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In vitro regeneration of tuberose through petals and immature flower buds

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ABSTRACT

Unlike many other bulbous flower crops, where *in vitro* propagation techniques have been perfected, tuberose does not have enough background studies. A study was carried out to establish aseptic cultures and to standardize *in vitro* propagation protocol with petal segment and immature flower bud as explants. The explants pre-treated with carbendazim 0.1% + mancozeb 0.1% and 8-HQC (200 mg l^{-1}) for 2½ h and surface sterilized with $HgCl_2$ (0.1%) + NaOCl (1.0%) for 8 min., respectively were found to be the best in getting aseptic culture. The best medium for shoot multiplication was MS medium supplemented with 6.0 mg/l BAP + 0.5 mg/l NAA + 0.7 mg/l 2, 4-D + 0.5 mg/l TDZ for both petals (4.0) and immature flower bud (4.33) enhanced shoot multiplication. Irrespective of explants, maximum roots were observed on half-strength MS medium + 1.0 mg/l IBA. The *in vitro* regenerated micro-plant lets were successfully acclimatized in glass jar with polypropylene cap.

Key words: Bulb, flower bud, *in vitro* regeneration, petal, tuberose.

INTRODUCTION

Tuberose (*Polianthes tuberosa* Linn.) belonging to the family Amaryllidaceae is one of the important bulbous flower crops of tropical and subtropical regions. Among the commercially grown flowers in India, tuberose occupies a prime position owing to its popularity as a cut flower, loose flower, for perfumery as well as its potential as source of secondary metabolites.

Ornamental flowering bulbs (genotypes) are heterozygous and are primarily propagated vegetatively. Conventional propagation methods have some bottlenecks such as difficulty in producing true-to-the-type bulbs as well as spread of diseases from stock plants infected with bacteria, fungi, mycoplasma, viroids, viruses, etc. Therefore, under such a situation *in vitro* propagation technique has several advantages like rapid mass multiplication of new cultivars, maintenance of disease-free stock, year round production of plantlets and storage of plantlets in a small space. In tuberose, mild mosaic virus (TMMV) has been recognized, as a major problem based on the serological and biological distinction.

Nowadays due to increasing temperature in northern plains of India the problem of nematodes is spreading very rapidly, therefore to tackle these problems *in vitro* propagation is one of the best approach. Unlike many other bulbous flower crops such as narcissus, *lillium* and tulip for which *in vitro* propagation techniques have been perfected, tuberose does not have enough background studies for regeneration through tissue culture. Since the genetic variability is limited in tuberose, hence there is ample scope for exploitation of somaclonal variation and genetic engineering. Therefore, standardizing the protocol for *in vitro* propagation of tuberose is prerequisite for micro-propagation of elite types. For large scale exploitation in both Single and Double types we attempted regeneration of plantlets using various explants.

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MATERIALS AND METHODS

The present investigation was carried out at the Division of Floriculture and Landscaping and Central Tissue Culture Laboratory of National Research Centre on Plant Biotechnology, IARI, New Delhi, during 2007 and 2008. The healthy plants of tuberose cultivars *viz.*, Prajwal (Single type) and Vaibhav (Double type) grown at Research Farm of Division of Floriculture and Landscaping, New Delhi were selected for the experiment. The explants used in this experiment flower bud and petal segment 10-15 mm and 10-12 mm (Fig. 1a & b) respectively were excised from newly emerged flower spike. The explants were then subjected to pre-treatment, using fungicides namely, carbendazim (0.1%), mancozeb (0.1%) and bactericide 8-HQC (200 mg/l) in different combinations for 2-4h, followed by 3 times distilled water wash. The pre treated explants were taken to the laminar air-flow chamber and surface sterilised using two agents namely; mercuric chloride (0.1%) and sodium hypochlorite (1.0%), for 6-8 min., followed by 3 times distilled water wash. The

