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Cultivar and environmental conditions affect the morphogenic ability of barley (*Hordeum vulgare*) scutellum-derived calli

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The morphogenic ability of immature scutella-derived cultures of ten barley cultivars was screened. Plants were field-grown during two seasons. All cultivars were able to produce callus and regenerate green plants. The embryogenesis and morphogenic ability of scutella was independent of callogenesis. The best responding cultivars in the first season were Golden Promise, Kosan and Novum (52.2–57%), in the second Amulet, Orbit and Kosan (24.4–40%). Differences between cultivars were statistically significant in both experiments. Despite the same regeneration protocol, the regeneration frequencies differed significantly between experiments in the two years probably because of the environmental conditions variability during the two seasons. Our results are consistent with previous reports indicating genotype-dependent plant morphogenesis and the effect of environmental factors on somatic embryogenesis and regeneration of barley.

Key words: *Hordeum vulgare*, environmental factors, genotype, somatic embryogenesis, plant regeneration.

Introduction

Techniques of genetic engineering for crop improvement are dependent on efficient *in vitro* regeneration systems. Plant regeneration *in vitro* is possible via organogenesis or somatic embryogenesis. In barley (*Hordeum vulgare* L.) somatic embryogenesis is the most frequently used way of regeneration and micropropagation (Thomas & Scott, 1985; Lührs & Lörz, 1987; Bregitzer, 1992). Production and the quality of barley cultures vary with the explant origin (Dale & Deambrogio, 1979). Different explants have been used for the induction of embryogenic calli (apical meristems, mature and immature embryos, immature inflorescences, mesocotyls, leaf bases), however immature embryos give the best results as explant sources.

Morphogenesis of barley *in vitro* is influenced by many factors, such as media composition, genotype, environmental conditions of the donor plant and their mutual interactions. In a number of species the plant regeneration *in vitro* is genetically determined. In barley, six loci influencing plant morphogenesis from callus were identified (Komatsuda et al., 1995; Mano et al., 1996; Bregitzer & Campbell, 2001). Genotype (Hanzel et al., 1985; Lührs & Lörz, 1987; Bre-

Table 1. Temperature and precipitation in 1999 and 2000.

	average temperature		precipitation	
	°C		mm	
	1999	2000	1999	2000
April	11.8	14.6	46.2	19.2
May	16.2	17.9	32.4	34.3
June	18.8	19.7	144.9	38.1

GITZER, 1992; CASTILLO et al., 1998), as well as environmental factors of the donor plant strongly influence somatic embryogenesis and plant regeneration in this species (DAHLEEN, 1999; BREGITZER & CAMPBELL, 2001).

The objective of our study was to evaluate the morphogenic ability of immature scutella-derived cultures of 10 barley cultivars registered in Slovakia. The purpose was to identify cultivars with high morphogenic ability and to describe the effect of both genotype and environmental conditions of the donor plant, on the regeneration of barley *in vitro*.

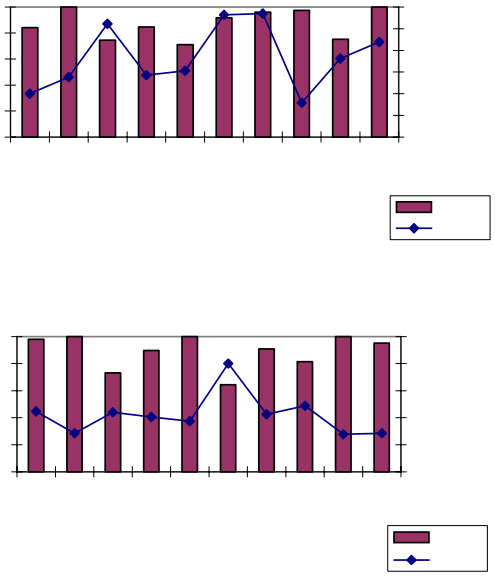

Fig. 1. The dependence of scutella callogenic frequency (%C) and regeneration frequency (%RCL) of 10 barley cultivars on the WL medium (A – year 1999, B – year 2000).

Material and methods

Ten cultivars of barley were used for the experiment: 3 Slovak (Kompakt, Kosan, Zlatan), 6 Czech (Amulet, Galan, Jubilant, Novum, Orbit, Perun) and a model cultivar Golden Promise. All cultivars were field grown during the seasons 1999 and 2000 (Tab. 1).

