Callus induction and regeneration efficiency of spring barley cultivars registered in the Czech Republic

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ABSTRACT

Immature zygotic embryos are frequently used for induction of cell cultures *in vitro* and for genetic transformation. The effect of three synthetic auxins: 2,4-dichlorophenoxyacetic acid (2,4-D), 3,6-dichloro-2-methoxybenzoic acid (dicamba) and 4-amino-3,5,6-trichloropicolinic acid (picloram) on induction and subsequent regeneration capacity of barley. *In vitro* culture was studied in 12 spring barley cultivars registered in the Czech Republic and a variety of Golden Promise, with known high regeneration efficiency. The callus formation frequency and number of green regenerants were influenced significantly both by genotype and auxin. Most cultivars formed statistically significantly a higher mean number of regenerants (1.95) after the callus induction on the medium with 2.4-D as compared to the media with picloram and dicamba. Only two cultivars (Nordus and SG-S-261) did not respond to the used auxins differently. The highest average number of regenerants (from all three auxins) was obtained with Golden Promise (2.7 plants per one cultivated scutellum). From the set of genotypes used in our study, the cultivars Atribut, Forum, and Scarlett with the mean number of regenerants (2.09–1.57) would be the most suitable cultivars for further transformation use. They differ statistically significantly from the cultivars Nordus, Amulet, Akcent, SG-S-252, Orbit, and Granát (0.42–0.92) which had the lowest mean number of regenerants.

Keywords: immature zygotic embryos; auxin; 2,4-D; picloram; dicamba; spring barley

The regeneration of differentiated cereal plant cells from callus remains a major limiting step in obtaining high numbers of cereal clones or independent transgenic cereal lines (Eudes et al. 2003). Barley (*Hordeum vulgare* L.) is one of the recalcitrant cereal crops with only a limited number of tissues suitable for *in vitro* culture and plant regeneration (Ganeshan et al. 2003). Barley improvement through genetic transformation and *in vitro* techniques requires establishment of an efficient and reproducible plant regeneration system (Dahleen and Bregitzer 2002, Chang et al. 2003). These techniques have the potential to assist in breeding improved barley cultivars. Somatic embryos are derived from a single cell whereas organogenic sectors are multicellular in origin. To reduce the chances of recovering chimeric transgenic plants, somatic embryogenesis is preferred (Ruíz et al. 1992, Steven and Kasha 1994). Regenerated plants are expected to have the same genotype as the donor plant. However, in some cases phenotypic and cytological variants have been found among regenerated barley plants (Karp et al. 1987). In barley differences in the production of embryogenic calluses and regenerated plants have been observed when cultures were initiated from leaf base/apical meristems, mature embryos (Ganeshan et al. 2003), immature inflorescences (Thomas and Scott 1985) immature zygotic embryos (Lührs and Lörz 1987, Ruíz et al. 1992, Chang et al. 2003) and immature scutella (Tingay et al. 1997). But immature zygotic embryos are currently the most reliable and efficient target tissue for *in vitro* regeneration of cereals, this has already been well documented in some reports by Dahleen and Bregitzer (2002) and Chang et al. (2003).

The genotype of donor plants and growth characteristics of induced calluses are the most important factors affecting plant regeneration (Bregitzer 1992, Castillo et al. 1998). The composition of the media, including growth regulators, is another important factor influencing culture initiation and plant regeneration of immature embryos. Phytohormones are crucial to establishing optimal culture conditions since they play a pivotal role in producing relatively undifferentiated callus tissue from differentiated tissues such as an embryo (Jiang et al. 1998). The auxin 2,4-dichlorophenoxyacetic acid (2,4-D), by itself or in combination with cytokinins, has been widely used to enhance callus induction

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and maintenance (Castillo et al. 1998). Both are required for cell division and during the *in vitro* culture, the balance between the two hormones determines what developmental tissue types are formed from the cell divisions (Jiang et al. 1998). Other strong auxins such as dicamba, picloram and or combination of these auxins have been used as alternatives (Conger et al. 1983, Lührs and Lörz 1987, Przetakiewicz et al. 2003). Most studies develop induction and regeneration systems for the model genotype Golden Promise which has high regeneration efficiency, but is agronomically obsolete (Wan and Lemaux 1994, Tingay et al. 1997) or for genotypes grown in Canada, U.S., Spain (Baillie et al. 1993, Castillo et al. 1998, Chant et al. 2003).

