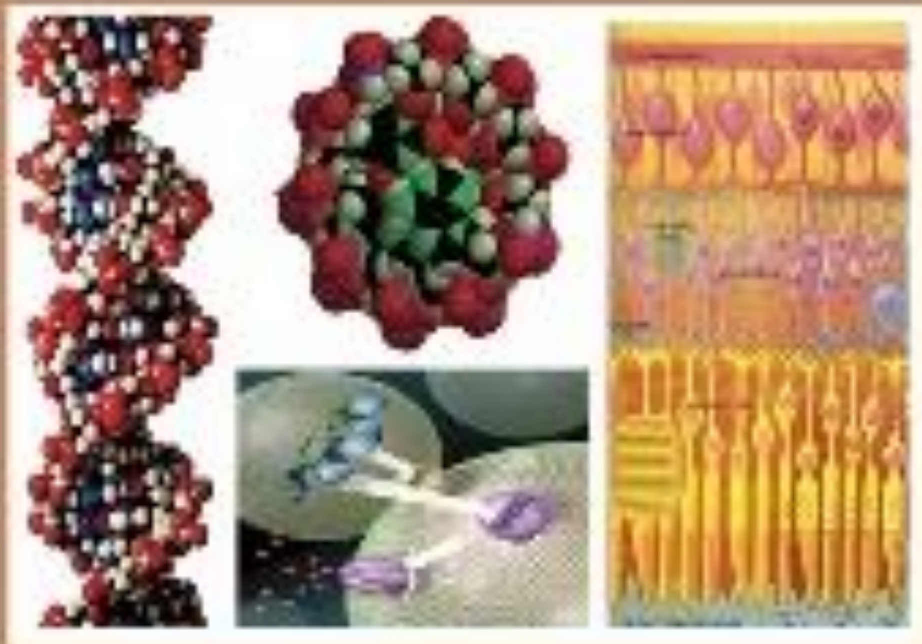
C

EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY



ISSN
2090-0767

WWW.EAJBS.EG.NET

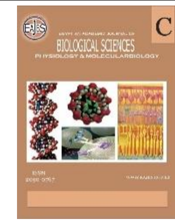
Vol. 16 No. 2 (2024)

.

Citation: *Egypt. Acad. J. Biolog. Sci. (C. Physiology and Molecular biology) Vol. 16(2) pp267-295 (2024)*
DOI: 10.21608/EAJBSC.2024.391693

Egypt. Acad. J. Biolog. Sci., 16(2):267-295 (2024)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



The Impact of Five *Glycine max* Genotype Polymorphism on The Genetic Structure of Their F1 Hybrids and Some Yield Traits

Mona H. El-Hadary [1*], Shimaa S. El-Sherbini [1], and Samar A. Omar[2]

[1]Department of Botany and Microbiology, Faculty of Science, Damanhour University, El-Baheria Governorate, Egypt.

[2]Department of Genetics, Faculty of Agriculture, Tanta University, Gharbia governorate, Egypt.

***E-mail:** Drmona3000@sci.dmu.edu.eg ;

ARTICLE INFO
Article History
Received:8/10/2024
Accepted:13/11/2024
Available:17/11/2024

Keywords:
Soybean, Genetic distance, Hybrid Superiority, Inbreeding Depression, Cluster Analysis.

ABSTRACT

Driving superior hybrids by breeding programs needs assessment of various genetic resources for detecting promising parents. The present study aimed to assess the polymorphism impact of five parental *Glycine max* genotypes on the genetic structure of the F1 hybrid to detect compatible crosses with distinguishable superiority compared to parents regarding some yield traits. Protein, RAPD, and ISSR patterns revealed the *de novo* presence/absence of alleles and genetic similarities (GS) among parents and hybrids. Results mentioned polymorphism among parental genotypes with an average of 24.2%, 100%, and 73.8% for protein profile and RAPD and ISSR markers, respectively. Giza111 had moderate polymorphism, segregation, and GS, while Line30 and D89-8940 were highly polymorphic segregated genotypes. Giza111 were compatible parents for crossing with Line30 and D89-8940, giving rise to the superiority of hybrids regarding most tested yield traits. Line30 X Giza111 recorded the highest percentage of polymorphism with one marker band specific to the cross. Giza111 and Giza83 had the lowest polymorphism and were incompatible parents due to the too-narrow genetic distance that led to inbreeding depression for the tested yield traits. Toano was an incompatible parent, causing inbreeding depression, and needing molecular markers set for efficient characterization. For hybrid superiority, the study suggested Giza111 as a good parent when crossed with Line30 or D89-8940, followed by Giza83 crossing with either Line30 regarding branch number, pod number, 100-seed/plant, and seeds/plant, or D89-8940 regarding the number of branches and 100-seed/plant.

INTRODUCTION

Glycine max (soybeans) supplies 25% of edible oil and two-thirds of the global protein concentrates (Mishra *et al*., 2024). Soybeans have multiple uses in human and animal foods and industry because of enrichment with essential, nonessential, and proteinogenic sulfur-containing amino acids (Singer *et al*., 2019). The information about the soybean genome facilitated genetic and genomic research since having 20 chromosomes and 46,430 protein-coding genes (Wang and Komatsu 2017). Soybean breeding programs focused on improving traits related to yield, abiotic stress tolerance, disease resistance, oil, fatty acids, amino acids quality, isoflavones, and seed composition enriching with prominent levels of protein (Singer *et al*., 2019).

Citation: *Egypt. Acad. J. Biolog. Sci. (C. Physiology and Molecular biology) Vol. 16(2) pp267-295 (2024)*
DOI: 10.21608/EAJBSC.2024.391693

Genetic diversity is crucial for plant breeding to improve crops (Swarup *et al*., 2021). The source of diversity is the genetic difference in DNA sequence in a population of individuals whose expression evolves variant observable traits (Swarup *et al*., 2021). Molecular screening and breeding in soybean varieties has a platform that includes molecular identification, genome sequencing, and novel forms of alleles responsible for expressing essential traits like protein content, salinity, and domestication traits (Valliyodan *et al*., 2017).

Protein is a brief of the DNA coding region enabling population discrimination by qualitative assay. Protein profiling is a rapid marker for constructing phylogenetic relations among genotypes (Singh *et al*., 2017; Sihmar *et al*., 2020; El-Hadary *et al.,* 2022). Also, Tadesse *et al.* (2018) were able to cluster 112 genotypes of *Lepidium sativum* L. from different regions into five clusters using SDS-PAGE to determine genetic diversity and relationships. Tadesse recommended protein profiling as a rapid and reliable method for genetic diversity studies.

DNA markers like RAPD and ISSR are effectively used for determining the genetic evaluation, gene cloning and breeding of crops, cultivar identification, tagging, and marker-aided selection of agronomically available genes (Pendergast *et al*. 2021), linkage map construction, and the mapping of quantitative traits loci (Hirao *et al*., 2019).

RAPD is an essential tool in DNA typing, genetic variability assessment among species, within species, and among populations, studies of phylogenetic relationships, genome mapping, and plant breeding applications (Verma *et al*., 2009; Zanaty *et al*., 2013). RAPD amplifies random DNA segments to detect polymorphism based on nucleotide sequence differences at the priming sites that result from point mutations, structural rearrangements, insertions, deletions, and inversions (Welsh and McClelland, 1990). RAPD techniques are simple, non-radioactive, low cost, and require a small quantity of DNA and the ability to analyze polymorphism without previous knowledge about the DNA sequence of the organism (Amiteye, 2021; Hromadová, 2023). So, RAPD is efficient in comparative purposes based on the similarity of fragments as an indicator of homology (Rieseberg, 1996) and identifying markers related to drought tolerance (Pakniyat and Tavakol, 2007). RAPD assessed genetic variations and germplasm diversity in soybeans (Mathpal *et al.,* 2024), common beans (Hromadová *et al.*, 2023,) and other plant species (Verma *et al*., 2017).

ISSRs are dominant markers that detect polymorphisms in microsatellite and inter-microsatellite loci, and neither require prior information on DNA sequences or large amounts (Amiteye, 2021). ISSR markers sense any polymorphism in the eukaryotic genome because of the lack of mutational constraints in ISSR as a part of the noncoding regions of the genome (Wolfe *et al*., 1998). The polymorphisms generated by ISSR were enough to differentiate accessions of the closely related genotypes and cultivar identification in plant species (Joshi *et al*., 2000; Hou *et al*., 2005, Zhao *et al*., 2006; El-Gawwad *et al*.,2009). ISSR markers enabled the evaluation of genetic variation within collections of cultivated plants (Sonnante and Pignone, 2001) and drought-tolerant crops (Zhao *et al*., 2006).

A combination of RAPD and ISSR markers was suggested for determining genetic variance and species relationship among lens collection (Durán and Vega, 2004), thirty-eight accessions of *Citrullus colocynthis* L. (Verma *et al*., 2017), and 32 accessions of *Stephania rotunda Lour* using 14 RAPD and 14 ISSR primers (Ninh *et al*., 2024).

Plant Breeding concerns the genetic improvement of plants for human benefit by developing new distinguishable cultivars regarding yield, disease resistance, and drought or salinity tolerance (Ethiopian Institute of Agricultural Research, 2024). Plant breeders have various mating designs to select from to investigate the genetic

properties of plant populations. The diallel mating design concerns the possible crosses among a group of genotypes (Sprague and Tatum, 1942). The partial diallel genetic design estimates components of genotypic variance in cross-pollinating populations (Comstock and Robinson, 1948). Kempthorne and Curnow (1961) demonstrated the partial diallel mating design for the increasing number of parents used in a diallel. A partial diallel requires fewer crosses per parent than does a regular diallel. Paschal and Wilcox (1975) used a partial diallel mating design with 12 parents to determine the magnitude of heterosis, components, and other agronomic characters of the yield and to determine the relative importance of general combining ability (GCA) and specific combining ability (SCA) in hybrids of the tested parental adapted cultivars and exotic strains of *G. max* (L.) Merr.

Understanding gene mechanisms controlling dominance levels helps plant breeders to increase yield (El-Abssi, 2019). For example, molecular markers enable the determination of the dormancy of parents by cross similarity to this parent more than the other one (El-Abssi, 2019). Also, significant correlations between traits result in additive and/or variety heterosis effects due to the genes controlling certain traits that work as the same or in coupling phase linkage (Siles *et al*., 2004, Abd El-Aty *et al*., 2023). Thus, there is a relationship between genetic diversity and heterosis in faba beans (Abd EL-Mageed, 2018).

Using previously identified drought-related primers, the present study aimed to identify good parents of five genotypes of G. max with compatible crosses that were observable superiority over parents in terms of certain yield traits based on similarities found in protein, RAPD, ISSR, and combined RAPD-ISSR patterns.

MATERIALS AND METHODS

1-Plant Materials:

The Food Legumes Research Department, Field Crops Research Institute-Agriculture Research Centre, Itay EL-Baroud, EL-Behaira governorate, the Egyptian Agricultural Ministry, Egypt provided us with seeds of five *G. max genotypes* and a field for constructing our experiment. The five *G. max genotypes* belong to two origins: (i) Egyptian origin; Giza111 (Pedigree, Crawford*Celest); Giza83 has white seeds (Pedigree, Selection from MBB-133) and Line30 has purple seeds (Pedigree Crawford*L62-1686), (ii) American origin; Toano has purple seeds (Pedigree, Ware*Essex) and D89-8940 has white seeds.

2-Experimental Conditions:

Two groups of *G. max* seeds were grown under field conditions. One group was germinated to obtain fresh green leaves of one-month-old seedlings for conducting genetic polymorphism investigations. The other group of *G. max* seeds were planted following a Randomized Complete Block Design (RCBD) with nine replications for yield traits investigations. The five parental genotypes underwent a half-diallel cross-mating design to obtain hybrids for yield traits investigations. The five parental genotypes were sown in three planting dates to avoid the difference in flowering time between them and to ensure enough hybrid seeds. The parents were going under manual crossing to obtain all the possible cross combinations (without reciprocals). The plot size was one ridge in the parents and their F1. Each ridge was three meters long and 70 cm apart. Seeds were planted on one side of the ridge at 20 cm hill spacing with one seed per hill. The wet planting method (Herati) was applied, with all the other recommended cultural practices.

3-Genetic Polymorphism Analysis:

Polymorphism analysis of parents and hybrids intended genomic DNA extraction from fresh young leaves of one-month-old seedlings. Fine powder of samples ground in liquid nitrogen undergoing genomic DNA extraction by the easy extraction kit (EZ-10 Spin Column Genomic DNA Minipreps Kit, plant) followed by an RNase-A treatment. The extracted DNA quality was tested using 0.8 % agarose gel for long strands and the absence of smears. The purified DNA was quantified and adjusted to

a working concentration of 50 ng/ µl to prepare the sample for DNA amplification polymorphism analysis using primer sets related to RAPD and ISSR. The primers are selected according to some previous studies (Table 1) to investigate polymorphism among genotypes regarding drought tolerance.

Table 1: A list of twelve RAPD and fifteen ISSR primers, denoting nucleotides number, sequences, and annealing temperatures for detecting polymorphism among five *Glycine max* parental and F1 genotypes.

