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BREAKING OF JUDAS TREE SEED DORMANCY BY PLANT HORMONE TREATMENTS

Abstract: The study deals with the influence of growth regulators on the germination of Judas tree (*Cercis siliquastrum* L.) seed which is double dormant. We simultaneously tested seeds prepared in the conventional procedure: scarification + stratification and scarified seeds treated with phytohormones from the groups of gibberellins, auxin and cytokinins. The results indicate a positive effect of gibberellic acid (GA), as well as some combinations of this phytohormone with others. Recommendations for practice are to combine the conventional procedure with GA. The procedure may shorten the duration of stratification; the application of GA should follow stratification because the temperature of 4°C does not provide growth regulators activity. The study results can serve as the base for easier generative reproduction of this valuable woody ornamental species which could have a wide use in changed climate conditions.

Key words: *Cercis siliquastrum* L., seed dormancy, growth regulators, scarification, stratification, temperature

ОТКЛАЊАЊЕ ДОРМАНТНОСТИ СЕМЕНА ЈУДИНОГ ДРВЕТА РЕГУЛАТОРИМА РАСТЕЊА

Извод: У раду је истраживан утицај регулатора растења на клијање семена јудиног дрвета (*Cercis siliquastrum* L.) које показује двоструку

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дормантност. Истовремено је тестирано семе припремљено на уобичајени начин: скарификација + стратификација и скарификовано семе третирано регулаторима растења из групе гиберелина, ауксина и цитокинина. Резултати указују на позитивне ефекте гиберелинске киселине (GA), као и на неке комбинације овог регулатора растења са другим испитаним. Препоруке за праксу су комбинација уобичајене процедуре са GA. Поступак скраћује време стратификације тако што се GA аплицира пре стратификације јер температура од 4°C не обезбеђује активност регулатора растења. Резултати могу да послуже као основа за лакше генеративно размножавање ове важне декоративне врсте која може да нађе ширу примену у промењеним климатским условима.

Кључне речи: *Cercis siliquastrum* L., дормантност семена, регулатори растења, скарификација, стратификација, температур

1. INTRODUCTION

In addition to the more widespread use of growth regulators in the vegetative propagation of ornamental trees and shrubs, which is reflected in the application of auxin for rooting cuttings of various types, as well as, micropropagation, plant growth regulators can be used for troubleshooting in the germination of species that have a dormant embryo or double seed dormancy (embryo + seed coat). The germination of tree seeds appears to be controlled by a variety of external and internal factors. Plant growth regulators figure prominently among these factors, but the range of mechanisms by which such control is edited may vary considerably—from physical to metabolic.

Bewley & Black (1985) reported a list of chemicals that have been proven to eliminate seed dormancy. It belongs to respiratory inhibitors, oxidants, nitrates, nitrites and growth regulators.

Levels of endogenous plant growth regulators are believed to play a major role in overcoming seed dormancy of different species with variable success. The most commonly used are gibberellins, cytokinins and ethylene. Gibberellins have the widest range of effect. Gibberellins (GAs) promote the induction of cell wall hydrolases and thereby promote endosperm weakening and endosperm rupture (micropilar endosperm in particular). Abscisic acid (ABA), acting as antagonist of GAs, inhibits the induction of cell wall hydrolases and thereby inhibits endosperm weakening and endosperm rupture. GA promotes and ABA inhibits the embryo growth potential (Müller *et al.*, 2006). Work of Fernandez *et al.* (1997) shows strong variations in endogenous GAs during dormancy release in beech seeds (*Fagus sylvatica* L.) and the capacity of non-dormant seeds to carry out metabolic conversions when labeled GA₂₀ was injected into the seeds. These findings support a dynamic role of GAs in the release of dormancy. Similar results obtained with *Chionanthus virginicus* L. seed where GA has shown helpful in overcoming endodormancy which is confirmed with species (beside Mechanical dormancy). Different concentrations of GA₃ had different influence. The lower one (100 mgL⁻¹) has shown

