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Direct Multipropagation through Organogenesis from Nodal Explants of *Anoectochilus formosanus* Hayata

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ABSTRACT

To establish direct multipropagation through organogenesis from nodal explants of *Anoectochilus formosanus* Hayata, the nodes were cultured on LS medium containing various concentrations of 6-benzyladenine(BA). High plant regeneration and adventitious bud formation were obtained from supplemented with 4.0mg/l of BA. Plant height was promoted by adding 0.3% activated charcoal. Plantlet regeneration capacity from nodes was depended on nodal parts on the stem, upper position was the best comparing with intermediate and lower.

Key words: *Anoectochilus formosanus* Hayata, multipropagation, organogenesis, charcoal, node.

INTRODUCTION

Anoectochilus formosanus Hayata plants are perennial medicinal plants that belongs to the family Orchildaceae. *Anoectochilus* is a *Anoectochilus* genus and distributed in India, the Himalayas, East-south Asia and Indonesia etc(Chen et al., 1989; Choi & Rha, 1986).

It has 1-3 dipalmitin, palmitic acid and β-D-glucopyranoside (Ito et al., 1994) and acts as the anti-inflammatory, antipyretin, hemo static, diuresis and cardiotonics for medicine(Choi & Rha, 1986).

A. formosanus Hayata has been normally propagated from seed, but the percentage of germination is so low(Ito et al., 1994). In relation to this, it is important for the conservation of the useful genetic plant recourses in the course of the rapid industrial development nowadays. Especially, the modern' s concern on the herb medicine concentrates on ensuring the useful components which can be got from the plants.

we conducted an basic experiment to establish for rapid multipropagation system through direct organogenesis from nodal explants of *Anoectochilus formosanus* Hayata *in vitro*.

MATERIALS AND METHODS

Healthy wild *Anoectochilus formosanus* Hayata was collected from department of agricultural management, National Giayee College in Taiwan.

Explants collected from the field were thoroughly washed in running tap water and then surfaced disinfected using 0.5% sodium hydrochloride(NaClO) solution(v/v) for five minutes. After three times washing with sterile distilled water, explants were cut 10mm in size and were inoculated onto LS medium(Linsmaier and Skoog, 1965) containing 3% sucrose for 70 days. The medium was gelled using 0.8% agar. In activated charcoal treatment, we also added 3.0% activated charcoal in LS basic medium and cultured for 150 days. All materials inoculated on the media were subcultured with same fresh medium by one month interval.

The explants were cultured on LS medium that was supplemented with different concentrations of 6-benzyl-aminopurine(BA) (1.0, 4.0, 6.0, and 8.0mg/l) Three

parts of nodal explants on the stem were also used to determine their efficiency for adventitious bud formation and plant regeneration in *Anoectochilus formosanus* Hayata. All the explants on the media were incubated at a constant temperature of 25±℃ and a 16-h photoperiod under 2,000 lux.

The experiments were conducted by two replicates with 30 or 90 pieces per treatment. Treatment means were compared at the level p=0.05 by Duncan' s Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Effect of BA

Plant regenerated and adventitious bud formed from nodes placed on media that contained BA, regardless of the concentration of BA in *Anoectochilus formosanus* Hayata (Table 1). Out of different BA concentrations used regenerated plants, especially, 4.0mg/l of BA was most effective concentration for adventitious bud formation and plant regeneration. The ratio of direct adventitious bud formation from nodal explants was the lowest at the 1.0mg/l treatment with 13.3% and highest at the 4.0mg/l with 96.6%. At the 6.0mg/l and 8.0mg/l treatments, it showed 73.3% and 20.0% for bud formation ratio, respectively. This results were similar to other reports with BA concentrations(Kato & Ozawa, 1979; Nakano et al., 1994), but it was inconsistent with good response, at low concentration, 1mg/l of BA, in *Angelonia salicariefolia*(Debergh and Wael, 1997).

The regeneration of shoot through direct organogenesis from nodal explants is illustrated Fig. 1A-C. The total number of plantlet regeneration was also the most in the 4.0mg/l treatment with 242 plants and followed by 6.0mg/l and 8.0mg/l treatment with 144 and 43, respectively. But, at the 1.0mg/l , it regenerated only 7 plants from 30 nodal explants. In addition, it was better for the inducement of the multiple stem to use only the BA *Populous* hybrid(Chun et al., 1986).

