

IN-VITRO REGENERATION AND DEVELOPMENT FOR THE CONSERVATION AND PROPAGATION OF TOMATO PLANT (*SOLANUM LYCOPERSICUM*) AND CURRANT TOMATO (*S. PIMPINELLIFOLIUM*) FROM TWO DIFFERENT EXPLANTS

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Abstract. While the contribution of stable tomato cultivars to ecological balance and environmental preservation has been recognized, two tomato cultivars have developed methods for tissue culture. For two tomato cultivars *Solanum lycopersicum* and *Solanum pimpinellifolium*, the regeneration capacity of two types of explants (cotyledons and stem node segments) was compared. Explants were cultured on five different regeneration media (½ MS and BAP) for stem node and MS for callus induction with a combination of 6-benzylaminopurine (BAP) and α-naphthaleneacetic acid (NAA). It was found that ability to regenerate was substantially dependent on both the cultivars and the type of explant. Stem nodes, followed by cotyledons, were the best explants to induce shoot regeneration. It was noticed that the best formulation of the medium for this regeneration from cotyledon explants used was MS with 1 mg/L BAP and MS+1BAP+0.25 NAA mg/L for callus induction in *S. pimpinellifolium* and for stem node explant the best formulation used was ½ MS and 7 mg/L BAP in *S. lycopersicum* was observed. All these data raised the possibility that to preserve and propagate wide hybrids of tomato materials *in vitro*, the regeneration ability of two explants was compared in *Solanum lycopersicum* and *S. pimpinellifolium*.

Keywords: tomato, explants, benzyloaminopurine (BAP), α-naphthylacetic acid (NAA), stem node

Introduction

Tomato (*Solanum lycopersicum*, 2n=2x=24), is an economically important crop worldwide. Moreover, this vegetable appears as a model plant for the introgression of agronomically crucial genes into the genome of dicotyledonous crop plants (Bhatia et al., 2004). The introduction of qualitative traits into commercial tomato cultivars is substantial, the aim being to ameliorate their nutritional value, productivity, abiotic stress resistance and application in molecular farming (Gerszberg et al., 2015). These crop species exhibited

extraordinary nutritional value that's why it is considered to be preventive food (Raiola et al., 2014).

Recently, this crop has gained huge popularity due to its anti-cancer and antioxidant characteristic (Khuong et al., 2013). Elaboration of the aforementioned system is essential for positive results in a broad range of techniques, including micro propagation, mutation selection, somatic hybridization or germplasm preservation of the ecological balance (Benson, 2000).

Numerous studies pointed out that the process of tomato regeneration via the organogenesis pathway is influenced by various elements, including media composition, environmental conditions, genotype, explant origin and its age (Ishag et al., 2009; Rashid and Bal, 2010; Zhang et al., 2012; Wayase and Shitole, 2014). So far, several procedures were developed concerning *in vitro* tomato plants regeneration from diverse explants through organogenesis and tomato transformation (Gerszberg et al., 2015).

These findings pointed out the intractability of *S. lycopersicum* explants (fragmentary or even complete incapability to react to *in vitro* culture impulses) (Bhatia et al., 2004). Consequently, the improvement of a suitable recovery system exploiting tissue culture techniques of *S. lycopersicum* plants has a great potential if we consider the fact that various genotypes differ in regards to their morphogenic potential.

Traditional methods for tomato breeding can be expensive and tedious due to the time and facilities required by each breeding generation and to the problems with the selection of suitable standards for cultivating (Gerszberg et al., 2015). Therefore, as an alternative, the creation of effective regeneration protocols is an essential step for exploiting of tissue or cell culture for genetic amelioration.

Tomato *in vitro* culture has been used successfully in various biotechnological applications including clonal propagation of high-value commercial cultivars, virus-free plants, and genetic transformation (Hanus-Fajerska, 2005; Li et al., 2011; Yarra et al., 2012; Namitha and Pradeep, 2013).

The current research was focused on the impact of different media formulations on the regeneration of two tomato inbred lines of *S. lycopersicum* and *S. pimpinellifolium*. We used two inbred lines for *in vitro* culture to establish the best system of tissue culture in tomato, to conserve F₁ plants from wide hybridization for the balance of ecology.

