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Factors Affecting the Production of *In Vitro* Plants from the Nodal Pieces of Chinese Yam (*Dioscorea opposita* Thunb)

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Abstract

This study was carried out to establish The regeneration of healthy seedlings from the nodal segment culture of Chinese yam (*Dioscorea opposita* cv. Danma), cultivated in Korea. Different explants such as leaves, petioles, roots and nodal pieces, excised from the *in vitro* grown seedlings of Chinese yam, were cultured on MS medium supplemented with various combinations of growth regulators. All the growth regulators used induced plantlet regeneration from the nodal segments at a high frequency, while there was no induction of shoot or callus from leaf, petiole or root tissues. The medium supplemented with 0.01 mg/L NAA, 0.5 mg/L BA, 0.5~1.0 mg/L kinetin and without plant growth regulator was effective for shoot development of buds from the nodal segment culture. The concentration of BA and NAA was an important factor in the bud induction of buds from the nodal segments of Chinese yam. Nodal segments cultured on the medium containing 1.0 mg/L NAA and 0.5~1.0 mg/L BA gave the best response to bud formation. The addition of GA_3 to the culture medium suppressed shoot induction and growth, while it increased microtuber formation. The shoot growth and microtuber formation were also affected by medium strength and solidity. The MS basal medium containing 1 g/L gelrite was suitable for microtuber formation from the nodal segment of Chinese yam.

Key words: Chinese yam, growth regulator, micropropagation, node culture

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Introduction

Yams (*Dioscorea* spp.) produce edible tubers, bulbils, and rhizomes of considerable economic importance. They are major source of carbohydrate in many tropical and subtropical countries (Onwueme 1978). Many wild *Dioscorea* species are medicinally important for the commercial production of steroidal drugs (Coursey 1967 ; Datta and Datta 1981).

The traditional methods of growing yams are inefficient and are associated with viruses, nematodes and other diseases that result in decreased yields. An increase in yam production can come from improved cultural methods, improved cultivars by selection and breeding, and by improved propagation methods such as *in vitro* propagation. The *in vitro* culturing of defoliated nodal explants on suitable agar nutrient media has been used in the regeneration of edible and medicinal yams (Sylvia et al. 1995). The regenerative behaviour of the explants in culture depends on the species (Ammirato 1984), the age of the parent plant (Mantell et al. 1978) and the level of concentration of auxins/cytokinin in the medium (Uduebo 1971). Factors such as inorganic ammonium, growth regulator supplements, propagule type and a subculture period affecting the *in vitro* establishment of *D. cayenensis* and *D. trifida* were tested by Sylvia et al. (1995). However, the genus *Dioscorea* showed large differences in its response depending on the type of explant, cultivars and concentrations of plant growth regulators. Therefore, this study was carried out to determine the appropriate concentrations of plant growth regulators for establishment of the healthy seedlings of Chinese yam (*D. opposita*), cultivated in Korea.

Materials and Methods

Plant materials

Different types of explants (leaves, petioles, roots and nodal pieces) used in this study were obtained from the *in vitro* grown seedlings of Chinese yam (*D. opposita*) cv. Danma. Stock plants were grown in the 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 solution (2%) for 15 min. The explants were thoroughly washed with sterile distilled water (3 times). We obtained young seedlings from the explants after 40 days of culturing *in vitro* on MS (Murashige and Skoog 1962) basal medium. Petioles (1 cm), roots (1 cm) and leaf segments (5 mm×5 mm) were excised from the *in vitro* grown seedlings derived from vine cuttings. For nodal segment culture, nodal segments of 1.5 cm in length consisting of a single node and a portion of petioles with axillary bud, were obtained from the middle region of vigorously growing stems, and used as explants.

