BIOLOGIA PLANTARUM 44 (1): 157-159, 2001

BRIEF COMMUNICATION

Regeneration of immature inflorescences of barley *in vitro*

M. HAVRLENTOVÁ*, J. FARAGÓ and M. NEŠŤÁKOVÁ

Research Institute of Plant Production, Bratislavská cesta 122, SK-921 68 Piešťany, Slovak Republic

Abstract

Two spring barley cultivars, Golden Promise and Galan, were screened for callus induction and shoot regeneration from cultured immature inflorescences. Genotype Galan have better regeneration capacity in *in vitro* conditions than Golden Promise.

Additional key words: *Hordeum vulgare*, somatic embryogenesis, plant regeneration.

A high rate of regeneration from callus is a prerequisite for using tissue cultures as a tool in crop improvement (Goldstein and Kronstad 1986). Several types of explants have been used as a source of embryogenic calli. Immature embryos are a conventionally used somatic explant source in cereal tissue culture. In recent years, some works describe successful embryogenic callus induction and regeneration from immature inflorescences and Rout and Lucas (1996) consider the immature inflorescences as an excellent source of a young meristematic tissue. In addition, the composition of the medium and culture conditions are very important for successful *in vitro* regeneration in cereals.

In the present study we describe an *in vitro* regeneration capacity of immature inflorescence tissues of two barley cultivars. In the world literature there is only one work about the use of immature inflorescences of *Hordeum vulgare* for embryogenic calli production (Thomas and Scott 1985).

Two spring cultivars of barley (*Hordeum vulgare* L.), well-known cultivar Golden Promise and a cultivar of Slovak provenience Galan, were grown in a naturally lit greenhouse (mean day/night temperature of 13/5, 16/7, and 23/10 °C in March, April, and May, respectively, and air humidity of 50 to 70 %). As a source material for immature inflorescences the stem tips with the inflorescence was surface-sterilized in 96 % ethanol followed by 10 min in 0.1 % $HgCl_2$ solution. The samples (2 - 3 mm in length) were aseptically removed under a microscope and cut into 1 mm pieces for explant culture. Two media for callus induction were used: Thomas and Scott (1985, hereafter referred to as the medium A) and Brettell *et al.* (1980, hereafter referred to as the medium B). Both media were supplemented with 2.5 mg dm^{-3} 2,4-dichlorophenoxy-acetic acid (2,4-D). The media were autoclaved at 121 °C for 30 min. For callus initiation cultures were maintained for 8 weeks in darkness at 25 °C. After the callus induction explants were transferred to the regeneration medium. The regeneration medium was that of Murashige and Skoog (1962) basal medium (MS). All the calli were cultured under 16-h photoperiod (irradiance of 50 μmol m^{-2} s^{-1}) and temperature of 23 ± 2 °C for 4 weeks. Regenerated plantlets were transferred to the fresh MS medium to initiate rooting. Plants with emerging roots were transferred to soil and grown to maturity in the greenhouse.

Golden Promise had the highest frequency of embryogenic calli induction (1.6 %) on the medium A. On the medium B, the callus induction was 40.6 % but no embryogenic callus has been observed (Table 1). For the cultivar Galan the best results were obtained using the medium B. The callus initiation was 50.0 % and the frequency of embryogenic calli was 27.4 %. 23.4 % of these calli regenerated plants. An average number of regenerated shoots per initial explant was 0.5 (but 11.8 % of all regenerants were albinotic plants). On the medium A, cv. Galan callogenesis was 48.4 %. The induction of embryogenic calli was 10.9 %. All embryogenic calli

Received 20 March 2000, *accepted* 25 August 2000.
Fax: +421 838 7726306, e-mail: havrlentova@vurv.sk

regenerated plants. A total number of plants per explant were 0.1.

In general, it has been known for a long time that plants of several species of *Gramineae* may efficiently regenerated *in vitro* via callus culture. In recent years the successful cultivation of immature inflorescences of *Setaria italica* (Vishnoi and Kothari 1995), sorghum (Brettell *et al.* 1980, Wen *et al.* 1991), rice (Rout and Lucas 1996) and tritordeum (Barcelo *et al.* 1994) was described. Only one work about the cultivation of barley immature inflorescences is known (Thomas and Scott 1985). This work reported 39.1 % frequency of embryogenic calli induction for spring barley cv. Prior, and the existence of morphologically normal green and albinotic regenerants was stated. Under the same conditions we have obtained the highest percentage of embryogenic calli (10.9 %) for the cv. Galan. The medium of Brettell *et al.* (1980) described for cultivation of immature inflorescences of sorghum showed better results for the same cultivar. From 64 cultivated inflorescence segments 17 calli (27.4 %) were embryogenic. Brettell *et al.* (1980) observed 77.0 % of embryogenic calli of sorghum inflorescences and Wen *et al.* (1991) described 29.0 % of *Sorghum bicolor* calli differentiated to shoots and roots. However, in immature inflorescence tissue culture of rice on a modified MS medium approximately 120 plants were regenerated from a single immature inflorescence (Rout and Lucas 1996).

