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Direct shoot organogenesis from cultured stem disc explants of tuberose (*Polianthes tuberosa* Linn.)

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A protocol has been established to propagate four genotypes of tuberose *viz*: Phule Rajni, Shringar, Prajwal, and Mexican Single through direct *in vitro* organogenesis from stem disc explants. During the present investigation, culture medium WH.3Td.5N (WH + 0.3 mg. l^{-1} TDZ + 0.5 mg.l^{-1} NAA + 20.0 g.l^{-1} sucrose + 7.5 g. l^{-1} agar) was found to be more responsive for shoot proliferating ability (98.90%) and shoot (s) per explant (11.48). Induction medium WH3N.5Td (WH + 3.0 mg.l^{-1} NAA + 0.5 mg.l^{-1} TDZ + 20.0 g.l^{-1} sucrose + 7.5 g. l^{-1} agar) was proved well for enhancing mean shoot length (5.56 cm). In terms of *in vitro* rooting response, culture medium WH2I (WH + 2.0 mg.l^{-1} IBA + 10.0 g.l^{-1} sucrose + 7.5 g. l^{-1} agar) was found consistently superior for all culture phases, *i.e.* root proliferating efficiency (96.24%), number of root (s) per responding shootlet (14.20), and average root length (7.56 cm). Among the four cultivars Mexican Single was found significantly superior followed by Prajwal, Phule Rajni and Shringar for the most of the attributes. Regenerated plantlets were established successfully in the field after hardening. Phenotypically normal plants were regenerated from the stem disc explants.

Key words: *Polianthes tuberosa*, stem disc culture, organogenesis, and plantlet regeneration

Introduction

Tuberose (*Polianthes tuberosa* Linn.) originated in Mexico belongs to family Amaryllidaceae is one of the important bulbous ornamental crop in India and being grown in tropical and subtropical areas. Commercially it is being grown in West Bengal, Karnataka, Tamilnadu and Maharashtra and Madhya Pradesh. Among the commercially grown flowers in India, tuberose occupies a

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prime position owing its popularity as a cut flower, loose flower, to flower loving people because of its prettiness, elegance and sweet pleasant fragrance, for perfumery as well as its potential as source of secondary metabolites. Tuberose can be propagated by seed as well as vegetatively by bulbs and bulblets. However, the 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 cultivars set seed and seeds are difficult to germinate. 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 increased vase life of tuberose, modern molecular biology and genetic engineering techniques need to be exploited fully for tailoring transgenic plants. For achieving *per se* goals, an efficient and reproductive regeneration system from explant cultures must be available.

In tuberose, various explants have been tried to produce regenerable cultures *via in vitro* morphogenesis are shoot tips (Hutchinson *et al*., 2004), bulb scale (Muralidhar and Mehta,1982; Bose *et al.,* 1987; Khan *et al*., 2000; Rajasekaran *et al.,* 2000; Nazneen *et al*., 2003; Mishra *et al*., 2006) rhizome (Sangavai and Chellapandi, 2008), leaf disc (Bindhani *et al*., 2004), root (Narayanaswamy and Prabhudesai, 1979) and anther (Gi and Tsay, 1989). However, the regeneration frequencies in all above experiments were found considerably moderate.

Even if, a few studies have been conducted in some laboratories in India and abroad to obtain prolific *in vitro* culture system of tuberose with limiting regeneration potential, no work has been done so far in M.P. on these aspects. In view of this, an experiment was conducted to select the most responding cultivar, cytokinin-to-auxin ratio and other physical factors exhibiting higher *in vitro* morphogenesis by means of stem disc culture with four cultivars of tuberose *viz:* Phule Rajni, Shringar, Prajwal, and Maxican Single, which has a high commercial value in Indian flower market.

Materials and methods

Explant material

All the experiments for the present experimentation were conducted at the Horticultural Biotechnology Laboratory, KNK College of Horticulture, Mandsaur, Jawaharlal Nehru Agricultural University, Jabalpur (M. P., India). Four cultivars of *Polianthes tuberosa* were selected for the study of stem disc culture. Three cultivars *viz:* Phule Rajni, Prajwal and Shringar were procured from Udaipur Agriculture University, Rajasthan, while, cultivar Mexican

Single was collected from Bahaduri Farm, KNK, College of Horticulture, Mandsaur (M. P.).

Culture media

To begin with a preliminary experiment, three different fortifications of basal media *viz*: MS (Murashige and Skoog, 1962), WH (White, 1963) and B_5 (Gamborg *et al*., 1968) were venerated to find out better *in vitro* response. During the preliminary investigation, WH basal medium was found more receptive than MS and B_5 media (data not presented); hence, for later experimentations basal WP medium was used. All initial culture media were made using readymade basal WP medium (HiMidia^{TM}) and supplemented with three different sets of plant growth regulators to fortify WP basal media. In first set: three different auxins, *namely*: 2, 4-D, NAA and 2, 4, 5-T (alone), in second set: three diverse cytokinins *viz*: BAP, Kn and TDZ (alone) and in third set: two different cytokinins (TDZ and BAP) in combinations with an auxin (NAA) in varying concentrations to attain the best *in vitro* response. Apart from WP basal macro and micro salts, vitamins, all initial media was supplemented with 20.0 $g.l^{-1}$ sucrose and the final volume was made to 1000 ml and pH was adjusted to 5.8 ± 0.1 with 1N KOH solution. After adjusting the pH, agar powder @ 7.5 $g.l^{-1}$ was added to the media as a solidifying agent. Warm culture media, still in liquid state was poured into baby food bottles (50-60 ml / bottle) and culture tubes (15-20 ml/ tube) followed by autoclaving at 121^{o}C under 15 psi pressures for 25 minutes. Readymade basal media, plant growth regulators and other ingredients were procured from Hi-media Laboratories, Mumbai, India.

