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Organ Regeneration from Anther Callus of Sweet Potato[1]

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Abstract : Callus was induced from anthers of *Ipomoea batatas* Poir. by culturing on Murashige and Skoog basic medium supplemented with 2 mg/l each of 2,4-D, IAA and kinetin or 2 mg/l each of NAA and kinetin. Embryoids or shoots could be regenerated directly from ABA-containing medium. A large number of roots could be induced by subculturing these embryoids to ½ strength of MS medium with 0.1 mg/l NAA, 0.5 mg/l kinetin and 7.5 mg/l adenine sulfate, a few buds were observed from the nodule-like tissue of the growing root after 2-4 months in the same medium.

Sweet potato is one of the most important micellaneous crops in Taiwan. Due to the self-imcompatibility of this cross-pollinated crop, it is very difficult to obtain inbred lines by using the convensional pedigree breeding method. If haploid plant can be obtained by anther culture, then complete homozygous diploid plant can be produced by colchicine treatment and inbred line maintained by vegetative propagation. Complete homozygous plants will open up the possibility to convert sweet potato from a tuber-propagated crop to a seed-propagated one with the advantages of obtaining virus-free plants and the utilization of heterosis by hybridization. This paper describes the organ regeneration from anther-derived callus of sweet potato.

Varieties Tainung Hsin 31, Tainan 15, Tainung 57, Simon 1, Golden, Bud mutant-1 and Bud mutant-2 of *Ipomoea batatas* Poir. were selected as experimental materials. Immature flower buds measuring 1 cm in length excised from plants were sterilized in 70% alcohol for 1 min. After peeling off two outmost sepals, the flower buds were sterilized in 1% sodium hypochloride in a ultrasonic apparatus for 10 min. Two more sepals were then peeled followed by sterilizing in 0.5% sodium hypochloride for 5 min and washing several times with autoclaved water. Anthers with pollen grains in the uninuclear stage were then removed from flower buds and inoculated in 25 x 120 mm test tubes containing 10 ml of nutrient medium. Basic composition of the culture medi-

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um was similar to that reported by Murashige and Skoog (1962) with 2 mg/l each of 2,4-D, IAA and kinetin or 2 mg/l each of NAA and kinetin. The medium was solidified with 0.8% phytagar and the pH was adjusted to 6.0 before autoclaving for 15 min at 121°C. Anthers were cultured in the dark for callus induction. The produced callus were then kept under 1,500 lux white flurorescent light with a 16 hr photoperiod at 27±1°C for organ formation.

Light yellow callus appeared from the top of anthers and the cut end of filaments about 3-5 weeks after inoculation. Callus formed at the cut end of filaments was discarded because it was somatic in nature. About 20-30 days after callus initiation, they were transferred to medium containing various concentrations of adenine, kinetin, BA, ABA and IAA individually or in combination for organ formation testing. It was found that 0.1-1 mg/l ABA was the only treatment capable to promote shoot formation from var. Tainan 15 (Fig. 1). No positive reponse of shoot differentiation was observed from other treatments and other varieties, whereas a few roots was produced in different kinds of medium. Most of the root induced were originated from embryoids appeared on the surface of the cultured callus. A second transfer to a new medium was essential to prevent the embryoids from browning and senescence. By subculturing these embryoids to ½ strength of MS medium with 0.1 mg/l NAA, 0.5 mg/l kinetin and 7.5 mg/l adenine sulfate, many roots were induced and a few buds were observed from the nodule-like tissue of the growing roots after 2-4 months in the same medium (Figs. 2-4).

Induction of callus from anthers of sweet potato has been reported by Tsay and Lin [5], Kobayashi and Shikata [1], and Tsay and Tseng [6]. For organ regeneration, Yamaguchi and Nakajima reported that abscisic acid played important role in shoot-differentiation from tuber-derived callus of sweet potato. [7] Tsay and Tseng described that ABA stimulated the embryoid formation from anther callus of sweet potato. [6] We found that ABA not only promoted the embryoid formation from anther callus but also induced the direct initiation of shoot from callus cells. Kobayashi and Shikata reported that shoots and plantlets could be obtained through the culture of root segments originated from anther callus. [1] Sehgal stated that MS medium supplemented with 10-20 mg/l adenine or 0.1-0.5 mg/l kinetin promoted root and shoot formation from leaf-derived callus of sweet potato. [3] Sehgal also reported that anther-derived callus of sweet potato obtained on MS medium supplemented with adenine (10-20 mg/l) and 1 mg/l 2, 4-D showed extensive nodule formation with the ability of full plant regeneration on transferring to MS medium. [4] Similar result was obtained in this experiment as buds were differentiated from the growing roots originated from the anther callus-derived embryoids.

