Plant Hormone Gibberellin Induces Decline of Viability in Isolated Larch Shoot Buds

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Žiauka J. and Kuusienė, S. 2009. Plant Hormone Gibberellin Induces Decline of Viability in Isolated Larch Shoot Buds. *Baltic Forestry*, 15 (1): 13–22.

Abstract

The development of larch (*Larix* sp.) short shoots *in vitro* was investigated using isolated axillary buds. The explants were collected from two mature larch trees possessing different genotypes: 30-year-old European larch (*Larix decidua* Mill.) and 42-year old hybrid larch (*Larix kaempheri* Carr. x *Larix decidua* Mill.) derived after crossing between Japanese larch and European larch. Isolated short shoot buds were planted onto MS nutrient medium supplied with distinct plant growth regulators. The negative effect of some kinds of plant hormones was noted. Gibberellins GA_3 and, especially, $GA_{4/7}$ caused strong decline of viability in isolated shoot primordia. Negative influence of auxin indole acetic acid was also noted, though in less extensive rate. The explants of the investigated hybrid larch tree were far more resistant to negative effect of these plant hormones than the explants collected from the European larch tree. The certain role of developing primordia of axial needles in stimulation of chlorophyll loss was confirmed in European larch explants. Cytokinin zeatin when supplied to the nutrient medium together with gibberellin significantly promoted the negative effect of gibberellin (but this effect of zeatin was noted only in European larch explants). It was also confirmed that *in vitro* developed larch shoots can act on new-planted explants from a distance if they share the same space for gas interchange (*in vitro*). Short shoots previously treated with auxin or gibberellin were significantly more sufficient for the induction of needle browning on newly developing shoots that shared the common space for gas interchange. The synergistic effect of gibberellin and other plant hormones, including gaseous plant growth regulator ethylene is under discussion.

Key words: axillary bud, explant development, *in vitro* culture, needle browning, plant hormones, short shoot

Introduction

Plant growth regulators gibberellins form a distinct group of "classical" plant hormones. Not all gibberellin-like compounds can play essential role in plant development but only few of them. The main forms of gibberellin that are known for their role in regulation of plant physiological processes are GA_1, GA_3, GA_4, GA_7. Bioactive gibberellins control seed germination, stem elongation, leaf expansion, trichome development, and flowering (Olszewski *et al*. 2002).

Promoting diverse developmental processes gibberellins variously interact with other plant growth regulators and effect on plant growth and development is usually obtained when gibberellin treatment is combined with auxin or cytokinin treatment (Pearce *et al*. 1987). Gibberellin interaction with auxin is quite well investigated. Natural auxin indole-3-acetic acid promotes formation of bioactive gibberellin through regulation of particular gene expression (O'Neill and Ross 2002). Gibberellins in turn promote auxin distribution, as it was demonstrated in Japanese horse-chestnut (*Aesculus turbinata*) seedlings (Du *et al*. 2004), and therefore are essential for broad-exploded action of auxin. Compared to gibberellin, auxin is able to act on much higher number of plant genes including also the genes inducible by gibberellin (Sundberg 2006). Gibberellin relation to ethylene and cytokinins can be hardly explained according to direct metabolic links. But the investigation of effects caused by plant growth retardants that act inhibiting formation of gibberellins has revealed that decline of gibberellin biosynthesis cause an increase in the cytokinin content and a decrease in ethylene production (Rademacher 2000).

Most investigations of gibberellin effect on developmental processes and gibberellin interaction with other growth regulators in woody plants were carried out through *in vivo* experiments. The positive effect of gibberellins on shoot elongation and vegetative bud development was estimated in conifer seedlings (Little and MacDonald 2003, MacDonald and Little 2006). Gibberellin mixture $GA_{4/7}$ was found as successful flow-

ering stimulator in conifers (Eysteinsson and Greenwood 1995, Almqvist 2003).

Previous research of gibberellin effect on the development of isolated larch short shoots (Žiauka and Kuusienė 2007) revealed that gibberellin (GA_3) is somewhat able to stimulate the development of basal needles under particular conditions, while it usually causes the total break of development of axial needle primordia (in developing larch shoot axial needles are distributed at the shoot axis which does not elongate in short shoots and can be practically identified with shoot apex). But the attempts to carry out full-scale research of gibberellin influence on larch shoot development *in vitro* met a serious problem because European larch explants rapidly lost their viability on the nutrient medium containing gibberellin. Thus this research was carried on with the purpose to find possible explanations for the mechanism of a negative action of gibberellin considering its interactions with other plant growth regulators.

Materials and methods

Abbrevations

EL – European larch; GA – gibberellin; HL – hybrid larch; IAA – indoleacetic acid

Plant material and growth conditions

Explants from two distinct larch trees were investigated. 30-year old European larch (*Larix decidua* Mill.) tree (EL) from the Girionys park (Kaunas district, Lithuania) and 42-year old hybrid larch tree (HL) from the seed-derived larch plantation of Vaišvydava (Kaunas district, Lithuania) were chosen for the research. The hybrid larch was derived after crossing between Japanese larch and European larch (*Larix kaempheri* Carr. x *Larix decidua* Mill.). Prepared axillary buds were used as explants during this research.

