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Genetic Diversity of Soybean Genotypes revealed by Agro–morphological and SSR markers

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Increasing the diversity of the soybean germplasm base could introduce new genes affecting agronomic traits. In this study, we demonstrated the differences of genetic diversity level among 24 soybean genotypes and adapt an augmented design to screen and select the superior entries among 24 soybean germlasm and to calculate similarity parameters. Likewise, to elucidate the relationships based on molecular markers among new promising lines and introduced genotypes with improved Egyptian commercial cultivars using SSR markers, to use this information in future breeding programs. The results exhibited significant differences among the tested genotypes for all studied characters. This provides evidence for the possibility to carry out a sufficient selection program on the basis of these traits using the studied genotypes. Thirteen out 14 SSR primer pairs amplify polymorphic SSRs from all of these genotypes, a total of 42 alleles were produced. The polymorphic information content (PIC) among genotypes varied from 0.55 (satt001) to 0.88 (satt173) with 2 and 5 alleles respectively. However, Satt005 produced only one monomorphic band. The used SSR primer pairs successfully distinguished most of soybean genotypes, with the exception of a pair of closely related breeding lines from the same cross. The genetic relationships among genotypes based agro–morphological analysis not completely agreed with known pedigrees. However, phylogenetic tree based on SSR confirmed the separation of soybean genotypes into six clusters and were more clearly separated. These results suggest that SSR markers are efficient for measuring genetic diversity and relatedness as well as identifying varieties of soybeans.

Key words: *Glycine max*, polymorphism, phylogenetic tree, SRAP, SSR

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the most frequently cultivated crops worldwide. It is important for both protein meal and vegetable oil in addition it is rich in lysine and vitamins and is used for both human and animal consumption as well as for industrial purposes, such as biofuels (Hartman *et al*., 2011). One of the pre–requirements for soybean successful breeding strategies for biotic and or a biotic stress is the complete understanding of the genetic diversity of this crop. The augmented designs were proposed by Federer (1956) to permit the early assessment of many new varieties when no replicated trial is possible due to paucity of seed material and limitations of introduced or lines seeds, it has been used in field screening trials of soybean (Spehar, 1994). Genetic relationships among number of tested genotypes can be measured by similarity using number of quantitative characters which meaning that the differences among characters of tested genotypes attributed to the genetic divergence of these genotypes in soybean Iqbal *et al*. (2008) and Ojo *et al*. (2012). Several methods have been used to investigate the genetic variation in soybean. Morphological and agronomic traits have been employed (Perry and McIntosh, 1991; Sneller *et al*., 1997). As in other major crops, genetic diversity of soybean grown is very narrow (Brown–Guedira *et al*. 2000), and has been decreasing at an alarming rate. The narrow genetic base of soybean cultivars has been confirmed in many studies based on pedigree analysis (Delannay *et al*., 1983; Gizlice *et al*., 1994) or molecular markers (Narvel *et al*., 2000; Thompson *et al*., 1998; Khatab and Morsy, 2012).

At present, the use of exotic germplasm in soybean cultivar development generally has been limited to a small number of introductions (introduced) that have served as sources of genes for resistance to biotic and/or a biotic stress. To efficiently broaden the genetic base of soybean cultivars a detailed insight into genetic diversity of soybean resources is required. Such insight could be achieved through molecular characterization using DNA markers, which are more informative, stable and reliable, compared to pedigree analysis and traditionally used biochemical markers. Microsatellites or simple sequence repeat markers (SSR) are being extensively used in

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genome studies, marker assisted selection, and cultivar identification and are well–known for their versatility in providing a quick assay and for their highly informative data (Prioli *et al*., 2002; Abe *et al*., 2003; Wang *et al*., 2006; Fu *et al*., 2007; Tantasawat *et al*., 2011). It have been widely applied in the genetic diversity studies of the soybean germplasm (Meesang *et al*., 2001; Abe *et al*., 2003; Fu *et al*., 2007; Li *et al*., 2008; Yoon *et al*., 2009; Zhenbin *et al*., 2014). Genetic relationships among accessions are helpful for designing future breeding efforts for crop improvement (Wang *et al*., 2006). Complete description of existing certified soybean varieties and patterns of genetic diversity could facilitate introgression of diverse germplasm into the current commercial soybean genetic base (Tara *et al*., 2006).