stimulative effect while the higher one (1000 mgL^{-1}) has shown the inhibitor effects (Grbić et al., 2005). However, there are some species (*Malus sylvestris* Mill.) whose seed gibberellin is not affected, while cytokinins have a positive impact. Cytokinins reversed the inhibitory effect of ABA (similar with GAs when the ABA is in lower concentrations), but sometimes cause abnormal germination (inhibition of radicle emergence). It is known that the effect increases in phytohormone coactions with other factors or in the joint action with other phytohormones. Kinetin with low light intensity is more effective, and ethylene + gibberellin in the presence of light. Experiments with *Lactuca sativa* 'Grand Rapids' seeds indicates a positive effect of GA, negative when the GA and ABA together, and again positive when cytokinins added to the previous combination (Kahn, 1968). Reports of Tamura et al. (2006) suggest that ABA and GA are involved in the control of seed germination by temperature, but how these hormones mediate the high-temperature signal remains unknown.

The aim of this study was to examine the effects of plant growth regulators (GA_3 , BA, and IBA), either single or in combination with other regulators and temperature (as one of the key factors in overcoming dormancy) on seed of Judas tree (*Cercis siliquastrum* L.).

Judas tree or redbud is a small tree to large shrub with several stems, 4-6 m tall and wide (10-13 m exceptionally) from Mediterranean Europe and Western Asia. Species belong to Caesalpiniaceae family. Habit is funnel-shaped becoming umbrella like: slow-growing, occasionally with runners.

Leaves are up to 13 cm, often broader than long, round-reniform with blunt apex or occasionally with shallow notch, dull green; pale yellow from the end of October to November. The inflorescences are conspicuous with soft fragrance, crimson-pink flower clusters appear directly on the stem and branches (cauliflory), before leaves shoot, 2-3 weeks in April. The flowers are pollinated by bees, attracted by nectar. Fruits are brownish, 7-10 cm long flat leathery legume, lasting through the winter time. Nitrogen-fixing root is flat, forming runners when injured.

The species prefers deep, moderately dry, not too nutritious, well drained soil with high chalk content; demands a position in full sun or partial shade, hot and protected areas as sensitive to frost, and is good for urban areas (hardiness zone 7a).

Seeds of Judas tree show germination obstacle which is very frequent in species from Caesalpiniaceae family mainly caused by physical dormancy, but the seed of Judas tree possess double dormancy: hard test and physiological dormancy (Stilinović et al., 1983, 1985). The seeds of *C. siliquastrum* have a coat-imposed dormancy due, in part, to the hardness and impermeability of the seed coat (Riggio Bevilacqua et al., 1985) and, in part, to the presence in the endosperm of ferulic acid as a chemical inhibitor (Martinucci et al., 1985). Bound ferulic acid in the endosperm of *Cercis siliquastrum* L. Embryos in such genera as *Cercis* (Fabaceae) are still non-deep dormant and thus require a few weeks of cold stratification, i.e. after physical dormancy is broken and seeds imbibe water, before they will germinate (Baskin and Baskin, 2004).

2. MATERIALS AND METHODS

Legumes with seeds of Judas tree were collected from a green space of New Belgrade (44.815789° - 44.814115° N; 20.411993° - 20.415791° E). Basic morphological characteristics and quantitative indicators of fruits and seeds collected in New Belgrade are shown in Table 1.

Table 1. Some morphological characteristics and quantitative data of fruit and seed

Табела 1. Неке морфолошке карактеристике и квантитативни показатељи плодова и семена

Properties Особина	Minimum	Average Сред. вр.	Maximum
Legume / Махуне			
Length (cm) / Дужина (cm)	6.70	9.29	13.80
Width (cm) / Ширина (cm)	1.30	1.67	1.80
Weight (g) / Маса (g)	0.20	0.38	0.50
Number of seeds / Број зрна	4.00	8.25	14.00
Seed per 100 kg of fruit (kg) / Фактор екстракције	53.56	/	55.28
Seed / Семе			
Weight of 1000 seeds (g) / Апсолутна маса (g)	/	22.90	/

