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Genotype specific shoots regeneration from different explants of tomato (*Solanum lycopersicum* L.) using TDZ

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ABSTRACT

Thidiazuron (TDZ) is among the most active cytokinin like substances and induces greater in vitro shoot proliferation than other cytokinins used in plant. Stem node and cotyledonary node explants of 2 extensively cultivated Tomato cultivars - MHTM and Shalimar were cultured on Murashige and Skoog (MS) media supplemented with various concentrations of TDZ. The present study was conducted to develop a rapid and efficient, genotypic specific shoot regeneration system suitable for the transformation of tomato (Solanum lycopersicum L.) using TDZ. Cotyledonary nodes and leaf nodes after the initial callus stage regenerated prolific adventitious shoots via organogenesis. Cotyledonary nodes showed a higher shoot formation capacity than stem nodes. MS medium supplemented with 3.40µM TDZ produced the highest frequency of shoot formation from cotyledonary nodes in both genotypes. Regenerated shoots were rooted on MS medium with and without IBA. Rooted plants were finally transferred to soil in pots.

Key Words: Thidiazuron, *in vitro*, shoot regeneration, cotyledonary node.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a major vegetable crop that has achieved tremendous popularity over the last century [Bhatia et al, 2004] and is a model species for introduction of agronomically important genes into dicotyledonous crop plants [Wing et al, 1994]. Establishment of an efficient tissue culture protocol is an essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement. Efficient plantlet regeneration in tomato was reported from meristems [Mirgis et al, 1995], leaf [Behki and Lesley 1976, Kartha et al, 1976., Padmanabhan et al, 1974] stems, anthers [Zamir et al, 1990] and hypocotyls [Ohki et al, 1978]. *In vitro* regeneration through organogenesis and somatic embryogenesis can be used

for multiplication of genetically identical clones and it is an integral part of genetic transformation procedures. Kumar et al., [2011] [30] has studied the importance of in vitro regeneration for aloe vera using cytokinins and auxins. Thidiazuron (TDZ), a substituted phenyl urea, is known to regulate varied morphogenetic responses, such as breaking of bud dormancy in apple [Steffens and Stutte, 1989] regeneration and multiple shoot formation in soybean [Shan et al, 2005] and somatic embryogenesis in peanut and African violet respectively [Murthy,1995 and Mithila et al, 2003].

A number of studies have reported that Thidiazuron is more potent than BA in *in vitro* regeneration of shoots in dicotyledonous species [Singh et al, 2003]. A study on cotton showed that addition of TDZ and silver nitrate to shoot initiation medium increased the number of multiple shoots formed on the proximal end of hypocotyls explants [Ouma et al, 2004]. Although, some information is available on the morphogenesis of tomato, the techniques have not been developed to a level at which they can be utilized in large-scale multiplication of commercially important cultivars. Despite the potential and vast amount of the research undertaken on this subject, plant tissue culture has not become an integral part of tomato-breeding programme [Bhatia et al, 2004]. The effect of TDZ on any explants of tomato has not been studied so far. The objective of this study was to optimize the protocol for rapid and simple genotypic specific shoot regeneration from cotyledonary nodes and leaf nodes of popular tomato cultivars based on Murashige and Skoog basal medium with B5 vitamins and using Thidiazuron and also to compare it with the regeneration results produced by other two important cytokinins – BAP and Kinetin. Kumar et al., [2011] [31] has studied the effect of hormones on shoot induction in *Solanum trilobatum* using different hormonal combinations.

MATERIALS AND METHODS

The Tomato seed Cultivar - MHTM were obtained from the commercial seed shop, Coimbatore and Cultivar - Shalimar was obtained from DIHAR, Leh, India. Surface-sterilization was performed by immersion of seeds into a solution of 0.1% (w/v) mercuric chloride for 3 min and then by four rinses in sterile distilled water. Thereafter, the seeds were allowed to germinate in tissue culture tubes containing 10mL of half-strength MS medium containing the MS salts [Murashige and Skoog, 1962], 100mg L^{-1} myoinositol, 2mg L^{-1} thiamine-HCl, 0.5mg L^{-1} pyridoxine-HCl, 0.5mg L^{-1} nicotinic acid, and 2% (w/v) sucrose. The regeneration medium was solidified with 0.6% (w/v) agar. Cultures were cultivated initially for two days in dark at 18 ±1°C temperature and then they were maintained under photoperiod of 16h illumination with light intensity of 50µmol m^{-2} s^{-1} (25°C) and 8h dark (20°C).

