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THE EFFECT OF EXPLANT SOURCES AND GROWTH REGULATORS ON CALLUS INDUCTION AND REGENERATION IN DIFFERENT TOMATO CULTIVARS.

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ABSTRACT

Three cultivars of tomato (*Solanum lycopersicum* L.)i.e. Rio Grande, Moneymaker and Gala were evaluated for their tissue culture responses on different types of media containing different formulations of growth regulators. Among the three cultivar tested on eight different callus induction media (CIM) formulations, Rio Grande was the most responsive cultivar showing 93.3% callus induction responses on CIM1, having 1 mgL^{-1} BAP and 0.1 mgL^{-1} NAA, followed by Moneymaker with 46.67% callus induction responseson CIM7 containing 0.5 mgL^{-1} BAP, 0.5 mgL^{-1} NAA, 0.5 mgL^{-1} IAA and 1 mgL^{-1} Kinetin fromhypocotyl explants.Among the eight different media compositions, CIM1 resulted in maximum callus induction showing 34.1% and 27.23% average callus induction from hypocotyls and cotyledons' explants, respectively. Successfully proliferated callus were transferred to 7 types of shoot induction media (SIM). Shoot induction was observed on both SIM1 having 1 mgL^{-1} BAP and 0.1mgL^{-1} NAA and SIM2 having 2 mgL^{-1} BAP and 0.2 mgL^{-1} NAA but SIM1 showed maximum number of shoots primordia from calli. Calli with shoot primordia were transferred to shoot multiplication media i.e. MS media containing 2 mgL^{-1} IAA, 0.2 mgL^{-1} BAP, all the calli showed shoot multiplication. Rootinduction media i.e. MS or ½ MS media containing 2 mgL^{-1} IBA were used to induce rooting in the regenerated plantlets.Rio Grande and Moneymaker produced roots successfully and were successfully acclimatized to greenhouse after well developed rooting system.

Keywords: Solanum lycopersicum L., callus induction, regeneration, growth regulators, Auxin, Cytokinins

Abbreviations:SIM- Shoot Induction Media; RIM- Root Induction Media; MS- Murashige and Skoog medium (1962); BA-Benzyladenine; IAA-Indoleaceticacid; NAA-α-naphthaleneaceticacid.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important member of *Solanaceae* family consisting of 98 genera and300-400 species (Muller*et al.*,2005, Rick, 1980). It is a diploid species with a basic set of 12 chromosomes and a genome size of 950 Mb (Arumuganathan *et al.*, 1991). It is well-known as a highly nutritive food and major vegetable crop that has been cultivated across the globe from tropical to sub-tropical and temperate areas (Atherton and Rudich, 1986). The chemical composition of tomato fruit includes vitamins, carotenoids, polyphenols, fatty acids and cholesterol, which account for its antioxidant property(Abushita*et al.*, 2000; Raiola *et al.*, 2014; Rao and Agarwal, 1999). Itis a commonly grown vegetable crop with 160 million ton global production (FAOSTAT, 2011). Regarding biotechnological approaches, it is not only used to produce virus free and disease free plants via *in vitro* cultures but it isused as a model for genetic transformation (Ling*et al.*, 1998; Moghaleb*et al.*, 1999; Namitha and Negi, 2013; Paduchuri *et al.*, 2010). Several kinds of explants including hypocotyls, cotyledon, leaf petiole, internodes and epicotyls have been used for tomato cultivars (Gubis*et al.*,2003). Hypocotyls or cotyledonshave been used specifically in tomato to induce regeneration (Raziuddin*et al.*, 2004). Nature of explants can greatly influence the process of callus induction and regeneration (Takashina*et al.*, 1998). The regeneration potential in tomato is highly dependent on growth regulators, genotype, and type of explants used. Shoots regenerated from callus arehighly dependent on factors like type of explant, media composition, growth regulators used in media, solidifying agent, temperature, intensity of light and duration of photoperiod (Reed, 1999; Sheeja *et al.*, 2004).

