ナガイモ茎頂の形態形成に及ぼすオーキシン,サイトカイニンおよび窒素量の影響

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Effects of Auxin, Cytokinin and Nitrogen Concentration on Morphogenesis of Tissue-cultured Shoot Apex of Chinese Yam (*Dioscorea opposita* Thunb.)

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Summary

Most shoot apices of Chinese yam (*Dioscorea opposita* Thunb. cv. Nagaimo) developed into shoots in modified Murashige-Skoog (MS) medium in which the nitrogen level was reduced to one tenth of the recommended concentration and containing 0 to 0.1 mg·liter^{-1} NAA and 0.1 mg·liter^{-1} BA. Bases of shoot apices developed friable calli in a medium supplemented with 1.0 mg·liter^{-1} NAA. The composition of the basal medium had little effect on shoot formation, but morphogenic calli were produced by excised apices on a medium having 1 to 2 mg·liter^{-1} BA and low concentration of NAA. Morphogenic callus resembled shoot primordium in that adventitious buds were formed on its surface. When morphogenic calli were subcultured on MS medium containing 0 to 0.1 mg·liter^{-1} BA and 0.01 to 0.1 mg·liter^{-1} NAA, a large number of plantlets were regenerated.

Introduction

Shoot apices have been cultured to obtain virus-free plants for a wide range of crop species (5, 9, 12). Some viruses infect *Dioscorea* species (3, 8, 11). However, numerous virus-free plants have been isolated and maintained through shoot apex culture (4, 6, 8, 12).

For clonal propagation by shoot apex culture, it is desirable to obtain plantlets arising from lateral buds and to avoid those regenerated from the basal callus because the latter may produce somaclonal variants (17).

In the present paper, the effects of auxin, cytokinin, nitrogen concentration and different basal medium on shoot formation from shoot apices and calli formed on cut surfaces of shoot apices of *Dioscorea opposita* were examined.

Recently, shoot apex culture has been used for multi-propagation because its use has resulted in useful morphogenic responses, i.e., the induction of bud-multiplying body (14), embryogenic callus (2) and morphogenic callus (10). The formation of morphogenic callus by apices of Chinese yam was recognized in some media, thus creating a potential for multi-shoot propagation. Therefore, the effects of auxin and cytokinins on the formation of morphogenic calli and on shoot regeneration from them were pursued.

Materials and Methods

Shoots were obtained from pieces of tuber of *Dioscorea opposita* cv. Nagaimo (about 150 g) in a greenhouse. When the stem elongated to approximately 1 m, 5 cm-stem tips were excised and sterilized with 70% ethanol for 10 sec. and 1% sodium hypochlorite for 15 min. They were rinsed with sterile distilled water 2 to 3 times. The shoot apices originating from lateral buds which included 2 or 3 leaf bud primordia were excised and cultured on a solid medium.

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Experiment 1. Shoot formation

Shoot apices were cultured on MS medium and modified MS medium in which the concentrations of ammonium and potassium nitrate were either kept at the recommended level or reduced to one tenth the level. The concentrations of α-naphtaleneacetic acid (NAA) were adjusted to 0, 0.1 and 1.0 mg·liter^{-1}, whereas those of 6-benzyladenine (BA) were kept at 0.1 and 1.0 mg·liter^{-1}; sucrose and gellan gum concentrations were 20 g and 2 g per liter, respectively. Thus the trial consisted of two levels of nitrogen, three levels of NAA, and two levels of BA in a 2×3×2 factorial arrangement.

Half-strength MS medium (1/2 MS) or White's medium having a known concentrations of NAA and BA were used to test the effect of a basal medium on shoot formation.

Experiment 2. Morphogenic callus production and shoot proliferation from it

The formation of morphogenic callus was investigated by culturing shoot apices on MS medium supplemented with four levels each of NAA and BA as shown in Table 1. By subculturing shoot apices on the medium containing kinetin, 6-γ, γ-dimetylallylamino prine (2ip) and zeatin for BA, the effectiveness of different cytokinins on the induction of morphogenic callus was examined. These morphogenic calli were subcultured on solid MS medium supplemented with four levels of NAA and three levels of BA for shoot regeneration.

