ACTA BIOLOGICA CRACOVIENSIA Series Botanica 51/2: 119–125, 2009

EFFECT OF ETHEPHON AND GIBBERELLIN A_3 ON *AMARANTHUS CAUDATUS* SEED GERMINATION AND α- AND β-AMYLASE ACTIVITY UNDER SALINITY STRESS

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Received July 27, 2008; revision accepted October 15, 2009

This study assessed the effects of different doses of ethephon and gibberellin A_3 on germination and α- and β-amylase activity in *Amaranthus caudatus* seeds exposed to different levels of salt stress. NaCl at 25 and 50 mM only delayed germination; at 75, 100 and 125 mM it caused 50%, 90% and 99.5% inhibition of *Amaranthus caudatus* seed germination. Both ethephon and GA_3 (0.01, 0.1, 0.3 mM) effectively counteracted inhibition of seed germination under salinity. The stimulatory effect of ethephon appeared earlier, and the seeds were more sensitive to ethephon than to GA_3. Ethephon enabled seed germination in the presence of all NaCl concentrations (75, 100, 125 mM) even after 24 h. GA_3 alleviated inhibition caused by 75 and 100 mM NaCl until 48 h and did not affect reduction of germination caused by NaCl at 125 mM. NaCl (100 mM) reduced α- and β-amylase activity and seed germination after 14 h, and enhanced α-amylase activity after 20 h, although germination was reduced. Ethephon and GA_3 increased α- but not β-amylase activity under salt stress during the first 14 h of incubation.

Key words: *Amaranthus caudatus* seeds, α-, β-amylase activity, ethephon, germination, gibberellin A_3, NaCl.

INTRODUCTION

Salinity is one of the major environmental stresses for plants (Greenway and Munns, 1980). Based on their ability to grow on salt medium, plants, including crop species, are traditionally classified as glycophytes, showing the effects of salt at concentrations less than 50 mM, or halophytes which can complete their life cycles at 500 mM (Flowers, 1985; Maas, 1986). Although glycophytes generally are more sensitive to saline stress, they range widely in tolerance between species and varieties (Greenway and Munns, 1980). Plant growth is affected by salinity at all stages of development, but sensitivity varies greatly at different stages (Caravajal et al., 1998; Akram et al., 2002; Akinci et al., 2004). Germination is a critical stage in the growth cycle of plant species; it determines plant establishment and final crop production (Pearen et al., 1997). Increasing salinity generally reduces germination of glycophytes (Basalah and Mohammad, 1999; Almansouri et al., 2001; Akinci et al., 2004; Jamil et al., 2006; Siddiqui et al., 2006). This reduction is caused by two components of salt stress: osmotic effects due to restricted supply of water, creating water stress, and ionic effects due to seed ion uptake and/or accumulation (Ungar, 1996; Dodd and Donovan, 1999; Almansouri et al., 2001). Both components of salt stress can reduce seed germination by limiting water absorption (Dodd and Donovan, 1999; Duan et al., 2004), altering the mobilization of stored reserves (Lin and Kao, 1995), or other metabolic and molecular responses (Ramagopal, 1990).

α-mylases (E.C. 3.2.1.1), which randomly attack the internal α-D-(1→4) O-glucosidic linkages, and β-amylases (E.C. 3.2.1.2), which hydrolyze α-D-(1→4) O-glucosidic linkages from the non-reducing end, are enzymes that play a major role in the mobilization of insoluble starch granules (Duedahl-Olesen et al., 2000). Under salinity, decreased α-amylase activity has been reported in *Hordeum distichum* (Tipirdamaz et al., 1995), *Triticum aestivum* (Siddiqui et al., 2006), *Triticum durum* seeds (Almansouri et al., 2001), *Vigna radiata* cotyledons (Promila and Kumar, 2000) and

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Abbreviations: DNSA – dinitrosalicylic acid reagent; GA_3 – gibberellin A_3.

PL ISSN 0001-5296

Cicer arietinum seedlings (Kaur et al., 1998), and increased β-amylase activity in *Triticum durum* seeds (Almansouri et al., 2001).

Plant growth regulators such as exogenous ethylene (Li et al., 1995; Basalah and Mohammad, 1999; Zhenguo and Jundi, 2001) or gibberellic acid (GA_3) (Kumar and Singh, 1996; Kaur et al., 1998; Basalah and Mohammad, 1999) have been shown to alleviate salinity-induced inhibition of seed germination of glycophytic plants. Reversal of the inhibitory effect of NaCl on seed germination in *Hordeum distichum* (Tipirdamaz et al., 1995) and on seedling growth in *Cicer arietinum* (Kaur et al., 1998) due to exogenous GA_3 has been attributed to stimulation of α -amylase activity.