Current year twigs were collected from the middle one-third of the crown of selected larch trees. The gatherings for distinct experiments followed from the first half of November till the second half of January. After removal of needles the twigs were cut into short pieces (1-2 cm, each segment with an unburst short shoot bud). Segments of twigs were soaked for 3 minutes in 75 % ethyl alcohol and then for 4 minutes in 0.1 % solution of silver nitrate. After disinfection all covering tissues, including bud scales, were removed from woody cores using sterile pincers. Prepared explants with naked green shoot buds were placed in plastic Petri dishes (55 mm in diameter) or vitreous test-tubes (20 mm in diameter and 150 mm in height). Three explants were placed in every Petri dish of 55 mm in diameter. MS nutrient medium (Murashige and Skoog 1962) containing 30 $g \cdot l^{-1}$ sucrose (pH 5.5 before autoclaving) and 8.5 $g \cdot l^{-1}$ phytoagar was used for cultivation of larch shoot primordia. Nutrient medium was usually supplied with plant growth regulators (obtained from ICN Biochemicals GmbH, Germany). GA_3 and $GA_{4/7}$ (soluted in ethyl alcohol and diluted with distilled water), IAA and zeatin (both soluted in sodium hydroxide and diluted with distilled water) were added to the medium after autoclaving before the sterilized medium congealed. Syringe driven filters Millex (pores 0.22 μm) were used for sterilization of plant hormones. Hormone-free MS medium was used for the control in different experiments.

Larch explants were cultivated in the room fitted for cultivation of plant tissue cultures. Automatically regulated white-light illumination (irradiance 30 μmol m^{-2} s^{-2}) was used for established photoperiod of 16 hours. Temperature in the cultivation room rose to 25 ºC during the light phase and decreased to 18 ºC during the dark phase. Every experiment was made in three repetitions under same conditions.

Investigation of the effect caused by different gibberellins on the viability of larch-shoot primordia

Explants were collected from the European larch tree. Seven variants of nutrient medium with different gibberellin content were used for cultivation of isolated shoot primordia: 1) without plant growth regulators (hormone-free); 2) GA_3 0.01 $mg \cdot l^{-1}$ (0.03 μM); 3) GA_3 0.05 $mg \cdot l^{-1}$ (0.15 μM); 4) GA_3 0.30 $mg \cdot l^{-1}$ (0.90 μM); 5) $GA_{4/7}$ 0.01 $mg \cdot l^{-1}$; 6) $GA_{4/7}$ 0.05 $mg \cdot l^{-1}$; 7) $GA_{4/7}$ 0.30 $mg \cdot l^{-1}$.

Seven experimental sets containing 24 explants each were obtained. The percentage of browning explants was evaluated after 25 days of cultivation *in vitro*.

Comparison of development between the explants of two investigated larch genotypes, independently from exogenous hormone treatment

Explants were collected from both the European larch tree (EL) and the hybrid larch tree (HL) in the first half of December and were cultivated on hormone-free nutrient medium (two experimental sets, each containing 42 explants). After 25 days of cultivation the percentages of explants with particular developmental features were evaluated. These developmental features were the following: a) most needles fully elongated, b) several fully elongated needles, c) elongating needles, d) sprouting axial needles. Each of this features was estimated distinctly (see Figure 1 for example) and independently from other features.

Investigation of gibberellin effect on explants collected from different larch trees

Explants were collected from both the European larch tree (EL) and the hybrid larch tree (HL). Explants from each tree were divided into three experimental sets

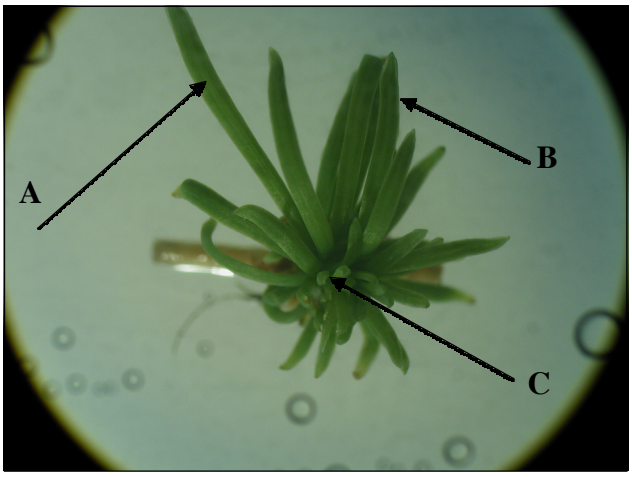

Figure 1. A larch explant with following developmental features: several fully elongated needles (A), elongating needles (B), sprouting axial needles (C)

(six experimental sets in total). Each set contained 42 explants. Distinct experimental sets were cultivated on different nutrient media in Petri dishes: 1) hormone-free; 2) GA_3 0.05 mg·l^{-1} (0.15 µM); 3) $GA_{4/7}$ 0.05 mg·l^{-1}.

