



Evaluation of Water Stress Tolerance of Soybean Using Physiological Parameters and Retrotransposon-Based Markers

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Abstract

Drought is one of the most important environmental stresses that severely reduce plant growth and crop productivity. This study was carried out to investigate the difference in the response of six soybean cultivars (Giza 21, 22, 35, 82, 83 and 111) under water stress and the genetic difference between these cultivars using retroelements technique. The results showed that drought stress caused reduction in morphological criteria, photosynthetic pigments, starch, phospholipids, glycolipids, pectin, cellulose and lignin in shoots of all soybean cultivars except Giza 22 and Giza 83. On the other hand, there was a considerable increase in root length, soluble sugars, proline, glycine betaine, total lipids and hemicellulose contents in the shoots of the soybean cultivars in response to water stress. The soybean cultivars Giza 22 and 83 were more drought tolerant than the other cultivars while Giza 21 and Giza 111 were the most sensitive. Inter-primer binding sites (iPBS) and inter-retrotransposon amplified polymorphism (IRAP) techniques were used to fingerprint the six soybean cultivars using a set of eight primers. The techniques successfully tagged each cultivar with specific bands and detected molecular genetic markers related to drought tolerance in soybean.

Keywords Photosynthetic pigments · Osmolytes · Retroelements · IRAP · IPBS

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Bewertung der Toleranz von Sojabohnen gegenüber Wasserstress mittels physiologischer Parameter und Retrotransposon-basierter Marker

Zusammenfassung

Trockenheit ist eine der wichtigsten Umweltbelastungen, die das Pflanzenwachstum und die Pflanzenproduktivität stark reduzieren. Diese Studie wurde durchgeführt, um den Unterschied in der Reaktion von sechs Sojasorten (Giza 21, 22, 35, 82, 83 und 111) unter Wasserstress und den genetischen Unterschied zwischen diesen Sorten mittels Retroelement-Technik zu untersuchen. Die Ergebnisse zeigten, dass Trockenstress zu einer Verringerung der morphologischen Kriterien, der photosynthetischen Pigmente sowie zu einer Reduktion von Stärke, Phospholipiden, Glycolipiden, Pektin, Cellulose und Lignin in den Sprossen aller Sojasorten außer Giza 22 und Giza 83 führte. Auf der anderen Seite kam es als Reaktion auf Wasserstress zu einer beträchtlichen Zunahme der Wurzellänge und des Gehalts an löslichen Zuckern, Prolin, Glycinbetain, Gesamtlipiden und Hemicellulose in den Sprossen der Sojabohnensorten. Die Sojasorten Giza 22 und 83 waren trocken-toleranter als die anderen Sorten, während Giza 21 und Giza 111 am empfindlichsten waren. Die iPBS- (inter-primer binding site) und die IRAP-Technik (inter-retrotransposon amplified polymorphism) wurden verwendet, um einen genetischen Fingerabdruck der sechs Sojabohnensorten unter Verwendung eines Satzes von acht Primern zu erstellen. Mithilfe dieser Techniken konnten jeder Sorte erfolgreich spezifische Banden zugeordnet werden. Außerdem wurden molekulargenetische Marker entdeckt, die mit der Trockentoleranz von Sojabohnen in Zusammenhang stehen.

Schlüsselwörter Photosynthetische Pigmente · Osmolyte · Retroelemente · IRAP · IPBS

Introduction

Water deficit is caused due to the absence of water or moisture which helps the plant in the growth, development and differentiation (Zhu 2002). In arid and semi-arid zones the inadequacy of water available for irrigation frequently exposes plants to drought (Wang et al. 2005). Water stress affects the activity of some enzymes and causes the accumulation of proteins and compatible solutes i. e., sugars, glycine betaine and amino acids (Gong et al. 2005). In addition, it causes inhibition of the crop productivity and alters the metabolism of lipids which help in the membrane synthesis (Ford and Barber 1983). Water stress also caused reduction in linolenic acid, glycolipid and phospholipid contents of leaf tissues (Wilson et al. 1987).

