INT. J. BIOL. BIOTECH., 15 (3): 505-512, 2018.

LIGHT INFLUENCES SEED GERMINATION RESPONSES OF *CYPERUS CONGLOMERATUS* ROTTB. UNDER BOTH SALINE AND NON-SALINE CONDITIONS

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ABSTRACT

Light acts as an important cue for the seeds of halophytes. However, generally little is known about the effects of light quality (color) and quantity (intensity) on seed germination of halophytes. This study hence examined seed germination responses of a halophytic sedge *Cyperus conglomeratus* Rottb. (Cyperaceae) to various photoperiod (12h light/12h dark and 24h dark), light quality (white, red, yellow, blue and green cellophane paper) and light intensity (100, 75, 50, 25 and < 25%) treatments under both non-saline (distilled water) and saline (50 and 100 mM NaCl) conditions. Seeds of *C. conglomeratus* were positively photoblastic and germinated maximally under 12h light/12h dark photoperiod as compared to 24h darkness under both non-saline and saline conditions. High salinity decreased seed germination significantly (p < 005). Seed germination was influenced by light quality, as relatively higher germination was observed under white, red, and yellow light as compared to blue and green light treatments. Variation in light quantity also affected seed germination of test species, which decreased with decline in light intensity. These results thus indicate that the seeds of *C. conglomeratus* are positively photoblastic, whose germination is influenced by variation in both light quality and quantity.

Key-words: Halophyte, Light, Photoblastism, Photoperiod, Salinity, Seed germination

INTRODUCTION

Light has been recognized as germination controlling factor for a variety of plants, particularly of those inhabiting stressful habitats (El-Keblawy and Gairola, 2017; Vieira *et al*., 2017). Based on seed germination responses to presence/absence of light, plants can be grouped into three categories: i) species with an absolute light requirement for seed germination (i.e. positive photoblastism; Baskin and Baskin, 1998; 2014; Colbach *et al.*, 2002), ii) species germinating similar in both light and dark (i.e. neutral photoblastism; Baskin and Baskin, 2014; Wei *et al*., 2008) and iii) species requiring dark for optimal seed germination (i.e. negative photoblastism; Baskin and Baskin, 2014; Benvenuti *et al*., 2004). Hence, seed germination responses of plants are diverse and considered important from view point of both ecology and conservation (El-Keblawy and Gairola, 2017; Fenner and Thompson, 2005; Gul *et al*., 2013; Vieira *et al*., 2017).

The behavior of halophytic seeds are species specific to different environmental conditions (Gul *et al*., 2013). Baskin and Baskin (1995) reported that out of 41 halophytes, seed germination of 20 was up-regulated in light, 10 species required dark for optimal germination, while seed germination of 11 species was indifferent in the absence/presence of light. In addition, several reports are available to describe interactive effects of light and salinity during germination of different halophytic seeds. For instance, salinity tolerance of germinating seeds of *Limonium stocksii* was higher in the presence of light than in dark (Zia and Khan, 2004). Therefore, a thorough research is required to describe the role of light signals to understand the aspect of seed ecology of halophytes.

Seed germination is the first photomorphogenesis stage of the plants' life cycle, which is regulated by at least four distinct families of photoreceptors (McWatters and Devlin, 2011), which include phytochromes, cryptochromes, phototropins and unidentified ultraviolet B (UV-B) photoreceptor (Jiao *et al.*, 2007; Pierik *et al*., 2009). These photoreceptors are involved in regulating various plant processes including seed germination (Hofmann, 2014), seedling de-etiolation, phototropism, shade avoidance, circadian rhythms and flowering time (Chen *et al*., 2004) based on information such as presence/absence, intensity, duration and spectral quality of the light (Vinterhalter and Vinterhalter, 2015). It has been established that 680-730nm of wavelength percept through the family members (PHY) of phytochrome (PHY), whereas phototrophins and cryptochromes perceive and respond to the UVA and blue regions (Chen *et al*., 2004). Five phytochrome photoreceptors (PHYA to PHYE) have already been reported in *Arabidopsis thaliana*, out of which PHYB and PHYE reportedly mediate germination, however underlying mechanisms are generally obscure (Dechaine *et al*., 2009). This kind of information is generally absent about the seeds of non-crops.

