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Screening for salinity tolerance in tomato during germination using in vitro approach- A Review

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Abstract
One of the major challenges faced by India is to meet the food requirements of its fast-growing population within limited resources. Loss of cultivable land due to salinity is a threat to food security. Screening of germplasm in presence of salt stress is an important step towards selection of tolerant varieties for their possible cultivation in saline land thus increasing productivity and ensuring food supplies. Tomato, the second most consumed vegetable worldwide is moderately sensitive to salinity at all stages of development including seed germination, vegetative growth and reproduction as a result of which the yield is considerably compromised under salt stress. Plant tissue culture offers a promising and cost-effective tool for rapid screening of germplasm for salt tolerance in limited space, under disease free conditions, throughout the year irrespective of seasonal variations. The present review discusses the importance of screening for salinity tolerance in tomato during seed germination and early seedling stage through in vitro approach.

Keywords: tomato, salinity, seed germination, in vitro, screening

Introduction

Existence of human life is attributable to the agrarian resources such as cereals, legumes, vegetables, spices, condiments, beverages etc that gratify our nutritional and health requirements. Majority of these agronomical crops continue to suffer the brunt of Nature in the form of various abiotic stresses such as cold, salinity and drought, biotic stresses like pathogen, insect, herbivore and rodent infestations [1]. Salinity is one of the leading abiotic stresses threatening the sustainability of agriculture world-wide. Continuous increase in saline land is a major cause of concern for our country's food security. Presently 6.74 million ha of land in India is saline which is expected to increase further making almost 50% of the productive land salt- affected and unfit for cultivation by 2050 [2]. Increment in saline land is either natural or man-made. Natural increment in saline areas is due to weathering of rocks that release soluble salts containing chlorides of sodium, calcium, magnesium, potassium along with sulfates, bicarbonates, carbonates and nitrates. Amongst them, sodium chloride is the major contributor towards salinity as it is most soluble and generally released in large amounts. Coastal areas undergo salinization due to the deposition of oceanic salts carried by wind and rain [3]. As the sea level is rising due to climate change, the coastal and inland areas are becoming more vulnerable to salinization [4]. Anthropogenic activities like land clearing, inappropriate agriculture practices such as irrigation with inferior quality and/or brackish water, excessive irrigation in dry areas resulting in evapotranspiration [1], inadequate drainage and disproportionate use of chemical fertilizers result in secondary salinization [2]. One of the major reasons of salinization in India is irrigation with poor quality ground water in the inland plains of arid/semiarid regions and infiltration of sea water in the humid regions of the coastal plains [5]. Saline soils are characterized by high amounts of soluble salts having electrical conductivity (ECe) of 4 dS m^{-1} (equivalent to approximately 40 mM NaCl) or more. The growth and productivity of vegetables is significantly hampered at ECe less than 4 dS m^{-1} as being glycophytes most of them are susceptible to salt stress [6]. Soil salinity is detrimental to plant growth because 1) it reduces the ability of plant roots to take up water creating water deficit within the plant, 2) it results in ion toxicity due to excessive accumulation of Na^+ and Cl^- ions, 3) it leads to nutritional disorders as nutrient uptake is reduced [7, 8, 9]. Tomato, *Solanum lycopersicum* L., a member of family Solanaceae is a versatile vegetable that is moderately sensitive to salinity throughout its ontogeny [10], the threshold limit being 2.5 dS m^{-1} [6]. The consumption of tomato berries is continuously increasing worldwide as they are a storehouse of several dietary nutrients which promote wellness such as minerals, vitamins (C, E) and antioxidants (lycopene, β-carotene) [11]. India is the second largest producer of tomato after China with the area under tomato cultivation accounting to 8.12 lakh hectares leading towards the production of 20.57 million tons of tomato in 2020 contributing around 11% of the world's total production [12]. In order to meet the growing demands of tomato fruits both in the domestic and international market, cultivation of tomato needs to be enhanced. However, the cropping areas and fresh water resources are gradually declining in developing countries like India owing to increased population load, urbanization and industrialization. Therefore, to increase productivity, marginal or salt affected areas are being diverted towards farming and inferior quality water containing dissolved salts is being utilized for irrigation purposes [13]. Soil salinity increases the time required for germination and reduces the germination percentage in tomato resulting in irregular stand establishment and reduced crop productivity [14, 10]. Consequently, screening of tomato germplasm for rapid and uniform seed germination in presence of salt stress is a significant step to boost the production of this indispensable vegetable by utilizing the unproductive salt affected land.