The goal of our research was to develop a regeneration system for the selected cultivars of barley grown in the Czech Republic that would be efficient and suitable for transformation using a biolistic method. Based on the study the influence of three synthetic auxins on the callus induction and subsequent plant regeneration was observed and discussed.

MATERIAL AND METHODS

Plant material

Twelve selected genotypes of spring barley registered in the Czech Republic: Akcent, Amulet, Atribut, Forum, Granát, Orbit, Viktor, Nordus, Orthega, Scarlett, breeding lines SG-S-252, SG-S-261, and genotype Golden Promise, were used to study the callus induction and subsequent plant regeneration. The seed was provided by the ARI Kroměříž, Ltd.

The donor plants were grown under controlled conditions in soil in growth chambers. The first week the plants were left in a cold chamber at 6°C at an 8-h photoperiod. Then the plants were transferred to the growth chamber at 12/10°C day/night temperature and a 12-h photoperiod for one month. Afterwards, the temperature was increased to 19/17°C day/night at a 16-h photoperiod with light levels of 500 μmol/m^2/s. The plants were treated with commercial N:P:K fertilizer and pesticide application once a week. Immature spikes were harvested 10–15 days after anthesis when the immature embryos were approximately 1.5–2.0 mm in length. The immature seed was removed and surface sterilised first by washing in 70% ethanol for 1 min, rinsed with sterile water and then with 6% solution of sodium hypochlorite with drops of TWEEN for 20 min and subsequently grains were rinsed with sterile water three times. The immature embryos were dissected from immature caryopses. Coleoptile and coleorhiza areas were removed from each immature embryo using forceps and a scalpel.

Culture condition

Scutellums were placed side up on the callus induction medium CI (Wang and Lemaux 1994) containing the basal salts of Murashige and Skoog (1962) supplemented with 350 mg/l myo-inositol, 690 mg/l proline, 1 mg/l thiamine-HCl, 1 mg/l casein hydrolysate, 30 g/l maltose, 1.25 mg/l $CuSO_4$, solidified with 3.5 g/l phytagel and one of three synthetic auxins: 2,4-D (2.5 mg/l), dicamba (2.5 mg/l) or picloram (2.0 mg/l), pH was adjusted to 5.8. Fifty scutellums of each cultivar were cultured on petri dishes with a medium always containing one of the three above described synthetic auxins. Resulting calluses were cultured in the dark at 25°C and transferred at 2 weeks intervals to fresh induction medium. The callus induction frequency was assessed 4 weeks after culture establishment and it was evaluated as the percentage of scutellums forming calluses/number of scutellums plated. Calluses were divided into small pieces after they had been cultured for 4 weeks in the dark and transferred to a modified regeneration medium FW (Harwood et al. 1995), containing 20 g/l maltose, 1.25 mg/l $CuSO_4$ but without phytohormones. Regeneration plates were cultivated at 25°C in the light (12 hours per day). Plantlets were counted and discarded. Less-

Table 1. Analyses of variance of number of regenerants

Sources of variability	*d.f.*	*SS*	*MS*
Varieties	12	934.775	77.898***
Auxins	2	635.088	317.544***
Interaction varieties × auxins	24	485.739	20.239***
Residuum	1 911	4 095.188	2.143
Total	1 949	6 150.789	3.156

***$P \geq 0.001$

developed green plantlets and green shoots were transferred to a fresh regeneration medium. The number of plantlets regenerated from one primary explant (one immature scutellum) was counted after 4–6 weeks of culturing on the regeneration media.

The ploidy level of regenerated plants was evaluated by flow-cytometric determination of nuclear DNA measured on the flow cytometer PARTEC II.

