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Gene Expression Analysis of Egyptian and Yemeni Sorghum Cultivars under Salinity Stress Using Isozymea and SDS-PAGE

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Abstract: This investigation was done to find out some molecular markers in some sorghum cultivars to compare between their responses to salt stress. Three concentrations of NaCl (0 ppm, 6000 and 8000 ppm) were used to irrigate the seventeen cultivars during 60 days of growth period, planted in sand pots in greenhouse. To determine the most tolerant and sensitive cultivars, three growths related traits [plant height (Ph), shoot fresh weight (SFW) and shoot dry weight (SDW)] were measured and statistically analyzed. Three Yemeni cultivars (Demasques-1298, Saif and Haiq) were the most tolerant cultivars whereas the Egyptian cultivars (TX430, TX436 and TX2864) were the most sensitive. Electrophoresis of the total soluble proteins using SDS-PAGE and six isozymes (alpha-Esterase, beta-Esterase, Peroxidase, Polyphenol Oxidase, Amylase and Superoxide Dismutase) were used to study gene expression of the tolerant and sensitive sorghum cultivars under control and salinity stress (0 and 8000 ppm NaCl, respectively). The difference of gene expression was scored for (between and within) tolerant and sensitive cultivars. Analysis of the total proteins using SDS-PAGE revealed that, ten out of 31 bands were polymorphic with ratio of 32.26% polymorphism. Analysis of the six isozymes banding patterns showed a variety of polymorphism ranged from 100% to 42.86% for Amylase and Peroxidase respectively. Among the six isozymes banding patterns, Superoxide dismutase had a maximum number of bands (16 bands) with moderate polymorphism (50%) and two out of eight polymorphic bands could considered as positive markers for the tolerant sorghum cultivars. Therefore, we can depend on the protein and enzymes patterns to choice the most tolerance cultivars using the electrophoresis pattern of isozymes.

Key words: Electrophoresis · Isozymes · Protein · Salinity · Sorghum

INTRODUCTION

Salinity is one of the repressive abiotic factors that limiting the useful farming lands [1-3]. It has reached a level of 19.5% of all irrigated land and 2.1% of dry-land agriculture worldwide [4]. The plants that grow in saline soils have diverse ionic compositions and a range in concentrations of dissolved salts. Production of the economic crops is decreased in response to salt stresses [5]. Plant species differ in their salt tolerance depending on their genetic makeup ranging from high to low levels of salts in the soil. Sorghum (*Sorghum bicolor* L.) belongs to the grass family and was domesticated in different areas of Africa. It is a major crop of the world with various uses. Sorghum was grown primarily as a source of sugar for syrup and is normally used animal feed. Sorghum is known to be an annual C4 plant of African (tropical) origin and is well adapted to semiarid and arid tropic regions, being highly biomass productive and water efficient. It is the fifth most important cereal crop in the world after wheat, rice, corn and barley [6]. Sorghum possesses a variety of anatomical, morphological and physiological features that enable it to survive in water-limited environments. Sorghum is a salt tolerant crop; it is more tolerant of salinity than maize but is less tolerant than barley [7]. There are significant differences between genotypes of sorghum grown in salt stress conditions [8, 9]. The tolerance to salt stress is a complex process that involves morphological physiological and biochemical modifications. Survival and growth under

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saline environments are the result of adaptive processes such as ion transport and compartmentalization, synthesis and accumulation of organic solutes, bearing to an osmotic adjustment [10]. Many researchers investigated details of the physiology and biochemistry of salt tolerance and also looked at methods to screen overall plant performance that could be used in breeding programs [11-13]. Exogenous application of abscisic acid (ABA) was able to increase plant adaptive response to various environmental conditions. The resurrection plant, Craterostigma plantaginem can tolerate extreme dehydration. However, in vitro propagated callus derived from this plant has a strict requirement for exogenously applied ABA in order to survive severe dehydration, Desikan [14]. The plant hormone abscisic acid as a stress signal increased as a result of water stress and play important role in the regulation of plant responses from the whole plant level and the cellular level. Tabatabaei and Anagholi [15] study the effect of salinity on germination stage of seven sorghum genotypes a controlled experiment was con ducted in Agricultura l Research Center, Yazd, Iran, showed that genotype could be considered as a salt tolerant genotype at least at germination stage.

