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EMBRYOID FORMATION AND PLANTLET REGENERATION FROM ANTHER CALLUS OF SWEET POTATO[1]

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Abstract

Callus was obtained from anthers of *Ipomoea batatas* Poir. by culturing them on two basal media supplemented with various amounts of auxins and 6-furfurylaminopurine (kinetin). 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin were found to be more effective than indole-3-acetic acid (IAA) for callus induction. High concentration of 2,4-D over 10 mg/l suppressed callus induction. Differences in the production of callus among varieties were also observed. Embryoid formation was obtained when callus was cultured on MS medium supplemented with 0.1-1 mg/l abscisic acid (ABA) in one variety, subsequent differentiation to plants was possible when embryoids were transferred to the same medium plus 1 mg/l IAA and 4 mg/l kinetin. Plant regeneration was obtained only from var. Tainung Hsin No. 31 among the three varieties tested.

Introduction

Since the original discovery of Guha and Maheshwari (1964, 1966, 1967) that haploid plants of *Datura innoxia* could be produced by *in vitro* culture of anthers, successful achievements have also been made with many other species.

Induction of callus from anthers of *Ipomoea batatas* Poir. (sweet potato) has been reported by Tsay and Lin (1973). For organ regeneration, Kobayashi and Shikata (1975) reported that shoots and plantlets could be obtained through the culture of root segments originated from anther callus. Yamaguchi and Nakajima (1973) found that abscisic acid (ABA) played an important role in organ formation from root tuber-derived callus. Sehgal (1975) reported that Murashige and Skoog (1962) medium supplemented with 10-20 mg/l adenine or 0.1-0.5 mg/l kinetin promoted roots and shoots formation of leaf-derived callus of sweet potato. Sehgal (1978) also reported that anther-derived callus of sweet potato obtained on MS media supplemented with adenine (10 - 20 mg/l)

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and 2, 4-D (1 mg/l) showed extensively nodular and with the ability for full plant regeneration on transferring to MS medium. This paper reports the induction of callus from anthers of sweet potato and the regeneration of plants from the anther-derived callus by passing the embryoid formation. Cultural conditions have also been discussed.

Material and Methods

Varieties Tainan No. 15, Tainung Hsin No. 31, Hsin Chu No. 1, Kuoshon Tsu and Sheng Hsin Wei of *Ipomoea batatas* poir. were selected as experimental materials. Immature flower buds measuring 0.8-1.0 cm in length were excised from plants for culture. Anthers from these buds contained mostly uninucleate pollen grains. The flower buds were sterilized in 10% calcium hypochlorite solution for 20 minutes and washed several times in autoclaved distilled water. Anthers were then removed from flower buds and inoculated in 20 x 100 mm test tubes containing 10 ml of nutrient medium. Basal composition of the culture medium was similar to that reported by Blaydes (1966) or Murashige and Skoog (1962). Various concentrations of IAA, 2,4-D, kinetin, ABA, N^6-($\triangle^2$-isopentenyl)-adenine (2ip), 6-benzyladenine (BA) and yeast extract were tested individually or in combination for their effects on callus induction and organ formation. The medium was solidified with 1% Bacto-agar and the pH was adjusted to 6.0 before autoclaving for 15 minutes at 121°C. Cultures for callus induction were kept in darkness at 27±1°C, whereas those for organ formation were under 1,500 lux white fluorescent light with a 16 hr photo-period at 25 - 27°C.

Results

Light yellow cell clusters (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. Table 1 showed that the composition of the culture medium had a pronounced effect on the frequency of callus production. With various concentrations of IAA, 2, 4-D and kinetin, basal medium of Murashige and Skoog had been found superior to that of Blaydes in the induction of callus. Table 1 also showed that kinetin and 2, 4-D were more effective than IAA in inducing callus from anthers. However, concentrations of 2, 4-D higher than 10 mg/l appeared to suppress callus formation. The most effective medium for callus formation from anthers, as tested in this experiment, was Murashige and Skoog basal medium supplemented with 2 mg/l each of 2, 4-D, IAA and kinetin.

