In vitro flowering and fruiting in tomato (*Lycopersicon esculentum* Mill.)

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Abstract. *In vitro* flowering and fruiting were induced in plants regenerated from calli of leaf explants of *Lycopersicon esculentum* var. Pant 11. MS medium supplemented with 2 mg/l BAP displayed the best response followed by MS with 2 mg/l BAP, 1 mg/l ABA and 0.5 mg/l IAA. Flower buds were developed both directly from callus and regenerated plantlets from leaf explants under 16 h (39.3 μmol m⁻² s⁻¹) photoperiod. They opened only under continuous dim light (2.2 μmol m⁻² s⁻¹) within 116 days of initial culture. Flowering was not observed in dark. Average number of flower buds per explant was found to be 10.8. Maximum pollen fertility was 71.4 % with an average pollen diameter of 0.52 mm and thickness 0.26 μm. Fruits were set *in vitro* within 162 days following self-pollination. Subsequently leaf and stem explants from seven other varieties viz. KS118, PP2, Le 3704, Le 79, No. 324, CI 9 d 0-0-6-3 and Pant 5 were evaluated on MS with 2 mg/l BAP for bud induction. All the varieties produced flower buds *in vitro*. However, only KS118 could produce fully bloom flowers. Profuse callus induction and simultaneous shoot bud regeneration were observed in all the varieties.

Keywords. *In vitro* flowering, fruiting, *in vitro* culture, *Lycopersicon esculentum*

INTRODUCTION

*In vitro* flowering bears immense importance in selective hybridization especially in using pollens from rare stocks. It also facilitates the understanding of physiology of flowering and largely depends upon the level and interaction of endogenous phytohormones, sugars, minerals, phenolics, (Tanimoto and Harada, 1981a; Tanimoto, 1981 b) quality and quantum of light, length of photoperiod etc. during *in vitro* culture (Taha, 1997). Differential light intensity was found to be required for shoot induction and flower initiation (Taha, 1997). This is a rare process of importance in crop plants, mainly due to its high genetic purity (Stephan and Jayabalan, 1998). *In vitro* flowering was reported in citrus (Moss, 1969), cauliflower (Vandana et al., 1995), maize (Mandal et al., 2000), coriander (Stephan and Jayabalan, 1998), bamboo (Nadgauda et al., 1990; Madhulika et al., 2000) etc. In tomato, the unilateral incompatibility among self-compatible (subgenus *Eulycopersicon*) and self-incompatible (subgenus *Eriopersicon*) species of the genus, *Lycopersicon* seriously limits genetic recombination through hybridization. Furthermore, less pollen formation and low fertility status are other serious hurdles in this endeavour (Hogeboom, 1972; Rick, 1983). *In vitro* fertilization is a feasible proposition to overcome this incompatibility barrier (Hogeboom, 1972). It also enables the production of precious hybrid seeds in off-season too. Hence *in vitro* flowering and fruiting may significantly contribute to genetic improvement of tomato involving rare stocks, not accessible through conventional plant breeding. Tomato is highly compatible to *in vitro* culture of various tissues and organs. Different types of responses like callus induction, regeneration of whole plantlets, roots and pseudo-fruit differentiation have been reported in tomato (Kartha et al., 1976, Tal et al., 1977; Kut and Evans, 1982; Kurtz and Lienieberger, 1983; Locky, 1983; Zorzoli et al., 1993 a,b) In general, media with varying BA concentrations (1-5 mg/l) in combination with IAA was found to be suitable for shoot regeneration (Kartha et al., 1976). The objective of this study was to identify media suitable for *in vitro* flower formation and to compare the genotypes. The present communication reports successful *in vitro* flowering and fruit formation in tomato for the first time.

