

EFFECTS OF SALINITY, TEMPERATURE, LIGHT AND DORMANCY REGULATING CHEMICALS ON SEED GERMINATION OF *SALSOLA DRUMMONDII* ULBR.

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Abstract

Salsola drummondii Ulbr. is a perennial halophyte found in salt deserts of southern Balochistan, Pakistan. Experiments were conducted to study the effects of salinity (0, 200, 400, 600, 800 and 1000 mM NaCl), thermoperiod (10/20, 15/25, 20/30 and 25/35°C), light (12-h photoperiod and dark) and dormancy regulating chemicals (DRCs) on germination, recovery and viability of the seeds of *S. drummondii*. Seeds of *S. drummondii* germinated quickly in distilled water at different temperature regimes and increases in salinity decreased seed germination. Interestingly, few seeds could even germinate in 1000 mM NaCl treatment, which is about twice as high as seawater salinity. Seeds were partially photoblastic and showed relatively higher germination under 12-h photoperiod than in dark. Seeds showed poor recovery of germination from salinity and particularly when germinated in dark. Germination inhibition at high salinity (800 mM NaCl) under 12-h photoperiod was partially alleviated by the exogenous application of different DRCs, particularly fusicoccin. Moreover, all the DRCs, except GA₄₊₇, ameliorated germination of salt stressed seeds under complete darkness and GA₄ and fusicoccin were most effective. Our study shows that seeds of *S. drummondii* are highly tolerant to salinity and variation in temperature but partially photoblastic nature indicate that seeds will not germinate if buried under the soil. Seed germination under saline conditions can be improved by the use of DRCs particularly by application of fusicoccin.

Key words: Fusicoccin, Germination recovery, Halophyte, Salt desert, Seed germination, Seed viability.

Introduction

Soil salinity is considered an important factor influencing seed germination of halophytes (Gulzar *et al.*, 2007; El-Keblawy & Shamsi, 2008; El-Keblawy *et al.*, 2011; Gul *et al.*, 2013; Zeng *et al.*, 2014). Salinity tolerance of halophytes during seed germination varies considerably among species; however *Salsola* species are among those with high salt tolerance (Khan & Gul, 2006). Wei *et al.* (2008) reported extremely high salinity tolerance for *S. affinis* seeds which could germinate (2%) in 2000 mM NaCl solution. Seeds of *S. nitraria* (Chang *et al.*, 2008), *S. ferganica* (Wang *et al.*, 2013) and *S. iberica* (Khan *et al.*, 2002) could germinate in up to 1000 mM NaCl concentration, while *S. imbricata* germinated in up to 800 mM NaCl (Mehrun-Nisa *et al.*, 2007).

Variation in thermoperiod and photoperiod significantly affect seed germination of halophytes under natural conditions (Khan & Gul, 2006). Seeds of subtropical halophytes generally germinate optimally at the temperature regime of 20/30°C (Khan & Ungar, 1997a and 1998; Gulzar *et al.*, 2001). For example, seeds of *Limonium stocksii* could germinate in 500 mM NaCl at optimum temperature of 20/30°C but not at 10/20 and 25/35°C (Zia & Khan, 2004). However, seeds of some species preferably germinate at cooler (10/20°C; e.g. *S. imbricata*; Mehrun-Nisa *et al.*, 2007) or warmer (25/35°C; e.g. *Desmostachya bipinnata*; Gulzar *et al.*, 2007) temperature regimes. Effect of light on seed germination is also quite variable and varies with species (Gul *et al.*, 2013). Gul *et al.* (2013) indicated that some halophytes failed to germinate in dark while in some others light had little effect on seed germination. Similarly, Baskin and Baskin (1995) reported that out of 41 halophytes, seed

germination of 20 species was promoted in presence of light, 10 species germinated better in dark, while 11 species germinated equally well in both light and dark. These reports indicate that the salinity tolerance and germination responses of halophyte seeds greatly depend on combinations of thermoperiod and photoperiod in saline conditions.

Halophyte seeds are reported to remain viable under high temperature and salinity stress and readily germinate when conditions becomes more favorable (Ungar, 1995; Khan & Ungar, 1997a and 1998, Pujol *et al.*, 2000). However, this recovery response varies from poor recovery in some halophytes such as *Zygophyllum simplex* (Khan & Ungar, 1997b) to almost complete recovery in many such as *Suaeda fruticosa* (Khan & Ungar, 1998) and *Limonium stocksii* (Zia & Khan, 2004). In addition, temperature regimes also influence recovery responses of halophytes (Khan & Ungar, 1997; Zia & Khan, 2004; Gulzar *et al.*, 2007; El-Keblawy & Shamsi, 2008; Ahmed & Khan, 2010). Seed germination under saline condition may be improved by exogenous application of dormancy regulating chemicals (DRCs) such as gibberellins, cytokinins, thiourea and fusicoccin (Khan & Ungar, 2002; Gul & Khan, 2003; Mehrun-Nisa *et al.*, 2007).