Shoot apexes, nodes and root segments have been used as explant source for callus induction and regeneration (Jatoi*et al.*, 2001) with varying degrees of potential responses. The first step in plant tissue culture is callus induction which is dependent on several factors (George *et al.*, 2008). The second important step in plant tissue culture after callus induction is plant regeneration, which is influenced by several factors and is usually highly genotype dependent. Various concentrations and combinations of growth regulators have been used to initiate callus and regeneration such as BAP and NAA, IAA and Kinetin etc., in *in vitro* studies of tomato (Chen*et al.*, 1999).Keeping in view, the genotype dependency and the critical role of precise hormonal formulations for successful tissue culture responses. The present study attempts to evaluate the tissue culture responses of three different common tomato cultivars with the primary focus to identify the most promising genotype that can be utilized as a valuable tool in

subsequent tissue culture based tomato improvement programs.

MATERIALS AND METHODS

Plant material: Forsurface sterilization, seeds of selected tomato cultivars were washed with tap water three times followed by washing with distilled water. The seeds were subsequently dipped in 70% ethanol for two to three minutes. After the removal of ethanol, seeds were treated with sodium hypochlorite containing2–3 drops of Tween-20 for 5 minutes. The seeds were finally rinsed four times with sterilized distilled water and kept on Whatman No.1 sterilized filter paper for drying.

Approximately30-40 seeds were placed in sterile jars on solidified ½Xstrength MS media (Murashige and Skoog, 1962) for germination in the dark for 3 days at 22±2ºC in growth chamber and then transferred to long day conditionsi.e.16hours light and 8 hours dark, respectively.

Explants' culture: Seeds germinated after 7-9 days were ready to culture. The hypocotyls and cotyledons were cut into small pieces of about 3-4 mm segments and transferred to the callus induction media.Callus induction frequency was calculated as follows:

$$\text{Callus Induction frequency} = \frac{\text{Number of calli-producing explants}}{\text{Total number of explants cultured}} \times 100$$

Callus induction: Callus induction media was prepared using full MS media with 3% sucrose and supplemented with different concentrations of growth regulatorsat pH 5.7. In this experiment eight sets of treatments(Table 1) were applied for callus induction. Three replications per treatment were used for each cultivar with each replication containing 15 ex-plants. The ratio of growth regulators were increased from BAP 1-3 mgL^{-1} and NAA 0.1-0.3 mgL^{-1} in CIM1 to CIM3 media, while in CIM4 media BAP was replaced with 0.5 mgL^{-1} kinetin and NAA with 1 mgL^{-1} IAA (Table 1). In CIM 5 media the auxins were replaced with GA_3, whereas, CIM8 consisted of only 3 mgL^{-1} 2, 4-D. CIM6 and CIM7 consisted of BAP and kinetin in combination with NAA alone (CIM6) or NAA and IAA (CIM7).

Table 1.Concentrations of growth regulators used for callus induction.

Treatments (T)	BAP (mgL^{-1})	NAA (mgL^{-1})	IAA (mgL^{-1})	GA3 (mgL^{-1})	Kinetin (mgL^{-1})	2,4 D (mgL^{-1})
CIM1	1	0.1	-	-	-	-
CIM2	2	0.2	-	-	-	-
CIM3	3	0.3	-	-	-	-
CIM4	-	-	1	-	0.5	-
CIM5	0.5	-	-	1.5	-	-
CIM6	0.3	4	-	-	0.3	-
CIM7	0.5	0.5	0.5	-	1	-
CIM8	-	-	-	-	-	3

Shoot induction: Full MS media supplemented with sucrose 3%, BAP 0.5 mgL^{-1} along with IAA 2.5 mgL^{-1} and 0.6% agar was used for shoot and leaf multiplication. The pH of the media was adjusted to 5.7 ± 0.1. Three replications per treatment were used for each cultivar. In total 25-45 calli were initially evaluated for shoot induction and finally for each replication 7 well proliferated calli were selected for further sub-culturing.

