

Research Paper

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Stimulatory and inhibitory effects of light on *Cereus repandus* (Cactaceae) seed germination are strongly dependent on spectral quality

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Abstract

In small seeds, light often promotes germination and longer-term exposure to darkness reduces light sensitivity. In cacti inhabiting harsh environments, a rapid response to light exposure is potentially advantageous for seedling establishment. We exposed dark-imbibed seeds of the cactus *Cereus repandus* to doses of red (RED) light and far-red (FR) light. The seeds exhibited positive photoblastism to RED light. Although the initial levels of germination varied between seed lots, the sensitivity to increasing the RED dose did not. As little as 5 min per day for 4 d was sufficient to saturate the light requirement for germination. The effects of RED light were reversed by FR exposure as long as the interval between RED and FR did not extend to 2 d, by which time the seeds had ‘committed’ to germinate. Dark incubation for 1–2 weeks prior to RED exposure reduced light sensitivity in two seed lots, such that RED only promoted around 20% germination. Phytochrome is assumed to mediate the reversibility of the RED:FR response. High sensitivity to light spectral quality suggests that seeds of *C. repandus* are able to germinate quickly in high-quality microsites, but seed burial or shading may commit the seeds to form a soil seed bank. The light characteristics of the germination trait in this species are typical of many small seeded species of the drylands.

Introduction

The promotion of germination by light is a common feature of small seeds of many plant families (Millberg et al., 2000; Barzani et al., 2012; Baskin and Baskin, 2014), including cacti (Flores et al., 2011). Seed of six species of Argentine cacti (Lindow-Lopez et al., 2018) and five species of Southern Chihuahuan desert cacti (Mascot-Gomez et al., 2020) hardly germinate or fail to germinate in the dark. Such photoblastism is known for seeds of the cacti *Carnegiea gigantea* (Alcorn and Kurtz, 1959), *Cereus griseus* (Williams and Arias, 1978), *Ferocactus histrix* (del Castillo, 1986) and at least 250 other taxa in the family (Rojas-Aréchiga et al., 1997; Nobel, 1988; Barrios et al., 2020). For example, out of the 30 taxa of cacti occurring in north-eastern Brazil, 22 taxa all from the Cactoideae subfamily were classified as positive photoblastic with no germination in darkness, while the other eight taxa from the Opuntioideae (five taxa) and Pereskioideae (three taxa) subfamilies were indifferent to light (Meiado et al., 2016).

Evidence suggests that seeds of barrel cacti, which tend to be smaller, have a greater preference for germination in the light than the usually larger-seeded columnar species, which may germinate equally well with or without light (Rojas-Aréchiga et al., 1997; Flores et al., 2011). For temperate woodland herbs, seeds with masses >1.5 mg tended to have less preference for light germination (Jankowska-Blaszczuk and Daws, 2007). In contrast, this smaller *versus* larger seed light preference threshold does not appear to apply consistently in the cacti. While two larger-seeded (5–10 mg) *Pachycereus* species germinate equally well in the light and dark (Yang, 1999), in 55 species of the tribe Cacteae, there was evidence of positive photoblastism with seed mass <1.5 mg (45 species) and >1.5 mg (10 species), with no germination under dark conditions (Rojas-Aréchiga et al., 2013). Moreover, in 136 cactus taxa, there is strong covariance between relative light germination (RLG) and log₁₀ seed mass (Flores et al., 2011).

In comparative studies, seed positive photoblastism is often measured as RLG (Millberg et al., 2000) rather than germination percentage as seed lots can differ in their dormancy level as a result of inter-seasonal variation in environments during seed maturation and other factors. In a study of six cactus species of the Sonoran desert, four smaller-seeded species in *Stenocereus* and *Ferocactus* had RLG of 1 and two larger-seeded *Pachycereus* species had RLG of 0.51 (Yang, 1999). In another study of four accessions of three cactus species (*Coryphanta clavata*, *Mammillaria compressa* and two varieties of *Ferocactus latispinus*)

from southern Chihuahuan Desert (Mexico), with seed masses between 0.39 and 1.6 mg, three out of the four seed accessions germinated higher in light and only *C. clavata* had a RLG of 0.5 (Flores et al., 2016). For 17 cactus species from north-western Córdoba province in Argentina, the RLG was 1 for eight species, and always ≥ 0.65 , irrespective of seed mass (Sosa Pivatto et al., 2014). Similarly, among 55 species of Cactaceae tribe, seed RLG was 1, suggesting that the photoblastic response is phylogenetically fixed and coupled with environmental cues that fine tune the germination response (Rojas-Aréchiga et al., 2013). Overall, in the cacti, RLG has been estimated to range from 0.441 (*Copiapoa gigantea*) to 1 (around 60% of 196 seed lots studied among 136 taxa) (Seal et al., 2008; Flores et al., 2011).

In many plant species, RLG decreases with increasing seed mass, for example, in 27 temperate forest herbs (Jankowska-Blaszczuk and Daws, 2007), neotropical pioneer species (Pearson et al., 2002) and in 136 cactus taxa (Flores et al., 2011). As light only penetrates into the soil by a few millimetres (Tester and Morris, 1987) and light quality will be attenuated towards higher wavelengths (Bliss and Smith, 1985), a light requirement for germination acts as a depth sensing mechanism to enable small seeds to germinate only when close the surface, or from the soil seed bank after tillage or erosion of the upper soil layer (De la Barrera and Nobel, 2003).

