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Research Article **BOTANY**

Genetic Diversity Assessment of Egyptian Soybean Varieties Based on SDS-PAGE and ISSR Fingerprinting

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ABSTRACT

Two-thirds of the world's overall production of protein feed ingredients, along with all other main oils and fish meals, are produced from soybeans. Genetic variability and relationships among genotypes can be examined using SDS-PAGE of seed storage proteins. In this study, seven Egyptian soybean varieties were assessed for variation in whole seed proteins using SDS-PAGE, which showed an overall of 11 protein bands with diverse molecular weights. To distinguish these soybean genotypes, precise bands of their seed storage protein profiles can be used as markers. In addition, using of five ISSR primers for genome fingerprinting produced a total of 23 ISSR bands which ranged between 990 bp and 248 bp. Of which ten ISSR bands were polymorphic, 6 bands were monomorphic, and 7 bands were unique. SDS-PAGE profiles and ISSR markers revealed the genetic relatedness among the seven soybean genotypes. Cluster analysis based on ISSR data separated the soybean varieties into several sub-clusters, which indicated that ISSRs are more differentiating than did SDS-PAGE. These results could greatly assist in the identification, breeding and preservation of the soybean germplasm.

Introduction

One of the most important legume crops in the world is soybean (*Glycine max* L. Merrill), an annual plant in the *Fabaceae* family **(Ferguson and Gresshoff, 2009)**. Besides that, soybean is the most widely consumed oil crop worldwide and provides high-quality oil, phospholipids, hormones, and antioxidants **(Choi and Rhee, 2006)**. It also contains phenolic compounds, tocopherols, organic acids, carbohydrates, and essential fats **(Chung *et al*., 2017)**. Moreover, it is a competitive alternative for crop rotation in Africa at the domestic level due to its capacity to fix nitrogen, which aids in boosting soil fertility **(Obua *et al*., 2020)**.

Fingerprinting and characterization of germplasm using proteins and molecular markers has received particular attention as a result of its growing usage in crop improvement and the choice of desired genotypes for breeding crop plants. Also, the use of genetic markers and protein profiles is effectively employed to address the taxonomic and evolutionary issues in a number of field crops **(Das and Mukarjee, 1995; Ghafoor *et al*., 2002; Bisen *et al*., 2015)**. Genetic variability based on seed storage proteins has been described for numerous crops, including lima beans **(Gill-Langarica *et al*., 2011)**, *Phaseolus vulgaris* **(Ferreira *et al*., 2000)** and chickpeas **(Ghafoor *et al*., 2002; Sahu *et al*., 2012; Vinu *et al*., 2013)**. Furthermore, investigations of seed storage proteins not only clarify genetic transgression and the phylogenetic relationship of the accessions but also aid in the identification and characterization of variation in crop varieties, cultivars, and their wild relatives.

For plant breeders, molecular markers are extremely helpful in isolating, finding, and assessing markers-linked genes impacting particular phenotypes **(Kehinde *et al*., 1997, Vinu *et al*., 2013)**. Many legume species, including *Trifolium* **(Badr, 1995)**, *Lupinus* **(El-Shazly *et al*., 2006)**, and *Vigna*, have been successfully resolved and characterized using variations in seed storage proteins as indicated by electrophoresis **(Ghafoor and Ahmad, 2005; Igwe *et al*., 2017)**.

Molecular markers have been used in many studies that assess the genetic variability of soybean **(Tomar *et al*., 2011; Bisen *et al*., 2015; Tiwari *et al*., 2019)**. In order to analyze the genetic diversity of soybeans, a large number of molecular markers were employed. These studies found low genetic

diversity in soybean cultivars **(Jo *et al*., 2021)**, moderate genetic variability **(Nkongolo *et al*., 2020)**, or high genetic variability **(Torres *et al*., 2015, and Kujane *et al*., 2019)**. Because they offer distinct genetic information for each species regardless of age, physiological conditions, or environmental influences, molecular markers are trustworthy sources of genetic variability (**Kalpana *et al*., 2012; Tiwari *et al*., 2019)**. The quickness and independence from development stage-specificity of the molecular markers are advantages **(Chander *et al*., 2021)**. Also, they were frequently utilized in breeding and diversity studies of legumes and were used to estimate both intra- and inter-genomic diversity **(Sahu *et al*., 2012; Reddy *et al*., 2013; Mishra *et al*., 2014; Sunil, 2015; Tiwari *et al*., 2019)**.