Statistical analysis

The regeneration efficiency was assessed as the mean of the number of green plants/number of cultured scutellums. The variables of total plants/scutellum were transformed ($x' = \sqrt{x + 3/8}$) (Chloupek 1996). The final means in Tables 2 and 3 are non-transformed. The analysis of these variables was carried out using the programme UNISTAT, the two-way analysis of variance (ANOVA). Tukey's test (at $P \leq 0.05$) was used for multiple mean comparisons.

RESULTS

Successful barley regeneration efficiency is affected by a number of factors such as genotypes, medium composition including growth regulators and conditions of culture.

The length of 1.5–2.0 mm of immature zygotic embryos, used by us, proved to be the best for callus induction and plant regeneration. No albino plants were detected among the obtained regenerants.

We found out that the frequency of callus induction varied from 85 to 100% in dependence on the genotype and auxin used. In five of the 13 barley cultivars the callus induction on the medium with dicamba was higher, in three cultivars it was higher on the medium with 2,4-D, in five cultivars no differences among the used auxins were observed. The structure of calluses depended on genotype and auxin used and it ranged from hard, compact, nodular, yellowish to soft, watery, loose, friable, creamy white to translucent. The callus formed after the induction on the medium with 2,4-D was crumbly, intensively growing, and the formation of somatic embryos was observed, the character of callus after dicamba was compact, hard, deep yellow and the formation of somatic embryos was observed as well. A soft, watery and frequently rooting callus was observed after picloram in most varieties (Figures 1 and 2).

The number of green regenerants was assessed 4–6 weeks after culture establishment. According to the Table 1 showing the analysis of variance

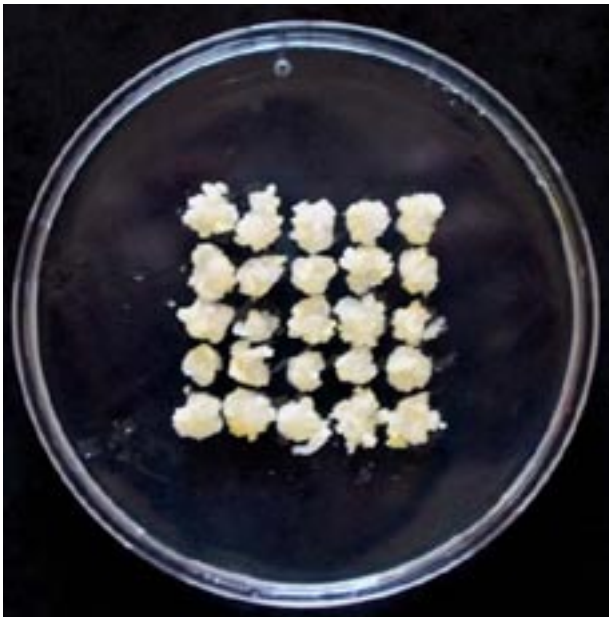

Figure 1. Somatic embryogenesis from scutellum of zygotic embryos of spring barley (cv. Viktor), 2 weeks after cultivation on CI medium (with dicamba)

the variability of a number of green regenerants was significantly influenced by both genotype and auxin used. Interactions of the genotype and auxin concentration was statistically significant too, it means that each cultivar responded to the auxin used in the medium uniformly.

Golden Promise with the average number of regenerants (average for all auxins) 2.70 plants per one cultivated scutullum, Atribut (2.09), Forum (1.97) and Scarlett (1.57) differed significantly from the cultivars Nordus, Amulet, Akcent,

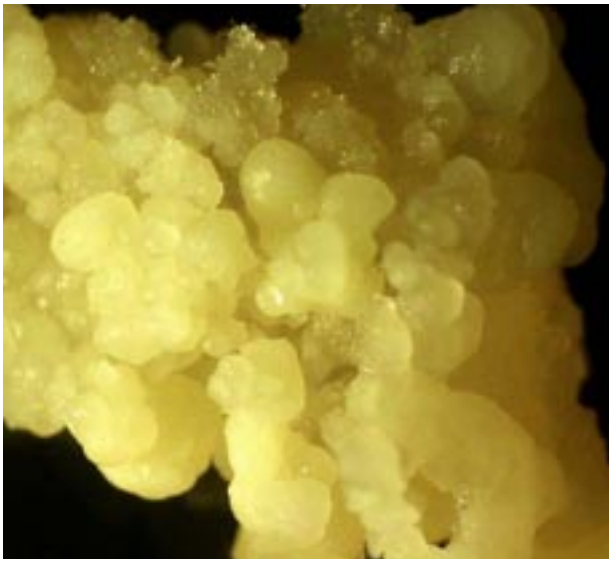

Figure 2. Embryogenic callus derived from scutelum of immature zygotic embryo (cv. Viktor)