Salsola drummondii Ulbr. is a leaf succulent perennial xerohalophyte from family Amaranthaceae, which is commonly found in salt deserts of Saharo-Sindian region specifically eastern parts of Arabian Peninsula, southern Iran and southern to central Pakistan (http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=242100179). Large populations of *S. drummondii* are found in salt deserts of Balochistan, Pakistan (Khan & Qaiser, 2006), where it grows in association with *Suaeda*

fruticosa, *Aerva javanica* var. *bovei*, *Prosopis juliflora* and several desert grasses. There are several economic usages of this plant. For instance, cylindrical to terete leaves of *S. drummondii* are burnt to obtain soda ash by locals, different plant parts are medicinally important (Gilani *et al.*, 2010) and leaves could serve as forage (Qureshi *et al.*, 1993). However, information about salinity tolerance and germination ecology of *S. drummondii* seeds is not known. We proposed to answer following questions regarding the seed germination of *S. drummondii*: 1) How tolerant seeds are at germination stage? 2) Whether salinity tolerance of *S. drummondii* at seed germination is temperature and light dependant, 3) Can seeds maintain viability when exposed to high salinity and temperature stress? and 4) Can salinity tolerance during germination be improved by the application of DRCs.

Materials and Methods

Seed collection and study site: Mature seeds of *Salsola drummondii* were collected from a salt desert located at Winder, Balochistan (24°25'07.16" N and 66°37'32.38"E) during February 2011. Seeds were separated from perianths, surface sterilized by using 1% commercial bleach (Sodium hypochlorite), rinsed with distilled water, air dried and stored in clear plastic Petri plates. Freshly collected seeds were used in germination experiments.

Seed germination responses: Seed germination was carried out in programmed incubators with alternating temperature regimes of 10/20, 15/25, 20/30 and 25/35°C, where low temperatures represent temperatures of 12-h dark and higher temperatures with 12-h light period (25 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 400-700 nm, Philips cool-white fluorescent lamps). Germination was carried out in tight fitting plastic Petri plates (5 cm Φ), which were placed in large glass Petri plates to prevent evaporative loss of the test solutions. There was 5 ml of test solution in each Petri plate. Six NaCl (0, 200, 400, 600, 800, and 1000 mM) concentrations were used with four replicates of 25 seeds each per treatment. Germination was recorded (Protrusion of embryonic axis; Bewley & Black, 1994) every alternate day for 20 days. Rate of germination was calculated according to the method of Khan & Ungar (1984). Seed germination was also carried out to study effect of dark on seed germination in response to increasing NaCl concentrations at various temperature regimes by placing Petri plates in dark-plastic bags. Germination of dark-treated seeds was noted once after 20 d.

Seed recovery responses: Recovery from high salinity stress was recorded by transferring the seeds to distilled water under similar experimental conditions and the germination was recorded on every alternate day for another 20d. Recovery from dark was also studied by transferring the Petri plates with seeds to 12-h photoperiod keeping other conditions unchanged.

Seed viability responses: All the un-germinated seeds at the end of recovery experiments were tested for their viability using 2, 3, 5 - triphenyl tetrazolium chloride (TTC) test (MacKay, 1972; Bradbeer, 1998).

Seed responses to dormancy regulating chemicals: Effects of different dormancy regulating chemicals (DRCs) for alleviating salinity and light effects on seed germination of *S. drummondii* were studied under 12-hour photoperiod and in complete dark at 20/30°C. Solutions of GA₃ (10 μM), GA₄ (10 μM), GA₄₊₇ (10 μM), thiourea (100 μM), kinetin (3 mM) and fusicoccin (5 μM) were added in germination media (0 and 800 mM NaCl). Concentrations of DRCs used in this study were determined in a preliminary study (Data not given). Germination was recorded every alternate day for 20 days in case of 12-hour photoperiod treatments, while once after 20 days for dark treatment.

Statistical analyses: The data was transformed using arcsine transformation before the statistical analysis. Analyses of variance (ANOVA) were performed to determine the significance of the effects of NaCl, temperature and light on seed germination, recovery, viability and mortality data. Bonferroni post-hoc tests ($p < 0.05$) were carried out to determine significant difference between individual means. Student *t*-test ($p < 0.05$) was used to compare DRC treatments with control. Statistical analyses were done by using SPSS for windows version 11 (Anon., 2001).

Results

Seed germination responses: Analysis of variance indicates that NaCl treatments had significant ($p < 0.001$) effect on both rate and final seed germination of *S. drummondii* (Table 1). About 80% seeds germinated in distilled water at all temperature regimes studied indicating little effect of temperature on seed germination under non-saline conditions. However, higher temperature regime (25/35°C) delayed the seed germination compared to cooler thermoperiod (Fig. 2). Increases in NaCl concentration linearly decreased rate and final germination and few seeds germinated at 1000 mM NaCl (Figs. 1 and 2). Seed germination under 12-hours photoperiod was significantly higher compared to dark at favorable temperature regimes and no differences were recorded in other treatments.

