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Plant regeneration from seedling explants of common bean (*Phaseolus vulgaris* L.)

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ABSTRACT For shoot induction and plant regeneration in bean we used MS media+BA and NAA. Multiple shoot induction was obtained in case of *P. vulgaris* cv. Főnix and Maxidor. The efficiency of regeneration from intact seedling (IS) and cotyledonary node (CN) explants was compared. The optimum treatment for the induction of multiple shoot formation was the culturing of (IS) on MS-based media+BA and NAA. Multiple shoot induction on dry bean (CN) cultured on full MS medium+1mg/l BA and 0.1mg/l NAA was feasible. Shoots that were 2cm in length or longer and having 2 trifoliate leaves were responsible for rooting ability, and root development. The method can be applied in transformation experiments.

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KEY WORDS

Phaseolus vulgaris
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The lack of regeneration procedures has slowed the improvement of dry bean, soybean, and pea via tissue culture selection and plant transformation (McClean and Grafton 1989). Difficulties in obtaining plants from somatic cells in *Phaseolus* species have hampered the production of transgenic plants by the application of the most common methods available for the introduction of foreign DNA into cells (Genga et al. 1990). Due to the lack of highly efficient protocols for plant regeneration from somatic tissues and protoplasts, genetic transformation of bean is not feasible to date by conventional methods such as *Agrobacterium* spp or DNA uptake by protoplasts (Allavena and Bernacchia 1991). Shoot and plant regeneration in *P. vulgaris in vitro* has been attempted repeatedly, however the frequency of shoot regeneration obtained in these reports was still limited, namely about four to eight shoots per explant (Malik and Saxena 1992). Thus, a logical approach to plant regeneration of bean in culture is to use tissue explants consisting of totipotent cells.

We report here an attempt to develop a regeneration procedure for dry bean which utilises (IS) and (CN) tissue; and to show that the new shoot development appears to be of an adventitious nature. In the present work, the successful establishment of *in vitro* requirements for plant regeneration from (IS) and (CN) explants are reported.

Materials and Methods

Seeds of *P. vulgaris* cv. Főnix and Maxidor were obtained from the breeder (Prof. I. Velich). The seeds were first immersed in concentrated sulphuric acid for 60 s, and washed twice with sterile water. They were then washed once with ethanol for 1min, soaked in a 1% solution of sodium hypochlorite (20% Clorox) for 20 min, and washed (5 changes) with sterile distilled water. One seed was germinated under aseptic conditions in a glass tube containing 10 ml of the culture medium, and incubated under 16h photoperiod in growth cabinet. The germination medium containing MS major and minor salts (Murashige and Skoog 1962), B5 vitamins without hormones. The regeneration medium contained full the MS contents+1 mg/l benzyladenine(BA) and 0.1 mg/l naphthaleneacetic acid (NAA). Seed germination medium and explant culture medium adjusted to pH 5.7 before being autoclaved. After 10-12 days, the roots were removed and residual seedlings were cut into different parts: whole seedling without roots, or (CN) tissue and cultured on MS media+1 mg/l BA or 0.1 mg/l NAA. The efficiency of regeneration from (IS) and (CN) explants was compared by counting the number of buds and shoots.

Axillary shoots, 1 cm in length or longer, were saved and transferred to half-strength MS without plant growth regulators, in order to induce root formation and plantlet development.

Results and Discussion

An effective and reproducible procedure for the regeneration of shoots and plants from cell and tissue cultures is essential in studies involving gene transfer. Somewhat greater success in regeneration of bean from organ cultures has been achieved in the last years (McClean and Grafton 1989; Franklin et al. 1991; Malik and Saxena 1991). The frequency of shoot regeneration obtained in these reports was still limited.

In our experiments, multiple shoot and bud formation on bean (IS) cultured on MS media+1 mg/l BA and 0.1 mg/l NAA was obtained. Malik and Saxena (1991) observed that leaf explants, consisting of the petiole and a portion of the lamina, produced shoots when cultured in the presence of BAP. Although regeneration occurred only from petiolar tissue, the presence of an attached portion of lamina was essential for shoot formation.

This observation prompted us to investigate the role of morphological integrity of the donor plant on subsequent shoot formation. Thus, (IS) of the seedlings was maintained in part of this experiment and the another (CN) were cultured

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on media containing BA and NAA. The efficiency of formation of shoots from (IS) was compared with that of (CN) explants. The regeneration percent of (IS) and (CN) was 100%. The number of buds and shoots produced by (IS) was significantly higher than that from the (CN) explant. Our results are similar to that of (Malik and Saxena 1992).Our recent experiment suggest that full-strength MS may be optimum for common bean regeneration.

We found that most shoot buds originated from the meristematic zones from the axillary (CN). The most frequent location was in the nodal region where buds began to appear about 7 days after explant preparation. Buds normally appeared in clusters and the clusters were distributed randomly in the nodal region. We can assume that younger cells might respond better to exogenously applied hormonal stimuli, and BA and NAA proved to be particularly suitable in stimulating shoot formation.

Regenerated shoots were subsequently tested for rooting ability on half strength medium without plant growth regulators for all common bean shoots. The results obtained indicated that the big shoots that were 2cm in length or longer and having 2 trifoliate leaves were responsible for rooting ability, and root development.

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