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Exogenous Proline Induced Changes in SDS-PAGE Protein Profile for Salt Tolerance in Wheat (*Triticum aestivum* L.) Seedlings.

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ABSTRACT

The present investigation was an attempt to verify the role of exogenous application of proline in inducing salt tolerance in wheat (*Triticum aestivum* L.) seedlings. NaCl (0, 50, 150 and 300 mM) concentrations were supplemented to the germination MS medium in combination with proline (0 and 50 ppm). Exogenous proline alleviated the reduction in germination percentage, seedling length, fresh and dry weights as well as photosynthetic pigments caused by NaCl stress. NaCl stress (300 mM) blocked the synthesis of a 98 KDa polypeptide that was restored by the addition of proline. Proline increased the synthesis of 112 and 48 KDa polypeptides at all NaCl concentrations. High NaCl concentrations alone induced the synthesis of new polypeptides of molecular weights 93 and 62 KDa. Proline was successful in protecting protein turnover machinery and up-regulating stress protective proteins.

Key words: Wheat, Growth, Salt tolerance, Proline, SDS-PAGE proteins.

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INTRODUCTION

Salinity is a widespread environmental stress for crop plants. It is common in arid and coastal regions (Baccio et al., 2004). Up to 20% of the irrigated arable land in arid and semiarid regions is already salt affected and is still expanding (Mühling and Läuchli, 2003). Salinity stress, the most adverse factor of plant growth and productivity (Amor *et al*., 2005; Baek *et al*., 2005; Raja *et al*., 2005), triggers a wide variety of plant responses, ranging from changes in growth rates and crop yields to altered gene expression and cellular metabolism. Plants develop a plethora of biochemical and molecular mechanisms to cope with salt stress. Biochemical pathways leading to products and processes that improve salt tolerance are likely to act addiditively and probably synergisticalllly (Lyengar and Reddy, 1996). Soluble protein contents of leaves decrease in response to salinity (Alamgir and Ali, 1999). SDS-PAGE analysis of proteins in peanut (*Arachis hypogaea* L.) showed induction (127 and 52 KDa) and/or repression (260 and 38 KDa) in the synthesis of polypeptides in plants grown under NaCl stress (Hussanein, 1999). In wheat, the content of 26 kDa protein increased in NaCl treated plants, while, the contents of 13 and 20 KDa proteins decreased and the 24 KDa proteins disappeared with NaCl treatment (Elshintinawy and Elshourbagy, 2001*).*

Proline accumulation is important for osmotic adjustment under abiotic stress conditions; it is believed that high level of proline can be beneficial to stressed plants (Hyun *et al*., 2003). In addition, supraoptimal level of proline could be beneficial to salt-stressed plants through stabilization of proteins, prevention of heat denaturation of enzymes and as a hydroxyl radical scavenger (Hsu *et al*. 2003). Moreover, proline improves the salt tolerance by protecting protein turnover machinery and up-regulating stress protective proteins. Although a variety of roles have been suggested for proline, its physiological role in seed germination under salt stress has received far less attention. Therefore, this work was designed to verify the influence of exogenous application of proline on germinating wheat seedlings grown under saline conditions, with a special emphasis on the role of SDS-PAGE proteins.

MATERIALS AND METHODS

Plant Material

Certified salt-sensitive wheat (*Triticum aestivum* L. cv. Gemmeza 7) grains were obtained from the Agricultural Research Center, Giza, Egypt.

Seed Germination

Wheat grains were floated on distilled water for 10 minutes, then collected and surface-sterilized by immersing in 5% Clorox for 5 minutes, washed three times with sterilized distilled water, then allowed to germinate on a hormone-free Murashig and Skoog (MS^{-1}) medium (Murashig and Skoog, 1962).

Treatments

NaCl (0, 50, 150 and 300 mM) concentrations were supplemented to the germination medium in combination with proline (0 and 50 mg/L). In all media

combinations, the pH was adjusted to 5.7±1 before autoclaving. Each jar contained 6 wheat grains and replicated 3 times. All jars were kept in a fluorescent white light-illuminated incubator at 27º C (Lercari *et al*., 1986). The fresh and dry weights of the two-week-old seedlings were estimated and recorded.

Estimation of Photosynthetic Pigments

Chlorophyll-A, Chlorophyll-B, and carotenoids were estimated ($ugml^{-1}$) in the fresh foliage leaf of the 2-week-old wheat seedlings. One gram of fresh leaf tissue was extracted by grinding in 10 ml of 80% acetone. The mixture was then centrifuged for 5 min. at 3000 rpm. The supernatant was used for spectrophotometric determination according to the method of Lichtenlhaler (1987).

Protein Electrophoresis

Extraction of total protein

Total protein extracts were prepared by extracting appropriate weight from the frozen fresh plant material in 0.125 M tris/borate, pH 8.9. All the obtained extracts were kept at 4º C for 24 h, and then centrifuged at 10,000 rpm for 20 min. The supernatants were used for electrophoresis.

Gel electrophoresis

Sodium dodcyl sulphate polyacrylamide gel electrophoresis (SDS-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 technique. The gel was scanned with an appropriate Gel-pro-Analyzer.