Different wavelengths (i.e. quality) of light affect seed germination differently (Bewley and Black, 1994; Vieira *et al*., 2017). Generally, red (630 – 740) and yellow (570 – 580) colored light enhance seed germination in many species (De-Oliveira and Macedo, 2015; Strydom *et al*., 2017), whereas green (520 – 570nm) (Strydom *et al*., 2017) and blue (450 - 495 nm) light reduce seed germination (Hofmann, 2014). Seeds of *Tabebuia rosea* responded to all common light colors such as red, blue, blue-red and green but red wavelength resulted in highest percent germination (Basto and Ramirez, 2015). Red to far-red ratio is also known to have key role in germination of several species but the order of irradiation is critical to regulate percent seed germination (Kolodziejek *et al*., 2017). In addition, light intensity can also influence seed germination (Zhang *et al*., 2015). For instance, Li *et al*. (2015) reported that strong light inhibited seed germination, whereas weak light increased percent germination. However, such information about the seeds of halophytes particularly those from warm subtropical regions are generally missing.

Cyperus conglomeratus Rottb (a.k.a. Rasha, Thanda, Tunda in Arabic) is a tufted perennial sedge (Family Cyperaceae) found commonly on sandy soils and dunes of the coastal areas from Northern dry Africa through Middle East to Indo-Pak region (Khan and Qaiser, 2006; http://www.efloras.org/ florataxon.aspx?flora_id=5&taxon_id=242101114). It is among few species forming monospecific stands on sand dunes (Ksiksi *et al.*, 2007). This monocot xero-halophyte is a good fodder for animals (El-Keblawy, 2003; El-Keblawy *et al.*, 2009). Sedges have also been reported to use for making ropes, baskets and mats and also as fuel (Simpson and Inglis, 2001). Many aspects such as ethnobotany, phytochemistry and community ecology of sedges are well documented (Bryson and Carter, 2008), however information about its salt tolerance and seed germination ecology is non-existent. Furthermore, information about the effects of spectral quality and intensity of light on its seed germination is also absent. This study was therefore designed to answers following questions: i) Are seeds of *C. conglomeratus* light sensitive during seed germination? ii) Does salinity modulate photoblastic effects? iii) If wavelengths of light affect seed germination differently? and iv) Can light intensity/quantity affect seed germination of *C. conglomeratus*?

MATERIALS AND METHODS

Seed collection and study sites

Seeds of *C. conglomeratus* were collected from the sand dunes of Hawks Bay, Karachi during fall of 2015. Seeds were separated from inflorescence manually and surface sterilized by using 0.1% (v/v) sodium hypochlorite, as detailed in Zia and Khan (2003). Surface sterilized seeds were then dry stored in clear plastic plates at room temperature (~25^{o}C) until used. Fresh seeds were used in germination experiments within four weeks of collection.

Experiment 1: Examining the effects of presence/absence of light on seed germination

Experiments to investigate the light mediated response (presence/absence of light) on seed germination of *C. conglomeratus* were carried out in programmed germination incubators (Percival, USA). Experiments were conducted either under 12h night/ 12h day (hereafter ‘light’) or 24h dark (hereafter ‘dark’) photoperiod at 20/30^{o}C night/day thermoperiod (i.e. common thermoperiod for optimal germination of subtropical halophytes; Gul *et al*., 2013). Clear lid petri dishes containing 7 mL of different NaCl solutions (0, 50 and 100 mM; based on preliminary trials) to determine the effect of salinity on germination. For dark treatments, petriplates were wrapped in a dark photographic envelop. Under 12-h light photoperiod seed germination was recorded on every alternate day for 20 days and for dark treatment once at 20th day (Ahmed and Khan, 2010). At least four replicates (25 seeds each) were used per treatment. Emergence of the radicle was considered as germination (Bewley and Black, 1994).