Seed recovery responses: Un-germinated seeds of *S. drummondii* from different NaCl treatments under 12-hours photoperiod showed low (~10 to 20%) recovery, when transferred to distilled-water with little or no difference among temperature treatments (Table 2; Fig. 3). While, un-germinated seeds from dark treatments did not recover at any of the temperatures (Table 3; Fig. 4).

Seed viability responses: Only ~10-30% of the un-germinated from different salinity treatments under 12-hours photoperiod after recovery experiment, were viable and about 50% were dead (Fig. 3). High temperature (25/35°C) and NaCl (≥ 600 mM) treatments increased the percentage of dead seeds under 12-hour photoperiod. Seed death under 12-hours photoperiod was relatively lesser and most un-germinated/un-recovered seeds were viable, especially at 20/30°C (Fig. 4).

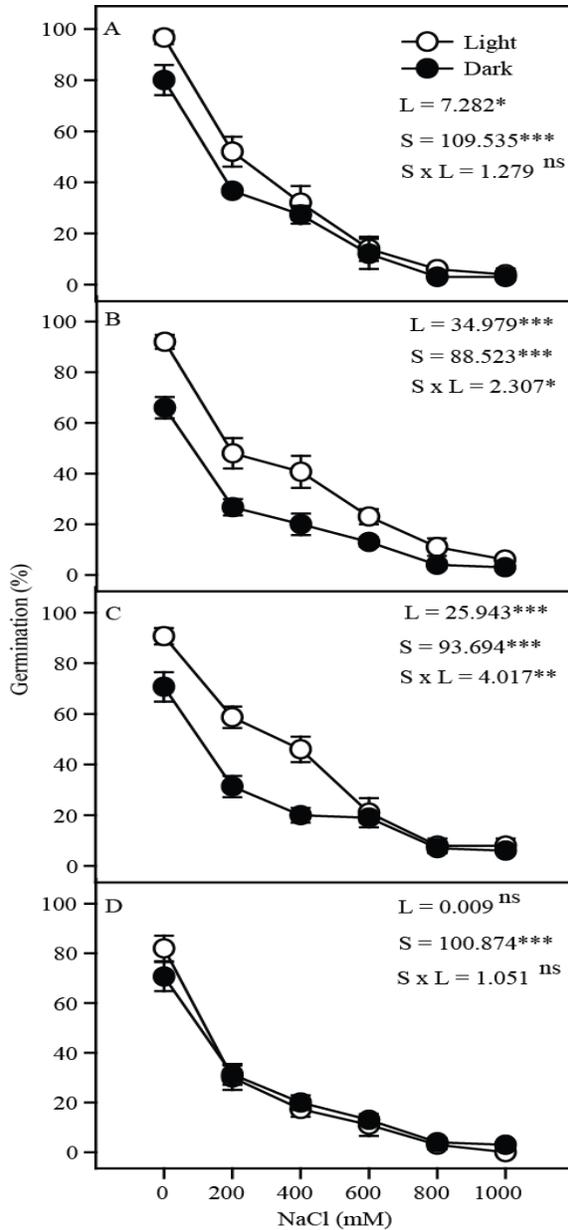


Fig. 1. Effect of salt, light/dark and temperature treatments on the seed germination of *Salsola drummondii*. A. 10/20°C, B. 15/25°C, C. 20/30°C and D. 25/35°C. Circles represent mean±standard errors. *F*-Values were obtained from analysis of variance (ANOVA) by using L (light/dark treatments) and S (NaCl treatments) as variables. Where, * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ and ns = non-significant.

