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Soybean Field Evaluation for Resistance to Cotton Leaf Worm (*Spodoptera Littoralis*) Confirmed by SSR Markers

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ABSTRACT

Insect resistant soybean {*Glycine max* (L.) Merr.} cultivars could reduce pesticide use in controlling insects, resulting in less risk to the environment. This study was conducted to develop an effective field and SSR analysis screening procedurs to screen 15 soybean genotypes . A field experiment was conducted for their resistance , and showed that genotypesH105 andH153 recorded the highest insect resistance and low defoliation rating of 0.0 and 0.1 respectively, followed by H10L10A, L162, and H1L1. Genotype AGS-129 showed highest insect susceptibility and high defoliation rating of 4.0. Genotypes H10L10A, H 153, and H1L1 recorded the highest seed yield values due to the number of branches plant , number of pods plant , and 100-seed weight indicating that these genotypes could be recommended particularly with high resistance to cotton leaf worm. A total of 13 SSR primer pairs were used to generate 39 bands (alleles). Nearly 92% (12) of the primer pairs amplified SSR alleles of expected size and 11 SSR loci were polymorphic. The polymorphic SSRs were successfully used to differentiate among 15 genotypes. These markers could be particularly useful for genetic differentiation cotton leaf worm resistance.

Key words, defoliation, cotton leaf worm, genotypic variance, soybean resistance for cultivars, SSR.

INTRODUCTION

Soybean (*Glycine max* (L.) gives more than 61% of the world vegetable oil production about 336 million mega grams (Mg) of seeds (USDA, 2017) and such levels could be increased if pest control is strengthened (Oerke, 2006). To alleviate pest outbreak growers must fight phytophagous arthropods, which can cause considerable decrease in crop productivity (Zaluck*iet al*., 2009).

In Egypt, soybean is attacked by about twenty different major insect pests especially cotton leaf worm (*Spodoptera littoralis*), which is a main leaf feeding insect, and a major constraints to soybean production. The National Legume Research Program (NLRP) has been successful in releasing five high yielding soybean cultivars (Giza21, Giza22, Giza35, Giza83, and Giza111) with acceptable resistance to cotton leaf worm derived from crosses involved Celest , Forrest, and MBB-80-133 as sources of resistance previously identified for cotton leaf worm. Some elite breeding lines were recently identified for high resistance to cotton leaf worm, but they are late maturing genotypes under Egyptian conditions. These elite breeding lines were initiated from crosses between Egyptian soybean cultivars and resistant cultivars. The most economical way to deal with these insect-pests to avoid yield losses is to cultivate insect resistant cultivars (EL-Garhy *et al*., 2015), in order to avoid using pesticides and minimize environmental pollution (Lucas,2012). The development of soybean cultivars with insect resistance has been an objective of several breeding programs during the 1970s (All *et al*., 1999).This occurred after the identification of three plant introductions of (1) PI 171451"Kosamame", (2) PI 229358 "Sodendaizu" and (3) PI 227687 "Miyako White" resistant to the Mexican bean beetle *Epilachnavarivestis* Mulsant (Van Duyn*et al.* 1971 and 1972) and other insect pests (Lambert and Kilen ,1984a and b). However full resistance levels are not acquired in progenies derived from crosses with adapted high-yielding cultivars or poor agronomic qualities (Kilen and Lambert,1986 and Lambert and Tyler, 1999).

Many resistance evaluations are conducted in field nurseries . Field experiments are more close to natural

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conditions, but are of limited effect on high numbers of genotypes . Inadequate infestations of the desired species often occur in field studies (All *et al*., 1989). Integrated pest management may be used against insect attacks(Ismail and Morshedy. 2009**)**. One of the methods used for this purpose is developing pest-resistant cultivars (Gallo *et al*.,2002).

Soybean yield loss through defoliation depends on levels and stages of defoliation (Todd and Morgan, 1972, Gazzoni and Moscardi, 1998 and Gazzoni *et al*., 1998) .Levels of less than one third of defoliation decreases soybean yield (Turnipseed, 1972) and defoliation during the early growth stages cause little effect (Board *et al*., 1994). Soybean is sensitive to defoliation at beginning of bloom and the most sensitive stage is R5 at fully developed leaves (Fehr *et al*., 1981). Defoliation decreases canopy photosynthesis, loss of leaf storage material, and shortening of effective grain filling period (Board, 2004). Gregorutti *et al*.(2012) obtained linear relationship between defoliation and yield loss, although yield response does not always reflect the degree of light interception (Board, 2004).