Surface sterilization of donor plant

Stem disc explants were washed under running tap water for 2 hours for the removal of adhering soil particles. Then the discs were placed into 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 a treatment of aqueous solution of two different surface sterilizing agents *i.e.* $HgCl_2$ or Ca $(OCl)_2$ (alone) in different concentrations as well as in combinations of $HgCl_2$ or Ca $(OCl)_2$ with Bavistin® (BASF, Germany) in varying concentrations for different durations with initial vacuum of 100 psi. Finally stem disc was rinsed 4-5 times with sterile double distilled water prior to inoculation.

Stem disc excision and plating technique

The stem disc was excised measuring 5–10 mm in diameter with young active shoot bud was dissected comprising at least one bud and cultured flat with bud side up in baby food bottles containing culture medium.

Culture conditions

Baby food bottles containing cultures sealed with Lab film (Parafilm®) were incubated under complete darkness at 25 ± 2°C for one week. Later *in vitro* cultured explants were subjected to photoperiod regime of 16 hours light/8 hours dark cycle at an intensity of 2000-lux luminance provided by Photo-synthetically Active Radiation lamps at 25±2 ^{0}C and 60% RH.

Regeneration of plantlets

After 28-35 days of initial culturing, cultures were sub cultured on same medium (initial medium) for regeneration of plantlets. Multiple shoots obtained from direct organogenesis (auxiliary bud proliferation) were transferred to elongation medium which was WP basal medium supplemented with 1.0 mg.l^{-1} GA_3, 15.0 g.l^{-1} sucrose and 7.5 g.l^{-1} agar powder. Cultured baby food bottles/culture tubes were subjected to 25 ± 2^{o}C temperature and photoperiod regimes of 60 µ mol m^{-2} s^{-1} luminance provided by cool fluorescent tubes for 16 hr.

In vitro rooting of regenerants

When root formation was not obtained on regeneration medium, plantlets were subsequently transferred to WP rooting medium supplemented with different concentrations of IBA, NAA, Kn and TDZ, 10.0 g. l^{-1} sucrose and 7.5 g.l^{-1} agar powder. For rooting, reduced level of sucrose was applied on the basis of work conducted by various scientists as well as preliminary experiments of this laboratory.

Acclimatization of regenerants

The plantlets obtained after root initiation were carefully separated from the medium of the culture vessels using forceps to avoid damaging them and were thoroughly washed with running tap water to remove the adhering agar and were planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. Root trainers with transplanted plants were transferred under

30±2°C and 60 ± 5% Relative Humidity for 10-25 days in an Environmental Growth Chamber for hardening. Latter these regenerants were transferred to the Net House for 30-35 days for acclimatization. Finally, acclimatized plants were transferred to field.

Experimental design and analysis of data

The experiment was laid out in factorial Completely Randomized Design. Experiment had two factors, first genotypes and second different culture media combinations. Design was used to find out the significance of genotype, culture medium combination and their interactive effect. Each treatment was consisting of two replications. Per replication approximately 80-100 stem discs were excised and cultured on each media. The arc-sine transformation was made before the analysis of data, since all data were in percentage. The data were analyzed as per method suggested by Snedecor and Cochran (1967).

Results and discussion

The goal of *in vitro* culture of tuberose is to develop steadfast protocol for achieving plant regeneration in higher frequencies from stem disc cultures in order to use them for mass clonal propagation of desirable genotypes or cultivars.

Decontamination of explants obtained from underground parts has been reported to be very difficult task by several workers (Seabrook 1990; Hol and Vander linde, 1992). In present study sequential application of 70% ethanol for 30 seconds, 1% bavistin in combination with 0.1% $HgCl_2$ for 10 minutes were found to be more effective sterilants for tuberose stem disc explant resulting 78.36% aseptic culture and 73.36% survival of explant (Table1). Consequently, this combination for surface sterilization was applied throughout the experiment. Treatments of single sterilant either by Ca $(OCl)_2$ or Hg Cl_2 was not found effectual for the establishment of aseptic culture with good percentage of survival. Further increase in exposure time to sterilants led to kill the explants. One probable reason may be due to metal contamination of mercury present in $HgCl_2$ proving phytotoxic for the survival of the explant. The results are in conformity with Mishra *et al*. (2006).

Stem disc explants of four tuberose cultivars were cultured on various fortifications of WH medium. The first response of cultured explants was similar after 2-3 days and mostly independent from genotype and culture media. All explants became swollen and no response was evident during first few days (Fig. 1 A). After 4 days of culture, shoot initiation was observed from

the most of the explants. Firstly, shoot initiation started from shoot bud (Fig.1 B) then multiple shoots were initiated (Fig.1B-D). From cultured stem disc, mostly plants regenerated *via* direct organogenesis (without callus formation). Shoot proliferation started approximately 10 days from initial culturing (Fig. 1E-F). However, the duration varied from culture to culture and in a few cases shootlets were proliferated after 21 days of initial culturing. With time, these structures formed multiple shoots (Fig.1 G) and consequently elongated. Regenerated shoots alone were also counted as plantlets as they gave rise to complete plants after rhizogenesis on root initiating medium (Fig.1 H-I). Root trainers with transplanted plants were transferred for 10-25 days in an Environmental Growth Chamber for hardening (Fig.1J). Later these regenerants were transferred to the Net House for 25-30 days (Fig.1K) and field (Fig.1L) for acclimatization. The plants, after survival in the net house conditions, were evaluated visually on the basis of their appearance. Although the traits were not scored quantitatively, regenerated plants were found phenotypically normal and true to the type.