Table 1. Influence of the culture medium on embryogenic and regeneration capacity (percentage of callogenesis, percentage of embryogenic calli after 8 weeks of callus induction, percentage of calli regenerated shoots after 4 weeks of subcultivation, total number of regenerated plants, number of albinotic plants, number of plants per cultured explant) of two barley cultivars: Golden Promise and Galan (embryogenic callus - callus with embryogenic, green structure; callus regenerated shoots - callus with regenerants minimum 0.5 cm in length).

Barley cultivar Culture medium	Golden Promise medium A	Golden Promise medium B	Galan medium A	Galan medium B
No. of explants cultured	64	64	64	64
Callogenesis [%]	53.10	40.6	48.4	50.0
Embryogenic calli [%]	1.60	0	10.9	27.4
Calli regenerated shoots [%]	1.60	0	10.9	23.4
Total number of regenerated plants	3.00	0	7.0	34.0
Number of albinotic plants	0	0	0	4.0
Number of plants per explant	0.05	0	0.1	0.5

Generally, the immature inflorescence tissue of the Slovak cv. Galan had better regeneration capacity in *in vitro* conditions than the inflorescence tissue of the cv. Golden Promise that is commonly used in tissue cultures. Further improvement in callus induction and shoot regeneration can probably be made by modifying the callus induction and plant regeneration media and culture conditions. Useful can be the use of other Slovak cultivars, some of them seem to be more suitable for somatic embryogenesis from scutellum explants than the conventionally used Golden Promise (Kubranová *et al.* 1999).

References

Barcelo, P., Hagel, C., Becker, D., Martin, A., Lörz, H.: Transgenic cereal (tritordeum) plants obtained at high efficiency by microprojectile bombardment of inflorescence tissue. - Plant J. **5**: 583-592, 1994.

Brettell, R.I.S., Wernicke, W., Thomas, E.: Embryogenesis from cultured immature inflorescences of *Sorghum bicolor*. - Protoplasma **104**: 141-148, 1980.

Goldstein, C.S., Kronstad, W.E.: Tissue culture and plant regeneration from immature embryo explants of barley, *Hordeum vulgare*. - Theor. appl. Genet. **71**: 631-636, 1986.

Kubranová, M., Faragó, J., Nešťáková, M.: Factors affecting somatic embryogenesis from scutellum explants of barley (*Hordeum vulgare* L.). - Biologia **54** (Suppl. 7): 23, 1999.

McElroy, D.: The industrialization of plant transformation. - Biotechnol. Annu. Rev. **4** [Mühlbach, H.-P. (ed.): Use of Plant Cell Cultures in Biotechnology]: 113-176, 1998.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1962.

Rout, J.R., Lucas, W.J.: Characterization and manipulation of embryogenic response from *in vitro*-cultured immature inflorescences of rice (*Oryza sativa* L.). - Planta **198**: 127-138, 1996.

Ruiz, M.L., Rueda, J., Pelaez, M.I., Espino, F.J., Candela, M., Sendino, A.M., Vasquez, A.M.: Somatic embryogenesis, plant regeneration and somaclonal variation in barley. - Plant Cell Tissue Organ Cult. **28**: 97-101, 1992.

Thomas, M.R., Scott, K.J.: Plant regeneration by somatic embryogenesis from callus initiated from immature embryos and immature inflorescences of *Hordeum vulgare*. - J. Plant

Physiol. **121**: 159-169, 1985.

Vishnoi, R.K., Kothari, S.L.: Somatic embryogenesis and efficient plant regeneration in immature inflorescence culture of *Setaria italica* (L.) Beauv. - Cereal Res. Commun. **24**: 291-297, 1996.

Wen, F.S., Sorensen, E.L., Barnett, F.L., Liang, G.H.: Callus induction and plant regeneration from anther and inflorescence culture of *Sorghum*. - Euphytica **52**: 177-181, 1991.