Physiological and molecular genetic studies for cotton leaf worm (*Spodoptera littoralis*) tolerance on six Egyptian soybean cultivars

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ABSTRACT

Cotton leaf worm (*Spodoptera littoralis*) is considered one of the most destructive agricultural pests. Six soybean cultivars (Giza-21, Giza-22, Giza-35, Giza-82, Giza-83 and Giza-111) were grown under natural infection with cotton leaf worm. The effect of two elicitors, methyl jasmonate and sodium nitroprusside on enhancing the ability of susceptible cultivars to tolerate (*Spodoptera littoralis*) was studied. Giza-35 and Giza-111 showed tolerance performance under natural infection compared to Giza 22 and Giza 82 as sensitive ones, while Giza 83 and 21 showed moderate tolerance. Both treatments positively affected seed yield and its components and fatty acid composition. Extracted fatty acids showed great changes in treated plants comparing with the untreated controls. Plants treated with the two elicitors showed an increase in Linoleic acid and Linolenic acid fatty acids and decrease in Palmitic acid and Palmitolic acid content. Treatment with methyl jasmonate was found to be more effective than sodium nitroprusside and enhanced resistance of the susceptible cultivars. Eight IRAP and iPBS retrotransposon-based markers were used to detect genetic differences among studied soybean cultivars and to develop molecular genetic markers for cotton leaf worm infestation. The technique successfully identified soybean genotypes in addition to nineteen molecular markers related to soybean tolerance.

Key words: Soybean, Methyl jasmonate, Sodium nitroprusside, Fatty acids, IRAP-iPBS.

Introduction

Soybean crop (*Glycine max* L) is a very important economic crop belongs to leguminosae, it is attacked by cotton leaf worm (*Spodoptera littoralis*) which considered the major pest throught out its growing season (**Massouda *et al*., 2014).** Soybean crop is accounting for 58% of the world oil-seed production; it is the largest oil seed crop worldwide in terms of production and consumption **(SoyStats, 2011).** Soybean is a significant source of fatty acids, proteins, vitamins, minerals, amino acids and other nutrients for both humans and animals, it has other industrial importance as feedstocks and combustible fuels **(Maltas *et al*., 2011).** In Egypt, soybean production in 2016 was 35000 ton **(http://www.fao.org/faostat/en/#data/QC).**

Cotton leaf worm (*Spodoptera littoralis*) is considered one of the most destructive agricultural lepidopterous pests. It can attack numerous economically important crops all over the year **(Abouelghar *et al.*, 2013).** Chemical pesticides were effectively used against insect pests but are associated with a number of drawbacks including high costs and concerns about environmental pollution and food safety, for these reasons, plants can be treated with elicitors to induce resistance to herbivores (**Mohamed and Abd-El Hameed, 2014)**. Several environmental manipulations can be attained by employing chemical insecticides but still the developing of tolerant cultivars is the best choice.

Jasmonic acid (JA) and its methyl ester (MeJa) are cyclopentanone compounds which act as signal transduction molecule in plant defense reactions, induces secondary metabolites and is an important phytohormone that is involved in signaling wound responses **(Howe, 2004 and Deng, 2005).** Because of the wide natural distribution of JA and their effects on many physiological processes in plants they have been proposed as naturally occurring plant growth regulators **(Mohamed and Latif 2017).** Nitric oxide (NO) is a small, highly diffusible, gaseous free-radical and a ubiquitous bioactive molecule **(Lamattina *et al*., 2003).** Nitric oxide at the lower concentration can serve as a signal in plant developmental, hormonal and stress responses **(Akladious and Mohamed 2017).** NO donor molecules, such as sodium nitroprusside produces nitric oxide which is a lipophilic gas that is favorable because of its relatively low cost **(Filippou *et al*., 2013)** and plays an important role in regulating the response of numerous plants to a variety of stressors and stimulate plant defense responses **(Garcia-Mata and Lamattina, 2007 and Klessig *et al*., 2000).**

Molecular marker assay is playing a vital role in plant biology and in molecular breeding, different DNA-based marker technologies have been developed to indicate polymorphism by assaying subsets of the total amount of DNA in a genome. DNA fingerprinting is useful for identification, determination of family relationship, linkage mapping, phylogenetics, systematics, conservation, molecular ecology, localization of disease loci and determination of genetic variation, (**Golenberg *et al*., 1990**). Variation in genome size is often attributed to repetitive DNA (**Flavell *et al.*, 1974**). Transposable elements constitute a major portion of the repetitive

DNA of plant genomes, contributing significantly to genome size variation (**Vicient *et al.*, 1999**). Soybean genome contains up to 40 to 60% repetitive DNA (**Gurley *et al.*, 1979**). Plants have high transposon percentages in proportion with their genome size; *Arabidopsis thaliana* contains 14% transposon sequence (genome size equals125 Mb), while 80% of *Hordeum vulgare* genome contains TEs (genome size equals 5300 Mb), *Glycine max* contains 76% TEs sequences out of its 1,115 Mbp genome **(Gozukirmizi *et al.*, 2015).** Retrotransposons are mobile genetic elements which transpose replicatively through RNA intermediates. They are found in all major eukaryote divisions and comprise major fractions of the genomes of plants (**SanMiguel *et al.*, 1996; Pearce *et al.*, 1996**). In both monocot and dicot angiosperms, LTR retrotransposons comprise highly heterogeneous populations, whose members frequently span different genera (**Voytas *et al.*, 1992**). Retrotransposons based markers are used in a variety of applications, including DNA fingerprinting, measurement of genetic diversity, phylogenetic relationship studies, genetic mapping, genes analyses, genome evolution, population structure, and cladistic relationships have been applied successfully in some plant genera and species (**Zein *et al.*, 2010**). Retrotransposons are also an ideal target for developing molecular marker techniques because of their amplification mechanism and sequence characteristics. There are different types of transposon based marker techniques. Some of them are; Inter-Retrotransposon Amplified Polymorphism (IRAP) and Inter Primer binding sits (iPBS) (**Gozukirmizi *et al.*, 2015).**

This work is aimed to study the effectiveness of two elicitors, methyl jasmonate and sodium nitroprusside , for controlling cotton leaf worm infestation under field condition to test their effects on yield and seed fatty acid composition and to use retrotransposon-based marker techniques (IRAP and iPBS) to detect molecular markers for cotton leaf worm tolerance in soybean.

