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Molecular responses of Bt transgenic corn (*Zea mays* L.) plans to salt (NaCl) stress

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Abstract

Non-transformed and Bt transgenic crop lines of a hybrid (YieldGard 2) corn (*Zea mays* L.) plants in the 4th leaf stage were subjected to 0, 50, 100 and 150 mM NaCl. Significant ($P \geq 0.05$) reduction in growth of non-traformed plants was recorded under almost all levels (50, 100 and 150 mM) of salt (NaCl) stress; while, the growth of the Bt transgenic corn plants showed no significant changes under the same levels of salt stress. Chlorophyll a contents were reduced at 150 mM NaCl only; but did not change in the BT transgenic corn plants. Chlorophyll b was not responsive to NaCl treatments in both non-transformed and Bt transgenic corn plants. Chlorophyll stability index (CSI) were always higher in the Bt transgenic than in the non-transformed corn plants under all salinity levels. The analyses of SDS-PAGE revealed relative stability in the patterns of protein bands in the Bt transgenic corn plants under salinity stress. The sum of optical densities of protein bands was higher in the Bt transgenic corn plants. In response to salinity stress, a group of polypeptide (76.96, 59.38, 41.56, 33.5 and 31.26 KDa) were newly synthesized in both non-transformed and Bt transgenic plants. Salt-susceptible polypeptides of molecular weights 325.47, 32.64 and 24.17 KDa were found only in non-transformed corn plants and completely disappeared under all level of salt stress; while two polypeptides of molecular weights 38.59 and 30.61 KDa were totally inhibited in all salt-stressed corn plants. The synthesis of another four polypeptides (298.81, 99.82, 20.79 and 19.43 KDa) was solely specific to the Bt transgenic corn plants. Stability of chlorophyll pigments and molecular weights of salt stress responsive proteins are key genetic determinants of salt stress in Bt transgenic corn plants.

Keywords: Bt Transgenic corn, CSI, salt stress, SDS-PAGE, proteins.

Introduction

Soil salinity, one of the major abiotic stresses reducing agricultural productivity, affects large terrestrial areas of the world. The damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations. It is estimated that 20% of the irrigated land in the world is presently affected by salinity (Yeo, 1999). Reduction in growth and yield are surely the most conspicuous physiological responses of the crop to excessive salts in the media. One of the two main approaches that have been adopted to improve salt tolerance of crops is the generation of transgenic plants to introduce novel genes or to alter expression levels of the existing genes to affect the degree of salt stress tolerance (Yamaguchi and Blumwald, 2005). For verifying the molecular basis of salinity tolerance in genetically modified (transgenic) plants, proteomic changes should be identified and detected. In contrast to the genome, the proteome is not static but rather responsive to many internal and external factors (Zhu *et al*., 1995). Thus, identifying differentially regulated proteins would assist in the analysis of gene

Table 1. Mean vegetative and reproductive growth parameters of non-transformed and Bt transgenic corn (*Zea mays* L.) plants under salt (NaCl) stress.

Corn crop line	Treatment	Growth parameters								
		Plant height (cm)	Root length (cm)	No. of leaves/plant	Leaf length (cm)	Leaf width (cm)	Shoot F. Wt. (g)	Root F. Wt. (g)	Shoot D. Wt. (g)	Root D. Wt. (g)
Non-transformed	C	69.6	38.3	7.0	50.7	3.8	11.78	2.290	1.209	0.429
	50	55.5	19.7	6.0	41.7	1.9	6.03	0.651	0.696	0.179
	100	48.0	20.2	5.0	35.3	2.0	5.51	0.629	0.716	0.144
	150	41.3	11.9	4.0	31.5	1.8	3.58	0.658	0.535	0.090
Bt transgenic	C	58.4	21.8	6.0	45.3	3.2	8.22	1.240	0.816	0.150
	50	56.5	19.3	5.0	37.6	2.8	6.73	1.899	0.846	0.101
	100	54.0	18.8	5.0	36.8	2.3	6.26	1.890	0.755	0.207
	150	48.0	17.0	5.0	34.4	2.2	5.88	1.517	0.814	0.155
LSD ($P \geq 0.05$)		15.25	13.90	1.65	11.41	1.27	4.32	1.177	0.346	0.192

C, 50, 100, 150 = Plants treated with 0, 50, 100 and 150 mM NaCl, respectively.

