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Research article

IN VITRO EVALUATION OF TOMATO GENOTYPES FOR SALT TOLERANCE AT SEEDLING STAGE

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ABSTRACT: Laboratory experiments were conducted to examine the effect of salinity onthree tomato cultivars at seedling stage. The seeds of Arka Vikas, PKM-OP and YVU-1tomato cultivar were treated with 0.02M, 0.04M, 0.06M, 0.08M, 0.10M, 0.12M, 0.14M, 0.16M, 0.18M and 0.20M NaCl concentration. The percentage of germination, primary root length and shoot length were evaluated for the assessment of effect of different salt concentrations.The cultivars showed enhanced shoot and root growth at lower salt concentration and declined shoot and root growth at higher salt concentration. Complete seed germination was observed up to 0.06M salt concentration but the seed germination rate varied among three cultivars.At higher salt concentration(>0.08M)theseed germination percentage was reduced. All the cultivars at0.02Mand 0.04M NaClconcentration showed enhanced seedling growthin comparison with control and highest shoot and root length was observed in all cultivars at 0.04M NaCl. Allcultivars showedsignificant reduction in germination percentage and seedling growth rate at 0.10M concentration.Seed germination oftomato cultivarswas poor above 0.16M NaCl concentrations and complete inhibition was observed at greater than 0.20M salt concentration. Hybrid cultivar PKM-OP showed better tolerance to NaClthan other cultivars.

Key words: Tomato, salinity, NaCl, Germination, Salt stress.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most important vegetable cropgrown all over the world and it is a good model crop for conducting fruit ripening studies. Tomato has a relatively small diploid genome (n=12) with 950 Mbp genome size and contained hundreds of mapped traits and molecular markers [1]. In 2009, whole-genome sequence of tomato was completed by shotgun sequencing approach. In addition to genome sequencing, a tomato EST database was also developed, which has more than 28,000 non-redundant sequences derived from fruits at different stages of development (http://www.tigr.org/tdb/lgi/).

Plenty of literature is available on salt tolerant germplasm of crops under a colossal range of soil, climatic and salinity conditions. The salt-specific or ion-excess stress, defined as excessive amounts of salt (sodium and chloride) that enters in to the plant transpiration stream which can be toxic and cause injury to the cell [2, 3]. Salinity affects the growth of plants by affecting the availability, transport, and partitioning of nutrients such as K^+, Ca^{++}, Mg^{++} and NO_3^-due to competition of Na^+ and Cl^- with them[3, 4, 5, 6, 7]. High salt concentration in the soil not only affects the plant growth but also interfere with activity of soil's microbial population.

Plants are able to survive in saline conditions, by excluding a large propotion of salt while taking up of water continuously.Based on tolerance to salinity plants can be categorized into two groups i.e. halophytes and glycophytes. Halophytes possess the ability to survive salt shocks and can grow at the higher salt concentrations of more than 200mM [8, 9, 10, 11], while glycophytes cannot survive at these conditions.

Salt tolerant plants follow different adaptive strategies, by which the plants can remain functional regardless of inside ionic stress, such as avoidance through ion exclusion (potentially as a result of low membrane ion permeability), through ion inclusion (possible compartmentalisation) and osmotic stress tolerance [12, 13]. In some plant species, Ion exclusion is one of the important salt tolerant mechanisms, in which plant root excludes most of the ions like sodium and chloride dissolved in the soil solution, and escape from build-up of salts in shoots at toxic level [12, 13, 14, 15, 16, 17].

Field trials to screen the salt tolerance level of plants at different stages requires considerable time and labour, therefore development of standardized screening methods are required to save time and inputs [18]. In the present investigation we determine the germination percentage and seedling growth of the Arka Vikas, PKM-OP and YVU-1 under varied salt (NaCl) stress conditions.

