EFFECTS OF GINSENG AND GINSENOSIDES ON CALLUS INDUCTION AND ORGAN FORMATION IN ANTHER CULTURE OF RICE

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Abstract: Responses of cultured anthers to ginseng and plant hormones on browning and callus induction, and responses of microspore-derived calli to ginseng and ginsenosides, Rg_1, and Rb_1, on organ formation in rice (*Oryza sativa* L.) were studied. Results obtained from these studies indicate that anther browning was attributed to plant hormones and that ginseng had little effect on it. Kinetin and α-naphthaleneacetic acid were important factors for the induction of microspores to form calli in cultured anthers while ginseng had no effect on callus induction at the concentrations tested. Ginsenosides Rg_1 and Rb_1 promoted organ formation in calli derived from microspores. Rg_1 was more effective than Rb_1 in promoting plantlet regeneration while Rb_1 was effective in promoting root formation, indicating that their mode of actions in regulating organ differentiation may be somewhat different.

INTRODUCTION

The dry root of ginseng (*Panax ginseng* C.A. Meyer) has long been used as tonic by the Chinese people. Effect of ginseng on animals (Bittles *et al.*, 1979) and plants have also been studied. Hui and Zee (1980, 1981) reported that ginseng promoted plantlet formation from cotyledon and hypocotyl explants of *Brassica oleracea* and from leaf disks of *Peperomia viridis*. In addition, ginsenoside Rg_1 was shown to promote mitosis, shorten cell cycle and increase DNA synthesis in root tip cells of *Allium cepa*, but ginsenoside Rb_1 had opposite effects (Ng and Chao, 1981). Similar effects were observed with tomato root tips in that the growth and incorporation of radioactive precursors of DNA, RNA and proteins were enhanced by Rg_1 (Chao and Yung, 1984).

The objectives of this study were to elaborate (1) responses of cultured anthers to ginseng on browning and callus induction and (2) responses of callus derived from microspores to ginseng and ginsenosides, Rg_1 and Rb_1, on organ formation in rice (*Oryza sativa* L.).

MATERIALS AND METHODS

The plant material used in this study was *Oryza sativa* L. cv. Tainung 67. Samples of white ginseng were kindly supplied by Korea Ginseng Centre, Hong Kong. Purified ginsenosides Rg_1 and Rb_1 were generously provided by Professor

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S. Shibata of the Meiji College of Pharmacy, Tokyo. Crude samples of these two ginsenosides were obtained from the Korea Ginseng and Tobacco Research Institute, Seoul.

The procedures of anther culture and medium used have been described previously (Tsay *et al.*, 1986). Ginseng powder was added to the mediun before autoclaving. Anther cultures were kept in the dark at 26°C.

Calli were transferred to a medium composed of N_6 inorganic salts and MS organic substances supplemented with 1 mg/1 α-naphthaleneacetic acid (NAA) and 4 mg/1 kinetin (Chen *et al.*, 1982) about 10 days after their emergence. In one experiment, ginseng powder, purified Rg_1, or Rb_1 was added to the medium. In the second experiment, the medium was supplemented with crude Rg_1 or Rb_1. Callus cultures were incubated at 26°C under 16 h daily illumination with 1000 lux cool light.

RESULTS AND DISCUSSION

Anther browning and callus induction

Anthers cultured on the basal medium containing ginseng (200 or 400 mg/1) but without plant hormones did not turn brown after 21-22 days of culturing; however, 10.9-16.7% of anthers did so on the medium supplemented with hormones whether ginseng was added or not (Table 1). These results indicate that browning of rice anthers might be attributed to plant hormones rather than ginseng. Although the mechamism of anther browning has not been established, it has been suggested that anther browning may be caused by toxic quinones which are oxidation products of phenolic compounds released from injured tissues (Loomis and Battaile, 1966). However, whether or not rice anther browning induced by plant hormones in this study was the result of quinone formation remains unknown.

In tobacco, rice, and several other species, there is a negative correlation between anther browning and the ability of anthers to produce embryoids or calli.