To date, there is little information are available regarding genetic varia¬tion in Egyptian soybean. Recently Khatab and Morsy (2012) used ISSR and morphological traits used to evaluate genetic diversity in six Egyptian soybean genotypes and reported that narrow genetic diversity were found among the studied genotypes. However, more and intensive work still needed to draw clear image about most common soybean genotypes grown in Egypt using more informative markers and more genotypes. The objectives of the present study were to adapt an augmented design to screen and select the superior entries among 24 soybean germlasm and to calculate similarity parameters among soybean genotypes using cluster analysis and elucidate the relationships based on molecular markers among new promising lines with improved Egyptian commercial cultivars using SSR markers, to use this information in future breeding programs.

MATERIALS AND METHODS

Plant Materials

The materials consisted of 24 genotypes of soybean (20 tested genotypes; cultivars, introduced genotypes and promising lines; and four check varieties being Giza 35, Giza 83, Giza 111 and Crawford) for yield and some agronomic traits. The experiment was grown in augmented design at Sakha Research Station during the 2015 season with six blocks. The experimental plot consisted of 2 ridges, 3 m long and 70 cm apart, spacing between plants at 15 cm. At harvest, five guarded plants were randomly taken from each plot to measure plant height (cm), number of branches per plant, number of pods per plant and 100–seed weight (g). Seed yield was determined using the full plot area and then converted to the unit of ton/fed. The details of pedigree and some seed proper-

Table 1. Name, Pedigree and some morphological properties for 24 soybean genotypes

No.	Genotypes	Pedigree	Maturity group	Stem Termination	Flower color	Flower date (day)
1	Giza 21	Crawford × Celest	Ⅳ	Indeterminate	Purple	39
2	Giza 22	Crawford × Forrest	Ⅳ	Indeterminate	Purple	41
3	Giza 35	Crawford × Celest	Ⅲ	Indeterminate	Purple	39
4	Giza 82	Crawford × Mable Presto	Ⅳ	Indeterminate	Purple	37
5	Giza 111	Crawford × Celest	Ⅳ	Indeterminate	Purple	41
6	Clark	Lincoln × Richland	Ⅳ	Indeterminate	Purple	37
7	Holladay	N77–179(N70–1549 × N72–3213) × Johanston	Ⅵ	Determinate	Purple	59
8	Crawford	Williams × Columbus	Ⅳ	Indeterminate	Purple	41
9	H_{30}	Crawford × L62–1686	Ⅳ	Indeterminate	Purple	41
10	H_{32}	Giza 21 × L86K–73	Ⅳ	Indeterminate	White	40
11	H 105	Giza 35 × Lamar	Ⅴ	Indeterminate	Purple	42
12	H_{113}	Giza 21 × Major	Ⅴ	Indeterminate	Purple	34
13	H 127	D89–8940 × Giza 82	Ⅲ	Indeterminate	Purple	30
14	H 155	Giza 83 × Giza 21	Ⅳ	Indeterminate	Purple	34
15	H 162	Toano × (L86K–73 × Toano)	Ⅴ	Indeterminate	Purple	42
16	H_1 L_1	DR 101 × Giza 22	Ⅴ	Indeterminate	Purple	44
17	H_2 L_3	Clark × Ware	Ⅵ	Indeterminate	Purple	53
18	H_6 L_1	Giza 83 × Ware	Ⅳ	Indeterminate	Purple	46
19	H_2 L_{24}	Crawford × Celest	Ⅳ	Indeterminate	Purple	44
20	H_{11} L_8	Ware × L86K–73	Ⅵ	Indeterminate	Purple	50
21	H_{11} L_{145}	Giza 111 × HC83–123–9	Ⅴ	Indeterminate	Purple	47
22	H_{15} L_5	Crawford × D79–10426	Ⅳ	Indeterminate	Purple	40
23	HC83–123–9	Pixie × PI 229358	Ⅵ	Determinate	Purple	47
24	AGS–129	Shish Shish × SRF400	Ⅵ	Indeterminate	Purple	51

ties for the studied genotypes are presented in Table (1).

