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SEED GERMINATION OF *GENTIANA CRUCIATA* L.*

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Seeds of *Gentiana cruciata* are light requiring. Germination in darkness can be induced by gibberellic acid and N-substituted phtalimide AC-94,377. Gibberellin-induced germination takes place in a broade temperature range (10-30°C) while hypocotyl elongation is more sensitive to temperature with an optimum at 19°C. Additional percent of germination of light-induced seeds can be obtained by gibberellic acid and AC-94,377 application but not by fusicoccin. Light-induced germination is potentiated by potassium nitrate. However, the adition of potassium nitrate two weeks after the onset of imbibition is ineffective while the same treatment with gibberellic acid brings about an additional increase in germination. Prolonged imbibition in darkness at 24°C decreases, while at 4°C increases, percent of germination of light-induced seeds.

Key words: *Gentiana cruciata,* germination, light, plant growth substances

* Dedicated to Prof. Mirjana Nešković on the occasion of her 45th universary of scientific work

Ključne reči: *Gentiana cruciata,* biljne supstance rastenja, klijanje, svetlost

INTRODUCTION

Gentiana cruciata is Eurasian species which grows on dry meadows and pastures, on sunny slopes, among bushes, and at forest edges up to 1500 m altitude mainly at carbonate substrates. Like some other species of this genus, it contains bitter compounds and for that reason it can be used as an alternate source of pharmacologically important substances. In a popular medicine it is used as substitute for *Gentiana lutea.* Specific bitter substances are found in all tissues of the plant and thus, whole plants are collected, during blossoming, and used as a drug – *Gentianae cruciatae herba.* The fruit contains numerous small seeds of ellipsoidal shape, up to 1.5 mm long with fine net like seed coat of a dark color. In many species of *Gentiana* genus, the embryo is poorly

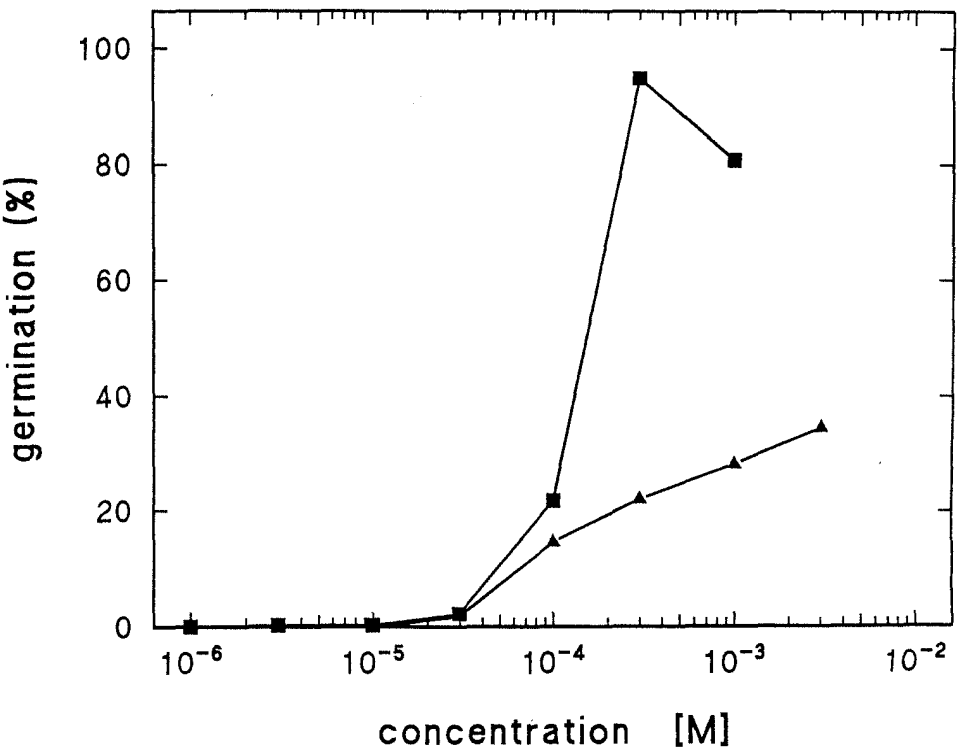

Fig. 1. – The effect of gibberellic acid and AC-94,377 on the germination of *Gentiana cruciata* seeds in darkness
Seeds were imbibed in GA_3 (squares) or AC-94,377 (triangles) solutions and left in darkness to germinate. Germination was scored 2 weeks after the onset of imbibition.

