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DIRECT SHOOT ORGANOGENESIS FROM STEM DISC EXPLANTS OF TUBEROSE (*Polianthes tuberose* Linn.)

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ABSTRACT

Direct shoot organogenesis from stem disc explants of tuberose (*Polianthes tuberose* L.) was tried with the objectives to study the response of different culture media and cultivars for induction and multiplication of shoot and root and to identify suitable culture media composition and cultivars for micropropagation of tuberose during the year 2015-16 in FCRD at College of Agriculture, Nagpur. The stem disc explants of three tuberose varieties namely Nagpur local 1, Nagpur local 2 and Amravati local 1 were inoculated in White's basal media fortified with 28 different compositions of BAP, Kinetin, BAP with NAA and Kinetin with NAA and the shoots of 3-5 cm length were transferred for root differentiation in White's media fortified with 12 different compositions of IAA and NAA. Significant variation for varieties, media composition and variety × media interaction were observed for all the characters related to shoot and root differentiation studied. The highest frequency of shoot (53.3%), early shoot formation (11.11 days), more number of shoots per responding explants (5.00 shoots), more number of shoot elongation (2.3 shoots) and highest average shoot length (5.0 cm) was observed in S_{20} (WH + 4 mg^{-1} BAP + 0.5 mg^{-1} NAA). Nagpur local 1 in S_{20} (WH + 4 mgl^{-1} BAP + 0.5 mgl^{-1} NAA) performed better for shoot proliferating explants (60 %), days for shoot proliferation (8.50 days) and shoot elongation (2.5 shoots). Full strength White's media supplemented with 2 mgl^{-1} IAA was observed to be the best treatment for root differentiation as it resulted in highest root proliferation (58.7 %), early root formation (12.72 days) and maximum root length (5.73 cm). Maximum survival rate was recorded in Nagpur local 1 (53.84%), followed by Nagpur local 2 (40.90%). This study lead to the conclusion that Nagpur local 1 could be successfully micropropagated with White's media supplemented with 4 mgl^{-1} BAP and 0.5 mgl^{-1} NAA for shoot proliferation followed by rooting in White's media supplemented with 2 mgl^{-1} IAA.

(Key words: Direct shoot, stem disc and tuberose)

INTRODUCTION

Maharashtra is one of the leading flower producers in the country. The flower spikes are in great demand in global markets throughout the year. It is also a very good source for essential oil industry (Kharde and Kshirsagar, 2014).

Tuberose is conventionally propagated by means of bulbs which are produced by the mother plant. However, this method is very slow with low multiplication rates following 'Faibonacci rules *i.e*. one bulb can proliferate only 1.6 $annum^{-1}$ (approximate) and a single bulb grows into 1000 bulbs in 16 years. Therefore, requirement of planting material is more in this method because only one plant can be obtained from a bulb and this method is rather slow. Moreover, only single cultivar set seed and seeds are difficult to germinate. Tuberose is attacked by number of pest including nematodes. During the hot month insects such as aphids, spiders, mites and thrips affect tuberose. Fungal and bacterial disease are common under moist growing condition (Jyothi *et al*., 2008).

Production of tuberose is greatly constrained by the bulb quality, physiological decline and accumulation of pathogen. To meet the growing demand, massive *in vitro* propagation through tissue culture is the only option. Furthermore, to increase the oil content, flower colour, quality and increase in vase life of tuberose, tissue culture techniques need to be exploited. Plant tissue culture is a practice used to propagate plants under sterile condition, often to produce clones of a plant which realised on the fact that many plantlets have the ability to regenerate a whole plants.

In comparison to conventional breeding; modern plant biotechnology and genetic engineering has the potential to reduce the time needed for traditional breeding (Azaidi and Khosh, 2007) but is not possible without the presence of a robust *in vitro* protocol (Aida *et al.,* 2014). Thus, under such situation, *in vitro* propagation technique

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proves a highly potential tools with a several benefits like rapid mass multiplication of new cultivars, ease in maintenance of disease-free stock, year round production of plantlets and storage of plantlets in a small space (Bhojawani and Razdan, 2002). Unlike many other bulbous flower crops such as Marcissus, *Lilium* and *Tulip* for which *in vitro* propagation techniques have been perfected, tuberose does not have enough background for regeneration through tissue culture (Kadam *et al*., 2009). Keeping the above consideration in view, an investigation was taken up, to study the establishment of aseptic culture and standardization of *in vitro* shoot multiplication, rooting and acclimatisation protocol for three local cultivars of tuberose.

