Changes in growth and nitrogen assimilation in maize plants induced by NaCl and growth regulators

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Abstract

Experiments were conducted to determine the interactive effects of salinity and certain growth regulators on growth and nitrogen assimilation in maize (*Zea mays* L. cv. GS-2). 100 mM NaCl inhibited the biomass accumulation, chlorophyll and carotenoid contents in leaves, nitrate content and uptake and nitrate reductase activity. The application of kinetin, ascorbic acid and 10 and 50 μM abscisic acid in the first experiment and 50 and 100 μM abscisic acid in the second experiment induced a substantial increase in the above parameters, the effect was highest with abscisic acid in salinized as well as non-salinized plants.

Additional key words: salinity, nitrate reductase activity, *Zea mays* L.

Introduction

Salinity effects on crops are well documented, although the mechanisms involved are not fully understood (Flowers *et al.* 1977, Greenway and Munns 1980). Addition of NaCl in the nutrient medium reduces growth (*e.g.*, Lewis *et al.* 1989), nitrate uptake and transport (*e.g.* Klobus *et al.* 1988, Gouia *et al.* 1994) as well as nitrate reductase (NR) activity (Rao and Gnanam 1990, Lips *et al.* 1990, Kanjebaeva and Rakova 1995) in many plant species.

Growth regulators have been implicated in mediating changes in many plant processes including some aspects of nitrate assimilation (Kumar *et al.* 1993, Kamínek *et al.* 1994, Pandey and Srivastava 1994). Soaking seeds in ascorbic acid increases *in vivo* NR activity in *Zea mays* L. (Asthana and Srivastava 1978). Similarly, cytokinin application affects NR activity in many species (Trekova *et al.* 1992, Kamínek *et al.* 1994, Pandey and Srivastava 1994). Although the abscisic acid is usually considered as growth inhibitor, some reports indicated that it is essential for normal growth and development (Addicott 1984) and also it acts as a signal of salinity stress

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(*e.g.*, Davies and Zhang 1991). As far as the N assimilation is concerned, ABA has been reported to have no effect on NR activity.

The present investigation was undertaken to study the responses of maize plants to the interaction between salinity and certain growth regulators, particularly ABA. It is expected that the study will provide some insight into the possible mechanism of action of salinity on nitrogen assimilation.

Materials and methods

Plants and experimental conditions: Maize (*Zea mays* L. cv. GS-2) seeds were surface sterilized with 1 % sodium hypochlorite solution and sown in plastic pots containing 2 kg sterilized sandy loam soil. After emergence of seedlings, each pot was irrigated with 1/2 strength Hoagland's solution containing 6 mM NO_3 as sole N source, with or without NaCl (control). Seedlings grew under controlled conditions (12-h photoperiod and an irradiance of 60 W m^{-2}).

In the first experiment, plants were treated with three growth regulators, *i.e.*, ascorbic acid, kinetin and abscisic acid (10 and 50 μM). In the second one, plants were treated only with abscisic acid (50 and 100 μM). Three replicates for each treatment were maintained.

Two week-old plants were harvested, divided into roots and shoots, and dried in a hot-air oven at 60 °C for 48 h.

Pigment composition: Total chlorophyll and carotenoid contents in leaves were estimated in 80 % acetone extracts by a *Beckman DU* spectrophotometer (Nyon, Switzerland) at 664 and 440 nm, respectively, according to Strain and Svec (1966).

Nitrate content: Nitrate concentration in plant tissues was determined spectrophotometrically using salicylic acid nitration method (Cataldo *et al.* 1975).

Nitrate uptake: Intact plants were taken from each treatment and kept in nitrate solution (6 mM) for 4 h. Nitrate contents in these plants were determined by a nitrate ion meter *Model 290* (*Orion Res. Inc.*, Boston, USA). The difference in nitrate content before and after immersion was considered as uptake by plants.

