

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

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ABSTRACT

Direct shoot organogenesis from stem disc explants of tuberose (*Polianthes tuberosa* 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 S₂₀ (WH + 4 mg⁻¹ BAP + 0.5 mg⁻¹ NAA). Nagpur local 1 in S₂₀ (WH + 4 mg⁻¹ BAP + 0.5 mg⁻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 mg⁻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 mg⁻¹ BAP and 0.5 mg⁻¹ NAA for shoot proliferation followed by rooting in White's media supplemented with 2 mg⁻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 $25 \pm 2^\circ\text{C}$ 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 ₁ S ₁ | 40.0 (39.23) | 11.250 | 3.250 | 2.0 | 3.0 |
| V ₁ S ₂ | 40.0 (39.23) | 12.780 | 3.330 | 1.0 | 2.3 |
| V ₁ S ₃ | 40.0 (39.23) | 11.250 | 3.750 | 1.0 | 1.4 |
| V ₁ S ₄ | 40.0 (39.23) | 12.000 | 3.250 | 1.0 | 3.8 |
| V ₁ S ₅ | 29.5 (32.90) | 13.665 | 3.665 | 1.0 | 6.0 |
| V ₁ S ₆ | 40.0 (39.23) | 10.050 | 3.415 | 1.0 | 3.9 |
| V ₁ S ₇ | 40.0 (39.23) | 12.500 | 3.250 | 1.0 | 3.1 |
| V ₁ S ₈ | 60.0 (50.77) | 18.000 | 3.500 | 1.3 | 2.4 |
| V ₁ S ₉ | 40.0 (39.23) | 15.000 | 3.650 | 0.8 | 3.5 |
| V ₁ S ₁₀ | 60.0 (50.77) | 10.000 | 3.250 | 1.3 | 3.4 |
| V ₁ S ₁₁ | 40.0 (39.23) | 11.250 | 3.500 | 1.0 | 3.6 |
| V ₁ S ₁₂ | 40.0 (39.23) | 15.250 | 4.250 | 1.3 | 3.6 |
| V ₁ S ₁₃ | 29.5 (32.90) | 11.250 | 4.750 | 1.0 | 3.4 |
| V ₁ S ₁₄ | 29.5 (32.90) | 15.250 | 3.500 | 0.5 | 2.0 |
| V ₁ S ₁₅ | 50.0 (45.00) | 12.250 | 4.650 | 1.3 | 5.7 |
| V ₁ S ₁₆ | 60.0 (50.77) | 11.750 | 3.250 | 1.0 | 4.5 |
| V ₁ S ₁₇ | 60.0 (50.77) | 14.250 | 3.000 | 1.8 | 6.3 |
| V ₁ S ₁₈ | 40.0 (39.23) | 14.250 | 3.750 | 1.8 | 4.8 |
| V ₁ S ₁₉ | 29.50 (32.9) | 16.665 | 3.500 | 1.0 | 3.4 |
| V ₁ S ₂₀ | 60.0 (50.77) | 8.500 | 4.000 | 2.5 | 4.9 |
| V ₁ S ₂₁ | 40.0 (39.23) | 12.000 | 6.900 | 1.3 | 8.0 |
| V ₁ S ₂₂ | 60.0 (50.77) | 16.665 | 3.415 | 1.0 | 3.4 |
| V ₁ S ₂₃ | 40.0 (39.23) | 15.165 | 3.250 | 1.3 | 4.3 |
| V ₁ S ₂₄ | 40.0 (39.23) | 14.500 | 4.000 | 1.0 | 5.0 |
| V ₁ S ₂₅ | 40.0 (39.23) | 14.915 | 3.415 | 1.3 | 2.4 |
| V ₁ S ₂₆ | 50.0 (45.00) | 16.750 | 3.500 | 0.8 | 4.0 |
| V ₁ S ₂₇ | 40.0 (39.23) | 15.250 | 3.500 | 0.8 | 2.8 |
| V ₁ S ₂₈ | 60.0 (50.77) | 15.500 | 3.500 | 1.0 | 4.3 |
| V ₂ S ₁ | 60.0 (50.77) | 9.750 | 4.930 | 1.0 | 5.1 |
| V ₂ S ₂ | 20.0 (26.57) | 23.665 | 3.500 | 1.3 | 1.9 |
| V ₂ S ₃ | 60.0 (50.77) | 17.750 | 3.330 | 1.5 | 3.9 |
| V ₂ S ₄ | 40.0 (39.23) | 14.750 | 3.000 | 1.3 | 3.2 |
| V ₂ S ₅ | 29.5 (32.90) | 20.500 | 3.250 | 1.0 | 3.2 |
| V ₂ S ₆ | 60.0 (50.77) | 17.665 | 3.250 | 1.0 | 3.8 |
| V ₂ S ₇ | 40.0 (39.23) | 17.750 | 3.500 | 1.2 | 3.5 |
| V ₂ S ₈ | 40.0 (39.23) | 13.000 | 3.665 | 1.0 | 4.3 |
| V ₂ S ₉ | 40.0 (39.23) | 17.000 | 3.250 | 1.0 | 3.5 |
| V ₂ S ₁₀ | 40.0 (39.23) | 18.800 | 3.250 | 1.1 | 5.2 |
| V ₂ S ₁₁ | 40.0 (39.23) | 19.000 | 3.500 | 0.5 | 1.9 |
| V ₂ S ₁₂ | 20.0 (26.57) | 16.500 | 3.500 | 1.0 | 3.3 |
| V ₂ S ₁₃ | 70.5 (57.10) | 19.750 | 3.375 | 1.0 | 1.6 |
| V ₂ S ₁₄ | 60.0 (50.77) | 20.250 | 3.250 | 1.5 | 7.8 |
| V ₂ S ₁₅ | 40.0 (39.23) | 12.500 | 3.750 | 0.3 | 1.2 |
| V ₂ S ₁₆ | 20.0 (26.57) | 14.915 | 3.000 | 1.5 | 6.5 |
| V ₂ S ₁₇ | 29.5(32.90) | 16.800 | 3.750 | 0.4 | 1.6 |
| V ₂ S ₁₉ | 40.0 (39.23) | 15.250 | 3.750 | 1.0 | 4.1 |
| V ₂ S ₂₀ | 29.5 (32.90) | 16.250 | 3.250 | 0.5 | 2.5 |
| V ₂ S ₂₁ | 40.0 (39.23) | 12.500 | 7.750 | 3.5 | 6.3 |
| V ₂ S ₂₂ | 70.5 (57.10) | 17.750 | 3.250 | 1.3 | 4.1 |
| V ₂ S ₂₃ | 20.0 (26.57) | 17.250 | 4.600 | 0.3 | 1.4 |
| V ₂ S ₂₄ | 40.0 (39.23) | 11.000 | 3.250 | 0.8 | 2.8 |
| V ₂ S ₂₅ | 40.0 (39.23) | 12.500 | 3.250 | 0.5 | 2.2 |
| V ₂ S ₂₆ | 40.0 (39.23) | 12.415 | 3.250 | 1.0 | 6.4 |
| V ₂ S ₂₇ | 60.0 (50.77) | 16.000 | 2.750 | 2.0 | 5.6 |
| V ₂ S ₂₈ | 40.0 (39.23) | 12.000 | 3.000 | 0.8 | 2.7 |

