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Assessment of Genetic Diversity Among Some Peanut Cultivars (*Arachis hypogaea* L.) by ISSR Markers

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ABSTRACT

One of the products of agriculture, which promotes national development, is the peanut (*Arachis hypogaea* L.). Over time, the food business sector's needs and demand for peanuts grew. However, the demand for peanuts cannot be met by the existing level of national output, hence imports have significantly increased. Peanuts are a crop that is both locally consumed and exported in Egypt. In order to prevent genetic deterioration in local Egyptian peanut cultivars and to improve their beneficial active components, peanuts are reproduced by breeding some cultivars from other parts of the world. To evaluate its genetic diversity, advanced plant breeding programs rely on morphological, biochemical, cytological, and molecular markers. The genetic diversity of four peanut cultivars (Arachis hypogaea L.) grown in Egypt—Giza-5, Giza-6, Turky, and Masr-1 was examined in this work using the ISSR molecular marker. According to the data analysis, the ISSR marker can be used to assess the genetic diversity among the four peanut cultivars under investigation because it showed a polymorphism percentage of 55.8% and six distinct band numbers. To sum up, the ISSR approach shows promise in identifying cultivars and assessing strains' genetic purity.

Keywords: Genetic diversity, peanut (*Arachis hypogaea* L.), molecular markers, ISSR

1. Introduction

One of the most significant industries promoting national growth is agriculture. Rosidi and Daryono (2020) provide the climatic, geographic characteristics, and supporting area. Peanuts have a long history that begins with the Incas of ancient Peru. As part of their religious rituals, they were the first to grow wild peanuts and present them to the sun god (Arya *et al.,* (2016*).*

Peanuts are a major crop that is farmed all over the world. Although peanuts are mostly utilised commercially to produce oil, their byproducts also include a variety of other useful substances, such as proteins, fibres, polyphenols, antioxidants, vitamins, and minerals, which can be employed as functional ingredients in a variety of processed meals (Arya *et al.,* 2016).

Molecular, biochemical, and morphological markers can be used to quantify genetic diversity and genetic similarity levels in populations. DNA sequences on the genome that are utilised to determine the similarities and differences between accessions are known as molecular markers (Yang and Associates, 2015).

One of the significant uses of biotechnology is molecular methods, which can be used to speed up the plant breeding process. Molecular markers based on DNA are helpful instruments that offer a somewhat impartial assessment of genetic variation. The idea behind PCR-based marker techniques is to use primers appropriate for the species and variations required to detect the features in order to amp up the DNA area between the primers' binding sites.

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One of the key molecular marker techniques for identifying genetic variation is the interprimer binding site (iPBS) method, which Kalendar *et al.* (2011) define as an intermediate primer binding site based on a retrotransposon.

Because they are distributed throughout the genome, have sequences with similar structural characteristics, and take part in genome-wide arrangements, retrotransposons are appropriate for use as molecular markers (Kalendar and Schulman, 2014). In several species, iPBS primers have been successfully employed to assess genetic diversity (Žiarovska *et al.,* 2019; Bonchev and Vassilevska-Ivanova 2020; Samarina *et al.,* 2021; Olejnik *et al.,* 2021).

A PCR technique called inter simple sequence repeat (ISSR) amplifies DNA segments between two identical microsatellites by repeating portions in opposing orientations. The primer sequence utilised is 16–25 bp long. The benefits of ISSR include high polymorphism and repeatability, nocodominance, the elimination of the requirement to know the precursor sequence beforehand, and random, affordable, easy, quick, and efficient locus sampling.

The ISSR molecular marker was used in this study because it offered a number of benefits over alternative molecular markers. Compared to RFLP, ISSR molecular markers were simpler and less expensive, and they were more sensitive to genetic diversity. According to Rosidi and Daryono (2020), ISSR produced multilocus and polymorphism patterns without requiring genomic sequence information. ISSR has been accessible and extensively utilised for mapping, diversity, and phylogenetic research since 1994 (Zietkiewicz *et al*., 1994).

Additionally, ISSR markers have been effectively used for genetic diversity studies in a variety of crops, including rice Blair *et al*., (1999), blackgram Souframanien and Gopalkrishna, (2004), barley Fernandez *et al*., (2002), chickpea Iruela *et al*., (2002), fennel *(Ramadan et al., 2019),* cotton (Shoaib *et al*., 2020), citrus (EL-gengaihi *et al.,* 2020), and coleus (Shoaib *et al.,* 2020).