MATERIALS AND METHODS

This experiment was conducted during the year 2015-2016 in the plant tissue culture laboratory at Agricultural Botany Section, College of Agriculture, Nagpur. Stem disc explants of three tuberose varieties were collected from field and washed under running tap water for 2 hours followed by 3 to 4 times washing with distilled water and then surface sterilized with 100 ml double distilled water containing 2-3 drops of surfactant Tween 20 for 10-15 minutes to remove the fine particles. The clean discs were then treated with 90% (v/v) ethanol for 30 seconds followed by treatment of aqueous solution of mercuric chloride (0.1%) with bavistin (1%) for 10 minutes. Finally stem discs were rinsed 4-5 times with sterile double distilled water prior to inoculation. The surface sterilized and stem disc explants were placed aseptically on White's basal media fortified with 28 different combinations of growth regulator (BAP, Kinetin, BAP with NAA and Kinetin with NAA) with sucrose (2%) and Agar (1%) as mentioned in table 1. The pH of White's basal media was adjusted to 5.8 prior to autoclaving. The cultures were maintained at a temperature of 25±2^{0}C and 16 hrs. Photoperiod. Shoots of 3 to 4 cm. height were separated and transferred to root differentiation in White's media fortified with 12 different compositions of IAA and NAA as mentioned in table 2. Observations on shoot proliferating explants (%), days for shoot proliferation, number of shoots per responding explants, number of shoot elongated per responding explants, mean shoot length (cm), root proliferating shoot lets (%), days for root formation and mean root length (cm) were recorded. The experiment for all the traits was conducted in FCRD and mean value of five cultures were used in duplex for statistical analysis (Panse and Sukatme, 1954).

RESULTS AND DISCUSSION

Shoot regeneration

An attempt was made in this study to select the most responding cultivar to different growth hormones ratio and other physical factors exhibiting higher *in vitro* morphogenesis by means of media standardization for the cultivars of tuberose *viz*. Nagpur local 1, Nagpur local 2 and Amravati local 1 which have a commercial value in flower market. As far as the three varieties were concerned Nagpur local 1 showed better results as compared to Nagpur local 2 and Amravati local 1 for all the five shoots differentiation related traits, *viz.,* shoot proliferating explants (%), days for shoot proliferation, number of shoot per responding explants, number of shoots elongated per responding explants and Mean shoot length (cm). This lead to the inference that Nagpur local 1 might have possessed higher endogenous hormone concentration that may lead to better growth. In accordance to this results Panigrahi *et al*. (2013 a and b) also reported Phule Rajni to show better results as compared to Calcutta Double. It can be inferred here that *in vitro* morphogenesis efficiency is highly specific to the variety used. Different varieties showed different levels of performance related to *in vitro* morphogenesis.

The various growth aspects related to shoot differentiation of tuberose as influenced by different concentrations of BAP, Kinetin either alone or in combination with NAA under *in vitro* conditions have been studied in detail. In this study, the highest frequency of shoot produced was 53.3 %, when explants were cultured in White's media supplemented with 4 mg^{-1} BAP + 0.5 mg^{-1} NAA. Early shoot formation (11.11 days), more number of shoots per responding explants (5.00 shoots), more number of shoot elongation (2.3 shoots) and highest average shoot length (5.0 cm) was also observed in the same media ie. S_{20} (WH + 4 mg^{-1} BAP + 0.5 mg^{-1} NAA). Another treatment S_{21} (WH + 5 mg^{-1} BAP + 0.5 mg^{-1} NAA) also exhibited better performance in all the traits related to shoot differentiation ie. 50.2 % shoot proliferating explants, 11.66 days for shoot formation, 4.38 number of shoots per responding explants, 1.4 number of shoot elongation and 5.0 cm shoot length. From these observations it is evident that higher concentration of BAP alone were not found effective for inducing higher number of shoot and length of shoots. From the observations it is also evident that increased level of BAP was antagonistic for all the parameters observed during the entire experiment. This may be due to the presence of endogenous cytokinin in cultures to higher levels of cytokinin in tuberose. This antagonistic effect was over come by the addition of auxin NAA at lower concentration. Thus, BAP in combination with kinetin was found suitable for shoot induction.

