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# Forest seed treatment with brassinosteroids to increase their germination under stress conditions

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**ABSTRACT**: The effect of different concentrations of brassinosteroids (1:10, 1:100, 1:210) on the germination of sycamore (*Acer pseudoplatanus* L.) and ash (*Fraxinus excelsior* L.) seeds was investigated in stress conditions. The product Lexin (a mixture of humic acid, fulvic acid and auxin), which also supports seed germination, was selected as a reference treatment. The results show that the most effective is the lowest concentration of brassinosteroids which gave nearly the same effect as the reference product Lexin. The data also suggest the positive effect of pretreatment of sycamore and ash seed in pure water both on germination and germination energy.

**Keywords**: Lexin; hydration; sycamore seed; ash seed

Brassinosteroids (BRs) belong among the major plant hormones involved in many processes of plant growth and development (Zheng et al. 2014). Although the first plant steroid with regulatory activity was isolated from the pollen of rape (*Brassica napus*) in 1979, the phytohormones are biologically active substances that were classified relatively recently in 2000. Currently, more than 100 brassinosteroids have been detected and they are classified into sixth classes of phytohormones (Procházka et al. 1998; Fujioka et al. 2003; Reyes Guerrero et al. 2014). Many brassinosteroids were first prepared synthetically and subsequently detected in plants (Fujioka et al. 2003). However, Brassinosteroids have been isolated from many other plants. New BRs are identified in plants either by classical procedures (isolation of very small amounts and proof structure) or by spectroscopic evidence when they occur in very small quantities. Currently, these substances are often demonstrated as immunoassays (Müssig et al. 1999; Kohout 2001) and they occur in the reproductive organs of plants (Procházka et al. 1998).

Brassinosteroids ensure many very important functions during the plant life. The anti-stress function is of particular interest which allows the plants to develop normally even under stress conditions, such as low temperature, lack of nutrients, water shortage etc. (Fujioka et al. 2003; Kamlar et al. 2010). Brassinosteroids increase the efficiency and rate of photosynthesis as well as chlorophyll content in etiolated plants, promote cell growth and total biomass, accelerate and improve the growth of young plants, support the membrane stability, osmoregulation and foetal development (Anuradha et al. 2007). The changes in the formation of dry matter production of ethylene and abscisic acid levels were detected as the response of plants to the stress in the form of dryness situations. Brassinosteroids reduced the level of abscisic acid and ethylene production in drought stressed plants after five days from the beginning of stress conditions (Zhang et al. 2008). Many experiments also show that the application of brassinosteroids to seed improves both its germination and vigour (Procházka et al. 2013, 2014).

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Temperatures over 40°C, and/or thermal stress, increase the production of oxygen radicals in plants. Brassinosteroids significantly reduce these oxygen radicals mainly by superoxide dismutase (SOD), which stimulates the activity (even at 25°C, but especially at 40°C). The increase of SOD activity after treatment with 24-epibrassinolide indicates that this application may reduce the potential toxicity limit of oxygen radicals (Mazorra et al. 2002).

Tree seeds need the minimum basic environmental conditions to germinate. A necessary prerequisite is to have enough storage compounds in the seed during germination until the growing seedling is able to photosynthesize. Seed vitality is an important factor in environmental stress conditions. Seed vitality can also be understood as the level of seed tolerance to unfavourable conditions during germination and emergence, or internal stability during storage (Hosnedl 1995; Pazderů 2013).