Table 1. Effects of ginseng and plant hormones on browning of cultured rice anthers

Medium*	Plant hormones (mg/l)		Ginseng powder (mg/1)	No. of anthers cultured	Browning anthers	
	NAA	kinetin			No.	%
1	—	—	200	850	0	0
2	—	—	400	850	0	0
3	2	1	—	900	111	12.3
4	2	1	50	900	110	12.2
5	2	1	100	800	125	15.6
6	2	1	200	850	93	10.9
7	2	1	400	750	125	16.7
8	2	1	800	850	130	15.3

* Basal medium: N_6 inorganic salts and MS organic substances.

Table 2. Effects of ginseng and plant hormones on callus formation from cultured rice anthers

Medium*	Plant hormones (mg/l) NAA	kinetin	Ginseng powder (mg/1)	No. of anthers cultured	Amthers producing callus No.	%
1	—	—	200	750	42	5.6
2	—	—	400	800	48	6.0
3	2	1	0	650	277	42.6
4	2	1	50	500	199	39.6
5	2	1	100	600	263	43.8
6	2	1	200	450	175	38.9
7	2	1	400	550	220	40.0
8	2	1	800	550	213	38.7

* Basal medium: N_6 inorganic salts and MS organic substances.

Anthers turning brown at an earlier stage of culturing reduced their abilities to form embryoids or calli than those tanned at a later stage (Mii, 1976; Tsay, 1981). However, in this study, treatments with plant hormones, which induced browning, promoted callus formatoin. About 43% of the anthers grown on the control medium containing hormones (medium 3) produced calli (Table 2). Addition of ginseng (50-800 mg/1) to the control medium (media 4-8) did not promote callus induction. On the other hand, in the absence of plant hormones, only about 6% of anthers produced calli on the media supplemented with ginseng (200 or 400 mg/1) (media 1 and 2). These results indicate that NAA and kinetin were important factors for the induction of microspores to form calli in cultured rice anthers while ginseng had no effect at the concentrations tested.

Organ formation

Two experiments, one using purified and the other crude ginsenosides, were conducted to study their effects on organ formation in calli. Formation of both shoots and roots occurred in a number of calli. Some calli, however, produced roots only. A close examination of the results obtained from treatments with ginseng and purified ginsenosides (Table 3) revealed that: (1) there was no marked difference in the ability of organ formation between calli treated and those not treated with ginseng; (2) calli treated with Rg_1 showed a greater ability to differentiate into green plantlets than did calli not treated with this ginsenoside; and (3) calli treated with Rb_1 showed a greater ability to differentiate into roots than did calli not treated with this ginsenoside. These results indicate that both ginsenosides promoted organ formation in rice calli but their mode of actions may be somewhat different; Rb_1 appears to be more effective than Rg_1 in promoting root formation while Rg_1 is more effective than Rb_1 in promoting plantlet regeneration.

In the second experiment when only crude Rg_1 and Rb_1 were tested, the number of calli forming green or albino plantlets could not be counted separately because a number of calli produced both green and albino plantlets. Nevertheless, calli

Table 3. Effects of ginseng, purified Rg_1 or Rb_1 on differentiation of callus derived from cultured rice anthers

Treatment	No. of calli	Calli differentiated into: Green plantlet No.	%	Albino plantlet No.	%	Root only No.	%	Total No.	%
Control*	58	9	15.5	8	13.8	10	17.3	27	46.6
Ginseng (mg/l)									
25	56	10	17.9	8	14.2	9	16.1	27	48.2
50	62	6	9.7	4	6.5	16	25.8	26	41.9
100	34	7	20.6	2	5.9	6	17.6	15	44.1
200	63	8	12.7	12	19.0	9	14.3	29	46.0
Total	215	31	14.4	26	12.1	40	18.6	97	45.1
Rg_1 (mg/l)									
4	58	13	22.4	8	13.8	11	19.0	32	55.2
Rb_1 (mg/l)									
2	61	12	19.7	11	18.0	13	21.3	36	59.0
4	49	6	12.2	6	12.2	14	28.6	26	53.1
8	63	10	15.9	7	11.1	14	22.2	31	49.0
Total	173	28	16.2	24	13.9	41	23.7	93	53.8

