550 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0.3 |
| 530 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 470 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 430 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 380 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 350 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 320 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 267 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0.2 |

**Table 9. Primer ISSR-7, molecular weight (bp) and presence or absence bands for ten of mung bean (*Vigna rediata* L.) genotypes.**

| ISSR-7 | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MW | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
| 820 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0.4 |
| 630 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0.4 |
| 450 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 410 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.1 |
| 320 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 300 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 280 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0.4 |
| 228 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0.5 |

**Table 10.Primer ISSR-9, molecular weight (bp) and presence or absence bands for ten of mung bean (*Vigna rediata* L.) genotypes.**

| ISSR-9 | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MW | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
| 810 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 |
| 700 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0.2 |
| 570 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 540 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0.2 |
| 410 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0.7 |
| 380 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 340 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 300 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 252 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 |

**Table 11. Primer ISSR-10, molecular weight (bp) and presence or absence bands for ten of mung bean (*Vigna rediata* L.) genotypes.**

| ISSR-10 | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MW | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
| 1200 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0.2 |
| 1050 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0.1 |
| 930 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0.2 |
| 800 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0.2 |
| 650 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 620 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 510 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 490 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 420 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0.3 |
| 370 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 300 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 |
| 230 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 210 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 183 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.3 |

**Table 12.Primer ISSR-14, molecular weight (bp) and presence or absence bands for ten of mung bean (*Vigna rediata* L.) genotypes.**

| ISSR-14 | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MW | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
| 790 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0.4 |
| 610 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0.6 |
| 500 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0.4 |
| 370 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 340 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0.3 |
| 310 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 270 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 250 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0.6 |
| 210 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 180 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 170 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0.3 |
| 150 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0.4 |
| 140 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0.6 |

**Table 13. Primer ISSR-19, molecular weight (bp) and presence or absence bands for ten of mung bean (*Vigna rediata* L.) genotypes.**

| ISSR-19 | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MW | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
| 1500 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0.2 |
| 1050 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0.6 |
| 930 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 700 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0.2 |
| 660 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0.5 |
| 590 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0.5 |
| 500 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0.4 |
| 460 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 |
| 400 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0.6 |
| 360 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0.6 |
| 300 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 250 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 200 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 180 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 160 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |

**Table 14. Primer ISSR-20, molecular weight (bp) and presence or absence bands for ten of mung bean (*Vigna rediata* L.) genotypes.**

| ISSR-20 | Genotypes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MW | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
| 660 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 620 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0.4 |
| 560 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.2 |
| 500 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0.6 |
| 410 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 340 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 300 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 250 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |
| 200 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.0 |

**Table 15. Ten ISSR primers present in this study, monomorphic bands (MB), polymorphic bands (PB), total bands (TB), unique bands, percentage of polymorphic bands (PB%) and mean of band frequency.**

| Primers | MB | PB (with.unique) | PB (without.unique) | TB | Unique bands | PB% | Mean of band frequency |
|---|---|---|---|---|---|---|---|
| ISSR- 1 | 4 | 8 | 7 | 12 | 1 | 67 | 0.7 |
| ISSR- 3 | 6 | 11 | 10 | 17 | 1 | 65 | 0.6 |
| ISSR- 5 | 5 | 6 | 5 | 11 | 1 | 55 | 0.6 |
| ISSR- 6 | 6 | 3 | 3 | 9 | 0 | 33 | 0.7 |
| ISSR- 7 | 3 | 5 | 4 | 8 | 1 | 63 | 0.6 |
| ISSR- 9 | 4 | 5 | 4 | 9 | 1 | 55 | 0.6 |
| ISSR- 10 | 7 | 7 | 6 | 14 | 1 | 50 | 0.6 |
| ISSR- 14 | 5 | 8 | 8 | 13 | 0 | 62 | 0.7 |
| ISSR- 19 | 6 | 9 | 9 | 15 | 0 | 60 | 0.6 |
| ISSR- 20 | 6 | 3 | 3 | 9 | 0 | 33 | 0.8 |
| Total | 52 | 65 | 59 | 117 | 6 | 543 | 6.5 |
| Average | 5.2 | 6.5 | 5.9 | 11.7 | 0.6 | 54.3 | 0.65 |

**Similarity matrices based on ISSR markers:**

The relationships among the ten genotypes of mung bean (*Vigna rediata* L.) used in this study were estimated by a UPGMA cluster analysis of genetic similarity matrices. The composition of clusters obtained using ISSR markers were presented in Fig. 3 and Table 16. Cluster analysis using ISSR data grouped the ten mung bean genotypes into two main groups with Jaccard's similarity coefficient ranging from 0.0 to 1.0**.** The similarity exhibited among genotypes ranged from 0.76 to 0.88. The maximum value (0.88) was recorded between L1 and L2, while the minimum value (0.76) was recorded between L2, L9 and L4, L5. Data resulted in this study offered a medium level of similarity between (L1, L8), (L1, L9), (L2, L5), (L3, L9) and (L4, L8).