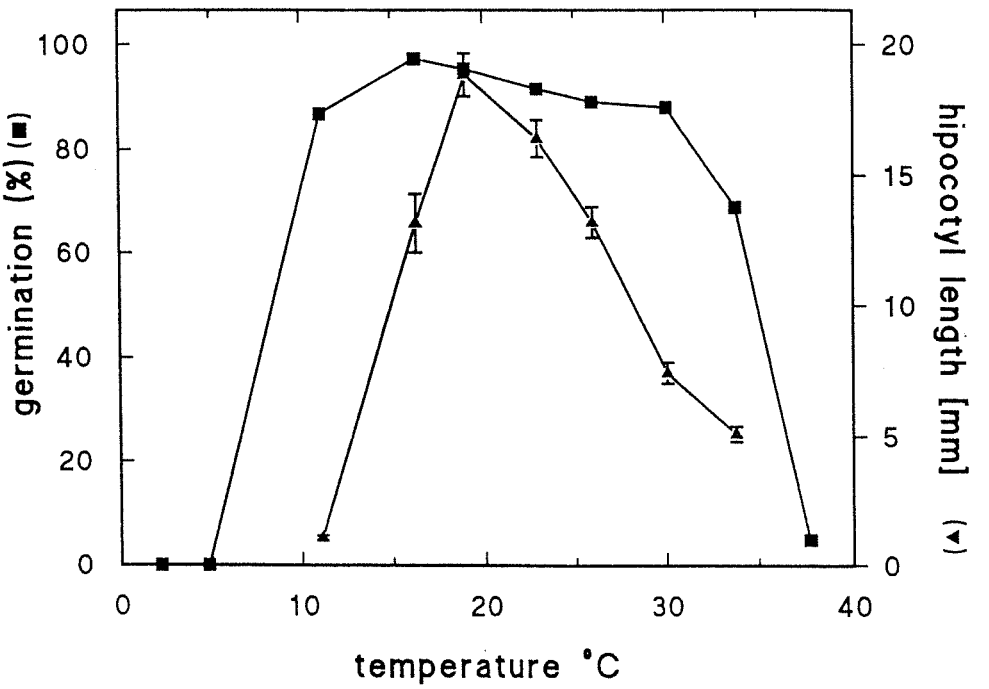

Fig. 2. – The effect of constant temperatures on GA_3 induced seed germination and hypocotyl elongation
Seeds were germinated in 1 mM GA_3 solution (squares) and left in darkness at indicated temperature. Germination was scored 3 weeks after the onset of imbibition. For hypocotyl elongation (triangles) seedlings germinated in light at 25°C were transferred to corresponding temperatures and grown for additional period of time. At the end of this period, hypocotyl length was measured. Each point represents the mean value for 40 plantlets.

developed and some species require prolonged chilling to germinate (Nikolaeva et al., 1985). According to Kinzel (1913) seeds of *G. cruciata* are light-requiring. In the present paper we have tested temperature requirements in differnet phases of germination, as well as the effects of gibberellic acid, N-substituted phtalimide AC-94,377 and fusicoccin on the germination of *Gentiana cruciata* seeds.

MATERIAL AND METHODS

Seeds of *Gentiana cruciata* were collected in October 1991 at the slopes of mountain Koritnik near the village Krstec, Serbia, Yugoslavia. Plant material was