Morsy *et al* .(2011)obtained a highly significant and positive correlation between seed weight plant^{-1} and each of number of pods plant^{-1}, number of branches plant^{-1}, and plant height, but a negative non-significant one with the100-seed weight . Lin. *et al* (1996) used RFLP, RAPD, and AFLP markers to develop a linkage map of soybean markers. Rector et at. (1999) used 139 to constructed genetic linkage map to identify QTLs associated with maize earworm resistance and. Narvel (2000) used SSR markers to study soybean genome. Characterizing insect-resistant or susceptible soybean cultivars and genetic diversity among them are a central concern for soybean breeding (Fahmy and Salama, 2002). Finding a molecular marker helps in identifying genetic divergent accessions (Lee *et al*., 2008 and Li *et al*., 2009). In this concern microsatellite or simple sequence repeats (SSR) markers are useful, due to their effectiveness in genealogy analysis and assessment of genetic diversity (Narvel *et al*., 2000 and Kuroda et al., 2009). Many DNA markers in soybean were used with different techniques (Caetano *et al*., 1991; Prabhu and Gresshoff, 1994; Chowdhury*et al*., 2000; Brik and Sivolap, 2001a & b; Khatab and Morsy 2012) .Khatab *et al* (2016) used SSR markers to assess genetic variation of Egyptian soybean gene pool.This method is an alternative choice to others for obtaining highly reproducible markers and can be used for differentiating soybean resistance to cotton leaf worm.

Thus, The current study aims to (1) use agronomic and molecular approaches to do genetic diversity for 15 soybean genotypes and (2) determining the genetic relationships between cotton leaf worm resistance and susceptible genotypes to generate associated marker(s) using SSR markers.

MATERIALS AND METHODS

Field Screening

Fifteen soybean genotypes (five resistant, three moderately resistant ,four susceptible and three highly susceptible) were used in an open-field screening experiment using larval antibiosis screening techniques. The genotypes were grown during the two seasons of 2017 and 2018 at Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt using a randomized complete block design with three replicates. The pedigree, maturity group, flower colour, steam termination and insect resistance of the tested genotypes are shown in Table1.

Each plot consisted of six ridges, 70 cm apart and 4 m long. Seeds were inoculated with their specific rhizobia then seeded at density of 20 plants m^{-1}. All agricultural practices were conducted as recommended for Sakha. Ten guarded plants were randomly taken from each plot to measure plant height ,number of branches plant^{-1}, number of pods plant^{-1}, 100-seed weight ,and Seed yields (kgha^{-1})

An early "trap" cultivar of Crwaford, was planted next to the soybean genotypes to attract and encourage the buildup of a resident cotton leaf worm population. Field screening was done by allowing a natural cotton leaf worm population to feed. Defoliation ratings were taken at the beginning maturity i.e. growth stage RT (Fehr and Caviness, 1977) of the latest maturing line (H 105) on a scale of 0 = no feeding to 4 = heavy defoliation of > 30% (Rufener *et al*., 1987).

Estimating Defoliation Damage

In soybean, field scouting to assess insect populations is based on the number of insects per row, insects per plant, sweep net samples, or the level of defoliation. The percent of defoliation is determined by the amount of leaf tissue loss based on visual inspection of randomly selected plants. Examples are provided in Figure 1 as guidelines of loss for individual leaflets. Actual defoliation made for pest management are based on leaf area lost over the whole plant (Mc Carville *et al*., 2010).

The growth stage of the soybean plant is important when making pest management decisions. Under most conditions, moderate defoliation early in the season has little effect on the final soybean yield. As plants reach the flowering and pod-filling stages, defoliation causes greater threat to yield.

