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THE EFFECT OF PLANT GROWTH REGULATORS ON CALLUS INDUCTION AND SOMATIC EMBRYOGENESIS OF HYBRID TOMATO

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Abstract

Efficient tissue culture system is important for transformation of important genes in hybrid tomato cultivars. The present study was undertaken to develop an efficient tissue culture system for hybrid tomato cultivar Peto-86. The young primary leaves and stems were inoculated into five different MS media having different concentrations of plant growth regulators in different combinations for callus induction, somatic embryogenesis and for both direct and indirect regeneration. Maximum callus induction frequency 90% was achieved with MS media containing 2,4-D 4 mg L^{-1} and BAP 0.5 mg L^{-1}. The direct somatic embryogenesis was found highest on MS media supplemented with 2,4-D 4 mg L^{-1} and BAP 0.5 mg L^{-1}. Maximum indirect regeneration frequency 87% was achieved from primary leaves explants with MS media containing IAA 0.5 mg L^{-1} and BAP 3 mg L^{-1} and highest direct regeneration frequency 77% was obtained from primary leaves explants with MS media containing NAA 1 mg L^{-1} and BAP 3 mg L^{-1}. The high concentration of 2,4-D increased callus induction and somatic embryogenesis frequencies while the high concentration of BAP increased regeneration frequency. An improved tissue culture system of hybrid tomato cultivar Peto-86 was established and it may be recommended for further transformation experiments.

Key words: Hybrid tomato, Callus Induction, Regeneration, Somatic embryogenesis, Tissue culture.

Introduction

Tomato (*Solanum lycopersicum* L.) is the second most popular vegetable crop in world after potato and grown all over the world for multiple purposes (Bhatia *et al.,* 2004). For *In vitro* studies tomato is the crop of choice due to its low chromosome number i.e., 2n=24 and a lot of store data about tomato genetics (Chaudhry *et al.,* 2001). Due to high food value the demand of tomato is increasing day by day but biotic and abiotic stresses like salinity, drought, heat, microbial diseases and nutrient deficiencies are the major factors that decrease its productivity and yield (Zhu, 2002; Mathur *et al.,* 2008). Therefore an efficient and quick *In vitro* tissue culture system is very important for continuous improvement in tomato through transformation of agronomical important genes (Wing *et al.,* 1994).

Tissue culture system is prerequisite step before transformation (Hussain *et al*., 2011) and the development of an improved tissue culture system is important for tomato cultivars grown in all over the world. Efficient callus induction and regeneration system has been tested for different tomato cultivars in early study (Costa *et al.,* 2000; Venkatachalam *et al*., 2000) but it is very difficult to develop a single tissue culture protocol for all genotype (Compton & Veilleux, 1991; Ahmed *et al*., 2011). The different source of explants gives different response to plant growth regulators used in combination or separately (Chen *et al.,* 1999; Gubis *et al.,* 2004). The regeneration response varies with different types of cultivars used and types of plant growth regulators used in culture medium (Praveen & Rama Swamy, 2011, Hussain *et al.,* 2013; Khan *et al.,* 2014; Abbassi *et al*., 2011). An efficient tissue culture system has been established for many cultivars by using proffer source of explants and plant growth regulators used. The enhanced callus induction and regeneration can be obtained by using optimum level of cytokines, auxin and other plant growth regulators in MS media (Jabeen *et al.,* 2005; Sheeja *et al*., 2004; Gubis *et al.,* 2004; Hussain *et al.,* 2011; Hussain *et al*., 2013).

Hybrid tomato cultivar Peto-86 is commonly grown in all over the world including Pakistan for its better yields and large fruit size, but still it shows low tolerance against biotic and abiotic stresses such as microbial diseases, frost, salinity, heat and drought. There is need of further improvement through transformation of important genes against biotic and abiotic stresses. The hybrid tomato cultivar Peto-86 lack a proffer tissue culture system therefore, the present work was conducted to develop a quick and efficient tissue culture system for hybrid tomato cultivar Peto-86. The resulted efficient tissue culture protocol will be useful for further transformation experiments in hybrid tomato cultivar Peto-86.

