Short Communication

Shoot Regeneration from the Callus of Immature Primary Leaves in Mungbean *Vigna radiata* (L.) WILCZEK

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Immature primary leaves of mungbean, varieties Pag-asa-1, Pag-asa-3, M79-9-82 and MG-50-10A obtained from seeds imbibed for eight hours produced soft and friable calli on media containing half-strength MS inorganic salts, full-strength MS vitamins, 50 mg/l yeast extract, 1.0~2.0 mg/l L-proline, 0.1~1.0 mg/l NAA and 6-BA, and 3% sucrose. Calli from Pag-asa-1 and MG-50-10A regenerated shoots at about six weeks after transfer onto the regeneration medium which consisted of the same MS basal medium supplemented with 1.0 mg/l 6-BA. Scanning electron microscopic observations revealed that the soft and friable calli consisted of clusters of small rounded cells and loosely arranged large, elongated, or lobed cells. The clusters of small and rounded cells formed a thin cuticle layer on the surface, and then leaf initials developed from this organized group of cells during culture onto the regeneration medium. Shoots were considered to arise from the clusters of small rounded cells based on the morphological similarity of their cells.

KEY WORDS: *Vigna radiata*, shoot regeneration, callus, immature primary leaf

Introduction

In legumes, successful plant regeneration from leaflet-derived callus has been reported in *Stylosanthes scabra* (GODWIN *et al.* 1987), *Lupinus luteus*, *L. hartwegii* and *L. angustifolius* (SATOR 1985), *Pisum sativum* (MROGINSKI *et al.* 1981), *Arachis hypogaea* (MROGINSKI and KARTHA 1981), *Glycine max* (WRIGHT *et al.* 1987) and *Vigna angularis* (OZAKI 1986). Plant regeneration has been successfully obtained from explanted cotyledons in mungbean (GULATI and JAIWAL 1990; MENDOZA and FUTSUHARA 1990), however, plant regeneration from callus derived from primary leaves has not been reported so far in this drought resistant pulse crop.

Plant regeneration from calli and identification of totipotent cells are especially important because protoplast culture, somatic cell fusion, and delivery of foreign DNA to develop genetically engineered plants could be anticipated. This study describes the cultural conditions required for inducing soft and friable calli capable of undergoing shoot organogenesis in mungbean primary leaves. The morphological characteristics of organogenic and non-organogenic calli are also described based on scanning electron microscopic observations.

Materials and Methods

Dry seeds of mungbean, *Vigna radiata* (L.) WILCZEK, from one green-seeded variety MG-50-10A, and three yellow-seeded varieties namely, M79-9-82, Pag-asa-1 and Pag-asa-3 were sterilized as described previously (MENDOZA and FUTSUHARA 1990). The seeds were washed thoroughly with sterile distilled water and imbibed in water-humidified glass flasks for 8, 16, and 24 hours in the dark at 30°C. Thereafter, the immature primary leaves of

Received October 23, 1991.

Accepted December 13, 1991.

about 3 mm in size were excised and cultured on media containing half or full-strength MS inorganic salts (MURASHIGE and SKOOG 1962), MS vitamins, 50 mg/l yeast extract, 3% sucrose, 0, 1.0, 2.0 and 4.0 mg/l L-proline, 0.1~1.0 mg/l NAA (naphthalene acetic acid), and 0.1, 1.0 and 2.0 mg/l 6-BA (6-benzylaminopurine). The media were adjusted to pH 5.8, solidified with 0.6% agar, and then autoclaved at 121°C under 1.2 kg/cm² pressure for 15 minutes. The cultures were incubated in the dark or under continuous exposure to white light provided by fluorescent tubes for twenty eight days for callus initiation. The induced calli were not subcultured, and together with the explants, they were transferred onto a regeneration medium which consisted of half-strength MS inorganic salts, MS vitamins, 3% sucrose and 1 mg/l 6-BA and incubated under continuous light exposure. The regenerated plantlets were excised and then transferred onto half-strength MS medium for further growth and elongation.

