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Plant Regeneration through Callus of Korean Native Seosanjong of *Zingiber officinale* Rosc.

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ABSTRACT

Embryogenic callus cultures of Korean native Seosanjong of ginger(*Zingiber officinale* Rosc.) were induced through stem explants taken from *in vitro* shoot-tip cultures. Among the four concentrations of 2,4-D tested in Murashige and Skoog medium, 0.5 and 1 mg/L of 2,4-D was most effective in inducing embryogenic callus. Leaf explants did not express any new morphogenetic response in all 2,4-D concentrations tested. Plantlets transferred to hormone-free MS medium were developed and successfully acclimatized under greenhouse.

Key Words **:** ginger, plantlet, tissue culture, 2,4-dichlorophenoxy acetic acid(2,4-D)

INTRODUCTION

Ginger is a monocotyledonous perennial herb, an important tropical crop, has been used around the world as a condiment and also for its medicinal materials. Important ingredients contained are zingerone, shogaol, gingerol, refined oil and other things of ginger rhizomes. It is exclusively propagated vegetatively by rhizomes. Because ginger does not produce seeds, it is very difficult to breed new genotypes through sexual propagation. Thus, most of the crop improvement programmes of this species are confined to evaluation and selection of naturally occurring clonal variations.

Biotechnological approaches for crop improvement require efficient regeneration of crops from tissue culture. Ginger is mostly confined to propagation from shoot-tip culture(Noguchi and Yamakawa, 1988; Roh et al., 1996). In a vegetatively propagated crop like ginger, the risk of systemic infections with rootknot nematodes, bacterial wilt, virus and *Fusarium* from the propagules are remarkably high. De Lange et al.(1987) successfully eliminated rootknot nematodes from heavily infected rhizomes through *in vitro* culture of shoot tips. Malamug et al. (1991) and Kackear et al.(1993) have also reported plant regeneration by organogenesis in ginger.

This study has been undertaken to demonstrate the relative importance of 2,4-D, different explant, and their interactions for *in vitro* plant regeneration from stem explant of Korean native Seosanjong of ginger.

MATERIALS AND METHODS

Plant material

Plant materials used in this experiment were leaf,

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stem and root explants(4~6 ㎜ long), obtained from *in vitro* regenerated plantlet through shoot tip culture of Korean native Seosanjong of ginger(*Zingiber officinale* Rosc.).

Culture conditions

Callus induction medium(CIM) consisting of Murashige and Skoog(1962, MS) salts, vitamins, 30 g/L sucrose, 2,4-D(0.1, 0.5, 1 and 3 ㎎/L) were prepared, and solidified with Gelrite 2 g/L after adjustment of pH to 5.8. The media were autoclaved for 15 min at 1.2㎏/㎠, and then 30 *㎖* was dispensed into petri dishes(Ø 87×15 ㎜). Ten explants(leaf, stem and root) were cultured per vessel, each treatment was replicated 5 times.

Callus cultures were maintained by subculturing on MS medium containing 1.0 mg/L 2,4-D(2,4-dichlorophenoxy acetic acid). The culture condition in the growth chamber was maintained 25±2℃ air temperature, a 60 μmol · m^{-2} · s^{-1}, photosynthetic photon flux(PPF) with a 16 h photoperiod using white fluorescent lamps.

Transplantation

The regenerated plantlets obtained after 10 weeks of culture on appropriate medium were transferred to 60 mL hormone-free basal MS medium in 500 mL mayonnaise vessels. The plantlets could be succesfully transferred to the plastic pot(Ø 9.0×7.5 cm) in greenhouse bed after initial hardening for 2 weeks under high humidity(> 80% relative humidity) conditions.

