In Vitro Regeneration of Three Gladiolus Cultivars Using Cormel Explants

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ABSTRACT

In vitro technique was dedicated to alleviate the technical problems in conventional propagation of gladiolus for healthy planting material production in marketable quantities. Two experimental series were conducted in non factorial and factorial designs respectively. Cormels of three gladiolus cultivars namely Kaifa, Clara and Nabila were inoculated into free hormone media for plantlet establishment and dormancy breaking observations among genotypes. The established plantlets were then deflasked into solidified MS + 0.5 mg/l NAA with various BA concentrations (1, 2, 3 and 4 mg/l). A randomized complete experiment with seven replications was designed to accomplish these subsequent experiment to determine optimal BA for shoot-leaves initiations and complete plantlet formations. The results showed that in free hormone media, the dormancy breakings of cormel were varied among cultivars tested. The variation among cultivars was also detected on the level of BA for optimum shoot and leaf formations. 2 mg/l BA gave highest shoot and leaves formations for cv. Nabila while higher concentrations needed by other two cultivars. Thus, BA concentrations ranged from 2–3 mg/l were recommended shoot inductions on in vitro propagation of gladiolus.

Keywords : In vitro regeneration, gladiolus, cultivar, cormel explants

INTRODUCTION

Gladiolus (*Gladiolus grandiflorus* L.) is an important bulbous ornamental plant valued for its attractive spikes. It has long lasting flower stalks, attractive colors and numerous forms that these make its an all-time favorite for flower lovers and is said to be the queen of bulbous flowers (Nagaraju *et al*. 2002). The genus *Gladiolus* covered more than 180 species, yet only 20 of them were used for ornamentals purposes. Nowadays, more than 10,000 cultivars have been developed and commercially grown all over the world (Roy *et a*l 2006)

The conventional propagation of gladiolus in the field nowadays has faced some problem due to the slow growth and low multiplication rate of cormel and disease attacks. Gladiolus is usually propagated using cormel that was formed as daughter bulb from primary corm. These cormels were not able to be directly forced to flower, since gladiolus plant was naturally groan as biennial. The cormel should be planted for about 3 to 4 growing cycles to reach adequate size (corm) and then, flower forcing could be made up. The involvement of *Fusarium oxisporum* fsp. *gladiolii* were also known to have impacts on the growth and survivals rates of the seedlings and resulted in inadequate availability of quality planting materials (Dantu & Bhojwani 1995). These low propagation rates hinder the introduction of new varieties or virus-free plants.

In vitro technique was expected to be a breakthrough in alleviating the technical problem in vegetative conventional propagation in gladiolus. The method guaranteed the identical reproduction of the parents tested and selected, and prevented genotypic alteration which would occur after the generative multiplication (Chen *et al*. 2002). Using various hormones such as 2,4-D, IAA and BAP, several authors have reported the successful micropropagation of gladiolus varieties (Misra & Singh 1999, Pathania *et al*. 2001, Priyakumari & Sheela 2005) and however, such reports generally underscore differential cultivar response.

Shoot induction from derived callus or direct regeneration of gladiolus in vitro culture is usually conducted using nutrient and hormone modification within the media. In this paper, the in vitro propagation in three national gladiolus cultivars released by IOCRI for elite planting material production was studied. Dormancy breaking of each cultivars and various BA concentrations within the media for direct shoot induction from cormel explants and complete plantlet formation were investigated.

METHODS

The research was conducted in the tissue culture laboratory at the Indonesian Ornamental Crops Research Institute from July 2007 to March 2008. A randomized complete experiment with seven replications was designed to accomplish the combination of two factors. The first factor was three commercial cultivars of gladiolus, namely Kaifa, Clara and Nabila. While the second factor was various BA concentrations (1, 2, 3 and 4) mg/l supplemented in MS + 0.5 mg/l NAA.

Plant material and explant preparation

Gladiolus cv. Clara, Nabila and Kaifa were newly released cultivars resulted from IOCRI breeding activities. The collected cormels of the three accessions were replanted into 30 x 40 cm porous plastic tray with sterile carbonized rice husk as the media and maintained in the growth chamber and provided by 24 °C day/18 °C night temperature and 16 h photoperiod for 1 week. Every two days during growth chamber maintenance, systemic fungicide and bactericide were applied to reduce endogenous potential contaminants. The cormels were then served as the source of explants.

The explants were collected by pealing the sticky, thin cormel-coated layer skin. The pealed cormels were then disinfected in 0.01% pa. sodium hypoclorite with 2 drops of wetting agent (Tween 20/100 ml) for 2 minutes. The leaves were then, sterilized with 80 % ethanol for 1 minute and rinsed twice with autoclaved sterile water.