Amaranthus (Amaranthaceae) is a glycophyte and C_4 dicotyledonous mesophyte crop plant (Wang et al., 1999). *Amaranthus caudatus* var. *atropurpureus* is one of several ornamental forms of this group of plants, widely grown all over the world. Exogenous ethylene and gibberellin A_3 were found to stimulate *A. caudatus* seed germination (Kępczyński and Karssen, 1985; Kępczyński et al., 1988). There is no information related to germination of *A. caudatus* seeds under salinity.

The aim of this study was to determine whether inhibition of seed germination by NaCl and its counteraction by ethephon and gibberellin are related to the effects on α- or β-amylase activity during seed imbibition and germination.

MATERIALS AND METHODS

PLANT MATERIAL

The experiments used *Amaranthus caudatus* cv. *atropurpureus* seeds harvested in 2003 and stored dry at -20°C. These seeds were obtained from the W. Legutko Ornamental and Vegetable Seed Station, Kobylin, Poland.

GERMINATION OF SEEDS

Seeds were incubated in 5 cm Petri dishes on one layer of filter paper moistened with 1.5 ml distilled water or the same volume of water solutions of 25–125 mM NaCl at 25 mM intervals. Seed germination was checked at 21, 24, 36, 48, 72 and 84 h incubation in water and/or NaCl. To determine the effect of ethephon or GA_3 under salinity stress seeds were incubated in the presence of those regulators at 0.01, 0.1 and 0.3 mM and NaCl at 75, 100 or 125 mM. Seed germination was determined at 24 and 48 h incubation. Five replicates of 50 seeds each were made.

The seeds were incubated in darkness at 25°C, and checked under green safelight (0.5 μmole m^{-2} s^{-1}). A seed was regarded as germinated when its radicle was ~1 mm in length.

α- AND β-AMYLASE ACTIVITY

For determination of α- and β-amylase activity, three replicates of 100 seeds each incubated in the presence of ethephon or GA_3 at 0.1 mM and at 100 mM of NaCl for 12, 14 or 24 h were used. Before enzyme extraction, germination percentage and fresh weight were determined.

α-amylase activity was measured according to Black et al. (1996). Seeds were homogenized in an Eppendorf tube with 4 ml ice-cold 20 mM TRIS-maleate buffer (pH 6.2) containing 1 mM $CaCl_2$. The homogenate was centrifuged at 12,000 g for 5 min. The clear supernatant was used for the α-amylase activity assay. Buffer (1.2 ml) with 1.2 ml enzyme extract was incubated for 2 min at 37°C. To 2.4 ml diluted enzyme extract, 0.6 ml suspension (25 mg ml^{-1}) of Phadebas blue starch (Pharmacia, Uppsala, Sweden) was added, vortexed and incubated with shaking for 30 min at 37°C. The reaction was stopped by adding 0.6 ml 0.5 M NaOH. The reaction mixture was centrifuged at 8000 g for 5 min and the absorbance of the supernatant was measured at 620 nm. A calibration curve using barley malt α-amylase was prepared. Enzyme activity was expressed in units g^{-1} FW; one unit is equivalent to the amount of enzyme liberating 1 mg maltose from starch at 37°C and pH 6.2.

β-amylase activity was measured by the method of Bernfeld (1955). Seeds were homogenized with 4 ml ice-cold 16 mM sodium acetate buffer, pH 4.8. The homogenate was centrifuged at 12,000 g for 15 min, and the supernatant was used for determining β-amylase activity. Enzyme extract (0.5 ml) was added to 0.5 ml 1% potato starch in 16 mM sodium acetate buffer equilibrated at 37°C for 2 min, vortexed and incubated with shaking for 5 min at 37°C. Then 0.5 ml 3,5-dinitrosalicylic acid (DNSA) reagent was added to the reaction mixture and it was boiled for 5 min. Absorbance at 540 nm was read after adding 4.5 ml distilled water. The DNSA reagent consisted of 1% 3,5-dinitrosalicylic acid, 0.4 M NaOH and 1 M potassium sodium tartrate. A standard curve using maltose solution was prepared. β-amylase activity was expressed in units g^{-1} FW; one unit is defined as the amount of enzyme that liberated 1 mg maltose from starch in 5 min at 37°C at pH 4.8.