The conventional way to eliminate complex forms of seed dormancy is a combined procedure of seed coat wounding, and, after that, seed exposure to positive temperatures close to zero, and high humidity (stratification) affects the dormant embryo. The seed of Judas tree, according to previous studies, corresponds to chemical scarification (H_2SO_4 30 min) combined with stratification at +2°C for 30 days (Stilinović *et al.*, 1983, 1985). The same conventional procedure was used for the control treatment in our studies. A study, individual and combined effects of tree growth regulators to speed up germination, was conducted in order to simplify the sowing pre-treatment. Mechanically scarified seeds were soaked for 24 hours in the following solution: GA_3 (500 $mgL^{-1}/1.44 mM$), BAP (200 $mgL^{-1}/8.88 mM$), GA_3+BAP (500+200 $mgL^{-1}/10.32 mM$), GA_3+IBA (500+500 $mgL^{-1}/11.28 mM$), and BAP+IBA (200+500 $mgL^{-1}/18.72 mM$). The seeds were placed in the sand at temperatures of +20°C and +4°C, after the treatment. Auxin IBA, although not in the group of phytohormones that affect overcoming seed dormancy is involved in the experiments due to the outstanding synergies BA/IBA often manifested in a balanced differentiation of tissues *in vitro*, and to prevent the potential impact of BAP on the formation of abnormal seedlings.

Since the Judas tree is not covered by the International Rules for Seed Testing by the International Seed Testing Association (ISTA, 1999), germination was carried out by the procedure for *Gleditsia triacanthos* L., species closest to Judas tree. The germination

test was carried out in a cabinet germinator, in sand (4x100 seeds per treatment). The photoperiod was 16/8 (light - dark). The testing lasted 28 days, while germinative energy (GE), calculated on the 10th day based.

The result was presented by means of nine indicators of germination, some of which reflect only the quantitative value of germination (germinative capacity (GC), real germination (RG), and germinative energy (GE)), others only the dynamics (mean germination period (MGP), germination intensity (GI), coefficient of the rate of germination (CRG), and the coefficient of uniformity of germination (CUG), and still others refer to both groups (germination value by Czabator (GV (Cz)) and germination value by Djavan-shir (GV (MC)). The first five indicators are widely used and common, and their formulas are available in references. The coefficient of uniformity of germination and germination value by Czabator and Djavan-shir have been calculated according to the formulas from the papers by Bewley & Black (1985), Czabator (1962) and Djavan-shir & Pour-beik (1976).

The conclusions on treatment efficiency were made on the basis of statistically analyzed obtained data using the Statgraphics program, version 5.0 (STSC Inc. and Statistical Graphics Corporation, 1994-2000, USA). The significance of differences between the mean values was determined by the analysis of variance (ANOVA, $p < 0.05$) and the method of Duncan's multiple range test.

3. RESULTS AND DISCUSSION

The effect of phytohormones in combination with lower temperature (+4°C) did not affect germination, and all variants of phytohormone treatments have yielded negative results. Germination was completely absent (BA+IBA 0%, GA₃+IBA 0%) or was below 2% (GA₃ 1.5%, GA₃+BAP 0.75%, BAP 0.25%). In our trials, low temperatures during stratification affected the removal of endogenous dormancy of seeds, if low temperatures are combined with phytohormones that showed an inhibitory effect. Exposure of imbibed seeds to low temperature promotes seed germination of many plant species. Experience with some species, such as *Arabidopsis* mutants, shows that some mutants are up-regulated and others are down-regulated by red light, which promotes seed germination. In addition to light, In *Arabidopsis*, expression of GA₂₀-oxidase genes, GA₂₀ox1 and GA₂₀ox2, and GA₃ox1 was reported to be up-regulated by low temperature in darkness, and germination of GA₃ox1 mutant seeds are not stimulated by low temperature (Yamauchi et al., 2004). During germination of *Arabidopsis* seeds, GA₂₀ox genes, GA₂₀ox1, GA₂₀ox2, and GA₂₀ox3, and GA₃ox genes, GA₃ox1 and GA₃ox2, are involved in GA synthesis (Ogawa et al., 2003). Suppression of all these GA₂₀ox and GA₃ox genes at supraoptimal temperature may be responsible for the suppression of GA synthesis. Mitchum *et al.* (2006) reported that seeds of the GA₃ox1 GA₃ox2 double mutant do not germinate in the light at 22°C and their germination is rescued by the application of bioactive GA. Therefore, the suppression of GA₃ox1 and GA₃ox2 genes at high temperature should be critical for

