Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 5 Issue 4 April 2021

Research Article

Development of a Novel *In-vitro* Protocol for Micro propagation of Tomato Male Sterile Line (Shalimar FMS-1) of Kashmir Valley India

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Received: March 03, 2021

Published: March 16, 2021

Abstract

The present study aimed to develop a novel, efficient and cost effective protocol for *in-vitro* micro propagation of tomato male sterile line and consisted of two experiments viz., Standardization of protocol for sterilizing the explants for *in vitro* micro propagation of tomato male sterile line (Shalimar FMS-1) and development of protocol for *in vitro* shoot and root regeneration in tomato male sterile line in a single medium. Among various sterilization treatments, sodium hypochlorite (1.0%) for 45 seconds showed the highest survival percentage of explants (100% for hypocotyl and 91.66% for single node cutting) and minimum contamination (16.17% for hypocotyl and 17.22% in case of single node cutting). Among twenty-seven modified MS media treatment combinations, the treatment combination T-14 (MS medium supplemented with calcium D pantothenate 2 mg l^{-1} + calcium chloride 440 mg l^{-1} + gibberillic acid 0.4 mg l^{-1}) resulted in maximum root and shoot regeneration, recorded maximum shoot and root length. The protocol developed is therefore proposed for micropropagation of tomato male sterile line Shalimar FMS-1 from the tomato explants in a single modified MS medium overcoming the requirement of two MS media, one for root regeneration and the other for shoot regeneration thus saving more than 50% of the time and media required for microplant regeneration This protocol overcomes the inefficient, expansive, time consuming and cumbersome nature of already available protocols which require two media (rooting and shooting media) while this proposed protocol ensures complete root and shoot regeneration from an explant (single node cutting/ hypocotyl) in a single modified MS medium.

Keywords: Hypocotyl; Microplant; Sterilization; Single Node Cutting; Tomato

Introduction

Tomato (*Solanum lycopersicum* L.), (2n=2×=24) belonging to family Solanaceae an important commercial vegetable crop grown across the globe. The crop is world's largest vegetable crop after potato and sweet potato in area and production and tops the list of canned vegetables. The total global area under tomato is 4.78 million hectares with a yearly production of 177 million metric tonnes [1]. In India it is grown on an area of 789 thousand hectares with annual production of 19759 thousand metric tonnes [1]. In Jammu and Kashmir State, it is grown over an area of 2.28 thousand hectares with an annual production of 52.96 thousand metric tonnes [2].

Even though India is the second largest tomato producer in the world next to China, its productivity lies far below the average productivity due to number of reasons ranging from climatic conditions to technologies employed in crop management but the most important being the non-availability of high yielding varieties/hybrids suitable to a particular region. Hybrids have spread over the entire world and appear to be the primary reason for high productivity. However, there are several constraints in hybrid adoption; the main factor is high cost of hybrid seed as production of hybrid seed involves skilled labour for manual emasculation and pollination. The use of male sterile lines avoids manual emasculation reduces the labour and cost necessary for F_1 hybrid seed production.

The maintenance of functional male sterile line in field is difficult, laborious and costly as it involves forced self-pollination for its maintenance which involves removal of anthredial cone and artificial pollination. To overcome this laborious process, tissue culture is an ideal alternative. Available literature shows that the regeneration protocols have been made available for cultivated tomato using different explants viz., cotyledons, hypocotyl, leaf, pedicel, peduncle, stem sections and inflorescence for organogenesis employing different plant growth regulators and requirement of two types of MS media, one for shoot regeneration and thereafter subsequent transfer of regenerated shoot to other type of MS media for root regeneration which is quite cumbersome and extremely time consuming. This study reports for the first time a novel highly efficient modified MS media for microplant regeneration in tomato male sterile line Shalimar FMS-1.

Materials and Methods

The present investigation on studies on micro propagation of tomato male sterile line (Shalimar FMS-1) was carried out in the Tissue Culture Laboratory, Division of Vegetable Science, and Genome Engineering Laboratory of Division of Plant Biotechnology, SKUAST-K during 2018-19.

Plant material/explants

Plants of tomato male sterile line Shalimar FMS-1 from the Experimental Field, Division of Vegetable Science and Polyhouse were selected for the explant collection (Mother Block) from disease free juvenile branches. For taking hypocotyl as explants, the seeds were germinated on half MS media in the Divisional Potato Tissue Culture Lab.

