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ORIGINAL ARTICLE

Optimization of multiple shoot induction and plant regeneration in Indian barley (*Hordeum vulgare*) cultivars using mature embryos

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KEYWORDS

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Abstract Barley is the fourth most important crop in the world. Development of a regeneration system using immature embryos is both time consuming and laborious. The present study was initiated with a view to develop a regeneration system in six genotypes of Indian barley (*Hordeum vulgare*) cultivars as a prerequisite to transformation. The mature embryos were excised from seeds and cultured on MS medium supplemented with high and low concentrations of cytokinins and auxins respectively. The MS medium containing 3 mg/L N^6-benzylaminopurine (BA) and 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) was found to be the most effective for multiple shoot formation in HOR7231 cultivar that could produce 12 shoots per explant. The other cultivars HOR4409 and HOR3844 produced a minimum number of adventitious shoots (1.33 and 1.67 respectively) on MS medium supplemented with 1 mg/L BA and 0.3 mg/L 2,4-D. The elongated shoots were separated and successfully rooted on MS medium containing 1 mg/L indole-3-acetic acid (IAA). The response of different barley cultivars was found to be varying with respect to multiple shoot production. This is the first report of multiple shoot induction and plantlet regeneration in Indian cultivar of barley which would be useful for genetic transformation.

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1. Introduction

Barley is an annual cereal grain crop that comes from the family of *Poaceae* and is the fourth most important crop in the world after wheat, maize and rice (faostat.org). It is consumed as a major food and feed for animals as well as in the brewing industry (Ritala et al., 1993). Barley seeds are unique as they accumulate up to 15% protein by dry weight and be stored for a period as long as 10 years (Tanasienko et al.,

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2011) and (Ritala et al., 2008). Considering its importance, there is a requirement for development of transgenic barley with new properties (Wan and Lemaux, 1994). Existence of high potent in vitro regeneration system is a prerequisite for efficient genetic transformation (Havrlentová et al., 2001). The regeneration system is supposed to be fast growing with stable plantlets suitable for hardening (Sood et al., 2011). Barley is one of the most difficult crops for in vitro tissue culture as it is a well known recalcitrant species like other monocots. Several explants of barley have been considered for their regeneration and transformation capacity and successful attempts have been reported including immature embryos (Wan and Lemaux, 1994), microspores (Jahne et al., 1994), protoplasts (Funatsuki et al., 1995), shoot meristematic cultures (Zhang et al., 1999), ovules (Holme et al., 2006). Other explants which were used for in vitro regeneration of cereal plants include: young leaves and leaf tissues (Saalbach and Koblitz, 1978) and (Lu et al., 1983), anthers (Haydu and Vasil, 1981), immature inflorescences (Havrlentová et al., 2001) and (Desai et al., 2004), nodal explants (Sharma et al., 2007), leaf base and apical meristems (Ganeshan et al., 2003) and (Ramesh et al., 2009).

Most regeneration protocols for barley are based on immature embryos in controlled conditions and the procedure for generation of donor plants is time consuming. Moreover, reproducible in vitro regeneration system even with immature embryos has mostly been restricted to a few cultivars (Li et al., 2009) like Golden Promise that is not commercially valuable. Nearly all the commercially important cultivars are poor in regeneration (Ganeshan et al., 2003) and have not been explored for in vitro studies (Chauhan and Kothari, 2004). On the other hand, explants like mature embryos and leaf base/apical meristems have several advantages over immature tissues as they are easier to handle, available throughout the year in a large quantity without any problems of seasonal effect on regeneration (Sharma et al., 2005a).

Direct regeneration of plants from explants is a faster and a time saving approach for obtaining whole plants without the callus interphase that can increase somaclonal variation (Zapata et al., 1999). Direct shoot induction and regeneration have been reported for some important cereals including oat (Zhang et al., 1996), maize (Zhang et al., 2002), sorghum (Baskaran and Jayabalan, 2005), barley (Sharma et al., 2004) and wheat (Sharma et al., 2005b). As the regeneration ability varies between genotypes, it is essential to screen different varieties for the response to in vitro culturing as it is a key step for genetic manipulation.

The objective of the present study was to investigate the regeneration capacity of barley cultivars originating from India using mature embryos as explant by direct shoot induction in order to use them for future molecular breeding programs.

2. Materials and methods

2.1. Preparation of explants

Barley seeds of six Indian varieties were provided by Gene Bank Department of Leibniz Institute of Plant Genetics and Cultivated Plant Research (IPK) – Germany. The accession numbers are shown in Table 1.

Seeds were dehusked and after washing with tap water surface sterilized by soaking in 70% ethanol for 1 min, rinsing with sterile distilled water, soaking in 0.1% (w/v) mercuric chloride solution for 7 min followed by rinsing with sterile distilled water (10 times). The seeds were kept in 250 ml conical flasks containing sterile water for 24 h at 4 °C. Mature embryos were excised from seeds by a scalpel and cultured on media.

2.2. Media composition, preparation and establishment of cultures

The seeds were sterilized after dehusking as described above and were incubated on direct shoot induction media (DSIM) for induction of multiple shoots. DSIM was MS basal media by Murashige and Skoog (1962) supplemented with high level of BA and low concentration of 2,4-D as shown in Table 1.

The cultures were kept in light at 22 ± 1 °C in 16/8 h of light/dark cycle regime with subculturing one in two weeks. Counting of multiple shoots was done for each explant after it was subcultured twice. The main shoots were removed during subculturing. Shoots which emerged from mature embryos directly without callus interphase were separated and transferred on rooting media. The regenerated plantlets were transferred on MS media containing 1 mg/L IAA for rooting.

2.3. Statistical analysis

In the direct shoot induction study the number of shoots per explants and efficiency of regeneration were recorded and ANOVA was conducted to investigate differences in above the parameters. All Statistical analysis was carried out by using DSAASTAT (available at: http://www.unipg.it/~onofri/DSAASTAT/DSAASTAT.htm).

3. Results and discussion

Mature embryos of six barley varieties were excised from 24 h imbibed surface sterilized seeds as initiation explants for direct embryogenesis on six different media containing different concentrations of BA and 2,4-D. Mature embryos started to germinate few days after culturing. The main shoots were cut to promote adventitious shoot initiation within the first week in order to retard the growth of primary shoots (Fig. 1A and

Table 1 Barley genotypes and combination of different media used in this study.

Cultivars used	HOR 3844, HOR 3272, HOR 4505, HOR 7231, HOR 4409, HOR 3838					
Levels of BAP and 24D in mg/L	1B + 0.3 D (1 mg/L BAP + 0.3 mg/L 2,4-D)	1B + 0.5D D (1 mg/L BAP + 0.5 mg/L 2,4-D)	2B + 0.3D D (2 mg/L BAP + 0.3 mg/L 2,4-D)	2B + 0.5D D (2 mg/L BAP + 0.5 mg/L 2,4-D)	3B + 0.3D D (3 mg/L BAPP + 0.3 mg/L 2,4-D)	3B + 0.5D D (3 mg/L BAP + 0.5 mg/L 2,4-D)