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Effect of paclobutrazol and sucrose on *in vitro* cormel formation in gladiolus

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Studies with excised shoots of six gladiolus cultivars viz., Bellariana, Blue Moon, Cream White, Friendship, Her Majesty and Top Brass indicated that paclobutrazol enhanced early cormel initiation and development. The interaction between paclobutrazol and sucrose was significant for cormel size. Supplementation of 10 mg L^{-1} paclobutrazol and 120 g L^{-1} sucrose to Murashige and Skoog's medium favored formation of bigger cormels. Among the cultivars response to exogenous sucrose supplementation varied significantly and absence of paclobutrazol produced longer leaves, roots and smaller cormels.

Key words: Gladiolus, cormel, paclobutrazol, sucrose

Introduction

The gladiolus is said to be the queen of bulbous flowers. It is rated as the most popular flower in the world from the commercial viewpoint (COHAT 1993). Conventionally it is propagated mainly by corms, cormels and seeds (NAGARAJU and PARTHASARATHY 1997). Rapid *in vitro* techniques have been reported for *in vitro* multiplication (ZIV 1979; LOGAN and ZETTLER 1985; DANTU and BHOJWANI 1987, 1992; NAGARAJU and PARTHASARATHY 1995; NAGARAJU et al. 1996). The transplanting and reestablishing of aseptically propagated plants under non aseptic conditions is still one of the main problem in the micropropagation of many plants including the gladiolus (ZIV 1979, SENGUPTA et al. 1984). Gladiolus plants obtained *in vitro* develop a small corm at the base of the shoot (ZIV 1979, SUTTER 1986) and the ultimate growth of corm is dependent on supplementation with sucrose (DANTU and BHOJWANI 1987) and exogenous hormones. Paclobutrtazol reduces stem elongation in several ornamental species (COULSTON and SHEARING 1985) and enhances storage organ development (ZIV 1989, STEINITZ and LILIEN-KIPNIS 1989) when grown in media enriched with sucrose. The present studies were undertaken in order to ascertain the optimum concentration of paclobutrazol and sucrose for *in vitro* cormel production of gladiolus cultivars and field performance of *in vitro* grown cormels.

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Materials and methods

Shoots from *in vitro* grown plants of six gladiolus cultivars viz. Bellariana, Blue Moon, Cream White, Friendship, Her Majesty and Top Brass were excised aseptically, trimmed to 1 cm long and were cultured in MURASHIGE and SKOOG (1962) medium containing paclobutrazol (PP_{333}) (0, 5 and 10 mg L^{-1}) and sucrose (30, 60, 90 and 120 g L^{-1}). A single explant was cultured in each culture tube with 30 culture tubes per treatment. 10 culture tubes formed one replication. The media contained 0.8 per cent agar and pH was adjusted to 5.8 prior to autoclaving at 15 lbs for 20 minutes. The cultures were incubated at 25 ± 1 °C under a photoperiodic regime of 16 hours light and 8 hours dark at an intensity of 3000 lux at culture level. Observations were recorded 14–15 weeks after culture initiation. The cormels formed *in vitro* were harvested once the leaves had dried. The *in vitro* cormels of Blue Moon, Friendship and Her Majesty were soaked overnight in solution containing 1 mg L^{-1} BAP and planted in pots containing equal parts of leaf mould, farmyard manure and garden soil. Compared with field grown cormels, various morphological, floral, corm and cormel parameters were analysed by factorial design (SNEDECOR and COCHRAN 1976).

Results and discussion

Favourable effects of growth inhibitors on corm formation were reported by COURDUROUX (1967) and EL-ANTABLY et al. (1967). The effect of paclobutrazol on cormel development was therefore investigated. There was significant response to paclobutrazol as evi-

Tab. 1. Effect of paclobutrazol and sucrose (amount added to the medium) on cormel formation *in vitro*. Data pertain to mean of 6 varieties.