Table 2. The means (for three types of auxins) of regenerants for varieties

Varieties	Means	Differences
Nordus	0.42	a
Amulet	0.45	a
Akcent	0.51	a
SG-S-252	0.58	a
Orbit	0.64	a
Granát	0.92	a
Viktor	0.97	ab
SG-S-261	1.04	ab
Orthega	1.05	ab
Scarlet	1.57	bc
Forum	1.97	c
Atribut	2.09	cd
G. Promise	2.70	d

Means with various letters are statistically different ($P \leq 0.05$)

SG-S-252, Orbit, Granát which had a statistically lower average number of regenerants (0.42–0.92) per one cultivated scutellum as compared to the above mentioned varieties. The variety Orthega (1.05), Viktor (0.97) and the line SG-S-261 (1.04) had a medium regenerant number, but not a statistically significantly different from the cultivar Scarlett and the cultivars with the lowest number of regenerants (Table 2).

Most of varieties produced the most regenerants when the calluses induced on the medium contained 2.4-D [Figure 3, i.e. Golden Promise (4.20 regenerants per one scutellum), Forum (4.0), Atribut (3.33), Scarlett (3.22)]. Although statistically significant difference between the effect of dicamba and picloram on regeneration was not recorded in an average for all varieties, the statistically significantly higher number of regenerants was determined in three varieties (Atribut, Orthega, Granát) when using dicamba versus picloram. Only in the variety Nordus and a newly bred variety SG-S-261, no statistically significant difference between the numbers of regenerants obtained after induction on the medium with different auxins was observed. Significant difference in the number of regenerates was recorded in the only variety of all thirteen studied varieties (Atribut) according to the auxin used in the medium. Different reactions of some varieties in the number of regenerants can be attributed to their statistically significant interaction with auxins used (Table 1).

This suggests that the most regenerants in an average for all varieties were obtained after the callus induction on the medium with 2,4-D, it was statistically significant as compared to dicamba or picloram (Table 3, Figure 4). The number of regenerants obtained after callus induction on these two auxins was not statistically significantly different.

Ploidity of the obtained regenerants was analysed in the framework of the experiment. Number of changes had occurred in the course of somatic embryogenesis and plant regeneration under *in vitro* conditions. Approximately 300 regenerated plants that were measured had a diploid number of chromosomes ($2n = 2x = 14$). Aneuploid, and polyploid chromosome numbers was not detected in any plant.