Sterile explant culture establishment and plantlet formation

After quick drying with sterile papers, the bottom side of pealed cormels were cut slightly to facilitate explants contact with the media. For about 4 – 5 cormel explants were inoculated into free hormone media per culture flasks for 18 days for ensuring no contamination existed in the cultures and examination of dormancy breaking. The existence of excessive globose callus formation on higher BA concentrations in our previous experiment (unpublished), the cultured explants were then, deflasked into lower BA concentrations on media comprised of MS + 0.5 mg/l NAA + (1, 2, 3, and 4) mg/l BA and incubated in 16 h long day. The observation of the complete plantlets were conducted after 60 days and the plantlets were then, transferred outside for acclimatization.

RESULTS AND DISCUSSION

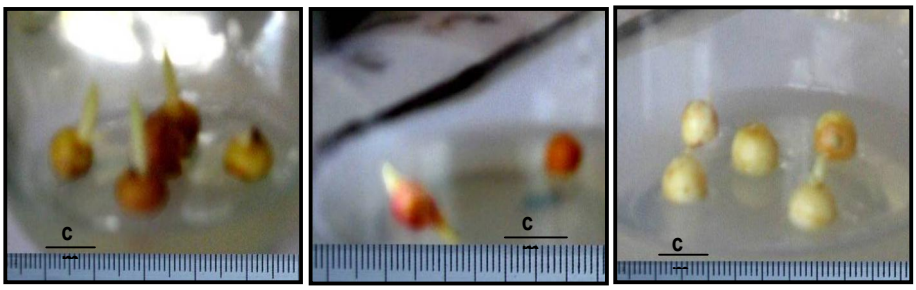
Dormancy breaking of cormel in free hormone media

The dormancy breaking of cormel explants in hormone–free MS media were different among the cultivar tested. The dormancy breaking was signalized by the initial shoot development emerging from the cormel tip.

Table 1. Shoot initiation and shoot length after 18 days incubation from cormel explants of three gladiolus cultivars on MS free hormone media.

No.	Gladiolus cultivars	Parameter evaluated*)	
		Shoot initiation (DAP)**)	Shoot length after 18 days (cm)
1.	Nabila	10.6[a]	1.52b
2.	Clara	15.2[b]	0.62a
3.	Kaifa	16.5[b]	0.43a

Note : *) values followed by different letter in the same column differ significantly at LSD 5 %.
**) DAP : days after planting .



(a) (b) (c)

Figure 1. Cormel explants with shoot intiation of three gladiolus cultivars; (a) Nabila, (b) Clara and (c) Kaifa in 18 days after planting on MS free hormone media.

Table 1 showed the earliness shoot initiation and length from cormel explants of gladiolus cultivars tested. cv. Nabila showed more progressive initial shoot development than the rest two cultivars, cv. Kaifa and Clara. The differences in shoot initiation of cormel explants among the gladiolus cultivars might reveal to the different further shoot growth performance as indicated in 18 days after planting. Cv. Nabila performed successive dormancy breaking and promoted earlier shoot initiation, consequently, longer shoot than other two cultivars (Table 1). The performance of plantlets derived cormel explant of three gladiolus cultivars in 18 days after planting was presented in Figure 1.

Effect of different BA concentrations on plantlet development of three gladiolus cultivars

For about 1.5 cm-long shoot emerged from cormel explants of the cultivars tested in MS free hormone media collected by horizontally slicing the cormel and inoculating them into media treatment of solidified MS + 0.5 mg/l NAA supplemented by various BA concentrations. After 60 days, the complete plantlets were observed and recorded. Table 2 showed the analysis of variance of the effect of BA concentrations within the media of MS + 0.5 mg/l NAA to the plantlet performance of three gladiolus cultivars tested.

The analysis of variance in Table 2 revealed that the number of shoots and leaves on plantlet among gladiolus cultivars were significantly different. However, the interaction effects of BA concentration and the cultivars were also significant. The discussion were then, focused on the interaction effect on the treatments being applied. The summary of averages of the interaction of BA concentration and gladiolus cultivars was presented in Table 3.

Table 3 showed that number of shoots and leaves was varied among the gladiolus cultivars on the BA concentrations of 1 to 2 mg/l and 1 to 3 mg/l respectively. The optimum BA level for the peak vegetative organ formations among cultivars was also fallen into different concentrations. Plantlet of cv. Nabila produced highest shoot and leaf formations on the BA concentration of 2 mg/l, while cv. Kaifa and Clara showed their most productivity in 3 mg/l BA. However, in general, the number of shoot and leaves of plantlet decreased when higher BA was applied.

The different BA levels among gladiolus cultivars to promote optimum shoot and leaf formations indicated that each cultivar had different capacity to take advantages from a defined environment. These characteristic was genotipictly indigenous that different genetic constructions might perform different phenotypic performance as a results of their interactions with the environment (Torabi-Giglou & Hajieghrari 2008).