* Medium composed of N_6 inorganic salts and MS organic substances supplemented with 1 mg/l NAA and 4 mg/l kinetin.

treated with a crude ginsenoside, either Rg_1 or Rb_1, showed a higher frequency of plantlet regeneration compared with those without the ginsenoside treatment (Table 4). However, a small fraction of Rg_1-treated calli produced shoots only, and some Rb_1-treated calli produced roots only. Thus, crude and purified ginsenosides appear to produce a similar effect on promoting organ formation in cultured rice anthers.

Hui and Zee (1980, 1981) showed that addition of ginseng to the medium supplemented with plant hormones increased the frequency of plantlet regeneration from cotyledon and hypocotyl explants of broccoli and from leaf disks of

Table 4. Effects of crude Rg_1 and Rb_1 on differentiation of callus derived from cultured rice anthers

Treatment	No. of calli	Calli differentiated into: plantlet No.	%	Root only No.	%	Shoot only No.	%	Total No.	%
Control*	29	7	24.1	4	13.8	2	6.9	13	44.8
Rg_1 (mg/l)									
3	28	17	60.7	0	0.0	1	3.6	18	64.3
6	30	21	70.0	0	0.0	1	3.3	22	73.3
Total	58	38	65.5	0	0.0	2	3.4	40	68.9
Rb_1 (mg/l)									
3	28	12	42.9	4	14.3	0	0.0	16	57.2
6	28	14	50.0	8	28.6	0	0.0	22	78.6
Total	56	26	46.4	12	21.4	0	0.0	38	67.8

* Medium composed of N_6 inorganic salts and MS organic substances supplemented with 1 mg/l NAA and 4 mg/l kinetin.

Peperomia. In the present study, the addition of this tonic to the medium supplemented with hormones neither increased the percentage of callus formation from cultured rice anthers (Table 2) nor improved the ability of organogenesis of the callus (Table 3). However, ginsenosides Rg_1 and Rb_1 promoted organ formation. Both ginsenosides showed hormone-like action but their effects appeared to be somewhat different. Rg_1 produced a similar effect in promoting both shoot and root differentiation, while Rb_1 preferentially enhanced root formation. Different effects of these two gensenosides on mitosis, cell cycle and DNA synthesis in onion root tip cells have been reported by Ng and Chao (1981). Changes in the cytokinin/auxin ratio have been shown to affect the development and differentiation of tobacco organs (Skoog and Miller, 1957).

In conclusion, this study indicates that treatment of rice anther cultures with NAA and kinetin, while produced a browning effect, promoted callus formation. Although ginseng power had little effect on callus formation, ginsenosides were effective in organ induction. Rg_1 was more effective than Rb_1 in promoting plantlet regeneration, and Rb_1 was effective in inducing root formation.

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人參與人參皂甙對水稻花藥內癒合組織之誘導與分化的影響

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摘 要

將單核小孢子期的水稻花藥與由其誘發之癒合組織培養於 N_6 無機鹽與 MS 有機物之培養基，加以不同劑量之 NAA 與 kinetin 及人參粉末，人參皂甙 Rg_1 或 Rb_1 等物，從而觀察花藥之褐化，癒合組織之誘導及癒合組織之分化。結果簡述如下：

1. 花藥之褐化似與植物荷爾蒙有關，人參對花藥之褐化沒有影響。
2. NAA 與 kinetin 爲誘導水稻花藥產生癒合組織之要素，人參劑量如在 50-800 mg/l，則並無增進癒合組織之作用。
3. 人參粉末亦無增進癒合組織分化作用。
4. 人參皂甙 Rg_1 與 Rb_1 能促進癒合組織之分化。
5. Rg_1 能增進小植株之形成而 Rb_1 則促進根的形成。