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**ARTICLE**

# Regeneration ability of wheat (*Triticum aestivum* L.) embryos after bombardment with a particle gun

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**ABSTRACT** The plant regeneration ability of the spring wheat variety Cadenza from the UK and the winter variety Mv Emese from Hungary was studied over the course of two years. The calli were regenerated with 10 hours illumination in the first year and 14 hours illumination in the second, and the external environment was not completely excluded, light being admitted through a window. The level of plant regeneration was evaluated 7–8 weeks after the isolation of the scutella. Both genotypes exhibited considerable fluctuation in plant regeneration over the two years, but no significant difference was observed in the mean plant regeneration ability of Mv Emese and Cadenza as a function of the two illumination periods
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**KEY WORDS**

particle bombardment,
*bar*,
plant regeneration,
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Breeders are able to improve the agronomic traits of wheat (*Triticum aestivum* L.) by exploiting the genetic variability of the species itself and by crossing it with closely related species (Bedő et al. 1998; McIntosh 1998). From the breeding point of view the aim of transformation is to create new varieties with better yield potential, resistance or breadmaking quality by improving these traits in varieties well adapted to the given climatic conditions (Lazzeri et al. 1997; Vasil 1998, Pellegrineschi et al. 2000). The first reports of successful wheat transformation were published by Vasil et al. (1992), who opened up the way for the introduction of genes into the wheat genome which could not be incorporated by natural means. Over the past 15 years many authors have reported on the development of transgenic genotypes.

In many cases the initial material used for biolistic gene transformation is the scutellum isolated from immature embryos, the uncut side of which is bombarded (Sparks and Huw 2004). In cereal species the DNA is either introduced into the plant material a few hours after isolation (Barro et al. 1997; Pellegrineschi et al. 2002) or 2–8-day calli are transformed (Vasil et al. 1992; Patori et al. 2001; Sparks and Jones 2004).

One major criterion for plant transformation is the existence of a tissue culture system with good *in vitro* plant–cell–plant regeneration (Shewry and Jones 2005). The regeneration ability of wheat callus is a quantitative trait, the genes responsible for it being located on various chromosomes (Galiba et al. 1986; Ben Amer et al. 1997). The regeneration ability, and thus the efficiency of transformation, depends to a great extent on the genotype (Fennel et al. 1996; Varsheny and Altpeter 2002), on the plant organ used for callus formation (Barro et al. 1999; Folling and Olesen 2001) and on the tissue culture conditions (He et al. 1989; Barro et al. 1999; Rákszegi et al. 2003). There have been many reports of studies on the tissue culture conditions and the composition of the nutrient media used for plant regeneration (Rasco-Gaunt et al. 1999; Zhang et al. 1999). Depending on the concentration and ratio of the plant hormones applied, either shoot or root regeneration may be initiated first (Dudits and Heszky 2003). The nutrient media most frequently used for species belonging to the *Triticeae* genus are MS (Murashige and Skoog 1962) and variants of this. Shoot regeneration requires a fairly high concentration of cytokinin, with or without auxin (Barro et al. 1998), while 5–10 mg/l zeatin has also been found to have a positive effect on regeneration. Root regeneration takes place on hormone-free medium (Barro et al. 1999; Sparks and Jones 2004). High concentrations of sugars or sugar alcohols in the nutrient medium, or the presence of various metal ion additives (e.g. silver) may increase the efficiency of plant regeneration in the case of bread wheat.

Wheat is the most sensitive to the environmental variables of temperature and photoperiod during the vegetative period (Slafer and Rawson 1994). Various recommendations can be found for the light conditions, ranging from 20 µmol $m^{-2}$ $s^{-1}$ with a 16-h photoperiod (Tamás et al. 2004) to 250 µmol $m^{-2}$ $s^{-1}$ with 12-h daylength (Sparks and Huw 2004). Vectors supplied with a ubiquitin promoter, such as pAHC20 or pAHC25, have a high level of expression in monocotyledonous plants (Alan et al. 1995), so they are widely used for wheat transformation.

Our aim was to study the regeneration reaction of two wheat varieties with different growth habit.