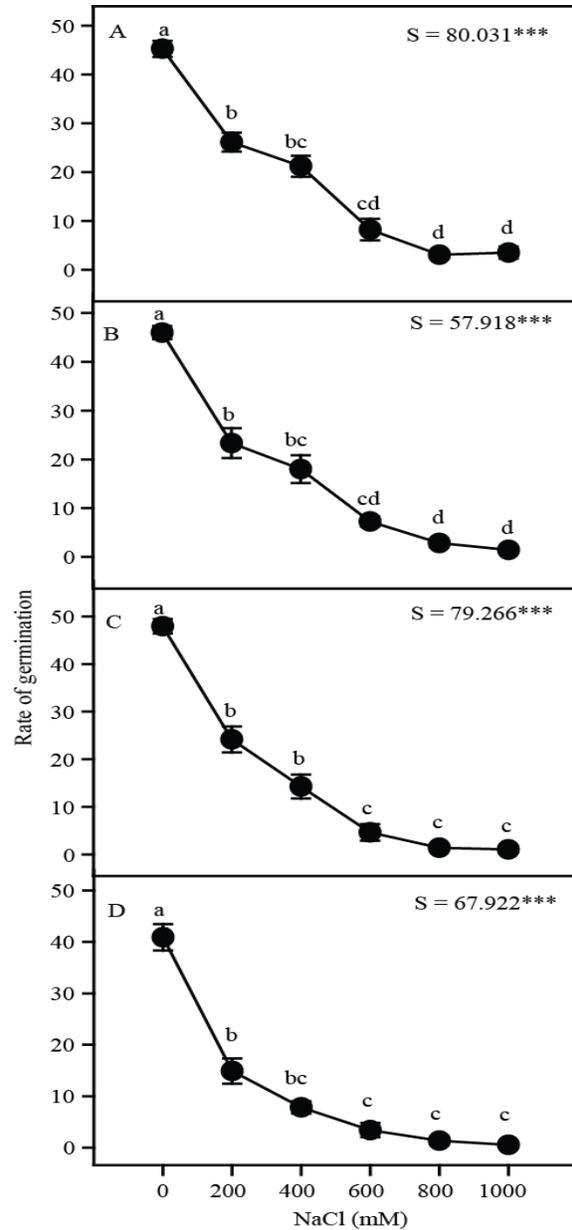


Fig. 2. Effect of salt, light/dark and temperature treatments on the rate of seed germination of *Salsola drummondii*. A. 10/20°C, B. 15/25°C, C. 20/30°C and D. 25/35°C. Each circle represent mean±standard errors. Symbols having same letter are not significantly different ($p < 0.05$) among means (Bonferroni test). *F*-Values were obtained from Analysis of variance (ANOVA) by using S (NaCl treatments). Where, *** = $p < 0.001$.

Table 1. Two-way analysis of variance (ANOVA) indicating significance of the individual and collective effects of various experimental factors on the percentage of germinated (G), recovered (R), viable (V) and dead (D) seeds when recovered from salt. Where, number represents F-values.

Treatments	G	R	V	D
T	8.215***	18.843***	55.702***	31.214***
S	202.602***	82.650***	95.237***	46.983***
S x T	1.924*	7.405***	8.904***	1.953*

* = $p < 0.05$ and *** = $p < 0.001$

Table 2. Two-way analysis of variance (ANOVA) indicating significance of the individual and collective effects of various experimental factors on the percentage of germinated (G), recovered (R), viable (V) and dead (D) seeds when recovered from temperature. Where, number represents F-values.

Treatments	G	R	V	D
T	5.784**	26.522***	18.858***	14.871***
S	41.267***	7.502***	23.999***	22.393***
S x T	0.436 ^{ns}	6.392***	3.059*	0.511 ^{ns}

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ and ns = Non-significant

Table 3. Two-way analysis of variance (ANOVA) indicating significance of the individual and collective effects of various experimental factors on the percentage of germinated (G), recovered (R), viable (V) and dead (D) seeds when recovered from dark. Where, number represents F-values.

Treatments	G	R	V	D
T	3.759*	-	139.178***	48.030***
S	101.920***	-	72.379***	30.006***
S x T	1.044 ^{ns}	-	4.866***	3.210**

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ and ns = Non-significant

Seed responses to dormancy regulating chemicals: All the DRCs used, inhibited seed germination of *S. drummondii* in absence of salinity under both 12-hours photoperiod and dark (Fig. 5), however a significant ($p < 0.05$) improvement in germination was observed at 800 mM NaCl by all DRCs under 12-hours photoperiod (Fig. 5A, B, E and F). Fusicoccin improved germination (> 4 folds) of salt stressed seeds more than any other DRC under 12-hours photoperiod (Fig. 5B and F). All the DRCs, except GA₄₊₇, improved seed germination of salt stressed seeds under complete darkness and this improvement was highest in case of GA₄ and Fusicoccin applications (Fig. 5C and D).

Discussion

Salt desert halophytes of subtropical environment generally lack innate dormancy (Gul *et al.*, 2013). Similarly, innate dormancy was also absent in seeds of *S. drummondii* which germinated well in distilled water at all temperature and light regimes. This finding is in accordance with the results obtained for other *Salsola* species such as *S. imbricata* (Mehrun-Nisa *et al.*, 2007), *S. affinis* (Wei *et al.*, 2008), *S. nitraria* (Chang *et al.*, 2008), *S. ferganica* (Wang *et al.*, 2013) and *S. iberica* (Khan *et al.*, 2002). Lack of innate dormancy hence appears an adaptation of *Salsola* species to germinate quickly upon availability of adequate moisture after rains. Fast germination rate of *S. drummondii*, as reported for *S. affinis* and *S. imbricata* too, also supports this hypothesis (Mehrun-Nisa *et al.*, 2007; Wei *et al.*, 2008).