Description of shalimar FMS-1

(Accession No: IC 573425) used as female parent in the development of two tomato indeterminate hybrids viz., Shalimar Tomato Hybrid-1 and Shalimar Tomato Hybrid-2 with an outstanding yield of potential of 1320 quintals per hectare and 995 quintals per hectare respectively [3].

Nutrient media

MS (I-V) medium supplemented with 3% sucrose, 0.8 percent agar and different concentrations and combinations of calcium D pantothenate (CDP), calcium chloride ($CaCl_2$) and gibberellic acid (GA_3) were added as variables, then sterilized using an autoclave at 121˚C and 1.05 kg/cm^2 (15 psi) for 20 minutes. And all the aseptic manipulations like surface sterilization, preparation and inoculation of explants were carried out. The cultures were generally incubated at 24 ± 2 ˚C in an air conditioned culture room with a 16/8-hour light/dark regime.

Experimental parameters

A variety of experiments were conducted during the present investigation aimed at the development of a protocol for *in vitro* propagation of tomato (*Solanum lycopersicon)* male sterile line Shalimar FMS-1. Details regarding the methodologies adopted are given in the sections that follow.

Experiment-1: Standardization of protocol for sterilizing the explants for *in vitro* micro propagation of tomato male sterile line (Shalimar FMS-1)

The success of plant tissue culture protocol depends on explant sterilization Selection of sterilizing agent and time period of exposure is also critical because the living material should not lose

Figure 1: Main steps in micro propagation of tomato male sterile line (Shalimar FMS-1).

their biological activity and only contaminants should be eliminated during sterilization. Past investigations suggest that sodium hypochlorite (NaOCl) is the best choice for surface sterilization as it is readily available and can be diluted to proper concentrations. However, a balance between concentration and time must be determined empirically for each type of explant because of phytotoxicity. Ethanol is also used as powerful sterilizing agent but also extremely phytotoxic. Therefore, very short exposure of few minutes or seconds is generally given to explants. Surface sterilization with heavy metal salts is another way of sterilization in which Mercuric chloride ($HgCl_2$) is the famous one and always been a major choice but because of is toxicity and unsafe nature for both researcher and environment it is usually replace by other disinfectants. Details of surface sterilization procedure for explants are given in the following section.

Single node cutting and hypocotyl segments

Single node and hypocotyl segments were procured from the selected plants from vegetable field, polyhouse and the laboratory. These were separated with the help of a sharp scalpel. Explants on arrival in the laboratory were washed with running tap water 2 to 3 times and thereafter final 2 to 3 rinses with double distilled water.

For standardization of protocol for sterilization of explants (hypocotyl and single node cutting) the mercuric chloride and sodium hypochlorite for surface sterilization of explants were used as mercuric chloride @ 0.01%, 0.05%, 0.10%, 0.20% and sodium hypochlorite @ 0.5%, 1.0%, 1.5% for the time duration of 30,45 and 60 seconds totaling 21 treatment combinations.

Experiment- 2: Development of protocol for *in vitro* shoot and root regeneration in tomato male sterile line in a single medium

For developing a protocol for *in vitro* shoot and root regeneration (micro plant regeneration) of tomato male sterile line using various explants (hypocotyl and single node cutting), the treatment combinations were used as shown in table (Table 1).

S. No.	Treatment	Medium Composition	CDP (mg/l)	$CaCl_2$ (mg/l)	GA_3 (µg/l)
01.	T_1	MS salts + 3% Sucrose +0.8% Agar	1.50	352	300
02.	T_2	MS salts + 3% Sucrose +0.8% Agar	1.50	352	400
03.	T_3	MS salts + 3% Sucrose +0.8% Agar	1.50	352	500
04.	T_4	MS salts + 3% Sucrose +0.8% Agar	1.50	440	300
05.	T_5	MS salts + 3% Sucrose +0.8% Agar	1.50	440	400
06.	T_6	MS salts + 3% Sucrose +0.8% Agar	1.50	440	500
07.	T_7	MS salts + 3% Sucrose +0.8% Agar	1.50	528	300
08.	T_8	MS salts + 3% Sucrose +0.8% Agar	1.50	528	400
09.	T_9	MS salts + 3% Sucrose +0.8% Agar	1.50	528	500
10.	T_{10}	MS salts + 3% Sucrose +0.8% Agar	2.00	352	300