In accordance with this result media supplemented with BAP in combination of NAA has been reported to promote shoot differentiation by Mishra *et al*. (2006), Jyothi *et al.* (2008) and Kadam *et al*. (2009) in tuberose. The quality of shoots and overall growth response was better in these growth regulator combinations. Surendranath *et al*. (2016) also reported the antagonistic effect of increased level of BAP for all the parameters studied in tuberose.

The results of different characters related to shoot differentiation as influenced by interaction between genotypes and media composition revealed significant variation among the interaction. It was observed that none of the interaction showed consistent superiority for all the

Table 1. Effect of different compositions of White's media and tuberose varieties on different traits related to shoot differentiation

Treatments	Shoot proliferating explants (%)	Days for shoot proliferation	Number of shoots per responding explants	Number of shoots elongated per responding explants	Shoot length (cm)
Main treatments					
A] Varieties					
Nagpur local 1	45.2(42.21)	13.4966	3.712	1.2	3.9
Nagpur local 2	41.4(40.08)	15.8950	3.586	1.1	3.6
Amravati local 1	40.7(39.62)	17.2502	3.421	0.9	3.2
SE (d)±	**0.7024**	**0.4600**	**0.087**	**0.1**	**0.2**
CD(5%)	**1.397**	**0.9166**	**0.172**	**0.2**	**0.3**
B] Media composition					
S_1 - WH + 0.1 mg/L BAP	46.6 (43.07)	14.333	3.810	1.3	3.7
S_2 - WH + 0.5 mg/L BAP	33.0 (35.01)	16.982	3.377	0.9	2.2
S_3 - WH + 1 mg/L BAP	42.9 (40.96)	14.110	3.527	1.2	3.7
S_4 - WH + 2 mg/L BAP	40.0 (39.23)	16.250	3.167	1.3	4.4
S_5 - WH + 3 mg/L BAP	39.3 (38.85)	16.055	3.305	0.9	3.8
S_6 - WH + 4 mg/L BAP	46.6 (43.07)	12.682	3.405	1.0	3.5
S_7 - WH + 5 mg/L BAP	40.0 (39.23)	13.917	3.467	1.1	3.2
S_8 - WH + 0.1 mg/L KIN	39.3 (38.85)	14.985	3.555	1.1	3.0
S_9 - WH + 0.5 mg/L KIN	46.6 (43.07)	19.417	3.383	0.9	4.3
S_{10} - WH + 1 mg/L KIN	39.3 (38.85)	18.017	3.333	1.1	4.4
S_{11} - WH + 2 mg/L KIN	40.0 (39.23)	18.000	3.417	0.8	2.4
S_{12} - WH + 3 mg/L KIN	33.0 (35.01)	18.250	3.750	1.0	3.0
S_{13} - WH + 4 mg/L KIN	50.2 (45.18)	14.860	3.792	1.1	3.4
S_{14} - WH + 5 mg/L KIN	42.9 (40.96)	15.110	3.500	0.9	4.3
S_{15} - WH + 0.1 mg/L BAP + 0.5 mg/L NAA	50.0 (45.00)	15.417	3.883	1.0	3.7
S_{16} - WH + 0.5 mg/L BAP + 0.5 mg/LNAA	39.3 (38.85)	18.222	3.383	1.0	4.4
S_{17} - WH + 1 mg/L BAP + 0.5 mg/L NAA	42.9 (40.96)	17.850	3.333	1.0	3.6
S_{18} - WH + 2 mg/L BAP + 0.5 mg/L NAA	46.7 (43.07)	16.167	3.667	1.1	3.5
S_{19} -WH + 3 mg/L BAP + 0.5 mg/L NAA	33.0 (35.01)	19.555	3.333	1.0	3.0
S_{20} - WH + 4 mg/L BAP + 0.5 mg/L NAA	53.3 (46.92)	11.110	5.000	2.3	5.0
S_{21} - WH + 5 mg/L BAP + 0.5 mg/L NAA	50.2 (45.18)	11.667	4.383	1.4	5.0
S_{22} - WH + 0.1 mg/L KIN + 0.5 mg/L NAA	45.9 (42.70)	18.472	3.888	0.7	2.4
S_{23} - WH + 0.5 mg/L KIN + 0.5 mg/L NAA	46.7 (43.07)	11.888	3.250	0.9	3.7
S_{24} - WH + 1 mg/L KIN + 0.5 mg/L NAA	33.0 (35.01)	12.750	3.867	0.8	3.1
S_{25} -WH + 2 mg/L KIN + 0.5 mg/L NAA	40.0 (39.23)	12.943	3.305	0.9	3.7
S_{26} -WH + 3 mg/L KIN + 0.5 mg/L NAA	50.0 (45.00)	17.967	3.333	1.1	3.9
S_{27} -WH + 4 mg/L KIN + 0.5 mg/L NAA	40.0 (39.23)	13.000	3.333	0.8	2.5
S_{28} - WH + 5 mg/L KIN + 0.5 mg/L NAA	39.3 (38.85)	15.350	3.300	0.8	3.3
SE (d) ±	**2.1460**	**1.4079**	**0.264**	**0.2**	**0.5**
CD (5%)	**4.268**	**2.8003**	**0.525**	**0.5**	**1.1**

