Journal of Applied Sciences

ISSN 1812-5654

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 5 (4): 655-658, 2005
ISSN 1812-5654

Amino Acids in Grains of Barley Plants as Affected by Benzyl Adenine and Salinity from Diluted Sea Water

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Abstract: Grains of barley (*Hordeum vulgare* L.) Cv. Giza 124 were treated by benzyl adenine and grown under different salinity levels (2000, 4000 and 6000 ppm of diluted seawater) where the concentration of different amino acids were determined. Phenyl alanine, serine, valine and glutamic acids increased in grains of plants subjected to 2000 ppm salts and tended to decrease with the higher salt levels. Proline, isoleucine and tyrosine concentrations increased by increasing salt concentration in the irrigation water up to 4000 ppm and then lowered in grains of plants treated with 6000 ppm salt solution. Threonine, serine, isoleucine, leucine, tyrosine and glutamic acids increased, however, proline and phenyl alanine concentration were progressively increased by spraying benzyl adenine at the rate of 50 ppm. On the other hand arginine, lysine, histidine and aspartic acids showed a significant decrease. Benzyl adenine application led to decrease in the concentration of all amino acids in grains irrigated by saline water 2000 ppm salts and significant increase at 6000 ppm compared to the control (tap water).

Key words: Barley, salinity, seawater, benzyl adenine, grains, amino acids

INTRODUCTION

Salinity is threatening civilization by persistently reducing the area for crop cultivation. To achieve optimal food production under salinity stress condition the most appropriate logical choice is growing salt-tolerant varieties using a suitable technology that saves the properties of grain yield. The most evident effect of salinity is disturbances in growth and growth is affected by phytohormones. Phytohormones are the signals sent between root and shoot, triggering responses to external stress[1]. Plant growth regulators (plant hormones) play an important role in growth and development of root and its response to external stimuli[2,3].

The endogenous hormonal balance has an important regulating role in the response of plants to salinity and it may be possible to ameliorate the endogenous balance by application of exogenous hormones. In various crop species, a decrease in protein level in salt stressed plant parts is attributed to a decrease in protein synthesis and availability of amino acid as denaturation of the enzymes involved in amino acid and protein synthesis and translocation. Treatments with hormones can counteract, to a certain extent, the adverse effect of salinity. Despite the limited information regarding the relation between salinity, amino acid concentration in grains cereals and growth regulators.

Thus the present study planned to study the effect of salinity stress in addition to Benzyl adenine application on amino acid concentration in barley grains.

MATERIALS AND METHODS

A pot trial was executed on Barley variety Giza 124 grown in clay loam soil during 2000/2001 at the greenhouse of the National Research Centre, Dokki, Cairo, Egypt.

Metallic pots of 35 cm diameter and length of 50 cm were filled with 20 kg of air dried clay loam soil.

The inner surface of pots were coated with three layers of bitumen to prevent direct contact between the soil and metal Barley grains (*Hordeum vulgare* L.) Cv. Giza 124 were sown on late November at the rate of ten seed pot^{-1}. All pots received recommended dose of NPK fertilizers (2.29 g of calcium superphosphate 16% P_2O_5, 1.14 g potassium sulphate, 48.5% K_2O and 6.86 g ammonium sulphate 20.5% N) in two equal portions, once on sowing and the other two weeks later. After two weeks

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plants were thinned to three seedlings pot^{-1}, seedlings were subjected to different salinity levels (sea water dilution) 2000, 4000, 6000 ppm of 30,000 ppm.

Each pot was replicated four times and arranged in a complete randomized design. A foliar application of Benzyl adenine was used of 50 ppm which was sprayed twice during plant life cycle.

The design of the experiment was as follows:

- Control (using tap water).
- Dilution of sea water to 2000, 4000, 6000 ppm.
- Tap water+Benzyl adenine.
- Salinity levels+foliar application of Benzyl adenine 50 ppm.

At harvest, grains were collected for estimation of amino acid concentration µg/mL the method was described by the AOAC[4] using LC 2000 amino acid analyzer. The means of data were statistically analyzed using Chi-square test according to Snedecor and Cochran[5].

RESULTS AND DISCUSSION

Salinity stress affected free amino acids in barley grains and the use of benzyl adenine didn't show a clear trend for amino acid constituent.

Obtained results, showed that high salinity level led to accumulation of (aspartic, glutamic, proline, leucine, tyrosine, histidine, lysine, NH_4 and arginine) while threonine, serine, glycine, cystin, alanine, valine, isoleucine and phenyl alanine didn't show any significant increase in grains of barley (Table 1). Rabe[6] found that a number of N-containing compounds (NCCs) accumulate in plants subjected to environmental stress. The most frequently accumulating NCCs include amides (glutamine and asparagines), amino acids (arginine, proline, citrulline, ornithine) and the amine putrescine. Any stress condition causing glucose depletion and/or reduced growth or impaired plant health will result in NH_3-NH_4 accumulation early in the stress period. The detoxification process in which excess of free ammonia in the cells is removed results in the accumulation of NCCs. The specific NCCs that accumulate are determined by the plant species affected and the nature of stress.