On the other hand, Hasegawa(Hasegawa, 1979) observed that shoot development is less effective with BA and NAA combination than with sole BA in *Anoectochilus formosanus* Hayata.

Effect of activated charcoal

Effect of activated charcoal on the plant height of *Anoectochilus formosanus* Hayata was in Table 2. The effect of activated charcoal was good response with activated charcoal medium comparing with non-adding activated charcoal medium for plant height, regardless of BA concentrations. Shoots produced on activated charcoal medium were of high quality, and the longest shoots with 1.0mg/l of BA. It will be a good source as a material for direct multipropagation *in vitro*.

The plant height tended to decrease in proportion to increasing BA concentration with activated charcoal in

Table 1. Effect of concentration of BA for direct plantlet regeneration from nodal explants of *Anoectochilus formosanus* Hayata

BA Concentration (mg/l)	No. of nodal explant cultured	Adventitious bud forming stem explants		Total no. of regenerated plantlets	No. of plantlets regeneration per explant
		No.	%		
1.0	30	4	13.3	7	1.8c
4.0	30	29	96.6	242	8.3a
6.0	30	22	73.3	144	6.6bc
8.0	30	6	20.0	43	7.1b

Mean separation within colum by DMRT at 5% level.

Table 2. Effect of activated charcoal for plant height on different concentration of BA

Treatment	BA(mg/l)			
	1.0	4.0	6.0	8.0
	Plant height (cm)			
(-)Activated charcoal(Control)	3.9	3.3	3.0	2.7
(+)Activated charcoal	4.4	3.7	3.5	2.2

the medium. There is no confirmed orthodox about the mechanism of the AC effect on the medium, but it conferred that the AC had the function to secret secondary products from the tissue(Wang and Huang, 1976), and to control the supply of any growth regulator(Reinert and Bajaj, 1977). Recently, activated charcoal frequently promotes the *in vitro* growth and organogenesis of plant tissues by the absorption of compounds from the culture medium and /or from the container atmosphere(Druart and Wulf, 1993).

Effect of various parts of nodal explant

The result of adventitious bud formation and plant regeneration from nodes on the stem of *A. formosanus* Hayata were in Table 3. More regenerated plants were produced by used different parts of nodal explant cultured on activated charcoal medium with 4.0mg/l. The variable number of plantlets produced by nodes may derived from the different parts on the node in stem. Nodes on the stem used for material were divided into three parts; upper, intermediate and lower.

Both of adventitious bud formation and plant regeneration were the most from upper part of nodal explants, and followed by intermediate and lower part. Especially, upper part of nodal explants formed adventitious buds with 100%. It may be reason of the softness of plantlet tissue(texture)(Chun et al., 1986; Datta and Datta, 1984). As the similar results, Chen et al.(from Debergh and Wael, 1977) reported that the adventitious bud formation ratio was 100% in the bulbil, 84% in the root tip and 48% in the unripe seed in *Pinellia ternata*(Choi and Rha, 1986). According to Debergh and Wael(from Kuku et al., 1977), the

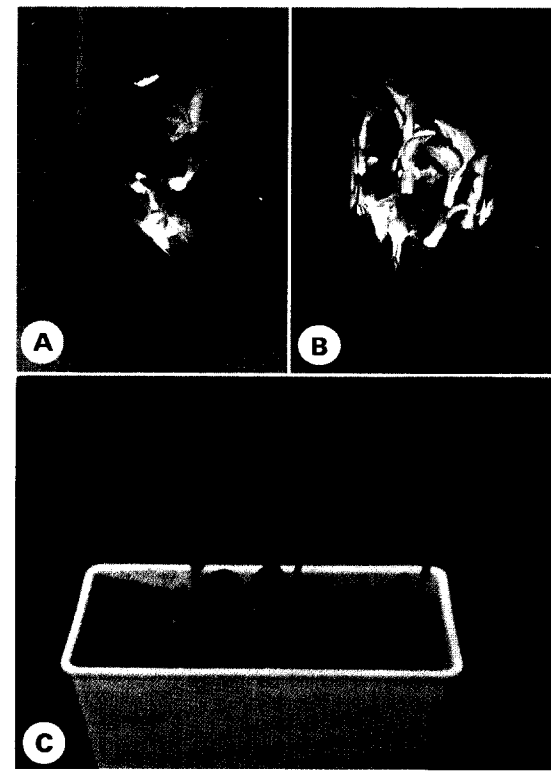

Fig. 1A-C. Plant regeneration of *Anoectochilus formosanus* Hayata. A-B : Direct regenerated plantlets from nodal explants on LS medium with 1.0mg/l and 4.0mg/l of BA. C : Fully grown regenerants in soil.

