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Potential of Benzyl Adenine, Naphthalene Acetic Acid and Sucrose Concentration on Growth, Development, and Regeneration of New Shoot and Cormel on Gladiolus

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ABSTRACT

Young adventitious shoots from corm of Gladiolus were used as explants and cultured on MS medium supplemented with combination of various concentration (0, 0.1, 0.5, 1, 2 mg/l) BA or kinetin and (2% ,3% and 4%) sucrose. In this experiment, potential of BA to get the highest average plantlets by culturing explants on MS medium supplemented with 0.1 - 0.5 mg/l BA and 3% sucrose within 12 weeks. MS medium with free BA and kinetin got the highest height of plantlets. When transferred plantlets to MS medium supplemented with various concentration of NAA and sucrose, potential of NAA which got the highest average number of cormels by culturing them on MS medium supplemented with 0.1- 0.5 mg/l NAA and 3% sucrose, and gave the highest fresh weight, also.

1. Introduction

The gladiolus was known as sword lily. Commercial gladiolus cultivations improved by the low multiplication rate of corm were first developed from crosses of several species native to the

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Mediterranean area of Europe. Later discovery of African species led to crosses, which produced the forerunners of the attractive large-flowered types we know today. Tissue culture technique has been used successful for propagation. *In vitro* propagation techniques were known for securing rapid multiplication (Misra and Singh, 1999; Pathania et al., 2001). This paper, hence, aims at development of an efficient mass propagation of *in vitro* plantlets to form cormel, regeneration from callus to plantlets of Gladiolus and studied effect of light color on growth and differentiation to new shoots.

2. Materials and Method

Sprouts of new shoots from corm about 0.5-1 cm were used as explants. Before culture, the explants surface sterilization were done with running tap water and washed with liquid detergent, then soaked with alcohol 70% for 15 sec. and surface sterilization with 10% Clorox for 15min, followed by 5% Clorox for 15 min. then washed three times with sterilized distilled water for 5 min. each., then cut the end of explants which contracted with Clorox off. Explants were cultured on MS (1962) medium supplemented with combination of various concentration (0, 0.1, 0.5,1, 2 mg/l) BA, (0, 0.1, 0.5,1, 2 mg/l) kinetin and 2%, 3% and 4% sucrose. All cultures incubated at 25±2°C and 16 hours photoperiod with illumination provided an intensity of 60 µmolm-2 sec-1 (TLD 18 w/18 lm Phillips Holland). The explants were subcultured every 3 weeks into the same medium for four times.

Plantlets height about 2–3cm cultured on MS medium supplemented with combination of various concentrations of (0.0.1.0.5.1.0 and 2.0 mg/l) NAA and (2% 3% and 4%) sucrose. All culture were incubated at 25±2°C and 16 hours photoperiod with illumination provided an intensity of 60 µmolm-2 sec-1 (TLD 18 w/18 lm Phillips Holland). New cormels regenerated at the distil end of plantlet after cultured for two weeks and collected them after cultured for 6 weeks. The number of cormels and fresh weight per explants were recorded.

3. Statistical analysis

Experiments were set up in Completely Randomized Design (CRD) with 27 treatments; each treatment consisted of 20 replicates for the first and 15 treatments and 20 replicates in the second experiments. The test of statistical significance was done by applying Duncan's Multiple Range

Test (DMRT) at 5% confidence level using SAS statistical software, Release 6.03 (SAS Institute Inc., Cary, NC).

4. Result

After cultured explants on MS medium supplemented with combination of various concentration (0, 0.1, 0.5, 1, 2 mg/l) BA or kinetin and (2% , 3% and 4%) sucrose for 27 treatments. It was found that concentration of BA or kinetin and sucrose on explants regenerated to new shoot and developed to plantlet were highly significant different ($p \leq 0.01$) as shown in Table 1.

Table 1: Influence of sucrose, BA and kinetin concentration in MS medium on the average height and number of new shoots in gladiolus after cultured for 12 weeks.

