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Somatic Embryogenesis and Plant Regeneration in Shoot Apical Meristem Cultures of an African Local Variety Cassava (*Manihot esculenta* Crantz)

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아프리카 재래종 카사바의 경단분열조직 배양에 의한 체세포배발생과 식물체 재분화

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Shoot apical meristem dome explants from cassava plants (Ghanaian local variety) produced somatic embryos at a frequency of 32% when cultured on MS medium supplemented with 2 mg/L 2,4-D. Somatic embryo segments formed secondary embryos at frequencies of up to 93% when cultured on medium containing 1 mg/L 2,4-D for 2 to 3 weeks. Since the somatic embryos were not capable of converting into plantlets, adventitious shoot were induced from the sliced embryo segments by culturing them on medium containing 0.1 to 5 mg/L BA. After 8 weeks of culture, numerous shoots formed on the segments at frequencies up to 100%. The shoots were rooted and successfully transplanted to potting soil.

Key words: adventitious shoot formation, Ghanaian local variety

Cassava is one of the most important crops in the tropics of which storage roots constitute a major source of calories to 500 million people in over 60 countries of Africa, Southeast Asia, the Far East, Latin America, and Oceania (Roca, 1984). In these regions, it is a staple food crop for low-income people. Because of vegetative propagation, cassava can hardly escape from diseases caused by systemic organisms transmissible through successive cuttings such as bacterial blight and African mosaic disease. In addition, the storage roots contain cyanide toxins and are highly perishable after harvest.

Biotechnology research is urgently needed to solve the problems mentioned in the above. Although plant regeneration via organogenesis or somatic embryogenesis has been reported in a few varieties of the crop (Tilquin 1979; Shahin and Shepard 1980; Stamp and Henshaw, 1982, 1987; Mathews et al., 1993; Raemakers et al., 1993; Szabados et al., 1987; Stamp, 1987), all of them are South American varieties, which are of limited agronomic interest. So far, no African varieties have been reportedly regenerated in spite of its enormous significance in the tropics. This paper describes culture conditions for high frequency somatic embryogenesis and plant regeneration of one of the African varieties which have previously been claimed to be recalicitrant to regeneration (Hodgson, 1992).

MATERIALS AND METHODS

Plant Material

Twenty-five-cm-long stems were excised from cassava (*Manihot esculenta* Crantz; Ghanaian local variety) plants grown in a greenhouse, potted to be rooted, and maintained in the phytotron at 27°C day/22°C night under a 16-h photoperiod.

Culture Media and Culture Conditions

The basal medium used throughout the experiments was Murashige and Skoog's (1962) medium containing 100 mg/L myo-inositol, 0.4 mg/L thiamine HCl, 30 g/L sucrose, and 0.8% Bacto-Agar at pH 5.8. Twenty-five-ml medium was dispensed into a plastic Petri dish (87 × 15 mm). Unless mentioned otherwise, all of the cultures were incubated at 25° C in the dark. In case they were cultured in the light, about 1,000 lux cool-white fluorescent lamps at a 16-h photoperiod were provided.

Somatic Embryo Formation

Axillary shoot-tips, 1- to 2-cm-long, were excised from plants and disinfested with 1% NaOCl for 10 min before rinsing 3 times with sterile distilled water. After the immature leaves and leaf primordia were removed, the meristem domes were severed from shoot-tips with a hypodermic needle (Monoject) mounted on a 50 μL microsyringe (Hamilton) under a dissecting microscope with a magnification of 40 × as previously described (Liu et al., 1989). Five explants each were placed upright on medium containing either at 0.5, 1, 2 or 4 mg/L 2,4-D in a Petri dish which was then wrapped with Parafilm. Four Petri dishes containing explants for each treatment were cultured. The frequency of somatic embryo formation was determined in the aid of a dissecting microscope after 6 weeks of culture.

Secondary Embryo Formation

To induce secondary embryos, somatic embryos at various developmental stages (<1, greater than 1 and <2, or greater than 2 and < 3 mm in length) were longitudinally cut into two halves in equal. At least 3 Petri dishes were cultured per treatment with 10 explants per dish containing MS medium with 1 mg/L 2,4-D. Data were collected after 3 weeks of culture. Likewise, 1- to 2-mm-long somatic embryos were transversely cut into three parts (upper, middle, and lower). Sixteen explants per treatment (part) were placed onto a Petri dish and cultured in the same conditions.