Molecular Analysis

SSR–PCR Amplification

DNA was isolated by CTAB method (Doyle and Doyle, 1990). The primers used were used in this study and the nucleotide sequences of the primers are listed in Table 2. PCR reaction for SSR analysis were done in a volume of 20 μl using 40 ng genomic DNA, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 10 pmol of each primer (forward and reverse) and 0.5 U *Taq* polymerase. PCR conditions were as follow: 96°C for 1 min, 35 cycles of 96°C for 30 s, 55–57°C (according primer annealing Tm) for 30 sec, 72°C for 45 sec) and 72°C for 7 min. The reproducibility of the amplification products was checked twice for each primer. After amplification, a 10 μl aliquot of the amplified SSR samples was combined with 2 μl of a loading buffer (0.4% (w/v) bromo–phenol blue and analyzed directly on 2% (w/v) agarose gels in 1 × TAE buffer (10 mM Tris–Borate, 1 mM EDTA) containing 0.5 μg per ml of ethidium bromide. A 100 bp DNA ladder was used as a size marker to compare the molecular weights of amplified products. After electrophoresis, the gels were documented using Gel Documentation System.

Data Analysis

Agro–morphological analysis, ANOVA belong to augmented design was carried out according to the procedure outlined by Federer (1956), the resulted mean square error is used to estimate four orders of least significant differences (LSD). Genotypes were clustered using un–weighted pair group method using arithmetic average as outlined by Kovach (1995).

Molecular analysis, the amplified bands from SSR were scored under the heading of total scorable fragments. For each of the defined loci, SSR allelic composition was determined for each genotype. Polymorphism information content (PIC) values which indicating the ability to distinguish between genotypes for each primer was determined according to the formula described by Anderson *et al*. (1993). Cluster analysis was based on similarity matrix obtained with un–weighted pair group method using arithmetic average (UPGMA), and the relationships between genotypes were displayed as dendrogram calculated based on Jukes–Cantor coefficient using PAST program adapted by Hammer *et al*. (2001).

RESULTS AND DISCUTION

Genetic diversity of soybean was assessed using both quantitative and qualitative traits and their cluster analyses are shown in Table 3, it showed the mean values of 20 tested genotypes (adjusted after discarding the block effect) and four check varieties for seed yield and some related characters. The results exhibited significant differences among the tested genotypes for all studied characters. This provides an evidence for the possibility to carry out a sufficient selection program on the basis of these traits using the studied genotypes. The results clearly indicated that the tested genotypes differed significantly in plant height, genotypes H30 and H113 gave the tallest plants and recording 118.50 cm while Holladay and HC83–123–9 had the shortest plants recording 58.5 cm. The short genotypes are preferable with the aim of applying mechanical management of agricultural practice.

Regarding the number of branches per plant, H162 produced the highest number of branches (4.3) and ranked the first over all tested genotypes and check cultivars followed by Clark and H15L5 (4.05). Increasing the number of branches per plant means that the leaf surface would be more capable to enhance photosynthentic activity which translated in seed formation. Genotypes; H6L1 and H2L24 recorded the lowest number of branches