SDS-PAGE and ISSR marker were chosen because of their reliability and usefulness in characterizing the genetic makeup of plant germplasm **(Ghafoor *et al*., 2002; Vinu *et al*., 2013)**. Genetic similarity information can be used for making choices about the selection of the best genotypes for crop improvement or to be utilized as parents in the crossbreeding of new varieties. Therefore, the aim of the current study was to determine the genetic variability of the seven soybean cultivars frequently grown in Egypt by assessing the protein and ISSR profiles.

Material and methods

Plant material

Seeds of the seven Egyptian soybean cultivars were obtained from the Agricultural Research Center (ARC), El-Doki, Giza, Egypt. The procured cultivars are designated Crawford, Giza21, Giza22, Giza35, Giza82, Giza83, and Giza111.

SDS-PAGE seed storage proteins

100 mg of seeds powder was mixed with 0.5 ml of 0.03 M Tris-HCl solution (pH 8.0), an extraction buffer containing 5 µl of ß-mercaptoethanol, to extract the total seed proteins. Total seed proteins were separated using slab-type SDS-PAGE in 12% polyacrylamide gel as described by Laemmli (1970). After taking a photo of the gel, bands were either present or absent and were given a score of 1 or 0, respectively. The molecular weights of protein bands were determined using Lab Image program version 2.7 (Kapelan GmbH, Germany) by comparison with the standard protein markers.

ISSR analysis

The ISSR fingerprinting procedure was performed according to the method described by **Dogan *et al*., (2007)**. From all of the soybean samples, five ISSR

primers (Operon Nippon EGT CO. LTD.) were examined for the ability to produce polymorphic products. The sequence information of the primers and their characteristics are mentioned in Table (1). PCR amplification was conducted using Bio-Rad Thermo-cycler according to the following cycle profile: an initial denaturation at 94°C for 4 min was followed by 40 cycles of 45 sec. at 94°C for denaturation, 45 sec. at a temperature depending on each primer constituent for annealing, and 45 sec. at 72°C for extension, followed by a final extension at 72°C for 7 min. The temperature was then set at 4°C until the removal of the PCR tubes.

The PCR amplicons were separated by electrophoresis on agarose gel (1.5%) containing ethidium bromide (0.5 µg/ml) in 1 × TAE buffer at 100 V **(Gaafar *et al.*, 2017)**. Using a Gel Works 1D advanced gel documentation system (UVP, UK), the ISSR fingerprinting was displayed and photographed under UV light. Using a 100 bp DNA ladder (Fermentas, Germany) as a DNA standard, the size of each band was estimated. Lab Image software version 2.7 was used to determine the molecular size of the ISSR bands (Kapelan GmbH, Germany).

Table (1): Names and sequence information of the ISSR primers used for fingerprinting of soybean varieties as well as total number of amplified alleles, polymorphic alleles, unique alleles, and % of polymorphism.

Primer name	Sequence (5`-3`)	No. of amplified alleles	No. of polymorphic alleles	No. of unique alleles	% of polymorphism
844-B	5`- (CT)8 GC-3`	5	2	2	80
862	5`- (AGC)6 -3`	5	4	0	80
HB-10	5`- (GA)6 CG-3`	4	2	1	75
HB-14	5`- (CTC)3 GC-3`	4	0	3	75
UBC-827	5`- (AC)8 G-3`	5	2	1	60
	Total	**23**	**10**	**7**	**Average = 74**

Cluster analysis

Unweighted pair group method with arithmetic average (UPGMA) and the SAHN package of NTSYS-pc 2.10 were used to create the phylogenetic tree analysis based on Jaccard's similarity coefficients (JS) **(Rohlf, 2000)**.

