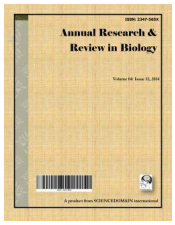

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# Establishment and Regeneration of Callus Cultures in Tomato (*Solanum lycopersicum* L.) from Various Explants

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author AK collected the literature, carried out the experiments and performed the statistical analysis. Author Shilpa assisted the experiments and wrote the first draft of the manuscript and author RK designed the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The present investigation aimed at *in vitro* establishment, multiplication and regeneration of plantlets from tomato callus using leaf, internode and root explants from *in vitro* geminated seedlings of cv. Solan Vajr.
**Study Design:** Completely randomized design (CRD).
**Place and Duration of Study:** Department of Biotechnology, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India -173230, between 2013 and July 2015.
**Methodology:** Murashige and Skoog (MS) medium given in 1962 was used to carry out tissue culture experiments. Seeds of Tomato cv. Solan Vajr were sterilized using bavistin and sodium hypochlorite before proceeding for germination. Different concentrations of 6- Benzylaminopurine (BAP) and Naphthalene acetic acid (NAA) were tried for callus establishment, multiplication and regeneration.

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**Results:** Treatment of leaf explant with 0.1% (w/v) bavistin for ten minutes and 0.5% (v/v) sodium hypochlorite for three minutes was the most effective giving 68.05% uncontaminated cultures and 60.94% survival of seedlings. MS medium supplemented with 1.2 mg/l $GA_3$ gave the optimum seedling germination of 32.33%. MS medium supplemented with 3.0 mg/l NAA+ 1.0 mg/l BAP gave maximum growth of callus. Leaf and root explants gave 95.21% and 81.11% callus induction than internode (100%). The medium found best for callus growth was also found most suitable for callus multiplication and regeneration.
**Conclusion:** This study standardized protocols for callus initiation from different types of explants. Internode was found the best explant for callus induction in tomato cv. Solan Vajr. Protocol was also successfully established for multiplication, maintenance and regeneration from callus. This *in vitro* generated callus can further be used for cell selection studies.

*Keywords: Seedlings; explants; medium; callus; cultures.*

## 1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.), belonging to family Solanaceae, is a widely grown vegetable all over the world [1]. Tomato is rich in vitamins (vitamin A and C), minerals and lycopene. It is an excellent source of antioxidants that help to reduce the risk of many types of cancers [2]. But tomato is susceptible to many diseases caused by bacteria, fungi and viruses. Conventional breeding programs including extensive intermating and screening campaigns help breeders to improve cultivars, however, this is limited by inherent difficulties, open pollination, high level of heterozygosity and poor fertility of $F_1$ hybrids. As biotechnology represents the modern techniques to improve the existing cultivars through tissue culture, genetic engineering and marker assisted studies, thus, an alternative to conventional breeding, one approach for obtaining useful genetic variation is to select for somaclonal variants generated by tissue culture techniques [3]. Before proceeding this kind of work, it is necessary to generate efficient protocols for establishment and multiplication of cultures. Keeping this in view present study was undertaken for the establishment of callus cultures in cv. Solan Vajr of tomato from suitable explants.

## 2. MATERIALS AND METHODS

MS medium given by Murashige and Skoog [4] was used for tissue culture experiments.

### 2.1 Plant Material

Tomato cv. Solan Vajr is susceptible to many diseases but commercially is high yielding was selected for the experiments. Seeds of Solan Vajr were procured from the Department of Vegetable Science, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP) which were used for raising *in vitro* seedlings.

### 2.2 Sterilization and Germination of Seeds

Seeds of tomato were dipped overnight in distilled water. The seeds were then washed under running tap water for one hour followed by a treatment with 5% (v/v) aqueous solution of teepol for 20 minutes and rinsed 4 to 5 times with distilled water. In the laminar air flow chamber the seeds were treated with 0.1% bavistin followed by 0.5% sodium hypochlorite for 3 to10 minutes. The seeds were washed thrice with autoclaved distilled water after every treatment to remove the traces of sterilants before transferring to culture medium.

The sterilized seeds were cultured on solid MS basal medium supplemented with different concentration of $GA_3$ ranging from 0.1 to 1.5%. The cultures were then incubated in culture room at 25 ± 2°C initially in dark, later on under 16 hours photoperiod. Well developed seedlings were obtained within 15 to 25 days of culturing which were later used as source for cotyledons, leaves, internodal segments and root explants of 0.5 to 1.0 cm size.