thermo inhibition. Interestingly, the expression of GA₂₀ox1, GA₂₀ox2, and GA₃ox1 has been reported to be up-regulated by low temperature (Yamauchi *et al.*, 2004). Thus, GA synthesis is likely to be regulated by a wide range of temperature through modulation of GA₂₀ox and GA₃ox gene expression. The expression of GA₃ox genes in lettuce and Arabidopsis is well known to be photoreversibly regulated by light through phytochrome (Toyomasu *et al.*, 1998; Yamaguchi *et al.*, 1998). Understanding the cross talk between light and temperature signals on the regulation of GA₃ox gene expression may give insight into how plants transduce multiple signals and how plants respond to complex environmental stimuli (Toh *et al.*, 2008). Studies with hormone-deficient Arabidopsis mutants have indicated that the release of dormancy can occur in the absence of GA-biosynthesis but this involves changes in the sensitivity of seeds to gibberellins (Karssen and Groot, 1987).

Studies of Gastaldo *et al.* (1982) conducted on *Cercis siliquastrum* seeds treated with kinetin confirm that this hormone does not interrupt dormancy in either whole seeds or those decoated at the radical pole. Seeds totally decoated or decoated at the cotyledon pole only demonstrated atypical germination linked to cotyledon growth, permitting the embryo to escape the inhibitory action present in the endosperm; this does not occur when the cotyledon surface is experimentally reduced. In our research, seed treated with BAP did not germinate, but in combination with GA₃ shows that relative germination (RG) does not differ significantly from the same indicator of stratified seeds. In combination with IBA it provides a much lower value, but in both cases there was no abnormal germination.

The results of experiments conducted at +20°C are shown in Table 2 and Figure 1 (non-scarified seed, scarified seed, and seed treated with BAP did not germinate, which is not shown).

Table 2. Parameters of seed germination after different phytohormone treatments at +20°C
Табела 2. Показатељи клијавости семена после различитих третмана регулаторима растења на +20°C

Treatment Третман	GC Кг	RG Ка	GE Ек	MGP СМК	GI Ик	CRG КК	CUG КЗК	GV (Cz)	GV (Dj)
GA ₃	60 ^a	63 ^a	1.5 ^b	18.3 ^b	580 ^b	5.47 ^b	0.05	5.09 ^b	9.57 ^b
GA ₃ +IBA	42 ^c	46 ^b	0.0 ^b	22.3 ^c	236 ^c	4.48 ^d	0.07	2.22 ^c	3.28 ^c
GA ₃ +BAP	32 ^d	37 ^c	0.0 ^b	19.5 ^c	272 ^c	5.13 ^{bc}	0.08	1.42 ^{cd}	2.76 ^{cd}
BAP+IBA	10 ^e	12 ^d	0.0 ^b	21.2 ^d	70 ^d	4.73 ^{cd}	0.20	0.15 ^d	0.27 ^d
scar+strat	51 ^b	51 ^b	49 ^a	4.6 ^a	1195 ^a	21.97 ^a	0.19	14.31 ^a	20.20 ^a

* Values followed by the same letter did not differ significantly (P < 0.05) according to Duncan's multiple range test / Вредности изнад којих је исто слово према Данкановом тесту вишеструких опсега значајно се не разликују (P < 0,05).

