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Effect of sterilization treatments on *in vitro* culture establishment of tomato (*Solanum lycopersicum* L.)

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Abstract
Sterilization of explants is a pre-requisite step for carrying out any plant tissue culture strategy. The type of chemicals used as fungicides viz. Carbendazim & Fenbendazole etc. and surface sterilants viz., sodium hypochlorite, calcium hypochlorite, hydrogen peroxide and mercuric chloride, etc. are being used in tissue culture to decontaminate the explants tissues. These sterilants have their adverse effect depending upon the type of explants, nature of sterilants and their specific duration when applied individually or in combination. In the present study, the effect of fungicide (Carbendazim) and surface sterilant (sodium hypochlorite) was documented on different type explants of tomato cv. Solan Lalima. During sterilization treatment, 0.2% Carbendazim for 10 min followed by different durations of 10% sodium hypochlorite viz., 8 min for true leaf (77.08%) and epicotyl (66.66%), 10 min for hypocotyl (74.30%), 6 min for cotyledon (61.80%) and 20 min for seeds (77.77%), respectively was found to be effective for getting maximum survival rate *in vitro*. Hence, the reported sterilization treatments could be useful for carrying out any biotechnological strategies such as *in vitro* cell line selection and development of transgenics against various biotic and abiotic stresses as well as for other qualitative parameters in tomato crop improvement programme.

Keywords: Explants, sterilants, *in vitro*, tissue culture, tomato

Introduction
Tomato (*Solanum lycopersicum* L., *Solanaceae*) is a one of the most important vegetable crops grown throughout the world with an estimated global production of over 160 million tonnes. It possesses significant quantities of mineral and vitamins, so it is regarded as 'poor man's orange. It is an essentially nutritive and high economic value crop which can be grown in short period of time with high productivity. Besides, it is a rich source of minerals (iron), vitamins (A and C), organic acid, essential amino acids, dietary fibers and can be used in preserved foodstuffs like pastes, sauces, pulps, juices, ketchup and used for culinary purposes and also used as a flavoring ingredient in soups, meat or fish dishes (Namitha and Negi, 2013) [10]. Due to the anti-oxidative and anti-cancer properties of lycopene, tomato consumption as well as production is still increasing (Gerszberg and Hnatuszko-Konka, 2017) [5].
India ranks second in the world's total tomatoes production after China. According to National Horticulture board of India, tomato covers an area of 80.9 million ha with an annual production of 22.33 million metric tonnes in 2017-18. However, according to Ministry of Agriculture and farmer's welfare of India, in Himachal Pradesh the annual production of tomato is 489.96 MT from area of 11.08 thousand ha in 2016-17.
From the socioeconomic outlook, tomato is well-known as 'Red Gold' in the farming community due to its prominent economic status. However, its productivity is weakened by a wide range of biotic and abiotic stresses which are most important constraints for plant yield and productivity. Abiotic stress conditions such as drought, salinity and heat have been studied in agricultural plants due to the economic losses that they cause every year worldwide (Martinez *et al*,. 2018) [8]. Likewise, biotic stresses caused due to pathogen attack viz., bacteria, fungi, virus and nematodes etc. also stimulate adverse effects on plants. Among these, fungi are the main and prevalent pathogens that infect a wide range of host plants, causing destruction and economic loss in tomato either in the field, poly house, storage or transportation.

The most important fungal rots reported all over the world with varying intensities on tomato includes *Alternaria* rot, *Phytophthora* rot, Buckeye rot, Anthracnose ripe rot and *Fusarium* rot (*Fusarium* spp.) (Wani, 2011) [14].

Therefore, there is an urgent need for biotechnological interventions to increase the productivity of this crop. Culture of plant cell, tissues *in vitro* is an integral part of plant biotechnology, which has been be exploited for *in vitro* regeneration and genetic improvement of this crop. Tissue culture is a prospective technology for obtaining the desired characteristics of variant which can lead towards the excepted outcome while the probability depend on the accessibility of an effective selective agent (Rai *et al.,* 2011) [11]. Therefore, biotechnology interventions like *in vitro* cell line selection and genetic transformation strategies seem to be an appropriate alternative for improving such qualitative parameters. The *in vitro* culture of the tomato has been successfully used in the different biotechnological applications including the clonal propagation of high-value commercial cultivars, virus-free plants and genetic transformation (Namitha and Negi, 2013) [10].

However, maintaining an aseptic or sterile condition has been identified as a pre-requisite step in successful tissue culture procedures (Badoni and Chauhan, 2010) [1]. The chemicals used as fungicides viz. Carbendazim & Fenbendazole etc. and surface sterilants such as sodium hypochlorite, mercuric chloride, calcium hypochlorite, ethanol and hydrogen peroxide etc. have been reported toxic to the explants used in plant tissue culture. Therefore, their appropriate minimal concentration and exposure time of sterilants to the explants is an essential step before culturing to achieve maximum survival rate. Keeping in view the above considerations, the present study was undertaken to see the effect of different sterilization treatments durations on different type of explants in tomato cv. Solan Lalima for establishing indirect and direct regeneration that could be useful *in vitro* cell line selection and transgenic strategies against various biotic and abiotic stresses.