Materials and Methods

A field experiment was conducted in the Agricultural Research Centre (ARC) experimental farm, Giza, Egypt during 2014 and 2015 summer seasons. Day temperature ranged from 28 to 45°C with an average of 36.7 ± 3.1°C while that at night was 22.3 ± 2.2°C. Daily relative humidity averaged 43.5 ± 4.6%, in a range between 31.1 and 57.3%. Soybean seeds cultivar (Giza-21, Giza-22, Giza-35, Giza-82, Giza-83 and Giza-111) were obtained from (ARC), Giza, Egypt. Soybean seeds were selected for uniformity, the selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water and left to dry at room temperature (25°C) for about 1 hr. Rhizobial inoculants were applied as peat slurry containing 10^7*Rhizobium*/g. Soybean seeds were sown in the field on the 12th June apart in rows 60 cm and hills were spaced 20 cm. Thinning was done before first irrigation to secure two plants/ hill. The soil had a clay loam texture (sand 20%, silt 25% and clay 55%). Experiment was laid out in randomized complete block design (RCBD) with three replications; plot area was 21 m^2 (4.2 m× 5.0 m). Thirty days after sowing (DAS) the first group was sprayed with MeJa (20 µM), the second group was sprayed with SNP (500 µM) and the third group was sprayed with distilled water and served as control. The treatment was repeated for three times with 4 days interval. At maturity (120 DAS) ten plants were randomly chosen from each replication and the following parameters were studied; number of pods/ plant, number of seeds/ plant, fresh and dry weight of pods and seed index and biochemical components in yielded seeds (total soluble proteins, total soluble sugars, reducing sugars and fatty acid composition). Leaf defoliation (percentage of the leaf area destroyed by the pests) was measured as an indicator for insect lesion; the accumulative damage caused by the defoliator larvae of each of 10 randomly chosen leaves was recorded, percentage of infestation was calculated according to the formula given by **Kasopers 1965**.

Biochemical analysis

Fresh samples (1g) were grounded in 80% aqueous ethanol and the mixture was boiled for 10 min and then centrifuged at 2000 rpm for 10 min. The supernatant was collected and the pellets were re-extracted in 5 ml of 80% ethanol. The supernatants of both extractions were combined and completed to 50 ml by measuring flask with ethanol 80% **(A.O.A.C 1984)**.

Determination of total soluble protein

Seeds of soybean plants (0.5 g fresh seeds) was grounded in 5 ml phosphate buffer pH 6.5 and then centrifuged at 6000 g for 10 minutes. The supernatant is the protein extract. The residue was washed with 2 ml of distilled water. The supernatant and the washing were combined to give the total soluble proteins. The total soluble proteins content was measured by using Folin- Cicalteu reagent according to **Lowry *et al.*, (1951)** and modified by **Hartree (1972)**.

Determination of carbohydrate fractions

Seeds of soybean plants (1g) were grounded in 80% aqueous ethanol and the mixture was boiled for 10 min and then centrifuged at 2000 g for 10 min. The supernatant was collected and the pellets were re-extracted in 5 ml of 80% ethanol. The supernatants of both extractions were combined and completed to 50 ml by measuring flask with ethanol 80% **(A.O.A.C 1984)**.

Total soluble sugars were determined in ethanolic extract using the phenol sulphuric method according to **Dubois *et al*., (1956)** and modified by **Dey (1990).** Reducing sugars were determined in the ethanolic extract using dinitrosalicylic acid method according to

Miller (1959). Non reducing sugars were calculated by difference between the total soluble sugars and reducing sugars. Starch was estimated according to **(Rose *et al*., 1991)** by perchloric acid method**.**

Determination of fatty acids

Lipids were extracted according to **Kates and Eberhardt (1957).** The methyl esters of fatty acids were prepared according to the method of **Glass (1971).**The methylated samples were subjected to analysis by GLC Agilent technologies 6890 N Network GC system Oven. FAME condition was: Initial temp. 50 ºC, Initial time 2 min. Inlet temp.: 250 ºC. Detector temp.: 280ºC, Flame Ionization Detector (FID), Flow: 1.5 ml/min. Column: HP-5 (5% phenyl methyl siloxane) L = 30 m, D = 320 µm. Flame thickness = 0.25 µm. Carrier gas: N_2 30 ml/min, H_2 30 ml/min and Air 30 ml/min.

Retrotransposon-based markers

Genetic diversity among the studied cultivars was carried out using inter-retrotransposon amplified polymorphism (IRAP) and inters primer binding site (iPBS) marker systems **(Fig. 2).** Eight IRAP and iPBS primers were applied on six soybean cultivars. The codes and sequences of the tested primers are listed in **Table (1).** Genomic DNA isolation and quantification from young leaves of six soybean genotypes was performed according to the method advised by **http\\www.primerdigital.com/DNA**. PCR reactions were conducted as follows: 95°C for 3 min hot start; 35 cycles (95°C for 20 sec, Annealing temperature (according to each tested primer as listed in **Table (1)** for 30 sec and 72°C for 90 sec); final extension at 72°C for 5 min. Electrophoresis was performed on 1.2% agarose gel in 1xTHE buffer at constant voltage of 70 Volts for 14 hours. Electrophoresis Gene Ruler^{TM} DNA ladder mix (Thermo scientific) 100-10,000 base range was diluted with 1x gel loading buffer to final concentration 25ng/µl and were used as ladder DNA. Gels were visualized by Alpha Innotech Gel Imager 2000 Multimage Light Cabinet AlphaImager Gel Documentaion.

Table 1. List of the tested primers used for DNA profiling of soybean genotype.