The percentage of browning explants was estimated after 20 days of cultivation. Then explants from all experimental sets were transplanted onto fresh hormone-free nutrient medium (in test-tubes). After 20 days the rate of needle discoloration was assessed. Here and in following experiments a particular system for estimation of the rate of needle discoloration was used. The discoloration rate was estimated according to the proportion of browning (discolourated) needles among the total needle content of particular explant (short shoot). The mark was given in such a way:

0 – 20% browning needles = 1 (green shoot)
20 – 40% = 2
40 – 60% = 3
60 – 80% = 4
80 – 100% = 5 (yellow/brown shoot).

Comparison of gibberellin and auxin effects on senescence induction in larch explants

Explants were collected from both the European larch tree (EL) and the hybrid larch tree (HL). Explants from each tree were divided into four experimental sets (eight experimental sets in total). Each set contained 20 explants. Two-stage cultivation passed on these combinations of nutrient medium:

1) hormone-free, 2) hormone-free
1) IAA 2.0 µM, 2) hormone-free
1) hormone-free, 2) GA_3 0.15 µM
1) IAA 2.0 µM, 2) GA_3 0.15 µM

Explants were cultivated in Petri dishes during the first stage of cultivation and in test-tubes during the second. Each stage of cultivation lasted 25 days and after 50 days the rate of needle discoloration was assessed according to the system set forth above.

Investigation of the axial zone significance for gibberellin-induced senescence in larch short shoots

Explants were collected from European larch tree. They were divided into eight experimental sets (each set contained 16 explants). The explants of one half of the sets were planted reversely (upside-down) on the nutrient medium in Petri dishes and the explants of another half were planted normally. During the first stage of cultivation explants developed either on hormone-free medium or on the medium supplied with 3.5 µM IAA. After 24 days they were transplanted (all normally) either onto hormone-free medium either onto medium supplied with 0.15 µM GA_3 (in test-tubes). Thus two-stage cultivation passed on these combinations of nutrient medium:

1) hormone-free (planted normally or reversely), 2) hormone-free
1) IAA 3.5 µM (planted normally or reversely), 2) hormone-free
1) hormone-free (planted normally or reversely), 2) GA_3 0.15 µM
1) IAA 3.5 µM (planted normally or reversely), 2) GA_3 0.15 µM

After 22 days the rate of needle discoloration was assessed according to the system set forth above (according to the proportion of browning needles among the total needle content of particular explant).

Investigation of cytokinin influence on gibberellin-induced decline of viability in larch-shoot primordia

Explants were collected from both European larch (EL) and hybrid larch (HL) trees in the first half of January. Explants from each tree were divided into four experimental sets (eight experimental sets in total). Each set contained 30 explants. Distinct experimental sets were cultivated on different nutrient media in Petri dishes: 1) without plant growth regulators (hormone-free); 2) GA_3 0.15 µM; 3) GA_3 0.15 µM, zeatin 3.6 µM; 4) zeatin 3.6 µM.

The percentage of explants with expanding needles and the percentage of browning explants were estimated after 24 days of cultivation.

Investigation of gaseous signal influence on senescence induction in larch short shoots

Explants were collected from the European larch tree (EL). They were divided into seven experimental sets. Each set contained 16 explants. The first set of explants was planted onto hormone-free medium in test-tubes. All other sets were also planted onto hormone-free medium but each explant in these sets was supplied with a co-dwelling additional larch explant.

This additional explant shared the same space with the main explant as it was planted onto hormone-free medium in slightly smaller test-tube (16 mm in diameter and 150 mm in height) that was embedded into larger (standard) test-tube (20 mm in diameter) with new-planted EL explant. Thus both explants shared the common space inside the smaller test-tube and this space was restricted to both as the one test-tube was tightly embedded into another and its bottom-side was pressed with the cover of another test-tube and the cover was fixed with parafilm. Additional explants (short shoots) were orientated upside-down in double test-tubes therefore the rack on which the rest containing test-tubes was laid was covered with aluminium foil for reflection of light. Additional explants differed by their origin and had underwent different hormonal treatment during two previous stages of cultivation until they had developed into short shoots. These explants were the same investigated in the experiment that was carried out on purpose to compare the effects of gibberellin and auxin on senescence induction in larch explants. Thus the experimental sets differed according to the previous cultivation (two stages) of complementary short shoots:

Control (without complementary explant)

1) hormone-free, 2) hormone-free (both EL and HL explants)

1) IAA 2.0 µM, 2) hormone-free (both EL and HL explants)

1) hormone-free, 2) GA_3 0.15 µM (HL explants only)

1) IAA 2.0 µM, 2) GA_3 0.15 µM (HL explants only).