Seed tolerance to abiotic stresses during germination could be increased by different methods of hydration treatment. These methods consist in a partial or complete soaking of seeds in water or in a solution of various substances, for example in a solution of brassinosteroids. During hydration seeds receive such a quantity of water that is sufficient to start metabolic processes, but it does not allow the radicle to break through the seed envelope. The swollen seed increases its metabolism, accelerates the activity of enzymes in general, and especially of enzymes working on a partial correction of biochemical processes occurring during seed aging. Other documented changes are the removal of toxic metabolites accumulated in the course of aging and the repair of damaged cell membranes (Kaya et al. 2006; Pazderů 2013). Li et al. (2005) mentioned the possibility of practical use of brassinosteroids also in forestry where little knowledge and experience with the application of those hormones have been available so far. Li et al. (2002) examined the effects of brassinolide on seed germination of Chinese red pine (*Pinus tabuliformis*) and black locust (*Robinia pseudoacacia*). The seeds of Chinese red pine improved their germination by 23.1%, germination energy by 14.9% and germination time was shortened by 24 h after brassinosteroid application. The seeds of black locust increased their germination by 10.9%, germination energy by 15.9% and the time of germination was shortened by 32 h after brassinosteroid application. The authors also investigated the influence of brassinolide on the ailanthus (*Ailanthus altissima*). The application led to an increase in germination by 17.6% compared to the control, germination energy by 18.8% and germination time was shortened by 34 h. Brassinosteroids have always been applied in very low concentrations (0.1–0.4 mg·l$^{-1}$).

Štranc et al. (2013) and Procházka et al. (2014) discovered in their experiments that the application of brassinosteroids to field crops provides very similar results to those obtained by the application of the commonly used product Lexin (solution of humic acid, fulvic acid and auxin). It should be noted that the product Lexin is one of the most effective formulations which these authors tested.

The main purpose of the contribution is to evaluate the effects of brassinosteroids on sycamore and ash seed germination capacity and to check the best concentrations which could be effective.

## MATERIAL AND METHODS

The role of brassinosteroids in the pre-sowing treatment of sycamore and ash seed was investigated in germination tests which were preceded by seed hydration in the solution of these substances. The used substance is labelled as 4154, a synthetic analogue of natural diluted 24-epibrassinolide (2α,3α, 17β-trihydroxy-5α-androstan-6-one), which is further called a brassinosteroid. Lexin, which is highly effective in agricultural practice, was used as a reference product. Lexin is a concentrate of humic acids, fulvic acids and auxins. Dry seeds and seeds hydrated in water only were used as controls and at the same time to differentiate the effect of hydration from the effect caused by brassinosteroid and Lexin. Detailed information on the various options of hydration is shown in Table 1. The total volume of solution for hydrating the seeds was 250 ml due to their quantity.

Table 1. Overview of seed treatment variants

| Tree species | Seed treatment variants |
|---|---|
| Sycamore (*Acer*) | untreated |
| | only water (hydration) |
| | BR 1:10 |
| | BR 1:100 |
| | BR 1:210 |
| | Lexin (0.05% solution) |
| Ash (*Fraxinus*) | untreated |
| | only water (hydration) |
| | BR 1:10 |
| | BR 1:100 |
| | BR 1:210 |
| | Lexin (0.05% solution) |

BR – Brassinosteroid was supplied by the Institute of Organic Chemistry and Biochemistry, Academy of Sciences, and it was diluted with water just before seed soaking

Table 2. The air temperature and light conditions applied in an air-conditioned box during 24 h (light conditions: dark)

| No. of hours | 3 | 2 | 4 | 2 | 4 | 1 | 8 |
|---|---|---|---|---|---|---|---|
| Temperature (°C) | 9 | 2 | 3 | –1 | 4 | 20 | 30 |

Sycamore and ash seeds were supplied by a specialized unit for seed treatment of Lesy ČR in Týniště nad Orlicí. The seeds were already stratified. Germination was determined according to the Czech Standard (CSN 48 1211). Seed germination was 42% for ash and 12.5% for sycamore. Sycamore and ash seeds were thoroughly cleaned of all adverse plant residues to eliminate blight. Solutions for seed hydration were prepared just before use. The seeds were emerged into prepared solutions for 24 h. The next step was to put the seeds into germination boxes where distilled water (30 ml) was added. Each variant had four replications and all of them were conditioned. Distilled water was regularly added when necessary to avoid their drying. The air-conditioned box was in the regime of 16 h at 20°C in the dark and 8 h at 30°C under the light conditions. As soon as the seeds started germinating (after 7 days), the dark phase was set to stress conditions based on low temperatures to simulate spring frosts which could be quite frequent in our conditions. A general scheme of setting the air-conditioned box under stress conditions is shown in Table 2. Stress conditions were applied for a period of 56 days. A standard germination test for sycamore is only 21 days but for the reason of one experimental design, the same time period was applied (56 days).

