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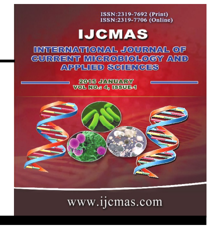

Original Research Article

In vitro screening: An effective method for evaluation of commercial cultivars of tomato towards salinity stress

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A B S T R A C T

The present investigation was undertaken for in vitro screening of seeds of five commercial cultivars of tomato (Abhinav, Rohini, TO1389, N2535 and Naina) in presence of NaCl mediated salinity stress. Seeds were germinated on half strength Murashige and Skoog's medium supplemented with different concentrations of NaCl (0, 40, 60, 80 and 100 mM). Under the influence of salt stress the days required for germination increased while the germination percentage declined. The shoot/root length and fresh/dry weight of seedlings reduced with increasing concentration of NaCl stress. Based on seed germination and seedling growth the cultivars were ranked as tolerant (Abhinav and Rohini), moderately tolerant (TO1389 and N2535) and sensitive (Naina) towards salt stress. This study forms the basis for rapid evaluation of large number of commercial cultivars of tomato towards salinity stress under disease free, uniform and controlled conditions in limited space and time throughout the year.

Keywords

Salt stress, Tomato, Tissue culture, Cultivars, Screening

Introduction

Salinity significantly limits crop productivity leading to negative impact on food security. Nearly 20% of the world's agricultural land is affected by salinity (Chinnusamy et al., 2005). The world population is expected to exceed 9 billion by 2050 (www.unfpa.org). To fulfil the growing demand for food, the production needs to be enhanced using limited resources of land and water. Annual production of tomato in India ranged around 17.5 million tonnes in the year 2012 (FAO Statistical Database, 2014). Tomato is a "protective supplementary food" endowed with the richness of vitamins, minerals and antioxidant lycopene. India is the fifth largest producer of tomato (*Lycopersicon esculentum* Mill.) and Maharashtra is amongst the major contributor's. However, in Maharashtra about 6 lacks hectare of land is salt affected which includes some of the main tomato producing districts (www.cssri.org). Tomato is cultivated using costly hybrid seeds and most of the commercial cultivars are susceptible to salinity at seed germination and early seedling growth (Foolad, 2004). Therefore, screening towards salinity stress during these stages is important for better germination in direct seeded crop and enhanced adaptability of the seedlings in saline environment after transplantation (Li et al., 2011). Field screening is critical as salinity levels vary with geographical locations, soil types and environmental conditions (Arzani, 2008). In vitro screening is a promising and cost effective tool for rapid evaluation of germplasm under controlled, uniform, disease free conditions, in limited space throughout the year (Rai et al., 2011). Moreover, seedlings germinated in vitro are akin to in vivo plants and are expected to respond to saline stress in a manner similar to them (Mills and Tal, 2004; Shibli et al., 2007). Earlier studies on genotypic variation for salinity tolerance in tomato were performed using Hoagland's solution in vivo (Alian et al., 2000; Dasgan et al., 2002; Singh et al., 2012) and seldom in vitro (Amini and Ehsanpour, 2006; Mohamed et al., 2011). The present study was undertaken with the objective of screening five commercial cultivars of tomato grown in Maharashtra towards NaCl mediated salinity stress in vitro.

Materials and Methods

Seeds of five commercial cultivars of tomato i.e., Abhinav, TO1389 (Syngenta Seeds), Rohini (Indo-American hybrid seeds), N2535 (Namdhari Seeds) and Naina (Monsanto Seeds) were procured from Certified Seed Agencies (Pune, Maharashtra, India). Seeds were surface sterilized with 0.1% $HgCl_2$ for 5 min., rinsed thrice with sterile distilled water, briefly blotted and placed on half strength hormone free Murashige and Skoog's medium (Murashige and Skoog, 1962) supplemented with different concentrations of NaCl (0-100 mM), sucrose (3%, w/v) and gelrite (0.2%, w/v) in culture tubes (25 x 100 mm). Non saline medium served as control. The pH of the medium was adjusted to 5.8±0.1 before autoclaving at 121ºC and 1.1 Kg cm^{-2} for 20 min. The cultures were kept in dark for germination. Germinated seedlings were incubated at 25±4°C under 16:8 h (light:dark) photoperiod maintained by cool white fluorescent tube light of 32 µmol m^{-2} s^{-1} intensity. There were ten replicates per treatment containing two seeds each. Each experiment was repeated thrice. The time required for initiation of germination and the germination percentage were recorded at 24 hrs interval. Completion of germination (at higher stress levels) took nearly three and a half weeks and further seedlings were given two more weeks to stabilize. Therefore observations on seedling vigour (shoot/root length and fresh/dry weight) were recorded after six weeks of culture. Seedlings were harvested and weighed immediately for determination of fresh weight (FW). Dry weight (DW) was recorded from oven dried (60°C for 48 h) seedlings.