Primer Symbol	Nucleotides Number bp	RAPD Primers Sequence (5`to 3`)	Annealing Temperatures (˚C)	Primer Symbol	Nucleotides Number bp	ISSR Primers Sequence (5`to 3`)	Annealing Temperatures (˚C)
M1	10	AGG GGT CTT G	32	SH1	10	AGACAGACGC	32
M2	10	CAA TCG CCG T	32	SH2	17	AGAGAGAGAGAGAGAGC	52
M3	10	CTG CTG GGA C	36	SH3	11	AACAACAACGC	32
M4	10	GTG AGG CGT C	36	SH4	13	GACGACGACGACG	44
M5	10	TTG GCA CGG G	36	SH5	11	GTGGTGGTGGC	38
M6	10	CAG GCC CTT C	36	SH6	16	TTGTTGTTGTTGTTGC	44
M7	10	GGT GAC GCA G	36	SH7	15	ACGACGACGACGAAC	48
M8	10	GAT GAC CGC C	36	SH8	14	CACACACACACAAG	42
M9	10	TGC TGC AGG T	32	SH9	18	ATGATGATGATGATGATG	48
M10	10	CCA GCA GCT T	32	SH10	16	AGAGAGAGAGAGAGAC	48
M11	10	AAT CGG GCT G	32	SH11	14	GTGTGTGTGTGTGC	44
M12	10	GTG ATC GCA G	32	SH12	17	GTGTGTGTGTGTTGTCC	52
				SH13	11	CACCACCACGC	38
				SH14	11	GAGGAGGAGGC	38
				SH15	11	GTGGTGGTGCG	38

3.1- RAPD Analysis:

Twelve ten-mer oligonucleotide primers were used for RAPD-PCR, according to Islam *et al.* (2013) in a study for drought tolerance (Table 1). A reaction volume of 25 µl including one µl of 10 µM primer and three µl of 50 ng/ µl genomic DNA was prepared for each primer, following the instruction of GoTaq® Green master Mix, 2x (Promega) using PCR amplification. The amplification ran for four min at 94 °C and then 35 cycles of 1 min at 94 °C, 2 min at 32 °C or 36 °C (according to the primer), and 2 min at 72 °C, followed by a final extension at 72 °C for 5 min by using (MyGene®–MG96G) programmable thermal cycler. The PCR amplicons were electrophoresed on 2 % (w/v) agarose gel against DNA ladders (Gene Direx 100-1500 bp and 100-5000 bp).

3.2-ISSR Polymorphism Analysis:

The study used fifteen ISSR-PCR primers (Table 1) that were repetitively mentioned in previous studies for drought tolerance in rapeseed (Nemati *et al.*, 2012), soybean, peanut (Baloch *et al.*, 2010), and barley (Akladious and Abbas, 2020). Each selected primer was recruited in 25 µl reaction volume for PCR amplification containing one µl of 10 µM primer and three µl of 50 ng/ µl genomic DNA, following the instruction of MyTaqTM Red Mix, 2x BIOLINE. Amplification was programmed for a 5-min initial denaturation step at 94 °C followed by 35 cycles of denaturing at 94 °C for one min, annealing at 32 °C- 52 °C (according to each primer) for one min, extension step at 72 °C for one min, and a final extension step at 72 °C for 5 min by using (MyGene®–MG96G) programmable thermal cycler. The amplification products were electrophoresed on 1.5 % (w/v) agarose gel against a DNA ladder (100 bp to 5000 bp) to estimate the molecular sizes of the amplified fragments.

3.3-Protein Profiling:

The protein was extracted from fresh green leaves using the method of Dure et al. (1981). The concentration of the extracted protein was quantified according to Bradford (1976). The total protein patterns of parents and crosses were developed by SDS-PAGE following Laemmli (1970).

4-Assessment of Yield Traits:

Yield traits assessment considered the number of branches, pod number, seeds/plant, and 100-seed weight. Yield traits were calculated as an average of ten individuals for each parent, and F1 cross.

5-Data Analysis and Phylogenetic Tree Construction:

The electrophoretic patterns of RAPD, ISSR, and protein underwent documentation according to the presence or absence of the bands for parental genotypes and their F1 hybrids to determine parentage of polymorphism, genetic similarity (GS), and constructing phylogenetic trees. The polymorphism percentage calculation was as follows:

$$\% \text{ Polymorphim} = \frac{\text{Polymorphic DNA fragments x100}}{\text{No.of loci}}$$

The GS matrix was generated from the binary data matrix of one and zero for each marker based on the paired group, according to the Jaccard coefficient of the Past software. The genetic relationships estimation among parental genotypes and hybrids according to the Jaccard similarity increased with the value till it reached one to measure GS between genetic sequences among all the tested genotypes. The generated similarity matrix enabled the construction of dendrograms by the unweighted pair group method of arithmetic averages known as UPGMA (Sneath and Sokal, 1973) and sequential agglomerative hierarchical nested (SAHN) clustering, using Past software (version 2.17) of Hammer et al. (2001). Polymorphism calculations depended on the absence or presence of bands expressed in Zero or one, respectively.

The statistical analysis of yield traits was conducted by IBM SPSS Statistics for Windows, (Version 20.0. Armonk, NY: IBM Corp) using the Unpaired Student T-test to compare two groups in the quantitative data.

RESULTS

1-DNA Screening for Polymorphism Among Parents and F1 Hybrids Based on RAPD:

The RAPD pattern for parental genotypes (Fig. 1) and hybrids (Fig. 2) ranged from 204 bp to 3180 bp. The scored pattern showed 100 % polymorphism in both parents and hybrids (Table 2). Parental *G. max* genotypes showed 188 flanked loci with 155 unique loci and 33 polymorphic ones. The F1 hybrid showed 140 loci with 75 unique and 65 segregated (polymorphic) out of them. The total flanking average of the primers was 27.37 fragments/primer, 15.3 in parents, and 11.7 fragments/primer in hybrid. RAPD primer flanked several segments up to 510 among the parental genotypes and F1 hybrids with a flanking average of 39.23 fragments/genotype (Table 3).

The primer efficiency was maximum with M2 and minimum with M3 among parents and hybrids (Table 3). Line30 and D89-8940 had the highest number of flanked fragments, followed by Giza83, D89-8940 x Giza83, and Line30 x Toano, while the hybrid D89-8940 X Toano had the lowest number of flanked segments (Table 3). Table 4 showed that out of the 510 flanked fragments, there were 237 loci for parents (82 polymorphic; 155 unique) and 273 loci for F1 hybrids (198 polymorphic; 75 unique loci). The polymorphism average was 16.4 fragments/genotype in parents, with an average of 31 marker fragments/genotype (Table 4). The polymorphism average in F1 hybrids was 24.8 fragment/genotype with an average of 9.4 unique band/ genotype. Although the RAPD primers detected 180 loci in parents and 140 loci in hybrids (Table 2), parents and hybrids exerted 237 loci in parents and 273 loci in hybrids (Table 4) due to sharing some loci through polymorphism between genotypes. However, the number of loci was less in crosses than in parents with RAPD primers.

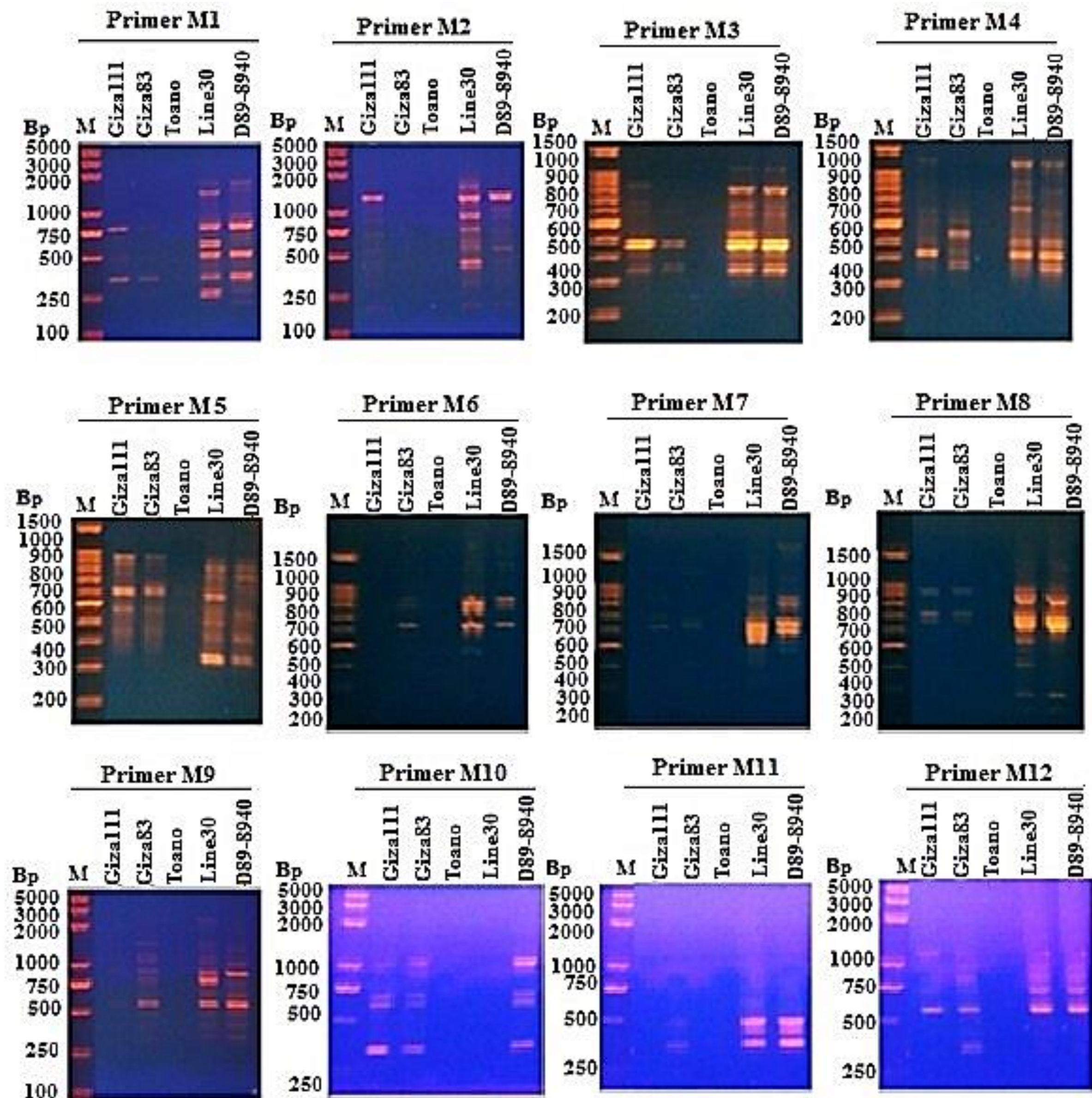


Fig. 1: DNA profiles of five *Glycine max* parental genotypes by using twelve RAPD primers against DNA ladder (M).

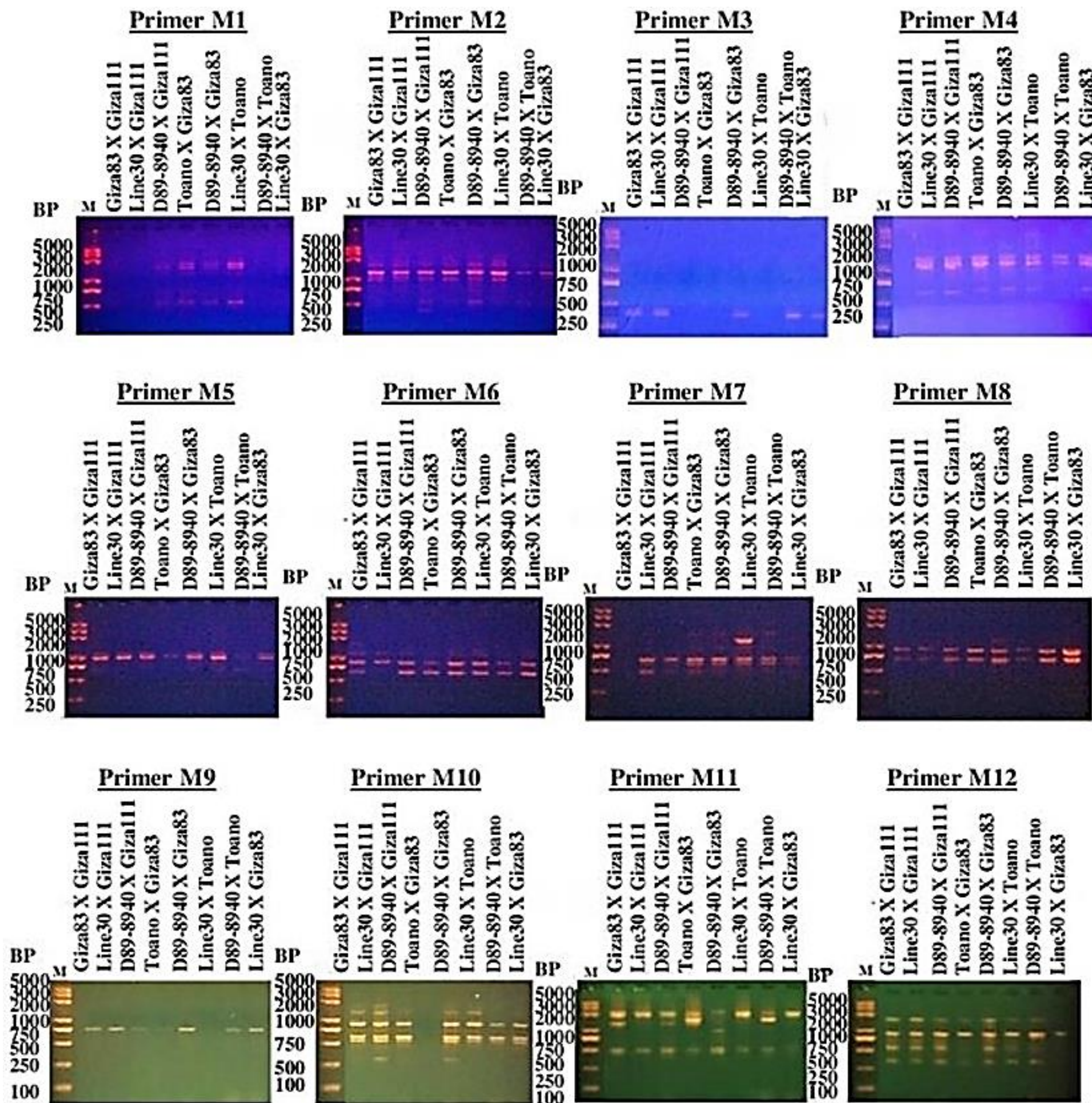


Fig. 2. DNA profiles of eight F1 hybrids of *Glycine max* by using twelve RAPD primers against DNA ladder (M).