In addition to reacting to the presence/absence of light, small seeds may also be responsive to light quantity; too much light (high irradiance reaction, HIR) can be inhibitory (Carta et al., 2017). Often the inhibitory effects of light are associated with light spectral quality, specifically the ratio between red (RED; 660 nm) and far-red (FR; 730 nm) light (RED:FR). Whereas under leaf canopies and leaf litter the filtering of RED light results in a RED:FR of around 0.2 or lower, unfiltered daylight has a ratio of around 1.2 (Federer and Tanner, 1966; Daws et al., 2002). Small seeds (<1.5 mg) of temperate woodland herbs prefer high-quality microsites with the RED:FR ratio of >0.5 (Jankowska-Blaszczuk and Daws, 2007). For neotropical pioneer species, the seed germination preference for light quality matches the habitat preference of the adult plant, for example, gap size, with smaller seedlings being less likely to be able to compete in shade conditions (Daws et al., 2002). Thus, the requirement of small seeds of cacti, and other species, for germination in the light can be seen as an important means of preventing germination at times and in places that are unfavourable for seedling establishment.

Habitat conditions can change and the process of germination under favourable light conditions can be adversely affected by a shift in light spectral quality. A decreased ratio of RED:FR may then result in the establishment of induced or secondary dormancy (Bewley and Black, 1994; Baskin and Baskin, 2014) as a result of the dark reversion of the FR, active form of phytochrome, P_{FR} , to the red receptive form, P_R . Germination stimulation and the counterbalancing inhibition by alternating illuminations, and the reversibility of these triggers, were first observed by Borthwick et al. (1952). Interestingly, seeds of some cacti are known to be insensitive to FR; when applied for 12 h d^{-1} at a constant 25°C , germination occurred in all seven cactus species studied (Rojas-Aréchiga et al., 1997.) However, for some of the species, the germination level in FR light was significantly lower than that in both the RED and white light treatments. Detailed information on how cactus seeds react to light in terms of germination stimulation and possible subsequent desensitization to light signals by dark incubation is not available (Flores et al., 2011)

C. repandus (L.) Mill. formerly *C. peruvianus* (L.) Miller is a non-arborescent cactus that grows to around 1 m tall and produces edible fruits of commercial importance (Mizrahi, 2014). The species has a water-use efficiency of about 10 times higher than C3 fruit tree species; it can tolerate very high temperatures and low relative humidities and is an ideal fruit crop for semi-arid zones (Mizrahi, 2014). The seeds are relatively small (3.2 mg) and have a RLG of 1, similar to that of two other *Cereus* species (Yang, 1999). The temperature control of germination has been elucidated, with an average base temperature for the germination rate, T_b , across percentiles of 10.4 ± 0.3 (SD) $^\circ\text{C}$ and a thermal time for 50% germination (θ_{50}) of around 50°C d (Yang, 1999). These are germination trait features that are similar to those of the relatives *Polaskia chende* and *P. chichipe*, which have T_b of 11.2 and 12, and θ_{50} of 65 and 54°C d , respectively (Ordoñez-Salanueva et al., 2015). *C. repandus* is, thus, an ideal model in which to study the sensitivity of cactus seed to light spectral quality. In this paper, we explore (1) the requirement of *C. repandus* seed germination for light; (2) the differential sensitivity of the seeds to RED and FR light dose among seed lots; (3) the reversibility of the RED light response by FR light; and (4) the kinetics of dark-induced desensitization to the light triggering of germination.

Materials and methods

Seed accessions

Seeds from mature fruits of *Cereus repandus* (L.) Mill. (syn. *Cereus peruvianus*) – the Peruvian apple cactus – were provided by the breeding programme of the Ben Gurion University of the Negev, Israel (Batches 1 and 2; B1, B2). Batch 3 (B3) seed was purchased from a commercial supplier (Whitestone Gardens Ltd, UK). Seed dry weight was measured after oven drying (103°C , 17 h) of 15 individual seeds per seed lot. After receipt, the seeds were kept at 45% relative humidity (RH) and 15°C in heat-sealed aluminium foil laminate bags. Seed RH was measured at 21°C using a Michell series 4020 dewpoint hygrometer (B1, B2) or a Rotronic hygrometer (B3) after a 30 min period. Times of storage under these conditions before use varied from a 1 (B2, B3) to 2 years (B1).

Light-mediated germination

An initial screen for germination in light and darkness was carried out at 26°C , within the sub-optimal temperature range for germination rate (Yang, 1999), using 50 seeds ($n = 2 \times 25$) per treatment. Seeds were sown on 1% (w/v) agar-water in Petri dishes and incubated in the presence of light (12 h d^{-1} , warm white fluorescent light at around $15 \mu\text{mol m}^{-2} \text{ s}^{-1}$) or the absence of light, achieved by wrapping dishes in aluminium foil. Germination (>1 mm radicle emergence) was recorded under very low-level green light (Pritchard and Manger, 1990). The number of germinated seeds was recorded regularly over a 2- to 3-week period until no more germination occurred.