Result and Discussion

Germination period was prominently influenced by salinity and it varied with different cultivars. The average seed germination was delayed by 3 days at 40 mM, 6 days at 60 mM, 11 days at 80 mM and 13 days at 100 mM NaCl (Table 1). Abhinav and Rohini germinated almost after 9 days while TO1389 and N2535 required 16 days to germinate in presence of 80 mM stress. Cultivars which germinate early at higher salinity are considered more vigorous and can serve as potential parents in salinity tolerance breeding programmes (Singh et al., 2012). Seed germination was 100% in cultivars Abhinav and Rohini, 90% in TO 1389, 80% in N2535 and 70% in Naina on NaCl free medium (Table 1). Out of the five cultivars, only two (Abhinav, 33.33 % and Rohini 15.54 %) showed germination at 100 mM NaCl. Amongst the cultivars germinated on 80 mM stress percent decline in germination was least in Abhinav (24.50%) and maximum in N2535 (93.79%). Similar response was obtained by Cuartero and Munoz (1999) in tomato where decline in germination percentage became evident at 80 mM NaCl stress and only few genotypes germinated at higher levels of stress with reduced germination percentage. The average seed germination ranged from 88.14±5.80 % on control to 9.77±6.61 % at 100 mM NaCl (Table. 1).

Salinity significantly decreased the seedling growth leading to reduction in shoot /root length and fresh/dry weight. The shoot length of the seedlings was reduced by 66.54% and 72.36% in Abhinav and Rohini at 100 mM NaCl. The average shoot length on control was 9.56±1.21 cm while it was 1.43±0.91cm on 100mM stress showing 85.04% reduction (Table 2). Amongst the cultivars germinated on 80 mM stress the decline in shoot length of the seedling was: Abhinav, 47.91%; Rohini, 48.74%; TO1389, 63.22% and N2535, 67.55%. Salinity usually has a negative impact on shoot length of seedlings as reported by Dash and Panda, (2001) and Vijayan et al., (2004). The average root length on control was 4.05±0.56 cm whereas on 100mM NaCl it was 0.91±0.56 cm showing a reduction of 77.64% (Table 2). The inhibition in root length at 80 mM stress was: Abhinav, 35.58%; Rohini, 38.61%; TO1389, 57.35% and N2535, 63.16%. Reduction in root length due to salinity is mainly because of limited cell growth owing to low water potential in the external environment, nutritional imbalance and ion toxicity (Alizadeh et al., 2010). The ratio of root to shoot growth was higher in seedlings germinated in presence of salt stress as compared to control. The average root to shoot growth ratio on control was 0.42±0.02 and it was 0.65±0.07 on 100 mM stress showing an increment of 54.41% (Table 2). The increment in ratio of root to shoot growth at 80 mM NaCl stress was in the following order: Abhinav, 23.67%; Rohini, 19.76%; TO1389, 15.96 and N2535, 13.52%. The shoot growth is reduced due to salinity induced water deficit so a greater proportion of plants assimilates can be allocated to the root system which supports its growth hence the ratio of root to shoot growth increases (Taiz and Zeiger, 2010, Maggio et al, 2007; Parida and Das, 2005). In control the average fresh weight was 1.88±0.38 g whereas at 100 mM stress it was 0.16±0.11 g exhibiting a reduction of 91.72%. Reduction in fresh weight at 80 mM stress was as follows: Abhinav, 62.70%; Rohini, 66.51%; TO1389, 79.27% and N2535, 86.52% (Figure 1). The average dry weight on control was 0.109±0.02g while it ranged at 0.017±0.01g at 100 mM NaCl showing a decline of 84.71%. The least reduction in dry weights was evident in Abhinav (69.25%) and Rohini (78.85%) at 100 mM stress showing their salinity tolerant nature at the seedling stage (Figure 2). Decline in the fresh/dry weights of the seedlings with increment in salt stress have been reported earlier in tomato (Mills and Tal, 2004; Amini and Ehsanpour, 2006), tobacco (Niknam et al., 2006), blackgram (Dash and Panda, 2001) etc.

The relevance of this study is in rapid screening of commercial tomato cultivars, recommending tolerant cultivars to farmers for cultivation in salt affected fields and incorporating them into breeding programmes.