Plants can adapt or tolerate stress by the accumulation of osmoprotectant compounds such as trehalose, polyamines and proline which help the plant to cope abiotic and biotic stress (Mohamed and Akladios 2014; Ashry and Mohamed 2011). Genotypes differ in their response to environmental stresses in proportion with their difference in the genetic based antioxidant systems resulting in tolerant and sensitive genotypes in the same species (Sairam et al. 1998). Drought stress affects growth and yield of soybean cultivars depending on the sensitivity of cultivated varieties and duration of water deficit (Frederick et al. 2001).

Long terminal repeat-retrotransposons (LTR-RTs) are the most considerable genomic components in plant genomes (Du et al. 2010). In the last few years, the most of the plant LTR-RTs have been amplified (Xiang et al. 2016). Soybean (*Glycine max* L. Merr.) is the most important crop in the world and belongs to leguminosae

family. Plants of this family contain a large amount of protein and are used as food and animal feed worldwide (Graham and Vance 2003). Recently, soybean has been sequenced due to its economic value (Schmutz et al. 2010). Du et al. (2010) characterized LTR-RTs in the soybean genome, their work resulted in 950 Mb mapped to the 20 soybean chromosomes. The retrotransposon markers have been successfully applied to the analysis of genetic diversity in different species such as sunflower (Vukich et al. 2009), flax (Smýkal et al. 2011) and brassica (Nouroz et al. 2015).

Transposable elements (TEs) are DNA sequences that move around the genome. There are two classes of TEs: retrotransposons and DNA transposons (Wicker et al. 2007). Transposable elements have two properties: (1) its ability to move within a genome from one place to another, (2) the ability to double their copy via transposition. Under stress conditions, the expression and mobility of TEs are different (Grandbastien et al. 2005).

Molecular markers are essential in plant breeding and biodiversity applications, LTR-RTs are used as molecular markers. Inter-primer binding sites (iPBS) technique is based on the virtually universal presence of a tRNA complement as a reverse transcriptase primer binding site (PBS) in LTR-RTs (Kalendar et al. 2010). The inter-retrotransposon amplified polymorphism (IRAP) method displays insertion polymorphisms by amplifying the segments of DNA between two retrotransposons and need for prior sequence information to design suitable primers (Flavell et al. 2003). RE based techniques were previously used to detect molecular markers for soybean (Sandhu et al. 2017).

This study was aimed to test the effect of water stress on some morphological, physiological and biochemical parameters of the six Egyptian soybean cultivars, to study the genetic variation among soybean cultivars and to detect molecular genetic markers related to drought stress tolerance in soybean using retrotransposon-based molecular markers.

Materials and Methods

Plant Material and Growth Conditions

A pot experiment was conducted in a wire house at the Faculty of Education, Ain Shams University, Egypt during the period from 5 June to 30 July 2016. This experiment was conducted under following environmental conditions (day length 12–14h, temperature 30–33 °C and humidity ~65%). Soybean seeds (*Glycine max* L. Giza 21, Giza 22, Giza 35 Giza 82, Giza 83 and Giza 111) were obtained from the Agricultural Research Centre, Egypt. Seeds of the same size and colour were selected and the surface sterilized in distilled water and left to dry at room temperature (25 °C). Seven seeds/pot (30cm diameter and 40cm depth) were sown containing equal amounts of soil. The soil characteristics were: sandy loam in texture, sand 80%, silt 15.9%, clay 4.1%, pH 7.7, EC 0.4 dSm⁻¹ and organic matter 0.60%. Rhizobial inoculants were applied as peat slurry containing 10⁷ *Rhizobium*/g. Seven seeds were sown per pot and were thinned to four after two weeks from planting. Five pots for each treatment were used as replicates. Soybean plants were grown with normal water supply until 25th day from sowing and then were divided into two groups: (i) the first group receives 80% maximum holding capacity (well-watered), (ii) the second group receives 25% of maximum holding capacity (drought). After they were 55 days old, five plants were randomly chosen from each treatment and used to determine morphological criteria. Shoots were used for the biochemical analyses.

Biochemical Analyses

Determination of Photosynthetic Pigments

Carotenoids and total pigments were determined in soybean leaves. The spectrophotometric method recommended by Vernon and Seely (1966) was used.

Determination of Soluble Sugars and Starch Content

Soluble sugars and starch content in shoots of soybean were determined according to Dubois et al. (1956) using phenol

sulfuric acid reagent. The absorbance of spectrophotometer was determined at 485 nm.