Table 2. RAPD primer total efficiency for flanking DNA markers fragments, shared polymorphic fragments among the parental and eight F1 hybrids genotypes of *Glycine max* as developed against DNA molecular marker.

Primer	DNA size (bp)	Markers DNA Fragments			Polymorphic DNA Fragments			Polymorphism (%)
		Parents	F1 Hybrids	Total	Parents	F1 Hybrids	Total	
M1	379-1709	16	7	23	3	5	8	100%
M2	376-2746	22	8	30	1	15	16	100%
M3	243-1132	15	0	15	2	1	3	100%
M4	250-3180	21	5	26	1	4	5	100%
M5	204-933	14	3	17	5	4	9	100%
M6	409-1609	9	6	15	1	9	10	100%
M7	526-1685	15	6	21	2	3	5	100%
M8	227-1118	16	6	22	1	5	6	100%
M9	393-2489	20	1	21	4	3	7	100%
M10	405-1774	2	21	23	4	4	8	100%
M11	410-2219	1	7	8	3	4	7	100%
M12	385-1759	4	5	9	6	8	14	100%
Total	204-3180	155	75	230	33	65	98	100%
Average		12.92	6.25	19.2	2.75	5.41	8.17	100%

Table 3. The total number of amplified DNA fragments in parental and F1 hybrids genotypes of *Glycine max* using twelve RAPD primers.

Primers	Parents					F1 Hybrids								Total
	Giza111	Giza83	Toano	Line30	D89-8940	Giza83 x Giza111	Line 30 x Giza111	D89-8940 x Giza111	Toano x Giza83	Line30 x Giza83	D89-894 x Giza83	Line30 x Toano	D89-8940 x Toano	
M 1	5	2	0	7	8	0	0	5	6	0	5	6	0	44
M2	7	0	0	11	8	9	8	10	9	5	8	9	3	87
M3	5	3	0	7	6	1	1	0	0	1	1	0	1	26
M4	5	5	0	6	7	0	3	3	3	3	3	5	2	45
M5	5	5	0	8	6	2	2	2	1	2	2	2	1	38
M6	0	3	0	3	5	4	2	4	3	4	4	4	2	38
M7	4	4	0	4	7	0	2	1	3	2	2	3	2	34
M8	3	3	0	7	5	2	2	2	2	2	3	2	2	35
M9	3	9	0	11	9	1	1	1	1	1	1	0	1	39
M10	4	4	0	0	4	4	6	4	0	3	5	4	3	41
M11	0	2	0	4	3	3	2	3	4	1	3	2	3	30
M12	5	5	0	5	5	5	5	5	3	1	6	4	4	53
Total	46	45	0	73	73	31	34	40	35	25	43	41	24	510

Table 4. Number of polymorphic and unique DNA fragments; and polymorphism percentage per parental and F1 hybrids genotypes of *Glycine max* as developed by RAPD pattern using twelve primers against DNA molecular marker.

Genotype/ F1 hybrids	DNA ladder (100- 5000bp)	Polymorphic DNA Fragments	Marker DNA Fragments	Polymorphism (%)
Giza111	302- 1501	16	30	100
Giza83	304- 1798	22	23	100
Toano	0	0	0	0
Line30	204- 3180	21	52	100
D89-8940	265- 2012	23	50	100
Total loci in parents= 237		**82**	**155**	
Average loci in parents		**16.4**	**31**	
Giza83 x Giza 111	304- 1798	21	10	100
Line30 x Giza111	204- 3180	25	9	100
D89-89-40 x Giza111	265- 2012	32	8	100
Toano x Giza83	255-2001	29	6	100
Line30 x Giza 83	204- 3180	14	11	100
D89-89-40 x Giza83	265- 2012	29	14	100
Line30 x Toano	204- 3180	31	10	100
D89-89-40 x Toano	265- 2012	17	7	100
Total loci in hybrids= 273		**198**	**75**	
Average loci in hybrids		**24.8**	**9.4**	
Total loci in parents and hybrids = 510		**280**	**230**	
Average in parents and hybrids		**21.54**	**17.69**	**100**

2-DNA Screening for Polymorphism Among Parents and F1 Hybrids Based on ISSR:

The ISSR primers exerted a pattern for *G. max* parental genotypes with 310 loci, 200 unique loci, 17 monomorphic loci, and 93 polymorphic loci, with a polymorphism average of 82.8% (Fig. 3). The flanking averages of *G. max* parental genotypes were 20.6 fragments/primer and 104.8 fragments/genotype ((Table 5 and Table 6). The most efficient primer with parental genotypes was SH1, while SH5 and SH15 had the least efficiency. The parental genotypes exerted 524 flanked fragments: 200 unique, 85 monomorphic, and 239 polymorphic DNA

fragments with a polymorphism average of 73.8%/genotype.

Toano, Line30, and Gia83 had a distinguishable number of polymorphic DNA fragments in the ISSR electrophoretic pattern (Table 6). Line30 had the highest number of unique fragments (44 fragments).

ISSR Profile of hybrids using 15 ISSR primers showed 1697 fragments (Fig. 4 and Table 7) with 1041 polymorphic DNA bands (61.4%) between male and female parents. The total number of polymorphic DNA bands was 562, a percentage of 33.2%, among F1 hybrids and their respective female parents. This result remarks the efficient distinguishing of the true F1 hybrids from the self-pollinated progeny of female parents. The total number of scorable DNA bands amplified by the 15 ISSR primers in each cross combination varied from 205 (D89-89-40 x Giza111) to 222 (Line30 x Toano), with an average of 212.1 bands (Table 7).

The percentage of polymorphism between male and female parents could reveal the genetic difference between male and female parents in each cross combination. When considering all 15 primers, the highest percentage of polymorphism between male and female parents was found in the D89-89-40 x Giza83 cross (67.1%), followed by the crosses D89-89-40 x Giza111 (66.8%) and Line30 x Giza111 (64.8%) as was shown in Table 7.

The number of scorable DNA bands amplified by each primer ranged from 5 (SH11) to 20 (SH82 and SH7) by an average of 14.737 bands/primer. The size of amplified products ranged from 364 to 4680 bp (Table 8). The SH9 primer flanked the highest percentage of polymorphism between male and female parents (80.0%), followed by the SH4 primer with (76.7%) polymorphism. Similarly, these primers displayed the highest percentages of polymorphism between the F1 hybrid and female parents since SH4 and SH9 detected a polymorphism of 43.3 % and 43%, respectively (Table 8).

All the primers could effectively differentiate between male and female parents in all crosses, distinguished true F1 hybrids from the self-pollinated progeny of female parents except SH10 in the cross D89-89-40 x Giza111, SH11 in the cross Giza83 x Giza111 and SH12 in the cross Line30 x Giza83 which give similar results in both male and female parents (Table 9). The other primers had no polymorphic bands for F1 hybrid-female parents with specific crosses such as SH2, SH3, SH5, and SH15 with the cross D89-89-40 x Toano, SH10 with the cross D89-89-40 x Giza83, SH13 with the cross Line30 x Toano and finally SH12 and SH14 with the cross Toano x Giza83.

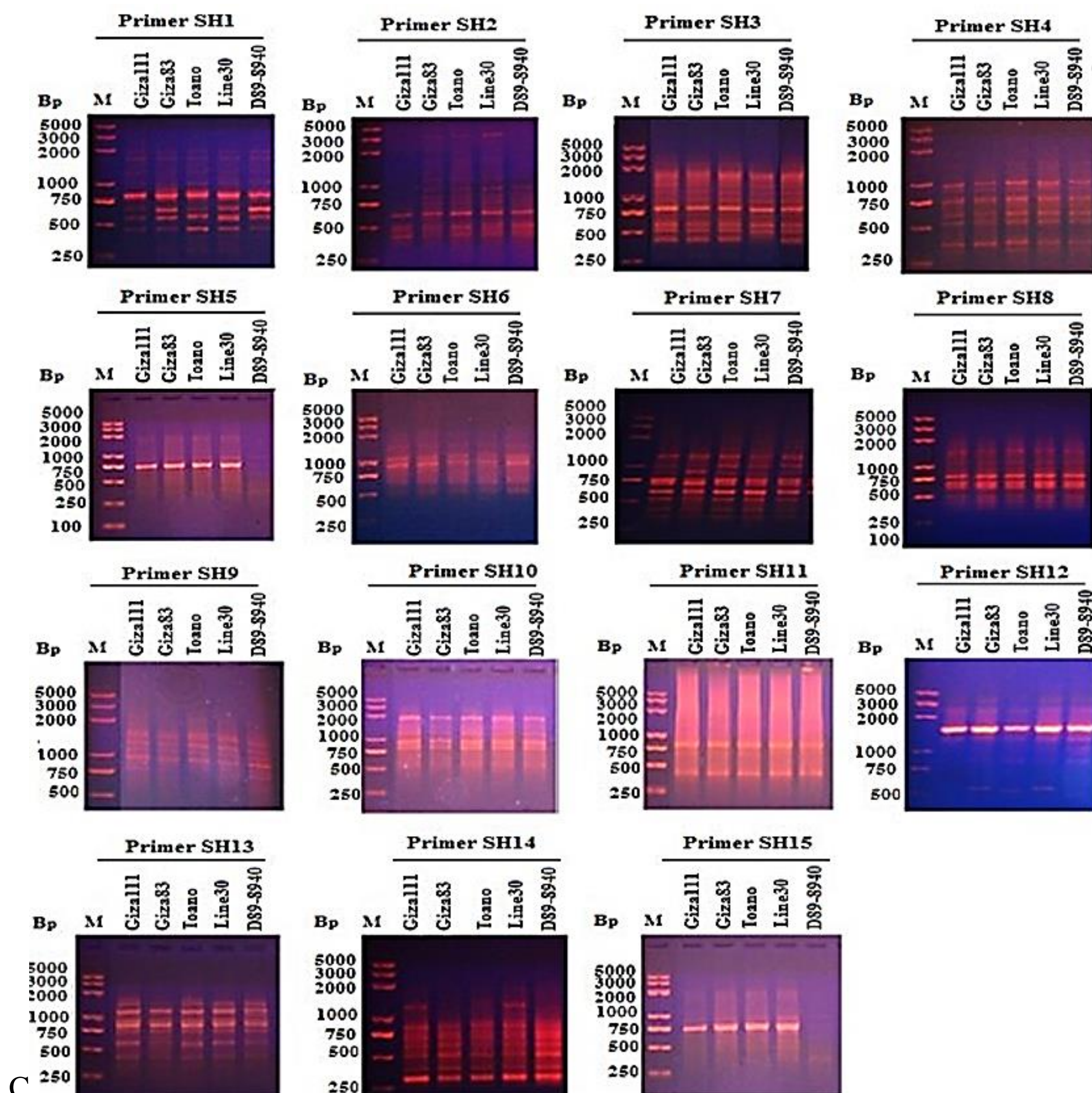


Fig. 3. DNA profiles of five *Glycine max* parental genotypes by using fifteen ISSR primers against DNA ladder (M).

Table 5. Number of loci, monomorphic, polymorphic, and marker DNA fragments; and polymorphism percentage per primer for five *Glycine max* genotypes' pattern as developed by fifteen ISSR primers against DNA molecular marker.

Primer	DNA ladder (100 - 5000bp)	Number of loci	Monomorphic DNA fragments	Polymorphic DNA fragments	Marker DNA fragments	Polymorphism (%)
SH 1	374 – 2025	13	1	12	12	92.3
SH 2	380 – 4086	11	0	11	15	100
SH 3	504 – 1614	10	1	9	12	90
SH 4	400 – 2596	5	1	4	24	80
SH 5	422 – 2045	3	1	2	26	66.7
SH 6	539 – 1258	5	0	5	15	100
SH 7	500 – 1626	8	4	4	15	50
SH8	470 – 1934	9	2	7	8	77.8
SH9	571 – 2140	6	0	6	15	100
SH 10	451 – 1976	6	1	5	4	83.3
SH 11	399 – 595	4	1	3	2	75
SH 12	675 – 3558	7	0	7	5	100
SH 13	426 – 1482	11	2	9	8	81.8
SH 14	393 – 1822	9	2	7	13	77.8
SH 15	422 – 2045	3	1	2	26	66.7
Total		110	17	93	200	
Average		7.3	1.1	6.2	13.3	82.8

Table 6. Number of monomorphic, polymorphic, and marker DNA fragments; and polymorphism percentage per genotype for five *Glycine max* genotypes' pattern as developed by fifteen ISSR primers against DNA molecular marker.