During present investigation basal WH medium was used throughout the experiment, as this was found more responsive as compared to MS and B_5 in course of preliminary experiments. As per studies conducted so far, composition of culture media does not seem to play major role in *in vitro* response as much as the type and concentration of plant growth regulators. The analysis of variance presented in Table 2-5 revealed that there were highly significant ($p<0.01$) differences exist among the response of genotypes, culture media combinations as well as their interactions in terms of overall shoot proliferating efficiency, number of shoot (s) per explant, mean shoot length, root proliferating efficiency, number of root (s) and mean root length. It indicates the presence of considerable amount of variability amongst the different culture media combinations, genotypes as well as their interactions. Furthermore, although the genotypes varied highly significantly ($p<0.01$) for the various aspects regarding *in vitro* responses, the non-significant difference were also documented for interaction of genotypes with different culture media combinations fortified with auxins alone in diverse concentrations for shoot proliferating ability and for number (s) of shoots per responding explant, culture media fortified with cytokinins alone as well as supplemented with auxins and cytokinins in combinations (Table 2-4).

Effects of different auxins in varying concentrations on *in vitro* are presented in Table 2. Higher shoot proliferating efficiency, Number (s) of shoot(s) per responding explant and mean shoot length with culture media fortified with NAA WH3N (59.93%, 3.99 and 2.04 cm respectively) and WH2N (57.16%, 3.97 and 1.98 cm respectively) and low *in vitro* response on

media devoid of NAA such as WH5D (17.13%, 2.21 and 1.84 cm) and WH5T (19.50%,1.37 and 1.08 cm respectively) reveal that 2.0 - 3.0 mg.l^{-1} NAA proliferated shoot (s) in higher frequencies (45-60%), Number (s) of shoot(s) per responding explant (3.97-3.99) and mean shoot length (1.98-2.04 cm) however, levels above 3.0 mg.l^{-1} reduced the response.

In terms of shoot proliferating ability, numbers of shoots and mean shoot length, culture media fortified with auxins (2, 4-D or 2, 4, 5-T) as alone in varying concentrations performed poorly as compared to media supplemented with NAA suggested that auxin NAA is more effective among three auxins tested. Culture medium altered with 2,4-D (17-41%) and 2, 4,5-T(19-41%) produced shoot (s) in lower to moderate frequencies, number (s) of shoot(s) per responding explant in range of 1.94 - 3.20 with 2,4-D and 1.10-1.74 with 2,4,5-T and mean shoot length with 2,4-D in range of 1.23 cm to 1.72 cm and 1.08 cm to 1.25 cm with 2,4,5-T depending upon concentrations.

In vitro response of different added cytokinins is presented in the Table 3. Varying concentrations of BAP, Kinetin and TDZ supplemented in the medium exhibited higher *in vitro* response as compared to culture media fortified with auxins alone. For shoot proliferating ability, culture media WH.3Td (94.37%), WH.4Td (93.44%) and WH.5Td (92.85%) with *at par* performance proved remarkably superior as compared to other media combinations fortified with BAP or Kn. Culture medium fortified with BAP as sole proliferated shoot (s) in range of 69-89% and kinetin (64-74%) in comparative lower frequencies. Higher number (s) of shoot(s) per explant was exhibited by culture media WH.3d (7.75) followed by WH3B (6.77).The shootlets of higher length were recovered from culture media fortified with TDZ irrespective of all tested concentration in range of 3.23- 3.63cm.

Regeneration of multiple shoots from diverse explants cultures of tuberose has been reported earlier in response to cytokinins BA (Khan *et al*.., 2000; Nazneen *et al*., 2003; Mishra *et al*., 2006; Kadam *et al*.,2009), Kn (Bose *et al*., 1987; Rajasekaran *et al*., 2000; Kadam *et al*.,2009) and TDZ (Hutchinson *et al*., 2004; Kadam *et al*.,2009). Both adenine (BA and Kn) and phenyl urea derivatives (TDZ) of cytokinins were used in the present study for shoot regeneration. In present investigation, culture media such as WH.3Td, WH.4Td and WH.5Td fortified with lower concentration of TDZ were responded well (more than 90% explants proliferated shoots) suggested that a lower concentration of TDZ is required for this purpose. TDZ was found more effective at lower concentrations (0.1-0.5 mg.l^{-1}) as compared to BA and Kn (they were found more effective at the concentration ranging from 2.0 -3.0 mg.l^{-1}). The concentration of BAP above 4.0 mg.l^{-1} were not found effective for inducing higher number of proliferated shoots and length of shoots were

inhibited due to inhibitory effect of BAP which result into bushy appearance due to development of excessive achlorophyllous tissues at base. The results are in conformity with Mishra *et al*. (2006). Effectiveness of TDZ at lower level perhaps, was due to induced accumulation of endogenous cytokinins, as reported earlier by Murthy *et al*. (1995). TDZ also affected auxin transport in hypocotyl tissues of *Pelargonium* and others (Murch and Saxena, 2001) and promoted regeneration frequency by altering the levels of abscisic acid, ethylene and perhaps proline (Murch and Saxena 2001). In the present study, TDZ was found to be unresponsive when supplemented into media at the concentration beyond to 2.0 $mg.l^{-1}$. This finding is in accordance with the findings of Huetteman and Preece (1993) who also reported that TDZ at higher concentrations inhibit shoot elongation in many species. Similar experiences were addressed by Hutchinson *et al*. (2004) and Kadam *et al*. (2009) in tuberose. However, the exact mechanism of TDZ induced shoot proliferation in plants is not all that clear.

Low to moderate results from initial studies to establish plant growth regulator type and concentrations revealed that auxins as well as cytokinins alone are not effective for achieving higher *in vitro* response. Therefore, combinations of an auxin and a cytokinin were considered for achieving the best *in vitro* response.

Combined effects of different added auxins and cytokinins in varying concentrations on various culture phases are presented in Table 4. Inoculation media WH.3Td.5N (98.90%) followed by two similar responding culture media WH.5Td.5N (94.95%) and WHTd.5N (94.18%) proved remarkably superior for shoot proliferating efficiency and number (s) of shoot (s) per explant.

