Biology of Salt Tolerant Plants
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Chapter 6

FACTORS AFFECTING THE GERMINATION OF SUMMER AND WINTER SEEDS OF HALOPYRUM MUCRONATUM UNDER SALT STRESS.

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Seeds of *Halopyrum mucronatum* (L.) Stapf. are produced twice during a season. Seeds produced during May (Summer seeds) are black and heavier and those which were produced during November (winter seeds) are brown and lighter. Seeds collected from each season were germinated under various salinity and temperature regimes. Summer seeds showed no effect of salinity concentrations used at higher temperatures whereas, at lower temperature regimes germination was subtantially inhibited by the high salinity concentration. Winter seeds were sensitive to higher salinity at all temperature regimes tested and this effect was lower at a moderate thermoperiod (30 - 20C). Application of thiourea was succesful in completely alleviating the inhibitory effect of salinity in summer seeds at the lower (10 -25C) thermoperiod.

INTRODUCTION

Halopyrum mucronatum (L.) Stapf. is a perennial grass distributed along the coast from Egypt to Mozambique and Madagascar, through Arabia to Pakistan, India and Sri Lanka (Jafri, 1966). It is a major component of dune vegetation through much of its range. It reproduces vegetatively by means of stolons and sexually by caryopses. It has an extensive partially underground stolon system which yearly produces new individuals. *H. mucronatum* flowers twice a year from April to May and September through November. Few seedlings were found after dispersal despite substantial seed production (Noor and Khan, unpublished data). Our field observation at Sandspit beach along the Karachi coast, Pakistan indicated that germination was primarily dependent on incident of sufficient monsoon rains which occurs somewhere from July through September.

Sandy beaches are the most physically stressful places in which a plant can begin its life. Salt spray, sand burial, dryness, high light intensity, high temperature, wind exposure, soil salinity and nutrient deficiency are among the major stresses imposed in coastal dune environment (Hesp, 1991). Variation in life cycle and germination strategies are common adaptations to such stresses. An adaptive ability to alter flowering time to maximize seed out put may enhance plant viability and germination (Ungar 1991). Certain species may have a physiological polymorphism, which is characteristic of some halophytes (Okusanya and Ungar, 1983). Seeds produced during summer and winter could be physiologicaly different. Dormancy of seeds may also be related to seed polymorphism, where germination ability differs between seed morphs (Ungar, 1991). The effect of salinity on germination varies considerably with temperature (Fowler, 1986; Badger and Ungar, 1987; Morgan and Myers, 1989; Myers and Morgan, 1989; Romo, 1990; Khan, 1991; Khan and Rizvi, 1994). Several studies have reported on seed germination and establishment of perennials in sand dunes (Maun, 1981; Hester and Mendelssohn, 1989; Blits and Gallagher, 1991; Zhang and Maun, 1991; Mariko et al., 1992). However no published data is available about germination ecology of *H. mucronatum*.

Some nitrogenous compounds such as nitrate, nitrite and thiourea are known to stimulate the germination of seeds (Esashi et al., 1979; Aldasoro et al., 1981). Thiourea counteracts the effect of

ABA and decreases the level of cytokinins in plant tissue. These adverse hormonal changes occur when plant tissues are subjected to water stress by drought, salinity or supraoptimal temperatures (Kabar and Baltepe, 1990). Treatment with thiourea has been shown to be highly effective in alleviating the inhibition of germination by salt or high temperature stress (Esashi et al., 1979).

Although perennial grasses reproduce both sexually and vegetatively, they rely more on the establishment of seedlings to maintain and expand their populations (Zhang, 1989; Zhang and Maun, 1991). Thus it is essential to understand the germination ecology of this species to maintain and manage dune systems. Our objective was *i)* to quantify the morphological differences of summer (produced in May) and winter (produced in November) seeds, *ii)* to determine their light requirement, *iii*) to determine the effect of salinity and various temperature regimes on germination and *iv)* to determine the ability of thiourea to alleviate the effect of salinity.

MATERIALS AND METHODS

Caryopses were collected at maturity from plants of *Halopyrum mucronatum* growing on dunes of Sands spit beach, Karachi, Pakistan. They were collected on May 1993 and November 1993 (referred to as summer and winter seeds respectively in the rest of the paper). Hulled seeds were cleaned and stored in refrigerator prior to use.