The above media were adjusted to pH 5.5 with 0.1 N NaOH or HCl before being autoclaved at 120°C for 15 min. Shoot apex was cultured in a test tube containing 10 ml of medium and kept at 25°C under a 16/8 hr day/night photoperiod and illuminated with 3 to 4 thousand lx provided by florescent lamps. The morphogenic responses by the shoot apices were evaluated after 3 months.

Results

1. Shoot formation

Shoot formation was observed in the medium supplemented with 0.1 mg·liter^{-1} BA and more than 60% of apices developed into shoots in all NAA concentrations (Fig.1), whereas, no shoot was formed when the BA concentration was raised to 1.0 mg·liter^{-1}. More shoots were developed in a medium with the reduced ammonium and potassium nitrate concentrations than at the recommended MS formulation.

Two pathways to shoot formation from shoot apex were recognized (Fig.2) : a) a direct shoot development in 80% of apices cultured on a medium having 0 to 0.1 mg·liter^{-1} NAA, and especially, in that which the N content was reduced, b) shoot differentiation via callus induced from the bases of excised shoot apices. In a medium supplemented with 1.0 mg·liter^{-1} NAA, the apices tended to develope into calli from which adventitious buds differentiated and grew into shoots. Although no shoots developed directly from apices kept in a

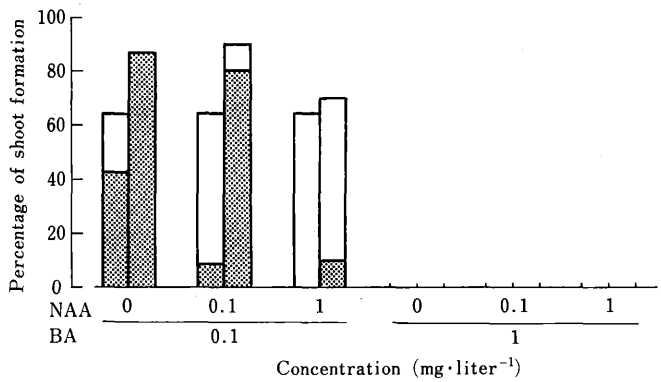

Fig. 1. Percentages of apices which developed shoots as a function of NAA, BA and nitrogen concentration. Two nitrogen rates are: 1×(left bar) and 1/10×(right bar). Shaded bars represent development from shoot apices directly.

medium containing 1.0 mg·liter^{-1} of BA, the cut bases formed morphogenic calli having a large number of adventitious buds.

Although more shoots were regenerated under the reduced N level plot, they had fewer leaves and shorter stem length than did those shoot produced in a medium with the higher N level (Fig.3). The frequencies of direct shoot formation by 1/2 MS, MS and White's media were 50, 34 and 15%, respectively (Fig.4). White's medium was not suitable for micro-propagation of *D. opposita.*

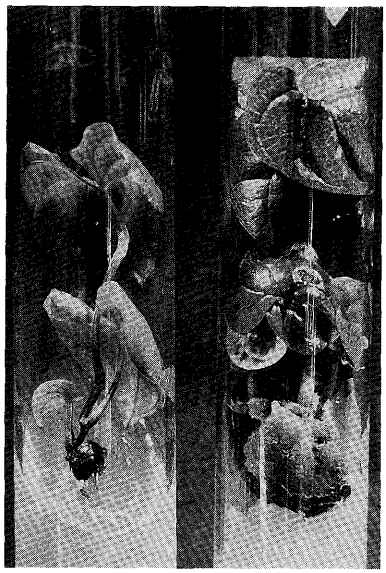

Fig. 2. Shoot derived from excised shoot apex (left, direct shoot formation) and that differentiated from a friable callus (right), a product of a cultured apex.

