Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231– 6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2016 Vol. 6 (4) October-December, pp. 17-23/Rani et al.
Research Article

EFFECT OF GROWTH REGULATORS ON *IN VITRO* ORGANOGENESIS OF THREE CULTIVARS OF TOMATO *(LYCOPERSICON ESCULENTUM* MILL.)

Anshu Rani, Priyanka, Varun Kumar, Sandeep Tripthi and *Sandeep Kumar
Department of Botany, NIET, NIMS University Rajasthan, Jaipur, India
**Author for Correspondence*

ABSTRACT
An efficient protocol for organogenesis from stem explants in cultivars of Tomato (Angoorlata, K5-7, A3ad-8). The stem explants excised from four weeks old *in vitro* grown seedling were cultured on MS medium supplemented with 1.0 –5.0 mg/l BAP and 0.1–3.0 mg/l NAA. Highest percentage of response for callus induction was recorded in hypocotyls explants at 0.5mg/l NAA + 2 mg/l Kn. Maximum regeneration frequency (85%) and number of shoots per callus (12 shoots) were observed on MS media supplemented with 2.5 mg/l BAP. The shoots were shifted to MS medium containing IBA (1 to 3 mg/l mg/l) for rooting and all responded positively to rooting. The regenerated plants were acclimatized and maintained in green house then transferred to the field.

Keywords: *In Vitro, Organogenesis, Growth Regulators, Lycopersicon esculentum* (MILL.*), Hypocotyl*

INTRODUCTION
Tomato *(Lycopersicon esculentum* Mill.*)* is an important *Solanaceous* vegetable crop grown throughout the world for its versatile uses. It is one of the most important protective foods as it possesses appreciable quantities of vitamins and minerals and sometime rightly referred to as poor man's orange (Devi *et al.,* 2008). Tomato belongs to *"Solanaceae"* family and botanical name of Tomato is "*Solanum lycopersicon"*.
Tomato is a genus of about 7500 species of herbs and shrub in the nightshade family. It is a perennial, annual plant and native to tropical region in Europe and Asia.
Tomato originated from South America. It was spread to the Caribbean, Philippines, Asia and Europe by the Spnish. It is grown throughout the country where irrigation water and arable land are available (Abdelmageed *et al.,* 2004).
It is popular because of its high nutritive value and diversified uses hundred gram of edible parts of tomato contains 0.9 grams protein, 0.1 gram fat, 3.5 grams carbohydrates, 15-20 calorie energy, 500-1500 IU vitamin "A", 0.1 mg thiamine, 0.02 mg riboflavin, 0.6 mg niacin, 20-25 mg vitamins, 6-9 mg calcium and 0.1-0.3 mg iron (Uddin *et al.,* 2004).
Leaves stem, and green unripe fruit of the tomato plant contain small amount of the toxic alkaloid tomatine. They also contain solanine a toxic alkaloid found in potato leaves and other plants in the nightshade family.
Tomato is a favorable food crop for *In vitro* studies due to its low chromosomal no i.e., 2n=2x=24 and due to comprehensive knowledge of tomato genetics (Chaudary *et al.,* 2001).
Tissue culture is an important tool in biotechnology, which can be used to improve productivity of crop via rapid availability of superior planting stock (Bhatia *et al.,* 2004; Tiwari *et al.,* 2013).
In vitro regeneration of cultivated tomato has been a subject of research because of the commercial value of the crop and its amenability for further improvement via genetic manipulation (Evans, 1989). *In vitro* plant regeneration has been found to depend on many factors, of which most important are: genotype explant, composition of basic medium, growth regulators, gelling agent, light intensity and quality, photoperiod, temperature, cultivation vessels and vessel covers (Reed, 1999; Leblay *et al.,* 1991).
Tissue culture techniques of tomato were successfully used for selection of tolerant cell lines for various biotic and a biotic stresses under laboratory condition, as it needs comparatively less effort and fewer resources than selection of tomato genotypes using traditional procedures under field condition.

MATERIALS AND METHODS

Plant Material

Seeds of tomato cultivars for the study will obtain from Chandra Shekhar Azad University of Agriculture Science and Technology, Kanpur.