Root induction: Root induction media was prepared by adding IBA 2 mgL^{-1}, 3% sucrose along with agar 0.6-0.7% in both half and full strength of MS media.

Growth conditions: Growth chamber temperature was set at 22 ± 2ºC with subsequent exposure to light and dark for 16 and 8 hours, respectively for the growth of plants.

Acclimatization: The plantlets with well developed roots were transferred to Jiffy pellets (http://www.jiffypot.com/) and kept in a beaker covered with plastic sheets having few holes for aeration at 22 ± 2ºC. The beakers were placed at 25ºC in growth room with 16and 8hours consecutive light and dark photoperiod. After one week, plants were transferred to green house.

Statistical analysis: Data obtained was subjected to statistical analysis. The experimental unit consisted of Completely Randomized Design (CRD) with three replications. Least Significant Difference (LSD) test was applied to look for the statistical differences among the means for different parameters. For the analysis of variance Statistix 8.1 software was used.

RESULTS

Callus induction responses of the cultivars on different callus induction media: Hypocotyls and cotyledons of 10-12 days old seedlings were cut into 3-4mm segments and subsequently transferred to eight

different callus induction media (CIM)with three replications, containing different formulations of growth regulators to check the effect of these two most commonly used explants for callus induction.

Based on the overall response of hypocotyl explants of the three cultivars on all treatment, cultivar Rio Grande showed the maximum callus induction responses(93.3%)on CIM1 formulation (Fig. 1A and Table 2). The same cultivar also showed good resultswith 60% and 53.3% callus induction responses on CIM 2 and CIM 3 media, respectively (Fig. 1C and Table 2). However, the responses of hypocotyl explants of Rio Grande on other media formulations (CIM5 to CIM8) were poor and statistically different from the first three CIM formulations. On the other hand, the cultivar Moneymaker showed fairly good callus induction response on CIM7 (46.67%), followed by CIM6 (44.44%), CIM4 (42.22%) and CIM5 (40%) when hypocotyls were used as explants. The responses of hypocotyls explants of Moneymaker were fair (28.89%) on CIM8. However, CIM1, CIM2 and CIM3 which were the best media formulations in terms of callus induction for Rio Grande, turned out to be least responsive compositions in case of cultivar Moneymaker as evident from the significantly lower callus induction responses on these three media formulations. The cultivar Gala turned out to be the least responsive cultivar which showed very low callus induction responses on CIM1-3 media and no responses at all on CIM4-8 (Table 2). Analysis of variance revealed highly significant differences ($P \leq 0.01$) among cultivars, treatments and cultivars x treatment interactions in terms of callus induction using hypocotyls as explants (Table 5).

Although, cotyledon explants showed slightly lower responses compared to hypocotyls explants, a similar pattern in terms of callus induction was exhibited in case of cultivar Rio Grande which showed 70% callus induction frequency on CIM1 (Fig. 1B and Table 3) media followed by CIM2 (60%, Fig. 1D) and CIM3 (56%) formulations (Table 3). The responses of Rio Grande on CIM4-8 were statistically different from the rest of the media formulations. Likewise, the cotyledons of Moneymaker showed 53.3%, 48.89%, 31.11% and 11.1% callus induction responses on CIM4, CIM5, CIM6 and CIM7, respectively (Table 3). Irrespective of the explants source, the cultivar Gala proved to be the least responsive cultivar as evident from the poor (CIM1-3) or no (CIM4-8) callus induction responses of the cotyledon explants in our tested media compositions. Analysis of variance revealed highly significant differences ($P \leq 0.01$) among cultivars, treatments and cultivars x treatment interactions in terms of callus induction using cotyledons as explants (Table 5).