All other experiments were also performed with seeds on agar-water at 26°C . Before and after light treatment (RED or FR), the Petri dishes were placed in aluminium foil-wrapped containers to exclude light. Light treatments were applied to seeds with the Petri dish lids removed. Red (RED) and far-red (FR) light were generated from a Rank Aldis Tutor No. 2 slide projector using a 655–665 nm (RED) or a 725–735 nm (FR) interference filter. The RED or FR was transmitted through a porthole in the side of a constant

temperature (26°C) incubator and deflected through 90° onto the seeds using a glass mirror.

Imbibition time and light sensitivity

Seeds were rehydrated in the dark for varying periods up to 3 d. Immediately before illumination, five seeds of each sample were removed for moisture content (MC) assessment (after surface drying on laboratory tissue paper). Then, the seeds were given various light treatments ($n = 3 \times 25$ seeds per dose). For B3 seeds, with higher initial germination, the RED light dose given was 25 min. For B2 seeds, 100 min RED light was applied. The dishes were re-wrapped and kept at 26°C for germination and scored only under very low-level green light.

Differences in RED light sensitivity between seed batches were explored by imbibing seeds in the dark for 3 d, followed by the exposure of the seeds to RED light for up to 1000 min. Per dose, duplicate dishes of 25 seeds were re-wrapped and kept at 26°C for germination and scored only under very low-level green light.

Loss of RED sensitivity

The loss of RED light sensitivity in seeds was investigated in different ways. Seeds of B2 were imbibed at 26°C in the dark for periods up to 18 d and then exposed to RED light for 100 and 1000 min. Also, B3 seeds were given two different short doses of RED light per day, followed by being held in the dark for the rest of the day. In this case, B3 seeds were rehydrated at 26°C in the dark for 3 d and then treated with RED illumination for either 1 or 5 min daily for a total of 20 min RED light, that is, the treatments were applied over 20 and 4 d, respectively. All post light treatment germination at 26°C was assessed under very low-level green light.

Reversibility of RED by FR

The reversibility of the RED light effect by FR light was assessed in two ways. Seeds of B2 were hydrated for 3 d, irradiated with RED for 1000 min and then exposed to FR for up to 150 min immediately thereafter. Also easier to germinate B3 seeds were hydrated for 3 d, and exposed to RED for 60 min. Then after periods up to 1 d in the dark, the seeds were exposed to FR for 10 min. Dishes were re-wrapped and kept at 26°C for germination and scored only under very low-level green light. Two \times 25 seeds were used for each of the treatments.

Data analysis

The effects of light and darkness on germination were compared statistically using a two-sample test for the equality of proportions using S-Plus 2000 software (MathSoft, Inc.). RLG calculation followed the method of Millberg et al. (2000) as follows: $RLG = Gl / (Gd + Gl)$, where Gl is the germination percentage in light and Gd is the germination percentage in darkness.

All dose dependencies of seed germination to illumination were subjected to probit analysis (to accommodate variation in seed numbers and responders) in Generalized Linear Interactive Modelling (GLIM), with fits for convergence and parallel responses subjected to F -statistics (Crawley, 1993).

Because all light treatments involved duplicates (mainly) or triplicates (rarely) of 25 seeds, the error bars on the figures are