Experiment 2: Studying the effects of light quality (color) on seed germination

To test the effects of different colors (red, yellow, blue, and green) of light on germination, petri-plates were wrapped in two layers of colored cellophane paper and placed in germination chamber with normal white fluorescent light with wavelength 400-700 nm, as described by Dissanayake *et al*. (2010). Seed germination was recorded after 20 days.

Experiment 3: Examining the effects of light quantity (intensity) on seed germination

To expose seeds to variable intensities (i.e. quantity) of incident light, variable layers of medical guaze were used to cover the petriplates, as described by Wang *et al.* (2012). Following five light intensities were used: no layer of medical guaze (light intensity = 25 μmol m^{-2} s^{-1}), one layer of medical guaze (light intensity = 17 μmol m^{-2} s^{-1}), two layers of medical guaze (light intensity = 14 µmol m^{-2} s^{-1}), three layers of medical guaze (light intensity = 11 µmol m^{-2} s^{-1}), four layers of medical guaze (light intensity = 9 µmol m^{-2} s^{-1}). A light meter was used to measure transmitted light intensity. Seed germination was recorded once after 20 days.

Parameters studied

Following germination parameters were recorded:

(i) Final germination percentage was calculated as indicated by Bewley and Black (1994).

FGP = (number of germinated seeds after 20days/number of total seeds) × 100.

Statistical Analyses

The significant effects of salinity, presence/absence of light, light quality, light quantity and their interactions on seed germination were determined by using analysis of variance (ANOVA). While, a post hoc Bonferroni ($p < 0.05$) test was performed to compare treatment means. Statistical analyses were performed with the help of SPSS version 11.5 for Windows and graphs were plotted using Sigma Plot version 12.5.

RESULTS

Effects of presence/absence of light on seed germination

Seeds of *C. conglomeratus* germinated maximally in the presence of light (12h light/12h dark photoperiod) than in complete darkness (24h dark; Fig. **1**). Salinity affected the seed germination of *C. conglomeratus* significantly (see ANOVA; Table 1). As salinity increased, the percent germination of the test species decreased under both light and dark photoperiods (Fig. **1**). Reduction in seed germination was greater in dark than light under both non-saline and saline conditions (Fig. **1**).

Effects of light quality (color) on seed germination

Light quality had a significant effect on seed germination *C.conglomeratus* (Fig. **2**; Table **2**). Seed germination was higher under red followed by white and yellow cellophane paper treatments under both non-saline (control) and saline treatments, whereas blue and green colors inhibited percent germination under the aforementioned conditions. High salinity significantly inhibited percent germination in all colors, except red and yellow light where comparable germination was recorded in saline and non-saline conditions (Fig. **2**; Table **2**).