Sucrose conc. (%)	Conc. (mg/l)	BA (mg/l)		Kinetin (mg/l)	
		Plant height(cm.)**	No. of new shoots per explants (shoot.)**	Plant height(cm.)**	No.of new shoots per explants (shoot.)**
2%	0	4.54 ± 0.29 d	2.4 ± 0.24 e	4.54 ± 0.29 d	2.4 ± 0.24 b
	0.1	7.43 ± 0.25 c	4.4 ± 0.40 cd	8.54 ± 0.16 b	4.2 ± 0.49 a
	0.5	7.32 ± 0.42 c	5.6 ± 0.24 b	8.54 ± 0.07 b	4.4 ± 0.40 a
	1	7.18 ± 0.18 c	5.4 ± 0.24 b	8.18 ± 0.05 b	3.8 ± 0.58 a
	2	6.96 ± 0.27 c	4.4 ± 0.24 cd	7.88 ± 0.08 c	3.8 ± 0.58 a
3%	0	9.60 ± 0.30 a	4.8 ± 0.20 bcd	9.60 ± 0.30 a	4.0 ± 0.45 a
	0.1	8.18 ± 0.10 b	6.6 ± 0.24 a	8.46 ± 0.20 b	3.6 ± 0.40 a
	0.5	7.40 ± 0.21 c	7.0 ± 0.32 a	8.46 ± 0.16 b	4.4 ± 0.24 a
	1	7.18 ± 0.12 c	5.2 ± 0.20 bc	8.18 ± 0.09 b	4.4 ± 0.24 a
	2	7.02 ± 0.04 c	4.8 ± 0.37 bcd	7.94 ± 0.04 c	4.2 ± 0.49 a
4%	0	9.86 ± 0.09 a	4.2 ± 0.37 d	9.86 ± 0.09 a	3.8 ± 0.37 a
	0.1	8.12 ± 0.10 b	5.4 ± 0.40 b	8.56 ± 0.17 b	5.0 ± 0.32 a
	0.5	7.56 ± 0.10 bc	5.6 ± 0.40 b	8.48 ± 0.16 b	4.4 ± 0.40 a
	1	7.26 ± 0.10 c	4.8 ± 0.20 bcd	8.24 ± 0.10 b	4.8 ± 0.37 a
	2	7.14 ± 0.30 c	4.4 ± 0.24 cd	7.82 ± 0.13 c	4.2 ± 0.49 a

** Average mean Highly Significant different at $P \leq 0.01$

a,b,c Means followed by the same letters are not significantly different ($p < 0.05$) using Duncan New Multiple Range Test (DMRT's test.)

After cultured explants on MS medium supplemented with combination of different concentration BA or kinetin and sucrose for 12 weeks. It was found that explants cultured on MS medium supplemented only 3% and 4% sucrose with free BA or kinetin gave the highest average plant height about 9.6-9.86 cm. as shown in Figure 1. When compared the average number of plantlets per explants of each treatments in statistic. It was highly significant different ($p \leq 0.01$)

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among them. At MS medium supplemented with 3% sucrose and 0.1 - 0.5 mg/l BA gave the highest average number of plantlets per explants about 6.6-7.0 plantlets (Figure 2a). The second was MS medium supplemented with 0.1–0.5 mg/l BA and 4% sucrose about 5.4 -5.6 plantlets (Figure 2b)) and these were higher than kinetin concentration. Number of plantlets in MS medium supplemented with combination of 0.1-2.0 mg/l kinetin and 2%, 3% and 4% sucrose were not significant different in each other as shown in Table 1.

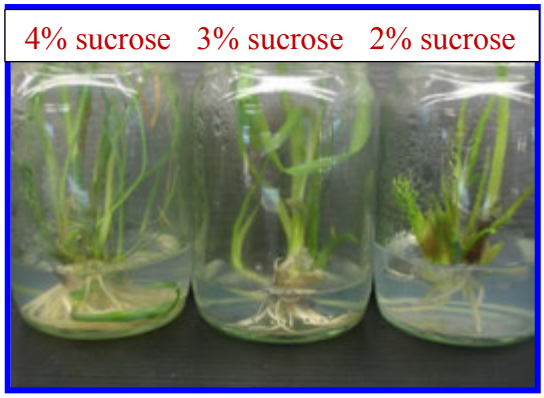
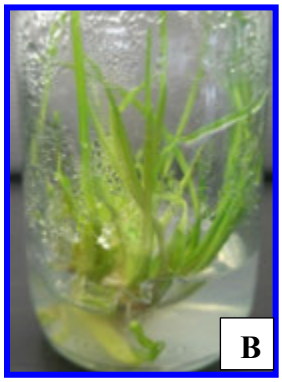


Figure1: Height of Gladiolus plantlets which cultured on MS medium supplemented various concentration of sucrose after cultured for 12 weeks.