Materials and methods

Plant materials

The present research work was conducted at Department of Horticulture, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University Multan, Pakistan. Two different inbred lines of *Solanum lycopersicum* cv. Micro-Tom (2n = 24) and *S. pimpinellifolium* line WVa700 (2n = 24) were used as a plant material in this study. The seeds of these varieties were surface sterilized with 70% (v / v) ethanol for 1 minute; subsequently they were dipped in a commercial bleach solution comprising 5.5% (w / v) of sodium hypochlorite for 10 minutes accompanied by 5 times rinsed with sterilized distilled water (3 minutes for each).

Culture conditions

The seeds were placed in a sterilized glass jar containing MS medium and then transferred to the growth room under controlled conditions at a temperature of 25±1°C

under 16 hours of photoperiod light intensity (1500 lux) and relative humidity (60-70%). Sterilized seeds were placed in jars on the seed germination medium. Four seeds were inoculated in each jar. The culture was then incubated in the incubation room until seed germination. It was observed that seeds began to grow in the dark and were later transferred to light.

The explants, both cotyledon and stem node, were taken from sterile seedlings (young seedlings) and placed on two media types: MS with different concentrations of benzylaminopurine (BAP i.e. cytokinin) and α - naphthaleneacetic acid (NAA i.e. auxin) for callus induction and $\frac{1}{2}$ MS and BAP with different concentrations for stem node induction respectively to conserve F₁ plants as shown in (Table 1 and Table 2). Experiment was repeated three times, with a total of 150 explants (50 explants per replicate).

Table 1. Composition of culture media used for stem node induction and plant regeneration of *Solanum lycopersicum* and *Solanum pimpinellifolium*

Medium	Composition
MS ₁	without plant growth regulators
MS ₂	$\frac{1}{2}$ MS + 1 mg/L BAP
MS ₃	$\frac{1}{2}$ MS + 3 mg/L BAP
MS ₄	$\frac{1}{2}$ MS + 5 mg/L BAP
MS ₅	$\frac{1}{2}$ MS+ 7 mg/L BAP

MS= Murashige and Skoog medium, BAP= benzyloaminopurine

Table 2. Composition of culture media used for callus induction and plant regeneration of *Solanum lycopersicum* and *Solanum pimpinellifolium*

Medium	Composition
MS ₁	without phytohormones
MS ₂	1 mg/L BAP + 0.25 mg/L NAA
MS ₃	1mg/L BAP + 0.50 mg/L NAA
MS ₄	2mg/L BAP + 0.25 mg/L NAA
MS ₅	2 mg/L BAP + 0.50 mg/L NAA

BAP= benzyloaminopurine, NAA= α - naphthaleneacetic acid

Experimental data for stem node induction and callus bud induction was collected after 15, 30 and 45 days, respectively. All the regeneration parameters (e.g. callus bud induction frequency and axillary bud frequency or growth features) were evaluated constantly. Explants of the two varieties were grown on 5 different media to evaluate their regeneration capacity.

Statistical analysis

The data collected was statistically analyzed following the analysis of the variance (ANOVA) technique and the mean differences were assessed by Duncan's Multiple Range Test (DMRT) using the statistical computer package program MSTATC (Gomez and Gomez, 1984).

Results

The purpose of this study was to investigate the performance of the two tomato explants, one explant (cotyledon) for callus induction and another explant (stem node) for axillary bud induction from two different cultivars i.e. inbred lines of *S. lycopersicum* cv. (Micro-Tom) ($2n = 24$) and *S. pimpinellifolium* line (WVa700) ($2n = 24$) were used as parents. The seed germination rate on MS media was 80% where 1.5% of the seeds were contaminated and the remainder was unable to grow. The impact of different levels and combinations of PGRs in both cultivars was noted in MS and $\frac{1}{2}$ MS media for tomato explant for callus proliferation and stem node induction.

In the present experiment, the capability of two types of tomato explants (cotyledons and stem nodes) for the regeneration through indirect organogenesis was tested. Both explants cut from tomato seedlings (young seedlings) were cultured on various media: $\frac{1}{2}$ MS+BAP (at different concentrations) for stem node and BAP (cytokinin), together with NAA (auxin) (at different concentrations) for cotyledon for the production of callus and multiple shoots (Table 1 and Table 2).

Generally, callus induction was noticed in the aforementioned type of explant culture. Among the 5 media tested, no substantial variation was observed in the frequency of callus induction except *S.pimpinellifolium* at BAP, together with NAA (at different concentrations) for cotyledon for the production of callus but the highest callus bud induction frequency was noticed with the concentration of MS+ 1BAP+0.25 NAA (Fig. 1).