DATA ANALYSIS

Germination data are expressed as means ±SD from five replicates; amylase activity data are expressed as means ±SD from three replicates. Statistical treatment of the results employed

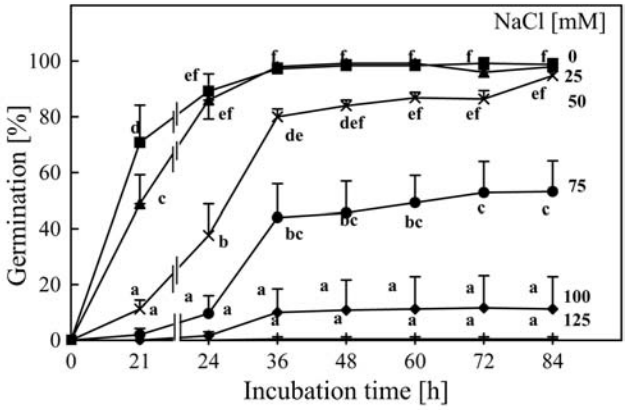

Fig. 1. Effect of NaCl on *Amaranthus caudatus* seed germination at different intervals of incubation at 25°C in the dark. Means from 5 replicates. Points with different letters differ significantly at $p \leq 0.05$ by two-way ANOVA with Duncan's test of arcsine-transformed data.

Statistica for Windows ver. 7.1 (StatSoft Inc., Tulsa, Oklahoma, USA). Two-way ANOVA was used to determine the effect of each NaCl concentration at each time of incubation. Three-way ANOVA was used to compare the effects of plant growth regulator (PGR) concentration, salinity and incubation time. Differences between means were considered significant at $P \leq 0.05$ by Duncan's multiple range test. Prior to analyses the data were checked for normality of distribution with the Shapiro-Wilks test. Germination data were arcsine-transformed to ensure homogeneity of variance. Amylase activity data were log(x)-transformed.

RESULTS

Germination of *Amaranthus caudatus* seeds in distilled water reached maximum (~100%) at 36 h (Fig. 1). NaCl at 25 and 50 mM delayed germination without any significant effect on the final germination percentage, but seeds exposed to 50 mM NaCl reached maximum germination later than in the control (Fig. 1). Higher concentrations of NaCl significantly inhibited germination, manifested in delayed initiation of germination and reduction of the final germination percentage. NaCl at 75, 100 and 125 mM reduced germination by 50%, 90% and 99.5% versus the control after 84 h.

Ethephon alone at 0.01, 0.1 and 0.3 mM significantly increased the germination percentage versus the control after 24 h incubation (Fig. 2a). Combined application of ethephon with NaCl (75, 100 and 125 mM) significantly reduced the inhibitory effect of salinity on seed germination after 24 h (Fig. 2a), but ethephon had less of an ameliorating effect on inhibition in the 125 mM NaCl treatment than at lower salinity. After 48 h, seed germination in treatments containing ethephon with NaCl reached 90–98% (Fig. 2b).

The germination percentages for GA_3-treated seeds (0.01, 0.1, 0.3 mM) were similar to those of the controls at 24 and 48 h (Fig. 3). Salinity-induced inhibition (NaCl concentrations as in Fig. 3a) was not alleviated during 24 h incubation by any applied GA_3 treatment (Fig. 3a). GA_3 did significantly alleviate inhibition induced by NaCl at 75 and 100 mM after 48 h (Fig. 3b). We found that GA_3 could not improve germination to any significant extent in the 125 mM NaCl treatment at 48 h.

NaCl at 100 mM had no significant effect on α-amylase activity, as compared to the control at 12 h, when germination was not noted in any combination (Fig. 4a, numbers above columns). After 14 h, NaCl at 100 mM slightly but significantly decreased enzyme activity and visibly reduced seed germination versus the control. Although the inhibitory effect of NaCl was apparent in the germination percentages, seeds showed clearly higher α-amylase activity than in the control after 20 h. When ethephon and GA_3 were used alone at 0.1 mM, α-amylase activity significantly increased after 12 and 14 h, and germination increased after 14 h incubation (Fig. 4a). Combined application of ethephon and GA_3 under salinity yielded significantly higher α-amylase activity versus the control and NaCl alone after 12 and 14 h incubation.

GA_3 significantly increased β-amylase activity versus the control after 12 h incubation (Fig. 4b). NaCl significantly decreased it at 14 h. Combined treatment with ethephon and NaCl or with GA_3 and NaCl did not affect β-amylase activity during the incubation period.