Experimental sets involving EL explants treated with GA_3 were not presented because the loss of viability caused by gibberellin did not allow us to establish sufficient experimental sets. The rate of needle discoloration was assessed in all experimental sets after 30 days of co-cultivation.

Statistics

Bias of value expressed in percentage (S_p) was used for statistical verification of reliability of obtained results. It was calculated by formula:

$$S_p = \pm\sqrt{(p(100-p)/n)}$$

here p – value of an analysed parameter expressed in percentage, n – sample size.

The standard error of the mean obtained from the set of ordinary values (numbers) was calculated after the sample standard deviation. Quantiles of the normal distribution and the sample mean and standard error was used to calculate confidence intervals for the mean. The following expressions were used to calculate the upper and the lower 95% confidence limits, where x is equal to the sample mean, s is equal to the standard error for the sample mean, and 1.96 is the .975 quantile of the normal distribution:

upper 95% limit = $x + (s*1.96)$

lower 95% limit = $x - (s*1.96)$

Results

Effect of different gibberellins on the viability of European larch buds

It was observed that even minimal concentration (0.01 $mg \cdot l^{-1}$) of gibberellin mixture $GA_{4/7}$ caused a very strong negative response of isolated European larch shoot primordia. The processes of senescence were followed by such visible features as browning of needles and their primordia. When more considerable $GA_{4/7}$ concentrations were used almost all explants planted on the media of that type lost their viability (Figure 2). The effect of GA_3 on developing larch shoots was also straight negative but the effect of this gibberellin form was not so total and the significance of concentration for the negative response of larch explant was observed. Minimal GA_3 concentration induced only slight increase of explant browning as compared to hormone-free medium, whereas on the medium containing 0.05 $mg \cdot l^{-1}$ (0.15 µM) GA_3 rather more than half of explants turned browning and the highest one of investigated GA_3 concentrations caused total majority of browning explants. Of interest is the fact that negative effect of $GA_{4/7}$ was usually followed by reduced needle development while GA_3 had no significant effect and even the highest concentration of GA_3 caused only slight reduction of needle development.

Comparison of development between the explants of two investigated larch genotypes, independently from exogenous hormone treatment

The primary *in vitro* development of larch explants collected from different larch trees was quite different (Table 1). The buds collected from tree HL (hybrid

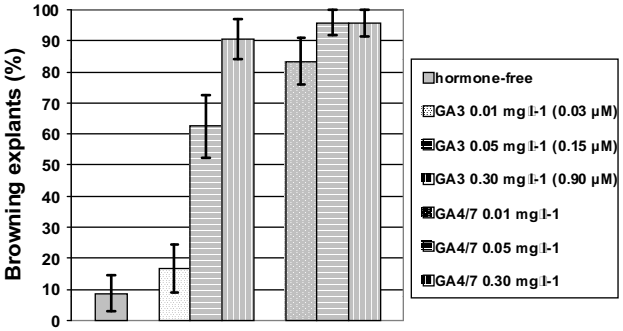

Figure 2. Effects of different gibberellins on the viability of European larch shoot primordia after 25 days of cultivation *in vitro*. Biases of estimated values are shown in error bars

Table 1. Developmental differences between explants collected from the hybrid larch tree (HL) and the European larch tree (EL) estimated after 25 days of cultivation *in vitro* on hormone-free medium

Explants with particular developmental features, %	HL	EL
most needles fully elongated	71.1±6.8	2.3±2.2
several fully elongated needles	95.5±3.1	18.2±5.8
elongating needles	95.5±3.1	72.7±6.7
sprouting axial needles	37.8±7.3	95.5±3.1

larch) rapidly developed into short shoots and most of them had intensively developing (elongating) needles while development of needles on explants collected from tree EL (European larch) was much slower. HL explants were able to sprout well-developing basal needles but the development of axial needles was mostly delayed. By contrast, on hormone-free medium almost all EL explants sprouted axial needles (although not intensively elongating) but the development of basal needles was not so intensive.

Effect of gibberellins on explants collected from different larch trees

The loss of viability followed by explant browning was the main effect caused by gibberellins, both GA_3 and $GA_{4/7}$ (Figure 3). But, interestingly, such primary negative effect of gibberellin was characteristic only of EL explants while HL explants seemed quite resistant (none of applied gibberellins had significant effect on the viability of HL explants after 20 days).