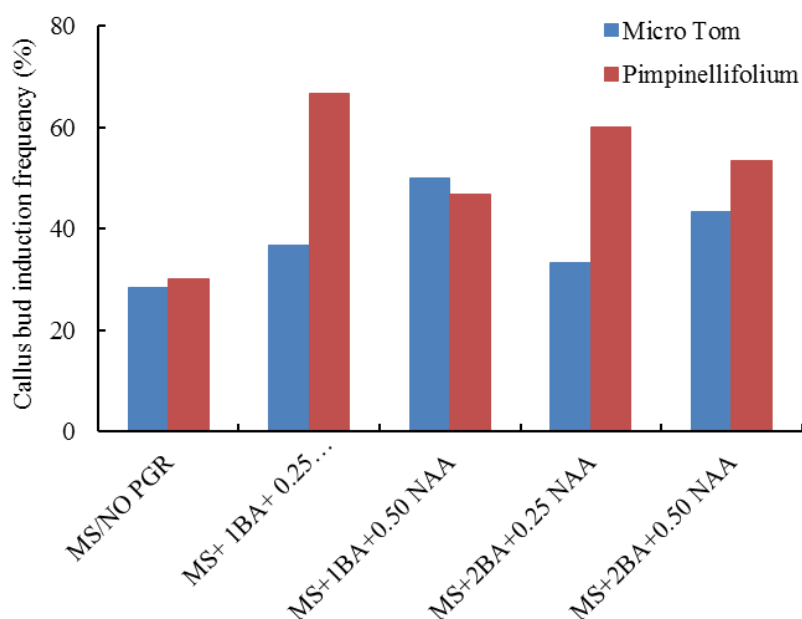


Figure 1. Callus bud induction frequency of *Solanum lycopersicum* cv. Micro-Tom and *S. pimpinellifolium* line WVa700

Callus formation began within ~12 days and shortly thereafter buds occurred. The origination of calli and shoots was noticed in the middle part of explants and on cut edges. In terms of morphology, the callus was fragile and characterized by a pale yellow or green color. In general, stem node explant showed high frequency of axillary bud regeneration in comparison to the callus originating from cotyledons because callus

induction took a long time to regenerate while stem node usually took less time. Shoot regeneration frequency changed with medium composition, the variety and also with explant type.

Despite the fact the plant regeneration could be caused with BAP only, combining with NAA enhanced the frequency of plant regeneration significantly. Based on our results, MS with 1mg/L BAP and 0.25 mg/L NAA is the best formulation of the medium for regeneration from the cotyledon explants used in this research. The regeneration of plants from axillary buds or shoots has been shown to be the most generally applicable and reliable method of *in vitro* true-to-type propagation (Fig. 2).

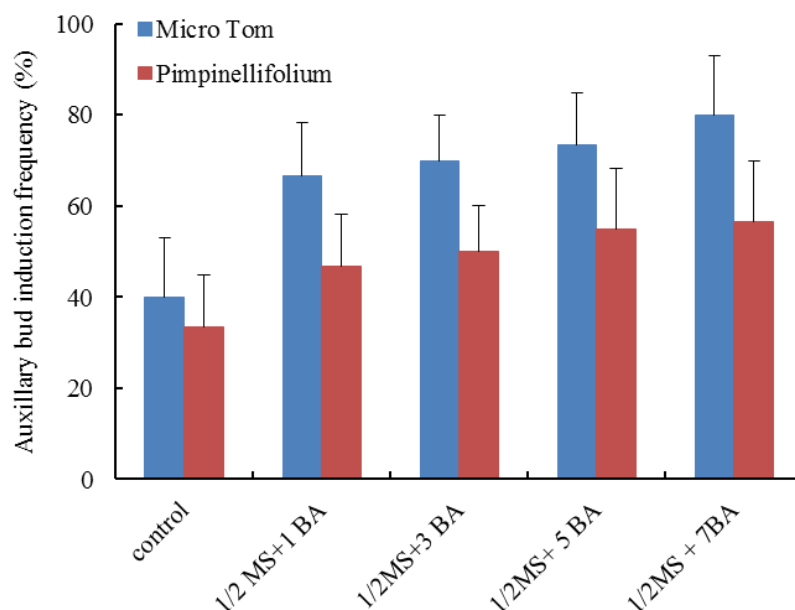


Figure 2. Axillary bud formation percentage of *Solanum lycopersicum* cv. Micro-Tom and *S. pimpinellifolium* line WVa700

We observed high regeneration axillary bud frequency from the stem node explants used in this study with the following concentrations $\frac{1}{2}$ MS and 1BA, $\frac{1}{2}$ MS and 3BA, $\frac{1}{2}$ MS and 5BA and $\frac{1}{2}$ MS and 7BA.

