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Research article

STUDIES ON THE EFFECT OF VARIOUS STERILANTS AND CULTURE CONDITIONS ON *IN-VITRO* SEED GERMINATION IN TOMATO (*SOLANUM LYCOPERSICUM*)

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ABSTRACT: Studies on the effectiveness of various sterilants and culture conditions on *in-vitro* seed germination in tomato (*Solanum lycopersicum* L.) cv. PKM-1 revealed that among three sterilants used, surface sterilization of seeds with 5 % NaOCl for 20 minutes was found to be more effective resulting in high germination rate and contamination free cultures. Similarly among the different media and culture conditions considered in the present experiment, MS medium without sucrose with dark incubation for three days recorded lower no. of days (3.3 days) for initiation of germination with 96 % germination. These findings could be well exploited for further development of quick regenerative and transformation protocols for the tomato cv.PKM-1.

Key Words: Toamto, *in-vitro* seed germination, sterilants and MS medium.

INTRODUCTION

Tomato (*Solanum lycopersicum* L. 2n=24) is one of the most important vegetable crop in India and is a rich source of minerals, vitamins and organic acids . In India, it is cultivated in an area of 5.20 Lakh ha with a production and productivity of 90.06 Lakh tonnes and 17,800 kg/ha respectively. In Andhra Pradesh, it accounts for 0.765 Lakh ha with a production and productivity of 14.3 Lakh tonnes and 19,000 kg/ha, respectively. Keeping in mind the future demands of tomato for the burgeoning population, there is an immediate need to improve the production and productivity of tomato utilizing the advances in the areas of biotechnology and tissue culture. To exploit the advances in these areas for the tomato crop improvement, development of regeneration and transgenic protocols needs immediate attention. Tomato is very amenable to tissue culture and highly responsive to *in vitro* cultures. Establishment of efficient tissue cultures involves the surface sterilization or disinfection of explants that carry a wide range of microbial contaminants. Removing contaminants from the surface of the explant is of prime concern (Hartmann *et al.*, 1997). Disinfection requires the use of chemicals that are toxic to microorganism but non toxic to plant material. In order to find an optimized protocol for sterilization of a specific tissue, three factors have to be taken into consideration *viz.*, sterilizing chemical, its concentration and the treatment duration. Similarly, the culture conditions and media not only affect germination, but also effect the uniform growth of seedlings. The prime conditions decides for optimized seedling production are light, darkness and medium with or without carbon source. Since, the effect of sterilants, media and culture conditions are mostly genotype specific, standardization of these to the specific genotype is highly essential to proceed further for any *in-vitro* culture experiments. Though several protocols were developed for different varieties, work on regeneration protocol for PKM-1 is so far has not been accomplished. PKM-1 is an adaptable high yielding cultivar widely grown in A.P for its high acidity and is ideally suitable for long distance transport. Hence, keeping these points in view the present investigation has been undertaken to standerdize the best concentration and time for a better sterilant and best culture conditions for *in-vitro* seed germination of tomato cv.PKM-1.

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MATERIAL AND METHODS

The seeds of tomato cv. PKM-1 procured from Dept. of Horticulture, TNAU, Coimbatore were used for the present investigation. The seeds were immersed in sterile double distilled water for 15 minutes and treated with Bavistin 1% solution for 20 minutes followed by thorough rinsing with sterilized water. One drop of Tween-20 was added to the seeds and shaked thoroughly for 5 min and thoroughly rinsed with sterile distilled water for 4-5 times. The seeds were taken in to laminar air flow cabinet, and treated with different concentrations of various surface sterilants for different intervals of time as mentioned in the Table 1 with occasional swirling. They were washed with 4-5 changes of sterile distilled water and were treated with 70% ethyl alcohol for 30 sec followed by washing for 4-5 times with double distilled water. After standardizing the sterilization protocol, the seeds were inoculated on various combinations of media and light/dark incubation viz., MS medium + light, ½ MS medium + light, MS medium + Dark incubation, ½ MS medium + Dark incubation, MS medium without Sucrose + Light, ½ MS medium without sucrose + Light, MS medium without sucrose + Dark incubation and ½ MS medium without sucrose + Dark incubation. The cultures were incubated in a culture room whose temperature was maintained at 25 ± 2°C. The observations were recorded after inoculation of seeds on the medium on visual basis on mean time taken for initiation of seed germination, no. of seeds germinated and Germination frequency (%).