Immature caryopses (12–15 days post-anthesis) were treated with ethanol for 1 min, with 0.1% $HgCl_2$ solution for 10 min, and rinsed five times in sterile distilled water for 1, 5 and 10 min. Immature embryos (1–2 mm) were aseptically excised from caryopses and scutella were placed on callus inducing medium. Twenty-five embryos were plated in a Petri dish, 100 embryos were isolated in each cultivar. Medium WL (WAN & LEMAUX, 1994) was supplemented with 2.5 mg L^{-1} Dicamba, 60 g L^{-1} maltose and solidified with 3.5 g L^{-1} Phytagel. The media were adjusted to pH 5.8 and autoclaved for 20 min at 120 °C. Only Dicamba was filter-sterilised and added after autoclaving. Explants were cultivated for 28 days at 27 °C in the dark. Induced calli were dissected into 5 mm pieces and transferred onto the regeneration medium (= induction medium without Dicamba, maltose reduced to 30 g L^{-1}). Cultures were maintained in 16h photoperiod, light intensity approximately 50 μmol m^{-2} s^{-1}, at 25/20 °C for 4 weeks. Then regenerated shoots (longer than 5 mm) were counted. Characteristics evaluated were: callogenic frequency (C), regeneration frequency (percentage of callus lines with minimum one regenerated shoot from all callus lines, RCL), number of regenerated shoots (REG), number of regenerated shoots in a regenerating callus line (REG/RCL), regeneration efficiency (number of regenerated shoots per explant, RE). Data were analysed with Wilcoxon test, percentage after transformation *via* analysis of variance (ANOVA).

Results and discussion

All cultivars were able to produce callus and regenerate plants on WL medium. Dicamba (2.5 mg L^{-1}) instead of usually used 2,4-D was applied, because it was found more suitable for barley regeneration (KUBRANOVÁ et al., 1999). From the comparison of callogenesis and regeneration frequency we have concluded, that embryogenesis and morphogenic ability are independent of the callogenic frequency (Fig. 1). GOLDSTEIN & KRONSTAD (1986) reported negative relationship between the fresh weight of barley callus and plant regeneration. Also in experiments of BREGITZER (1992) the ability of the genotype to form callus was not correlated with the ability to regenerate green plants.

Table 2. Callogenesis and plant morphogenesis of 10 barley cultivars from immature scutella in two years (1999 and 2000, SD-statistical difference, %C-callogenic frequency, REG-number of regenerated shoots, REG/RCL-number of regenerated shoots per regenerating callus line, *−95%, **−99%).

Cultivar	*%C*		*REG*			*REG/RCL*		
	1	2	1	2	SD	1	2	SD
Amulet	84.21 ± 1.58	97.92 ± 3.26	38 ± 0.8	34 ± 0.56	–	2.37 ± 0.05	1.62 ± 0.03	–
Galan	100 ± 0	100 ± 0	37 ± 1.1	15 ± 0.31	*	2.85 ± 0.09	1.25 ± 0.03	*
GP	74.44 ± 5.05	73.17 ± 28.12	129 ± 2	19 ± 0.5	**	3.68 ± 0.06	1.46 ± 0.04	**
Jubilant	84.62 ± 7.26	89.77 ± 7.09	61 ± 1.1	25 ± 0.5	–	2.77 ± 0.05	1.56 ± 0.03	–
Kompakt	71.01 ± 9.27	100 ± 0	35 ± 1	25 ± 0.45	–	2.33 ± 0.07	1.47 ± 0.03	*
Kosan	91.67 ± 6.27	64.29 ± 36.17	102 ± 2	39 ± 1.04	*	3.29 ± 0.06	2.17 ± 0.06	–
Novum	95.88 ± 4.36	90.8 ± 11.2	160 ± 1.8	25 ± 0.49	**	3.02 ± 0.03	1.47 ± 0.03	**
Orbit	97.44 ± 4.92	81.4 ± 17.52	15 ± 0.7	63 ± 1.22	–	2.5 ± 0.11	3.32 ± 0.06	–
Perun	75.32 ± 7.22	100 ± 0	86 ± 2	24 ± 0.64	**	4.1 ± 0.09	2.67 ± 0.07	*
Zlatan	100 ± 0	95.06 ± 5.5	56 ± 1.1	16 ± 0.36	**	2.24 ± 0.04	1.45 ± 0.03	**