Results and Discussion

Protein analysis

Variation in banding patterns of the seven soybean varieties was studied using SDS-PAGE (Fig. 1a, Table 2). SDS-PAGE protein profiles of the seven soybean varieties showed total of 11 protein bands, with different molecular weights. These 11 protein bands were classified as following: six polymorphic bands which were found in some varieties and absent in others and five monomorphic bands which were of molecular weights 82, 59, 28, 24, 12 and 45 kDa and found in all 7 soybean varieties (Giza21, Giza22, Giza35, Giza82, Giza83, Giza111 and Crawford). Three of the six polymorphic bands with molecular weights 146, 96 and 37 kDa appeared in varieties Giza21, Giza22, Giza35, Giza82 and Giza83, while they were absent in the two varieties Giza111 and Crawford. Meanwhile, two polymorphic bands of molecular weights 75 and 45 kDa appeared only in the two varieties Giza111 and Crawford. However, the last polymorphic band of molecular weight 15 kDa was found only in the varieties Giza21 and Giza35 and were absent in the rest of the 7 soybean varieties (Fig. 1a, Table 2).

It has been demonstrated that protein electrophoresis is an effective approach for examining genetic structure and polymorphism **(Parker *et al*., 1998)**, and the SDS-PAGE technique is especially regarded as a consistent method since storage proteins are relatively neutral to environmental variations **(Javid *et al*., 2004; Singh *et al*., 2017)**. In addition, biochemical markers could be used to estimate a precise genetic diversity index **(Akhtar, 2001; Rabbani *et al*., 2001; Igwe *et al*., 2017)**.

Also, to solve the taxonomic and developmental issues of numerous crop plants, seed storage proteins have been utilized as genetic markers produced using electrophoresis **(Das and Mukarjee, 1995; Ghafoor *et al*., 2002; El-Shazly *et al*., 2006; Vinu *et al*., 2013)** From the previous results, the SDS-PAGE technique exploits the variation amongst soybean varieties besides the repeatability of their chromatographic profiles and can illustrate seed protein composition with appropriate accuracy to differentiate varieties. In this study and based on

expression of the protein bands, presence or absence, we evaluated the genetic diversity of seven soybean varieties which demonstrated that a protein band of 15 kDa was present only in two particular varieties (Giza111 and Crawford).

Table (2): Molecular weights (kDa), presence and absence (0) of the protein bands of SDS-PAGE pattern of 7 soybean varieties.

Band no.	M.W. (kDa)	Soybean variety							Band type
		Giza 111	Crawford	Giza 21	Giza 22	Giza 35	Giza 82	Giza 83	
1	146	0	0	1	1	1	1	1	P
2	96	0	0	1	1	1	1	1	P
3	82	1	1	1	1	1	1	1	M
4	75	1	1	0	0	0	0	0	P
5	59	1	1	1	1	1	1	1	M
6	45	1	1	0	0	0	0	0	P
7	37	0	0	1	1	1	1	1	P
8	28	1	1	1	1	1	1	1	M
9	24	1	1	1	1	1	1	1	M
10	15	0	0	1	0	1	0	0	P
11	12	1	1	1	1	1	1	1	M

1: presence, **0:** absence, **M:** monomorphic, **P:** polymorphic.

In the current study and based on protein data cluster analysis, Figure (1b) showed two main clusters, one contained the two varieties Giza111 and Crawford which showed almost identical protein profiles (Fig. 1a), while the rest five soybean varieties were included in the second main cluster. Also, protein cluster analysis indicated that Giza111 and Crawford are very closely linked genotypes (Fig. 1b). The phylogenetic tree, based on variation in protein profiles, revealed very low genetic distance among the examined genotypes reflecting high similarity of genes responsible for the seed storage proteins, which is very obvious particularly in the sub-clusters.

