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Standardization of Protocol for *In-Vitro* Multiplication of Tomato (*Lycopersicon esculentum* L.)

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Abstract

The standardization of protocol for *in vitro* multiplication of tomato (*Lycopersicon esculentum* L.) was undertaken during 2007-08. The planting material (seed) of the variety BT-10 of tomato was collected from the market. The best surface sterilants for the seed explant was the treatment with bavistin at 0.1% and tetracyclin at 0.1% for 20 minutes, 70% alcohol for 30 seconds followed by 0.1% $HgCl_2$ for 2 minutes. The sterilized seeds were inoculated in the agar medium for germination. After 10—12 days the seeds were germinated. Excised cotyledons, hypocotyl and roots from the seedling were cultured on MS (1962) medium supplemented with different concentrations of 2-4-D. MS medium in combination with 2-4-D (2.5 mg/liter) proved to be more efficient in inducing callus in tomato. The efficient regeneration of shoot buds and their conversion into shoots were significantly high on MS medium supplemented with BAP at 0.5 mg/liter. MS media supplemented with IBA at 2.0 mg/liter produced roots earlier at 10 days after inoculation with maximum number of roots per shoot and longer root. Therefore, MS medium supplemented with IBA 2 mg/liter have been considered as the best medium for rooting in tomato. This investigation will be helpful in transformation technique in this crop for development of varieties resistant to biotic and abiotic stresses.

Key words : *Lycopersicon esculentum*, Callus, Shoot regeneration, Root initiation, *In vitro* multiplication.

Tomato (*Lycopersicon esculentum* L.) is one of the most popular and versatile vegetable crop grown universally. In India tomato occupies an area of 5.028 lakh ha with the production of 8.126 million tonnes. The tomato is a plant in the family Solanaceae. The tomato is native to central, south and southern North America from Mexico to Argentina. It is grown in almost every country of the world. It is susceptible to a number of diseases and insect pests. Development of protocols for *in vitro* selection can provide new advances for the production of stress (biotic and abiotic) tolerant cultivars. Techniques have to be optimized for the production of haploid and somatic hybrids. The morphogenesis response seems to be highly dependent on plant growth regulators used in the media, which is again the cultivar and genotypic specific. Somatic embryogenesis in tomato is still at its infancy, and efficient procedures for large-scale production via somatic embryogenesis are yet to be developed. Genetic stability of the tissue culture raised plants also needs to be addressed. The use of a combination of molecular and conventional breeding techniques could be the option for the development of cultivars resistant to biotic and abiotic stresses. So the present research work was undertaken with the objective of the standardization of the procedure for sterilization of seed for *in vitro* germination and procedure for callus induction, shoot regeneration and root formation in tomato for *in vitro* multiplication.

Methods

The present investigation on the standardization of protocol for *in vitro* multiplication of tomato (*Lycopersicon esculentum* L.) was undertaken at the tissue Culture Laboratory of Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar during 2007-08. The seed (planting material) of the variety BT-10 of tomato was collected from the market. Seeds were washed in tape water twice. Then the seeds of tomato were given

Table 1. Effect of different sterilants on the level of Contamination and Survival of tomato.

Treatments (%)	Fungul infection (%)	Bacterial infection (%)	Death (%)	Aseptic culture (%)	Survival
T_1—Bavistin 0.1	4.25	52.39	21.54	43.36	21.82
T_2—Tetracyclin 0.1	43.28	5.23	20.34	51.49	31.15
T_3—Bavistin 0.1 + tetracyclin 0.1	5.12	4.98	9.28	89.9	80.62
T_4—T_3 +70% alcohol for 30 second	2.78	3.5	5.64	93.72	88.08
T_5—T_5 + 0.1 $HgCl_2$ for 2 minutes	0	0	3.56	100	96.44
SE ±	0.02	0.03	0.05	0.68	0.07
CD (P = 0.05)	0.1	0.12	0.18	1.44	0.28

different treatments like : T_1–Bavistin at 0.1% for 20 minutes, T_2– Tetracyclin at 0.1% for 20 minutes, T_3—Bavistin at 0.1% and tetracyclin at 0.1% for 20 minutes, T_4—Bavistin at 0.1% and tetracyclin at 0.1% for 20 minutes and 70% alcohol for 30 seconds, and T_5—Bavistin at 0.1% and tetracyclin at 0.1% for 20 minutes, 70% alcohol for 30 seconds, and 0.1% mercuric chloride solution for two minutes and then washed with sterile dilled water twice. The culture were kept at 22 ± 2C in an air conditioned room with a 16 hour photoperiod (3,000–3,200 lux) supplied by fluorescent tubes and with 80% relative humidity. The observation on the percentage contamination (fungal + bacterial), percentage of aseptic culture [100–(fungal contamination % + bacterial contamination %)] and percentage of survival [100–(fungal contamination % + bacterial contamination % + death of explants %)] were recorded at regular intervals.

After treating the seeds in the best sterilants, 6 to 7 grams of Agar powder were added to one liter of hot distilled water and then it was mixed thoroughly. Ten ml of those mixtures was added to each culture tube. The culture tubes were closed with cotton plugs and were sterilized in autoclave at 15 psi and 121 C for 20 minutes. After cooling, the treated seeds were inoculated into the culture tube and were kept at 22 C for germination. Culture media for callus formation was prepared using different concentrations of 2-4-D (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/liter) with the basal medium of modified Murashige and Skoog (1). The autoclaved medium was kept in a laminar air flow bench for cooling. The excised hypocotyl, cotyledon and root segments from the seedling 10—15 days old were inoculated and kept in the dark for callus formation. Embryogenic callus was subsequently transferred to shoot regeneration medium. Embryogenic calli were cultured in MS medium with different concentration of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/liter) with the basal MS medium for shoot proliferation study in tomato. Healthy plants of about 2 cm height were placed in the root induction media. Phytohormones of different concentrations (IBA—1.0, 2.0 and 3.0 mg/liter, IAA—2.0 mg/liter and IAA—2.0 + NAA 2.0 mg/liter) were applied along with normal MS

Table 2. Effect of 2, 4-D on callus induction in tomato.