C] Interactions

Treatments	Shoot proliferating explants (%)	Days for shoot proliferation	Number of shoot per responding explants	Number of shoots elongated per responding explants	Shoot length (cm)
V_1S_1	40.0 (39.23)	11.250	3.250	2.0	3.0
V_1S_2	40.0 (39.23)	12.780	3.330	1.0	2.3
V_1S_3	40.0 (39.23)	11.250	3.750	1.0	1.4
V_1S_4	40.0 (39.23)	12.000	3.250	1.0	3.8
V_1S_5	29.5 (32.90)	13.665	3.665	1.0	6.0
V_1S_6	40.0 (39.23)	10.050	3.415	1.0	3.9
V_1S_7	40.0 (39.23)	12.500	3.250	1.0	3.1
V_1S_8	60.0 (50.77)	18.000	3.500	1.3	2.4
V_1S_9	40.0 (39.23)	15.000	3.650	0.8	3.5
V_1S_{10}	60.0 (50.77)	10.000	3.250	1.3	3.4
V_1S_{11}	40.0 (39.23)	11.250	3.500	1.0	3.6
V_1S_{12}	40.0 (39.23)	15.250	4.250	1.3	3.6
V_1S_{13}	29.5 (32.90)	11.250	4.750	1.0	3.4
V_1S_{14}	29.5 (32.90)	15.250	3.500	0.5	2.0
V_1S_{15}	50.0 (45.00)	12.250	4.650	1.3	5.7
V_1S_{16}	60.0 (50.77)	11.750	3.250	1.0	4.5
V_1S_{17}	60.0 (50.77)	14.250	3.000	1.8	6.3
V_1S_{18}	40.0 (39.23)	14.250	3.750	1.8	4.8
V_1S_{19}	29.50 (32.9)	16.665	3.500	1.0	3.4
V_1S_{20}	60.0 (50.77)	8.500	4.000	2.5	4.9
V_1S_{21}	40.0 (39.23)	12.000	6.900	1.3	8.0
V_1S_{22}	60.0 (50.77)	16.665	3.415	1.0	3.4
V_1S_{23}	40.0 (39.23)	15.165	3.250	1.3	4.3
V_1S_{24}	40.0 (39.23)	14.500	4.000	1.0	5.0
V_1S_{25}	40.0 (39.23)	14.915	3.415	1.3	2.4
V_1S_{26}	50.0 (45.00)	16.750	3.500	0.8	4.0
V_1S_{27}	40.0 (39.23)	15.250	3.500	0.8	2.8
V_1S_{28}	60.0 (50.77)	15.500	3.500	1.0	4.3
V_2S_1	60.0 (50.77)	9.750	4.930	1.0	5.1
V_2S_2	20.0 (26.57)	23.665	3.500	1.3	1.9
V_2S_3	60.0 (50.77)	17.750	3.330	1.5	3.9
V_2S_4	40.0 (39.23)	14.750	3.000	1.3	3.2
V_2S_5	29.5 (32.90)	20.500	3.250	1.0	3.2
V_2S_6	60.0 (50.77)	17.665	3.250	1.0	3.8
V_2S_7	40.0 (39.23)	17.750	3.500	1.2	3.5
V_2S_8	40.0 (39.23)	13.000	3.665	1.0	4.3
V_2S_9	40.0 (39.23)	17.000	3.250	1.0	3.5
V_2S_{10}	40.0 (39.23)	18.800	3.250	1.1	5.2
V_2S_{11}	40.0 (39.23)	19.000	3.500	0.5	1.9
V_2S_{12}	20.0 (26.57)	16.500	3.500	1.0	3.3
V_2S_{13}	70.5 (57.10)	19.750	3.375	1.0	1.6
V_2S_{14}	60.0 (50.77)	20.250	3.250	1.5	7.8
V_2S_{15}	40.0 (39.23)	12.500	3.750	0.3	1.2
V_2S_{16}	20.0 (26.57)	14.915	3.000	1.5	6.5
V_2S_{17}	29.5(32.90)	16.800	3.750	0.4	1.6
V_2S_{19}	40.0 (39.23)	15.250	3.750	1.0	4.1
V_2S_{20}	29.5 (32.90)	16.250	3.250	0.5	2.5
V_2S_{21}	40.0 (39.23)	12.500	7.750	3.5	6.3
V_2S_{22}	70.5 (57.10)	17.750	3.250	1.3	4.1
V_2S_{23}	20.0 (26.57)	17.250	4.600	0.3	1.4
V_2S_{24}	40.0 (39.23)	11.000	3.250	0.8	2.8
V_2S_{25}	40.0 (39.23)	12.500	3.250	0.5	2.2
V_2S_{26}	40.0 (39.23)	12.415	3.250	1.0	6.4
V_2S_{27}	60.0 (50.77)	16.000	2.750	2.0	5.6
V_2S_{28}	40.0 (39.23)	12.000	3.000	0.8	2.7