Normally, the distance between varieties demonstrates the strength of seed protein data to reflect genetic diversity in soybean genotypes, where

the highest uniformity in number of bands reflects the genetic relationship and the developmental processes, which could be used as criteria for, selection between plants that are rich in storage proteins **(Duran *et al.*, 2005; El-Shazly *et al.*, 2006; Smykal *et al.*, 2008, and Vinu *et al.*, 2013)**. The seed storage protein profile generally produces some variation and may therefore be an effective approach for assessing the level of genetic diversity. In light of this, SDS-PAGE marker data revealed numerous sub-groupings and a significant level of genetic variation **(Tadesse *et al.*, 2018).**

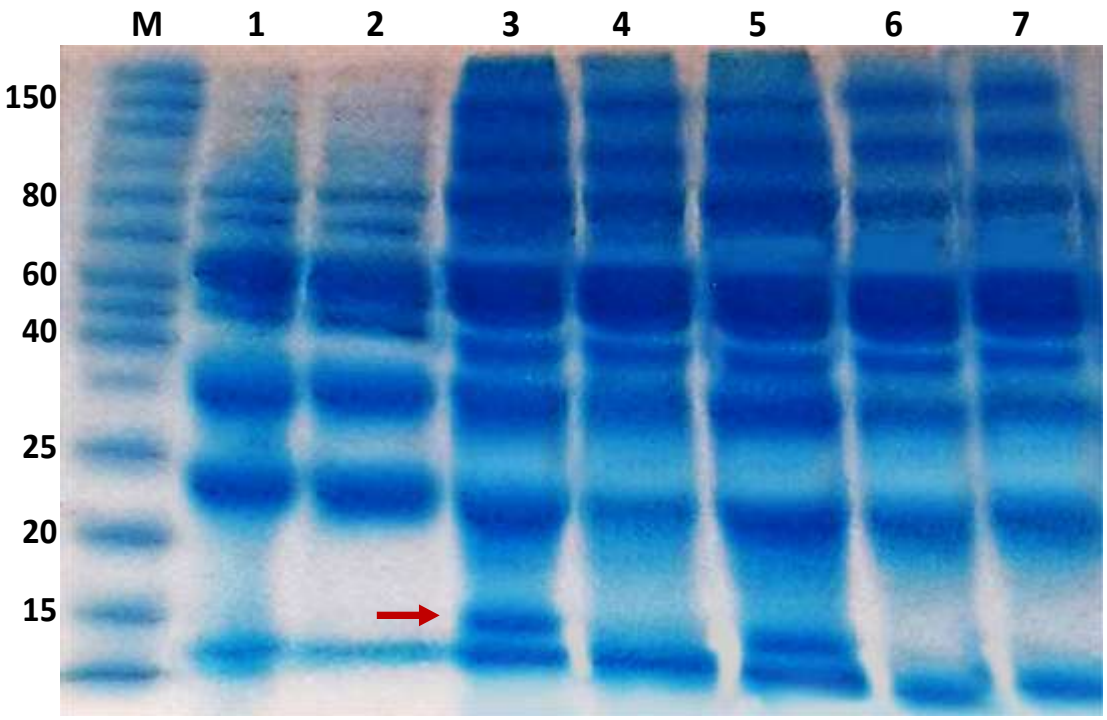

Fig. (1a): SDS-PAGE profiles of seven Egyptian soybean varieties. M= protein marker (kDa). 1= Giza111, 2= Crawford, 3= Giza21, 4= Giza22, 5= Giza35, 6= Giza82 and 7= Giza83. Arrow indicates a polymorphic band.