RESULTS & DISCUSSION

Proline is a proteinogenic amino acid with an exceptional conformational rigidity, and is essential for primary metabolism. Proline accumulation has been reported during conditions of drought (Choudhary *et al*.,2005), high salinity (Yoshiba *et al*.,1995), high light and UV irradiation (Saradhi *et al*., 1995), heavy metals (Schat *et al*.,1999), oxidative stress (Yan *et al*.,2009), and in response to biotic stresses (Fabre *et al*.,2004; Haudecoeur *et al*.,2009).

As shown in Table 1, NaCl (50, 150, 300 mM) reduced the germination % of wheat grains by 12, 48, 53 %, the seedling length by 16, 22, 29 %, the fresh weight by 50, 54, 34% and the dry weight by 38, 49, 59 %, respectively. These inhibitions were mostly alleviated by providing proline (50 ppm) to the MS growth media containing NaCl. The osmoprotective function of proline was discovered first in bacteria, where a causal relationship between proline accumulation and salt tolerance has long been demonstrated (Csonka *et al*.,1988;1991). Such data led to the assumption that proline accumulation in stressed plants has a protective function, which has been emphasized in numerous reviews (Hare *et al*.,1997; Verbruggen *et al*., 2009).

The estimated photosynthetic pigments (Table 2) revealed improvement in chlorophyll-A contents with the added proline (50 ppm) at all NaCl concentrations. Chlorophyll-B was less responsive to the exogenously added proline to the MS growth medium. Carotenoid contents increased with the added proline only at the low concentration of NaCl (50 mM). In general, the total chlorophyll contents were significantly enhanced in response to the exogenously incorporated proline in the growth MS medium. Previous studies found that stabilization of proteins and protein complexes in the chloroplast and cytosol, protection of the photosynthetic apparatus and enzymes involved in detoxification of ROS are an important, but not the only function of proline accumulation during stress. The enhanced rate of proline biosynthesis in the chloroplasts can contribute to the stabilization of redox balance and maintenance of cellular homeostasis by dissipating the excess of reducing potential when electron transport is saturated during adverse conditions. Moreover, proline oxidation can regulate mitochondrial ROS levels and influence programmed cell death. As well as modulating responses to abiotic and biotic stresses, proline appears to function as a metabolic signal that regulates metabolite pools and redox balance, controls the expression of numerous genes and influences plant growth and development.

The SDS-PAGE analysis (Figure 1 & Table 3) of total proteins revealed both qualitative and quantitative changes in the average optical density (O. D.) of protein banding in response to NaCl stress and exogenously added proline. NaCl stress (300 mM) blocked the synthesis of a 98 KDa polypeptide that was restored by the addition of proline. Proline increased the synthesis of 112 and 48 KDa polypeptides at all NaCl concentrations. High NaCl concentrations alone induced the synthesis of new polypeptides of molecular weights 93 and 62 KDa.

For a long time, proline was considered as an inert compatible osmolyte that protects subcellular structures and macromolecules under osmotic stress (Csonka *et al*.,1991; Kavi *et al*.,2005). Proline has been shown to function as a molecular chaperone able to protect protein integrity and enhance the activities of different enzymes. Examples of such roles include the prevention of protein aggregation and stabilization of M4 lactate dehydrogenase during extreme temperatures (Rajen *et al*.,1994) , protection of nitrate reductase during heavy metal and osmotic stress (Sharma *et al*., 2005) and stabilization of ribonucleases and proteases upon arsenate exposure (Mishrai *et al*., 2006). Proline is used for protein synthesis, has protective functions as an osmolyte, contributes to the maintenance of the redox balance, can regulate development and is a component of metabolic signaling networks controlling mitochondrial functions, stress relief and development.

Several studies have attributed an antioxidant feature to proline, suggesting ROS scavenging activity and proline acting as a singlet oxygen quencher (Smirnoff *et al*.,1989; Matysik*et al*., 2002). As an alternative to direct ROS scavenging feature, proline can protect and stabilize ROS scavenging enzymes and activate alternative detoxification pathways. In salt-stressed tobacco cells, proline increased the activities of methylglyoxal detoxification enzymes, enhanced peroxidase, glutathion-S-transferase, superoxide dismutase and catalase activities, and increased the glutathione redox state (Hoque *et al*.,2008; Islam *et al*.,2009) . In the desert plant *Pancratium maritimum*, catalase and peroxidase were found

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to be stabilized by proline during salt stress (Khedr *et al*.,2003). The application of system biology approaches might help to understand regulation of proline-dependent and proline-mediated signaling in plants.

Table 1: Germination percentage and vegetative growth parameters of wheat (*Triticum aestivum* L.) seedlings subjected to NaCl (50, 150 and 300 mM) or NaCl + 50 ppm proline on MS medium or MS medium + 50 ppm proline (values are means of 3 replicates ± SD).

