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In Vitro Propagation o. *Stevia rebaudiana* Bertoni

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스테비아의 器內培養과 增殖에 관한 硏究

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ABSTRACT

This study was undertaken to know the possibility of in vitro propagation of Stevia through axillary bud culture and the results indicated that: (1) Addition of NAA (0.01-0.05 mg/l) alone on Murashige-Skoog basal medium promoted shoot differentiation and growth rate. And also additional of kinetin of 0.5-1.0 mg/l alone showed the same trend as that of NAA: (2) Addition of both NAA (0.01-0.05 mg/l) and kinetin (0.5-1.0mg/l) to MS medium promoted better shoot formation. (3) Shoot differentiation and growth were better on the full salt strength of MS medium (1X MS) than that of half strength (1/2 MS), while their effects were reversed for root differentiation

INTRODUCTION

Among several potential application of in vitro culture in agriculture, rapid clonal multiplication of specific genotypes is the most often sought as an alternative in propagation when conventional methods permit only slow increase in clonal plants. There are many reports indicating that plants could be propagated through meristem tip culture.[1-8, 10)]

Stevia is a perennial herb native to the mountainous areas of Paraguay. Stevioside contained in the leaves of Stevia is utilized as a natural sweetener. So far conventional stem cutting method is only way of maintain the uniformity of the variety or the genotypes. If plants could be regenerated directly from the meristematic tissue, this might be advantageous over the techniques through callus formation since plants regenerated from callus were liable to exhibit genetic aberrations. Plants could be produced from explants without callus state.[11, 12)]

This study was undertaken to investigate the possibility of uniform and rapid clonal propagation through axillary bud culture.

MATERIALS AND METHODS

Stem with 2-3 axillary buds were predisinfected by immersing in 70% ethanol for 10 seconds followed washing with sterilized-distilled water 2-3 times, and then surface-sterilized in 20% Chlorax bleach (5.7% sodium hypochlorite) solution for 10 minutes followed by 2-3 times rinses in sterilized-distrilled water.

Surface-sterilized stem sections were again cut into approximately 5mm in length, and placed on Murashige-Skoog (MS) medium with various concentrations of NAA and kinetin. From the shoots differentiated from axillary buds, the number of leaves and nodes, and shoot height were recorded. For economical clonal propagation, full and half

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salt strength of MS medium were investigated with different NAA and kinetin combinations. To know the possibility of mass production of clones in a short time, nodes with 5mm in length from the first differentiated shoot were continuously subcultured up to third subculture generations on the MS medium, and then put them on root formation medium.

RESULTS AND DISCUSSION

Shoots from axillary buds were differentiated within two weeks on all media regardless of the concentration of NAA and kinetin used, however, shoot growth was better on the media with the concentration of NAA ranged from 0.01 to 0.1mg/l throughout third generations when NAA alone to be applied, and when kinetin was the only source of growth substances, shoot differentiation was better on the concentration ranged from 0.5 to 1.0mg/l throughout all subculture generations (Table 1).

Callus formation was good on the MS medium with 1mg/l NAA but shoot growth rate was poor on it. Especially the number of node and leaf, and shoot height were reduced than that of low concentration of NAA. High levels of kinetin (5 and 10mg/l) brought abnormal shoot growth such as longer leaves than normal plants, curled leaves and three leaves per node (Plate 1).

The combinational effects of NAA and kinetin was shown in Table 2. As indicated in Table 1, the concentration of NAA ranged from 0.01 to 0.05mg/l combined with kinetin concentration ranged from 0.5 to 1.0mg/l showed good combinational effects on node and leaf number increase,

Table 1. The effects of NAA and kinetin on growth of shoots differentiated from axillary bud in Stevia.