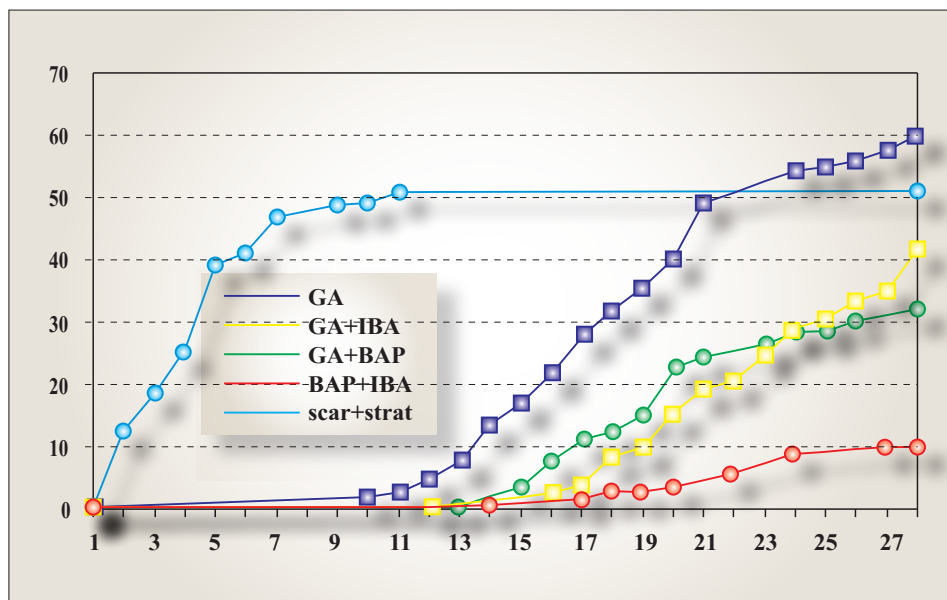


Diagram 1. Germination curves (GC) of Judas tree seeds treated in different ways
Графикон 1. Техничка клијавост семена јудиног дрвета третираног различитим поступцима

3.1. Quantitative indicators of germination

Germinative capacity and Real germination clearly show the best results with seeds treated with gibberellic acid, and then followed by seeds treated with conventional procedure (scarification + stratification) and then, seed treated with the combination of GA_3 +IBA with or without significant differences from the conventional procedure, depending on whether the number of germinated seeds expressed on the basis of all seed placed on germination (GC), or on the basis just of sound ones (RG). Germinative energy (GE) of seeds treated with standard procedure is significantly higher than in other treatments (Tab. 2; Fig. 1). The results of germinative energy (GE) in comparison to GC and RG show differences. Lower values of GE compared with GC point out germination after the 10th day which is the base for GE. A longer period of inactivity during the test is a characteristic of a seed with partially overcome dormancy, so the standard procedure showed that GE was by more than 47% higher than the GE of seeds treated with GA_3 . In other treatments there were no germinated seeds after the 10th day of the test (Tab. 2).

Gebre and Karam (2004) obtained similar results with various methods for breaking dormancy and enhancing the germination of *Cercis siliquastrum* seeds investigated. It was found that imbibed seeds that were treated with 1.4 mM GA_3 exhibited 48% germination, whereas those stratified for 16 weeks exhibited 77% germination. It was also reported that treating imbibed seeds with KNO_3 or thiourea failed to induce germination

and imbibition occurred only when the seeds were scarified mechanically, with water at 80°C or with H₂SO₄, but none of the imbibed seeds germinated although seed viability was 80%. Significant difference between germination of treated seed with GA₃ and stratified seeds in our studies may be explained by the shorter duration of stratification (30 days), and genetic differences related to the provenance of the seeds, as well as different environmental factors during seed maturation. On the other hand, 60% (CG) and 63% (RG) germination is sufficient for it to be considered successful. Significant practical benefit is shortening of the presowing treatment duration by avoiding stratification.

3.2. Indicators of germination dynamics

Better results of MGP and GI were obtained with a conventional procedure also, indicating a better dynamic of germination than to hormone treated seed. Within these treatment order does not change. Treatment with GA₃ showed a significant difference compared to the GA₃+BAP, GA₃+IBA and BAP+IBA (Tab 2.). Better results with stratified seeds can be taken only in part because the first germinated seeds in this treatment received 31 days after the beginning of stratification, and in seeds treated with GA first germinated grains were observed 11 days after immersion in phytohormone solution. The germination intensity reflected identical tendencies as the previous parameter (Tab 2.).