The callus were usually transferred to MS medium containing 2 mg/l each of 2, 4-D and IAA in order to produce enough material for testing of organ formation. They were then transferred to medium containing various concentrations of auxin, cytokinin and ABA for plant regeneration. The effects of these plant growth regulators on organ and embryoid formation were shown in Table. 2. It was clear that ABA was the only substance capable to promote the formation of embryoids. No positive response was

Table 1. *The effects of auxin, cytokinin and basal medium on callus formation among varieties.*

Basal Medium	IAA	2, 4-D	Kinetin	Varieties			
				Tainan No. 15	Taniung Hsin No. 31	Kuoshon Tsu	Sheng Hsin Wei
	mg/1	mg/1	mg/1				
Balydes	2	2	2	++	+++	+	++
Blaydes	2	5	2	++	++	+	+
Blaydes	2	10	2	–	–	–	–
Blaydes	2	15	2	–	–	–	–
Blaydes	2	20	2	–	–	–	–
Blaydes	2	0	2	–	–	–	–
Murashige & Skoog	2	2	2	+++	++++	++	+++
Murashige & Skoog	2	2	0	++	–	+	–
Murashige & Skoog	0	2	2	++	++	++	++
Murashige & Skoog	2	0	2	+	+	–	–

++++, +++, ++, +, – : Degree of callus formation; very good, good, poor, very poor and no response, respectively.

observed from treatments with other plant growth regulators. The optimal concentration of ABA for embryoid formation was 1 mg/l among the three concentrations tested. Varietal difference in organ and embryoid differentiation existed, as Tainung Hsin No. 31 was the only variety showing embryoids formation upon the addition of ABA into medium. Processes of plant regeneration from anther-derived callus were illustrated in Figs. 1-4.

Discussion

The importance of 2, 4-D and kinetin for inducing callus from cultured anthers of sweet potato had been described by Kobayashi and Shikata (1975) and Tsay and Lin (1973). They reported that both 2, 4-D and kinetin were necessary for callus induction and that responses were different among varieties. Similar results were also obtained from this study.

It was found that ABA stimulated embryoid formation from anther-derived callus of sweet potato. Among the three concentrations tested, 1 mg/l was most suitable for this purpose. The effects of ABA on embryoid formation also varied among three varieties. Tainung Hsin No. 31 showed a high percentage of root and embryoid formation, whereas only roots were observed for varieties Tainan No. 15 and Hsin Chu No. 1 regardless of the kind and concentration of plant growth regulators added to the culture medium.

Table 2. *The effects of auxin, cytokinin and abscisic acid on embryoid and root formation*

Murashige & Skoog medium supplemented with	Tainan No. 1515		Tainung Hsin No. 31		Hsin-Chu No. 1	
	Root	embryoid	Root	embryoid	Root	embryoid
ABA 0.01 mg/1	–	–	+	+	–	–
ABA 0.1 mg/1	–	–	++	++	–	–
ABA 1 mg/1	+	–	++	+++	+	–
IAA 2 mg/1 + 2ip 0.015 mg/1	–	–	++	–	–	–
IAA 2 mg/1 + 2ip 0.1 mg/1	–	–	+	–	–	–
IAA 2 mg/1 + 2ip 1.0 mg/1	–	–	+	–	–	–
IAA 2 mg/1 + Kinetin 0.1 mg/1	–	–	++	–	+	–
NAA 2 mg/1 + BA 0.05 mg/1	–	–	–	–	+	–
Kinetin 1 mg/1 + 0.4% YE	–	–	+	–	–	–

ABA: Abscisic acid.
IAA: Indole-3-acetic acid.
NAA: Naphthaleneacetic acid.
2ip: N^6 – ($\triangle^2$ – Isopentenyl)-adenine.
BA: 6-Benzyladenine
YE: Yeast extract.

+++, ++, +, – : Degree of root and embryoid formation
+++: Good
++: Fair
+: Poor
–: No response.

It is possible that cytokinin may be present in anther-derived callus of sweet potato. As reported by Yamaguchi and Nakajima (1973), callus from root tuber of sweet potato contained abundant endogenous cytokinins. The amount of those endogenous cytokinins varied genetically with varieties and also with storage duration of root tuber. They also studied the hormonal regulation of adventitious bud and root formation and postulated that the positive effect of ABA on organ formation might be due to an antagonist effect with the endogenous cytokinins. They further stated that higher concentrations of ABA were necessary to form adventitious bud when root tuber-derived callus contained more cytokinin. On the other hand, a low concentration of ABA was sufficient for initiating bud differentiation when cytokinin level in callus was low. Antagonism between cytokinins and ABA in various plant species had also been reported (Khan, 1968; Khan and Downing, 1968; Valadon and Mummery 1971).

Based on the above hypothesis, it could be suggested that in the present experiment, ABA regulated the effect of endogenous cytokinin in the anther-derived callus of sweet potato and thus stimulated the formation of embryoids. Varietal difference in response to ABA might be attributed to genetically varied cytokinin levels present in the callus of different varieties.