The browning of EL explants had one general feature: the developing short shoots started to brown exactly from the axial zone and brown axial needles were circled by yet green basal needles. However, when HL explants from all three experimental sets were transplanted onto the fresh nutrient medium without plant growth regulators, the negative effect of gibberellin treatment during the first subcultivation became significant. The explants that were cultivated on hormone-free medium maintained all green needles during the second subcultivation (rate of needle discoloration = 1) whereas the explants transplanted from the medium containing GA_3 or $GA_{4/7}$ started browning. The difference from the group of explants that did not undergo gibberellin treatment was significant after 20 days: the average rate of needle discoloration was 3.86±0.27 in the set of explants treated with 0.05 mg·l^{-1} (0.15 μM) GA_3 during the first subcultivation and it was 4.38±0.18 in the set treated with 0.05 mg·l^{-1} $GA_{4/7}$.

Comparison of gibberellin and auxin effects on senescence induction in larch explants

This experiment was performed on purpose to investigate the role of auxin in needle browning generally and especially in needle browning caused by gibberellin. The results indicated a certain role of IAA in needle discoloration but this negative effect of auxin was demonstrated only with European larch (EL) explants (Figure 4). Hybrid larch (HL) explants were much more able to maintain green colour of the needles than EL explants and even auxin treatment during the first subcultivation had no negative effect on HL explants. GA_3 treatment during the second cultivation caused great increase of needle browning in the sets of EL explants, independently whether their first subcultivation passed on the medium with IAA or not. The negative influence of gibberellin on the needle colour was significantly more considerable than that of auxin.

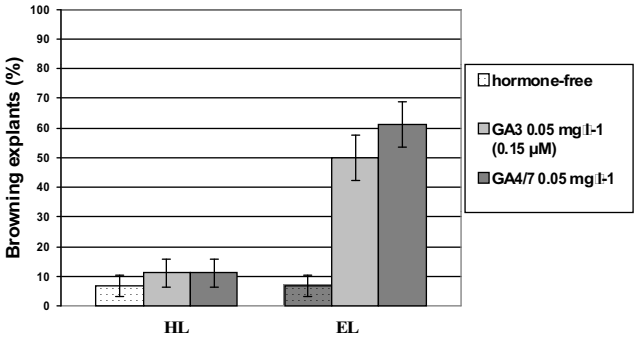

Figure 3. Effect of gibberelins on explants collected from different larch trees (the European larch tree – EL and the hybrid larch tree – HL) after one stage of cultivation (20 days *in vitro*). Biases of estimated values are shown in error bars

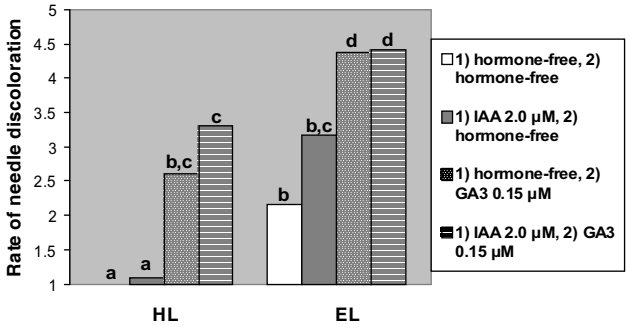

Figure 4. Gibberellin and auxin effects on discoloration of larch short shoots after two stages of cultivation *in vitro* (25 days + 25 days). Different lower case letters above the columns indicate the means with non-overlapping confidence intervals within 95% confidence limits

Needle discoloration caused by gibberellin was clearly demonstrated not only with EL explants but also with HL explants although not in such a total rate. Interestingly, IAA treatment during the first subcultivation had some influence on the response of HL explants to GA_3 during the second subcultivation as the rate of needle discoloration was even more increased.

Significance of axial zone for gibberellin-induced senescence in larch short shoots

The purpose of this experiment was to analyse the role of the axial zone of *in vitro* developing larch shoot in the response to exogenously applied gibberellin. Also the effect of exogenous auxin on shoot senescence was further investigated. The role of the axial zone was estimated planting explants reversely on the medium of the first subcultivation (the axial zone of the reversely planted explants totally diminished). The results revealed that axial zone played a certain role promoting the loss of chlorophyll in needles. All experimental sets of explants that were reversely planted for their first subcultivation demonstrated better ability to maintain green needles during the second stage of cultivation (Figure 5), only the difference between the groups of explants that were cultivated on hormone-free medium during both stages of cultivation was not so significant. The explants that underwent the first subcultivation in reverse position showed increased resistance to gibberellin-induced chlorophyll degradation.

Cytokinin influence on gibberellin-induced decline of viability in larch shoot primordia

The results were quite different according to particular larch tree which served as donor of explants (both EL and HL explants were investigated). Zeatin totally delayed needle development in explants from both donor trees, independently whether it was applied to the nutrient medium together with gibberellin or alone (Figure 6). The particular role of zeatin acting together with gibberellin was revealed comparing the proportion of browning explants among different experimental sets. Gibberellin significantly increased the number of browning explants among both HL and EL explants (Figure 7). Zeatin when applied together with GA_3 had no significant effect on the viability of HL explants and the number of browning explants was even slightly decreased, as compared to the set of HL explants that were treated with GA_3 only. But combined zeatin and gibberellin treatment strongly increased the number of browning EL explants and this increase was significantly bigger than that caused by GA_3 alone. Zeatin alone had no negative effect on the viability of larch explants.