Hormones (mg/l)	1st generation			2nd generation			3rd generation		
	No. of node	No. of leaf	Shoot height (cm)	No. of node	No. of leaf	Shoot height (cm)	No. of node	No. of leaf	Shoot height (cm)
NAA									
0.00	–	–	–	1.1	2.4	0.8	2.3	4.3	3.4
0.01	4.1	8.1	3.2	4.7	9.3	6.9	4.5	8.7	6.5
0.05	4.9	10.4	4.5	5.7	8.8	5.8	4.0	10.3	6.2
0.10	4.2	8.1	6.2	5.0	11.8	4.5	4.0	7.3	5.6
0.50	5.5	11.3	3.9	3.7	8.0	5.1	4.2	11.4	5.0
1.00	1.7	2.7	1.2	2.3	4.7	1.3	1.5	1.1	1.0
Kinetin									
0.5	4.0	8.3	3.8	3.5	7.0	3.5	4.1	8.4	4.1
1	5.0	10.0	4.4	4.5	9.3	4.4	3.7	8.5	4.6
5	3.5	6.7	1.1	3.5	7.0	1.9	4.2	8.0	0.7
10	4.0	6.9	1.2	4.0	4.0	0.8	4.4	*	0.6
LSD 5%	0.86	1.19	0.47	0.95	1.15	0.45	0.73	0.96	0.51
1%	1.16	1.60	0.63	1.28	1.55	0.61	0.98	1.29	0.68

*Severe abnormal plants.

and better shoot growth throughout all subculture generations. Combined effects of higher concentrations of NAA (0.5-1.0mg/l) and kinetin (5-10mg/l) retarded shoot growth as shown in Plate 1. The results in this study indicated that low concentration of NAA and high concentration of kinetin maximized shoot formation instead of callus formation. In general, low concentration of NAA and kinetin was suitable for clonal propagation when stems with axillary buds were used as explants.

To know the effects of salt strength on the node and leaf numbers, shoot growth and rooting efficiency, both full and half salt strength of MS medium were employed, and the results was shown

Table 2. Combinational effects of NAA and Kinetin on growth of shoots differentiated from axillary buds in Stevia.

Hormones (mg/l)		1st generation			2nd generation			3rd generaion		
NAA	Kinetin	No. of node	No. of leaf	Shoot height (cm)	No. of node	No. of leaf	Shoot height (cm)	No. of node l	No. of lead	Shoot height (cm)
0.01	0.5	2.5	5.8	7.0	3.0	6.0	7.1	6.5	13.3	8.1
	1	2.5	5.0	5.2	5.5	12.5	5.2	8.0	15.0	7.6
	5	3.0	6.0	3.8	2.5	7.5	3.4	9.7	15.0	4.8
	10	3.0	6.0	3.6	4.1	8.3	3.2	8.5	18.0	4.5
0.05	0.5	3.0	6.0	6.2	3.0	7.5	3.2	5.8	11.8	7.6
	1	3.0	6.2	6.7	6.0	11.5	4.3	8.8	17.3	6.3
	5	2.0	4.5	3.5	5.5	9.0	2.9	*	*	2.5
	10	2.0	4.0	2.4	3.5	8.5	2.1	*	*	2.7
0.1	0.5	3.0	6.0	5.4	2.5	6.0	3.9	7.0	14.3	6.0
	1	3.7	4.6	2.4	3.0	6.5	2.1	6.8	12.6	6.4
	5	2.3	4.3	4.2	3.0	5.0	2.1	8.0	14.8	6.2
	10	3.3	6.3	3.5	4.0	8.0	2.3	2.9	5.6	4.2
0.5	0.5	3.3	6.3	4.0	3.5	8.5	2.5	5.4	11.4	3.1
	1	3.0	2.8	3.8	4.7	10.0	3.8	6.5	13.0	3.8
	5	3.0	8.0	3.4	3.0	7.0	3.2	4.2	8.8	1.7
	10	4.0	8.0	2.4	2.5	5.5	1.2	3.2	5.8	1.9
1	0.5	3.3	6.3	2.4	2.0	5.5	1.2	3.8	7.8	1.4
	1	3.0	6.0	4.6	1.0	7.0	0.7	3.6	8.0	2.9
	5	3.0	7.0	2.6	4.0	8.0	1.8	4.6	9.8	1.2
	10	4.0	9.0	2.5	9.0	5.0	0.7	4.0	8.6	1.3
LSD	5%	0.96	1.08	0.94	0.99	1.56	1.04	1.60	2.94	1.07
	1%	1.28	1.44	1.25	1.31	2.07	1.38	2.12	3.90	1.41

*Severe abnormal plants.

