ECOPHYSIOLOGY OF SEED GERMINATION IN NATIVE AND EXOTIC LABIATES OF BALOCHISTAN

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ABSTRACT

The effect of light and temperature on seed germination was investigated in three species of Labiatae found in Balochistan namely, Lallemantia royleana Benth. Ocimum basilicum L. and Ziziphora tenuior L. The experiments were performed at constant temperature under white light, darkness, and far-red light. The role of phytochrome in seed germination was also investigated, for this purpose the imbibed seeds were irradiated with pulses of red and far-red light of various durations. The seeds of L. royleana germinated optimally at 20 °C and were capable of germinating within a range of 15 to 25 °C, however at 15 and 25 °C a slight suppression in germination was observed under far-red light. The white light resulted in promotion of seed germination of O. basilicum while the germination was suppressed under darkness and far-red light in cooler temperatures, i.e., below 25°C. The optimum temperature for seed germination of Z. tenuior was observed to be 15 °C. The seed germination in the species was suppressed by far red light at warmer temperatures whereas white light promoted the germination. A single red light pulse was unable to promote the seed germination in all three species, however, intermittent pulses of red light promoted the seed germination in L. royleana and Z. tenuior this effect was reversed when red light was followed by far-red light. On the basis of this reversal effect the involvement of phytochrome in the seed germination of L. royleana and Z. tenuior is proposed.

Key words: Balochistan, far-red light, Labiatae, phytochrome, seed germination.

INTRODUCTION

The effect of light and temperature on seed germination was investigated in three species of Labiatae found in Balochistan namely, Lallemantia royleana Benth. Ocimum basilicum L. and Ziziphora tenuior L. The two species namely L. royleana and Z. tenuior are the wild herbs growing in some parts of Balochistan, while an exotic variety “Greek Yevani” of O. basilicum was examined in this study. The Labiatae is an important family with about 210 genera and 3200 species. It is cosmopolitan in distribution, especially abundant in the Mediterranean region. Various species of Labiatae have been reported in different localities of Pakistan which include Chitral, Waziristan, Parachinar, Rawalpindi, Sibi, Makran, Quetta, Zobe, Zairat and Kalat (Hedge, 1990). The plants are predominantly annual or perennial herbs and sometimes shrubs. The seeds are enclosed in one seeded nutlets and generally exhibit dormancy (Ellis et al., 1985). Seeds sense a number of environmental signals and tend to germinate when these signals provide indication of favorable conditions for seedling establishment and completion of the life cycle. The light environment of the seeds, which is perceived by photoreceptors, plays a widely acknowledged role in this regard. The control of seed germination by light is one of the earliest documented processes; this control is manifested through phytochrome which is known to be a small family of photoreceptors (Casal & Sánchez, 1998). Phytochromes possess two photo-interconvertible forms: the red light (r) absorbing form (Pr) and the far-red light (Fr) absorbing form (Pfr). Absorption maxima of Pr and Pfr are around 660 and 730 nm, respectively. The plants examined in the present study have economic and ecological significance yet very little is known about how light and temperature affect the germination of these plants; such information plays a significant role in predicting the regeneration of these species in nature. Most of the scientific work carried out on the family relates to allelopathic effects of the plants and medicinal importance of the family (Jambere et al., 1995) with few exceptions where ecophysiology and effect of light on seed germination of Labiatae has been explored (Ellis et al., 1985; ISTA 1993; Thanos & Doussi, 1995; Thanos et al., 1995). The information regarding temperature and influence of quality of light on seed germination of the species under study is scarce, therefore these parameters are investigated in present work and results have been
correlated with conditions prevailing in their respective habitat.

**MATERIALS AND METHODS**

The seeds were obtained from Arid Zone Research Center (AZRC) Quetta, Pakistan. Following the method adopted by Doussi & Thanos (2002) the germination tests were performed with 5 replicates of 20 seeds per petri dish of 9 cm diameter. Each petri dish was lined with two layers of filter paper and moistened with 8 ml of distilled water. The criterion of seed germination was visible radicle protrusion. Final germination percentage and T₅₀ was calculated. T₅₀ was used to determine the speed of germination; T₅₀ is the number of days taken to germination of 50% of all germinated seeds (Saatkamp et al., 2011).

**Light sources**

The red and far-red broad band irradiations were produced by a bank of tube lights (Philips 20W); the light was filtered through a combination of colored Plexiglas sheets. The red light was obtained by using single 3 mm thick red Plexiglas filter, while far-red was obtained by using two 3 mm thick blue and one 3 mm thick red Plexiglas filter. The φ, ζ and I under identical light conditions were measured with a spectroradiometer (Licor 1800, LICOR, USA) by Saeed (2000). (The φ denotes phytochrome photostationary state ratio which is \[ \frac{[\text{Phytochrome in fr form}]}{[\text{Phytochrome in total}]} \], the ζ denotes 660/730 nm photon ratio, while I is the total fluence \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The values of \( \phi \), \( \zeta \) and \( I \) are given in table 1. In case of exposure to a single pulse of light the seeds imbibed for 12 h in darkness were exposed for only one time to a 15 min of red light and 15 min red light followed by 15 min far-red light before incubation in growth chamber under dark. For intermittent pulses the treatment mentioned above was repeated every 24 h during the course of experiment, after each irradiation the seeds were returned to growth chamber. All manipulations of seeds were carried out under dim, green safe light (constructed by filtering the light of one fluorescent tube, 10 W Philips, through two Plexiglas sheets, one red-orange, and one green).