Shoot and bud induction

All explant cultures - leaves, petioles, roots and nodal pieces - were placed on fresh initiation media (MS with 30 g/L sucrose and 2 g/L gelrite) containing 0.5 mg/L 2,4-D, 0.5 mg/L kinetin, 0.5 mg/L BA and 0.5 mg/L BA + 0.5 mg/L kinetin for one month. All solutions and media used were autoclaved at 121℃ under 1.2 kg/cm^3 pressure for 20 min. The plantlets were grown in a petridish containing 20 ml media. The cultures were transferred to a growth room with a 16 h photoperiod at 26±1℃, with light provided by cool white fluorescent tubes (1,200 lux intensity). Plant generation from various kind of explants was checked after 30 days.

The media used for induction of shoots and buds from the nodal segments included 1/2 MS supplemented with 30 g/L sucrose and 2 g/L gelrite. Details on the growth regulator supplements of the medium were 0.5～2.0 mg/L 2,4-D, 0.01～1.0 mg/L NAA, 0.5～1.0 mg/L BA, 0.5～1.0 mg/L kinetin, and 1.0～2.0 mg/L GA_3 individually. Eight nodes with and without leaves were transferred to media with various growth regulators and cultured for two months.

Microtuber induction

For the purpose of microtuber induction, 2,4-D, BA, kinetin, GA_3, (0.5～2.0 mg/L), and NAA (0.01～1.0 mg/L) were used individually. The nodal segments were grown in glass tube containing 20 ml media at 26±1℃, 16 h photoperiod. Microtuber formation was also recorded on different medium strength and solidity. Eight nodal pieces were cultured in bottle containing 80 ml of half and full strength medium with 0～2 g/L gelrite. The cultures in liquid medium (0 g/L gelrite) were suspended at 120 rpm. The number of nodal segments with microtubers was recorded after 60 days of being cultured.

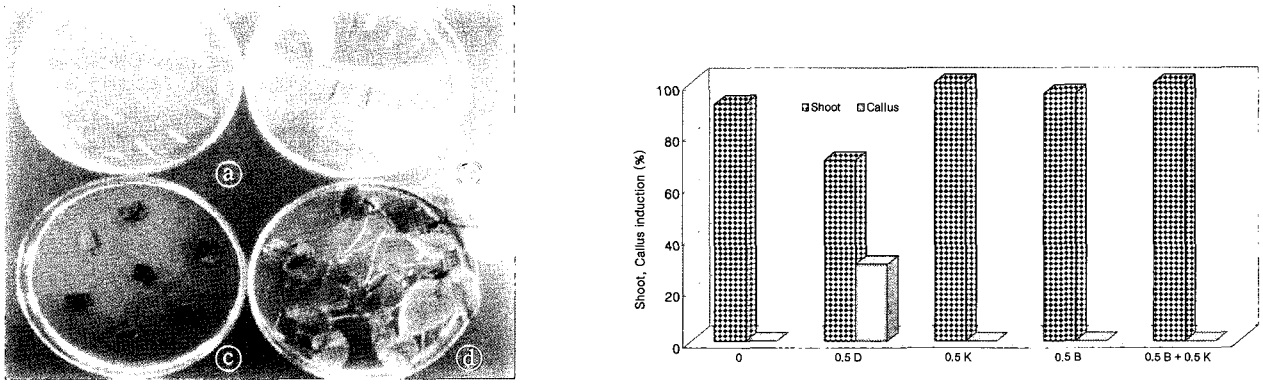
Growth parameters

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

Results and Discussion

Organ formation from explants

For the organ induction from tissue of Chinese yam, different types of explants (leaf segments, petioles, roots and internodal stem pieces), excised from the *in vitro* grown seedlings, were cultured on a MS medium supplemented with 0.5 mg/L 2,4-D, 0.5 mg/L kinetin, 0.5 mg/L BA, 0.5 mg/L BA + 0.5 mg/L kinetin or without growth regulators. All of the growth regulators induced shoots from the nodal segments at high frequency (above 70%). However, no shoot or callus was induced from the leaf, petiole and root tissues (Figure 1). Conventional methods of yam propagation by seed and planting sets are slow and not effective for the rapid proliferation of disease-free planting stocks. To overcome such problems, *in vitro* methods like nodal segment culturing, indirect organogenesis, and somatic embryogenesis, have been implemented (Mantell et al. 1978 ; Ng 1992 ; Twyford and Mantell 1996). The method of clonal propagation of *Dioscorea* plants through tissue culture can also be of great help in a breeding program by speeding up the selection process (Chaturvedi and Sinha 1979).