Results and Discussion

This study utilizing immature primary leaves of mungbean obtained from seeds imbibed for eight, sixteen and twenty four hours showed that the physiological state of the explant affected callus formation. Addition of 4 mg/l L-proline and 2 mg/l 6-BA on full or half-strength MS basal medium resulted to necrosis of the explants. In the remaining media, explants from seeds imbibed for sixteen and twenty four hours produced roots, soft calli that produced excessive roots, or compact calli which failed to regenerate plants when transferred onto the regeneration medium. Explants obtained from seeds imbibed for eight hours also tended to produce callus with roots when cultured in darkness but soft calli were formed when cultured under exposure to light. Hence, the calli that did not undergo rhizogenesis were more suitable because the presence of roots usually inhibited shoot organogenesis. It was observed that varieties differed in their regeneration response. No regeneration was observed among the callus produced in varieties M79-9-82 and Pag-asa-3. Regenerable calli were induced in Pag-asa-1 and MG-50-10A in a limited number of media that were supplemented with 1.0~2.0 mg/l L-proline (Table 1). It was observed that within seven days after culture, the explants cultured on the media listed in the Table formed soft and friable calli at the base, in the petiolar area. The calli proliferated and scanning electron microscopic observation after twenty eight days of culture revealed the presence of small and rounded cell clusters as well as loosely arranged larger and elongated cells forming the callus (Fig. 1). Regeneration was attained at about six weeks after transfer of the explant with its callus onto the regeneration medium. Scanning electron microscopic observations showed that shoot buds had possibly differentiated from the clusters of small rounded cells based on the morphological similarity of the cells (Fig. 2). Shoot differentiation appeared to be preceded by the formation of a thin cuticle layer on the surface of the clusters of callus cells characterized by a small and rounded structure. Most shoots developed from the callus formed at the base of the explant but regeneration also occurred in other areas (Figs. 3 and 4). Shoot elongation required the transfer of shoots onto hormone-free MS medium and the process was slow. Root initiation was difficult to attain even for a period of sixty days on hormone-free MS medium, instead calli developed on the base of the plantlets (Fig. 5). In *Vigna angularis*,

Table 1. Callus induction and shoot organogenesis from 50 primary leaf explants of two mungbean varieties. The calli with the explants were transferred onto regeneration medium containing half-strength MS inorganic salts, MS vitamins, 3% sucrose and 1.0 mg/l 6-BA.

Callus induction media components in mg/l ¹⁾				Pag-asa-1			MG-50-10A		
NAA	6-BA	L-proline	No. of induced calli	No. of regenerable calli	No. of regenerated plants ²⁾	No. of induced calli	No. of regenerable calli	No. of regenerated plants ²⁾	No. of regenerated plants ²⁾
0.1	0.1	1.0	48	6	17 (2.8±1.4)	45	3	5 (1.6±0.4)	5
0.1	0.1	2.0	45	6	7 (1.1±0.3)	48	4	5 (1.2±0.4)	5
1.0	0.1	1.0	41	4	6 (1.5±0.5)	42	4	5 (1.2±0.4)	5
1.0	0.1	2.0	36	3	3 (1.0±0.0)	31	2	2 (1.0±0.0)	2
1.0	1.0	1.0	36	2	5 (2.5±0.5)	24	2	2 (1.0±0.0)	2
1.0	1.0	2.0	37	2	2 (1.0±0.0)	24	2	2 (1.0±0.0)	2

¹⁾ Basal medium is composed of 1/2 MS inorganic salts and MS vitamins

²⁾ Figures in parenthesis indicate mean values ± S.D.

Fig. 1. Morphology of organogenic callus twenty eight days after callus induction. Scale bar = 250 μ m

Fig. 2. Initial stages of shoot organogenesis (arrows) on the calli six weeks after transfer onto regeneration medium (o, organogenic callus; no, non-organogenic callus). Scale bar = 250 μ m

Fig. 3. Regenerated shoots on the callus six weeks after culture on the regeneration medium. Scale bar = 250 μ m

Fig. 4. Structure of the callus and regenerated shoots observed under a dissecting microscope.

Fig. 5. Elongated shoots sixty days after culture on hormone-free MS medium.

plantlets regenerated from compact calli and roots were subsequently developed from them (OZAKI 1986). For unknown reasons the inability to form roots among regenerated plants were also similarly observed in *Lupinus hartwegii* and *L. luteus* (SATOR 1985). The developmental signals and conditions regulating root formation in regenerated plantlets still remain to be elucidated.

Pag-asa-1 showed better regeneration response than MG-50-10A when calli were induced on a medium containing half-strength MS inorganic salts, 0.1 mg/l NAA, 0.1 mg/l 6-BA and 1 mg/l L-proline. Varietal differences on plant regeneration *in vitro* in crops is widely documented. From these results it is concluded that the choice of the variety, the physiological state of the explant, media components, and conditions during culture are important factors when considering plant regeneration from somatic cells.

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緑豆 (*Vigna radiata* (L.) WILCZEK) 初生葉カルスからのシュート再分化

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緑豆品種 Pag-asa-1, Pag-asa-3, M79-9-82, MG-50-10A の浸漬後8時間の種子から得られた未熟の初生葉を基本培地として無機塩の濃度を1/2としたMS改変培地を用い, 50mg/l Yeast extract, 1.0-2.0mg/l L-proline, 0.1-1.0mg/l NAA, 0.1-1.0mg/l 6-BA, 3% ショ糖および0.6% 寒天を添加した固型培地で培養したところ柔らかくフライアブルなカルスが誘導された。Pag-asa-1とMG-50-10A から得られたカルスは基本培地に1.0mg/l 6-BA, 3% ショ糖, 0.6% 寒天を添加した再分化培地に移植後, 6週間でシュートを再分化した。このような柔らかくフライアブルなカルスを走査型電子顕微鏡によって詳細に観察した結果, 小さい丸い細胞と粗に配列し大きく伸長した, 葉状の細胞の塊から構成されているのが認められた。シュートは小さく丸い細胞群の上に薄いクチクラ層が形成された後, この部分から再分化した。