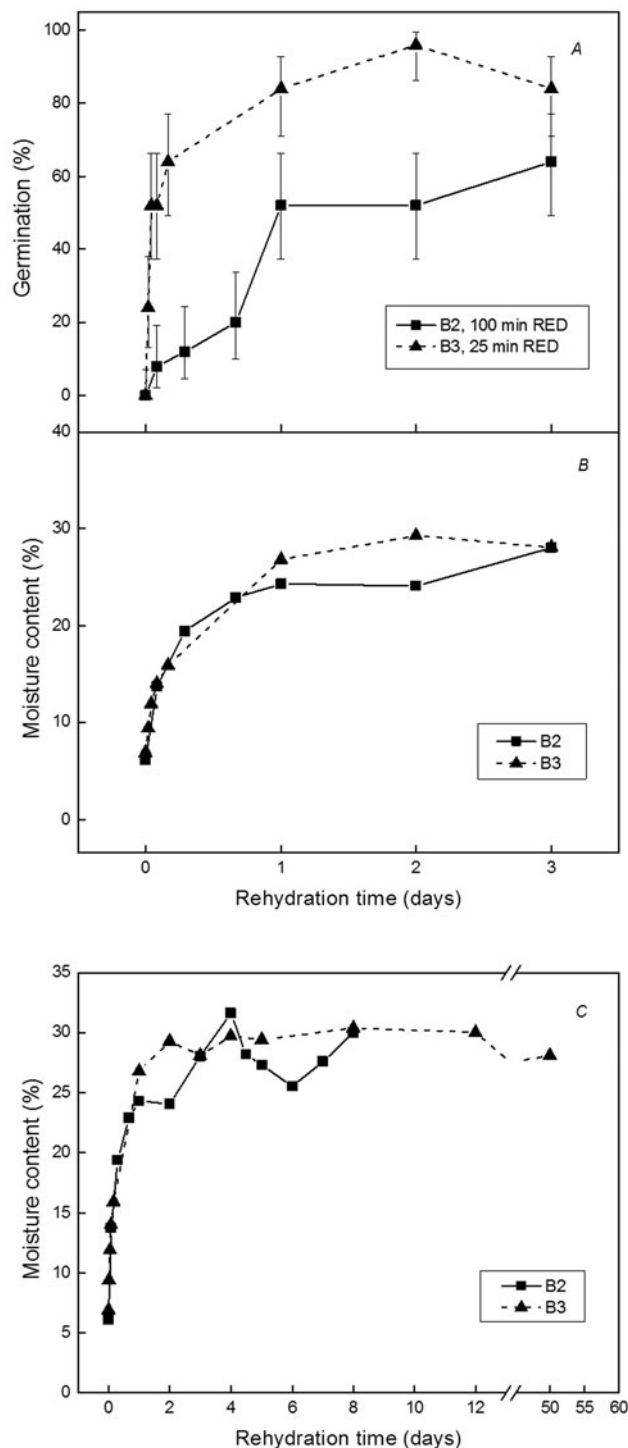


Fig. 1. Effects of imbibition time and exposure time to RED light on the germination of *C. repandus* B2 (■) and B3 (▲) seeds at 26°C. (A) Mean seed germination after imbibition and exposure to 100 min (B2 seeds) and 25 min (B3 seeds) RED light. Binomial confidence limits (95%) are shown for the total responders based on three replicates of 25 seeds sown. (B,C) Changes in seed MC with shorter-term (≤ 3 d; B) and longer-term (≥ 8 d; C) imbibition at 26°C prior to RED illumination for B2 (■) and B3 (▲) seeds. The MC means are for a single determination per time point per seed batch.

shown as binomial confidence limits (95%) for the total seeds sown and responding. In Figs. 1B,C, the MC values are for single determinations per time point per seed batch.

Results and discussion

Relative light germination

Seeds of all three batches failed to germinate in the dark and reached 96% (B2) or 98% (B1, B3) germination in 12 h d^{-1} light. RLG is therefore 1 in all cases.

The triggering of seed germination in cacti by light, that is, positive photoblastism, is well known and is associated with a range of plant traits including shorter stature and lighter seeds. *C. repandus* is cylindrical and has relatively light seeds (3.2 mg). Other species in the tribe Cereeae also have light seeds, and a RLG averaging 0.89 ($n = 12$ species) (Yang, 1999; Seal et al., 2008; Flores et al., 2011). Small (0.62–0.77 mg) seeds of *Trichocereus terscheckii* also germinate in the light but not in the dark (Ortega-Baes and Rojas-Aréchigo, 2007). These species thus shows a preference for germination close to the soil surface, as light only penetrates to a depth of a few millimetres (Tester and Morris, 1987). The relationship between light preference for germination and smaller seeds has been reported previously for European herbs from a variety of habitats (Millberg et al., 2000; Jankowska-Blaszczuk and Daws, 2007), pioneer trees from semi-deciduous tropical forest and cacti, with around 60% of a broad range of seed lots among 136 taxa having RLG of 1 (Seal et al., 2008; Flores et al., 2011). However, less is known for light-responsive cactus seeds about the spectral requirements for the trigger, which has implications for understanding of the importance of nurse plants, and shading, in the natural regeneration process in cacti (Ordoñez-Salanueva et al., 2015). This specific requirement for light might also serve to stop the seed from germinating within the developing fruit before endozoochorous dispersal (Flores et al., 2011), although this is not the case in all cacti as vivipary has been reported for at least 53 species (Cota-Sánchez, 2004; Cota-Sánchez et al., 2011; Aragón-Gastélum et al., 2017).

Onset of RED light sensitivity is related to the seed imbibition period

The imbibition rates for B2 and B3 seeds were very similar, with MCs increasing rapidly to around 25% fresh weight (FW) basis by 24 h and remaining around 30% from 2 to >8 d. (Fig. 1). During water uptake, the sensitivity of seeds to RED light increased. Few seeds responded to RED when applied around 10% MC, about half responded at an average seed MC of 15%, and at 25% MC, all seeds capable of germination responded to RED. Thus, as the seeds were hydrated in the transitional region of sorption zones II and III, they became sensitive to light-stimulated germination, similar to the heat shock response of *Carica papaya* seeds (Wood et al., 2000). What was noticeable was the difference in RED light sensitivity among seed batches with B2 requiring 100 min RED to achieve around 60% germination compared with only 25 min for B3 to be saturated with RED and achieve higher germination (around 90%) (Fig. 1A).

Germination is dependent on RED light

With the sufficient dose of RED light seed germination in all three batches of *C. repandus* reached 88–96% (Fig. 2). However, the single dose required to promote a certain level of germination varied between batches, indicating different initial competencies for germination. While B2 required a dose of around 100 min RED to attain around 50% germination, the other two batches needed <10 min RED. Thus, the requirement for RED light for the

germination of B3 and B1 seed was more than one order of magnitude lower than B2. Pre-storage time could have impacted the germination response, as B1 had been stored the longest (2 years) and had the highest initial germination level without RED treatment (Fig. 2, closed circles). However, parallel RED dose-response lines for the three batches suggest that the variation in pre-storage time did not affect the overall sensitivity of seeds to increasing RED (Fig. 2).

