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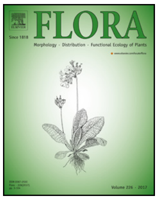

Role of chemicals in alleviating salinity and light related seed dormancy in sub-tropical grasses

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ABSTRACT

This study is based on the assumptions that annual grasses express more complex seed dormancy than perennials, while dormancy regulating chemicals (DRCs) help in recovering seeds from dormancy. The role of six such chemicals, viz. plant hormones – Kinetin, Fusicoccin; organic osmotica – Proline, Betaine; nitrogenous compounds – Nitrate, Thiourea, in alleviating effects of salinity and light on seed germination of three subtropical grasses (perennials: *Phragmites karka*, *Dichanthium annulatum*; and annual: *Eragrostis ciliaris*) was hence investigated. All seeds of *P. karka* and *D. annulatum* germinated in non-saline condition while in *E. ciliaris*, 60% seeds germinated in absence of salts, 10% were dead and 30% were dormant. Salinity reduced germination of the test species to following levels (% of non-saline control) under highest NaCl concentration used: *P. karka* (30% at 500 mM), *D. annulatum* (25% at 400 mM) and *E. ciliaris* (10% at 125 mM). The application of all DRCs alleviated conditional dormancy associated with salinity more in *P. karka* than *D. annulatum* but negligibly in the case of *E. ciliaris*. Absence of light inhibited seed germination of *P. karka* (partially) and *E. ciliaris* (completely) but under these conditions the application of DRCs increased germination of *P. karka* only. Nitrate was generally more effective than other DRCs in increasing seed germination both in salinity as well as in complete darkness. Seed germination was synergistically inhibited by salinity under dark conditions compared to their individual effects and was completely alleviated in *P. karka* and *D. annulatum* seeds by DRCs. In conclusion, the annual grass (*E. ciliaris*) used both innate and induced type of seed dormancy along with absolute light demand for successful seedling establishment while perennials (*P. karka* and *D. annulatum*) depended on enforced dormancy. Moreover, seed dormancy could be alleviated by DRCs in perennial grasses but not in the annual species.

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 is 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

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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 et 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 et 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 GA_3, kinetin, fusicoccin 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 (Hermann et al., 2007; Kucera et al., 2005). Similarly, nitrogenous compounds like nitrate and thiourea 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 Pakistan 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* (Forssk.) Stapf is a perennial fodder grass of up to 100 cm height occurring in arid/semiarid 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), Fusicoccin (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 $\mu mol\, m^{-2}\, s^{-1}$; 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).