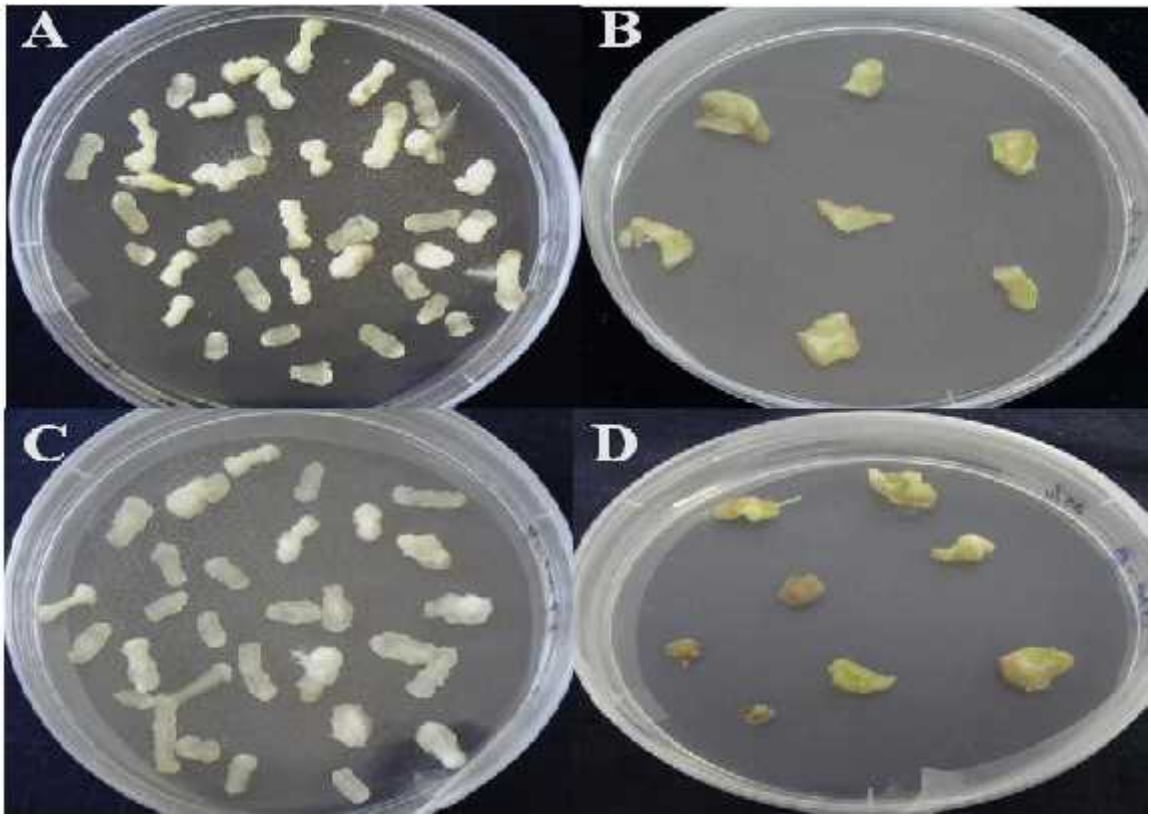

Figure 1. Callus induction on CIM 1 and CIM 2 media
A) Hypocotyls of Rio Grande; B) Cotyledons of Rio Grande showing response on CIM 1.

C) Hypocotyls of Rio Grande; D) Cotyledons of Rio Grande showing response on CIM 2.

Table 2.The response of cotyledon explants of*cv*. Rio Grande, Moneymaker and Gala on eight different formulations of growth regulators for callus induction. Least Significant Difference (LSD) values for cultivars and media compositions calculated by using statistical package statistix 8.1 were 0.7968 and 1.3012, respectively, while for interactions between genotypes and media compositions was 2.2538. Means (of three replications) of each category with different letters are significantly different at 5 % level of significance.