expression altered under salinity stress and involved in the mechanism of salt resistance (Zörb *et al*., 2004). Corn (*Zea mays* L.) is considered as a moderately salt-sensitive plant (Mass and Hoffman, 1977). Efforts to improve the crop performance under environmental stresses have not been that fruitful because the fundamental mechanisms for stress tolerance in plants remain to be completely understood. Bt transgenic corn is normal corn that contains one or more genes from the soil bacterium *Bacillus thuringiensis*, or Bt as in commonly used. The gene(s) allow the bacterium to produce one or more toxins that are toxic to certain insects, but are not toxic to mammals, including humans. Bt corn also contains genes unrelated to the pest-control function, including marker genes, which are used to select plants that have been successfully engineered, and genes that may be carried passively into the corn on the gene-splicing vector Therefore, the present investigation was conducted to verify and identify key genetic determinants of salt stress tolerance in Bt transgenic corn plants with a special emphasis on the role of SDS-PAGE protein profile in the mechanism of salt tolerance in corn plans.

Materials and methods

Plant Material

Seeds of both non-transformed and Bt transgenic crop lines of a hybrid (YieldGard 2) corn (*Zea mayes* L.) were kindly provided by Pioneer Hi-Bred International, Inc., USA.

Plantation

Seeds of both non-transformed (control) and B transgenic corn (*Zea mays* L.) crop lines were soaked (3 seeds/pot) in plastic pots (10 cm) filled with pre-sieved homogeneous garden soil (sandy loam). All planted pots were kept in the open garden in about 32/21 ºC day/night temperature and average relative humidity of 68% and irrigated regularly up to field capacity with pure water. After one week from soaking (4th leaf stage), pots were subdivided into two separate groups (non-transformed and Bt transgenic) and each group (3 replicates/treatment) was subjected to four salinity (NaCl) levels: 0, 50, 100 and 150 mM with a 120 ml doze/pot every other day for three more weeks.

Growth Parameters

By the end of the 4th week, corn plants were gently uprooted, cleaned from soil residues and prepared for measurements. Plant height, root length, shoot and root fresh and dry weights, leaf length and width and total number of leaves per plant were recorded.

Chlorophyll Pigments

The contents of chlorophyll a; chlorophyll b and total chlorophyll were estimated in the 4th expanded corn leaf in 80% acetone according to the procedure

Table 2. Chlorophyll contents (mg/g) in foliage leaves of non-transformed and Bt stransgenic corn (*Zea mays* L.) plants under salt (NaCl) stress.

Pigment	Non-transformed				Bt transgenic				LSD
	C	50	100	150	C	50	100	150	(P ≥ 0.05)
Chlorophyll a	0.5712	0.5671	0.5232	0.4560	0.4486	0.4131	0.3738	0.3669	0.0782
Chlorophyll b	0.2094	0.2205	0.1796	0.1518	0.1327	0.0663	0.0857	0.0620	0.1126
Total Chlorophyll	0.7768	0.7873	0.6949	0.6077	0.5811	0.4763	0.4595	0.4288	0.2541

C, 50, 100, 150 = Plants treated with 0, 50, 100, 150 mM NaCl

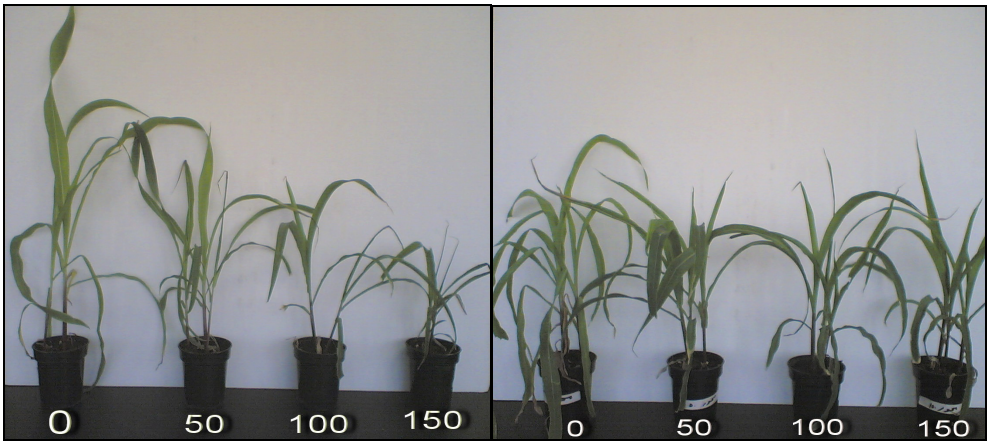

Fig 1. Growth responses of non-transformed (left) and Bt transgenic (right) corn (*Zea mays* L.) plants to salt (NaCl) stress: 0, control plants; 50, 100, 150, plants treated with 50, 100, and 150 mM NaCl, respectively.

