Role of chemicals in alleviating salinity and light related seed dormancy in sub-tropical grasses

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1. Introduction

Under harsh environmental conditions halophytes, particularly grasses, generally employ vegetative propagation as a short term strategy while they rely on seed germination for routine propagation to introduce new genotypes from evolutionary point of view (Gul et al., 2013). Seed germination, a very important stage in the life cycle of a plant, may however be affected by nature of the seed and/or environmental conditions. Seeds of agricultural crops which have been domesticated for many years germinate readily on imbibition while wild plants that face uncertain, often hazardous conditions, have to adopt suitable strategies — seed dormancy being one such tactic — for progeny perpetuation. The phenomenon of particular importance for halophytes that are routinely exposed to extreme climatic and soil conditions in their habitats. Seeds that do not germinate under optimal conditions but retain viability are considered as ‘innately dormant’ whereas those that do not germinate in adverse environments but have ability to do so when conditions are favorable are called ‘conditionally dormant’ (Baskin and Baskin, 1985).

Plants of sub-tropical vegetation have generally to face conditions of low rainfall and high temperatures which forces them to adopt suitable strategies to survive. The perennials are at advantage under these conditions because they do not depend entirely on seed germination but can also propagate vegetatively. The situation attains more significance for annuals like Eragrostis ciliaris which perpetuate through seed germination and have very limited time at their disposal to be successful. Consequently they have a greater need than perennials to rely on keeping some seeds dormant to keep the seed bank intact until the monsoon rains
arrive (June to August) that decrease soil salinity as well as lower the ambient and soil temperatures along with providing appropriate photoperiod. The presence of innate seed dormancy has been previously reported in *E. ciliaris* (El-Keblawy and Gairola, 2016).

Salinity, temperature and light are among the most important factors which govern these adaptive strategies and keep the seeds viable but restrict or delay their germination to avoid unfavorable conditions thereby regulating seedling establishment (Baskin and Baskin, 1998; Footitt al., 2013). Some halophytic grasses like *Aeluropus lagopoides*, *Desmostachya bipinnata*, *Urochondra setulosa* have the ability to germinate some seeds in up to 500 mM NaCl while a number of them (like *Triglochin maritima* and *Cyprus conglomeratus*) remain dormant but maintain viability and recover upon removal of salt stress (see Gul al., 2013 and references therein). Similarly, some grass species like *Sporobolus ioclados* (Khan and Gulzar, 2003) or *Urochondra setulosa* (Khan and Gulzar, 2003) require light for seed germination while others like *Panicum turgidum* (El-Keblawy et al., 2011) or *Halopyrum mucronatum* (Khan and Gulzar, 2003) have no such need. The response may vary under combination of stresses, e.g. germination inhibition of *Aeluropus lagopoides* and *Desmostachya bipinnata* seeds increased on exposure to salinity in absence of light (Gul et al., 2013).

Alteration in the endogenous hormonal profile during seed germination may change physiological, biochemical and molecular responses resulting in variable effects (Baskin and Baskin, 1998). The importance of dormancy regulating chemicals to alleviate the innate dormancy in grasses has recently been highlighted by El-Keblawy and Gairola (2016) who reported that exogenous application of plant hormones and some other organic compounds increased seed germination of sub-tropical halophytes under saline conditions. Plant hormones such as GA₃, kinetin, fuscoiccin may alter membrane functions (Ashley et al., 2006; Sastry and Shekhawat, 2001; Sawan et al., 2000), substitute for light and counter the effect of abscisic acid under saline stress (Herrmann et al., 2007; Kucera et al., 2005). Similarly, phytoregulators like nitrate and thiouracil are reported to alleviate salinity enforced dormancy (El-Keblawy, 2013; Khan and Ungar, 2001) while organic osmotica like proline and glycine-betaine may provide osmo-protection to alleviate seed dormancy under salinity stress (Song et al., 2005).

