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The role of hormones in regulating the germination of polymorphic seeds and early seedling growth of *Atriplex triangularis* under saline conditions

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Germination of the polymorphic seeds and seedling growth of *Atriplex triangularis* under various salinity, gibberellic acid and kinetin treatments were determined. Gibberellic acid (GA_3; 2.9 m*M*) promoted germination and growth at high NaCl concentrations (345 m*M*). Kinetin (4.7 μ*M*) stimulated germination at all salinities and seed sizes tested. GA_3 and kinetin generally increased seedling growth at all concentrations of salinity studied. Higher concentrations of kinetin were found to be inhibitory.

Additional key words – Dormancy, GA_3, halophyte, kinetin, polymorphism.

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Introduction

Variation in the dormancy level of seeds is of significant advantage to species growing in variable environments like salt marshes. The distribution of germinating seeds through time is considered to be of survival value since it provides more than one chance for establishment (Harper 1965, Baskin and Baskin 1976, Ungar 1984a). *Atriplex triangularis* seeds are polymorphic (Khan and Ungar 1984b) and this is reflected in their size, weight and germination response under field conditions. Seeds germinate from February through June but large seeds germinate very early in the season, while small seeds can germinate later and also become part of the persistent seed bank (Ungar 1984b). This germination pattern could be due to differential responses of the polymorphic seeds to both salinity and temperature and their interaction in affecting seed germination (Khan and Ungar 1984a). Biotic factors influencing germination include changes in internal hormonal balance and variation in the thickness of seed coats.

Seed dormancy induced by salt stress in halophytes can be alleviated by the application of GA_3 (Ungar and Binet 1975, Boucaud and Ungar 1976a, Ungar 1977, 1984a). However, kinetin alone is not reported to promote germination in halophytes. Higher concentrations of NaCl inhibit the growth of the halophytes *Suaeda* and *Salicornia* and this inhibition could be alleviated by the application of GA_3 (Boucaud and Ungar 1976b, Ungar 1978).

The effect of hormones on the germination and early seedling growth of *Atriplex triangularis* has not been previously reported. This study describes the influence of various concentrations of GA_3 and kinetin in alleviating dormancy induced by salt stress in the polymorphic seeds and early seedling growth of *Atriplex triangularis*.

Abbreviation – GA, gibberellic acid.

Materials and methods

Seeds of *Atriplex triangularis* Willd. were collected from a salt marsh at Rittman, Wayne county, OH, in October 1981 and stored at 4°C until used. Seeds were separated

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from inflorescences and sorted into large (> 2.0 mm), medium (1.5 to 2 mm) and small (< 1.5 mm). The seeds were surface sterilized with 0.5% (w/v) sodium hypochlorite for 1 min and then washed two or three times with distilled water. Germination was carried out in 50 × 9 mm Gelman no. 7232 tight-fitting plastic Petri dishes with 6 ml of test solution. Each dish was placed in a 9 cm diameter glass Petri dish as an added precaution against the loss of water by evaporation. Seeds were germinated in a growth chamber at alternating temperature regimes of 5–25°C based on a 24 h cycle, where 25°C coincided with a 12 h light period provided by Sylvania cool white fluoresecent lamps at 8.5 W m^{-2} and the 5°C coincided with a 12 h dark period. GA_3 concentrations of 29 μ*M*, 0.29 m*M*, 2.9m*M*, kinetin concentrations of 4.7 μ*M*, 47 μ*M*, 0.47 m*M* and NaCl concentrations of 0, 86, 172, 285, and 345 m*M* were used. Four replicates of 25 seeds were used for each treatment. Seeds were considered to be germinated with the emergence of the radicle. Germination was recorded on every alternate day for twenty days. Hypocotyl length, root length and dry weight were recorded after 20 days of growth. The velocity of germination was calculated using an index of germination velocity proposed earlier: G/t, where G = percentage of seeds germinated at 2 days intervals, t = total germination period (Khan and Ungar 1984a).

