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EFFECT OF LIGHT AND GROWTH SUBSTANCES ON SEED GERMINATION IN RAMONDA SERBICA PANČ. (GESNERIACEAE)

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Seeds of *R. serbica* have an absolute requirement for light in order to germinate. Irradiation during four consecutive days is required for minimum germination, but some seeds germinate after 11 days of light. The percentage of germination is increased by prolonging the daily light period from 1 to 24 h. Continuous red light is the most effective treatment for inducing germination. Continuous far red light induces germination in about 30% seeds. The involvement of phytochrome is indicated by the reversal of red light effect, when the daily light period is terminated by short far red irradiation, which is by itself ineffective. Gibberellins A_3 and A_7 cannot substitute for light in concentrations up to 300 mg l^{-1}. However, in continuous light they significantly accelerate germination. A synergistic action of light and gibberellins was evident when both were supplied at suboptimal doses.

Key words: *Ramonda serbica* P a n č., seed germination, red – far red light, gibberellins.
Ključne reči: *Ramonda serbica* P a n č., klijanje semena, crvena–tamno crvena svetlost, giberelini.

INTRODUCTION

Ramonda serbica P a n č. is a tertiary relict plant and an endemic species in the Balkan Peninsula, firstly described in the Balkan flora in the last century (P a n č i ć,

1874). The species has some interesting ecophysiological characteristics (K o š a n i n, 1939, V e l č e v *et al*, 1975), showing its adaptability to the changing climate conditions since the tertiary period. Seed germination of *R. serbica* has not been studied, although the germination control is also of great importance for the survival of species. In several genera belonging to the fam. *Gesneriaceae*, seed germination is light–dependent (F i d g o r, 1907). Because of the very small size of *R. serbica* seeds, it seemed likely that they too may be photoblastic. The purpose of the present work was to study the controlling factors in *R. serbica* seed germanation, in order to better understand the physiology of this interesting species.

MATERIAL AND METHODS

Seeds of *R. serbica* were collected in July 1982, in the Lazareva Reka canyon, near Bor in East Serbia. The seeds were stored until use at $-18^{\circ}C$, since it was found that they lose viability at room temperature within 3–4 months.

Seeds were sterilized in commercial sodium hypochlorite solution, containing about 0.25% active chlorine, for 10 min, washed with sterile water and germinated in 60 mm Petri dishes, on 1 layer of Whatman No. 42 filter paper, moistened with 2.5 ml of glass distilled water or the hormone solution to be tested.

Seeds were germinated at $23 \pm 2\ ^{\circ}C$. Light of different wavelengths was obtained by combining adequate light sources with plastic 3 mm Rohm and Haas (Darmstadt, FRG) filters. For red light a fluorescent tube Philips TL 20/15 was equipped with filter No. 501, maximal emission was at 660 nm, irradiance 1.6 $W \cdot m^{-2}$. Far red light was obtained from an incandescent 100 W bulb „Tesla", with red No. 501 and blue No. 627 filters and a 10 cm deep water layer. Maximal emission was at 730 nm, irradiance 5 $W \cdot m^{-2}$. Counting of germinated seeds and other manipulations were done using a magnifying lens, in a dim green safe light from a fluorescent tube Philips TL 20/17 and a filter No. 700.

One hundred seeds were put in each Petri dish. Germinated seeds were scored daily during the experiment and removed from the Petri dishes as soon as their radicles were visible. All experimental treatments were done in three replicates and each experiment was repeated at least twice. Results are presented as percentage of germination.

RESULTS

Seeds of *R. serbica* are very small, 1000 seeds weighing approximately 14 mg. The seed coat is brown, wrinkled and thorny. Microscopic observation showed that seeds harboured fully developed embryos, with discernible axis and cotyledons and a multilayered seed coat, surrounding the reduced endosperm tissue.