RESULTS AND DISCUSSION

In this study, we report organogenesis and callus formation from leaf, stem and root explants of Korean native Seosanjong of ginger. We also demonstrate the

Table 1. Effect of 2,4-D on the callus induction in various explants of Korean native Seosanjong of *Zingiber officinale* Rosc. for ten weeks cultured

Explants	2,4-D (㎎/L)	Explants producing callus (%)	Type of callus (%)[Z]			
			E	M	NE	Remarks
Leaf	0.1	0	-	-	-	-
	0.5	0	-	-	-	-
	1.0	0	-	-	-	-
	3.0	0	-	-	-	-
Stem	0.1	0	-	-	-	-
	0.5	83	6	27	67	R
	1.0	80	13	25	62	R
	3.0	85	0	0	100	R
Root	0.1	25	0	0	100	R
	0.5	63	0	0	100	R
	1.0	95	0	0	100	R
	3.0	50	0	0	100	R

[Z]E, embryogenic; M, mixed embryogenic and non-embryogenic callus; NE, non-embryogenic callus; -, no response; R, adventitious root formation.

Fig 1. Somatic embryogenesis and plant regeneration through stem explants of Korean native Seosanjong of *Zingiber officinale* Rosc. A, Induction of plantlet with embryogenic callus on stem explants cultured for ten weeks on MS medium supplemented with 1.0 mg/L 2,4-D; B, Root elongation of regenerated shoots on hormone-free MS medium; C, Regenerated plantlets growing in the pot.

relative importance of 2,4-D and different explant, and their interactions for *in vitro* plant regeneration from stem explant.

The effect of various concentrations of 2,4-D in inducing callus on leaf, stem and root explants of ginger is presented in Table 1. Among the four concentrations of 2,4-D and different explants, 1.0 mg/L 2,4-D and stem explant was the most effective in initiating embryogenic cultures and embryoid formation. At the optimum concentration of 1.0 mg/L 2,4-D, 95% of root explants gave calli. In those explants, callus formation was found in stem and root explants, while callus was not quite formed in leaf explants. The present culture of leaf explants is entirely different from the previous report that embryogenic callus formation of ginger was induced from young leaf explants taken from *in vitro* shoot cultures(Kackar et al., 1993). Although no supporting evidence was presented here, the callus may have been induced through young leaf explants.

The frequency of callus formation per stem explants ranged from 80% to 85% while root explants ranged from 25% to 95%. Stem explants gave embryogenic, non-embryogenic, mixed embryogenic and non-embryogenic callus in 0.5 and 1.0 mg/L 2,4-D, however all root explants were formed only non-embryogenic callus. The present results thus showed that 0.5 and 1.0 mg/L 2,4-D was very effective inducing and maintaining embryogenic callus of ginger. Our present results were similar to previous reports of ginger(Malamug et al., 1991; Kackar et al., 1993).

Plantlet through embryogenic callus of Korean native Seosanjong of *Zingiber officinale* Rosc. obtained in present study was by incubation of stem explants for ten weeks on MS medium supplemented with 1.0 mg/L 2,4-D(Figure 1A). The regenerated plantlets were transferred to 60 mL hormone-free basal MS medium in 500 mL mayonnaise vessels(Figure 1B). Ginger plantlets could readily be potted into a vermicullite : perlite (7 : 3, v/v) medium for acclimatization before planting in the plastic pot(Figure 1C).

Plantelt regeneration from callus may not be a consistent method of clonal propagation because of the risk of somaclonal variation induced *in vitro*. Besides, regeneration of plantlets through ginger callus is an important work which can be utilized in the application of biotechnology research in developing suitable germplasm of ginger.

REFERENCES

Dekkers, A.J., Rao, A.N., Goh, C.J. 1991. *In vitro* storage of multiple shoot cultures of gingers at ambient temperatures of 24-29℃ . Scientia Horticulturae 47:157-167.

Kackar, A., Bhat, S.R., Chandel, K.P.S., Malik, S. K. 1993. Plant regenration via somatic embryogenesis in ginger. Plant Cell, Tissue and Organ Culture 32:289-292.

Malamug, J.J.F., Inden, H., Asahira, T. 1991. Plantlet regeneration and propagtion from ginger callus. Scientia Horticulturae 48:89-97.

Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15:473-497.

Roh, K.H., Kim, T.S., Lee, J.H., Choi, I.L., Choi, Y.H., Jang, Y.S. 1996. *In vitro* propagation and tuberization of plantlet regeneration from shoot-tip culture in ginger. Korean J. Plant Tissue Culture 23: 129-134.

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