Out of 140 halophyte grasses reported worldwide (Glenn et al., 1999), 70 are found in India having commercial and ecological significance (Khan and Qaiser, 2006). *Phragmites karka* (Retz.) Trin. ex. Steud, a perennial grass, usually grows as pure populations (around 50 plants m⁻²) in flooded saline habitats (Konwar et al., 2009). It can attain a height of about 10 m with rapid growth rate (annual net primary productivity rate about 800 g m⁻² year⁻¹; Khatri and Barua, 2011) which makes it a good biofuel crop (Abideen et al., 2012). *Dichanthium annulatum* (Forsk.) Stapf is a perennial fodder grass of up to 100 cm height occurring in arid/semi-arid regions of the world and throughout the inland and coastal areas of Pakistan (Ashraf et al., 2006). *Eragrostis ciliaris* (L.) R. Br. is an annual grass of 5–60 cm height that is distributed widely in salt flats and usually develops after monsoon rains; it is readily grazed (Ashraf et al., 2006). A species-specific response has been reported when seeds of these species were germinated under varying salinity, temperature and photoperiod (Shaikh et al., 2013; Zehra et al., 2013). With the objective to explore the types and mechanism of seed dormancy in these annual and perennial grasses that help them to cope with harsh environmental conditions, the current study was conducted based on the following hypotheses: 1) Seeds of annual grasses have more developed dormancy mechanisms than those of perennial grasses, and 2) Dormancy can be alleviated with the external application of dormancy regulating chemicals (DRCs).

2. Materials and methods

2.1. Habitat and seed source

Mature seeds of *D. annulatum*, *E. ciliaris* and *P. karka* were collected during September 2009 (rainfall: 20 mm; minimum temperature: 26 °C; maximum temperature: 32 °C and Humidity: ≈55%) from salt flats of Karachi University campus (24°56'11.23'' N, 67°07'26.43'' E). Inflorescences were air dried in shade; seeds were separated from spikes, surface sterilized with 0.82% sodium hypochlorite for 1 min, thoroughly washed, air dried and used for the experiments without any storage.

2.2. Seed germination in distilled water (DW)

Seeds were germinated in plastic Petri plates (50 mm diameter × 9 mm height; 25 seeds/Petri plate in 4 replicates/treatment) containing 5 ml of distilled water (DW) (both with and without DRCs [plant hormones: Kinetin (0.05 mM), Fusicoiccin (5 μM); organic osmotica: Betaine (0.1 mM), Proline (0.1 mM); nitrogenous compounds: Thiourea (10 mM), Nitrate (20 mM)]) and placed in programmed incubators (Percival Scientific, Boone, Iowa, USA). Based on information from earlier studies (Shaikh et al., 2013; Zehra et al., 2013), seeds of *E. ciliaris* and *D. annulatum* were subjected to thermoperiods of 25/35 °C and of *P. karka* to 20/30 °C [higher temperature corresponded to 12 h light (25 μmol m⁻² s⁻¹; 400–700 nm) and the lower temperature to 12 h dark]. A set of petri-plates was placed in 12 h photoperiod, whereas another set was placed in complete dark. Final germination (emergence of the radical) was recorded after 20 d and un-germinated seeds were tested for viability by tetrazolium chloride.

2.3. Effect of salinity on seed germination

Based on our earlier studies (Shaikh et al., 2013; Zehra et al., 2013) seeds of all three test species were germinated in various concentrations of NaCl (25, 75, 100, 125, 150 mM for *E. ciliaris*; 100, 200, 300, 400, 500 mM for *D. annulatum* and *P. karka*) with and without DRCs, while using the same temperature regime and photoperiods as mentioned in Section 2.2. Germination was recorded after 20 d of experiment. Seeds that did not germinate under salinity and absence of DRCs were subjected to tetrazolium chloride test and all viable seeds were considered conditionally dormant.

2.4. Statistical analyses

Data were subjected to analyses of variance using SPSS Version 11.5 (SPSS, 2002) for windows (SPSS, Chicago, IL, USA). Bonferroni test (multiple range test) was performed to compare the significant differences among means. Variables were arcsine transformed for normality and homogeneity of variance when necessary. Means and standard errors were used to construct graphs by Sigma Plot for Windows ver. 10.0 (Systat Software, San Jose, CA, USA).

3. Results

3.1. Seed germination in distilled water

All seeds of *P. karka* and *D. annulatum* germinated in DW in 12 h photoperiod; however, seeds of *E. ciliaris* showed only 60% germination while out of remaining 40%, 10% were dead and 30% were dormant (Fig. 1). All DRCs failed to increase seed germination of *E. ciliaris* in 12 h photoperiod (data not shown).