Primers	Sequences	Tm°C	G:C
iPBS-2219	´5 gaacttatgccgatacca´3	51.5	44.4
iPBS-2394	´5 gagcctaggcca´3	48.5	66,7
iPBS-2399	´5 aaactggcaacggcgcca´3	63.4	61.1
IRAP-4341	´5 gtcccacagcttgggcaacag´3	63.7	61.9
IRAP-4361	´5 gtcgaccttcccggcatgaa´3	61.4	60
IRAP-4364	´5 atagcgccgagatgcatgct´3	59.4	55
IRAP-4368	´5 gatgttgcggtggatgtgtggtaagact´3	66.6	50
IRAP-4377	´5 cgtacccttaagggatcaaaacc´3	61.3	44

Statistical Analysis

All data collected were subjected to analysis of variance according to **Gomez and Gomez (1984)**, treatment means were compared using Duncan's Multiple Range Test (**Duncan 1955**) using MSTAT-C computer software package 1990. Data scoring for IRAP and iPBS fragments were treated as binary characters for similarity matrix development, cluster analysis was performed using NTSYs-pc version 2.11 software as described by **Rohlf (1993).**

Results and Discussion

Effect of MeJa and SNP on percentage of soybean defoliation

Percentages of soybean leaf area injured by cotton leaf worm (defoliation) are illustrated in **Fig. (1).** Giza-82 and Giza-22 proved to be the most susceptible genotypes for cotton leaf worm infestation; the percentage of defoliation was (34 and 28%). while, Giza-83 and Giza-21 showed moderate resistant, on the other hand, Giza-35 and Giza-111 were detected the lowest estimates and proved to be more resistant (18 and 13% respect.). It was found that treatment of soybean plants with MeJa or SNP enhanced resistance in susceptible genotypes and decreased mean percentage of defoliation comparing with untreated plants. In general, methyl jasmonate (MeJa) treatment achieved better results than SNP treatment. Results are obtained in agreement with **Zayed (2007)** who reported that Giza-111 is considered to be resistant genotype for cotton leaf worm based on consumed leaf area recorded comparing with Giza-82 and Giza-22 which are susceptible genotypes. It was also found that treatment of soybean plants with MeJa and/or SNP enhanced the resistance in susceptible genotypes as it decreased defoliation percentage comparing with untreated plants. These results are in conformity with the findings of **Thaler *et al.* (2001)** who found that the application of jasmonic acid caused reduction in the population of *Frankliniell aoccidentalis* and aphids in tomato field plots.

Effect of MeJa and SNP on yield and its components

Data in **Table (2)** were significant difference among control and treatments for each genotype in number of pods plant except for Giza-21 which

showed insignificant differences when treated with NSP while treating the same genotype with MeJa resulted in positive significant difference, it is also worthy to mention that no significant differences between the two treatments were observed except for Giza-111 which revealed positive and significant difference when treated with MeJa. Mean number of seeds per plant was increased significantly with both treatments (MeJa and SNP) and showed significant increases as compared with their control. MeJa effect was found to be better than that induced with SNP treatment in all soybean genotypes except Giza-111 **(Table 2).** There was insignificant difference in fresh weight of pods between all soybean genotypes except the most tolerant genotypes (Giza-35 and Giza-111) which showed significant increase in fresh weight/plant. MeJa treatment induced significant increase in pods fresh weight in all genotypes except Giza-83 when compared to its respective controls. On the other hand, SNP treatment induced significant increase in all genotypes except the two tolerant genotypes Giza-35 and Giza-111. By comparing the effects of the two treatments it was clear that no significant difference observed for mean fresh weight/plant in all soybean genotypes **(Table 2).**

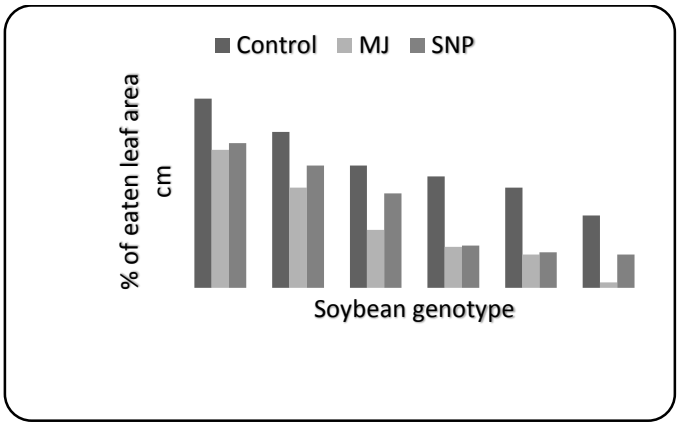

Figure (1) Effect of foliar spray of methyl jasmonate and sodium nitroprusside on soybean defoliation under natural cotton leaf infestation.

Results in **table (2)** showed insignificant difference in pods dry weight in all soybean genotypes. Both treatments (MeJa and SNP) showed insignificant effect in pods dry weight with an exception in Giza 21, Giza 35 and Giza 111 which induced a significant increase in pod dry weight and again the for mentioned genotypes revealed significant difference as a result of their response to treatments, MeJa was found to have positive and significant effects on means of seed dry weight/plant. Seed index in soybean cultivars showed significant differences under natural cotton leaf worm infection, the most sensitive genotypes Giza-82 and Giza-22 recorded the lowest seed indices, no significant differences were observed between the two treatments in all cultivars except for Giza- 111 where MeJa had positive and significant effects. Data showed that a significant increase in yield and its components was observed for tolerant genotypes as compared with the susceptible ones under natural infection with cotton leaf worm. These results are in accordance with **Myers *et al.*, (2005)** who stated that, insect infection can severely reduce soybean growth and yield by reducing the number of pods, number of seeds/pod and individual seed weight. Significant yield loss may be due to the diversion of photosynthesis in response to chewing insect infection **Myers *et al.*, (2005)**.