Intra-specific variability in seed lot quality with respect to the seed proportion that easily germinates is well known, and is often a function of the maternal environment during development, for herbaceous (Baskin and Baskin, 2014) and tree (Daws et al., 2004) seeds. In addition, B2 seed was stored for the shortest period of time (1 year) prior to experimental use and post-harvest handling can affect the depth of any primary dormancy. In the case of *Stenocereus queretaroensis* seeds, storage in the dark at 25°C and 42% RH increased germination competence to 85% after around 15 months (De la Barrera and Nobel, 2003), similar to that of B1 which had been stored dry for 2 years before use.

A linear relationship was observed between seed germination (on a probit scale) and exposure time to RED for the seed batches B1, B2 and B3 (Fig. 2). Fitting parallel lines did not significantly affect the goodness of fit compared to free-fitting [$F = 1.22$; tabulated $F(2, 28) 3.34$; $r^2 = 0.850$], such that probit germination increased by 1.571 ± 0.059 per log dose (min). This suggests that although the initial competency levels for germination were different (intercepts 4.266 ± 0.101 , 1.715 ± 0.124 and 3.752 ± 0.116 probit for B1, B2 and B3, respectively), the seed sensitivity (i.e. the distribution of responses per dose) to the RED dose was the same across seed batches of this species. Thus, germination capability increased by 1 probit per 0.64 log min of RED dose in all three seed batches.

High sensitivity to increasing the RED light level has been observed in the cactus *S. queretaroensis*, for which seed germination approximately doubled per log dose (De la Barrera and Nobel, 2003). As *C. repandus* seed germination increased about fourfold per log dose (Fig. 2), it appears that these seeds are relatively more sensitive to RED light than *S. queretaroensis*. The fine-tuning of the seed response to light is modulated by PIF (phytochrome[phy]-interacting factor) transcription factors, which are pivotal in cellular signalling and promote shade-avoidance through regulated gene expression (Leivar and Quail, 2011). When phy molecules are activated by light, the rapid degradation of PIF proteins is induced. Other pathways converge to regulate PIF, however, including the gibberellin pathway which contributes to the lowering of seed physiological dormancy. In *Arabidopsis*, for example, PIF1 promotes the expression of two gibberellic acid (GA)-repressor (DELA) genes that decrease the sensitivity of the seed to GA (Leivar and Quail, 2011).

Dark incubation reduces light sensitivity

The long-term retention of light-sensitive seeds in the dark often results in the desensitization to light (or the induction of secondary dormancy; Bewley and Black, 1994). To see if this was also the case for *C. repandus*, B2 seeds were left in the dark for greater than the usual period (3 d prior to RED). Two conditions of RED stimulation, 100 min and 1000 min, were applied and dark incubation times of up to 18 d were used.

Lengthened dark imbibition decreased light sensitivity significantly (Fig. 3). For example, germination was reduced from around 92 to 20% when dark incubation time increased from 2

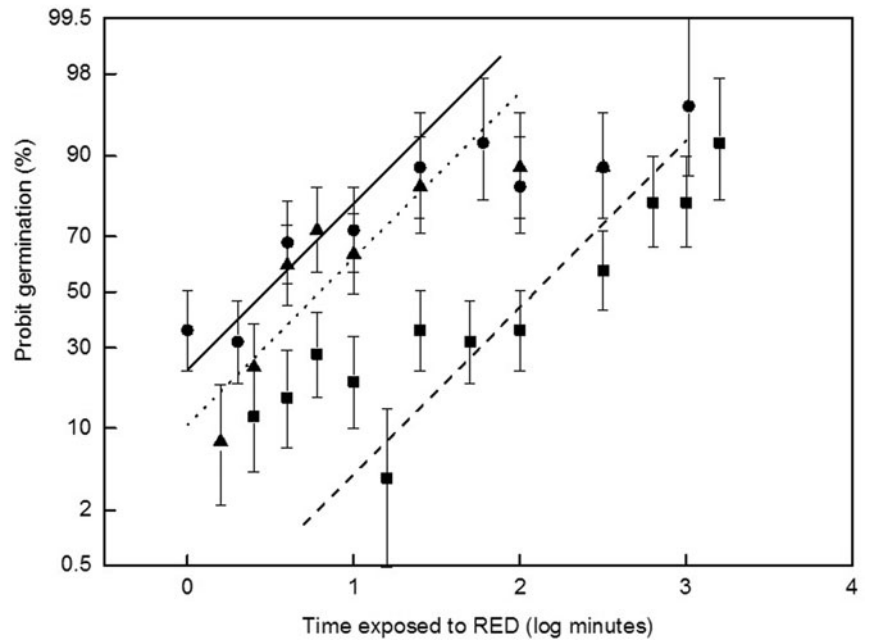


Fig. 2. Effects of RED light dose (log min) on seed germination (probability scale) for *C. repandus* B1 (●), B2 (■), B3 (▲). Probit germination increased by 1.571 ± 0.059 per log dose (min) for each seed batch, which had zero dose germination levels. Further explanation for fitting parallel lines is given in the text. Binomial confidence limits (95%) are shown for the total responders based on two replicates of 25 seeds sown per light dose.