Genetic variation and relationships between subspecies and botanical varieties of cultivated peanuts were evaluated using molecular techniques (Halward *et al*., 1991; Hopkins *et al*., 1999; He *et al*., 1999; Raina *et al*., 2001; He *et al*., 2003; Guohao *et al*., 2003; Ferguson *et al*., 2004; Krishna *et al*., 2004; He *et al*., 2005; Gimenes *et al*., 2007; Azzam *et al*., 2007; Varshney *et al*., 2009; Kamdar *et al*., 2014 and 'Arya *et al*., 2016). The purpose of this study was to build a potent tool for genetic diversity among certain peanut cultivars *(Arachis hypogaea L.)* and to characterise four distinct cultivars in terms of molecular features.

2. Materials and Methods

2.1. Plant materials

Four peanut cultivars *(Arachis hypogaea* L.*)* Giza-5, Giza- 6, Turky and Masr -1) which cultivated in Egypt were examined in this work for the study of their molecular diversity.

2.2. Molecular analysis

2.2.1. DNA isolation

Young and fresh leaves of the three cultivars of peanut were collected and soaked in liquid nitrogen for DNA extraction, and the DNA was extracted by Cetyl trimethyl-Ammonium Bromide (CTAB) method (Doyle and Doyle 1990).

2.2.2. Polymerase chain reaction (PCR) condition for ISSR

Five ISSR primers were selected and used for the molecular analysis; HB-08, HB-09, HB-11, HB-12 and HB-13, the sequences base of these ISSR primers were listed in tables (1). ISSR analysis was performed as described by Adawy *et al.* (2004) and Hussein *et al.* (2006). Each 25 µl of ISSR amplification reaction consisted of 2 µl of 10× PCR buffer, 1.6 µl of 50 mM $MgCl_2$, 1.6 µl of 10 µM of each forward and reverse primer, 2.5 µl of 2 mMdNTPs, 25 ng template DNA and 0.25 µl of 5U Taq-DNA polymerase. The PCR was carried out with the initial cycle at 94°C for 2 min, 5 cycles of 94°C for 30 s, 35°C for 30 s and 72°C for 1 min, another 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1 min, and the final extension at 72°C for 5 min. PCR products were separated on 1.2 % agarose gel in 1X TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 115 volt for 2.5-3 h. The fragment

patterns were photographed under UV light for further analysis and A 100 bp standard DNA ladder as the molecular standard.

2.3. Data analysis

Dice similarity coefficients were used to calculate the bands found by the photo Capt MW program (Dice, 2004). The Gel Works ID advanced software UVP-England Program was used to create the similarity matrices. The SPSS software version 19 was used to analyse the associations between species as shown by dendrograms.

Table 1: List of the ISSR primer names and their nucleotide sequences were used in the study of four peanut cultivars (*Arachis hypogaea* L.).

No	Name	Sequence	No	Name	Sequence
1	HB-08	5`GAG AGA GAG AGA GG3`	**4**	HB-12	5´CAC CAC CAC GC 3`
2	HB-09	5´GTG TGT GTG TGT GC 3`	**5**	HB-13	5´CAC CAC CAC GC 3`
3	HB-11	5`GTG TGT GTG TGT TGT CC 3`			

3. Results and Discussion

The genetic variation between the four peanut cultivars under investigation was investigated in the current study using five specific ISSR primers. The information gleaned from each primer was explained as follows: The five fragments produced by primer HB-08, which ranged in size from 300 to 880 pb, are shown in Table (2) and Fig. (1). Three of them were monomorphic, with molecular sizes of 880, 800, and 540 bp, while two of them were polymorphic, with molecular sizes of 350 and 300 bp. With the exception of the Turky cultivar, all cultivars contained bands at molecular weights of 350 and 300 bp. For this cultivar, it might therefore be seen as a negative sign.

Table 2: Total bands number was detected by primer HB-8, between the four peanut (*Arachis hypogaea* L.).