Table 2. Primers name, sequences and core motif

primer name	sequence(5'—>3') Farward	sequence (5'—>3') Reverse	Core motif
Satt001	AAAGTCTTTAAAAGTGTGTCTTA	TTAAAAGAAAAATGCAACAT	(ATT)25
Satt002	TGTGGGTAAAATAGATAAAAAT	TCATTTTGAATCGTTGAA	(ATT)25
Satt005	TATCCTAGAGAAGAACTAAAAAA	GTCGATTAGGCTTGAAATA	(ATT)19
Satt009	CCAACTTGAAATTACTAGAGAAA	CTTACTAGCGTATTAACCCTT	(ATT)14
Satt030	AAAAAGTGAACCAAGCC	TCTTAAATCTTATGTTGATGC	(ATT)21
Satt031	TTCCACTTTGTATCACTTTC	TGACTGTAAAAGAACAGATAAA	(ATT)12
Satt173	TGCGCCATTTATTCTTCA	AAGCGAAATCACCTCCTCT	(ATT)18
Satt181	TGGCTAGCAGATTGACA	GGAGCATAGCTGTTAGGA	(ATT)18
Satt324	GTTCCCAGGTCCCACCATCTATG	GCG TTT CTT TTA TAC CTT CAA G	(ATT)19
Satt250	CGCCAGCTAGCTAGTCTCAT	AATTTGCTCCAGTGTTTTAAGTT	(ATT)16
Satt268	TCAGGGGTGGACCTATATAAAATA	CAGTGGTGGCAGATGTAGAA	(ATT)17
Sat_036	GCGACTCCAAGTTTTTTTTGTTT	GCGGGAGTTAGAGGAAGAGAACA	(AT)19
Sat_168	TGTGGATAAAAGAGCATTCAAAATG	GCGATCCTTGTTTATCTCAAAAAAGTGT	(AT)15
Sat_185	GCGGCTGGAGAAAACCTTTTATG	GCGAATAAAAACCGAGAATGATTT	(AT)31

Table 3. Mean values of 20 tested genotypes (adjusted after discarding the block effect) and four check varieties for seed yield and some related characters

Genotypes	PH*	NOB*	NOP*	100–SW*	SY*
Tested genotypes					
Giza 21	108.50	3.05	148.75	20.78	1.80
Giza 22	103.50	2.05	167.75	16.17	2.52
Clark	78.50	4.05	126.75	16.89	0.68
Holladay	58.50	2.05	40.75	18.91	1.88
H30	118.50	2.30	45.00	21.09	0.70
H32	103.50	2.30	81.10	18.75	1.25
H 105	113.50	3.30	109.25	16.95	1.38
H113	118.50	2.30	115.00	21.17	0.91
H 127	79.75	2.30	113.75	17.43	1.69
H 153	79.75	3.30	102.75	18.55	2.33
H 162	104.75	4.30	69.75	20.20	1.81
H 1 L 1	94.75	2.30	92.75	18.95	1.69
H2 L3	109.75	3.30	138.25	18.13	2.12
H6 L1	114.75	1.30	94.25	16.99	2.35
H2 L24	114.75	1.30	147.25	17.96	2.57
H11 L8	104.75	3.30	38.25	18.96	1.93
H11 L145	108.50	3.05	82.25	17.30	1.19
H15 L5	108.50	4.05	119.25	18.37	2.81
HC83–123–9	58.50	2.05	62.25	11.27	1.87
AGS 129	103.50	2.05	169.25	19.18	2.54
Check cultivars					
Giza 35	94.00	2.60	72.00	18.65	2.15
Giza 82	96.00	2.80	52.60	16.80	1.31
Giza 111	100.00	2.40	68.00	16.35	2.35
Crawford	109.00	3.40	89.40	18.72	2.05
LSD values					
Among check cultivars	4.68	0.83	13.77	1.27	0.26
gi vs gj (same block)	13.22	2.36	38.96	3.58	0.73
gi vs gj (different blocks)	14.78	2.63	43.56	4.01	0.82
Check vs tested genotypes	10.84	1.93	31.93	2.94	0.60

*PH, plant height; NOB, number of branches per plant; NOP, number of pods per plant; 100–SW, 100–seed weight (g) and SY, Seed yield.

(1.30) per plant. Genotypes; AGS 129 and Giza 22 significantly surpassed all tested and check genotypes considering number of pods per plant recording 169.25 and 167.75 branches, respectively. However, the lowest number of pods/plant were produced by H11L8 (38.25), Holladay (40.75) and H30 (45.0). Results in Table 2, also showed that the heaviest weights of 100 seeds were obtained by H113 (21.17 g) followed by H 30 (21.07 g) and Giza 21 (20.78 g) while, the minimum seed index were obtained by HC83–123–9 (11.27 g). With respect to seed yield, results revealed that genotypes H15L5, H2L24, AGS 129 and Giza 22 gave the heaviest seed yield recording 2.81, 2.57, 2.54 and 2.52 ton/fed, respectively indicating their magnitude as promising genotypes and they could be recommended to be used in breeding programs of soybean. Similar results were observed by Hassan *et al*. (2001) and (2002), Mohamed and Morsy (2005), Iqbal *et al*. (2008).

