

Effect of salinity, temperature, and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. *stocksii*

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Seed germination and early seedling growth of *Atriplex griffithii* var. *stocksii* from seeds collected from a saline desert habitat in Karachi, Pakistan, were studied. The seeds of *A. griffithii* did not germinate under high salt stress (516 mM NaCl). Best germination percentages were obtained in distilled water. Seed germination was stimulated in light at cooler alternating temperature (25:10°C) and inhibited at warmer temperature (30:15, 30:20, and 35:25°C) regimes. Early seedling growth showed responses towards salinity and temperature that were similar to the germination responses. Both GA₃ and kinetin alleviated salinity-induced germination inhibition, and in most cases germination was stimulated by growth regulator treatments. A low concentration of kinetin (0.46 μM) and a high concentration of GA₃ (28.9 μM) significantly promoted early seedling growth at all salinity treatments.

Key words: *Atriplex griffithii* var. *stocksii*, seed germination, early seedling growth, salinity, temperature, growth regulators.

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Les auteurs ont étudié la germination et le développement des plantules provenant de graines de l'*Atriplex griffithii* var. *stocksii* récoltées dans un habitat désertique salin dans la région de Karachi au Pakistan. Les graines de l'*A. griffithii* ne germent pas sous l'influence de tous les stress salins utilisés (516 mM NaCl). On observe les meilleurs pourcentages de germination dans l'eau distillée. Les régimes de basses températures alternées (25:10°C), avec lumière, stimulent la germination des graines alors que les températures élevées (30:15, 30:20 et 35:25°C) l'inhibe. Les premiers développements des plantules sous l'influence de la salinité et de la température ressemblent à ceux observés pendant la germination. Dans la plupart des cas, les régulateurs de croissance GA₃ et kinétine stimulent la germination et diminuent les effets inhibiteurs de la salinité. De faibles concentrations de kinétine (0,46 μM) et des concentrations élevées de GA₃ (28,9 μM) stimulent fortement la croissance des plantules avec tous les traitements de salinité.

Mots clés : *Atriplex griffithii* var. *stocksii*, germination des graines, début de la croissance des plantules, salinité, température, régulateurs de croissance.

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Introduction

Germination is a crucial stage in the life history of many plants, and salt tolerance during the germination stage is critical for the establishment of plants that grow in saline soil (Ungar 1978; Maranon et al. 1989; Mohammad and Sen 1990; Khan 1991). Laboratory investigations of seed germination indicate that seeds of most halophytes attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and early establishment phase (Khan and Ungar 1984a, 1984b; Khan and Weber 1986; Ungar 1987; Myers and Morgan 1989; Khan 1991). Seed germination occurs in a saline environment during a season of high precipitation, when soil salinity levels are usually reduced (Khan and Ungar 1986). Several studies show that seeds of many halophytes do not germinate when exposed to low water potential (Ungar 1978; Khan and Ungar 1986) and that when salinity stress is reduced germination occurs at levels equivalent to that of distilled water (Ungar 1978; Khan and Ungar 1984a, 1984b). Khan and Weber (1986) reported that seeds of *Salicornia pacifica* var. *utahensis* could germinate at 5% NaCl. Less tolerant species such as *Atriplex littorale* and *Atriplex hastatum* did not germinate at salinities above 1.5% (Rozema et al. 1983).

Salinity and temperature have a differential effect on the germination of halophytic seeds (Khan and Weber 1986; Ungar 1987, 1988; Khan et al. 1987; Morgan and Myers 1989; Ismail 1990; Khan 1991). Germination percentages of several *Atriplex*

species were greater in the range of 12–25°C at various salinity regimes but declined at higher temperatures beyond this range (Ignaciuk and Lee 1980; Khan and Ungar 1984a). Khan and Ungar (1984a) found that alternating temperatures of 25 and 5°C enhanced seed germination of *Atriplex triangularis* and increases in salinity (86–285 μM) decreased both the rate of and the total seed germination.

Variation in hormonal balance that is induced by salt stress is at least one of the causes of germination inhibition in halophytes (Ungar 1978). Seed dormancy induced by salt stress in halophytes can be alleviated by the application of gibberellic acid (Khan and Ungar 1985; Khan et al. 1987; Kabar 1987), kinetin (Khan and Ungar 1985, 1986; Khan et al. 1987; Tirmizi 1988), or a mixture of gibberellic acid and kinetin (Ungar 1978; Tirmizi 1988). However, the role of plant growth regulators in alleviating seed dormancy induced by salt stress is not clearly understood.

