International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 236-241

Received: 01-11-2018
Accepted: 05-12-2018

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Standardization of protocol for sterilization and *in vitro* regeneration in tuberose (*Polianthes tuberosa* L.)

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Abstract
Investigations on micro-propagation of four tuberose varieties *viz.,* Arka Nirantara, Shringar, Calcutta Double and Vaibhav were carried out at the Tissue Culture laboratory of the Horticultural College and Research Institute of TNAU, Coimbatore during 2014-2016. Two types of explants namely axillary bud and bulblets were used. Of these, axillary buds followed by bulblets were found to be the most suitable explants with respect to response for *in vitro* regeneration and multiplication. The best treatment for sterilization was treating the explants with 0.5% Bavistin solution along with teepol for two hours and 5% NaOCl for 10 minutes and for shoot regeneration and elongation are full strength MS medium fortified with BAP (2 mg/l) + NAA (0.5 mg/l). Efficient rooting was achieved with ½ MS medium fortified with 1 mg/l of IBA + 1 mg/l of NAA for all the four varieties. Rooted plantlets were hardened with pot mixture (1:1:1) which resulted in the highest rate of *ex vitro* survival. Experiments taken up to standardize methods to rescue microbial contaminated cultures indicated that slicing off the infected portion and treating with Bavistin was found to be the best for controlling fungal contamination, whereas, adding the antibiotic streptomycin in the medium was the best, for controlling bacterial contamination.

Keywords: tuberose, sterilization, regeneration, multiplication, shoot elongation, rescue

Introduction
Tuberose (*Polianthes tuberosa* L.), a member of family Amaryl lidaceae, is one of the most common and delightful plants in the garden. It occupies a prime position among the commercially grown flower crops in India, owing to its popularity as a loose flower, cut flower and source of a highly preferred enticing fragrance for perfumery. It produces tall spikes, bearing clusters of pure white flowers which emit an exquisite fragrance. Both single and double flowered types are under commercial cultivation in India. Single flowered varieties with high concrete content (0.08-0.14%) are most suitable as loose flowers and source of raw material for perfumery industry, while the double flowered varieties with lesser concrete are preferred in the cut flower trade in flower arrangements due to the density of flowers on its spike. Variegated types with golden yellow leaf margin are suitable for beautification of gardens. There are four types of tuberose flowers viz., Single, Double, Semi double and Variegated. Single is the popular variety among farmers. It excels other cultivars in fragrance and yield per unit area. The performance of any crop or variety largely depends upon its genetic makeup. Further, the performance of these crops depends upon climatic conditions of the region under which they are grown. As a result, cultivars, which perform better in one region, may not perform well in other regions of varying climatic conditions. Hence, it is very much important to collect and evaluate all the available genotypes in order to select suitable and high yielding genotypes.

Tuberose is conventionally propagated by means of bulbs which are produced by the mother plant. However, this method is very slow with lower multiplication rates, since as per the 'Fibonacci rules' (Nasreen Zaidi *et al.* 1994), one bulb can proliferate only 1.6 bulbs per annum (approximate), i.e. 600 bulbs grows into 1000 bulbs in one year. To meet the growing demand, of quality planting material of tuberose, massive *in vitro* propagation is the only panacea. Further, propagation by bulbs in soil media is the chief cause of spreading nematode infestation. The conventional method of propagation through bulbs is rather slow to meet the growing demand.

Materials and Methods

Plant materials

Four commercially important cultivars namely Arka Nirantara, Shringar, Calcutta Double and Vaibhav were involved in standardization of protocol for *in vitro* regeneration. The bulbs are collected from the field in college botanical garden TNAU, coimbatore. The explants used for *in vitro* regeneration are axillary buds and bulblets.

The explants were washed with teepol to remove the soil debris and pretreated with 0.5% bavistin and 1% streptomycin for 2 hours on rotatory shaker and thoroughly washed with distilled water. The explants were then surface sterilized by using 70% ethanol for 30 sec. followed by 0.2% $HgCl_2$ for 10min, 5% NaOCl (10 min) and 0.5% AgNO3 (15 min). The explants were then washed with sterile distilled water to remove trace of surface sterilants.