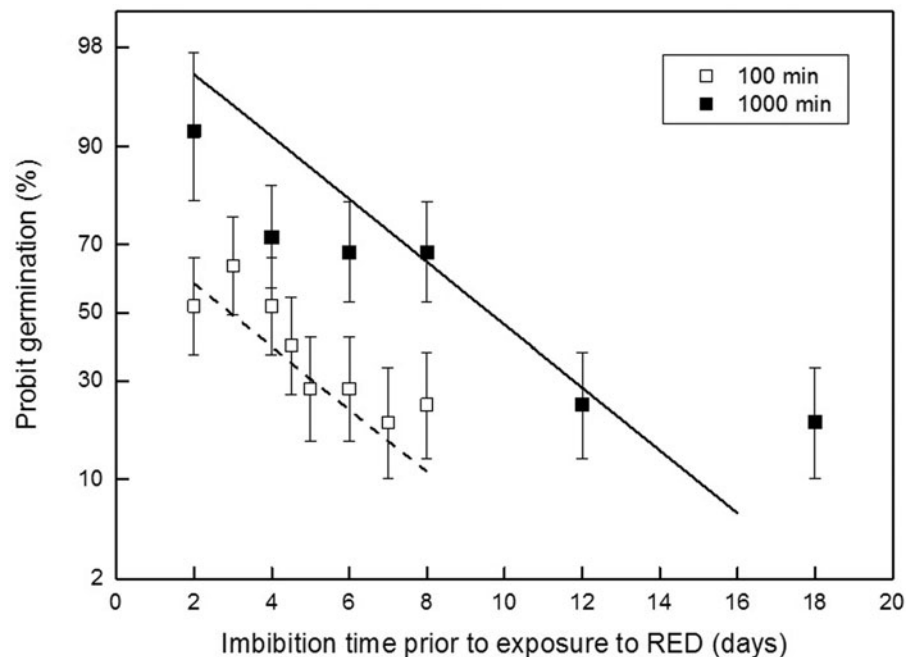


Fig. 3. Effect of dark incubation time prior to RED light irradiation on the germination (probability scale) of *C. repandus* B2. Seeds were imbibed for up to 18 d prior to the application of RED light for 1000 min (■) or 100 min (□). Binomial confidence limits (95%) are shown for the total responders based on two replicates of 25 seeds sown. The fitted parallel lines indicate a one probit reduction in germination per 0.242 dose of dark imbibition, that is, every 4.1 d.

to 18 d prior to irradiation with 1000 min RED. When seeds were only exposed to RED for 100 min, germination fell from 52% after the shortest dark incubation time of 2 d to 20% after the seeds were incubated in the dark for 8 d. A negative linear relationship between probit germination and dark incubation time was observed for both RED doses. GLIM analysis showed an insignificant difference between the parallel model and free-fitting models ($F = 0.79$; tabulated $F(1,10) = 4.96$; $r^2 = 0.863$), such that probit germination = $7.331 - 0.242x$ (1000 min RED) and probit germination = $5.709 - 0.242x$ (100 min RED). The reduced sensitivity of *C. repandus* seeds to RED light after long-term (1–2 weeks)

exposure to darkness suggests that buried seeds could enter the soil seed bank (Fig. 3).

The effect of multiple, short doses of RED light and long periods of darkness per day on seed germination was also assessed. B3 seeds were rehydrated in the dark for 3 d and exposed to 1 or 5 min of RED per day, cumulatively for 20 min, with germination proceeding in the dark. When 1 min of RED was provided daily, germination increased to around 40% by 15 d, but there was no further increase by the 20th day (Fig. 4). Germination promotion did not reach the full level for this seed lot. A germination increase was greater when 5-min daily exposures to RED were

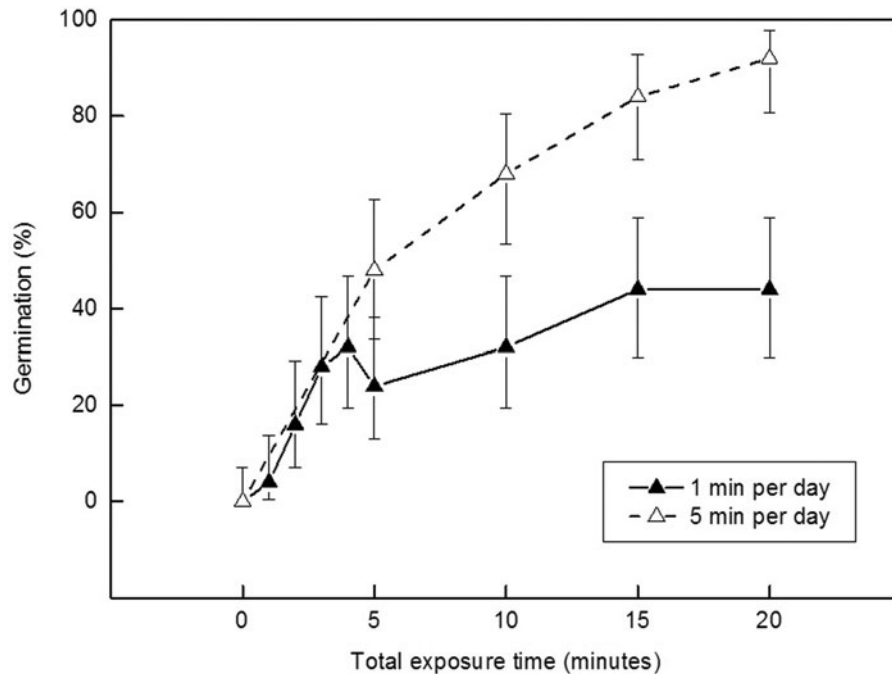


Fig. 4. Effect of cumulative exposure to RED light on the germination of B3 seeds. Seeds were imbibed in the dark for 3 d and then RED light applied for 1 min (\blacktriangle) and 5 min (\triangle) per day to total exposure time of 20 min. Thus, the experiments ran for 20 and 4 d, respectively. Binomial confidence limits (95%) are shown for the total responders based on two replicates of 25 seeds sown.