Genotype	DNA ladder (100 - 5000bp)	Monomorphic DNA fragments	Polymorphic DNA fragments	Marker DNA fragments	Polymorphism (%)
Giza83	312-1984	17	50	36	74.6
Giza111	310- 1990	17	44	40	72.1
Tono	315-1993	17	56	37	76.7
Line30	320- 1994	17	49	44	74.2
D89-8940	325-1982	17	40	43	70.2
Total	310- 1994	85	239	200	
Average		17	47.8	40	73.8

Table 7. Number of scorable DNA bands, percentages of polymorphism between male and female parents, and percentages of polymorphism between F1 hybrid and female parent for the fifteen ISSR primers in each *Glycine max* cross.

Crosses	No. of scorable DNA bands	DNA bands polymorphism (%)	
		Male-female parents	F1hybrid-female parents
Giza83 x Giza111	206	50.5	36.9
Line30 x Giza111	213	64.8	39.4
D89-89-40 x Giza111	205	66.8	37.1
Toano x Giza83	215	60.9	33.5
Line30 x Giza83	215	62.8	36.7
D89-89-40 x Giza83	207	67.1	29.9
Line30 x Toano	222	58.6	28.8
D89-89-40 x Toano	214	59.3	22.9
Total	1697	490.8	265.2
Average	212.1	61.4	33.15

Table 8. DNA size, number of scorable DNA bands, percentages of polymorphism between male and female parents, and percentages of polymorphism between F1 hybrid and female parent of *Glycine max* for each ISSR primer.

Primer	DNA size (bp)	No. of scorable DNA bands	% DNA bands polymorphism	
			Male-female parents	F1 hybrid-female parents
SH1	374-2025	17-19	64.4	28.1
SH2	370-4680	15-20	55.6	32.4
SH3	504-2347	15-16	69.6	20.0
SH4	400-3781	12-18	76.7	43.3
SH5	422-2045	12-17	73.1	32.8
SH6	526-1258	11-12	61.1	42.2
SH7	494-2276	17-20	44.9	42.9
SH8	470-1980	15-16	49.2	32.8
SH9	483-2140	12-13	80.0	43.0
SH10	364-1976	10	47.5	23.8
SH11	375-595	5-6	51.1	35.6
SH12	669-3558	12-14	35.9	24.3
SH13	426-1729	13-15	70.2	28.9
SH14	393-2011	14-17	60.9	35.8
SH15	422-2045	12-17	73.1	32.8
Average		14.73	60.9	33.2

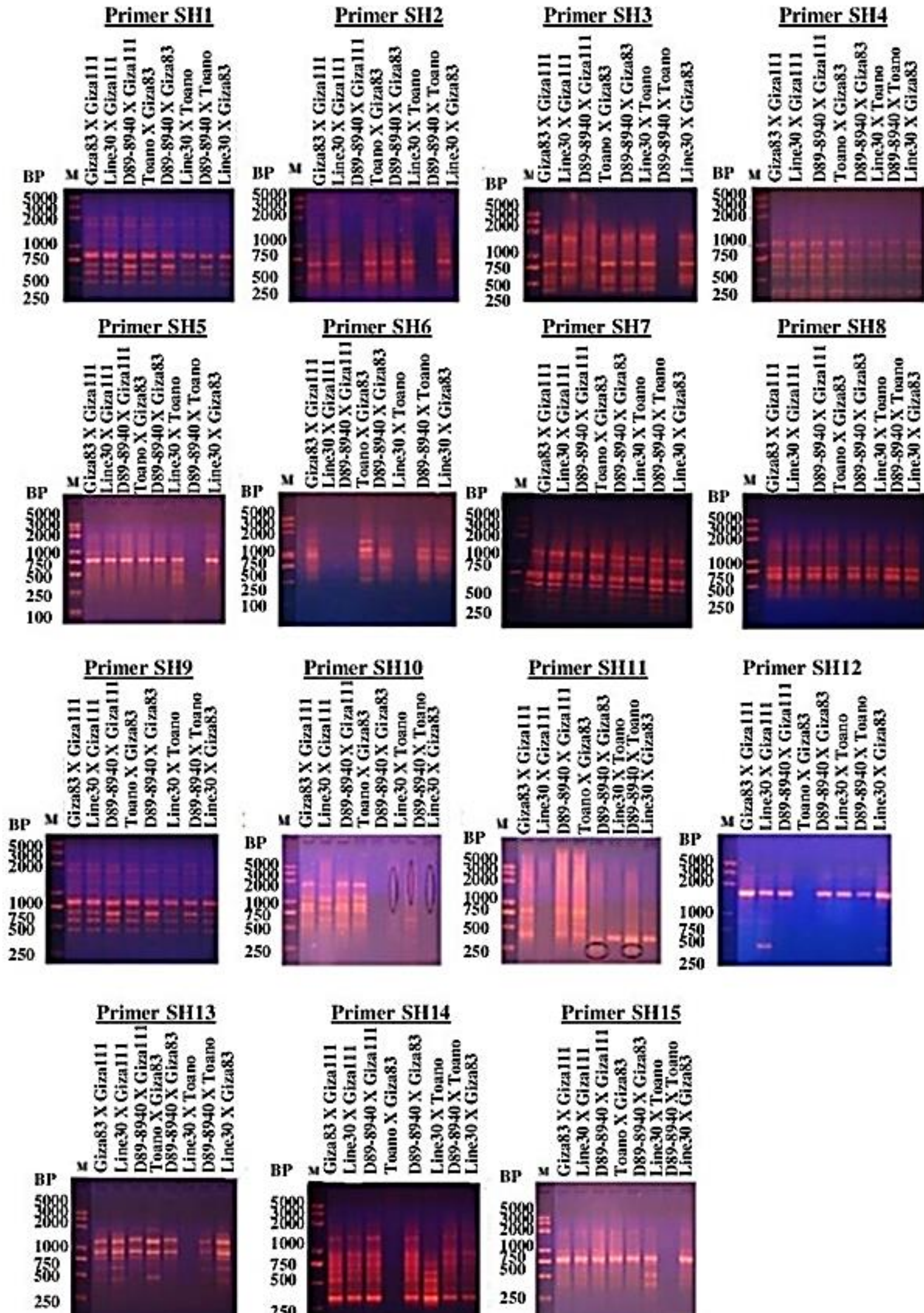


Fig. 4. DNA profiles of eight F1 hybrids of *Glycine max* by using fifteen ISSR primers against DNA ladder (M).

Table 9. Number of scorable DNA bands, polymorphic bands between male and female parents, polymorphic bands between F1 hybrid and female parent, and the percentages of polymorphism for each of them based on ISSR primers in each cross of *Glycine max.*

Crosses/primer	Scorable bands	Male-female parents		F1 Hybrid-female parents	
		Polymorphic bands	% Polymorphism DNA bands	Polymorphic Bands	Polymorphism DNA bands (%)
Giza83 x Giza111					
SH1	18	8	44.4	6	33.3
SH2	16	10	62.5	8	50.0
SH3	16	6	37.5	4	25.0
SH4	12	10	83.3	6	50.0
SH5	15	9	60.0	6	40.0
SH6	12	2	16.7	2	16.6
SH7	17	5	29.4	5	29.4
SH8	16	8	50.0	3	18.8
SH9	13	9	69.2	6	46.2
SH10	10	8	80.0	2	20.0
SH11	6	0	0.0	3	50.0
SH12	12	2	16.7	6	50.0
SH13	14	10	71.4	6	42.9
SH14	14	8	57.1	7	50.0
SH15	15	9	60.0	6	40.0
Line30 x Giza111					
SH1	19	15	78.9	3	15.8
SH2	15	13	86.7	9	60.0
SH3	15	13	86.7	5	33.3
SH4	15	13	86.7	8	53.3
SH5	16	12	75.0	5	31.3
SH6	11	2	18.2	4	36.4
SH7	19	9	47.4	7	36.8
SH8	16	10	62.5	5	31.3
SH9	12	8	66.7	8	66.7
SH10	10	2	20.0	5	50.0
SH11	5	3	60.0	0.0	0.0
SH12	12	2	16.7	6	50.0
SH13	15	11	73.3	6	40.0
SH14	17	13	76.5	8	47.1
SH15	16	12	75.0	5	31.3
D89-8940 x Giza111					
SH1	19	17	89.5	5	26.3
SH2	16	14	87.5	4	25.0
SH3	16	12	75.0	6	37.5
SH4	14	10	71.4	7	50.0
SH5	12	8	66.7	6	50.0
SH6	11	5	45.5	4	36.4
SH7	19	9	47.4	9	47.4
SH8	15	7	46.7	5	33.3
SH9	12	10	83.3	7	58.3
SH10	10	0.0	0.0	2	20.0
SH11	6	4	66.7	2	33.3
SH12	12	10	83.3	2	16.7
SH13	15	11	73.3	4	26.7
SH14	16	12	75.0	7	43.8
SH15	12	8	66.7	6	50.0
Toano x Giza83					

SH1	18	14	77.8	4	22.2
SH2	19	7	36.8	6	31.6
SH3	16	8	50.0	3	18.8
SH4	15	11	73.3	9	60.0
SH5	16	14	87.5	5	31.3
SH6	12	10	83.3	5	41.7
SH7	18	8	44.4	11	61.1
SH8	15	7	46.7	5	33.3
SH9	13	11	84.6	6	46.2
SH10	10	8	80.0	5	50.0
SH11	6	2	33.3	3	50.0
SH12	13	1	7.7	0.0	0.0
SH13	14	10	71.4	5	35.7
SH14	14	6	42.9	0.0	0.0
SH15	16	14	87.5	5	31.3
Line30 x Giza83					
SH1	17	11	64.7	7	41.2
SH2	19	13	68.4	6	31.6
SH3	15	13	86.7	4	26.7
SH4	15	13	86.7	5	33.3
SH5	17	11	64.7	7	41.2
SH6	11	9	81.8	6	54.5
SH7	18	8	44.4	9	50.0
SH8	16	10	62.5	7	43.8
SH9	13	9	69.2	4	30.8
SH10	10	6	60.0	1	10.0
SH11	5	3	60.0	3	60.0
SH12	14	0.0	0.0	5	35.7
SH13	13	7	53.8	2	15.4
SH14	15	11	73.3	6	40.0
SH15	17	11	64.7	7	41.2
D89-89-40 x Giza83					
SH1	17	13	76.5	3	17.6
SH2	20	6	30.0	6	30.0
SH3	16	12	75.0	1	6.25
SH4	14	12	85.7	6	42.9
SH5	13	9	69.2	5	38.5
SH6	11	9	81.8	5	45.5
SH7	18	8	44.4	8	44.4
SH8	15	9	60.0	4	26.7
SH9	13	13	100.0	6	46.2
SH10	10	8	80.0	0.0	0.0
SH11	6	4	66.7	2	33.3
SH12	14	10	71.4	1	7.1
SH13	13	9	69.2	4	30.8
SH14	14	8	57.1	6	42.9
SH15	13	9	69.2	5	38.5
Line30 x Toano					
SH1	19	11	59.7	6	37.5
SH2	18	10	55.6	7	38.9
SH3	15	13	86.7	2	13.3
SH4	18	10	55.6	6	33.3
SH5	17	13	76.5	5	29.4
SH6	11	7	63.6	6	54.5
SH7	20	10	50.0	9	45.0
SH8	15	5	33.3	4	26.7

SH9	12	10	83.3	6	50.0
SH10	10	4	40.0	2	20.0
SH11	5	3	60.0	1	20.0
SH12	13	1	7.7	2	15.4
SH13	15	11	73.3	0.0	0.0
SH14	17	9	53.0	3	17.6
SH15	17	13	76.5	5	29.4
D89-8940 x Toano					
SH1	19	5	26.3	7	36.8
SH2	19	6	31.6	0.0	0.0
SH3	16	10	62.5	0.0	0.0
SH4	17	13	76.5	5	29.4
SH5	13	11	84.6	0.0	0.0
SH6	11	11	100.0	6	54.5
SH7	20	10	50.0	6	30.0
SH8	14	4	28.6	7	50.0
SH9	12	10	83.3	0.0	0.0
SH10	10	2	20.0	2	20.0
SH11	6	4	66.7	2	33.3
SH12	13	11	84.6	1	7.7
SH13	15	11	73.3	6	40.0
SH14	16	8	50.0	7	43.8
SH15	13	11	84.6	0.0	0.0

3-Polymorphism Among Parents and F1 Hybrids Based on Protein Profiling:

Table 10 and Figure 5, show the protein of parents and hybrids. The parental protein pattern indicated 73 protein fragments. Toano had the highest number of fragments, (18 bands) followed by Line30 and D89-8940 (15 bands), while Giza83 had the lowest (11 bands). The polymorphism ranged from 9.1% to 33.3%, with a polymorphism average of 24.2%. Line30 recorded the highest percentage of polymorphism without distinguishable marker bands specific to the genotype. Toano counted the highest number of marker bands with molecular weights of 38KDa, 80 KDa, 128 KDa, and 142 KDa. Giza111 had a moderate number of polymorphic and makers bands.