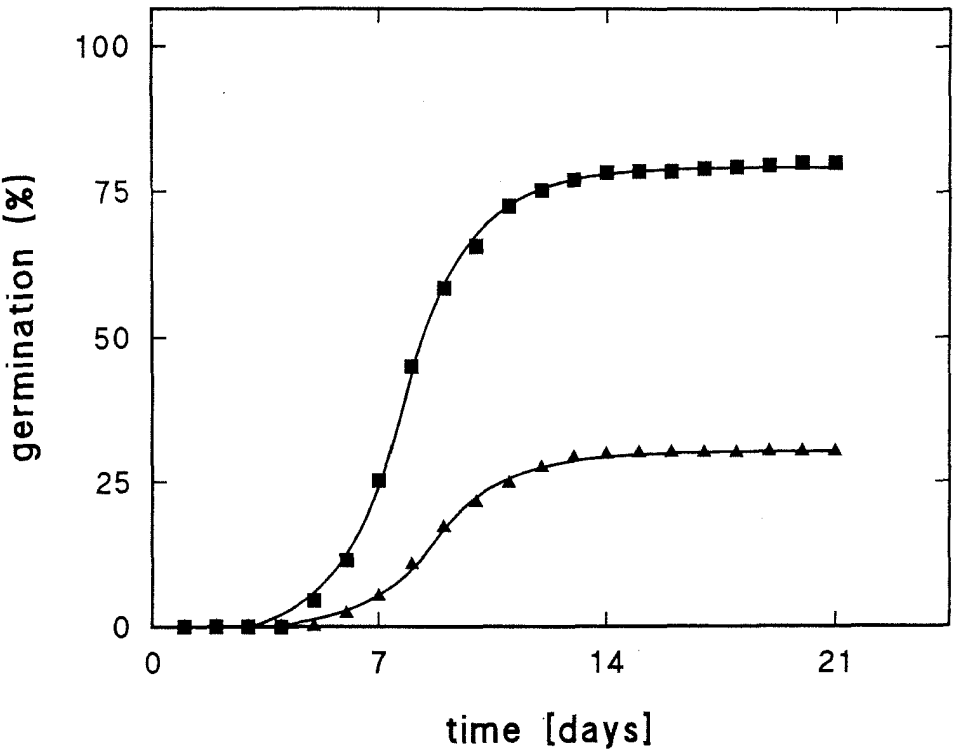

Fig. 3. – The effect of KNO_3 on light-induced germination of *Gentiana cruciata* seeds Seeds were germinated either in distilled water (triangles) or in 10 mM potassium nitrate solution (squares) in light. Germinated seeds were counted every day after the onset of imbibition.

taxonomically identified and saved in the Botanical Garden of the University of Belgrade. Lots of 100 seeds were sown in 6 cm diameter petri dishes containing 2 ml of distilled water or a substance to be tested and kept in darkness for 3 days at 25 or 3°C. Seeds were germinated a) in light; b) in light in the presence of gibberellic acid, AC-94,377 or potassium nitrate; and c) in darkness in the presence of gibberellic acid or AC-94,377. The germination temperature was 25°C, except in experiments where the influence of temperature was tested. In these experiments seeds were kept in darkness at different constant temperature in a termostat with temperature gradient (Autofrigor A.G., Zürich, Switzerland). White light was obtained from fluorescent tubes Tesla (20 W, 4500 K) at fluence rate 23,5 μmol $m^{-2}s^{-1}$ and red light from fluorescent tubes Philips (TL 20/15) combined with 3-mm plastic Röm & Hass filter No. 501 at fluence rate 5,5 μmol $m^{-2}s^{-1}$. Gibberellic acid was purchased from the Sigma

Company and AC-94,377 was obtained from American Cyanamide Company. All experiments were repeated 3 times with four replicates. The data are means of pooled results, and standard errors are not shown since they never exceeded 3%. Specific experimental protocols are given in figure legends.

RESULTS AND DISCUSSION

Preliminary experiments confirmed that *Gentiana cruciata* seeds require light to germinate (Kinzel, 1913). Germination in darkness can be induced by gibberellic acid and N-substituted phtalimide AC-94,377 (Fig. 1). It is well known that exogenous compounds can modify temperature requirements for germination (Reynolds and Thompson, 1971). In blueberry seeds the range of optimal temperatures is wider for GA_3- and AC- than light-induces seeds (Giba et al., 1993). The high percent of germination of *G. cruciata* seeds induced by gibberellic acid was obtained at range of