In vitro shoot regeneration and multiplication

For *in vitro* shoot multiplication, the explants were cultured on MS medium alone as control and MS medium with different concentrations of cytokinins and auxins (BAP, NAA). The effects of different plant growth regulators were tested on multiple shoot induction, shoot elongation, and were recorded periodically. The axillary bud and bulblet cultures were incubated in a culture room with physical parameters maintained as given below:

- Temperature : 25 ± 2 ^oC
- Humidit: 70 to 80%
- Light source: White fluorescent lamps
- Light intensity: 2500 to 3000 Lux
- Photoperiod: 16/8 hr light/dark

In vitro root induction

When the micro shoots had attained sufficient growth, (i.e. 5 leaves with 10cm height) the explants were trimmed to 6 cm and then they were transferred to rooting media which was half strength MS (1/2 MS) medium supplemented with auxins (IBA, NAA). The cultures were examined every week and were recorded on the basis of visual observations.

Hardening of plants

For hardening, at first the tissue culture derived healthy rooted plantlets were removed from the agar medium, washed thoroughly under running tap water and transferred to earthen pots containing sterilized media composition. To preserve moisture, the potted plantlets were covered with transparent polythene bag and placed in the green house. After one month, plantlets were transferred to the main field.

Statistical analysis

The experiment design was Completely Randomized block Design and Factorial (FCRD) as per the standard procedures of Gomez and Gomez (1984). Observations recorded as percentage were subjected to angular transformation. Analysis was carried out with AGRES software package and MS Excel spreadsheet. The means of different experiments were compared using DMRT (Duncan's Multiple Range Test) at the 5% level.

Results and Discussion

Disinfection of explants

Of the various sterilants tried (S_4) combination of 0.5% Bavistin along with teepol for two hours followed by 5% NaOCl for 10 min, 70% ethanol wash for 30 seconds and then with 0.1% $HgCl_2$ for 10 minutes resulted in the maximum survival percentage (Table 1). The use of NaOCl and $HgCl_2$ in combination as effective sterilants for disinfection of axillary buds and bulblets explants of tuberose has been strongly justified earlier by Ali *et al.* (2014) [4].

Table 1: Effect of surface sterilization on explants of tuberose

Surface sterilization (%)			
Treatments	**Contamination**	**Survival**	**Mortality**
S_1: 0.5% bavistin (2 hours) + streptomycin 1g/l (2 hours) + teepol (2 hours) + 70% ethanol (1 min) + 0.1% $HgCl_2$ (10 min)	44.44 (41.80)	55.55 (48.20)	0.00 (0.827)
S_2: 0.5% bavistin (2 hours) + 8HQC (0.33g/l) (2 hours) + teepol (2 hours) + 70% ethanol (1 min) + 0.1% $HgCl_2$ (10 min)	33.33 (35.15)	66.66 (54.84)	0.00 (0.827)
S_3: 0.5% bavistin (2 hours) + teepol (2 hours) + 5% NaOCl (10 min) + 70% ethanol (1 min) + 0.1% $HgCl_2$ (10 min)	19.40 (26.03)	80.55 (63.94)	0.00 (0.827)
S_4: 0.5% bavistin (2 hours) + teepol (2 hours) + 0.5% $AgNo_3$ (15 min) + 70% ethanol (1 min) + 0.1% $HgCl_2$ (10 min)	58.33 (51.44)	38.88 (38.55)	0.00 (0.827)
Mean	38.88 (38.61)	60.41 (51.38)	0.00 (0.827)

Note 1: values in the parenthesis are arcsine transformed value

Reported that combination of 0.3% Bavistin along with 0.4% 8-HQC for three hours followed by 70% ethanol wash for 30 seconds and then with 0.25% NaOCl and 0.5% $HgCl_2$ for 10 minutes and 2 minutes respectively resulted in the maximum survival percentage. Bisen and Tiwari (2006) [14] also demonstrated the use of combinations of $HgCl_2$ and NaOCl as effective sterilants in guava axillary buds. The effective of use of 8-HQC as a bactericide is well justified by Wouter and Perik (1990) in cut roses.