Seeds of *S. drummondii* were highly salt tolerant and could germinate in up to 1000 mM NaCl, which is about twice as saline as seawater. Seeds of other *Salsola* species such as *S. affinis* (Wei *et al.*, 2008), *S. nitraria* (Chang *et al.*, 2008), *S. iberica* (Khan *et al.*, 2002), *S. ferganica* (Wang *et al.*, 2013) and *S. imbricata* (Mehrun-Nisa *et al.*, 2007) could also germinate at or above 800 mM NaCl, indicating that seeds of *Salsola* species are highly salt tolerant during germination. However, seeds of co-occurring species such as *Suaeda fruticosa* (Khan &

Ungar, 1998; Hameed *et al.*, 2006), *Desmostachya bipinnata* (Gulzar *et al.*, 2007) and *Aerva javanica var. bovei* (Khan *et al.*, Unpublished data) failed to germinate beyond 500 mM NaCl. High salinity tolerance during germination in comparison to co-occurring species could be advantageous for *S. drummondii* at least during early developmental stages to colonize saline gaps of the community.

Temperature had little or no effect on seed germination of *S. drummondii*, however, rate of germination at 25/35°C was lower. Wei *et al.* (2008) also reported that seed germination of *S. affinis* occurred in a wide range (5 to 30°C) of temperatures, constituting an “opportunistic” germination strategy to produce seedlings whenever conditions are favorable for seedling growth. In contrary, seeds of most subtropical perennial halophytes germinate optimally at 20/30°C (Khan & Gul, 2006). However, some species like *S. imbricata* prefer cooler (10/20°C; Mehrun-Nisa *et al.*, 2007) temperatures for germination and a few like *D. bipinnata* germinate better at warmer (25/35°C; Gulzar *et al.*, 2007) temperatures. Broader “temperature window” for germination as compared to other co-occurring plants, could possibly be another adaptation of *S. drummondii* seeds to colonize study site promptly whenever adequate moisture is available.

Light is one of the key factors which determine the timing of germination in seeds of many halophyte species by facilitating the conditional dormancy to protect the seedlings from the extreme environment (Qu *et al.*, 2008; Gul *et al.*, 2013). Light can control the seed germination responses independently as well as it can operate in combination with salinity and temperature (Gul *et al.*, 2013). Light requirements of salt desert halophytes for seed germination are quite variable and inconclusive (Khan & Gul, 2006). Some halophytes like *Haloxylon recurvum* and *Zygophyllum simplex* germinated better in presence of light (Khan & Ungar, 1997), many such as *Lasiurus scindicus* and *Panicum turgidum* (El Keblawi *et al.*, 2011) germinate better in complete dark and others like *Atriplex stocksii* (Khan & Ungar, 1997) germinate

equally well under light as well as dark. Seeds of *Limonium stocksii* germinated equally well in light and dark under non-saline conditions but dark was relatively more inhibitory under high salinity treatments (Zia & Khan, 2004). In contrast, seeds of *Suaeda salsa* germinated better in dark than light under saline conditions, while were light/dark insensitive under non-saline conditions (Li *et al.*, 2005; Song *et al.*, 2008). It is noteworthy that seeds of *S. drummondii* germinated better under 12-hours photoperiod than in complete dark under no/low (≤ 400 mM) salinity and moderate temperature (15/25 and 20/30°C) treatments. This could help recruiting seedlings at or near soil surface under no/low

salinity stress conditions, so that their survival chances are maximized (Pons, 1992; El-Keblawy & Al-Shamsi, 2008). However, difference between germination in light and dark diminished at high (≥ 600 mM) salinity and extreme (10/20 and 25/35°C) temperatures in this study. This could also be an adaptive strategy of our test species to take advantage of delayed summer monsoon, which may not dilute soil salts to low levels. Such harsh conditions could be an opportunity for salt desert halophytes to produce some seedlings either at soil surface or in low light cracks or litter covers, as even low rains occur once in a year(s) in arid regions.