Adventitious Shoot Formation

To induce adventitious shoots, 1- to 3-mm-long secondary embryos were sliced into halves and 12 halves each were placed with the cut surfaces down on medium containing 0.1, 0.5, 1, 2, or 5 mg/L BA and cultured in the light. In addition, 12 intact secondary embryos each were also placed horizontally on medium and cultured in the same manner. After 8 weeks of culture, the frequency of adventitious shoot (or bud) formation was determined.

Plant Regeneration

Secondary embryo explants forming adventitous shoots (or buds) were transferred onto the basal medium to elongate the shoots. One- to 3-cm-long shoots were excised and placed onto the basal medium to root. The regenerated plantlets were transplanted to potting soil and grown in the phytotron.

Statistical Analysis

Data were subjected to statistical analysis using the GLM procedure, if necessary.

RESULTS AND DISCUSSION

After two weeks of culture, the meristem dome explants enlarged two- to threefold in diameter. Except for the explants on medium containing 0.5 mg/L 2,4-D which were not able to grow further, most of the explants on medium containing 1 to 4 mg/L 2,4-D produced slimy callus. After 4 weeks of culture, globular to heart-shaped somatic embryos were protruded through the slimy callus covering the explants (Fig. 1A). The frequency of somatic embryos formation increased as the concentration of 2,4-D in medium increased up to 2 mg/L, wherein it was highest (32%). However, the frequency declined as the concentration increased further (Fig. 2). The developmental process in morphology was similar to previously described in cultured shoot apical meristem dome explants of sweet potato (Cantliffe et al., 1987, Liu et al., 1989). Histological examination of the torpedo-shaped embryos revealed a bipolar organization with an integrated shoot-root axis (Fig. 1B).

Up to 93% of the longitudinally sliced halves of somatic embryo, 1 to 2 mm in length, formed numerous secondary embryos (Fig. 1C; Table 1). When three parts of transversely sliced somatic embryos were cultured, the highest frequency (88%) of secondary embryo formation was obtained from upper segments (Table 1).

Secondary somatic embryos underwent development into mature embryos when transferred onto the basal medium. Most of the mature embryos had a trumpet-shaped aberrant cotyledon. Although cultured in a proloned period, none of