Cluster analysis

Results of cluster analysis are displayed in Table 4 and graphically illustrated in dendrogram Fig. 1. Results indicated that the lowest similarity level (less than 30) was recorded between two nodes (1 and 24) equaled 22.64. The next smallest similarity levels (less than 50) were obtained between genotype 1 (Giza 21) and each one of the three genotypes being Clark, Holladay and H30. Also, the similarity level between Giza 21 and Giza 22 was 53.36. Four pairs of genotypes revealed similarity levels are less than 70 being (Giza 21and H162),

(Giza 21and Giza 35), (Giza 21and Crawford) and (Giza 22 and H6 L1) recording 62.71, 63.77, 68.41 and 68.74, respectively. From dendrogram, it is obvious that four pairs of genotypes (H 105 and H11 L145), (H32 and H1 L1), (Giza 35 and Giza 111) and (Crawford and H2 L3) are closely related to each other where the similarity levels among them were more than 80 recording 86.69, 85.81, 80.83 and 80.54, respectively. On the other hand, the remainder similarity levels among the pairs of genotypes ranged between 70 and 80.

Table 4. Cluster analysis to classify 24 genotypes of soybean based on agro–morphological traits

No. of clusters	Similarity level	Clusters jointed		New cluster	No. of entries in new cluster
23	86.69	11	21	11	2
22	85.81	10	16	10	2
21	80.83	3	5	3	2
20	80.54	8	17	8	2
19	78.87	19	23	19	2
18	77.54	4	11	4	3
17	77.15	2	19	2	3
16	76.65	10	13	10	3
15	75.46	8	22	8	3
14	73.75	3	10	3	5
13	72.91	15	20	15	2
12	71.98	9	12	9	2
11	71.04	8	14	8	4
10	70.83	3	4	3	8
9	68.74	2	18	2	4
8	68.41	1	8	1	5
7	63.77	1	3	1	13
6	62.71	1	15	1	15
5	53.36	1	2	1	19
4	49.37	1	9	1	21
3	46.34	1	7	1	22
2	45.22	1	6	1	23
1	22.64	1	24	1	24

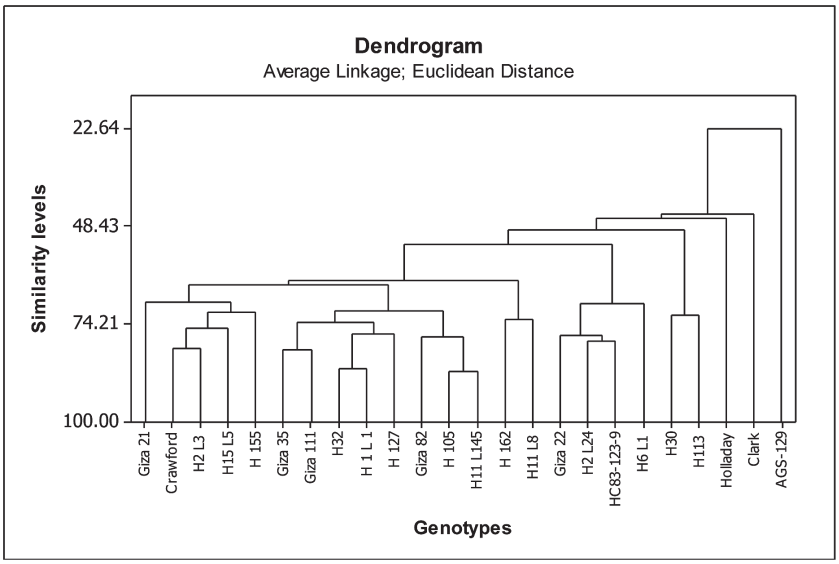

Fig. 1. Similarity levels for 24 soybean genotypes calculated by cluster analysis based on agro–morphological traits.