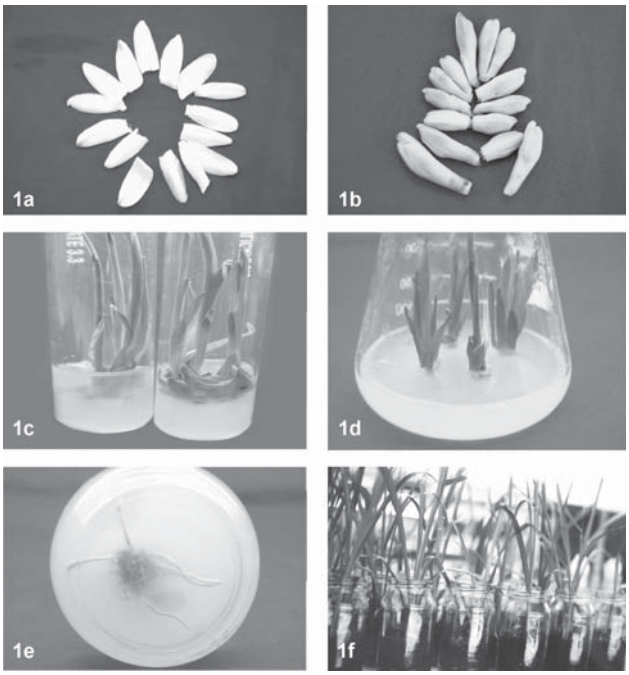

Fig. 1(a). Petal segment explant; 1(b) Immature flower bud explant; 1(c) Shoot multiplication by petal segment explant; 1(d): Shoot multiplication by immature flower bud explant; 1(e) Root initiation; 1(f) Acclimatized plants in glass jars.

decontaminated explants were cultured on medium (Murashige and Skoog, 12) supplemented with 30g of sucrose. The observations *viz*., per cent microbial contamination and per cent survival were recorded after 21 days of culture period.

The initiated micro-shoot tip from the immature flower bud and petal segments were sub-cultured on multiplication media containing BAP (2.0-6.0 mg/l) in combination with NAA (0.2-0.5 mg/l), IAA (0.2-0.5 mg/l), 2,4-D (0.5-0.7 mg/l) and TDZ (0.5 mg/l) and the observations on average shoot number and mean shoot length (cm) were recorded. The regenerated multiple shoots were separated and placed in different rooting media comprising of half-strength MS medium with different auxins *viz.,* IBA and NAA (1-2 mg l^{-1}) alone and the observations on rooting per cent, days to root initiation, root number and mean root length (cm) were recorded. The multiplication rate per explant was determined 60 days after culture. Acclimatization of *in vitro* raised tuberose plantlets was done by washing the roots with sterile distilled water to dislodge the adhering agar and then rooted plantlets were transferred to sterilized potting mixture comprising of peat + soilrite (1:1 v/v) in glass jars with polypropylene cap and in plastic pots with polythene cover. The plants were moistened regularly and after 30 days they were transferred to glasshouse.

RESULTS AND DISCUSSION

For the present investigation, explants were excised from freshly emerged flower spike of the field grown plant. Usually, the explants collected from field grown plants harbour microbes. Results obtained in the present study reveals that per-treatment of explants with 0.1 per cent each of carbendazim, mancozeb and (200 mg/l) of 8-HQC for 2h gave the maximum culture survival, 90.83 and 87.5 % in case of immature flower bud and petal segment, respectively in both the cultivars. Surface sterilents, their levels and duration of exposure is known to influence the *in vitro* culture establishment. Further, combinations of sterilents are also known to influence the culture establishment. Results obtained in the present study advocate that exposure of explants to $HgCl_2$ (0.1%) and NaOCl (1.0%) for 8 min. was best for surface sterilization, which gave maximum culture survival, *i.e.* 92.50 and 90.83% with petal and immature flower bud explants, respectively in both the cultivars.

Multiple shoot formation is the most crucial phase in large scale multiplication of plants. In the present study, the medium supplemented with 6.0 mg/l BAP + 0.5 mg/l NAA + 0.7 mg/l 2,4-D + 0.5 mg/l TDZ was found to be most suitable (Fig. 1c & d) in induction of callus and good quality shoots with better multiplication rate. This treatment gave higher average number of shoots (4.33 and 4.00) in immature flower bud and petal segment explants of cv. Prajwal. The same treatment gave higher average number of shoots (3.33 and 3.67) in petal segment and immature flower bud explants of cv. Vaibhav (Tables 1 & 2). The concentrations of BAP lower than 6.0 mg/l were not found effective for inducing higher rate of proliferation.

Use of auxin for root induction has been reported in several bulbous ornamentals. Proper root initiation may help the future performance of *in vitro* raised plantlets. In this study, among different treatments tried, half-strength MS medium supplemented with 1 mg/l IBA was observed to be the best treatment with highest root number (Fig. 1e), in Prajwal and Vaibhav with respect to immature flower bud and petal segment explants. The success of a tissue culture technique primarily depend on the *ex vitro* survival of the *in vitro* rooted micro-shoots followed by their adaptation to the field conditions. Hence, two different strategies were studied and it was evident that glass jars filled with peat: soilrite (1:1) and covered with polypropylene cap was the most effective in both the cultivars. The other strategies in which plastic pots covered with polyethylene also yielded satisfactory survival.

It is well established fact that bactericide along with a fungicide have great efficiency in controlling systemic infections. Shield *et al.* (14) suggested the combined use of different chemicals in controlling microbial

Table 1. Effect of growth regulators on *in vitro* multiplication and shoot quality of tuberose on petal segment.