Materials and Methods

All the experimental work was carried out in Plant Genomics and Biotechnology lab at National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agricultural Research Center (NARC), Islamabad, Pakistan.

Plant material: Seeds of local hybrid tomato cultivar Peto-86 were obtained from the Horticultural Research Institute (HRI), National Agricultural Research Center (NARC), Islamabad, Pakistan.

Sterilization and germination of seeds: The overnight soaked mature seeds of tomato cultivar Peto-86 were surface sterilized by washing with 70% ethanol for 1-2 minutes. The seeds were than treated with the five

different concentrations (10, 20, 30 40 and 50%) 0.8% (v/v) "Clorox" bleach (sodium hypochlorite) for 20 min followed by 3-4 times rinsed with double distilled water for 5 minutes. The seeds were dried in Petri dish containing sterilized filter paper. The surface sterilized seeds were transferred to the MS media (Murashige & Skoog, 1962) and the PH of media was adjusted 5.7 to 5.8. The culture tubes were shifted to growth room at 25 ± 2ºC under light (16/8 hours photoperiod) conditions for 2-3 weeks. After 2-3 weeks seeds germination were noted and data was recorded.

Callus induction: The young primary leaves and stems were used as source of explants for callus induction. The primary leaves and stems explants were cuts in small pieces about 3-5 cm long from the young 2-3 weeks old regenerated plantlets and were kept on five different callus induction media (CM1-CIM5) containing different combination of plant growth regulators at different levels (Table 1).

Somatic embryogenesis: Direct somatic embryogenesis was induced from 10 days old primary leaves explants on five different somatic embryogenesis media (SEM1-SEM5) containing different concentrations of plant growth hormones at different combination (Table 2). The different stages of embryo formation and percent somatic embryogenesis were recorded.

Regeneration: The primary leaves and stems were cuts into small pieces and were inoculated on five different direct and indirect regeneration media (RM1-RM5) supplemented with different concentration and combination of plant growth regulators (Table 3a and 3b). The inoculating flask and test tubes were shifted to growth room and were kept for 3-4 weeks at 25°C and 16 hours photoperiod.

Results

Tissue culture condition was optimized for hybrid tomato cultivar Peto-86 by using primary leaves and stems as source of explants. An optimized protocol for callus induction, somatic embryogenesis, direct and indirect regeneration was obtained by using different levels of plant growth regulators in different combinations. The following important findings were noted from the optimization of protocol for callus induction, somatic embryogenesis and for both direct and indirect regeneration of hybrid tomato cultivar Peto-86.

Sterilization is important steps in tissue culture to completely remove unwanted microbes which negatively affect the tissue culture process. The effects of different Clorox (sodium hypochlorite) concentrations on seed germination were noted. Results of seed germination after surface sterilization with Clorox showed that the Clorox (10%) concentration showed maximum 90% seed germination in Peto-86. The low concentration of Clorox (10%) increased germination percentage while high concentrations of Clorox (20, 30, 40, and 50%) decreased contamination rate but adversely affected seed germination frequency (Fig. 4). The maximum seed germination percentage 95% was recorded in Peto- 86 (Fig. 1, Table 4).

Table 1. List of combination of media used for callus induction in hybrid tomato cultivar Peto-86.

Media	Composition
CIM1	MS, 2, 4-D 3 mg L^{-1}, pH 5.7-5.8
CIM2	MS, 2, 4-D 3 mg L^{-1}, BAP 1 mg L^{-1}, pH 5.7-5.8
CIM3	MS, 2, 4-D 4 mg L^{-1}, BAP 0.5 mg L^{-1}, pH 5.7-5.8
CIM4	MS, IAA 1 mg L^{-1}, BAP 2 mg L^{-1}, pH 5.7-5.8
CIM5	MS, IAA 1 mg L^{-1}, Kinetin 1 mg L^{-1}, pH 5.7-5.8

Table 2. List of combination of media used for Somatic embryogenesis in hybrid tomato cultivar Peto-86.