Our data showed the highest axillary bud regeneration frequency (80%) in *S. lycopersicum* (Micro-Tom) and (56.6%) in *S. pimpinellifolium* (WVa700). Moreover, by comparing two cultivars, highest germination rate was observed in Micro-Tom and lower in Pimpinellifolium (WVa700). It has therefore been noted that $\frac{1}{2}$ MS and 7 mg/L BA in Micro-Tom are the best formulation of the medium used for this stem node explant regeneration (*S.lycopersicum*) (Fig. 2).

According to recent findings, in callus induction, *S. pimpinellifolium* (WVa700) showed high callus bud induction and multiple shoot regeneration as compared to *Solanum lycopersicum* (Micro-Tom) (Fig. 1) while in stem node induction, *Solanum lycopersicum* (Micro-Tom) showed high axillary bud regeneration frequency over *S.pimpinellifolium* (WVa700) (Fig. 2 and Fig. 3).

Plants with a well-developed root system were moved to pots with a soil and perlite compound (3:1) and subsequently successfully acclimated and grown in a glasshouse. All *in vitro* plants were characterized by normal phenotypic appearance.

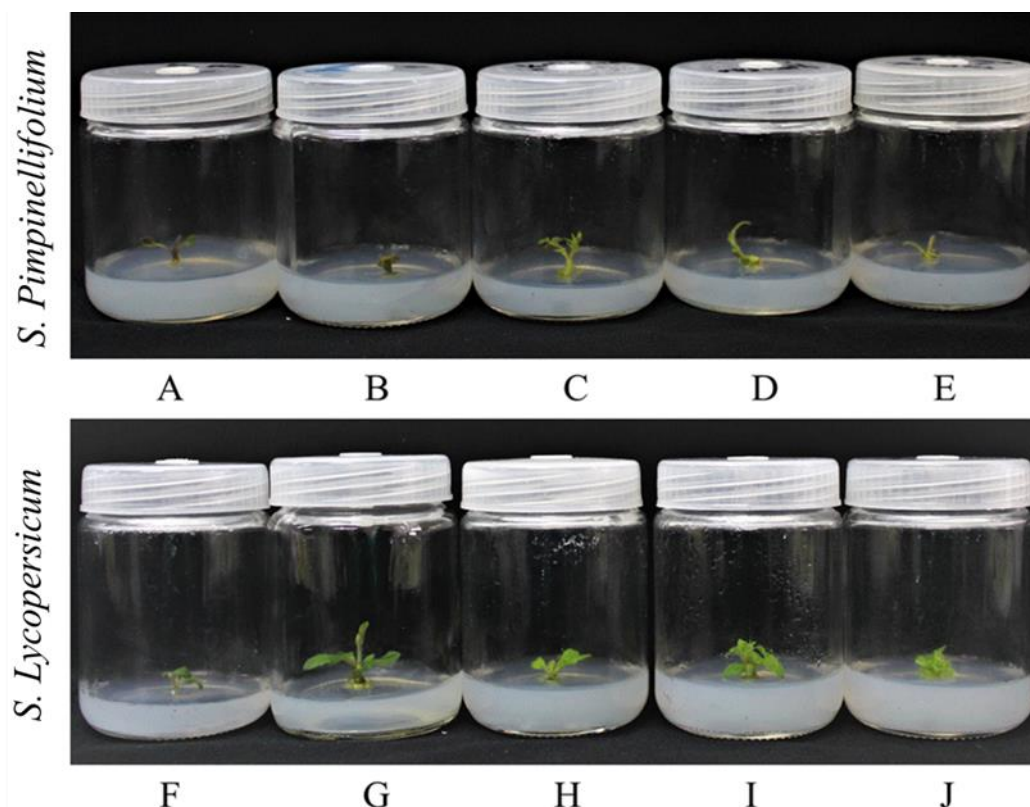


Figure 3. Axillary bud regeneration from stem node explant of *Solanum lycopersicum* cv. Micro-Tom, *S. pimpinellifolium* line WVa700, **A.** control, **B.** $\frac{1}{2}$ MS + 1BA, **C.** $\frac{1}{2}$ MS + 3BA, **D.** $\frac{1}{2}$ MS + 5BA, **E.** $\frac{1}{2}$ MS + 7BA for *S. pimpinellifolium* (WVa700), **F.** control, **G.** $\frac{1}{2}$ MS + 7BA, **H.** $\frac{1}{2}$ MS + 5BA, **I.** $\frac{1}{2}$ MS + 3BA, **J.** $\frac{1}{2}$ MS + 1BA, for *S. lycopersicum* (Micro-Tom)