The peak number of shoots and leaves produced by plantlet of the three gladiolus cultivars was not really different in particular level of BA. These referred to the number of shoots and leaves of cv. Nabila in 2 mg/l BA with those of cv. Clara and Kaifa on 3 mg/l (Table 3). These conditions indicated that BA concentrations ranged from 2 to 3 mg/l were the most favorable level of BA in MS medium + 0.5 mg/l NAA for shoot and leaf formations.

Table 2. Variance analysis of BA concentration effects on the shoot development and plantlet formation of three gladiolus cultivars.

No.	Source of variance	Plantlet performance after 60 days culture	
		Number of shoots	Number of leaves
1.	Replication	ns	Ns
2.	Gladiolus cultivars (A)	*	*
3.	BA concentrations (B)		
	- linier	**	**
	- quadratic	*	*
	- cubic	ns	Ns
4.	Interaction A x B	*	*
	CV (%)	9.03	7.94

Notes: ns: not significant,
**: significantly different at 1 %
* : significantly different at 5% F test.

Similar finding was reported by Emek & Erdag (2007) on the study of somatic embryogenesis induction from the leaf explants that higher BA concentration than 3 mg/l in almost all cases, reduced the vegetative organ development. The plantlet performance of the three gladiolus cultivars after 60 days subculture was presented on Figure 2.

Cormlet formation and root induction were conducted through omitting BA from MS medium and increasing NAA concentration up to 2 mg/l according to Sinha and Roy (2002). After 45 days subculture, however, no differences on the number of roots in plantlets among the cultivars tested (data not presented). The cormlet and rooted plantlets of three gladiolus cultivars were presented on Figure 3.

Table 3. Interaction effect of BA concentration and gladiolus cultivars on number of shoots and leaves of plantlets after 60 days culture.

BA concentration *) (mg/l)	Gladiolus cultivars **)		
	Nabila	Clara	Kaifa
	Number of shoots		
1	16.42[b] X	15.47[ab] X	13.21[a] X
2	25.87[b] Y	18.12[a] XY	18.45[a] Y
3	21.21[a] Y	24.73[a] Y	22.97[a] Z
4	18.34[a] XY	21.97[a] XY	19.34[a] YZ
	Number of leaves		
1	31.26 [b] X	25.73[ab] X	24.17[a] X
2	41.49 [b] Z	30.21[a] XY	30.15[a] X
3	32.78 [a] XY	39.54[b] Z	36.56[ab] Y
4	30.28 [a] X	34.79[a] YZ	35.12[a] XY

Notes : *) Values followed by different lower cased-letters in the same row differ significantly at LSD 5 %.
**) Values followed by different capitalized letters in the same column differ significantly at LSD 5 %.

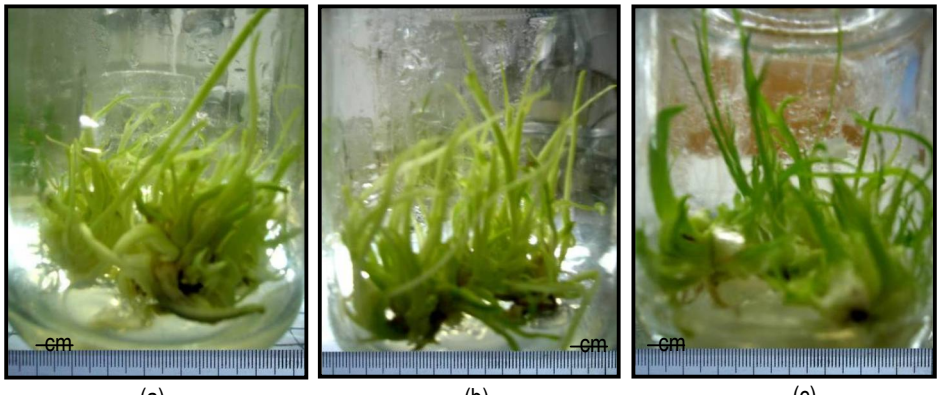


Figure 2. Plantlet performance of gladiolus cultivars: (a) cv. Nabila on 2 mg/l BA, (b) cv. Clara and (c) cv. Kaifa on 3 mg/l BA supplemented in MS + 0.5 mg/l NAA after 60 days culture.

Figure 3. Rooted plantlet with cormlet formation of gladiolus cultivars: (a) Nabila, (b) Clara and (c) Kaifa in 45 days after subculture on MS + 2 mg/l NAA.

CONCLUSION

The dormancy periods of cormel were varied among cultivars tested. Cv. Nabila showed earlier dormancy breaking viewed from shorter shoot initiation from cormel tip and longer shoot in free hormone media.

Each gladiolus cultivar needs different levels of BA for optimum shoot and leaf formations. Cv. Nabila showed the highest number of shoots and leaves on 2 mg/l BA supplemented in MS + 0.5 mg/l NAA, while those of cv. Clara and Kaifa performed the best in higher BA concentration, 3 mg/l. Omitting BA and increasing NAA concentration up to 2 mg/l in MS medium induced root development with cormlet formation in all gladiolus cultivars within 45 days.

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