The protein pattern of the crosses showed a lower loci number than the parents. The F1 hybrid protein pattern indicated 131 protein fragments. Line x Toano had the highest number of fragments (19 bands), followed by 18 bands for D89-8940 X Toano, D89-8940 X Giza83, and Line30 X Giza111. Giza83 X Giza111 had the lowest among the hybrids, equalled eleven. The polymorphism ranged from 54.5 % to 70.6 %, with a polymorphism average of 65.7%. Line30 X Giza111 recorded the highest percentage of polymorphism with one marker band specific to the cross. Giza83 X Giza111 had the lowest polymorphism ratio without any specific marker band. Line X Toano and D89-8940 X Giza83 recorded the highest number of marker bands represented by three bands.

Table 10. Number of monomorphic, polymorphic, and marker protein fragments and polymorphism percentage per genotype of five *Glycine max* and their F1 hybrids.

Genotype	Monomorphic protein fragments	Polymorphic protein fragments	Marker protein fragments	Polymorphism (%)
Giza83	10	1	0	9.1
Giza111	10	2	2	16.7
Tono	10	4	4	28.6
Line30	10	5	0	33.3
D89-8940	10	5	0	33.3
Total in parents	**50**	**17**	**6**	
Average in parents	**10**	**3.4**	**1.2**	**24.2**
Line30 X Giza111	5	12	1	70.6
D89-8940 X Giza111	5	10	0	66.7
Line30 X Giza83	5	11	0	68.8
Line30 X Toano	5	11	3	68.8
D89-8940 X Toano	5	11	2	68.8
Giza83 X Giza111	5	6	0	54.5
Toano X Giza83	5	10	1	66.7
D89-8940 X Giza83	5	10	3	66.7
Total in F1 hybrids	**40**	**81**	**10**	
Average in hybrids	**5**	**10.1**	**1.25**	**65.7**

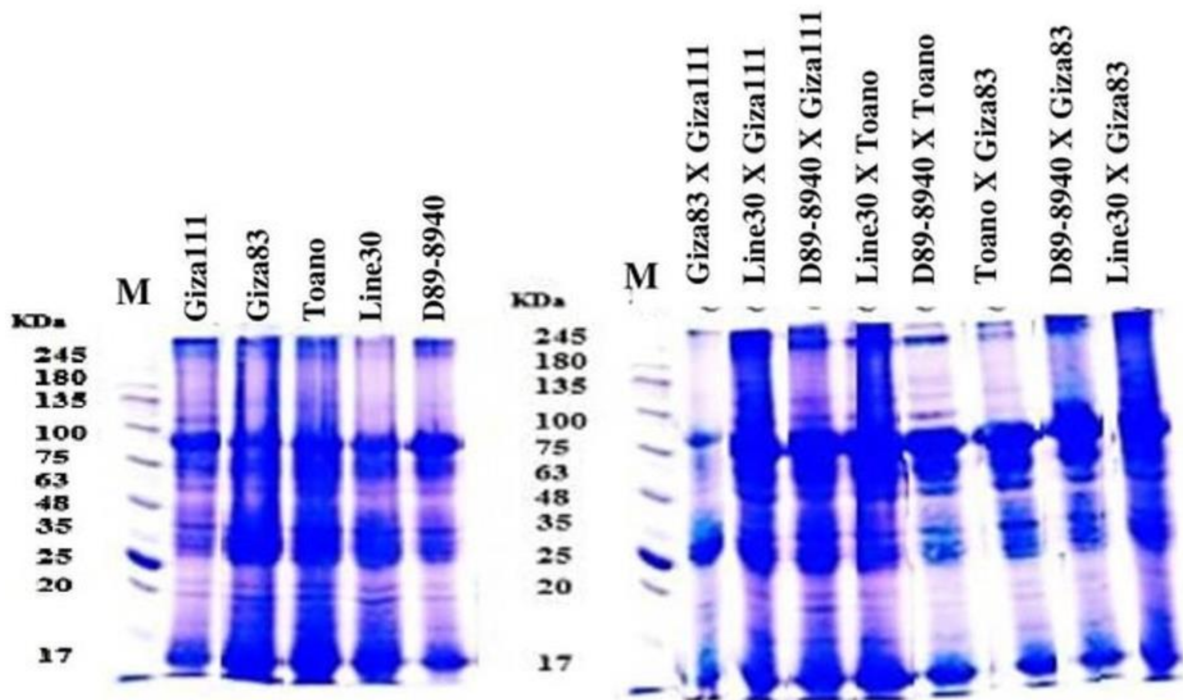


Fig. 5. Protein profile of five *Glycine max* parental genotypes and their eight F1 hybrids against protein molecular marker (M)

4-The Genetic Relationships Among Parental Genotypes:

4.1. The GS with the derived dendrogram among parental genotypes based on RAPD:

The GS among parental genotypes based on the RAPD-PCR pattern ranged from 0.038 to 0.13 among the parental genotypes by a mean similarity of 0.7 (Table SI 1). The dendrogram based on RAPD classified parents into three groups (Fig. 6-a). Group I had the highest GS (0.038), including Giza111 and Giza83 on one clade, and Group III had the lowest GS (0.13), including Line30 and D89-8940. The latter parents had the least GS to Giza111.

Group II clustered Toano separately at zero coefficients on the phylogenetic dendrogram since Toano exerted zero bands at the RAPD pattern despite the good DNA quality (long strands and absence of smears) that enabled the flanking by ISSR primers.

Table SI 1. Similarity and distance indices (Jaccard) among five *Glycine max* parental genotypes based on twelve RAPD primers.

Genotype	Giza111	Giza83	Toano	Line30	D89-89-40
Giza111	1	0.13	0	0.038	0.038
Giza83		1	0	0.059	0.10
Toano			1	0	0
Line30				1	0.11
D89-8940					1

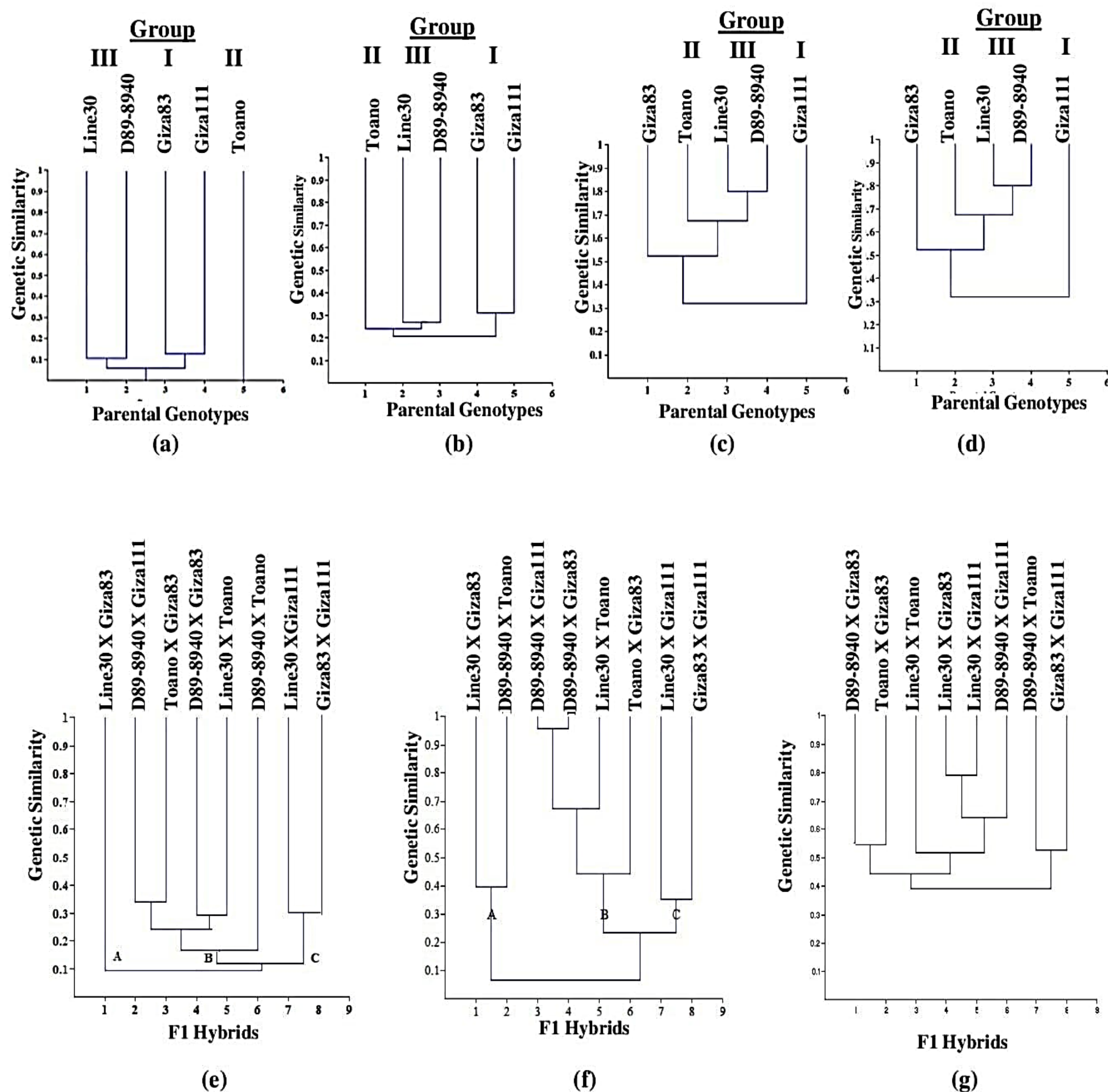


Fig. 6. UPGMA phylogenetic dendrograms representing the genetic distance based on: (a) twenty RAPD primers, (b) fifteen ISSR primers, (c) twenty-seven primers for the combined RAPD and ISSR patterns, (d) protein profile for Glycine max parental genotypes; and for their eight hybrids based on (e) twenty RAPD primers, (f) fifteen ISSR primers, (d) protein profile.

4.2. The GS with the derived dendrogram among parental genotypes based on ISSR:

The GS based on the ISSR-PCR pattern ranged from 0.17 to 0.31 based ISSR-PCR pattern (Table SI 2) among the parental genotypes by a mean similarity of 0.24. The highest detected GS by ISSR-PCR primers was between Giza83 and Giza111 (0.31), while the lowest GS was between Giza83 and D89-8940 (0.17).

The ISSR-phylogenetic dendrogram diverged into two clades representing three groups (Fig. 6-b). One included Giza111 and Giza83, which had a similarity of 0.31 and represented group I. The other clade had two sub-clades; Toano occupied a separate sub-clade and represented group II, while the other sub-clade included Line30 and D89-8940, representing group III.

Table SI 2. Similarity and distance indices (Jaccard) among five *Glycine max* parental genotypes based on fifteen ISSR.

Genotype	Giza111	Giza83	Toano	Line30	D89-8940
Giza111	1	0.31	0.21	0.19	0.20
Giza83		1	0.25	0.21	0.17
Toano			1	0.26	0.22
Line30				1	0.27

Combined RAPD and ISSR molecular Markers of Parents:

The combined RAPD-ISSR pattern flanked 355 marker loci out of 761 loci (Table SI 3). The combined RAPD-ISSR pattern revealed that the highest number of segregated loci was in Gia83 (72%) and Line30 (70%), while the highest number of unique fragments was in Line30 only (96 marker loci). Combined RAPD and ISSR similarity indices showed that the GS ranged from 0.45 to 0.54.

The phylogenetic dendrogram constructed based on similarity indices for the combined pattern of RAPD and ISSR markers showed a divergence of the studied genotypes into two clades (Fig. 6-c). The first clade represented Giza111 (group I). The other clade carried Giza83 on one subclade. The other sub-clade had two sub-subclades, one carried Toano (group II) while the other subclade diverged into Line30 and D89-8940 (group III).

Table SI 3. Similarity and distance indices (Jaccard) among five *Glycine max* parental genotypes based on combined RAPD and ISSR pattern, using twenty-seven primers.

Genotype	Giza111	Giza83	Toano	Line30	D89-8940
Giza111	1	0.54	0.53	0.45	0.46
Giza83		1	0.55	0.47	0.47
Toano			1	0.51	0.50
Line30				1	0.47
D89-8940					1

4.3. The GS with the derived dendrogram among parental genotypes based on protein profiling:

Protein similarity and distance indices among parental genotypes showed a similarity range of 0.44882 to 0.53616 (Table SI 4 and Fig. 6-d). The constructed phylogenetic dendrogram based on proteins clustered genotypes into two clades; the first clade carried Giza111 (group I). The second clade carried Giza83 on one subclade, while the other subclade branched into Toano (group II) on one sub-subclade, and Line30 and D89-8940 (group III) on the other branch.

Table SI 4. Similarity and distance indices (Jaccard) among five *Glycine max* parental genotypes based on protein profiling.