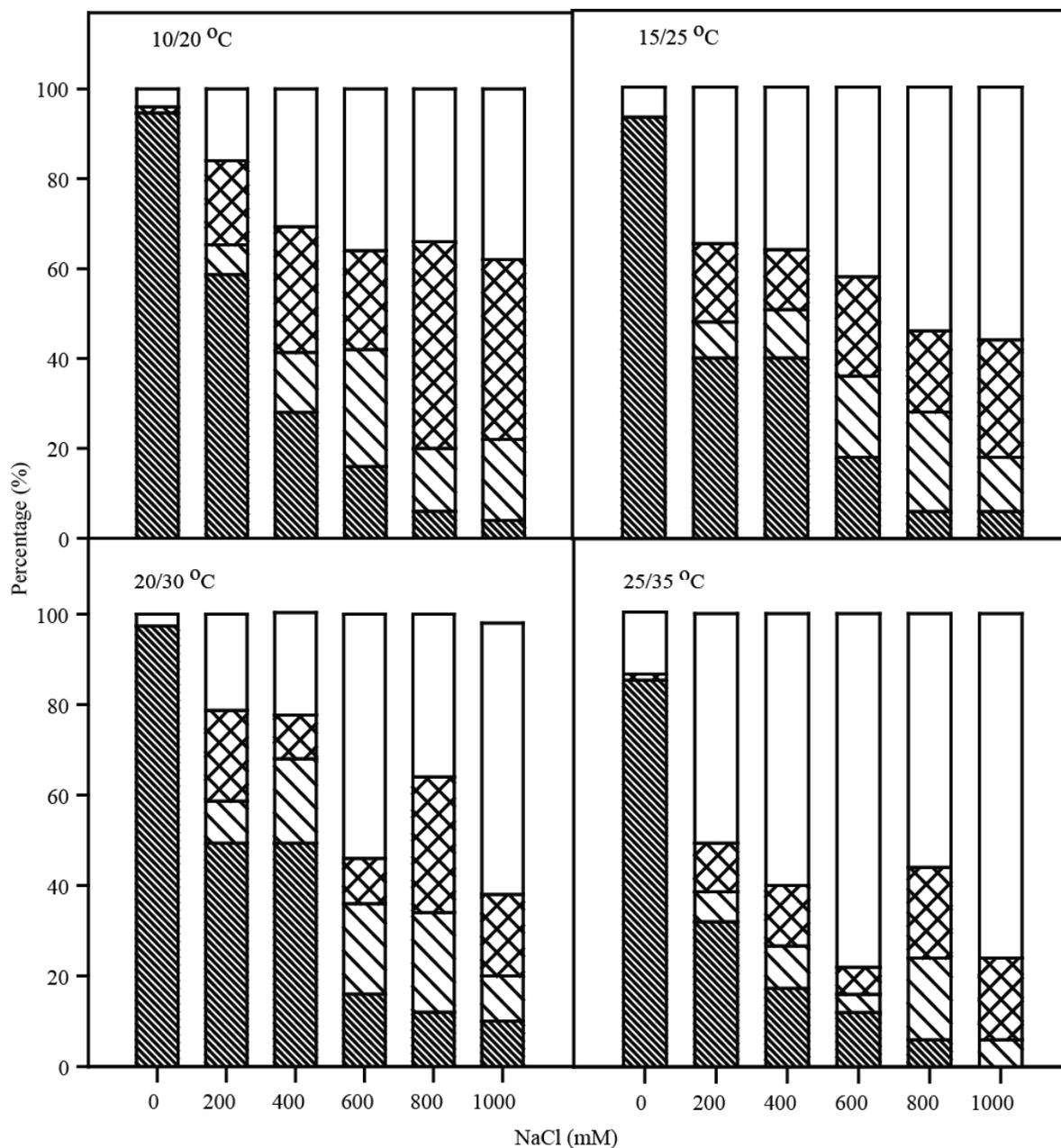


Fig. 3. Percentage of germinated (▨), recovered (▩), viable (▧) and dead (□) seeds of *Salsola drummondii* treated with various concentrations of NaCl under 12 h photoperiod at different temperature regimes.