Determination of Proline Content

Free proline in shoots of soybean plants was determined using the method of Bates et al. (1973) and then the absorbance was determined by Uv-visible spectrophotometer at 520 nm.

Determination of Glycine Betaine

Glycine betaine in shoots of soybean plants was determined according to Grieve and Grattan (1983). The absorbance was measured at 365 nm.

Determination of Lipid Components

Lipids were extracted three times from air dried shoots of soybean plants according to Navari-Izzo et al. (1989). Glycolipids were determined by measuring monosaccharide content by the phenol sulfuric acid reagent (Hodge and Hofreiter 1962). Phospholipids were determined according to Woods and Mellon (1941).

Determination of Cell Wall Fraction

Cell wall fraction was determined according to Bishop et al. (1958) with modification by Dever et al. (1968).

Genetic Relationships Among Soybean Cultivars

IRAP and iPBS techniques were the molecular markers of choice in the present study to reveal differences among soybean cultivars and to detect molecular markers related to water stress. A number of primers were tested for suitable amplifications. Five IRAP and three iPBS primers designed based on LTR-sequences were informative, their sequence GC:AT ratios and suitable annealing temperature are listed in Table 1. Genomic DNA was isolated from leaves of the six soybean cultivars using CTAB methods (Kidwell and Osborn 1992). Amplification was carried out in 20 µl reaction mixture containing 5 ng Templat DNA, 2 µl 10x Dream *Taq* buffer, 0.4 µl dNTPs, 2 µl IRAP primer, 0.2 µl Dream *Taq* polymerase (5 U/µl). The amplification conditions were one initial denaturation at 95 °C for 3 min followed by 35 cycles each consists of 95 °C for 20s, 60 °C for 30s, 72 °C for 90s, followed by a final extension at 72 °C for five minutes. 10 µl IRAP-PCR products were loaded with 1% agarose gel in 1xTHE buffer, stained with ethidium bromide and fractionated at constant voltage of 40 V for 16h. Both monomorphic and polymorphic bands were scored visually.

Table 1 List of retrotransposon-based primers tested against soybean cultivars

Primer type	Primer code	Primer sequence	GC:AT ratio	T _m (°C)
iPBS	iPBS-2219	5'gaactatgccgatacca'3	44.40	51.40
	iPBS-2394	5'gagcctaggcca'3	66.70	40.00
	iPBS-2399	5'aaactggcaacggcgcca'3	61.10	58.20
IRAP	IRAP-4341	5'gtcccacagcttgggcaacag'3	61.90	63.70
	IRAP-4361	5'gtcgaccttcccggcatgaa'3	60.00	61.40
	IRAP-4364	5'atagcggcgagatgcatgct'3	55.00	59.40
	IRAP-4368	5'gatgttgcggtggatgtgtgtaagact'3	50.00	66.60
	IRAP-4377	5'cgtacccttaaggatcaaaacc'3	44.00	61.30

Table 2 Effect of water stress on morphological criteria of different soybean cultivars (55 days old)

Cultivars	Treatment	Shoot length (cm)	Root length (cm)	FW of shoots (g)	DW of shoots (g)	FW of roots (g)	DW of roots (g)
Giza 21	Control	27.7d ^a	14.3bc	4.16cd	2.04ab	1.12b	0.52d
	Drought	22.7e	11.5d	2.70e	1.66de	1.09c	0.38e
Giza 111	Control	28.8d	15.5bc	4.21cd	1.99ab	1.10b	0.58cd
	Drought	21.0e	12.0d	2.24e	1.69de	1.07c	0.36e
Giza 35	Control	36.3b	15.3bc	5.87ab	1.72cde	1.13b	0.79bc
	Drought	29.3d	12.0d	4.11cd	1.67de	1.08c	0.53d
Giza 82	Control	31.5c	16.0bc	5.43ab	1.76cd	1.12b	0.64bc
	Drought	28.2d	12.0d	3.95d	1.71cde	1.08c	0.50d
Giza 22	Control	40.3a	16.5bc	6.79a	2.30a	1.14b	0.89b
	Drought	37.8ab	20.5a	5.69ab	2.05ab	1.20a	0.94a
Giza 83	Control	36.3b	13.0cd	5.31bc	1.98bc	1.14b	0.85b
	Drought	34.8bc	17.0ab	4.44cd	1.75cd	1.19a	0.96a

FW fresh weight, DW dry weight

^aMean values ($n=5$) in the same column for each trait followed by the same lower-case letter are insignificantly different according to Duncan's multiple range test at $P \leq 0.05$

Statistical Analysis

The results were analyzed according to Gomez and Gomez (1984). The treatments were compared using Duncan Multiple Range Test (Duncan 1955) using MSTAT-c computer software package 1990.