Cultivars	% Callus induction								% Mean cultivars
	CIM1	CIM2	CIM3	CIM4	CIM5	CIM6	CIM7	CIM8	
Rio Grande	93.3^{a}	60^{ab}	53.3^{bc}	9^{ef}	9^{ef}	3^{f}	2^{f}	0^{f}	28.70^{a}
Moneymaker	2.22^{f}	0^{f}	4.4^{f}	42.22^{bcd}	40^{bcd}	44.44^{bcd}	46.67^{bcd}	28.89^{cde}	26.38^{a}
Gala	7^{f}	22.22^{de}	21.1^{de}	0^{f}	0^{f}	0^{f}	0^{f}	0^{f}	6.29^{b}
% Meantreatments	34.17^{ab}	27.41^{abc}	26.27^{bc}	17.07^{bcd}	16.33^{bcd}	15.81^{bcd}	16.22^{cd}	9.63^{d}	

Table 3:The response of cotyledon explants of cv. Rio Grande, Moneymaker and Gala on eight different formulations of growth regulators for callus induction. Least Significant Difference (LSD) values for cultivars and media compositions calculated by using statistical package statistix 8.1 were 0.7841 and 1.2805, respectively, while for interactions between genotypes and media compositions was 2.2179. Means (of three replications) of each category with different letters are significantly different at 5 % level of significance.

Cultivars	% Callus induction								% Mean cultivars
	CIM1	CIM2	CIM3	CIM4	CIM5	CIM6	CIM7	CIM8	
Rio Grande	70^{a}	60^{ab}	56^{ab}	0^{f}	2.2^{ef}	2^{ef}	3^{ef}	0^{f}	24.03^{a}
Moneymaker	5^{f}	0^{f}	22.22^{de}	53.3^{ab}	48.89^{abc}	31.11^{bc}	11.1^{def}	8.89^{def}	22.59^{a}
Gala	6.7^{ef}	20^{cd}	20^{cd}	0^{f}	0^{f}	0^{f}	0^{f}	0^{f}	5.84^{b}
% Mean treatments	27.23^{a}	26.67^{a}	32.74^{a}	17.8^{bc}	16.69^{bc}	11.03^{bcd}	4.7^{cd}	2.96^{d}	

Shoot and roots induction responses of the cultivars on different media compositions: Callus induction responses of the fully proliferated calli were evaluated on seven different shoot induction media (SIM) compositions with three replications for each treatment. The first three shoot induction media i.e. SIM1, SIM2 and SIM3 were similar to CIM1, CIM2 and CIM3, respectively. In SIM4 and SIM5 media formulations, NAA was replaced with IAA and BAP with Kinetin (Table 4). The last two shoot induction media formulations i.e. SIM6 and SIM7 were composed of IAA and BAP. Among all these tested combinations, shoot primordia appeared on the calli on SIM1and SIM2 media only after 28 days of sub-culturing(Fig. 2 and Table 3).

Although the calli showed good secondary blotting and greening, no shoots appeared on calli on SIM3 media containing BAP 3 mgL^{-1}, NAA 0.3 mgL^{-1} (Fig. 3). Shoot primordia were obtained from 8 calli and after being transferred to shoot multiplication media i.e. MS media containing 2 mgL^{-1} IAA, 0.2 mgL^{-1} BAP, all the calli successfully showed shoot multiplication and very good differentiation (Fig. 4). The same treatment was also used by substituting BAP with kinetin but results were not promising (data not shown). The same shoot multiplication treatment, applied to the green calli without shoot primordia didn't result in induction of shoots (Fig. 3).

The differentiated shoots were shifted to root induction media (RIM) containing 2 mgL^{-1} IBA in both ½ strength MS and full strength MS medium with sucrose 3% and 0.8% agar as solidifying agent for rooting. Profuse rooting was observed on root induction media at ½ strength MS in contrast to the full strength MS medium. All the differentiated shoots that were transferred to the ½ strength MS medium with 2 mgL^{-1} IBA have developed roots (Fig. 5).