### 2.3 Callus Establishment, Multiplication and Regeneration

Different concentrations and combinations of plant growth regulators viz., NAA (0.5 to 3.0 mg/l) and BAP (0.5 to 1.0 mg/l) were tried on callus induction using different explants viz., leaves, internodal segment and root explants. The cultures were incubated initially under dark for one week and then at 25 ± 2°C under 16 hour photoperiod. Fresh green callus was cut and multiplied on callus establishment medium. The

optimum growth of the callus was determined on the basis of type of callus, change in color and size of callus. Finally the best performing medium is selected on which the callus growth was optimum. Sub-culturing of callus was continued on standardized MS medium complemented with 3.0 mg/l NAA and 1.0 mg/l BAP for further multiplication. Different concentrations of BAP (0.25 to 3.50 mg/l) and NAA (0.05 to 0.5 mg/l) were attempted for shoot regeneration.

## 3. RESULTS AND DISCUSSION

### 3.1 Sterilization of Seeds

Best treatment for surface sterilization of seeds was found out to be treatment comprising of 0.1% (w/v) bavistin for 10 minutes and 0.5% (v/v) sodium hypochlorite for 3.0 minutes which resulted in 66.40% uncontaminated cultures and 60.94% survival of the seeds. With further increase in treatment duration per cent survival of cultures decreased from 60.94% to 45.42%. Lowest uncontaminated cultures (6.21%) was recorded in the seeds that were treated with 0.1% bavistin for 3.0 minutes followed by 0.5% sodium hypochlorite for 3.0 minutes. From this it was observed that higher duration of treatment with sterilant can prove lethal and results in lower survival of seeds. Several workers earlier used the different sterilants such as 0.2% bavistin for 7 minutes followed by 0.1% $HgCl_2$ for three minutes [5], 20% chlorax treatment [6], 0.20% solution of SAAF (Carbendazim 12% + Mancozeb 63%) treatment for 10 minutes [7].

### 3.2 *In vitro* Establishment of Seedlings

The *in vitro* establishment of seedlings was attempted on MS basal medium as well as on MS medium supplemented with different concentrations of $GA_3$. Observations were recorded for five weeks of seed culturing, at an interval of a week and experiment was repeated thrice. MS basal medium + 1.2 mg/l $GA_3$ was the best treatment showing 32.53% germination of seeds within 20-25 days of culturing , followed by 1.0 mg/l $GA_3$ which gave 25.58% seed germination after 20-25 days of culturing (Fig. 1.).

### 3.3 Establishment of Callus Cultures

For callus induction various explants namely leaf, internode and root were excised from the *in vitro* grown 20 days old seedlings (Fig. 2). The explants were cut into small pieces and cultured on to the MS medium supplemented with various concentrations and combinations of growth regulators i.e. NAA (0.5-2 mg/l) and BAP (0.5 mg/l) (Table 1). Callus initiation was observed after about one week of culturing from the cut ends of the explants. The surface of the explant was covered with callus in four weeks of culturing. Data presented in the Table 1 shows that root was the least responding explant for callus induction. 95.21%, 100.00%, 81.11% callus induction was observed on MS medium supplemented with 3.0 mg/l NAA and 1.0 m/l BAP in leaves, internode and root respectively. It was observed that internode was the best responding explant and conferred maximum callus formation (100.0%) in treatment $TC_7$ i.e. MS basal medium+3.0 mg/l NAA+ 1.0 mg/l BAP. Concentration of NAA higher than BAP proved best as is substantiated in earlier studies [7-9].

In the present study juvenile explants gave good response, though no comparison had been made with mature explants for callus induction. Similarly better results for *in vitro* callus induction from juvenile explants were reported by Murkute et al. [10] where they obtained better callus induction and proliferation from cotyledon explant (juvenile explant) than from leaf explant (mature explants) of *Punica granatum* L. cv. Ganesh on MS medium containing 1.0 mg/l BA and 0.5 mg/l NAA. Verma et al. [11] also reported better response of cotyledonary explant than other explants for callus induction and shoot regeneration. Higher per cent callus induction from cotyledon explant of tomato than leaf explants on MS medium supplemented with 1.0 mg/l of zeatin, 1.0 mg/l of IAA and 2.0 mg/l of BAP was also observed by Majoul et al. [12].