2. Induction of morphogenic callus and shoot proliferation

Production of morphogenic calli from basal cuts of shoot apices was observed in a medium supplemented with 0.1 to 2 mg·liter^{-1} BA and 0 to 1.0 mg·liter^{-1} NAA. (Table 1). The external appearance of morphogenic callus differed from that of a friable one. The former was compact and differentiated adventitious buds on its surface. Most morphogenic calli grew to about 4 mm in diameter and differentiated shoots after a 3-month incubation period; some grew to be 6~9 mm in diameter in a medium containing 0.1 mg·liter^{-1} BA and 1 mg·liter^{-1} NAA or only 1.0 mg·liter^{-1} BA (Table 1, Fig. 5-1,2,3). But most of morphogenic calli formed in media containing 1 to 2 mg·liter^{-1} BA did not produce shoots.

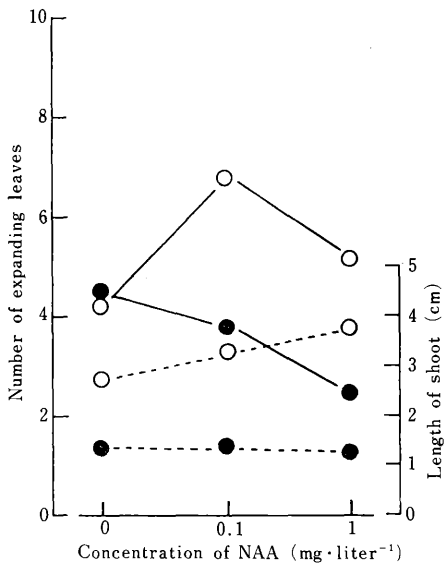

Fig. 3. The number of leaves (——) and shoot length (- - - -) derived from excised Chinese yam apices cultured on the Murashige-Skoog basal medium as a function of NAA and nitrogen concentration. The medium contained 1×(○) and 1/10×(●) of the recommended N level, plus 0.1 mg·liter^{-1} BA.

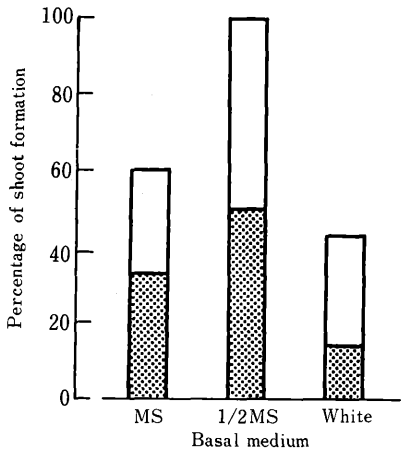

Fig. 4. Effects of different basal media containing 0.1 mg·liter^{-1} NAA and 0.1 mg·liter^{-1} BA on shoot formation. Shaded columns represent the percentages of apices which formed shoot from lateral bud (direct shoot formation). Open columns represent the percentages of apices which differentiated shoot via friable calli.

Table 1. Effect of BA and NAA on induction of morphogenic callus and differentiation of shoot.

Concentration (mg·liter^{-1})		No. of shoot apices cultured	Morphogenic callus		No. of shoots differentiated from morphogenic calli
BA	NAA		No. of calli formed	Size[z]	
0	0	7	0	−	0
0	0.01	7	0	−	0
0	0.1	10	1	+	3
0	1	8	0	−	2
0.1	0	7	2	+	1
0.1	0.01	7	1	+	0
0.1	0.1	8	3	+	1
0.1	1	7	3	++	3
1	0	7	7	++	0
1	0.01	7	5	+	0
1	0.1	7	4	+	0
1	1	8	4	+	0
2	0	5	4	+	0
2	0.01	6	3	+	0
2	0.1	8	0	−	0
2	1	7	6	+	0

[z] 3～5 mm (+); 6～9 mm (++)

Table 2. Effectiveness of different cytokinins on the production of morphogenic callus[z].