Media	Composition
SEM1	MS, 2,4-D 2 mg L^{-1} pH 5.7-5.8
SEM2	MS, 2,4-D 2 mg L^{-1}, BAP 0.5 mg L^{-1}, pH 5.7-5.8
SEM3	MS, 2,4-D 3 mg L^{-1}, BAP 0.5 mg L^{-1}, pH 5.7-5.8
SEM4	MS, 2,4-D 4 mg L^{-1}, BAP 0.5 mg L^{-1}, pH 5.7-5.8
SEM5	MS, IAA 2 mg L^{-1}, BAP 0.5 mg L^{-1}, pH 5.7-5.8

Table 3(a). List and combination of media used for indirect regeneration in hybrid tomato cultivar Peto-86.

Media	Composition
RM1	MS, BAP 3 mg L^{-1}, pH 5.7-5.8
RM2	MS, NAA 0.5 mg L^{-1}, BAP 2 mg L^{-1}, pH 5.7-5.8
RM3	MS, IAA 0.5 mg L^{-1}, BAP 3 mg L^{-1}, pH 5.7-5.8
RM4	MS, IAA 0.5 mg L^{-1}, Kinetin 1 mg L^{-1}, pH 5.7-5.8
RM5	MS, IAA 0.5 mg L^{-1} , Kinetin 2 mg L^{-1}, pH 5.7-5.8

Table 3(b). List and combination of media used for direct regeneration in hybrid tomato cultivar Peto-86

Media	Composition
RM1	MS, BAP 3 mg L^{-1}, pH 5.7-5.8
RM2	MS, NAA 1 mg L^{-1}, BAP 3mg L^{-1}, pH 5.7-5.8
RM3	MS, IAA 1 mg L^{-1}, BAP 3 mg L^{-1}, pH 5.7-5.8
RM4	MS, IAA 1 mg L^{-1}, Kinetin 1 mg L^{-1}, pH 5.7-5.8
RM5	MS, IAA 1 mg L^{-1}, Kinetin 2 mg L^{-1}, pH 5.7-5.8

Table 4. Seed germination in hybrid tomato cultivar Peto-86.

Genotype	Non germinated	Germinated		Germination %
		Contaminated	Non contaminated	
Peto-86	6	4	110	95

Total numbers of seeds used were 120

Table 5. Primary leaves derived direct somatic embryogenesis in hybrid tomato cultivar Peto-86.

Media	Callus initiation (%)	Embryo formation (days)	Embryogenesis (%)
SEM1	30	28	12
SEM2	53	20	22
SEM3	65	17	56
SEM4	90	13	82
SEM5	15	30	7

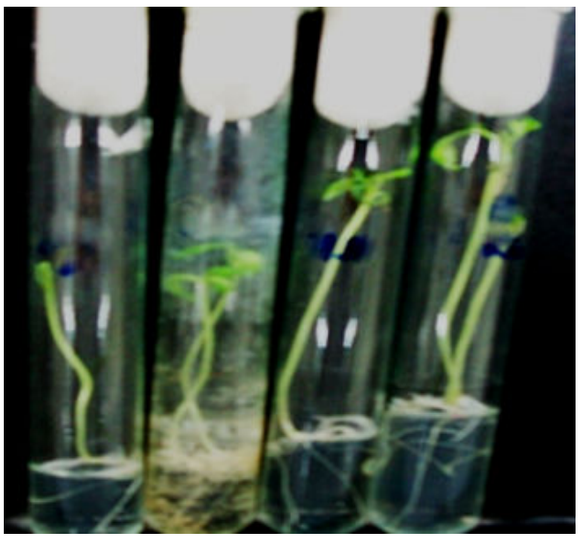

Fig. 1. 2-Weeks old *In vitro* seedling of hybrid tomato cultivar Peto-86.