Seed Surface Sterilization and Seed Germination

The seeds were immerse in distilled water and treated with Bavistin 1% solution for 45 minutes followed by thoroughly rinsing with sterilized water.

One drop of Tweeen-20 were add to the seeds and soaked thoroughly for 5 minutes and thoroughly rinsed with sterile distilled water for 4 to 5 times.

The seeds were take into laminar air flow cabinet and treated with 70% ethyl alcohol for 30sec followed by treatment with (0.1%) $HgCl_2$ for 3 minutes and then washing for 4 to 5 times with double distilled water.

Sterilized seeds were inoculate in test tubes containing MS medium (Murashige and Skoog, 1962) without hormones and transferred in the dark room for germination. Germinated seedling served as explants source for tissue cultured experiments.

Culture Media and Inoculation

Seeds were germinated on full strength MS medium at 5.7 pH by keeping them initially in dark for 6 days at 26±1°C and then maintained less than 16h photoperiod, with day night temperature 25°C±2 respectively.

Callus Induction

The cotyledons and hypocotyls segments of about 1 cm in length were taken from 3 – 4 weeks old *in vitro*, seedling.

These were utilized as explants source for callus induction on MS medium supplemented with various concentrations (0.5 to 4 mg/l) of BAP, (0.1 to 3 mg/l) NAA and (0.5 to 4 mg/l) kinetin, *in vitro* were excised under aseptic condition.

Induction of Organogenesis and Multiplication of Shoots

Explants sources of cultivated tomato were induced for callus and proliferation on medium containing MS medium supplemented with different combination and concentration BAP (0.5 to 5 mg/l), Kn (1 to 3 mg/l), NAA(0.5 to 1.5 mg/l) and IBP(0.5 to 3 mg/l) for organogenesis.

Explants cultures were maintain in the dark for three weeks. After eight weeks, clean and healthy cultures were removed from the jars for growth analysis.

Initiation of Rooting

The elongated shoots were excised individually and transferred to MS Medium supplemental with different concentration of IAA (0.5 to 2 mg/l), IBA (0.5 to 3 mg/l) and NAA (0.5 to 2 mg/l) for rooting.

RESULTS AND DISCUSSION

Explants sources of cultivated tomato were induced for callus and proliferation on medium containing MS medium supplemented with different combination and concentration BAP (0.5-3mg/l), kinetin (1-5mg/l) and NAA (0.5mg/l - 2mg/l) for organogenesis. Explants cultures were maintain in the dark for four weeks (Figure 1, Graph 1).

Cotyledons and hypocotyls segments of old *in vitro* plant were excised under aseptic condition. Induction of callus from three varieties of tomato were used different concentration of growth regulator were individually such as BAP (1 to 5mg/l), IAA (0.5 to 2.5mg/l), Kinetin (0.5 to 4mg/l), and NAA (0.5 to 3mg/l) with MS medium.

Established cultured consisted of a mass of immature shoot primordial and differentiated leafy shoot. The variations were observed for shoot length in Ks-7 were recorded maximum no. of shoots in MS media with (1.5mg/l) BAP and (2.0 mg/l) Kn, Angoorlata were recorded maximum no. of shoots MS media with (2.0mg/l Kn + 1.5 mg/l BAP) and A3adT-8 were recorded maximum no. of shoots MS media supplemented with 3.0mg/l Kn and 2.0mg/l BAP. The shoots emerged from between scale leaves and from tissue produced from the base of callus.

New shoots developed adventitiously and formation of callus was rare (Figure 3 and 4, Graph 2 and 3). Yield of shoots from all cultivars was recorded from the date of initiation. A cumulative yield for the first 8 weeks was given in previous report.

Yield of shoots varied for each of the cultivar tested (Figure 1). The highest yields were obtained for KS-7, this cultivar gave 14 multiple shoots per single initial shoot. Moderate yields were obtained from A3adT-8 and Angoorlata gave low yields.