Band NO	MW bp.	Cultivars			
		Giza-5	Giza- 6	Turky	Masr -1
1	880	1	1	1	1
2	800	1	1	1	1
3	540	1	1	1	1
4	350	1	1	0	1
5	300	1	1	0	1
Total		**5**	**5**	**3**	**5**

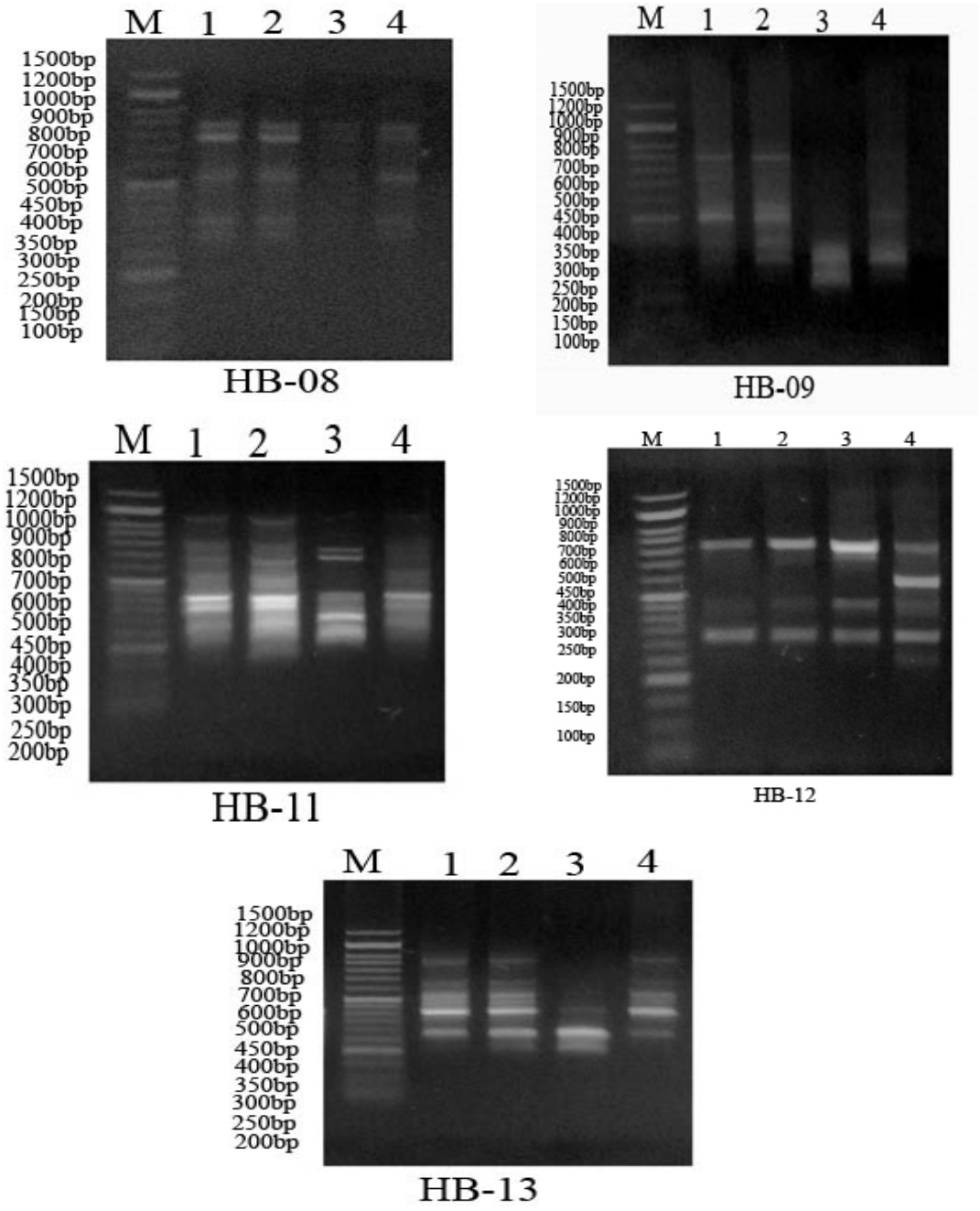

Fig. 1: ISSR banding patterns of the four peanut cultivars (*Arachis hypogaea* L.) with five ISSR primers. M: marker, 1: Giza-5, 2=Giza- 6, 3= Turky and 4=Masr -1.

Table (3) and Figure (1) display the seven fragments that were found for primer HB-09, which ranged in size from 300 pb to 900 pb. One of these fragments was monomorphic, with a band at 400 bp molecular size, while the other eight were polymorphic. With the exception of the Turky cultivar, all cultivars included molecular-sized pieces that were 900 bp, 680 bp, 580 bp, and 500 bp. As a result, these can be seen as unfavourable indicators for this cultivar. Similarly, the 450 bp fragment has been found in all cultivars but is not present in cultivar Masr-1. For this cultivar, they therefore regarded it as a negative signal. However, only the cultivar Turky had the band with a molecular size of 300 bp. Consequently, they are seen as a favourable indicator of

Table 3: Total bands number was detected by primer HB-09, between the four peanut (*Arachis hypogaea* L.).