Weight and size of seeds- Summer and winter seeds of *H. mucronatum* were found to be black and brown in color, respectively. Five replicates of 100 seeds each were used to determine seed weight. Size of 500 seeds of each type were measured with an Area meter (CI-201 CID, Inc. USA).

Germination - Germination experiments were conducted in June 1993. All seeds used were surface sterilized in 0.52% sodium hypochlorite for 1 min. followed by thoroughly rinsing with distilled water. Seeds were presoaked in distilled water or respective test solutions for 4h. Germination was carried out in test tubes of 20 mm in diameter and 180 mm in length. Seeds were placed on Whatman No. 1 filter channels (3.5 x 16 cm strip of filter paper folded to form a channel). Filter channels were moistened with either 1.5 ml of test solution or distilled water. All tubes were sealed with parafilm. Five replicates with 20 seeds each were used. Germination was carried out for 20 days.

Light and darkness: The effect of light on germination was studied by placing a set of seeds with distilled water and test solutions in a 12 h light-12 h dark photoperiod in a programmed incubator (2000 lux Sylvania cool-white fluorescent lamps). Another set of tubes with seeds were sealed with two layers of black paper and a layer of aluminum foil to exclude light. Both treatment had an alternating day/night temperature of 25-10°C. Percentage germination was recorded at the end of the 20 day treatment.

Salinity-temperature: The effect of salinity and temperature were determined by germinating seeds in 0, 50, 100, 150, 200 mM NaCl solutions at an alternating day/night thermoperiod of 25-10, 20-30, 25-30, and 25-35°C. These four alternating temperatures represent the average seasonal temperature regimes in Karachi. Germination was monitored at 2-day intervals for 20 days.

Thiourea: Thiourea (10 mM) was used in all NaCl treatments (0, 50, 100, 150, 200 mM) and alternating temperature regimes to study its effect in alleviating inhibition of germination caused by salinity. Germination was recorded at 2-day intervals for 20 days.

RESULTS

Morphology of seeds - *Halopyrum mucronatum* flower twice a year from April to May and September through November. Seeds reached maturity in May and November. These seeds will be referred to as summer and winter seeds, respectively. Black summer seeds were significantly heavier than brown winter seeds (Table 1). There was no significant difference between the area of the seeds.

Table 1. Colour, weight and size of two seasonal seeds of *H. mucronatum.*

Time of Seed Production	Colour	Weight (mg 100 seed^{-1})	Size (cm^2)
May (Summer)	Black	280 ±08.9	0.03 ±0.01
November (Winter)	Brown	214	0.03

	±04.4	±0.01

Germination

Light-darkness **-** Seed germination of winter and summer seeds showed no effect of light at low salinity treatments (Fig. 1). However, presence of light significantly (P = 0.001) increased the inhibition of germination at higher salinities tested.

Fig. 1. Effect of light on germination percentage of *H. mucronatum* under different salinities at 25-10°C.

Salinity-temperature **-** Summer and winter seeds varied in their response to increasing salinity under different alternating temperature regimes (Fig 2 and 3). Summer seeds were very sensitive to low alternating thermoperiod (25 - 10C). Germination was progressively delayed, as well as inhibited, with increases in salinity and few seeds germinated at 5% NaCl treatment (Fig 2 and 4). At the moderate thermoperiod (30-20C) there was no delay and germination inhibition at lower salinity, but significant inhibition and delay was noted at the higher salinity. However, at higher thermoperiod (25-30, 25-35C) there was some delay but no germination inhibition even at high salinity treatments (Fig. 2 and 4).

Fig. 2 Rate of germination of *Halopyrum mucronatum* summer seeds in 0 (λ), 50 (ν), 100 (σ), 150 (τ), and 200 (υ) mM NaCl at various alternating temperature regimes.

Fig. 3 Rate of germination of *Halopyrum mucronatum* winter seeds in 0 (λ), 50 (ν), 100 (σ), 150 (τ), and 200 (υ) mM NaCl at various alternating temperature regimes.

Seeds which matured during winter showed no effect of salinity at low salinity and temperature regimes (Fig. 3 and 4). However, at salinity treatments higher than 50 mM, there was a substantial decline in germination percentage. This decline was more substantial at higher and lower thermoperiods.

Fig. 4. Final germination percentages of winter and summer seeds at various alternating temperature regimes.