Treatment	Prajwal	Vaibhav	Mean	Prajwal	Vaibhav	Mean
	Average shoot number	Average shoot number		Average shoot length (cm)	Average shoot length (cm)	
$MSMp_1$	0.00	0.00	0.00	0.00	0.00	0.00
$MSMp_2$	2.33	1.67	2.00	0.68	0.59	0.64
$MSMp_3$	4.00	3.67	3.83	4.00	3.70	3.85
$MSMp_4$	1.67	1.33	1.50	1.30	1.20	1.25
$MSMp_5$	2.67	2.33	2.50	2.87	2.40	2.63
$MSMp_6$	1.00	1.00	1.00	1.13	0.63	0.88
$MSMp_7$	1.33	1.00	1.17	1.27	1.20	1.23
Mean	1.86	1.57	1.72	1.61	1.39	1.50
CD at 5%	Cultivar	0.30		Cultivar	0.17	
	Treatment	0.57		Treatment	0.32	
	Treatment × Cultivar	0.81		Treatment × Cultivar	0.46	

$MSMp_1$= (MS + No hormone), $MSMp_2$ = 4.0 mg/l BAP + 0.2 mg/l NAA + 0.5 mg/l 2, 4-D + 0.5 mg/l TDZ, $MSMp_3$ = 6.0 mg/l BAP + 0.5 mg/l NAA + 0.7 mg/l 2, 4-D + 0.5 mg/l TDZ, $MSMp_4$ = 4.0 mg/l BAP + 0.2 mg/l IAA + 0.5 mg/l TDZ, $MSMp_5$= 6.0 mg/l BAP + 0.5 mg/l IAA + 0.5 mg/l TDZ, $MSMp_6$ = 2.0 mg/l BAP + 0.2 mg/l IAA+ 0.5 mg/l TDZ, $MSMp_7$= 2.0 mg/l BAP + 0.5 mg/l IAA + 0.5 mg/l TDZ.

Table 2. Effect of growth regulators on *in vitro* multiplication and shoot quality of tuberose on immature flower bud.

Treatment	Prajwal	Vaibhav	Mean	Prajwal	Vaibhav	Mean
	Average shoot number	Average shoot number		Average shoot length (cm)	Average shoot length (cm)	
$MSMf_1$	0.00	0.00	0.00	0.00	0.00	0.00
$MSMf_2$	2.00	1.33	1.67	0.57	0.53	0.55
$MSMf_3$	4.33	3.33	3.83	3.67	3.53	3.60
$MSMf_4$	1.67	1.33	1.50	1.30	1.23	1.27
$MSMf_5$	2.33	1.67	2.00	3.07	2.93	3.00
$MSMf_6$	1.00	1.00	1.00	1.23	0.77	1.00
$MSMf_7$	1.50	1.00	1.25	1.33	0.93	1.13
Mean	1.88	1.38	1.63	1.60	1.42	1.51
CD at 5%	Cultivar	0.37		Cultivar	0.20	
	Treatment	0.70		Treatment	0.38	
	Treatment × Cultivar	0.99		Treatment × Cultivar	0.54	

$MSMf_1$= MS + No hormone (control), $MSMf_2$ = 4.0 mg/l BAP + 0.2 mg/l NAA + 0.5 mg/l 2, 4-D + 0.5 mg/l TDZ, $MSMf_3$ = 6.0 mg/l BAP + 0.5 mg/l NAA + 0.7 mg/l 2, 4-D + 0.5 mg/l TDZ, $MSMf_4$ = 4.0 mg/l BAP + 0.2 mg/l IAA + 0.5 mg/l TDZ, $MSMf_5$ = 6.0 mg/l BAP + 0.5 mg/l IAA + 0.5 mg/l TDZ, $MSMf_6$ = 2.0 mg/l BAP + 0.2 mg/l IAA+ 0.5 mg/l TDZ, $MSMf_7$ = 2.0 mg/l BAP + 0.5 mg/l IAA + 0.5 mg/l TDZ.

infections in tobacco cultures. Furthermore, it has been proposed that these compounds should be used at non-phytotoxic levels to obtain the desired results. The conventional disinfection method (Bapat and Narayanaswamy, 1) had to be adopted to reduce the level of contamination present on the tissue. The phytotoxicity of sterility depending upon the nature of tissue, age and type of plant parts. Mercuric chloride has been one of the most used sterilent (George, 6), while sodium hypochloride is also efficient for different plant species. Uses of mercuric chloride and sodium hypochlorite have been reported by several other workers for effective surface sterilization of explants. However, duration varied with cultivars (Bora and Paswan, 2; Dilta *et al.,* 4). In the present study, combined application of sterilents has been proved to

be more effective. Earlier, efficiency of dual sterilent has been suggested by Gupta and Durzon (7), and Hennerlg *et al*. (8).