DISCUSSION

The low concentrations of NaCl only delayed germination, and the high concentrations affected the final percentages of *Amaranthus caudatus* seed germination. Similar trends have been reported in other crop seeds, including *Amaranthus paniculatus* (Jamil et al., 2006), *Medicago sativa* (Basalah and Mohammad, 1999), *Triticum durum* (Almansouri et al., 2001) and *Vigna radiata* (Mohammed, 2007). Our results clearly demonstrate the ability of exogenous ethephon and GA_3 to alleviate inhibition of *A. caudatus* seed germination under salinity. The stimulatory effect of ethephon in the presence of NaCl was seen earlier than that of GA_3, and ethephon was found to be more effective than GA_3. Other authors have described alleviation of the detrimental effects of salinity on seed germination by ethylene and/or GA_3 in seeds of crops including *Lactuca sativa*, *Triticum aestivum*, *Cicer arietinum*, *Medicago*

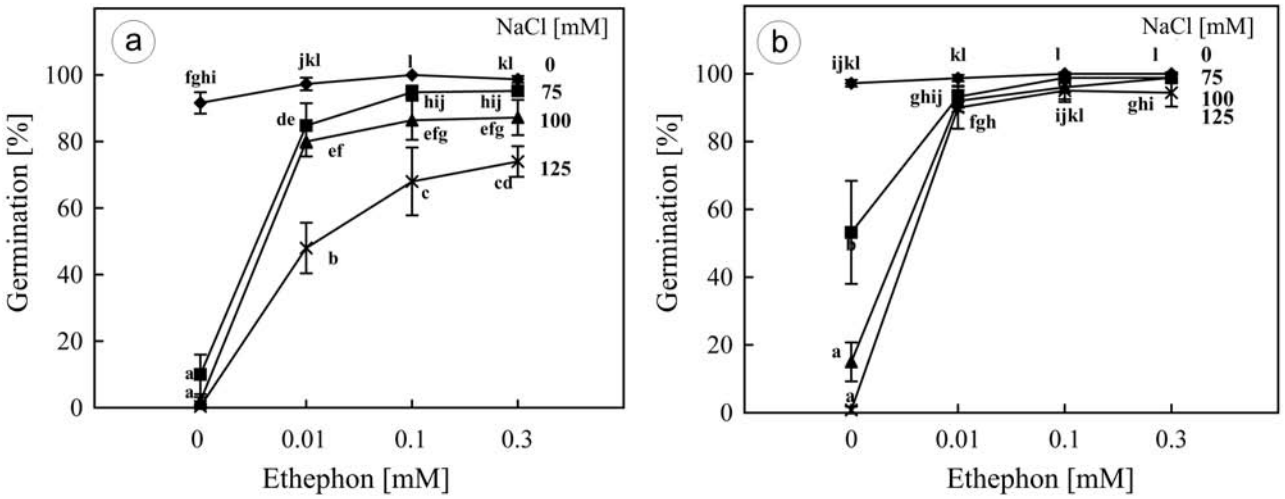

Fig. 2. Effect of ethephon on *Amaranthus caudatus* seed germination in the presence of NaCl after 24 h **(a)** or 48 h (**b**) incubation at 25°C. Means from 5 replicates. Points with different letters differ significantly at $p \leq 0.05$ by three-way ANOVA with Duncan's test of arcsine-transformed data.