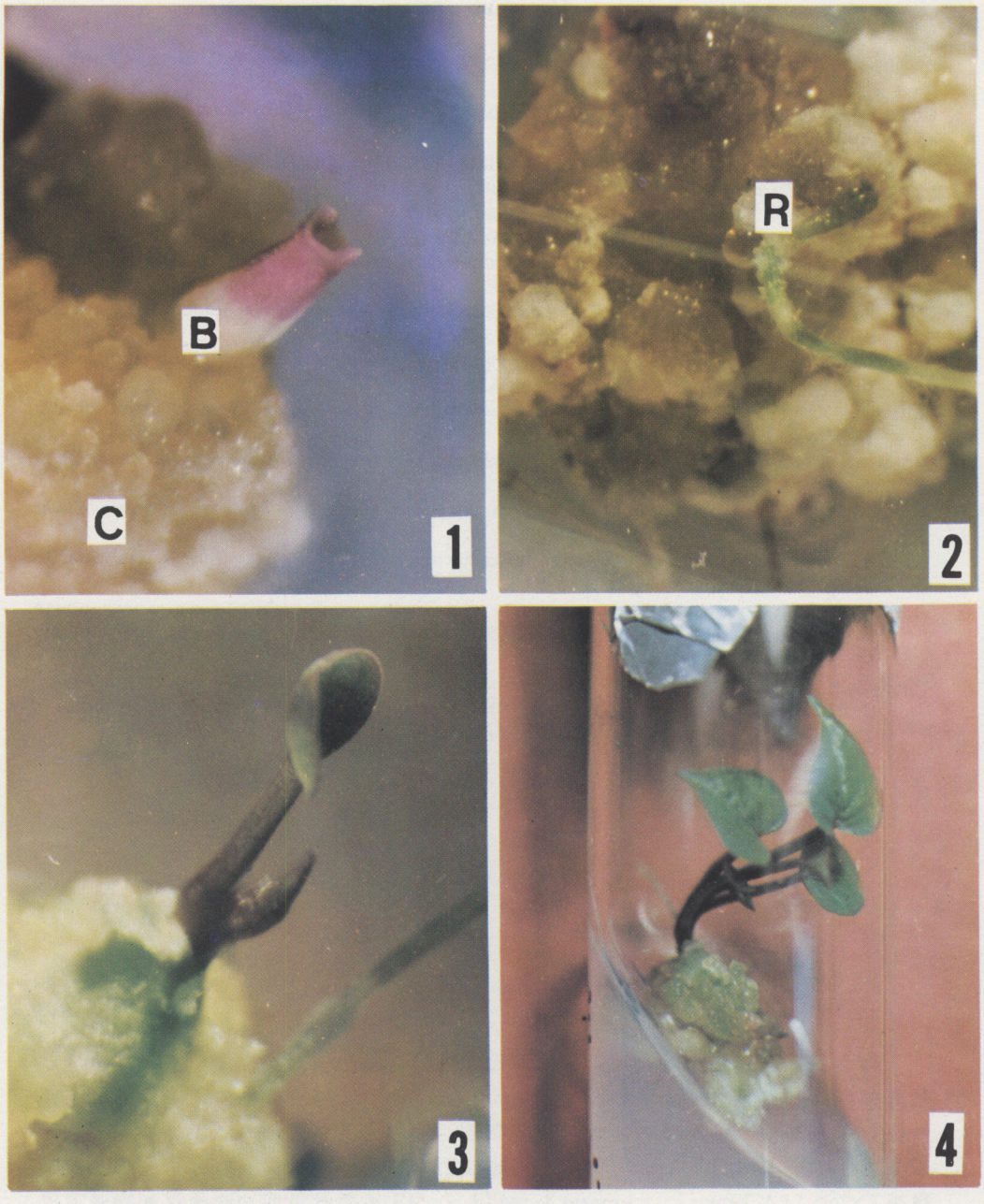

Fig. 1–4: Plant regeneration from anther-derived callus of sweet potato.

1. Anther-derived callus (C) with embryoid (B) on MS medium supplemented with 1 mg/1 ABA.
2. Anther-derived callus with root (R) on the same medium as Fig. 1.
3. Leaf initiated from embryoid which had been transferred from embryoid induction medium to MS medium supplemented with IAA 1 mg/1 and kinetin 4 mg/1.
4. Anther-derived plant of Tainung Hsin No. 31.

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甘藷花葯癒傷組織植物體之誘導

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將甘藷花葯培養於含有不同植物荷爾蒙的培養基中，可誘導癒傷組織的形成；對癒傷組織的誘導，2,4－D及kinetin比IAA重要，過高濃度的2,4－D（超過10 mg/l）可抑制癒傷組織的形成。癒傷組織的形成，品種間差異極大。MS基本培養基配合0.1－1 mg/l ABA可誘導癒傷組織形成胚狀體的分化；將分化後的胚狀體移植於含有IAA 1 mg/l，kinetin 4 mg/l之MS培養基可成功的誘致植物體形成。在受測三品種中，只台農新31號形成植物體，所形成之植物體其染色體未經檢查。