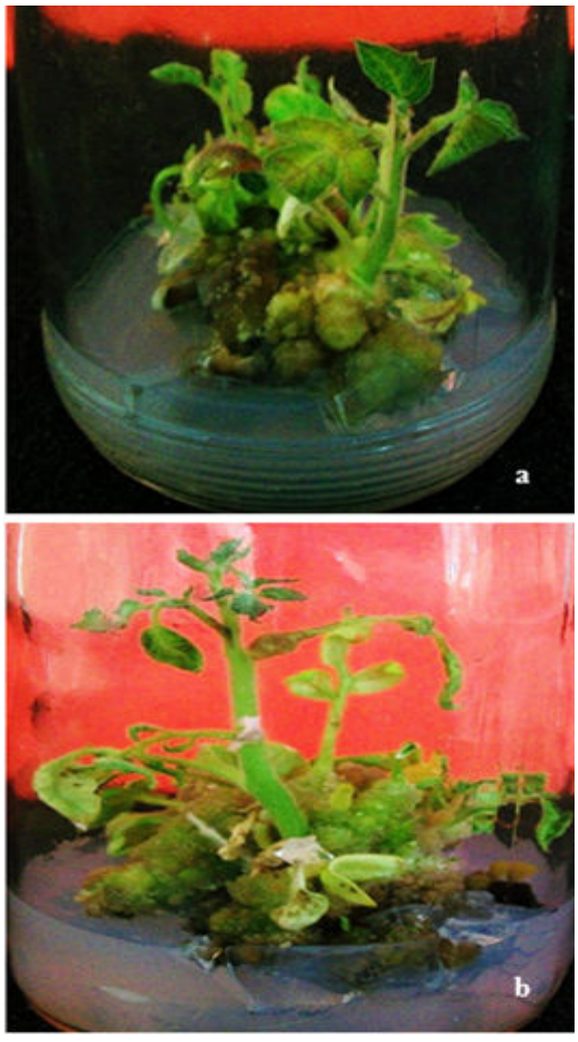

Fig. 2. Direct somatic embryogenesis in hybrid tomato cultivar on SEM4 (a) Early young embryo (b) Mature embryo.

The callus induction response of hybrid tomato cultivar Peto-86 was changed in various callus induction media (CIM1-CIM5) having different combination of hormones. The primary leaves explants of Peto-86 gave maximum callus induction frequency 90% on CIM3 and 71% on CIM2 (Fig. 5a). The callus was green and soft on CIM2 and CIM3 as compared to other three types of media used. The stems derived callus induction frequency was highest 85% on CIM3 (Fig. 5b). The callus quality was good on CIM3 followed by CIM2 while its quality was poor on CIM1 and CIM4.

The direct somatic embryogenesis protocol from young primary leaves explants was optimized. The maximum early young and mature embryo was induced on SEM4 (Fig. 2a, 2b). The maximum somatic embryogenesis 82% was achieved on SEM4 in 13 days followed by 56% on SEM3. The quick somatic embryogenesis was obtained on SEM4 in 13 days while the slowest somatic embryogenesis was noted on SEM5 after 30 days (Table 5).

The genotype Peto-86 showed different response to the different regeneration media (RM1-RM5). The maximum indirect regeneration 87% was noted from primary leaves explants on RM3 while it was 82% from stems explants (Fig. 6a, 6b). The direct regeneration was obtained without callus induction and its regeneration frequency was highest 70% from primary leaves explants on RM2 and highest stems derived explants regeneration frequency was recorded 60% (Fig. 6c, 6d). The regeneration frequency was lower on RM1, RM4 and RM5 and the quality was also very poor on these media (Fig. 3a-e).

Discussion

The establishment of an efficient tissue culture system for local hybrid tomato genotypes is important task because without a proffer callus and regeneration system the genetic transformation is not possible. Therefore we have established an efficient tissue culture system for local hybrid tomato cultivar Peto-86 by using different combination of media for callus induction, somatic embryogenesis and regeneration.

Surface sterilization of seeds is important step in tissue culture used to remove unwanted microbes from seeds which is inoculating on MS media for germination of plantlets. Seeds which were surface sterilized with 10% Clorox showed maximum regeneration percentage 90% in Peto-86. The similar Clorox concentration was used for seed germination by Chaudhry *et al.,* (2004). Our results are not in line with Shah *et al*., (2014) that obtained highest germination rate with 50% Clorox concentration in three important tomato cultivars Riogrande, Money maker and Roma. The germination frequency varies with genotype used Jaebok *et al.* (2001). Our findings show contradiction with the findings of Reda *et al.* (2004) who archived maximum regeneration with 5% Clorox. This deviation from our findings is due to different genotypes used. Our findings are is in agreement with Hussain *et al.* (1990) who obtained maximum germination frequency in tomato after 7-10 days of seeds inoculation on MS media.