Effect of MeJa and SNP foliar application on seed chemical components

Seed chemical components of soybean cultivars treated with MeJa and SNP are shown in **table (3)**. From the resulted data it is obvious that there were significant differences among studied genotypes for all traits (total soluble protein, total soluble sugars, reducing sugars and starch content) under natural conditions. Significant and positive differences were observed (P> 5%) between MeJa and SNP treatments for total soluble protein compared to its respective control, on the other hand, there were significant differences on the effects of both treatments for all. Generally, treatment with MeJa revealed positive increase in total soluble protein than SNP for all soybean cultivars. Treatment with both MeJa and SNP was found to have positive and significant effects on total soluble sugars for all genotypes compared to its respective control. Comparison between the effects of both treatments was significant (P> 0.05) with slight increase in the effect of MeJa on all cultivars. Regarding seed reducing sugars content, significant differences were obtained when soybean cultivars were sprayed with MeJa compared to its respective control, the same trend was observed for SNP treatment. Differences between both treatments was found to be significant and positive for all genotypes in spite of the fact that treatment with MeJa showed more increase in reducing sugars content than SNP.

Table 2 . Effect of foliar spray with MeJa and SNP on yield attributes of soybean cultivars under natural infestation with cotton leaf worm.

Genotypes	Treatments	No. of pods/ plant	No. of seeds/ plant	F. wt. of pod (g)	D. wt. of pod (g)	Seed index (g)
Giza 82	Control H_2O	22.4 g	40.8 p	0.72 d	0.26 c	9.44 f
	MeJa 20 µM	70.8 bc	125.0 f	1.09 a-c	0.33 c	15.89 de
	SNP 500 µM	55.5 b-e	89.0 k	1.01 a-c	0.30 c	14.16 de
Giza 22	Control H_2O	23.4 fg	46.4 o	0.92 cd	0.27 c	9.56 f
	MeJa 20 µM	74.6 b	127.3 e	1.12 a-c	0.34 bc	16.28 a-d
	SNP 500 µM	58.2 b-e	98.4 j	1.02 a-c	0.30 c	15.08 c-e
Giza 83	Control H_2O	25.8 fg	54.5 n	0.93 cd	0.29 c	12.67 e
	MeJa 20 µM	76.0 b	130.3 d	1.14 abc	0.34 bc	17.76 a-c
	SNP 500 µM	59.2 b-d	116.2 i	1.03 a-c	0.30 c	15.36 c-e
Giza 21	Control H_2O	33.6 e-g	69.2 m	0.94 cd	0.29 c	13.28 de
	MeJa 20 µM	76.8 ab	130.2 d	1.19 ab	0.46 ab	18.60 ab
	SNP 500 µM	61.0 b-d	119.0 h	1.05 a-c	0.31 c	15.64 b-e
Giza 35	Control H_2O	38.6 d-g	74.3 l	0.97 bc	0.29 c	13.88 de
	MeJa 20 µM	80.2 ab	142.7 c	1.23 a	0.47 a	18.64 ab
	SNP 500 µM	65.0 bc	123.3 g	1.06 a-c	0.32 c	15.68 b-e
Giza 111	Control H_2O	47.8 c-f	180.0 a	0.99 bc	0.30 c	14.12 de
	MeJa 20 µM	101.8 a	169.9 b	1.23 a	0.49 a	18.86 a
	SNP 500 µM	69.20 bc	130.5 d	1.07 a-c	0.33 c	15.76 b-e

*Different letters indicate a significant difference at p≤ 0.05 according to Duncan's multiple tests.
*F.wt. = fresh weight, D.wt= dry weight, seed index= weight of 100 dry seeds

Table 3. Effect of foliar spray with MeJa and SNP on harvested soybean seed composition under natural infestation with cotton leaf worm.

Genotypes	Treatments	Total soluble protein mg/g	Total soluble sugars mg/g	Reducing sugars mg/g	Starch mg/g
Giza 82	Control H_2O	45.9 n	11.45 l	0.516 e-g	0.763 k
	MeJa 20 µM	101.0 fg	23.10 c-e	0.640 c-e	2.520 e
	SNP 500 µM	84.8 j	19.01 hi	0.280 hi	1.323 hi
Giza 22	Control H_2O	50.8 n	13.31 k	0.106 j	0.887 k
	MeJa 20 µM	111.0 de	23.38 b-e	0.720 b-d	3.663 d
	SNP 500 µM	87.0 ij	20.45 gh	0.386 gh	1.827 g
Giza 83	Control H_2O	58.5 m	16.27 j	0.127 ij	1.143 j
	MeJa 20 µM	114.2 d	24.0 b-d	0.773 bc	3.750 cd
	SNP 500 µM	90.6 ij	20.78 g	0.466 fg	1.820 g
Giza 21	Control H_2O	64.6 l	17.56 ij	0.146 ij	1.190 ij
	MeJa 20 µM	122.4 c	24.47 bc	0.810 b	3.810 c
	SNP 500 µM	91.6 hi	21.47 fg	0.500 e-g	2.110 f
Giza 35	Control H_2O	71.2 k	18.28 i	0.190 ij	1.457 h
	MeJa 20 µM	148.6 b	24.79 b	1.207 a	4.027 b
	SNP 500 µM	97.4 gh	22.38 ef	0.517 e-g	2.070 f
Giza 111	Control H_2O	65.0 l	18.57 i	0.270 hi	1.387 h
	MeJa 20 µM	162.9 a	27.22 a	1.347 a	5.720 a
	SNP 500 µM	105.8 ef	22.46 d-f	0.577 d-f	2.520 e

*Different letters indicate a significant difference at p≤ 0.05 according to Duncan's multiple tests.