Genotype	Giza111	Giza83	Toano	Line30	D89-89-40
Giza111	1	0.49041	0.53616	0.53616	0.47229
Giza83		1	0.49685	0.44882	0.48442
Toano			1	0.54996	0.50959
Line30				1	0.46043
D89-8940					1

5. The Genetic Relationships Among F1 Hybrids:

5.1 The GS with the Derived Dendrogram among F1 Hybrids Based on RAPD:

The GS of hybrids based on the RAPD-PCR pattern ranged from 0.037 to 0.34 (Table SI 5). The dendrogram of the tested hybrids with RAPD-PCR primers (Fig. 6-e) classified the hybrids into three major groups. The highest GS was between Toano x Giza83 and D89-89-40 x Giza111, which occupied the same sub-sub-clade. The least GS was between Line30 x Giza83 and Giza83 X Giza111.

The dendrogram showed Line30 x Giza83 on clade (A) while the other clade branched into two sub-clades (B and C). The hybrids Line30 x Giza111 and Giza83 x Giza111 diverged from subclade (C). The sub-clade (B) diverged into two sub-subclades; D89-89-40 x Toano was located on one while the other sub-subclade carried the remaining hybrids.

3.5.2 The GS with the derived dendrogram among F1 hybrids based on ISSR

The GS of the Fi hybrids based on ISSR ranged from 0.005 to 0.9575 (Table SI 6). The least GS of the Fi hybrids was between the hybrid Line30 x Giza83 on the sub-clade (A) and Line30 x Giza111 on the sub-cade (C) as a divergence from a different clade (Fig. 6-f). The greatest GS was between the hybrid D89-8940 x Giza83 and D89-8940 x Giza111, which diverged from clade (B).

The dendrogram based on ISSR-PCR primers classified hybrids into three main groups. The first group included a bifurcated clade (A) occupied by Line30 X Giza 83 and D89-8940 x Giza111. The second clade diverged into a sub-clade occupied by Line30 X Giza111 and X Giza 83 x Giza111 on the sub-clade (C) while the remaining hybrids are on the sub-clade (B).

3.5.3 The GS with the derived dendrograms among F1 hybrids based on protein profile

The GS ranged from 0.33977 to 0.67974. the highest GS was between the cross Line30 X Giza111 and Line30 X Giza83 (Table SI 7), which occupied the same sub-clade as lineage on the dendrogram (Fig. 6-g). The hybrid Giza83 X Giza111and Line30 X Giza111, and the hybrid D89-8940 X Giza83 and Line30 X Toano had the least GS.

The protein profile dendrogram of F1 hybrids branched the crosses into two clades: the first included D89-8940 x Toano and Giza83 X Giza111. The other clade diverged into two subclades: one carried D89-8940 X Giza83 and Toano X Giza83, and the other diverged into two sub sub-clades; one represented Line30 X Toano while the other included the remaining crosses.

Table SI 5. Similarity and distance indices (Jaccard) among eight F1 hybrids of *Glycine max* based on twelve RAPD primers.

Crosses	Giza83 x Giza111	Line30 x Giza111	D89-8940 x Giza111	Toano x Giza83	Line30 x Giza83	D89-8940 x Giza83	Line30 x Toano	D89-8940 x Toano
Giza83 x Giza111	1	0.3	0.18	0.16	0.037	0.072	0.091	0.078
Line30 x Giza111	0	1	0.19	0.11	0.072	0.12	0.14	0.055
D89-8940 x Giza111			1	0.34	0.083	0.24	0.25	0.10
Toano x Giza83				1	0.11	0.22	0.27	0.16
Line30 x Giza83					1	0.079	0.12	0.14
D89-8940 x Giza83						1	0.29	0.22
Line30 x Toano							1	0.18
D89-8940 x Toano								1

Table SI 6. Similarity and distance indices (Jaccard) among eight F1 hybrids of Glycine *max* based on fifteen ISSR.

Crosses	Giza83 x Giza111	Line30 x Giza111	D89-8940 x Giza111	Toano x Giza83	Line30 x Giza83	D89-8940 x Giza83	Line30 x Toano	D89-8940 x Toano
Giza83 x Giza111	1	0.35	0.155	0.3975	0.35	0.0675	0.3875	0
Line30 x Giza111		1	0.055	0.195	0.005	0.4525	0.155	0.005
D89-8940 x Giza111			1	0.475	0.0275	0.9575	0.6175	0.0175
Toano x Giza83				1	0.0175	0.3925	0.4625	0.0175
Line30 x Giza83					1	0.01	0.4775	0.3975
D89-8940 x Giza83						1	0.7325	0.0425
Line30 x Toano							1	0.16
D89-8940 x Toano								1

Table SI 7. Similarity and distance indices (Jaccard) among eight F1 hybrids of *Glycine max* based on protein profiling.

Crosses	Giza83 x Giza111	Line30 x Giza111	D89-8940 x Giza111	Toano x Giza83	Line30 x Giza83	D89-8940 x Giza83	Line30 x Toano	D89-8940 x Toano
Line30 X Giza111	1	0.57634	0.67974	0.42256	0.40086	0.39977	0.4937	0.40086
D89-8940 X Giza111		1	0.57634	0.49363	0.4691	0.40086	0.46981	0.33977
Line30 X Giza83			1	0.46891	0.4453	0.37983	0.49363	0.40086
Line30 X Toano				1	0.42265	0.40086	0.42265	0.33977
D89-8940 X Toano					1	0.51962	0.40086	0.40086
Giza83 X Giza111						1	0.37983	0.46891
Toano X Giza83							1	0.49363
D89-8940 X Giza83								1

6. The Yield Traits:

The yield traits of *G. max for* both parents and F1 hybrids mostly showed differences with varied significance degrees (Fig. 7). The parent Giza111 was distinguishable regarding the number of pods, 100-seed weight (g), and seed yield/plant. Yield traits showed distinguishability of the parent Giza83 regarding the number of branches, followed by Toano and Giza111. Despite D89-8940 having the lowest yield traits and seeming a parent with low values, it resulted in superior hybrids regarding the number of branches and the 100-seed weight when crossed by Giza83 and the number of pods by crossing Giza111.

Giza111 and Line30, followed by Toano and Giza83, had the highest 100-seed weight (g). Consequently, the crosses Line30 x Toan, Line30 x Giza83, and D89-8940 x Giza83 had the highest 100-seed weight. Giza111 was the best parent regarding the seed yield/plant and exerted a distinguishable combination with Line30 in the cross Line30 x Giza111. On the other hand, Giza111 was the worst combination with Giza83 in the cross Giza83 x Giza111.

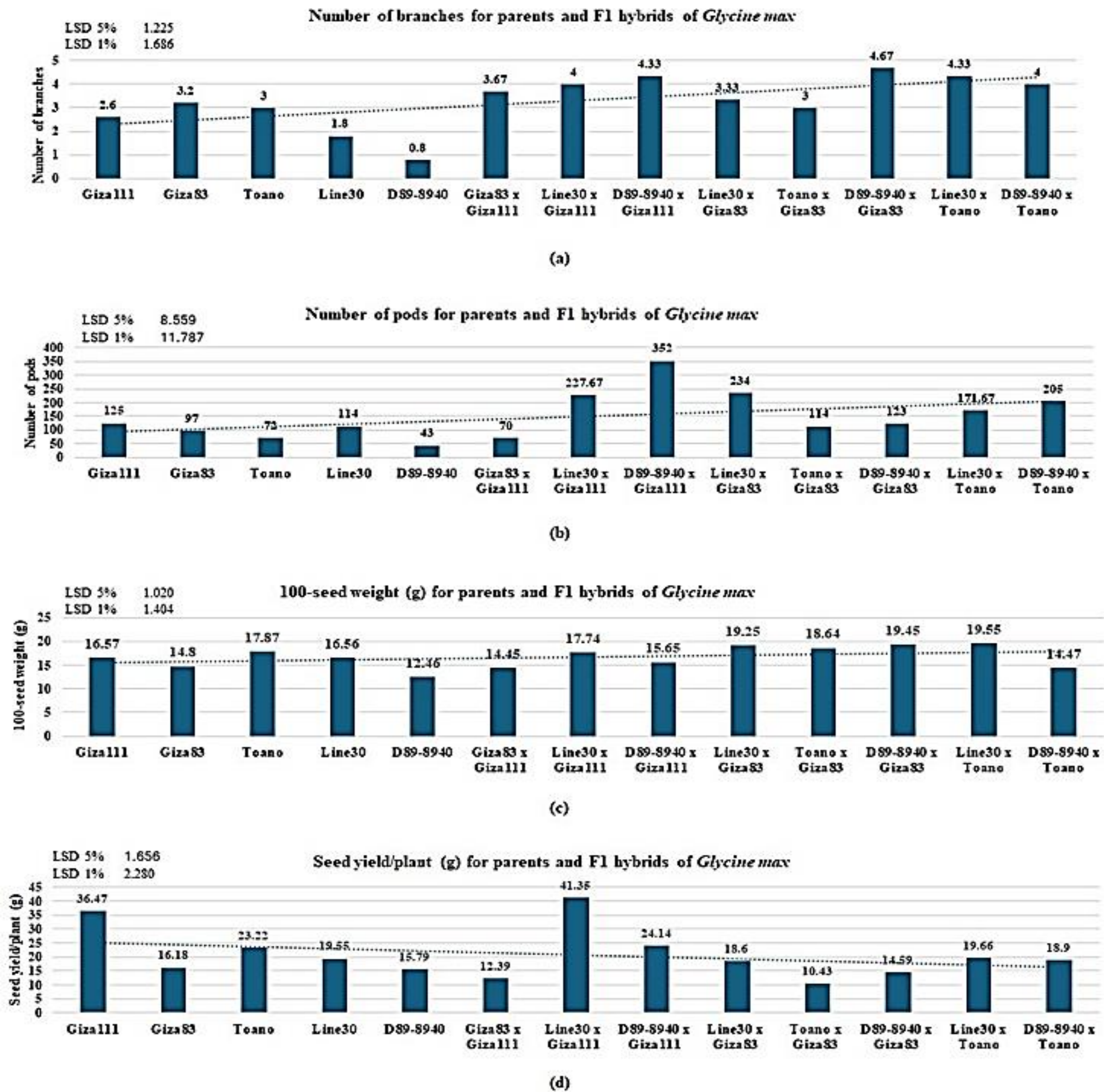


Fig. 7. Productivity of five parental Glycine max genotypes and their F1 hybrids expressed in terms of (a) number of branches, (b) number of pods, (c) weight of 100-seed yield; and (d) number of seeds per plant

DISCUSSION

The harsh environmental conditions need sustainable efforts to characterize promising genotypes. Constraining these good parents for breeding programs enables improving crops. Accordingly, this study concerned molecular genotyping and yield evaluation of five *G. max* genotypes and their F1 hybrids, using primers almost used for drought tolerance assessment.

1-Genotyping and Assessment of the Parental Genotypes:

Protein profiling detected 24.2% polymorphism among *G. max* parental genotypes. Protein phylogenetics was a rapid tool for determining genetic diversity and discriminating-related varieties (Singh *et al*., 2017; Tadesse *et al*., 2018; Sihmar *et al*., 2020; El-Hadary *et al*., 2022). Comparative molecular screening of the genomic resources for diverse germplasm could provide marker genotyping platforms and predict the translational genomics in soybeans to design a strategy for improving drought tolerance (Valliyodan *et al*., 2017) since protein is a brief of the DNA coding region. Combining

different biochemical and molecular markers regardless of specificity is more efficient for an inclusive clustering of genotypes. For example, the phylogenetic dendrogram drawn by protein profiling and combined RAPD-ISSR was almost similar due to protein translation as a final product for the targeted genes in all DNA coding regions.

RAPD and ISSR of the parental soybean genotypes detected 100% and 73.8% polymorphism, respectively. Accordingly with our result, Valliyodan *et al.* (2017) reported the ability of molecular markers for genotyping soybeans regarding drought tolerance by compatible markers. Also, RAPD efficiency for detecting genetic variability in *Trichodesma indicum* populations was remarked (Verma *et al.*, 2009) depending on fragment size for comparing loci (Rieseberg, 1996). Using a set of molecular markers for screening the studied genotypes was assumed to be more effective than a sole marker. For instance, Toano had a highly polymorphic protein pattern with the highest number of unique fragments and exerted 76.7% polymorphism and 73 marker loci with ISSR but had no flanked regions by any recruited RAPD primer for the same extracted genomic sample. Thus, the similarity of Toano to other genotypes might be limited to the transcribed proteins and ISSR region since it has the highest number of polymorphic ISSR fragments (56 loci) and protein marker fragments (four bands). This result was emphasized by the mostly limited similarity of Toano to the other genotypes. Contrastingly, the similarity of the other recruited genotypes had spread along the chromosome according to the RAPD pattern. Toano might be highly polymorphic along the whole chromosome except in ISSR regions. These results agree with the literature that cited ISSR efficiency for assaying diversity among soybean genera (Baloch *et al.*, 2010), closely related genotypes in Oryza (Joshi *et al.*, 2000), and wheat (EL-Gawwad *et al.*, 2009).

The screening with combined RAPD-ISSR clusteredvLine30 and D89-8940 together and suggests them as a well polymorphic genetic pool due to the enrichment with both segregated and marker loci that attributed to using drought-tolerant associated primers as described by previous studies (Islam *et al.*, 2013; Akladious and Abbas, 2020). This result agrees with Costa *et al.* (2016) results that clarified genetic relationships among closely related orchard grass species by more than biochemical and molecular markers like combining RAPD-ISSR (Guasmi *et al.*, 2012; Verma *et al.*, 2017; Ninh *et al.*, 2024). On the other hand, the individual patterns of RAPD and ISSR clustered Giza111 and Giza83 together with a high GS that nominated them as a bad parental combination due to the narrow genetic distance between them.