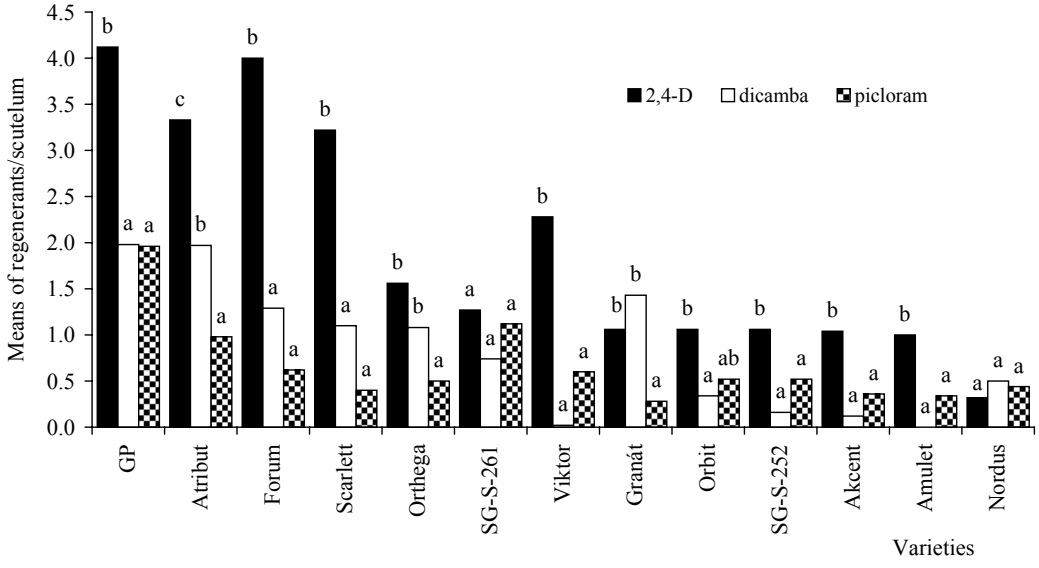

Figure 3. Average number of regenerants for particular varieties on particular auxins; means with various letters are statistically different ($P \leq 0.05$)

Table 3. Mean number of regenerants (for all varieties) on particular auxins

Growing regulator	Mean	Significance
Picloram	0.66	a
Dicamba	0.83	a
2,4-D	1.95	b

Means with various letters are statistically different ($P \leq 0.05$)

DISCUSSION

Chang et al. (2003) found out that small immature zygotic embryos (0.5–1.5 mm) had 100% callus formation and a higher efficiency of plant regeneration than those with large size, which corresponds with the results of our study. No albino plants occurred among the regenerants obtained from embryos sized 1.5–2.0 mm. Chang et al. (2003) did not record the occurrence of albino plants at the size of explants 0.5–1.0 mm, while at the size of immature embryos 2.6–3.0 mm approximately 50% of the regenerants plants were albino plants. These results clearly showed that the development stage of the explant, i.e. the size of the embryos, was a primary determinant of the number of the albino plants generated. Bregitzer and Campbell (2001) and Wang et al. (2002), reported that the regeneration of albino plants *in vitro* was quite common in some cereal crops and might be under genetic control. Bregitzer et al. (1998) and Dahleen and Bregitzer (2002) also reported a reduction in the frequency of albino plants by modified medium components and improved media. This indicates that the frequency of albino plant regeneration might be affected by multiple factors.

In our case the frequency of callus induction depended on genotype and auxin used and ranged from 85 to 100%. The highest initiation of callus was on the medium with dicamba in five varieties and on the medium with 2,4-D in three varieties. Similarly, Jiang et al. (1998) tested two barley cultivars Golden Promise and the recalcitrant commercial cultivar Galena and found out that two auxins (dicamba and 2,4-D) alone were the best of all tested phytohormones because callus induction frequencies were nearly 100%. Bregitzer (1992) also observed that callus formation was affected by genotype, medium, and their interaction. Genotype was the primary source of the variation.

In the present study the character of callus was dependent on the variety and auxin. Yellowish and crumbly calluses originated on the medium with 2,4-D; creamy white to transparent, compact and soft calluses originated on the medium with dicamba.