This tissue grew well, was really sub-cultured and appeared to bear masses of shoot primordia (Figure 2). Experiments with KS-7 showed shoot formation was stimulated by culturing shoots in low concentration of agar/liquid medium (coconut coir).

Culture flasks were inoculated with 2-3 shoots each bear 5nodes. After 6weeks, flasks were filled with shoots which appeared to develop from axillary buds.

The effect of a range of BAP concentrations on shoot development in the absence of cytokinin, shoots obtained were relatively few in no and large in size.

The elongated shoots were excised individually and transferred to MS Medium supplemental with different concentration of IAA, IBA and NAA for rooting.

The initiations of roots from three varieties of tomato were used different concentration of plant growth regulators.

In KS-7 were recorded maximum no. of roots in MS media with (1.5mg/l) NAA and (3.0 mg/l) IBA, *Angoorlata* were recorded maximum no of Roots MS media with (1.0mg/l NAA + 2 mg/l IBA) and A3adT-8 were recorded highest no. of Roots MS media supplemented with (1.5mg/l) NAA and (3.0mg/l) IBA.

Most of the reports about adventitious regeneration in tomato deal with induction of regeneration in hypocotyl or cotyledon explants (Rashid and Bal, 2010).

Studying the *in vitro* organogenesis in tomato cv. S-22, using 10-12 d cotyledonary explants, Vikram *et al.*, (2011) obtained highest callus induction on media containing BAP 3 mg/L and multiple adventitious shoots on MS media with 0.1 mg/L IAA and 2.5–5.0 mg/L BAP, which support our results, wherein we obtained multiple shoots from hypocotyl and cotyledon explants on MS media supplemented with BAP and IAA.

Various combinations of auxins/cytokinins have been used to study their effect on regeneration (Rao *et al.*, 2005) (Gubis *et al*., 2004), observed that zeatin (1mg/l) and IAA (0.1 mg/l) supplemented media had 100% regeneration frequency with hypocotyl explants in all genotypes studied. However, we obtained a regeneration frequency of 85%, 64% and 55% for hypocotyls explants in media with auxin and cytokinins combination respectively.

The result of this study demostrates the callus induction and shoot regeneration for three cultivars of Tomato with MS media and different combination of plant growth regulators. For further multiplication the callus were transferred on the multiplication medium the physical appearance of all calli was friable and ranging from yellow to brown (Kumar *et al.*, 2003, 2004).

In previous reports genotypic various variation was observed among the tomato cultivers in terms of callus and regeneration responses (Bhatia *et al*., 2005).

All the earlier reports shows that the good and fasterresponse of callus are formed only in medium having equal combination of auxin and cytokinins (Chaughary *et al.*, 2004). The auxin and cytokinin (1/4) proportion we were used in the present study study (0.5mg/l NAA + 2mg/l BAP) increased the callus induction significantly and higher freque.

Tomato regeneration is genotype, explant and media dependent. Callus induction was observed in both hypocotyls and cotyledon explants.

The present investigation showed that the medium containing BAP (2mg/l) and IAA (0.1mg/l) are better organogenesis and regeneration was observed on MS medium supplemented with NAA 1.5mg/l), Kn (3mg/l), BAP (5mg/l) for hypocotyls explants.

This study is a baseline to carry further research on this tomato variety for improvement by using gene transfer technology.

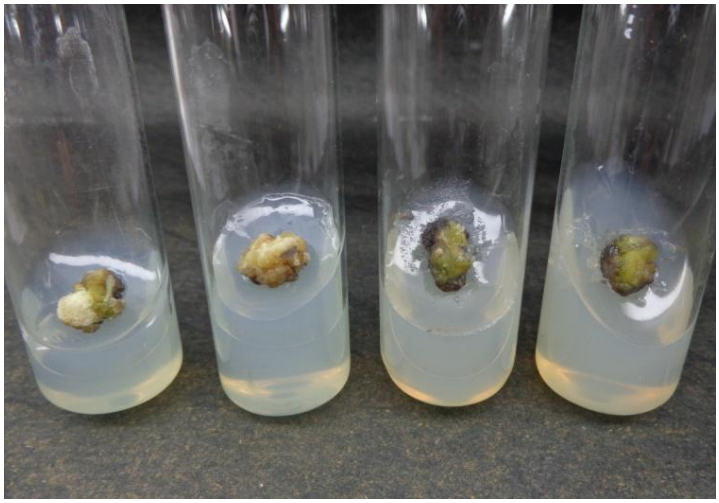


Figure 1: Initiation and Multiplication of Callus on MS Media with Cytokinins and Auxins