2. Genotyping and Assessment of the F1 Hybrids:

Protein and RAPD profiles certified the success of hybridization processes since the hybrid profile showed variation from the parental profile, documented by the lower number of fragments in hybrids than parents even by using the same primers. Also, ISSR proved the hybridization process by detecting molecular differences between hybrids and their respective female parents. ISSR markers verified eight soybean F1 crosses, selected after excluding crosses with low field performance based on displaying morphological characteristics like those of their respective female parents. These results contribute to parental selection in future soybean breeding programs. ISSR markers evaluated the genetic variation within collections of cultivated plants (Sonnante and Pignone, 2001).

Our results confirm that ISSR markers are efficient tools for discriminating hybrids from the self-pollinated progeny of female parents in controlled crosses. ISSR effectively fingerprinted and differentiated plants with highly similar morphological characteristics. The hybridity status was verified by comparing amplified polymorphic bands between F1 hybrids and female parents. These results agreed with the results of Bianco *et al.* (2011) that ISSR markers are

effective in seed purity estimation and prediction of F1 hybrid characteristics in several plants, including artichokes.

Akladious and Abbas (2020) used SH4 and SH9 primers for association with water stress tolerance in barley genotypes. Thus, the highest percentage of polymorphism between male and female parents and between F1-hybrids and female parents in our study was with SH9 primer followed by SH4. A comparison of allelic patterns between parental and hybrid genotypes showed that some alleles (375 bp with primer SH11) were present in D89-89-40 x Toano and D89-89-40 x Giza83 despite the absence from parental genotypes. In contrast, some bands were present in parental genotypes but were absent in the hybrid plants. For example, the allele of 4086 bp was absent in D89-89-40 x Giza83 and Line30 x Giza83 but was present in both its parental genotypes with primer SH2. Also, many alleles disappeared in other hybrids like Line30 x Toano, D89-89-40 x Toano, and Line30 x Giza83 with primer SH10. Other hybrids did not give any product with primers.

Different allelic patterns between parents and hybrids that produce novel allelic patterns could be due to nucleotide changes in flanking regions for primer annealing, chromosomal crossing over during meiosis, and loss of primer attachment pair sites in each genotype (Sheidai *et al.*, 2008). This phenomenon has also been reported in other plant species hybrids (Sushir *et al.*, 2008).

3. Yield Traits and Hybridization Compatibility with Regards to Genetic Similarities:

RAPD and ISSR revealed the highest similarity between Giza111 and Giza83, so they were non-compatible parents due to the narrow genetic distance (GD) between them. Giza83 x Giza111 crosses were not distinguishable regarding all tested yield traits with values less than parents. Our results agreed with the quantitative genetic theory that heterosis requires GD between parents at heterotic quantitative trait loci since a positive association between heterosis and GD even varied in strength (Würschum *et al.,* 2023). Also, results agreed with the theory of optimal mating distance (OMD), which predicts that the GD between parents is neither too small nor too large for maximizing heterosis and hybrid fitness while avoiding genetic incompatibility (Wei and Zhang, 2018).

Giza111 was a promising parent since possessing the most distinguishable yield traits and the highest GD from Line30 and D89-8940 according to all the used markers. Giza111 had the highest number of pods and seed yield/plant compared to other parents. Thus, the crosses Line30 x Giza111 and D89-8940 x Giza111 had the highest seed yield/plant and pod number compared to their parents. These results referred to the adequate combining ability due to the considerable GS between Giza111 and the parental genotypes Line30 or D89-8940. The additive effect of Giza111 suggested its dominance regarding yield/plant and number of pods over all the other parents according to the derived hybrid results, offering hybrid superiority.

The distinguishable number of branches in Giza83 enabled hybrids to reach values higher than their parents (hybrid superiority), suggesting Giza83 dominance over the other parent in the crosses Line30 x Giza83 and D89-8940 x Giza83. Line30 dominance over Giza83 was suggested regarding the number of pods, 100-seed/plant, and yield/plant since Line30 x Giza83 had better results than the parent Giza83. Our results agreed with the results of Würschum *et al*. (2023) that endorsed the phenotypic superiority of a hybrid over both parents based on the dominance model that proposes hybrid complementation for the prejudicial alleles present in the parents at many loci by dominant alleles from the other parent. On the other hand, it was unclear whether heterosis results from dominance, overdominance, or positive intergenic epistasis (Lippman and Zamir, 2007). Siles *et al.* (2004) and Abd El-Aty *et al*. (2023) added that the significant correlations between traits cause additive and/or variety heterosis effects for genes controlling grain yield, plant height, and spike length in either the same or in coupling phase linkage. Some parents had an adverse effect

regarding certain traits like seed/plant that significantly diminished in Toano X Giza83 to reach the minimum among parents and hybrids. This result suggested the species incompatibility (Seidel *et al.,* 2008; Corbett-Detig *et al*., 2013; Cutter, 2012). The species incompatibility was described by inbreeding and outbreeding depression (Lynch, 1991) as a converse of heterosis that led to a vigor decrease of the hybrid due to close relatives mating or when deleterious recessive alleles becoming homozygous, resulting in a hybrid necrosis phenomenon (Bomblies and Weigel 2007; Li and Weigel 2021).

The phenotypic superiority or inbreeding depression in hybrids might be due to gene action with each other and the new appearance or disappearance of some alleles in the hybrid to satisfy hybrid complementation for some deleterious alleles, as the ISSR pattern showed. For example, D89-89-40 x Giza83 exerted *de novo* presence of an allele of 375 bp with primer SH11 but the absence of an allele of 4086 bp with primer SH2 compared to parents, accompanied by hybrid superiority to the number of branches, number of pods, and 100 seed/plant but inbreeding depression of seeds/plant. This result could suggest a somewhat relation between the presence/absence of the mentioned alleles and the hybrid superiority/inbreed depression regarding the tested yield traits. Also, the *de novo* presence of an allele of 375 bp with primer SH11 in D89-89-40 x Toano but the absence of an allele of 4086 bp with primer SH2 in Line30 x Giza83 compared to parents, accompanied by hybrid superiority to the tested yield traits. Moreover, the varied number of fragments between parents and hybrids indicated the *de novo* presence or absence of some alleles according to protein and RAPD patterns. This result is explained by the potential occurrence of dominance, overdominance, or positive intergenic epistasis to satisfy heterosis and hybrid vigor (Lippman and Zamir, 2007). and hybrid complementation (Würschum *et al.,* 2023).

Conclusion

This work reported a high polymorphism among five *G. max* genotypes, and their F1 hybrids with GS ranged from too narrow to moderate based on patterns of RAPD, ISSR, RAPD+ISSR, and protein profiling. Breeding incidence was ensured by ISSR pattern scoring for hybrids, which relied on polymorphic bands between male and female parents and polymorphic bands between F1 hybrid and female parents. Line30 and D89-8940 had the highest number of unique loci, while Giza111 had a moderate number according to RAPD, ISSR, and protein profile. Toano, Giza83, and Line30 had the highest polymorphism according to ISSR, while Line30 and D89-8940 had the highest polymorphism according to RAPD and protein.

The GS among parental genotypes can play a relative indicator for excluding some parents with high similarity following optimal mating distance theory. For example, Giza83 X Giza111 was a bad cross due to the highly similar entities between the parents. However, the combination of parents with moderate GS entities like Giza111 and enriched with segregated and marker loci like Line30 and D89-8940 represented a well polymorphic genetic pool for hybrid superiority compared to parents regarding yield traits. This study suggested some hybrid complementation by *de novo* presence or absence of specific alleles compared to parents as derived from the ISSR pattern of D89-89-40 x Giza83, Line30 x Giza83, Line30 x Toano, and D89-89-40 x Toano. The superiority of some hybrids derived from parents with low performance (D89-8940) was satisfied when crossed by good parents (Giza111 or Giza83) regarding some yield traits.

The study recommends Giza111 as a compatible parent for crossing with Line30 or D89-8940 due to the superiority of hybrids over parents regarding the branch number, pod number, 100-seed/plant, and seeds/plant. Also, the crossing of Giza83 with Line30 regarding all tested traits and with D89-8940 regarding the number of branches and 100-seed/plant resulted in hybrid superiority. The study concludes that Toano mostly was an

incompatible parent with other parents, causing inbreeding depression, suggesting Toano was a parent of too far GD based on the markers set. Giza111 and Giza83 were not compatible parental combinations. Giza111 was a compatible parent with Line30 or D89-8940. Giza83 followed Giza111 in compatibility with Line30 or D89-8940.

Declarations:

Ethical Approval: The study neither use human nor animals at any research stage, so there was no need for any ethical approval.

Author Contributions: Conceptualization suggestion and project administration were done by Mona H. El-Hadary and Samar A. Omar; methodology, software, formal analysis, investigation, resources, data curation, software and funding acquisition, validation, were carried out by Mona H. El-Hadary, Samar A. Omar, and Shimaa S. El-Sherbini; original draft preparation, was written by Mona H. El-Hadary, and Shimaa S. El-Sherbini; writing—review, editing, and visualization were done by Mona H. El-Hadary, supervision, was done by Mona H. El-Hadary and Samar A. Omar**.**

Conflict of interests: The authors declare no conflicts of interest.

Funding: This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of Data and Materials: All data used to conduct this study is provided within the manuscript and as supplementary material.

Acknowledgements: We would also like to thank Prof. Dr. Adel El-Garhy Mohamad, Food Legume Research Section-Field Crops Research Institute (Itay El Baroud station)-Agriculture Research Centre– Egypt for their guidance while holding the experimental work in lands of Itay El Baroud station.

REFERENCES

Abd El-Aty, M.S., El-Hity, M.A., Abo Sen, T.M., El-Rahaman, I.A.E.A., Ibrahim, O.M., Al-Farga, A., and El-Tahan, A.M. (2023). Generation Mean Analysis, Heterosis, and Genetic Diversity in Five Egyptian Faba Beans and Their Hybrids. *Sustainability,* 15, 12313. https://doi.org/10.3390/ su151612313.

Abd EL-Mageed A. M. (2018). Genetic Diversity among Faba Bean Hybrids in relation with heterosis using molecular and agronomic data. Egypt. *Egyptian Journal of Agronomy,* 40 (3): 237- 249. https://agro.journals.ekb.eg/article_19515.html

Akladious, S.A., and Abbas, S.M. (2020). Inter simple sequence repeat (ISSR) markers and some physiological attributes of barley genotypes to drought and potassium nutrition. *Life Science Journal,* 17(9): 84-98. http://www.lifesciencesite.com. Doi:10.7537/marslsj170920.10.

Amiteye, S. (2021). Basic concepts and methodologies of DNA marker systems in plant molecular breeding. *Heliy,* 7: e7: e08093. D**oi**. 10.1016/j.heliyon. 2021.e08093

Baloch, F.S., Kurt, C., Arioglu, H., and Ozkan, H. (2010). Assaying of diversity among soybean (*Glycin max* (L.) Merr.) and peanut (*Arachis hypogaea* L.) genotypes at DNA level. *Turkish Journal of Agriculture and Forestry,* 34: 285-301. https://doi:10.3906/tar-0907-281

Bianco, C. L., Fernández, J. A., Migliaro, D., Crinò, P., and Egea-Gilabert, C. (2011). Identification of F1 hybrids of artichoke by ISSR markers and morphological analysis. *Molecular Breeding,* 27: 157-170. Doi**:** 10.1007/s11032-010-9418-0

Bomblies, K., and Weigel, D. (2007). Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nature Reviews Genetics,* 8(5): 382-93. Doi. 10.1038/nrg2082.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* 72(1-2): 248-254. https://doi.org/10.1016/0003-2697(76): 90527-3.

Comstock, R. E., and Robinson, H. F. (1948). The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. *Biometrics,* 4: 254-266. PMID: 18108899.

Corbett-Detig, R.B., Zhou, J., Clark, A.G., Hartl, D.L., and Ayroles, J.F. (2013). Genetic incompatibilities are widespread within species. *Nature,* 504:135-137. Doi. 10.1038/nature 12678

Costa, R., Pereira, G., Garrido, I., Tavares-de-Sousa, M. M., and Espinosa, F. (2016). Comparison of RAPD, ISSR, and AFLP molecular markers to reveal and classify orchardgrass (*Dactylis glomerata* L.) germplasm variations. *PLoS ONE,* 11(4), e0152972. https://doi.org/10.1371/journal.pone.0152972

Cutter, A.D. (2012). The polymorphic prelude to Bateson–Dobzhansky–Muller incompatibilities. *Trends in Ecololgy and Evolution,* 27:209–218. https://doi.org/10.1016/j.tree.2011.11.004

Durán, Y., and Vega, M.P. (2004). Assessment of genetic variation and species relationship in a collection of lens using RAPD and ISSR. *Spanish Journal of Agricultural Research,* 2(4): 538-544. **D**oi. https://doi.org/10.5424/sjar/2004024-110

Dure, L., Greenway, S. C., and Galau, G. A. (1981). Developmental biochemistry of cotton seed embryogenesis and germination: changing messenger ribonucleic acid populations as shown by *in vitro* and *in vivo* protein synthesis. *Biochemistry,* 20: 4162-4168. https://doi.org/10.1021/bi00517a033

El-Abssi, M., and Rabie, H., Awaad, H., and Qabil, N. (2019). Combining ability of earliness, yield, quality and chocolate spot disease for faba bean. *Bioscience Research,* 16: 3584–-3594. https://www.isisn.org/Bioscience_Research.htm

EL-Gawwad, M., EL-Rabey, H., Khalaf, R., EL-Hadary, M., and EL-Halafawy, K. (2009). Cytological and molecular characterization of some wheat (*Triticum aestivum* L.) cultivars. *Egyptian Journal of Genetics and Cytology,* 38(2): 235-250. http://www.journal.esg.net.eg/index.php/EJGC/article/view/16

El-Hadary, M.H., Elsaied, H.E., Khalil, N.M. and Mikhail, S.K. (2022). Molecular taxonomical identification and phylogenetic relationships of some marine dominant algal species during red tide and harmful algal blooms along Egyptian coasts in the Alexandria region. *Environmental Science and Pollution Research,* 29, 53403–53419. https://doi.org/10.1007/s11356-022-19217-8.