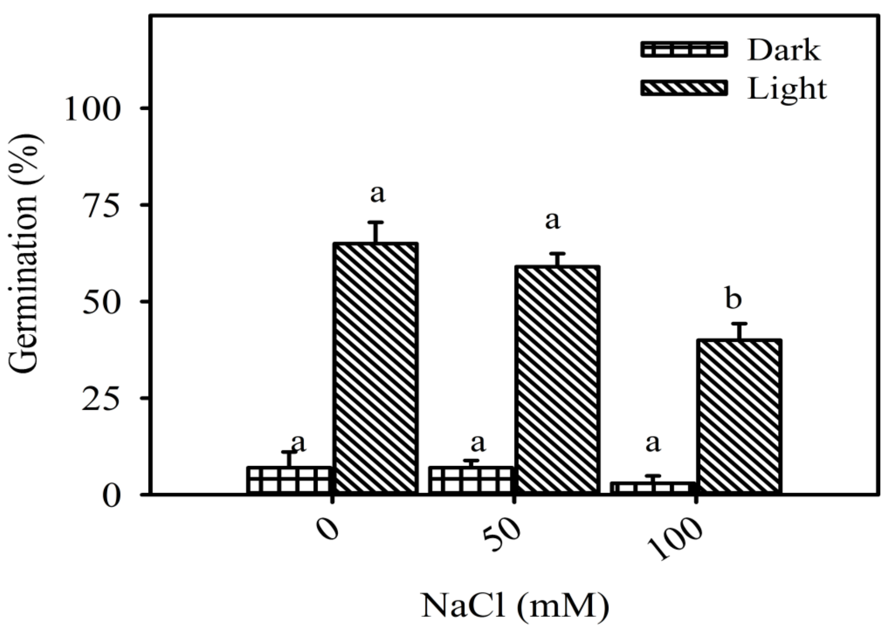

Fig. 1. Effects of presence/absence of light and NaCl (salinity) on percentage seed germination of *C. conglomeratus*. Bars represent mean ± S.E. (n = 4). Similar bars with same alphabet are not significantly ($P < 0.05$; Bonferroni Test) different from each other.

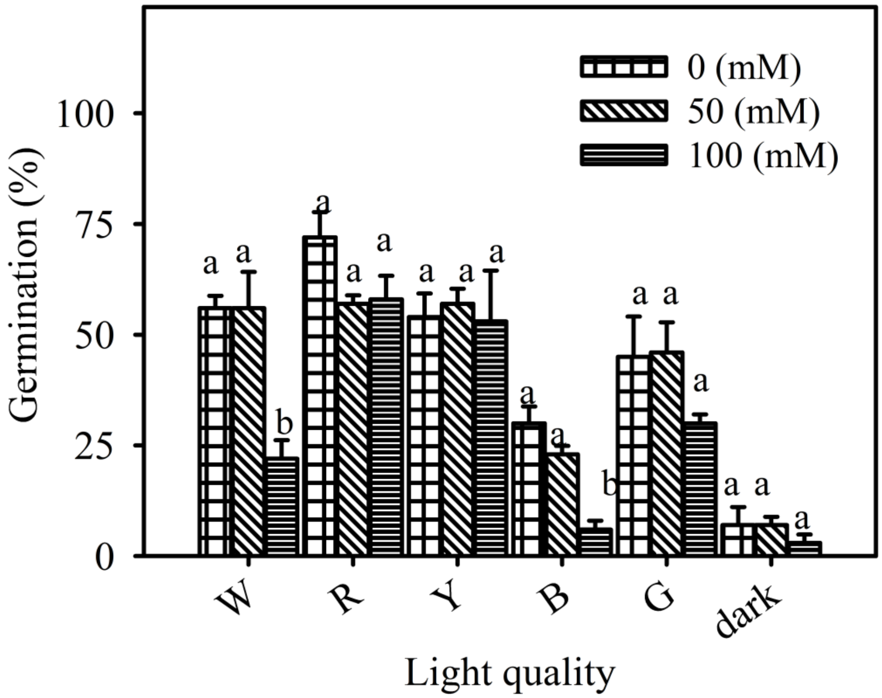

Fig. 2. Effects of light quality and NaCl (salinity) on percentage seed germination of *C. conglomeratus*. Bars represent mean ± S.E. (n = 4). Bars for each light color with same alphabet are not significantly (P < 0.05; Bonferroni Test) different from each other.

