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Influence of media and growth regulators on adventitious shoot production in gerbera

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Abstract : Gerbera *(Gerbera jamesonii* (H.) Bolus) is a cut flower with increasing commercial importance. It grows well in tropical and subtropical zones. An experiment was conducted at Horticultural Research Station, Yercaud during 1998-2000 to standardize the protocol for production of adventitious shoots from *in vitro* leaf explants of gerbera cv. YCD. 1. The *in vitro* derived leaf explants were inoculated in three media *viz.* MS, G and ½ MSH supplemented with combinations of KIN at 3.0, 4.0 and 5.0 mg l^{-1} with GA at 0.5 and 1.0 mg l^{-1}. Culturing the leaf explants in MS medium supplemented with KIN 5.0 mg l^{-1} with GA 0.5 mg l^{-1} recorded the highest percentage as well as the earliest formation of adventitious shoots. Among the three media, MS medium was the best.

Key words: Gerbera, Tissue culture, Media, Growth regulators, Adventitious shoots.

Introduction

Gerbera, a cut flower has a high demand in the domestic as well as the international market. The conventional method of propagation of gerbera is through division of clumps, which is very slow and insufficient to meet the growing demand for planting materials. Production of gerbera plants through tissue culture is the only alternative to get rapid multiplication.

The prime requirement in any multiplication programme is the maintenance of genetic purity. Reynoird *et al.* (1993) stated that due to the absence of phenotypic variation in the regenerants during their vegetative and regenerative phase, *in vitro* gerbera shoot production via regeneration from leaves could be proposed as a useful propagation technique. In order to standardize the protocol for adventitious shoot production in gerbera, an experiment was conducted at the tissue culture lab, Horticultural Research Station, Yercaud during 1998-2000.

Materials and Methods

Shoot tip explants collected from field grown plants of YCD.1 were cultured in gerbera multiplication medium (Murashige and Skoog, 1962) supplemented with Kinetin (KIN) 10 mg l^{-1} and Indole Acetic Acid (IAA) 0.1 mg l^{-1}. This produced numerous miniature divisions or small outgrowth of shoots. These shoots were separated and recultured in fresh medium to obtain multiple shoots. The *in vitro* derived leaf explants were inoculated in three media *viz.* MS, G and 1/2 MSH supplemented with combinations of KIN at 3.0, 4.0 and 5.0 mg l^{-1} with GA at 0.5 and 1.0 mg l^{-1}.

Ten explants were inoculated per treatment and replicated. thrice. They were kept in the culture room maintained at 14:10 hours of light and dark cycle. Observations on the percentage of adventitious shoot formation were recorded 35 days after inoculation. The days taken for adventitious shoot formation was recorded as and when the shoots were initiated.

Results and Discussion

The percentage of adventitious shoot production and the days taken for the formation of adventitious shoots on leaf explants of gerbera cv. YCD.1 as influenced by three media *viz.* MS. G and 1/2 MSH supplemented with the combination of growth regulators *viz.* KIN at 3.0, 4.0 and 5.0 mg l^{-1} with GA at 0.5 and 1.0 mg l^{-1} are presented in Tables 1 and 2 respectively.

Percentage of adventitious shoot production in leaf explants

The percentage of adventitious shoots produced ranged from zero to 86.67 in MS medium, zero to 80.00 in G medium and zero to 63.33 in 1/2 MSH medium. No shoot formation was observed in any of the media without growth regulators. The variation in the percentage of adventitious shoot formation varied significantly among the three media. The mean highest percentage of adventitious shoot production was recorded in MS medium (59.05) followed by

Table 1. Effect of media and growth regulators on the percentage of adventitious shoot production in leaf explants of gerbera cv. YCD 1

Concentration of growth regulators (mg l^{-1})	Percentage of adventitious shoot production			
	MS	G	½MSH	Mean
KIN 3.0 + GA 0.5	52.78 (63.33)	46.92 (53.33)	43.08 (46.67)	47.59 (54.44)
KIN 4.0+ GA 0.5	56.99 (70.00)	54.78 (66.67)	46.92 (53.33)	52.90 (63.33)
KIN 5.0 + GA 0.5	68.86 (86.67)	63.93 (80.00)	52.78 (63.33)	61.86 (76.67)
KIN 3.0 + GA 1.0	48.85 (56.67)	39.15 (40.00)	35.22 (33.33)	41.07 (43.33)
KIN 4.0 + GA 1.0	54.78 (66.67)	43.08 (46.67)	39.15 (40.00)	45.67 (51.11)
KIN 5.0 + GA 1.0	56.99 (70.00)	46.92 (53.33)	43.08 (46.67)	49.00 (56.67)
Control	0.91 (0.00)	0.91 (0.00)	0.91 (0.00)	0.91 (0.00)
Mean	48.60 (59.05)	42.24 (48.57)	37.31 (40.47)	
	SEd	CD (0.05)	CD (0.01)	
G	1.40	2.78	3.68	
M	1.30	2.57	3.41	
G x M	3.42	6.81	9.02	

(Figures within parenthesis indicate transformed values)

Table 2. Effect of media and growth regulators on the days taken for adventitious shoot production in leaf explants of gerbera cv. YCD. 1