11.	T_{11}	MS salts + 3% Sucrose +0.8% Agar	2.00	352	400
12.	T_{12}	MS salts + 3% Sucrose +0.8% Agar	2.00	352	500
13.	T_{13}	MS salts + 3% Sucrose +0.8% Agar	2.00	440	300
14.	T_{14}	MS salts + 3% Sucrose +0.8% Agar	2.00	440	400
15.	T_{15}	MS salts + 3% Sucrose +0.8% Agar	2.00	440	500
16.	T_{16}	MS salts + 3% Sucrose +0.8% Agar	2.00	528	300
17.	T_{17}	MS salts + 3% Sucrose +0.8% Agar	2.00	528	400
18.	T_{18}	MS salts + 3% Sucrose +0.8% Agar	2.00	528	500
19.	T_{19}	MS salts + 3% Sucrose +0.8% Agar	2.50	352	300
20.	T_{20}	MS salts + 3% Sucrose +0.8% Agar	2.50	352	400
21.	T_{21}	MS salts + 3% Sucrose +0.8% Agar	2.50	352	500
22.	T_{22}	MS salts + 3% Sucrose +0.8% Agar	2.50	440	300
23.	T_{23}	MS salts + 3% Sucrose +0.8% Agar	2.50	440	400
24.	T_{24}	MS salts + 3% Sucrose +0.8% Agar	2.50	440	500
25.	T_{25}	MS salts + 3% Sucrose +0.8% Agar	2.50	528	300
26.	T_{26}	MS salts + 3% Sucrose +0.8% Agar	2.50	528	400
27.	T_{27}	MS salts + 3% Sucrose +0.8% Agar	2.50	528	500

Table 1: Treatment combination detail for micro-plant regeneration.

CDP= Calcium D Pantothenate, $CaCl_2$ = Calcium Chloride, GA_3 = Gibberellic Acid.

Medium composition and concentration were based on preliminary studies.

Results and Discussion

The results of present investigation, "Development of a novel *in-vitro* Protocol for Micro propagation of Tomato Male Sterile Line (Shalimar FMS-1). are presented under the following headings and sub-headings.

Standardization of protocol for sterilizing the explants for *in vitro* micro propagation of tomato male sterile line (Shalimar FMS-1)

The explants hypocotyl and single node segments were subjected to twenty-one different sterilization regimes using Murashige and Skoog, 1962 (MS) as the basal medium. Effect of various sterilization regimes (concentration) and explants on percent survival and percent contamination (Table 2) was highly significant.

The highest mean percentage of survival to an extent of 96.29 and 86.11 percent was obtained by treating the explants (hypocotyl and single node cuttings) with sodium hypochlorite (1.0%). The lowest mean survival percent of 33.33 and 55.55 percent was obtained by treating the explants (hypocotyl and single node cuttings) with mercuric chloride 0.20% and 0.01% respectively. Maximum percentage of surviving explants was 100 and 91.66 percent when the hypocotyl and single node cutting explants were treated with sodium hypochlorite (1.0%) for 45 seconds respect-ively. The highest mean percent contamination to the extent of 54.65 and 56.93 percent was obtained by treating the explants (both hypocotyl and single node cuttings) with mercuric chloride (0.01%). The lowest mean percent contamination to the extent 16.17 and 17.22

percent was obtained by treating the explants (both hypocotyl and single node cuttings) with sodium hypochlorite (1.0%). The lowest percent contamination of 11.92 and 12.22 percent was observed when the explants (both hypocotyl and single node cuttings) were treated with sodium hypochlorite 1.0 percent for 45 seconds. The present study on the effect of different surface sterilants and sterilization regimes revealed that maximum survival percentage (100% for hypocotyl and 91.66% for single node cutting), minimum mortality percentage (0.00% for hypocotyl and 8.33% for single node cutting) and least contamination (11.92% in hypocotyl and 12.22% in single node cutting) was observed when both single node cutting and hypocotyl explants were treated with 1% sodium hypochlorite for 45 seconds (Table 2). These results are in close conformity with those of [4] in tomato, who found that the sterilization treatment with 2% hypochlorite for 10 minutes, 70% ethanol for 1 minute and 3.5% hypochlorite for 20 minutes without the ethanol treatment gave optimum sterilization of the explants of three tomato cultivars [5]; who reported treatment of explants with 5.25% sodium hypochlorite with tween 20 for 15 minutes proved effective surface sterilization procedure for maximum survival of explants with minimum tissue injury [6], who worked on development of efficient *in vitro* callus induction and plant regeneration protocol for different polish tomato cultivars and found that surface sterilization in 70% (v/v) ethanol for 1 minute; then treatment in a 30% solution of commercial bleach containing 5.5% (w/v) of sodium hypochlorite for 10 minutes proved efficient sterilization procedure and avoided contamination also.