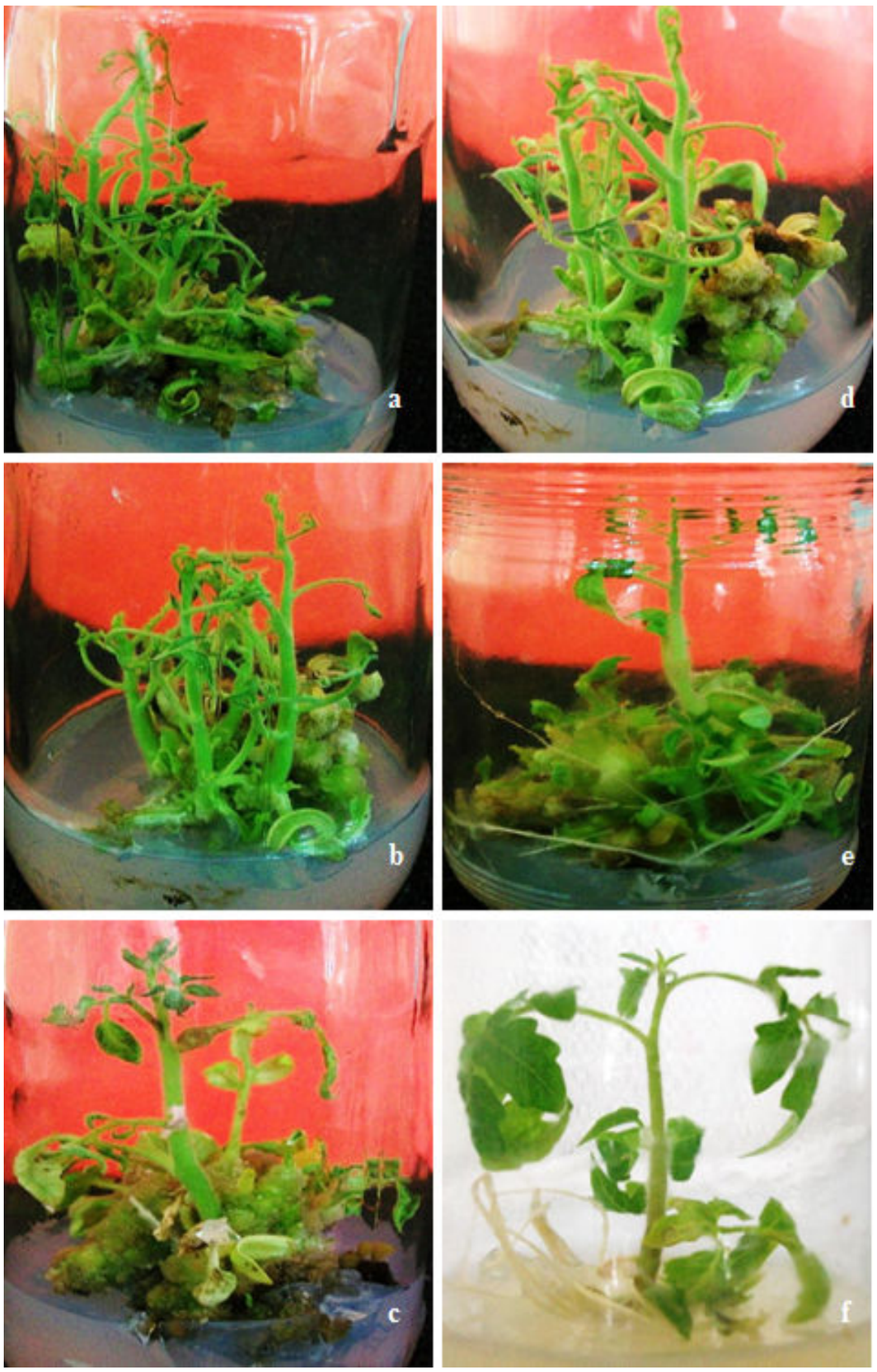

Fig. 3. Regeneration of hybrid tomato cultivar Peto-86 on different regeneration medium (RM1-RM5): (a-e), Regeneration on RM1-RM5, RM3 show best regeneration follow by RM2 (f) Direct regeneration on RM3. RM3 show best and quick regeneration potential as compared to other regeneration media.

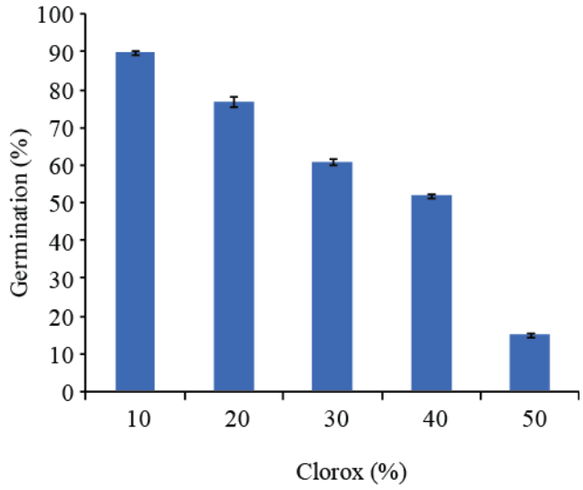