Cotyledonary node explants were excised from 2-3cm long 2 week-old seedlings and stem nodes were obtained from 6 week old plant lets. The explants were cultured on MS, 3% sucrose, 0.7% agar and 1.13, 2.27, 3.40, 4.54 and 9.08µM TDZ, 4.44 and 8.88 µM BAP (Table 1) and 9.3, 23.25 and 46.5 µM Kinetin along with 11.42 µM IAA. A Stock solution of 4.54 mM TDZ was prepared either by using dimethyl sulphoxide (DMSO) or 50% ethanol as solvent.

The media were adjusted to pH 5.8 prior to autoclaving and 10mL of these media were dispensed into each of culture tubes. Regeneration of explants was assessed after six weeks. The following

parameters were evaluated: the frequency of regeneration (No. of regenerating explants/No. of plated explants) X 100 and the number of shoots and shoot primordia/explant plated. The experiments were repeated two times and data were analysed at 5% significance level using Duncan's Multiple Range Test.

Rooted plantlets were acclimatized in growth cabinets under relative humidity of 90% during the first 7 days, which was decreased gradually thereafter to 40%, until they were established in a greenhouse. A control was planted without treatment both for shoot regeneration and rooting.

RESULTS AND DISCUSSION

In vitro regeneration of two tomato cultivars, 'Shalimar' and 'MHTM' were standardized up to acclimatization stage. Figures **1-5** show different stages of *in vitro* development of tomato plant for MHTM cultivar and figures **6-10** shows the corresponding developmental stages of cultivar 'Shalimar'. **Table 1** and **table 2** show the results of *in vitro* response of 'MHTM' and 'Shalimar' cultivars respectively. *In vitro* seed germination was successful with sterile cotton soaked with ½ MS liquid within 10 days of inoculation (**fig.1 & fig.6**). Shoot formation from the cotyledonary node explants were obtained with an average 20 days time period (**fig. 2 & fig.7**). Shoot elongation and rooting were completed within 20 days after shoot formation (**fig.3 & fig. 8**). Plant let acclimatization in soil: organic manure, 1:1 mixture were done first in paper tea cups and then transferred to clay pots (**fig.4, 5 & fig. 9, 10**). Significant differences among the genotypes, explants and media for frequency of culture response and number of shots per explant were observed. Genotypic differences were not observed for day to shoot initiation, in case of Tomato cultivars tested, even though earlier workers have reported such differences [Lalage 2008]. Genotypic differences with respect to shoot regeneration from tomato callus have been reported earlier [Lalage et al 2008., Locy 1983.,Garcia and Luque 1988., El-Farash 1993 and Selvi, 1993] . Between the two explants tried (**Table 1& 2**) the cotyledonary node regenerated more number of shoots per explant than stem node in both the cultivars. Such inter – explant differences for shoot regeneration in tomato have been recognized by earlier workers [Locy, 1983., El-Farash et al, 1993., Selvi, 1993., Lu et al 1997 and Moghaleb et al, 1980].The regeneration efficiency obtained in our study is comparatively higher than that of earlier reporters [Lalage et al, 2008 and Muhl Bach, 1980].

TDZ is among the most active cytokinin – like substances and it induces greater *in vitro* shoot proliferation than many other cytokinins in many plant species. It is very soluble in DMSO with slight solubility in water [Noram technical bulletin 1987]. Tomato is one of the most studied higher plants because of its importance as a crop species, and of several advantages for genetic, molecular and physiological studies [Mc Cormick et al, 1986].

We found that cotyledonary node explants were more responsive than stem nodes on all TDZ concentrations (Tables 1 and 2; $p< 0.05$). We have obtained a similar frequency of shoot regeneration from cotyledonary node explants using TDZ, which has not been reported previously. Significance difference was observed in the response of Tomato explants in various concentrations of TDZ and that of BAP or Kinetin. Lower concentrations of TDZ showed more response than 4.44 μM and 8.88 μM BAP. [Nogueira et al., 2001] observed high regeneration frequency of 92% or 85% on cotyledonary explants of tomato genotype Santa Clara or its natural

mutant Firme, respectively. Osman et al, 2010 has studied the effect of TDZ on cotyledon and hypocotyl of tomato cultivar Omdurman, with different regeneration method and combination of hormones. Our study also observed similar regeneration frequency irrespective of the cultivar and method of regeneration.

