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THE EFFECT OF PLANT GROWTH REGULATORS ON CENTAURY (*CENTAURIUM ERYTHRAEA* RAFN.) SEED GERMINATION

NADA MIJAJLOVIĆ[1], D. GRUBIŠIĆ[2,3], Z. GIBA[3] and R. KONJEVIĆ[3]

[1]*"Eko-lab" Quality Management Section Ltd.*; [2]*Siniša Stanković Institute for Biological Research, University of Belgrade,* Bulevar Despota Stevana 142, 11060 Belgrade; [3]*Institute of Botany, Faculty of Science, University of Belgrade,* Takovska 43, 11000 Belgrade, Serbia and Montenegro

Abstract - Centaury seeds are light-requiring. Long-term red light irradiation caused more than 80% of seeds to germinate. Seeds did not germinate in darkness. Gibberellic acid and GA_7 can replace light, but N-substituted phtalimide AC 94,377 was ineffective. Light-induced germination was inhibited by abscisic acid and growth retardants such as ancymidol, tetcyclacis, and paclobutrazole. Growth retardant-caused inhibition can be overcome by the addition of gibberellic acid.

Key words: *Centaurium erythraea*, centaury, germination, gibberellins, growth retardants, light

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INTRODUCTION

Centaurium erythraea Rafn. (centaury), like some other species from the Gentianaceae family, is a medicinal plant whose aerial parts are used in folk medicine as a drug. The crude drug "Centaurii herba" has been described in pharmacopoeias of many countries in Europe. The whole herb is appetite-stimulating, aromatic, bitter, cholagogic, diaphoretic, digestive, emetic, weakly febrifugal, hepatic, stomachic, and tonic. The plant is also used for preparation of commercial beverages. It grows in open woods, meadows, and dry grasslands, often on chalky soils. It is in flower from June to October, and the seeds ripen from August to October. The scented flowers are hermaphroditic (have both male and female organs) and are pollinated by bees, flies, and beetles. Centaury is widely spread in Europe. However, due to heavy harvesting by tearing out, centaury has become a rather threatened plant species. Although it is not critically endangered, some measures should be applied to protect centaury. For example, areas severely devastated by previous harvests should be repopulated. Production of "Centaurii herba" on plantations is another way of protection. The simplest and easiest way of repopulating or planting would be by means of seed sowing. However, knowledge about centaury seed germination is rather scarce. Germination of these seeds is treated from the ecological point of view in the paper of Silvertown (1980). It is also mentioned in a survey of basic requirements for germination of dormant seeds (Nikolaeva *et al*. 1985). The present paper describes the effect of some plant growth regulators on the germination of *Centaurium erythraea* seeds.

MATERIAL AND METHODS

Seeds and seed manipulation

Seeds collected from different localities in Western Serbia (neighborhood of the town of Šabac) were used throughout these studies. Lots of a hundred seeds each were placed in Petri dishes 6 cm in diameter with 1 ml of distilled water or test solution. The fungicide Nistatin was supplied at a concentration of 500 mg L^{-1} in order to prevent fungal infections. The seeds were kept in darkness until germination score, or after 24 h of imbibition in darkness were continuously irradiated with red light until the end of experiment. All experiments were repeated at least two times, each with four replicates, at a constant temperature of $t = 25 \pm 0.2\,^0C$. Germination was scored 7 days after the onset of imbibition.

Light sources

Red light (λ = 660 nm) was provided by Philips TL 20/15 red-light fluorescent tubes with a 3-mm plastic Rohm and Haas (Darmstadt, Germany) filter No. 501 producing a fluence rate 5.76 μmol m^{-2} s^{-1} at the seed level. Light was measured with a Li-Cor (Lincoln, Nebr., U.S.A.) LI-1905A quantum sensor and a 660/730 SKR 110 sensor (SKYE Instruments Ltd., Liandrindod Wells, Powys, Wales, U.K.).

Chemicals

Gibberellic acid (GA_3) was purchased from the Sigma Company, U.S.A; GA_7 from Serva, Germany; ancymidol (α-cyclopropyl-α-(4-methoxy-phenyl)5 pyrimidine methanol) from Eli Lily and Co., Indianapolis, Ind., U.S.A.); tetcyclacis [(5-(4-chloro-phenyl)-3,4,5,9,10 -pentaaza-tetra-cyclo-5,4,1,$0^{2,6}$,$0^{8,11}$-dodeca-3,9-diene)] from BASF, Germany; paclobutrazol [(2RS, 3RS)-1-(4-chloro-phenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol] from ICI, Bracknell, Berks., England. N-substituted phtalimide, AC 94,377 [1-(chlorophtalimido)cyclohexanecarboximide] was obtained from American Cyanamide Co., USA.

