Histological Study of Callus Formation and Root Regeneration from Mung Bean (Vigna radiata W.)

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We have established a reproducible culture system for callus formation and root development from juvenile stem segments of mung bean (Vigna radiata). In particular, we have studied the influence of plant growth regulators. Induction of calli from young stem explants was very effective on MS inorganic salts supplemented with 0.5 mg/L 2,4-D and 1.0 mg/L kinetin. In regenerating adventitious roots from callus tissues, we found that a combination of 0.75 mg/L NAA, 1.5 mg/L kinetin, and MS salts resulted in 20% efficiency. Histological examination showed that callus tissues originated from out-growths of the cambium rings through de-novo meristematic activity. Those rings were localized outside the vascular cambium. Adventitious roots that developed from root primordia originated from the center of the Callus masses. These primordia produced tracheid-like cells, which then became meristemoid cells for the cambium. Newly formed adventitious roots had the typical tetrarche actinostele type.

Keywords: meristemoid, organogenesis, rooting, Vigna radiata

Extensive research has been conducted on organogenesis in plant tissue culture ever since adventitious root and shoot inductions were first reported from the calli of carrot (Nobecourt, 1939) and tobacco (White, 1939). Anatomical and histological studies of organ differentiation have been reported for many species, using different explant types. During organogenesis, callus-derived adventitious organs originate from three regions of the cambium: external phloem, internal phloem, and callus tissue (Sterling, 1951).

Adventitious roots develop from inside the callus because of the influence of shoots preformed within the callus cells (Paterson and Rost, 1981; Gladfelter and Phillips, 1987; Wagley et al., 1987). However, it has also been reported that independent of those effects from the shoots, the meristematic nodules are induced from serial divisions of certain cells within the callus under special culture conditions, and differ from the adventitious roots (Steward et al., 1958). Soh et al. (1980) have shown that root primordia from the adventitious roots of Phaseolus vulgaris develop from divisions within the vascular cambial cells and phloem parenchyma. The derivatives of those primordia then penetrate the bundle sheath and cortex, finally elongating toward the epidermis or cork cells. However in peanuts, the adventitious roots arise from the epidermal cells rather than, the vascular cambium (Atreya et al., 1984).

As evident from these conflicting reports, the origins of adventitious shoots and roots, as well as the developmental process for these two organs during organogenesis, have not yet been established. Different explant types and culture conditions can result in various pathways. Although many papers have been published on cytodifferentiation and root development (Haissig, 1988; McCown, 1988), the results have been confusing and sometimes contradictory because nearly every study has dealt with different species at several developmental stages, all tested under widely varying experimental conditions (Haissig, 1986; Gonzalez et al., 1991).

The objective in this study was to determine, the optimum conditions for callus induction and adventitious root formation from young stem explants of mung bean (Vigna radiata W.). Moreover, we used cytohistological observations to evaluate the callus origin, the initial cells of the adventitious roots, and the process for their formation.

MATERIALS AND METHODS

Plant Material

Seeds of mung bean (Vigna radiata W.) were planted in flats containing commercial potting-mix soil, and germinated in a growth chamber.
Callus Induction and Organ Development

Seven-day-old stems were surface-sterilized with a 2% sodium hypochlorite solution for 10 min. After rinsing three to four times with sterile distilled water, 2-mm-long segments were placed on Murashige and Skoog (1962; MS) inorganic salts. Calli were induced in the dark at 25°C, then subcultured to fresh MS inorganic salts every four to five weeks. The MS medium was supplemented with 30 g/L sucrose, 100 mg/L myo-inositol, 10 g/L agar, and various concentrations and combinations of 2,4-D, IAA, and NAA (auxins) as well as kinetin and, BAP (cytokinins). Adventitious roots were induced later from calli that were maintained in a root-inducing medium.

Cytohistological Observations

Young stem explants and calli grown for 12, 24, 36, 48, 60 or 72 h, or for 5 or 7 d following subculture, were histologically examined. The adventitious roots that differentiated from the callus tissue at two, three, and four weeks after culture were fixed in a formalin-acetic acid-alcohol (FAA) solution at room temperature for 12 h. After dehydration in an alcohol series, they were transferred to xylene and embedded in pure paraffin. The paraffin blocks were sectioned using a rotary microtome set at 10 µm thick. After being double-stained with hemalum and safranin, the sections were observed and photographed using light microscopy.

RESULTS

Optimized Media for Callus Induction and Organ Development

The most effective combination of growth regulator concentrations for callus induction was 0.5 mg/L 2,4-D and 1.0 mg/L kinetin in an MS medium (Fig. 1). However, media supplemented with 0.75 mg/L NAA and 1.50 mg/L kinetin produced better results for root regeneration from callus (Fig. 2).

Figure 1. Effects of various auxin and cytokinin combinations on callus and root formation from the young stem of V. radiata after four weeks of culture.

Figure 2. Effects of various concentrations of NAA and kinetin on root formation from the young stem of V. radiata after four weeks of culture.