Screening Approaches The screening approaches adopted for selection of germplasm in presence of salinity stress are

either based on field screening or controlled environment screening such as hydroponics or in vitro screening [9]. Agriculture field represents a varied landscape owing to changing climatic and edaphic conditions. Climatic factors such as rainfall, light intensity, wind velocity, temperature, relative humidity, air composition keep fluctuating under field conditions. The edaphic conditions also vary depending upon the soil type and geographic location. Salinity levels are quite heterogeneous within the agriculture field so it is difficult to expose the plants at a particular level of stress throughout their growth period [8]. Moreover, a combination of stress factors along with salinity such as drought, temperature, light intensity etc. may act simultaneously in the field conditions adversely affecting the precision and repeatability of field screening [15]. Screening of tomato germplasm is generally not effective in field conditions as tomato plants develop their root system in less saline regions of the soil [16]. Therefore, solution based hydroponic system or artificial media based in vitro screening methods are preferred. Hydroponic system using solution culture is beneficial over field screening as it provides controlled environmental conditions and appropriate monitoring of nutrient levels. However, replenishing the nutrient solution at frequent intervals is not only expensive but also time consuming [17]. In vitro methods provide highly controlled and stable experimental conditions free from soil or other ecological complications for selection of salt tolerant germplasm [18]. Artificial media based in vitro methods help in fast screening of vast germplasm in limited space, under steady, consistent, disease-free conditions throughout the year irrespective of seasonal variations [19]. Generally, two in vitro approaches are adopted for obtaining salt tolerant plants: a) selection of salt tolerant cell lines followed by regeneration of plants and b) in vitro screening of germplasm using various culture systems such as seeds, seedlings, shoot apex, hypocotyl or cotyledon etc in presence of salt stress [8]. In vitro screening offers a convenient, quick and economical method for germplasm evaluation because seedlings germinated in vitro are analogous with those germinated in vivo and are therefore expected to respond to saline stress in similar manner [18]. Additionally, in vitro techniques also help in understanding the physiological and biochemical changes associated with salt tolerance [20]. In this review the advancements made towards in vitro screening of tomato germplasm at seed germination and early seedling stage are discussed in the light of the literature compiled from several published sources.

Tomato and salt stress Tomato is moderately sensitive to salinity during seed germination, vegetative growth and reproduction therefore, soil salinity is a grave problem for tomato cultivation [21, 22]. Salinity causes a delay in the onset of germination and reduction in germination percentage in commercial cultivars of tomato [22]. Several studies were undertaken to screen tomato cultivars for salinity tolerance during germination using in vitro approach (Table 1). Earlier investigation on tomato focused on understanding the comparative effect of salinity at both organizational levels i.e., callus and whole plant level [23, 24]. In vitro grown tomato plants and their corresponding calli obtained from root, stem leaves were compared for growth, organic and inorganic solutes, nitrogen assimilation enzymes in presence of salt stress [25]. The possibility of using in vitro shoot apex culture to assess salt tolerance of cultivated (*L. esculentum*) and wild (*L. pennellii*) tomato species and its comparison with callus cultures was investigated [26]. The findings suggested that rooting parameters are most useful traits for rapid evaluation and screening of tomato species. Effect of salinity stress on organogenesis was studied using leaf explants and shoot apex culture [27]. Upon exposure of in vitro grown seedlings of wild (*L. pennellii*) and cultivated (*L. esculentum*) tomato species to increased ventilation in growth vessel their response to salinity became closer to the ex-vitro grown plants stressing the role of proper ventilation during culture conditions [28]. Further advancement in research suggested water agar medium as better substratum for seed germination in presence of NaCl as compared to high salt containing Murashige and Skoog's (1962) basal medium. [29, 30] On exposing in vitro grown shoot apices to high salt stress reduction in shoot length, number of leaves, shoot and root fresh and dry weights was observed [31]. Response of tomato micro shoots to induced salinity and its relationship with headspace ethylene accumulation was studied. Elevated salinity levels enhanced ethylene accumulation in headspace resulting in leaf epinasty, reduction in growth and soluble protein content. However, membrane injury, electrolyte leakage, raffinose and total sugars increased in presence of stress [18]. The exposure of tomato plantlets to NaCl during in vitro conditions improved their adaptability towards salt stress when compared to the original plants [32]. Seed germination declined in presence of both NaCl and Na_2SO_4, the effect being more pronounced on full MS medium as compared to half MS medium [33]. In presence of NaCl stress, cotyledon explants showed better shoot regeneration compared to hypocotyl explants but their growth parameters and chlorophyll content declined [34]. When the shoot tips obtained from in vitro germinated seedlings were exposed to NaCl stress, reduction in shoot and root growth was observed but the root growth was more susceptible compared to shoot growth [35, 36]. In presence of salinity stress delay in seed germination, reduction in germination percentage and seedling growth became evident amongst different cultivars [37-41]. Morphological and anatomical changes were also observed during seed germination and early growth stages in presence of salt stress [42]. Upon testing different salts such as Na_2SO_4, NaCl and $CaCl_2$ for seed germination and callus induction in tomato, Na_2SO_4 proved most detrimental [43]. Besides seedlings, shoot apex and callus cultures were also successfully utilized for rapid screening of tomato genotypes [44]. These studies proved that in vitro screening is a valuable tool for rapid diagnostics of varietal variation in tomato towards salinity stress.