In this study we discriminated 17 sorghum cultivars depending on their salinity tolerance to study gene expression at protein level under salinity, using SDS-PAGE and isozymes.

MATERIALS AND METHODS

Sorghum Cultivars: Eleven Egyptian sorghum cultivars (TX430, TX436, TX2737, TX2817, TX2794, TX2864, TX2862, TX2783, 93MR732, Sh-2 and Dorado) grains were kindly provided by Agronomy Department, Agriculture Research Center, Giza, Egypt and six Yemeni cultivars (Negawhite, DemasquesB81-990, DemasquesB81-1298, Beine, Saif&Haiq) were kindly provided by Faculty of Agri. Sanaa university, Yemen.

Salinity Experiment: The seventeen sorghum cultivars were assessed for their salt tolerance according to the performance of some growth-related traits as plant height (Ph), shoot fresh weight (SFW) and shoot dry weight (SDW). The cultivars were grown in sand pots in the greenhouse in Dept. Genetics, Fac. of Agri. Ain Shams Univ. and subjected to salt stress at 0, 6000 and 8000 ppm of NaCl supplemented by (10^{-5}M) abscisic acid (ABA) up to 60 days of planting Somasundaram *et al*. [16].

SDS-PAGE: Total soluble protein was extracted from 0.1 g of fresh leaves of the selected most tolerant and sensitive cultivars under concentrations of 0 (control) and 8000 (stress) ppm NaCl and applied for electrophoreses as described by Laemmli [17].

Isozymes: Six isozymes (*alpha-Esterase*, *beta-Esterase*, *Peroxidase*, *Polyphenol Oxidase*, *Amylase* and *Superoxide Dismutase*) were used to study differences of gene expression in sorghum cultivars under salinity stress Ediga *et al*. [18]. The differences of gene expression were scored in both of: 1) within each tolerant and sensitive sorghum cultivars. 2) Between the most tolerant and sensitive groups.

Statistical Analysis: Data were statistically analyzed by SPSS13 software package using two-way ANOVA (cultivars vs. treatments) and Tukey multiple comparison test to determine the most tolerant and sensitive cultivars to salinity stress.

RESULTS AND DISCUTION

Salinity Experiment: Seventeen sorghum cultivars (11Egyptian) and (sixYemeni) were tested for their salinity tolerance. After 60 days of irrigation with (0, 6000 and 8000 ppm NaCl supplemented with ABA). The treats Ph, SFW and SDW were measured as stress-related traits as shown in Fig. 1. The Yemeni cultivars (Demasques-1298, Saif and Haiq) were selected at concentration of 8000 ppm NaCl as the most tolerant cultivars, whereas the Egyptian cultivars (TX430, TX436 and TX2864) were the most sensitive at the same salt concentration as in Table 1. It is known that increasing salinity concentration decreased the survival percentage, transplant length, leaves number/ transplant, leaf erea, leaves content of Nm P, K, Ca and chlorophyll as observed by Shaheen *et al* [19] in the study on olive. Somasundaram *et al*. [16] is found that, these growth regulators can be used as stress ameliorating agents in this crop plant, as they increase the antioxidant enzyme activities and thereby providing stress tolerance ability to the plant. ABA treatment alone increased the peroxidase and superoxide dismutase (SOD) activity in stem and lives of plant. Padmanaban *et al*. [20] reported that the expression of isozyme could be differed with specific tissues or environment with circadian rhythm of the particular genotype.