Materials and Methods

Plant material

The certified seed material of tomato cv. Solan Lalima was procured from the Department of Vegetable Science, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (H.P). The seeds were raised *in vivo* in the glass house (nursery). 15-20 days old seedlings were used for procuring explants viz., true leaf, hypocotyl, cotylendon, epicotyl and seeds for the establishment of cultures *in vitro*.

Sterilization treatment

Initially, the explants were washed with teepol for 20 minutes with running tap water to remove dust and other debris stuck with explant surface. After that, surface sterilization was carried out inside the laminar air flow. The explants were treated with fungicides i.e. Carbendazim 0.2% (w/v) and surface sterilant 10% (v/v) of sodium hypochlorite (NaOCl) for specific durations. These explants were washed for 2-3 minutes with autoclaved distilled water thrice to remove traces of chemicals used in sterilization treatment and further blotted on filter paper before culturing on particular MS (Murashige and Skoog, 1962) [9] medium and incubated at 25±2 ^{0}C for 16/8 hours photoperiod under 1.5-2.0 kilo lux light intensity. The experiments were repeated thrice and data was observed under completely randomized design (CRD).

Results and Discussion

Surface sterilization of explants is an important precondition in any tissue culture experiment to minimize the chances of contamination. Since, surface sterilization requires the use of chemicals that are, toxic to microorganism but nontoxic to plant material preferably at low concentrations, and in order to find an optimized protocol for sterilization of a specific tissue, three factors were to be taken into consideration viz., sterilant, its concentration and the treatment duration.

It was evident from the above study (Table 1, Fig. 1) that treatment T5 was found to be the best for surface sterilization of true leaf explants having maximum rate of survival (77.08%) followed by T7 (65.97%) among all the 13 treatments used while minimum rate of survival of true leaf was recorded in T13 (9.72%). However, from same set of observation, it was recorded that T6 was found to be the best treatment for sterilization of hypocotyl explants having maximum survival rate (74.30%) followed by T5 (63.19%) in total 13 treatments. The minimum rate of survival of hypocotyl was recorded in T13 (11.80%). Furthermore, it was observed that T5 was found to be the best treatment for sterilization of epicotyl explants with maximum survival rate (66.66%) followed by T6 (53.47%) among all the 13 treatments and minimum survival rate of epicotyl was recorded in T13 (9.02%). Similarly, it was also recorded that the T4 was found to be the best treatment for sterilization of cotyledon explants having maximum survival rate (61.80%) followed by T5 (58.33%) out of all the 13 treatments. The minimum survival rate of cotyledon was recorded in T13 (6.94%).

In vitro sterilization of tomato seeds was also carried out inside the laminar air flow to study the effect of sterilization treatment i.e. Carbendazim 0.2% (w/v) and followed by sodium hypochlorite 10% (v/v). It was observed that treatment T11 was found to be best for surface sterilization of seeds out of total 13 treatments. The maximum survival rate of seeds was recorded in T11 (77.77%) followed by T10 (63.19%) while minimum survival rate of seeds after surface sterilization was shown in T2 (4.16%). Likewise, different researchers had also used these sterilants in case of tomato like viz. seeds were surface sterilized by dipping in 70% ethanol for 1 min, followed by immersion in 3% sodium hypochlorite for 15 min, and three rinses in sterile distilled water, by immersion for 1 min in 70% (v/v) ethanol followed by 30 min in 25% (v/v) commercial bleach, 5% NaOCl for 20 minutes-seeds, 15% NaOCl solution for 10 minutes (Sherkar and Chavan, 2014) [12], 20% NaOCl for 15 minutes, 4% NaOCl for 6-8 minutes and were washed thrice with autoclaved sterile distilled water, respectively. In contrast to our observations, many researchers have previously recorded different treatment combinations and concentrations of sterilants for different durations such as 70% ethanol for one minute followed by soaking in 4% NaOCl (Soundarajan, 2015), 70% ethanol for 30 minutes and 20% NaOCl for 20 minutes (Manawadu *et al.,* 2014) [7], 70% ethanol for 10 seconds followed by continuous shaking (Chandra *et al.,* 2013) [3] and 0.1% $HgCl_2$ for 3 minutes, respectively.