*__Table 1:__ Screening for salinity tolerance in tomato during germination using in vitro approach.

Sr. No	Plant	CV/ Sp./ G/	Medium	Range of stress (NaCl)	Explant	Effect	Reference
1.	*L. esculentum*	1 CV	-	25-50 mM	In vitro plants, Callus	Response of in vitro grown plants & their corresponding calli compared in presence of NaCl. RGR of root calli & shoot biomass of in	Bourgeais-Chaillous & Gilles

						vitro plants increased; RGR of shoot calli & root biomass of in vitro plants decreased in presence of NaCl. Except for organic acid, the total soluble sugars, reducing sugar, total amino acids & proline content comparable both in calli & in vitro plants under stress.	Guemier (1992) [25]
2.	*L. esculentum, L. pennellii*	2 Sp.	Half MS	0, 35, 70, 105,140, 175, 210 mM	In vitro shoot apex culture, Callus	Increment in NaCl reduced leaf & root number *in L. esculentum* as compared to *L. pennellii.* NaCl reduced K^+ in shoots as compared to callus, increased proline both in shoots & callus.	Cano, *et. al.* (1998) [26]
3.	*L. esculentum*	2 CV	MS	0, 46, 86, 129, 172 mM	Shoot apex culture, Shoot organogenesis from leaf	NaCl hampered root development as compared to shoot, reduced regeneration frequency of shoot form leaf.	Mercado, *et. al.* (2000) [27]
4.	*L. esculentum, L. pennellii*	2 Sp.	MS	100 mM	Seedlings	Improved ventilation reduced leaf abnormalities related to hyperhydricity, increased K^+, Na^+ & Cl^- accumulation in shoots of both tomato species as compared to restricted ventilation in presence of NaCl.	Mills & Tal (2004) [28]
5.	*L. esculentum*	4 CV	Water agar +/- sucrose, MS +/- sucrose	0,40, 80, 120, 160 mM	Seeds	Water agar medium proved better compared to MS for seed germination in presence of NaCl. NaCl decreased seed germination, DW, chlorophyll content, altered acid phosphatase activity & soluble protein content.	Amini & Ehsanpour (2006) [30]
6.	*L. esculentum*	6 CV	-	0, 25, 50, 75, 100 mM	Hypocotyl, Shoot apices	NaCl declined callus FW, DW, RGR, increased proline & Na^+ content & reduced K^+ content. NaCl at higher levels reduced shoot length, number of leaves, shoot / root FW /DW.	Mohammed, *et. al.* (2007) [31]
7.	*L. esculentum*	2 CV	MS	0, 50, 100, 150, 200 mM	Microshoots	NaCl enhanced headspace ethylene which increased leaf epinasty. NaCl decreased growth, leaf cell sap osmolarity, leaf tissue viability, shoot soluble protein, K^+/Na^+ ratio, macronutrients & micronutrients, increased Na^+ content, electrolyte leakage, membrane injury, raffinose & total sugars.	Shibli, *et. al.* (2007) [18]
8.	*L. esculentum*	1 CV	MS	0, 25, 50, 100 or 150 mM	Cotyledon	NaCl reduced FW, DW in shoots & roots, lowered Na^+ level, increased proline, anthocyanin, enzyme activities of PAL (phenylalanine ammonia lyase), TAL (tyrosine ammonia lyase), CI (chalcone isomerase) & K^+ level.	Hassan, *et. al.* (2008) [32]
9.	*L. esculentum*	1 CV	Half MS & MS	0, 40, 80, 120 mM Na_2SO_4; 0, 50, 100, 150 mM NaCl	Seeds, Pollens	Seed germination better in half MS compared to MS in presence of NaCl. Pollens failed to germinate beyond 50mM & 30mM NaCl & Na_2SO_4 respectively.	Yokas, *et.al.* (2008) [33]
10.	*L. esculentum*	2 CV	MS	0, 25, 50, 75 mM	Hypocotyl & cotyledon explants	NaCl reduced growth traits & chlorophyll content.	Mohamed, *et. al.* (2011) [34]
11.	*L. esculentum*	12 CV	Half MS	0, 50, 75, 100 mM	Shoot tips obtained from in vitro germinated seedlings	NaCl lowered shoot & root growth.	Osman, *et. al.* (2011) [35]
12.	*Solanum lycopersicum*	4 CV	Agar (seeds), Half MS (seedlings)	0, 50, 100, 150 mM	Seeds & seedlings	NaCl delayed seed germination, reduced plant growth, K^+ content, photosynthetic rate, increased Na^+ content.	Sholi (2012) [37]
13.	*S. lycopersicon*	2 CV	Agar	0, 50, 100, 150 mM	Seeds	NaCl delayed seed germination, reduced seedling height, development of root & leaves, vascular system in root & stem. NaCl increased thickness of cortical region of stem while decreased that in root.	Al-Tardeh & Iraki (2013) [42]
14.	*S. lycopersicum*	3 CV	Agar	0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20 M	Seeds	Germination percentage & seedling growth significantly reduced at 0.1M & completely inhibited beyond 0.2 M NaCl	Basha, *et. al.* (2015) [38]
15.	*S. lycopersicum*	5 CV	Half MS	0, 40, 60, 80, 100 mM	Seeds	NaCl increased the days required for germination, reduced germination percentage,	Seth & Kendurkar