Yamaya and Matsumoto[7] found that growing barley under NaCl stress (50 mM) accumulated aspargine in barley seedling roots by 28 % xylem sap by 48% and leaf blades 32%, but did not affect proline accumulation, other amino acids (glutamate, alanine and serine) were also increased by NaCl stress but glutamine was decreased in the xylem sap.

Yasseen *et al.*[8] estimated proline accumulation in the endosperm and radicals of barley as NaCl concentration increased in the growing medium. They concluded that proline conc./mg degraded endosperm protein increased consistently with increasing salinity, indicating that conversion of some amino acids to proline could be taking place. Also Sanda *et al.*[9] proved that proline has bifunctional role in the acclimatization to high salt stress, an osmoregulant role in the light and as a substrate for dark respiration to supply energy to perform the compartmentation of ions into the vacuole in the dark. Similarly Fedine[10] observed proline accumulation in barley seedling leaves treated with 150 mM NaCl which was promoted in the light and suppressed in the dark.

Table 1: Variation of amino acid concentration ($\mu g\ mL^{-1}$) in barley grains grown under salinity stress in addition to Benzyl adenine foliar application

		Salinity			Salinity + BA					
Amino acids	Tap water	2000	4000	6000	Tp + BA	2000 + BA	4000 + BA	6000 + BA	Chi-square	
Aspartic	14.51	13.62	11.53	16.14	8.84	7.85	44.68	31.78	62.42	**
Threonine	2.78	5.21	5.07	2.37	4.04	3.23	10.03	5.83	8.75	NS
Serine	4.75	8.21	7.92	4.68	6.40	5.11	16.67	9.13	13.85	NS
Glutamic	19.15	65.82	57.56	21.49	45.52	41.94	54.24	40.38	44.02	**
Proline	3.37	25.49	22.77	3.87	24.51	16.31	10.31	10.18	38.17	*
Glycine	5.08	7.47	7.10	5.62	5.66	4.44	15.78	11.18	13.31	NS
Cystin	---	---	---	0.0084	0.0048	0.00113	0.0004	0.00058	0.03	NS
Alanine	5.10	7.38	6.79	5.52	5.04	4.64	14.90	10.62	11.80	NS
Valine	0.86	1.86	0.03	2.07	2.76	2.13	5.89	2.59	9.10	NS
Isoleucine	1.57	2.79	3.65	2.05	2.10	1.71	5.06	4.30	4.05	NS
Leucine	5.08	8.95	2.40	7.24	7.29	5.98	15.30	13.58	15.8	*
Tyrosine	2.22	5.31	8.50	2.67	9.17	3.32	5.37	5.39	10.9	*
P. alanine	2.48	9.66	5.03	3.22	6.98	5.76	8.77	7.70	7.37	NS
Histidine	6.27	7.44	7.16	7.24	5.83	5.01	18.67	13.37	17.99	**
Lysine	5.57	4.28	4.06	6.72	3.20	2.72	18.54	12.40	29.49	*
NH_4	12.37	39.21	33.72	15.48	24.64	24.31	31.69	28.05	21.83	*
Arginine	6.76	5.03	4.50	8.49	3.44	2.61	6.96	18.14	24.10	*

Tp = Tap water, BA = Benzyl adenine, Tabulated = 14.07, calculated at 5% level. **: highly significant *: significant

However, using benzyl adenine as a foliar application, under salinity stress, significantly increased the accumulation of aspartic, threonine, serine, glycine, alanine, valine, isoleucine, leucine, phenyl alanine, histidine lysine at 4000 ppm while arginine increased at 6000 ppm.

On the other hand, benzyl adenine application reduced glutamic acid, proline, tyrosine and NH_4 under the same salinity level 4000 ppm. Some of the researchers has supported the use of benzyl adenine, other did not recommend its application.

Kuiper *et al.*[11] found that the addition of BA made salt resistant varieties behave as salt sensitive varieties, as they concluded that addition of BA under saline condition inhibited growth to some extent and enhanced cytokinin concentration. Also, Ma *et al.*[12] mentioned that growth regulator did not alter protein yield, lysine and amino acid composition in barley grains.

Kuiper *et al.*[13] showed that BA treatment adversely affected growth of sensitive barley cultivar, while for salt resistant cultivar, addition of BA temporarily overcame the sensitive one. However, Shariatoanahi and Dobsirashraf[14] found that addition of 2 mg BA L^{-1} calluses produced 28 barley cultivars tolerant to salinity. Also, Zhu *et al.*[15] deduced that salinity increased leaf putrescine levels and application of BA decreased leaf putrescine and increased leaf spermine. While, Leinhoss *et al.*[15] approved that water stress increased plant content of glycine betaine, trigonelline, putrescine and lignin. Treatments with BA reduced accumulation of the these compounds in grains of barley. Also, Stewart *et al.*[17] and Riazi *et al.*[18] found that salt shocked barley plant treated with BA inhibited proline accumulation when added at the start or after salt treatment.

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