Results

Changes on Morphological Criteria

The results in Table 2 show that the six soybean cultivars varied in their response to water stress. Water stress significantly decreased shoot and root lengths in all soybean cultivars except in Giza 22 and Giza 83 which showed insignificant effects in shoot length and significant increase in root length compared with well watered plants. In addition, fresh weights of shoots and roots were significantly decreased in stressed plants of all soybean cultivars except Giza 22 and Giza 83 which showed insignificant effects in

shoots fresh weight and significant increase in roots fresh weight compared with well watered plants.

Moreover, no significant effect in shoots dry weight of stressed plants was observed in all soybean cultivars except Giza 21 and Giza 111 which showed a significant decrease compared with well watered plants. Roots dry weight was significantly decreased in all soybean cultivars but significantly increased in cultivars Giza 22 and Giza 83 when compared with control plants.

Changes in Biochemical Components

Data in Table 3 show that total photosynthetic pigments content was significantly decreased in some soybean cultivars (Giza 21, Giza 111, Giza 35, and Giza 82) but significant increased in the two genotypes Giza 22 and Giza 83 compared with control plants. In addition, water stress caused insignificant difference in carotenoids content in leaves of all soybean cultivars except in Giza 22 and Giza 83 cultivars which showed significant increases compared with well watered plants. Starch content in shoots of all soybean cultivars was significantly decreased

Table 3 Effect of water stress on biochemical components of different soybean cultivars (55 days old)

Cultivars	Treatment	Total photosynthetic pigments (mg/g)	Carotenoid (mg/g)	Starch (mg/g)	Total soluble sugars (mg/g)	Proline (mg/g)	Glycine betaine Umol/g (FW)
Giza 21	Control	0.69d ^a	0.15bcd	31.9bc	23.1e	0.39de	2.83g
	Drought	0.58e	0.17bcd	24.9de	28.3bc	0.59bc	3.43ef
Giza 111	Control	0.75d	0.13d	35.0bcd	24.0de	0.31e	2.23g
	Drought	0.51e	0.15bcd	19.9e	28.6bc	0.53bc	3.37ef
Giza 35	Control	0.82c	0.12d	37.0ab	26.1cd	0.36e	2.57g
	Drought	0.74d	0.13cd	26.3cde	29.3b	0.50cd	4.03cde
Giza 82	Control	0.83c	0.17bcd	41.7ab	24.2de	0.35e	3.43ef
	Drought	0.75d	0.19abc	26.8cde	29.5b	0.50d	4.20bcd
Giza 22	Control	0.95bc	0.15cd	48.3a	29.7b	0.61bc	3.77de
	Drought	1.18a	0.21ab	31.2b–e	34.9a	0.78a	4.87ab
Giza 83	Control	0.88cd	0.15cd	42.0ab	26.0cd	0.56c	4.53bc
	Drought	1.12ab	0.23a	27.4cde	29.9b	0.69ab	5.57a

^aMean values ($n=5$) in the same column for each trait followed by the same lower-case letter are nonsignificantly different according to Duncan's multiple range test at $P \leq 0.05$

Table 4 Effect of water stress on lipid components of different soybean cultivars (55 days old)

Cultivars	Treatment	Total lipids mg/g	Glycolipids mg/g	Phospholipids mg/g
Giza 21	Control	21.0gh ^a	28.2cd	1.33b
	Drought	23.9f	23.7e	0.73c
Giza 111	Control	19.3h	28.7cd	0.77c
	Drought	22.3fg	22.6e	0.40c
Giza 35	Control	19.3h	29.0bc	1.30b
	Drought	26.7de	26.5cde	0.77c
Giza 82	Control	24.8ef	28.3cd	2.57a
	Drought	28.9cd	23.7e	1.33b
Giza 22	Control	44.8b	29.5bc	0.63c
	Drought	47.6a	33.3a	1.47b
Giza 83	Control	22.0fgh	25.0de	1.67b
	Drought	30.3c	32.2ab	2.33a