The first group consisted of two sub-groups and included four mung bean genotypes, while the second group consisted also of two sub-groups but included six mung bean genotypes. These results, suggested that cluster analysis of ISSR markers used genetically to distinguish between mung bean genotypes, Therefore, a large number of primers were suggested to be used. These results were in agreement with those obtained by (Koch *et al*., 1999; Singh *et al*. 2011 and kaur *et al*. 2016). Generally, there is genetic diversity between genotypes as well as a high level of genetic similarity that can be used as a selection tool in breeding programs.

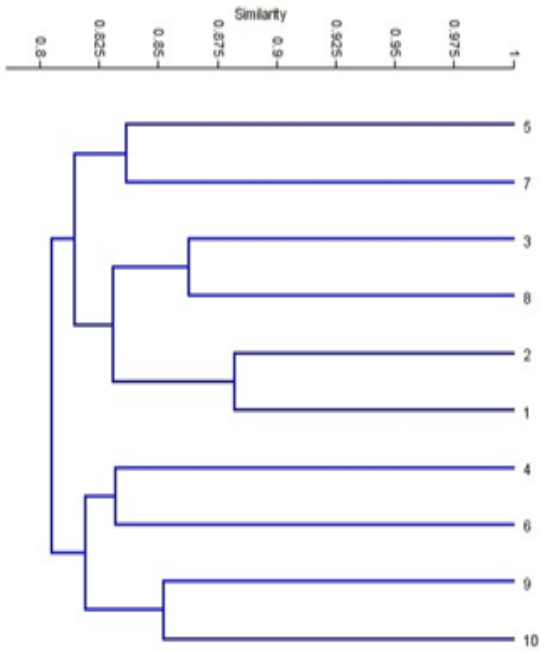


**Fig. 3. Dendrogram constructed with UPGMA from ten studied genotypes of mung bean using ISSR technique based on jaccard similarity coefficient.**

**Table 16. Similarity matrices based on Jaccard similarity coefficients of ISSR markers of ten studied genotypes of mung bean.**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.000 | | | | | | | | | |
| 2 | 0.88 | 1.000 | | | | | | | | |
| 3 | 0.81 | 0.83 | 1.000 | | | | | | | |
| 4 | 0.77 | 0.80 | 0.83 | 1.000 | | | | | | |
| 5 | 0.83 | 0.82 | 0.81 | 0.76 | 1.000 | | | | | |
| 6 | 0.83 | 0.81 | 0.80 | 0.83 | 0.81 | 1.000 | | | | |
| 7 | 0.84 | 0.83 | 0.79 | 0.78 | 0.84 | 0.83 | 1.000 | | | |
| 8 | 0.82 | 0.87 | 0.86 | 0.82 | 0.80 | 0.84 | 0.79 | 1.000 | | |
| 9 | 0.82 | 0.76 | 0.82 | 0.85 | 0.79 | 0.84 | 0.85 | 0.80 | 1.000 | |
| 10 | 0.79 | 0.78 | 0.84 | 0.79 | 0.80 | 0.80 | 0.79 | 0.80 | 0.85 | 1.000 |

ISSR technique is simple, speedy, reliable, exact, and synchronous in detection of polymorphism at many sites in genome using small amount of DNA, ISSR technique is helpful for genetic analysis of different plants.

It was found from this study that the similarity ratio between the genotypes was large and the percentage of differences between them.

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## الوصف الجزيئي لبعض التراكيب الوراثية لفول المونج (*Vigna rediata* L.) باستخدام تقنية ISSR

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أجريت هذه الدراسة لتقييم عشرة تراكيب وراثية من فول المونج لعشر خصائص زراعية هامة. تم الحصول على عشرة تراكيب وراثية من فول المونج من محطة أبحاث بهتيم التابعة لمعهد بحوث المحاصيل الحقلية بالجيزة في عام ٢٠١٨م. وقد تم زراعة التجربة فى تصميم قطاعات كاملة العشوائية بأربعة مكررات ، وتم الاختيار على أساس عشر صفات زراعية هامة هي ارتفاع النبات (سم) وعدد الفروع ، عدد القرون المخصبة لكل نبات ، عدد القرون في كل عقدة ، طول القرون (سم) ، عدد البذور لكل قرن ، عدد القرون لكل نبات ، عدد البذور لكل نبات ، وزن البذور لكل نبات (جم) ووزن ١٠٠ بذرة (جم). أشارت النتائج إلى وجود اختلافات متوسطة الأهمية بين التراكيب الوراثية ووجد تنوع وراثي بين الصفات المدروسة ، مما يتيح فرصة للتحسين الوراثي. ثم تم عمل التحاليل الجزيئية باستخدام تكرار التسلسل البسيط (ISSR) لتقييم التنوع الوراثي وكذلك التعرف علي درجة القرابة الوراثية واستخدمت لذلك عشرة بادئات على عشرة تراكيب وراثية من فول المونج. واستنتج من الدراسة أن العدد الإجمالي من الحزم الكلية كان ١١٧ حزمة، منهم ٦٥ حزمة متعددة و ٥٢ حزمة أحادية. كما أظهرت النتائج أيضا أن هناك درجة عالية من التشابه الوراثي تراوحت بين ٠,٨٨ و ٠,٧٦ حيث كانت الأعلى ٠,٨٨ بين التركيبان الوراثيان (١ و ٢) والأقل ٠,٧٦ بين التراكيب الوراثية (٤ و ٥) و (٢ و ٩).