Fig. 4. Effect of different concentration of Clorox on seed germination frequency.

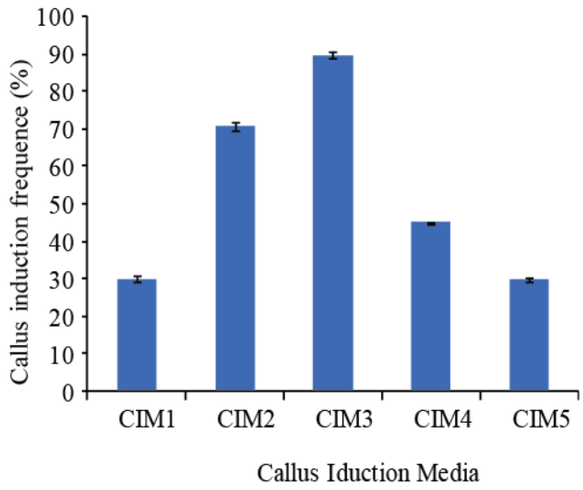

Fig. 5(a). Primary leaves derived callus induction on different callus induction media (CIM1-CIM5).

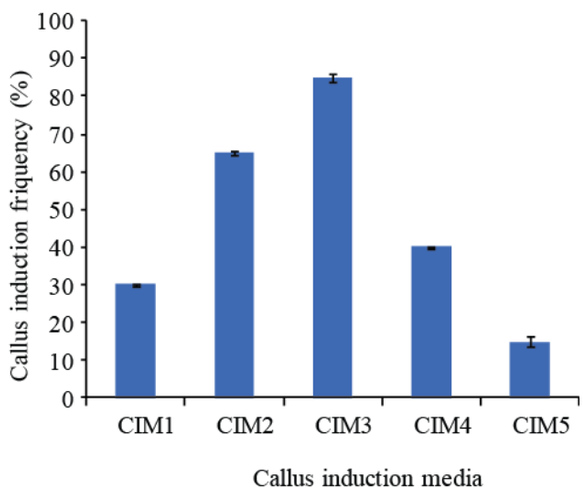

Fig. 5(b). Stems derived callus induction on different callus induction media.