From the previous results, it could be concluded, based on similarity levels, that genotypes 24, 6, 7, 9, 2, 15, 3 and 8 have low similarity levels (dissimilarity) with genotype 1 (Giza 21) and may produce good results if they are crossed with. It is noteworthy that cluster analysis considered a valuable tool for subdividing number of genotypes in groups including similarity and dissimilarity genotypes which would help the breeder to plan an effective breeding program. These results are in harmony with those obtained by Iqbal *et al*. (2008) and Ojo *et al*. (2012).

Molecular analysis

Polymorphism was revealed in the banding patterns across the set of the studied 24 soybean genotypes. Among the used 14 SSR primer pairs, all of of them generated polymorphic bands. For a total of 14 primers 42 bands were obtained of which (92.85%) were polymorphic and (7.15%) were monomorphic bands as shown in Figure 2. The number of microsatellite alleles of used markers ranged from one to five alleles of which Satt173 and Satt181 markers produced the highest numbers of alleles (5 alleles) for both primers while Satt005 produced the lowest numbers of alleles (one allele) as a monomorphic band as shown in Table 5. Polymorphic information content (PIC) values were varied from 0.55 to 0.88, the highest value belong to Satt173 (PIC 0.88) with five alleles which amplify core motif (ATT)18, while Satt001 showed the lowest PIC value (PIC= 0.55) with three alleles, generally, SSR core motifs (ATT) gave higher allele numbers and PIC values than motifs (AT). Hence, primer Satt173 is highly informative in the present study; this indicated that the primer (Satt173) might be an effective and useful tool to determine the genetic differences among the soybean accessions and to study the phylogenetic relationship. The PIC observed in the present study is comparable to those reported by Gyu *et al*. (2008) who found lower PIC ranged from 0.43 to 0.82. A slightly higher SSR diversity was reported by Fu *et al*. (2007), who found 6.3 alleles per locus (included null alleles)

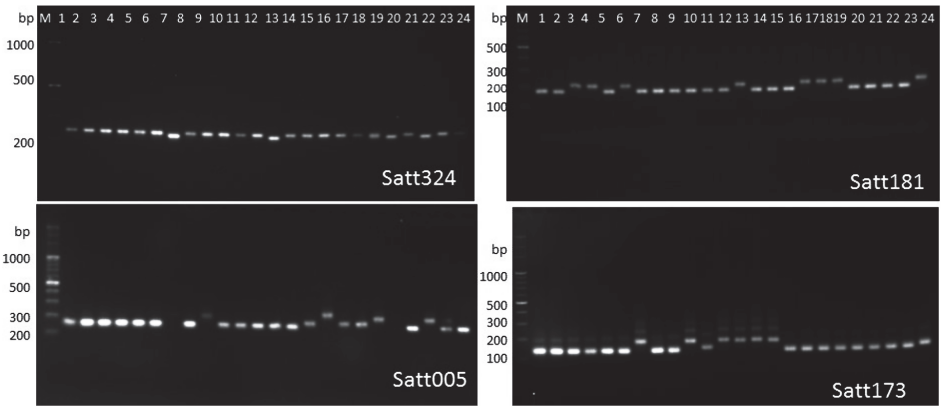

Fig. 2. DNA patterns of primer SSR; M, marker 100bp 1–24 soybean genotypes as shown in Table 1.

Table 5. Simple sequence repeat (SSR) No., of bands, Polymorphic, expected allele size, band size range and polymorphic information content (PIC)

primer name	Core motif	Number of bands	Polymorphic (%)	Expected allele size (bp)	Band size range	PIC
Satt001	(ATT)25	3	100.0	117	120–135	0.55
Satt002	(ATT)25	2	100.0	127	125–140	0.58
Satt005	(ATT)19	1	00.0	141	150	0.00
Satt009	(ATT)14	3	100.0	163	158–250	0.77
Satt030	(ATT)21	2	100.0	164	170–180	0.61
Satt031	(ATT)12	2	100.0	122	140–190	0.63
Satt173	(ATT)18	5	100.0	197	160–200	0.88
Satt181	(ATT)18	5	100.0	214	190–220	0.73
Satt324	(ATT)19	4	100.0	250	220–230	0.70
Satt250	(ATT)16	4	100.0	202	190–210	0.81
Satt268	(ATT)17	4	100.0	236	230–250	0.74
Sat_036	(AT)19	3	100.0	142	145–150	0.60
Sat_168	(AT)15	2	100.0	157	150–160	0.59
Sat_185	(AT)31	2	100.0	230	200–240	0.76

and an average polymorphic information content of 0.63 among 45 Canadian soybean cultivars and 37 exotic germplasm accessions analyzed at 37 SSR loci.