Total soluble starch showed that significant differences among studied soybean cultivars, significant positive differences were observed among cultivars when treated with both MeJa and SNP treatments compared to its respective controls, significant differences for the two treatments were observed for all cultivars with an increase in starch content when soybean was treated with MeJa. Total protein, total soluble sugars, reducing sugars and total starch were decreased significantly in the susceptible genotypes comparing with the tolerant ones. On the other hand, MeJa and SNP treatments showed a

significant increase in yield attributes and components as compared with untreated plants. Similar results was reported By **Wilen *et al.*, 1991** who found that MeJa enhanced protein content in rapeseed and an increase in mRNA was detected comparing with untreated plants. **Sultana *et al.* (2001)** reported that the yield contributing characters of rice plants were increased by applying JA to stressed and unstressed plants. **Raouf *et al.*, (2012)** showed that significant increase in essential oils of *Agastache foeniculum* was induced after 24 hours of treatment with 0.1 mM of MeJa. SNP is currently being applied to plants exposed to stressful conditions in order to improve growth and yield **(Farooq *et al.*, 2009).** The tolerant soybean genotypes contained higher amounts of protein. The synthesis and accumulation of a variety of storage proteins have been shown to be closely related to plant defense since several of these proteins present entomotoxic properties such as α-amylase and proteinase inhibitors, lectins and globulins **(Franco *et al.*, 2002).**

Effect of MeJa and SNP on seed saturated fatty acids composition

Total content and composition of saturated fatty acids of the six soybean cultivars are tabulated in **table (4).** Saturated fatty acids were much abundant in the tolerant and moderate genotypes as compared with the susceptible ones under natural infestation with cotton leaf worm. Both treatments (MeJa and SNP) caused an increment in saturated fatty acids as compared with their respective controls. Total saturated fatty acids were found to be divergent among soybean cultivars; the susceptible cultivars showed much more saturated fatty acid content than the tolerant ones.

Effect of MeJa and SNP on seed unsaturated fatty acids composition

Data recorded for unsaturated fatty acids composition is presented in **table (5).** Unsaturated fatty acids showed a wide variation in the six soybean genotypes. Generally, linoleic acid (C 18:2) and linolenic acid (C18:3) recorded lower values in the susceptible genotypes (Giza 82 and Giza 22) , Giza-111 recorded the highest values for (C 18:3) under different treatments. In addition, MeJa and SNP treatments induced increase in C 18:2 as compared with untreated plants with some exceptions in **table (4).** Both treatments were found to enhance Oleic acid (C 18:1) content compared to their respective untreated controls with an exception in Giza-83 and Giza-35 when treated with SNP. On the other hand, Arachidi acid (C 20:0) recorded higher values in the moderate and tolerant genotypes than in the susceptible ones. The total unsaturated fatty acids were lower in the sensitive genotypes (Giza-22 and Giza-82) as compared with the tolerant ones (Giza-35 and Giza-111). These findings are In harmony with **Howe (2004)** who reported that once plant tissues are damaged by chewing insects releasing of linolenic acid from intracellular membrane lipids of the affected tissues tacks place. Linolenic acid C18:3 is converted through octadecanoid pathway to green leaf volatiles like jasmonic acid **(Unsicker *et al.*, 2009)** which considered as indirect inducible defense chemical that has been reported to deter insect attack in different plant systems **(Wang *et al.*, 2008). Hyun *et al.*, (2008)** supported the same observations, they concluded that when plant tissues are damaged by herbivores or mechanically C18:3 fatty acids are released from the chloroplast membrane through the action of phospholipases, linolenic acid is oxidized to cause the accumulation of JA in herbivore-wounded plants **(Smith *et al.*, 2009). Tooker and De Moraes (2009)** showed that, a tobacco bud worm caterpillar *Heliothis virescens* feeding caused an increase in the levels of linoleic and linolenic acid in damaged leaves compared to the undamaged controls. Arachidic acid were found to play an important role in wax and cuticle formation which are the first barrier in plant defense **(Kachroo and Kachroo, 2009).** Fatty acids play a vital role in membrane fluidity and they are involved in controlling plant defense against pathogens and pests **(Heldt, 2005).** The modulation of membrane fluidity and stability by regulating seed oil fatty acid composition may allow plants to adapt to various stresses **(Upchurch, 2008). Goldhaber-Pasillas *et al.*, (2014)** who reported the polyunsaturation of fatty acids has proven to be correlated to adaptation when plants are challenged in response to biotic and abiotic stress.

Retrotransposon based markers

A set of eight IRAP and iPBS primers based on the conservative regions of retrotrarsposones were used for this study (**Fig. 2**). Retrotransposons based primers successfully distinguished soybean cultivars with 142 bands, out of which 24 were monomorphic and 118 were polymorphic **Table (6) and Figure (2),** Thirty seven unique markers were distinguished that characterized their respective genotypes with twenty three positive and fourteen negative specific markers **(Table 6).** These results are inagreement with **Brown-Guedira *et al.*, 2000**, **Wang *et al.*, 2006a, Mulato *et al.*, 2010 and Kumawat *et al.*, (2015)** who succeeded in distinguishing a larg set of soybean accessions using simple sequence repeat markers (SSRs). 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 (**Satyavathi *et al.*, 2006**).

Retrotransposon based markers succeeded to distinguish tolerant and sensitive cultivars with 19 markers resulted from five out of the eight tested primers (**Table 7**), four primers distinguished sensitive genotypes (Giza-22 and Giza-82) with positive markers ie iPBS-2399, IRAP-4314, IRAP-4364 and IRAP-4361. On the other hand the two sensitive genotypes were distinguished with positive markers generated from the two IRAP primers IRAP-4341 and

IRAP-4377. It worthy to mention that IRAP-4377 generated the highest number of markers (13 markers) that positively identified the two tolerant genotypes while there were completely absent in the two sensitive ones, this primer is considered highly informative in distinguishing tolerant form sensitive soybean cultivars.

The genetic diversity has a great significance for planning an efficient breeding programme for crop improvement (**Chandra *et al.*, 2013**). The assessment of genetic diversity is not only important for crop improvement efforts but also for the efficient management and protection of available genetic variability. Molecular profiling has been the preferred choice for breeders as these are more reliable, authentic and less influenced by environmental fluctuations (**Vinu *et al.*, 2013**). Several retrotransposons have been shown to be highly polymorphic for insert location within plant species **(Porceddu *et al.*, 2002).** These properties have been exploited in several molecular marker systems for genetic analysis in a range of cereal grass and grain legume species **(Porceddu *et al.*, 2002).** The effectiveness of IRAP, REMAP, SSR, and ISSR markers were investigated to assess genetic diversity among and within eight *Medicago sativa* L. populations. IRAP markers generated the maximum proportion of polymorphic loci per primer (**Mandoulakani *et al.*, 2012)**.