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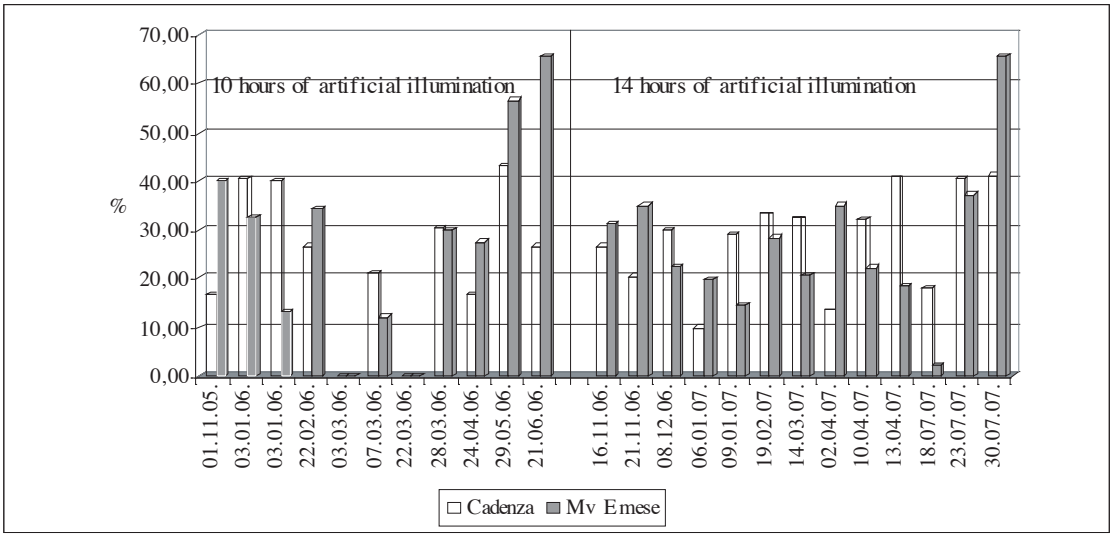


**Figure 1.** Percentage values of plant regeneration for the winter wheat Mv Emese and the spring wheat Cadenza under 10 and 14 hours of artificial illumination.

## Material and Methods

After germination, donor plants of the Cadenza spring variety were vernalised at 4°C for 2 weeks and those of Mv Emese winter variety for 6 weeks. The seedlings were then planted out and raised under constant phytotronic conditions at a day/night temperature of 18/16°C with 16-h daylength. Immature grains were collected 12–14 days after flowering, after which the scutella were isolated and the nutrient media prepared as described by Sparks and Huw (2004). The scutella were placed on medium designed to induce callus formation. After keeping them in the dark for two days, the scutella were transformed using a PDS-1000/He particle gun according to the manufacturer's instructions. A 28 Hgmm vacuum was created in the chamber and the helium gas was injected into the space above the macrocarrier at a pressure of 650 psi. The distance between the Petri dish containing the scutella and the macrocarrier was 5.5 cm. Gold particles with a diameter of 0.6 µm were suspended in distilled water at a density of 20 mg/ml and coated with pAHC25 plasmid DNA as described by Sparks and Huw (2004).

pAHC25 is a complete cassette containing the *bar* selection marker gene coding for the phosphinotricin acetyltransferase (PAT) enzyme, isolated from the microorganism *Streptomyces hygroscopicus*, and the *uidA* reporter gene coding for ß-glucoronidase, isolated from *E. coli*. The PAT enzyme inactivates the total herbicide phosphinotricin (PPT), which was applied to the nutrient media as a selection agent.

Two or three hours after bombardment the scutella were placed in fresh Petri dishes at an equal distance from each other and incubated in the dark at 23°C for three weeks. Scutella exhibiting callus formation were then transferred to shoot regeneration medium and kept in the light for a further three weeks.

Root and shoot regeneration were induced by illumination with cool white light at low intensity (20 µmol $m^{-2}$ $s^{-1}$) at a constant temperature of 23°C. In the first year of the experiment, between September 2005 and June 2006, a daylength of 10 hours was applied. In the second year (September 2006 to June 2007) this was increased to 14 hours. The external environment was not completely excluded: light was allowed in through a window facing north-east and measuring 0.72 m². After the sixth week calli exhibiting plant regeneration were placed on shoot regeneration medium containing 2 mg/l phosphinotricin for selection and the efficiency of plant regeneration was evaluated in the $6^{th}$–$7^{th}$ week. Plant regeneration was expressed as the percentage of calli producing shoots compared with the number of embryos isolated. The regeneration values of embryos isolated on the same day were grouped according to the date when they were transferred to the light. In both years approximately 1500 embryos of each genotype were isolated and bombarded.