Table1.Pedigree, maturity group, flower colour, steam termination and insect of resistance the tested soybean genotypes

No.	Genotype	Pedigree	Maturity group	Flower color	Steam termination	insects resistance*
1	H 105	Giza 35 x Lamar	V	Purple	Indeterminate	Resistant
2	H 153	Giza 83 x Giza 21	V	Purple	Indeterminate	Resistant
3	L 162	Toano x (L86-K-73 x Toano)	V	Purple	Indeterminate	Resistant
4	$H_1 L_1$	DR 101 x Giza 22	V	Purple	Indeterminate	Resistant
5	$H_{10} L_{10A}$	Ware x Holladay	V	Purple	Determinate	Resistant
6	Giza 21	Crawford x Celest	IV	Purple	Indeterminate	M†
7	Giza 111	Crawford x Celest	IV	Purple	Indeterminate	M†
8	Giza 35	Crawford x Celest	III	Purple	Indeterminate	M†
9	HC83-123-9	Pixie x PI 229358	V	Purple	Determinate	Susceptible
10	H_5L_5	$H_2 L_{12}$ xClark	IV	Purple	Indeterminate	Susceptible
11	$H_2 L_{24}$	Crawford x Celest	IV	Purple	Indeterminate	Susceptible
12	$H_{15} L_{17}$	Crawford x D79-10426	IV	Purple	Indeterminate	Susceptible
13	AGS-129	Shish Shish x SRF400	V	Purple	Indeterminate	Highly Susceptible
14	Giza 82	Crawford x Mable Presto	III	Purple	Indeterminate	Highly Susceptible
15	Giza 22	Crawford x Forrest	IV	Purple	Indeterminate	Highly Susceptible

M†: moderate resistance to insects..* Resistance based on field evaluation during 2017 and 2018 seasons

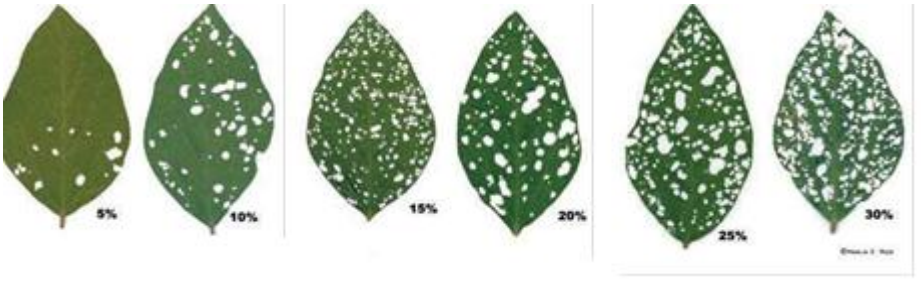

Figure 1.Estimations of percent defoliation in soybean. *Reprinted from McCarville,.et al*. 2010 A combined analysis of variance was computed over the two seasons.

Levene test (1960) was used to satisfy the assumption of homogeneity of variances before running the combined analysis.

DNA extraction and amplification

DNA was isolated from fresh leaves using CTAB method according to Doyle and Doyle (1990). Thirteen SSR primers were used in the PCR reactions. Data on expected sequence bands from fresh leave sand core motif of SSR primers are shown in Table 2.

The PCR reactions were prepared in a total volume of 25 µL containing approximately 50 ng DNA, 200 µMdNTPs, 5 µM primer, 0.5 units of *Taq* polymerase and 10X *Taq* polymerase buffer. PCR cycling was carried out according to the following program : one cycle at 94 °C for 5 min, then 35 cycles as follows: 1 min at 94 °C for denaturation, 40 sec at 54~58°C for annealing (according to primer) and 45 sec, at 72 °C for extension. Then the reaction was incubated at 72°C for 7 min and kept at 4°C. The PCR products were separated by electrophoresis using 2% agarose gel in 0.5 x TBE buffer against ngml DNA Ladder as a size marker. Bands were detected with Red safe staining and documented on Gel Documentation.

PCR amplifications were compared with each other and DNA fragments were scored as a binary data. The electrophoretic patterns of the reproducible banding patterns of each primer produced by SSR were chosen for analysis. Each band was scored as present (+) or absent (-), and pair wise comparisons between individuals were made to calculate the Jukes-Cantor coefficient using PAST program (Paleontological Statistics Version 1.94b) adopted by Rohlf (2000). Cluster analysis was performed to produce a dendero-gram using the un-weighted pair-group method with

Table 2. Primer sequence, and core motif of SSR primers the tested soybean genotypes