MATERIAL AND METHODS

Solanum lycopersicum, cultivar Arka Vikas seeds were obtained from Indian Institute of Horticulture Research (IIHR), Bangalore. The seeds of YVU-1 were collected from local farmers of Kadapa district, Andhra Pradesh, India and the germplasm is being maintained at Yogi Vemana University. PKM-OP are hybrid seeds and are collected from local traders. Lots of 30 seeds of each variety were placed in 7% agar media in tissue culture bottles. The seeds were germinated in the7% agar supplemented with 0.02M, 0.04M, 0.06M, 0.08M, 0.10M, 0.12M, 0.14M, 0.16M, 0.18M and 0.20M NaCl concentration, to determine the tolerance of the germination to the salt. The germination percentagesshoot and root lengthsof three replications of 30 seeds i.e total 90 seeds were recorded.Seeds were surface sterilized in 4% Sodium hypochlorite under gentle shaking for 15 minutes at room temperature followed by extensive washing by autoclaved distilled water until traces of sodium hypocholorite removed.The seeds were inoculated in to the culture bottle andwere tightly sealed with screwed covers in order to avoid contamination and water losses during the incubation and then seeds were incubated at 25 ± 2^0C in continuous white light for 10 days.The seed germination percentage, seedling shoot length and root length were noted after 10 days. For each harvest, the sensitivity of the germination was calculated as the ratio of the germination percentage under the salt (with stress) and to that of control (without stress).ANOVA was carried out to test the variation at 0.05 significance among the three cultivars. Newman-Keuls Multiple Comparision, Post-HOC test were carried out for Pairwise comparison among all groups of cultivars.

RESULTS AND DISCUSSION

Most of the agriculture crops are sensitive to saline condition. The conventional agriculture in these salt-affected sites is generally economically unviable and may result significant reduction in crop yield and also increase the expenses in conventional farming. The effect of salinity on germination of Arka Vikas, PKM-OP and YVU1 cultivars in agar medium is showed in Figure 1. The descending lines with varied slopes indicate the germination percentage is differed among all three cultivars at different salt concentrations (Figure 1). The seeds of the varieties could not germinate in the agar medium containing more than 0.20M concentration. The primary observation of the present study reveals that with the increasing concentration of NaCl from 0.08M onwards, the germination percentage got decreased in all the cultivars (Figure1). Highest percentage of germination was observed in the following series- control, 0.02M, 0.04M, 0.06M, and 0.08M, but the germination rate showed mixed results in these salt concentrations. These observations suggest that the entire seed germination observed at 0.02M, 0.04M and 0.06M salt concentration but the rate of seed germination varied and reduction in seed germination percentage was noted at higher salt concentrations(>0.08 M). In *Alfalfa*, the seeds germination rate was reduced and not germinated due to increase in osmotic pressure[19]. Ashraf et al., (1986) [20] reported that germination and seedling stage of the plants are more sensitive to salt stress than in other stages. Foolad and Lin (19970 [21] reported that the tomato seed germination rate was mainly affected by osmotic rather than ionic. Shannon and Grieve (1999)[22] also reported that the lower salt concentration affects only the germination rate but not the total percentage of germination. Salt stress causes osmotic stress ionic imbalance which reduce or inhibit the seed germination [3, 23]. The inhibition of seed germination was more with the increasing levels of NaCl in *Brassica juncea* and *Vigna mungo*[24, 25].Similarly, decrease in germination of seeds of several plant species with the increasing salt concentration has been reported by several authors [26, 27]. Miri and Mirjalili (2013) [28] reported that with increasing salt concentration the seeds of cone flower germination were decreased.