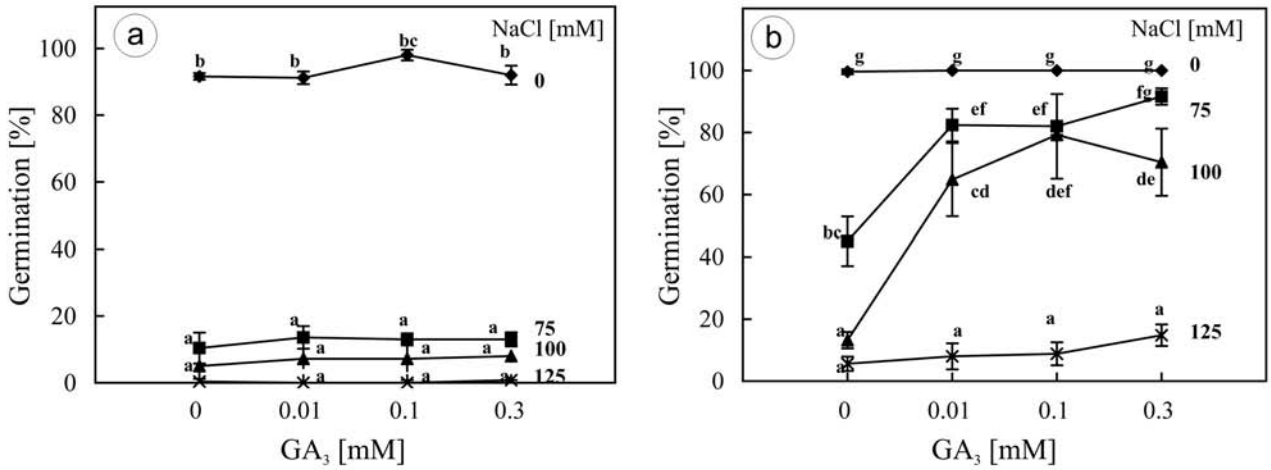

Fig. 3. Effect of GA_3 on *Amaranthus caudatus* seed germination in the presence of NaCl after 24 h **(a)** or 48 h **(b)** incubation at 25°C. Means from 5 replicates. Points with different letters differ significantly at $p \leq 0.05$ by three-way ANOVA with Duncan's test of arcsine-transformed data.

sativa and *Vigna radiata* (Khan and Huang, 1988; Li et al., 1995; Kumar and Singh, 1996; Kaur et al., 1998; Basalah and Mohammad, 1999; Zhenguo and Jundi, 2001; Mohammed, 2007). In *Cucumis melo, Lycopersicon esculentum* and *Spinacia oleracea*, salinity decreased ethylene biosynthesis during seed germination (Zapata et al., 2004). Gibberellin content was reduced under salinity in *Hordeum vulgare* and *Lactuca sativa* seeds (Kabar and Baltebe, 1989). Salinity-induced reduction of plant growth hormone levels has been shown to be enough to inhibit germination (Boucaud and Ungar, 1976). It is possible that NaCl at least partially lowers the ethylene or gibberellin levels in *A. caudatus* seeds, since endogenous ethylene and gibberellins have been shown to be needed for germination of these seeds (Kępczyński and Karssen, 1985; Kępczyński et al., 1988). The mechanisms by which ethylene and/or gibberellins promote seed germination may involve alleviation of the inhibitory effects of abscisic acid or phenolic compounds, whose production is induced by salt stress (Dhingra and Varghese, 1985;