No.	primer	Forward sequences	Reverse sequence	Core motif
1	Satt030	AAAAAGTGAACCAAGCC	TCTTAAATCTTATGTTGATGC	(ATT)21
2	Satt031	GCGGTGAATATCCATCAGCCATGAAATTATA	GCGTGCCCATTATTTGTTGGATATTTGTTTG	(AT)23
3	Sat_036	GCGACTCCAAGTTTTTTTTGTTT	GCGGGAGTTAGAGGAAGAGAACA	(AT)19
4	Satt173	CCGGTCCAATCTTTATTCAAAC	CCAAGCGAAATCACCTCCTCT	(TAT)18
5	Satt181	TGGCTAGCAGATTGACA	GGAGCATAGCTGTTAGGA	(ATT)18
6	Satt250	CGCCAGCTAGCTAGTCTCAT	AATTTGCTCCAGTGTTTTAAGTT	(ATT)16
7	Satt268	TCAGGGGTGGACCTATATAAAATA	CAGTGGTGGCAGATGTAGAA	(ATT)17
8	Sat_168	TGTGGATAAAAGAGCATTCAAAATG	GCGATCCTTGTTTATCTCAAAAAAGTGT	(AT)15
9	Satt324	GTTCCCAGGTCCCACCATCTATG	GCGTTTCTTTTATACCTTCAAG	(ATT)19
10	Satt659	GCGGCTCAACTTCGTGTAACAAG	GCGCATCGGTAACTATCTAATATTCGTA	(ATT)
11	Satt220	GAG GAG GAT CCC AAG GTA ATA AT	GCG CAT GGA GAA AAG AAG AG	(ATT)18
12	Satt175	GAC CTC GCT CTC TGT TTC TCA T	GGT GAC CAC CCC TAT TCC TTA T	(ATT)16
13	Sat_199	GCG CAA TTT GAC TAT TTT TAG CTG TTG	GCG CGA TAT AAG ATG ATT TTT ATT GAT	(AT)22

arithmetic average (UPGMA) according to Taran *et al*(2005).

RERSULTS AND DISCUSSION

Field Screening

Significant differences among soybean genotypes (Table 3) were obtained in the field screening. All three high susceptible insect genotypes showed defoliation ratings ranging from 3.8 to 4.0 (AGS-129, Giza 82 and Giza 22), indicating heavy insect feeding. The five high resistant promising genotypes, had defoliation rating of 0.0 to 0.3 (H 105, H153, L 162, H1 L1 and H10 L10 A), indicating extreme light feeding by cotton leaf worm. However H105 derived from cross 'Giza 35 x Lamar' exhibited high resistance to defoliation, with its resistance genes being derived from the Lamar with resistance to a wide range of foliar feeding insects (Hartwig *et al*., 1990). Giza 35 was a resistant commercial cultivar in Egypt .

Genotype H153 exhibited high resistance to defoliation, and derived its resistance genes from the cross Giza 83 x Giza 21; Giza 83 was selected from MBB-133-9, and MBB-133-9 was selected from the cross " Union x L 76-0038" (Williams x PI 171451).The PI171451 carries genes resistant to the Mexican bean beetle and other foliar feeding insect pests (Elden *et al*., 1992) .The Giza 21 is a resistant commercial cultivar in Egypt and both H105 and H153 exhibited high resistance more than HC 83-123-9 ; selected from the cross Pixie x PI229358 and carries a antibiosis type of resistance to insects similar to soybean introduction PI229358, and determinate line with high level of insect resistance (Cooper and Hammond ,1988). The three Egyptian commercial cultivars Giza 21, Giza 111 and Giza 35 had defoliation rating from 0.8 to 1.4, indicating extreme feeding by cotton leaf worm. The other soybean genotypes had intermediate susceptible defoliation ratings ranging from 1.7 (HC83-123-9) to 2.8 (H_2 L_{24}).

Field performance

Field performance of soybean genotypes over the two seasons is presented in Table 4. The results reveal significant differences among the genotypes for all studied characteristics. Genotypes Giza 111, H 105, and Giza 21 were the tallest exhibiting heights (cm plant^{-1}) of 118.2, 113.9, and 113.6 respectively while HC-83-123-9 was the shortest (53.7 cm plant^{-1}). The current results are similar to those obtained by Eisa *et al* (1998), Hassan *et al* (2001 and 2002) and Morsy *et al*., (2011).

