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Effects of Ginseng on Rice Anther Culture[1]

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Abstract : The effects of ginseng powder on anther browining, callus formation and organ regeneration of rice anher cultutre were studied. Results indicated that ginseng powder at 500—1,000 ppm reduced the rate of browning of anther culture and increased the callus-forming ability and breeding effeciency.

Introduction

Utilization of haploid plants from anther culture has been recognized as a valuable tool to produce inbred lines of crop species for many decades (2, 6). After several years of work on rice anther culture, an efficient and reliable procedure has been established in our laboratory[4,5]. Although more than 20,000 anther-derived green plants can be produced in a year, it still has rooms for improvement.

There are two major ways of increasing the breeding efficiency (number of green plants porduced per 100 cultured anthers), i. e., increasing the callus-forming ability of cultured anthers and preventing the cultured anthers from turning brown. The latter has been found to be the most important factor for successful initiation of callus[19]. It was found that earlier anther browning was associated with low ability of callus formation or plant regeneration[11,14,15,16]. Our preliminary results showed that ginseng powder delayed the browning of both cultured rice anthers and anther-derived callus. This paper reports the effects of ginseng powder on the callus forming and plant regeneration abilities of rice anther culture.

Material and Method

Oryza sativa L. cv. Tainung 67 was used in this study. Rice culms were excised from field-grown plants when the distance between the ligules of the uppermost and second leaves was about 5cm. Unemerged panicles with flag leaf were kept at 10±1°C for one week before culturing the anthers. To prevent dessication and to maintain pollen viability during cold-shock pretreatment, the base of the panicles was wrapped with wet tissue paper and placed inside a plastic bag. Collection and sterilization of

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inflorecence was done according to Chen and Chen [3] except that 6% calcium hypochloride was replaced by 0.5% sodium hypochloride. Five levels of ginseng powder (GP) (0, 250, 500, 1,000 and 1,250 ppm) were added into Chen *et al.* [5] medium for callus induction. Ten-day-old callus was tranferred to MS medium supplement with 3% sucrose, 170 mg/l $NaH_2PO_4 \cdot H_2O$, 40mg/l adenine sulfate, 1mg/l NAA and 4mg/l kinetin for plant regeneration. Anthers were maintained at 25±1°C under complete darkness for callus induction and under 16-h daily illumination with 1,500 lux fluorescent light for plant regeneration. Browning of anthers was examined once a week during culture.

Results

Anthers cultured in GP-free medium turned brown more rapidly than anthers cultured in GP-containing medium during the period of seven weeks in culture (Fig. 1). Most of the anthers turned brown at 3—5 weeks in cultured on GP-free medium, whereas anthers cultured on 500—1,000 ppm GP-containing medium browned after five weeks in culture. The speed of browning in 1,250 ppm GP-containing medium was similar to that in 250 ppm GP-containing medium, indicating that GP at high concentration might exhibit some toxic effects.

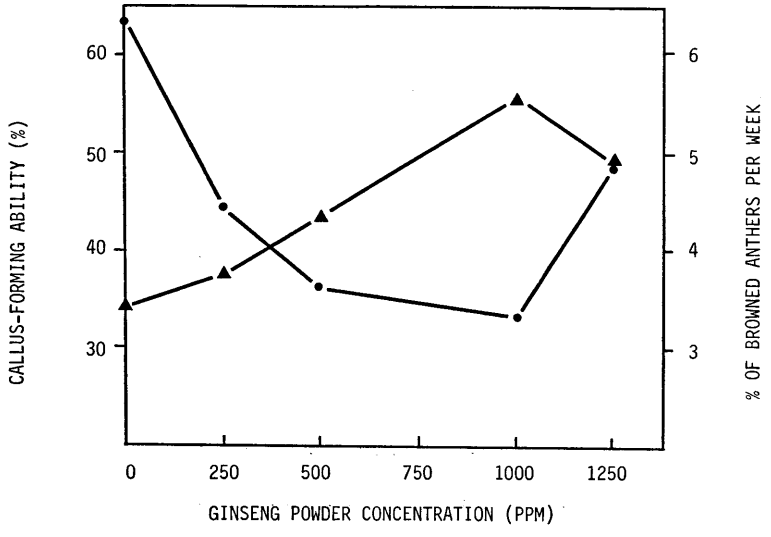

Fig. 1. Effect of ginseng powder on the average rate of rice-anther browning during 3 —5 week and callus-forming ability in rice anther culture

▲▲ : Callus-forming ability

●● : Anther-browning rate

Higher percentage of callus-forming ability was associated with higher concentration of GP, up to 1,000 ppm, in medium (Fig. 1). In other words, the lower the anther browning rate, the higher the callus-folming ability. The highest GP-containing medium (1,250 ppm) caused quick browning and lower callus-forming ability. The callus browning speed during 3—5 weeks of culture can be used as an index for callus forming ability

In rice anther culture. The earlier the anthers turned brown, the less the callus produced. The callus-forming ability peaked at 55.8% in 1,000 ppm GP-containing medium. The decreased of callus-forming ability at 1,250 ppm GP-containing medium was probably due to toxic effect at such high concentration.