Developing a protocol for *in vitro* shoot and root regeneration in a single medium

The results pertaining to effect of different media on root and shoot regeneration percentage, days to root, shoot and microplant initiation, root, shoot and microplant length (cm) (hypocotyl and single node cuttings) are presented in the tables 3. Highest root regeneration percentage to the extent of 91.66 and 83.33 percent was obtained when the explants (both hypocotyl and single node cuttings) were cultured on T_{14} medium consisting of MS I-V medium supplemented with (calcium D pantothenate 2 mg l^{-1} + calcium chloride 440 mg l^{-1} + gibberellic acid 0.4mg l^{-1}), Lowest root regeneration percentage of 25 percent was obtained when hypocotyl explants were cultured on T_2, T_3, T_{13} and T_{20} medium respectively. The lowest root regeneration percentage of 8.33 percent was obtained from single node cuttings when cultured on T_{20} and T_{23} respectively. The maximum days to root initiation 8 were observed when hypocotyl explants were cultured on T_{18} medium while in case of single node cuttings the maximum number of days to root initiation 15 were taken in medium T_6, T_{10}, and T_{23} respectively. The minimum days to root initiation by explants were 3 in case of hypocotyl and 9 in case of single node cuttings and was observed in T_{14} medium. Highest root length to the tune of 7 cm and 6.40 cm in both hypocotyl and single node cuttings was observed on T_{14} medium whereas the length of roots was small 1.90 cm and 1.60 cm in hypocotyl and single node cuttings respectively when cultured on T_{13} medium. Highest shoot regeneration percentage to the extent of 91.66 and 83.33 percent was obtained when the explants (both hypocotyl and single node cuttings) were cultured on T_{14} medium consisting of MS I-V medium supplemented with (calcium D pantothenate 2mg l^{-1} + calcium chloride 440 mg l^{-1} + gibberellic acid 0.4mg l^{-1}), Lowest shoot regeneration percentage of 16.66 percent was obtained when hypocotyl explants were cultured on T_{18} medium. The lowest root regeneration percentage of 8.33 percent was obtained from single node cuttings when cultured on T_{20} and T_{23} medium respectively. The maximum days to shoot initiation 11 were observed when hypocotyl explants were cultured on T_{23} medium while in case of single node cuttings the maximum number of days to shoot initiation 18 were taken in medium T_{10}, T_{20} and T_{23} respectively. The minimum days to shoot initiation by explants were 5 in case of hypocotyl and 11 in case of single node cuttings and was observed in T_{14} medium. Highest shoot length to the tune of 11 cm and 10.20 cm in both hypocotyl and single node cuttings was observed on T_{14} medium whereas the length of roots was small 2.0 cm and 1.70 cm in hypocotyl and single node cuttings respectively when cultured on T_{23} medium. The maximum days to microplant regeneration 23 were observed when hypocotyl explants were cultured on T_{23} medium while in case of single node cuttings the maximum number of days to microplant regeneration 28 were taken in medium T_{23}. The minimum days to microplant regeneration by explants were 12 in case of hypocotyl and 17 in case of single node cuttings and was observed in T_{14} medium. Highest microplant length to the tune of 18 cm and 16.60 cm in both hypocotyl and single node cuttings was observed on T_{14} medium whereas the length of roots was small 4.0 cm and 3.30 cm in hypocotyl and single node cuttings respectively when cultured on T_{23} medium.