Plate 1. Abnormal plants were observed at high concentration of kinetin in 3rd generation of subculture.

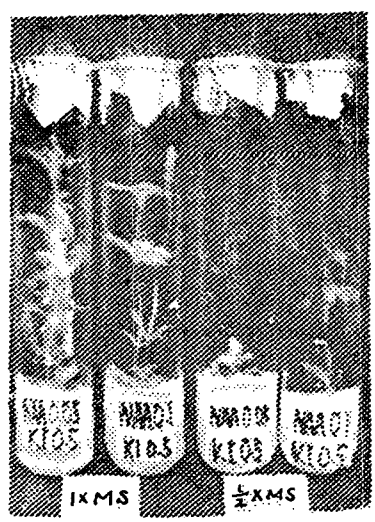

Plate 2. The growth of shoots dependending on MS salt strength.

Table 3. The effects of different concentrations of the Murashige and Skoog salt strength on growth of shoots differentiated from axillary bud culture in combination treatments of NAA and Kinetin after two weeks.

Hormones (mg/l)		1 X M S			1/2 S M S		
NAA	Kinetin	No. of node	No. of leaf	Shoot height (cm)	No. of node	No. of leaf	Shoot height (cm)
0.01	0.5	2.5	8.5	7.0	2.0	4.0	5.0
	1	2.5	5.0	5.2	2.0	4.0	2.8
	5	3.0	6.0	3.8	2.0	4.0	1.7
	10	3.0	6.0	3.6	2.5	4.5	2.2
0.05	0.5	1.5	6.0	6.2	2.0	4.0	1.5
	1	2.0	6.2	6.7	2.0	6.0	1.3
	5	2.0	4.5	3.5	2.0	4.0	1.0
	10	2.0	4.0	2.2	1.0	2.0	1.1
0.1	0.5	3.0	6.0	5.4	2.0	4.0	2.0
	1	1.7	4.6	2.4	3.0	6.0	1.8
	5	2.3	4.3	4.2	2.5	5.0	2.2
	10	3.3	6.3	3.5	2.0	4.0	6.9
0.5	0.5	3.3	6.3	4.0	2.0	5.0	2.7
	1	3.0	2.8	3.8	2.0	4.0	0.9
	5	3.0	8.0	3.4	2.3	2.8	1.2
	10	4.0	8.0	2.4	2.0	5.0	1.2
1	0.5	3.3	6.3	2.4	3.0	6.0	0.8
	1	3.0	6.0	4.6	2.0	4.0	0.4
	5	3.0	7.0	2.6	2.0	4.0	0.5
	10	4.0	9.0	2.5	2.0	4.0	0.5
LSD	5%	0.96	1.06	0.68	0.50	0.75	0.60
	1%	1.28	1.40	0.90	0.67	0.99	0.80

Table 4. The effects of different concentration of MS salt strength on root initiation of shoots differentiated from axillary bud culture in combination treatment of NAA and Kinetin after 25 days.

Hormones(mg/l)		% of rooting		Hormones(mg/l)		% of rooting	
NAA	Kinetin	1X MS	1/2S MS	NAA	Kinetin	1X MS	1/2X MS
0.01	0.5	20	20	0.1	5	0	0
	1	0	20		10	0	0
	5	0	0	0.5	0.5	60	60
	10	0	0		1	20	80
0.05	0.5	0	40		5	0	00
	1	0	35		10	0	0
	5	0	0	1	0.5	90	80
	10	0	0		1	80	60
0.1	0.5	0	60		5	0	0
	1	0	50		10	0	00

		1X MS	1/2X MS
LSD	5%	3.60	6.20
	1%	4.80	8.25

in Table 3 and 4. Node and leaf numbers were not much different between full and half salt strength, however, shoot growth rate was much higher on full strength than that of half strength (Table 3).