The suitability of $HgCl_2$ as an ideal surface sterilant for disinfection of underground plant parts has been reported by various workers such as Hussain (1975) in gladiolus, Bhaskar (1991) [12] in banana and Babu *et al.* (2004) [7] in ginger.

Effect of different concentrations of (BAP and NAA) on Shoot regeneration and proliferation

Among the growth regulator treatments, M_1 [(MS medium + BAP (2 mg l^{-1}) + NAA (0.5 mg l^{-1})**]** recorded the lowest number of 4.92 days taken for bud emergence. It was observed that bulblets proved to be superior to axillary buds by registering the least number of 5.20 days taken for leaf emergence. Among the four cultivars, Arka Nirantara proved to be superior by registering less number of 5.87 days taken for bud emergence (Table 2). Regeneration of multiple shoots from diverse explant cultures of tuberose has been reported earlier in respect of response to cytokinins viz., Kinetin (Bose *et al*. 1987) [16] and TDZ (Hutchinson *et al*., 2004) [33]. In the present study, BAP 2 mg/l in combination with NAA 0.5 mg/l has reduced the time required for greening and days for

axillary bud emergence while simultaneously increasing the number of shoots obtained in four varieties namely, Arka Nirantara, Shringar, Calcutta Double and Vaibhav, as compared to the other treatments tried. When the concentration of BAP increased in the media for axillary bud culture, its role seemed to be of least importance as evident from the data. The axillary buds became dwarf without any growth.

The above results are in confirmation with the observations made by Surendarnath (2012), where in BAP 4 mg/l, in combination with NAA 0.5 mg/l has reduced the time required for greening and days for axillary bud emergence while simultaneously increasing the number of shoots obtained in both the cultivars Prajwal and Suvasini.

Effect of different concentration of BAP and NAA on shoot multiplication

Different concentration of BAP and NAA showed significant difference on shoot multiplication present in table 3. The height number shoots per explant (5.73) observed in M_1 [MS medium + BAP (2 mg l^{-1}) + NAA (0.5 mg l^{-1})].

Reported that the number of shoots produced in tuberose increased with increase in concentration of BAP. However, in the present study, a different trend was observed wherein the number of shoots produced was the highest with 2 mg/l of BAP and a further increase in the level of BAP to 5 mg/l resulted in decline in the number of shoots regenerated, indicating certain kind of antagonistic effect of higher levels of BAP (>2 mg/l) for both the types of explants used. These results are in conformity with the findings of Gajbhiye *et al.* (2011) [28] in tuberose.

Table 2: Effect of growth regulators on days taken for bud emergence (E1: axillary bud) and leaf emergence (E2: bulblets)

Days taken for bud emergence													Grand mean
Treatment	Arka Nirantara			Shringar			Calcutta Double			Vaibhav			
	E1	E2	Mean	E1	E2	Mean	E1	E2	Mean	E1	E2	Mean	
M_1: BAP 2mg/l + 0.5mg/l NAA	3.46	4.78	4.12	5.63	4.34	4.99	7.14	4.49	5.82	5.1	4.4	4.75	4.92
M_2: BAP 3mg/l + 0.5mg/l NAA	4.23	4.97	4.60	6.12	5.04	5.58	7.23	4.51	5.87	5.1	4.51	4.81	5.22
M_3: BAP 4 mg/l +0.5mg/l NAA	6.57	5.18	5.88	7.89	6.34	7.12	8.48	5.72	7.10	7.1	5.85	6.48	6.64
M_4: BAP 4mg/l	7.43	5.29	6.36	8.55	5.29	6.92	9.27	5.75	7.51	6.9	5.9	6.40	6.80
M_5: control (basal MS medium)	9.53	5.77	7.65	11.24	6.03	8.64	11.58	6.08	8.83	8.8	6.14	7.47	8.14
Mean	6.24	5.20	5.72	7.89	5.41	6.65	8.74	5.31	7.03	6.60	5.36	5.98	6.34

