Adv. Hort. Sci., 2016 30(1): 13-21 DOI: 10.13128/ahs-18697

DNA changes in cotton (*Gossypium hirsutum* L.) under salt stress as revealed by RAPD marker

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Key words: cotton, genomic template stability, RAPD, salt stress.

Abstract: Random amplified polymorphic DNA (RAPD) analysis was applied to evaluate DNA changes among four upland cotton (*Gossypium hirsutum* L.) varieties [Niab 78 (N78), Deir-Ezzor 22 (DE22), Deltapine 50 (DP50) and Aleppo 118 (A118)] grown under non-saline conditions (control) and salt stress (200 mM NaCl) for seven weeks. Changes in RAPD profiles were measured as genomic template stability (GTS%). The highest estimated GTS% value was recorded for the two sensitive varieties, DP50 (79.1%) followed by A118 (58.2%); whereas, the lowest value was recorded for the two other tolerant varieties DE22 (36.7%) followed by N78 (26.4%). Based upon the data presented, RAPD marker could be used as potential tool for early identification of cotton tolerance to salt stress.

1. Introduction

Cotton is an economically important plant grown world-wide as a principal source of staple fiber and vegetable oil. A great deal of effort has been made to improve cotton cultivation and characteristics by breeders. Cotton is one of the major fiber crops in Syria, with a cultivated area of 125,000 ha, a production of 470,000 t of seed cotton, and lint production at 160,000 t. Yarn spinning capacity is estimated at 180,000 t (USDA, 2011). Salinity tolerance is a complex trait that involves physiological, biochemical, cellular, and genetic strategies. At present, out of 1.5 billion ha of cultivated land around the world, about 77 million ha (5%) is affected by excess salt content (Moradi *et al*., 2011). There is evidence that high salt concentrations cause an imbalance of the cellular ions resulting in ion toxicity and osmotic stress, leading to the generation of reactive oxygen species (ROS) which alter cellular metabolism causing lipid peroxidation, protein denaturing, and DNA mutation (Dat *et al*., 2000; Davenport *et al*., 2003; Implay, 2003). Moreover, salt stress causes nuclear deformation and subsequent nuclear degradation (Katsuhara and Kawaski, 1996). Structural changes of nuclei caused by salt stress have been previously reported as well (Werker *et al*., 1983).

At present, there are several methods (physiological, biochemical, and molecular) available for detecting different kinds of DNA damage but with some limitations. Recently, molecular markers have been successfully applied to detect DNA damage induced by different abiotic stresses, particularly salinity. Among others, RAPD technique has been well documented as a sensitive means of detecting DNA damage and shows potential as a reliable and reproducible assay for the detection of DNA fragmentation and chromosomal mutations (Citterio *et al*., 2002).

The RAPD marker has been extensively applied for salinity tolerance screening in plant breeding programs, such as in date palm (*Phoenix dactylifera* L.) (Kurup *et al*., 2009), aquatic plants *Hydrilla verticillata* and *Ceratophyllum demersum* (Gupta and Sarin, 2009), in *Euplotes vannus* (Protozoa, Ciliophora) (Zhou *et al*., 2011), and in *Acacia Senegal* (Daffalla *et al*., 2011); in cotton (Dojan *et al*., 2012) and in fish full-sib Nile tilapia (*Oreochromis niloticus*), Blue tilapia (*Oreochromis aureus*) and their diallel interspecific hybridization (El-Zaeem, 2012); and recently, also in soybean (*Glycine max* L.) (Khan *et al*., 2013).

RAPD bands can be scored for genomic template stability (GTS) evaluation to detect various types of DNA damage and mutations (rearrangement, point mutations, small insertions or deletions of DNA and polyploidy changes) which suggests that RAPD bands may potentially form the basis of novel biomarker

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Received for publication 28 May 2015
Accepted for publication 20 December 2015

assays for the detection of DNA damage and mutations in the cells of bacteria, plants, and animals (Savva, 1998; Atienzar *et al*., 1999; Tanee *et al*., 2012). It is well documented that genomic template stability ratios (GTS) were calculated. GTS implies qualitative measure reflecting changes in RAPD profiles. Changes in RAPD and profiles were expressed as reductions in GTS in relation to profiles obtained from control samples (Gupta and Sarin, 2009; Aly, 2012; Dojan *et al*., 2012; Tanee *et al*., 2012).