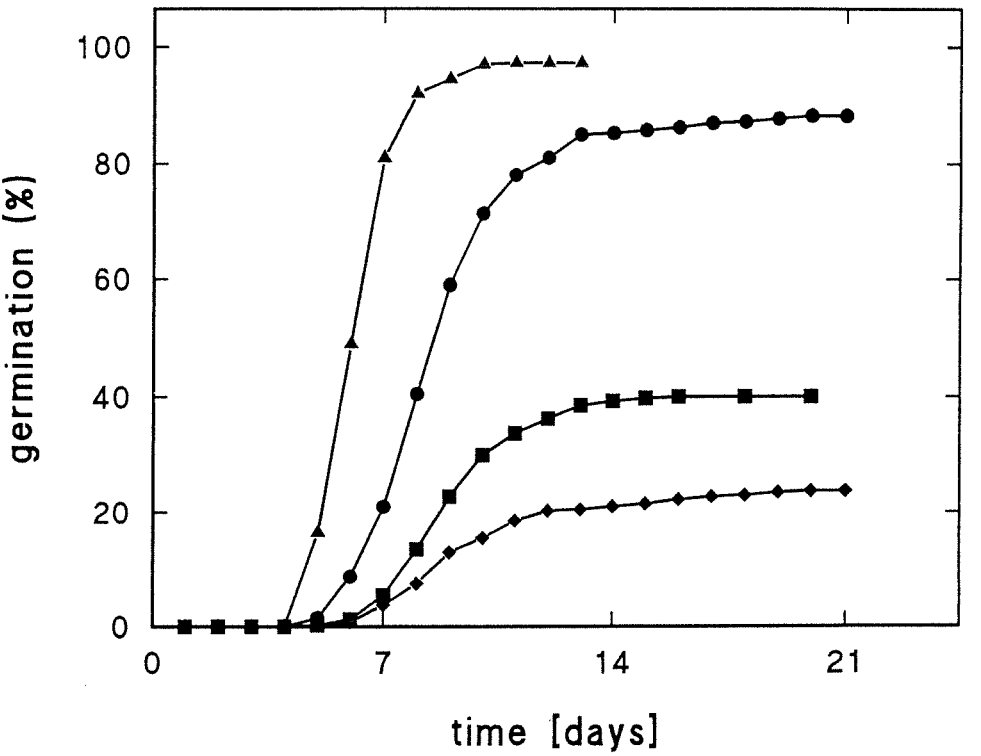

Fig. 4. – The effect of GA_3 AC-94,377 and fusicoccin on light-induced germination of seeds
Seeds were germinated in 1 mM GA_3 solution (triangles), 1 mM AC-94,377 (circles), distilled water (squares) or in 0.01 mM fusicoccin. Germinated seeds were counted every day.