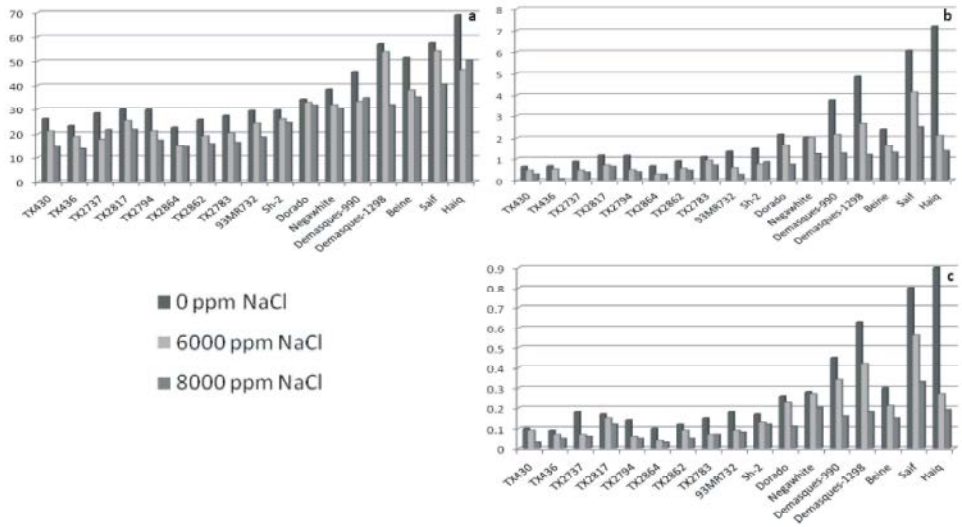

Fig. 1: Means of plant height (a), shoot fresh weight (b) and shoot dry weight (c) of the seventeen sorghum cultivars under (0, 6000 and 8000 ppm NaCl).

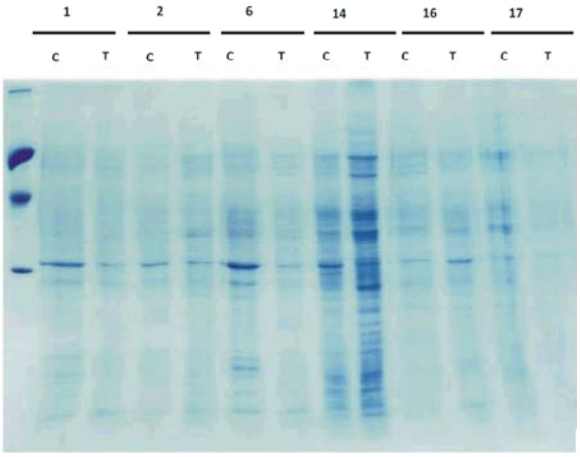

Fig. 2: SDS-PAGE profile of total protein fraction of the tolerant (1, 2 and 6) and sensitive (14, 16 and 17) sorghum cultivars under control and salinity stress (0 and 8000 ppm, respectively) combined with ABA.

Analysis of SDS-PAGE: Analysis of the total proteins using SDS-PAGE revealed 313 total bands through 31 different bands ranged from (sixto 161 kDa). The tolerant cultivar Demasques-1298 showed all the 31 different bands under salinity whereas the band 161 kDa was disappeared in the negative control (0 ppm NaCl). The other cultivars showed less bands than Demasques-1298 where the number of bands ranged from 23 to 29 for the other five sorghum cultivars. From the 31 different bands only ten of them were polymorphic showing a percent of 32.26% polymorphism as showed in Table 2 and Fig. 2. This result in agreement with Bavei *et al*. [21] as reported about salinity stress on sorghum. Electrophoresis has been widely used for separating proteins from all cereals. Sorghum proteins play an important role in the utilization of sorghum and its nutritional properties and SDS-PAGE has been a major tool used to study sorghum proteins Sharmila *et al*. [22].

Table 1: Means of PH (cm), SFW (g) and SDW (g) for the 17 sorghum cultivars under control and salinity stress.