used. A single 5-min treatment on the first day resulted in 48% germination, with the trajectory for germination promotion similar to that of a 1-min daily dose (Fig. 4). However, four daily 5-min treatments (i.e. 20 min cumulatively) raised germination to 92%, close to the maximum for this seed lot (Fig. 4).

A single dose of 100 min of RED light are sufficient to saturate the light requirement in B3 seeds, which then germinate to a high level in the dark (Fig. 2). The seeds are clearly highly sensitive to RED light, as the dose effect can be reduced to just 20 min at 5 min a day for 4 d. However, shortening the exposure to RED light to 1 min, a day only partially promotes subsequent germination (Fig. 4). Nonetheless, the seeds show signs of a low fluence response (Cone et al., 1985; De Petter et al., 1988), similar to that of *S. queretaroensis* seeds that respond over a 10-d period to a minimum light level of $0.15 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 10 h d^{-1} (De la Barrera and Noble, 2003). Such sensitivity to high-quality light acts as a gap-sensing mechanism that also enables the rapid emergence of the seedling (Daws et al., 2002). When the daily RED dose is insufficient to stimulate all seeds to germinate (i.e. <5 min), the long periods of darkness between RED applications are presumed to be sufficient to permit the reversion of the active form of phytochrome (P_{FR}) to the inactive form (P_{R}), and some of the seeds fail to germinate potentially because of the induction of secondary dormancy (Cone et al., 1985; De Petter et al., 1988). Similarly, seeds of eight out of 11 species of cacti did not germinate to a significantly higher level when the same set of seeds was transferred from dark to light, suggesting that darkness had triggered secondary dormancy (Flores et al., 2006). The molecular framework for such rapid and time-lapse responses to light could be modulated by the interaction between de-etiolated 1 (DET1), which is an evolutionary conserved protein and central repressor of light-induced germination, constitutive photomorphogenic 10 (COP10), long hypocotyl in FR 1 (HFR1) and PIF1 (Shi et al., 2015).

As postulated for *S. queretaroensis* (De la Barrera and Nobel, 2003), only with erosion of the soil surface or tilling would *C.*

repandus seeds likely accumulate sufficient light to germinate. This response has the advantage that the seeds could await rainfall in a habitat, in which rainfall events are far apart. Eventual emergence therefore depends on the seeds having the ability to survive. Seeds used in this study, which were stored for up to 2 years before experimentation, nonetheless had initial (on receipt) germination competency of at least 96%, indicating, as with *S. queretaroensis* (De la Barrera and Nobel, 2003), that the seeds have good longevity. Nonetheless, it would be interesting to know precisely how temperature and humidity affect seed longevity in *C. repandus*.

RED light effect is reversible by FR light

The involvement of phytochrome in the RED light response was confirmed by reversing the effect with FR light using seed lots B2 and B3.

For B2 seeds, the greater the dose of FR given to seeds immediately after 3-d hydration followed by exposure to RED for 1000 min, the less effect RED had on germination. While control (1000 min RED) seeds reached 80% germination, irradiation with FR for 1.5 min (0.18 log min), 15 min (1.176 log min) and 150 min (2.18 log min) reduced the germination to 48, 34 and 26%, respectively. GLIM analysis revealed a significant ($r^2 = 0.986$, $P < 0.05$) dependency of (decreased) germination on the FR of -0.488 (± 0.215) probit per log min, suggesting that FR was very effective at increasing the requirement for RED. Numerous PIFs are known to repress light responses in plants, including PIF8 (Oh et al., 2020). PIF8 protein accumulates more in *Arabidopsis* in FR light than in darkness or RED light through a mechanism that includes the inhibition of COP1 by phyA (Oh et al., 2020). In seeds, the FR light response depends on phyA being transported from the cytoplasm to the nucleus by shuttle proteins FAR RED ELONGATED HYPOCOTYL 1 (FHY1) and FHY1-like (FHL) (Sheerin and Hiltbrunner, 2017). In the nucleus, light-activated phytochromes suppress the E3