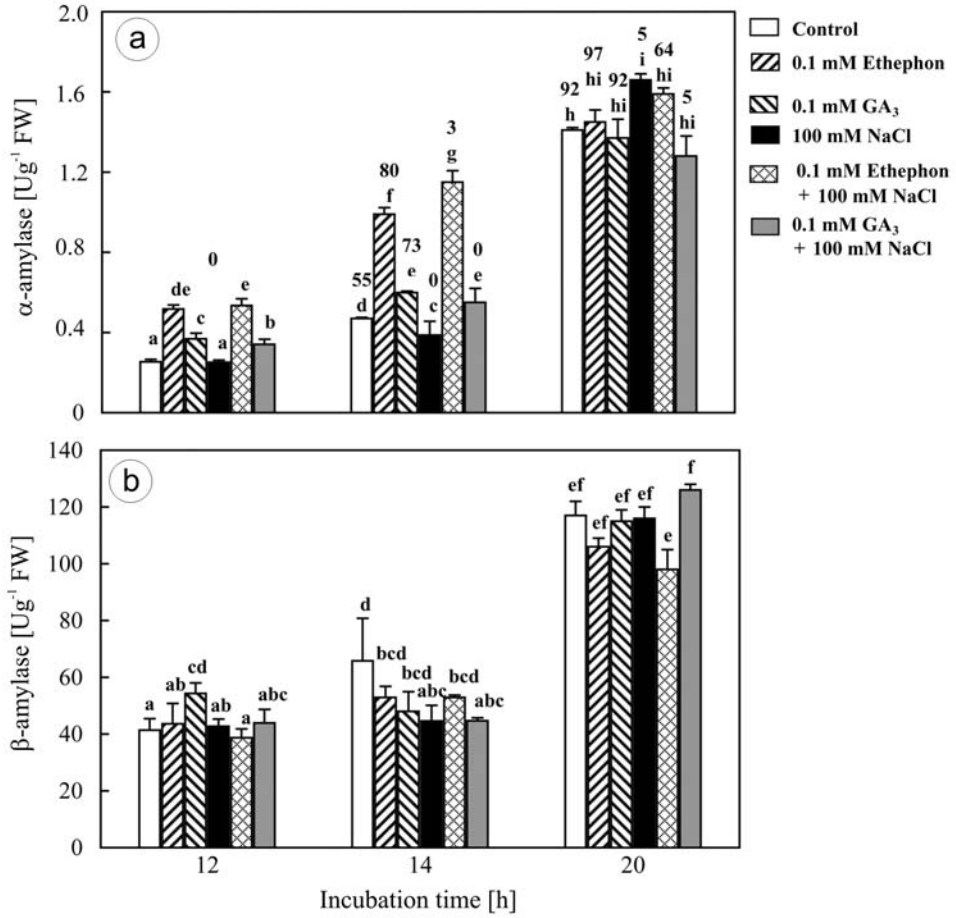

Fig. 4. Effect of 100 mM NaCl in the presence of 0.1 mM ethephon or GA_3 on α-amylase **(a)** or β-amylase **(b)** activity in *Amaranthus caudatus* seeds after different intervals of incubation at 25°C. Means from 3 replicates. Numbers above columns indicate seed germination percentage (nontransformed data). Columns with different letters differ significantly at $p \leq 0.05$ by three-way ANOVA with Duncan's test of log(x)-transformed data.

Ayaz et al., 2000; Khan and Ungar, 2002; Xiong and Zhu, 2003). Counteraction of exogenous ABA-inhibition of *A. caudatus* seed germination by ethylene or GA_3 (Rudnicki and Dzięcioł, 1971; Kępczyński, 1986) might support that suggestion, but such conclusions cannot be verified without thorough study of the endogenous levels of plant hormones in *A. caudatus* seeds under salt stress.

The stimulatory effect of exogenous ethylene and GA_3 on α-amylase activity up to 14 h incubation suggests that their mode of action in regulating *A. caudatus* seed germination involves control of key enzymes responsible for starch degradation. The increase of β-amylase activity by GA_3 at 12 h may indicate that exogenous gibberellin controls both major enzymes responsible for starch metabolism during early imbibition by *A. caudatus* seeds. Salt stress reduced both α- and β-amylase activity only at 14 h. The effect of NaCl on α-amylase activity at 20 h was the opposite. Based on these data it seems unlikely that the stress effect on *A. caudatus* seed germination occurred via inhibition of α- and β-amylase activity. It should be mentioned that increased α-amylase activity in *A. caudatus* seeds did not always lead to stimulation of germination in our study. This suggests that *A. caudatus* seed germination is not directly dependent on α-amylase activity. However, the increase of α-amylase activity due to ethylene and GA_3 during the first 14 h of *A. caudatus* seed germination may help the seeds to overcome the inhibition exerted by salinity stress. α-amylase activity in *Triticum aestivum* seedlings was stimulated under salinity (El-Fouly and Jung, 1972). In these seeds, salinity may induce increased respiration (Kasai et al., 1998). The energy requirement for basal metabolism increases in stressed seeds, and this could explain higher α-amylase activity independently of the effect on germination in *A. caudatus* seeds. The increase of α-amylase activity could be associated with an

adaptive strategy for saline conditions. In *Oryza sativa* and *Gossypium vitifolium*, enhanced α-amylase activity, increased total free sugar concentration and reduced starch content were suggested as means of achieving accumulation of solutes, reducing the osmotic potential under salt stress conditions (Dubey and Singh, 1999; Ashraf et al., 2002). Soluble sugars are listed among the compatible organic solutes responsible for osmotic adjustment and maintenance of turgor (Kameli and Losel, 1995; Agarwal et al., 1999; Prado et al., 2000). In *A. caudatus*, alleviation of NaCl-induced inhibition of seed germination by both ethephon and GA_3 was not linked to regulation of β-amylase activity.

Our results clearly show the detrimental effect of NaCl on *A. caudatus* seed germination. These seeds showed higher sensitivity to exogenous ethylene than to GA_3 under salinity stress. The mechanism by which NaCl inhibited *A. caudatus* seed germination was not mediated by reduction of α- and β-amylase activity. Exogenous ethylene and GA_3 increased α- but not β-amylase activity during early imbibition and germination under salinity, but their ameliorating effects on seed germination inhibition did not occur through a direct effect on those metabolic activities. The NaCl-induced increase in α-amylase activity in the seeds might be an adaptive mechanism for managing stress conditions.

ACKNOWLEDGEMENTS

This study was supported in part by grant No. N 310151935 from the Polish Ministry of Science and Higher Education.

REFERENCES

Agarwal RM, Pandey R, and Gupta S. 1999. Certain aspects of water stress induced changes and tolerance mechanisms in plants. *Journal of Indian Botanical Society* 78: 255–269.