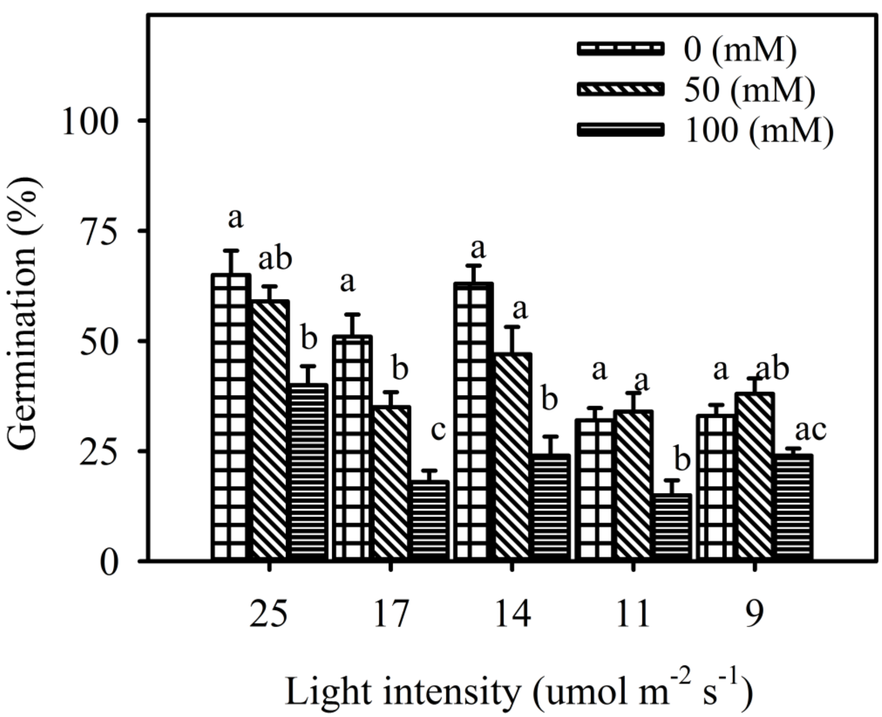

Fig. 3. Effects of light quantity (intensity) and NaCl (salinity) on percentage seed germination of *C. conglomeratus*. Bars represent mean ± S.E. (n = 4). Bars for each light intensity with same alphabet are not significantly (P < 0.05; Bonferroni Test) different from each other.

Table. 1. Two-way analysis of variance indicting effects of photoperiod, salinity and their interaction on seed germination of *C. conglomeratus*.

Factor	**df**	**MS**	**F value**	**P value**
Photoperiod (P)	1	14406.00	254.22	< 0.0001
Salinity (S)	2	468.66	8.27	< 0.001
P x S	2	234.00	4.12	< 0.01

Table. 2. Two-way analysis of variance indicting effects of light quality, salinity and their interaction on seed germination of *C. conglomeratus*.

Factor	**df**	**MS**	**F value**	**P value**
Light quality (Q)	5	5550.62	53.716	< 0.0001
Salinity (S)	2	1416.22	13.70	< 0.0001
Q x S	10	311.95	3.01	< 0.0001

Table. 3. Two-way analysis of variance indicting effects of light quantity, salinity and their interaction on seed germination of *C. conglomeratus*.

Factor	**df**	**MS**	**F value**	**P value**
Light quantity (I)	4	1431.26	22.24	< 0.0001
Salinity (S)	2	3250.06	50.51	< 0.0001
I x S	8	168.06	2.61	< 0.01

Effects of light quantity (intensity) on seed germination

Light intensity also affected seed germination of *C. conglomertatus*. Seed germination of test species decreased with reductions in light intensity (Fig. **3**; Table **3**). Reduction in germination owing to decline in light intensity was greater under saline as compared to non-saline condition (Fig. **3**; Table **3**).