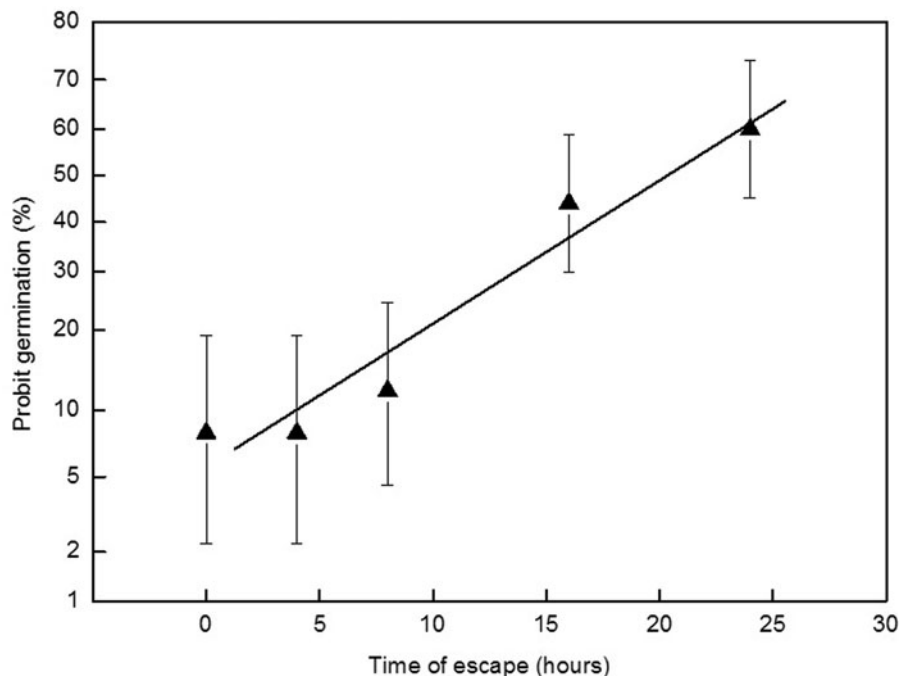


Fig. 5. Effect of FR light on *C. repandus* B3 seed germination (probability scale). After rehydration for 3 d, seeds were exposed to RED for 60 min and, then after a range of escape times (hours), exposed to FR light for 10 min. Responsivity to FR light decreased by one probit of germination per 0.078 dose, that is, 12.8 h. Binomial confidence limits (95%) are shown for the total responders based on two replicates of 25 seeds sown. A data point at 48 h and 100% germination is not shown in the graph but was included in the probit analysis, by convention, as 99.9% germination.

ubiquitin ligase complex CUL4/DDB1^{COP1/SPA} and inactivate PIF, although additional mechanisms must exist that shift the action of phyA to FR (Sheerin and Hiltbrunner, 2017). For example, in *Arabidopsis* seeds, SPATULA gene expression is repressed under FR light in a PIF1-dependent manner (Vaistij et al., 2018).

As FR clearly desensitized the seeds to RED light, we next investigated how quickly seeds could escape the effect of FR completely (Fig. 5). B3 seeds were hydrated for 3 d in the dark and then given 60 min RED light, which in the control promoted germination to >90% and then subjected seeds to 10 min FR light up to 2 d afterwards. The application of FR within 10 min of RED was sufficient to reduce germination to <10% (Fig. 5). As the time between the RED and FR pulse increased the reversibility of RED by FR was reduced, such that applying FR 24 h after RED failed to stop germination in 60% of the seeds. However, when 48 h were allowed to elapse between RED and FR, FR failed completely to stop the progress of germination; the seeds reached 100% (data point not shown in Fig. 5). The dependency of germination on escape time since RED illumination was described for B3 seed as: Germination (probability) $3.41 (\pm 0.019) + 0.078 (\pm 0.011)$ dose, in hours ($r^2 = 0.945$, $P < 0.01$) (Fig. 5).

Presumably, *C. repandus* seed burial or deep shading by a nurse plant cumulatively reduces seed sensitivity to light and supports the formation of a soil seed bank, as is relatively common in small seeds of many cacti, for example, 0.6 mg seeds of *P. chende* (Flores et al., 2011; Ordoñez-Salanueva et al., 2017). However, if the seeds receive sufficient RED light and time to initiate the germination process before burial, the stimulatory effects of RED light are irreversible by FR light. This time interval is evidently around 1–2 d (Fig. 5). At 26°C in the light (12 h d⁻¹), *C. repandus* seed germination is visible within 3 d (Yang, 1999), and germination processes prior to radicle emergence must be far advanced by 2 d, as the seeds will have been at full hydration from 24 h onwards (Fig. 1). The commitment to complete germination quickly is a feature of small seeds of desert plants in order to

maximize the likelihood of seedling establishment (Baskin and Baskin, 2014). This temporal control of the interplay between the effects of RED and FR light on cactus seed germination involving phytochrome might also be temperature-dependent. The biologically active Pfr state can convert to Pr in a light-dependent thermal relaxation process called dark reversion (Mancinelli, 1994). As phyB inactivation is proportional to temperature in the dark, phytochromes potentially also function as thermal timers that integrate temperature information overnight (Jung et al., 2016). Although much is known about how temperature influences cactus seed germination (Yang, 1999; Seal et al., 2017; Barrios et al., 2020), the possible modulating and concurrent role of phytochromes in both the thermal and photon responses of cactus seeds appears to be unexplored.

Conclusion

In conclusion, the germination in three seed lots of *C. repandus* are revealed to be highly sensitive to RED light in a classical phytochrome-mediated response that is reversible by FR light. The preference for high RED light may enable the small seeds to germinate preferentially in high-quality microsites, rather than open sites within which seedlings can die faster than under nurse plants due to high solar gain (Flores et al., 2004). As seeds used in this study were obtained from a breeding programme of the Ben Gurion University of the Negev, Israel, and from a commercial supplier, some differences in response could be possible for wild-collected material.

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