Influence of gaseous signal on senescence induction in larch short shoots

This experiment was carried out on purpose to investigate whether the development and viability of larch explants can be influenced by other larch explants that share the same space for gas exchange but have no direct contact even through the nutrient me-

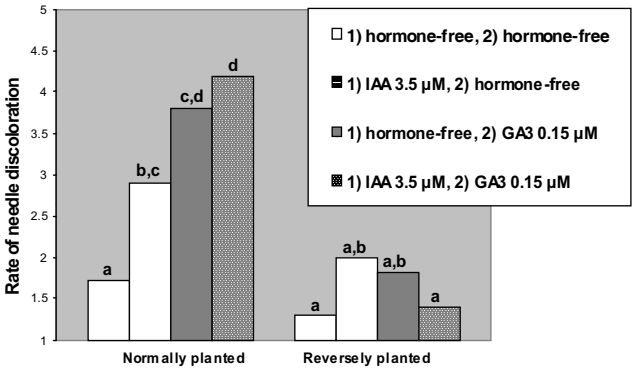

Figure 5. Discoloration of European larch short shoots under different cultivation conditions (results after two stages of cultivation *in vitro*: 24 days + 22 days). Different lower case letters above the columns indicate the means with non-overlapping confidence intervals within 95% confidence limits

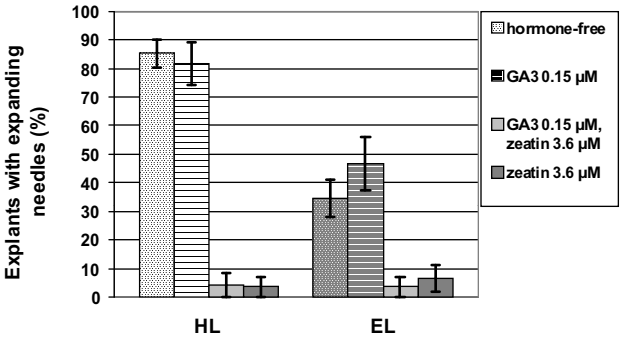

Figure 6. Larch needle development under gibberellin and cytokinin treatment (results after 24 days of cultivation *in vitro*). Biases of estimated values are shown in error bars

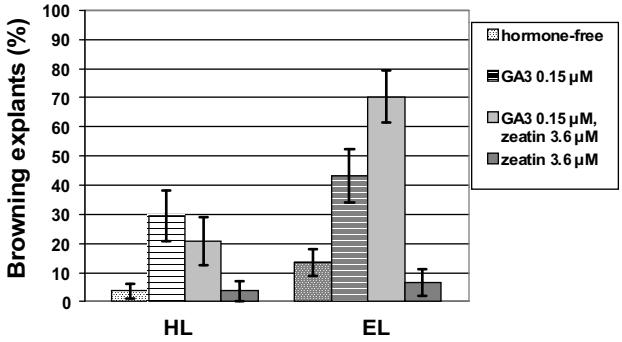

Figure 7. Browning of larch explants under gibberellin and cytokinin treatment (results after 24 days of cultivation *in vitro*). Biases of estimated values are shown in error bars

dium. The results revealed that one larch explant can act on another through the space that they share for gas exchange. The EL explants showed quite different needle development according to the space they were cultivated. Almost all new planted explants expressed intensive needle development (elongation) when cultivated alone. The needle development of newly planted EL explants was strongly delayed when they shared the common space with another already developed larch short shoot. Interestingly, the level of this delay depended also on the type of additional explant. When cultivated together with HL explants new-planted explants expressed significantly better needle development than sharing the same space with EL explants. A similar tendency was stated observing needle browning of short shoots developed from new-planted EL axillary buds. Most of explants cultivated without an additional explant maintained all green needles 30 days after introduction into tissue culture (Figure 8). Sharing the space with HL short shoots increased the rate of needle discoloration, and sharing it with EL short shoots caused even more extensive needle browning than that caused by HL explants (significant difference from the buds cultivated without an additional explant). An interesting note was the fact that needle discoloration of new-developed EL short shoots clearly depended on the hormonal treatment which additional explants underwent before. When EL explants were cultivated together with HL short shoots that had undergone IAA treatment during the first stage of their cultivation *in vitro* needle browning of new-developed EL short shoots was increased. The same tendency was observed in the case when additional HL explants had been previously cultivated on the medium containing GA_3 during the second stage of their cultivation, even in more extensive rate (the needle-discoloration rate of together cultivated EL explants was significantly increased). The significance of the previous hormonal treatment on the ability of certain larch short shoot to influence the viability of other explants was demonstrated, too, when EL explants were used as additional short shoots. The EL short shoots grown on the medium supplied with IAA during the first stage of cultivation caused more extensive needle browning of new-planted EL explants than EL short shoots previously cultivated on hormone-free medium only.