Discussion

The seeds of both cultivars *Solanum lycopersicum* and *S. pimpinellifolium* were germinated on plant growth regulator-free MS medium. Fifteen days after germination, seedlings were harvested for use as explant. The use of *in vitro* methods is useful for many cultivars and the individuals who rely on them. It has been noted that plant regeneration by *in vitro* technologies is usually associated with the existence of variability among regenerants (Duncan, 1996).

In our previous studies, phenotypic traits of parents and their hybrids in terms of plant height (6.866 cm for M_W and 5.966 cm for W_M) at 6 leaf stage, plant height (56.66 cm for M_W and 74.33 cm for W_M) at 8 leaf stage (70 days old leaf), fruit shape index (0.953 for M_W and 0.985 for W_M) and single fruit weight (3.622 g for M_W and 4.352 g for W_M) were observed at 6 leaf stage (45 days old leaf) respectively (Fig. 4 and Fig. 5) (Raza et al., 2017). The present experiment was performed to conserve F₁ generation used in our previous studies by using *in vitro* technique.

Plant growth regulators (PGRs); have influenced the morphogenic response by modifying various physiological processes. In the case of tomato regeneration, a broad range of different phytohormones (e.g. BAP, zeatin, 2iP (isopentenyl adenine), KIN (Kinetin), IAA, NAA (α -Naphthaleneacetic acid) IBA (Indole-3-butyric acid), 2, 4-D (2, 4-Dichlorophenoxyacetic acid) at varying concentrations have been used so far (Koleva Gudeva and Dedejski, 2012; Jehan and Hassanein, 2013; Koul et al., 2014).

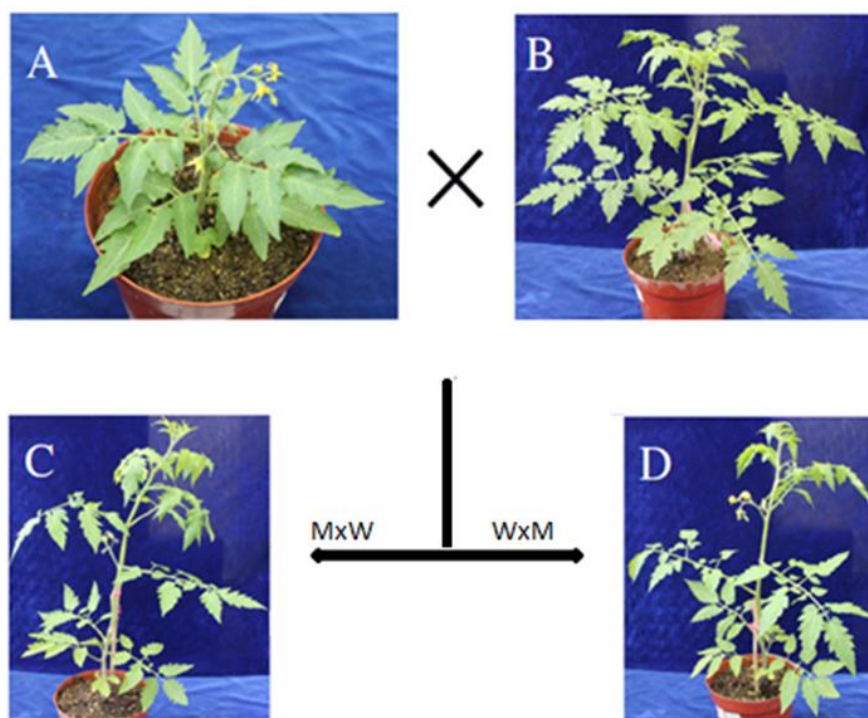


Figure 4. Parents and their reciprocal hybrids, **A.** *Solanum lycopersicum* cv. Micro-Tom, **B.** *S. pimpinellifolium* line WVa700, **C.** Micro-Tom_WVa700 i.e. (Micro-Tom X WVa700) **D.** WVa700_Micro-Tom i.e. (WVa700 X Micro-Tom)