During the present study, medium supplemented with NAA in combination with cytokinins (TDZ or BA) has shown to promote shoot bud differentiation. Similar results were reported by Mishra *et al*. (2006), Jyothi *et al*. (2008) and Kadam *et al*. (2009) for bulb scale culture of tuberose. The quality of shoots and the overall growth response was better in these growth regulators combinations. A comparatively lower response was recorded when cytokinins was used separately in the medium. Much lower results were documented with supplementation of auxins alone into medium. A review of literatures indicates that addition of either 2,4-D or NAA in the culture medium improved the response in a number of species including tuberose in terms of overall shoot growth. We observed that 0.3 $mg.l^{-1}$ TDZ solely in WH medium proliferated maximum shoots (94.37%) and higher shoots per explant (7.75). On the other hand, addition of 0.5 mg. l^{-1} NAA and 0.3 mg. l^{-1}TDZ elevated all

Table 1. Effects of different surface sterilizing and antifungal agents on recovery of aseptic culture in tuberose.

Treatments	Concent-ration (%)	Exposure Time	Aseptic culture (%)	Survival of explants (%)
$Ca(OCl)_2$	10	10	**12.19^{k}**	**23.17^{m}**
$Ca(OCl)_2$	10	15	**13.15^{k}**	**31.31^{jk}**
$Ca(OCl)_2$	10	20	**33.22^{h}**	**42.28^{h}**
$Ca(OCl)_2$	15	10	**25.31^{j}**	**47.22^{g}**
$Ca(OCl)_2$	15	15	**29.41^{I}**	**54.30^{f}**
$Ca(OCl)_2$	15	20	**35.39^{h}**	**62.17^{d}**
$Ca(OCl)_2$	20	10	**33.11^{h}**	**33.29^{ij}**
$Ca(OCl)_2$	20	15	**40.27^{f}**	**46.29^{g}**
$Ca(OCl)_2$	20	20	**45.19^{e}**	**25.21^{lm}**
$HgCl_2$	0.1	5	**60.17^{c}**	**68.31^{b}**
$HgCl_2$	0.1	10	**46.14^{e}**	**66.18^{bc}**
$HgCl_2$	0.2	5	**46.26^{e}**	**64.26^{cd}**
$HgCl_2$	0.2	10	**36.28^{gh}**	**59.33^{e}**
Bavistin +$Ca(OCl)_2$	1+10	20	**39.23^{fg}**	**48.10^{g}**
Bavistin+ $Ca(OCl)_2$	1+15	20	**48.28^{de}**	**59.13^{e}**
Bavistin +$Ca(OCl)_2$	1+20	20	**51.12^{d}**	**26.07^{l}**
Bavistin+ $HgCl_2$	1+0.1	10	**78.36^{a}**	**73.36^{a}**
Bavistin+ $HgCl_2$	1+0.2	10	**75.30^{ab}**	**63.15^{d}**
Bavistin + $HgCl_2$	2+0.1	10	**73.16^{b}**	**35.40^{hI}**
Bavistin+ $HgCl_2$	2+0.2	10	**63.14^{c}**	**29.27^{k}**
Mean			44.23	47.89
CD (0.05)			3.29	2.93

$Ca(OCl)_2$: Calcium hypochlorite, $HgCl_2$: Mercuric chloride
Values within column followed by different letters are significantly different at 5% probability level.

the *in vitro* responses considerably. Maximum shoot proliferating ability (98.90%) and higher numbers of shoots per explant (11.48) have been achieved with the combined application of auxin and cytokinin. Much lower results were documented with application of 0.5 $mg.l^{-1}$ NAA (alone) in the medium. The Present study also pointed out the synergistic effect of both auxins (NAA) and cytokinin (BAP) favors profuse growth of multiple shoots. Recovery of shoots in higher numbers on medium supplemented with combination of an auxin with a cytokinin as compared to supplementation of an auxin as well as a cytokinin

separately perhaps due to occurrence of direct as well as indirect organogenic mode of regeneration concurrently.

Shoot with higher length were attained on culture media WH4N.5B (5.81cm), WH3N.5Td (5.56 cm) and WH5N.5B (5.27 cm) with *at par* performance. For mean shoot length, culture media WH4N.5B and WH3N.5Td (containing a higher concentration of an auxin in combination with lower concentration of cytokinins) performed better as compared to nutrient media WH5B.5N and WHTd.5N (containing a higher concentration of cytokinins in combination with lower concentration of an auxin) as well as culture media fortified with auxin or cytokinin (alone) that suggested that higher concentration of an auxin in combination with a lower concentration of a cytokinin enhance shoot length.

During present investigation, rhizogenesis frequencies were higher when shootlets were transferring to the rooting medium. Shootlets of tuberose were transferred on twenty different fortifications of basal WH medium for induction of *in vitro* rooting (Table 5). Culture medium MS2I was found consistently superior for all culture phases, *i.e*. root proliferating efficiency (96.24%), number of root (s) per responding shootlets (14.20), and average root length (7.56 cm). In general, auxins like IBA, IAA as well as NAA were effective for inducing *in vitro* rooting. In tuberose, IBA (Rajasekaran *et al*., 2000; Krishnamurthy *et al*., 2001; Mishra *et al*., 2006; Kadam *et al*. 2009) and NAA (Nazneen *et al*., 2003; Mishra *et al*., 2006) were found effective for inducing *in vitro* rooting. In present research, full strength WH medium supplemented with 2.0 $mg.l^{-1}$ IBA was found to be optimum for inducing *in vitro rooting*. The results clearly indicated that rooting of *in vitro* shoots of tuberose required lower to moderate concentrations of IBA responded better as compared to culture media fortified with NAA, Kn or TDZ. Medium supplemented with higher auxins enhanced the intensity of callus induction, while, the number of roots produced per shoot and root growth reduced considerably. Similar response has also been addressed by Mishra *et al*. (2006) in tuberose. Auxins promoted adventitious root development on intact plants as well as excised stems. Among auxins, IBA was the most effective than any other synthetic auxins in the most of the cases apparently because it is not destroyed by IAA oxidase or other enzymes and therefore persists longer. These results are in conformity with the earlier findings of Rajasekaran *et al*. (2000), Krishnamurthy *et al*. (2001), Mishra *et al*. (2006) and Kadam *et al*. (2009) for *in vitro* rooting response of tuberose.