Table 3: Effect of growth regulators on number of multiple shoots per explant (axillary bud & bulblet)

No. of multiple shoots per explants													Grand mean
Treatments	Arka Nirantara			Shringar			Calcutta Double			Vaibhav			
	E_1	E_2	Mean	E_1	E_2	Mean	E_1	E_2	Mean	E_1	E_2	Mean	
M_1: BAP 2mg/l + 0.5mg/l NAA	7.36	5.82	6.59	6.42	5.13	5.78	5.49	4.56	5.03	6.04	5.00	5.52	5.73
M_2: BAP 3mg/l + 0.5mg/l NAA	6.12	4.92	5.52	5.17	4.40	4.79	4.72	3.15	3.94	5.09	3.68	4.39	4.66
M_3: BAP 4 mg/l +0.5mg/l NAA	4.69	2.79	3.74	3.59	2.08	2.84	2.68	1.79	2.24	3.28	2.07	2.67	2.87
M_4: BAP 4mg/l	5.66	3.93	4.80	4.82	3.26	4.04	3.58	2.38	2.98	4.65	3.27	3.96	3.95
M_5: control (basal MS medium)	2.85	1.40	2.13	2.46	1.31	1.88	1.19	1.11	1.15	1.29	1.14	1.22	1.60
Mean	5.34	3.77	4.56	4.49	3.24	3.87	3.53	2.60	3.07	4.07	3.03	3.55	3.76

Table 4: Shoot length in different growth regulators (cm) in tuberose

Shoot elongation in length (cm)													Mean elongation
Treatments	Arka Nirantara			Shringar			Calcutta Double			Vaibhav			
	E_1	E_2	Mean	E_1	E_2	Mean	E_1	E_2	Mean	E_1	E_2	Mean	
T_1: BAP 2mg/l + 0.5mg/l NAA	10.33	11.23	10.78	9.80	10.77	10.28	8.90	8.80	8.85	9.97	10.77	10.37	10.07
T_2: BAP 3mg/l + 0.5mg/l NAA	9.03	10.50	9.77	8.17	8.37	8.27	7.30	7.83	7.57	8.70	9.50	9.10	8.68
T_3: BAP 4 mg/l + 0.5mg/l NAA	7.73	8.60	8.17	7.20	7.53	7.37	6.30	6.47	6.38	7.27	8.27	7.77	7.42
T_4: BAP 4mg/l	1.43	2.70	2.07	1.30	2.50	1.90	1.10	2.33	1.72	1.17	3.67	2.42	2.03
T_5: control (basal MS medium)	3.30	5.77	4.53	3.57	5.37	4.47	2.93	3.80	3.37	3.50	5.10	4.30	4.17
Mean	6.37	7.76	7.06	6.01	6.91	6.46	5.31	5.85	5.58	6.12	7.46	6.79	6.47