Table.1 Effect of NaCl stress on days required for germination and germination percentage in different cultivars of tomato in vitro

Cultivars	NaCl (mM)									
	0		40		60		80		100	
	DRG	G (%)	DRG	G (%)	DRG	G (%)	DRG	G (%)	DRG	G (%)
Abhinav	6.88	100.00	7.67	99.50	11.25	99.10	14.54	75.50	20.40	33.33
Naina	11.87	70.00	17.74	20.91	24.32	5.72	----	0.00	----	0.00
N2535	9.65	80.54	12.65	50.79	19.00	20.60	25.55	5.00	----	0.00
TO1389	9.20	90.15	11.76	50.8	18.09	40.08	23.77	10.38	----	0.00
Rohini	8.85	100.00	9.33	99.6	14.10	75.08	18.23	50.52	23.73	15.54
Mean	9.29	88.14	11.83	64.32	17.35	48.12	20.52	28.28	22.07	9.77
SE	0.80	5.80	1.72	15.38	2.23	17.24	2.53	14.81	1.18	6.61

DRG – Days required for germination; G (%) – Germination Percentage

Table.2 Effect of NaCl stress on seedling growth in different cultivars of tomato in vitro

Cultivars	NaCl (mM)														
	0			40			60			80			100		
	SL	RL	R:S	SL	RL	R:S	SL	RL	R:S	SL	RL	R:S	SL	RL	R:S
Abhinav	13.15	5.20	0.40	11.12	4.98	0.45	9.05	4.20	0.46	6.85	3.35	0.49	4.4	2.55	0.58
Naina	5.56	2.06	0.37	3.84	1.48	0.39	2.65	1.05	0.40	0.00	0.00	----	0.00	0.00	----
N2535	9.09	3.8	0.42	6.32	2.8	0.44	4.7	2.1	0.45	2.95	1.4	0.47	0.00	0.00	----
TO1389	10.06	4.15	0.41	7.42	3.35	0.45	5.8	2.7	0.47	3.7	1.77	0.48	0.00	0.00	----
Rohini	9.95	5.05	0.51	7.51	4.2	0.56	6.4	3.82	0.60	5.1	3.10	0.61	2.75	2	0.72
Mean	9.56	4.05	0.42	7.24	3.36	0.46	5.72	2.77	0.47	3.72	1.92	0.51	1.43	0.91	0.65
SE	1.21	0.56	0.02	1.17	0.60	0.03	1.05	0.58	0.03	1.14	0.61	0.03	0.91	0.56	0.07

SL - Shoot Length; RL - Root Length; R: S –Root: Shoot Growth Ratio

Figure.1 Effect of NaCl stress on seedling fresh weight in different cultivars of tomato

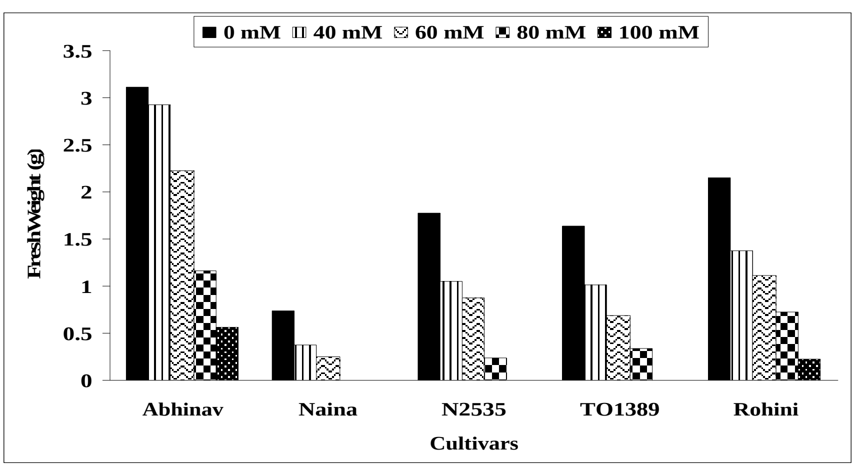
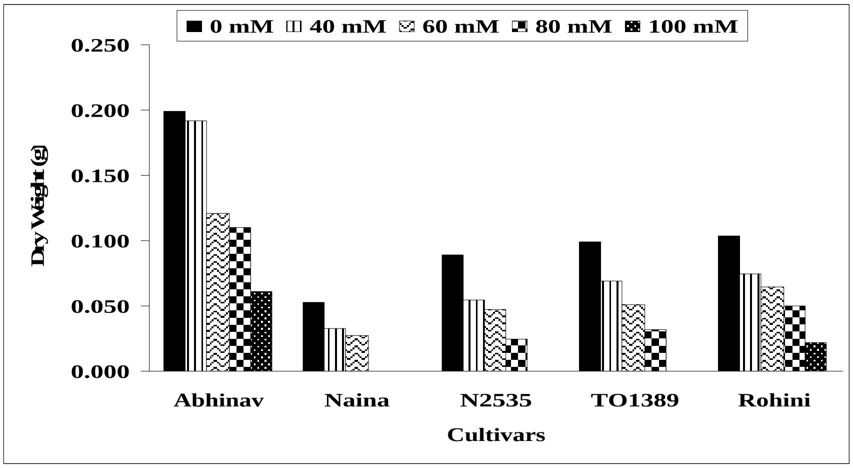


Figure.2 Effect of NaCl stress on seedling dry weight in different cultivars of tomato



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