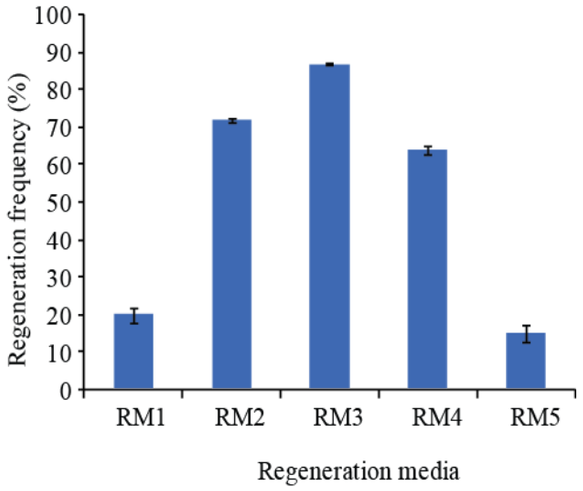

Fig. 6(a). Primary leaves derived indirect regeneration on different regeneration media (RM1-RM5).

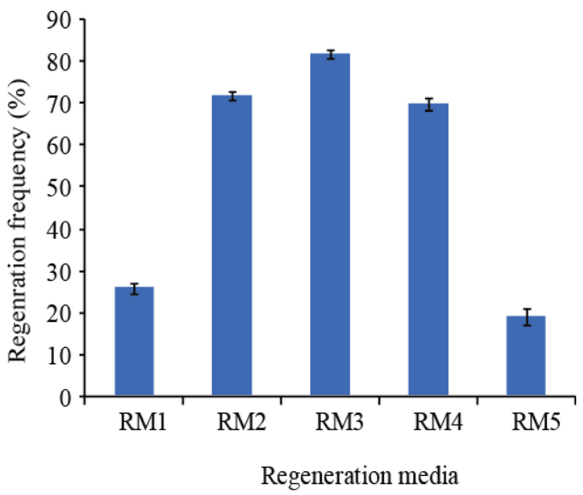

Fig. 6(b). Stems derived indirect regeneration on different regeneration media (RM1-RM5).

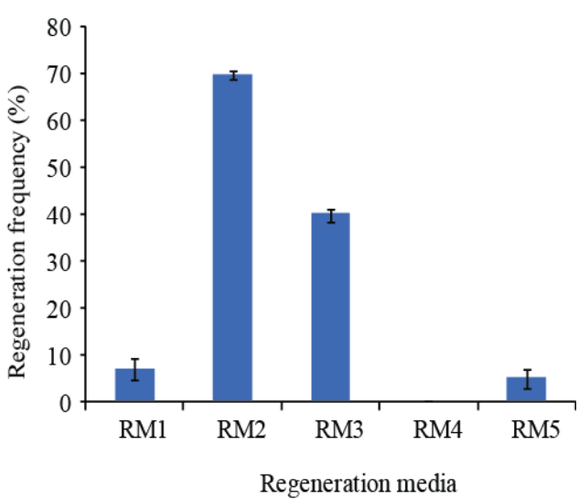

Fig. 6(c). Primary leaves derived direct regeneration on different regeneration media (RM1-RM5).

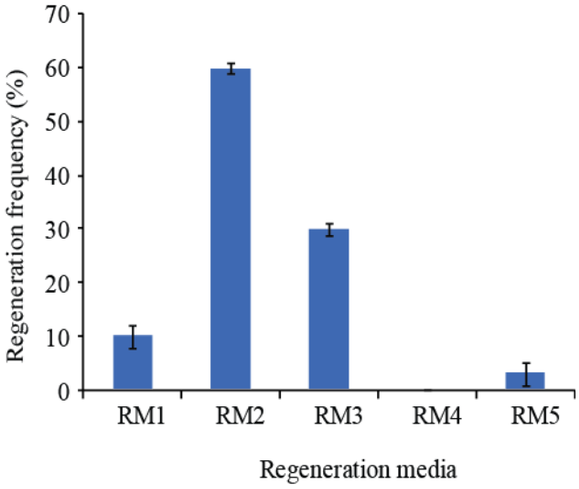

Fig. 6(d). Stems derived direct regeneration on different regeneration media (RM1-RM5).