## Results

The plant regeneration ability of the genotypes Mv Emese and Cadenza, isolated at the same time, was evaluated when exposed to 10- and 14-hour illumination (Fig. 1). The values were grouped according to the date when the cultures were placed in the light. In the case of 10-hour illumination the plant regeneration of Mv Emese exhibited greater deviation over the course than that of Cadenza (Fig. 1), with values ranging from 0–65.6% for Mv Emese and from 0–43.0% for Cadenza. Both genotypes had the lowest regeneration ability

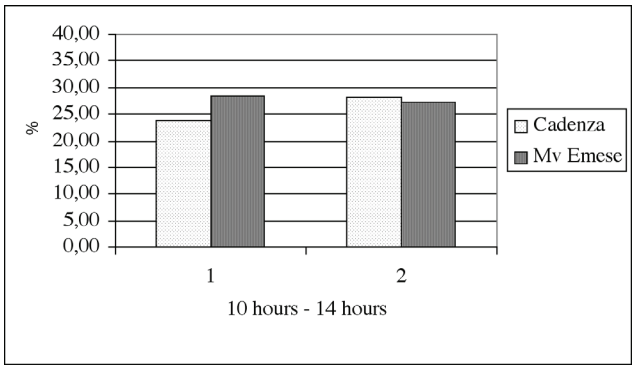


**Figure 2.** Mean plant regeneration from scutella of the wheat varieties Mv Emese and Cadenza under 10 and 14 hours of artificial illumination.

in March, when no plants were regenerated from the calli in two of the four experiments set up for each variety. The highest percentage of plant regeneration was recorded for Cadenza calli in May and for Mv Emese calli in June.

In the case of 14-hour illumination (Fig. 1) the difference between the minimum and maximum values of plant regeneration was again greater for Mv Emese (2.2–65.6%) than for Cadenza (10.0–41.3%), the lowest values being recorded in July for Mv Emese and in January for Cadenza, while the largest numbers of plants were regenerated from both Mv Emese and Cadenza calli in July. Greater differences were observed between the mean plant regeneration values of the two genotypes in the case of 10-hour illumination than for 14-hour illumination (Fig. 2), with values of 23.8% for Cadenza and 28.4% for Mv Emese at 10 hours illumination and 28.3% for Cadenza and 27.2% for Mv Emese at 14 hours.

## Discussion

A comparison was made of the plant regeneration ability of the spring wheat variety Cadenza (UK) and the winter wheat variety Mv Emese (Hungary). Cadenza has been used for years as a donor plant for wheat transformation, while Mv Emese was found by Tamás et al. (2004) to have good plant regeneration ability.

The experiments were carried out under the same conditions in two years, the only difference being the length of illumination. The regeneration ability of the two varieties did not differ significantly at illumination periods of 10 and 14 hours, though under 10 hours of artificial illumination the values recorded for Mv Emese exhibited greater differences than those of Cadenza. Both the maximum and mean plant regeneration values of Mv Emese were higher than those of Cadenza at this level of illumination. A larger number of plants could be regenerated from isolated scutella of Mv Emese wheat than from Cadenza under short-day illumination, when the external environment was not completely excluded. When the artificial illumination was increased to long-day conditions there was an improvement in the plant regeneration of Cadenza, while that of Mv Emese did not change to any great extent. In the case of 10-hour illumination in spring, both genotypes were incapable of plant regeneration in some cases, while this was not observed for 14-hour illumination. Both varieties exhibited maximum regeneration ability in summer in both daylenght.

The greatest level of plant regeneration was observed in June and July for Mv Emese calli and in May and July for Cadenza calli. Data from the literature confirm that plant regeneration is strongly dependent on the genotype (Varsheny and Alpenter 2002).

No significant difference was observed between the plant regeneration levels of isolated, callus-forming scutella of the two varieties despite the fact that one was a winter variety and the other a spring variety. The plant regeneration of both genotypes exhibited mean values of 24–28% in both years.

Cadenza is a more suitable donor plant for transformation as it only requires a few days of vernalisation, compared with six weeks for Mv Emese, which thus lengthens the time required for producing donor plants. Under the climatic conditions of Eastern Central Europe, however, Mv Emese is well adapted, so it can be recommended for use as a donor plant in transformation studies.

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