| | | | | | |
|--------------------------------|---------------|---------------|--------------|------------|------------|
| V ₃ S ₁ | 40.0 (39.23) | 22.000 | 3.250 | 1.0 | 3.1 |
| V ₃ S ₂ | 40.0 (39.23) | 14.500 | 3.300 | 0.5 | 2.4 |
| V ₃ S ₃ | 29.5 (32.90) | 13.330 | 3.500 | 1.0 | 5.9 |
| V ₃ S ₄ | 40.0 (39.23) | 22.000 | 3.250 | 1.5 | 6.3 |
| V ₃ S ₅ | 60.0 (50.77) | 14.000 | 3.000 | 0.8 | 2.3 |
| V ₃ S ₆ | 40.0 (39.23) | 10.330 | 3.550 | 1.0 | 2.9 |
| V ₃ S ₇ | 40.0 (39.23) | 11.500 | 3.650 | 1.0 | 3.0 |
| V ₃ S ₈ | 20.0 (26.57) | 13.955 | 3.500 | 1.0 | 2.3 |
| V ₃ S ₉ | 60.0 (50.77) | 26.250 | 3.250 | 0.9 | 5.9 |
| V ₃ S ₁₀ | 20.0 (26.57) | 25.250 | 3.500 | 1.0 | 4.5 |
| V ₃ S ₁₁ | 40.0 (39.23) | 23.750 | 3.250 | 0.8 | 1.9 |
| V ₃ S ₁₂ | 40.0 (39.23) | 23.000 | 3.500 | 0.8 | 2.0 |
| V ₃ S ₁₃ | 20.0 (26.57) | 13.580 | 3.250 | 1.3 | 5.2 |
| V ₃ S ₁₄ | 40.0 (39.23) | 9.830 | 3.750 | 0.8 | 3.1 |
| V ₃ S ₁₅ | 60.0 (50.77) | 21.500 | 3.250 | 1.3 | 4.2 |
| V ₃ S ₁₆ | 40.0 (39.23) | 28.000 | 3.900 | 0.5 | 2.2 |
| V ₃ S ₁₇ | 40.0 (39.23) | 22.500 | 3.250 | 0.8 | 2.8 |
| V ₃ S ₁₈ | 60.0 (50.77) | 19.000 | 3.500 | 0.5 | 1.6 |
| V ₃ S ₁₉ | 40.0 (39.23) | 25.750 | 3.250 | 1.6 | 3.2 |
| V ₃ S ₂₀ | 60.0 (50.77) | 12.330 | 3.250 | 0.9 | 4.0 |
| V ₃ S ₂₁ | 40.0 (39.23) | 5.250 | 3.000 | 1.5 | 3.0 |
| V ₃ S ₂₂ | 60.0 (50.77) | 21.500 | 3.650 | 0.8 | 2.3 |
| V ₃ S ₂₃ | 60.0 (50.77) | 9.500 | 3.250 | 0.7 | 4.0 |
| V ₃ S ₂₄ | 20.0 (26.57) | 11.250 | 4.350 | 0.8 | 2.2 |
| V ₃ S ₂₅ | 40.0 (39.23) | 11.500 | 3.250 | 0.4 | 2.3 |
| V ₃ S ₂₆ | 40.0 (39.23) | 21.150 | 3.750 | 0.5 | 2.3 |
| V ₃ S ₂₇ | 40.0 (39.23) | 11.750 | 3.500 | 0.8 | 2.0 |
| V ₃ S 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 ₁ R ₁ | 63.0 (52.50) | 12.250 | 6.050 |
| V ₁ R ₂ | 75.0 (60.00) | 10.375 | 5.600 |
| V ₁ R ₃ | 63.0 (52.50) | 9.000 | 5.190 |
| V ₁ R ₄ | 63.0 (52.50) | 14.250 | 6.600 |
| V ₁ R ₅ | 34.2 (35.78) | 11.750 | 5.500 |
| V ₁ R ₆ | 37.2 (37.50) | 13.000 | 5.250 |
| V ₁ R ₇ | 50.0 (45.00) | 12.000 | 4.950 |
| V ₁ R ₈ | 50.0 (45.00) | 13.000 | 5.405 |
| V ₁ R ₉ | 25.0 (30.00) | 14.500 | 3.900 |
| V ₁ R ₁₀ | 42.0 (40.38) | 10.165 | 4.950 |
| V ₁ R ₁₁ | 37.2 (37.50) | 12.000 | 4.350 |