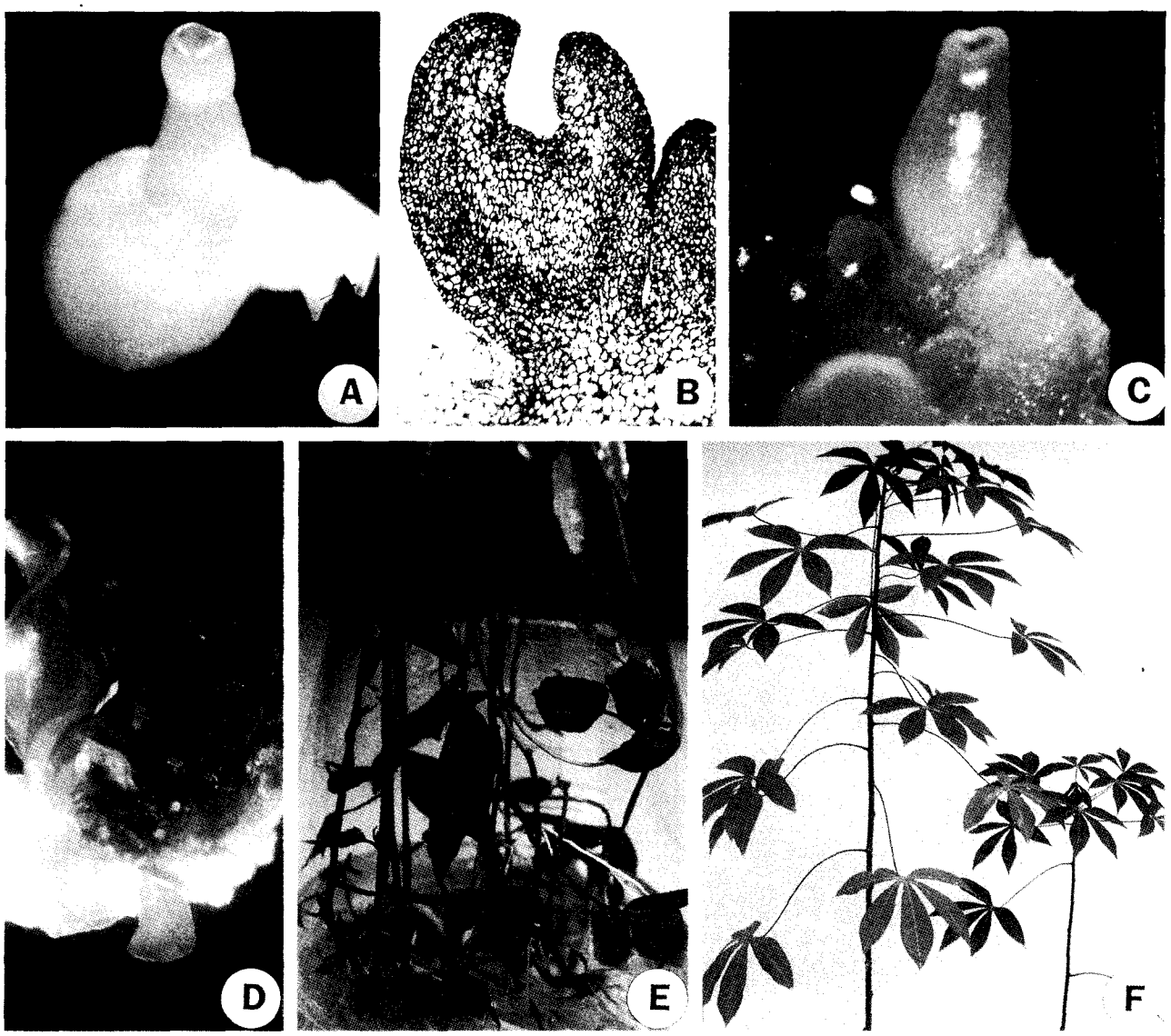

Figure 1. Somatic embryogenesis and plant regeneration in cassava. A: Somatic embryo arising from shoot apical meristem dome explant: B: Longitudinal section of a torpedo-shaped somatic embryos: C: Secondary somatic embryos developed from transversely sliced somatic embryo: D: Adventitious shoot formation on sliced somatic embryo: E: Plants regenerated from somatic embryo segments via organogenesis: F: Plants after 4 months of transfer to potting soil.

them were capable of converting to plantlets. However, when transferred onto the medium containing 1 mg/L GA_3, about 70% of them were rooted. However, only a few of them formed leafy shoots, whereas most of them remained quiescent probably due to the embryo dormancy. To overcome the embryo quiescence, adventitious shoots were induced from secondary embryos. Adventitious shoots (or buds) formed on the epidermal layers of embryo axes and cotyledons exposed to the air (Fig. 1D). Shoots were readily elongated when the explants were cultured on medium containing low concentrations of BA. However, all of the buds that formed on the explants cultured on medium containing high concentrations of BA remained stunted while staying on medium. As the concentration of BA increased up to 1 mg/L, the frequency of adventitious shoot formation increased and embryo halves produced adventitious shoots at a frequency of about 15% greater than intact ones (Fig. 3). Moreover, the frequency of adventitious shoots reached 100% when they were cultured on medium containing 0.5 to 1 mg/L BA. Upon transfer onto the basal medium, the stunted shoots or buds were elongated. Over 20 plants were successfully transferred to potting soil and grown to maturity in the growth chamber (Fig. 1F).

Although a few genotypes of cassava have been regenerated

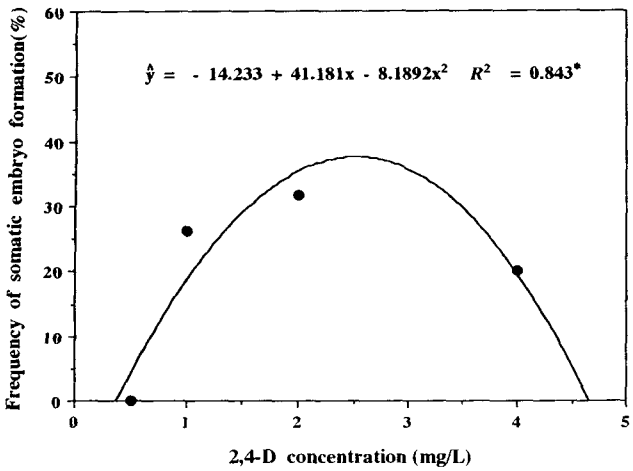

Figure 2. Changes in the frequency of somatic embryo formation from apical meristem explants of cassava cultured on media containing various concentrations of 2,4-D.
*Significant at $P \leq 0.05$.