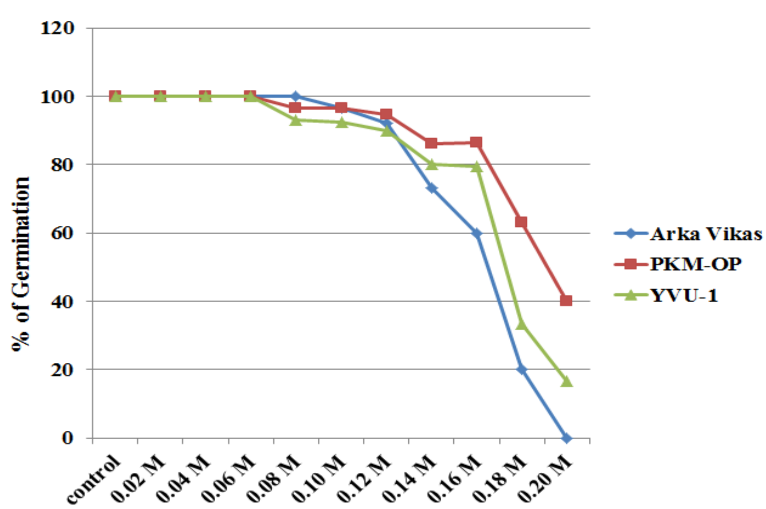
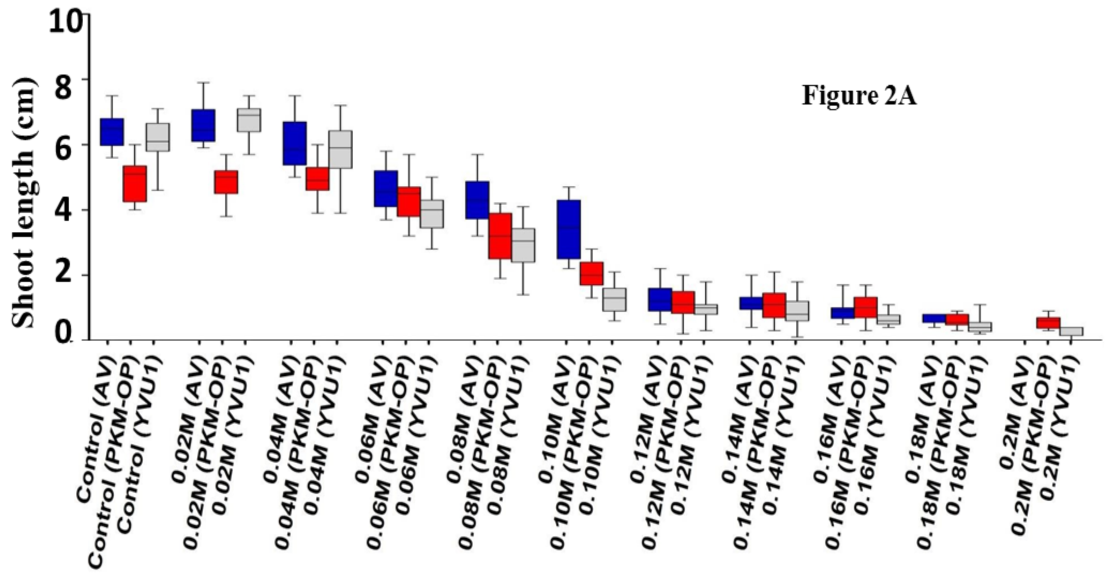


Figure 1: Effect of NaCl on seed germination in Arka Vikas (AV), PKM-OP and YVU-1incubated at different salt concentrations in agar medium for 10 days.

The mean and standard error bars of shoot and root length in Arka Vikas, PKM-OP and YVU-1 cultivars showed marked differences at various salt concentrations ($P<0.05$) (Figure 2A and Figure 2B). Interestingly the highest shoot and root length was found at 0.02M followed by 0.04M NaCl and then control. The shoot and root mean length values decreased in all cultivars as the salinity level increased from 0.04M Nacl concentration. ANOVA test reveals a significant variation among three cultivars in their shoot and root lengths with increase in salt concentration ($F_{(332)}$; $P<0.05$). The POST-HOC results revealed that there is no significant difference among Arka Vikas, PKM-OP and YVU-1 in control ($P<0.05$). But a significant difference among three cultivars was observed at 0.02M NaCl and 0.10M NaCl concentration in shoot and root lengths (Figure 2A and Figure 2B). Significant difference was found between YVU and PKM-OP at 0.06M concentration but notin other cultivars (Figure 2A and Figure 2B). Significant difference ($P<0.05$) was observed in shoot and root length among all three cultivars at 0.08M and 0.12M NaCl concentration. But at 0.04M concentration ($P<0.05$) a significant difference was observed between PKM-OP and YVU, Arka Vikas and YVU, but not in PKM-OP and Arka Vikas ($P>0.05$) (Figure 2A and Figure 2B).