V_3S_1	40.0 (39.23)	22.000	3.250	1.0	3.1
V_3S_2	40.0 (39.23)	14.500	3.300	0.5	2.4
V_3S_3	29.5 (32.90)	13.330	3.500	1.0	5.9
V_3S_4	40.0 (39.23)	22.000	3.250	1.5	6.3
V_3S_5	60.0 (50.77)	14.000	3.000	0.8	2.3
V_3S_6	40.0 (39.23)	10.330	3.550	1.0	2.9
V_3S_7	40.0 (39.23)	11.500	3.650	1.0	3.0
V_3S_8	20.0 (26.57)	13.955	3.500	1.0	2.3
V_3S_9	60.0 (50.77)	26.250	3.250	0.9	5.9
V_3S_{10}	20.0 (26.57)	25.250	3.500	1.0	4.5
V_3S_{11}	40.0 (39.23)	23.750	3.250	0.8	1.9
V_3S_{12}	40.0 (39.23)	23.000	3.500	0.8	2.0
V_3S_{13}	20.0 (26.57)	13.580	3.250	1.3	5.2
V_3S_{14}	40.0 (39.23)	9.830	3.750	0.8	3.1
V_3S_{15}	60.0 (50.77)	21.500	3.250	1.3	4.2
V_3S_{16}	40.0 (39.23)	28.000	3.900	0.5	2.2
V_3S_{17}	40.0 (39.23)	22.500	3.250	0.8	2.8
V_3S_{18}	60.0 (50.77)	19.000	3.500	0.5	1.6
V_3S_{19}	40.0 (39.23)	25.750	3.250	1.6	3.2
V_3S_{20}	60.0 (50.77)	12.330	3.250	0.9	4.0
V_3S_{21}	40.0 (39.23)	5.250	3.000	1.5	3.0
V_3S_{22}	60.0 (50.77)	21.500	3.650	0.8	2.3
V_3S_{23}	60.0 (50.77)	9.500	3.250	0.7	4.0
V_3S_{24}	20.0 (26.57)	11.250	4.350	0.8	2.2
V_3S_{25}	40.0 (39.23)	11.500	3.250	0.4	2.3
V_3S_{26}	40.0 (39.23)	21.150	3.750	0.5	2.3
V_3S_{27}	40.0 (39.23)	11.750	3.500	0.8	2.0
V_3S_{28}	20.0 (26.57)	18.750	3.150	1.0	4.0
SE (d)	**3.7170**	**2.4380**	**0.458**	**0.4**	**0.9**
CD (5%)	**7.392**	**4.8502**	**0.910**	**0.8**	**1.8**

Table 2. Effect of different compositions of White's media and tuberose varieties on different traits related to root differentiation