Nitrate reductase activity: NR activity was determined in leaf and root samples separately following the methods published earlier. In the roots, both isoforms (NADH:NR and NADPH:NR) were determined, and in the leaves only NADH:NR was assayed. The enzyme was extracted in 0.1 M phosphate buffer (pH 7.5) containing 1 mM EDTA, 5 mM cystein, 0.5 % bovine serum albumin and was assayed *in vitro* following the reduction of nitrate to nitrite using either NADH (Srivastava and Ormrod 1984) or NADPH (Shankar and Srivastava 1997) as a reductant.

Results

Biomass production: The dry masses of root and shoot in maize plants were substantially decreased in response to salinity, in control as well as in plants treated with growth regulators, however, the salinity caused reduction was higher in control plants (Table 1). Similarly, the root/shoot ratio was also variable following the decrease in dry masses. On the other hand, the growth regulators stimulated biomass accumulation. The effect of kinetin was comparatively higher than that of other regulators applied (Table 1).

Table 1. Root and shoot dry masses, DM [mg plant^{-1}] in maize plants as affected by NaCl and growth regulators [mM] (means ± SE; $n = 5$).

NaCl	Growth regulators		Root DM	Shoot DM	Total DM	Root/shoot
0			85 ± 8	385 ± 30	470 ± 46	0.22
100			50 ± 7	282 ± 25	332 ± 33	0.18
0	ascorbic acid	0.01	90 ± 9	398 ± 35	488 ± 48	0.23
100	ascorbic acid	0.05	65 ± 8	290 ± 30	355 ± 31	0.23
0	ABA	0.01	108 ± 9	435 ± 38	543 ± 50	0.25
100	ABA	0.05	80 ± 8	315 ± 29	395 ± 38	0.25
0	kinetin	0.01	92 ± 9	400 ± 28	492 ± 44	0.23
100	kinetin	0.05	70 ± 7	301 ± 30	371 ± 35	0.23

Pigment composition: Total chlorophyll contents declined due to the presence of NaCl in nutrient medium (Table 2). The reduction was also seen in carotenoid content although to a lower extent. The salinity caused reduction in pigment contents was substantially lower in plants fed with any growth regulator (Table 2) and this effect became more evident when ABA was supplied to those plants (Table 2).

Table 2. Pigment contents [mg g^{-1}(DM)] in maize plants as affected by NaCl and growth regulators [mM] (means ± SE; $n = 5$).

NaCl	Growth regulators		Chlorophyll *a*+*b*	Carotenoids
0			12.25 ± 0.81	5.75 ± 0.05
100			10.07 ± 0.52	5.75 ± 0.05
0	ascorbic acid	0.01	13.38 ± 0.92	6.10 ± 0.07
100	ascorbic acid	0.05	11.17 ± 0.52	5.73 ± 0.05
0	ABA	0.01	14.45 ± 1.20	6.55 ± 0.06
100	ABA	0.05	11.89 ± 0.93	5.80 ± 0.06
0	kinetin	0.01	13.90 ± 1.11	6.30 ± 0.06
100	kinetin	0.05	11.10 ± 0.61	5.78 ± 0.05

Nitrate contents: Nitrate content was substantially reduced in roots and shoots of plants under 100 mM NaCl. However, this reduction was more marked in shoots than in roots. The effects of ABA on nitrate content was higher than those of other growth regulators in both salinized as well as non salinized plants (Tables 3 and 4).

Nitrate uptake: Salinity inhibited the nitrate uptake to a large extent. ABA moderated the salinity induced reduction in respective N uptake rates more substantially than other growth regulators (Table 3). Moreover, abscisic acid at both concentrations promoted NO_3 uptake rates.

Table 3. Nitrate contents, NC [μmol(NO_3) g^{-1}(DM)] and uptake rates [μmol(NO_3) g^{-1}(DM)] in maize plants as affected by NaCl and growth regulators [mM] (means ± SE; n = 5).