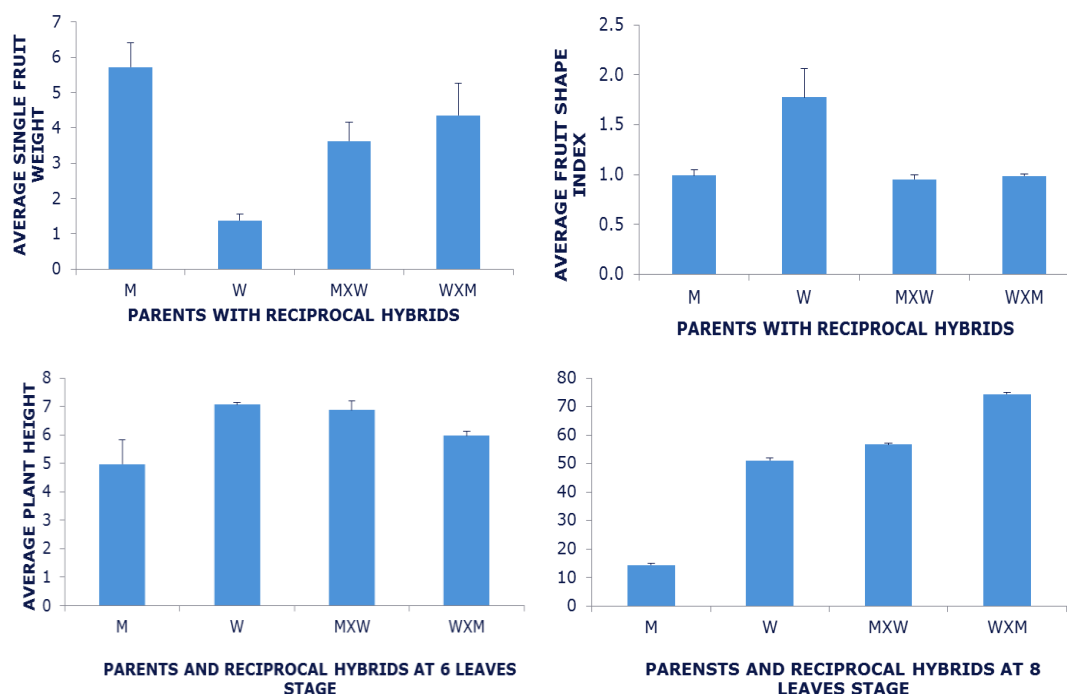


Figure 5. Phenotypic traits of parents *Solanum lycopersicum* cv. Micro-Tom, *S. pimpinellifolium* line WVa700, and their reciprocal hybrids (Micro-Tom X WVa700) and (WVa700 X Micro-Tom)

Moreover, the nature and concentration of PGRs used in the medium proven to be dependent on the cultivar being cultured and especially on the auxin or cytokinins applied (Koleva Gudeva and Dedejski, 2012; Bahurupe et al., 2013; Jehan and Hassanein, 2013; Koul et al., 2014; Wayase and Shitole, 2014). Majority of *in vitro* methods depend on induction of the regeneration process exploiting different explants cultured *in vitro*. Various types of explants were used by others: seed-cut cotyledon, hypocotyl, leaf, stem sections, nodes, internodes, pedicels, petioles, apical meristem, shoot apex/tip and inflorescences for organogenesis (Gerszberg et al., 2015).

A substantially higher range of BA (22.2 μ M) was used for *Hedeoma multiflorum* micro propagation (Koroch et al., 1997). Slightly lower values were recorded for *S. lycopersicum* (Micro-tom) (Fig. 1). In many tree species, a range of auxin in combination with cytokinin played a vital role in multiple shoot regeneration (Vengadesan et al., 2002; Giri et al., 2004; Anis et al., 2011). Moreover, addition of low levels of auxin along with cytokinin is known to increase shoot numbers in many plant species like *Wrightia tinctoria* (Purohit et al., 1994).

Conclusion

The present study revealed an acceptable frequency of regeneration from two explants (cotyledons and stem node segments). Development of an effective *in vitro* regeneration protocol for crop species could be extremely beneficial in the process of cultivating and creating new varieties or in breeding lines in a relatively short period of time and for genetic improvement by exploiting biotechnology strategies. It is known that there are more than ten thousand tomato cultivars, and it is therefore impossible to establish a universal tomato regeneration protocol.

Hence, creating a tissue culture protocol for selected commercially important varieties is fully justified. This step should be preceded by wide screening of the above-mentioned varieties of morphogenic potential. Our study would provide novel valuable information about this very important issue. It can be concluded from the above findings that the regeneration protocol developed in this study is simple, reproducible and applicable.

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