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In vitro regeneration of *Lycopersicum esculentum* Mill.

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An *in vitro* regeneration system for *Lycopersicum esculentum* variety CO2 was developed to produce transgenic plants. The transformation and regeneration protocol was based upon indirect organogenesis from seedling-derived explants method. Initially the seed was inoculated in basal medium to produce aseptic explants. Callus were produced from aseptic explants through use of MS medium supplemented with 2,4,Dichlorophenoxy acetic acid at various concentration ranges from 0.01-0.1 mg/l. Regeneration of callus was achieved when it is transferred to medium supplemented with 6-benzyl aminopurine, Indole acetic acid and Naphthalene acetic acid alone and in combination. Callus induction was observed on MS medium augmented with 0.1 mg/l 2,4-D. Calli which were separated from the primary culture and subcultured on fresh medium containing MS supplemented with 0.6 mg/l NAA and 0.4 mg/l IAA also showed increased biomass. Regeneration of callus was achieved when MS medium supplemented with 0.5 mg/l BAP. Regenerated plantlets having secondary and tertiary roots were considered to be fully regenerated and were transferred to plastic pots for hardening in a mixture containing Red soil sand and vermiculate.

Key words: Callus induction, Plantlet regeneration, Shooting, Rooting.

The word tomato may refer to the plant *Solanum lycopersicum* or the edible, typically red, fruit that it bears. Having originated in America, the tomato was spread around the world following the Spanish colonization of the Americas, and its many varieties are now widely grown, often in greenhouses in cooler climates. The tomato is consumed in diverse ways, including raw, as an ingredient in many dishes and sauces, and in drinks. While it is botanically a fruit, it is considered a vegetable for culinary a purpose which has caused some confusion. The fruit is rich in lycopene, which may have beneficial health effects. Prior to general introduction of this trait tomatoes were able to produce more sugar during the process of ripening and were sweeter and more flavorful. Plant tissue culture is a collection of techniques used to maintain plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture (Durzan *et al.,* 1984) is widely used to produce clones of a plant in a method known as micro-propagation (Ichimura *et al.,* 1995). Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, (Georg *et al.,* 1984) including: The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits. Modern plant tissue culture is performed under aseptic conditions under HEPA filtered air provided

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by a laminar flow cabinet. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting material (explants) in chemical solutions (usually sodium or calcium hypochlorite or mercuric chloride) is required.

Materials and Methods

Aseptic plant preparation

The cherry type of *L. esculantum* variety CO-2 were washed thoroughly in running tap water and surface sterilized with detergent Tween 20 for 15 minutes. Wash again with running tap water and rinsed with commercial fungicide (Bavastin). Wash the seeds with running tap water for 15 minutes and taken to laminar air flow chamber aseptically. Then it was rinsed with double distilled water followed by 70% alcohol for 30 seconds, 0.1% mercuric chloride ($HgCl_2$) for 3-5 min and rinsed five times with sterile distilled water. The surface sterilized seeds were cultured in test tube containing 1000ml Murashige and Skoog (MS) medium supplemented with 0.8% agar, 3% sucrose and 1% myoinositol. Care must be taken to avoid contamination while transferring seeds to culture tubes. The pH of the medium was adjusted to 5.7 using 0.1N NaOH or HCl, prior to autoclaving.

Explant Preparation and Plant Regeneration

3-4cms of stems were excised from 25 days old aseptic *L. esculantum* plants and inoculated in MS medium supplemented with 2,4-Dichlorophenoxyacetic acid at different concentration (0.02, 0.04, 0.06, 0.08 and 0.10 mg/l) for induction of callus. Well grown callus was subcultured at regular interval in the media having same concentrations of hormone. After 8 weeks callus was cultured on shoot regeneration medium comprised of MS salts, 0.8% agar, 3% sucrose and 6-benzyl aminopurine at different concentrations (0.1, 0.3, 0.5, 0.7 and 0.9). After weeks of culture, well grown greenish callus were collected and used as explants for regeneration and transformation experiments.

All cultures were maintained at 25±2°C under 16/8 h (day/night) photoperiod with provided by cool white fluorescent lamps at an intensity of 35-40_mol m-2 s-1.6. Multiple shoots were obtained from the callus when MS medium supplemented with 6-benzyl aminopurine. Well-grown plantlets separated were washed thoroughly in running tap water before being transplanted in plastic pots containing sterilized soil and vermiculite (1:1). Plants were covered with transparent polyethylene bags to maintain adequate moisture and transferred to the greenhouse for acclimatization. Plantlets were then transferred to pots containing soil : compost (1:1), grown to maturity and then seeds were collected.

Result and Discussion

Aseptic explants

Surface sterilized seeds of *Lycopersicum esculentum* was *inoculated* in MS basal medium, it began to germinate after sixth day. Well grown, 25 days old plants were used as explants for callus induction and multiple shoot induction. Surface sterilization of seeds was done by washing with Tween 20, followed by Bavistin for the removal of the fungal spores. Ethanol was sprayed on the seed and left for 15-20 seconds. Traces of spirit were removed by washing with autoclaved distilled water (thrice). 0.1% $HgCl_2$ were used as the sterilant before inoculation into the medium. The sterilized seeds were transferred in sterilized petri plates. Seeds were inoculated in test tubes containing MS medium and were transferred in dark room for germination. Germinated seedlings served as explants source for tissue culture experiments. Hypocotyl segments (1-2 cm) and leaf discs (5×5cm2) of 18-21 day old *in vitro* plants were excised under aseptic conditions.