described by Sadasivam and Manickam (1991). The absorbance of the extract was read at 645, 663 and 652 nm against the solvent as blank. Pigments were determined as mg /g fresh weight residue. Chlorophyll stability index (CSI) was calculated by combining chlorophyll a+b contents in corn leaf before and after salinity stress following the formula noted by Kumari et al. (2004): CSI % = Chlorophyll before stress – chlorophyll under stress / chlorophyll under stress × 100

Protein Electrophoresis

Preparation of total protein

Total protein extracts were prepared by extracting appropriate portion of the frozen plant material with 0.125 M tris/borate (pH = 8.9). All extracts were kept for 24 h at 4 ºC and then centrifuged at 10,000 rpm for 20 min. The supernatants were used for electrophoresis.

Gel electrophoresis

SDS Polyacrylamide Gel Electrophoresis (PAGE) was carried out with gel slabs according to the method of Laemmili (1970). Protein subunit bands were stained with Coomassie blue R-250 by standard techniques. The gel was scanned using Gel-Pro-Analyzer.

Statistical Analysis

All parameters were statistically analyzed by multiple comparison procedure at p≤ 0.05 using t-test and mean separation by least significant difference (LSD) (Steel and Torrie, 1980).

Results

The Statistical analysis (P ≥ 0.05) of the growth parameters (Table 1) of corn plants revealed significant reduction in plant growth of non-transformed plants under almost all levels (50, 100

Table 3. Comparative analysis of average optical density (O. D.), molecular weight (M.Wt.) and relative front (R_f) of SDS-PAE protein profile of non-transformed and Bt transgenic corn (*Zea mays* L.) plants under salt (NaCl) stress

Band number	Treatment & O. D.								R_f	Mol. Wt. (KDa)
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**		
1	201.75	-	-	-	-	-	-	-	0.02	325.47
2	-	-	-	-	-	-	-	179.89	0.04	298.81
3	127.5	84.49	-	151.83	141.54	-	-	144.23	0.10	187.87
4	-	-	-	-	-	103.50	93.16	-	0.12	184.49
5	-	-	-	149.44	148.45	-	-	-	0.13	149.39
6	-	102.16	-	-	152.78	131.15	117.10	-	0.18	104.81
7	153.66	-	96.85	147.28	-	-	-	-	0.19	101.46
8	-	-	-	-	-	-	-	208.46	0.22	99.82
9	-	-	-	143.50	137.22	-	-	-	0.24	88.89
10	171.96	113.59	99.72	136.94	135.27	120.98	116.44	235.31	0.25	81.95
11	-	105.17	96.92	133.00	125.06	109.21	121.54	-	0.26	76.96
12	-	125.53	136.15	167.99	150.74	128.92	167.04	-	0.35	59.38
13	208.19	172.96	193.35	198.27	161.54	161.10	193.02	244.73	0.38	50.73
14	-	116.97	105.46	120.31	108.67	102.63	124.89	-	0.45	41.56
15	207.17	-	-	-	-	-	-	237.61	0.48	38.59
16	157.21	90.24	76.54	84.38	79.84	74.91	86.56	188.99	0.53	34.85
17	-	96.16	76.89	86.13	78.15	78.04	86.57	**-**	0.56	33.50
18	146.92	**-**	**-**	**-**	**-**	**-**	**-**	**-**	0.59	32.64
19	-	89.80	73.03	95.07	84.60	80.49	91.10	**-**	0.62	31.26
20	116.28	-	-	-	-	-	-	147.97	0.65	30.61
21	92.64	67.39	-	74.59	64.07		-	-	0.72	27.36
22	-	-	-	-	-	60.91	75.30	119.15	0.76	26.63
23	70.14	-	-	-	-	-	-	-	0.82	24.17
24	-	53.55	38.74	-	-	-	-	88.89	0.86	22.85
25	-	-	-	-	-	54.32	-	-	0.89	22.11
26	-	-	-	-	-	-	67.13	-	0.90	21.97
27	-	-	-	-	-	-	-	73.92	0.94	20.79
28	-	-	-	-	-	-	-	89.52	0.99	19.43
Total O. D.	**1653.4**	**1218.0**	**0993.7**	**1687.9**	**1567.9**	**1206.2**	**1339.9**	**1958.7**		
Band/Lane	**11**	**12**	**10**	**13**	**13**	**12**	**12**	**12**		

1 and 8= untreated (control) non-transformed and Bt transgenic plants, respectively 2, 3, 4 and 5,6,7 = non-transformed and Bt transgenic plants treated with 50, 100, 150 mM NaCl, respectively.

and 150 mM) of salt (NaCl) stress; while, the growth parameters of the Bt transgenic corn plants showed no significant changes under the same levels of salinity stress. However, the growth parameters of controlled (0.0 NaCl) plants of Bt transgenic corn were always lower than those of non-transformed plants.

