Medium optimization for efficient somatic embryogenesis and plant regeneration from immature inflorescences and immature scutella of elite cultivars of wheat, barley and tritordeum

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Summary
Media have been developed for somatic embryogenesis and plant regeneration from immature inflorescences and immature scutella of elite cultivars of wheat, barley and tritordeum. For wheat and tritordeum inflorescences, regeneration from embryogenic calluses induced on medium with picloram was almost twice as efficient as regeneration from cultures induced on 2,4-dichlorophenoxyacetic acid (2,4-D). The addition of zeatin at 5 or 10 mg l⁻¹ to regeneration media had a positive effect on regeneration. For scutella, the highest frequencies of embryogenesis (85%) and regeneration (50%) was obtained using an induction medium containing 2 mg l⁻¹ of 2,4-D and half concentration of aminoacids. The morphogenetic capacities of 19 different cultivars of wheat, barley and tritordeum were compared, and clear differences were found both between explants and genotypes. In wheat, embryogenic capacity from inflorescences (average of 92%) was higher than from immature scutella (average of 62%). However, shoot regeneration from scutella was clearly higher than from inflorescences (averages of 63% and 18% respectively). Frequencies of regeneration in wheat and barley varied widely among the cultivars tested and in both species no difference was found between spring and winter varieties.

Introduction
The transformation of all major cereals has now been achieved opening the way for the genetic engineering of new transgenic plants with modified agronomic traits, such as herbicide resistance, biotic and/or abiotic stresses resistance and grain quality and composition (Lazzeri & Shewry, 1994). However, in most species cereal transformation is still strongly genotype-dependent and efficiency is largely determined by the ability of the tissue to regenerate whole plants (Maddock et al., 1983; Mathias & Simpson, 1986; Fennell et al., 1996). In addition, explant source (Thomas & Scott, 1985; Vasil, 1987), and medium composition (Elena & Ginzo, 1988; He et al., 1989) are major factors influencing embryogenic response and regeneration. For wheat and barley, transgenic plants have been obtained using immature embryos as explant source (Vasil et al., 1993; Weeks et al., 1993; Becker et al., 1994; Nehra et al., 1994; Wan & Lemaux, 1994), but immature inflorescence is an alternative target for transformation. In the amphiploid tritordeum inflorescence tissues are highly responsive in vitro and are amenable to transformation (Barcelo et al., 1989, 1994). Advantages of using inflorescence tissue versus scutella are that explants are harvested from younger plants reducing glasshouse/growth chamber requirements and that physiological status of donor plants appear to have less influence on explant response in culture.

The aim of the present study was to establish standard media for embryogenesis induction and plant regeneration from immature inflorescences and immature scutella of commercial varieties of wheat and barley in order to develop efficient culture procedures for transformation experiments. Tritordeum was included...
in the experiments as a standard for inflorescence cultures.

Material and methods

Plant material

Eight current European cultivars of bread wheat (Triticum aestivum L.) and seven of barley (Hordeum vulgare L.) plus model tissue culture genotypes from wheat, barley and tritordeum (fertile amphiploid between Hordeum chilense Roem. et Schultz. and Triticum turgidum L. conv. durum, Martin & Sanchez-Monge, 1982) were grown in a greenhouse with supplementary light providing a 16 h photoperiod, with a day/night temperature regime of 16–18/14 °C.

In vitro culture

Immature scutella (0.5–1.5 cm in length) and immature inflorescences approximately 0.5 cm in length were used as explant for in vitro culture. Caryopses and tillers containing immature inflorescences were harvested and explants isolated as described by Barcelo and Lazzeri (1995). Ten immature scutella or ~1 mm inflorescence segments were placed in a petri dish containing induction medium (see below and Table 1), and a minimum of six petri dishes per genotype per explant were prepared. After three weeks at 26 °C in darkness, embryogenic capacity was assessed by counting the number of explants producing embryogenic callus. They were then transferred to regeneration medium (see below and Table 1) and cultured for a further three weeks. Regeneration capacity was assessed as the number of calluses producing shoots/number explants.

Immature inflorescence cultures

Preliminary experiments to examine the effects of the different hormones on embryogenesis and regeneration from immature inflorescences and immature scutella were made. As a result of these experiments inflorescences from wheat, barley and tritordeum were induced on L7AA medium supplemented with 2 mg l⁻¹ of either 2,4-Dichlorophenoxyacetic acid (2,4-D) or picloram. Embryogenic calluses were transferred to L7VD0.1 medium for regeneration.

To test the effect of different concentrations of picloram in induction medium immature tritordeum inflorescences were induced on L7AA basal medium supplemented with 2, 4, 6 or 8 mg l⁻¹ of picloram and regenerated on L7VD0.1 medium.

In a third experiment, the influence of zeatin on regeneration was tested inducing immature inflorescences from tritordeum on the best induction medium from the above experiments (4 mg l⁻¹ picloram) and transferred to regeneration medium (L7VD0.1) containing 0, 2.5, 5 or 10 mg l⁻¹ zeatin.

Immature scutellum cultures

Immature scutellum cultures from wheat cultivars ‘Pavon’ and ‘Florida’ were induced on four different induction media: MSLSD1, MSLSD2, MSLSAA/2D2 and MSLMD2, differing in their auxin, aminoacid and sugar composition (Table 1). Embryogenic calluses were transferred to L7VD0.1 medium supplemented with 5 mg l⁻¹ zeatin.

To study the effects of sugar on regeneration, wheat scutella from cultivars ‘Pavon’ and ‘Florida’ induced on MSLSAA/2D1 medium were regenerated on two regeneration media L7VD0.1 and L7VSD0.1 differing in their sugar composition (Table 1). Both media were supplemented with 5 mg l⁻¹ zeatin.

Inflorescence and scutellum cultures from elite cultivars of wheat and barley

Inflorescences and scutella from elite wheat and barley cultivars (listed in Table 3) were cultured on the optimal induction and regeneration media identified in previous experiments: L7AA medium supplemented with 4 mg l⁻¹ picloram for inflorescence cultures from wheat, barley and tritordeum, and MSLSAA/2 medium supplemented with 1 mg l⁻¹ 2,4-D for scutellum cultures of wheat and tritordeum. Barley scutella were induced on L1C7 medium supplemented with 2 mg l⁻¹ picloram (Nobre et al., 1997). All cultures were regenerated on L7VD0.1 medium supplemented with 5 mg l⁻¹ zeatin.

Statistical analysis

The Tukey (HSD) pairwise comparison of means was used to determine significant differences in embryogenesis and regeneration capacities from wheat and barley varieties. Data were transformed using the arcsin function.