Atriplex is an essentially cosmopolitan genus containing more than 417 species (Osmond et al. 1980), many of which are important as wildlife cover and food plants. Most species are adapted to grow in saline–alkaline soils or extremely arid situations. *Atriplex griffithii* Moq. var. *stocksii* Boiss. (Chenopodiaceae) is a short, robust perennial shrub that occurs primarily in Mediterranean, South Asian, and Southeast Asian regions. In Pakistan, this species occurs in coastal as well as inland areas. Typically, *A. griffithii* var. *stocksii* is restricted to areas of moderate salinity. Our study was conducted in a population of *A. griffithii* that occurs on an inland salt flat located on the Karachi University campus where it is the

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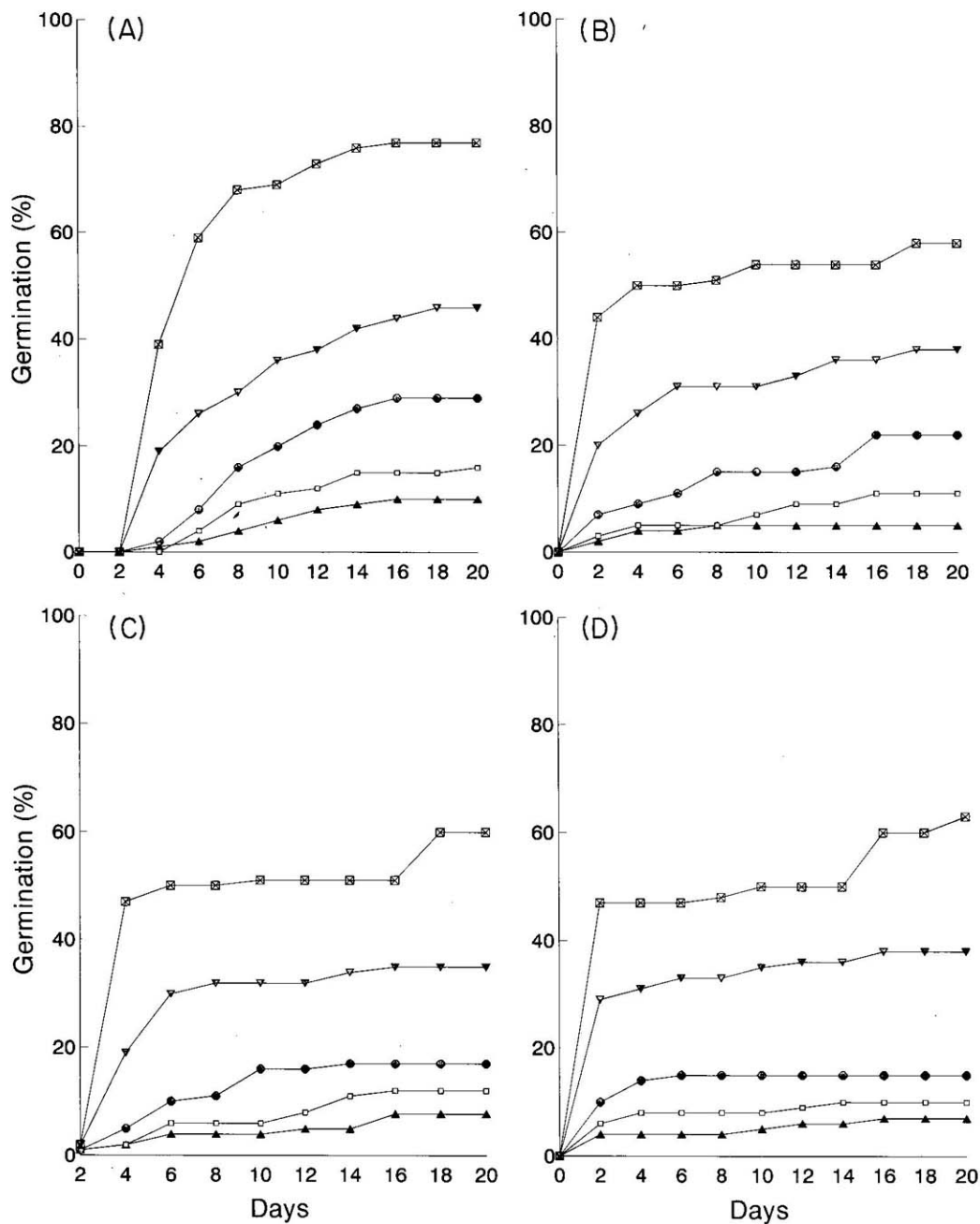