The differentiated plantlets with mature shoots and roots were transferred to the Jiffy pellets for better acclimatization. The plantlets in Jiffy pellets were placed in a beaker covered with perforated plastic sheet for aeration. Covering from the beakers were removed after one week and the plants were kept in the glass house for one more week (Fig. 6). Those plants that survived in the greenhouse were then transferred to the pots having 1:1:1 ratio of sand, clay and farm yard manure.

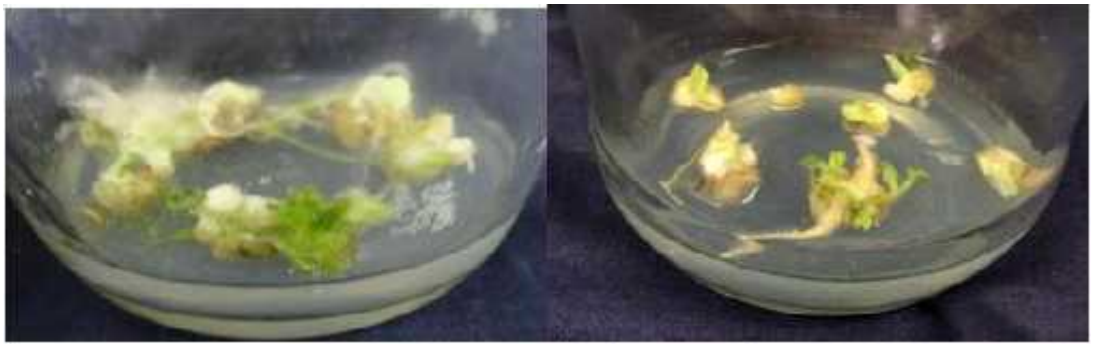

Figure 2. Shoot induction on SIM media
Shoot induction on SIM 1 media 28 days after sub- culturing.

Figure 3.Calli on shoot induction media
Response of calli cultured on MS media containing 3 mgL-1 BAP and 0.3 mgL-1 NAA.

Table 4.Shoot induction media containing varying concentrations of IAA, BAP, NAA and Kinetin used for the induction of shoots from callus obtained from callus induction media. Three replications were used for each experiment.

Shoot induction media	IAA (mgL-1)	BAP (mgL-1)	NAA (mgL-1)	Kinetin (mgL-1)	No of leaf/ plantlets	Out of calli
SIM1	-	1	0.1	-	6	45
SIM2	-	2	0.2	-	3	45
SIM3	-	3	0.3	-	0	45
SIM4	2	-	-	0.1	0	25
SIM5	2.5	-	-	0.1	0	25
SIM6	2	0.1	-	-	0	25
SIM7	2.5	0.1	-	-	0	25

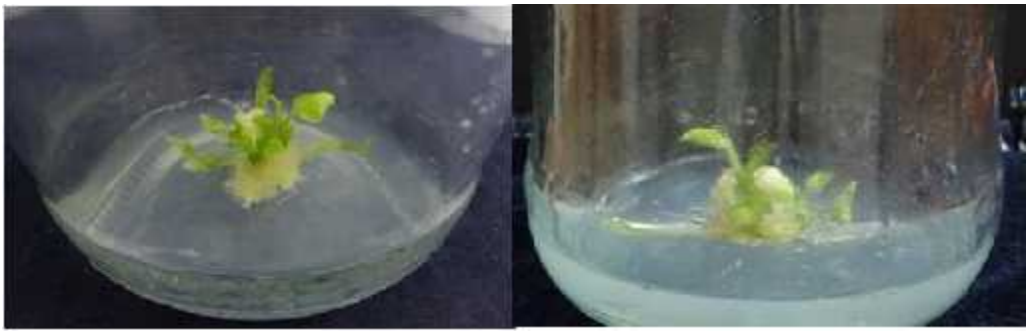

Figure 4. Shoot multiplication on shoot induction media
Response of from calli with shoots primordia on shoot multiplication media. Maximum number of shoots were observed for *cv.* Rio Grande.