Ethiopian Institute of Agricultural Research, Chiro National Sorghum Research and Training Center, Chiro, Ethiopia (2024). Genetic Variability and Its Benefits in Crop Improvement: A review Temesgen Begna and Temesgen Teressa. *Middle East Journal of Agriculture Researchers, 13*(1): 128-136. Doi. 10.36632/mejar/2024.13.1.6

Guasmi, F., Elfalleh, W., Hannachi, H., Fères, K., Touil, L., Marzougui, N., Triki, T., and Ferchichi, A. (2012). The use of ISSR and RAPD markers for genetic diversity among south Tunisian barley. *ISRN Agronomy,* e952196, 10p. https://doi.org/10.5402/2012/952196.

Hammer, Ø., Harper, D. A. T., and Ryan, P. D. (2001). Past: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica,* 4(1): 4-9. http://palaeo- electronica.org/2001_1/past/issue1_01.htm.

Hirao, T., Matsunaga, K., Hirakawa, H., Shirasawa, K., Isoda, K., Mishima,

K., Tamura, M., Watanabe, A. (2019). Construction of genetic linkage map and identification of a novel major locus for resistance to pine wood nematode in Japanese black pine (*Pinus thunbergii*). *BMC Plant Biology,* 15;19(1): 424. Doi. 10.1186/s12870-019-2045-y. PMID: 31615405; PMCID: PMC6792208.

Hou, Y.-C., Yan, Z.-H., Wei, Y.-M., and Zheng, Y.-L. (2005). Genetic diversity in barley from west China based on RAPD and ISSR analysis. *Barley Genetics Newsletter,* 35: 9-22. e: https://jsrs.journals.ekb.eg/

Hromadová, Z., Gálová, Z., Mikolášová, L., Balážová, Ž., Vivodík, M., and Chňapek, M. (2023). Efficiency of RAPD and SCoT Markers in the Genetic Diversity Assessment of the Common Bean. *Plants (Basel),* 25;12(15): 2763. Doi: 10.3390/ plants12152763. PMID: 37570917; PMCID: PMC10420954.

Islam, M., Ali, M., Guswami, P., Ullah, S., Hossain, M., Miah, M., and Prodhan, S. H. (2013). Assessment of genetic diversity among moderately drought tolerant landraces of rice using RAPD markers. *Journal of Bio Science and Biotechnology,* 2(3): 207-213. ISSN: 1314-6246. http:// www.jbb.uni-plovdiv.bg

Joshi, S., Gupta, V., Aggarwal, R., Ranjekar, P., and Brar, D. (2000). Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus Oryza. *Theoretical and Applied Genetics ,* 100(8), 1311-1320. https: //doi.org/10.1007/s001220051440

Kempthorne, O., and Curnow, R. (1961). The partial diallel cross. *Biometrics* 17: 229-250.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227(5259): 680-685. https://doi.org/ 10.1038/227680a0

Li, L. and Weigel D. (2021). One hundred years of hybrid necrosis: hybrid autoimmunity as a window into the mechanisms and evolution of plant–pathogen interactions. *Annual Review of Phytopathology,* 59: 213-237. Doi. 10.1146/annurev-phyto-020620-114826

Lippman, Z. B., and Zamir, D., (2007). Heterosis: Revisiting the magic. *Trends in Genetics,* 23: 60–66.

Lynch, M. (1991). The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution,* 45(3): 622-629. Doi. 10.1111/j. 1558-5646. 1991.tb04333. x. PMID: 28568822.

Mathpal, S., Joshi, R., Bhatt, B., Sahoo, S., and Joshi, S. (2024). Randomly Amplified Polymorphic DNA (RAPD) Molecular Markers as a Valuable Tool to Confirm the Genetic Diversity in Soybean Germplasms (*Glycine max*). *Indian Journal of Agricultural Research,* A-6176 [1-7]. Doi.10.18805/IJARe.A-6176

Mishra, R., Tripathi, M. K., Sikarwar, R. S., Singh, S.Y., and Tripathi, N. (2024).Soybean (*Glycine max L. Merrill*): A Multipurpose Legume Shaping Our World. *Plant Cell Biotechnology and Molecular Biology,* 25(3-4): 17–37. https://doi. org/10.56557/pcbmb/ 2024/v25i3-48643

Nemati, M., Asghari, A., Sofalian, O., Rasoulzadeh, A., and Chamanabad, H. M. (2012). Effect of water stress on rapeseed cultivars using morpho-physiological traits and their relations with ISSR markers. *Journal of Photochemistry and Photobiology B: Biology,2*(1): 55-66 ISSN: 2008-5168.

Ninh, T.T., Nguyen, T.P., Dang, H.T., Duc H. C., Dinh, T.S., Nguyen, X. C., Pham, T. D., Nguyen1, and Thi Hue Nong, T.H. (2024). Assessment of genetic

diversity of *Stephania rotunda* Lour. collected in Northern Vietnam using RAPD and ISSR markers. *Australian journal of crop science.* 18(07):374-381. https://doi.org/10. 21475/ajcs.24.18.07.pne19

Pakniyat, H., Tavakol, E. (2007). RAPD markers associated with drought tolerance in bread wheat (*Triticum aestivum* L.). *Pakistan Journal of Biological Sciences.* 10(18):3237-9. Doi. 10.3923/pjbs.2007.3237.3239. PMID: 19090135

Paschal, E. H., and Wilcox, J. (1975). Heterosis and combining ability in exotic soybean germplasm. *Crop Science,* 15: 344-349. Doi.10. 2135/ cropsci1975.0011183x0015000300 19x

Pendergast IV, T.H., Qi P., Odeny, D.A. , Dida, M.M. Devos, K.M. (2021). A high-density linkage map of finger millet provides QTL for blast resistance and other agronomic traits. *The Plant Genome,* 15 (1): e20175. https://doi.org/10.1002/ tpg2.20175

Rieseberg, L. H. (1996). Homology among RAPD fragments in interspecific comparisons. *Molecular Ecology,* 5(1): 99-105. https://doi.org/10. 1111/j.1365-294X.1996.tb00295.x

Sambrook, J, Fritsch, E.F., and Maniatis, T. (1989). Molecular cloning, a laboratory manual, 2nd edn. Cold Spring Harbor: Cold Spring Harbor laboratory press. https://doi.org/ 10. 1002/jobm.3620300824

Seidel, H.S. Rockman, M.V., Kruglyak, L. (2008). Widespread genetic incompatibility in *C. elegans* maintained by balancing selection. *Science,* 319:589-594. Doi. 10.1126/science.1151107. PMID: 18187622; PMCID: PMC2421010.

Sihmar, M., Sharma, J.K., Santal, A.R., and Singh, N.P. (2020). Seed storage protein phylogenetics of Indian wheat genotypes belong to *Triticum aestivum*, *T. dicoccum* and *T. durum*. *Indian Journal of Biotechnology,* 19,17-27.

Siles, M.M., Ken Russell, W., Baltensperger, D.D., Nelson, L.A., Johnson, B., Dale van, Vleck L., Jensen S.G, Hein, G. (2004).Heterosis for grain yield and other agronomic traits in *Foxtail Millet*. *Crop Science,* 44(6). https://doi.org/10.2135/ cropsci2004.1960

Singh, B.K., Singh, A.K., Hotti, A. H., Kumar, J., and Singh, S.K. (2017). Diversity analysis through SDS-PAGE of seed storage protein of pea genotypes. *Research in Environment and Life Sciences,* 10(5): 499-452. ISSN: 0974-4908. http://rels. comxa.com.

Singer, W.M., Zhang, B., Rouf Mian, M.A., and Huang, H. (2019). Soybean amino acids in health, genetics, and evaluation. In: Soybean for human consumption and animal feed. Ed. Sudarić, A. https://www.intechopen.com/chapters/69396. Doi. 10.5772/intechopen. 89497

Sneath, P.H.A., and Sokal, R.R. (1973). Numerical taxonomy: The principles and practice of numerical classification. San Francisco, W.H. Freeman, and Company, USA.

Sonnante, G., and Pignone, D. (2001). Assessment of genetic variation in a collection of lentils using molecular tools. *Euphytica,* 120: 301-307. Doi:10.1023/A:1017568824786

Sprague, G.F., and Tatum, L.A. (1942). General vs. specific combining ability in single crosses of corn. *Agronomy Journal,* 34: 923-932. https://doi.org/10.2134/agronj1942. 00021962003400100008x

Sushir, K.V., Mehetre, S.S., Patil, S.C., and Kamdi, S.R. (2008). RAPD and cytogenetic study in F1 and F2 of interspecific cross between *Gossypium arboreum*× *Gossypium anomalum*. *Cytologia, 73*: 213-219.

Swarup, S., Cargill, E.J., Crosby, K., Flagel, L., Kniskern, J., and Glenn K.C. (2021). Genetic diversity is indispensable for plant breeding to improve crops. *Crop Science,* 61: 839–852. doi.org/10.2134/ agronj 1942.00021962003400100008x**.**

Tadesse, L., Mekbib, F., Wakjira, A., and Tadele, Z. (2018). Genetic diversity of seed storage protein in the Ethiopian garden cress (*Lepidium sativum* L.). *African Journal of Biotechnology,* 17(37): 1152-1161, D**oi**. 10.5897/AJB2018.16587.

Valliyodan, B., Ye, H., Song, L., Murphy, M., Shannon, J. G., & Nguyen, H. T. (2017). Genetic diversity and genomic strategies for improving drought and waterlogging tolerance in soybeans. *Journal of Experimental Botany,* 68(8):1835-1849. https://doi.org/10.1093/jxb/er w433.

Verma, N., Koche, V., Tiwari, K., and Mishra, S. (2009). RAPD analysis reveals genetic variation in different populations of *Trichodesma indicum* -A perennial medicinal herb. *African Journal of Biotechnology,* 8(18): 4333-4338. ISSN 1684–5315. http:// www.academicjournals.org/AJB

Verma, K.S., Ul Haq, S., Kachhwaha, S., Kothari, S.L. (2017). RAPD and ISSR marker assessment of genetic diversity in *Citrullus colocynthis* (L.) Schrad: a unique source of germplasm highly adapted to drought and high-temperature stress. *3 Biotech,* 7(5):288. **D**oi. 10. 1007/s13205-017-0918-z. PMID: 28868215; PMCID: PMC5570720.

Wang, X., and Komatsu, S., (2017). Improvement of soybean products through the response mechanism analysis using proteomic technique. *Advances in Food and Nutrition Research.* 82: 117-148. https://doi. org/10.1016/bs.afnr.2016.12.006

Wei, X., Zhang, J. (1018). The optimal mating distance resulting from heterosis and genetic incompatibility. *Science Advances,* 7;4(11): eaau5518. Doi. 10.1126/ sciadv. aau5518. PMID: 30417098; PMCID: PMC6221538.

Welshh J., and McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research,* 25;18(24):7213-8. Doi. 10.1093/nar/18.24.7213. PMID: 2259619; PMCID: PMC332855.

Wolfe, A.D., Xiang, Q.-Y., and Kephart, S.R. (1998). Diploid hybrid speciation in Penstemon (*Scrophulariaceae*). *Proceedings of the National Academy of Sciences,* 28;95(9):5112-5. Doi. 10.1073/ pnas. 95.9.5112. PMID: 9560237; PMCID: PMC20222.

Würschum, T., Zhu, X., Zhao, Y., Jiang, Y., Reif, J.C., Maurer, H.P. (2023). Maximization through optimization? On the relationship between hybrid performance and parental genetic distance. *Theoretical and Applied Genetics,* 12;136(9):186. Doi. 10. 1007/s00122-023-04436-5. PMID: 37572118; PMCID: PMC10423127.

Zanaty, A., El-Hadary, M.H., Ismail, M., and Gmal, A. (2013). Genetic diversity of wheat genotypes based on RAPD relative to F1 hybrid performance. *International Journal of Agronomy and Plant Production (IJAPP),* 4 (5): 1098-1107. Available online at http:// www.ijappjournal.com. ISSN 2051-1914 © 2013 Victor Quest Publications, United Kingdom; London.

Zhao, W.G., Zhang, J.Q., Wangi, Y.H., Chen, T.T., Yin, Y.L., Huang, Y.P., Pan, Y.L., and Yang, Y.H. (2006). Analysis of genetic diversity in wild populations of mulberry from western part of Northeast China determined by ISSR markers. *Journal of Genetics and Molecular Biology*, 17: 196-203.