J. Plant Biotechnology(2004) Vol. 6(2). pp. 103~106

Journal of Plant Biotechnology

The Effects of Growth Regulators and Medium Strength on the Shoot and Bud Formation from the Shoot Apex of Chinese Yam (*Dioscorea opposita* Thunb)

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Abstract

Plantlet regeneration from the shoot apex was studied in three different genotypes of the chinese yam (*Dioscorea opposita* Thunb) cv. Jangma and Danma, Dunggunma. The effects of plant growth regulators and inorganic salts concentration of the culture medium on bud induction and shoot growth were examined. The combinations of 0.2 mg/L BAP + 0.2 mg/L kinetin, 0.01 mg/L NAA + 0.2 mg/L kinetin and a single treatment of 0.2 mg/L BAP were equally effective for bud and shoot formation from the shoot apices in the three cultivars. Auxin (2,4-D, NAA) treatment enhanced calli formation from the cultured apices. Also, the shoot apices of the cv. Dunggunma produced more callus and buds on the culture medium (MS) containing 0.05 mg/L NAA and 0.5 - 1.0 mg/L BAP. Lower salt strength of medium inhibited shoot elongation but did not have much effect on the shoot and bud induction from the shoot apices. These results will be useful to obtain disease-free plants of the Chinese yam.

Key words: Chinese yam, growth regulator, micropropagation, shoot apex

Introduction

Yams are monocots and dioecious plants propagated by tuber sets, a practice which increases the risk of the spread of disease. The *Dioscorea* species are severely attacked by several diseases caused by fungis, viruses and nematodes (Asolkar and Chadha 1979). The infection from the Chinese yam necrotic mosaic virus (ChYNMV) and the Japanese yam mosaic virus (JYMV) is one of the major problems in the production of *D. opposita*. In order to produce plantlets free from viruses, information on the morphological development of excised shoot apices and their ability to develop into plantlets is required. The culture of the excised shoot apex may be used for large-scale clonal propagation as well as for virus elimination (Mantell et al. 1989).

The method of clonal propagation of the *Dioscorea* plants through tissue culture has led to the successful application of micropropagation of desired economically important crops. The method can also be of great help in a breeding programme by speeding up the selection process (Chaturvedi and Sinha 1979). Some success has also been achieved in the somatic embryogenesis of the *Dioscorea* species, which is faster and more economical. The establishment of a tissue culture method for different genotypes of the *Dioscorea* species may be applied for the maintenance of the genotypes over years without any change in the genetic make up. This leads to the establishment of the "Tissue Bank". In such a case the maintenance of genotype is easy. There space requirement is less, and it is also a novel method for germplasm preservation (Chaturvedi and Sinha 1979). Grewal et al. (1977) cultured 0.5 mm-long apical meristems of the *D. deltoidea* and were able to regenerate a large numbers of plants. Chaturvedi et al. (1977) compared the apical meristems and single-node leaf cuttings and found that they had nearly the same rate of growth under one set of nutrient conditions. Plants which are produced by

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Received Apr. 12, 2004; Accepted May. 27, 2004

nodal or meristem culture show a high degree of uniformity (Ammirato 1984).

In the present study, plant regeneration through multiple bud induction from the shoot apex was observed in three genotypes of the *D. opposita* Thunb cultivated in Korea. The effects of plant growth regulators and inorganic salts concentration of culture medium on bud induction and shoot growth were examined.

Materials and Methods

Plant materials and surface sterilization

Stock plants of *Dioscorea opposita* cv. 'Jangma' (long spindle type tuber), 'Danma' (short cylinder type tuber) and 'Dumgunma' (round type tuber) were grown in field of The Institute of Bioresources, Gyeongbuk Provincial Agricultural Technology Administration, Andong, Korea. Vine cuttings with several axillary buds were collected in late July and 3 cm long cuttings with one or two buds were prepared by removing leaves. The explants were surface sterilized by dipping the tissue in 70% ethanol for 30 sec followed by immersing in a NaOCl (2%) for 15 min. The explants were then thoroughly washed with sterile distilled water (3 times).