Thickness of the small, medium and large seed coats was measured. These seed coats were obtained from the germinated seeds and transferred to 5% sodium hydroxide solution for 24 h and then transferred to 70% alcohol for 24 h to remove wax layers. After these treatments,seed coats were passed through a series of xylene solutions and embedded in paraffin. Ten μm thick sections were cut with a microtome and permanently mounted. Photographs of the seed coat were taken with a Zeiss Ultraphot III B. Seed coat thickness was measured from negatives and the actual thickness was calculated.

Statistical analysis was done using SAS statistical packages on a IBM 370 computer. LSD value are significant at $P < 0.05$.

Results

Effect of GA_3 and salinity on germination

Although a range of growth regulator concentrations was used, only the optimal concentration for promotion of germination (2.9 m*M*) and seedling growth (29 μ*M*) are reported (Tabs 1–4). Germination of small, medium and large seeds was inhibited with an increase in media salinity above 172 m*M* NaCl (Tab. 1). This salt-induced inhibition was significant in small seeds ($P < 0.0001$, LSD = 7.19). The index of germination velocity also shows a similar pattern of inhibition in the rate of germination. GA_3 was only stimulatory to large seeds in distilled water. At 345 m*M* NaCl, GA_3 treatment promoted germination only in small seeds (Tab. 1). The velocity of germination remained unchanged at lower salinities for all seed sizes, but at higher salinities the velocity of germination of small and large seeds was significantly promoted by GA_3 ($P < 0.0001$). The interaction between salinity, GA_3 and seed size was found to be very highly significant ($P < 0.001$) for percentage germination, but data for velocity of germination was not significant ($P < 0.64$).

Effect of GA_3 and salinity on growth

All salinity concentrations significantly inhibited ($P < 0.0001$, LSD = 1.79) hypocotyl growth. The inhibitory effect was stronger for seedlings grown from small seeds than for those grown from medium and large seeds (Tab. 2). At low salinities, GA_3 significantly ($P < 0.0001$, LSD = 1.60) inhibited hypocotyl growth, whereas at 345 m*M* NaCl hypocotyl length was increased by GA_3 treatments. Root length was also inhibited significantly ($P < 0.0001$, LSD = 1.04) with an increase in salinity; this inhibition was also more strongly expressed in small seeds (Tab. 2). Root length of seedlings grown from all seed sizes was inhibited by the addition of GA_3 to the medium. At higher salinities GA_3 generally promoted root growth, while at lower salinities it was inhibitory. This promotion was greater for

Tab. 1. Effect of GA_3 (2.9 m*M*) and salinity on the percentage and velocity of germination of polymorphic seeds; mean ± SE; A, Percentage germination after 20 days; B, Velocity of germination (max. = 50).

NaCl (m*M*)	Additions (m*M*)	Seed size					
		Small		Medium		Large	
		A	B	A	B	A	B
0	None	97±2*	37±2	94±2	34±1	99±1	36±1
	2.9 m*M* GA_3	95±2	39±2	89±1	32±2	99±1	40±1
172	None	70±9	22±3	63±7	21±3	100±0	25±3
	2.9 m*M* GA_3	77±11	32±6	47±14	17±5	25±4	30±1
345	None	8±2	2±1	33±13	11±4	33±9	7±1
	2.9 m*M* GA_3	27±5	6±2	30±7	11±5	36±3	18±1

Tab. 2. Effect of salinity (0,172,345 mM NaCl) and GA_3 (29 μM) on the hypocotyl length (mm ± SE), root length (mm ± SE) and dry weight (mg ± SE) of *Atriplex triangularis*; mean ± SE-

