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Short Communication

DEVELOPMENT OF *IN VITRO* ESTABLISHMENT AND MULTIPLICATION TECHNOLOGY FROM GERBERA FLOWER BUDS

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INTRODUCTION

The floriculture industry in Sri Lanka has been identified as an economically viable enterprise. Gerbera (*Gerbera jamesonii* Bolus), commonly known as transvaal daisy, barberton daisy or African daisy, is an important commercial flower grown throughout the world in a wide range of climatic conditions. Gerbera belongs to the family Asteraceae; is a popular cut flower and has a high market demand, which is used for many kinds of decorations. Gerbera ranks fifth in the international cut flower market (Tjia, 2001). Despite high demand exists for gerbera flowers in Sri Lanka, non availability of high quality planting materials has been identified as the major constraint in the industry. Gerbera is generally propagated by division of suckers or clumps. Propagation by division of suckers or clumps gives true to type plants, but the multiplication rate is very low (Yapa, 2001). Application of tissue culture techniques could be helpful in this situation if the desired multiplication rates would be achieved. Consequently, this study was under taken at Agriculture Research Station, Sita Eliya to develop a locally adoptable protocol for *in vitro* planting material production of Gerbera enabling the increase of high quality planting material production.

MATERIALS AND METHOD

Plant materials and explants

Four varieties of Gerbera namely Rosaline, Ice Burge, Black Jack and Brilieury collected from the Botanical Gardens, Hakgala, Sri Lanka was used for the study. The collected plants were established in a glasshouse and explants were collected from healthy mother plants. Flower bud, capitulum, leaf blade and petiole were used as explants, for *in vitro* propagation (see Table 1 for details of ex plant conditions).

Table 1. **Types of explants and their status.**

Type of ex plant	*Conditions*
Leaf blade	7 days old leaves, 1 cm^2 pieces
Leaf petiole	Young leaf petiole, 0.5-1 cm long pieces
Capitulum	Fully developed inflorescence used with removal of disc florets and ray florets
Flower bud	3-5 days old flower buds with complete inflorescence

Nutrient Media

Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was used with 4 % sucrose and 0.7 % Agar. pH of the medium was maintained between 5.6-5.8. Culture vessels containing nutrient media were autoclaved at a pressure of 1.06 kg cm^2 and 121 ^{0}C for 20 minutes. The experiment was conducted in a culture room maintained at 25 ± 2 ^{0}C, 16 hours of light at 2,500-3,000 Lux and 8 hours dark. The explants were thoroughly washed under running tap water and immersed in a mild teepol solution for 30 minutes, followed by 70% alcohol for 1 minute, 20% Clorox for 10 minutes. Immersed explants were washed thoroughly with sterilized double distilled water. Experiment was carried out with 10 replicates and each vessel contained 4 explants and they were subjected to the following establishment media during the first six weeks. Number of calli calculated was recorded.

1. MS + 2 mg/l (Benzyle Amino Purine) BAP + 2 mg/l 2.4-D
2. MS + 2.5 mg/l BAP +0. 2 mg/l IAA (Indol Acitic Acid)
3. MS + 3 mg/l BAP + 0.1 mg/l IAA

Well developed calli were transferred to the following three shoot induction media and shoots per explants were recorded.

1. MS + 1 mg/l BAP + 0.01 mg/l NAA
2. MS + 1 mg/l BAP + 0.2mg/l GA_3
3. MS + 3 mg/l BAP + 0.1mg/l NAA

Six weeks after, well developed plantlets were isolated and established in Gerbera multiplication medium with MS+ 2 mg/l BAP, 0.1 mg/l GA_3 (Pierik *et al*., 1973). *In vitro* multiplication ability was tested using nodal cuttings and stem cuttings of the plantlets. Completely developed plants were transferred to 3:1 sand:coir dust medium and time taken to flower initiation and flower characteristics were recorded.

RESULTS AND DISCUSSION

Calli initiation was observed in explants of leaf blade and flower buds with the medium containing MS + 3 mg/l BAP + 0.1 mg/l IAA. No calli formation was observed with MS + 2 mg/l BAP + 2 mg/l 2.4-D. However, Kumar *et al.* (2004) has identified the later as a suitable callus initiation media. Calli formation ability was different depending on the variety and explants used and well developed callus (more than 3 mm width) was observed only with flower buds. Variety Roseline produced the highest number of flower buds and calli per explants. The result of the experiment clearly indicates the suitability of flower bud as explants than the others explants used.

Table 2. Mean number of calli initiated from different explants of different varieties after 6 weeks.

	Variety											
Type of ex plant	*Roseline*			*Ice Burge*			*Black Jack*			*Brileury*		
	Establishment Media											
	1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
Leaf Blade	-	-	2	-	-	2	-	-	2	-	-	-
Petiole	-	-	-	-	-	-	-	-	-	-	-	-
Capitulum	-	-	-	-	-	-	-	-	-	-	-	-
Flower bud	-	1	7	-	-	4	-	-	4	-	-	3

Note: 1=MS + 2 mg/l (Benzyle Amino Purine) BAP + 2 mg/l 2.4-D; 2=MS + 2.5 mg/l BAP +0. 2 mg/l IAA (Indol Acitic Acid); 3=MS + 3 mg/l BAP + 0.1 mg/l IAA

Table 3. Mean number of shoots initiated per callus from flower buds of different varieties after 6 weeks.

	Variety			
Initiation Media	*Roseline*	*Ice Burge*	*Black Jack*	*Brilileury*
1. MS + 1mg/l BAP + 0.01 mg/l NAA	3	1	2	1
2. MS + 1 mg/l BAP + 0.2 mg/l GA_3	3	2	2	3
3. MS + 3 mg/l BAP + 0.1 mg/l NAA	10	5	6	6

Note: 1=MS + 1 mg/l BAP + 0.01 mg/l NAA; 2=MS + 1 mg/l BAP + 0.2mg/l GA_3; 3=MS + 3 mg/l BAP + 0.1mg/l NAA

Highest number of shoots per callus (10 plantlets) was observed in medium 3 in variety Roseline. Other varieties showed comparatively lower number of shoot formation in the same medium. It has been also shown elsewhere that each genotype has a specific range of optimum growth regulator concentration (Deepaja, 1999). After 6 weeks, well

developed plantlets were isolated and established in Gerbera multiplication medium MS+ 2mg/l BAP, 0.1 mg/l GA_3 and *in vitro* multiplication was carried out using nodal cuttings and stem cuttings of the initiated plantlets. Nodal cuttings produced more plants than that of the stem cuttings, irrespective of the sub culture (Table 3).

Table 3. Number of plants obtained from regular sub culturing.

		No of plants
No of Sub-culturing	*Shoots*	*Nodal cuttings*
1	2	6
2	3	5
3	3	6
4	2	8
5	3	7

Results of the study revealed that the nodal cutting were the best plant part for *in vitro* multiplication. Flower characteristics observed in completely developed plants were similar to that of the mother plant stock characters. This shows that the *in vitro* propagation was successful and no variation has been detected between mother plant and *in vitro* planting material. Hence, this multiplication technique can be used efficiently for the production of high quality planting materials within a shorter period of time and achieve national targets.

CONCLUSIONS

Gerbera plants can be successfully produced *in vitro* by using MS + 3 mg/l BAP + 0.1mg/l IAA medium for calli establishment and MS + 3 mg/l BAP + 0.1mg/l NAA medium as plant initiation medium. Flower bud was the best ex plant and nodal cuttings were the most effective plant for *in vitro* multiplication of gerbera.

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