Table 5. Statistical probability values for ANOVA comparing differences for different categories. The probability values are based on the average of three replications.

SOV	Probability values		
	Hypocotyl callus induction	**Cotyledon callus induction**	**Shoot induction**
Cultivars	0.0000 **	0.0000 **	0.1549 NS
Treatments	0.0016 **	0.0000 **	0.0000 **
Cultivar. x treatments	0.0000 **	0.0000 **	0.0553 NS

**= significant at 1% probability level, NS= non-significant

Figure 5. Root induction in the regenerated shoots on root induction media
Rooting was observed in Rio Grande on ½ strength MS media supplemented with IBA 2 mgL^{-1}

Figure 6. Acclimatization of regenerated plantlets after rooting on soil.
After one week of rooting, plantlets with developed roots from rooting media were transferred to the Jiffy pellets.

DISCUSSION

Factors effecting callus induction responses in tomato: Choice of appropriate explant is an important determinant in tissue culture responses along with the age of the seedling and nature of explants as they can strictly affect callus induction and regeneration (Takashina *et al*, 1998). Hypocotyls and cotyledons of 10-12 day old tomato *in vitro* seedlings were used as explant sources. The younger plants in general responded better than the older ones which is evident from the use of six day old *in vitro* seedlings of tomato for callogenesis and organogenesis responses (Hu and Philips, 2001; Reda *et al.*, 2004). In our experiment best callus induction response were observed for hypocotyls explants which is in line to many of the previous studies where hypocotyls explants have been reported to be superior to cotyledon explants in different tomato cultivars (Osman *et al.*, 2010 ;Yasmeen, 2009). Cotyledonary explants were also used by many researchers while working on tomato (Sharma *et al.*,

2009; Wu *et al.*, 2006) and in fact some have rather reported cotyledons to be superior to other explants like stems, hypocotyls and leaves for tomato shoot organogenesis (Ling *et al.*, 1998). Although many different types of explants such as epicotyls, leaf petiole and internodes have also been used in tomato (Plastira and Perdikaris, 1997; Gubis *et al.*, 2003) but hypocotyls and cotyledons were by far the most commonly used explants in tomato (Jatoi *et al.*, 1995; Soniya *et al.*, 2001; Park *et al.*, 2003; Raj *et al.*, 2005; Roy *et al*, 2006). The choice of explants have also a close relevance to the nature of the study, for example, cotyledons have been most frequently used explants in genetic transformation for better transformation and regeneration potential (Frary and Van Eck, 2005; Sigareva *et al.*, 2004; Ellul *et al.*, 2003; Ling*et al.*, 1998;). In addition tothe explants source, callus formation is also dependent on many other sources like the culture media composition and genotype of the plant (Guillermo*et al.*, 2003). In our studies, Rio Grande was the most tissue culture responsive cultivar whereas Moneymaker and Gala were low and poor responsive cultivars, respectively. The differences in terms of callus induction frequenciescan be attributed to their genotypes which has a significant impact oncallus initiation and regeneration (El-Farash*et al.*, 1993; Lu*et al.*, 1997).