Therefore, this investigation aimed to detect DNA changes induced by NaCl application by monitoring the RAPD profiles of control and stressed plants in four upland cotton (*Gossypium hirsutum* L.) varieties grown in Syria.

2. Materials and Methods

Plant materials and growth conditions

Two local varieties were selected on the basis of their wide-ranging tolerance towards salinity: Deir-Ezzor 22 (DE22) as salt-tolerant and Aleppo 118 (A118) as salt-sensitive variety (Saleh, 2011). These two varieties were compared with two introduced cotton varieties, Niab 78 (N78) (known as salt-tolerant) and Deltapine 50 (DP50) (known as salt-sensitive) under 0 and 200 mM NaCl for seven weeks. Seeds of upland cotton (*G. hirsutum* L.) were provided by the General Commission for Scientific Agricultural Research of Syria (GCSAR).

Seeds were soaked in distilled water for 24 h and then planted in pots filled with a 1:2 (v/v) mixture of perlite:peatmoss. Germination was carried out in a greenhouse at 18°C, 12 h photoperiod, and relative humidity of 80%. Seedlings were allowed to grow in a greenhouse under controlled conditions (temperature 25°C, 12 h photoperiod, and relative humidity 80%). Seedlings were irrigated with tap water for one week before the initiation of NaCl treatments. Salt stress application was carried out by adding NaCl (200 mM) to the water. Plants were irrigated twice a week with water with or without salt. All solutions were changed twice a week. The same environmental conditions were maintained during the experiment. The experiment (five replicates/treatment) was carried out in the greenhouse for seven weeks.

Genomic DNA extraction

Plant genomic DNA was extracted from young leaves (bulk of five plants/variety) including the control and stressed plants (200 mM NaCl) using CTAB (cetyltrimethylammonium bromide) protocols described by Doyle and Doyle (1987) with minor modifications.

Leaf tissue (150 mg) was ground in liquid nitrogen and the powder was transferred to a 2 ml Eppendorf tube, mixed with 900 µl of extraction buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 0.0018 ml β-mercaptoethanol, 2% CTAB), and incubated at 65°C for 20 min. One volume of a chloroform:isoamyl alcohol mix (24:1, v/v) was added and centrifuged at 12,000 *g* for 10 min at 4°C. The aqueous phase was transferred to a fresh tube, and the DNA was precipitated with an equal volume of cold isopropanol and kept at -20°C for 10 min. It was then centrifuged at 12,000 *g* for 10 min at 4°C, the supernatant was discarded, and DNA was spooled out and washed with 1 M ammonium acetate and 100% ethanol. The cleaned DNA pellet was air dried and dissolved in 100 µl of 0.1X TE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Finally 5 µl of RNase (10 mg ml^{-1}) were added and incubation for 30 min at 37°C was applied. DNA concentration was quantified by DNA Spectrophotometer at 260/280 nm and adjusted to final concentration of 10 ng μl^{-1}. DNA was stored at -80°C until needed.

RAPD marker

Twenty-three RAPD primers from Operon Technologies Inc. (USA) and three primers from the University of British Columbia were tested to detect DNA changes in stressed plants, and their respective controls, for four cotton varieties.

RAPD marker was performed as described by Williams *et al*. (1990) with a minor modification. PCR amplification reaction was carried out in 25 µl reaction volume containing 1xPCR buffer, 2 mM $MgCl_2$, 0.25 mM dNTPs, 25 pmol primer, 1.5 U of Taq DNA polymerase and 30 ng template DNA. PCR amplification was performed in a T-gradient thermal cycler (Bio-Rad; T Gradient) programmed to fulfill 42 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step (1 min at 94°C), an annealing step (2 min at 35°C), and an extension step (for 2 min at 72°C). A final extension cycle was performed for 7 min at 72°C. The PCR products were separated on a 1.5% ethidium bromide-stained agarose gel (Bio-Rad) in 0.5xTBE buffer. Electrophoresis was performed for 3 h at 85V and visualized with a UV transilluminator. Band sizes were determined by comparison with a 1 kb DNA Ladder Mix, ready for use (Fermentas).