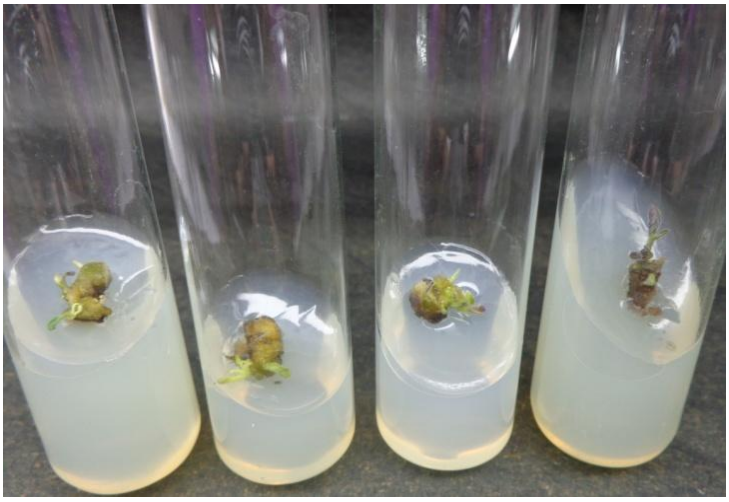


Figure 2: In-Vitro Induction of Organogenesis of Three Cultivars of *Solanum lycopersicon*

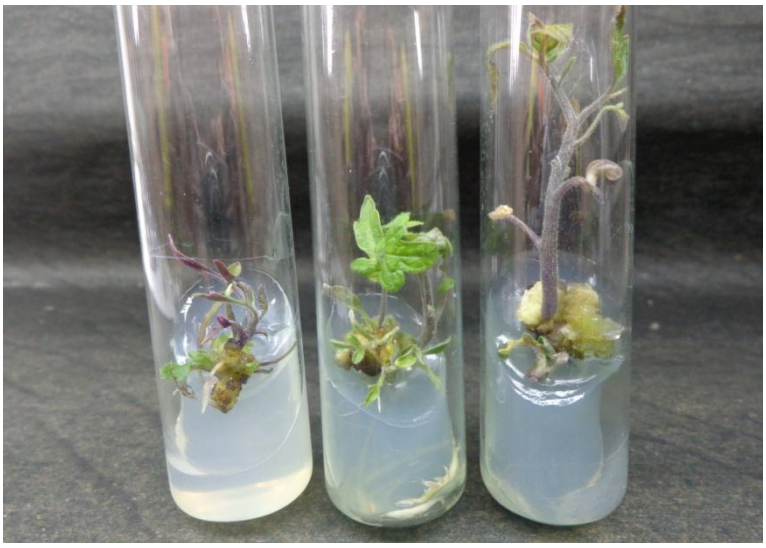
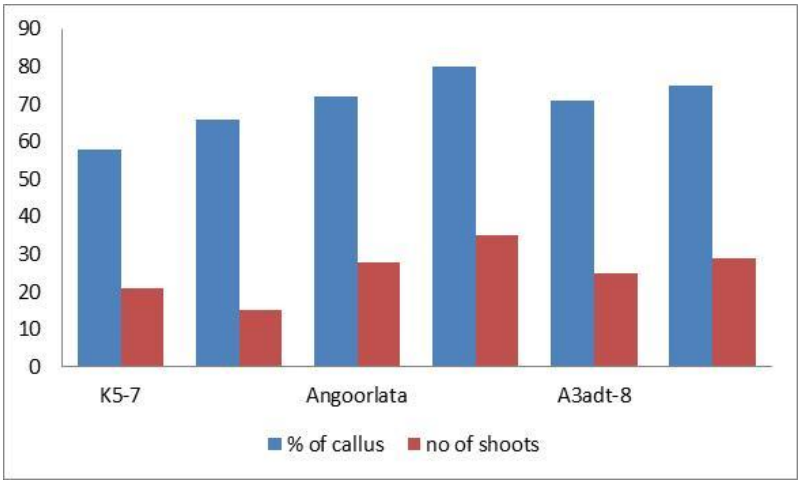
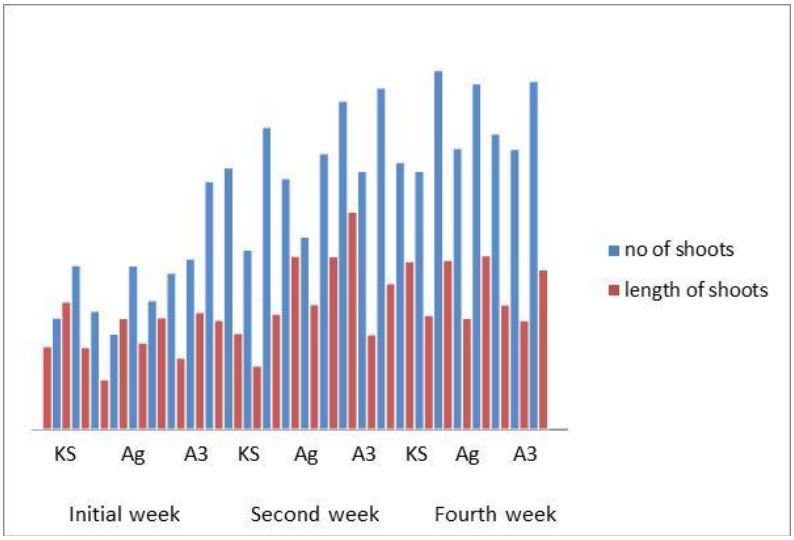