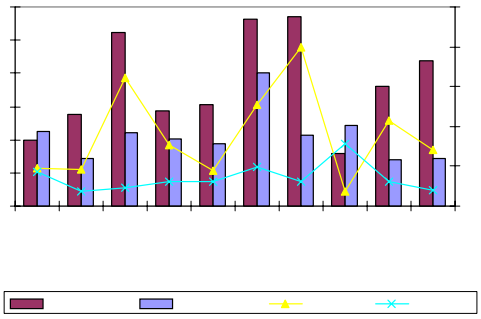

Fig. 2. Effect of genotype and vegetation seasons (1999 and 2000) on the regeneration frequency (%RCL) and regeneration efficiency (RE) of barley scutella-derived callus cultures.

The results from the season 1999 are presented in Table 2 and Fig. 2. Explants of all cultivars formed calli after four weeks of cultivation. Differences were statistically significant. Cultivars Galan, Kosan, Novum, Orbit and Zlatan showed callogenic frequencies higher than 90%. Regeneration frequency varied from 15.79 to 56.99%. The best responding cultivars were Novum, Kosan and Golden Promise (GP). Differences were significant between three cultivars. Regeneration efficiency ranged from 0.15 to 1.6 with significance (cultivars Golden Promise, Kosan and Novum regenerated more than one shoot per explant). Moreover, the effect of the genotype was considerable also in the number of regenerated shoots per regenerating callus line.

Explants of all cultivars tested produced calli and regenerated shoots in the season 2000. No statistical differences in callogenic and regeneration frequency in this experiment were found. The highest morphogenic values were observed in cultivars Kosan, Orbit, Amulet and Golden Promise (GP) (Fig. 2). Number of regenerated shoots in regenerating callus lines varied between 1.25–3.32 (Tab. 2). Significant differences were estimated in the regeneration efficiency (0.15–0.63). In this experiment none of the cultivars used regenerated more than one shoot per explant. Cultivars Amulet, Kosan and Orbit regenerated with higher frequency in comparison with the model cultivar Golden Promise.

Genotypic control of morphogenesis is described for various cereals (maize, wheat, oat, etc.). We obtained different regeneration frequencies and efficiencies in 10 cultivars of barley. In the work of Hanzel et. al. (1985) 45 of 91 genotypes formed callus and 8 of them regenerated with a frequency lower than 15%. Goldstein & Kronstad (1986) reported a regeneration efficiency 0.1–2.1 of shoots per explant in 19 of 20 genotypes. Bregitzer (1992) evaluated morphogenesis of 15 American genotypes. All of them were able to regenerate with frequencies 1–22%. Also other studies described genotype-dependent *in vitro* morphogenesis of barley (Lührs & Lörz, 1987; Castillo, 1998; Dahleen, 1999).

In both seasons the same regeneration protocol was used. Despite of this fact, the comparison indicates significant differences in shoot initiation depending on the season. Differences in regeneration efficiency occurred between the two years in 6 of 10 barley cultivars. In most of the cultivars better morphogenic ability in the first season has been observed. It confirmed the impact of donor

plant environment on *in vitro* morphogenesis. The summer 2000 was warmer with less rainfall compared with 1999.

Different climatic conditions in two years has been proposed to cause various frequencies of embryogenic callus induction in the barley cultivars tested (LÜRZ & LÖRZ, 1987). DAHLEEN (1999) indicated significant effect of genotype, planting date and donor plant environment on *in vitro* morphogenesis of barley and proposed that the impact of donor plant growth environment on regeneration can be as large as the effects of the genotype and media composition. Morphogenic responses varied within some barley lines between experiments of BREGITZER & CAMPBELL (2001). They found significant influence of growth chamber conditions. According to their model (based on QTLs associated with the morphogenesis), environmental factors could play a major role in the morphogenesis of green plants than genetic factors.

The objective of this study was to evaluate the morphogenic ability of 10 barley cultivars. Nine of them are registered in our country. We observed genotype and environmental effect on the morphogenesis in callus cultures. Statistically significant differences were found between cultivars and vegetation season of the two years. Our results are consistent with previous reports published that indicate genotype-dependent morphogenesis of callus cultures and also an influence of environmental factors on this process.

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