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MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum* Mill.), var. Pant 11, grown widely in the humid tropics of Andaman and Nicobar Islands, India was employed for the present study. Seeds were soaked in 2% (w/v) Bavistin solution (carbenzazim) with 5% (v/v) Teepol for 15 minutes for decontamination. They were surface sterilized using freshly prepared 0.1% HgCl₂ for 5 minutes. Seeds were washed three times with sterile distilled water, blot dried and implanted onto half strength Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) with 3% sucrose and solidified with 0.8% agar. To facilitate callus induction 2 mg/l 6-Benzylamino Purine (BAP) was used. The pH of the medium was adjusted to 5.8±0.02 prior to autoclaving at 1.05-kg/cm² pressure and 121°C for 20 minutes. Leaf and stem explants from 15 days old *in vitro* grown seedlings were used for *in vitro* culture. They were cut into ~1cm² pieces and cultured aseptically. Cultures were incubated under 16/8 h light/dark cycles. Light intensity of 39.3 µmol m⁻² s⁻¹ received from cool white fluorescent lamps was maintained (Philips, India). A minimum of 15 replicates was used in each treatment and each experiment repeated thrice. After 30 days of culture about 0.5 g calli were transferred onto MS with various hormones at different doses singly or in combination, viz T1 2 mg/l BAP; T2 2 mg/l BAP+0.5 mg/l gibberellic acid (GA); T3 2 mg/l BAP +1.0 mg/l GA + 0.5 mg/l IAA; T4 2 mg/l BAP +1.5 mg/l GA + 0.5 mg/l IAA; T5 2 mg/l BAP +2.0 mg/l GA + 0.5 mg/l IAA; T6 2 mg/l BAP +1 mg/l Abscisic acid (ABA) + 0.5 mg/l IAA; T7 2 mg/l BAP +1 mg/l ABA + 0.5 mg/l IAA; T8 2 mg/l BAP +10µM AgNO₃ and T9 2 mg/l BAP+20µM AgNO₃. After bud formation the cultures were shifted to continuous light of low intensity (2.2 µmol m⁻² s⁻¹.) for flower induction both *in vitro* and *in vivo* conditions. Anthers were removed and anthers squashed in distilled water, centrifuged and stained in I-KI solution (Glenner, 1968). Diameter of randomly selected 50 pollens in each microscopic field was recorded and mean value expressed in µm. Pollen fertility was calculated in terms of percent fertility using the following formula:

\[
\text{Fertility} \% = \frac{\text{Number of fertile pollens}}{\text{Total number of pollens counted}} \times 100
\]

To assess flower induction potential and to confirm earlier findings, leaf and stem explants of seven other varieties (PP2, Pant 5, KS118, No: 324, CI 9 d 0-0-6-3, Le 3704 and Le 79) were cultured following the same protocol on MS with 2 mg/l BAP. Simultaneous evaluation of *in vitro* culture response such as callus induction and regeneration of whole plantlets and shootlets were done. Data were statistically analysed.

RESULTS AND DISCUSSION

Callus induction was observed within 8-10 days of culture from leaf explants of Pant 11 on all the treatments evaluated. The explants swelled up and calli started growing from the cut surface. Calli remained healthy and showed plantlet regeneration from both leaf and stem explants. T1 was found to regenerate maximum number of whole plantlets, shoots and flower buds (Table 1). Furthermore, T4 and T7 were found to be promising for induction of shootlets, while T5 and T6 showed best response for flower bud induction as well as whole plantlet development. It was observed that presence of GA reduced the number of buds/explant. This was contradictory to the earlier observations of Yang (2000) in *Panax ginseng*. However, T5 was found to be the best among all treatments involving GA singly or in combination producing calli with very good health. T1 and T3 induced profuse callus over the entire surface of the plantlet. T4 completely inhibited flower bud induction and simultaneously developed brownish calli and yellowish plantlets. Similarly increased levels of ABA decreased the number of buds/explant. This is corroborative to the observations made in *Murraya paniculata* (Taha, 1997). It indicates the presence of higher levels of endogenous auxins in tomato explants used in this study. BAP was found to promote bud formation as observed in tobacco (Smulders et al., 1990) and maize (Mandal et al., 2000) playing an important role not only as a growth regulator, but also as a factor regulating floral organ formation of regenerated plantlets. Only in T1, T2 and T6 blooming of flower buds was observed, the best response being in T1. It is mentionable that in T6 efficient rooting was induced in tomato plantlets due to the presence of AgNO₃ similar to observations in *Brassica campestris* (Palmer, 1992). Thus presence of gibberellic acid and ABA inhibited the overall efficiency in terms of plantlet regeneration, callus and flower bud induction in our study. Induced flower buds attained full bloom only when the cultures were transferred to low light intensity regime (2.2 µmol m⁻² s⁻¹) within a period of 4 days. It was also observed that under 16/8 h light/dark cycle buds failed to bloom and died off. Therefore day length plays a crucial role in flower induction both *in vitro* and *in vivo* possibly due to altered photosynthetic turnover on flowering (Pierik, 1967). Cultures maintained under dark did not produce flowers and/or buds. This is in agreement with earlier observations (Pierik, 1967 and Taha, 1997). Days required for flower induction and fruit set were however, found to be more under *in vitro* condition in contrast to *in vivo* conditions.