Paclobutrazol (mg L^{-1})	Sucrose (g L^{-1})	Total culture weight (mg)	Cormel				Leaf Length (mm)	Root Length (mm)
			Length (cm)	Breadth (cm)	No.	Weight (mg)		
0	30	370.1	4.7	4.4	2.0	143.3	20.3	61.5
	60	570.7	5.9	5.6	2.3	230.4	19.1	62.8
	90	761.4	5.9	5.8	2.5	242.9	19.2	60.3
	120	519.4	6.8	5.6	2.7	223.7	15.5	71.9
5	30	757.8	8.3	7.4	2.1	363.8	6.7	30.9
	60	918.5	8.4	7.4	1.9	444.9	5.6	33.1
	90	996.3	7.5	6.7	2.3	465.6	6.9	49.0
	120	943.6	8.0	6.8	2.6	420.7	4.2	32.2
10	30	1044.8	7.9	7.5	3.1	567.8	5.3	23.9
	60	1285.5	8.5	7.1	2.6	537.8	5.5	25.2
	90	1322.9	9.2	8.5	3.8	591.8	4.9	31.8
	120	1379.2	8.9	8.1	3.6	739.6	5.0	19.7
S Em ±		131.59	0.572	0.407	0.341	45.637	1.565	5.704
CD (0.05)		NS	1.601*	1.139*	NS	NS	NS	NS

* – Significant at 5%, NS – not significant

denced from the effect of paclobutrazol on cormel formation (Fig. 1). Higher culture weight and bigger cormels were recorded at increased levels of paclobutrazol (10 mg L^{-1}) as indicated by higher cormel weight, increased length and breadth of cormels. Further, plant growth analysis revealed that the inclusion of paclobutrazol in the growth medium inhibited the growth of *in vitro* plants, especially in relation to plant height, leaf weight and root length. However, the absence of paclobutrazol resulted in elongated leaves and the formation of smaller cormels. A decrease in stem elongation is natural due to the anti--gibberellin activity of paclobutrazol (GRAEBE 1987, RADEMACHER et al. 1984). Reduction in plant height in chrysanthemum and sugar beet was also reported by RITCHIE et al. (1991).

The mean response of six gladiolus cultivars to various levels of paclobutrazol was shown to be (Tab. 1) highly significant for culture weight, cormel number as well as weight, leaf characters and root length. The total culture weight among the varieties at different levels of paclobutrazol varies from 278.6 mg in Her Majesty at 0 mg L^{-1} paclobutrazol to 1864.8 mg in Friendship at 10 mg L^{-1} paclobutrazol. Though Cream White recorded 1717.90 mg, it was statistically at par with Friendship. A similar trend was also recorded for cormel number (1.1 to 4.8) as well as weight (104.0 to 857.4 mg L^{-1}). The cormel length and breadth among the varieties ranged from 4.6 to 9.5 mm and 4.0 to 8.6 mm. Addition of paclobutrazol enhanced cormel weight in all the cultivars. Similarly, a greater number of cormels was also recorded in the presence of paclobutrazol. Significant interaction between paclobutrazol concentration in the culture medium for shoot size and height was also reported by KLOCK (1998).

The data presented in figure 2 showed significant variations among the varieties for all the characters studied. Maximum culture weight (1363.0 mg), bigger cormels (8.33 × 8.22 cm), and longer leaves as well as weight were recorded in Cream White. However, Friendship (1217.1 mg) was statistically at par with Cream White for culture weight and cormel length as well as leaf length and leaf weight. Such a variation among the varieties might be due to the combined influence of paclobutrazol in the medium as well as the genetic nature of the plant material used. The differentiation and development of storage organs is most probably regulated by the level of endogenous gibberellins, similar to that indicated for potato tuberization (TIZIO 1969, VREUGDENHIL and STRUIK 1989). Paclobutrazol shifted assimilate allocation preferentially towards cormel development (STEINITZ et al. 1991).