Texture and color of callus varied with the concentration of growth regulators and type of explant. Most of the leaf and internode calli were green in color, compact in nature and vigorous in growth pattern, while light green, cream colored and friable callus was obtained in case of root explant (Table 1). Best responding medium for obtaining best green and friable callus was MS basal medium supplemented with 3.0 mg/l NAA+ 1.0 mg/l BAP.

There are several studies which reported callus initiation from leaf segment, cotyledon and hypocotyl explants of tomato e.g. Leaf disc [12, 13], hypocotyl and cotyledon [14-16]. Some earlier studies also reported callus initiation on

MS basal medium supplemented with different combinations of auxins and cytokinins e.g. MS +2.0 mg/l IAA+1.0 mg/l Kn [14], MS + 0.5 mg/l IAA+2 mg/l 2ip [15], MS+2.0 mg/l BAP and 0.2 mg/l NAA [17].

For present work some of the earlier protocols were also tried but all these protocols did not work for callus initiation in tomato cv. Solan Vajr. Medium No. 7 (Table 1) was successfully used for callus induction and maintenance, proving that tissue culture response is genotype specific [13]. The quality of callus was green, pale yellow and compact in nature.

### 3.4 Multiplication of Callus and Regeneration of Shoots

The callus formed from the cut ends of the initial explants were separated and subcultured on the previously optimized medium given in Table 2. $TC_7$ medium i.e. the one supplemented with 3.0 mg/l NAA+ 1.0 mg/l BAP was found to be best medium for callus multiplication. Hence, $TC_7$ medium was alone subsequently used for the multiplication and maintenance of the callus cultures.

For shoot regeneration the calli were cut into small pieces and cultured on MS medium supplemented with different concentrations of BAP and NAA. At low concentrations of BAP i.e from 0.25-0.75 mg/l, callus did not respond for shoot regeneration and turned brown. However, when callus cultured on higher concentration of BAP the callus turned into green nodular form. Slightly raised nodulation of green color were observed on callus on MS medium containing 1.50, 1.75 and 3.0 mg/l BAP (Fig. 3). These results were supported by the findings of Oceania et al. [18] and Papry et al. [19] who obtained shoot multiplication on MS medium containing 3.0 mg/l BAP and 2.0 mg/l BAP, respectively.

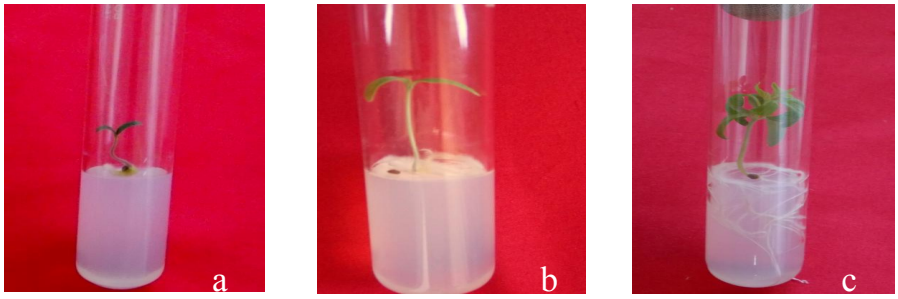


**Fig. 1. *In vitro* establishment of seedlings on MS basal + 1.2 mg/l $GA_3$ after (a)10 days, (b)15 days and (c) 25 days**

**Table 1. Response of different explants for callus induction after 30 days of incubation on MS medium supplemented with different concentrations of plant growth regulators**