Light had a significant effect on seed germination of *P. karka* and *E. ciliaris* in non-saline condition. Germination inhibition of *P. karka* was ≈30% in complete dark as compared to 12 h light (Fig. 1).
Almost no seed of *E. ciliaris* germinated in 24 h dark while response of *D. annulatum* seeds remained unchanged in both photoperiods (Fig. 1). Nitrate was the only DRC which increased seed germination of *P. karka* in dark, whereas no positive effect of any DRC was found in *E. ciliaris* (Fig. 2).

### 3.2. Seed germination in saline medium

Seed germination of all species was inhibited by salinity at 12 h photoperiod, with *P. karka* being the most salt tolerant among test species (Fig. 3). Under the higher NaCl concentration used for individual species, *P. karka* showed 30% germination at 500 mM while 5% seed of *D. annulatum* and *E. ciliaris* germinated in 500 and 125 mM respectively (Fig. 3). All un-germinated seeds were viable and dormant except 10% dead seeds in *E. ciliaris* (data not shown). Salinity-imposed conditional dormancy in *P. karka* and *D. annulatum* seeds was partially alleviated (≈30%) with application of kinetin and fusicoccin (Fig. 4). Organic osmota (Betaine, Proline) and nitrogenous compounds (Thiourea, Nitrate) increased seed germination up to 60% and 40% in *P. karka* and *D. annulatum* respectively at 500 mM NaCl, however seed germination of *E. ciliaris* was <20% in 150 mM NaCl (Fig. 4). Among all DRCs, the effect of nitrate was more pronounced on seed germination of test species, especially at higher NaCl concentrations (Fig. 4).

Seed germination of *P. karka* and *D. annulatum* was inhibited >50% under salinity in 24 h dark treatment, while seeds of *E. ciliaris* failed to germinate in dark (Fig. 3). Seed germination of *P. karka* at 400 mM NaCl was almost completely alleviated by all DRC used except for fusicoccin (Fig. 5). In contrast, all DRC’s significantly increased seed germination of *D. annulatum* compared to control in 24 h dark (Fig. 5). Among used DRCs, fusicoccin and proline increased seed germination to less than 10% in *E. ciliaris* under complete dark and salinity (Fig. 5).

### 4. Discussion

In the present study, partial innate dormancy was observed in seeds of *E. ciliaris* (annual) that could be due to immature embryo as also reported in *Eragrostis rigida* (Ernst et al., 1991) or it could be a strategy to maintain a seed bank under harsh environmental conditions like some other sub-tropical grasses (Gul et al., 2013). El-Keblawy and Gairola (2016) found complete innate dormancy
Fig. 4. Germination percentages of Phragmites karka, Dichanthium annulatum and Eragrostis ciliaris seeds in different NaCl solutions with and without dormancy regulating chemicals (DRCs) at 12 h photoperiod. UC is the untreated control. Values are means ± S.E (n = 4). Bars with the same letters indicate no significant difference (P > 0.05) between treatments across NaCl concentrations. Results from ANOVA on the interaction effects of salt concentrations (“S”) and dormancy regulating chemicals (“DRCs”) are shown (df = 4,6).

Fig. 5. Germination percentages of Phragmites karka, Dichanthium annulatum and Eragrostis ciliaris seeds in NaCl solutions with and without dormancy regulating chemicals (DRCs) at 24 h dark photoperiod. UC are the untreated controls (of 12 h light: 12 h dark and 24 h dark photoperiods, respectively). Values are means ± S.E (n = 4). Bars with the same letters indicate no significant difference (P > 0.05) between treatments for each NaCl concentration. Results from ANOVA on the interaction effects of salt concentrations (“S”) and dormancy regulating chemicals (“DRCs”) are shown (df = 1,6).
in *E. ciliaris* seeds (collected during the month of April and stored at room temperature for two months) from a population located in the Arabian Peninsula. The difference in the intensity of innate dormancy in *E. ciliaris* is possibly the result of temporal and environmental variation in seed development to maturation time. Fresh seeds of perennial species (*D. annulatum* and *P. karka*) did not have any dormancy which could assist them to make a transient seed bank after seed dispersal and allow them to germinate in a short window after rainfall.