RAPD data analysis and Genomic Template Stability (GTS) estimations

DNA changes induced in treated plants compared to their respective controls were screened by RAPD assay. The polymorphism was calculated in relation to the appearance of new bands and disappearance of bands in treated plants, compared to control band patterns.

Genomic template stability (GTS%) was calculated as follows:

$$GST\% = (1- a/n) \times 100$$

where (a) is the average number of changes in the DNA profile and (n) the number of total bands in the control. Polymorphism observed in RAPD profiles included disappearance of a normal band and appearance of a new band in comparison to the cotrol RAPD profiles (Atienzar *et al*., 2002).

3. Results

A set of 26 random10-mer primers was used to detect the DNA changes among four cotton varieties under salt stress application compared to their respective controls. RAPD fragment sizes ranged from 200 to 3000 bp. The generated band characteristics for the four varieties (including control and stressed plants) are summarized in Table 1. The total number of characteristic bands (common observed bands in control and stressed plants for the four examined varieties) was 29. The amplification products produced from 26 RAPD primers are listed in Table 2 in terms of loss or appearance of new bands (number and size) under salt stress compared to their respective controls for each variety separately. The RAPD analysis carried out on the four cotton varieties produced a number of distinct fragments which varied according to each tested primer. Twelve of the 26 RAPD primers (OPA02, OPB05, OPC08, OPD08, OPD20, OPJ07, OPK13, OPK17, OPR12, OPY10, UBC132 and UBC159) produced polymorphic bands under saline conditions for the four tested varieties (Table 2).

Figure 1 shows the amplification products using OPA02, OPB17 and OPY10 RAPD primers with template DNA from the four varieties under control and saline conditions (200 mM NaCl).

Changes in DNA pattern induced by NaCl treatment in the four tested cotton varieties were detected based on estimated genomic template stability (GTS%) (Table 3). In this respect, it was found that the highest GTS% was recorded in salt-sensitive cotton, whereas the lowest was found among salt-tolerant varieties (Table 3).

Table 1 - Characteristic bands identified for the four tested cotton varieties using 26 RAPD primers

Primer name	Sequence (5' - 3')	Characteristic bands (number and size)
OPA02	TGCCGAGCTG	(1) 950
OPA04	AATCGGGCTG	(3) 250, 550 & 1600
OPB05	TGCGCCCTTC	(1) 1500
OPB17	AGGGAACGAG	0
OPC08	TGGACCGGTG	(2) 500 & 800
OPC13	AAGCCTCGTC	(1) 450
OPD08	GTGTGCCCCA	(1) 1200
OPD20	GGTCTACACC	0
OPE07	AGATGCAGCC	(3) 550, 650 & 800
OPE15	ACGCACAACC	0
OPG11	TGCCCGTCGT	(3) 700, 1200 & 2100
OPJ01	CCCGGCATAA	0
OPJ07	CCTCTCGACA	0
OPK12	TGGCCCTCAC	0
OPK13	GGTTGTACCC	0
OPK17	CCCAGCTGTG	0
OPQ01	GGGACGATGG	(3) 450, 600 & 1000
OPQ18	AGGCTGGGTG	(3) 650, 750 & 1200
OPR09	TGAGCACGAG	(1) 250
OPR12	ACAGGTGCGT	(1) 300
OPT18	GATGCCAGAC	(2) 1200 & 1350
OPW17	GTCCTGGGTT	(1) 2000
OPY10	CAAACGTGGG	(1) 650
UBC132	AGGGATCTCC	0
UBC159	GAGCCCGTAG	(1) 1600
UBC702	GGGAGAAGGG	(1) 400
Total		29

4. Discussion and Conclusions

Detection of DNA changes in cotton via salt stress was assessed using RAPD marker system. As shown in Table 1, characteristic bands ranged between 0 (OPB17, OPD20, OPE15, OPJ01, OPJ07, OPK12, OPK13, OPK17 and UBC132) and 2 (OPC08 and OPT18), whereas the highest number (three) was yielded by OPA04, OPE07, OPG11, OPQ01 and OPQ18 RAPD primers (Table 1). Our findings reveal that nine out of the 26 tested RAPD primers generat-

Table 2 - Markers identified by 26 RAPD primers for the four tested cotton varieties under salt stress compared to their respective controls. DNA changes induced by NaCl treatment using RAPD marker as described by loss or appearance of new bands (number and size) under salt stress compared to their respective controls for each variety separately