Band NO	MW bp.	Cultivars			
		Giza-5	Giza- 6	Turky	Masr -1
1	900	1	1	0	1
2	680	1	1	0	1
3	580	0	1	0	1
4	500	1	1	0	1
5	450	1	1	1	0
6	400	1	1	1	1
7	300	0	0	1	0
Total		**5**	**6**	**3**	**5**

Table (4) and Fig. (1) provide clarification on the eight fragments that primer HB-11 discovered, which ranged in size from 250 pb to 1120 pb. Two polymorphic and six monomorphic at molecular sizes of 1120 bp, 720 bp, 680 bp, 400 bp, 350 bp, and 280 bp. With the exception of the Turkey cultivar, all cultivars had molecular-sized pieces, measuring 560 bp. This might be seen as a negative indication for the Turky cultivar in this case. Otherwise, only cultivar Giza-6 had the band with a molecular size of 250 bp. In this manner, they regarded it as a positive identifier for the cultivar Giza-6.

Table 4: Total bands number was detected by primer HB-11, between the four peanut (*Arachis hypogaea* L.).

Band NO	MW bp.	Cultivars			
		Giza-5	Giza- 6	Turky	Masr -1
1	**1120**	1	1	1	1
2	**720**	1	1	1	1
3	**680**	1	1	1	1
4	**560**	1	1	0	1
5	**400**	1	1	1	1
6	**350**	1	1	1	1
7	**280**	1	1	1	1
8	**250**	0	1	0	0
Total		**7**	**8**	**6**	**7**

Additionally, seven distinct fragments, ranging in size from 250 pb to 840 pb, were identified in primer HB-12. These were explained in table (5) and figure (1); four of them were polymorphic and three of them were monomorphic with molecular sizes of 840, 480, and 280 bp. The 800 bp fragment was found in the Giza-6 and Turky cultivars, but it was not present in the Giza-5 or Masr-1 cultivars. Over time, only cultivar Masr-1 displayed the bands with molecular diameters of 620 bp, 600 bp, and 250 bp. These are therefore regarded as positive indicators of the Masr-1 cultivar.

Similar to primer HB-13, seven bands with molecular sizes ranging from 300 pb to 960 pb were visible, as seen in table (6) and figure (1). Two of these bands were monomorphic, with molecular sizes of 500 bp and 400 bp, while the other five were polymorphic. In every cultivar but cultivar Turky, pieces with molecular weights of 960 bp, 640 bp, 600 bp, and 520 bp were present. They therefore regarded the Turky cultivar's negative indicators. Similarly, only cultivar Giza-5 lacked the band with a molecular size of 300 bp. They are therefore regarded as a negative indication for the cultivar Giza-5.

Table 5: Total bands number was detected by primer HB-12, between the four peanut (*Arachis hypogaea* L.).

Band No	MW bp.	Cultivars			
		Giza-5	Giza- 6	Turky	Masr -1
1	840	1	1	1	1
2	800	0	1	1	0
3	620	0	0	0	1
4	600	0	0	0	1
5	480	1	1	1	1
6	280	1	1	1	1
7	250	0	0	0	1
Total		**3**	**4**	**4**	**6**

Table 6: Total bands number was detected by primer HB-13, between the four peanut (*Arachis hypogaea* L.).

Band No	MW bp.	Cultivars			
		Giza-5	Giza- 6	Turky	Masr -1
1	960	1	1	0	1
2	640	1	1	0	1
3	600	1	1	0	1
4	520	1	1	0	1
5	500	1	1	1	1
6	400	1	1	1	1
7	300	0	1	1	1
Total		**6**	**7**	**3**	**7**

Generally speaking, the genetic polymorphism among the four peanuts in question was examined using the generated monomorphic/polymorphic bands, unique bands, percentage, and average of polymorphism using five chosen ISSR primers, which are shown in Table (7). The amplified bands ranged in size from around 250 bp (after primers HB-11 and HB-12) to 1120 bp (following primer HB-11), as shown in table (7). With an average of 6.8 amplicons per primer, the number of bands varied from 5 bands (primer HB-08) to 8 bands (primers HB-11).

Table 7: Primers name, total bands number, monomorphic bands number, polymorphic bands number, unique bands number and percentage of polymorphism as revealed by 5 ISSR primers between the four peanut (*Arachis hypogaea* L.)