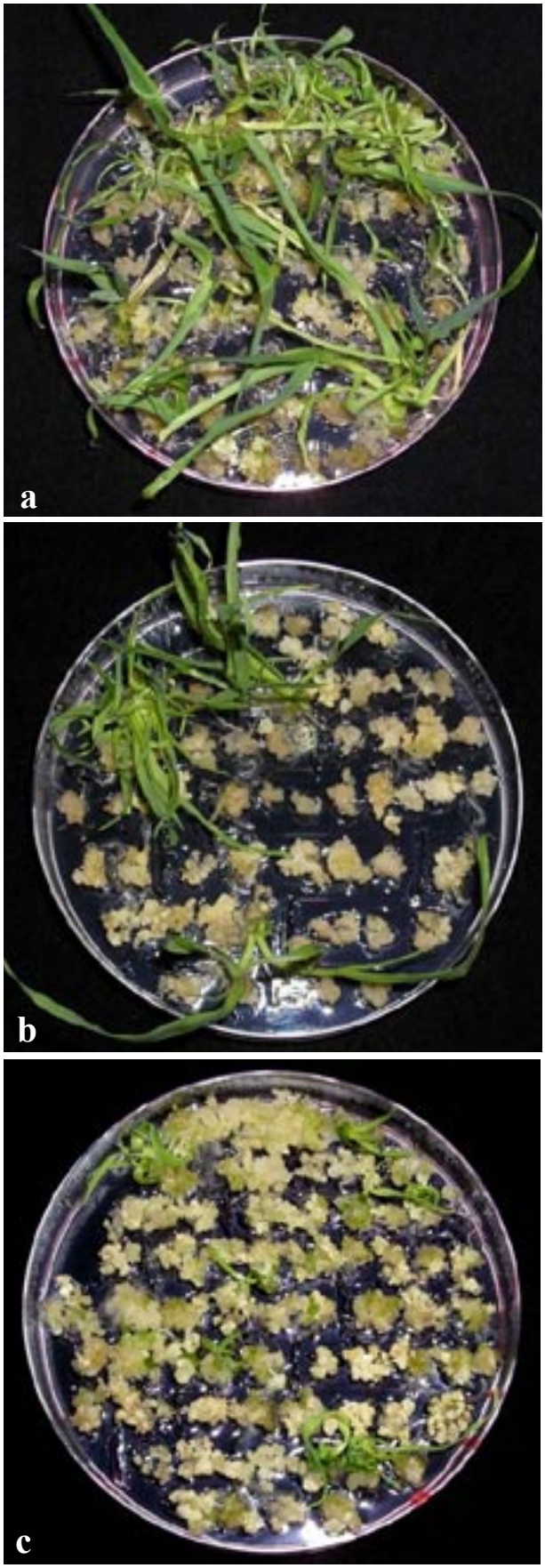

Figure 4. Green plant regeneration of Czech cv. Viktor; scutelum were cultivated on CI induction medium with auxins (a = 2,4-D, b = picloram, c = dicamba)

The same type of callus was also observed by Ruíz et al. (1992), Bregitzer et al. (1995) and Castlillo et al. (1998).

The use of 2,4-D in induction medium has proven a statistically significant effect on a bigger amount of regenerated plants than the use of dicamba or picloram in the most varieties tested by us. Bregitzer et al. (1998) indicated that the number of green plants per embryo was significantly influenced by the genotype and the interactions of genotype with the concentrations of copper and with 2,4-D. The concentration of 2,4-D (2.5 mg/l) used by us corresponds with the results of Chang et al. (2003), i.e. low concentrations (1.5 mg/l) of dicamba or 2,4-D were less efficient for optimal regeneration than the higher concentrations (3 mg/l). Baillie et al. (1993) as well, obtained maximum regeneration rates with 2.5 mg/l 2,4-D. Steven and Kasha (1994), however, raised the 2,4-D concentration to 5 mg/l for callus induction, more somatic embryos were induced on 5 mg/l 2,4-D than 2 mg/l. But there was not any significant difference in green plant regeneration. Lührs and Lörz (1987) suggested that the induction of embryonal callus depended on the concentration of particular auxins contained in the induction medium; 2,4-D, dicamba, picloram and 2,4,5-T proved useful here.

Przetakiewitz et al. (2003) obtained the best response of seven out of eight barley cultivars to dicamba with 2,4-D or dicamba alone. The results of our study, however, suggest that the use of dicamba alone lead to a lower regeneration capacity in the average of barley genotypes tested by us. Conformably with this author we found out statistically significantly lower regeneration after using picloram alone. But Trifanova et al. (2001) reported that plant regeneration from barley transgenic callus was difficult to obtain when 2,4-D was used, dicamba may thus could be a better choice for transgenic barley plant regeneration.

Although there was not any statistically significant difference between the effect of dicamba and picloram on regeneration in all variety averages, we determined statistically significantly higher number of regenerants with the use of dicamba versus picloram in three varieties (Atribut, Orthega, Granát). Similar results are presented by Castillo et al. (1998) for varieties grown in Spain.