Primer name	N78		DE22		DP50		A118		Total polymorphic bands
	C	T	C	T	C	T	C	T	
OPA02	9		8		9		9		
-		(4) 400, 650, 1800 & 2500		(3) 650, 800 & 2500		(2) 1800 & 2500		(2) 1800 & 2500	16
+		(2) 600 & 1500		(3) 500, 600 & 900		ND		ND	
OPA04	3		3		3		3		
-		ND		ND		ND		ND	5
+		(3) 400, 500 & 1000		(2) 400 & 500		ND		ND	
OPB05	5		5		4		5		
-		(4) 450, 650, 1000 & 2000		(3) 450, 1000 & 2000		(1) 900		(1) 2000	14
+		(3) 500, 700 & 1100		(2) 500 & 1100		ND		ND	
OPB17	7		9		8		8		
-		(5) 600, 700, 1100, 1200 & 1800		(4) 350, 800, 1500 & 1800		ND		(5) 400, 600, 800, 1000 & 1800	20
+		(2) 400 & 650		(2) 700 & 1100		ND		(2) 450 & 750	
OPC08	4		5		5		4		
-		(2) 1850 & 1900		(1) 1200		(1) 1850		ND	10
+		(2) 300 & 900		(2) 1350 & 1900		ND		(2) 1850 & 1900	
OPC13	6		6		5		5		
-		(1) 1000		(2) 500 & 1000		ND		ND	12
+		(5) 300, 800, 900, 1100 & 1350		(4) 300, 1100, 1350 & 1500		ND		ND	
OPD08	4		3		4		3		
-		(2) 450 & 700		(2) 450 & 750		(1) 900		(2) 450 & 800	15
+		(2) 650 & 1350		(4) 500, 600, 800 & 1350		(1) 800		(1) 600	
OPD20	6		4		2		2		
-		(4) 650, 850, 1100 & 1850		(3) 700, 900 & 1350		(1) 800		(1) 300	13
+		(1) 1350		(1) 800		(1) 300		(1) 200	
OPE07	8		8		8		8		
-		(5) 1000, 1300, 1800, 2300 & 3000		(1) 1800		ND		ND	7
+		(1) 1100		ND		ND		ND	
OPE15	5		4		6		6		
-		(1) 200		(2) 800 & 1800		ND		ND	8
+		(4) 800,1300, 1500 & 1800		(1)1500		ND		ND	
OPG11	3		3		3		3		
-		ND		ND		ND		ND	2
+		(1) 600		(1) 600		ND		ND	
OPJ01	7		6		7		7		
-		(5) 400, 600, 850, 1200 & 2000		(3) 400, 600 & 850		ND		(2) 600 & 2000	20
+		(4) 350, 500, 950 & 1800		(4) 350, 500, 950 & 1800		ND		(2) 500 & 800	
OPJ07	3		4		2		3		
-		(3) 500, 800 & 1600		(4) 300, 500, 700 & 1600		(2) 550 & 1350		(1) 1100	21
+		(3) 700, 900 & 1200		(3) 550, 1000 & 1200		(2) 450 & 1600		(3) 450, 550 & 650	

T(-) loss bands, (+) gains bands, (ND) no differences.

to be continued

Table 2 (continued)