Primer Name	Total Band	Mono. Band	Poly. band	Unique Band	Poly. %
HB-08	5	3	2	-	40 %
HB-09	7	1	6	2	85 %
HB-11	8	6	2	1	25 %
HB-12	7	3	4	3	57 %
HB-13	7	2	5	-	71 %
Total	**34**	**14**	**19**	**6**	**55.8 %**

Each of the five primers identified polymorphic patterns, yielding 34 amplicons with a polymorphism level of 55.8%. Following primer HB-09, the maximum proportion of polymorphism was 85%, while primer HB-11 produced the lowest percentage of polymorphism, 25%.

In an average of 2.8 monomorphic bands per primer, the number of monomorphic fragments ranged from 1 (primer HB-09) to 6 (following primers HB-11). However, in the average of 3.8 polymorphic fragments per primer, the number of polymorphic bands varied from 2 with primers HB-8 and HB-11

to 6 with primer HB-09. Additionally, with an average of 1.2 unique amplicons per primer, the number of unique bands ranged from 0 with primers HB-08 and HB-13 to 3 with primer HB-12.

The four cultivars under study were split The four cultivars under study were split into two major groups based on the ISSR analysis results, as shown in figure (2). Cultivar 3 was part of the first group, while cultivars 1, 2, and 4 made up the second. Cultivars 1 and 2 were included in the first subgroup of the second group, whereas cultivars 4 were included in the second.

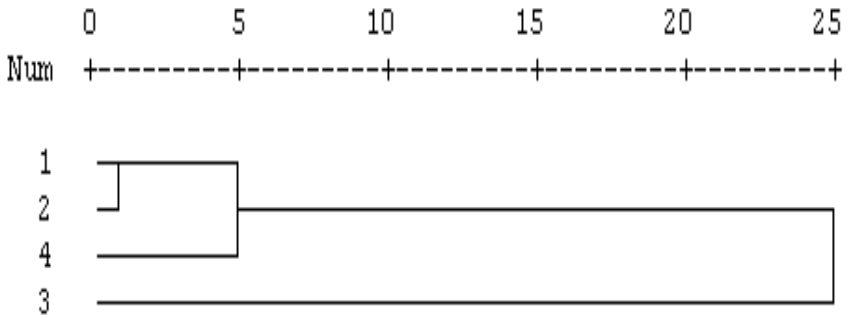

Fig. 2: Dendrogram represents the genetic relationships among the four peanut cultivars using UPGMA cluster analysis generated from of ISSR marker.

The ISSR method was simpler, quicker, and required no particular locus; instead, the primer would look throughout the genome for any location that had microsatellite reasons (Fang and Roose, 1997). A different method for examining polymorphism based on the existence of microsatellites across genomes is ISSR (Raina *et al.,* 2001; Xiang *et al.,* 2020).

In this regard, the ISSR analysis method has been effectively applied to the study of genetic diversity for many plant and crop species, including Artemisia capillaries and *Phaseolus vulgaris* L. Galvan *et al.,* (2003). Manica-Cattani *et al.,* (2009), Achillea millefolium Farajpour *et al.,* (2012), Shafie *et al.,* (2009), and Lippia albaill. Bahmani *et al.,* (2012) used seven ISSR primers to analyse 25 ecotypes of *F. vulgare* Mill. Shojaiefar *et al.,* (2015) examined the differences in 18 fennel populations using 8 ISSR primers. Mostafa and Abou Alhamd (2015) identified salt-tolerant mutants of *F. vulgare* using six ISSR primers. Using several markers, Omid and Kalantar (2016) investigated 30 ecotypes of *F. vulgare* Mill. According to Gostimskii *et al.,* (2005), the ISSR technique holds promise for

ISSR markers were initially used to distinguish between closely related plant varieties, according to Wolfe (2005). The genetic diversity and population structure of Distylium are investigated by Xiang *et al.,* (2020). Chinense was examined using ISSR and SRAP markers both within and between eight populations. The results showed that ISSR and SRAP are highly useful methods for a number of applications, such as DNA fingerprinting, map building, gene tagging, genomics, germplasm identification, and genetic diversity assessment. According to Mondal *et al.,* (2008), RAPD and ISSR approaches were used to compare the molecular profiles of 19 peanut cultivars and breeding lines. Rosyidi and Daryono (2020) use the ISSR marker, which can generate eight polymorphic bands, to examine genetic differences between common peanuts.

4. Conclusion

As a result of this research, ISSR markers will be helpful in the study of genetic diversity, the identification of germplasm collections, and the identification of superior parents to create a high-yielding variety. Lastly, the benefits and universality of ISSR markers are increasingly sought after.

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