Code No.	Cultivars	PH	SFW	SDW
1	TX430	20.48	0.46	0.07
2	TX436	18.59	0.42	0.07
3	TX2737	22.59	0.57	0.10
4	TX2817	25.65	0.83	0.15
5	TX2794	22.80	0.67	0.08
6	TX2864	17.30	0.40	0.06
7	TX2862	20.29	0.63	0.09
8	TX2783	21.37	0.90	0.10
9	93MR732	24.07	0.74	0.12
10	Sh-2	26.63	1.02	0.14
11	Dorado	33.04	1.51	0.20
12	Negawhite	33.59	1.75	0.25
13	Demasques-990	37.96	2.40	0.32
14	Demasques-1298	47.63	2.91	0.41
15	Beine	41.63	1.76	0.22
16	Saif	54.04	4.23	0.56
17	Haiq	51.10	3.56	0.45

Analysis of Isozymes Electrophoresis: Electrophoretic pattern of *α-Esterase* isozyme revealed 75 total bands in nine different bands. All the nine bands were observed in the sensitive cultivar TX436 under control treatment and

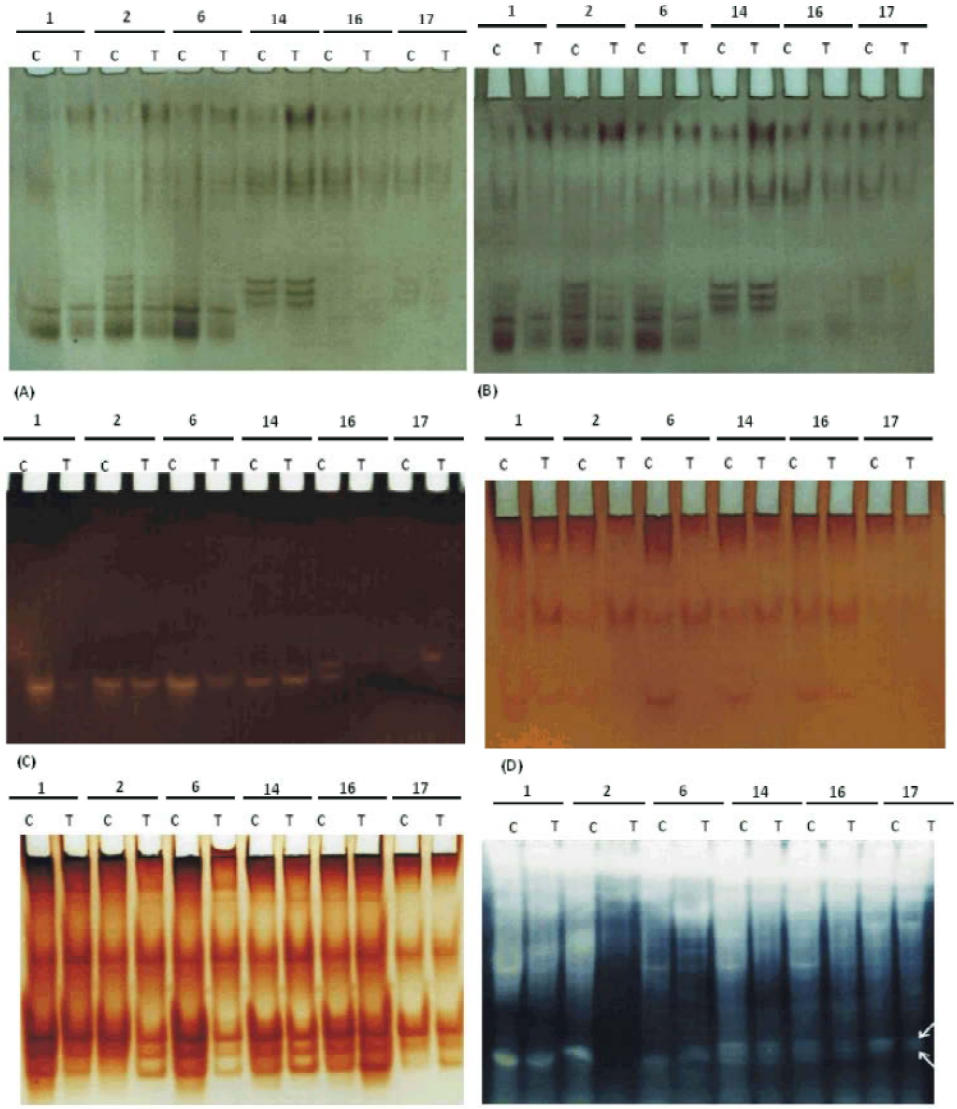

Fig. 3: Native PAGE profile of some isozymes fortolerant (1, 2 and 6) and sensitive (14, 16 and 17) sorghum cultivars under salt stress (0 and 8000 ppm) combined with ABA: (A) *α-esterase*, (B) *β-esterase*, (C) *amylase*, (D) *Polyphenoloxidase*, (E) *peroxidase* and (F) *Superoxide dismutase*.

Table 2: Monomorphic, polymorphic, total number of bands and polymorphism percentages for the electrophoresis of the most tolerant and sensitive sorghum cultivars using SDS-PAGE and six isozymes under 0 and 8000 ppm NaCl

	Monomorphic bands	Polymorphic bands	Different bands	Total bands	Polymorphism percentages (%)
SDS-PAGE	21	10	31	313	32.26
α-Esterase	4	5	9	75	55.56
β-Esterase	4	6	10	90	60
Peroxidase	4	3	7	78	42.86
Polyphenol Oxidase	1	2	3	27	66.67
Amylase	0	2	2	14	100
Superoxide Dismutase	8	8	16	165	50

two of them are absent under stress. The other cultivars ranged in band number from eight to only four bands with 55.56% polymorphism as in Fig. 3(A). *β-Esterase* electrophoretic analysis showed ten different bands and about 90 total bands with 60% polymorphism as in Fig.3 (B), *Amylase*in Fig.3 (C) and *Polyphenol Oxidase*in Fig.3 (D) showed the less total band numbers which were 14 (only two different bands) and 27 (three different bands),

respectively. *Peroxidase*isozyme analysis displayed a moderate total number of bands which was 78 include seven different bands as showed in Fig.3 (E).