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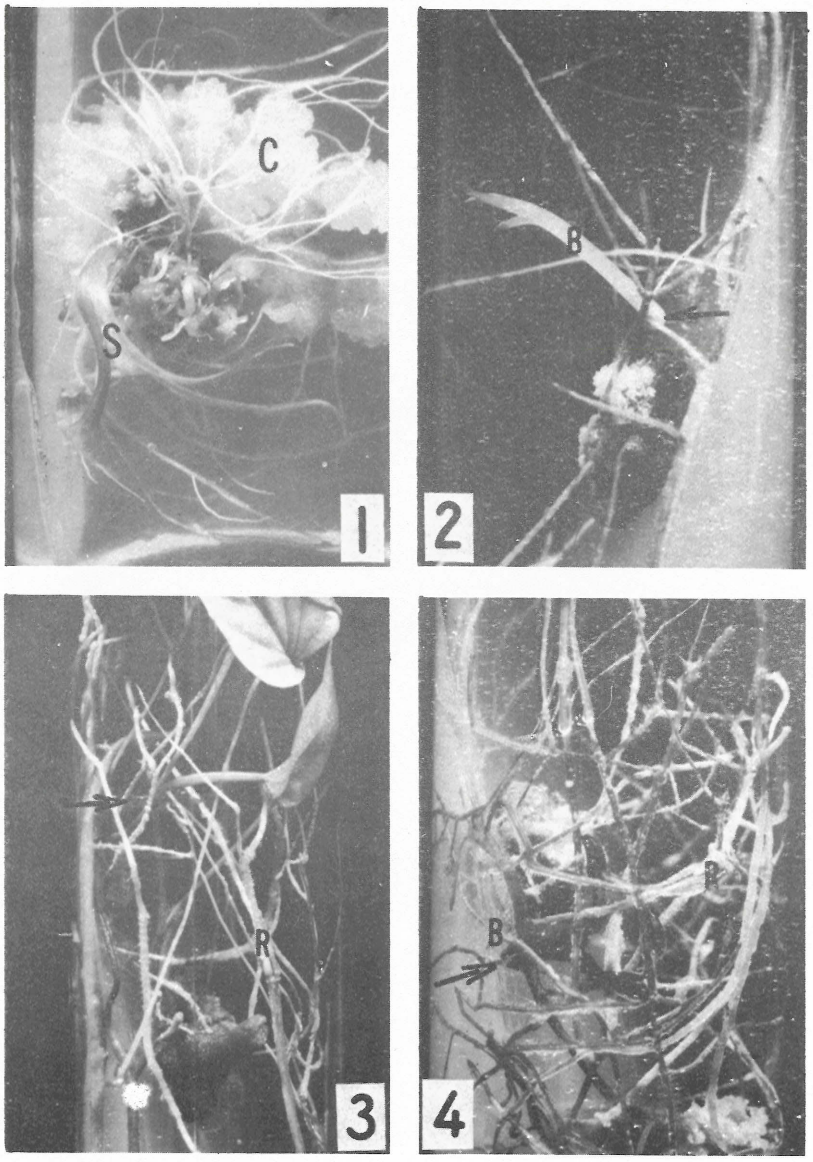

Fig. 1. Shoot(S) initiated directly from anther-derived callus(C) cultured in ABA-containing medium (var. Tainan 15).

Figs. 2-4. By subculturing the embryoids to ½ strength of MS medium with 0.1 mg/l NAA, 0.5 mg/l kinetin and 7.5 mg/l adenine sulfate, many roots (R) were induced, a few buds (B) were observed from the nodule-like tissue of the growing root (arrow) after 2-4 months (var. Tainung Hsin 31).

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含有 2,4-D, IAA 及 Kinetin 各 2 mg/l 或 NAA 及 Kinetin 各 2 mg/l 之 MS 基本鹽類培養基，可誘導甘藷花葯產生癒傷組織。含有 Abscisic acid 之 MS 基本鹽類培養基，可直接誘導癒傷組織形成擬胚體或芽體，將擬胚體繼代培養於含有 NAA 0.1 mg/l, kinetin 0.5 mg/l 及 adenine sulfate 7.5 mg/l 之 ½濃度 MS 基本鹽類培養基可誘導多量的根形成，繼代培養這些生長中的根經 2—4 個月後，可自根之瘤狀部形成少數芽體。

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