Regarding number of branches/plant, H_{10} L_{10} A, genotypes gave the highest number of branchesplant^{-1}being 4.5, 3.7 and 3.5, respectively compared with H_{15} L_{17} which recorded the lowest number of branchesplant^{-1}1.7. On the other hand L153, H_1 L_1, H_{10} L_{10} and Giza 111 showed the highest number of podsplant^{-1} (160.1, 156.5, 155.5, and 153.4, respectively, with no significant differences among them) lowest number (63.7 and 68.8 respectively). These results are similar to obtained by Eisa *et al* (1998), Hassan *et al* (2001 and

2002) and Morsy *et al* (2011). The heaviest value of the 100-seed weight (20.79 and 19.82 g) were given by H_{10} L_{10} A, H_1 L_1 and H153 genotypes compared with HC 83-123-9 genotype that gave the lowest 100-seed weight (15.17 and 15.46g).

Results show that H_{10} L_{10}, L 153 and genotypes surpassed the other genotypes for seed yield recording 7.066 and 6.900 Mg ha^{-1} respectively while Giza 82 and HC83 123-9 genotypes gave lowest yields of 3.238 and 2.974Mg ha^{-1}respectively. This finding is in agreement with those reported by Hassan *et al* (2001 and 2002), Mohamed and Morsy (2005), Hamdi *et al* (2008) and Morsy *et al*., (2011).

Correlation matrix

The correlation coefficients among all pairs of studied characters of soybean over the two seasons are given in Table 5.

Table3. Defoliation rate for the 15 soybean genotypes

No.	Soybean genotype	Field defoliation rating score †
1	H 105	0.0
2	H 153	0.1
3	L 162	0.3
4	H_1 L_1	0.2
5	H_{10} L_{10} A	0.3
6	Giza 21	1.4
7	Giza 111	1.2
8	Giza 35	0.8
9	HC83-123-9	1.7
10	H_5L_5	2.2
11	H_2 L_{24}	2.8
12	H_{15} L_{17}	2.5
13	AGS-129	4.0
14	Giza 82	3.8
15	Giza 22	3.8

†Rating scores (*Mc Carville, .et al*. 2010) : zero= no noticeable feeding to 4 = >30% defoliation,

Table 4. Mens of yield and yield components for the tested soybean genotypes

No.	Soybean genotype	plant height (cm)	Branches plant^{-1}	Pods plant^{-1}	100-Seed weight (g)	Seed yield (Mgha^{-1})†
1	Giza 35	98.3	3.1	86.5	18.38	4.428
2	Giza 82	100.2	2.5	72.9	15.46	3.238
3	Giza 21	113.6	3.3	102.7	18.91	5.547
4	Giza 111	118.2	3.5	153.4	18.97	5.700
5	Giza 22	103.7	2.9	97.6	18.27	4.260
6	H_5L_5	108.3	2.7	73.8	16.47	3.641
7	H_2 L_{24}	110.3	2.5	73.2	17.54	4.140
8	H_{15} L_{17}	108.7	1.7	69.5	16.81	3.948
9	HC83-123-9	53.7	2.5	63.7	15.17	2.974
10	AGS-129	108.9	3.7	68.8	17.74	4.219
11	H_{10} L_{10} A	83.9	4.5	155.5	20.79	7.066
12	H_1 L_1	93.7	2.9	156.5	19.84	5.988
13	L 162	103.7	3.4	69.5	18.68	5.155
14	H 105	113.9	3.2	83.5	18.52	5.028
15	H 153	78.3	3.1	160.1	19.82	6.900
L.S.D. $_{0.05}$		9.48	0.31	9.58	1.09	0.408

†: Mg (mega gram) = 10^6 g (i.e. metric ton)

Table 5.Correlation matrix for yield-related traits in soybean crop

	Defoliation	Plant Height	No. of BranchesPlant^{-1}	No. of Pods Plant^{-1}	100-Seed Weight	Seed Yield
Seed Yield	-0.628**	0.109**	0.603**	0.834**	0.931**	1
100-Seed Weight	-0.665**	0.119**	0.696**	0.789**	1	
No. of Pods Plant^{-1}	-0.644**	-0.14**	0.492*	1		
No. of BranchesPlant^{-1}	-0.377*	0.011**	1			
Plant Height	0.279	1				
Defoliation	1					

* and ** : Significant and highly Significant at probability levels 0.05 and 0.01, respectively.

The results show highly significant positive correlations between seed yield and each of plant height (0.109), number of branches plant^{-1} (0.603), number of pods plant^{-1} (0.834), and 100-seed weight (0.931). There was a highly significant negative correlation between yield and defoliation (-0.628). It is suggested that seed yield may be raised through selection for plant height, number of pods and branches per plant, and the 100-seed weight.