Regulation of both organ differentiation and growth in tissue culture plants by interplay of cytokinins and auxins has reported by several workers beginning with Miller and Skoog (11). Multiple shoot formation is the crucial phase in large scale multiplication of plants. It is the efficiency of this phase that determines the ultimate yield of fully differentiated plantlets. In our study growth regulator 2,4-D has found good in callus induction and higher level of cytokinin in combination with lower level of different auxins were found most suitable for multiple shoot induction. This work is supported by Kanwar and Kumar (10) who found MS medium supplemented with 2,4-D to be better for callus induction in leaf and petal explants of gerbera. They also suggested that 4 mg/l of BAP along with lower levels of auxins which has found in close relation with our study. To support present findings Bose *et al.* (3) suggested that good plantlets production in tuberose was achieved in adventitious buds from callus when cultured on MS medium supplemented with BA in combination with different auxins at lower level. Surinder and Jitender (15) stated that the maximum callus induction and growth in petal explant was observed on MS medium supplemented with lower level of 2,4-D and 3 mg/l of BAP along with 0.5 mg/l IAA and NAA in the medium had shown good regeneration and proliferation capacity.

In the present investigation, root induction occurred in the medium supplemented with different growth regulators with half-strength MS medium. However, the best medium for rooting was half-strength MS + 1 mg/l IBA. A similar observation of maximum root length in medium with IBA has been reported (Hussey *et al*., 9; Gabryszewaska and Hempel, 5) in *Alstromeria*. The results of the present study are supported by Patil *et al.* (13) where they proved that half-strength MS medium was best for rooting of gladiolus cv. American Beauty, which was also influenced by phyto-hormone. In the present study the two cultivars differed in their *in vitro* response. Single type cultivar Prajwal exhibited higher regeneration and better multiplication than in Double type cultivar Vaibhav.

REFERENCES

1. Bapat,V.A. and Narayanaswamy, S. 1976. Growth amd organogenesis in explanted tissue of *Amaryllis* in culture. *Bull. Torrey Bot. Club*, **103**: 53-56.

2. Bora, S. and Paswan, L. 2003. *In vitro* propagation of *Heliconia psittacoram* through axillary bud. *J. Orn. Hort.* **6**: 11-15.

3. Bose, T.K., Jana, B.K. and Moulik J., 1987. A note on the micropropagation of tuberose from stem scale section. *Indian J. Hort*. **44**: 100-1.

4. Dilta, B.S., Sehgal, O.P., Sharma, B.P. and Sharma, D.R. 2000. *In vitro* multiplication of *Lilium* hybrids. **In:** *Book of Abstracts of National Seminar on Hi-tech Horticulture*, held at Bangalore, pp. 35-35.

5. Gabryszewaska, E. and Hempel, M. 1985. The influence of cytokinin and auxins on *Alstromeria* in tissue culture. *Acta Hort.* **167**: 295-300.

6. George, E.F. 1993. *Plant Propagation by Tissue Culture Part-1*. *The Technology.* Exegetic Limited. Edington, England, 574 p.

7. Gupta, P.K. and Durzon, D.J. 1985. Shoot multiplication from nature tree of Douglas fir (*Pseudotsugo menziesii*) and sugar pine (*Pinas lambertiana*). *Pl. Cell Rep.* **4**: 177-79.

8. Hennerlg, M.J., Upton, M.E., Furlong, P.A., Harris, D.P., James D.J. and Eaton, R.H. 1988. Microbial contamination of *in vitro* cultures of apple rootstocks M 26 and M 29. *Acta Hort.* **225**:129-37.

9. Hussey, G., Milton J. and Lumsden, P.C. 1979. *In vitro* propagation of *Alstroemeria*. *John Innes Institute Seventeenth Annual Report,* 56 p.

10. Kanwar, J.K. and Kumar, S. 2008. *In vitro* propagation of *gerbera* – A review. *Hort. Sci.* **35**: 35-44.

11. Miller, C.O. And Skoog, F. 1957. Chemical regulation of growth and organ formation in plant tissue culture *in vitro. Expt Biol.* **11**: 118-30

12. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. *Physiol. Plant.* **15**: 473-97.

13. Patil, M.S., Dinakara, Adiga, J., Reddy, B.S., Kulkarni, B.S., Hegde, L. and Mulge, R. 2004. *In vitro* conservation of gladiolus (*Gladiolus hybridus Hort*.) cv. American Beauty. *J. Orn. Hort.* **7**: 292-99.

14. Shields, R., Robinson, S.J. and Angslow, P.A. 1984. Use of fungicides in a plant tissue culture. *P. Cell Rep.* **3**: 33-36.

15. Surinder, K. and Jitender, K.K. 2006. Regeneration ability of petiole, leaf and petal explants in gerbera cut flower cultures *in vitro. Folia Hort.* **18**: 57-64.

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