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Media standardization for pre-hardening and hardening of *in vitro* regenerated plantlets of gerbera cultivars

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Abstract

Experiment was carried out to standardize media for pre-hardening and hardening of *in vitro* regenerated plantlets taking two cultivars of gerbera namely Red Star and Jallisse. During hardening, soil, sand, FYM and coco peat mixture with a proportion of 1:1:1:1, 1:1:1:2,1:1:2:1, 1:2:1:1, ½:1:1:1 , ½:1:1:2, ½:1:2:1, ½:2:1:1, respectively and control (only soil) were tried for transplanting the regenerated plants. Among these, soil: sand: FYM: coco peat in ½:1:1:2, ½ :2:1:1 and ½:1:1:1 proportion for *cv.* Jallisse and ½:1:1:2 proportion for *cv.* Red star were found to be most promising combinations for 100% survival of regenerated plants.

Key words: Gerbera, pre-hardening, hardening, MS liquid medium

Introduction

Gerbera, the magnanimous flower, is used as fresh in exhibition, flower arrangement, floral decorations, high class bouquets and also as dry flower. Its commercial cultivation is gaining momentum day by day as it provides high profit from small holdings. The main purpose for propagation of ornamental plants is for its aesthetic value. Thus the improvement for quality attributes such as flower colour, longevity and form, plant shape and the creation of novel variants are important economic goals. So, tissue culture techniques are much more preferred in gerbera as in this genus, genetic variability is relatively limited, which makes it difficult to breed for new flower colours and patterns. There is also limited genetic material that can be used to improve resistance to biotic and abiotic stress (Orlikowska *et al*., 1999). In order to produce high quality uniform blooms, rapid clonal multiplication is the need of the hour. The earlier works indicated that, depending on cultivars as well as local conditions, the requirement for response, multiplication and rooting vary considerably. Micropropagation on large scale can be successful only when plants after the transfer from culture to the soil show high survival rates and the cost involved in the process is low. A heterotrophic mode of nutrition and poor mechanism for water loss control further renders micropropagated plants vulnerable to transplantation shocks. Therefore, transfer of individual plantlets to a potting mixtures and their acclimatization under greenhouse conditions require the application of various methods to harden the plants for transplantation (Kanwar and Kumar, 2008). It is therefore, necessary to standardize media for pre-hardening and hardening to get high survival rate with high quality high value hybrids of gerbera.

Materials and methods

Regenerated plants with healthy root systems of 2 to 3 cm length were washed with sterile distilled water to clear of all the adhering gel. The plantlets were transferred to ¼ strength of MS liquid medium bottles/tubes (Plate 1 and 2) and covered with glass beaker or polythene sheet to maintain humidity for 2 days for pre-hardening.

Then the plantlets were transferred to the small plastic tea cups containing sterilized soil : sand: FYM: coco peat in different proportions (Table 1). The pots were covered with glass beaker or polythene sheet (Plate 3), for maintaining high humidity and irrigated regularly with ¼ strength liquid medium for initial 4 days and kept in greenhouse for hardening for 28 days. Three replications per treatment and 10 pots per replication *i.e.*, 30 pots per treatment were used.

Table 1. Details of proportions of soil mixture for hardening of plantlets

Treatments	Soil	Sand	FYM	Coco peat
T_1 (Control)	Only soil	-	-	-
T_2	1	1	1	1
T_3	1	1	1	2
T_4	1	1	2	1
T_5	1	2	1	1
T_6	½	1	1	1
T_7	½	1	1	2
T_8	½	1	2	1
T_9	½	2	1	1

Observations recorded up to 30 days of pre hardening and hardening were:

Days to establishment: It is the number of days taken by the plantlet to establish in the hardening media after planting.

Days to new leaf formation: It is the days taken to initiate new leaf after planting.

Percentage of survival: It was calculated as: (Number of plants survived/Total number of plant hardened) x 100

Plant height: It is the length measured after the plants are fully established.

Plantlets were transferred to black polythene bags containing soil, sand, FYM and coco peat in their best proportion as per variety and kept in net house for 10 days. After thorough hardening the plantlets were ready for marketing or planting.