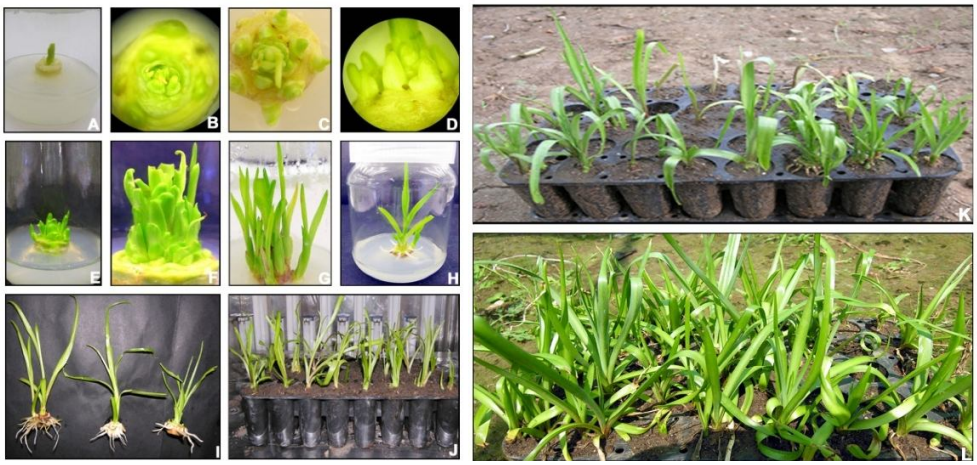

Fig. 1. Plant regeneration from stem discs: **A.** Stem disc after 2 days in culture; **B.** Initiation of multiple shoots after 4 days; **C.** Initiation of multiple shoots after 7 days; **D.** Initiation of multiple shoots after 10 days; **E.** Proliferation of multiple shoots after 10-15 days; **F.** Proliferation of multiple shoots after 15-20 days; **G.** Formation of multiple shoots after 20-25 days; **H.** Gammo-rhizogeensis *in vitro*; **I**. Rooted shootlets; **J**. Regnerants transferred in Environmental Growth Chamber after 40-45 days; **K.** Regnerants transferred in Net House after 50-55 days of rooting and **L**. Regnerants transferred in Field after 60-70 days.

In terms of the genotypic response to *in vitro* culture considerable variability was observed among four cultivars (Table 2-4). Genotype Mexican Single was found consistently superior to others for the most of attributes investigated. Genotype Mexican Single proliferated shoot(s) in higher frequencies (82.80%), number of shoots (6.20) and shootlet of higher length (5.63). The lowest performance was exhibited by genotype Shirngar. However, genotypes Phule Rajni and Prajwal performed in between them. In various other investigations on tuberose genotypic differences have also been observed for various explant cultures by Jyothi *et al.* (2008) and Kadam *et al*. (2009).
During present investigations interactions of genotype with culture medium also varied considerably. More than 92% explants proliferated shoots from all genotypes on either culture media WHTd.5N or on WH.5Td.5N/ WH.3Td.5N/ WH.2Td.5N. Interactions of all cultivars with medium also exhibited similar results for number (s) of shoot per responding explant, either culture medium WH2Td.5N or WHTd.5N or both were found more responsive (More than 9 shoot (s) were initiated) from all the genotypes. This trend was also followed by various genotypes on different nutrient media for mean shoot length, where, either culture medium WH4N.5B or WH3N.5Td or both induced shoots of more than 5 cm long. This reveals that in addition to different responses of genotype for culture medium, specific genotype does not necessarily respond in

the similar manner to each of diverse culture media tested. This suggests that a particular genotype selected for advance work can be cultured on the most suitable medium to obtain maximum response. Also, the possibility exists for improvement of *in vitro* efficiency of a particular genotype by further modifying the culture medium.

During present study, it was shown that under appropriate conditions stem disc explant cultures of tuberose as other flower crops gave rise to higher number of shoots *via* direct organogenesis. So this route of morphogenesis can be used to obtain multiple plants. The obtained regeneration frequency and survival rate were higher than that described by earlier findings, which has immediate potential for breeding and biotechnological studies.

Table 2. Effects of different auxins (alone) in varying concentrations on *in vitro* response of stem disc cultures in tuberose.