A. plantlets on MS medium supplemented with 0.5 mg/l BA and 3% sucrose



B. plantlets on MS medium supplemented with 0.5 mg/l BA and 4% sucrose

Figure 2: Number of plantlets cultured on MS medium supplemented with 0.5 mg/l BA and 3% and 4% sucrose after 12 weeks.

4.1 Number of cormels

After cultured small plantlets about 2 – 3 cm. in MS medium supplemented with combination of various concentration (0, 0.1, 0.5, 1.0, and 2.0 mg/l) NAA and (2%, 3% and 4%) sucrose. New cormels regenerated on the distal end of stem after cultured for two weeks and collected them after cultured for 6 weeks. When compared their mean in statistic, this showed that the average number

of cormels and their fresh weight per plantlets were highly significant different ($p \leq 0.01$) as shown in Table 2.

Table 2: Effect of different concentration of sucrose combined with NAA (Naphthaline acetic acid) on number of cormels and fresh weight of cormels per explants after cultured for 8 weeks.

Sucrose conc. (%)	NAA (mg/l)	Number of cormel** (cormel per explants)	Fresh weight of cormel** (mg. per explants)
2%	0	4.1 ± 0.37 cd	138.33 ± 0.42 f
	0.1	4.8 ± 0.20 bc	141.60 ± 0.51 def
	0.5	4.4 ± 0.24 cd	142.00 ± 0.55 de
	1	4.4 ± 0.24 cd	141.20 ± 0.37 ef
	2	4.4 ± 0.24 cd	142.60 ± 0.40 d
3%	0	5.4 ± 0.24 ab	141.20 ± 0.49 ef
	0.1	5.8 ± 0.20 a	144.00 ± 0.32 c
	0.5	5.6 ± 0.24 a	144.20 ± 0.37 c
	1	4.8 ± 0.20 bc	145.80 ± 0.37 b
	2	4.8 ± 0.20 bc	141.00 ± 0.45 ef
4%	0	4.4 ± 0.24 cd	140.75 ± 0.25 ef
	0.1	4.2 ± 0.20 cd	145.80 ± 0.58 b
	0.5	4.2 ± 0.20 cd	146.20 ± 0.37 b
	1	4.2 ± 0.20 cd	148.60 ± 0.24 a
	2	3.8 ± 0.20 cd	149.00 ± 0.44 a

** average mean Highly Significant different at $P \leq 0.01$

a,b,c Means followed by the same letters are not significantly different ($p < 0.01$) using Duncan New Multiple Range Test (DMRT's test.)

The average number of cormels on MS medium supplemented with 0.1–0.5 mg/l % NAA and 3% sucrose was the highest about 5.8-5.6 cormels and their fresh weight about 144–144.2 mg per explants, respectively. On MS medium supplemented with 1–2 mg/l NAA and 4% sucrose gave the highest average fresh weight of cormels per explant about 148.6–149.0 mg and the average cormel was the lowest about 4.2- 3.8cormels, respectively.