Variable	Additions	Seed size								
		Small			Medium			Large		
		0	172	345	0	172	345	0	172	345
Hypocotyl length	None	25.3±0.5	7.4±0.7	0.0±0.0	27.2±1.8	13.8±1.7	3.5±0.9	31.6±0.5	27.5±2.2	7.5±0.5
	29 μM GA_3	26.5±1.4	22.6±4.0	6.5±1.7	29.1±1.4	13.6±0.7	8.0±1.0	43.6±1.8	30.1±2.9	7.4±1.6
Root length	None	15.9±0.8	3.5±0.7	0.0±0.0	26.3±2.4	2.8±0.5	2.2±0.3	26.3±2.5	6.2±0.4	6.1±1.8
	29 μM GA_3	16.0±0.5	6.7±1.8	3.4±0.1	24.6±1.4	3.8±0.8	3.1±0.2	20.4±4.4	9.4±1.04	3.2±0.8
Dry weight	None	0.4±0.02	0.1±0.01	0.0±0.00	0.6±0.08	0.5±0.08	0.5±1.04	1.8±0.09	2.0±0.09	0.4±0.15
	29 μM GA_3	0.3±0.03	0.3±0.09	0.4±0.29	0.6±0.11	0.6±0.01	0.2±0.03	1.5±0.17	1.7±0.26	0.3±0.09

seedlings grown from the small and medium seeds than for those from large seeds.

Dry weight of seedlings derived from the large seeds was about five times that of the seedlings obtained from small seeds (Tab. 2). Generally, low salinity stimulated an increase in seedling dry weight but these differences were not statistically significant (LSD = 0.10). Seedlings exposed to higher salt concentrations had significantly ($P < 0.0001$) lower dry weight than those at lower salt concentrations. GA_3 inhibited dry weight production of seedlings from small seeds at low salinity. Dry weights of seedlings from small seeds in 172 and 345 mM NaCl were significantly ($P < 0.001$) promoted by GA_3 treatments. The interaction between GA_3, salinity and seed size was very highly significant in affecting dry weight ($P < 0.0001$), hypocotyl length ($P < 0.0001$) and root length ($P < 0.0001$) of plants.

Effect of salinity and kinetin on germination

Kinetin generally caused an increase in the percentage and velocity of germination at all seed sizes in salinities of 172 mM and higher (Tab. 3), but was more stimulatory in saline conditions for small seeds than for medium and large seeds. For example, small seeds that were germinated in 285 mM NaCl had 39% germination and a velocity index of 14, but inclusion of 47 uM kinetin in the germination medium yielded a percentage germination of 77% and a velocity index of 24. Individual effects of salinity ($P < 0.0001$, LSD = 1.43), seed size ($P < 0.0001$, LSD = 1.11), kinetin ($P < 0.0001$, LSD = 1.28) and their interaction ($P < 0.0001$) were very highly significant in affecting the percent and velocity of germination.

Effect of salinity and kinetin on growth

Kinetin (4.7 μM) generally caused an increase in hypocotyl length in medium and large seeds, and this promotion was marked in highly saline medium (Tab. 4). Root length of seedlings at all salinities was significantly ($P < 0.0001$, LSD = 0.72) promoted by addition of kinetin to the medium. Dry weight of seedlings at all salinity levels generally increased with the inclusion of kinetin (Tab. 4). The interaction between kinetin, salinity and seed size in affecting dry weight ($P < 0.0001$), hypocotyl length ($P < 0.001$) and root length ($P < 0.01$) was very highly significant.

Thickness of seed coat

Testa of all sizes of seeds were one cell thick. The thick-

Tab. 3. Effect of kinetin and salinity on the percentage and velocity of germination of the polymorphic seeds of *Atriplex triangularis*; mean ± SE; A, Percentage of germination after 20 days; B, Velocity of germination (max. = 50).

NaCl (mM)	Additions (μM)	Seed size					
		Small		Medium		Large	
		A	B	A	B	A	B
0	None	99±1	40±1	97±3	37±2	98±2	42±2
	4.7 μM kinetin	100±0	41±1	100±0	40±1	100±0	40±0
172	None	46±2	20±1	57±4	19±1	61±8	22±2
	4.7 μM kinetin	90±2	32±1	72±4	22±2	84±7	29±3
345	None	7±2	3±0	11±1	3±0	22±3	9±1
	4.7 μM kinetin	69±5	19±2	39±4	8±0	39±4	13±1

Tab. 4. Effect of salinity (0,172,345 mM NaCl) and kinetin (4.7 μM) on the hypocotyl length (mm ± SE), root length (mm ± SE) and dry weight (mg ± SE) of *Atriplex traiangularis* seedlings grown from polymorphic seeds; mean ± SE.