The contents of chlorophyll a in the expanded leaf of non-transformed corn plants were significantly reduced by the highest NaCl treatment (150 mM) only; but did not significantly change in the Bt transgenic corn plants (Table 2). However, chlorophyll b was not responsive to NaCl treatments in both non-transformed and Bt transgenic corn plants. On the other hand, the values of chlorophyll stability index (CSI) were always higher in the Bt transgenic than in the non-transformed corn plants under all salinity levels (Fig. 3).

The analyses of SDS-PAGE protein profiles of either non-transformed or Bt transgenic corn plants revealed both qualitative (Table 3) and quantitative (Figs. 2 & 4) changes in the patterns of protein bands in

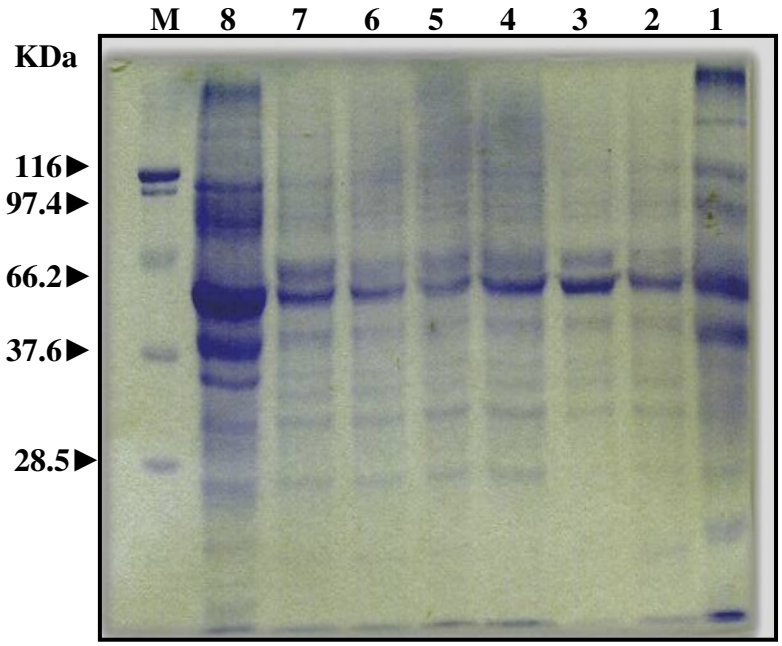

Fig 2. Electrophotograph of SDS-PAGE of total proteins of non-transformed (1, 2, 3, 4) and Bt transgenic (8, 5, 6, 7) corn (*Zea mays* L.) plants subjected to 0, 50, 100 and 150 mM salt (NaCl) stress. M, molecular weight markers used on polyacrylamide gel.

response to salinity stress. The total number of protein bands (Table 3) showed relative stability in the Bt transgenic corn plants under salinity stress, but showed fluctuations in the non-transformed plants. The sum of optical densities of protein bands (Table 3) was higher in the Bt transgenic corn plants. In response to salinity stress, a group of polypeptide (76.96, 59.38, 41.56, 33.5 and 31.26 KDa) were newly synthesized in both non-transformed and Bt transgenic plants. Salt-susceptible polypeptides of molecular weights 325.47, 32.64 and 24.17 KDa were found only in non-transformed corn plants and completely disappeared under all level of salt stress; while two polypeptides of molecular weights 38.59 and 30.61 KDa were totally inhibited in all salt-stressed corn plants. The synthesis of another four polypeptides (298.81, 99.82, 20.79 and 19.43 KDa) was solely specific to the Bt transgenic corn plants (Table 3).

Discussion

Twenty eight years ago Epstein (1980) described the technical and biological constraints to solving the problem of salinity. Although there has been some success with technical solutions to the problem, the biological solutions have more difficult to develop because a pre-requisite for the development of salt-tolerant crops is the identification of key genetic determinants of stress tolerance (Yamaguchi and Blumwald, 2005).

The non-significant ($P \geq 0.05$) growth responses of Bt transgenic corn plants to salt stress proved the magnitude of salt tolerance in those plants. Earlier supporting works (Munns, 1993) reported that growth parameters were found to be perfect indicators for screening salt tolerance in plants.