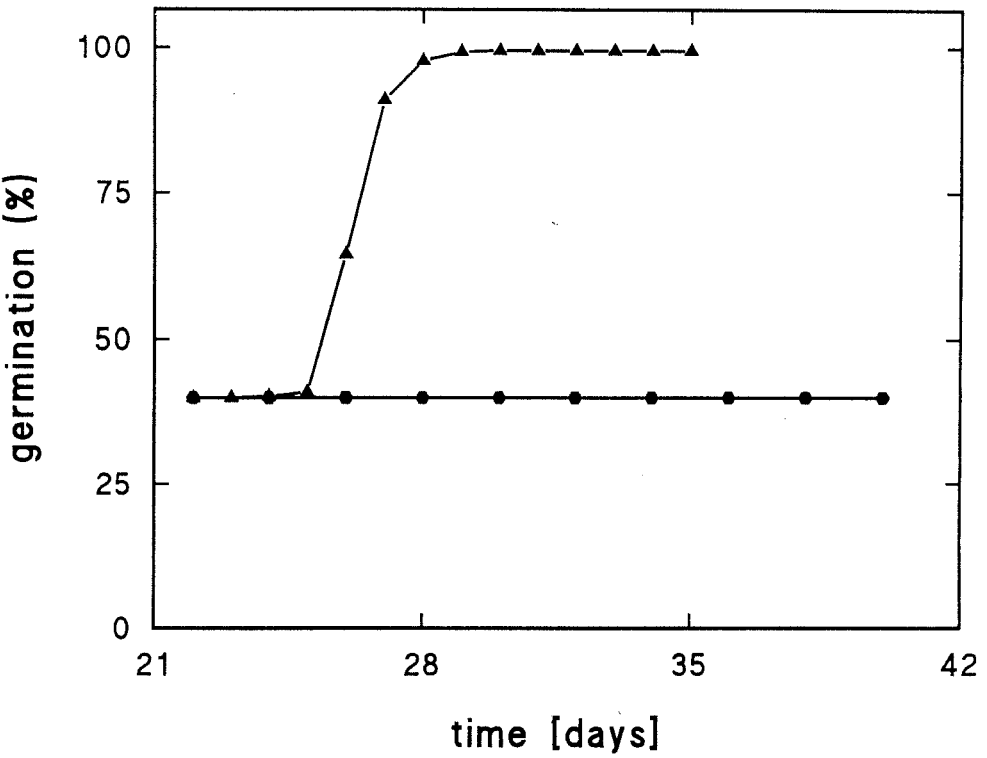

Fig. 5. – The effect of delayed application of GA_3 and KNO_3 on light-induced germination
Seeds were imbibed and germinated in light and water for 3 weeks. At the end of this period they were supplemented either with 1 mM GA_3 (triangles) or 10 mM KNO_3 (circles) and left in light for indicated period of time. Germinated seeds were counted every day.

temperatures between 10 and 30°C (Fig. 2). In *G. lutea* seeds temperature for high germination ranged from 19 to 24°C (unpublished data). Hypocotyl elongation of germinated *G. cruciata* seedlings was more sensitive to tremperature with an optimum at 19°C (Fig. 2). Continuous irradiation with red light induced germination up to 30% after 14 days. Further irradiation did not increase percent germination. Incubation of seeds in potassium nitrate solution, from the onset of imbibition, potentiated light-induced germination (Thompson, 1969). However, in both cases the plateau was reached after two weeks (Fig. 3). When the seed were incubated in gibberellic acid or AC-94,377 from the onset of imbibition, rate and percentage of germination were increased in light. Fusicoccin did not have this effect (Fig. 4). The same effect of gibberellic acid is evident even in GA_3 was added when the maximum of germination

induced by light was reached (21 days). Interestingly, potassium nitrate which stimulates germination when added from the onset of imbibition (Fig. 3) did not show the same effect if its application was delayed like in case of GA_3 (Fig. 5). Thus, stimulatory effect of KNO_3 was obvious only if it was present from the onset of light, while GA_3 additionally induced germination in light regardless of when it was administered. Similar difference between GA_3 and potassium nitrate effects was found in the germination of *Paulownia tomentosa* seeds (Grubišić and Konjević, 1990).

Percent of light induced germination of *Gentiana cruciata* seeds was determined by previous imbibition history. If the seeds were imbibed in darkness at 4°C for different period of time prior to transfer to light, number of germinated seeds was increasing