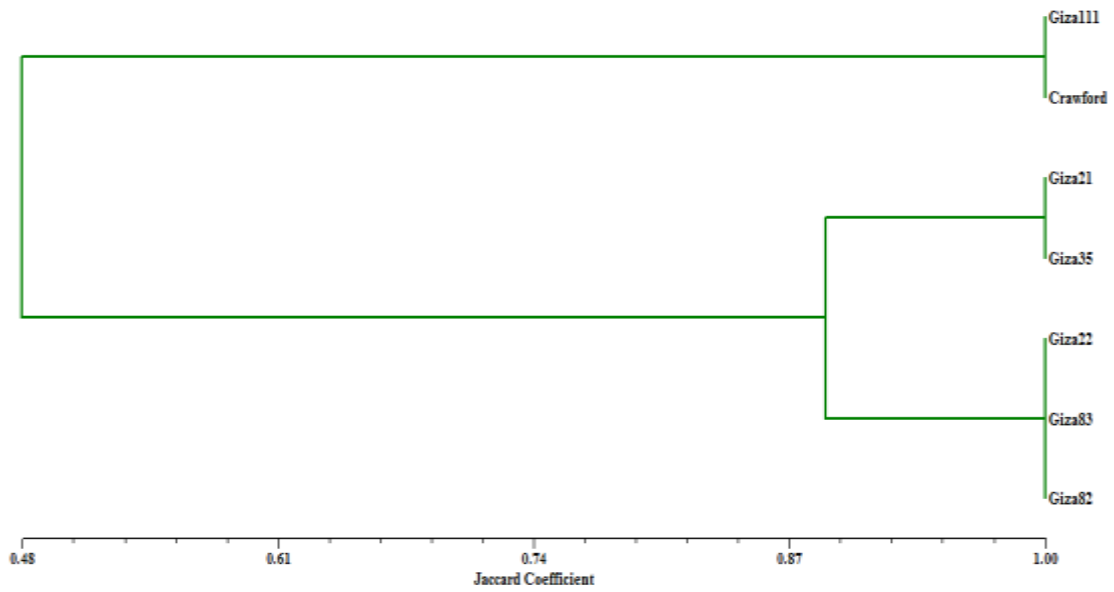

Fig. (1b): Phylogenetic tree showing the relationship among seven Egyptian soybean varieties based on seed storage protein data analyzed with NTSYS-pc ver. 2.1.

ISSR analysis

In this investigation, five ISSR primers produced a total of 10 polymorphic, 6 monomorphic, and 7 unique bands (Table 1). The ratio of polymorphism varied between 60% and 80 % and the average polymorphism of the five ISSR primers was 74%. The primer 844-B amplified 5 ISSR bands, two were unique bands (714 and 496 bp), one is monomorphic with molecular size of 373 bp and found in all 7 soybean varieties, while 2 polymorphic bands (433 and 550 bp) were found only in Giza35, Giza82, Giza111 and Crawford. However, primer 862 amplified only one monomorphic and 4 polymorphic bands which ranged in size between 700 to 382 bp (Fig. 2a, Table 1).