Table 5: Effect of growth regulators on days taken for rooting

Days taken for rooting in tuberose													Mean days for rooting
Treatments	Arka Nirantara			Shringar			Calcutta Double			Vaibhav			
	E_1	E_2	Mean	E_1	E_2	Mean	E_1	E_2	Mean	E_1	E_2	Mean	
R_1: IBA 1mg/l	14.67	16.33	15.50	15.67	17.33	16.50	20.00	21.00	20.50	16.67	18.33	17.50	17.50
R_2: IBA1.5 mg/l	16.67	18.33	17.50	17.67	19.33	18.50	23.33	23.67	23.50	18.67	20.33	19.50	19.75
R_3: IBA 2mg/l	18.33	19.67	19.00	19.33	20.00	19.67	25.67	26.00	25.83	20.33	20.67	20.50	21.25
R_4: IBA 2.5mg/l	20.33	23.00	21.67	21.33	24.00	22.67	30.00	28.67	29.33	22.67	26.00	24.33	24.50
R_5: IBA 1mg/l+NAA1 mg/l	10.00	12.67	11.33	12.33	13.67	13.00	15.33	15.33	15.33	13.33	14.67	14.00	13.42
R_6: IBA 1.5mg/l+NAA1 mg/l	29.67	33.00	31.33	30.67	34.00	32.33	34.00	31.67	32.83	31.67	35.00	33.33	32.46
R_7: IBA 2mg/l+NAA1 mg/l	33.67	36.33	35.00	34.67	37.00	35.83	37.00	37.00	37.00	24.33	38.33	31.33	34.79
R_8: IBA 2.5mg/l+NAA1 mg/l	38.00	37.00	37.50	39.00	38.00	38.50	42.33	42.67	42.50	40.00	39.00	39.50	39.50
R_9: Basal MS (control)	24.67	28.33	26.50	25.67	29.33	27.50	24.00	31.33	27.67	26.67	30.33	28.50	27.54
Mean	22.89	24.96	23.93	24.04	25.85	24.94	27.96	28.59	28.28	23.81	26.96	25.39	25.64

Table 6: Effect of growth regulators on no. of roots per explants

No. of roots per explant in tuberose													Mean no. of roots/plant
Treatments	**Arka Nirantara**			**Shringar**			**Calcutta Double**			**Vaibhav**			
	E_1	**E_2**	**Mean**	**E_1**	**E_2**	**Mean**	**E_1**	**E_2**	**Mean**	**E_1**	**E_2**	**Mean**	
R_1: IBA 1mg/l	19.00	18.00	18.50	17.00	16.00	16.50	14.00	13.33	13.67	16.67	14.00	15.33	16.00
R_2: IBA1.5 mg/l	14.67	13.67	14.17	12.67	11.67	12.17	11.33	10.00	10.67	11.67	10.67	11.17	12.05
R_3: IBA 2mg/l	12.67	11.67	12.17	10.67	10.33	10.50	10.00	8.33	9.17	9.67	8.33	9.00	10.21
R_4: IBA 2.5mg/l	10.33	9.33	9.83	8.33	7.33	7.83	5.33	5.33	5.33	7.33	6.00	6.67	7.42
R_5: IBA 1mg/l+NAA1 mg/l	29.00	28.00	28.50	25.33	23.00	24.17	18.00	17.67	17.83	22.33	22.33	22.33	23.21
R_6: IBA 1.5mg/l+NAA1 mg/l	5.33	4.33	4.83	4.67	3.33	4.00	2.33	4.33	3.33	3.67	2.00	2.83	3.75
R_7: IBA 2mg/l+NAA1 mg/l	4.33	3.33	3.83	3.33	2.00	2.67	0.00	3.00	1.50	2.33	0.00	1.17	2.29
R_8: IBA 2.5mg/l+NAA1 mg/l	2.67	2.00	2.33	1.67	1.00	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.92
R_9: Basal MS (control)	8.00	7.00	7.50	6.67	5.67	6.17	3.67	6.00	4.83	5.67	4.33	5.00	5.88
Mean	11.78	10.81	11.30	10.04	8.93	9.48	7.19	7.56	7.37	8.81	7.52	8.17	9.08

Table 7: Effect of growth regulators on per cent of *ex vitro* survival in tuberose