Effect of light

Preliminary trials showed that the seeds do not germinate in total darkness and that short and single light treatments are not adequate to induce germination. Light requirement can be satisfied only with exposures during several consecutive days. The final percentage of germination depends on daily light duration, maximal response being

attained in continuous light (Fig. 1B). When seeds were exposed to continuous light for 1–15 days, the minimum number of days required for germination was 4, while the full response was reached after 11 days (Fig. 1A). In all groups germination becomes visible on the 6th day after the onset of irradiation. Upon the return to darkness, the germination ceases within the following 3 days (not shown). Therefore, the seed population of *R. serbica* represents a very heterogenous group in respect to the minimal light requirement; in a certain number of seeds germination under favourable conditions in a very slow process.

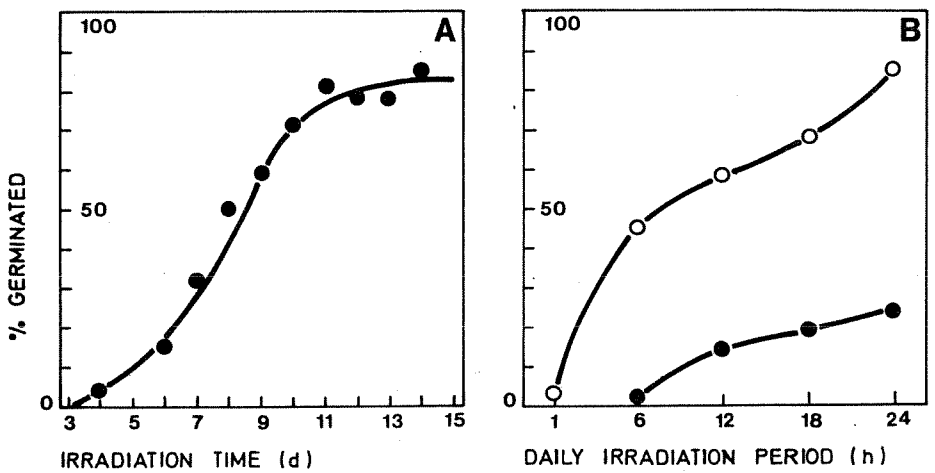

Fig. 1. – A. Effect of continuous irradiation on *R. serbica* seed germination. Seeds were exposed to continuous red light from the onset of inbibition and returned to darkness after 1–15 days. Germination scored after 18 days.
B. Effect of various daily light periods on seed germination in *R. serbica*. Seeds were irradiated every day with red (○) or far red (●) light for the indicated periods. Germinated seeds counted 15 days after imbibition.

Germination can be induced by red or far red light, the latter being less effective (Fig. 1B). The involvement of phytochrome is indicated in experiments in which a daily red light treatment was terminated by short exposure to far red light (Table 1).

Tab. 1. – Far red reversal of red light–induced germination of R. serbica seeds.

Daily light treatment	% of germination ± SE
6 h red	27.3 ± 1.8
6 h red + 10 min far red	6.7 ± 1.0
10 min far red	0

Seeds were irradiated every day as indicated. Germination scored after 15 days.

Effect of growth substances

Seeds were incubated in darkness for 15 days with GA_3, GA_7 and kinetin, in concentrations from 0.001 to 300 $\mu g\ ml^{-1}$. Neither gibberellins, nor kinetin substituted for light. However, under the conditions when continuous light induced only 50% germination, i.e. when the experiment lasted 9 days, gibberellins significantly accelerated germination (Fig. 2A). Abscisic acid inhibited light–induced germination (Fig. 2B).