Akinci IE, Akinci S, Yilmaz K, and Dikici H. 2004. Response of eggplant varieties (*Solanum melongena*) to salinity in germination and seedling stages. *New Zealand Journal of Crop Horticultural Science* 32: 193–200.

Akram M, Hussain M, Akhtar S, and Rasul E. 2002. Impact of NaCl salinity on yield components of some wheat accession/variety. *International Journal of Agriculture and Biology* 4: 156–158.

Almansouri M, Kinet JM, and Lutts S. 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant and Soil* 231: 243–254.

Ashraf MY, Sarwar G, Ashraf M, Afaf R, and Sattar A. 2002. Salinity induced changes in α-amylase activity during germination and early cotton seedling growth. *Biologia Plantarum* 45: 589–591.

Ayaz FA, Kadioglu A, and Turgut R. 2000. Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ctenanthe setowa* (Rose) Eichler. *Canadian Journal of Plant Science* 80: 373–378.

Basalah MO, and Mohammad S. 1999. Effect of salinity and plant growth regulators on seed germination of *Medicago sativa*. *Pakistan Journal of Biological Sciences* 3: 651–653.

Bernfeld P. 1955. Amylases α and β. In: Colowick SP and Kaplan NO [eds.], *Methods in Enzymology*, vol. 1, 149–158. Academic Press, New York.

Black M, Corbineau F, Grzesik M, Guy P, and Come D. 1996. Carbohydrate metabolism in the developing and maturing wheat embryo in relation to its desiccation tolerance. *Journal of Experimental Botany* 295: 161–169.

Boucaud J, and Ungar IA. 1976. Hormonal control of germination under saline conditions of three halophyte taxa in genus *Suaeda*. *Physiologia Plantarum* 36: 197–200.

Caravajal M, Amor FM, Fernandez-Ballester G, Martinez V, and Cerdia A. 1998. Time course of solute accumulation and water relations in muskmelon plants exposed to salt during different growth stages. *Plant Science* 138: 103–112.

Dhingra HR, and Varghese TM. 1985. Effect of salt stress on viability, germination and endogenous levels of some metabolites and ions in maize (*Zea mays* L.) pollen. *Annals of Botany* 55: 415–420.

Dodd GL, and Donovan LA. 1999. Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. *American Journal of Botany* 86: 1146–1153.

Duan D, Liu X, Khan MA, and Gul B. 2004. Effects of salt and water stress on the germination of *Chenopodium glaucum* L. seed. *Pakistan Journal of Botany* 36: 793–800.

Dubey RS, and Singh AK. 1999. Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolising enzymes in rice plants. *Biologia Plantarum* 42: 233–239.

Duedahl-Olesen L, Pedersen LH, and Larsen KL. 2000. Suitability and limitations of methods for characterisation of activity of malto-oligosaccharide-forming amylases. *Carbohydrate Research* 329: 109–119.

El-Fouly MM, and Jung J. 1972. Enzyme activity in wheat seedlings grown under different NaCl salinity levels and their interaction with growth regulators. *Biochemie und Physiologie der Pflanzen* 163: 492–498.

Flowers TJ. 1985. Physiology of halophytes. *Plant and Soil* 89: 41–56.

Greenway H, and Munns R. 1980. Mechanisms of salt tolerance in non-halophytes. *Annual Review of Plant Physiology* 31: 149–190.

Jamil M, Lee DB, Jung KY, Ashraf M, Lee SCH, and Rha ES. 2006. Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. *Journal of Central European Agriculture* 7: 273–282.

Kabar K, and Baltebe S. 1989. Effect of kinetin and gibberellic acid in overcoming high temperature and salinity (NaCl) stress on the germination of barley and lettuce seeds. *Phyton* 30: 65–74.

Kameli A, and Losel DM. 1995. Contribution of carbohydrates and solutes to osmotic adjustment in wheat leaves under water stress. *Journal of Plant Physiology* 145: 363–366.

KASAI K, MORI N, and NAKAMURA C. 1998. Changes in the respiratory pathways during germination and early seedling growth of common wheat under normal and NaCl-stressed conditions. *Cereal Research Communications* 26: 217–224.

KAUR S, GUPTA AK, and KAUR N. 1998. Gibberellin A_3 reverses the effect of salt stress in chickpea (*Cicer arietinum* L.) seedlings by enhancing amylase activity and mobilization of starch in cotyledons. *Plant Growth Regulation* 26: 85–90.

KĘPCZYŃSKI J. 1986. Inhibition of *Amaranthus caudatus* seed germination by polyethylene glycol-6000 and abscisic acid and its reversal by ethephon or 1-aminocyclopropane-1-carboxylic acid. *Physiologia Plantarum* 67: 588–591.