RESULTS

Seeds of centaury did not germinate in darkness. Continuous irradiation with red light induced germination reaching a maximum at about 80 h after the start of irradiation (Fig. 1). Germination in darkness can be induced by exogenously applied gibberellins. If the seeds were treated with gibberellins from the onset of imbibition, they germinated up to 95%, depending on the applied concentration and the type of gibberellin used. To be specific, GA_7 proved to be more effective than GA_3, inducing the same percent of germination at concentrations 10 times lower a those of GA_3. However, a physiological analog of gibberellins, the N-substituted phtalimide AC 94,377, was completely ineffective (Fig. 2). On the other hand, light-induced germination can be completely inhibited by the application of abscisic acid (Fig. 3). The complete arrest of germination was achieved

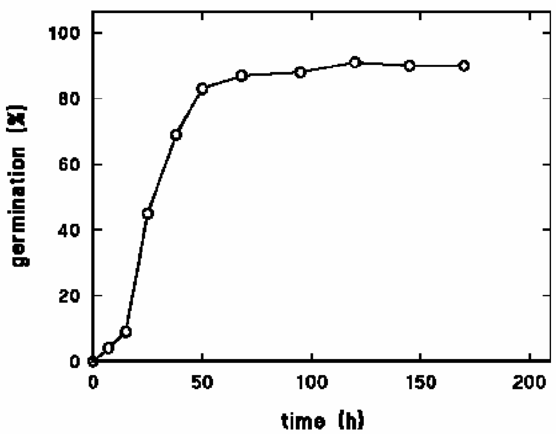

Fig. 1. Light-induced germination of *Centaurium erythraea* seeds. Seeds were imbibed for 24 h in distilled water in darkness at 25°C and then irradiated with continuous red light as indicated on the x axis.

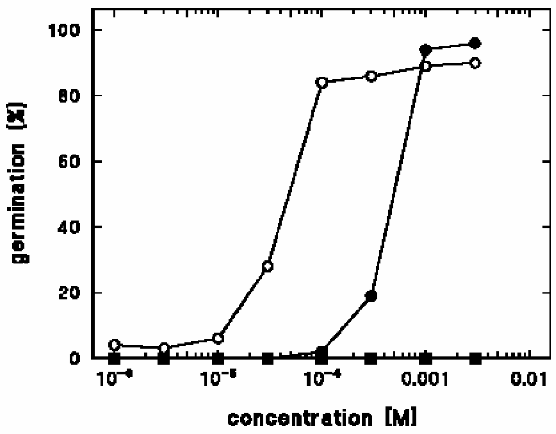

Fig. 2. The effect of GA_3 (˜), GA_7 (š), and AC 94,377 (**n**) on the centaury seed germination in darkness. Seeds were imbibed at indicated concentrations of growth regulators from the onset of imbibition. Germination was scored seven days after the onset of imbibition.

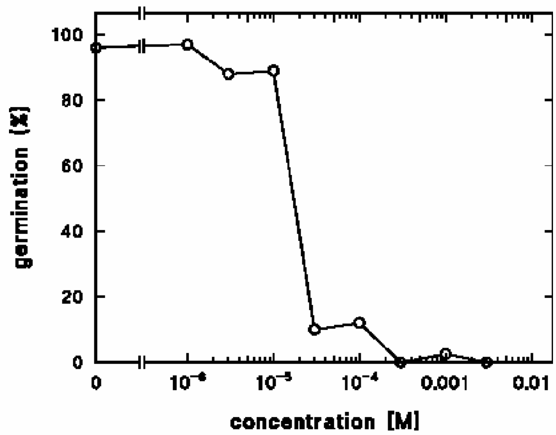

Fig. 3. The effect of abscisic acid on the germination of red light irradiated centaury seeds. Seeds were imbibed in different concentration of abscisic acid for 24 h in darkness and then irradiated with continuous red light. Germination was scored 7 days after the onset of imbibition.

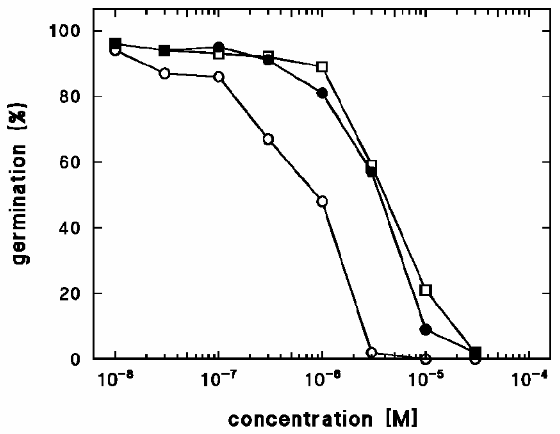

Fig. 4. Growth retardants inhibited germination of centaury seeds. Centaury seeds were imbibed in different concentration of tetcyclacis (š), ancymidol (˜), and paclobutrazol (£) for 24 h in darkness and then irradiated with continuous red light. Germination was scored 7 days after the onset of imbibition.

when ABA was present in the incubation medium in a concentration of 3 x 10^{-4} M.