Thiourea - Thiourea (10 mM) at low temperatures (25-10C) almost completely alleviated the effect of salinity (200 mM) on germination of summer seeds (Fig. 5). However, thiourea failed to alleviate germination in all other salinity and temperature treatments. In most other cases its effect was synergistic in inhibiting the germination induced by salinity.

Fig. 5. Effect of Thiourea on the germination of summer seeds at 25-10C alternating temperature regime.

DISCUSSION

Halopyrum mucronatum does exibit a temporal variation in flowering and seed production. The summer and winter seeds show a morphological as well as physiological dimorphism. Summer seeds are balck and heavy whereas, winter seeds are brown and lighter in weight. Area of summer and winter seeds, however, are not significantly different. Germination of halophyte seeds may be affected by parental environments to which plants are exposed (Ungar, 1987). However, the direct effect of the parent plant or seed responses vs that of physical environment to which seeds are exposed when they are still attached to the parent is difficult to distinguish (Ungar, 1991). Okusanya and Ungar, (1983) determined that *Spergularia marina* seeds collected from plants at different times during the growing season have different levels of dormancy, depending on the month of seed collection. Ungar (1988) reported that *S. marina* seeds collected from plants during the months of June and November are most dormant followed by August and September collection in their degree of dormancy.

Seeds of *H. mucronatum* did not require light for germination. They germinate equally well in light as well as dark. However, higher salinity concentrations inhibited germination significantly more in light as compared to dark treatments. Seeds of grasses like *Eriogonum abertianum* and *Eriastrum diffusum* (Baskin et al., 1993) and halophytes like *Spergularia rupicola* and *S. media* (Okusanya , 1979) and *S. marina* (Okusanya and Ungar 1983) do not germinate under dark conditions. *H. mucronatum* contrary to these reports could germinate on the surface of the soil or buried under the sand. Some germination of buried winter seeds was noticed during February 1994 at the depth of 20 to 30 cm but all of the seedlings died. Seedlings orginating from the seeds present on or near the surface after monsoon rains appeared to do well.

Winter and summer seeds of *H. mucronatum* do not have any innate dormancy because they germinated well in nonsaline treatments under all temperature regimes. However, introduction of salt into the medium induced dormancy in winter seeds and in summer seeds which were germinated at lower temperature regimes. Summer seeds germinated at higher temperature regimes did not show any significant effect of salinity in inhibiting germination. Major factors controlling the seed germination in coastal species are both tidal innundation and the abrasive and depositional effects of tidal action, as well as the salinity factors (Ungar, 1991). Salinity and temperature interact in their control of seed germination, and the greatest inhibition is usually found at the maximum and the minimum limits of tolerance of these two environmental variables (Khan and Rizvi, 1994; and Ungar 1995). The annual halophyte *Atriplex triangularis* varied in its capacity to germinate at higher salinity levels at different thermoperiods (Khan and Ungar, 1984). They reported that *A. triangularis* was least sensitive to salinity at optimal temperature for germination. Similar effects were reported for *Horedum jubatum* (Badger and Ungar, 1989), *Crambe abyssinica* (Fowler, 1991), *Puccinellia ciliata (*Myer and Couper, 1989*), Zygophyllum qatarense* (Ismail, 1990) and *Atriplex griffithii* (Khan and Rizvi, 1994).

Thiourea over came the effect of salinity in summer seeds at 10-25^{o}C alternating temperature regime. In all other temperature and salinity treatments thiourea either had no effect or had an inhibitory effect on germination. Thiourea is known to break dormancy and overcome the negative effect of temperature on germination (Esashi et al., 1979 and Aldasoro et al., 1981). It is also reported to inhibit germination in some seeds (Lip and Ballard, 1970).

Summer and winter seeds of *H. mucronatum* clearly show a morphological as well as physiological dimorphism. Black summer seeds were heavy as compared to brown winter seeds. They varied in their tolerance to salinity at various temperature regimes. Summer seeds are more tolerant to salinity at higher temperatures and induction of thiourea could reverse the inhibiting effect of salinity. In a subtropical maritime desert habitat which can be characterized by high temperature, salinity and drought except for monsoon periods, physiological dimorphism of *H. mucronatum* seeds provide alternate temporal and spatial conditions for germination and recruitment.

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