Various factors have been found to influence the induction of *in vitro* rooting and shooting in plant cell and tissue culture. Amongst

Survival percentage									Contamination percentage							
Treatment/ Concentration	Hypocotyl			Mean	Single node cutting			Mean	Hypocotyl			Mean	Single node cutting			Mean
	Time duration				Time duration				Time duration				Time duration			
	t_1: 30 sec	t_2: 45 sec	t_3: 60 sec		t_1:30 sec	t_2:45 sec	t_3:60 sec		t_1: 30 sec	t_2: 45 sec	t_3: 60 sec		t_1: 30 sec	t_2: 45 sec	t_3: 60 sec	
C_1: Mercuric chloride @ 0.01%	66.66 (8.21)	58.33 (7.69)	66.66 (8.21)	63.88 (8.04)	50.00 (7.12)	58.33 (7.69)	58.33 (7.69)	55.55 (7.50)	49.47 (7.09)	52.38 (7.30)	62.10 (7.94)	54.65 (7.44)	61.27 (7.88)	52.38 (7.30)	57.14 (7.59)	56.93 (7.59)
C_2: Mercuric chloride @ 0.05%	58.33 (7.69)	50.00 (7.07)	50.00 (7.07)	52.77 (7.28)	66.66 (8.21)	58.33 (7.69)	66.66 (8.21)	63.88 (8.04)	52.38 (7.30)	50.00 (7.14)	50.00 (7.14)	50.79 (7.19)	49.47 (7.09)	47.62 (6.96)	41.60 (6.52)	46.23 (6.86)
C_3: Mercuric chloride @ 0.10%	50.00 (7.12)	58.33 (7.69)	50.00 (7.12)	52.77 (7.31)	75.00 (8.70)	66.66 (8.21)	83.33 (9.17)	75.00 (8.70)	40.63 (6.36)	34.52 (5.89)	16.98 (4.23)	30.71 (5.49)	36.94 (6.15)	28.96 (5.46)	23.16 (4.90)	29.69 (5.50)
C_4: Mercuric chloride @ 0.20%	33.33 (5.83)	41.66 (6.51)	25.00 (5.05)	33.33 (5.79)	58.33 (7.69)	66.66 (8.21)	50.00 (7.12)	58.33 (7.67)	47.77 (6.93)	26.11 (5.18)	36.11 (6.03)	36.66 (6.05)	42.06 (6.54)	49.47 (7.09)	49.04 (7.05)	46.86 (6.89)
C_5:Sodium hypochlorite @ 0.50%	58.33 (7.69)	66.66 (8.21)	66.66 (8.21)	63.88 (8.04)	75.00 (8.70)	66.66 (8.21)	66.66 (8.18)	69.44 (8.36)	42.65 (6.56)	41.60 (6.52)	49.47 (7.09)	44.57 (6.72)	55.18 (7.49)	49.47 (7.09)	63.05 (8.00)	55.90 (7.52)
C_6: Sodium hypochlorite @ 1.0%	91.66 (9.62)	100.00 (10.05)	97.22 (9.90)	96.29 (9.85)	83.33 (9.17)	91.66 (9.62)	83.33 (9.15)	86.11 (9.31)	13.89 (3.82)	11.92 (3.56)	22.72 (4.85)	16.17 (4.08)	19.46 (4.46)	12.22 (3.61)	20.00 (4.50)	17.22 (4.19)
C_7:Sodium hypochlorite @ 1.5%	58.33 (7.69)	50.00 (7.12)	41.66 (6.51)	50.00 (7.10)	58.33 (7.69)	66.66 (8.21)	66.66 (8.21)	63.88 (8.04)	33.13 (5.83)	23.65 (4.83)	26.11 (5.18)	27.63 (5.28)	42.06 (6.54)	62.10 (7.94)	49.47 (7.09)	51.21 (7.19)
Mean	59.52 (7.69)	60.71 (7.76)	56.74 (7.44)		66.66 (8.18)	67.85 (8.26)	67.85 (8.25)		39.71 (6.23)	34.59 (5.81)	37.64 (6.06)		43.78 (6.59)	43.17 (6.49)	43.35 (6.52)	
	CD (p≤0.05) Concentration (C): 0.61 Time durations (t): NS C x t : NS				CD (p≤0.05) Concentration (C): 0.54 Time durations (t): NS C x t : NS				CD (p≤0.05) Concentration (C) : 0.66 Time durations (t): NS C x t : 1.15				CD (p≤0.05) Concentration (C) : 0.50 Time durations (t) : NS C x t : 0.86			

Table 2: Effect of different sterilization treatments on survival and contamination percentage of explants (hypocotyl and single node cutting) of tomato male sterile line Shalimar FMS-1.

Values under parenthesis are square root transformed values.