Further studies are warranted to reduce this time through manipulation of photoperiod and physico-chemical environments.

Profuse bud formation was observed both directly from the callus (Figure 1a) as well as from the plantlets (Figures 1b, 1c). A maximum of 13 buds was generated from a single piece of callus in the variety Pant 11. Fully developed, normal
### Table 1. Effect of various phytohormones in influencing *in vitro* culture response in tomato var. Pant-11

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phytohormone</th>
<th>No. of regenerated whole plantlets recovered/explant cultured</th>
<th>No. of shoots/explant</th>
<th>No. of flowerbuds/explant</th>
<th>No. of flowers/explant</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2 m.g/l BAP</td>
<td>1.4</td>
<td>5.1</td>
<td>10.5 (100)</td>
<td>0.6 (30)</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2 m.g/l BAP + 0.5 mg/l GA + 0.5 mg/l IAA</td>
<td>0.9</td>
<td>1.1</td>
<td>0.4 (40)</td>
<td>0.2 (10)</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2 m.g/l BAP + 1.0 mg/l GA + 0.5 mg/l IAA</td>
<td>0.5</td>
<td>1.4</td>
<td>0.8 (40)</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>2 m.g/l BAP + 1.5 mg/l GA + 0.5 mg/l IAA</td>
<td>0.2</td>
<td>1.3</td>
<td>1.6 (100)</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>2 m.g/l BAP + 2 mg/l GA + 0.5 mg/l IAA</td>
<td>0.4</td>
<td>2.4</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>2 m.g/l BAP + 1.0 mg/l ABA + 0.5 mg/l IAA</td>
<td>0.8</td>
<td>1.1</td>
<td>5.1 (100)</td>
<td>0.2 (10)</td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt;</td>
<td>2 m.g/l BAP + 2 mg/l ABA + 0.5 mg/l IAA</td>
<td>0.4</td>
<td>2.6</td>
<td>1.1 (40)</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;8&lt;/sub&gt;</td>
<td>2 m.g/l BAP + 10 µM AgNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.5</td>
<td>3.3</td>
<td>2.2 (100)</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;9&lt;/sub&gt;</td>
<td>2 m.g/l BAP + 20 µM AgNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.5</td>
<td>2.1</td>
<td>0.6 (20)</td>
<td>0</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td></td>
<td>0.78</td>
<td>2.22</td>
<td>1.85</td>
<td>0.126</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percent of explants with flower buds and/or flowers.