Salinity is known to cause seed dormancy either by osmotic or/and ionic effect (Kaya et al., 2006) which could disturb a balance among hormones (Atia et al., 2009). In the present study, seed germination among species was reduced in the following order: *E. ciliaris > D. annulatum > P. karka* with increasing NaCl concentration. The non-dormant seeds of *E. ciliaris* (annual) appear to have low tolerance to salinity (<150 mM) and entered into a phase of conditional dormancy. The responses of *E. ciliaris* seeds in salinity indicate the salt-sensitive nature of this grass. This is substantiated by the observation that seed germination of *E. ciliaris* generally occurs in nature immediately after heavy rainfalls that dilute salinity from the upper soil layer and support the plant to complete its short life span during favorable environmental conditions. The members of *Eragrostis* spp. have some slime on the seed surface that helps to absorb water during seed germination (Kreitschitz et al., 2009), but under salinity the prolonged retention of water around the seed may also restrict the availability of oxygen to the embryo with harmful consequences. Seeds of *P. karka* and *D. annulatum* maintained their viability and almost all seeds germinated when transferred from saline conditions to distilled water (Shaikh et al., 2013; Zebra et al., 2013). In the present study, seeds of *P. karka* and *D. annulatum* entered into conditional dormancy under saline conditions. Conditional seed dormancy in *E. ciliaris* could be due to its life form (annual) while in *P. karka* and *D. annulatum* it seems to be an adaptive response related to the water status of the soil habitat. In general, weeds have non-deep type physiological dormancy which can cycle back and forth between dormancy and non-dormancy depending on environmental conditions until they germinate. Conditional dormancy could directly regulate the populations of *E. ciliaris*, *D. annulatum* and *P. karka* seedlings in natural habitats and indirectly reduce competition among developing seedlings for nutrients and space (E. *ciliaris*, water (*D. annulatum*) and light (*P. karka*). Conditional dormancy linked to salinity could also help in maintaining the seed bank of test species by beginning germination at the onset of monsoon rains that reduces substrate salinity and helps in avoiding population extinction.

Photoblastic response is an ecological adaptation which prevents germination when seeds are buried deep in the soil, leaf litter or under a dense canopy. In the present study, seed germination of test species reduced under complete dark in the following order: *E. ciliaris > P. karka > D. annulatum*. Seeds of *E. ciliaris* showed an absolute light requirement which may be a part of complex dormancy mechanisms to regulate the germination time of this annual grass which may not be essential for most of the perennials. The absence of germination in complete dark is probably due to the presence of *E. ciliaris* seeds in the upper layer of sand dunes (personal observation). The light sensitive response of this annual grass favors to complete its life cycle in a short window of optimal environmental conditions (Gul et al., 2013). However, light seems important for seed germination of the perennial *P. karka* also which might help in controlling the density of seedlings under a dense canopy. Among test species, seed germination of *P. karka* and *E. ciliaris* appears more sensitive to light than higher salinity while in case of *D. annulatum*, NaCl solution inhibited more in comparison with complete dark treatment only. Increased sensitivity to light during seed germination under salinity has also been reported in other grasses (Khan and Gulzar, 2003).

Population establishment of the annual grass *E. ciliaris* purely depends on successful seed germination, therefore it is compelled to develop 1) innate seed dormancy to maintain a seed bank when rain-fall season is extended, 2) use conditional dormancy to retain seed viability in harsh environmental conditions (high salinity, cooler temperature; during September–April) and adopt light sensitivity to allow only those seeds to germinate which can complete the life cycle in a short period. In contrast, only conditional dormancy with partial light sensitivity in *P. karka* indicates the absence of any compulsion on seed germination in perennial grasses. Above findings are in agreement with our first hypothesis “seeds of annual grasses have more complex seed dormancy than perennials”.