DISCUSSION

Effects of presence/absence of light on seed germination

Seeds of *C. conglomeratus* germinated optimally in the presence of light (12h photoperiod) compared to dark. El-Keblawy *et al,* (2011) found similar results for the Arabian Gulf population of this species. This indicates that positive photoblastic nature is a species-level rather than population-specific attribute of *C. conglomeratus*. Similar positive photoblastic responses have been reported for the seeds of many other monocot halophytes such as *C. aerenarius* (Gulzar *et al.*, 2013), *Phragmites karka, Dichanthium annulatum* and *Eragrostis ciliaris* (Zehra *et al.*, 2012). This light dependent germination might be an adaptive strategy under harsh environmental conditions (Flores *et al*., 2016; Khan *et al*., 2017) to avoid formation of seedlings in deep soil layers, which could be lethal for seedling growth (Baskin and Baskin, 1998; El-Keblawy *et al*., 2011). The obligate requirement of light for the regulation of seed germination is often ascribed for the species found in disturbed habitatssuch as mobile sand dunes (Huang and Gutterman 1998; Koutsovoulou *et al*., 2014).

Seeds of *C. conglomeratus* germinated optimally in distilled water and low (50 mM NaCl) salinity and about 50% reduction in their germination occurred at high (100 mM NaCl) salinity in the presence of light, which is in accordance with the findings of El-Keblawy *et al.* (2011) on the Arabian Gulf population of this species. Likewise, many other monocotyledonous species such as with low salt tolerance such as, *Halopyrum mucronatum* (320mM NaCl; Ungar, 1974), *Puccinellia distans* (300mMNaCl; Harivandi *et al.*, 1982), *Panicum coloratum* (200mM NaCl; Perez *et al.*, 1998) and *P. turgidum* (200 mM NaCl; El-Keblawy, 2006) have also been shown to have low salinity tolerance. Hence, low salinity tolerance of monocotyledonous halophytes including of our test species during germination might be a common adaptive feature to confine seed germination only after sufficient rains, which would dilute salinity and drought for optimal seedling survival.

Effects of light quality (color) on seed germination

Light quality (color) had a significant effect on seed germination of *C. conglomeratus,* as white, red and yellow dominating light caused optimal, while blue and green light resulted in reduced seed germination. These results are in accordance with findings on *Ageratina adenophora* seeds, whose germination was higher under yellow, orange and red light and lower under blue and green light (Wang *et al*., 2012). Rattan and Tomar (2013) also reported that red and yellow light promoted seed germination of *Hippophae salicifolia* in comparison to blue and green light. In contrary, Basto and Ramirez (2015) reported that green light enhanced seed germination of *Tabebuia rosea*. Likewise, Patel *et al.* (2017) reported that blue light increased percent germination of *Vigna radiata*. Hence, it can be inferred that germination responses towards light quality might be species specific (Kanta and Rao, 2016).

Effects of light quantity (intensity) on seed germination

Seed germination of *C. conglomertatus* decreased with decline in light intensity. Similarly, seeds of *Carex* species (Kettenring *et al*., 2006). *Cassia fistula, Enterolobium saman, Delonix regia* (Aref, 2000) and *Quercus liaotungensis* (Xing-Fu *et al*., 2011) required full light during seed germination. In contrast, seeds of *Chromolaena odorata* (Ambika, 2007), *Pinus koraiensis* (Zhang *et al*., 2015), *Chrysophyllum albidum* (Onyekwelu *et al*., 2012) and *Copaifera oblongifolia* (Veloso *et al*., 2017) showed reversal effect in which high light intensity inhibited seed germination (Ambika, 2007). Hence, responses of seeds towards light intensity are variable and might be species specific. However, reduction in seed germination with decline in light intensity in our test species seems an important adaptation for the survival in dune environment, which would avoid germination of seeds upon burial in sand.

CONCLUSION

Results of this study indicate that: i) Seeds of *C. conglomeratus* are positively photoblastic (light requiring) during germination. ii) Dark caused reduction in germination under both non-saline and saline conditions. iii) White, red and yellow dominating light facilitate, while blue and green colors inhibit seed germination of test species. iv) Seed germination of *C. conglomeratus* decreases with reduction in light intensity.

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(*Accepted for publication June 2018*)