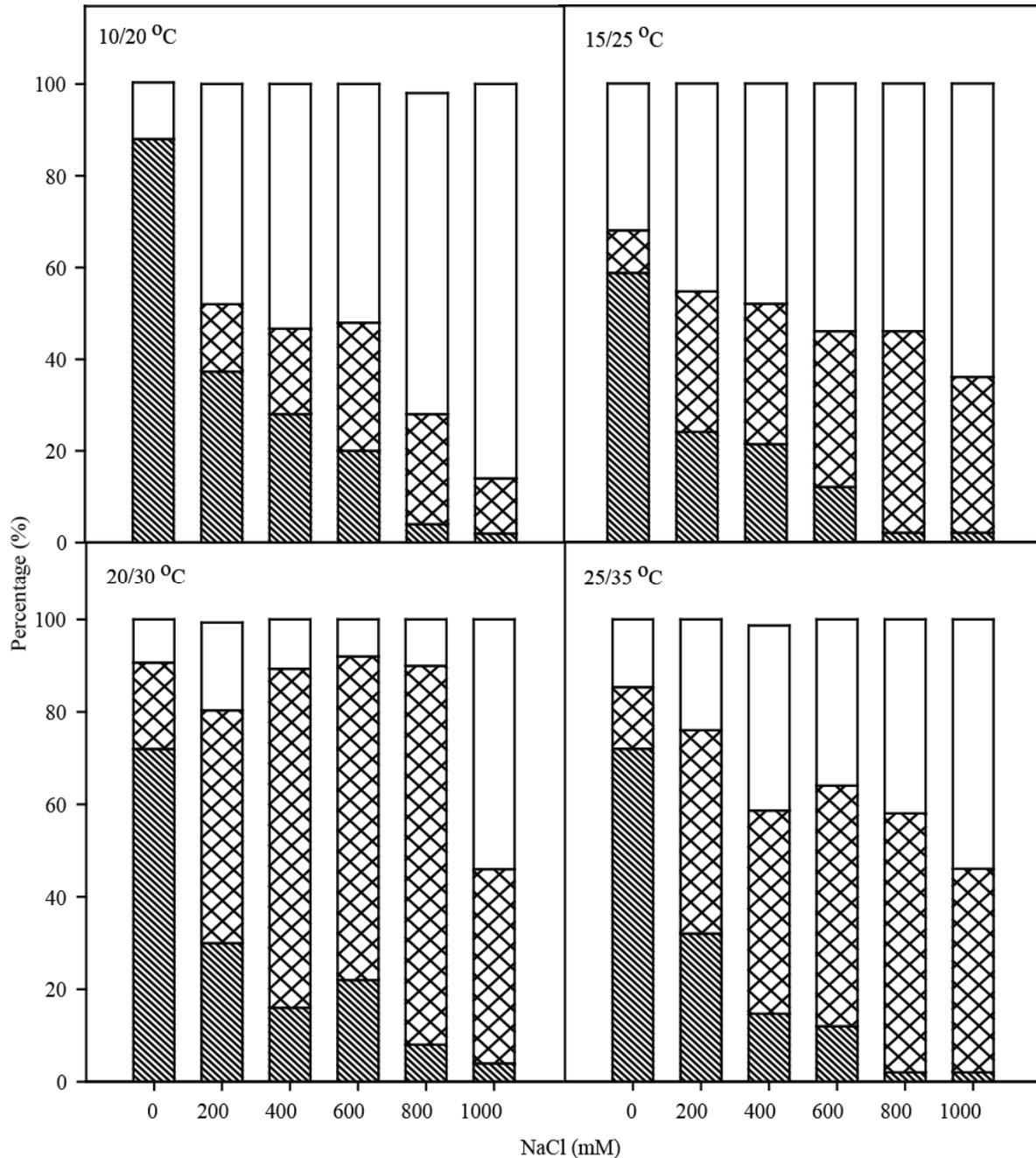


Fig. 4. Percentage of germinated (▨), recovered (▩), viable (▧) and dead (□) seeds of *Salsola drummondii* treated with various concentrations of NaCl at different temperatures under 24 h dark.

Most subtropical halophytes show high germination recovery after removal of salt (Khan & Gul, 2006). For example, *Arthrocnemum macrostachyum* (Khan & Gul, 1998), *Suaeda fruticosa* (Khan & Ungar, 1997) and *Limonium stocksii* (Zia & Khan, 2004) showed high recovery percentages after alleviation of salinity stress, indicating salinity enforced dormancy due to osmotic constraint. In contrast, seeds of *S. drummondii* showed low recovery of germination after alleviation of salt under 12-hours photoperiod at different temperatures. Similarly seeds of *S. imbricata* also showed poor recovery from

salinity when transferred to distilled water (Mehrun-Nisa *et al.*, 2007). Seed viability testing after recovery experiments indicated that the most un-germinated seeds were dead with $\leq 30\%$ having induced dormancy (un-germinated viable) under 12-hours photoperiod condition. It is also noteworthy that there was no germination recovery (enforced dormancy) in un-germinated seeds from complete dark and there was very high percentage of seeds with induced dormancy. Eco-physiological significance of this high induced dormancy and low seed mortality under dark treatment needs to be investigated.