^aMean values ($n=5$) in the same column for each trait followed by the same lower-case letter are nonsignificantly different according to Duncan's multiple range test at $P \leq 0.05$

compared to well watered plants. Compared to control plants, a drastic increase in total soluble sugars content was observed in shoots of all soybean cultivars. The most pronounced increase was recorded in soybean cultivars Giza 22 and Giza 83. Proline and glycine betaine content was significantly increased in shoots of all soybean cultivars compared to control plants. The most pronounced increase was recorded in cultivars Giza 22 and Giza 83.

Changes in Lipid Components

Total lipids content was significantly increased in shoots of all soybean plants compared to well watered plants. In addition, phospholipids and glycolipids content was significantly decreased in shoots of all soybean cultivars except

in the two cultivars Giza 22 and Giza 83 which showed significant increases compared with control plants (Table 4).

Changes in Cell Wall Components

Drought stress caused a significant decrease in cell wall components (pectin, cellulose and lignin) of shoots of all stressed soybean cultivars except the two cultivars Giza 22 and Giza 83 which showed significant increases in lignin content compared to unstressed plants. Hemicellulose contents were significantly increased under water stress (Table 5).

According to these results we may consider soybean cultivars Giza 22 and Giza 83 as tolerant cultivars and Giza 21 and Giza 111 as sensitive cultivars when exposed to water stress.

Table 5 Effect of water stress on cell wall components of different soybean cultivars (55 days old)

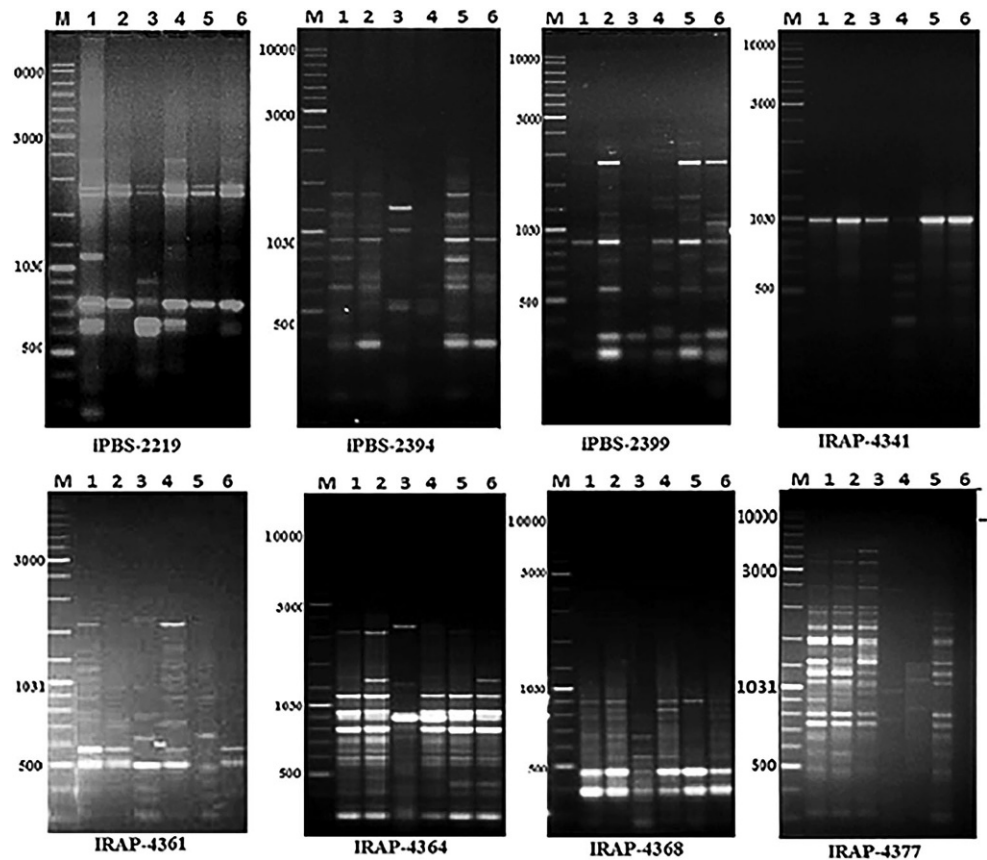
Cultivars	Treatment	Pectin mg/g	Cellulose mg/g	Hemicelluloses mg/g	Lignin mg/g
Giza 21	Control	24.5def ^a	39.3a	17.4f	96.4bcd
	Drought	17.9g	29.7bc	31.2ab	72.7ef
Giza 111	Control	28.1bc	25.0c	15.0f	105.9b
	Drought	21.8f	13.5d	25.3bcd	85.9de
Giza 35	Control	30.5ab	38.1ab	20.1def	89.3cd
	Drought	25.0c-f	25.9c	32.7a	64.7f
Giza 82	Control	31.6a	32.4abc	16.3f	92.7bcd
	Drought	25.4cde	24.1c	28.2abc	68.5f
Giza 22	Control	28.0bcd	36.5ab	24.1cde	87.4d
	Drought	22.9ef	23.0c	34.1a	124.3a
Giza 83	Control	28.7bcd	32.7b	18.5ef	83.8de
	Drought	22.5ef	23.7c	33.8a	103.0bc