NaCl	Growth regulators		Nitrate uptake	Root NC	Shoot NC
0			17.20 ± 1.60	36.05 ± 3.58	52.00 ± 5.00
100			11.84 ± 1.65	26.70 ± 2.57	34.45 ±3.40
0	ascorbic acid	0.01	19.20 ± 1.81	39.45 ± 3.90	60.15 ± 5.88
100	ascorbic acid	0.05	13.60 ± 1.25	27.15 ± 2.70	37.15 ± 3.65
0	ABA	0.01	23.80 ± 2.20	45.08 ± 4.50	66.30 ± 6.60
100	ABA	0.05	15.30 ± 1.40	32.07 ± 3.19	45.10 ±4.40
0	kinetin	0.01	20.40 ± 2.10	43.15 ± 4.30	61.15 ± 6.00
100	kinetin	0.05	13.60 ± 1.30	28.23 ± 2.75	43.05 ± 4.28

Table 4. Nitrate contents, *NC [μmol(NO_3) g^{-1}(DM)] and uptake rates [μmol(NO_3) g^{-1}(DM)] in maize* plants as affected by NaCl and ABA [mM] (means ± SE; n = 5).

NaCl	ABA	Nitrate uptake	Root NC	Shoot NC
0	0	16.30 ± 1.65	36.01 ± 3.51	45.15 ± 4.48
0	0.05	21.40 ± 2.12	43.15 ± 4.28	52.00 ± 4.80
0	0.10	23.25 ± 2.16	44.75 ± 4.37	52.85 ± 4.80
100	0	11.85 ± 1.25	23.25 ± 2.25	32.15 ± 3.00
100	0.05	13.95 ± 1.30	29.45 ± 2.73	38.65 ± 3.75
100	0.10	16.25 ± 1.58	30.10 ± 3.00	39.12 ± 3.80

***In vitro* nitrate reductase activity:** The presence of NaCl in the nutrient medium caused a decline in NR activity (NR:NADH) in both root and leaf (Tables 5 and 6). The higher levels of NR:NADPH than NR:NADH was found in plant roots, whereas in leaves, the NR:NADPH levels were non detectable. On both isoforms, the effect of growth regulators was promotive.

Discussion

The salinity depressed the growth, chlorophyll and carotenoid contents in leaves, nitrate uptake and accumulation as well as NR activity in 2-week-old maize plants. Similar reduction in growth (*e.g.* Flowers *et al.* 1977), N uptake (*e.g.* Klobus *et al.* 1988, Gouia *et al.* 1994) and NR activity (*e.g.* Srivastava 1980, Kohler *et al.* 1992, Kanjebaeva and Rakova 1995) have been reported in many crop species with certain exceptions (Misra and Dwivedi 1990). As NR is a substrate inducible (Beevers and Hageman 1969, Srivastava 1980), any factor may change the NR activity by restricting the nitrate availability. Saini *et al.* (1985) demonstrated that NR activity shows a positive interaction with KNO_3 and growth regulators. Moreover, it has also been indicated that the presence of salts (KCl and NaCl) has a strong synergistic interaction with ABA (Pesci and Buffagna 1986). In our exeriments, nitrate uptake was drastically inhibited by NaCl (100 mM) which might be the cause of reduced NR activity and biomass. This interpretation is, however, based on our previous studies on NR kinetics in maize (Khan and Srivastava, unpublished data) which indicated that NaCl inhibited NO_3 uptake in a non-competitive manner. Moreover, we also presume that the actions of applied growth regulators is at nitrate uptake level.

Table 5. Nitrate reductase (NR) activity [μmol(NO_2) g^{-1}(FM) h^{-1}] in maize plants as affected by NaCl and growth regulators [mM] (means ± SE; n = 5).

NaCl	Growth regulators		Root NR	Leaf NR
0			3.66 ± 0.35	5.55 ± 0.52
100			2.95 ± 0.28	3.70 ± 0.35
0	ascorbic acid	0.01	4.65 ± 0.40	5.80 ± 0.55
100	ascorbic acid	0.05	3.20 ± 0.30	4.85 ± 0.47
0	ABA	0.01	5.35 ± 0.50	6.45 ± 0.63
100	ABA	0.05	4.75 ± 0.45	5.20 ± 0.50
0	kinetin	0.01	4.40 ± 0.40	5.70 ± 0.55
100	kinetin	0.05	3.30 ± 0.40	4.05 ± 0.38

Table 6. Nitrate reductase (NADH:NR and NADPH:NR) activity [μmol(NO_2) g^{-1}(FM) h^{-1}] in maize plants as affected by NaCl and ABA [mM] (means ± SE; n = 5).