KĘPCZYŃSKI J, and KARSSEN CM. 1985. Requirement for the action of endogenous ethylene during germination of non-dormant seeds of *Amaranthus caudatus*. *Physiologia Plantarum* 63: 49–52.

KĘPCZYŃSKI J, KĘPCZYŃSKA E, and KNYPL JS. 1988. Effects of gibberellic acid, ethephon, and 1-aminocyclopropane-1-carboxylic acid on germination of *Amaranthus caudatus* seeds inhibited by paclobutrazol. *Journal of Plant Growth Regulation* 7: 59–66.

KHAN AA, and HUANG XL. 1988. Synergistic enhancement of ethylene production and germination with kinetin and 1-aminocyclopropane-1-carboxylic acid in lettuce seeds exposed to salinity stress. *Plant Physiology* 87: 847–852.

KHAN MA, and UNGAR IA. 2002. Influence of dormancy regulating compounds and salinity on the germination of *Zygophyllum simplex* L. seeds. *Seed Science and Technology* 30: 507–514.

KUMAR B, and SINGH B. 1996. Effect of plant hormones on growth and yield of wheat irrigated with saline water. *Annales Agriculture Research* 17: 209–212.

LI ZG, NI JD, and YU SW. 1995. Alleviation of inhibition of germination by ethylene in salt-stressed alfalfa seeds. *Acta Phytophysiologica Sinica* 21: 50–56.

LIN CC, and KAO CH. 1995. NaCl stress in rice seedlings: Starch mobilization and the influence of gibberellic acid on seedling growth. *Botanical Bulletin of Academia Sinica* 36: 169–173.

MASS EV. 1986. Salt tolerance in plants. *Applied Agricultural Research* 1: 12–26.

MOHAMMED AHMA. 2007. Physiological aspects of mungbean plant (*Vigna radiata* L. Wilczek) in response to salt stress and gibberellic acid treatment. *Research Journal of Agriculture and Biological Sciences* 3: 200–213.

PEAREN JR, PAHL MD, WOLYNETZ MS, and HERMESH R. 1997. Association of salt tolerance at seedling emergence with adult plant performance in slender wheat grass. *Canadian Journal of Plant Science* 77: 81–89.

PRADO FE, BOERO C, GALLARDO M, and GONZALES JA. 2000. Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* wild seeds. *Botanical Bulletin of Academia Sinica* 41: 27–34.

PROMILA K, and KUMAR S. 2000. *Vigna radiata* seed germination under salinity. *Biologia Plantarum* 43: 423–426.

RAMAGOPAL S. 1990. Inhibition of seed germination by salt and its subsequent effect on embryonic protein synthesis in barley. *Journal of Plant Physiology* 136: 621–625.

RUDNICKI R, and DZIĘCIOŁ J. 1971. The action of plant hormones on germination and amylase activity of *Amaranthus caudatus* seeds. *Bulletin Academy of Polish Science* 19: 291–295.

SIDDIQUI ZS, SHAUKAT SS, and ZAMAN AU. 2006. Alleviation of salinity-induced dormancy by growth regulators in wheat seeds. *Turkish Journal of Botany* 30: 321–330.

TIPIRDAMAZ R, DURUSOY M, and BOZCUK S. 1995. Effect of exogenous polyamines on α-amylase activity during seed germination under salt stress. *Turkish Journal of Botany* 19: 411–416.

UNGAR IA. 1996. Effect of salinity on seed germination, growth, and ion accumulation of *Atriplex patula* (*Chenopodiaceae*). *American Journal of Botany* 83: 604–607.

WANG Y, MENG YL, ISHIKAWA H, HIBINO T, TANAKA Y, NII N, and TAKABE T. 1999. Photosynthetic adaptation to salt stress in three-color leaves of a C_4 plant *Amaranthus tricolor*. *Plant and Cell Physiology* 40: 668–674.

XIONG L, and ZHU JK. 2003. Regulation of abscisic acid biosynthesis. *Plant Physiology* 133: 29–36.

ZAPATA PJ, SERRANO M, PRETEL MT, AMOROS A, and BOTELLA MA. 2004. Polyamines and ethylene changes during germination of different plant species under salinity. *Plant Science* 167: 781–788.

ZHENGUO LI, and JUNDI NI. 2001. Studies on inhibition mechanism of germination by ethylene in salt-stressed alfalfa seeds. *Chinese Journal of Applied and Environmental Biology* 7: 24–28.