### Table 2. Genotypic variation in *in vitro* flower induction

<table>
<thead>
<tr>
<th>Designation</th>
<th>Days to initial flower bud induction</th>
<th>Leaf explant</th>
<th>Stem explant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of shoots/explant</td>
<td>No. of flowers/explant</td>
<td>No. of flowerbuds/explant</td>
</tr>
<tr>
<td>PP2</td>
<td>65</td>
<td>6.7(2.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 (10)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>KS118</td>
<td>58</td>
<td>24(1.1)</td>
<td>0.6 (4)</td>
</tr>
<tr>
<td>PANT 11</td>
<td>65</td>
<td>24.8(6.8)</td>
<td>0.6 (30)</td>
</tr>
<tr>
<td>NO. 324</td>
<td>96</td>
<td>1.7(1.0)</td>
<td>0</td>
</tr>
<tr>
<td>Cl 9 d 0-0-6-3</td>
<td>138</td>
<td>3.5(1.7)</td>
<td>0</td>
</tr>
<tr>
<td>PANT 5</td>
<td>99</td>
<td>3.0(0.6)</td>
<td>0</td>
</tr>
<tr>
<td>Le 3704</td>
<td>109</td>
<td>1.33(0.3)</td>
<td>0</td>
</tr>
<tr>
<td>Le 79</td>
<td>65</td>
<td>10.2(3.0)</td>
<td>0.2 (20)</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td></td>
<td>1.63</td>
<td>0.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean no. of whole plants and shootlets regenerated/explant
<sup>b</sup> Percent induction of flowers
<sup>c</sup> Percent induction of flower buds

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Figure 1. *In vitro* flowering and fruiting in tomato. a) Multiple flower buds induced on MS with 2 mg/l BAP b) A fully grown plantlet bearing flower buds just before flowering. c) Fully bloomed flowers under *in vitro* culture. d) Stereo microscopic view of the LS of a young flower bud. e) Microscopic field showing I-KI stained fully fertile pollen grains. f) Microfruits in bearing. g) Fully ripened microfruit.
flowers were produced with 5 sepals, 5 petals and functional hypogynous ovary (Figure 1d). The flowers remained open for 18 days. Such an extended period offers ample scope for genetic manipulation via in vitro pollination. Histological sections under stereomicroscope showed fully developed anthers, ovary and associated floral parts (Figure 1d). Pollen fertility was found to be 71.4%. Pollens took dark brown stain in KI-I solution. Both filled and empty pollen grains were visible in the microscopic field (Figure 1e). The flowers, when shifted back to 16/8 h light/dark cycle, produced fruits within 162 days of initial culture of the explant (Figure 1f, 1g). Those micro fruits appeared to be normal with a diameter of ~1 cm and girth of ~3.1 cm within the culture tube.

Evaluation of seven other varieties showed that flower buds could be induced in all of them. The superiority of the leaf explants over stem pieces in inducing maximum flower buds was observed in all varieties except var. KS118, which showed five times more bud induction from stem explant. This disparity is probably attributed to the variation in endogenous hormone levels. Flower bud induction was obtained in all the varieties, earliest in KS118 within 58 days of culture and latest in Cl 9 d 0-0-6-3 within 138 days. The number of days taken for flower bud induction varied among varieties (Table 2). Only KS 118 produced flower buds from both stem and leaf explants. Pant 5 showed maximum flower buds from leaf explant derived calli but failed to produce flowers. Only Pant 11 and KS118 produced fully open flowers from leaf and stem explants derived regenerants, respectively and the former developed completely ripened fruits while the latter produced abnormal, small, cream coloured flowers with large sepals emerging directly from the main stem probably due to artifacts induced during in vitro culture. The other varieties produced small flower buds, which did not produce normal flowers and fruits.

Simultaneous evaluation of in vitro culture responses in terms of callus induction and plantlet regeneration showed that explants expanded rapidly and started initiating good healthy callus along the cut ends of stems and cut ends and midribs of leaf explants within 8-10 days of culture. Calli remained greenish except in Le 3704, Cl 9 d 0-0-6-3 and No. 324, where it was whitish. Simultaneous occurrence of callus and shoots was found as observed commonly in L. esculentum species (Hille, 1989). Profuse shoot bud induction and whole plantlet regeneration was observed in Pant 11, Pant 5, Le 79 and KS 118 (Table 2). The most in vitro culture responsive variety, Pant 11, which showed good flower bud induction, also showed the best response in terms of shoot bud regeneration and whole plantlet formation. The present study warrants further experimentation to achieve flowering and fruit set from those varieties, which failed to produce mature flowers. In addition, a shortened time period to obtain in vitro flowering and fruit set would be an added advantage in accomplishing in vitro pollination for rapid production of desired hybrids from rare stocks during off seasons too.

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REFERENCES