Plate 2 indicated that shoot growth was delayed and abnormal on half salt strength even though the hormonal concentration was the same. Full salt strength medium enhanced shoot formation while root formation was better on half strength (Table 4). Higher concentration of kinetin (5 and 10mg/l) inhibited root formtion regardless of salt strength of medium, however, higher concentration of NAA (0.5 and 1.0mg/1) tended promoting root formation even on full salt strength (Table 4). Root formation was maximal on half salt strength medium with hormonal combinations of 0.5mg/l NAA and 0.5mg/l kinetin. Meins and Skoog (1962) reported that reduced mineral salts of media promoted rooting in vitro culture.

Continuous subculture of axillary buds derived from original axillary bud can provides rapid and effective multilication of clones in a short time period. In general, each subculture generation took three weeks period. If four explants were taken from a derived plantlet and three weeks per subculture generations were concidered, then 4 x 10^{15} plants could be produced within a year.

摘　　要

自家不和合性 作物인 스테비아는 純度를 유지하기 위하여 삽목에 의해 번식하고 있으나 그 所要期間과 增殖率이 낮다. 본 실험에서는 腋芽로부터 완전한 식물체로 분화시켜 대량 증식을 위한 器內培養條件을 알고자 하였으며 그 결과를 요약하면 다음과 같다.

1. Murashige-Skoog (MS) 기본 培地에 NAA와 kinetin을 단독 처리할 때 腋芽로부터 shoot分化가 가장 좋은 경우는 NAA는 0.01-0.05 mg/ℓ 범위였고 kinetin은 0.5-1.0 mg/ℓ의 범위이었다.

2. MS培地에서 shoot分化는 NAA 0.01-0.05㎎/ℓ와 kinetin 0.5-1.0㎎/ℓ을 組合處理하였을 때 좋았다.

3. MS培地의 salt strength를 달리할 때 1×MS가 ½XMS보다 shoot分化와 生育에서 좋은 결과를 보였다. 그러나 root分化는 ½XMS에서 높았다.

4. 이상의 결과로보아 스테비아는 단시일 내에 많은 양을 증식시킬 수 있으며 shoot분화와 root 분화 시 배지의 염류농도와 홀몬농도를 달리하는 것이 효율적이다.

REFERENCES

1. Earle, E.D., and R.W. Langhas(1974) Carnation propagation from shoot tips cultured in liquid medium. Hortscience 10(6): 608-610.
2. Earle, E.D., and R.W. Langas(1974) Propagation of Chrysanthemum in vitro. I. Multiple plantlets from shoot tips and the establishment of tissue cultures. J. Amer. Soc. Hort. Sci. 99: 128-132.
3. Earle, E.D. and R.W. Langhas,(1974) Propagation of Chrysanthemum in vitro. II. Production, growth and flowering of plantlets from tissue cultures. J. Amer. Soc. Hort. Sci. 99:352-358.
4. Hackett, W.P., and J.M. Anderson(1967) Aseptic multiplication and maintenance of differentiation carnation shoot tissue derived from shoot apices. Hortscience 90:305-369.
5. Hackett, W.P, and James M. Anderson(1966) Aseptic multiplication and maintenance of differentiated carnation shoot apices derived from shoot tissues Amer. J. Hort. Sci. 90:365-369.
6. Jaacov Ben-Jaccov and Langhans, R.W.(1972) Rapid multiplication of Chrysanthemum plants by stem tip proliferation. Hortscience 7(3): 289-290.
7. Lazarte, J.E.(1982) In vitro propagation of *Epiphtllum chrysocardium*. Hortscience 17. 84-86.
8. Murashige, T.(1972) Propagation of Asparagus through shoot tip culture. I. nutient medium for formation of plantlets J. Amer. Soc. Hort. Sci. 97: 158-161.
9. Meins, F and F. Skoog(1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

10. Reinert, J.(1967) Propagation of Cattia by tissue culture of lateral bud meristem. Amer. Soc. Hort. Sci. 91: 664-671.
11. Raju, M.V.S. and E. Mann. Henrry(1969) Regenerative studies on the detached leaves of Echeveria elegans. Anatomy and regeneration of leaves in sterile culture. Can. J. Bot. 48: 1887-1891.
12. Tomes, D.T.(1979) A tissue culture procedure for propagation and maintenance of Lotus corniculatus genotypes. Can. J. Bot. 57:137.