Variable	Additions	Seed size								
		Small			Medium			Large		
		0	172	345	0	172	345	0	172	345
Hypocotyl length	None	25.0±2.3	11.1±2.9	0.0±0.0	21.4±1.3	12.3±0.9	0.0±0.0	35.6±1.1	26.0±1.0	6.5±0.7
	4.7 μM kinetin	18.8±1.4	7.5±2.2	4.6±0.4	26.3±0.7	16.9±2.8	16.5±0.5	40.4±0.8	30.9±0.3	8.5±0.8
Root length	None	16.7±1.2	3.6±0.5	2.1±0.1	16.7±1.3	3.3±0.6	2.0±0.2	28.5±1.1	8.5±0.8	5.0±0.8
	4.7 μM kinetin	19.2±1.6	5.1±0.3	2.1±0.1	19.9±6.4	4.8±0.2	4.0±0.5	30.7±2.4	11.0±1.0	6.4±0.5
Dry weight	None	0.4±0.05	0.2±0.03	0.1±0.01	0.7±0.04	0.4±0.07	0.1±0.06	1.9±0.09	2.0±0.19	0.5±0.13
	4.7 μM kinetin	0.4±0.03	0.4±0.13	0.4±0.04	0.8±0.06	0.4±0.15	0.0±0.00	2.8±0.13	2.8±0.10	0.6±0.08

ness of seed coats varied with the various seed sizes. The single large sclereids of the seed coat of seeds had the following diameters; small seeds-21.5 ± 0.37 µm, medium seeds-16.8 ± 0.45 µm and large seeds-13.6 ± 0.41 µm.

Discussion

Dormancy induced by high salt concentrations in halophytes could be alleviated by the application of GA_3 (Ungar and Binet 1975, Boucaud and Ungar 1976a, Ungar 1977, 1984a), but no such stimulation of germination was reported for kinetin treatments alone. However, in glycophytes, dormancies induced by salt, mechanical or osmotic stress were partially alleviated by exogenous application of kinetin (Boothby and Wright 1962, Odegbaro and Smith 1969, Kaufmann and Ross 1970, Hegarty and Ross 1979, Ross and Hegarty 1980, Bozcuk 1981).

Small seeds of *A. triangularis* have larger diameter epidermal cells than do the medium and large seed sizes. Osmond et al. (1980) has suggested that the mechanical resistance of these large epidermal cells in the species of *Atriplex* provides a resistance to germination and may induce dormancy in small seeds. Dormancy due to mechanical resistance of testa was closely associated with low levels of gibberellin-like activity in seeds of *Suaeda* spp. (Boucaud and Ungar 1973), and mechanically-induced dormancy was broken by an external application of GA_3 (Boucaud and Ungar 1976a).

High salt concentrations induce dormancy in seeds of many plant species (Heydecker 1977). Salinity was found to depress seed cytokinin levels but not the concentration of gibberellins (Boucaud and Ungar 1976a). However, dormancy induced by salinity, similar to that caused by emergence-restricting seed coats, was broken by an application of GA_3 but not by kinetin (Ungar 1977). Our results indicate that GA_3 and kinetin can break salt-induced dormancy in *A. triangularis,* suggesting that, when exposed to salt stress, seeds of various species of halophytes behave differently in response to an exogenous application of growth regulators.

Boucaud and Ungar (1976b) found that GA_3 and kinetin stimulated growth of *Suaeda maritima* var *macrocarpa* at all salinities. However, kinetin proved to be inhibitory to growth and elongation of *S. depressa* at higher salinities. Ungar (1978) found that GA_3 stimulated growth in height as well as dry weight production of *Salicornia europaea* at all salinities tested, while kinetin proved to be inhibitory. Growth of *A. triangularis* seedlings at high salinities was significantly increased with a GA_3 treatment. Kinetin (4.7 μM) also stimulated the growth of *A. triangularis* seedlings in all salinity treatments.

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