Cotton Bed Method

Plant regeneration was achieved in the cotton bed method. Sterile cotton wetted with media serves as a regeneration bed in the initial days of the plant germination. The healthy and disease free explants were collected from the culture for inoculation.

Direct Plant Regeneration of *Lycopersicum esculentum*

The *in vitro* grown micro shoots were inoculated on MS medium supplemented with BAP (0.5 mg/l) shown considerable result(Table 1). There are higher number of multiple shoot were recorded in the concentration. 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.

Regeneration of Plants Through Callus Pathway

Hypocotyl segments (1-2 cm) and leaf discs (5×5cm2) of 18-21 day old *In vitro* plants were excised under aseptic conditions. The excised explants were cultured on callus induction media. All the cultures were transferred to growth room for a period of almost 4-6 weeks. Compact callus was selected and used for regeneration Lu *et al*., (1997) at 25°C ± 2°C in growth room. Calli induced from the hypocotyls and leaf discs were shifted to different regeneration media. Costa *et al*., (2000) certain hypocotyls and leaf discs were directly regenerated on the same medium. As the tomato shoots began to regenerate (Afroz *et al*., 2009). Either directly from hypocotyls or from calli, they were transferred to rooting media. 0.10 mg/l of 2,4-D induced higher biomass of callus (Table 2). The continuous subculture of callus on MS media augmented with BAP and IAA induced (0.5+0.6 mg/l) high frequency of plant regeneration (Table 3). Jatoi *et al*., (2001) observed an increased callogenesis in two tomato hybrids cvs. Bornia and Royesta with increase in BAP from 5, 10, 20 and 30 µM. It appeared that the presence of a high concentration of BAP enhanced the growth of the plant. Although this combination was used for regeneration (Suprasanna *et al*., 2000). But in this particular variety it produced good callus with fresh green color. Root formation was observed in only one callus.

Table 1: Effect of BAP on shoot regeneration

S.No	Hormone concentration BAP mg/l	No. of explants inoculated	No. of explants responded	Response %	Plant regeneration (M±SD)
1	0.1	10	3	30%	4.207±6.742
2	0.3	10	5	50%	4.949±6.562
3	**0.5**	**10**	**8**	**80%**	**5.478±6.586**
4	0.7	10	6	60%	4.201±7.186
5	0.9	10	4	40%	4.985±6.386

Rooting and Hardening

The well developed micro shoots were inoculated in the rooting medium supplemented with BAP and NAA (1.2+0.6 mg/l) of plant growth regulators (Table 4). The will rooted plantlets were removed from media and washed with distilled water for the removal of agar particles. The cleaned plantlets were transferred in to the soil in the combination of red soil, sand and farmyard manure with ratio of 1:1:1.

Table 2: Effect of 2,4-D on induction of callus from stem explants

S.No	Hormone concentration 2,4-D mg/l	No. of explants inoculated	No. of explants responded	Response %	Plant regeneration (M±SD)
1	0.02	10	5	50%	0.061±3.664
2	0.04	10	6	60%	0.158±3.7
3	0.06	10	4	40%	0.079±3.274
4	0.08	10	7	70%	0.074±3.745
5	**0.10**	**10**	**10**	**100%**	**0.286±3.932**

Table 3: Effect of BAP and IAA on induction of callus and shoot regeneration

S.No	Hormone concentration BAP+IAA mg/l	No. of explants inoculated	No. of explants responded	Response %	Plant regeneration (M±SD)
1	0.5+0.4	10	4	40%	2.073±5.542
2	**0.5+0.6**	**10**	**8**	**80%**	**4.086±5.562**
3	0.6+0.8	10	5	50%	2.027±5.464
4	0.6+1.0	10	7	70%	3.241±5.64
5	0.7+1.2	10	6	60%	2.528±5.848

Table 4: Effect of BAP and NAA on induction of callus and root regeneration

S.No	Hormone concentration BAP +NAA mg/l	No. of explants inoculated	No. of explants responded	Response %	Plant regeneration (M±SD)
1	1.0+0.5	10	6	60%	3.641±4.64
2	**1.2+0.6**	**10**	**9**	**90%**	**4.231±4.356**
3	1.4+0.7	10	3	30%	2.334±4.936
4	1.6+0.8	10	5	50%	2.968±4.542
5	1.8+0.9	10	4	40%	2.834±4.432

Conclusion

The cherry type tomato was grown in the *in vitro* condition with various nutrients necessary for the growth. The *in vitro* grown shoots were inoculated on MS medium supplemented with BAP (0.5 mg/l) shown considerable result (Table 1). 0.1 mg/l of 2,4-D induced higher biomass of callus (Table 2). The continuous subculture of callus on MS media augmented with BAP and IAA induced (0.5+0.6 mg/l) high frequency of plant regeneration (Table 3). Multiple microshoots were formed with higher concentration of BAP. The well developed micro shoots were inoculated in the rooting medium supplemented with BAP and NAA (1.2+0.6 mg/l) of plant growth regulators (Table 4). The root induction was higher at higher concentration of NAA and the combination of results were effective. The cleaned plantlets were transferred into the soil in the combination of red soil, sand and farmyard manure with ratio of 1:1:1.

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