Cytokinin	Concentration (mg·liter^{-1})	No. of shoot apices cultured	No. of calli formed	
			Morphogenic	Non-morphogenic
BA	0.01	10	1	8
	0.1	10	8	0
	1.0	10	8	2
2ip	0.01	10	1	7
	0.1	10	5	5
	1.0	9	6	3
Zeatin	0.01	16	0	16
	0.1	16	6	5
	1.0	16	13	2
Kinetin	0.01	10	0	6
	0.1	10	0	7
	1.0	10	1	8

[z] MS medium was used for basal medium and 0.01 mg·liter^{-1} NAA was added to each medium.

Apices cultured in 1.0 mg·liter^{-1} of zeatin produced the most morphogenic calli followed by 0.1 to 1.0 mg·liter^{-1} BA and 2ip (Table 2). Kinetin was ineffective with respect to the formation of morphogenic callus. These calli generated shoots after 2 months of subculturing in all media tested except that which was supplemented with 1 mg·liter^{-1} BA and no NAA (Table 3, Fig. 5-4). Most shoots were proliferated from calli during a relatively short incubation period after being transferred to a medium supplemented with 0.01 to 0.1 mg·liter^{-1} NAA and 0 to 0.1 mg·liter^{-1} BA. Shoot proliferation continued in some morphogenic calli even after the experiments were considered terminated.

Discussion

Growth of shoot apex in *D. opposita* was regulated by the ratio between NAA and BA, and by the nitrogen concentration in the basal medium. The data in the present report indicated that shoot differentiation was favored by 0 to 0.1 mg·liter^{-1} BA, whereas, formation of morphogenic callus from excised shoot apices was promoted by 1 to 2 mg·liter^{-1} of BA.

Shoot proliferation and generation of new plantlets were accomplished by two pathways: a) forcing of shoots from shoot apices directly (without callusing) and b) shoot differentiation via callus

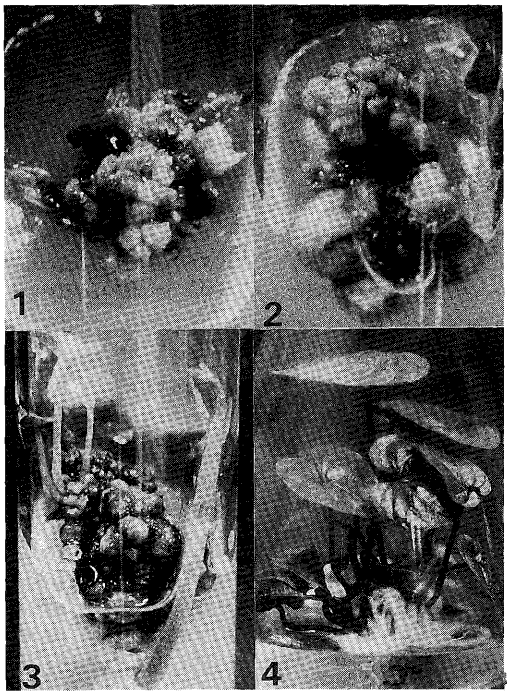

Fig. 5. External appearance of a morphogenic callus about 4 mm in diameter (1), and that of about 8 mm (2). A number of buds and shoots differentiating in the initial medium (3) and in a subculture medium (4). (See Table 1 for the composition of the medium).

Table 3. Effect of NAA and BA on shoot development and growth of morphogenic callus.

Concentration (mg·liter^{-1})		No. of morphogenic calli transferred	Morphogenic response			
NAA	BA		Shoot development	Growth of morphogenic callus	Change to non-morphogenic callus	Non-growth
0	0	9	2	0	0	7
0	0.1	8	3	2	1	2
0	1	4	0	0	0	4
0.01	0	8	6	0	0	2
0.01	0.1	9	5	0	0	4
0.01	1	11	3	1	0	7
0.1	0	10	4	0	1	5
0.1	0.1	11	5	0	2	4
0.1	1	12	5	0	2	5
1	0	6	2	1	1	2
1	0.1	7	3	0	2	2
1	1	6	1	0	0	5

from the basal cut surfaces of excised shoot apices.