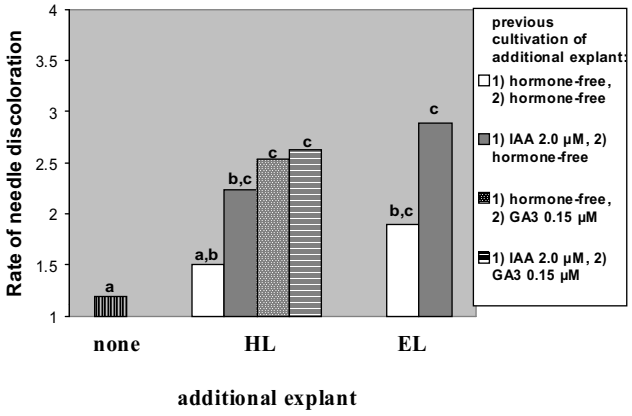

Figure 8. Influence of co-dwelling additional explant on discoloration of European larch short shoots (results after 30 days of cultivation *in vitro*). Different lower case letters above the columns indicate the means with non-overlapping confidence intervals within 95% confidence limits

Discussion and conclusions

The results obtained during this research indicate that gibberellins have great negative influence on the viability of larch short shoots developing from isolated buds. This influence is probably related with isolation of larch explants because other reports about gibberellin action on larch shoots carried out on *in vivo* material did not state its negative effect although it was claimed that gibberellin treatment together with root pruning caused a certain decrease of the chlorophyll content in black spruce (*Picea mariana* Mill.) needles (Smith and Greenwood 1997). But viability loss caused by gibberellins was almost total in larch short shoots that had developed *in vitro*. From two distinct larch genotypes that were investigated during this research one was more resistant to negative effect of gibberellin although this particular resistance was not absolute in any sense and explants started browning if once cultivated on the nutrient medium containing gibberellin. Such long-termed effect of gibberellin suggests that the loss of viability is promoted indirectly, but via long-living signal possibly mediated by other plant growth regulators, such as auxin and ethylene.

The concerted action of gibberellin and auxin is important for longitudinal growth, wood formation and some other developmental features in trees (Wang *et al*. 1997). These two plant hormones act on the level of each other. Auxin increases the level of bioactive gibberellins through regulation of the expression of the genes coding for GA 3-oxidase and GA 2-oxidase (the activity pattern of these enzymes causes the actual level of bioactive gibberellins) (O'Neill and Ross 2002). Gibberellin in turn also increases the actual level of auxin by promoting its distribution (Du *et al*. 2004), most likely through induction of polar auxin transport, as it was demonstrated in *Populus* (Björklund 2007). According to these facts, the suggestion can be made that the negative effect of gibberellin in isolated larch short-shoot buds is probably mediated by auxin. Ex-