The data recorded from the experiments were analysed following the method of Gomez and Gomez (1984) using one way ANOVA in Completely Randomized Design (CRD) with 3 replications each consisting of 10 pots.

Results

The data presented in Table 2 for hardening of the tissue culture derived plantlets of variety Jallisse revealed that various combinations of soil, sand, FYM and coco peat except the treatment T_1 (only soil) and T_2 (soil: sand: FYM: coco peat in 1:1:1:1) reduced significantly the days to establishment in the sterilized medium, being lowest (3.67) in T_7 (soil: sand: FYM: coco peat in ½ :1:1:2), T_8 (soil: sand: FYM: coco peat in ½: 1:2:1) and T_9 (soil: sand: FYM: coco peat in ½:2:1:1). Days to new leaf formation was significantly minimum in T_9 (soil: sand: FYM: coco peat in ½:2:1:1) remaining at par with all the treatments except control *i.e.*, only soil, T_4 (soil: sand: FYM: coco peat in 1:1:2:1) and T_5 (soil: sand: FYM: coco peat in 1:2:1:1). Significantly higher percentage of survival (100 %) was recorded in treatment T_6 (soil: sand: FYM: coco peat in ½:1:1:1), T_7 (soil: sand: FYM: coco peat in ½:1:1:2) and T_9 (soil: sand: FYM: coco peat in ½:2:1:1) remaining at par with T_3 (soil: sand: FYM: coco peat in 1:1:1:2). Whereas 100 % mortality was recorded in the control *i.e.*, T_1 (only soil). Height of the plantlets was significantly higher (12.67 cm) in T_7 (soil: sand: FYM: coco peat in ½:1:1:2) remaining at par with all the treatments except control *i.e.*, only soil, where no plant was available for recording the height. Hence, it has been concluded that the treatment T_7 (soil: sand: FYM: coco peat in ½:1:1:2), T_9 (soil: sand: FYM: coco peat in ½ :2:1:1) and T_6 (soil: sand: FYM: coco peat in ½:1:1:1) were the most promising combinations for hardening of the plantlets of *cv.* Jallisse (Plate 1,4).

The data presented in the Table 3 for the hardening of the plantlets of *cv.* Red Star revealed that significantly minimum days for establishment (3.33) was recorded in T_7 (soil: sand: FYM: coco peat in ½:1:1:2) remaining at par with T_9 (soil: sand: FYM: coco peat in ½:2:1:1) and T_5 (soil: sand: FYM: coco peat in 1:2:1:1). Days to new leaf formation was minimum (7.67) in T_7 (soil: sand: FYM: coco peat in ½ :1:1:2) remaining at par with T_4 (soil: sand: FYM: coco peat in 1:1:2:1) and T_5 (soil: sand: FYM: coco peat in 1:2:1:1). Percentage of survival of the plantlets during hardening was higher (100 %) in T_6 (soil: sand: FYM: coco peat in ½:1:1:1) and T_7 (soil: sand: FYM: coco peat in ½:1:1:2) remaining at par with T_9 (soil: sand: FYM: coco peat in ½:2:1:1). Whereas all the plantlets planted in only soil showed 100 % mortality during the process of hardening. Maximum plant height (12.27 cm) was recorded in T_7 (soil: sand: FYM: coco peat in ½ :1:1:2) remaining at par with all the treatments except T_1 (only soil) where plants were not available for recording the data. Considering all the biometric characters of plantlets during hardening, the treatment T_7 (soil: sand: FYM: coco peat in ½ : 1: 1: 2) was found to be best for hardening of the tissue culture plantlets of *cv.* Red Star (Plate 2, 5).