The constructed dendrogram tree revealed six main genetic clusters Figure 3. The first and second clusters comprise the highly diverged genotypes, H1L18, HC83–

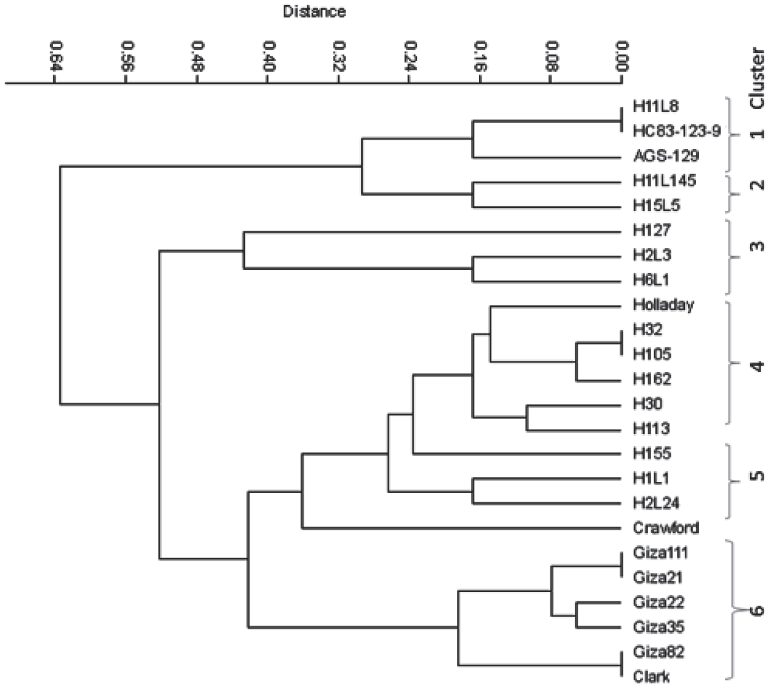

Fig. 3. UPGMA clusters analysis–based dendrogram depicting genetic relationships among 24 soybean genotypes using SSR markers.

Table 6. Distance index for SSR primer pairs based on Jukes and cantor for the studied 24 soybean genotypes