Treatments	Root proliferating shoot lets (%)	Days for root formation	Root length (cm)
Main treatments			
A] Varieties			
Nagpur local 1	48.8	12.149	5.207
Nagpur local 2	39.2	14.631	4.618
Amravati local 1	31.0	16.034	4.529
SE (d)	**2.6493**	**0.4591**	**0.1728**
CD5%	**5.3783**	**0.9320**	**0.3510**
B] Media composition			
R_1 - WH + 0.5 mg/L IAA	51.6 (45.96)	13.250	5.008
R_2 - WH + 1 mg/L IAA	57.0 (49.03)	12.902	5.067
R_3 - WH + 2 mg/L IAA	58.7 (50.00)	12.722	5.738
R_4 - WH + 3 mg/L IAA	40.3 (39.42)	16.250	4.482
R_5 - WH + 4 mg/L IAA	31.0 (33.85)	13.750	4.883
R_6 - WH + 5 mg/L IAA	44.6 (41.92)	15.167	4.592
R_7 - WH + 0.5 mg/L NAA	46.6 (43.07)	14.417	4.592
R_8 - WH + 1 mg/L NAA	31.0 (33.85)	15.667	5.007
R_9 - WH + 2 mg/L NAA	35.2 (36.35)	13.500	4.100
R_{10} - WH + 3 mg/L NAA	44.6 (41.92)	15.832	4.705
R_{11} - WH + 4 mg/L NAA	34.4 (35.96)	13.583	4.933
R_{12} - WH + 5 mg/L NAA	41.4 (40.01)	14.222	4.317
SE (d) ±	**5.2985**	**0.9183**	**0.3455**
CD (5%)	**10.7566**	**1.8640**	**0.7010**

C] Interactions

Treatments	Root proliferating shoot lets (%)	Days for root formation	Root length (cm)
V_1R_1	63.0 (52.50)	12.250	6.050
V_1R_2	75.0 (60.00)	10.375	5.600
V_1R_3	63.0 (52.50)	9.000	5.190
V_1R_4	63.0 (52.50)	14.250	6.600
V_1R_5	34.2 (35.78)	11.750	5.500
V_1R_6	37.2 (37.50)	13.000	5.250
V_1R_7	50.0 (45.00)	12.000	4.950
V_1R_8	50.0 (45.00)	13.000	5.405
V_1R_9	25.0 (30.00)	14.500	3.900
V_1R_{10}	42.0 (40.38)	10.165	4.950
V_1R_{11}	37.2 (37.50)	12.000	4.350
V_1R_{12}	47.0 (43.28)	13.500	4.750
V_2R_1	42.0 (40.38)	13.000	4.600
V_2R_2	45.0 (42.11)	12.580	5.175
V_2R_3	58.0 (49.61)	7.915	7.075
V_2R_4	22.4 (28.28)	20.000	3.715
V_2R_5	34.2 (35.78)	16.500	3.950
V_2R_6	55.0 (47.88)	14.500	4.400
V_2R_7	45.0 (42.11)	15.250	4.100
V_2R_8	22.4 (28.28)	19.000	5.040
V_2R_9	22.4 (28.28)	10.500	4.600
V_2R_{10}	42.0 (40.38)	17.165	4.315
V_2R_{11}	42.0 (40.38)	14.000	4.600
V_2R_{12}	45.0 (42.11)	15.165	3.850
V_3R_1	50.0 (45.00)	14.500	4.375
V_3R_2	50.0 (45.00)	15.750	4.425
V_3R_3	55.0 (47.88)	21.250	4.950
V_3R_4	37.1 (37.50)	14.500	3.130
V_3R_5	25.0 (30.00)	13.000	5.200
V_3R_6	42.0 (40.38)	18.000	4.125
V_3R_7	45.0 (42.11)	16.000	4.725
V_3R_8	22.4 (28.28)	15.000	4.575
V_3R_9	60.0 (50.77)	15.500	3.800
V_3R_{10}	50.0 (45.00)	20.165	4.850
V_3R_{11}	25.0 (30.00)	14.750	5.850
V_3R_{12}	32.3 (34.61)	14.000	4.350
SE (d)	**9.1774**	**1.5905**	**0.5984**
CD (5%)	**18.6310**	**3.2290**	**1.2150**