S. No	Treatment	Root regeneration percentage		Days to root regeneration		Root length (cm)		Shoot regeneration percentage		Days to shoot initiation		Shoot length (cm)		Days to microplant regeneration		Microplant length	
		Hypocotyl	Single node cutting	Hypocotyl	Single node cutting	Hypocotyl	Single node cutting	Hypocotyl	Single node cutting	Hypocotyl	Single node cutting	Hypocotyl	Single node cutting	Hypocotyl	Single node cutting	Hypocotyl	
1	T_2	25.00 (5.05)*	16.66 (4.11)**	6.00	14.00	3.00	3.00	25.00 (5.05)*	16.66 (4.11)**	9.00	17.00	6.40	4.40	18.00	27.00	9.40	7.40
2	T_3	25.00 (5.05)*	16.66 (3.68)**	5.00	13.00	2.50	2.50	33.33 (5.83)*	16.66 (3.68)**	8.00	15.00	4.70	3.70	19.00	24.00	7.13	6.53
3	T_4	41.66 (6.51)*	25.00 (4.89)**	5.00	12.00	2.40	2.70	41.66 (6.51)*	25.00 (4.89)**	8.00	14.00	6.40	5.50	20.00	22.00	8.80	8.20
4	T_6	41.66 (6.44)*	16.66 (4.11)**	5.00	15.00	4.00	3.90	50.00 (7.12)*	19.44 (4.41)**	6.00	17.00	10.00	7.00	17.00	26.00	14.00	10.90
5	T_7	58.33 (7.69)*	25.00 (5.05)**	5.00	13.00	4.00	3.70	58.33 (7.69)*	25.00 (5.05)**	6.00	15.00	4.50	3.60	17.00	23.00	8.50	7.30
6	T_9	50.00 (7.07)*	33.33 (5.83)**	4.00	11.00	5.00	5.50	50.00 (7.07)*	50.00 (7.12)**	6.00	13.00	9.93	5.46	19.00	22.00	15.00	10.96
7	T_{10}	50.00 (7.12)*	33.33 (5.83)**	6.00	15.00	3.50	3.30	55.55 (7.48)*	33.33 (5.83)**	8.00	18.00	8.00	6.00	21.00	25.00	11.50	9.50
8	T_{13}	25.00 (5.05)*	25.00 (5.05)**	3.00	13.00	1.90	1.60	25.00 (5.05)*	25.00 (5.05)**	5.00	16.00	3.10	2.70	22.00	24.00	5.00	4.30
9	T_{14}	91.66 (9.62)*	83.33 (9.17)**	3.00	9.00	7.00	6.40	91.66 (9.62)*	83.33 (9.17)**	5.00	11.00	11.00	10.20	12.00	17.00	18.00	16.60
10	T_{18}	33.33 (5.73)*	16.66 (4.11)**	8.00	11.00	2.00	2.00	16.66 (4.11)*	16.66 (4.11)**	10.00	14.00	4.00	3.00	21.00	24.00	6.00	5.00
11	T_{20}	25.00 (5.09)*	8.33 (3.05)**	6.00	14.00	2.00	1.80	33.33 (5.83)*	8.33 (3.05)**	8.00	18.00	4.00	2.80	22.00	26.00	6.00	4.60
12	T_{23}	33.33 (5.83)*	8.33 (3.05)**	7.00	15.00	2.00	1.60	33.33 (5.83)*	8.33 (3.05)**	11.00	18.00	2.00	1.70	23.00	28.00	4.00	3.30
CD ($p \leq 0.05$)		1.65	1.69	0.51	0.38	0.46	0.36	1.23	1.25	0.39	0.27	0.48	0.67	0.39	0.30	0.53	0.78

Table 3: Effect of various modified MS media on root, shoot and microplant regeneration %, days to root and shoot regeneration, root, shoot and microplant length.

* Values under parenthesis are square root transformed values.

** Values under parenthesis are arcsine transformed values.

these, the choice of growth medium, concentration and combination of growth regulators, and explants type are prominent ones and play a major role in standardizing a regeneration protocol for a plant species/cultivar [7]. The first step of initiating of *in vitro* rooting and shooting is to successfully adapt the plant tissue or explant to heterotrophic mode of nutrition (establishment stage). The culture establishment medium is useful for adaptation and stimulation of initial growth of explants and their subsequent development in microplants. Different types of nutrient media have been used for *Solanum* species tissue culture [8]. Out of the differ-

ent types of nutrient media (Woody Plant medium, Gresshoff medium) used for *Solanum* species tissue culture, MS medium has been found to be quite satisfactory for the micropropagation of *Solanum* species. In present study modified MS medium also proved to be satisfactory for the establishment, shooting and rooting of tomato male sterile line Shalimar FMS-1 using single node cutting and hypocotyl as explants.