Culture media	Genotypes														
	Shoot proliferating explants (%)					No. of shoot (s) per responding explant					Mean shoot length (in cm)				
	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean
WH.1D	30.06	36.26	34.12	28.20	32.16l	1.25	2.86	1.99	1.66	1.94c	1.15	1.58	1.36	1.47	1.39c
WH.5D	35.63	41.55	39.53	33.65	37.59j	1.38	3.09	2.50	1.48	2.11c	1.24	1.54	1.39	1.46	1.40bc
WHD	39.13	45.25	43.17	37.21	41.19h	1.33	2.94	2.91	1.77	2.23c	1.17	2.07	1.62	1.84	1.67b
WH2D	29.43	48.98	46.99	40.91	41.57h	3.15	4.15	2.36	3.16	3.20b	1.35	2.02	1.68	1.85	1.72ab
WH3D	25.83	31.96	29.83	23.97	27.89o	1.38	3.19	2.67	1.49	2.21c	1.20	1.52	1.36	1.44	1.38c
WH4D	17.48	23.56	21.43	15.61	19.52p	1.29	3.30	2.59	1.02	2.05c	1.18	1.44	1.31	1.37	1.32c
WH5D	15.10	21.16	19.07	13.19	17.13q	2.11	2.50	2.83	1.40	2.21c	1.21	1.25	1.23	1.24	1.23c
WH.1N	43.34	49.30	47.46	41.42	45.38g	1.21	3.10	2.48	1.78	2.14c	1.33	2.25	1.79	2.02	1.84a
WH.5N	46.89	52.90	50.98	44.99	48.94e	3.27	4.07	4.42	3.37	3.78b	1.48	2.25	1.86	2.05	1.91a
WHN	52.62	58.60	56.78	50.78	54.69c	3.56	4.35	4.12	3.47	3.87b	1.48	2.36	1.92	2.14	1.97a
WH2N	55.10	61.20	59.12	53.22	57.16b	3.97	4.21	4.34	3.30	3.97ab	1.58	2.31	1.94	2.12	1.98a
WH3N	58.57	63.63	61.75	55.78	59.93a	4.13	4.30	4.11	3.42	3.99a	1.53	2.45	1.99	2.22	2.04a
WH4N	49.68	55.80	53.72	47.84	51.76d	3.39	4.41	4.10	3.32	3.80b	1.35	2.19	1.77	1.98	1.82a
WH5N	45.02	51.08	49.00	43.06	47.04f	2.98	4.42	4.25	2.38	3.50b	1.42	2.11	1.76	1.93	1.80a
WH.1T	26.64	32.78	30.60	24.74	28.69n	0.82	1.85	1.45	0.31	1.10d	1.09	1.27	1.18	1.22	1.19c
WH.5T	28.02	34.10	32.25	26.28	30.16m	1.45	1.62	1.36	1.21	1.41d	1.10	1.33	1.21	1.27	1.22c
WHT	32.04	38.08	36.22	30.26	34.15k	1.58	1.78	1.10	1.32	1.44d	1.08	1.35	1.21	1.28	1.23c
WH2T	37.06	43.09	41.21	35.24	39.15i	2.11	1.76	1.50	1.30	1.74cd	1.10	1.36	1.23	1.29	1.24c
WH3T	39.66	45.78	43.70	37.82	41.74h	1.50	1.86	1.51	1.49	1.51d	1.06	1.41	1.23	1.32	1.25c
WH4T	26.45	32.58	30.50	24.63	28.54no	1.44	1.63	1.38	1.30	1.43d	1.16	1.21	1.18	1.19	1.18c
WH5T	17.02	23.92	20.98	16.08	19.50p	1.36	1.48	1.39	1.28	1.37d	1.01	1.15	1.08	1.11	1.08c
Mean	35.77c	42.45a	40.40b	34.51c		2.12c	2.99a	2.63b	1.96c		1.25c	1.73a	1.49b	1.61ab	
CD (0.05)															
Genotypes					1.68					0.33					0.17
Media					0.74					0.77					0.39
G x M					NS					1.55					0.79

Values within column followed by different letters are significantly different at 5% probability level.

Table 3. Effects of different cytokinins (alone) in varying concentrations on *in vitro* response of stem disc cultures in tuberose.

Culture media	Genotypes														
	Shoot proliferating explants (%)					No. of shoot (s) per responding explant					Mean shoot length (in cm)				
	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean
WH.1B	69.33	70.27	69.16	68.06	69.20i	2.77	2.95	2.79	2.87	2.84i	1.91	2.25	2.14	1.29	1.89d
WH.5B	71.45	75.47	74.06	70.09	72.76gh	2.80	2.98	2.86	2.92	2.89i	1.88	3.00	1.99	1.95	2.20cd
WHB	77.98	80.03	79.29	77.08	78.59f	3.73	3.86	3.77	3.81	3.79gh	2.44	3.21	2.45	1.49	2.39c
WH2B	84.03	88.33	85.02	83.13	85.12de	4.78	4.93	4.81	4.87	4.84f	1.84	2.75	3.16	2.04	2.44c
WH3B	87.48	92.42	89.46	86.16	88.88b	6.71	6.84	6.76	6.80	6.77b	2.17	3.86	2.34	1.43	2.45bc
WH4B	82.23	87.26	83.01	80.43	83.23e	4.97	5.15	5.05	5.10	5.06de	1.48	2.91	2.58	2.65	2.40c
WH5B	77.49	82.37	80.27	76.07	79.05f	4.48	4.68	4.55	4.61	4.58f	2.28	2.86	2.53	1.61	2.32c
WH.1Kn	64.38	69.49	66.23	62.38	65.62kl	2.39	2.59	2.44	2.51	2.48i	1.52	1.55	1.46	1.23	1.44d
WH.5Kn	67.16	71.47	68.02	65.17	67.95ij	2.41	2.62	2.53	2.57	2.53i	1.43	1.60	1.52	1.41	1.49d
WHKn	74.25	74.37	71.48	68.48	72.14h	2.46	2.67	2.56	2.61	2.57i	1.59	1.64	1.43	1.46	1.53d
WH2Kn	70.54	76.19	73.36	68.03	72.03h	3.69	3.82	3.72	3.77	3.75h	1.59	1.64	1.57	1.37	1.54d
WH3Kn	70.98	79.18	76.04	71.17	74.31g	4.82	4.96	4.88	4.92	4.89ef	1.61	1.70	1.65	1.64	1.65d
WH4Kn	67.27	71.43	67.19	62.19	67.02jk	4.58	4.72	4.60	4.66	4.64f	1.32	1.57	1.50	1.50	1.47d
WH5Kn	63.32	68.37	64.43	61.43	64.38l	2.51	2.66	2.54	2.60	2.57i	1.43	1.51	1.57	1.31	1.45d
WH.1Td	82.30	89.73	86.52	79.19	84.43e	3.50	3.65	3.58	3.61	3.58h	3.33	3.97	3.40	2.24	3.23a
WH.2Td	85.48	91.27	88.33	84.16	87.31c	3.92	4.14	3.98	4.06	4.02g	3.31	4.11	3.98	1.74	3.28a
WH.3Td	94.04	95.18	96.04	92.23	94.37a	7.66	7.87	7.71	7.79	7.75a	3.35	3.96	4.20	2.30	3.45a
WH.4Td	91.29	97.52	94.49	90.49	93.44a	6.10	6.23	6.14	6.18	6.16c	4.00	4.13	4.01	2.37	3.63a
WH.5Td	91.23	96.79	93.05	90.35	92.85a	5.26	5.38	5.41	5.39	5.36d	4.06	4.00	4.11	2.35	3.63a
WHTd	86.07	91.37	88.42	85.03	87.72bc	4.80	4.95	4.85	4.90	4.87f	4.11	4.00	3.95	2.10	3.54a
WH2Td	84.14	90.43	87.45	81.28	85.82cd	3.60	3.73	3.68	3.70	3.67h	3.25	3.98	3.36	2.21	3.20b
Mean	78.21c	82.80a	80.06b	76.31d		4.18a	4.35a	4.24a	4.29a		2.37b	2.86a	2.61ab	1.79c	
CD (0.05)															
Genotypes					0.88					0.19					0.35
Media					2.02					0.43					0.82
G x M					4.05					NS					1.64