Treatment	Germination %	Seedling		
		Length (cm)	F. Wt. (g)	D. Wt. (g)
1	71±1.73	16.16±0.19	0.25±0.008	0.163±0.057
2	42±2.65	15.00±0.45	0.23±0.012	0.133±0.070
3	38±2.65	13.66±0.64	0.33±0.004	0.103±0.080
4	74±1.73	17.30±0.42	0.35±0.006	0.221±0.011
5	57±3.00	17.26±0.36	0.27±0.006	0.183±0.011
6	47±2.00	14.20±0.60	0.15±0.021	0.092±0.015
7	80±2.64	19.20±0.44	0.50±0.072	0.260±0.011
8	90±2.83	21.33±0.68	0.51±0.062	0.290±0.063

1,2, 3 = NaCl-stressed (50, 150, 30 mM) wheat plants, 5, 6 = NaCl-stressed (50, 150, 30 mM) wheat plants + 50 ppm proline, 7 = MS medium, 8 = MS medium + 50 ppm proline

Table 2: Contents (µgml^{-1}) of photosynthetic pigments in leaves of wheat (*Triticum aestivum* L.) plants subjected to NaCl (50, 150 and 300 mM) or NaCl + 50 ppm proline. 7, MS medium; 8, MS + 50 ppm proline.

Treatment	Chlorophyll A	Chlorophyll B	Carotenoids	Total chlorophyll
1	0.050	0.065	0.018	0.116
2	0.041	0.055	0.014	0.097
3	0.041	0.088	0.013	0.092
4	0.063	0.058	0.024	0.121
5	0.054	0.115	0.026	0.169
6	0.059	0.093	0.028	0.152
7	0.054	0.084	0.035	0.138
8	0.055	0.081	0.036	0.136

1,2, 3 = NaCl-stressed (50, 150, 30 mM) wheat plants, 4, 5, 6 = NaCl-stressed (50, 150, 30 mM) wheat plants + 50 ppm proline, 7 = MS medium, 8 = MS medium + 50 ppm proline

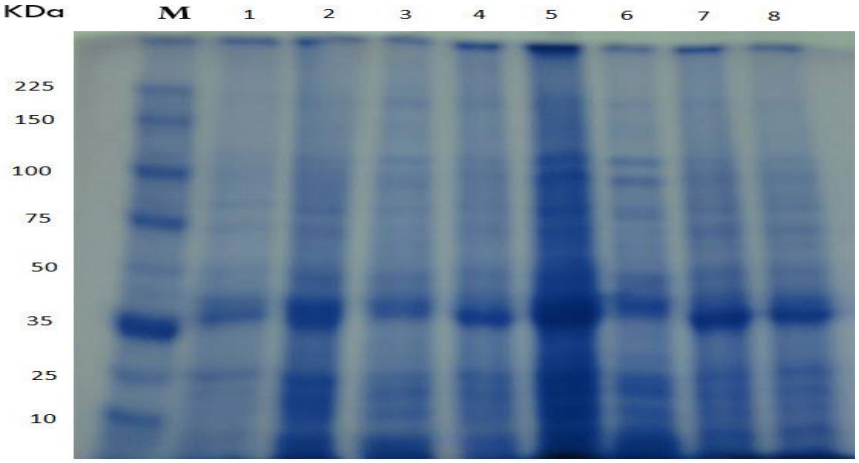

Figure 1. Electrophotograph of SDS-PAGE of total proteins of NaCl-stressed (1, 2, 3) and NaCl-stressed + 50 ppm proline (4, 5, 6) wheat (*Triticum aestivum* L.) plants subjected to 50, 150 and 300 mM NaCl. MS medium (7) , MS medium + 50 ppm proline (8) and M = molecular weight markers used on polyacrylamide gel.

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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 wheat (*Triticum aestivum* L.) plants subjected to NaCl or NaCl + proline.

Band Number	Treatment & Optical Density (O. D.)								R_f	Mol. Wt. (KDa)
	1	2	3	4	5	6	7	8		
1	07.50	08.12	15.11	13.19	07.29	O8.65	08.06	06.89	0.16	204.60
2	10.09	04.06	12.85	13.54	10.77	28.43	05.16	04.13	0.30	112.40
3	15.33	10.31	-	18.96	13.47	30.14	14.12	10.54	0.34	98.30
4	-	10.15	13.70	-	-	-	-	-	0.36	93.40
5	09.67	09.16	08.38	07.13	09.49	19.40	12.01	12.62	0.42	82.20
6	11.94	10.40	10.54	12.67	08.15	-	17.88	12.27	0.46	71.70
7	-	-	05.70	-	-	-	-	-	0.50	62.10
8	19.60	07.30	32.66	25.58	24.21	49.65	07.47	10.12	0.57	48.20
9	75.42	52.80	64.17	80.45	44.21	76.45	65.16	72.83	0.65	40.20
10	22.80	12.31	08.96	12.79	37.06	10.03	04.25	15.54	0.80	25.30
11	05.98	-	18.90	05.75	07.09	29.30	04.25	15.54	0.83	19.40
12	-	09.01	10.11	05.16	04.41	06.36	02.85	05.60	0.87	12.30
13	14.55	20.34	40.05	61.08	56.52	32,9	03.07	12.41	0.93	06.82
Band/Lane	10	11	12	11	11	10	11	11		

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