The banding pattern of the *Superoxide dismutase*isozyme showed the maximum number of bands through the all six isozymes. The total number of bands for *Superoxide dismutase* analysis was 165 bands in 16 different bands. Half of them (eight bands) were polymorphic bands with 50% polymorphism percentage as displayed in Table 2. Two of the eight polymorphic bands were presented in the tolerant cultivars (Demasques-1298, Saif and Haiq) only so they may be considered as biochemical markers for the tolerant sorghum cultivars as showed in Fig.3 (F). These results agree with the report of Ediga *et al.* [18] which explain that salinity stress, with increase in concentration and time period, the activities of antioxidative enzymes increased significantly when compared to their respective control plant.

Differential expression of isozymes has attributed with different developmental stages, tissue specificity, stress factors ect. Accordingly, the expression of isozyme could be differed with specific tissues or environment with circadian rhythm of the particular genotype and widely used to analyze the genetic diversity at intraspecies, interspecies and interspecific hybrid levels as reported by Padmanaban *et al*. [20].

The appearance of new bands and disappearance of the others, of different genotypes represented by different cultivars under salt stress would indicate either enhancement or repression of gene expression in these cultivars. This might alter the produced proteins in response to salt stress either on the transcription or post-transcription levels of gene expression. Similar conclusions were reached [23-26]. Our results are parallel with those of several authors, who used some isozymes and total protein pattern electrophoresis variations to evaluate some cultivars under different salt stress to be used as biochemical genetic markers for early evaluation [24, 27, 28].

CONCLUSION

Three Yemeni cultivars (Demasques-1298, Saif and Haiq) were the most tolerant cultivars whereas the Egyptian cultivars (TX430, TX436 and TX2864) were the most sensitive under salinity stress (6000 and 8000 ppm NaCl), SDS-PAGE revealed 32.26% polymorphism. Analysis of the six isozymes banding patterns showed a variety of polymorphism ranged from 100% to 42.86% for Amylase and Peroxidase respectively. From this study we can choice the most tolerant cultivars using the molecular markers which showed in our results of protein enzymes analysis.

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