^aMean values ($n = 5$) in the same column for each trait followed by the same lower-case letter are nonsignificantly different according to Duncan's multiple range test at $P \leq 0.05$

Table 6 Polymorphism estimates detected for the six soybean cultivars tested by three iPBS and five IRAP retroelements-based primers

Primers	Total No of bands	Polymorphic bands	Polymorphism %	Unique bands No. of bands	Unique bands			
					Positive Band size (bp)	Cultivar	Negative Band size (bp)	Cultivar
iPBS-2394	18	17	94	7	250	G-83	200	G-83
					500	G-111	620	G-83
					1030	G-83	–	–
					1250	G-83	–	–
					1700	G-22	–	–
iPBS-2399	20	17	80	4	150	G-21	–	–
					360	G-82	–	–
					600	G-22	–	–
					1070	G-21	–	–
					2100	G-82	–	–
iPBS-2219	20	17	80	2	230	G-111	–	–
					900	G-83	–	–
					–	–	–	–
IRAP-4341	12	10	92	1	250	G-83	–	–
IRAP-4361	15	15	100	4	350	G-82	400	G-82
					1650	G-83	500	G-83
IRAP-4364	21	13	65	8	500	G-82	150	G-21
					1230	G-82	640	G-82
					1310	G-21	940	G-82
					–	–	1100	G-82
					–	–	2100	G-82
IRAP-4368	14	9	65	6	500	G-82	600	G-35
					1030	G-22	680	G-35
					–	–	700	G-35
					–	–	900	G22
IRAP-4377	22	20	91	3	1030	G-82	1050	G-82
					2700	G-83	–	–
Total	127	108	84	35	–	–	–	–

Fig. 1 Electrophoretic banding patterns for the six studied soybean cultivars tested against eight iPBS and IRAP primer. (1 Giza-21, 2 Giza-22, 3 Giza-35, 4 Giza-82, 5 Giza-83, 6 Giza-111)



Molecular Tagging of Soybean Cultivars Using Retrotransposon-Based Markers

Five IRAP and three iPBS primers were applied to six soybean cultivars (Giza 21, Giza 22, Giza 35, Giza 82, Giza 83 and Giza 111) to determine the genetic difference. A summary of the accumulated results over the eight tested primers is tabulated in Table 6 and Fig. 1. A total number of 127 bands were produced; amongst, 19 were monomorphic and the remaining 108 were polymorphic. Primer IRAP-4377 gave the highest number of polymorphic bands (20 bands) while primers IRAP-4368 showed the lowest number (9 bands). The highest percentage of polymorphic bands was produced by primer IRAP4361 (100%) and the lowest was produced by both primers IRAP-4368 and IRAP-4364 (65% each). In addition, the eight primers produced 35 unique bands that specifically identified their respective genotypes; amongst 21 were positive and 14 were negative bands; primer IRAP-4364 gave the highest number of unique bands/primer (8 bands).