A B

Figure 1. Plantlet production from Chinese yam tissues.
A. Various cultured explants (ⓐroot, ⓑpetiole, ⓒleaf) and production of plantlets from nodal segmentⓓ after 30 days in a medium containing 0.5 mg/L kinetin and 0.5 mg/L BA.
B. Shoot and callus induction from nodal segment culture of Chinese yam.
Nondal segments were cultured for 30 days on a MS basal medium containing 0.5 mg/L 2,4-D (D), 0.5 mg/L BA (B), 0.5 mg/L kinetin (K) or hormone free (0).

Shoot and bud induction from nodal pieces

The medium with 0.01 mg/L NAA, 0.5 mg/L BA, 0.5～1.0 mg/L kinetin and without growth regulator were suitable for shoot production from the nodal segment culture. A high concentration of auxin (2,4-D, NAA), BA and the addition of GA_3 in the culture medium had adverse effect on shoot induction and elongation. The concentration of BA and NAA was an important factor for bud induction. The addition of 0.5～1.0 mg/L BA and 1.0 mg/L NAA provided enhanced in bud formation from the nodal segment of yams (Figure 2).

The production of shoots and buds from the nodal segment was effective for *in vitro* micropropagation of Chinese yams. In addition, the system using multiple buds is more practical than somatic embryogenesis, which has been achieved in *D. floribunda* (Ammirato 1984) and 'Nagaimo' Chinese yam (Nagasawa and Finer 1989), because of the low rate of plant regeneration from the somatic embryos. Although a few adventitious buds begin to develop into shoots when the incubation in the initial medium lasted for more than 2～3 months, vigorous shoot formation was promoted by transferring the clusters of adventitious buds to a fresh medium supplemented with a low concentration of auxin and cytokinin (Hiroyuki et al. 1995).

The shoot growth was also affected by medium strength and solidity (Table 1). The solidified medium inhibited shoot growth. A significant response to medium strength and solidity was not found for shoot formation. The low to medium strength and high to medium solidity, however, inhibited shoot elongation.

Microtuber induction from nodal pieces

Microtubers are considered as a useful means of international germplasm exchange as well as the propagation of planting material. The induction and growth of microtubers in *Dioscorea* have been found to be under the control of many factors such as cytokinins, auxin, abscisic acid, sugar concentration or photoperiod (Mantell and Hugo 1989 ; Jean and Cappadocia 1992 ; Ng 1992). In the present study, the addition of GA_3 to the culture medium increased microtuber formation (Figure 3). Microtubers were either directly formed at the axils of the explant or developed plantlet.

The microtuber formation was also affected by medium strength and solidity (Figure 4). Using a half strength medium, microtuber formation was inhibited on a medium which had a high concentration of gelrite. The full strength of MS basal medium containing 1 g/L gelrite was suitable for microtuber formation. Many *Dioscoreas* are cultivated for their tubers, which are rich sources of carbohydrates, and, in some species, the steroid diosgenin. In many *Dioscorea* species, the *in vitro* cultured shoots are able to produce microtubers under certain induction conditions and have a great potential for rapid multiplication and distribution of pathogen-free clonal material in the international yam germplasm exchange programmes.

