

Light and Phytochrome Involvement in *Rosa multiflora* Seed Germination

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Abstract. Seed germination percentage of multiflora rose (*Rosa multiflora* Thunb.) was much higher under continuous white light than in complete darkness. Red light was the most effective in inducing germination, and far-red light was ineffective. Exposure to red light for 1 min increased germination; this effect was saturated at an exposure of 2 min. The red-light effect was reversed by subsequent exposure to far-red light. The results indicate that rose seeds are positively photoblastic, and that the photoreceptor involved is most likely phytochrome.

Rose seed germination has been considered unaffected by light, whereas, seed germination in many other species is induced or inhibited by light (Bewley and Black, 1982). Even if germination of rose seeds is influenced by light, detecting significant differences in germination between illuminated seeds and those kept in darkness is difficult, because germination percentages are generally quite low. Therefore, it is still uncertain whether seeds of rose are photoblastic. Leaching achenes with activated charcoal or treating them with macerating enzymes greatly improves seed germination of *Rosa multiflora* and *R. hybrida* 'Inspiration' (Yambe et al., 1992; Yambe and Takeno 1992). By using a macerating enzyme to dramatically increase germination potential, we can more definitively examine the influence of light on germination of rose seeds. The present work examined the effect of light on seed germination of *R. multiflora* using achenes treated with Driselase, a common macerating enzyme.

Materials and Methods

Plant material. Mature cynarhodias (hips) of *R. multiflora* were harvested at the experimental farm of the Faculty of Agriculture, Tohoku Univ., Sendai, Japan, in Winter 1991-92. The harvested hips were stored at room temperature, and achenes were excised from the hips immediately before use. All experiments were conducted within a few months after harvest of the hips.

Enzyme treatment. The macerating enzyme used was Driselase (Kyowa Hakko Kogyo Co., Tokyo), a product of Basidiomycete fungi with activities as cellulase, pectinase, glucanase, xylanase, amylase, and others. The enzyme preparation was dissolved in 10 mM 2-(N-morpholino) ethansulfonic acid buffer (pH 5.0) at 1% (w/v). Fifty achenes were placed in a test tube containing 10 ml of enzyme solution. The dry achenes were placed in tubes wrapped with aluminum foil to prevent exposure to light before the start of imbibition. The tubes were rotated at 5 rpm on a rotary incubator at 30°C for 36 h. After enzyme treatment, the achenes were washed with distilled water to remove the enzyme. The achenes were then placed on filter paper in a 9-cm petri dish containing 4 ml distilled water. Three replicate dishes, with 50 achenes each, were used for each experimental lot. All the procedures after incubation in the

enzyme solution were done in complete darkness.

Light treatment. The achenes treated with the enzyme were immediately exposed to light for various lengths of time. White light was provided by cool white fluorescent tubes (20W FL20SW; Toshiba, Tokyo). Red and blue light were provided by combining a sheet of red and blue cellophane, respectively, and white fluorescent tubes. Far-red light was provided by combining sheets of blue and red cellophane and incandescent lamps (110V/110W; Toshiba) and a water layer (3 cm deep) as a heat-cut filter. Optical characteristics of the cellophane sheets used are shown in Fig. 1. The intensity of the light used in each experiment is described in the text. After the light treatment, the dishes were wrapped with aluminum foil and a light-proof plastic sheet and incubated at 25°C in darkness until germination percentage was determined. Each experiment was repeated at least twice.

Results and Discussion

Germination percentage was >60% under light, whereas germination in darkness was negligible (Fig. 2). These results clearly indicate that seeds of *R. multiflora* are positively photoblastic. Preliminary experiments with seeds of *R. hybrida* 'Inspiration' resulted in a similar conclusion (data not presented).

Red-light exposure resulted in the highest germination percentages (Fig. 3). Less than 10% of the seeds germinated in darkness. Germination percentages in the achenes exposed to far-red and blue light were similar to those for achenes maintained in darkness.

Exposure to red light for 1 min induced significantly higher germination than in the control; this effect was saturated at an

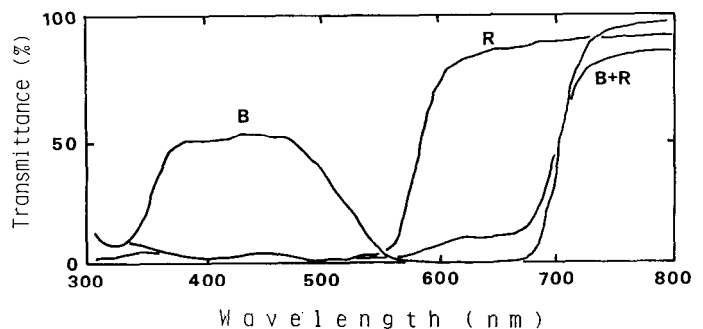


Fig. 1. Transmittance through the cellophane sheets used in the germination experiments. Blue (B) and red (R) cellophane sheets were used with a fluorescent lamp and a combination of blue and red cellophane sheets (B + R) was used with incandescent lamp and a water layer to provide blue, red, and far-red light, respectively (Wada, 1990).