The first check of the number of germinated seeds were done within the first seven days and then the germinated seeds were counted every seven days and it was repeated in such a way that the last check was on the 56$^{th}$ day. According to the Czech standard (CSN 48 1211) normally germinated seed had healthy roots and healthy cotyledon or healthy roots and partially damaged cotyledon and the capacity to produce a normal healthy seedling. The data were statistically evaluated by the General Linear Model (GLM ANOVA) method in the SAS system (v. 9.3, Carry, USA) at a significance level of $P \leq 0.05$. The significance of differences was evaluated by post-hoc Tukey's test.

## RESULTS AND DISCUSSION

The effect of brassinosteroids on seeds was tested in stress conditions and their vitality was evaluated by germination test. Stress conditions were simulated in the dark phase of the 24 h cycle as it usually occurs at night. The germination test has shown significant differences in the tolerance of two tree species seeds to stress conditions caused by frost. The hydration of sycamore and ash seeds using brassinosteroids has a great effect on seed germination, and even hydration in water has a significant influence on germination (Table 3, Fig. 2). It is also evident that the seed hydration in a solution significantly increased brassinosteroid effects on germination (Fig. 2). The positive benefits of seed hydration just in water were also described by Martiník and Palátová (2012). It should be noted that within the hydration process an appropriate (optimum) concentration of brassinosteroids should be used (in this case 1:210), because high concentrations could have in turn a reducing effect on seed germination. The negative effect of high concentrations of brassinosteroids was demonstrated for example by Procházka et al. (2012)

Table 3. Germination of ash (*Fraxinus*) and sycamore (*Acer*) seeds in stress conditions (number of observations used, $n$ = 8)

| Germination energy | Lexin | BRs 1:210 | BRs 1:100 | BRs 1:10 | Water | Untreated | MSD |
|---|---|---|---|---|---|---|---|
| *Fraxinus* | | | | | | | |
| GE1 | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.0000 |
| GE2 | 14.00$^{a}$ | 12.75$^{a}$ | 1.63$^{b}$ | 3.50$^{b}$ | 0.00$^{b}$ | 0.00$^{b}$ | 6.0782 |
| GE3 | 23.88$^{a}$ | 24.63$^{a}$ | 14.13$^{b}$ | 8.63$^{b}$ | 15.63$^{b}$ | 10.88$^{b}$ | 7.2339 |
| GE4 | 37.50$^{a}$ | 39.63$^{a}$ | 19.50$^{bc}$ | 12.38$^{c}$ | 26.75$^{b}$ | 19.00$^{c}$ | 7.2512 |
| GE5 | 49.63$^{a}$ | 51.38$^{a}$ | 35.38$^{b}$ | 21.75$^{c}$ | 37.75$^{b}$ | 20.00$^{c}$ | 7.9806 |
| *Acer* | | | | | | | |
| GE1 | 1.75$^{ab}$ | 2.00$^{a}$ | 0.00$^{c}$ | 0.29$^{bc}$ | 0.00$^{c}$ | 0.00$^{c}$ | 1.5550 |
| GE2 | 6.88$^{a}$ | 7.13$^{a}$ | 1.00$^{b}$ | 6.29$^{a}$ | 4.13$^{b}$ | 0.88$^{b}$ | 5.0866 |
| GE3 | 7.88$^{a}$ | 7.75$^{a}$ | 4.00$^{ab}$ | 7.57$^{a}$ | 5.25$^{ab}$ | 1.57$^{b}$ | 5.0413 |
| GE4 | 9.38$^{a}$ | 9.00$^{a}$ | 6.00$^{ab}$ | 8.29$^{a}$ | 5.88$^{ab}$ | 2.17$^{b}$ | 5.2127 |
| GE5 | 13.50$^{a}$ | 13.00$^{a}$ | 9.00$^{b}$ | 8.57$^{b}$ | 6.50$^{bc}$ | 3.33$^{c}$ | 3.9368 |