3.3. Complex indicators

The coefficients of germination favour the conventional procedure, followed by GA₃, and other procedures. The coefficient of uniformity of germination (CUG) did not show any statistically significant differences between the variants of the experiment (Tab. 2.).

4. CONCLUSIONS

The effect of phytohormones on overcoming seed dormancy of Judas tree is expressed differently.

GA₃ can be a complete replacement for the traditional procedure for preparing Judas tree seeds (based on the quantitative indicators of germination). Recommendations for practice are to apply GA₃ only, or a combination of the conventional procedure with GA. The procedure may shorten the duration of stratification; the application of GA should follow stratification because the temperature of 4°C does not provide growth regulators activity. Continued research should be directed towards the choice of optimal concentration, due to the fact that Hartmann *et al.* (1990) recommend using it for this purpose in the range of 200 to 1000 mgL⁻¹. In connection with this, need to continue research with GA₃ in combination with other phytohormones, including other GAs is necessary. BAP did not show a positive effect on germination, but no germination abnormalities too. Its combination with IBA exerts positive effects, which justifies the use of IBA in combination with the promoters of germination.

One more reason for additional trials are the findings of the research of post-germination events of Judas tree described by Rascio *et al.* (1998). It was found that the plantlets of *Cercis siliquastrum* that originated from GA₃-supplied seeds were taller than those from stratified ones. Moreover, they produced a greater number of leaves but a reduced root mass and had some difficulty in maintaining a good water balance.

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ОТКЛАЊАЊЕ ДОРМАНТНОСТИ СЕМЕНА ЈУДИНОГ ДРВЕТА РЕГУЛАТОРИМА РАСТЕЊА

Резиме

Поред све шире примене регулатора растења у вегетативном размножавању украсног дрвећа и жбуња, која се огледа у примени ауксина за оживљавање различитих типова резница као и у све комерцијалнијој микро пропацији, регулатори растења могу се

употребити и за отклањање сметњи у клијању врста које имају дормантан ембрион или двоструку дормантност (ембрион + семењача).

Циљ рада био је да се испитају утицаји регулатора растења, било изоловано или у комбинацији са другим регулаторима и температуром (као једним од пресудних фактора на превазилажење дормантности).

Класичан начин за отклањање сложених облика дормантности семена је комбиновани поступак у коме се озлеђује семењача, а затим излагањем семена позитивним температурама блиским нули и високој влажности (стратификација) утиче на дормантан ембрион. У циљу поједностављења сетвеног предtretмана извршена су истраживања појединачних или комбинованих утицаја три регулатора растења на убрзање клијавости. Механички скарификовано семе било је потапано 24 сата у следеће растворе: GA₃ (500 mg/L), BA (200 mg/L), GA₃+BA (500+200 mg/L), GA₃+IBA (500+500 mg/L) и BA+IBA (200+500 mg/L). По третману семе је стављано у песак на температуру од +20°C и на +4°C.

Дејство фитохормона у садејству са нижом температуром (+4°C) није се испољило. Све варијанте дале су негативне резултате. Клијање је потпуно изостало (BA+IBA 0%, GA+IBA 0%) или је било испод 10% (GA 1.5%, GA+BA 0.75%, BA 0.25%).

Техничка и апсолутна клијавост код огледа изведеног на +25°C јасно истичу семе третирано гиберелином као најбоље. Следи семе третирано класичним поступком (скар+страт), а онда, зависно да ли се број исклијалих зрна изражава на основу свих стављених на клијање или само пунозрних, семе третирано комбинацијом GA+IBA са или без сигнификантне разлике од класичног поступка. Енергија клијања семена (на основу 10. дана) третираног класичним поступком је неупоредиво већа него код осталих третмана. Бољи резултат СМК и Ик добијен је такође код класичног поступка, што указује на бољу динамику клијања у односу на хормонски третирано семе.