From statistically significant varietal differences in a number of regenerated plants determined in our study we can together with Bregitzer (1992) conclude that the genotype of donor barley plants is one of the decisive factors affecting regeneration *in vitro.*

Although the callus may maintain its regeneration ability for longer than six subcultures, Choi et al. (2001) present that prolonged subculturing could lead to a higher frequency of somatic mutation, especially with high concentrations of 2,4-D in the medium. In the regenerated plants obtained in our work, aneuploid or polyploid number of chromosomes was not recorded in the used auxin concentrations and two subcultures of induction.

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REFERENCES

Baillie A.M.R., Rossnagel B.G., Kartha, K.K. (1993): Evaluation of 10 Canadian barley (*Hordeum vulgare* L.) cultivars for tissue culture response. Can. J. Plant Sci., *73*: 171–174.

Bregitzer P. (1992): Plant regeneration and callus type in barley: effect of genotype and culture medium. Crop Sci., *32*: 1108–1112.

Bregitzer P., Campbell R. (2001): Genetic markers associated with green and albino plant regeneration from embryogenic barley callus. Crop Sci., *41*: 173–179.

Bregitzer P., Campbell R.D., Wu Y. (1995): Plant regeneration from barley callus: Effects of 2,4-dichlorophenoxyacetic acid and phenylacetic acid. Plant Cell Tiss. Org. Cult., *43*: 229–235.

Bregitzer P., Dahleen L.S., Campbell R.D. (1998): Enhancement of plant regeneration from embryonic callus of commercial barley cultivars. Plant Cell Rep., *17*: 941–946.

Castillo A.M., Egana B., Sanz J.M., Cistue L. (1998): Somatic embryogenesis and plant regeneration from barley cultivars grown in Spain. Plant Cell Rep., *17*: 902–906.

Chang Y., Zitzewitz J., Hayes P.M., Chen T.H.H. (2003): High frequency plant regeneration form immature embryos of an elite barley cultivars (*Horedum vulgare* L. cv. Morex). Plant Cell Rep., *21*: 733–738.

Chloupek O. (1996): Zemědělský výzkum. Academia, Praha.

Choi H., Lemaux P.G., Cho M. (2001): Selection and osmotic treatment exacerbate cytological aberration in transformed barley (*Hordeum vulgare* L.). J. Plant Physiol., *158*: 935–943.

Conger B.V., Hanning G.E., Gray D.J., McDaniel J.K. (1983): Direct embryogenesis from mesophyl cells of orchard grass. Science, *221*: 850–851.

Dahleen L.S., Bregitzer P. (2002): An improved media system for high regeneration rates from barley immature embryo-derived callus cultures of commercial cultivars. Crop Sci., *42*: 934–938.

Eudes F., Acharya S., Laroche A., Selinger L.B., Cheng K.J. (2003): A novel method to induce direct somatic embryogenesis, secondary embryogenesis and regenera-

tion of fertile green cereal plants. Plant Cell Tiss. Org. Cult., *73*: 147–157.

Ganeshan S., Båga M., Harwey B.L., Rossnagel B.G., Scoles G.J., Chibbar R.N. (2003): Production of multiple shoots from thiadiazuron-treated mature embryos and leaf-base/apical meristems of barley (*Hordeum vulgare*). Plant Cell Tiss. Org. Cult., *73:* 57–64.

Harwood W.A., Bean S.J., Chen D.-F., Mullineaux P.M., Snape J.W. (1995): Transformation studies in *Hordeum vulgare* using a highly regenerable microspore system. Euphytica, *85*: 113–118.

Jiang W., Cho M.J., Lemaux P.G. (1998): Improved callus quality and prolonged regenerability in model and recalcitrant barley (*Hordeum vulgare* L.) cultivars. Plant Biotechnol., *15*: 63–69.

Karp A., Steele S.H., Parmar S., Jones M.G.K., Shewry P.R., Breiman A. (1987): Relative stability among barley plants regenerated from cultured immature embryos. Genome, *29*: 405–412.