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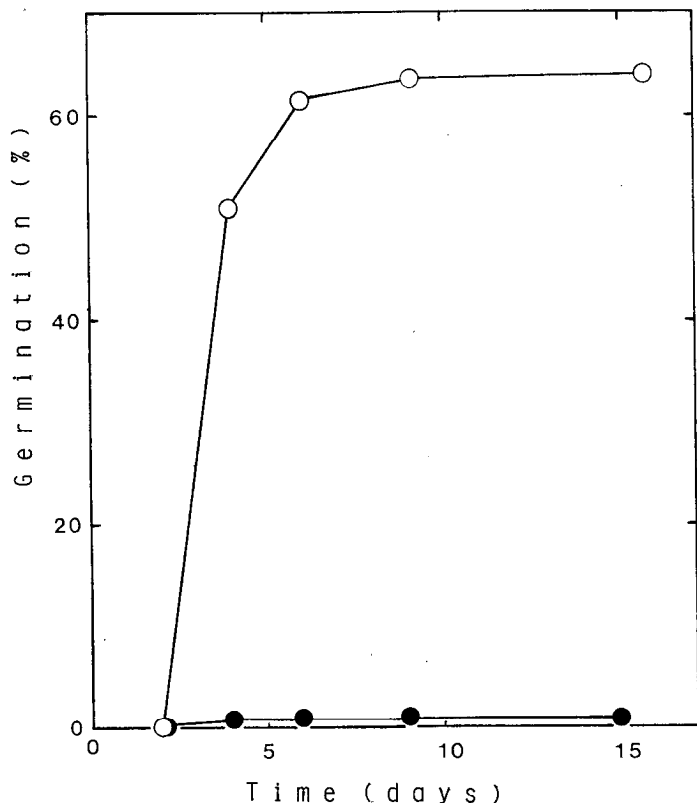


Fig. 2. Effect of light on seed germination of enzyme-treated achenes of *Rosa multiflora*. Achenes were treated with Driselase under continuous white light or in total darkness for 36 h. After treatment under light, seeds were placed in petri dishes and incubated at 25C under continuous white light ($2 \text{ W}\cdot\text{m}^{-2}$) (○). Achenes treated in darkness were placed in petri dishes and incubated at 25C in total darkness (●). Number of achenes used was 150 in each experimental lot.

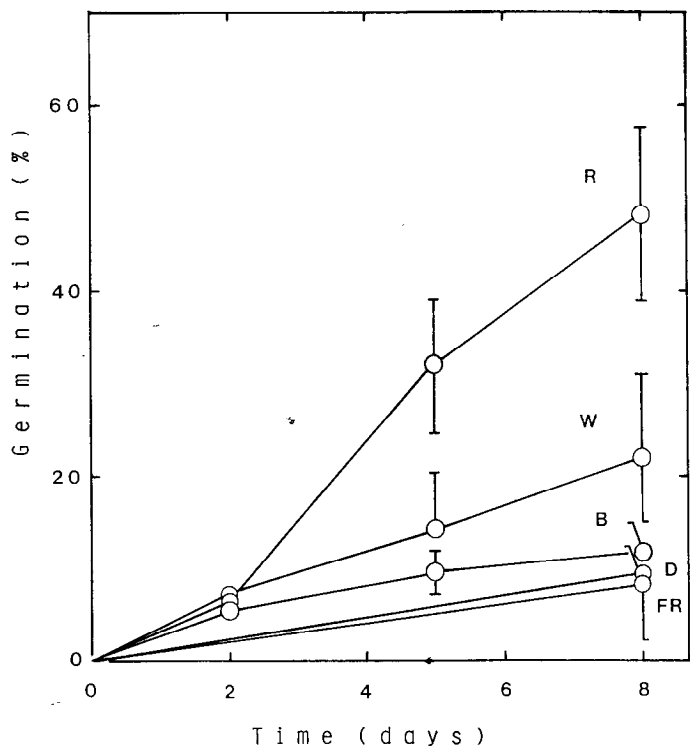


Fig. 3. Effect of exposure to light of different wave-lengths on seed germination of *Rosa multiflora*. Achenes previously treated with Driselase in darkness were exposed to continuous white (W), red (R), far-red (FR), or blue (B) light ($2 \text{ W}\cdot\text{m}^{-2}$ in all cases except FR, which was $15 \text{ W}\cdot\text{m}^{-2}$) or maintained in total darkness (D). Values are means \pm SE of three dishes each containing 50 achenes.

exposure of 2 min (Fig. 4). This result indicates that seeds of *R. multiflora* are quite sensitive to light. In preliminary experiments in which germination tests were conducted after preparing achenes in light, we could not detect any influence of light on rose seed germination (data not presented). The brief exposure to room light during achene preparation may have triggered germination even if the seeds were thereafter incubated in darkness. This may explain why rose seeds have been considered nonphotoblastic.