**Growth chamber**

The experiments were performed in plant growth chamber having three compartments; each equipped with four fluorescent tubes of 20 watts, the chamber maintained temperature within ± 1 °C.

**Statistical analysis**

The effects of light and temperature on the percentage germination were analyzed with ANOVA models using statistical package SigmaStat 3.5. Pairwise multiple comparison procedures were performed with the Tukey’s test.

<table>
<thead>
<tr>
<th>Light Condition</th>
<th>( \phi )</th>
<th>( \zeta )</th>
<th>I, ( \mu \text{mol m}^{-2} \text{s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Light</td>
<td>0.64</td>
<td>1.04</td>
<td>72.59</td>
</tr>
<tr>
<td>Far red Light</td>
<td>0.04</td>
<td>0</td>
<td>19.8</td>
</tr>
<tr>
<td>Red Light</td>
<td>0.58</td>
<td>1.08</td>
<td>45.2</td>
</tr>
</tbody>
</table>

**RESULTS**

The seeds of *L. royleana, O. basilicum* and *Z. tenuior* were incubated at constant temperatures of 15, 20, and 25 °C, and under white light, far-red light and darkness. The response of all three species varied in relation to experimental light and temperature conditions. Significant interaction was found between light and temperature among all three species (P < 0.001).

Time courses of seed germination of *L. royleana, O. basilicum* and *Z. tenuior* were obtained under darkness and 16h white light and far-red light. The final germination results are summarized in table 1.

**Seed germination of *L. royleana***

In *L. royleana* no significant difference in mean value of final germination percentage was observed among darkness, white light, or far-red light at 15 and 20 °C, where final germination percentage ranged from 54 to 71 %. On the other hand, at 25 °C a slight decrease was observed in the rate and final germination percentage under all three experimental light regimes, however this suppression of germination was not statistically significant (P > 0.05). The time course of seed germination of *L. royleana* is presented in Figures 1, 2, and 3.
Time courses of seed germination of *O. basilicum*.

Statistically significant interaction was found between various levels of light and temperature regime on the seed germination of *O. basilicum* (P <0.001). Similarly significant difference in mean final germination percentage was found with regard to various light and temperature regimes. The white light resulted in enhancement of seed germination of *O. basilicum* at all three tested temperature ranges; however, the germination speed was comparatively faster at 25 °C (T<sub>50</sub> value is 2.1). No germination was obtained at 15 °C under far-red light, while at 20 °C seed germination of *O. basilicum* was significantly suppressed under darkness and far-red light (figures 4 and 5).
The seeds of Z. tenuior germinated to their maximum capacity at 15 °C under white light and darkness (97 ± 2.0 % under both light conditions), with faster rate of germination (T₅₀ value 3.1 and 3.7 under white light and darkness respectively), while rate and final germination was significantly suppressed by far red light at 15 °C (figure 7). The germination at 20 °C was enhanced under white light; however darkness resulted in suppression of germination. The seed germination was totally inhibited by far-red light at 20 °C (figure 8). The seed germination of Z. tenuior was completely suppressed at 25 °C.

Seed germination after single and intermittent 15 min pulses of red and far-red light.

The results of seed germination after single 15 min pulse of light are represented in fig. 9 (A). The seeds of three species were incubated at 20 °C after irradiation with a) a single 15 min pulse of red light and b) a single pulse of red light followed by 15 min single pulse of far-red. A single 15 min pulse of red light was unable to replace the germination enhancement caused by white light in all three species, likewise a single 15 min pulse of far-red was unable to suppress the seed germination in the species, however slightly different results were obtained when seeds were irradiated with 15 min of intermittent red light followed by 15 min far-red light. In O. basilicum and Z. tenuior intermittent red light resulted in promotion of seed germination and this effect was slightly suppressed by intermittent pulse of far-red light these results are shown in fig. 9 (B).

DISCUSSION

Labiatae is predominantly a Mediterranean family and has characteristically been known to have relatively colder range of seed germination temperature ranging from 5 to 20 °C which has been described as "Mediterranean" temperature range (Thanos & Doussi, 1995). In present studies the species have exhibited varied responses to different germination conditions. The seeds of L. royleana were non photodormant. The seed germination in O. basilicum was promoted by light, while the light favored germination of Z. tenuior only at higher temperatures, whereas cooler temperature encouraged the germination regardless of light conditions.
Table 2. Final seed germination %, ± standard error and \( (T_{50}, \text{days}) \) under various temperatures and light regimes