Discussion

The success of tissue culture depends on the establishment of the *in vitro* produced plants under the natural conditions. Under *in vitro* conditions, the plantlets are heterotrophs. So they have to

Table 2. Effect of different growing media on hardening of regenerated plantlets of var. Jallisse (Duration –30 days)

Treatment	Composition				Days to establishment	Days to new leaf formation	Percentage of survival	Plant height during transfer
	Soil	Sand	FYM	Coco peat				
T_1	Only soil	-	-	-	-	-	-	-
T_2	1	1	1	1	4.67	10.00	86.66 (68.99)	12.40
T_3	1	1	1	2	4.00	9.33	90.00 (78.22)	12.40
T_4	1	1	2	1	4.00	11.00	80.00 (70.43)	12.40
T_5	1	2	1	1	4.33	10.67	86.66 (68.33)	12.27
T_6	½	1	1	1	4.33	9.67	100.00 (87.50)	12.50
T_7	½	1	1	2	3.67	8.67	100.00 (87.50)	12.67
T_8	½	1	2	1	3.67	9.67	83.33 (66.47)	12.33
T_9	1/2	2	1	1	3.67	8.33	100.00 (87.50)	12.20
LSD (P=0.05)					0.97	1.85	9.90	0.60

Table 3. Effect of different growing media on hardening of regenerated plantlets of var. Red Star (Duration –30 days)

Treatment	Composition				Days to establishment	Days to new leaf formation	Percentage of survival	Plant height during transfer
	Soil	Sand	FYM	Coco peat				
T_1	Only soil	-	-	-	-	-	-	-
T_2	1	1	1	1	4.33	9.33	86.66 (69.02)	11.45
T_3	1	1	1	2	4.67	10.67	90.00 (71.56)	11.77
T_4	1	1	2	1	4.33	8.67	80.00 (63.93)	11.60
T_5	1	2	1	1	4.00	8.67	86.66 (69.02)	11.70
T_6	½	1	1	1	4.67	9.67	100.00 (87.50)	11.83
T_7	½	1	1	2	3.33	7.67	100.00 (87.50)	12.27
T_8	½	1	2	1	4.33	10.67	83.33 (66.47)	11.57
T_9	1/2	2	1	1	3.67	9.33	100.00 (87.50)	11.50
LSD (P=0.05)					0.85	1.55	4.73	0.43

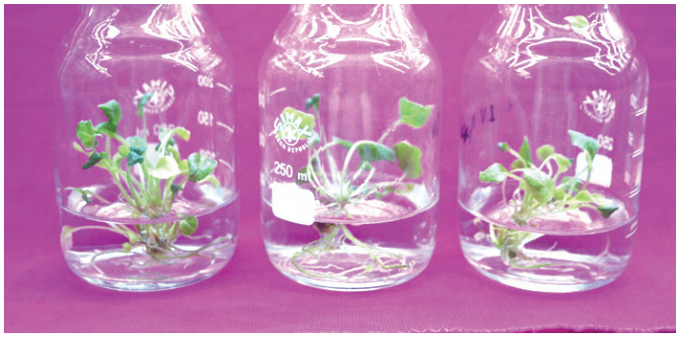

Plate 1. *In vitro* plantlets of *cv.* Jallisse cultured in 1/4th MS liquid medium during pre-hardening

Plate 2 *In vitro* plantlets of *cv.* Red Star cultured in 1/4th MS liquid medium during pre-hardening

Plate 3. Plantlet in plastic container immediately after transfer to soil media covered with beaker to maintain relative humidity