The yield components exhibited various trends of association among themselves. Highly significant negative correlations of plant height with number of pods per plant was observed (-0.140) The shortest genotypes (HC83-123-9 and H 153) showed low 100-seed values but produced more branches per plant .

Number of branches plant had highly significant positive correlation with number of podsplant^{-1}, 100-seed weight and seed yield explaining that genotypes produced more branches and pods per plant .Number of podsplant^{-1}showed highly significant positive correlation with each the 100-seed weight (0.789) and seed yield (0.834) but highly significant negative one with defoliation (-0.644).

Table 5 shows highly significant positive correlations between the100-seed weight and each of plant height (0.119), number of branchesplant^{-1} (0.696), number of pods plant^{-1} (0.789), and seed yield (0.931). Tall genotypes (such as Giza 111, H105 and Giza 21) showed high 100-seed weight and gave more branches and pods. The 100-seed weight showed highly significant negative correlations with defoliation (-0.665)

There was a highly significant negative correlation between defoliation and each of number of branchesplant^{-1} (-0.377), number of podsplant^{-1}(-0.644), 100-seed weight (-0.665), and seed yield(-0.628) and highly significant positive correlations with plant height (0.279). Seed yield was reported to decreases with increasing defoliation (Xiangjun *et al*., 2005, Lucas, 2012, and Gregorutti *et al*., 2012).

The current results agree with those reported by Akhter and Sneller (1996), Mohamed and Morsy (2005), Hamdi *et al* (2008), and Morsy *et al* (2011) .

Thirteen SSR primers were used, out of which twelve produced storable bands.The results showed that polymorphism levels differed from one primer to the other (350-3000 bp). There were some specific fragments which can be used to discriminate each soybean genotypes from the other. Markers which can be used are as follows :- PrimerSatt268 gave the lowest polymorphism percentage (25 %) and specific fragment (~2000bp) as positive marker for resistant genotypes. PrimersSatt220,250 ,173, 175, 181, 199, 30and Satt324 gave the highest polymorphism percentages with highest polymorphism percentage (100%) as shown in Figure 2.

In this study 39 polymorphic bands were amplified by 13 pairs of SSR primers, with an average of three bands for each primer. Moreover, primer Satt250 and Satt175 gave a clear distinct among tested genotypes and has banding pattern for resistance and susceptible genotypes as shown in Table 2.

The dendro-grams for the genetic relationships among the 15 soybean genotypes across all studied primers (Figure 3) indicate that the soybean genotypes can be separated into two clusters; cluster 1 includes two sub clusters: sub cluster1 includes five resistant genotypes (H105, L162, H153, H1L1 and H10L10A) ; sub cluster 2 includes four moderate genotypes (Giza111 , Giza 35 and Giza21) along with one susceptible genotype (HC83123-9). From these data it may be concluded that these genotypes may be moderate to resistance. Cluster 2 contains six genotypes (H5L5,H2L24, AGC129 , Giza 22 and Giza 82)all of which are susceptible or highly susceptible as shown in Figure 3 .

The results of cluster analysis (similarity index) show that highest similarity value 0.89, observed between Giza82 and Giza22 ; and between AGS129 and Giza22 as a highly susceptible genotypes. Similarly, another high similarity of was 0.82 occurred between

H153 and L162 as resistance genotypes. The lowest similarity value was 0.00 among most resistance and susceptible genotypes, as shown in Table 7.