Developing Molecular Markers Related to Water Stress Tolerance in Soybean Cultivars

The reported data showed that the two cultivars Giza 22 and Giza 83 are the most tolerant cultivars and Giza 21 and Giza 111 are the most sensitive ones. The electropherograms of the tolerant cultivars vs. sensitive ones were used to detect molecular markers. Electrophoretic banding patterns resulted from tested IRAP and iPBS primers were examined for markers related to drought tolerance in soybean. Nine bands co-segregated with either tolerant or sensitive cultivars and marked them, these bands resulted from iPBS-2389 and IRAP-4341, IRAP-4368 and IRAP-4377, the resulted band size of different markers are presented in Table 7. Primers IRAP-4361 and IRAP-4377 gave rise to three negative markers for drought tolerance, whereas iPBS-2399, IRAP-4341 and IRAP-4368 revealed one positive marker each that correlated to tolerant cultivars.

Discussion

Water stress caused a significant decrease in plant growth criteria of soybean plants which is similar to results reported by Abass and Mohamed (2011). In a previous study

Table 7 Developed drought tolerance molecular markers generated by different iPBS and IRAP primers

Primer type	Primer name	Band size (bp)	Tolerant cultivars	Sensitive cultivars
iPBS	2399	750	++	--
IRAP	4341	580	++	--
		280	--	++
		1100	--	++
		2800	--	++
	4368	590	++	--
		4377	480	--
	4377	580	--	++
		650	--	++

on Egyptian soybean cultivars, Mohamed and Akladios (2014) found that all growth parameters of soybean plants cv. Giza 22 and Giza 111 were significantly decreased under drought stress conditions and the cultivar Giza 22 was found to be more tolerant to drought stress than Giza 111. Also, Mohamed and Latif (2017) reported that drought stress caused reduction in the morphological criteria of soybean cultivars Giza 22 and Giza 35. Drought stress affects cell growth due to the reduction in turgor pressure and the soil water potential (Munns 2002). Root growth is less affected than shoot growth (Hsiao 1973) because the osmotic adjustment in the roots is more efficient than in the shoots (Ober and Sharp 2007). In addition, Jaleel et al. (2008) found that root growth in *Catharanthus roseus* increased under water stress. Shoot growth decreasing under drought stress might be due to decrease in cell elongation, cell turgor, cell volume and cell growth (Banon et al. 2006).

Total photosynthetic pigments decreased under water stress. These results are similar to Mohamed and Akladios (2014) who found that chlorophyll contents in soybean plants cv. Giza 22 and Giza 111 were significantly decreased under drought stress. The reduction of chlorophyll may be due to the degradation of chlorophyll enzymes (Sabater and Rodriguez 1978), the inhibition in the synthesis of photosynthetic pigments (Murkute et al., 2006), and the inhibition in the uptake of Mg (Sheng et al. 2008). Total photosynthetic pigments increased under water stress in the two soybean cultivars Giza 22 and Giza 83, these increases may be due to increase in leaf area (Benjamin and Nielsen 2006) which may help in the reduction of water loss and the increase in photosynthesis. Also, water stress caused the accumulation in carotenoids content in the leaves of soybean cultivars Giza 22 and Giza 83. Carotenoids are responsible for scavenging of singlet oxygen (Knox and Dodge 1985) and the decrease in carotenoids under water stress might also have contributed to the increased ROS, which further oxidized the photosynthetic pigments.

Drought stress stimulated soluble sugars and decreased starch content in shoots of all soybean cultivars. The increment in soluble sugars under drought stress was also doc-

umented by Abass and Mohamed (2011) in common bean plants. The increase in sugar concentration may be a result from the degradation of starch (Fischer and Höll 1991). Starch may play an important role in accumulation of soluble sugars in cells. Mohammadkhani and Heidari (2008) found that drought stress decreased starch content in maize shoots and roots. Under drought stress, the hydroxyl groups of sugars may replace for water to protect the interactions between membranes and proteins. Proteins and membranes of cells interact with hydrogen bond in sugars and prevent the denaturation of protein (Leopold et al. 1994).