Figure 3: Multiplication of Shoots of all Cultivars on MS Media with BAP

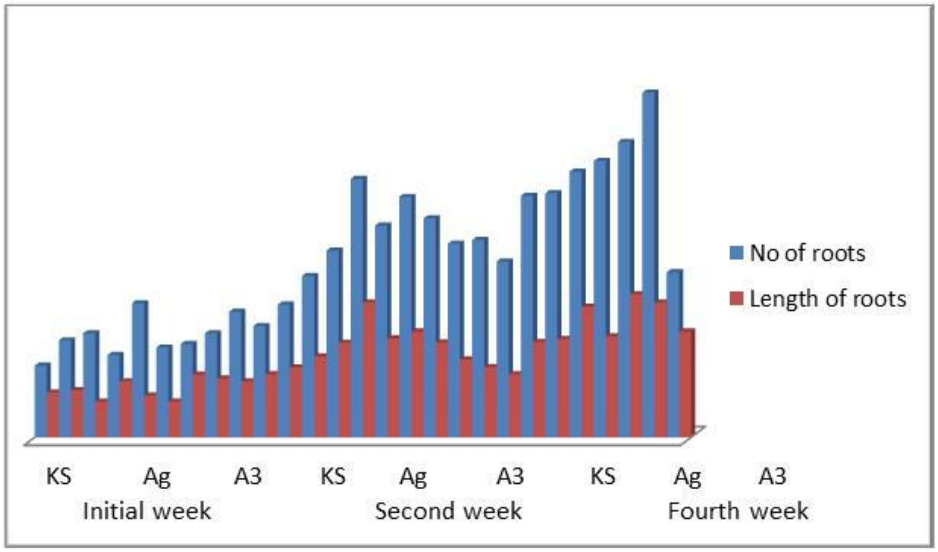
Figure 4: Initiation and Elongation of Root of all KS-7 on MS Media with NAA and IBA



Graph 1: Callus Induction and Regeneration of Cotyledon Explants of the Three Variety Tomato (*Lycopersicon esculentum*) on MS Media with Different Concentration of Growth Regulators



Graph 2: Multiplication of Shoots of Three Variety Tomato *(Lycopersicon esculentum)* on MS Media with BAP (0.5 to 5 mg/l) and Kn(1 to 3 mg/l)



Graph 3: Effect of Plant Growth Regulators on Multiplication of Roots on MS Media with NAA (0.5 to 1.5 mg/l) and IBA (1 to 3 mg/l)

REFERENCES

Abdelmageed AH, Gruda N and Geyer B (2004). Effects of temperature and grafting on the growth and development of tomato plants under controlled conditions. *Rural Poverty Reduction through Research for Development and Transformation* 5-7.