Primer name	N78		DE22		DP50		A118		Total polymorphic bands
	C	T	C	T	C	T	C	T	
OPK12	3		3		2		2		
-		(3) 700, 900 & 1350		(3) 800, 900 & 1350		ND		ND	11
+		(3) 850, 950 & 1100		(2) 500 & 1000		ND		ND	
OPK13	4		4		3		3		
-		(2) 650 & 1100		(2) 350 & 1100		(1) 200		(1) 490	20
+		(4) 450, 750, 1200 & 1350		(4) 500, 750, 1200 & 1350		(3) 300, 500 & 1100		(3) 700, 1200 & 1350	
OPK17	8		4		4		6		
-		(4) 400, 1500, 1850 & 2500		(1) 1850		(2) 700 & 1000		(4) 300, 400, 1000 & 1850	19
+		(3) 300, 350 & 800		(2) 350 & 1200		(2) 500 & 1500		(1) 1500	
OPQ01	9		4		4		4		
-		(5) 300, 800, 1100, 1200 & 1800		(1) 1100		ND		ND	10
+		(3) 200, 700 & 900		(1) 300		ND		ND	
OPQ18	7		3		6		6		
-		(4) 650, 900, 1350 & 1500		ND		ND		ND	9
+		(2) 550 & 1600		(2) 1500 & 1600		ND		(1) 1600	
OPR09	6		3		4		4		
-		(4) 400, 550, 1100 & 3000		(1) 600		ND		(1) 600	9
+		(2) 600 & 900		(1) 1000		ND		ND	
OPR12	11		10		7		7		
-		(6) 200, 450, 600, 1100, 2100 & 2250		(6) 250, 500, 800, 900, 1350 & 2250		(1) 900		(3) 200, 1200 & 1350	28
+		(4) 250, 500, 900 & 1800		(4) 700, 1000, 1100 & 1800		(2) 1350 & 2250		(2) 450 & 1000	
OPT18	5		8		10		10		
-		(1) 650		(2) 1800 & 2000		ND		ND	9
+		(4) 400, 500, 1000 & 2250		(1) 950		ND		(1) 2000	
OPW17	5		4		3		3		
-		(2) 600 & 1200		(3) 300, 900 & 1500		ND		ND	17
+		(5) 450, 500, 800, 1350 & 1500		(4) 300, 450, 500 & 800		ND		(3) 250, 450 & 800	
OPY10	4		5		4		4		
-		(1) 3000		(2) 1500 & 3000		(1) 400		(3) 400, 900 & 2500	12
+		(3) 400, 850 & 2500		(2) 400 & 2500		ND		ND	
UBC132	5		5		4		5		
-		(4) 1000, 1350, 1500 & 3000		(3) 550, 600 & 3000		(1) 1200		(3) 400, 1000 & 1200	17
+		(3) 600, 1200 & 1800		(3) 1000, 1350 & 1500		ND		ND	
UBC159	3		6		3		3		
-		(1) 500		(2) 1350 & 2000		(2) 500 & 700		(2) 650 & 2000	19
+		(5) 550, 700, 950, 1100 & 1350		(3) 300, 700 & 900		(2) 450 & 600		(2) 500 & 700	
UBC702	3		3		3		3		
-		(1) 800		(2) 750 & 800		ND		ND	7
+		(2) 600 & 2500		(1) 2500		ND		(1) 2500	

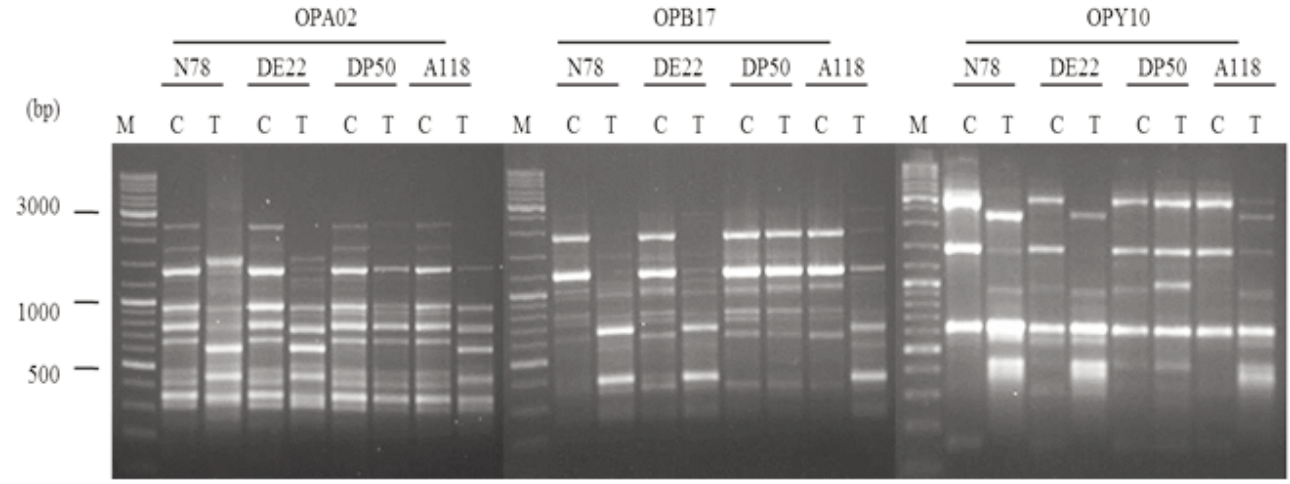

Fig. 1 - RAPD banding profiles generated by OPA02, OPB17 and OPY10 primers in the four tested cotton varieties showing DNA changes induced by NaCl application for seven weeks, C: Control, T: Treated plants. M: 1 kb DNA Ladder Mix, ready for use.