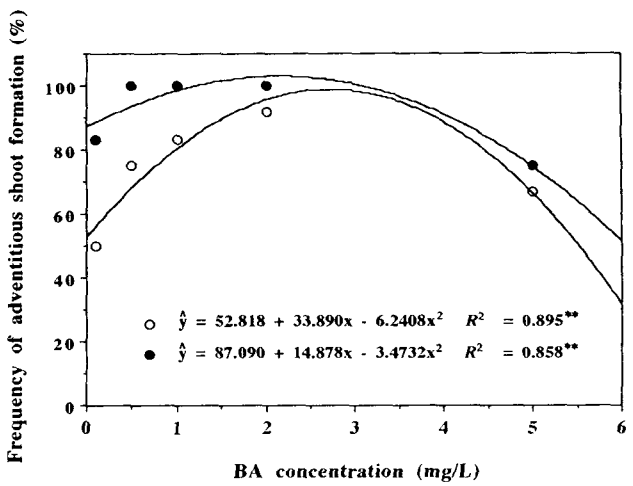

Figure 3. The frequency of adventitious shoot (or bud) formation on cassava intact (○) and sliced half (●) secondary embryos cultured on medium containing various concentrations of BA. Data were collected after 8 weeks of culture.
**Significant at $P \leq 0.01$.

Table 1. Frequency of secondary embryo formation on longitudinally or transversely sliced cassava somatic embryo segments cultured on medium containing 1 mg/L 2,4-D.[a]

Longitudinally sliced segments		Transversely sliced segments[c]	
Somatic embryo size[b]	Frequency of secondary embryo formation (%: ±S.E.)	Segment	Frequency of secondary embryo formation (%)
A	64.3 ± 5.7	Upper	88
B	93.3 ± 2.8	Middle	19
C	76.7 ± 9.0	Lower	0

[a]Data were collected after 3 weeks of culture.
[b]The number of replicates were 10 for 'A' (<1 mm in length), 8 for 'B' (greater than 1 mm and <2 mm), and 3 for 'C' (greater than 2 mm and <3 mm), respectively.
[c]One- to 2-mm-long somatic embryos were sliced and 16 segments each were cultured.

via organogenesis or somatic embryogenesis, all of them are South American varieties, which are of limited agronomic interest. The regeneration system described in this paper is almost the same as one previously developed for high frequency somatic embryogenesis in sweet potato (Liu et al., 1989). In this study we demonstrated that the system was successfully applicable to cassava, one of the African varieties which had previously been claimed to be recalcitrant to regeneration. In addition, this system does not seem to be applicable to a specific genotype of cassava as the one has been successfully applicable to a various genotypes of sweet potato (Liu et al., 1989: Min et al., 1994). Somatic embryos developed from shoot apical meristem explant may serve as a suitable material explants for transformation: a high frequency of transgenic secondary embryos of *Codonopis lanceolata* was obtained in as short as two weeks (unpublished) only in meristems. Such a method has previously been employed by Chavarriaga-Aguirre et al. (1992). Few somatic embryos produced from cassava explants were capable of "germination" in this study. To overcome the ingerminability of somatic embryos, we produced adventitious shoots on embryo segments. Such a diversion has not been reported in any species as far as we know. Raemakers et al. (1993) stimulated multiple shoot formation by culturing mature somatic embryos of cassava on medium containing high concentrations of BA, however the shoots were elongated from axillary buds of the somatic embryo. Embryonic tissue may be more competent to produce adventitious shoots than other nonembryonic tissues. Currently we are investigating to determine whether segments of ingerminable somatic embryos are capable of producing adventitious shoots readily in other species.

적 요

가나 재래종 카사바 식물체의 경단 분열조직을 2 mg/L 2,4-D가 함유된 MS 배지에서 배양하였을 때 32%가 체세포배를 형성하였다. 절단한 체세포배를 1 mg/L 2,4-D가 함유된 배지에 배양하였을 때는 최고 93%가 2차배를 형성하였다. 체세포배는 식물체로 전환하지 못하였으므로 종단으로 자른 체세포배를 0.1-5 mg/L BA가 함유된 배지에서 배양하여 부정아를 유도하였다. 8주 경과하였을 때 최고 100%가

부정아를 형성하였으며 이들은 발근된 후에 토양으로 이식하였다.

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