Far-red light exposure immediately after red light decreased percentage germination to the same level as in darkness (Table 1). The inhibitory effect of far-red light was reversed by a second exposure to red light. The second exposure to red light completely reversed the inhibitory effect of far-red light, even if the exposure to far-red light was as long as 90 min. Because red light was the most effective in inducing germination (Fig. 3) and its effect was reversed by far-red light (Table 1), the photoreceptor involved is most likely phytochrome as first established in lettuce seeds (Borthwick et al., 1952).

Exposure to blue light for a short period of time resulted in a slight promotion of seed germination over the dark control (Table 1). Red-light exposure immediately after blue light resulted in about the same percentage germination as exposure to red light alone. The effect of blue light was similar to that of far-red light (Fig. 3), and the effect of blue light was obscured by subsequent red light (Table 1), as previously reported (Borthwick et al., 1954; Yaniv and Mancinelli, 1968). Taken together, these results indicate that blue light was absorbed by phytochrome (Malcoste et al., 1972). Blue light is absorbed not only by Pfr but also by the Pr form of phytochrome. Thus, blue light absorbed by Pr may be responsible for the increase in seed germination over that in total darkness (Table 1).

The experiment described in Fig. 2 was conducted soon after

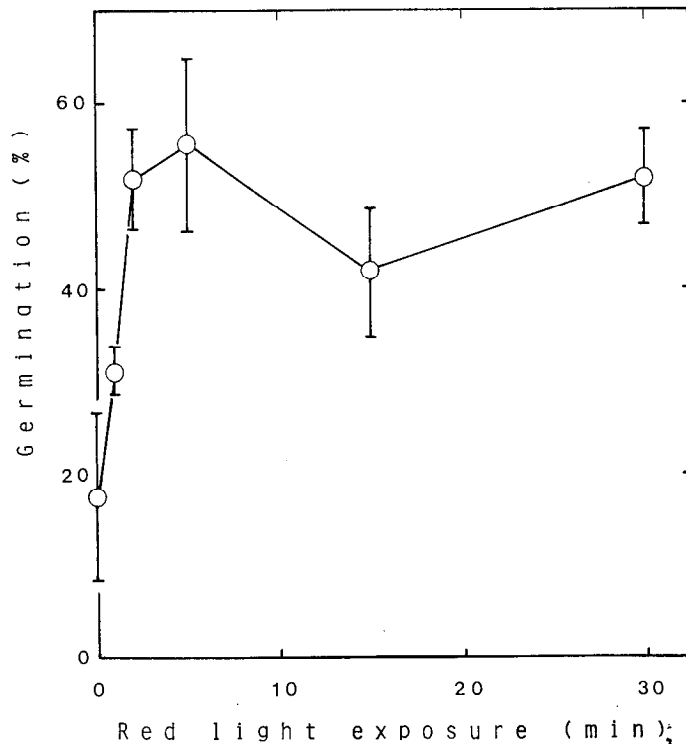


Fig. 4. Relationship between time of exposure to red light and seed germination of *Rosa multiflora*. Achenes treated with Driselase in darkness were exposed to red light ($5 \text{ W}\cdot\text{m}^{-2}$) for particular periods of time followed by the incubation in total darkness. Germination was recorded 8 days after light treatment. Values are means \pm SE of three dishes each containing 50 achenes.

Table 1. Effect of exposure to red, far-red and blue light on seed germination of *Rosa multiflora*. The achenes treated with Driselase in darkness were exposed to red (R; 5 W·m⁻²), far-red (FR; 15 W·m⁻²), or blue (B; 5 W·m⁻²) light for the particular lengths of time, as indicated in parentheses, and then maintained in darkness. Achenes maintained in total darkness (dark) served as the control. Percentage germination was recorded 8 days after light treatment. Values are means ±SE of three dishes each containing 50 achenes.

Light treatment	Germination (%)
Dark	13.2 ± 5.9
R (30 min)	62.8 ± 14.8
R (30 min)–FR (30 min)	17.6 ± 2.3
R (30 min)–FR (30 min)–R (30 min)	44.0 ± 12.1
R (30 min)–FR (60 min)–R (30 min)	59.5 ± 8.4
R (30 min)–FR (90 min)–R (30 min)	62.5 ± 8.3
B (30 min)	31.3 ± 13.0
B (30 min)–R (30 min)	54.1 ± 7.7
B (30 min)–R (60 min)	53.4 ± 12.6
B (30 min)–R (120 min)	58.7 ± 9.9

the hips were harvested, whereas the other experiments (Figs. 3 and 4, Table 1) were conducted a few months thereafter. Almost no germination was observed in darkness in the early experiment (Fig. 2), whereas about 10% or more seeds germinated in darkness in the later experiments, namely in the experiments with older achenes (Figs. 3 and 4, Table 1). This fact suggests that *R. multiflora* seeds are strictly photoblastic immediately after harvest, but this may change as the achenes age as occurs in *Eragrostis*

ferruginea (Fujii and Ishikawa, 1962). Generally, seed germination percentage is low and germination occurs sporadically in roses. Seed germination after prolonged incubation could occur irrespective of light condition. This may at least partially explain why rose seeds have been considered nonphotoblastic.

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