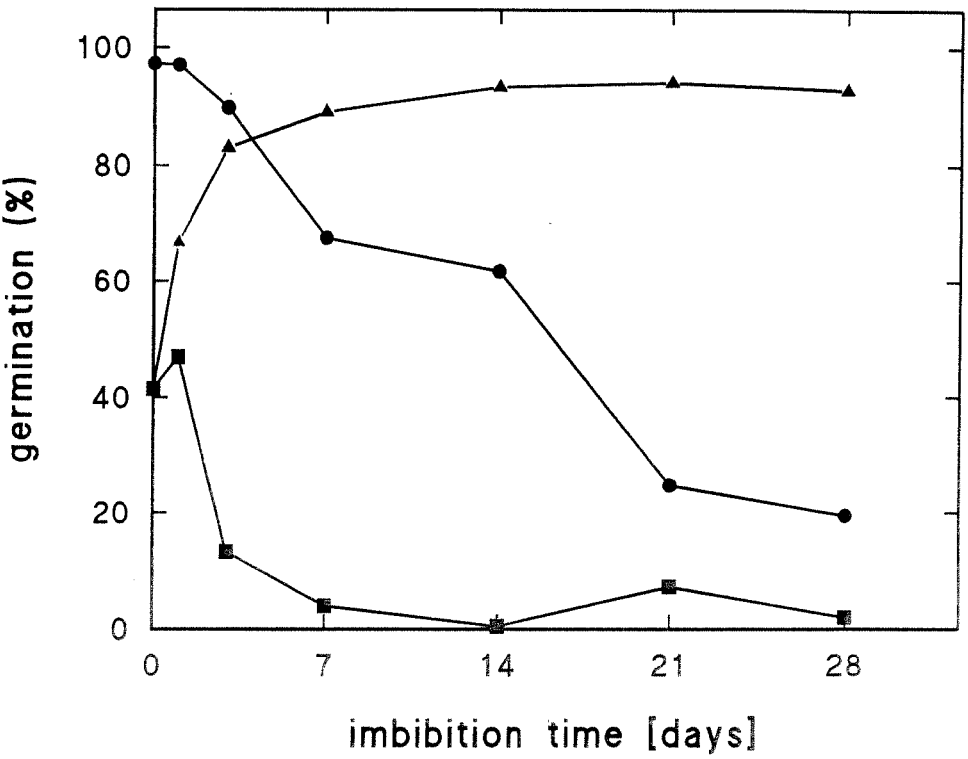

Fig. 6. – The effect of the imbibition time at constant temperatures on subsequent germination at 25°C
Seeds were imbibed in darkness at 4°C (triangles) or 25°C (squares) for indicated period of time and then transferred at 25°C to red light. Germination was scored after 3 weeks. Another set of seeds (circles) was imbibed in darkness at 25°C for indicated period of time, transferred at 4°C and left in darkness for two weeks. After that period, seeds were transferred to red light at 25°C and germination was scored 3 weeks later.

reaching the maximum after two weeks. On the countrary, the increasing time of imbibition at 24°C gradually decreased percent germination ultimately inducing scotodormancy after two weeks. Subsequent exposure of these seeds to low temperature for two weeks, prior to transfer to light, could overcome dormancy. The efficacy of low temperature treatment was inversely proportional to the time of imbibition at 24°C (Fig. 6).

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REFERENCES

Giba, Z., Grubišić, D. & Konjević, R. (1993): The effect of white light, growth regulators and temperature on the germination of blueberry *(Vaccinium myrtillus* L.). – Seed Sci. & Technol. *21*: 521-529.

Grubišić, D. & Konjević, R. (1990): Light and nitrate interaction in phytochrome-controlled germination of *Paulownia tomentosa* seeds. Planta *181*: 239-243.

Kinzel, W. (1913): Frost und Licht als beeinflussende Krafte bei der Samenkeimung, Stuttgart, 187S.

Nikolaeva, M.G., Rasumova, M.V. & Gladkova, V.N. (1985): Reference book on dormant seed germination, (M.F. Danilova, ed), pp. 158-160. – Nauka Publishers, Leningrad.

Raynolds, T. & Thompson, P.A. (1971): Characterization of the high temperature inhibition of germination of lettuce *(Lactuca sativa)*. – Physiol. Plant. *24*: 544-547.

Thompson, P.A. (1969): Effects of after -ripening and chiling treatments on the germination of species *Gentiana* at different temperatures. – J. Hort. Sci. *44*: 343-358.

Rezime

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KLIJANJE SEMENA *GENTIANA CRUCIATA* L.

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Semena *Gentiana cruciata* ne klijaju u otsustvu svetlosti. Indukcija klijanja u mraku se postiže giberelnom kiselinom i N-supstituisanim ftalimidom AC-94,377. Klijanje indukovano giberelinom se odvija u širokom temperaturnom opsegu (10-30°C) dok je izduživanje hipokotila osetljivije na temperaturu sa optimumom na 19°C. Procenat klijanja svetlom indukovanih semena se može povećati primenom giberelne kiseline i AC-94,377, ali ne i fuzikokcina. Klijanje indukovano svetlošću je pospešeno nitratima. Međutim, dodavanje nitrata dve nedelje posle početka imbibicije ostaje bez efekta mada isti tretman giberelnom kiselinom dovodi do povećanja procenta klijanja. Produžena imbibicija u mraku na 24°C smanjuje, dok na 4°C povećava procenat klijanja svetlom indukovanih semena.