Tab. 2. Performance gladiolus varieties of field and *in vitro* obtained cormels

Variety	Source	Plant height (cm)	Length (cm)		Leaf				Flower No.	Corm			Cormel No.	10 cormel wt (g)
			Spike	Stalk	Rachis	No.	Length	Width		Length (cm)	Width (cm)	Weight (g)		
Blue	Field	173.7	115.0	36.7	78.3	9.3	59.0	3.1	18.0	5.5	3.2	53.8	171.7	4.5
Moon	*In vitro*	136.7	105.3	46.7	58.7	7.3	44.3	3.9	12.0	5.3	3.1	60.1	35.3	4.3
Friend	Field	154.7	117.0	40.0	77.0	9.3	51.2	3.3	20.7	5.5	2.6	39.2	131.0	2.3
Ship	*In vitro*	129.3	85.0	32.3	53.3	7.7	54.3	3.8	14.0	6.7	3.5	96.8	34.3	4.4
Her	Field	149.3	117.0	30.7	86.3	9.6	45.1	3.1	23.3	6.2	2.8	76.8	86.3	2.9
Majesty	*In vitro*	132.0	96.3	39.3	57.0	7.3	49.0	3.5	14.3	5.1	3.0	42.7	16.0	5.3

Paclobutrazol created and maintained the conditions required for continuous cormel growth (ZIV 1989, STEINITZ and LILIEN-KIPNIS 1989, STEINITZ et al. 1991) and mediated shifts in assimilate partitioning (DAVIS et al. 1988).

The data on morphogenetic response of shoots of different cultivars to sucrose indicated a significant influence on cormel size and weight, leaf weight and root length. Though variation in culture weight was recorded at different sucrose supplementation levels, statistically it was insignificant. The data on mean weight of culture among the varieties ranged from 402.6 mg in Bellariana at 90 g L^{-1} sucrose to 1962.7 mg L^{-1} in Cream White. Sucrose did not improve leaf number significantly, and it ranged from 2 in Top Brass to 5.1 in Her Majesty at 90 g L^{-1} sucrose. Maximum cormel weight (711 mg) was recorded in Friendship at 120 g L^{-1} sucrose. The addition of sucrose to the medium had a positive effect

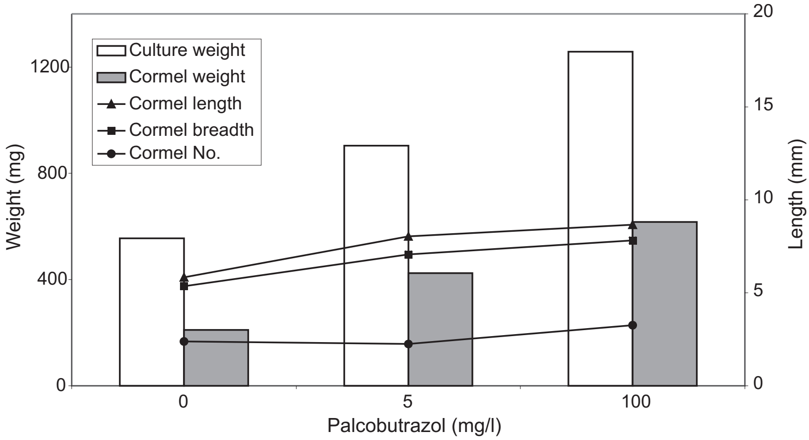

Fig. 1. Effect of paclobutrazol on cormel formation *in vitro*.