Exogenous application of hormones has been reported to increase seed germination of some sub-tropical halophytes under saline conditions (El-Keblawy, 2013). Species-specific effect of kinetin and fusicoccin on germination of several halophytic grasses under NaCl has been reported – they partially alleviated germination of *Aeluropus lagopoides* but had no effect on seeds of *Sporobolus ioclados* and *Urochondra setulosa* (Khan and Ungar, 2001). Kinetin could alleviate seed germination by protecting cell membranes from reactive oxygen species (Chaitanya and Naithani, 1998), increasing amylase activity (Kaur et al., 1998), improving water uptake (Sstry and Shekhawat, 2001) and enhancing ethylene biosynthesis to minimize ABA production (Hermann et al., 2007). Fusicoccin is reported to stimulate ATPase production which rapidly increases during the early phase of germination facilitating proton extrusion and K⁺ uptake (Ashley et al., 2006). It is also known to alleviate inhibitory effects of ABA on germination and embryo growth of de-coated Orobanche ramosa seeds (Evidente et al., 2006). In the present study, kinetin and fusicoccin helped in breaking dormancy of *D. annulatum* and *P. karka* seeds when exposed to salinity alone or under complete darkness, but had no effect on *E. ciliaris*. Our results indicate that plant hormones are involved in seed dormancy of perennials but do not have a major role in an annual grass. Such information indicates that the use of plant hormones is an effective idea to increase the recruitment of perennial grasses during saline agriculture.

Organic osmotics like proline and betaine are known to increase seed germination of some sub-tropical halophytes under harsh environments (Khan and Ungar, 2001). Both proline and betaine are involved in osmotic adjustment, reducing oxidative stress, membrane stabilization and protection of proteins in the cytoplasm (Song et al., 2005). In the present study, seed germination of *D. annulatum* and *P. karka* increased under salinity irrespective of presence or absence of light when treated with proline and betaine. However, both compounds failed to increase germination of *E. ciliaris* under salinity which indicates that germination inhibition is not due to water limitation as sufficient water uptake is possible because of the presence of slime around the seed (Kreitschitz et al., 2009).

Nitrogenous compounds have been extensively reported to break seed dormancy of sub-tropical species (El-Keblawy, 2013). Similarly, salinity enforced seed dormancy in test species was partially alleviated with the application of nitrate and thiourea. Thiourea and nitrate are reported to break seed dormancy by reducing the effect of ABA, increasing cytokinin concentration, and altering redox status (Srivastava et al., 2010). In addition, they promote seed germination under saline conditions by increasing cell elongation rate (Esashi et al., 1979), enhancing antioxidant defense system (Srivastava et al., 2010), controlling membrane kinetics for ion uptake (Aldasoro et al., 1981) and regulating enzyme activity and its turnover (Srivastava et al., 2010). The thiol (–SH) group in thiourea is implicated in activating enzymes during seed germination (Srivastava et al., 2010). Nitrate has also been reported as a hormone that naturally occurs in soils and regulates seed germina-
tion through phytochrome (Batak et al., 2002). Baskin and Baskin (1994) reported that nitrate can be used to break dormancy in seeds which require light for germination. For instance, seed germination of *Hypericum* species (light sensitive) is enhanced by exogenous KNO<sub>3</sub> in dark (Cirak et al., 2007). We also found similar results in *P. karka* which is distributed in nitrogen rich soils but not in *E. ciliaris* which is usually found in drier and nutrient poor habitats. Therefore, our second hypothesis “dormancy can be alleviated with the external application of DRC’s” is true for *D. annulatum* and *P. karka* but not for *E. ciliaris*. El-Keblawy and Gairola (2016) reported the failure of DRC’s in increasing germination of *E. ciliaris* in complete dark. Results indicate that the annual grass *E. ciliaris* has more complex seed dormancy than perennials.

5. Conclusion

The annual grass *Eragrostis ciliaris* used both partial innate and conditional dormancy which may help this annual (relatively salt sensitive) grass in maintaining a seed bank under harsh environmental conditions while perennials depended only on conditional dormancy as an adaptation in response to harsh environmental condition. Dormancy regulating chemicals failed to break salinity triggered conditional dormancy or increase germination under complete darkness in *E. ciliaris*. Chemical treatments helped in breaking salinity induced conditional dormancy to varying degree in both perennial grasses while light requirement during germination was compensated only in *Phragmites kurki*. Nitrate was generally more effective in alleviating adverse effects of salinity and light on germination. These results suggest that depending on their life form and variability in habitat, seeds of sub-tropical grasses have evolved different strategies to avoid/delay germination under unsuitable environmental conditions.

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