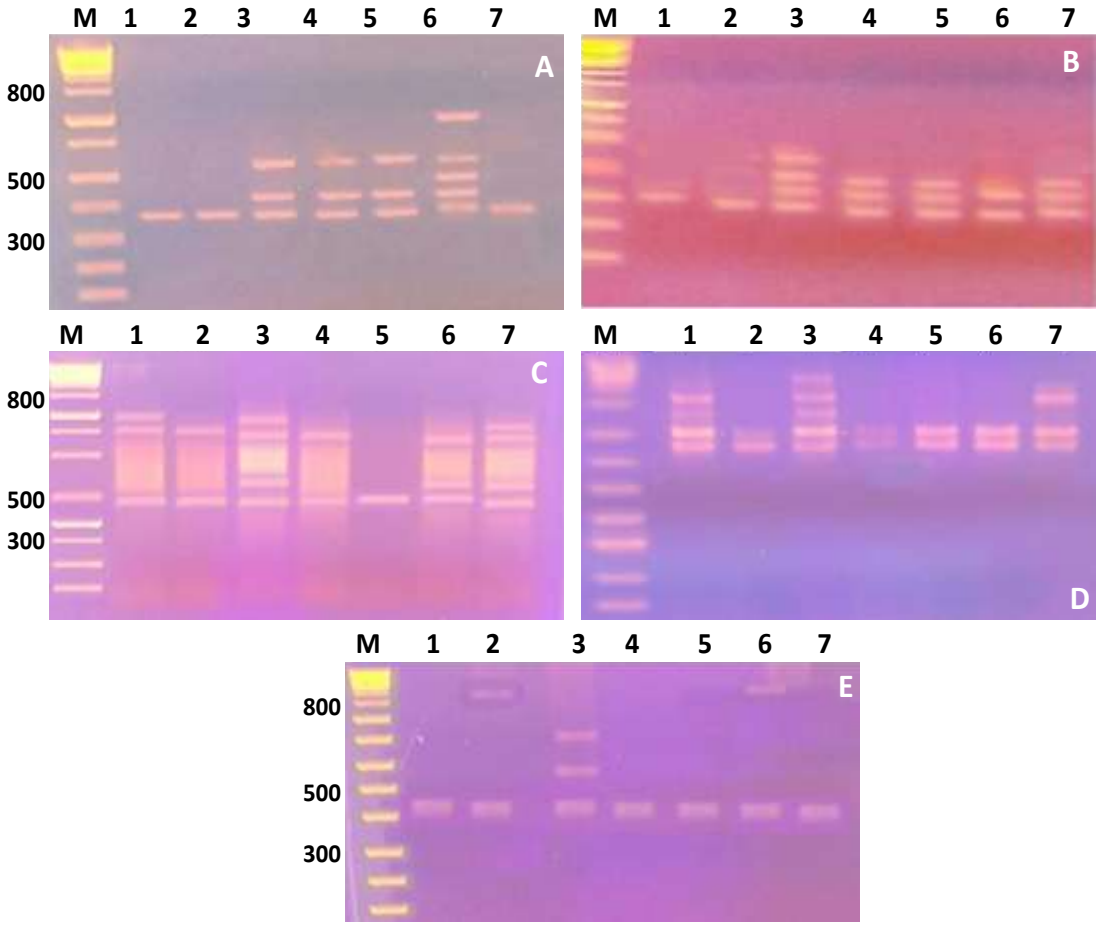

Fig. (2a): ISSR profiles of seven Egyptian soybean varieties revealed by fingerprinting using five different primers. M=100 bp ladder, 1= Giza83, 2= Giza22, 3= Giza35, 4= Giza82, 5= Giza111, 6= Crawford and 7= Giza21. A: primer 844-B, B: primer HB-0, C: primer 862, D: primer UBC-827, E: primer HB-14.

The ISSR fingerprinting using primer HB10 revealed four bands, which ranged in size between 248 to 429 bp. The primer UBC-827 showed five bands which ranged in size from 906 bp to 646 bp, the larger band was unique band with molecular size of 906 bp and was specific to variety Giza35. The last ISSR profiles revealed by primer HB14 showed four bands which ranged in size from 990 bp to 432 bp. No polymorphic bands were amplified with this primer, while one monomorphic band of molecular size 432 bp and three unique bands were shown in ISSR profile of size 990 bp in Crawford and 709 bp and 584 bp both in Giza21 (Fig. 2a, Table 1).

It has been reported that ISSR is a valuable type of marker that is utilized for detecting polymorphisms in DNA sequences. Also, it has been shown that ISSR markers are extremely variable, offer remarkable evidence on the genetic variability between varieties, and are beneficial tools for marker-assisted

selection of new genotypes **(Burstin *et al*., 2001; Costa *et al*., 2016)**.