Per cent of ex vitro survival in tuberose													Grand mean
Treatments	**Arka Nirantara**			**Shringar**			**Calcutta Double**			**Vaibhav**			
	E_1	**E_2**	**Mean**	**E_1**	**E_2**	**Mean**	**E_1**	**E_2**	**Mean**	**E_1**	**E_2**	**Mean**	
H_1	99.33 (86.09)	98.67 (83.47)	99 (84.78)	97.6 (81.26)	96.67 (77.54)	97.17 (79.40)	94.67 (76.66)	95.33 (77.54)	95 (77.10)	98.33 (82.67)	96.67 (79.51)	97.50 (81.09)	97.17 (80.59)
H_2	94.67 (76.66)	95 (77.12)	94.83 (76.89)	93 (74.69)	90.67 (71.92)	91.83 (73.30)	91 (72.56)	90.33 (71.92)	90.67 (72.24)	94.33 (76.32)	93.33 (75.05)	93.83 (75.68)	92.79 (74.53)
H_3	72.67 (58.48)	72.33 (58.27)	72.50 (58.37)	72 (58.06)	69 (56.17)	70.50 (57.11)	68 (55.55)	69 (56.17)	68.50 (55.86)	71.67 (57.85)	71.00 (57.42)	71.33 (57.63)	70.71 (57.25)
H_4	93.33 (75.29)	89.67 (71.26)	91.50 (73.27)	93.67 (75.49)	89 (69.74)	91.33 (72.62)	88 (69.74)	88 (69.740	88 (69.74)	89 (70.64)	88.67 (70.33)	88.83 (70.49)	89.92 (71.53)
H_5	79 (62.73)	79.67 (63.20)	79.33 (62.97)	78 (62.04)	79 (61.12)	78.50 (61.58)	77 (61.35)	76.67 (61.12)	79.67 (61.23)	79.67 (63.20)	78 (62.03)	78.83 (62.62)	78.38 (62.10)
Mean	87.80 (71.85)	87.07 (70.66)	87.43 (71.26)	89.08 (70.31)	84.87 (67.30)	86.98 (68.80)	83.73 (67.17)	83.8 (67.30)	83.80 (67.24)	86.60 (70.14)	85.33 (68.87)	86.07 (69.50)	86.07 (69.20)

H1: Pot mix (1:1:1 of cocopeat: sand: vermicompost); H2: Composted coir pith + pot mix; H3: Vermiculite; H4: vermiculite + pot mix; H5: composted coir pith

Table 8: Effect of surface sterilents on contaminated cultures for rescue in tuberose

Surface sterilization for rescueing	
Treatments	**Rescue %**
	Mean
Q_1: 70% EtOH (30 sec) + 0.6gl^{-1} streptomycin (20 min) + 0.5% bavistin	19.33 (26.08)
Q_2: 70% EtOH (30 sec) + 0.7gl^{-1} streptomycin (20 min) + 0.5% bavistin	32.33 (34.65)
Q_3: 70% EtOH (30 sec) + 0.8gl^{-1} streptomycin (20 min) + 0.5% bavistin	41.00 (39.82)
Q_4: 70% EtOH (30 sec) + 0.9gl^{-1} streptomycin (20 min) + 0.5% bavistin	51.67 (45.96)
Q_5: 70% EtOH (30 sec) + 1gl^{-1} streptomycin (20 min) + 0.5% bavistin + adding 1gl^{-1} streptomycin in media	83.67 (66.18)
Mean	45.60 (42.54)