Maintenance and culture environment

The MS medium (Murashige and Skoog 1962) supplemented with 30 g/L sucrose and 2 mg/L gelrite was used for clonal propagation. The pH of the medium was adjusted to 5.8 before autoclaving at 121℃ and 1.2 kg/cm^3 for 20 min. The cultures were kept at 26±1℃, and illuminated for 16 hr followed by 8 hr of darkness in a 24 hour cycle. Light intensity was maintained at 1,200 lux and humidity at 50%.

Shoot, bud and callus induction

Ten shoot apices were cultured in each petridish using 25 ml MS medium with various combinations of plant growth regulators for 40 days. For bud and shoot initiation, BAP (0.2-1.0 mg/L), NAA (0.01-0.05 mg/L), kinetin (0.2 mg/L) and 2,4-D (0.2-0.5 mg/L) were employed. The effects of the inorganic salts concentration of culture medium on bud induction and shoot growth were also examined. Shoot apices of cv Danma were cultured on a 1/4, 1/2 and a full strength MS medium supplemented with 0.2 mg/L BAP for 90 days.

Measurement

The parameters of growth such as callus induction, bud and shoot formation were recorded for all the experiments. Experiments were conducted in a random block design with a minimum of five replicates per treatment. The results were statistically analysed using Duncan's test.

Result and Discussion

The shoot apices excised from the three genotypes of *D. opposita* - Jangma, Danma, Dunggunma - were examined for their response to growth regulators (Table 1, Figure 1). The combination of 0.2 mg/L BAP + 0.2 mg/L kinetin, 0.01 mg/L NAA + 0.2 mg/L kinetin and a single treatment of 0.2 mg/L BAP were equally effective for bud and shoot formation from the cultures of the three genotypes. The combi-

Table 1. The effects of growth regulators on bud formation from the shoot apex in three genotypes of Chinese yams.

Plant growth regulators (mg/L)	Jangma		Danma		Dunggunma	
	Bud formation (%)	Bud forming degree	Bud formation (%)	Bud forming degree	Bud formation (%)	Bud forming degree
Control	-	-	-	-	90	+
BAP 0.2	94	+[a]	98	+	100	+
kinetin 0.2	20	+	22	+	100	+
BAP 0.2 + kinetin 0.2	88	++	96	++	90	++
2,4-D 0.2 + BAP 0.2	85	++	84	++	87	++
2,4-D 0.5 + BAP 0.5	68	+	73	+	56	+++
NAA 0.01 + kinetin 0.2	78	++	77	++	98	++
NAA 0.05 + kinetin 0.5	55	+	50	+	70	+
NAA 0.05 + BAP 0.5	24	+	24	+	60	+++
NAA 0.05 + BAP 1.0	20	+	15	+	77	+++

[a] Degree : - none, + rare, ++ moderate, +++ good.

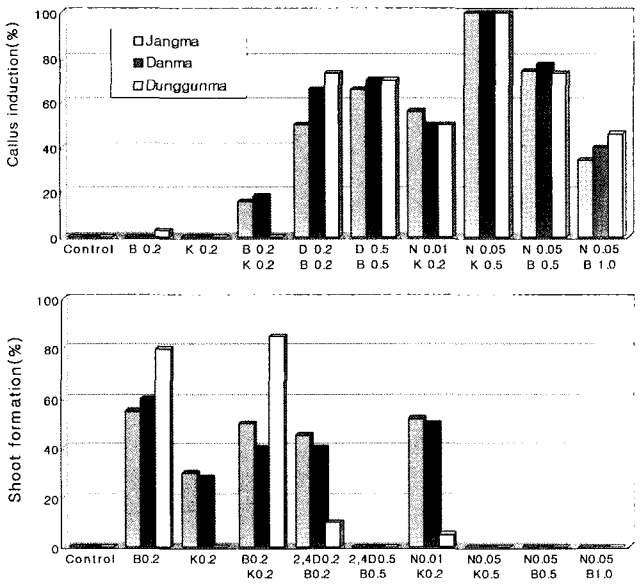

Figure 1. The effects of growth regulators on the callus and shoot formation from the shoot apex in Chinese yam. B, K, D and N mean BAP, Kinetin, 2,4-D and NAA, respectively. Shoot apices were cultured for 40 days on a MS basal medium containing various plant growth regulators.