NaCl	ABA	Root NADH:NR	Leaf NADH:NR	Root NADPH:NR
0	0	3.90 ± 0.38	5.74 ± 0.55	5.75 ± 0.55
0	0.05	4.10 ± 0.40	6.50 ± 0.64	6.50 ± 0.64
0	0.10	4.25 ± 0.41	6.70 ± 0.66	6.65 ± 0.65
100	0	3.05 ± 0.30	4.50 ± 0.44	4.85 ± 0.47
100	0.05	3.50 ± 0.34	5.10 ± 0.50	5.20 ± 0.50
100	0.10	3.75 ± 0.35	5.40 ± 0.52	5.30 ± 0.51

Plants exhibited a rise in nitrate uptake and reduction by growth regulators in general and ABA in particular. The reasons for the partial moderation of salinity effects may be due to use of N for synthesis of osmotically active compounds.

The growth regulators may also alleviate salinity stress by influence on the antioxidant system of the plants. The plants may respond to salinity by free radical generation (Rao and Ormrod 1996). Abscisic acid, and perhaps other growth regulators may either prevent the formation of free radicals or scavange the radicals generated.

Although there are numerous reports on the growth inhibition by ABA, the specific role of ABA has been questioned for root elongation (Mulkey *et al.* 1983, Pillet and Reveaud 1983). In addition, reports also confirmed that rapidly growing shoots contain high levels of endogenous ABA (Zeevart and Creelman 1990). The results of our second experiment demonstrate a marked impact of ABA in non-stressed plants. This enabled us to state that the response of ABA is rather complex, as it is likely influenced by several interactions, *e.g.*, with nutrients.

In conclusion, our data clearly demonstrate the salinity induced changes in growth and N assimilation in maize plants could be alleviated, at least in part, through application of certain growth regulators, particularly ABA.

References

Addicott, F.T.: Abscisic acid. - Praeger Press, New York 1984.

Asthana, J.S., Srivastava, H.S.: Effects of presowing treatment of maize seeds with ascorbic acid and salicylic acid on seed germination, seedling growth and nitrate assimilation in the seedling. - Indian J. Plant Physiol. **21**: 150-155, 1978.

Beevers, L., Hageman, R.H: Nitrate reduction in higher plants. - Annu. Rev. Plant Physiol. **20**: 495-522, 1969.

Cataldo, D.A., Haroon, M., Schrader, L.E., Youngs, V.L.: Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. - Commun. Soil Sci. Plant Anal. **6**: 71-80, 1975.

Davies, W.J., Zhang, J.: Root signal and the regulation of growth and development of plants in drying soil. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 55-76, 1991.

Flowers, T.J., Troke, P.F., Yeo, A.R.: The mechanisms of salt tolerance in halophytes. - Annu. Rev. Plant Physiol. **28**: 89-121, 1977.

Gouia, H., Ghorbal, M.H., Touraine, B.: Effects of NaCl on flows of N and mineral ions and on NO_3 reduction rate within whole plants of salt sensitive bean and salt tolerant cotton. - Plant Physiol. **105**: 1409-1418, 1994.

Greenway, H., Munns, R.: Mechanisms of salt tolerance in non-halophytes. - Annu. Rev. Plant Physiol. **31**: 149-190, 1980.

Kanjebaeva, S., Rakova, N.: Multiple forms of nitrate reductase and their role in nitrate assimilation in roots of wheat at low temperature or high salinity. - Physiol. Plant. **93**: 249-252, 1995.

Kamínek, M., Treková, M., Motyka, V., Gaudinová, A.: The role of cytokinins in the control of wheat grain development and the utilization of nutrients. - Biol. Plant **36** (Suppl.): 315, 1994.

Klobus, G., Ward, M.R., Huffaker, R.C.: Characteristics of injury and recovery of net NO_3 transport of barley seedlings from treatments of NaCl. - Plant Physiol. **87**: 878-882, 1988.