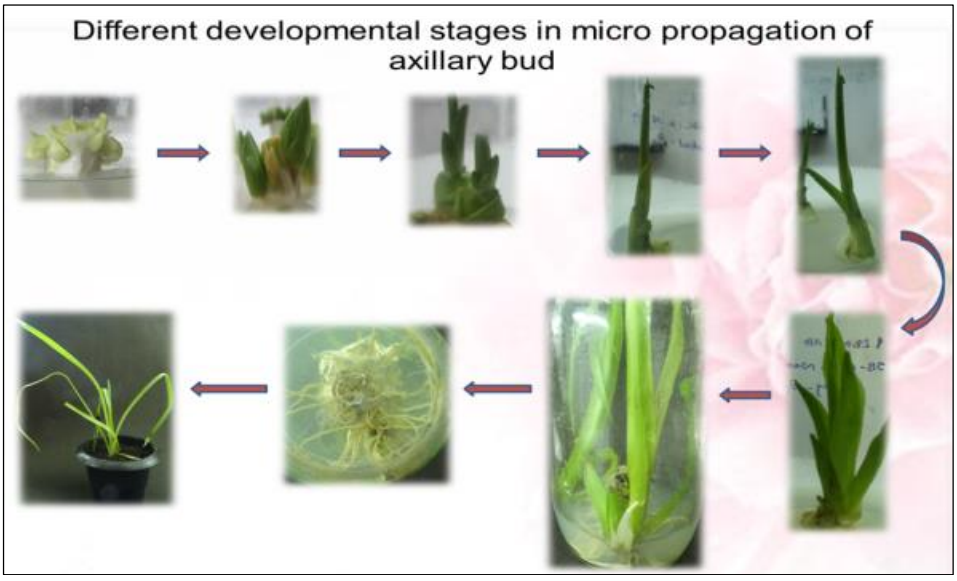

Fig: Different developmental stages in micro propagation of axillary bud

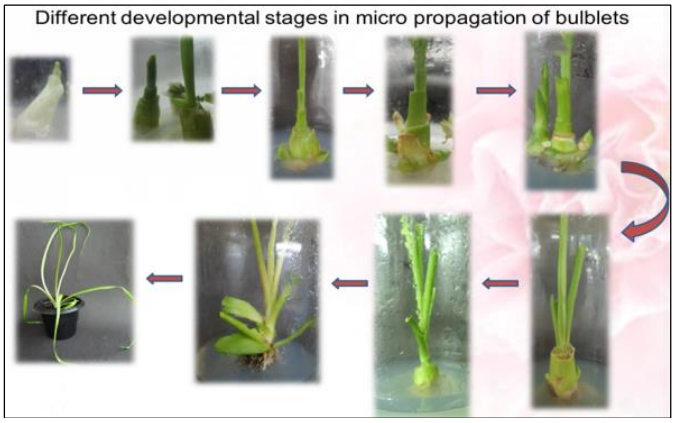

Fig: Different developmental stages in micro propagation of bulblets

Identification of viable explant

Among the two explants, axillary buds recorded more number of shoots per explant (7.36) and among the four cultivars, Arka Nirantara showed increased number of shoots per explant (4.56). Different explants of tuberose responded differentially to varying levels of cytokinins. Corroboration for such an observation comes from the similar findings of Ahmad *et al.* (2000) [2] with axillary buds of White Prosperity cultivar of Gladiolus.

Effect of different concentration of NAA and IBA on rooting

The role of auxins in inducing roots in plants both *in vitro* and *in vivo* has been demonstrated beyond doubt. Investigations in the field of plant tissue culture have shown that *in vitro* rooting could be successfully achieved by reducing salt concentration in the media, particularly in high salt media like MS and its derivatives. Half MS media has been successfully employed by many workers in crops propagated through specialized plant parts, including banana. In tuberose, half MS has been recommended by Krishnamurthy (2000), Sangavi *et al*. (2008) and Jyothi *et al*. (2008). The elimination of BAP in rooting medium as it inhibits rooting was properly adjudged by Nathan *et al*. (1992) and Hutchinson *et al*. (2004) [33] in *Heliconia* and tuberose respectively.

The *in vitro* rooting of regenerated shoot was presented in table 5. R_5 [½ MS medium + IBA (1 mg l^{-1}) + NAA (1mg/l)**]** recorded the lowest number of 13.40 days for root emergence. Among the two explants, axillary buds recorded the lowest number of 22.89 days taken for root emergence. Among the four varieties, Arka Nirantara recorded lesser number of 22.93 days for root emergence. The highest number of roots per explant (23.21) was recorded in MS media enriched with R_5 [½ MS medium + IBA (1 mg l^{-1}) + NAA (1 mg l^{-1})] table 6. Maximum root length of 12.31 cm was observed in MS media enriched with R_5 [½ MS medium + IBA (1 mg l^{-1}) + NAA (1 mg l^{-1})].