Compared to the lower chlorophyll a/b ratio in non-transformed corn plants, the high ratio of chlorophyll a/b as well as the stability of chlorophyll b content in Bt transgenic corn plants can be ranged as an index of salt tolerance, which might produce higher photosynthetic rate and consequently high yield (Raja Babo *et al*., 2005). Moreover, the chlorophyll stability index (CSI) is an important index for screening plant tolerance to abiotic stresses (Michael Gomaz and Rangasamy, 2002; Yagameena, 2004). In this investigation, the high values of chlorophyll

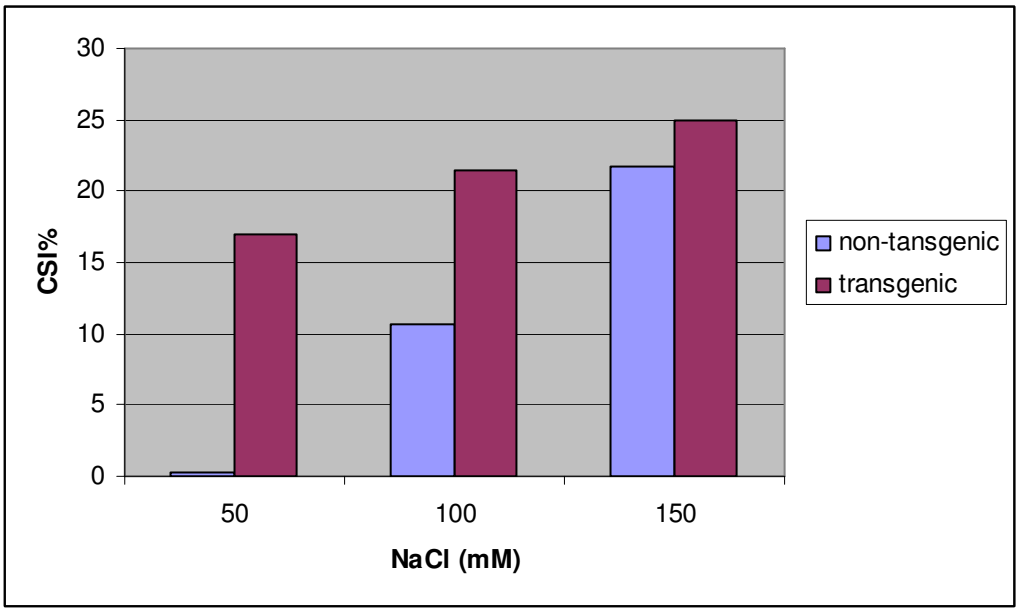

Fig 3. Chlorophyll stability index (CSI%) of non-transformed and Bt transgenic corn (*Zea mays* L.) plants in response to salt (NaCl) stress.

satiability index (CSI%) verified the tolerance of Bt transgenic corn plants to salinity stress, results were supported by the findings of Raja Babo *et al*. (2005).
Over the past few years, much attention has been concentrated on resolving the identity of salt stress proteins, in order to identify and understand the role of proteins in salt tolerance. Several workers have detected a number of proteins induced by salt, reflecting the complexity of biochemical and physiological responses accompanied with the biological changes of the adaptation process of salt tolerance. In this report, corn plants, non-transformed and Bt transgenic, responded to salt stress by both induction and repression in the synthesis of many polypeptides. In response to salinity stress, the newly synthesized proteins in common were 76.96, 59.83, 41.46, 33.5, and 31.26 KDa; while the suppressed proteins were 38.59 and 30.61 KDa. Quantitatively, the protein content in each band (band optical density) was always higher in the Bt transgenic corn plants. Other proteins (298.81, 99.82, 20.79, 19.43 KDa) were found only in Bt transgenic corn plants. Relevant salt stress proteins of 56.1, 70.8 and 93.8 KDa were reported in rice roots (Salekdeh, *et al*., 2002; de Souza Filho *et al*., 2003) and 18.0, 19.5, 21.0, 26.0, 34.0, 35.5, 37.0 and 58.0 KDa in tomato roots (Chen and Plant, 1999).

In conclusion, the data presented here revealed some of the key genetic determinants of salt stress tolerance in Bt transgenic corn plants, which could be partially attributed to the stability of photosynthetic pigments under salt stress. Moreover, this investigation reported molecular weights of some salt tress responsive proteins in Bt transgenic corn plants. It is necessary to further investigate the patterns of protein expression of these salt-induced protein bands as well as the specific polypeptides of Bt transgenic corn plants to enhance our understanding of salt tolerance in transgenic corn.

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