FIG. 1. Rate of germination of *Atriplex griffithii* seeds in 0 (□), 86 (▽), 172 (⊙), 285 (⊞), and 345 (▲) μM NaCl at (A) 25:10°C, (B) 30:15°C, (C) 35:20°C, and (D) 35:25°C alternating temperature regimes.

TABLE 1. Results of two-way analysis of variance of characteristics by salinity (S) and temperature (T) treatments

Dependent variable	Independent variable		
	S	T	S \times T
Germination	463.6***	22.5***	3.5***
Hypocotyl length	112.2***	143.5***	18.7***
Root length	4.7**	57.8***	3.9***
Dry weight	53.9***	40.0***	2.1*

NOTE: Numbers represent F -values; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 2. Index of germination velocity of *Atriplex griffithii* seeds at various salinity and temperature treatments

Salinity (mM)	25:10°C	30:15°C	35:20°C	35:25°C
0	30.7	26.3	23.6	26.1
86	16.3	16.0	14.3	17.3
172	10.6	7.7	6.4	7.2
285	4.8	3.8	3.8	4.3
345	3.0	2.2	2.4	2.7

dominant species (80–90% cover) and is associated with *Trianthema pentandra*, *Trianthema crystallina*, *Lactuca remotiflora*, and *Prosopis juliflora*. Flowering and seed set of *A. griffithii* occur after monsoon rains (July–August), and

seeds present in the soil from the previous year also germinate after rainfall. *Atriplex griffithii* reproduces predominantly through seeds in a habitat where vegetative reproduction is most common among shrubs and recruitment through seeds is a rare event.

TABLE 3. Germination percentages (mean \pm SD) of *Atriplex griffithii* seedlings after 20 days from various salinity, gibberellin A₃, and kinetin regimes

Growth regulator (μ M)	Salinity (mM)				
	0	86	172	285	345
Gibberellin A₃					
0.00	65.0 \pm 0.8	63.4 \pm 4.3	28.5 \pm 2.3	17.2 \pm 3.1	09.1 \pm 2.0
0.29	52.1 \pm 3.3	60.2 \pm 5.0	75.2 \pm 3.2	63.7 \pm 4.1	46.0 \pm 5.2
2.90	65.3 \pm 1.0	68.4 \pm 5.0	79.0 \pm 4.1	62.0 \pm 3.1	55.4 \pm 2.2
29.00	65.6 \pm 3.0	61.5 \pm 2.0	60.1 \pm 4.1	68.5 \pm 3.2	49.7 \pm 2.1
Kinetin					
0.00	65.0 \pm 0.8	63.4 \pm 4.3	28.5 \pm 2.3	17.2 \pm 3.0	09.1 \pm 2.0
0.46	60.2 \pm 4.9	68.2 \pm 3.0	71.2 \pm 2.3	72.6 \pm 3.1	47.0 \pm 5.2
4.60	65.3 \pm 1.0	74.7 \pm 4.0	73.7 \pm 2.1	74.3 \pm 3.0	43.4 \pm 1.4
46.60	77.4 \pm 2.9	64.4 \pm 3.0	61.1 \pm 3.8	68.5 \pm 3.2	89.7 \pm 2.1

The objective of this investigation was to determine the effect of temperature, salinity, and growth regulators on the germination and early seedling growth of *A. griffithii* var. *stocksii*.

Materials and methods

Seeds of *A. griffithii* var. *stocksii* were collected during January 1991 from salt flats situated on the Karachi University campus. Seeds were separated from inflorescence and were stored dry at 4°C. Germination and growth studies were started in May 1991.

Seeds were sterilized with 0.52% sodium hypochlorite (BDH) solution for 1 min and then washed 2 or 3 times with distilled water. Germination was carried out in test tubes that were 18 mm in diameter and 150 mm in length. Whatman No.1 filter paper strips 145 mm long and 50 mm wide were folded to form two depressions. Folded pieces of filter paper were placed in test tubes after placing 25 seeds in the depressions. Two millilitres of test solution was pipetted into the test tubes, which were then sealed with polyethylene sheets. The test tubes were placed in a small tray with the open side elevated slightly. Four replicates of 25 seeds each were used for all treatments. Emergence of the radicle indicated germination.