Shoots were obtained via the first pathway by utilizing a medium having less than 0.1 mg·liter^{-1} NAA and 1/10 × recommended MS nitrogen concentration. In the second pathway, the characteristics of calli was effected by plant growth regulator contained in the medium. Friable calli, which were formed in the media having 0.1 to 1.0 mg·liter^{-1} NAA, regenerated shoots without subculturing, whereas, morphogenic calli, which were induced to form adventitious buds, were promoted by high concentration of BA.

Until recently, nearly all regeneration of plantlets of *D. opposita* was accomplished by culturing calli derived from shoot apices (12). However, Kageyama et al. (7) reported that plantlet regeneration from shoot apices of *D. japonica* without going through the callus stage was possible. Their medium contained 0.01 to 0.1 mg·liter^{-1} NAA and 0.1 mg·liter^{-1} BA. Ninety percent of excised shoot apices of *D. opposita* was able to produce shoot directly in the medium containing only 0.1 mg·liter^{-1} BA (16). Kobayashi (8) suggested the following three methods to inhibit callus formation in tissue-cultured sweet potato: a) reducing the mineral concentration of MS basal medium to half-strength, b) reducing the nitrogen concentration of the same medium, or c) using White's medium.

Because there was no large difference between MS and 1/2 MS with respect to direct shoot formation, we believe that direct shoot formation is caused, not by a reduction of the quantity of total ions in the medium, but by a reduction in the N level. The resulting plantlets should be transferred to a MS basal medium because vegetative growth of the regenerated plants is suppressed by reducing the N level.

Morphogenic calli are induced in a medium supplemented with high cytokinin concentration. These calli have a large number of adventitious buds on their surface, so that shoot proliferation occurred upon their being transferred to a subculture medium containing NAA and BA. Moreover, the calli obtained resembles shoot primordium in its external appearance and in its volume (16). Likewise, Shinmori et al. (13) observed shoot primordium-like body in calli of *D. opposita* cv. Tsukune. They reported continuous differentiation of buds leading to shoot formation, whereas we did not observe it, at least, during our experimental period.

As to the combination between NAA and BA for morphogenic callus induction, a medium supplemented with 1 to 2 mg·liter^{-1} BA and a low concentration of NAA is optimal. There was a difference among the effectiveness of cytokinin to promote the formation of morphogenic callus. BA, zeatin and 2ip were more effective than kinetin.

Literature Cited

1. Ammirato, P.V. 1984. Yams. p.327-354. In: D.A. Evans, W.R. Sharp, P.V. Ammirato and Y. Yamada (eds.). Handbook of plant cell culture. Vol. 2. Macmillan Publishing Company, New York.
2. Ammirato, P.V. 1989. Recent progress in somatic embryogenesis. Int. Assoc. Plant Tissue Cul. Newsletter 57 : 2-16.
3. Fukumoto, F. and M. Tochihara. 1978. Chinese yam necrotic mosaic virus. Ann. Phytopath. Soc. Japan 44 : 1-5.
4. Gewal, S., S. Koul, U. Sachdeva and C.K. Atal. 1977. Regeneration of plants of *Dioscorea deltoidea* W. by apical meristem cultures. Indian J. Exp. Biol. 15 : 301-302.
5. Hu, C.Y. and P.J. Wang. 1983. Meristem, shoot tip and bud culture. p.177-227. In: D.A. Evans, W.R. Sharp, P.V. Ammirato and Y. Yamada (eds.). Handbook of plant cell culture. Vol.1. Macmillan Publishing Company, New York.
6. Kageyama, K., K. Yabe, T. Iida and S. Washida. 1988. Plant regeneration and acclimatization from meristem of yam (*Dioscorea japonica* Thunb.). Plant Tissue Culture Lett. 5 : 11-14. (In Japanese).
7. Kobayashi, H. 1982. Application of tissue culture in sweet potato. 1. Maintenance and multiplication of germplasm. Japan. J. Breed. 32 (Suppl. 2) : 13-14. (In Japanese).
8. Mantell, S.H. 1980. Apical meristem tips culture for eradication of flexous rod viruses in yam (*Dioscorea alata*). Tropical Pest Management 26 : 170-179.
9. Mori, K., E. Hamaya, T. Shimomura and Y. Ikegami. 1969. Production of virus-free plants by means of meristem culture. J. Cent. Agr. Exp. Sta. 13 : 45-110. (In Japanese).
10. Nagasawa, A. and J.J. Finer. 1988. Induction of morphogenic callus cultures from leaf tissues of garlic. HortScience 23 : 1068-1070.
11. Okuyama, S. and H. Saka. 1978. Yam Mosaic Virus. Sci. Rep. Fac. Agri. Ibaraki Univ. 26 : 29-34.
12. Oosawa, K., T. Kuriyama and Y. Sugahara. 1981. Clonal multiplication of vegetatively propagated crops through tissue culture. 1. Effec-