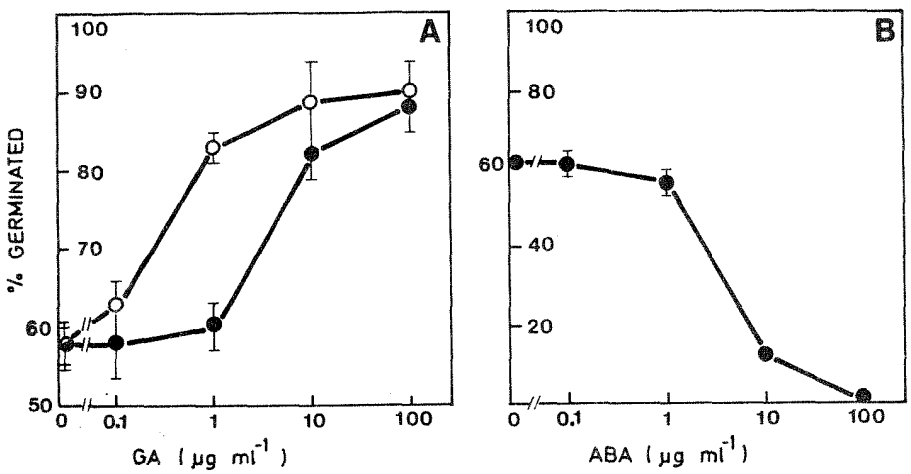

Fig. 2. – A. Dose–response curves for GA_3 (●) and GA_7 (○) effects on *R. serbica* seed germination. Seeds were imbibed and germinated in continuous red light, in GA solutions. Germination scored after 9 days.
B. Effect of abscisic acid on *R. serbica* seed germination in continuous red light. Germination scored after 15 days.

Synergistic effect of gibberellins and light was clearly shown in experiments where both factors were administered at suboptimal doses (Table 2).

Tab. 2. – Synergism between GA_3 ($\mu g\ ml^{-1}$) and light in R. serbica seed germination.

Light treatment	% Germination		
	H_2O	GA_3 0.1	GA_3 1.0
6 h red	26.6	31.3	58.3
6 h red + 10 min far red	7.0	26.6	35.6
12 h far red	26.6	43.6	51.0

Seeds were inbibed in water or GA_3 solutions and irradiated every day as indicated. Germination scored after 12 days.

DISCUSSION

The results of the present paper have confirmed the expectation that *R. serbica* represents another species of *Gesneriaceae* having light sensitive seeds. Similarly to the other genera (Fidgor, 1907), *R. serbica* requires a very long irradiation period. Isikawa and Tateda (1970) consider that the „long irradiation seeds" are those requiring more than 3×10^2 min (= 50 h) for germination. Seeds of *R. serbica* even surpass in light requirement all seeds ranked in that group. The light effect can hardly be designated as inductive, yet the involvement of phytochrome seems to be established according to the effective wavelengths and far red reversal of the red effect. The ecological significance of this long light requirement lies perhaps in the fact that *R. serbica* plants may in their natural habitats be exposed to severe drought, so that seeds are adapted to germinate only if the wet periods and light are of sufficient duration.

The inability of hormones, particularly gibberellins, to substitute for light requirement would suggest that light does not act through hormone changes. However, a synergism between low levels of P_{fr} and low doses of GA_3 is clearly shown. A similar effect in *Kalanchoe* seed germination is explained as the increase in physiological activity of P_{fr} by applied gibberellins (Fredericq *et al*, 1983). This explanation could also hold for *R. serbica* seeds, although generally higher levels of P_{fr} are required, than for *Kalanchoe* seed germination.

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Rezime

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UTICAJ SVETLOSTI I HORMONA NA KLIJANJE SEMENA RAMONDA SERBICA PANČ. (GESNERIACEAE)

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Za klijanje semena *Ramonda serbica* neophodna je svetlost. Osvetljenje tokom četiri uzastopna dana je minimalan uslov za klijanje, ali neka semena klijaju tek posle 11 dana na svetlosti. Procenat klijanja raste sa produženjem dnevnog perioda svetlosti od 1 do 24 sata. Neprekidno osvetljavanje crvenom svetlošću pretstavlja najefikasniji postupak za indukciju klijanja. Neprekidna tamno crvena svetlost indukuje klijanje oko 30% semena. Efekat crvene svetlosti se može poništiti kada se dnevni period osvetljenja završi kratkim izlaganjem tamno crvenoj svetlosti, što ukazuje da u ovom procesu učestvuju fitohrom. Giberelini A_3 i A_7 u koncentracijama do 300 mg l^{-1} ne mogu da zamene efekat svetlosti. Međutim, pri neprekidnom osvetljenju, giberelini značajno ubrzavaju klijanje. Sinergično dejstvo svetlosti i giberelina zapaženo je kada su oba faktora primenjena u suboptimalnim dozama.