Regarding culture media compositions, best responses in terms of callus induction for cotyledons and hypocotyls explants of tomato cultivars have been reported when MS media is supplemented with BAP and NAA (Kurtz and Lineberger, 1983; Chandel and Katiyar, 2000), which largely corroborates to our findings in the present studies especially for the tissue culture responsive cultivar Rio Grande. In our study, the highest callus induction frequency was observed for cultivar Rio Grande on CIM1 i.e. MS media supplemented with 1 mgL^{-1} BAP and 0.1 mgL^{-1} NAA which is in line to the previous reports where higher cytokinin to auxin ratio has been shown to enhanced callus formation and shoot regeneration in tomato (Jatoi *et al.*, 2001; Park *et al.* 2003). Similarly, other studies have also concluded that higher concentration of cytokinins with relatively lower concentrations of auxins were responsible for higher percentage of the callus formation (Capote *et al.*,2000; Hille*et al.*, 1989). The results of this study has indicated that the cultivar Rio Grande showed maximum callus formation as compared to all the other varieties followed by *cv.* Moneymaker are consistent with previous reports who had reported 100% callus induction in medium having BAP and IAA Jatoi *et al.*, (2001). The most widely used auxin in tissue culture is 2, 4-D for callus initiation in many species (Pal *et al.*, 2007). However, in this study, 2, 4-D was not found to be effective for callus induction in tomato. Among the eight different callus induction media formulations, CIM1 i.e. MS media supplemented with 1 mgL^{-1} BAP and 0.1 mgL^{-1} NAA, and CIM2 i.e. MS media supplemented with 2 mgL^{-1} BAP and 0.2 mgL^{-1} NAA were best media compositions in terms of callus induction showing on average 34.10% ± 0.51 and 27.41% ± 0.30 responses, respectively for hypocotyls explants. In case of cotyledons' explants CIM1 and CIM2 were again the best media compositions showing on average 27.23%± 0.37 and 26.67%± 0.31 callus induction responses, respectively. The low average values for callus induction in these cases are due to the recalcitrant nature of the two genotypes i.e. Money maker and Gala included in this study. Irrespective of the eight tested different media compositions, Rio Grnade was the most responsive cultivar in terms of callus induction showing on average 28.70% ± 0.35 and 24.03% ± 0.32 callus induction responses for hypocotyls and cotyledons explants, respectively. Here again the very poor or no responses of this particular cultivar on some of the callus induction media formulations contribute towards the overall low average values in terms of callus induction.

Factors effecting shoots and roots induction responses in tomato: In our study shoot induction was observed on MS media containing 1 mgL^{-1} BAP and 0.1 mgL^{-1} NAA or 2 mgL^{-1} BAP and 0.2 mgL^{-1} NAA which is in line to the previous reports (Gubis *et al*.,2003 and 2004;Sherkar and Chavan. 2014) but callus proliferation was very prominent. Maximum number of shoots were observed on the media having 2 mgL^{-1} BAP and of 0.2 mgL^{-1} NAA but not on the 3 mgL^{-1}BAPand 0.3 mgL^{-1} NAA. After shoot induction plant height and number of leaves or shoots were multiplied on the media supplemented with the 2 mgL^{-1}IAA and 0.2 mgL^{-1}BAP as described previously (Sherkar and Chavan, 2014). Given the fact that all the calli with shoot primordia, successfully showed shoot multiplication indicated that 2 mgL^{-1} IAA and 0.2 mgL^{-1}BAP generally works well as the genotypes used in our studies were different from the one used in previous studies.

Regenerated shoots were shifted to media containing 2 mgL^{-1} IBA in both full strength and half MS media for the rooting. Although rooting was observed in both cases, but longer and profuse rooting system was observed on ½ strength MS media containing 2 mgL^{-1} IBA which is in line to the previous studies (Sherkar and Chavan, 2014) obtained. Lower concentration of IBA i.e. 0.2 mgL^{-1} and the importance of half strength MS media for rooting of *in vitro* induced plants for tomato have been highlighted in other reports (Devi *et al.*, 2008). Our findings can be expected to provide useful guidelines for future tissue culture based tomato improvement projects especially involving tissue culture dependent genetic transformation steps.

Conclusion: Callus induction responses are highly genotype dependent as evident from the differential responses of cultivars on different callus induction media compositions. Among the three tested cultivars of tomato,

Rio Grande was found to be most responsive cultivar in terms of producing calli followed by money maker. Among the different callus induction media formulations, CIM1 was the best callus induction media formulation for cultivar Rio Grande. For shoot induction SIM1 and SIM2 were found to be best media compositions but SIM1 showed maximum number of shoots primordia from calli. Our data indicated that rooting can be successfully inducted by supplementing either full strength or half strength MS media with 2 mgL^{-1} IBA.

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