						shoot/root length, FW/DW of seedlings.	(2015) [39]
16.	*S. lycopersicum*	14 G	MS	0,50, 100, 200, 250 mM	Seeds	NaCl affected root length & plant weight.	Rashed, *et. al.* (2016) [36]
17.	*S. lycopersicum*, *S. peruvianum*, *S. pimpinellifolium*	12 G 3 Sp.	MS	0, 100, 200, 300 mM	Shoot apices & Callus	Shoot apices of *S. peruvianum* & *S. pimpinellifolium* developed roots in presence of NaCl, callus growth better in *S. peruvianum* & *S. pimpinellifolium* as compared to *S. lycopersicum. S. peruvianum* callus accumulated more Na^+ & Cl^-.	Zaki & Yokoi (2016) [44]
18.	*S. lycopersicum*	2 CV	MS	0, 20,40, 60, 80, 100 mM	Seeds	NaCl altered growth parameters, K, N, Na & Ca ions in leaves.	Al-Daej (2018) [40]
19.	*L. esculentum*	1 CV	Full & Half MS	50, 100, 150 mM (NaCl, $CaCl_2$); 40, 80, 120 mM (Na_2SO_4)	Seeds	NaCl decreased seed germination percentage, average seedling height, number of leaves, callus induction & regeneration percentage	Sultana, *et. al.* (2019) [43]
20.	*S. lycopersicum*	5 CV	Half MS	0, 35, 70, 105 mM	Seeds	NaCl reduced seed germination, growth & development.	Sane, *et. al.* (2021) [44]

*CV – Cultivar, Sp. - Species, G- Genotype, MS- Murashige and Skoog's Medium, RGR-Relative growth rate, DW-dry weight, FW-fresh weight

Conclusion

As salinity continues to be one of the major abiotic threats limiting productivity of tomato, this review highlights the role of in vitro screening of tomato germplasm in presence of NaCl mediated stress. In vitro methods provide the ease of germplasm assessment within uniform, disease-free environment irrespective of soil or climatic hindrances. Seed germination is the first stage when a plant encounters salt stress, therefore screening approaches adopted for salt tolerance during seed germination and early seedling growth in tomato are emphasized. Selecting tomato cultivars for efficient germination potential under saline environment is important for successful stand establishment and better adaptability in salt affected fields. This will help in enhancing the production of tomato, an indispensable ingredient in nearly every cuisine around the globe by bringing saline areas under cultivation. In addition, tolerant cultivars can be utilized in plant breeding or gene cloning programs and for investigating the salt tolerance mechanism.

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