The above results are also in line with reports of Bhatt *et al*. (2015) [13] who achieved rooting in Shringar with IBA 1mg/l + NAA 1 mg/l and Rajasekaran *et al.* (2000), who achieved rooting in tuberose with IBA 2 mg/l. Jyothi *et al*. (2008) and Kadam *et al*. (2010) also achieved the best results for rooting with the least number of days with IBA 1 mg/l in tuberose. Gajbhiye *et al.* (2011) [28] from his studies have shown that in different varieties of tuberose, rooting was achieved with IBA 3 mg/l, thus contradicting the fact that a higher level of IBA leads to callusing but not rooting. Dhande *et al*. (2012) [24] reported that for root induction in bhendi, MS medium supplemented with 1.0 mgl^{-1} of IBA resulted in 99% of rooting with a root length of 5.0cm.

Planting out and acclimatization

Among the media concentrations used, H_1 pot mix [vermicompost: sand: cocopeat @ 1:1:1**]** recorded the highest survival of 97.17 per cent followed by (92.79%) H_2 and the lowest survival of 70.71 per cent was recorded in H_3 table 7.

In the present study, pot mixture (sand: cocopeat: vermicompost) was found to be the most ideal hardening medium for acclimatization of the *in vitro* regenerated plantlets of tuberose varieties Arka Nirantara, Shringar, Calcutta Double and Vaibhav. Most of the earlier workers have also compared various hardening media for acclimatization of *in vitro* derived plantlets of tuberose namely, pot mixture (Bose *et al*. 1987, Kadam *et al*. 2010 and Gajbhiye *et al.* 2011) [16, 28], pot mix + cocopeat and cocopeat (Anu G Krishnan, 2004) [6] in tuberose. The results of the present study are in line with those of Jamwal *et al.* (2013) [35] who reported that during hardening a high success rate of 73.33 per cent of plantlet survival was found in mixture of sand, soil, FYM and vermicompost at equal proportion and Gajbhiye *et al.* (2011) [28] who also suggested that pot mixture alone or in combination with cocopeat could give the highest plant survival rates and leaf production.

Krishnamurthy (2000) recorded 100% survival in the pot mixture compared to other hardening mixtures tried. Nafees Altaf *et al.* (2009) also suggested pot mixture as the best rooting media for hardening of *in vitro* derived gerbera plants. In all the cases, plantlets before hardening were dipped in 0.1% Bavistin solution for 10 minutes (Bose *et al*. 1987) [16] as done in the present study.

Response of contaminated cultures to rescue treatments

The results of the rescue to save contaminated cultures are presented. After taking the plants from the media the plants were treated with 0.5%bavistin + streptomycin 1gm/l for 20 min so that plants can be saved from contamination.

The data on response of contaminated cultures to rescuing were found to be highly significant (Table 8).

Among the growth regulator treatments Q_5 is the best having highest rescue 83.67 (66.18) per cent while least per cent 19.33 (26.08) was observed in Q_1.

Microbial contamination by fungi or bacteria is one of the most serious and recurrent problems in plant tissue culture, raising the cost of large scale micropropagation. Hence, in order to cope up with it, different approaches have been adopted in the present study. Such approaches have already been reported by earlier workers which include addition of antibiotics in the media to reduce contamination from gram-ve bacteria in aster, mixing ClO_2 in the media solution (Cardoso *et al*. 2011) [20] in gerbera, use of PPM (Plant Preservative Mixture) in the medium or treating the contaminated cultures with PPM (Guri *et al.* 1998, Compton *et al*. 2001) [30, 22]. The present study has bought out some satisfactory outcome. The treatment involving addition of antibiotic @ 1gm/l of media eradicated almost 90% of the contamination (mostly bacterial) without any morphological influences on the growth of the plant. However, similar to antibiotic treatments, curative use of medium acidification may only suppress the growth of bacteria rather eliminate them completely.

The present study led to the inference that micropropagation of tuberose both (single and double types) using axillary buds as explants could be a viable and rapid means of producing

disease free true-to-type plants. It has been observed that around 425 plants can be obtained from one mother plant in a time period of approximately seven months. This multiplication ratio is 17 times higher than that which can be obtained through conventional propagation using bulbs.

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