The pair similarity coefficient among the six soybean cultivars ranged from 21 to 71% (**table 8**). The highest similarity value (71%) was observed between the two tolerant cultivars Giza-35 and Giza-111, while the lowest similarity value (21%) was observed between Giza-35 (tolerant) and Giza-82 (sensitive), genetic similarity was found to be 46 % between the two sensitive cultivars Giza-22 and Giza-82. The genetic base of soybean cultivars is considered to be extremely narrow **(Hymowitz 1970).** Cluster analysis performed using unweighted pair-group method of arithmetic means (UPGMA) expressing the relationships among studied soybean cultivars is illustrated in **figure (3).** The cluster analysis resolved the six soybean cultivars into two main clusters (A and B). The first, cluster (A) comprised only Giza-82 (sensitive), the second cluster (B) contains two sub-cluster (C and D) the first (C) have Giza-22 which is the second sensitive genotypes, the two sensitive genotypes were genetically correlated with 46% similarity and were located nearest to each other in the resulted dendrogram, cluster (D) was divided into two sub-clusters (E and F) sub-cluster E has Giza-83 (moderate) while sub-cluster (F) was divided into two sub-clusters (G and H) the first has Giza-21 (moderate) and the second has the most tolerant cultivars Giza-35 and Giza-111. Long terminal repeat-retrotransposons (LTR-RTs) are the most abundant genomic components in flowering plants, making up a large fraction of all plant genomes so far investigated (**Du *et al.*, 2010**). **Mulato *et al.*, 2010** investigated the genetic variation in 79 soybean (*Glycine max*) accessions using thirty SSR primer-pairs. All analyzed loci were polymorphic and 259 alleles were found. The genetic diversity observed was high and allowed the formation of five groups and several subgroups. A moderate relationship between genetic divergence and geographic origin of accessions was observed. Eleven SSR primer pairs could amplify polymorphic SSRs from 25 soybean genotypes. These eleven SSR markers successfully distinguished 23 of the 25 soybean genotypes, with the exception of a pair of closely related breeding lines from the same cross **Mulato *et al.*, 2010**. Genetic relationships among accessions are helpful for designing future breeding efforts for yield, quality and pest resistance improvement (**Wang *et al.*, 2006a**).

Table 4. Effect of foliar spray with MeJA and SNP on saturated fatty acid composition in soybean seeds under field condition of natural infestation with cotton leaf worm.

Fatty acids	Giza 82			Giza 22			Giza 83			Giza 21			Giza 35			Giza 111		
	Cont.	MeJA	SNP	Cont.	MeJA	SNP	Cont.	MeJA	SNP	Cont.	MeJA	SNP	Cont.	MeJA	SNP	Cont.	MeJA	SNP
C :8 Capryliac acid	0.00	0.00	0.20	0.00	0.00	1.51	18.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.87	1.19	0.00	0.88
C :10 Capric acid	0.00	0.00	0.05	0.00	0.00	0.66	0.82	0.00	0.49	0.00	0.00	0.00	0.00	0.00	1.40	1.67	0.00	1.06
C :11 Undecanoic acid	0.00	0.00	0.60	0.10	0.00	11.55	4.80	2.30	0.00	0.00	0.00	0.00	0.00	0.00	9.90	0.00	0.00	7.53
C :12 Lauric acid	0.00	0.32	1.43	0.44	0.50	1.84	1.10	5.36	4.40	2.00	1.60	0.24	0.11	0.48	2.89	2.49	0.27	1.59
C :13 Tridecanoic acid	0.00	0.00	0.07	1.32	1.29	3.28	2.36	3.09	0.16	1.84	2.71	1.17	0.00	2.14	2.70	6.73	0.46	2.44
C :14 0 Myrisitic acid	8.67	2.25	0.00	1.08	0.00	0.00	0.00	7.20	9.03	4.96	6.15	0.00	0.38	0.00	1.45	1.30	0.00	0.00
C :14 1Myristoleic acid	20.65	0.00	2.97	0.00	2.59	2.38	2.46	3.77	9.97	11.35	7.13	1.01	0.92	1.74	0.79	1.05	0.55	1.89
C :15 1Cis-10-pentadecanoic acid	8.37	0.86	2.22	0.68	1.73	0.00	2.13	3.77	6.77	5.84	0.00	1.11	0.00	1.83	0.43	0.00	0.50	1.12
C :16 0 Palmitic acid	9.65	13.48	16.36	0.83	1.63	0.00	1.35	9.84	0.00	4.49	3.32	0.89	0.46	0.00	0.00	0.00	0.33	0.63
C :17 0 Heptadecanoic acid	0.00	0.00	2.47	0.22	0.00	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.60	0.00	0.20
C :20 0 Arachidic acid	0.00	0.00	0.00	0.00	1.37	0.00	2.02	0.00	0.00	0.78	0.00	2.25	0.92	1.94	0.00	1.68	0.00	10.68
C :21 0 Henicosanoic acid	0.00	0.00	0.00	0.00	1.22	8.37	1.80	0.00	0.00	1.35	0.00	0.00	0.00	0.00	1.80	0.00	0.17	3.04
C :22 0 Behenic acid	0.00	0.98	0.00	0.00	0.00	3.49	0.00	0.00	0.00	1.96	0.00	0.00	0.00	0.00	2.96	1.90	0.00	1.59
C :23 0 Tricosanoic acid	0.00	0.00	0.55	51.34	0.43	0.00	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.96	1.77	0.11	0.73
% Saturated fatty acids	**47.33**	**17.89**	**26.93**	**56.01**	**10.76**	**34.06**	**35.64**	**35.32**	**31.22**	**33.80**	**20.91**	**5.13**	**2.78**	**8.14**	**28.34**	**20.37**	**2.38**	**33.38**

Table 5. Effect of foliar spray with MeJA and SNP on unsaturated fatty acid composition in soybean harvested seeds under field condition of natural infestation with cotton leaf worm.