Bhatia P, Ashwath N and David M (2005). Effect of genotype explants orientation and wounding on shoot regeneration in tomato. *In Vitro Cellular & Developmental Biology Plant* **41** 457-464

Chaudary Z, Feroz I, Ahmed W, Rashid H, Mirza B and Quraishi A (2001). Varietal response of *Lycopersicon esculentum* L. to callogenesis and regeneration. *Journal of Biological Sciences* **1**.

Chaudhry Z, Habib D, Rashid H and Qureshi AS (2004). Regeneration from various explants of *in vitro* seedling of tomato (*Lycopersicon esculentum* L., c.v. roma). *Pakistan Journal of Biological Sciences* **7**(2) 269-272.

Devi R, Dhaliwal MS, Kaur A and Gosal SS (2008). Effect of growth regulators on *in vitro* morphogenic response of tomato. *Indian Journal of Biotechnology* **7** 526–530.

Evans DA (1989). Somaclonal variation –genetic basis and breeding applications. *Trends in Genetic* **5** 46–50.

Gubis J, Lajchova Z, Farago J and Jurekov Z (2004). Effect of growth regulators on shoot inductionand plant regeneration in tomato (Lycopersicon esculentum Mill.). *Biologia, Bratislava* **59** 405-408.

Kumar S, Singh KK and Rai LK (2004). In vitro Propagation of a Endangered Sikkim Himalayan Rhododendron (R. maddeni) from Cotyledonary Nodal Segments. *Journal of American Rhododendron Society* **58**(2) 101-105.

Kumar S, Suri SS, Sonie KC and Ramawat KG (2003). Establishment of embryogenic cultures and somatic embryogenesis in callus cultures of guggul *(commiphora wightii arnott. Bhandari). Indian Journal of Experimental Biology* **41** 69-77.

Leblay C, Chevereau E and Raboin LM (1991). Adventitious Shoot Regeneration from In vitro Leaves of Several Pear Cultivars (Pyrus communis L.). *Plant Cell, Tissue and Organ Culture* **25** 99-105.

Rao KV, Kiranmayee K, Pavan U, Jaya Sree T, Rao AV and Sadanandam A (2005). Induction of multiple shoots from leaf segments, in vitro-flowering and fruiting of a dwarf tomato (Lycopersicon esculentum). *Journal of Plant Physiology* **162** 959-962.

Rashid R and Bal SS (2010). Effect of hormones on direct shoot regeneration in hypocotyl explants of tomato. *Notulae Scientia Biologicae* **2** 70-73.

Reed BM (1999). Design a micropropagation system: Workshop presentation from the 1998 SIVB Congress *on in vitro Biology*. *In Vitro Cellular & Developmental Biology – Plant* **35** 275-284.
Tiwari S, Arya A, Yadav P, Kumari P and Kumar S (2013). Enhanced *In vitro* Regeneration of Two Sugarcane Varieties COS 8820 and COS 767 through organogenesis. *Indian Journal of Fundamental and Applied Life Sciences* **3**(4) 17-26.
Uddin MF, Islaam SMA, Naznin S and Shiragi MHK (2004). Effect of variety and plant growth regulators in MS medium on callus proliferation from virus infected Tomato plant. *Asian Network for Scientific Information Biotechnology* **32** 181-186.
Vikram G, Madhusudhan K, Srikanth K, Laxminarasu M and Rama Swamy N (2011). Effect of plant growth regulators on in vitro organogenesis in cultivated tomato. *Journal of Research in Biology* **4** 263-268.