Explant

Shoot initiation

Multiple shoot

Shootlets for rooting

Root initiation

Rooted plantlet

Hardening of plantlet

Plate 1. Direct organogenesis of variety Nagpur local 1 in White's media with 4 mgl^{-1} BAP and 0.5 mgl^{-1} NAA for rapid shoot and leaf initiation and proliferation followed by rooting in White's media supplemented with 2 mgl^{-1} IAA.

five shoot differentiation related characters studied. However, Nagpur local 1 in S_{20} (WH + 4 mgl^{-1} BAP + 0.5 mgl^{-1} NAA) performed better for shoot proliferating explants (60 %), days for shoot proliferation (8.50 days) and shoot elongation (2.5 shoots). This was followed by Nagpur local 2 in S_{20} (WH + 4 mgl^{-1} BAP + 0.5 mgl^{-1} NAA) for number of shoots (7.75 shoots), shoot elongation (3.5 shoots) and shoot length (6.3 cm).

Thus, it is noticed from this study that variety Nagpur local 1 and Whites basal media fortified with 4 mgl^{-1} BAP and 0.5 mgl^{-1} NAA (S_{20}) can be identified as suitable variety and media composition for *in vitro* shoot propagation of tuberose, as it performed well in individual factors and also in their interactions.

Root regeneration

Among the auxin used in this study for root formation IAA was observed to be most effective than NAA. Proper root initiation helps in future performance of *in vitro* raised plantlets. In this study among the different treatments tried full strength White's media supplemented with 2 mgl^{-1} IAA was observed to be the best treatment as it resulted in highest root proliferation (58.7 %), early root formation (12.72 days) and maximum root length (5.73 cm). The traits related to root differentiation decreased with the absence of IAA concentration. In contrary to this result Panigrahi *et al*. (2013) reported that best root initiation was shown by the combination of NAA 0.5 mg^{-1} and IAA 3.5 mg^{-1} in tuberose. Surendranath *et al*. (2016) also observed that basal media fortified with IBA and NAA produced highest number of roots in tuberose. In this study IAA alone had a propounding influence in rhizogenesis. Ali *et al*. (2015) obtained maximum number of roots $plant^{-1}$ and longest roots in MS + 0.5 mgl^{-1} + 1 mg^{-1} KIN.

The results on different characters related to root differentiation as influenced by interaction between genotypes and media composition revealed significant variation among the interaction. It was noticed from the results that none of the interaction showed consistent superiority for all the three root differentiation related characters studied. However, Nagpur local 2 in R_3 (WH + 2 mgl^{-1} IAA) exhibited better performance for root proliferation (58%), days for root formation (7.9 days) and root lengths (7.07 days). This was followed by Nagpur local 1 in R_3 (WH + 2 mgl^{-1} IAA) for root proliferation (63%) and days for root formation (9.0 days).

Thus it is observed from this study that Nagpur local 2 and Nagpur local 1 and White's media fortified with 2 mgl^{-1} IAA can be identified as suitable varieties and media composition for *in vitro* root propagation of tuberose as it performed well in individual effect and their interactions.

Hardening and survival

The plantlets obtained after successful rhizogenesis were planted *ex vitro* for hardening. The plantlets were hardened under shade conditions. The response of plantlets for hardening is given. The number of shoots obtained $culture^{-1}$ in all the three varieties in 28 media combinations were not adequate enough to inoculate in the root induction media, which is clear from the observation on the number of elongated shoot buds $culture^{-1}$. This ultimately resulted in very few number of plantlets for hardening, because of which the data on plantlets obtained from all the treatments of each variety were pooled to estimate the survival rate. It was observed that maximum survival rate was recorded in Nagpur local 1 (53.84%), followed by Nagpur local 2 (40.90%) and Amravati local 1 (36.36%). The reduced plant recovery rate was mainly because of the less number of elongated shoots obtained for culturing in rooting media. Therefore, condition for successful elongation of shoots must be defined first to increase the frequency of plant recovery. In contrary to this result Naz *et al*. (2012) reported vigorous growth with 81% survival rate in sand in tuberose.

In conclusion, this experiment allowed the development of protocol for *in vitro* propagation of three genotypes of tuberose from stem disc explants. The results of the present investigations lead to the inference that Nagpur local 1 could be successfully micropropagated with White's media supplemented with 4 mgl^{-1} BAP and 0.5 mgl^{-1} NAA for rapid shoot and leaf initiation and proliferation followed by rooting in White's media supplemented with 2 mgl^{-1} IAA.

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