<table>
<thead>
<tr>
<th>Plant</th>
<th>15 °C</th>
<th>20 °C</th>
<th>25 °C</th>
</tr>
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<tbody>
<tr>
<td>Lallemantia royleana</td>
<td>Light: 4.8 (5.3), Darkness: 71 ± 3.3, Far-red: 45 ± 2.7</td>
<td>Light: 62 ± 4.2 (4.0), Darkness: 60 ± 6.5, Far-red: 59 ± 2.7 (3.3)</td>
<td>Light: 70 ± 4.2 (2.7), Darkness: 57 ± 4.3 (3.2), Far-red: 60 ± 2.4 (1.8)</td>
</tr>
<tr>
<td>Ocimum basilicum</td>
<td>Light: 4.6 (6.0), Darkness: 18 ± 2.5, Far-red: --</td>
<td>Light: 88 ± 7.6 (6.0), Darkness: 90 ± 8.0 (5.0), Far-red: 63 ± 5.0 (3.1)</td>
<td>Light: 97 ± 7.8 (5.0), Darkness: 97 ± 6.6 (4.9), Far-red: 65 ± 2.1 (2.4)</td>
</tr>
<tr>
<td>Ziziphora tenuior</td>
<td>Light: 2.0 (3.1), Darkness: 97 ± 2.0, Far-red: 65 ± 10.8 (1.82)</td>
<td>Light: 94 ± 7.9 (1.82), Darkness: 94 ± 5.0 (1.82), Far-red: 79 ± 3.7 (1.82)</td>
<td>Light: 15 ± 13.8 (6.11), Darkness: 15 ± 13.8 (6.11), Far-red: --</td>
</tr>
</tbody>
</table>

The seeds of *L. royleana* have a broad range of germination temperature. The species is additionally characterized by almost similar final germination at a variety of light and temperature conditions. No photodormancy was observed in seed germination of *L. royleana* as seeds were capable of germinating under 16 h white light, darkness, and under far-red light. This suggests that the species is adapted to germinate under a variety of conditions; for example in gaps where direct sunlight is available, and even if seed is buried. The tolerance to the far-red light further suggests that it may germinate under the shade of other plants where value of red to far-red ratio *i.e.* 660/730 nm photon ratio (R: FR) is low.

The white light promoted the seed germination of *Ocimum basilicum* in temperature range of 15-25 °C. The germination was slightly suppressed under far-red at 20 and 25 °C, but at lower temperature of 15 °C the suppression was more profound under darkness and far-red light, suggesting that the covered seeds of *O. basilicum* remain dormant at 15 °C. The effect of far-red light was more prominent under suboptimal temperature. The white light has been observed to promote the seed germination of *O. basilicum* under all temperature levels. The germination was much quicker at 25 °C under white light as indicated by lower value of \( T_{50} \). The requirement of white light for seed germination in *O. basilicum* may be an adaptation for regeneration in gaps, the seeds having requirement of white light for germination indicate that these species may be able to form a large persistent seed bank (Kettenring *et al.*, 2006).

Unlike earlier two species the germination of *Ziziphora tenuior* was favored by cooler spring
temperature. The seeds germinated to full capacity at 15 °C under white light and darkness. At 20 °C white light promoted germination, whereas far-red light has completely suppressed the germination. The seed germination was completely suppressed under all light regimes at higher temperature. While working on endemic Labiates of Crete, Thanos & Doussi (1995) have observed that suboptimal temperatures (which were, 5 and 25 °C in their study) inhibited germination in some species without inducing secondary dormancy. Further investigation is needed in case of Z. tenuior to exclude or to confirm the induction of such dormancy. The germination behavior of Z. tenuior resembles typical Mediterranean one; which is characterized by seed germination at lower temperature it also implies that seed germination may occur in the beginning of spring season which is marked by low temperature and available rainfall for seedlings to establish before the onset of dry and hot summer.

A single pulse of red light did not result in enhancement of germination in all three species. The seed germination in some instances is not enhanced by a single red pulse, and continuous light or repeated pulses are needed (Hsiao & Vidaver, 1984, Grubšič & Konjević, 1990). The seeds responded differently to the exposure to intermittent red light which slightly increased the seed germination of all three species. This effect was marked in Z. tenuior and O. basilicum and was not distinct in L. royleana. Though not completely reversed; this effect was however slightly suppressed by intermittent exposure to far-red light in O. basilicum and Z. tenuior. The results imply that germination of the two species will be inhibited under canopies having low R: FR. It was also observed that exposure to intermittent red light pulse did not seem to replace the effect of 16 h white light. One reason for that might be the beneficial effect of white beyond the effect of phytochrome, the other might be the lower fluence rate of red light (as proposed by Kettenring et al., 2006). The involvement of phytochrome in the seed germination of L. royleana and O basilicum is being proposed on the basis of effects of red light and reversal of this effect by far-red light.

The varied responses of species to different light and temperature conditions correspond to their respective climatic conditions. The seeds of L. royleana and O. basilicum have broad range of germination temperature and light requirement; L. royleana seems to be adapted to variety of habitat. The covered seeds of O. basilicum remain dormant at 15 °C. At higher temperature seed germination of Z. tenuior was suppressed which suggest the early emergence of seedlings in the field and light requirement suggest the inhibition of germination under low R: FR environment of under canopy.

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REFERENCES