In the present study, *in vitro* root and shoot regeneration leading to complete microplant development of tomato male sterile line (Shalimar FMS-1) in a single culture medium with the treatment combination of T_{14} (MS I-V medium supplemented with CDP @ 2 mg/l + $CaCl_2$ @ 44 mg/l + GA_3 @ 0.4 mg/l.) is reported. Among the different modified MS media having different combinations of growth regulators (calcium D pantothenate, calcium chloride, gibberellic acid), T_{14} medium MS I-V medium supplemented with CDP @ 2 mg/l + $CaCl_2$ @ 44 mg/l + GA_3 @ 0.4 mg/l. resulted in highest percentage of rooting(91.66% for hypocotyl and 83.33% for single node cutting), minimum days to root initiation (3 days in hypocotyl and 9 days in single node cutting), maximum root length (7.00 cm in hypocotyl and 6.40 cm in single node cutting), highest shoot regeneration (91.66% in hypocotyl and 83.33% in single node cutting), minimum days to shoot initiation (5 days in hypocotyl and 11 days in single node cutting), maximum shoot length (11 cm in hypocotyl and 10.20 cm in single node cutting), minimum days to microplant regeneration (12 in hypocotyl and 17 in single node cutting), maximum microplant length (18 cm in hypocotyl and 16.60 cm in single node cutting. Among the twenty-seven different growth regulator combinations in basal MS media, only twelve treatment combinations (T_2, T_3, T_4, T_6, T_7, T_9, T_{10}, T_{13}, T_{14}, T_{18}, T_{20}, T_{23}.) proved successful in culture establishment of explants while fifteen treatment combinations (T_1, T_5, T_8, T_{11}, T_{12}, T_{15}, T_{16}, T_{17}, T_{19}, T_{21}, T_{22}, T_{24}, T_{25}, T_{26}, T_{27}.) failed to evoke any response in terms of root and shoot initiation and microplant development.

Current work elucidates that sodium hypochlorite is more suitable surface sterilant than mercuric chloride as it resulted in maximum survival percentage, minimum mortality percentage and minimum contamination percentage of explants. Similar results have been obtained by [9-11] for sterilization of potato sprouts and shoot tips. Explant type has been found to significantly influence the regeneration response leading to complete microplant development *in vitro*. Micropropagation of *Solanum* species has been achieved using different explants viz., leaves [12-14] hypocotyl [13,15,16], Cotyledon [17-19] and inflorescence [20]. In the present study two types of explants viz., single node cutting and hypocotyl were used. The advantage with the single node cutting is that large number of explants can be taken from a single seed which develops in to a single plant while only a single hypocotyl segment can be taken from a single seed which drastically reduces the cost of *in vitro* microplant development of male sterile line. However, the main advantage with using hypocotyl is that it takes only 12 days for complete microplant development. Single node cutting with sub culturing is therefore recommended for micropropagation of male sterile line in tomato as it is economic as compared to using hypocotyl as explants.

Conclusion

From the study it is concluded that, various sterilization treatments yielded aseptic cultures but the sterilization regime of (1.0%) sodium hypochlorite gave highest percentage of aseptic cultures, highest percentage of surviving explants, minimum mortality and minimum contamination percentage. The percent survival response of hypocotyl explants was significantly higher than the single node cutting explants and their percent contamination was significantly lower than the single node cuttings. T_{14} medium consisting of MS I-V medium supplemented with (calcium D pantothenate 2mg l^{-1} + calcium chloride 44 mg l^{-1} + gibberellic acid 0.4mg l^{-1}) gave highest rooting percentage, minimum number of days to root initiation, maximum root length, gave highest shooting percent, minimum days to shoot initiation, maximum shoot length and highest microplant height, minimum days to microplant regeneration, maximum microplant diameter and maximum microplant weight. This shows that constituents of the medium especially the growth regulators have significant effect on the regeneration potential of explants. The shooting percent, rooting percent response of hypocotyl explants was significantly higher than the single node cutting explants. This shows that the morphogenetic response of different explants even from the same plant may vary.

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