tive balance of auxin and cytokinin in the medium and suitable explant part for mass production of plantlets in strawberry, garlic, scallion, welsh onion, yam and taro. Bull. Veg. & Ornam. Crops Res. Stn. Japan, Ser. A. No.9 : 1-46.
13. Shinmori, T. and Y. Naito. 1987. Multipropagation of *Dioscorea opposita* cv. Tsukune through tissue culture. Abst. Japan. Soc. Hort. Sci. Autumn Meet. 260-261. (In Japanese).
14. Pierik, P.L.M. 1987. In vitro culture of higher plants. p.159-167. Martinus Nijhoff Publishers, Dordrecht.
15. Takayama, S. 1983. Biotechnology. p.53-57. Nogyo Tosho, Tokyo. (In Japanese).
16. Tanaka, R. and H. Ikeda. 1983. Perennial maintenance of annual *Haplopappus gracilis* (2n=4) by shoot tip cloning. Jpn. J. Genet. 58 : 65-70.
17. Thanutong, P., I. Furusawa and M. Yamamoto. 1983. Resistant tobacco plants from protoplast-derived calluses selected for their resistance to *Pseudomonas* and *alternaria* toxins. Theor. Appl. Genet. 66 : 209-215.

ナガイモ茎頂の形態形成に及ぼすオーキシン，サイトカイニンおよび窒素量の影響

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摘　要

ナガイモ（*Dioscorea opposita* Thunb.）の茎頂培養において，カルス化による変異の出現を回避した個体再生法と大量増殖法を確立することを目的として，茎頂組織の形態形成に及ぼす培地中のオーキシン，サイトカイニン，窒素量および基本培地の影響について検討した．

MS 培地を基本として，0.1 mg·liter^{-1}以下の低濃度の BA 添加培地において高い割合でシュート形成が認められた．この場合，NAA 濃度が高くなるに従いカルス化が著しくなり，その後に再分化する傾向が認められた．NAA 濃度が0.1mg·liter^{-1}程度であれば，基本培地の窒素量を規定濃度の1/10に減じることで，約80%の茎頂がカルスを形成せずに直接シュートに発育した．窒素量を減少させると形成されたシュートの栄養生長が低下する傾向が認められた．基本培地の種類は直接的なシュート形成には大きな影響は認められなかった．BA 濃度を高くすると morphogenic なカルスが形成され，特に 1 ～ 2 mg·liter^{-1}の BA と低濃度の NAA が添加された培地において顕者であった．このカルスは外観が苗条原基に類似し，表面に多数の不定芽が存在して大量増殖への利用が期待されたが，詳しい特性はさらに検討する必要がある．

Morphogenic なカルスの形成は添加するサイトカイニンにより差異が認められ，BA，ゼアチンおよび 2 ip が効果的であった．Morphogenic なカルスを MS 培地を基本として 0.1mg・liter^{-1}以下の低濃度の BA と NAA が添加された培地に移植することでシュート形成が認められた．

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