In this study, several ISSR-unique markers were found that may be regarded as markers for genetic resource authentication and for the creation of property rights. The findings of the current study support the idea that ISSR markers can effectively discriminate between Egyptian soybean varieties, which agrees with **Mudibu *et al*., (2011) and Seyedimoradi and Talebi (2014)**, who revealed that ISSR is a valuable marker type that has been used for detecting polymorphisms in DNA sequences and stipulates precious markers for breeding novel lines of such imperative legume crops. In addition, this is consistent with the opinion that ISSR markers are powerful for investigating genetic variations within species and enable higher-stringency DNA amplifications than RAPD markers **(Ge *et al*., 2005; Seyedimoradi and Talebi, 2014)**. Similarly, SSR molecular markers have been used in the exploration of genetic variability, documentation of genotypes, and assessment of population make-up in soybean genotypes **(Bisen *et al*., 2015; Torres *et al*., 2015; Tiwari *et al*., 2019)**. Moreover, **Rayan and Osman (2019)** used SCoT markers to differentiate between some Egyptian soybean cultivars however these makers showed average of 49.11% polymorphism. In contrast, in this study ISSR showed higher average (75%) of polymorphism.

The relationship among the studied varieties based on ISSR fingerprinting is expressed by Jaccard similarity distance-based tree that composed of two main groups (Fig. 2b). The first one contained three cultivars Crawford, Giza111, and Giza83. The second group is large, comprised of the other 4 cultivars and differentiated into three subclusters. One of them contains only cultivar Giza21 and second contains two cultivars Giza22 and Giza35, while the third Giza82. The result of genetic diversity analysis based on the ISSR polymorphism indicates distinct grouping of soybean varieties.

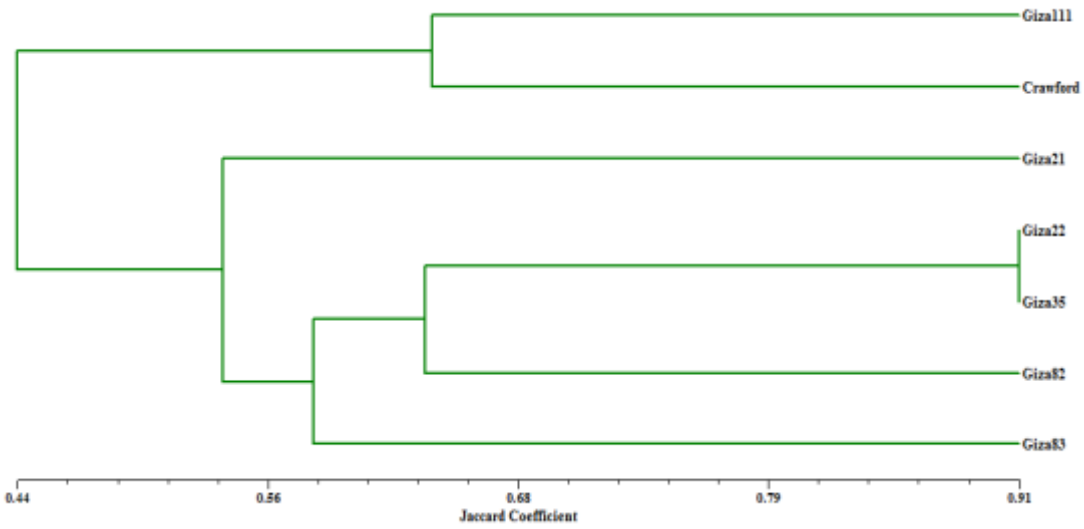

Fig. (2b): Phylogenetic tree showing the relationship among seven Egyptian soybean varieties based on ISSR data analyzed with NTSYS-pc ver. 2.1 software.