RESULTS AND DISCUSSION

Surface sterilization of seeds is an essential pre-requisite for seedling production in any tissue culture experiment to minimize the contamination. The data generated on the efficacy of the *in-vitro* seed germination with the three sterilants viz., mercuric chloride ($HgCl_2$), Hydrogen peroxide (H_2O_2) and Sodium hypochlorite (NaOCl) with different concentrations and for different durations were presented in Table 1. All the concentrations of $HgCl_2$ used for the surface sterilization of seeds *viz.*, 0.1, 0.2, 0.3, 0.5% for 5 minutes inhibited germination completely, except at 0.1% treatment which resulted in poor germination and infection. Application of mercuric chloride at different concentrations and time duration for surface sterilization of explants resulted in contamination free cultures and the germination was inhibited completely with increased concentrations. Such response might be due to bleaching action of two chloride atoms and also ions that combines strongly with proteins causing the death of organisms (Pauling, 1955). In contrast Sheeja and Mandal (2003), Bamel *et al.* (2007) and Singh *et al.* (2007) reported seedling production by using 0.1 % $HgCl_2$.

Among different combinations of H_2O_2 *viz.*, 10, 15, 20% treated for 15 min, lower concentrations i.e. 10 and 15% resulted in 80 and 70% germination but contamination of cultures was observed. Increased concentration i.e. 20% H_2O_2 resulted in contamination free cultures with 70% germination but seedling growth was not uniform. However, this survival rate was lower than the rate which could guarantee an aseptic culture establishment. The failure of above sterilants could be attributed to resistance or tolerance of microbial agents present on the surface of explant or due to the high load of contaminants on the explant surface. Hence, the above sterilants are not suitable for sterilizing the seed. Almost all the concentrations of sodium hypochlorite for 15 min resulted in contaminated cultures with lower rates of germination. But when the seeds were soaked for 20 min with occasional swirling in 5% concentration of sodium hypochlorite resulted in higher germination rate (95 %) with uniform seedling growth and reduced levels of contamination followed by 4 per cent for 20 min. When NaOCl was used as surface sterilant at lower concentration than 4 per cent resulted in contaminated cultures and when used at higher concentration than 5%, it resulted in the inhibition of germination. The reports of Newman *et al.* (1996) used NaOCl 2.5 per cent for 15 min; Gubis *et al.* (2003) 4 per cent sodium hypochlorite for 15 min; Reda *et al.* (2004) 3 per cent (v/v) sodium hypochlorite, also substantiate the role in deciding sodium hypochlorite as effective surface sterilant for sterilization of tomato seeds.

By and large, three sterilants used in the present study, surface sterilization of seeds with 5 % NaOCl for 20 minutes was found to be more effective resulting in high germination rate and contamination free cultures. Hence, the 5 per cent NaOCl was used for surface sterilization of seeds for conducting the study of effectiveness of media and culturing condintions experiment.

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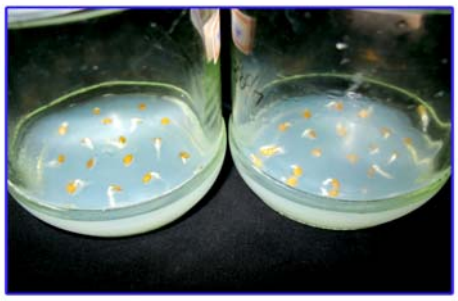

Plate 1: Germination of dark incubated seed in cultivar PKM-1

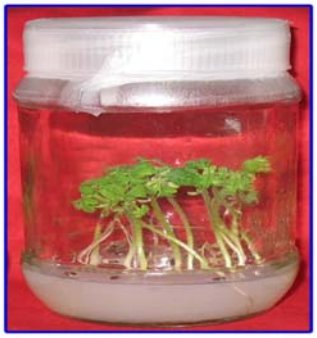

Plate 2: Seedlings of 10 days old

Table 1: Effect of various sterilants on sterilization of seeds of *Solanum lycopersicum* L. Cv. PKM-1

S.NO.	Sterilant	Concentration	Treatment duration min	Per cent of germination	Nature of response
1	$HgCl_2$	0.1	5	(50.76) 60	Germination delayed and contaminated.
2	$HgCl_2$	0.2	5	0	No germination
3	$HgCl_2$	0.3	5	0	No germination
4	$HgCl_2$	0.5	5	0	No germination
5	H_2O_2	10	15	(63.43) 80	Cultures contaminated
6	H_2O_2	15	15	(56.78) 70	Cultures contaminated
7	H_2O_2	20	15	(56.78) 70	No contamination but no uniform
8	NaOCl	3	15	(45.0) 50	germination
9	NaOCl	4	15	(33.21) 30	Cultures contaminated
10	NaOCl	5	15	(39.23) 40	Cultures contaminated
11	NaOCl	6	15	(39.23) 40	Cultures contaminated
12	NaOCl	3	20	(63.43) 80	Cultures contaminated
13	NaOCl	4	20	(71.56) 90	Cultures contaminated
14	NaOCl	5	20	(77.07) 95	Seedlings healthy and uniform and no
15	NaOCl	6	20	(65.64) 83	contamination
	(±) S.Em			1.93	Seedlings healthy and uniform and no
	C.D at			7.43	contamination
	5%				Seedlings healthy and uniform and no contamination