To determine the effect of temperature on germination and early seedling growth, seeds were incubated in a programmed, refrigerated incubator using 12 h light : 12 h dark (2000 lx Sylvania cool-white fluorescent lamps) with four thermoperiods, namely 25:10, 30:15, 35:20, and 35:25°C (light:dark).

Seeds were germinated in distilled water (neutral pH) and NaCl concentrations of 0, 86, 172, 285, and 345 mM. Germination was recorded on alternate days for 20 days. The rate of germination was estimated by using the Khan and Ungar (1984a) index of germination velocity = $\Sigma G/t$, where G is percentage of seed germination at 2-day intervals, and t is total germination period. The maximum value possible using this index with our data was 50 (i.e., 1000/20). The higher the value, the more rapid the rate of germination.

The gibberellin A₃ (GA₃) concentrations of 29, 2.9, and 0.29 μ M and kinetin concentrations of 0.46, 4.6, and 46.4 μ M were used with and without NaCl solution at a temperature regime of 25:10°C. Fifteen treatments were used for each hormone. Four replicates of 25 seeds each were used for each treatment and control. Germination was recorded on alternate days for 20 days. Root and hypocotyl length were measured and dry weights of seedlings were determined by drying the seedlings at 85°C for 48 h.

Germination, hypocotyl length, root length, and dry weight data were analyzed using the SPSSX ANOVA program (SPSS Inc. 1983). Percent germination data were transformed (arcsine) before statistical analysis.

Results

Effect of salinity and temperature on germination

Germination of *A. griffithii* seeds decreased with an increase in salinity (Fig. 1). Germination was reduced greatly in the

345- μ M NaCl treatment. Treatments higher than 345 μ M had little germination (data not shown). Alternating temperature regimes of 25:10°C had maximum germination at all salinities tested. Germination at other temperature regimes, i.e., 30:15, 35:20, and 35:25°C, had a similar pattern of germination. A two-way ANOVA of germination indicated significant main effects for salinity and temperature (Table 1). Interaction between salinity and temperature was also highly significant ($P < 0.0001$). One-way ANOVA of germination for each temperature regime revealed that salinity significantly affected germination of seeds grown at each temperature regime (25:10°C, $F = 151.6$, $P < 0.001$; 30:15°C, $F = 46.8$, $P < 0.001$; 35:20°C, $F = 29.84$, $P < 0.001$; 35:25°C, $F = 61.38$, $P < 0.001$).

Rate of germination in the various salinity treatments was usually higher at the 25:10°C temperature regime (Table 2). Germination velocity decreased with increases in salinity in all temperature treatments.

Effect of salinity and temperature on growth

Hypocotyl length, root length, and dry weight were greatest at the 25:10°C temperature regime at all salinity treatments. A two-way ANOVA of hypocotyl length indicated significant main effects and an interaction between salinity and temperature (Table 1). Similar results were also obtained for root length and dry weight. Increases in salinity and temperature significantly reduced the growth and biomass accumulation in *A. griffithii* seedlings.

Effect of salinity and growth regulators on germination

All concentrations of GA₃ (0.28, 2.80, and 28.80 μ M) and kinetin (0.46, 4.60, and 46.60 μ M) significantly promoted the germination of seeds in all salinity treatments (Table 3). Higher GA₃ and kinetin concentrations (46.6 μ M) alleviated the high-salinity (345 μ M) effects and promoted germination compared with the control. A two-way ANOVA of germination indicated significant main effects and their interaction between salinity and GA₃ (Table 4). Similar results were obtained for a two-way ANOVA of germination by salinity and kinetin treatments (Table 5).