Based on the Jaccard similarity distance coefficient, NTSYS-pc software employed cluster analysis to examine the genetic relationships among various soybean cultivars. The higher genetic distance among varieties was because of the existence of unambiguous alleles in all plants within these varieties. The achieved results propose that ISSR markers are more powerful for documentation of soybean varieties, evaluating genetic diversity, and estimating of genetic differentiation compared to seed storage proteins **(Ge *et al*., 2005; Sahu *et al*., 2012)**. Likewise, the genetic diversity and architecture of 148 soybean genotypes were also assessed employing 26 SSR markers **(Tiwari *et al*., 2019)**. Their results gave an extensive insight into the genetic makeup of these Indian soybean genotypes and suggested useful tool to improve the breeding strategies **(Tiwari *et al*., 2019)**. In this study, ISSR phylogenetic tree analysis showed that varieties Giza22 and Giza35 showed the highest similarity and were closely related to Giza82 than Giza83. Moreover, Giza21 clustered in the main cluster. This obtained result is similar to what was obtained by Rayan and Osman (2019). According to **Arslan *et al*., (2020)**, the overall number of bands produced by the ISSR primers ranged between 11.92 and 6.5 **(Aghaei *et al*., 2012)**. The polymorphism level, which was determined by counting the number of polymorphic bands produced by each primer, ranged from 60 to 80%. It is well known that the percentage of polymorphism produced by ISSR markers is higher than that of protein profiles; therefore, the phylogenetic trees

are different as they are based on the DNA markers yielded.

The vegetable soybean accessions likely have a limited genetic basis that is descended from a single ancestral gene because of the moderate amount of variation **(Shakoor *et al*., 2022)**. The degree of genetic variability found in this study is consistent with that found by other researchers who found significant levels of polymorphism (78.4 and 70.0%), as described by **Bisen *et al*., (2015) and Kumar *et al*., (2022)**. The diverse primers, marker methods, and accessions employed in various research may be the cause of conflicting findings on the polymorphism of soybeans, making the results difficult to compare. This highlights the significance of parental selection in breeding programs to minimize genetic relatedness and maintain genetic diversity **(Nkongolo *et al*., 2020)**.

In conclusion, the findings of this study exhibited that ISSR markers are beneficial in the evaluation of genetic relationships between soybean cultivars and documentation and could, consequently, be used for handling preserved germplasm, estimating seed purity in soybean assortments, and defending plant breeder rights. Moreover, the breeders might use the ISSR markers discovered in this work to assist in selecting the most variable cultivars (Giza35, Giza21 and Giza83), which could be employed as parents in any breeding program.

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تقييم التنوع الجيني لأصناف فول الصويا المصرية اعتمادا على أنماط البروتين وبصمات تكرارات البينية للتتابعات البسيطة

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يساهم فول الصويا بثلثي إجمالي إنتاج العالم من مكونات العلف البروتيني ، إلى جانب جميع الزيوت الرئيسية الأخرى ووجبات الأسماك. من المعروف انه يمكن استخدام SDS-PAGE لفحص التباين الجيني والعلاقات بين الأنماط الجينية لبروتينات البذور. في هذه الدراسة ، تم تقييم سبعة أنواع من فول الصويا المصري من حيث التباين في أنماط بروتينات البذور باستخدام تقنية SDS-PAGE ، والتي أظهرت إجمالي ١١ شريطا بروتينيًا بأوزان جزيئية متنوعة. و للتمييز بين هذه الأنماط الجينية لفول الصويا ، يمكن استخدام هذه الاشرطة الفريدة من انماط بروتين البذور الخاصة بهم كواسمات. بالإضافة إلى ذلك ، أظهر استخدام خمسة من بادئات ISSR لعمل بصمات وراثية إلى إنتاج ما مجموعه ٢٣ شريطا ISSR تراوحت بين ٩٩٠ و ٢٤٨ قاعدة. كانت منها عشرة متعددة الأشكال ، و ٦ أحادية الشكل ، و ٧ اشرطة كانت فريدة. كما كشفت انماط SDS-PAGE وواسمات ISSR عن الارتباط الوراثي بين الأنماط الجينية السبعة لفول الصويا. واظهر تحليل الشجرة اعتمادا على واسمات ISSR القدرة على فصل أصناف فول الصويا إلى عدة مجموعات فرعية ، مما يشير إلى أن واسمات ISSR أكثر تميزًا من SDS-PAGE. كما يمكن أن تساعد هذه النتائج بشكل كبير في تمييز وتربية وحفظ الأصول الوراثية لفول الصويا.