Values within column followed by different letters are significantly different at 5% probability level.

Table 4. Combined effects of different auxins and cytokinins on *in vitro* response for cultured dtem disc in tuberose.

Culture media	Genotypes														
	Shoot proliferating explants (%)					No. of shoot (s) per responding explant					Mean shoot length (in cm)				
	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean
WH.1Td.5N	87.21	90.09	89.19	86.14	**88.15^{d}**	5.86	6.54	6.40	5.80	**6.15^{d}**	5.04	4.54	4.11	5.25	**4.73^{b}**
WH.2Td.5N	91.52	94.68	93.56	90.60	**92.59^{c}**	5.84	6.55	6.88	5.80	**6.26^{d}**	4.79	4.26	5.05	5.09	**4.79^{b}**
WH.3Td.5N	98.84	99.04	98.93	98.82	**98.90^{a}**	10.60	12.24	12.12	10.98	**11.48^{a}**	4.60	4.55	5.11	5.04	**4.82^{b}**
WH.5Td.5N	94.90	95.09	94.96	94.85	**94.95^{b}**	9.89	9.49	11.29	9.86	**10.13^{b}**	4.59	4.28	6.03	4.58	**4.87^{b}**
WHTd.5N	94.13	96.20	94.23	92.16	**94.18^{b}**	8.90	9.50	9.28	8.43	**9.02^{bc}**	5.16	4.53	4.71	5.23	**4.90^{b}**
WH2Td.5N	79.49	84.51	82.51	75.59	**80.52^{f}**	7.58	8.69	9.02	8.02	**8.32^{c}**	4.94	4.78	6.39	4.82	**5.23^{b}**
WHN.5Td	57.73	63.65	70.24	55.76	**61.84^{n}**	6.31	7.01	5.86	6.29	**6.36^{d}**	3.81	6.77	4.86	4.75	**5.04^{b}**
WH2N.5Td	63.68	69.89	61.57	61.70	**64.21^{lm}**	4.62	5.10	5.54	3.84	**4.77^{e}**	3.89	6.79	5.75	4.39	**5.20^{b}**
WH3N.5Td	70.72	76.66	71.00	68.75	**71.78^{i}**	5.03	5.12	5.58	4.91	**5.16^{e}**	4.27	7.28	5.44	5.27	**5.56^{a}**
WH4N.5Td	67.04	73.17	67.82	65.08	**68.27^{k}**	5.46	4.99	4.29	3.89	**4.65^{e}**	3.97	6.84	5.92	3.87	**5.15^{b}**
WH5N.5Td	62.24	68.60	74.63	60.18	**66.41^{l}**	5.27	4.46	4.33	4.22	**4.57^{e}**	4.48	6.66	5.64	3.65	**5.10^{b}**
WH.5B.5N	68.10	74.20	72.07	66.23	**70.15^{j}**	5.36	4.38	3.94	4.15	**4.45^{e}**	3.99	5.00	7.17	3.90	**4.98^{b}**
WHB.5N	74.66	80.82	78.80	72.68	**76.74^{h}**	5.32	5.30	3.75	4.15	**4.63^{e}**	3.96	5.59	6.78	4.07	**5.10^{b}**
WH2B.5N	76.60	82.48	80.54	74.49	**78.52^{g}**	5.42	5.09	3.98	4.37	**4.71^{e}**	4.79	7.04	7.04	4.18	**5.53^{a}**
WH3B.5N	80.62	86.79	84.59	78.77	**82.69^{e}**	8.62	8.10	8.06	7.52	**8.07^{c}**	4.07	6.45	6.11	4.45	**5.53^{a}**
WH4B.5N	78.22	84.08	84.64	76.11	**80.76^{f}**	6.39	5.47	5.91	5.81	**5.89^{d}**	3.78	6.21	7.16	4.28	**5.36^{a}**
WH5B.5N	66.07	72.23	82.19	64.06	**71.13^{ij}**	5.22	5.80	4.76	4.62	**5.10^{e}**	3.64	5.71	4.78	3.42	**4.39^{b}**
WHN.5B	53.11	59.15	57.09	51.17	**55.13^{p}**	5.32	5.49	4.91	5.27	**5.24^{de}**	4.46	4.70	4.60	5.61	**4.84^{b}**
WH2N.5B	56.36	62.44	60.32	54.40	**58.38^{o}**	4.11	4.79	3.69	4.09	**4.17^{ef}**	4.32	4.62	4.95	5.48	**4.84^{b}**
WH3N.5B	60.99	66.89	64.90	58.88	**62.91^{mn}**	4.20	4.81	3.77	3.65	**4.10^{f}**	5.24	5.17	4.90	4.21	**4.88^{b}**
WH4N.5B	52.76	58.62	56.78	50.60	**54.69^{p}**	3.26	3.90	2.41	3.22	**3.19^{f}**	6.79	6.10	5.05	5.31	**5.81^{a}**
WH5N.5B	49.20	55.12	53.09	47.24	**51.16^{q}**	3.08	3.69	2.30	2.98	**3.01^{f}**	6.42	5.98	4.55	4.13	**5.27^{ab}**
Mean	**72.00^{c}**	**77.01^{a}**	**76.07^{b}**	**70.19^{d}**		**5.98^{ab}**	**6.20^{a}**	**5.82^{b}**	**5.53^{b}**		**4.59^{b}**	**5.63^{a}**	**5.55^{a}**	**4.59^{b}**	
CD (0.05)															
Genotypes					**0.64**					**0.50**					**0.38**
Media					**1.49**					**1.17**					**0.90**
G x M					**2.98**					**NS**					**1.80**