A one-way ANOVA of germination for each salinity treatment revealed that GA₃ significantly alleviated the inhibitory effects of high salt concentrations (86 μ M NaCl, $F = 3.2$ ns; 172 μ M NaCl, $F = 14.16$, $P < 0.001$; 285 μ M NaCl, $F = 12.46$, $P < 0.001$; 345 μ M NaCl, $F = 6.97$, $P < 0.01$). A one-way ANOVA of germination by kinetin treatment indicated similar effects (86 μ M NaCl, $F = 1.04$ ns; 172 μ M NaCl,

TABLE 4. Results of two-way analysis of variance of characteristics by salinity (S) and gibberellin A₃ (G) treatments

Dependent variable	Independent variable		
	S	G	S × G
Germination	377.3***	620.3***	129.1***
Hypocotyl length	157.2***	1755.5***	164.3***
Root length	908.3***	845.2***	298.0***
Dry weight	53.9***	111.4***	38.1***

NOTE: Numbers represent *F* values; *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

TABLE 5. Results of two-way analysis of variance of characteristics by salinity (S) and kinetin (K) treatments

Dependent variable	Independent variable		
	S	K	S × K
Germination	226.4***	526.6***	91.6***
Hypocotyl length	30.7***	669.4***	41.9***
Root length	96.2***	865.9***	108.2***
Dry weight	294.1***	239.7***	41.0***

NOTE: Numbers represent *F* values; *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

TABLE 6. Mean (± SD) hypocotyl length (mm), root length (mm), and dry weight (mg) of individual *Atriplex griffithii* seedlings after 20 days from various salinity and gibberellin A₃ regimes

Salinity (mM)	Gibberellin A ₃ (μM)			
	0.00	0.29	2.90	29.00
Hypocotyl length				
0	0.72±0.02	1.43±0.21	2.00±0.16	1.65±0.01
86	0.36±0.05	1.05±0.09	3.12±0.10	2.13±0.04
172	0.29±0.05	3.02±0.09	2.22±0.26	2.44±0.08
285	0.36±0.02	1.84±0.09	3.14±0.06	3.53±0.05
345	0.15±0.01	2.55±0.08	2.18±0.09	1.29±0.02
Root length				
0	0.51±0.01	0.15±0.01	0.64±0.04	0.66±0.03
86	0.43±0.02	0.50±0.04	0.28±0.02	0.68±0.04
172	0.28±0.02	1.40±0.07	1.15±0.09	0.88±0.02
285	0.36±0.02	0.99±0.07	1.74±0.03	2.28±0.10
345	0.29±0.03	1.12±0.04	0.74±0.05	1.04±0.06
Dry weight				
0	0.20±0.02	0.13±0.02	0.21±0.03	0.17±0.02
86	0.15±0.02	0.18±0.03	0.13±0.01	0.19±0.01
172	0.08±0.01	0.25±0.01	0.26±0.01	0.18±0.01
285	0.05±0.01	0.19±0.01	0.18±0.02	0.21±0.01
345	0.03±0.01	0.10±0.01	0.15±0.02	0.17±0.02

F = 19.84, *P* < 0.001; 285 μM NaCl, *F* = 4.48, *P* < 0.05; 345 μM NaCl, *F* = 27.22, *P* < 0.001).

Effect of salinity and growth regulators on growth

Hypocotyl length, root length, and dry weight were significantly promoted by both GA₃ and kinetin at all salinity treatments (Tables 6 and 7). This promotive effect is more pronounced at higher salinity concentrations. High concentrations of GA₃ (28.8 μM) and lower concentrations of kinetin

TABLE 7. Mean (± SD) hypocotyl length (mm), root length (mm), and dry weight (mg) of individual *Atriplex griffithii* seedlings after 20 days from various salinity and kinetin regimes

Salinity (mM)	Kinetin (μM)			
	0.00	0.46	4.60	46.00
Hypocotyl length				
0	0.72±0.09	1.52±0.29	1.30±0.01	1.60±0.01
86	0.36±0.03	1.61±0.02	1.37±0.02	1.49±0.01
172	0.29±0.02	2.19±0.19	1.97±0.10	2.06±0.08
285	0.24±0.01	1.71±0.07	2.03±0.10	1.49±0.24
345	0.08±0.01	3.04±0.19	1.36±0.24	1.72±0.07
Root length				
0	0.50±0.03	1.34±0.02	0.72±0.01	0.77±0.01
86	0.43±0.02	1.24±0.10	0.97±0.02	1.03±0.08
172	0.36±0.03	1.20±0.05	1.08±0.11	1.26±0.09
285	0.37±0.03	1.20±0.05	1.08±0.11	1.26±0.09
345	0.30±0.05	1.33±0.03	1.76±0.04	0.58±0.09
Dry weight				
0	0.20±0.02	0.24±0.12	0.20±0.02	0.21±0.01
86	0.15±0.02	0.25±0.02	0.20±0.01	0.17±0.01
172	0.09±0.01	0.21±0.02	0.22±0.02	0.15±0.01
285	0.04±0.01	0.26±0.01	0.21±0.02	0.28±0.02
345	0.03±0.01	0.12±0.02	0.07±0.01	0.05±0.02