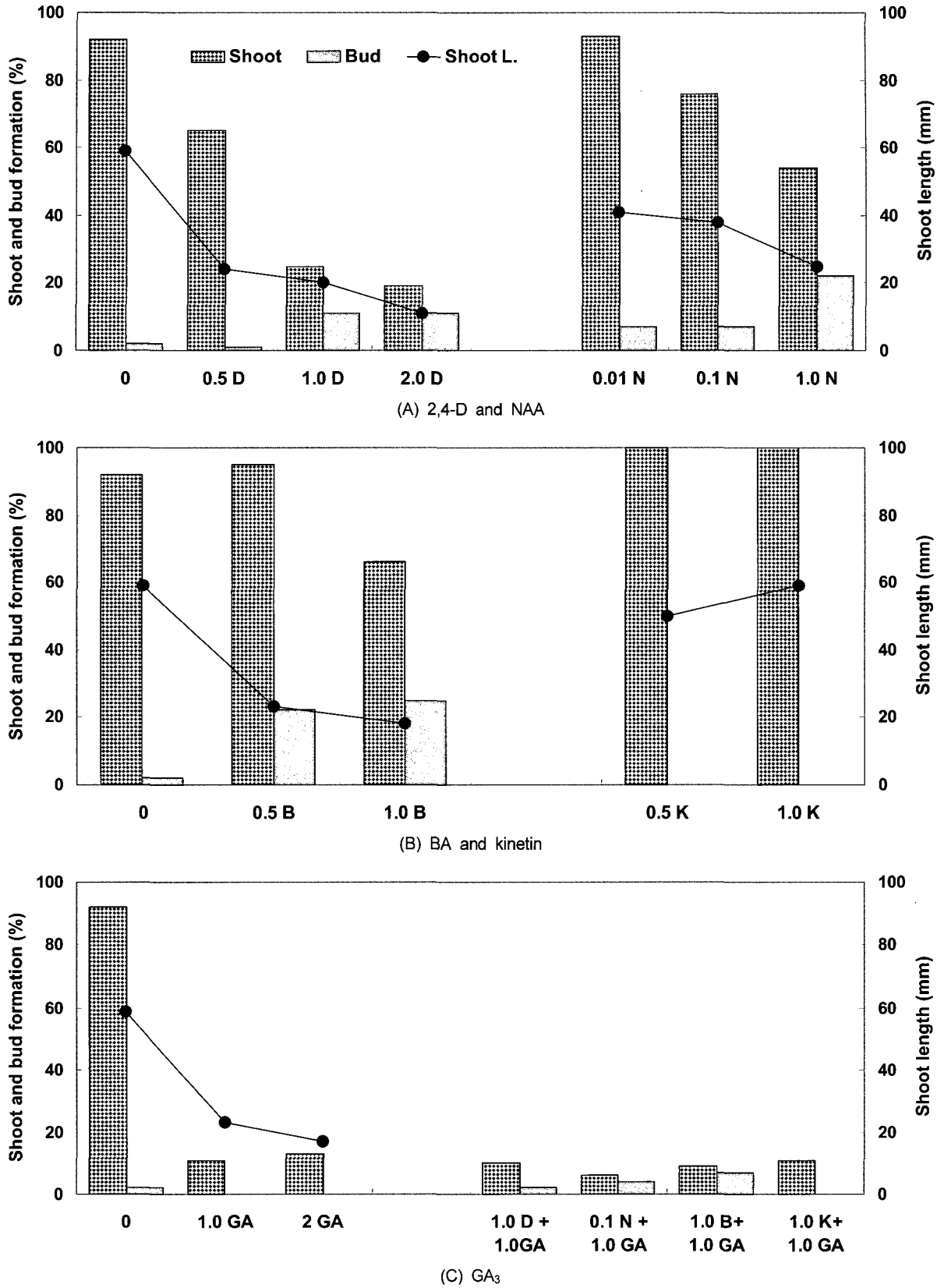


Figure 2. Effect of plant growth regulators (2,4-D and NAAⒶ, BA and kinetinⒷ and GA_3Ⓒ) on shoot formation and bud formation from a single nodal segment of Chinese yam. 0 (zero), D, N, B, K and GA mean hormone free, 2,4-D, NAA, BA, kinetin and GA_3, respectively.