shoot formation was the best in the midrib and leaf margin in the leaf of *Ficus lyrata*. It was explained that there is the endogenous growth substance in the plantlet tissue(Kuku et al., 1977). Since nodes proved an effective explant for multipropagation, most elongated shoots provided an effective protocol. we hope that the studies described in this paper will provide a useful preliminary for the development of techniques for the multipropagation

Table 3. Comparison of adventitious bud formation and plantlet regeneration among different parts of nodes on the stem from *Anoectochilus formosanus* Hayata on LS medium containing 4.0mg/l of BA

Nodal source	No. of nodal explants cultured	No. of adventitious bud forming stem explants	% adventitious buds induced	Plantlet regeneration**	
				Total	Per explant
Upper	90	90	100	769	8.5a
Intermediate	90	86	95.6	587	6.8b
Lower	90	69	76.7	471	6.8b

Mean seperation within coakum by DMRT at 5% level.

of other genotypes of *Anoectochilus formosanus* Hayata

LITERATURE CITED

Chen, C.C., T.G. Gau and H.S.Tsay. 1989. Studies on the callus formation and plant regeneration of *Pinellia Ternata*. J. Agric. Res. China 38:30-41.

Choi, J.S and E.U Rha. 1986. Studies on the mass propagation of *Pinellia ternata* Breit *in vitro*. Korean J. Crop Sci. 31:30-42.

Chun., Y.W., R.B. Hall and S.C Stephens. 1986. Influences of medium consistency and shoot density on *intro* shoot proliferation of *Populus alba x P. grandidentata*. Plant Cell Tissue Organ Culture 5: 179-185

Datta, K and S.K. Datta. 1984. Rapid clonal multiplication of *Angelonia salicariefolia* through tissue culture. Plant Cell Tissue Organ Culture 3:215-220.

Debergh and Wael. 1977. Mass propagation of *Ficus lyrata*. Acta hort. 78: 361-364.

Druart Ph and O De Wulf. 1993. Activated charcoal catalyses sucrose hydrolysis during autoclaving. Plant Cell Tissue Organ Culture 32: 97-99.

Hasegawa,. P.M. 1979. *In vitro* propagation of rose. HortSci. 14:610-612.

Ito, A., R. Kasai., K. Yamasaki and H. Sugimoto. 1994. Aliphatic and aromatic glycosides from *Anoectochilus koshunensis*. Phytochemistry 33: 1133-1137.

Kato, Y and N Ozawa. 1979. Adventitious bud formation on leaf and stem segments of *Heloniopsis orientalis* grown at various temperature. Plant and Cell Physiol. 20:491-497.

Kuku czanka, K., K. Klimaszewska and H. Pluta. 1977. Regeneration of entire plant of *Peperomia scandens* Ruiz from different parts of leaves *in vitro*. ACTA Hort. 78:365-369.

Linsmaier, E.M and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18: 100-127

Nakano, M., Y. Hoshino and M. Mii. 1994. Adventitious shoot regeneration from cultured petal explants of carnation. Plant Cell Tissue and Organ Culture 36:15-19.

Reinert, J and Y.P.S. Bajaj. 1977. Anther culture: Haploid production and it' s significance. In Applied and fundamental aspects of plant cell, tissue, and organ culture. Springer Verlag, Berlin, pp. 251-267.

Tsay, H.S., P.C. Lai and L.J Chen. 1982. Organ differentiation from callus derived from anther, stem and tuber of sweet potato. J. Agric. Res. China 31:191-198.

Wang, P.J and L.C. Huang. 1976. Beneficial effects of activated charcoal on plant tissue and organ culture. In Vitro 12: 260-262.