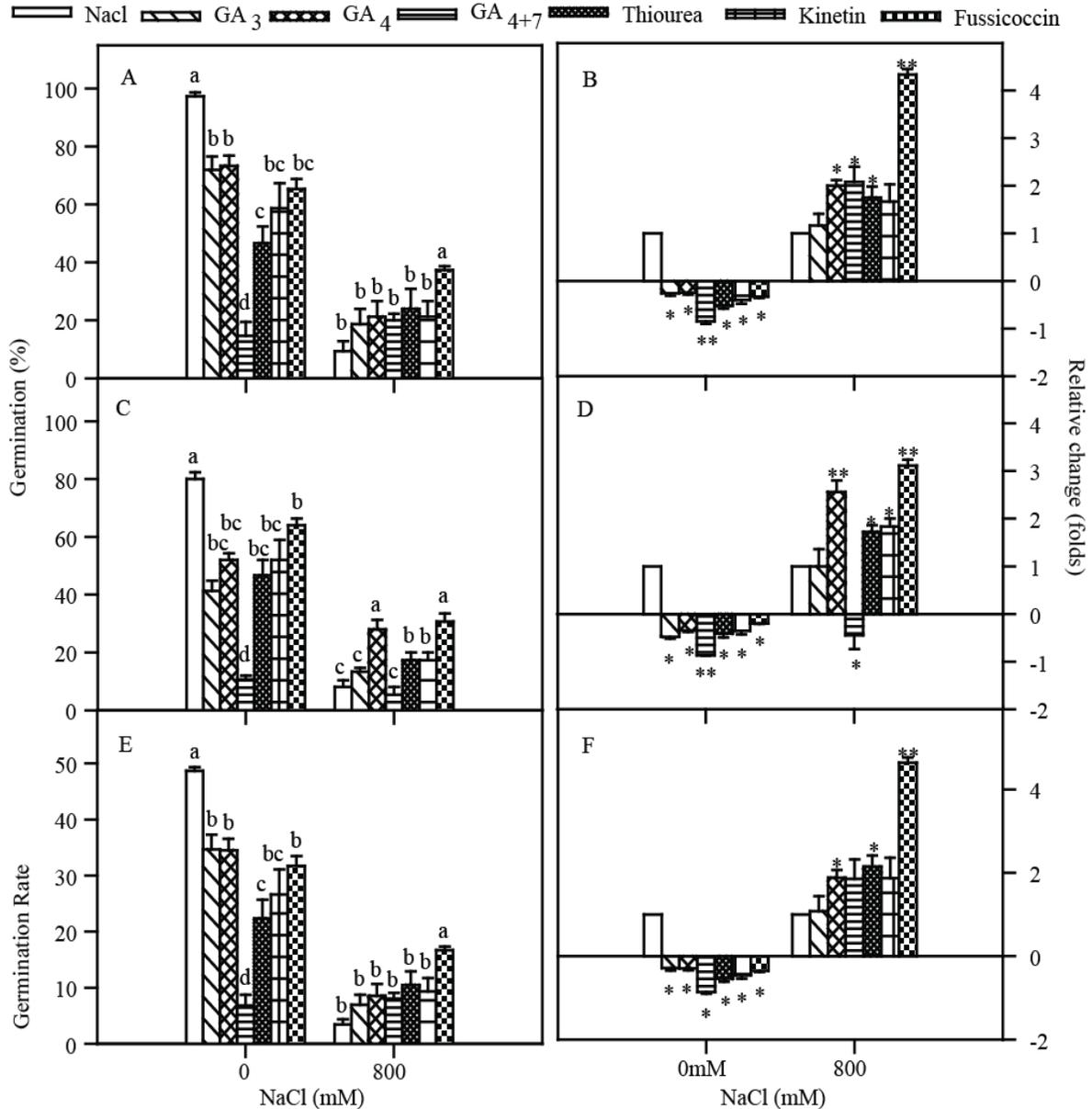


Fig. 5. Effects of GA_3 (10 μ M), GA_4 (10 μ M), GA_{4+7} (10 μ M), thiourea (100 μ M), kinetin (3 mM) and fusicoccin (5 μ M) on mean final germination of *S. drummondii* seeds in 12h photoperiod (A), 24h dark (C) and rate of germination (E) under control (0 mM NaCl) and saline condition (800 mM NaCl). Relative changes (folds) due to DRCs in mean final germination of *S. drummondii* seeds in 12h photoperiod (B), 24h dark (D) and rate of germination (F) are given in comparison to respective non-saline and saline controls. Bars represent mean \pm standard errors. Bars having same letter within each salt treatment are not significantly different ($p < 0.05$) (Bonferroni test). Asterisk (*) indicate significant ($p < 0.05$) difference between a DRC treatment and saline control (t-test).

Chemical treatments had differential effect on seed germination of *S. drummondii* under non-saline and saline conditions. All the DRCs inhibited seed germination under non-saline condition but significantly ($p < 0.05$) improved germination under highly saline (800 mM NaCl) conditions. Exogenous fusicoccin promoted seed germination of salt stressed seeds of *S. drummondii* more than any other DRC tested. Fusicoccin, a diterpene glycoside which was initially isolated as a toxin from fungus *Fusicoccum amygdali* (Ballio *et al.*, 1976), is widely reported to promote seed germination of halophytes

(Gul & Weber, 1998; Gul *et al.*, 2000; Khan *et al.*, 2002; Gul & Khan, 2003; El-Keblawy *et al.*, 2008) probably by enhancing cell elongation growth through ATPase mediated proton extrusion (Galli *et al.*, 1979; Marrè, 1979). Fusicoccin reversed the salinity induced germination inhibition in *Zygophyllum simplex* seeds completely (Khan & Ungar, 2002). According to Cocucci *et al.* (1990) fusicoccin reversed the inhibitory effects of salinity in *Raphanus sativus* seeds by enhancing H^+ extrusion and malic acid synthesis. While, Lutsenko *et al.* (2005) suggested that fusicoccin affects

the ionic balance particularly the K^+/Na^+ ratio. Fusicoccin and GA_4 could also improve germination of salt stressed *S. drummondii* seeds under complete darkness. Likewise, fusicoccin also alleviated dark enforced dormancy in *Allenrolfea occidentalis* (Gul et al., 2000). While, GA_4 was more effective in stimulating seed germination of *Arabidopsis thaliana* under dark as compared to GA_3 (Fei et al., 2004).

Our data indicates that the mature seeds of *S. drummondii* are non-dormant and partially photoblastic, which can germinate quickly in absence of salinity over a broad range of temperatures, constituting an "opportunistic" germination strategy to fully exploit brief rain periods. Seeds are highly salt tolerant and some seeds can germinate in 1000 mM NaCl solution. Germination inhibition under highly saline conditions can partially be alleviated by the exogenous application of different DRCs, especially fusicoccin. While, fusicoccin and GA_4 could ameliorate germination of salt stressed seeds under complete darkness.

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