| S. No. | NAA (mg/l) | BAP (mg/l) | Leaf callus induction (%) | Internode callus induction (%) | Root callus induction (%) | Characteristics of different callus | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Leaf | Internode | Roots |
| 1 | 0.5 | 0.5 | 10.40(18.79) | 16.79(24.18) | 0.00(0.00) | F+PY | F+PY | - |
| 2 | 1.0 | 0.5 | 22.71(28.45) | 30.61(33.57) | 19.43(26.13) | F+PY | F+PY | F+PY |
| 3 | 1.5 | 0.5 | 38.62(38.40) | 41.36(40.01) | 38.12(36.11) | C+PY | C+PY | F+PY |
| 4 | 2.0 | 0.5 | 49.56(44.73) | 50.66(45.36) | 35.55(38.58) | C+PY | C+PY | C+PY |
| 5 | 2.5 | 0.5 | 56.50(48.71) | 66.72(54.74) | 53.44(46.95) | C+PY | C+PY | C+PY |
| 6 | 3.0 | 0.5 | 75.39(60.24) | 78.34(62.24) | 73.44(58.96) | C+PY | C+PY | C+PY |
| **7** | **3.0** | **1.0** | **95.21(77.45)** | **100.00(90.00)** | **81.11(64.21)** | **C+N+LG** | **C+N+LG** | **C+N+LG** |
| 8 | 2.5 | 1.0 | 80.32(63.64) | 80.22(63.59) | 52.47(46.39) | C+N+LG | C+N+LG | C+LG |
| 9 | 2.0 | 1.0 | 70.47(57.06) | 72.63(58.44) | 62.21(52.05) | C+LG | C+N+LG | C+LG |
| 10 | 1.5 | 1.0 | 72.00(58.03) | 69.28(56.32) | 67.47(55.20) | C+LG | C+LG | F+LG |
| 11 | 1.0 | 1.0 | 56.67(48.81) | 57.16(49.09) | 52.32(46.31) | F+LG | F+LG | F+LG |
| 12 | 0.5 | 1.0 | 32.19(34.54) | 41.77(40.24) | 20.69(27.04) | F+LG | F+LG | F+LG |
| C.D | | | **1.72** | **1.26** | **1.404** | | | |
| S.E | | | **0.58** | **0.43** | **0.478** | | | |

*Parentheses values are arc sine transformed; C: Compact, N: Nodular, LG: Light green, PY: Pale yellow*

**Table 2. Effect of hormones on growth of callus during subcultures**

| Medium code | NAA (mg/l) | BAP (mg/l) | Level of growth |
|---|---|---|---|
| $TC_5$ | 2.5 | 0.5 | Fair |
| $TC_6$ | 3.0 | 0.5 | Fair |
| $TC_7$ | **3.0** | **1.0** | **Maximum growth** |
| $TC_8$ | 2.5 | 1.0 | Good |
| $TC_9$ | 2.0 | 1.0 | Good |
| $TC_{10}$ | 1.5 | 1.0 | Fair |


**Fig. 2. Different explants cultured for callus induction on MS basal +3.0 mg/l NAA+ 1.0 mg/l BAP (a) Leaf explants (b) Internode explants and (c) Root explants**

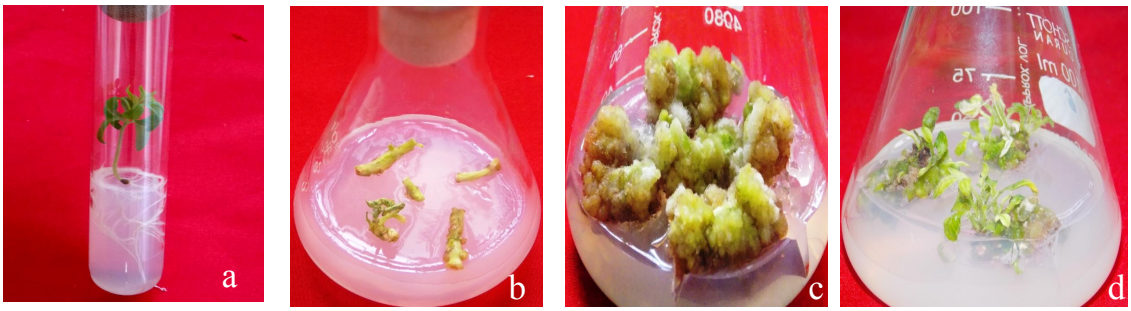


**Fig. 3. a) *In vitro* establishment of seedlings, b) callus induction from internode explants of 25 days old seedlings on MS basal supplemented with 3.0 mg/l NAA and 1.0 mg/l BAP, c) callus multiplication and d) shoot regeneration from the calli**

## 4. CONCLUSION

Crop improvement is primarily achieved by the production of better varieties using classical breeding techniques. Plant cell culture techniques will help to increase the efficiency of overall breeding procedures. With same aim this study focused on the development of efficient protocols to raise plants through tissue culture concluding that internode is the best explant among various other explant types for callus induction in tomato cv. Solan Vajr along with successful established for multiplication, maintenance and regeneration. These *in vitro* standardized protocols can further be used for raising true to type plants, somaclonal variation and cell selection studies.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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