Lührs R., Lörz H. (1987): Plant regeneration *in vitro* from embryogenic cultures of spring- and winter-type barley (*Hordeum vulgare* L.) varieties. Theor. Appl. Genet., *75*: 16–25.

Murashigee T., Skoog F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum, *15*: 473–497.

Przetakiewicz A., Orczyk W., Nadolska-Orczyk A. (2003): The effect of auxin on plant regeneration of wheat, barley and triticale. Plant Cell Tiss. Org. Cult., *73*: 245–246.

Ruíz M.J., Rueda J., Peláez M.I., Espino F.J., Candela M., Sendino A.M., Vázquez A.M. (1992): Somatic embryogenesis, plant regeneration and somaclonal variation in barley. Plant Cell Tiss. Org. Cult., *28*: 97–101.

Steven P.K., Kasha K.J. (1994): Optimizing somatic embryogenesis and particle bombardement of barley (*Hordeum vulgare* L.) immature embryos. In Vitro Cell. Dev. Biol., *30P*: 117–123.

Thomas M.R., Scott K.J. (1985): Plant regeneration by somatic embryogenesis from callus initiated from immature embryos and immature inflorescences of *Hordeum vulgare* L. J. Plant Physiol., *121*: 159–169.

Tingay S., McElroy D., Kalla R., Fieg. S., Wang M., Thornton S., Brettell R. (1997): *Agrobacterium tumefaciens*-mediated barley transformation. Plant J., *11*: 1369–1376.

Trifanova A., Madsen S., Olesen A. (2001): *Agrobacterium*-mediated transgene delivery and integration into barley under a range of *in vitro* culture conditions. Plant Sci., *161*: 871–880.

Wan Y., Lemaux P.G. (1994): Generation of large number of independently transformed fertile barley plants. Plant Physiol., *104*: 37–48.

Wang Z., Lehmann D., Bell J., Hopkins A. (2002): Development of an efficient plant regeneration system for Russian wildrye (*Psathyrostachys juncea*). Plant Cell Rep., *20*: 797–801.

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ABSTRAKT

Indukční a regenerační schopnost *in vitro* u odrůd jarního ječmene registrovaných v ČR

Nezralá zygotická embrya jsou nejčastěji používaným explantátem pro indukci somatických embryí a kalusů u ječmene. Odrůdy s vysokou indukční a regenerační schopností *in vitro* jsou pak velmi často využívány pro genetické transformace. U 12 odrůd jarního ječmene registrovaných v ČR a modelové odrůdy Golden Promise, vyznačující se vysokou indukční a regenerační schopností, byl sledován vliv tří syntetických auxinů: kyselina 2,4-dichlorfenoxyoctová (2,4-D), kyselina 3,6-dichloro-2-methoxybenzoová kyselina (dicamba), kyselina 4-amino-3,5,6-trichlorpikolinová (picloram) na indukční a následnou regenerační schopnost ječmene *in vitro*. Frekvence tvorby kalusů a počet zelených regenerantů byly signifikantně ovlivněny jak genotypem, tak auxinem. Statisticky významně vyšší průměrný počet regenerantů (1,95) tvořila většina odrůd po indukci kalusu na médiu s 2,4-D oproti médiím s picloramem a dicambou. Pouze dvě odrůdy (Nordus a SG-S-261) nereagovaly na použité auxiny rozdílně. Nejvyšší průměrný počet regenerantů (ze všech tří auxinů) byl získán u odrůdy Golden Promise (2,7 rostliny na jeden kultivovaný explantát). Z testovaných odrůd jarního ječmene byl nejvyšší počet regenerovaných rostlin získán u odrůd Atribu (2.09), Forum (1.97) a Scarlett (1.57), které se tak statisticky významně lišily od odrůd Nordus, Amulet, Akcent, SG-S-252, Orbit a Granát s nejnižším průměrným počtem regenerantů (0.42–0.92). Statisticky významná interakce odrůd s hormony se projevila v rozdílném počtu získaných regenerantů u odrůd v závislosti na použitém auxinu.

Klíčová slova: nezralá zygotická embrya; 2,4-D; picloram; dicamba; jarní ječmen

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