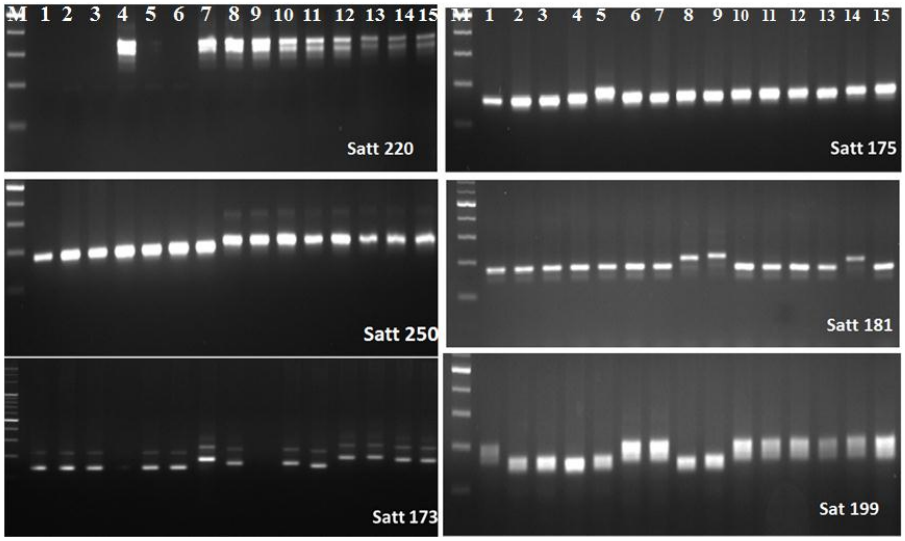

Figure 2. The SSR Profiles of 15 Soybean Genotypes

Table 6. Polymorphism of 13 SSR markers using 15 soybean genotypes

Primer	Total bands	Monomorphic bands	Polymorphic bands	% polymorphism
Satt220	8	0	8	100
Satt250	3	0	3	100
Satt173	8	0	8	100
Satt175	3	0	3	100
Satt181	3	0	3	100
Sat_199	6	0	6	100
Satt30	2	0	2	100
satt268	4	3	1	25
satt659	2	1	1	50
Satt‧31	1	1	0	0
Satt030	2	1	1	50
Sat_036	0	0	0	0
Satt324	3	0	3	100

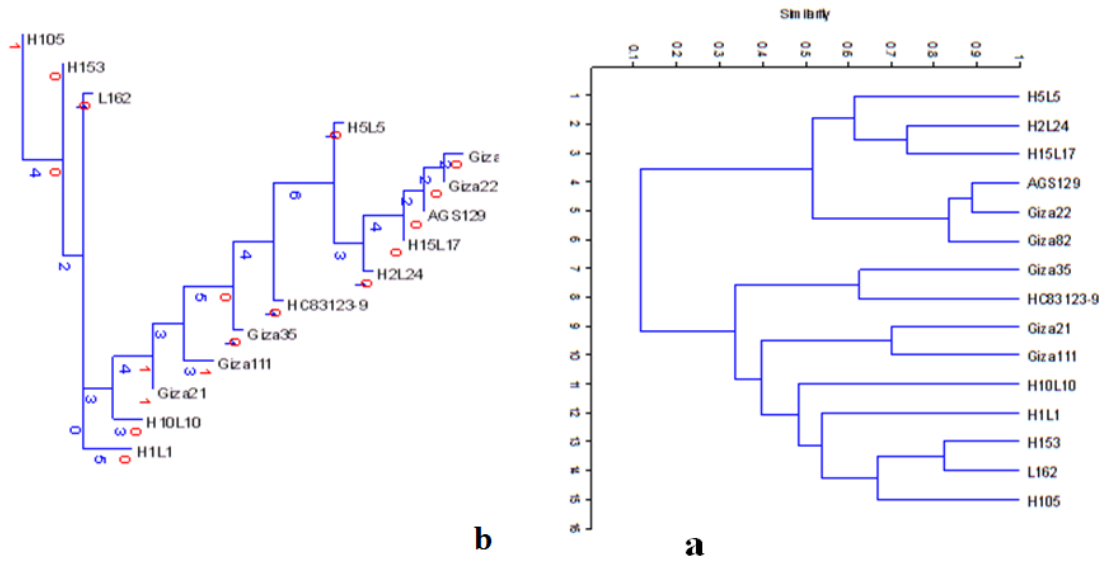

Figure 3 .Dendro-gram showing clustering pattern of all 15 genotypes of Soybean (a) rooted tree and (b) un-rooted tree using 13 SSR markers

Table7. Similarity matrix for 15 soybean using 13 SSR markers genotypes based on Dice