Note: Figures in parentheses represent arc sine transformed values

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Table 2 : Effect of media and culture conditions on *in vitro* seedling production

S. NO.	Medium / Condition	Mean time taken for initiation of germination in days	Mean no. of seeds germinated (Out of 10 seeds / bottle)	Per cent of germination	Nature of response
1	MS medium + Light	5.6	8.0	(63.43) 80	Healthy and non uniform seedlings.
2	½ MS medium + Light	6.3	7.3	(58.69) 73	Healthy and non uniform seedlings.
3	MS medium + Dark incubation	4.6	8.3	(65.64) 83	Healthy and uniform seedlings.
4	½ MS medium + Dark incubation	5.3	7.6	(60.66) 76	Healthy and uniform seedlings.
5	MS medium with out sucrose+ Light	5.0	8.3	(65.64) 83	Healthy and non uniform seedlings.
6	½ MS medium with out sucrose + Light	5.3	8.0	(63.43) 80	Healthy and non uniform seedlings.
7	MS medium with out sucrose + Dark incubation	3.3	9.6	(78.46) 96	Healthy and uniform seedlings.
8	½ MS medium with out sucrose + Dark incubation	4.3	8.3	(63.43) 83	Healthy and uniform seedlings.
	(±) S.Em	0.3	0.19	3.11	
	C.D at 5%	0.97	0.60	9.42	

Note: **1. The treatment of dark adoption means, media with seed kept for three days in dark followed by exposure to light until full seedling growth**

2. Figures in parentheses represent arc sine transformed value

The data on the effectiveness of MS basal medium with half strength and full strength without dark incubation (exposure to light) and with dark incubation (exposure to darkness for 3 days before exposed to light) to standardize better media for optimum seedling production in terms of time taken for initiation of germination and per cent of germination were presented in Table 2. The data revealed significant differences between the treatments. Among the different media used, MS medium without sucrose with dark incubation for three days recorded lower no. of days (3.3 days) for initiation of germination with 96 % germination (Plate 1 and Plate 2) followed by ½ MS without sucrose with dark incubation recorded 4.3 days for initiation with 83% germination and MS with dark incubation for three days recorded 4.6 days for initiation with 83% germination. All the treatments without sucrose with dark incubation for three days recorded lower no. of days taken for initiation of germination and highest percentage of germination with uniform and healthy seedlings. The culture conditions and media not only affect germination, but also effect the uniform growth of seedlings.

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The superiority of full strength MS medium over half strength MS medium in the present experiment might be due to quick depletion of the nutrients which are relatively low in full strength MS medium when compared to ½ strength MS medium. The results also revealed that seeds were germinated on MS medium without carbon source i.e. sucrose, denoting the less importance of sucrose for germination. In contrast, Bhatia (2005) reported that use of lower concentrations of sucrose (0.5-1.5%) along with full strength MS medium was optimum for plant growth in tomato. From the data obtained in the present investigation, it is evident that tomato *in vitro* seed germination and seedling growth is influenced by dark or light conditions. In seeds which were exposed to normal growth room conditions i.e. 16 hr light and 8 hr darkness till the growth of seedlings, both germination per cent as well as seedling growth was affected. However, when seeds were dark incubated for 3 days followed by exposure to normal growth room conditions showed higher germination per cent as well as seedling growth. The results are in accordance with Rao *et al.* (2007) who reported uniform and rapid seedling production when the seeds were kept in dark for 4 days compared to complete light incubation.

In conclusion, among the three sterilants used, surface sterilization of seeds with 5 % NaOCl for 20 minutes was found to be more effective resulting in high germination rate and contamination free cultures. Similarly among the different media and culture conditions considered in the present experiment, MS medium without sucrose with dark incubation for three days recorded lower no. of days (3.3 days) for initiation of germination with 96 % germination. These findings could be well exploited for further development of quick regenerative and transformation protocols for the tomato cv.PKM-1.

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