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Study on Mutant Induction of *Gladiolus* by *in vitro* Culture of Petals

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Abstract: Petals of *Gladiolus* 'Rose Supreme' were used to establish regeneration system. Developmental characteristics of petals were observed. A total of 94 plantlets of petal somaclonal line were marked in M_1 generation by ISSR molecular markers and traits were observed in M_3 generation. The results showed that the best medium to induce callus was MS+2, 4-D 4.0 mg·L^{-1}+6-BA 0.5 mg·L^{-1} and the best medium to induce somatic embryogenesis was MS+2, 4-D1.0 mg·L^{-1}+TDZ0.3 mg·L^{-1}. New petals could be formed from petal callus directly. Pigments of mother plant appeared on plantlets while they were formed from petals. Two mutants were obtained from petal somaclonal line in M_3 generation which demonstrated the correctness of ISSR analysis in M_1 generation.

Key words: *Gladiolus*, petal, variation

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Introduction

Gladiolus is a monocotyledonous bulb crop which ranks fifth in cut-flower sale worldwide (United States Department of Agriculture 1995-1996). *Gladiolus* is propagated by corms resulting in continual virus spread to progenies. Zhao (1999) reported petals tissue culture could get rid of virus effectively.

Petal tissue culture is different from shoot tip culture. It needs to experience the process of dedifferentiation and redifferentiation (Qiu, 1982; Qiu *et al*., 1983). Earle and Langhans (1974) first found the phenomenon that mutants were easy to occur from plantlets differentiated from petals. Skirvin and Janick (1976) found that the further explants existed from organ of differentiation or the longer time explants need to differentiate, the higher rate of somaclonal variation occurred. They suggested the potential use of clonal variation for improvement of horticultural plants. New varieties has been obtained by using petals *in vitro* culture which has been reported in many horticultural crops. Petal culture *in vitro* could be used as an effective auxiliary mean to breed new varieties (He, 1992). Keeping these in view, the present research used petal as explants to establish regeneration system, expecting to create new *Gladiolus* varieties.

Materials and Methods

Petal callus induction

Young inflorescences of 'Rose Supreme' in 5-leaf stage were used as initial explants. The inflorescences were cut from stem base, stripped out bracts. Young petals were surface disinfested with 75% (v/v) ethanol for 0.5 min, rinsed with sterile water, stirred for 5 min in 0.1% $HgCl_2$ with two drops of Tween 80 and rinsed three times with sterile water. Superfluous water was

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absorbed with sterile filter paper and young petals were cut into 3-5 pieces, inoculated on MS medium supplemented with different concentrations of auxins and cytokinins: (1) MS+2, 4-D 4.0 mg·L^{-1}+6-BA 0.5 mg·L^{-1}; (2) MS+NAA5.0 mg·L^{-1}; (3) MS+2, 4-D 1.0 mg·L^{-1}+KT2.0 mg·L^{-1}; (4) MS+ZT2.0 mg·L^{-1}. Cultures were maintained in a growth chamber at 25℃ under a 16-hlight photoperiod for 4 weeks, the situation of induction of callus on the medium was observed.

Induction of somatic embryo

Better loose texture of the callus was chosen and cut into squares of 0.5 cm. They were inoculated on MS solid medium supplemented with different concentrations of 2, 4-D, 6-BA, TDZ. Three factors 3 levels $L_9(3^4)$ orthogonal experimental design in Table 1 was used to select the best medium to induce somatic embryo. Total 40 explants were inoculated in each treatment. Cultures were maintained in dark chamber at 25℃ for 3 weeks, somatic embryo induction rate were calculated (somatic embryo induction rate=number of somatic embryo/number of explants).

Table 1 Factors and levels of experiment design

Factor	Level		
	1	2	3
2, 4-D (mg·L^{-1})	0.5	1	1.5
6-BA (mg·L^{-1})	–	0.5	1
TDZ (mg·L^{-1})	–	0.1	0.3

Developmental characteristics of petal plantlets and screening of mutants in M_1 generation

Petal somatic embryo calli were transferred to MS medium supplemented with 6-BA 0.5 mg·L^{-1} and NAA 0.1 mg·L^{-1} to induce shoots (Zheng, 2007).

Developmental characteristics of petal plantlets were observed during formation process of petal callus and shoots. Shoots were transferred to MS medium supplemented with IBA 1.0 mg·L^{-1}, PP_{333} 0.3 mg·L^{-1} and sucrose 60 mg·L^{-1} (Ma *et al*., 1994). Lids of the bottles were taken off in greenhouse for 2 days when the roots reached 1.5 cm in length or shoots had 1-2 euphylla. Medium on the roots was washed off. All the seedlings were transferred to soil in greenhouse and watered with 1/2MS nutrient solution every 10 days. Leaf-DNA of every single seedling was extracted for ISSR analysis by using primer (TGT GTG TGT GTG TGT GA) we had screened. The mutants were cultured with tags after preliminary genetic analysis.

Observation of traits in M_3 generation

Traits of mutants were observed in M_2 and M_3 generations in order to verify the accuracy of ISSR analysis in M_1 generation.

Results

Induction of petal callus

Although petal callus could be induced on each treatment according to Table 2, the induction results were different apparently. Red or white loose granular callus could be formed on treatment 1 (Fig. 1A). Red or brown callus could be formed on treatment 2 and treatment 3, but most callus could't develop into plantlets (Fig. 1B and C). White shoots could be formed earlier on treatment 4 than other three treatments, but these shoots were difficult to survive with subsequent culture (Fig. 1D). Therefore, treatment 1 was selected as the best medium to induce petal callus.

Petal callus could be induced on medium supplemented with 2, 4-D and 6-BA or KT hormone combination, but the induction result of 6-BA was better than that of KT. Callus also could be induced on treatment 2, but callus turned brown during subsequent culture. 2, 4-D 4.0 mg·L^{-1}+6-BA 0.5 mg·L^{-1} was the best hormone combination to induce petal callus. The color of the callus was red and white. Most of them grew rapidly (Fig. 1).

Induction of petal embryogenic callus

The results of 3 factor 3 level $L_9(3^4)$ orthogonal experimental design showed that the highest somatic