Fatty acids	Giza 82			Giza 22			Giza 83			Giza 21			Giza 35			Giza 111		
	Cont.	MeJA	SNP	Cont.	MeJA	SNP	Cont.	MeJA	SNP	Cont.	MeJA	SNP	Cont.	MeJA	SNP	Cont.	MeJA	SNP
C :16 1 Palmitolic acid	0.00	0.00	0.00	5.96	2.20	0.99	10.17	0.00	10.18	0.00	0.00	9.22	7.82	0.00	0.00	0.00	0.00	0.00
C :17 1 Cis-10-heptadecanoic acid	0.00	0.00	0.13	0.12	0.00	0.00	0.24	0.00	0.00	0.52	0.00	0.12	0.00	0.00	0.00	1.84	0.00	0.29
C :18 1 Oleic acid	22.47	71.29	65.38	33.32	70.78	38.37	35.44	34.15	29.75	28.14	34.75	48.84	51.22	53.11	14.93	0.00	0.00	1.85
C :18 2n Linoleic acid	0.00	0.00	0.00	0.00	0.00	2.31	7.29	25.55	24.03	25.61	29.11	26.64	30.23	31.30	37.90	0.00	0.00	28.32
C :18 3n3 Linolenic acid	0.00	8.00	6.70	3.30	13.34	15.72	4.70	2.58	3.69	4.29	12.03	6.18	6.56	6.66	0.00	63.10	96.19	29.13
C :20 36t Eicosadienoic acid	0.00	0.93	0.41	0.00	0.00	0.00	2.61	0.38	0.00	4.02	0.00	0.87	0.00	0.78	5.46	0.00	0.65	5.88
C :20 4 Arachidonic acid	0.00	0.00	0.00	0.38	1.61	0.95	0.00	0.00	0.40	0.00	0.00	0.71	0.90	0.00	5.14	5.07	0.00	0.00
C :20 5 Eicosapentaenoic acid	0.00	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	2.54	0.00	0.00
C :22 1 Erucic acid	3.02	1.89	0.00	0.91	1.31	7.61	1.89	2.02	1.13	2.84	3.20	0.64	0.49	0.00	8.23	7.07	0.78	1.15
% Unsaturated fatty acids	**52.67**	**82.11**	**73.07**	**44.00**	**89.24**	**65.94**	**64.36**	**64.68**	**68.78**	**66.20**	**79.09**	**94.87**	**97.22**	**91.86**	**71.66**	**79.63**	**97.62**	**66.62**

Table 6. Total number of bands, monomorphic, polymorphic, Positives and negative unique bands generated by testing IRAP and iPBS markers tested on six soybean cultivars.

primer	Monomorphic bansd	polymorphic bands	Total bands	Specific markers				Total markers/ primer
				Positive makers	genotypes	Negative marker	genotypes	
iPBS-2394	1	17	18	250	Giza83	200	Giza82	7
				500	Giza111	620	Giza83	
				1031	Giza83			
				1250	Giza83			
				1700	Giza22			
iPBS-2399	3	17	20	150	Giza21			6
				360	Giza82			
				600	Giza22			
				1070	Giza21			
				2100	Giza82			
				2200	Giza22			
IRAP-4341	2	10	12	250	Giza83			1
iPBS-2219	3	17	20	230	Giza111			2
				900	Giza83			
IRAP-4364	8	13	21	500	Giza82	450	Giza21	8
				1220	Giza82	640	Giza82	
				1310	Giza21	940	Giza82	
						1100	Giza82	
						2100	Giza82	
IRAP-4368	5	9	14	500	Giza82	600	Giza35	6
				1031	Giza22	660	Giza35	
						700	Giza35	
						900	Giza22	
IRAP-4377	2	20	22	1031	Giza82	1050	Giza82	2
				2700	Giza83			
IRAP-4361	0	15	15	350	Giza35	400	Giza82	2
				1650	Giza53	500	Giza83	
Grand Total	24	118	142	23		14		37

Table 7. Positive and negative unique bands identified for tolerant and sensitive soybean genotypes using IRAP and iPBS markers.

NO	Primer	Marker size (bp)	tolerance	sensitive
1	iPBS-2399	1200	--	++
2		1250	--	++
3	IRAP-4341	580	--	++
4		520	++	--
5	IRAP-4364	750	--	++
6		480	++	--
7		580	++	--
8		680	++	--
9		740	++	--
10		800	++	--
11		1100	++	--
12	IRAP-4377	1230	++	--
13		1400	++	--
14		1500	++	--
15		1600	++	--
16		1900	++	--
17		2000	++	--
18		3300	++	--
19	IRAP-4361	700	--	++

++ = positive marker for both genotypes, tolerant genotypes Giza-35, Giza-111 and sensitive genotypes Giza-22, Giza-82

Table 8. Genetic similarity matrix between the six soybean genotypes computed according to IRAP and iPBS data.

Genotypes	1. Giza21	2. Giza22	3. Giza35	4. Giza82	5. Giza83	6. Giza111
1. Giza21						
2. Giza22	0.50					
3. Giza35	0.60	0.57				
4. Giza82	0.39	0.46	0.21			
5. Giza83	0.53	0.42	0.60	0.36		
6. Giza111	0.67	0.61	0.71	0.34	0.58	