Kohler, K.H., Schmmerder, B., Sheikhany, H.: The effect of water stress on the germination and nitrate reductase activity in imbibing *Agrostemma githago* seeds. - J. Plant Physiol. **139**: 528- 532, 1992.

Kumar, P.A.M., Kumaran, L., Abrol, Y.P.: Hormonal regulation of nitrate reductase gene expression in *Hordeum vulgare*. - Indian J. exp. Biol. **31**: 472-473, 1993.

Lewis, O.A.M., Leidi, E.O., Lips, S.H.: Effect of nitrogen source on growth response to salinity stress in maize and wheat. - New Phytol. **111**: 155-160, 1989.

Lips, S.H., Leidi, E.O., Silberbush, M., Soares, M.I.M., Lewis, O.A.M.: Physiological aspects of ammonium and nitrate fertilisation. - J. Plant Nutr. **13**: 1271-1289, 1990.

Misra, N., Dwivedi, U.N.: Nitrogen assimilation in germinating *Phaseolus aureus* seeds under saline stress. - J. Plant Physiol. **135**: 719-724, 1990.

Mulkey, T.S., Evans, M.L., Kusumanoff, K.M.: The kinetics of abscisic acid action on root growth and gravitropism. - Planta **157**: 150-157, 1983.

Pandey, S., Srivastava, H.S.: Cytokinin and nitrate mediated expansion of *Leucaena leucocephala* cotyledons. - Indian J. Plant Physiol. **37**: 246-250, 1994.

Pesci, P., Beffagna, N.: Influence of exogenously supplied potassium and sodium salts on the abscisic acid induced proline accumulation in barley leaf segments. - Physiol. Plant. **87**: 123-128, 1986.

Pillet, P E., Rebeaud, J.E.: Effect of abscisic acid on growth and indole-3-acetic acid levels in maize roots. - Plant Sci. Lett. **31**: 117-122, 1983.

Rao, R.K., Gnanam, A.: Inhibition of nitrate and nitrite reductase activities by salinity stress in *Sorghum vulgare*. - Phytochemistry **29**: 1047-1049, 1990.

Rao, M.V., Ormrod, D.P.: Antioxidative enzymes: the missing link between physiological, biochemical and molecular responses. - Physiol. mol. Biol. Plants **2**: 99-100, 1996.

Saini, H.S., Bassi, P.K., Spencer, M.S.: Seed germination in *Chenopodium album*: further evidence for the dependence of the effects of the growth regulators on nitrate. - Plant Cell Environ. **8**: 707-711, 1985.

Shankar, N., Srivastava, H.S.: The responses of NADH and NAD(P)H specific nitrate reductase activity and nitrate and oxygen uptake to nitrate supply in maize seedlings. - Biol. Plant. **41**, in press, 1998.

Srivastava, H.S.: Regulation of nitrate reductase activity in higher plants. - Phytochemistry **19**: 725-791, 1980.

Srivastava, H.S., Ormrod, D.P.: Effect of nitrogen dioxide and nitrate nutrition on growth and nitrate assimilation in bean leaves. - Plant Physiol. **76**: 418-423, 1984.

Strain, H.H., Svec, W.F.: Extraction, separation, estimation and isolation of chlorophylls. - In: Vernon, L.P., Seeley, G.R. (ed.): The Chlorophylls. Pp. 21-26. Academic Press, New York 1966.

Treková, M., Kamínek, K., Zmrhal, Z.: Grain formation and distribution of nutrients in wheat plants after the application of the synthetic cytokinin (N-metahydroxybenzyladenosine). - In: Kamínek, M., Mok, E.W.S., Zažímalová, E. (ed.): Physiology and Biochemistry of Cytokinins in Plants. Pp. 241-244, SPB Academic Publishing, The Hague 1992.

Zeevart, J.A.D., Creelman, R.A.: Metabolism and physiology of abscisic acid. Annu. Rev. Plant Physiol. Plant mol. Biol. **39**: 439-475, 1990.