BRs – Brassinosteroids, MSD – minimum significant difference, means with the same letter are not significantly different

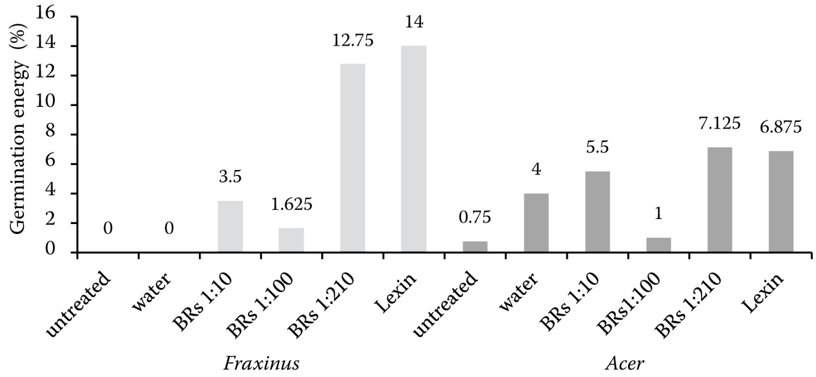


Fig. 1. Germination energy of ash (*Fraxinus*) and sycamore (*Acer*) seeds in experimental variant

in soybeans. Similar results were previously published by Kohout (2001).

Similarly Li et al. (2002) found that brassinosteroid application to seeds of Chinese red pine (*Pinus tabuliformis*) and to seeds of black locust (*Robinia pseudoacacia*) increased germination energy by 14.9% and 15.9%, respectively; the results of our experiments showed an increase of germination energy after the application of brassinosteroids by 12.8% for ash and 6.3% for sycamore. The influence of hydration on seed germination energy is shown in Fig. 1. The results document that the lowest concentrations of brassinosteroids gave almost the same effect as the reference product Lexin (Fig. 3).

It is evident that the brassinosteroid solution for seed hydration at the concentration of 1:210 significantly increased the seed germination under stress conditions: 4 times in sycamore and 2.5 times in ash when compared to untreated seed. The data show that the best working concentration of brassinosteroids was always the highest, i.e. 1:210. Many authors (Kohout 2001; Bečka et al. 2007; Kamlar et al. 2010) came to the conclusion that brassinosteroids also operate at very low concentrations. Similar conclusions were drawn by Li et al. (2008). Their experiments confirmed the higher germination energy of sorrel (*Rumex*) when the low concentration of natural brassinolide 0.01 mg·l$^{-1}$ was used in comparison with a concentration over 0.05 mg·l$^{-1}$. Hu et al. (2011) also discussed a suitable concentration in their paper. The authors proved the most suitable concentrations ranging from 0.025 to 0.050 mg·l$^{-1}$ for Camellia (*Camellia oleifera*) seeds.

The positive influence on seed hydration was also found by Gottwaldová et al. (2004). Their experiments showed that the soaking of fodder plant (*Melilotus*, *Phalaris*, *Malva* etc.) seeds in a solution of 24-epibrassinolide at concentrations of $10^{-9}$ M for two hours resulted in the increased germination by 3–8%.

The data suggest that the hydration of sycamore and ash seeds in pure water also showed a positive influence on germination and germination energy. Both tree species seeds proved a statistically significant increase in seed germination after hydration in water. Analogous results were obtained by Procházka et al. (2011). Their trials with soybean seed treatment using pure water showed not only an increase in the soybean seed emergence by 2%, but also a positive influence on the total number of plants with normally developed leaves per unit area.