The percentages of browned callus and callus forming plants after six-week culture in organ differentiation medium were shown in Table 1. Only 5% callus originated from GP-containing medium turned brown, whereas browning was as high as 12% for callus originated from GP-free medium. There was no difference in regeneration ability between callus originated from GP-free or GP-containing medium.

Table 1. Effect of ginseng powder on the differentation ability of anther-derived callus (cv. Tainung 67)

Ginseng powder concentration —ppm—	No. of callus cultured	Callus browning —%—	Callus forming plants (%)		
			green	albino	green+[1] albino
0	210	12.38a[2]	31.43a	13.33a	8.57a
250	140	5.71ab	30.00a	15.00ab	0.00b
500	104	4.81ab	33.65a	11.54a	9.62a
1,000	165	4.85b	33.33a	22.42b	3.64ab
1,250	166	5.42b	31.33a	21.08ab	6.02a

1. Green and albino plants produced simultaneously from single calli.
2. Means in each vertical column followed by same letter are not significatly different at 5% level using X^2-test

Table 2 showed the breeding efficiency of rice anther culture at five levels of GP-treatments. The breeding efficiency increased with GP concentration between 0 to 1,000 ppm. The major effect of GP was that it increased the percentage of anthers forming callus. Concentration of 500 and 1,000 ppm was most suitable for callus induction.

Table 2. Effect of ginseng powder on the breeding efficiency of rice anther culture (cv. Tainung 67)

Ginseng powder concentration —ppm—	Percent of anthers forming callus (A)	percent of callus forming green and green-albino plant (B)	Breeding efficiency (A×B)
0	34.11e[1]	40.00a	12.44
250	37.87d	30.00b	11.36
500	43.48c	43.27a	18.81
1,000	55.75a	36.97a	20.61
1,250	49.00b	37.35ab	18.30

1. Means in each vertical column followed by same letter are not significantly different at 5% levl using X^2-test.

Discussion

Oxidative browning is a common phenomenon in tissue culture [7,13,14,19]. The explants release a dark exudate which diffuses into the medium and causes oxidative browning and death of the plant [14]. One approach to circumvent this problem is to tranfer the explan frequently [9,12]. This method can not be applied to rice anther culture because transferring such a large number of cultured anthers is time consuming.

According to our experiment, anther browning can be classed into drastic browning resulted from mechenical injury and gradual browning due to anther senescence [16]. Our results showed that ginseng powder reduced both gradual browning and drastic browning. Anthers cultured on GP-free medium turned brown rapidly at the rate of 6.3% per week from the 3rd to 5th week of culture. On the contrary, the browning rate was below 5% per week on GP-containing medium. Although the specific action of ginseng powder is still unknown, this study shows that ginseng powder can be used as an additive to reduce anther browning during the first five weeks of culture and to obtain a higher breeding efficiency. These results are similar to those obtained by Tsay [19], i. e., the less the anther browned, the more the callus was formed.

Many researchers suggested that anther wall plays a role of supporting or transferring substances from medium to pollen grain or nursing the pollen grains [2,10,17,18,19]. Pelletier and Ilami [16] found that tobacco anthers which browned in the later period of culture possessed a higher potential for embryo formation from pollen grains than those having browned earlier. Mii [15] reported that the percentage of plantlet formation in anthers having browned during the first two weeks was low, whereas the frequency was high in long-lived anthers. Tsay [19] also reported that cultured tobacco anthers browned in later stage not only possessed higher potential for embryogenesis but also produced more plants. In rice, higher concentration or longer period of sterilant application accelerated the browning of cultured anthers and reduced the ability of callus formation [19].

Breeding efficiency determined by callus-forming and plantlet regeneration abilities is an important index in evaluating the value of anther culture in a breeding program. Our data showed that 1,000 ppm GP resulted in the higest breeding effeciency. Hui and Zee [8] also reported that both panax pseudo and panax ginseng powder at 500—1,000 ppm enhanced the plantlet regeneration of contyledon and hypocotyl explants of borccoli. The identity and mode of action of major components in the GP that are responsible for the enhancement in callus formation in rice anther culture need to be further studied.

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摘 要

本試驗目的在探討培養基內添加人參粉末對水稻花藥培養之花藥褐化、癒傷組織形成及植株分化的影響。試驗結果發現，培養基內添加500—1,000ppm濃度的人參粉末能够延緩花藥褐化速率，進而提高癒傷組織的形成能力及花藥培養之育種效率。

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