Proline and glycine betaine accumulated under water stress in all soybean cultivars but the most pronounced increase was reported in the two cultivars Giza 22 and Giza 83. These differences may be due to the regulation of proline dehydrogenase (PDH) which is considered as proline degrading enzyme under a variety of stress conditions (Szekely et al. 2008). The increase of proline in plants may be due to an increase in the synthesis of proline enzymes and a decrease in the degradation of proline (Delauney et al. 1993). Proline not only acts as an osmolyte but also helps in the stability of membranes and proteins and in scavenging free radicals under stress conditions (Iqbal 2009).

Glycine betaine is an osmoprotectant which plays an important role in the osmotic adjustment (Munns 2002), protecting the proteins from degradation (Bohnert and Jensen 1996), protecting the structure of membrane (Crowe et al. 1992), protecting the photosynthetic mechanism (Sakamoto and Murata 2002) and scavenging free radicals (Smirnoff and Cumbes 1989). Several reports found that glycine betaine was accumulated under drought stress in drought tolerant species as drought sensitive (Rhodes et al. 1987).

Water stress caused variable changes in lipid fractions of soybean plants. These results are similar to Al-Hakimi (2006) who found that drought stress caused a significant increase in the total lipid and glycolipid contents of shoots and roots of soybean plants but the phospholipids content was significantly decreased. The phospholipids content increased in the tolerant cultivars of soybean (Giza 22 and Giza 83). The increase in the plant growth in these cultivars

is attributed to increase in phospholipids content (Xue et al. 2007). On the other hand, Al-Hakimi (2003) reported that phospholipid and glycolipid contents in sunflower plants decreased with decreasing soil moisture content. Glycolipids are the most important compounds of photosynthesis and play an important role in the electron transport system and in the stabilization of thylakoids (Murphy and Woodrow 1983). The decrease in glycolipid contents may be due to the thinning of the chloroplast membranes and the reduction in the electron transport (Navari-Izzo et al. 1993).

Cell wall metabolism is an important component in cell division, elongation and plant growth (Al-Hakimi 2008). Al-Hakimi (2006) found that drought stress caused a significant decrease in the contents of cell wall components of shoots and roots of stressed soybean plants but hemicellulose contents of either shoots and roots were increased under drought stress.

Genetic diversity and relation between or within different individuals, species and populations has become an initial goal that might help breeders in constructing their crossing scheme. Several DNA techniques have been developed to determine genetic variability in different plants (Nybom et al. 2014). In the present investigation, retrotransposon-based techniques were used for tagging soybean cultivars. The technique successfully identified each cultivar with a specific banding pattern when tested against eight IRAP or iPBS primers. Different DNA-based marker technologies have been developed to study polymorphism using subsets of total genomic DNA (Kalendar and Schulman 2006). IRAP method has been exploited to study biodiversity in many plant genera and to determine genomic polymorphism (Kalendar et al. 2010, 2011).

Molecular genetic markers became a corner stone in plant breeding programs, where they help in choosing parents and detecting specific traits throughout a breeding program. Developing molecular genetic markers related to economically valuable traits is fundamental. In this study, several markers related either to tolerance or sensitivity to water stress in soybean were detected. Stress was found to activate the expression of plant transposable elements (Grandbastien et al. 2005; Latif and Mohamed 2016). Retro-elements were suggested as an important creative force in the genome evolution; driving processes such as mutation, recombination, genome expansion and adaptation of an organism to changing environmental conditions (Gogvadze and Buzdin 2009). Under stress, increased levels of TE transposition occur (Oliver and Greene 2009), this might accelerate the rate of genome restructuring and promote potentially useful genetic variability, it is expected that some progeny inherit a favorable adaptation traits to enable survival in the face of biotic or environmental challenges.

Conclusion

The results conclude that the soybean cultivars Giza 22 and 83 are more drought tolerant than the other cultivars, while Giza 21 and Giza 111 were the most sensitives. iPBS and IRAP techniques were used to fingerprint the six soybean cultivars using a set of eight primers. The technique successfully tagged each cultivar with specific bands and also successfully detected molecular genetic markers related to drought tolerance in soybean. We suggest using the tolerant cultivars Giza 22 and Giza 83 in areas which suffer from drought stress.

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Conflict of interest H. I. Mohamed, S. A. Akladios and N. A. Ashry declare that they have no competing interests.

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