Frequency and number of shoots regenerated from 'MHTM' cultivar of tomato on MS medium supplemented with different concentrations of TDZ , BAP and Kinetin + IAA				
Hormonal concentration (µM)	Type of explants			
TDZ	Cotyledonary node		Leaf node	
	Frequency (%)	number of shoots	Frequency (%)	number of shoots
1.13	80	2.9^{c}	80	2.4^{c}
2.27	80	2.9^{c}	90	3.2^{c}
3.40	100	11.8^{a}	100	7^{a}
4.54	100	11.4^{a}	100	6.4^{a}
9.08	90	11.2^{a}	95	5.4^{b}
BAP				
4.44	75	6.8^{b}	75	4.6^{b}
8.88	85	9.6^{b}	75	6.2^{a}
Kinetin + IAA				
9.3+0	55	2.6^{c}	50	2.5^{c}
23.25+11.42	55	3^{c}	50	2.6^{c}
46.5+11.42	65	6.2^{b}	60	5.8^{a}

Table.1. Shoot regeneration from different explants of tomato genotype MHTM after 6 weeks in culture on MS medium supplemented with various concentrations of TDZ and BAP. Each value is the mean of 3 replications with 5 explants each. Values within a column followed by different letters are significantly different at the 0.05 probability level using DMRT

Frequency and number of shoots regenerated from 'Shalimar' cultivar of tomato on MS medium supplemented with different concentrations of TDZ , BAP and Kinetin + IAA				
Hormonal concentration (µM)	Type of explants			
TDZ	Cotyledonary node		Leaf node	
	Frequency (%)	Number of shoots	Frequency (%)	Number of shoots
1.13	77	2.4^{d}	75	0.6^{d}
2.27	80	2.6^{d}	78	1.4^{d}
3.40	95	11^{a}	92	5.8^{a}
4.54	95	10.4^{a}	90	6.8^{a}
9.08	90	9.4^{a}	90	6.8^{a}
BAP				
4.44	87	7.8^{b}	90	5.2^{b}
8.88	90	9.4^{a}	85	6.6^{a}
Kinetin + IAA				
9.3+0	75	3^{d}	70	2.2^{c}
23.25+11.42	80	3.4^{d}	70	3.4^{c}
46.5+11.42	85	5.8^{c}	75	4.4^{b}

Table.2. Shoot regeneration from different explants of tomato genotype Shalimar after 6 weeks in culture on MS medium supplemented with various concentrations of TDZ, BAP and Kinetin. Each value is the mean of 3 replications with 5 explants each. Values within a column followed by different letters are significantly different at the 0.05 probability level using DMRT

Fig. 1: *In vitro* germinated seedlings of tomato cv. 'MHTM'
Fig. 2: *In vitro* shoot development of tomato cv. 'MHTM'
Fig. 3: Rooting of elongated shoots in MS media with IBA, tomato cv. 'MHTM'
Fig.4: Shoot grown in paper tea cup with soil: organic manure mixture for acclimatization
Fig.5: Acclimatized plantlet transferred and grown in clay pot , cv. 'MHTM'
Fig.6: *In vitro* germinated seedlings of tomato cv. 'Shalimar'
Fig.7: *In vitro* shoot development of tomato cv. 'Shalimar'
Fig.8: Rooting of elongated shoots in MS media with IBA, tomato cv. 'Shalimar'
Fig.9: Shoot grown in paper tea cup with Soil: organic manure mixture for acclimatization
Fig.10: Acclimatized plantlet transferred and grown in clay pot, cv. 'Shalimar'.

Root induction in regenerated shoots

Earlier it was recorded that, TDZ inhibits the root formation, of regenerated shoots. Our studies has shown that even though in the half MS media the shoots did not produce root, when sub cultured on to MS+IBA 4.9 µM and MS + IBA 9.8 µM they have produced roots within one week of culture period. The shoots which where regenerated in BAP as well as Kinetin + IAA readily produced roots when cultured in plane MS media for two weeks of time. The numbers of

roots were not significantly different in either in MS basal or in MS incorporated with IBA. All the rooted plants were grown in green house on 1:1 soil - vermiculite mixture.

In all experiments with two-selected tomato cultivars, any physiological disorders or morphological abnormalities such as hyperhydricity, excessive callus formation or productions of abnormally narrow leaves were not observed during *in vitro* shoot proliferation stage. Even medium devoid of auxins irrespective of cytokinin concentration produced adventitious roots on explants due to high endogenous auxins reported in tomato [Delange et al, 1974 and Shyluk and Constable 1976] .

CONCLUSION

In conclusion, the present study underlines the importance of lower concentrations of TDZ for high shoot regeneration from stem and cotyledonary nodes of tomato by organogenesis in respect of both the genotypes. The shoot regeneration and plantlet development method described here can be successfully deployed for transgenic tomato development as well as commercial production of elite cultivars.

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