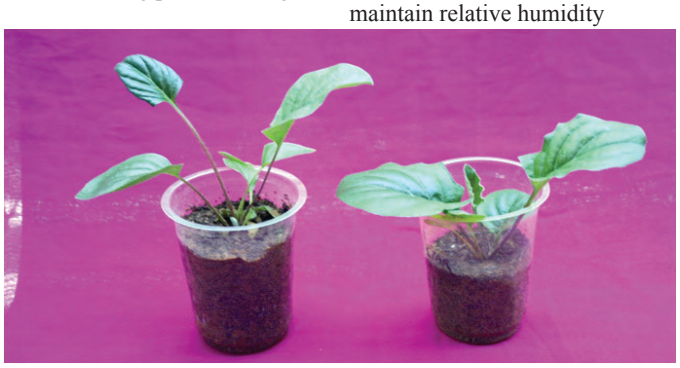

Plate 4. *In vitro* regenerated plantlets of *cv.* Jallisse during hardening

be gradually converted into autotrophs. The pre-hardening and hardening are the processes for making the *in vitro* raised plantlets into autotrophs and adapt them to the outside environment (Beura, 1998). The rooted plantlets were cultured on 1/4th MS liquid medium for pre hardening (2 days) in the culture room. Then, the plantlets were transferred to the soil mixture consisting of Soil: Sand: FYM: Coco peat in different proportions for further environmental acclimatization. Among all the combinations Soil: Sand: FYM: Coco peat in 1/2:1:1:2, ½:1:2:1 and ½:2:1:1 proportion were the most promising combinations for hardening the *in vitro* raised plantlets of *cv.* Jallisse (Table 2, Plate 4). In these combinations, percentage of survival was 100 %.

Early establishment with maximum plant height was observed in Soil: Sand: FYM: Coco peat in ½ :1:1:2 proportion. In cultivar Red Star, early establishment with new leaf formation, plant height and higher percentage of survival (100 %) was observed in Soil: Sand: FYM: Coco peat (1/2 :1:1:2) (Table 3, Plate 5). Hence,

Plate 5. *In vitro* regenerated plantlets of *cv.* Red Star during hardening

Plate 6. *In vitro* regenerated plantlets ready to transfer to black polythene containing media mixture

Plate 7. *In vitro* regenerated plantlets of cv. Jallisse and Red Star in black polythene ready for commercial cultivation

100 % survival was achieved in both the *cv.* Jallisse and Red Star in soil: sand: FYM: coco peat in ½:1:1:2 proportion. Petru and Matous (1984) successfully transferred the plantlets into sterilized peat :perlite (1:1) substrate and then into a standard horticultural substrate. Laliberte *et al*. (1985) transferred rooted plantlets to Jiffy-7 peat pellets, in glass covered acclimatization module and later to a mixture of 1 perlite:1 sphagnum moss in greenhouse with 95 % success. Reynoird *et al*. (1993) rooted *in vitro* shoots with ½ MS containing 0.25 µM NAA and acclimatized the regenerated plants in greenhouse under a plastic tunnel in trays containing peat: perlite (1:1) medium and achieved 100 % success. Parthasarathy and Nagaraju (1999) achieved a 90-100 % success in polythene bags containing equal amount of Soil: Sand: FYM. Kaur *et al*. (1999) obtained 100 % survival rate of *in vitro* shoots when transferred to pots filled with a mixture of Soil: Sand: Compost in 1:1:1 ratio. Posada *et al*. (1999) observed

50 % survival rate on the media with buried rice hull and coke Scoria under high humidity. Olivera *et al*. (2000) studied the effect of acclimatization on growth and plant development of gerbera under greenhouse conditions with 82.4 % survival of plantlets. Kumar and Kanwar (2005) achieved 50-60 % success in *cv.* Diablo in a mixture of Sand: FYM(1:1). Ray *et al*. (2005) reported that the plantlets micro-propagated in garden soil were uniform and identical to the mother plant with respect to growth characteristics and morphology. Kumar and Kanwar (2007) used barnyard manure : sand in 1:1 proportion in 10 cm pots for hardening the gerbera plantlets. Use of soil mixture consisting of Soil: Sand: FYM Coco peat (1:1:1:1) for acclimatization of the *in vitro* raised gerbera plantlets was reported by Patnaik (2007) in *cv.* Common Red and Nayak (2008) in *cv.* Red Star and Jallisse.

Hardening and acclimatization is one of the most important aspects of *in vitro* raised plantlets of commercial crops. Clonal multiplication or micropropagation on large scale can be successful only when plants after the transfer from lab to land show high survival rate and the cost involved in this process is low. Tissue culture plants generally show some structural and physiological abnormalities. A heterotrophic mode of nutrition and poor mechanism for water loss control further renders micro propagated plants vulnerable to transplantation shocks (Kumar and Kanwar, 2007). Hence, thorough and careful acclimatization is essential to overcome all these problems. In the present investigation, transfer of individual plantlets to potting mixtures of various combinations and their acclimatization under greenhouse condition for hardening showed 100 % success in hybrid cultivars of gerbera. Hence, the research findings will be helpful in mass multiplication of the plants to meet the growing global demands of this majestic flower.

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