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Research article

Physiological and molecular genetic studies on two elicitors for improving the tolerance of six Egyptian soybean cultivars to cotton leaf worm

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ABSTRACT

Cotton leaf worm (*Spodoptera littoralis*) is considered one of the most destructive agricultural pests in Egypt. 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 Giza-21 showed moderate tolerance. Both treatments positively affected seed yield and its components and fatty acid composition. Extracted fatty acids showed variable changes in treated plants compared 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.

1. 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 in Egypt (Massouda et al., 2014). The United States is the global leader in soybean production. In the crop year ended September 2016, the U.S. produced over 107 million tons of soybeans, the second largest producer, Brazil, had a soybean production volume amounting to 96.5 million tons (SoyStats, 2016). In Egypt, Soybean production is fluctuated reaching 35,000 tons in 2016 (http://www.fao.org/faostat/en/#data/QC). 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, induce secondary metabolites and is an important phytohormone that is involved in signaling wound responses (Howe, 2004; 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). Several defensive genes are activated after the application

Abbreviations: DNA, Deoxyribonucleic acid; IRAP, Inter retro-transposon Amplified Regions; iPBS, inter Primer Binding sites; LTR, the long terminal repeat; LTR-RTs, Long terminal repeat-retrotransposons; PBS, primer binding site; TEs, Transposable elements; tRNA, Transfer ribonucleic acid; Uv, ultraviolet; JA, Jasmonic acid; MeJA, methyl jasmonate; NO, Nitric oxide

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of jasmonic acid by the induction of systemic resistance against pests and by the release of volatile compounds which attract to insects infested plants (Thaler et al., 1996). The production of proteinase inhibitors and polyphenol oxidases and the decrease in the preference, performance, and abundance of insects are detected after treatment with jasmonic acid and methyl jasmonate in tomato (Thaler, 1999a; b), cotton (El-Wakeil et al., 2003) and winter wheat (El-Wakeil et al., 2010).

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; Klessig et al., 2000). In insects, nitric oxide plays roles in diverse physiological processes including reproduction, locomotion, olfaction, learning and memory, and host defense mechanisms (Müller, 1997; Davies, 2000). Recently, nitric oxide was discovered to be a potent fumigant against insects. Nitric oxide is highly effective against all insects at various life stages including egg and pupa (Liu, 2013).

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 40–60% 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 1115 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.

2. 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 h. 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. The experimental design was Randomized Complete Block Design with three replicates, plots containing different treatments were randomly distributed in each block, a plot consisted seven rows and separated from the next with tow non sown rows, the middle three rows were treated while two guarding rows each side were not treated to avoid cross-contamination between sprays, samples were obtained from the middle row of the treated ones. 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%). 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 four day 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).

2.1. Biochemical analysis

Fresh samples (1 g) 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).

2.1.1. Determination of total soluble protein

Seeds of soybean plants (0.5 g fresh seeds) were grounded in 5 ml phosphate buffer pH 6.5 and then centrifuged at 6000 g for 10 min. 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).

2.1.2. Determination of carbohydrate fractions

Seeds of soybean plants (1 g) 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

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