nation of 0.05 mg/L NAA + 0.5-1.0 mg/L BAP enhanced callus and bud formation from the shoot apices of the cv. 'Dunggunma' but the combination of NAA and BAP was not effective for the cv. 'Jangma' and 'Danma'. It is evident from this study that a different hormonal regime is required for each genotype of yam. This is not surprising as a wide diversity occurs among yam cultivars in their biochemical composition, storability, sprouting and growth in culture (Mitchell et al. 1989; Michell and Redway 1991; Mitchell and McLaughlin 1992; Asemoto et al. 1992; Muzac-Tucker et al. 1993; Wellington and Ahmad 1993).

For the production of *in vitro* plantlets, multiple buds induced in the culture medium were more effective than induction of morphogenic callus. In addition, the system using multiple buds is more practical in clonal propagation than that of somatic embryogenesis, which has been achieved in *D. floribunda* (Ammirato 1984) and 'Nagaimo' Chinese yam (Nagasawa and Finer 1989), but has low rate of plant regeneration. In this study, shoot apieces formed shoots,

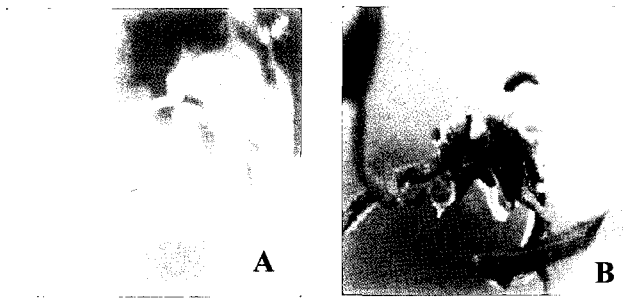

Figure 2. Shoot (A) and multi-propagules (B) formed at 40 days after the shoot apex culture of Chinese yam cv. 'Dunggunma'.

buds or calli in an initiation medium containing auxins and cytokinins (Figure 2) after 40 days. Multiple buds (clumps of adventitious buds with shoots) were obtained from the shoot apices of 'Dunggunma' Chinese yam (Figure 2-B). The propagation system through multiple bud induction from the shoot apex may be a useful method for the propagation of virus-free Chinese yams. In the present work, multiple buds that were clumps of adventitious buds or meristematic regions were obtained from the shoot apices of the cv. 'Dunggunma' Chinese yam. By transferring the multiple buds to a fresh medium, vigorous shoot development and plantlet regeneration were observed. This response was recognized to be an efficient method for the micropropagation of the cv. 'Jangma' and 'Danma' Chinese yam.

Effect of medium inorganic salts concentration in the bud and callus formation was observed in *D. opposita*. cv. 'Danma' (Talbe 2). Response of the salts concentration of medium was not significant on budding, shooting or the callus formation in half or full strength MS medium. Lower salt strength of medium inhibited bud and shoot induction but full strength medium was found to enhance the shoot elongation. Similar response was also observed in a previous study with a 'Nagaimo' Chinese yam (Sawada et al. 1958).

Acknowledgement

This study was supported by the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture

Table 2. The effects of inorganic salts strength of culture medium on the organ formation from shoot apex in a Chinese yam cv Danma.

Inorganic salts strength	Shoot formation (%)	Shoot length (cm)	Bud formation (%)	Callus formation (%)
One-fourth	80 b[a]	3.2 c	60 b	0
Half	100 a	4.5 b	75 a	0
Full	100 a	9.3 a	70 a	0

[a] Mean separation within columns by Duncan's multiple range test, $P \leq 0.05$.

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