In present study different callus induction media were used containing different types of hormones IAA, BAP and kinetin. The maximum callus induction from primary leaves explants were obtained on CIM3. The similar response for maximum callus induction and quality of callus induction was reported by Raj *et al. (2005)* who used 2 mg L^{-1} IAA and 1 mg L^{-1} BAP in local tomato cultivar Maskotka. Our results are also in agreement with Jatoi *et al.* (2001) reported maximum and green calli of tomato on callus induction media containing I mg L^{-1} BAP and 0.1 mg L^{-1} IAA. Skoog & Miller (1957) reported that different levels of IAA and BAP in MS media affect callus induction frequency in tobacco.

Stems derived Callus induction was highest on CIM2 containing 2, 4-D 3 mg L^{-1} and BAP 1 mg L^{-1}. Our results are in line with Reda *et al.,* (2004) who obtained maximum callus induction from leaf disk explants in tomato using high amount of 2, 4-D 3 mg L^{-1} along with BAP 2 mg L^{-1} in MS media. Our results showed that the callus induction response of genotype vary with different combination of hormones used in MS media and it is accord with the Lu *et al.* (1997) who reported that callus induction response vary among different genotype used for callus induction.

An optimum quick and efficient direct somatic embryogenesis protocol was established for important hybrid tomato cultivar Peto-86. The young primary leaves were used as source of explants for direct somatic embryogenesis. Maximum somatic embryogenesis was achieved on SEM4 containing 2,4-D 4 mg L^{-1} and BAP 0.5 mg L^{-1}. The highest concentration of 2,4-D with combination of low concentration of BAP in media increased the somatic embryogenesis frequency up to several fold. Ashakiran *et al.* (2011) reported that in MS media containing high concentration of 2,4-D 5 mg L^{-1} increase direct somatic embryogenesis in tomato cultivar Shalimar. Kilankaje & Girija (2011) achieved maximum direct somatic embryogenesis from cotyledons explants in tomato cultivar Shalimar with MS medium containing 5 mg L^{-1} 2,4-D. Our findings are also in accord with JayaSree *et al.* (2001) who obtained maximum direct somatic embryogenesis in potato cultivar Jyothi with MS media supplemented with high concentration of 2,4-D and BA.

An efficient regenerating system for hybrid tomato cultivar Peto-86 was established by using different growth regulator i.e., IAA, BAP, NAA, GA3 and kinetin in different combination. The maximum indirect regeneration was achieved from primary leaves explants on RM3 containing high concentration of BAP and low concentration of IAA while the direct regeneration frequency was also highest from primary explants on RM2 having high concentration of BAP and low concentration of NAA. Our results show contradiction with Ahsan *et al.* (2007) that used BAP 2 mg L^{-1} and IAA 0.5 mg L^{-1} in MS media for regeneration in tomato. This deviation from our findings is due to the genotypes difference. BAP at high concentration in MS media showed enhance regeneration with multiple shoot production in tomato Chandel & Katiyar (2000) and Soniya *et al.* (2001). Malik & Saxena (1992) suggested that media containing high level of cytokines increase regeneration frequency of *Pisum satvum*. Botau *et al.* (2002) reported that combination of IAA and BAP increased the regeneration of tomato. Li *et al.* (2005) reported that low concentration of IAA in combination with BAP increase large leaves formation in Zinnea plant. There are also some reports that BAP in combination with NAA increases regeneration potential in tomato Shahirari *et al*. (2006). This contradiction from our results is due to genotypes difference and different response of genotypes to plant growth regulators.

Conclusion

An efficient and quick tissue culture system has been developed for hybrid tomato cultivar Peto-86. The callus induction and regeneration frequency was high from the primary leaves explants as compared to stems explants. The high concentration of 2, 4-D in MS media increased callus induction frequency as well as direct somatic embryogenesis frequency from primary leaves explants up to several fold. The high concentration of BAP and low concentration of IAA or NAA in MS media significantly increased both direct and indirect regeneration frequency from both primary and stem explants. The young plantlets were transfer to glass house where these were acclimatized with natural environment. The resulted improved tissue culture protocol for hybrid tomato cultivar Peto-86 will be useful for further transformation experiments to produce new transformants and it will be used as model for tissue culturing of other hybrid tomato cultivars.

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