	H105	H153	L162	H_1L_1	H10L10	Giza 21	Giza 111	Giza 35	HC83 123-9	H5L5	H_2L_{24}	$H_{15}L_{17}$	AGS129	Giza 82	Giza 22
H105	1.00	0.75	0.59	0.35	0.50	0.47	0.21	0.13	0.13	0.00	0.00	0.00	0.00	0.00	0.00
H153		1.00	0.82	0.59	0.50	0.47	0.21	0.38	0.38	0.11	0.00	0.00	0.00	0.00	0.00
L162			1.00	0.67	0.59	0.56	0.30	0.35	0.47	0.21	0.11	0.11	0.00	0.00	0.00
H_1L_1				1.00	0.35	0.33	0.50	0.35	0.35	0.11	0.00	0.00	0.00	0.00	0.00
H10L10					1.00	0.59	0.32	0.25	0.38	0.22	0.22	0.24	0.24	0.12	0.24
Giza21						1.00	0.70	0.47	0.24	0.32	0.21	0.11	0.11	0.00	0.11
Giza 111							1.00	0.53	0.32	0.38	0.19	0.10	0.10	0.10	0.20
Giza35								1.00	0.63	0.44	0.11	0.00	0.00	0.12	0.00
HC831 23-9									1.00	0.56	0.33	0.24	0.12	0.24	0.12
H5L5										1.00	0.70	0.53	0.42	0.32	0.42
H_2L_{24}											1.00	0.74	0.53	0.42	0.53
$H_{15}L_{17}$												1.00	0.78	0.56	0.67
AGS 129													1.00	0.78	0.89
Giza82														1.00	0.89

Genetic variation has a considerable implication for planning an efficient breeding program for plant improvement (Chandra *et al*., 2013). Assessment of genetic variation is not only significant for crop improvement efforts but also for the efficient management and protection of available genetic variability. Molecular profiling is an ideal choice for breeders since they are more reliable, authentic and less affected by environmental instabilities (Vinu *et al*., 2013). These properties were exploited in several molecular marker systems for genetic analysis (Porceddu *et al*., 2002). The effectiveness of SSR markers assess genetic diversity among and within soybean cultivars (Kumawat *et al*., 2015). Genetic relationships among accessions are helpful for designing future breeding for yield, quality and pest resistance (Wang et al., 2006).Complete description of existing certified soybean cultivars and patterns of genetic diversity could facilitate introgression of diverse germplasm into the current commercial soybean genetic

base (Satyavathi *et al*., 2006). Genotypes H105, H153, H162, and H_1 L_1 had leaf *feeding* resistance to Cotton Leaf Worm (*Spodoptera littoralis* **)** insect, but they are late maturing genotypes under Egyptian conditions. Thus, using promising lines in crosses with early maturing with good agronomic types was an attempt to select progenies with good characters and resistance to cotton leaf worm.

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الملخص العربي

تقييم حقل فول الصويا لمقاومة دودة ورق القطن (*Spodoptera littoralis*) باستخدام SSR markers

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يمكن لأصناف فول الصويا المقاومة للحشرات (Glycine max (L.) Merr) أن تقلل من استخدام مبيدات الآفات في مكافحة الحشرات، مما يؤدي إلى تقليل المخاطر على البيئة. أجريت هذه الدراسة لتطوير ١٥ تركيبا وراثيا لفول الصويا المقاومة. أجريت تجربة حقلية على تساقط الأوراق بواسطة دودة ورق القطن، وأظهرت أن التراكيب الوراثية H105 و H153 سجلت أعلى مقاومة لدودة ورق القطن وانخفاض معدل تساقط الأوراق بنسبة 0.0أو0.1 علي التوالي، يليها H10L10A و H153 و H1L1 أعلى قيمة لمحصول البذور نتيجة لزيادة عدد الفروع فى النبات، وعدد القرون فى النبات، ووزن 100 بذرة مما يشير إلى أنه يمكن التوصية بهذه التراكيب الوراثية خاصة مع مقاومة عالية لدودة ورق القطن. تم استخراج 13 من ازواج SSR Primer للحصول على 39 حزمة (اليل). ما يقرب من 92% (١٣٢) من أزواج Primer قامت بتكبير الأليلات SSR allels ووجد ١١ موقع Polymorphic SSR loci. تم استخدام Polymorphic SSRs بنجاح للتمييز بين التراكيب الوراثية التى شملتها الدراسة (15 صنف). يمكن أن تكون هذه هذه الماركات مفيدة بشكل خاص للتمييز الوراثي فيما يتعلق بمقاومة دودة ورق القطن.

الكلمات المفتاحية: تساقط الأوراق، دودة ورق القطن، التباين الوراثي، أصناف فول الصويا المقاومة، SSR