Fig. 4 shows the effect of growth retardants on light-induced germination of centaury seeds. As can be seen, all of the applied retardants inhibited germination. The most effective was tetcyclacis. It prevented germination completely even at a concentration as low as 3 x 10^{-6} M. The inhibition of germination of centaury seeds caused

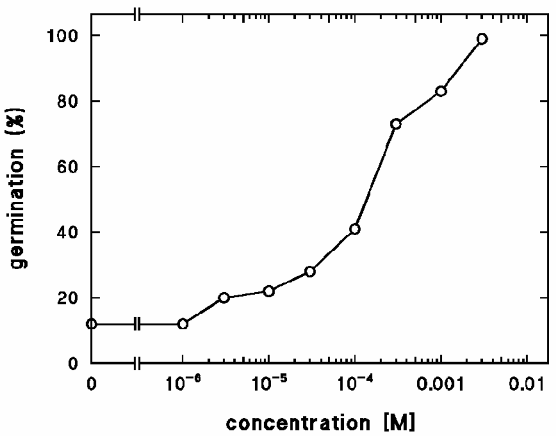

Fig. 5. The effect of gibberellic acid on paclobutrazol-inhibited germination of centaury seeds. Seeds were simultaneously imbibed in inhibitori concentration of paclobutrazol (3 x 10^{-5} M) and indicated concentration of GA_3. They were then irradiated with continuous red light. Germination was scored 7 days after the onset of imbibition.

by growth retardants can be overcome by gibberellic acid. Fig. 5 shows that the application of 3 x 10^{-3} M gibberellic acid to paclobutrazole (3 x 10^{-5} M)-inhibited seeds reverted germination to close to 100%.

DISCUSSION

Information about centaury seed germination is rather limited. To our best knowledge, apart from data on seed germination, in the survey of Nikolaeva *et al.* (1985) and on ecological aspects of germination (Silvertown, 1980), no other data on centaury seed germination can be found in the literature. As can be seen from our results, seeds of *Centaurium erythrea* are light-requiring. They did not germinate in darkness. However, gibberellins can replace light and induce maximum germination in darkness. Some 50 years ago, Lona (1956) established the role of gibberellins in control of seed germination by showing that the application of exogenous gibberellins promoted lettuce seed germination. In many other light-requiring seeds, gibberellins also stimulate germination in the absence of light (Borthwick *et al.* 1964; Grubišić, 1985; Grubišić *et al.* 1988, 1995). On the other hand, our results (Fig. 4), as well as the results of other authors (Gardner, 1983; Grubišić and Konjević, 1987; Grubišić *et al.* 1988), show that light-induced germination can be prevented by tetcyclacis, ancymidol, and paclobutrazol (inhibitors of gibberellin biosynthesis). All three retardants block gibberellin biosynthesis by interfering with oxidation steps in the conversion of *ent*-kaurene to *ent*-kaurenoic acid. These steps are catalyzed by so-called "mixed oxidases" (Coolbaugh and Hamilton, 1976; Dalziel and Lawrence, 1984; Rademacher *et al.* 1984; Rademacher, 2000).

Not all retardants were equally effective. Tetcyclacis arrested germination at a concentration 10 times lower than those of ancymidol and paclobutrazol. The higher level of inhibition obtained with tetcyclacis points to the possibility that this growth retardant may have a different site of action or some side effects (Grossman *et al.* 1985). However, the inhibition of centaury seed germination caused by growth retardants can be overcome by the application of exogenous gibberellins (Fig. 5). This was previously demonstrated in the case of *Paulownia tomentosa* seed germination (Grubišić *et al.* 1988). The findings presented here, together with those reported in the literature, suggest the possibility that light controls seed germination by regulating the endogenous gibberellin level (Toyomasu *et al.* 1998; Yamaguchi *et al.* 2002). However, these are only indirect data as far as germination of centaury seeds is concerned. A clearcut conclusion on this matter should be based on more detailed studies and in particular on analyses of the endogenous gibberellin level in light-stimulated and growth retardant-inhibited seeds.

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ЕФЕКАТ РЕГУЛАТОРА РАСТЕЊА НА КЛИЈАЊЕ СЕМЕНА КИЧИЦЕ (*CENTAURIUM ERYTHRAEA* RAFN.)

НАДА МИЈАЈЛОВИЋ[1], Д. ГРУБИШИЋ[2,3], З. ГИБА[3] и Р. КОЊЕВИЋ[3]

[1]*"Еко-лаб" Оделење за контролу квалитета*; [2]*Институт за биолошка истраживања "Синиша Станковић"*, *Универзитет у Београду, Булевар Деспота Стефана 142, 11060 Београд*, [3]*Институт за ботанику и ботаничка башта*, *Биолошки факултет*, *Универзитет у Београду, Таковска 43, 11000 Београд,* Србија и Црна Гора

Клијање семена кичице је зависно од светлости. Дуготрајно осветљавање црвеном светлошћу доводи до клијања преко 80% семена. Семена не клијају у мраку. Гиберелна киселина и GA_7 замењују потребу за светлошћу док је N субституисани фталимид AC 94,377 неефикасан. Клијање индуковано светлошћу инхибирају абсцисинска киселина и ретарданти растења као што су тетциклацис, анцимидол и паклобутразол. Инхибиција клијања изазвана ретандантима може да се превазиђе додавањем гиберелина.