5. Discussion

Direct micropropagation system through enhanced axillary bud development and organogenesis has been reported for different species of gladiolus (Begum and Hadiuzzaman, 1995; Grewal *et al*., 1995; Sen and Sen, 1995; Churvikova and Barykina, 1995; Gupta and Sehgal, 1997; Ziv *et al.*, 1997 and Hussain, et al. 2001). When cultured adventitious bud of gladiolus on MS medium and compared number of new plantlets regenerated in each combination of BA or

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kinetin and sucrose. The past works from Grewal *et al*.,(1995); Ahmad *et al*., (2000) reported on shoot regeneration only from uniform stage/size of any explant such as nodal buds. In this experiment, explants cultured on MS medium supplemented with 0.5 mg/l BA and 3% sucrose gave the highest average number of plantlets about 7.0 plantlets per bottle and MS medium supplemented only 3% and 4% sucrose and free BA or kinetin gave the highest average plant height about 9.6–9.86 cm. MS medium supplemented with 3% sucrose and 0.1–0.5 mg/l BA gave the highest average number of plantlets per bottle about 6.6-7.0 plantlets. The second was MS medium supplemented with 0.1–0.5 mg/l BA and 4% sucrose about 5.4–5.6 plantlets and higher than kinetin concentration. It was the same as Steinitz and Hannah (1989) reported that adventitious shoot regenerated after transferred to MS medium supplemented with 0.3 µM BA and 0.1µM NAA. De Bruyn and Ferreira (1992) reported the effect of different BA and sucrose concentrations as well as different temperatures on in vitro corm production of G. tristis was further investigated. The best production of shoots per explant was achieved on a medium containing 0.5-1.0 mg/ l BA and 6-9% sucrose. Hussey (1977) reported that cytokinins are also effective in production in *Gladiolus grandiflorus* .

On MS medium supplemented with 4% sucrose and 1 – 2 mg/l NAA got the lowest number of cormels about 4.2- 3.8cormels but their fresh weight were the highest about 148.6 – 149.0 mg per explants. As Ziv *et al*. (1970) and Steinitz and Lilien-Kinis (1989) reported that the gladiolus grew in media enriched with sucrose, it enhances storage organ(cormel) of the bulbous plants development. It was showed that high concentration of sucrose in the medium produced elongated leaves but small cormels. This confirms that the development of cormels need more food from photosynthesis (Ziv, 1979). Dantu and Bhojwani (1987) reported that the cormel induction and number of cormels was greatly affected by increasing levels of sucrose. The average number of cormels on MS medium supplemented with 0.1-0.5 mg/l % NAA and 3% sucrose gave the highest cormels about 5.8-5.6 cormels and their fresh weight about 144 – 144.2 mg per explant, respectively. MS medium supplemented with 1- 2 mg/l NAA and 4% sucrose gave the highest average fresh weight of cormels per plantlets about 148.6–149.0mg and the average cormel was the lowest about 4.2–3.8cormels, respectively. Van Aartrijk and Blom Barnhoorn (1980) reported that the regeneration of the various organs from the explants was found to be polarized and depended on the levels of growth substances added to the basal medium, best combination for organ initiation being 10 mg/l NAA and 0.5 mg/l kinetin. This result was the

same as Nagaraju *et al* (2002) showed that different concentration of sucrose supplementation levels in MS medium. Maximum cormel weight (mg per bottle) was recorded in Table 2 showed that 3% sucrose got the highest number of cormels confirmed by Roy *et al*. (2006) in "*Pacifica*"; Sinha and Roy (2002) in *Golden Wave.* It was found that rate of sucrose export from the leaf is reduced, carbohydrate accumulate in the organ. Taeb and Andrerson (1990) reported that sucrose appeared to be an important carbohydrate involved in the in vitro bulb development of tulips. In MS medium was found to be beneficial for in vitro cormel formation and new shoots.

The absence or low concentration of sucrose in MS medium resulted is very poor growth and formation of small average amount of cormel per explants, though it produced long leaves as well as root size, confirming finding in gladiolus cultivars (Ziv, 1979).

6. Conclusion

Potential of BA and sucrose to induce new shoots from young adventitious shoots from corm of Gladiolus and got the highest average plantlets per explants by culturing them on MS medium supplemented with 0.1-0.5 mg/l BA and 3% sucrose and the average plantlets about 6.6-7.0 plantlets per explants within 12 weeks. On MS medium with free BA or kinetin and only 3%-4% sucrose, gave the highest average plant height about 9.6-9.86 cm. MS medium supplemented with 0.1-0.5 mg/l NAA and 3% sucrose gave the highest average number of cormels at the distal end of plantlet about 5.8-5.6 cormels and their fresh weight about 144 mg per explants, respectively.

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