Table 1: Effect of sterilants on percent survival of different type of tomato cv. Solan Lalima explants

Treatment	Sterilants		Percent survival of explants*				
	0.2% Carbendazim (min)	10% sodium hypochlorite (min)	True leaf (%)	Hypocotyl (%)	Epicotyl (%)	Cotyledon (%)	Seeds (%)
T1	10	0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T2	10	2	27.08 (31.34)	16.66 (24.07)	20.13 (26.64)	22.22 (28.11)	4.16 (11.70)
T3	10	4	41.66 (40.18)	34.72 (36.08)	34.02 (35.66)	45.83 (42.59)	6.25 (14.45)
T4	10	6	59.72 (50.58)	46.52 (42.98)	49.30 (44.58)	61.80 (51.80)	10.41 (18.80)
T5	10	8	77.08 (61.37)	59.02 (50.17)	66.66 (54.71)	58.33 (49.77)	13.88 (21.85)
T6	10	10	51.38 (54.29)	74.30 (59.51)	53.47 (46.97)	52.08 (46.17)	20.13 (26.64)
T7	10	12	65.97 (45.77)	63.19 (52.62)	48.61 (44.18)	47.22 (43.38)	31.94 (34.39)
T8	10	14	44.44 (41.79)	52.77 (46.56)	43.75 (41.39)	41.66 (40.18)	40.97 (39.78)
T9	10	16	31.94 (34.39)	43.05 (40.98)	31.25 (33.97)	34.02 (35.66)	53.47 (46.97)
T10	10	18	26.38 (30.89)	34.02 (35.66)	20.13 (26.64)	27.08 (31.34)	63.19 (52.62)
T11	10	20	18.75 (25.64)	19.44 (26.14)	16.66 (24.07)	18.75 (25.64)	77.77 (61.84)
T12	10	22	14.58 (22.42)	15.97 (23.53)	13.19 (21.27)	15.27 (22.98)	65.27 (61.84)
T13	10	24	9.72 (18.14)	11.80 (20.07)	9.02 (17.45)	6.94 (13.24)	58.33 (49.77)
$CD_{0.05}$			1.53	1.61	1.42	1.57	1.53

*Data taken after 5-7 days of treatment; Number of explants taken = 30 per treatment; values in parentheses are transformed values

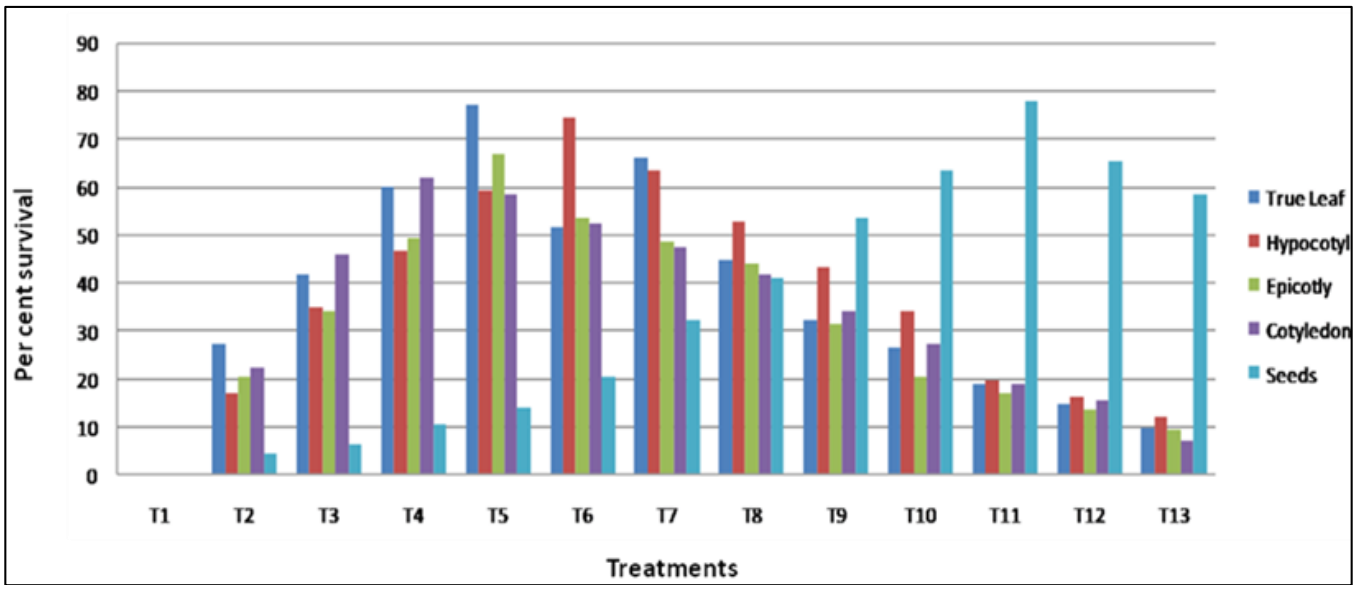

Fig 1: Effect of sterilants on percent survival of different explants of tomato cv. Solan Lalima

Conclusion

In the present study, it was revealed that sterilants, its concentration and the treatment duration is very critical factor while establishing any *in vitro* culture. Likewise, genotypic variation is also an important factor while tolerating exposure to any sterilants. Hence, the reported sterilization treatments could be useful for carrying out any biotechnological strategies such as *in vitro* cell line selection and development of transgenics against various biotic and abiotic stresses as well as for other qualitative parameters in tomato cv. Solan Lalima improvement programmes.

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