Treatments	Conc. of 2, 4-D (mg/l)	Days to callus induction	Callus induction (%)	Fresh weight of callus (g)
T_1	0.5	17	58.00	0.016
T_2	1.0	16	66.00	0.017
T_3	1.5	15	78.00	0.031
T_4	2.0	12	80.22	0.076
T_5	2.5	10	90.00	0.168
T_6	3.0	16	87.00	0.067
SE ±	–	0.06	0.18	0.68
CD (P=0.05)	–	0.18	0.48	1.68

Table 3. Effect of BAP on proliferation of shoot in tomato.

Treatments	Concentration of BAP (mg/l)	No. of multiple shoot/ explants	Days to shoot initiation
T_1	0.5	3.90	30
T_2	1.0	2.80	33
T_3	1.5	2.00	32
T_4	2.0	1.86	34
T_5	2.5	1.48	37
T_6	3.0	1.27	38
SE ±	–	0.13	0.16
CD (P = 0.05)	–	0.46	0.47

Table 4. Effect of phyto-hormones on root initiation in tomato.

Treatments (mg/l)	Days to root initiation	No. of roots	Length of root in cm
T_1—1.0 IBA	14	4.00	1.3
T_2—2.0 IBA	10	5.33	1.58
T_3—3.0 IBA	13	4.23	1.1
T_4—2.0 IAA	16	3.22	1.3
T_5—2.0 IAA+ 2.0 NAA	16	3.89	1.4
SE ±	1.2	0.05	0.24
CD (P = 0.05)	3.8	0.42	0.68

medium to study the root initiation in this crops. The observations for various characters like days after callus formation, callus induction percent, fresh weight of callus (g), days to shoot regeneration, no. of multiple shoots, days to root initiation, no. of roots and root length in cm were picked out by taking 10 culture tubes at a time. The data recorded from the experiment were analyzed following the method of Singh and Chowdhury (2) considering each culture tubes as a replication in each experiment by randomized block design.

Results and Discussion

The results of the experiments on surface sterilization of seeds as explants using different sterilants are presented in Table 1. The explants treated with bavistin 0.1% and plantomycin 0.1% for 20 minutes alone, washed with tap water and when inoculated in water+agar media, showed highest percentage of contamination by bacteria and fungus respectively. The contamination percent was reduced when the explants were treated with different surface sterilizing chemicals. The statistically analyzed data revealed that survival percentage of explants after treatment with several sterilants for different duration was highest (96.44% in seedlings) in T_5 comprising 0.1% bavistin for 20 minutes, 0.1% tetracycline for 20 minutes and 70% alcohol (0.5 minutes) followed by 0.1% mercuric chloride ($HgCl_2$) for 2 minutes. Total aseptic culture was maintained at T_5 treatment in this crop.

Sterilized seeds were inoculated in the plane agar medium. After 10—12 days the seeds were germinated. The effect of different hormones on callus induction in tomato is presented in Table 2. Callus induction and profuse proliferation was obtained in the MS medium supplemented with 2, 4-D at 2.5 mg/liter in tomato. However, the most commonly used auxin that had given rise to embryogenic callus was 2, 4-diclorophenoxyacetic acid (2, 4-D) with the concentrations ranging from 0.5 to 3.0 mg/liter and more frequently at 2 and 3 mg/liter. It has been generally observed that callus initiation and outgrowth require auxin elimination that is often necessary to obtain morphogenesis and production of organ. In the concentration of 2.5 mg/liter 2, 4-D, the weight of callus was high. The range of weight of proliferated callus was 0.16 to 0.168 g in tomato at different concentrations of 2-4-D. Induced callus percent ranged from 58.00 to 90.00. The days to callus induction varied from 10 to 17 days. These findings are in agreement with Ali et al. (3), Kagan-Zur et al. (4, 5).

Embryogenic calli were subsequently transferred to shoot regeneration medium. Efficient regeneration of shoot buds and their conversion into shoots were recorded on MS medium supplemented with 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/liter BAP. Effect of BAP on proliferation of shoots is presented in Table 3. The concentration of 0.5 mg/liter BAP along with normal MS medium produced significantly maximum number of shoots per explants. A further increase in concentration in BAP showed some adverse effect and subsequently reduced the number of multiple shoots.

Effect of IBA, IAA and NAA in rooting of elongated shoot derived from explants is presented in Table 4. The treatment T_2 (MS + 2.0 mg/liter IBA) exhibited root initiation on day 10 followed by treatment T_3 (MS + 3.0 mg/liter IBA) in tomato. The treatment T_2 was the best treatment which had taken least number of days for root initiation with highest number of roots per explants (i.e. 5.33 in tomato) and root length (1.58 cm in tomato). Therefore, taking these observations into consideration, MS medium supplemented with IBA 2 mg/liter has been considered as the best medium for rooting in tomato. This investigation will be helpful in transformation technique in this crop for development of varieties resistant to biotic and abiotic stresses.

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