	Giza 21	Giza 22	Giza 35	Giza 82	Giza 111	Clark	Holl–aday	Craw–ford	H30	H32	H105	H113	H127	H155	H162	H1 L1	H2 L3	H6 L1	H2 L24	H11 L8	H11 L145	H15 L5
Giza22	0.11	0.00																				
Giza35	0.05	0.05	0.00																			
Giza82	0.23	0.11	0.17	0.00																		
Giza111	0.00	0.11	0.05	0.23	0.00																	
Clark	0.23	0.11	0.17	0.00	0.23	0.00																
Holladay	0.23	0.38	0.30	0.57	0.23	0.57	0.00															
Crawford	0.30	0.47	0.38	0.69	0.30	0.69	0.17	0.00														
H30	0.47	0.47	0.57	0.69	0.47	0.69	0.17	0.38	0.00													
H32	0.30	0.17	0.23	0.30	0.30	0.30	0.17	0.38	0.23	0.00												
H105	0.30	0.17	0.23	0.30	0.30	0.30	0.17	0.38	0.23	0.00	0.00											
H113	0.30	0.30	0.38	0.47	0.30	0.47	0.17	0.38	0.11	0.11	0.11	0.00										
H127	0.69	0.47	0.57	0.30	0.69	0.30	0.47	0.82	0.57	0.23	0.23	0.38	0.00									
H155	0.47	0.47	0.57	0.69	0.47	0.69	0.30	0.38	0.23	0.23	0.23	0.11	0.38	0.00								
H162	0.38	0.23	0.30	0.38	0.38	0.38	0.11	0.30	0.17	0.05	0.05	0.17	0.30	0.30	0.00							
H1L1	0.69	0.47	0.57	0.69	0.69	0.69	0.30	0.38	0.38	0.23	0.23	0.38	0.38	0.23	0.17	0.00						
H2L3	0.82	0.57	0.69	0.57	0.82	0.57	0.38	0.69	0.30	0.30	0.30	0.47	0.47	0.69	0.23	0.47	0.00					
H6L1	0.99	0.69	0.82	0.69	0.99	0.69	0.47	0.82	0.57	0.38	0.38	0.57	0.38	0.57	0.30	0.23	0.17	0.00				
H2L24	0.38	0.23	0.30	0.38	0.38	0.38	0.23	0.47	0.30	0.17	0.17	0.30	0.47	0.47	0.11	0.17	0.38	0.30	0.00			
H11L8	0.82	0.82	0.99	0.82	0.82	0.82	0.57	0.69	0.69	0.69	0.69	0.69	0.69	0.47	0.57	0.47	0.82	0.69	0.82	0.00		
H11L145	0.69	0.47	0.57	0.47	0.69	0.47	0.47	0.57	0.57	0.38	0.38	0.57	0.38	0.38	0.30	0.23	0.47	0.38	0.30	0.30	0.00	
H15L5	0.82	0.82	0.99	0.82	0.82	0.82	0.57	0.69	0.69	0.69	0.69	0.69	0.69	0.47	0.57	0.47	0.57	0.47	0.57	0.23	0.17	0.00
HC83–123–9	0.82	0.82	0.99	0.82	0.82	0.82	0.57	0.69	0.69	0.69	0.69	0.69	0.69	0.47	0.57	0.47	0.82	0.69	0.82	0.00	0.30	0.23

123–9 and AGS–129 in cluster 1 as new exotic introduced, H11L145 and H15L5 in cluster 2, while the third, fourth and fifth clusters includes three promising lines. On the other hand, the last cluster includes the Egyptian cultivars, Giza 21, Giza 22, Giza 111, Giza 35, Giza 82 and Clark with low diversity. The low distance value (low ranged from 0.00 to 0.2) were recorded among the Egyptian cultivars indicating that these cultivars were closely related to each other and this is reflect their pedigree as shown in Table 6. On the other hand, the highest values 99.0% was recorded among the Egyptian cultivars and the exotic introduced in clusters 1 and 2 indicating that these cultivars were genetically distant than those exotic introduced genotypes.

In the present study, SSR markers were used to assess genetic variation of Egyptian soybean gene pool. This method provides an alternative choice to other system for obtaining highly reproducible markers. In fact several studies showed that domesticated soybeans have reduced genetic diversity, a changed distribution of alleles and in many other cases (Gizlice *et al*., 1994; Barakat, 2004; Min *et al*., 2010). On the other hand, (Brown–Guedira *et al*., 2000; Mulato *et al*., 2010) found a high genetic variation among some exotic and wiled soybean germplams. The genetic relationships among genotypes based agro–morphological analysis not completely agreed with known pedigrees. However, phylogenetic tree based on SSR confirmed the separation of soybean genotypes into six groups and were more clearly separated. In the SSR analysis described in the present study, all the accessions used were cultivars and promising lines and no wild soybean was analyzed. Moreover, when we excluded the diverged genotypes in cluster 4, 5 and 6 distance among the rest genotypes will be reduced. The information on the genetic diversity relationship from this study is propitious to develop novel soybean cultivars with good yield potential, resistant to biotic and abiotic stresses and accepted by soybean grower biased genetic transformation for cultivated soybean (Khatab and El–Banna, 2014) or mutation breeding (Khatab, unpublished data). It seems to be using introduces and exotic genotypes as distinct can be very useful for broadening the genetic base of soybean Egyptian cultivars. The results indicate that SSRs may constitute a relatively simple and efficient method for analyzing genetic variation in Egyptian soybean genotypes for future breeding programmers. To ensure sustaining breeding progress in the future, the introduction of new germplasm into these breeding programs, especially by the aid of molecular markers, is recommended.

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