ogenously applied auxin IAA caused significant loss of the viability in explants collected from the less resistant genotype EL although this effect was not so strong as that of gibberellin. However, HL explants remained quite resistant to auxin alone but when IAA was supplied to the nutrient medium before GA_3 treatment it caused more negative effect of gibberellin on HL explants. It can be also stated that EL explants were not only much more resistant than HL explants to both gibberellin and auxin but in general they expressed far weaker needle development and increased discoloration. Browning of EL explants firstly occurred exactly in the axial zone, and the needles closely circled around the non-developing shoot axis used to lose their green colour. This observation suggests that the degeneracy of *in vitro* developing EL shoots could be related with the axial zone and its needle primordia. It is a strong point towards auxin as possible factor acting to express the negative effect of gibberellin while some facts indicate that EL explants could be more capable in auxin production. EL explants extensively formed primordia of axial needles and expressed quite weak needle elongation. Auxin was reported in some other plant species as a necessary factor for formation of leaf primordium (Reinhardt *et al*. 2000) and that it is synthesized largely in shoot apical regions, probably not in the shoot apex itself but in the leaf primordia around the shoot apex (Woodward and Bartel 2005). This definition exactly fits the zone where natural browning of isolated larch explants firstly occurred. Also it was reported that auxin had a negative effect on leaf blade elongation in bean and *Arabidopsis* (Keller *et al*. 2004) and even on the expansion of larch needles (Žiauka and Kuusienė 2006). The experiment comparing explants with developing and lost (by reverse planting) axial zone confirmed that larch explants without axial needles were not able to respond to gibberelin treatment through totally increased rate of needle browning as explants with developing axial needles did. Suggestion about the role of auxin was indirectly supported also by the observation that cytokinin zeatin significantly increased the negative effect of gibberellin on the viability of sensitive EL explants but not on more resistant HL explants. It is known that the levels of free (active) auxin are increased after application of exogenous cytokinin (Nordström *et al.* 2004). Although the mechanism of these changes is not fully understood, cytokinin-induced inhibition of enzymes that conjugate free IAA into inactive IAA aspartate has been suggested. In that case cytokinin is able to increase the actual levels of active auxin if certain plant tissue is able to produce a high amount of auxin. Some suggestion could be made about the synergistic action of gibberellin and cytokinin zeatin decreasing the viability of larch explants: the primordia of axial needles produce high amounts of auxin, applied cytokinin preserves it from inactivation, and applied gibberellin promotes auxin distribution throughout the tissues of developing larch shoot. In general auxin is often defined as the critical plant hormone modulating diverse processes of plant developing (Vogler and Kuhlemeier 2003, Woodward and Bartel 2005). In many cases the physiologic action of auxin is closely related with its interaction with other plant growth regulators. Auxin is essential for production of ethylene in plant tissues because it regulates the expression of particular genes that encode ACC synthase, an enzyme which catalyses the critical step in ethylene biosynthesis (Chen *et al.* 2005). Ethylene is a plant growth regulator perhaps best known for its promotion of senescense. It can cause a serious loss of the chlorophyll content (Hörtensteiner 1999). In contrast to other plant growth regulators, ethylene is gas and can act even from the distance between two plants if they share the same restricted space for gas interchange. One of the experiments that were carried out during this research revealed that the patterns of development and viability of isolated larch explants can be regulated by gas interchange. When additional explants had undergone auxin or gibberellin treatment during the first stages of their cultivation they caused more significant decrease of the viability in new-planted EL explants that shared the common restricted space for gas interchange. This indicates that these differences are probably caused by more specific gas whose production is closely related with hormonal treatment. The most likely suggestion is that this gaseous factor should be ethylene. Ethylene involvement in the response of larch explants towards exogenously applied gibberellin can be supported by the data that ethylene production pattern can be very distinctive in conifers. It was proved that individual needles along a shoot of Scots pine (*Pinus sylvestris* L.) form very specific pattern of spatial distribution according to ethylene production (Ievinsh and Ozola 1998). Investigating embryogenic cell lines of black spruce (*Picea mariana* Mill.) it was stated that these cell lines varied widely according to their ability to produce ethylene and their need of ethylene for maturation (El Meskaoui and Tremblay 2001). We suggest that the lack of the viability in larch short shoots that develop from isolated buds is closely related with increased ethylene production. This can be one of the most serious factors limiting microvegetative propagation of larch species from isolated short shoot buds.

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Received 02 September 2008
Accepted 19 May 2009

ФИТОГОРМОН ГИББЕРЕЛЛИН ВЫЗЫВАЕТ ОСЛАБЛЕНИЕ ЖИЗНЕСПОСОБНОСТИ В ИЗОЛИРОВАННЫХ ПОЧКАХ ПОБЕГОВ ЛИСТВЕННИЦЫ

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Резюме

Изолированные пазушние почки были исползованы для изучения развития коротких побегов лиственницы (*Larix* sp.) *in vitro*. Экспланты были собраны от двух зрелых деревьев лиственницы с разными генотипами: от 30-летней лиственницы европейской (*Larix decidua* Mill.) и от 42-летней лиственницы гибридной (*Larix kaempheri* Carr. x *Larix decidua* Mill.), полученной вследствии скрещивания между лиственницей японской и лиственницей европейской. Изолированные почки коротких побегов были посажены на MS питательную среду, добавленную разными регуляторами роста растений. Было замечено негативное воздействие фитогормонов некоторых родов. Гиббереллины $ГК_3$ и, в особенности, $ГК_{4/7}$ вызвали сильное ослабление жизнеспособности изолированных зачатков побегов. Также было замечено негативное влияние ауксина индолилуксусной кислоты, хотя оно не было такое экстенсивное. Экспланты исследованной гибридной лиственницы были более стойкие против негативного воздействия фитогормонов, чем экспланты, собранные от дерева лиственницы европейской. Было подтверждено, что развивающиеся зачатки осевых хвой играют важную роль в потере хлорофила, замеченной в эксплантах лиственницы европейской. Цитокинин зеатин, при добавлении его в питательную среду вместе с гиббереллином, усилил негативное воздействие гиббереллина в значительной степени (но такое влияние зеатина было замечено лишь в эксплантах лиственницы европейской). Также было подтверждено, что *in vitro* развившиеся побеги лиственницы могут действовать на новосаженные экспланты через расстояние, если они разделяют то самое пространство для обмена газов (*in vitro*). Короткие побеги, предварительно воздействованные ауксином или гиббереллином, были в значительной степени более сильные факторы, вызывающие потерю хлорофила в хвоях новоразвивающихся побегов, посаженных в том самом изолированном пространстве для обмена газов. Синергичный эффект гиббереллина и других фитогормонов, в том числе и газообразного регулятора роста растений этилена, является темой дискуссии.

Ключевые слова: пазушная почка, развитие эксплантов, *in vitro* культура, обесцвечивание хвой, фитогормоны, короткий побег