Table 3 - Genomic Template Stability (GTS%) estimated by 26 RAPD primers for the four tested cotton varieties under salt stress compared to their respective controls

Primer name	Control	N78 200 mM NaCl	DE22 200 mM NaCl	DP50 200 mM NaCl	A118 200 mM NaCl
OPA02	100	33.3	25	77.8	77.8
OPA04	100	0	33.3	100	100
OPB05	100	40	0	75	80
OPB17	100	0	33.3	100	12.5
OPC08	100	0	40	80	50
OPC13	100	0	0	100	100
OPD08	100	0	100	50	0
OPD20	100	16.7	0	0	0
OPE07	100	25	87.5	100	100
OPE15	100	0	25	100	100
OPG11	100	66.7	66.7	100	100
OPJ01	100	28.6	16.7	100	42.9
OPJ07	100	100	75	100	33.3
OPK12	100	100	66.7	100	100
OPK13	100	50	50	33.3	33.3
OPK17	100	12.5	25	0	16.7
OPQ01	100	11.1	50	100	100
OPQ18	100	14.3	33.3	100	83.3
OPR09	100	0	33.3	100	75
OPR12	100	9.1	0	57.2	28.6
OPT18	100	0	62.5	100	90
OPW17	100	40	75	100	0
OPY10	100	0	20	75	50
UBC132	100	40	20	75	40
UBC159	100	100	16.7	33.3	33.3
UBC702	100	0	0	100	66.7
Mean	100	26.4	36.7	79.1	58.2

ed no characteristic bands for the for tested cotton varieties (Table 1). It is worth noting that primer OPR12 identified more polymorphisms (28) than any other primer tested (ranging between two for primer OPG11 and 21 for primer OPJ07) (Table 2). Whereas, the banding patterns produced by primers OPA04, OPC13, OPE07, OPE15, OPG11, OPK12 and OPQ01 were not polymorphic for varieties DP50 and A118 (Table 2).

Another investigation demonstrated varietal variation in salt tolerance among these cotton varieties based on various examined physiological indices (Saleh, 2011). According to the study, the DE22 variety could relatively be classified as salt tolerant variety to other tested varieties. Dojan *et al*. (2012) reported the potential of RAPD markers for the detection of DNA damage induced by NaCl in cotton.

Likewise, the RAPD marker has the potential to be applied in environmental pollution detection, e.g. Gupta and Sarin (2009) applied the same marker to detect pollution by cadmium (Cd) in two aquatic plants. Zhou *et al*. (2011) also used RAPD bands to indicate DNA damage in *Euplotes vannus* (Protozoa, Ciliophora) induced by nitrofurazone in marine ciliates. Previously, Aly (2012) used the same marker for genotoxic effect detection of Cd stress on Egyptian clover and Sudan grass plants.

Changes in RAPD profiles were also measured as Genomic Template Stability (GTS) and the data suggest noticeable genomic template instability (Table 3). Reduction in GTS values was observed under salt stress, compared to their respective controls for the four tested varieties (Table 3). Similarly, genetic instability induced by NaCl treatment of cotton was reflected by changes in RAPD profiles: disappearance

of bands and appearance of new bands occurred in the profiles in comparison to those of the controls (Fig. 1, Table 3). Our data supports the suggestion by Dojan *et al*. (2012) that detected DNA changes, induced by NaCl, using RAPD marker could be explained as previously reported by Atienzar *et al*. (1999).

It has been demonstrated that DNA damage levels could be reflected in GTS (Atienzar *et al*., 1999). The later investigation suggested that the loss of bands may be attributed to genomic rearrangements or to point mutations causing alterations in oligonucleotide priming sites, while appearance of new bands could be related to the presence of oligonucleotide priming sites which become accessible to oligonucleotide primers after structural changes (DNA mutation, deletions or homologous recombination).