The best results (in both cases) were obtained by hydration in a brassinosteroid solution at the concentration of 1:210 and in Lexin product. The treated seed always showed the highest germination energy and germination.

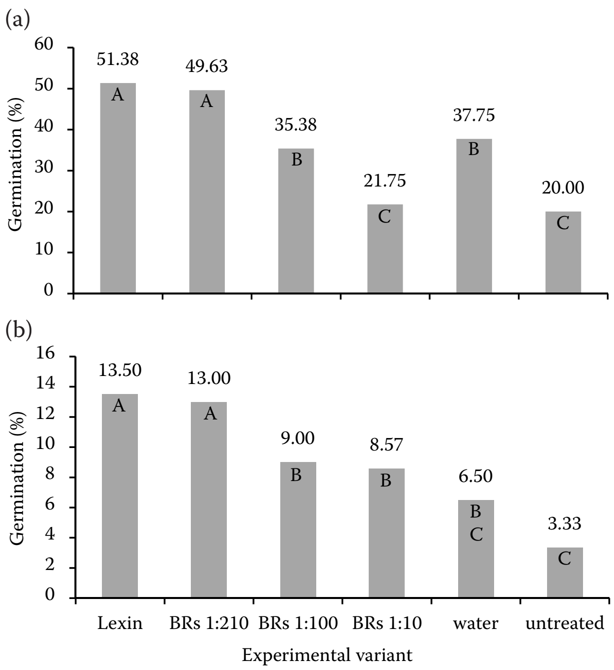


Fig. 2. Germination of ash (*Fraxinus*) (a) and sycamore (*Acer*) (b) seeds stress conditions

bars with the same letter are not statistically significantly different, minimum significant difference = 7.981 (a), 3.9368 (b)

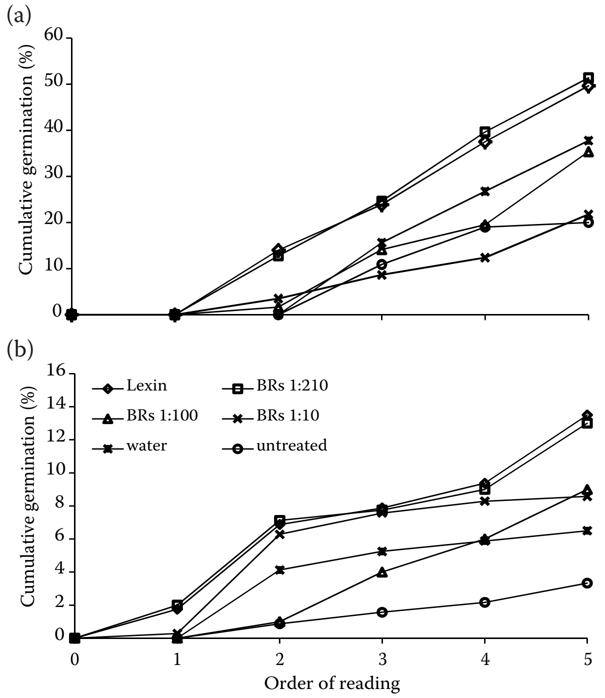


Fig. 3. Germination of ash (*Fraxinus*) (a) and sycamore (*Acer*) (b) seeds in stress conditions under different treatments expressed in cumulative curves

## CONCLUSIONS

The results proved the importance of pretreatment of sycamore and ash seeds for promoting germination capacity and germination in stress conditions.

The data suggest that the soaking of seeds of both species in a solution of brassinosteroids (substance 4154) has a positive effect on their germination energy and germination. The most effective brassinosteroid concentration is 1: 210, which proved to be the best of the three tested concentrations. Analogous good results were also obtained by the soaking of seeds in the product Lexin.

The results also confirmed that pure water used for seed hydration (as pretreatment) had also a positive effect both on the germination and germination energy.

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