| V ₁ R ₁₂ | 47.0 (43.28) | 13.500 | 4.750 |
| V ₂ R ₁ | 42.0 (40.38) | 13.000 | 4.600 |
| V ₂ R ₂ | 45.0 (42.11) | 12.580 | 5.175 |
| V ₂ R ₃ | 58.0 (49.61) | 7.915 | 7.075 |
| V ₂ R ₄ | 22.4 (28.28) | 20.000 | 3.715 |
| V ₂ R ₅ | 34.2 (35.78) | 16.500 | 3.950 |
| V ₂ R ₆ | 55.0 (47.88) | 14.500 | 4.400 |
| V ₂ R ₇ | 45.0 (42.11) | 15.250 | 4.100 |
| V ₂ R ₈ | 22.4 (28.28) | 19.000 | 5.040 |
| V ₂ R ₉ | 22.4 (28.28) | 10.500 | 4.600 |
| V ₂ R ₁₀ | 42.0 (40.38) | 17.165 | 4.315 |
| V ₂ R ₁₁ | 42.0 (40.38) | 14.000 | 4.600 |
| V ₂ R ₁₂ | 45.0 (42.11) | 15.165 | 3.850 |
| V ₃ R ₁ | 50.0 (45.00) | 14.500 | 4.375 |
| V ₃ R ₂ | 50.0 (45.00) | 15.750 | 4.425 |
| V ₃ R ₃ | 55.0 (47.88) | 21.250 | 4.950 |
| V ₃ R ₄ | 37.1 (37.50) | 14.500 | 3.130 |
| V ₃ R ₅ | 25.0 (30.00) | 13.000 | 5.200 |
| V ₃ R ₆ | 42.0 (40.38) | 18.000 | 4.125 |
| V ₃ R ₇ | 45.0 (42.11) | 16.000 | 4.725 |
| V ₃ R ₈ | 22.4 (28.28) | 15.000 | 4.575 |
| V ₃ R ₉ | 60.0 (50.77) | 15.500 | 3.800 |
| V ₃ R ₁₀ | 50.0 (45.00) | 20.165 | 4.850 |
| V ₃ R ₁₁ | 25.0 (30.00) | 14.750 | 5.850 |
| V ₃ R 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 mg l^{-1} BAP and 0.5 mg l^{-1} NAA for rapid shoot and leaf initiation and proliferation followed by rooting in White's media supplemented with 2 mg l^{-1} IAA.

five shoot differentiation related characters studied. However, Nagpur local 1 in S_{20} (WH + 4 mg^l⁻¹ BAP + 0.5 mg^l⁻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 mg^l⁻¹ BAP + 0.5 mg^l⁻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 mg^l⁻¹ BAP and 0.5 mg^l⁻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 mg^l⁻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^l⁻¹ and IAA 3.5 mg^l⁻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 mg^l⁻¹ + 1 mg^l⁻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 mg^l⁻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 mg^l⁻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 mg^l⁻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 mg^l⁻¹ BAP and 0.5 mg^l⁻¹ NAA for rapid shoot and leaf initiation and proliferation followed by rooting in White's media supplemented with 2 mg^l⁻¹ IAA.

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