(0.46 μM) significantly promoted seedling growth at all salt concentrations tested. A two-way ANOVA of hypocotyl length indicated significant main effects and their interaction between salinity and GA₃ treatments (Table 4). Similar results were obtained for root length and dry weight (Table 4). A two-way ANOVA of hypocotyl length indicated significant main effects and their interaction between salinity and kinetin treatments (Table 5). A two-way ANOVA of dry weight and root length shows similar effects for salinity and kinetin treatments.

Discussion

Recruitment of perennial halophytes through seed germination is not common in maritime tropical desert communities. Studies of several desert communities revealed that the predominant mode of reproduction is vegetative through rhizome growth rather than by seeds (Khan 1991; Zaman and Khan 1992; Aziz 1993). However, in an *A. griffithii* community relatively more seeds germinate in comparison with other perennial halophyte species.

Germination of *A. griffithii* decreased with an increase in salinity. Germination was substantially inhibited at 2% NaCl. Maximum germination percentages were obtained in the non-saline control. Germination of seeds of *Atriplex* spp. was reported to be inhibited by increases in salinity (Young et al. 1980; Ignaciuk and Lee 1980; Uchiyama 1981; Khan and Ungar 1984a, 1984b, 1985). In tropical saline desert conditions a substantial monsoon rainfall is required to decrease the salinity and to provide sufficient moisture to induce germination.

Salinity and temperature express their greatest effect at the extremes of these two environmental variables (Ungar 1978). With increase in salinity and temperature, there is a decrease in germination percentage, delay in germination rate, and decrease in seedling length. Ignaciuk and Lee (1980) reported that *Atriplex glabriuscula* and *Atriplex laciniata* germinated well under alternating temperature regimes in the laboratory.

They also reported a significant interaction between salinity and temperature in affecting germination. Khan and Ungar (1984a) reported that *A. triangularis* was least sensitive to salinity at optimal temperatures for germination and demonstrated a very highly significant ($P < 0.0001$) interaction between temperature and salinity in affecting germination. A similar interaction was reported for *Salicornia pacifica* var. *utahensis* (Khan and Weber 1986), *Cressa cretica* (Khan 1991), and *Hordeum jubatum* (Badger and Ungar 1989).

During the monsoon period the area around Karachi is covered with clouds and the average temperature is about 10–15°C cooler than an average summer day. Rainfall usually comes during the latter part of the monsoon. Availability of moisture with subsequent reduction in salinity and temperature could induce the germination of *Atriplex* seeds and may cause the relatively higher germination of *Atriplex* seeds in comparison with other halophytic shrubs present.

Early seedling growth is also significantly reduced with increased salinity. Seedling growth was highest in controls, which indicates that salinity is not necessary for optimal growth of *A. griffithii*. The low-temperature regime 25:10°C produced comparatively better seedling growth at all salinities. Billard and Binet (1975) showed a progressive decrease in dry weight with an increase in salinity in all *Atriplex* spp. studied. Similar results were obtained in the present study.

Plant growth regulators GA₃ and kinetin were effective in alleviating the inhibitory effect of salinity in high- and low-salinity treatments. At lower salinity these growth regulators not only alleviated the effect of salinity but also promoted germination and growth in comparison with the distilled water control. Khan (1991) reported that GA₃ alleviated the inhibitory effects of salinity on the germination of *C. cretica* seeds. Similar effects were also reported for *Salicornia pacifica* (Khan and Weber 1986), *A. triangularis* (Khan and Ungar 1985), and *Spergularia marina* (Ungar 1984). Kinetin is also found to alleviate the effect of salinity in the germination of *A. triangularis* (Khan and Ungar 1985) and *Salicornia pacifica* (Khan and Weber 1986). However, kinetin did not promote germination of *Spergularia media* (Ungar 1978), *Spergularia marina* (Ungar 1984), and *C. cretica* (Khan 1991).

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