Concentration of growth regulators (mg l^{-1})	Percentage of adventitious shoot production			
	MS	G	½MSH	Mean
KIN 3.0 + GA 0.5	22.33	24.00	25.67	24.00
KIN 4.0 + GA 0.5	21.67	23.33	24.00	23.00
KIN 5.0 + GA 0.5	20.00	21.67	22.67	21.45
KIN 3.0 + GA 1.0	23.67	25.00	26.33	25.00
KIN 4.0 + GA 1.0	22.00	23.33	24.00	23.11
KIN 5.0 + GA 1.0	21.33	22.67	22.00	22.00
Mean	21.83	23.33	24.22	
	SEd	CD (0.05)	CD (0.01)	
G	0.24	0.48	0.64	
M	0.24	0.48	0.64	
G x M	0.59	1.17	1.56	

G medium (48.57) while the lowest was observed in 1/2 MSH medium (40.47).

Among the combinations of growth regulators, KIN 5.0 mg l^{-1} with GA 0.5 mg l^{-1} produced the highest percentage of adventitious shoots production (76.67) followed by KIN 4.0 mg l^{-1} with GA 0.5 mg l^{-1} (63.33). The interaction between the media and combination of growth regulators indicated that the MS medium supplemented with KIN 5.0 mg l^{-1} GA 0.5 mg l^{-1}, produced the highest percentage (86.67) of adventitious shoot production.

Experiments on leaf explants excised from matured plants have resulted in root and callus formation (Pierik and Segers, 1973) and only incidental bud regeneration from intact leaves of *in vitro* derived leaves (Hedtrich, 1979). Engelke *et al* (1973) stated that GA_3 produced effects similar to auxin and hence a specific gibberellin/ cytokinin ratio should be arrived to induce adventitious shoot formation. Pierik *et al.* (1982) and Hempel *et al.* (1985) found that KIN was better than BA and recommended 5 to 10 mg l^{-1} of KIN for the production of quality shoots.

In this study, there was an increase in the percentage of adventitious shoot formation from 54.44 to 76.67 with an increase in the concentration of KIN from 3.0 to 5.0 mg l^{-1}. This clearly emphasizes the higher requirement of cytokinins for regeneration of adventitious shoots from leaves. Addition of GA_3 from 0.5 to 1.0 mg l^{-1} along with KIN 4.0 mg l^{-1} reduced the percentage of regeneration from 76.67 to 56.67, which might be due to the inhibitory action of GA_3 at higher concentration as reported by George (1993). Parthasarathy *et al.* (1996) reported similar findings.

The highest percentage (76.67) as well as the earliest formation (21.45 days) of adventitious shoots were reported with 5 mg l^{-1} KIN in combination with 0.5 mg l^{-1} of GA_3. Corroborative findings indicating higher concentration of KIN along with GA_3 as the best for adventitious shoot production was reported by Sree devi (1995).

Days taken for adventitious shoot production

The days taken for the formation of adventitious shoots ranged from 20.00 to 23.67, 21.67 to 25.00 and 22.00 to 26.33 in MS, G and 1/2 MSH media respectively. Among the three media, mean days taken for the earliest formation of adventitious shoots (21.83) was observed significantly in MS medium followed by G medium (23.33). However, in 1/2 MSH medium, the formation of adventitious shoots was highly delayed (24.22 days).

Out of the several combinations of growth regulators, the earliest formation of adventitious shoots was recorded in KIN 5.0 mg l^{-1} with GA 0.5 mg l^{-1} (21.45 mean days) followed by KIN 5.0 mg l^{-1}, with GA 1.0 mg l^{-1} (22.00 mean days). Whereas it was highly delayed (25.00 mean days) in the combination of KIN 3.0 mg l^{-1} with GA 1.0 mg l^{-1}. The interaction between the media and the combinations of growth regulators indicated that the MS medium supplemented with KIN 5.0 mg l^{-1} with GA 0.5 mg l^{-1} significantly recorded the earliest formation of adventitious shoots (20.00 mean days). But 1/2 MSH medium supplemented with KIN 3.0 mg l^{-1} with GA 1.0 mg l^{-1} slowed down the regeneration and took the highest number of days (26.33).

In most intact plants, tissues and organs take up nitrogen effectively and grow more rapidly in nutrient solutions containing both nitrate and ammonium ions. According to Drew (1987), adventitious organs may develop abnormally if ammonia was not included in the media. The present study on the influence of three media *viz.* MS, G and 1/2 MSH for adventitious shoot production indicated that the highest percentage (59.05) and the earliest formation (21.83 days) of adventitious shoots were found in MS medium followed by G and 1/2 MSH media. This might be due to the high proportion of ammoniacal nitrogen and also the high quantum of total nitrogen in MS medium than in the other two media. The proportion of nitrate to ammonia in MS medium is 66 : 34 and therefore, the total concentration of nitrogen in the medium and the ratio of nitrate to ammonia is considered to be an important factor for morphogenesis as reported by George (1993).

Conclusions

The leaf explants of gerbera cultivar YCD.1 when cultured in MS medium supplemented with KIN 5.0 mg l^{-1} with GA 0.5 mg l^{-1} recorded the highest percentage (86.67) as well as the earliest formation of adventitious shoots (20.00 days).

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