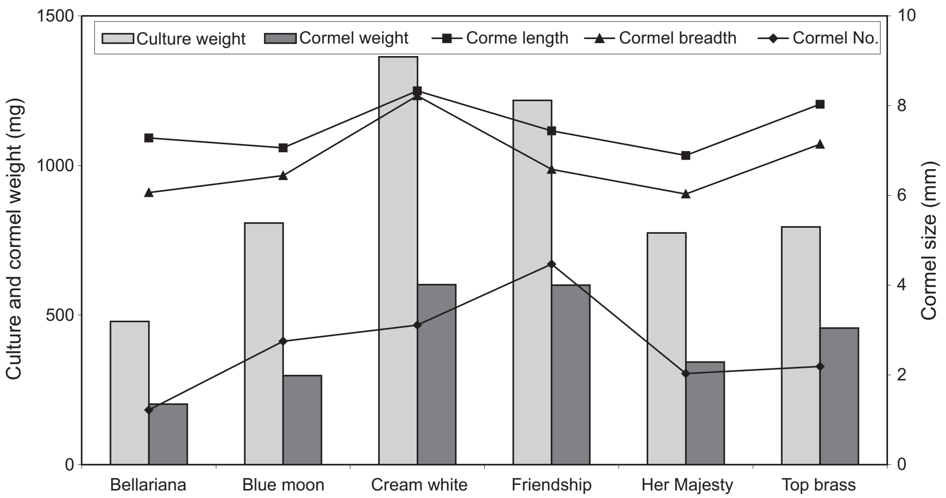

Fig. 2. Response of gladiolus varieties to *in vitro* cormel formation.

on culture weight, cormel number and weight confirming the earlier findings of TICHA et al. (1988) in tobacco and HUSSEY (1977), ZIV (1979) and SUTTER (1986) in gladiolus. HAZARIKA et al. (2000) found clear differences in starch content from citrus explants cultured in different levels of sucrose. CAPELLADES et al. (1991) observed a large deposition of starch in the chloroplasts of plantlets obtained on culture medium containing 5% sucrose as compared to medium containing 3% and 1% sucrose. AZCON-BIETO (1983) also found that when the rate of sugar export from the leaf is reduced, carbohydrates accumulate in the organ. TAEB and ANDERSON (1990) opined that sucrose appeared to be an important carbohydrate involved in the *in vitro* bulb development of tulips. Among the varieties, Bellaraiana and Cream White responded at 60 g L^{-1} sucrose compared to 120 g L^{-1} in other varieties for cormel weight. Such a variation among the species is due to the genetic nature of varieties for response to exogenous supplementation of sucrose, which is contrary to the earlier findings of DE BRUYN and FERREIRA (1992), and DANTU and BHOJWANI (1987).

Supplementation of paclobutrazol and sucrose (Tab. 1) in the medium was found to be beneficial for *in vitro* cormel formation. There was significant interaction between paclobutrazol and sucrose for cormel size. Supplementation of paclobutrazol improved the cormel weight in the presence of sucrose and maximum cormel weight was recorded in media supplemented with 10 mg L^{-1} paclobutrazol to which 120 g L^{-1} sucrose was added. The absence of paclobutrazol and low sucrose (3 %) in the medium resulted in very poor growth and formation of smaller cormels, though it produced long leaves as well as roots. The interaction of paclobutrazol and sucrose further indicated that the presence of paclobutrazol, irrespective of the levels of sucrose concentration, reduced the leaf as well as root size and sucrose was exclusively utilized for corm filling as indicated by the weight, confirming findings in gladiolus cultivars (ZIV 1979, SUTTER 1986) and in the gladiolus hybrid Kinneret (STEINTZ and YHEL 1982). The further presence of high sucrose in the medium produced elongated leaves but small cormels. This confirms that the development of longer leaves need not be related to the synthesis of more food by photosynthesis for cormel development (ZIV 1979).

The morphological response of plants obtained from field grown and *in vitro* obtained cormels (Tab. 2) indicated that plant height was higher in all the varieties obtained from plants of field-grown corms as compared to the plants grown from *in vitro* obtained corms. Similarly, spike length and number of flowers per plant were higher from plants grown using field grown corms. Though variations were recorded for various growth factors, phenotypically the plants obtained from field-grown as well as *in vitro* grown corms are similar. Therefore, for large-scale multiplication of true to type planting materials of selected varieties/ hybrids and the commercialisation of newly released hybrids, supplementation with 10 mg L^{-1} paclobutrazol and 60–120 g L^{-1} sucrose, according to variety/species/ hybrid is optimum.

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