Table 3 reveals that GTS% values decreased with salt application for the four tested varieties. Our data show that the highest estimated GTS value was recorded for the two sensitive varieties, DP5 (79.1%) followed by A118 (58.2%); whereas, the lowest was recorded for the two tolerant varieties, N78 (26.4%) followed by DE22 (36.7%) (Table 3).

Gupta and Sarin (2009) reported that the genomic template stability test was significantly affected by heavy metal stress, while Aly (2012) reported that GTS values decreased obviously with an increase in cadmium (Cd) concentration in Egyptian clover and Sudan grass seedlings. On the other hand, Tanee *et al*. (2012) used GTS to identify the *Vanda* species (Orchidaceae) of Thailand. Our results are in accordance with Dojan *et al*. (2012) who reported that there is positive correlation between GTS and other parameters (stem and leaf growth and stem length) under NaCl stress in cotton.

In this respect, the estimated GTS values in the current investigation were positively correlated with various physiological indices (biomass and leaf K^+/Na^+ ratio) tested under NaCl application in cotton (Saleh, 2011). Moreover, a positive relationship was also determined between GTS values and recent findings (Saleh, 2013) based on physiological indices (relative water content, osmotic potential and salt tolerance index) among the same tested varieties (Dojan *et al*., 2012).

Overall, the lowest estimated GTS values combined with the highest polymorphism level recorded for the tolerant varieties (N78 and DE22) (where, % polymorphic level was 68.5, 60.9, 21.3 and 36.4% for N78, DE22, DP50 and A118, respectively exposed to 200 mM NaCl for 7 weeks, using the same marker) could explain their salinity tolerance compared to the other tested varieties. However, the lowest estimated GTS value recorded for N78 and DE22 varieties could be attributed to genetic variation, inducing new protein in relation to salinity tolerance.

It has been successfully demonstrated that environmental constraint induced variation in DNA methylation pattern as a developed epigenetic mechanism after exposure to abiotic stress (Zhong and Wang, 2007; Peng and Zhang, 2009). Our findings could be supported by the data provided in Zhong and Wang (2007), where genotyping variation in wheat (*Triticum aestivum* L.) cultivar salinity tolerance was reported. In this respect, the study mentioned that the salt-sensitive wheat variety had a lower methylation rate compared to salt-tolerant ones.

Recently, Saleh (2013) reported that N78 and DE22 varieties showed a better protection mechanism against salinity damage than the other tested varieties, demonstrating variation in salt tolerance among cotton varieties based on physiological indices. Likewise, in the same investigation comparing the protein profiles between control plants and those salts treated using SDS-PAGE showed protein changes under salt treatment compared to their respective control. In this respect, the expression of ~19, ~21 and ~26 kDa for N78 and ~21 kDa protein for DE22, was highly increased by salt treatment, indicating that it could play a role in salt stress response. On the other hand, newly synthesized protein of ~30 kDa was recorded for both DE22 and N78 varieties under saline treatment which was not observed in their respective controls. The other two tested varieties (DP50 and A118) showed decreases in the same protein bands (~19, 26 and 34 kDa) under saline conditions, with respect to their respective controls, reflecting their sensitivity to salt stress. Salinity promotes the synthesis of salt stress-specific proteins; many of these proteins were suggested to protect the cell against the adverse effect of salt stress. Accumulation of these proteins is a common response to salt stress (Kong-Ngern *et al*., 2005; Metwali *et al.*, 2011).

It is worth noting that identified bands in DE22 and N78 (salt-tolerant varieties), which were not amplified in salt-sensitive varieties (DP50 and A118), could be related to gene(s) involved in salinity tolerance. These findings are in accordance with Kurup *et al*. (2009).

DNA variation could be exploited in plant breeding programs to improve salinity tolerance in germplasm. Overall, the lowest estimated GTS values for NB78 and DE22 varieties (known as salt-tolerant) reflect their highest polymorphic values. Based on the current results, the RAPD marker was useful to establish specific DNA markers associated with NaCl stress. Therefore, the RAPD marker could be used as useful tool in plant breeding programs for early identification of cotton tolerance to salt stress.

Acknowledgements

The author would like to thank Prof. Othman I, Director General of AECS and Prof. MirAli N, Head of Molecular Biology and Biotechnology Department for their support, and also to the Plant Biotechnology group for technical assistance.

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