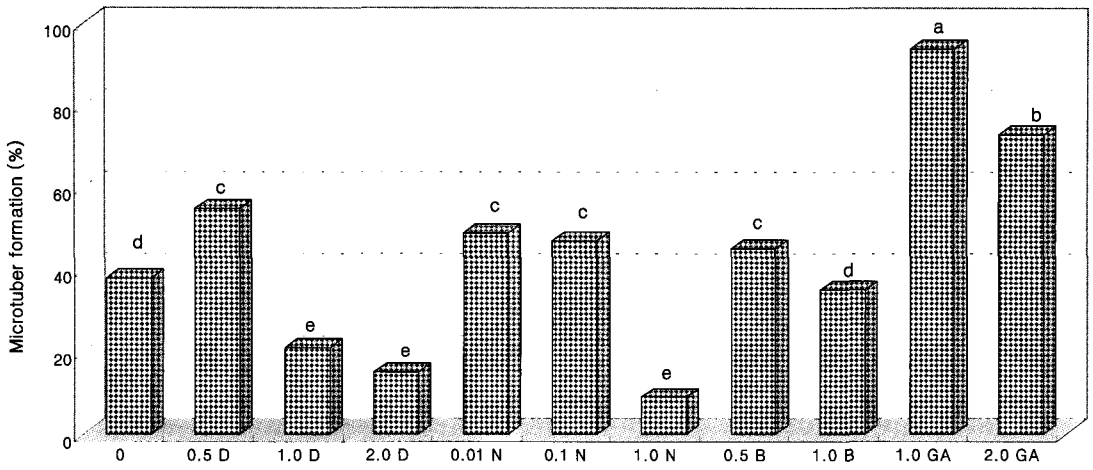


Figure 3. Effect of plant growth regulators on microtuber formation from a single nodal segment of Chinese yam. Mean separation within columns by Duncan's multiple range test, $P \leqq 0.05$.

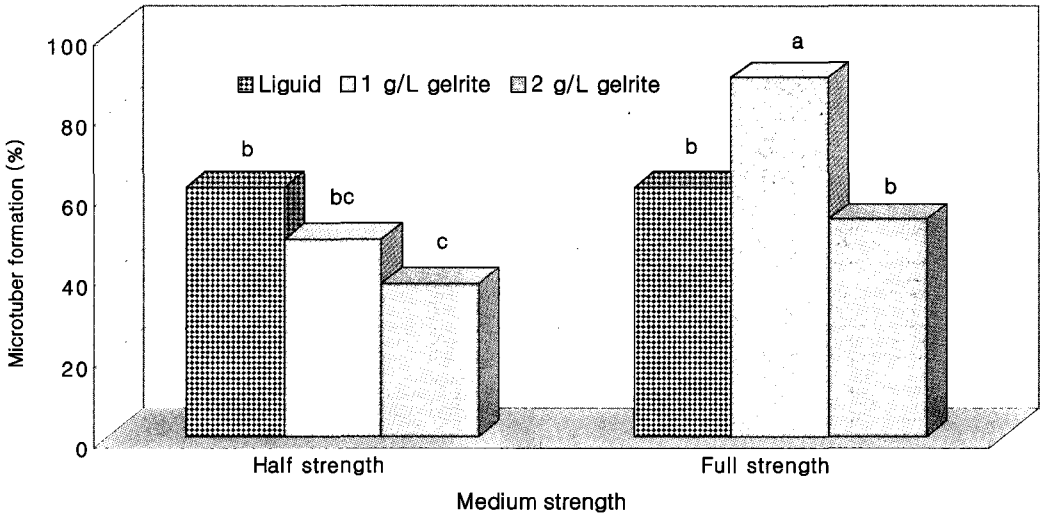


Figure 4. Effect of medium strength and solidity on microtuber formation from a nodal segment of Chinese yam. Mean separation within columns by Duncan's multiple range test, $P \leqq 0.05$.

Table 1. Effect of medium strength and solidity on shoot formation from a nodal pieces of Chinese yam.

Medium strength*	Conc. gelrite (g/L)	Shoot formation (%)	Shoot length (mm)
Half	0	94[ns]	110 a[z]
	1	98	61 c
	2	92	59 c
Full	0	98	125 a
	1	89	94 b
	2	88	70 c

[NS] Non-significant at the 5% level.

[z] Mean separation within columns by Duncan's multiple range test at, $P \leqq 0.05$.

* Nodal segment were incubated for 60 days on a half and a full strength MS medium without growth regulators.

Acknowledgement

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