Values within column followed by different letters are significantly different at 5% probability level.

Table 5. Effects of different plant growth regulators on *in vitro* rooting of shootlets in tuberose.

Culture media	Genotypes														
	Root proliferating shootlets (%)					No. of root (s) per shootlet					Mean root length (in cm)				
	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean	Phule Rajni	Mexican Single	Prajwal	Shringar	Mean
WH.5I	89.73	93.25	91.49	92.37	**91.71b**	09.59	14.95	10.29	07.55	**10.59b**	07.10	08.21	07.02	04.99	**6.83b**
WHI	90.60	95.01	92.80	93.90	**93.08b**	10.67	14.71	11.87	09.16	**11.60b**	06.62	09.38	07.15	05.60	**7.18ab**
WH2I	94.90	97.29	96.09	96.69	**96.24a**	14.62	17.58	13.26	11.35	**14.20a**	06.13	10.21	08.53	05.38	**7.56a**
WH3I	89.95	92.92	91.43	92.17	**91.62b**	09.47	13.88	10.88	05.97	**10.05bc**	06.24	09.58	07.13	05.53	**7.12b**
WH4I	85.54	89.59	87.56	88.57	**87.81c**	06.47	11.41	08.15	06.23	**08.06d**	06.58	08.48	07.23	04.87	**6.79b**
WH5I	86.99	88.11	87.55	87.83	**87.62c**	05.80	09.57	08.20	02.75	**06.58e**	06.38	07.94	06.99	05.41	**6.68b**
WH.5N	67.77	78.12	72.94	75.53	**73.59i**	04.77	09.17	08.10	04.11	**06.53e**	03.95	07.86	07.11	03.56	**5.62c**
WHN	67.34	81.84	74.59	78.21	**75.49hi**	04.48	09.68	07.69	04.30	**06.53e**	05.72	07.47	06.18	03.59	**5.74c**
WH2N	73.57	83.31	78.44	80.87	**79.05f**	04.26	10.10	08.67	03.69	**06.68e**	04.14	08.44	07.14	03.60	**5.83c**
WH3N	84.9	86.97	85.93	86.40	**86.06c**	07.95	08.52	08.78	07.85	**08.27cd**	04.10	08.43	08.11	03.00	**5.91c**
WH4N	69.05	85.03	77.04	81.03	**78.03fg**	02.80	12.85	10.35	01.48	**06.87d**	04.59	08.25	07.69	02.67	**5.80c**
WH5N	73.69	77.92	75.80	76.86	**76.06gh**	05.85	08.71	07.49	04.71	**06.69de**	03.41	08.30	07.80	03.25	**5.69c**
WH.5kn	69.81	71.37	70.59	70.98	**70.68jk**	03.62	06.46	05.80	03.83	**04.93e**	03.41	06.29	06.01	02.41	**4.53d**
WHKn	66.56	74.19	70.37	72.28	**70.85j**	03.44	07.19	06.33	02.38	**04.83e**	03.85	06.21	05.44	02.94	**4.61d**
WH2Kn	81.10	83.21	82.10	82.68	**82.28d**	05.89	08.62	08.77	04.69	**06.99d**	04.84	08.45	07.85	04.58	**6.43bc**
WH3Kn	67.83	69.46	68.60	69.05	**68.74k**	03.68	06.65	07.97	01.77	**05.02e**	03.83	06.28	04.98	03.59	**4.67d**
WH.1Td	76.89	78.14	77.51	77.82	**77.59g**	05.88	06.79	08.21	04.69	**06.39e**	04.26	09.56	08.21	04.13	**6.54b**
WH.2Td	78.54	80.63	79.58	80.10	**79.71ef**	04.25	11.26	09.52	02.88	**06.97d**	03.59	10.58	08.79	03.52	**6.62b**
WH.5Td	80.79	82.07	81.43	81.75	**81.51de**	07.78	07.01	07.47	07.58	**07.46d**	04.15	10.11	09.25	03.49	**6.75b**
WHTd	72.24	87.77	80.00	83.88	**80.97e**	06.69	08.09	09.41	03.21	**06.85d**	05.26	08.80	07.23	05.11	**6.60b**
Mean	78.39d	83.81a	81.10c	82.45b		06.40c	10.16a	08.86b	05.00d		04.90c	08.44a	07.29b	04.06d	
CD (0.05)															
Genotypes						0.93				0.84					0.36
Media						2.08				1.89					0.81
G x M						4.16				3.79					1.61

Values within column followed by different letters are significantly different at 5% probability level.

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