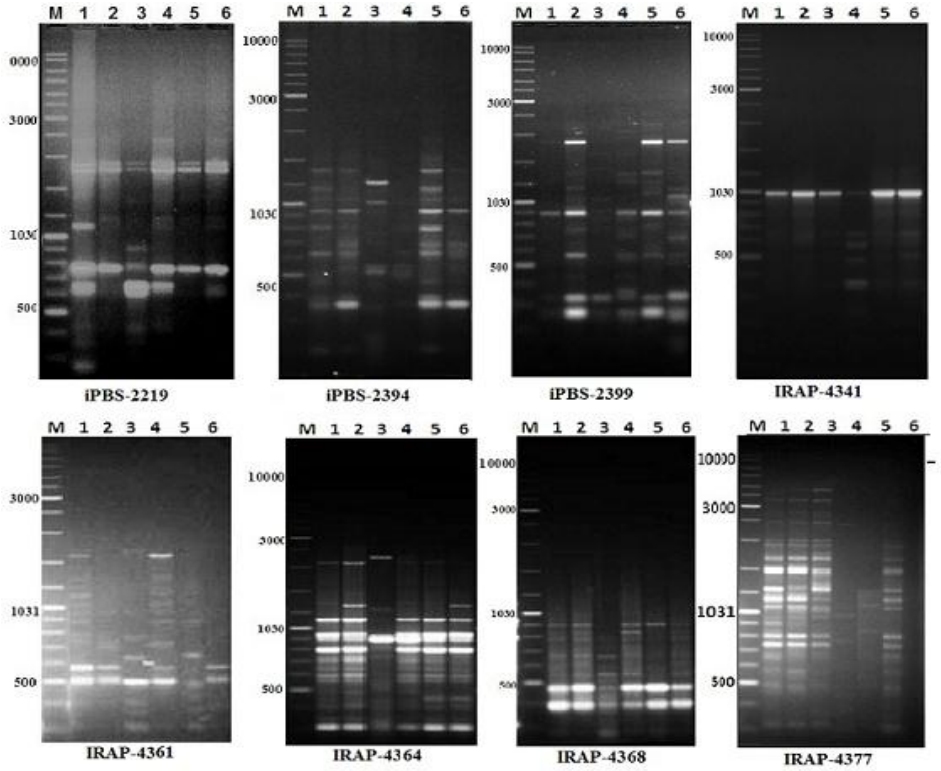

Figure (2): IRAP and iPBS PCR polymorphism of DNA for six soybean (1, 2, 3, 4, 5 and 6) genotypes using IRAP and iPBS primers, (**M**) refer to DNA ladder 10 000 pb DNA ladder

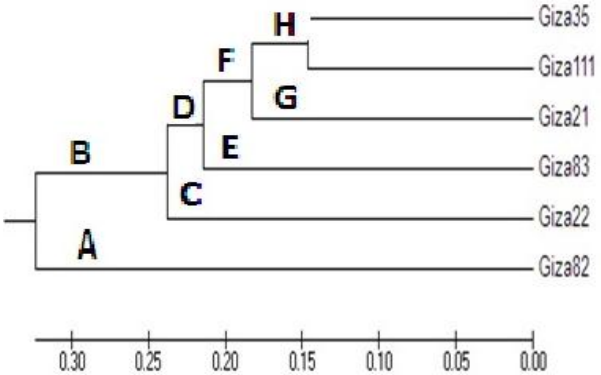

Figure 3. A dendrogarm for six soybean genotypes constructed from the IRAP marker data using un weighted pair group method with arithmetic mean (UPGMA).

Conclusion

From the results obtained in this investigation it could be concluded that methyl jasmonate and sodium nitroprusside treatments have positive effects on enhancing soybean tolerance to infestation with cotton leaf worm. Both treatments were found to induce significant decrease in leaf eaten area by cotton leaf worm which combined with significant increment in yield and yield components. In addition MeJa (20 µM) treatment recorded better results comparing with SNP (500 µM). Our results showed that, Giza-35 and Giza-111 genotypes were more tolerant to cotton leaf worm

infestation as compared with susceptible genotypes Giza-82 and Giza-22. Retotransposon-based markers (IRAP and iPBS) succeeded to differentiate tolerant and sensitive six cultivars.

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المستخلص العربى

١- تم دراسة اثر الاصابة بدودة ورق القطن علي ستة اصناف من نبات فول الصويا و هي جيزة ٢١، جيزة ٢٢، جيزة ٣٥، جيزة ٨٢، جيزة ٨٣، جيزة ١١١حيث تمت الزراعة و تقسيم كل صنف الي ثلاثة مجموعات المجموعة الاولي تمت معاملتها بالماء المقطر كمجموعة ضابطة اما المجموعة الثانية فقد تم معاملتها بميثيل جاسمونات (MeJA) المجموعة الثالثة تمت معاملتها بنيتروبروسيد الصوديوم (SNP) . اظهرت النتائج اختلافا واضحا في مقاومة الاصناف المختلفة للاجهاد الحيوي للاصابة بدودة ورق القطن حيث سجلت الاصناف جيزة ٢٢ و جيزة ٨٢ حساسية للاصابة اما جيزة ٢١ و جيزة ٨٣ فقد سجلت حساسية متوسطة في حين سجلت الاصناف جيزة ٣٥ و جيزة ١١١ مقاومة واضحة للاجهاد الحيوي بدودة ورق نبات القطن. كما اظهرت المجموعات الثانية و الثالثة تحسنا ملحوظا في الخواص المورفولوجية و كذلك بعض الخواص الفسيولوجية (محتوي الاصباغ، محتوي البروتينات الذائبة الكلية، محتوي السكريات الذائبة، محتوي الاحماض الامينية الحرة) مقارنة بالمجموعات الضابطة في جميع الاصناف. كذلك اظهرت نتائج تحليل محتوي الاحماض الدهنية في البذور تباينا بين المجموعات الثلاثة حيث لوحظ زيادة محتوي حمض لينوليك, لينولينيك و نقص في محتوي حمض بالمتيك و حمض بالموليتيك مقارنة في البذور المعاملة بميثيل جاسمونات و نيتروبروسيد الصوديوم مقارنة ببذور المجموعة الضابطة. اشارت نتائج دراسة العناصر المتنقلة الي امكانية التفريق بين الاصناف محل الدراسة بعدد من الواسمات الخاصة بكل صنف وكذلك أوضح الشكل الشجيري العلاقات الوراثية بين الاصناف وقد اظهرت تقاربا واضحا بين المتحملين جيزة ٣٥ و جيزة ١١١.

مفتاح الكلمات: فول الصويا ، الاجهاد الحيوي ، ميثيل جاسمونات، بنيتروبروسيد الصوديوم، مضادات الاكسدة، الدهون المؤكسدة، التانينات، الفلافونات و الفينولات.