The highest shoot and root length values were observed at 0.04M NaCl concentration and the lowest at 0.20M NaCl (Figure 1A and Figure 1B). The shoot and root length increased significantly in the PKM-OP seeds inoculated in 0.04M NaCl when compared with the control (Figure 2A and Figure 2B). The results reveals that YVU-1 seed inoculated in 0.02M and 0.04M NaCl concentration had highest shoot and root length as compared to those of control (Figure 2A and Figure 2B).



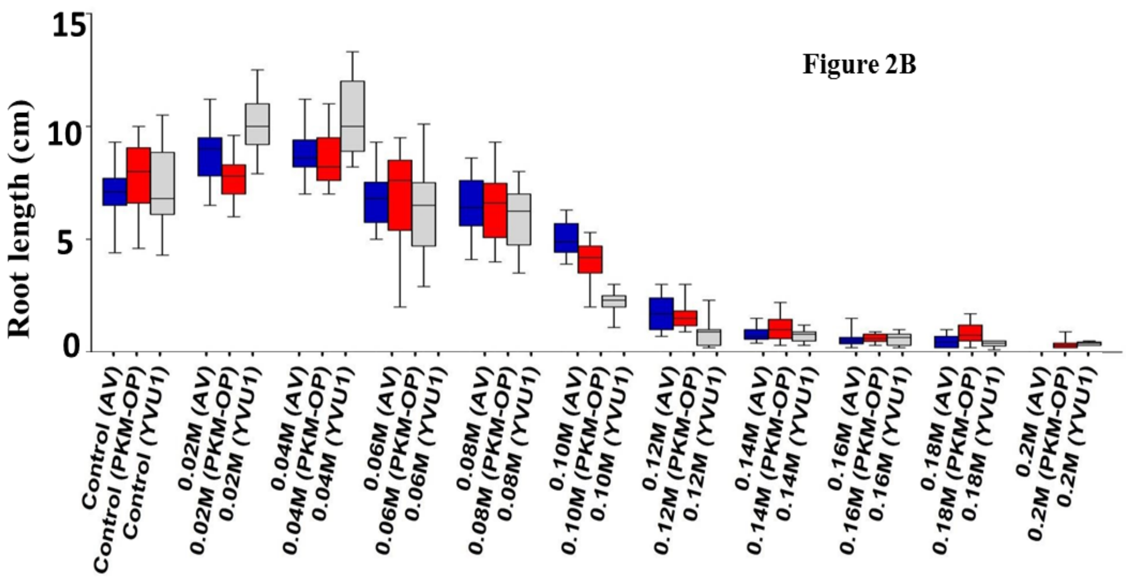


Figure 2A.Effect of increasing salt concentration on shoot length of Arka Vikas (AV), PKM-OP and YVU-1 under white light for ten days; Figure 2B.Effect of salt concentration on root length of Arka Vikas (AV), PKM-OP and YVU-1 under white light for ten days.

The box plots indicate that at higher than 0.04M NaCl concentration the shoot and root length got decreased drastically (Figure 2A and Figure 2B). The tolerance or sensitivity of the seed germination and seedling growth in the saline condition was a measure of the seed ability to withstand the effects of high concentration of soluble salts in the medium and it was observed that the forced stress conditions limited the water to reach the threshold level for the germination and then retarded the seedling growth [29]. This is in line with various reports stating that the seedling growth, dry weight and emergence were more sensitive than germination in the carrot [30], cucumber [31] and aubergine [32]. Hussain and Rehman (1997)[33] reported that roots of the plant seedling were more sensitive than the shoot. Foolad and Lin (1997) [21] examined the tomato seed germination in different ionic and non-ionic germination media with identical osmotic potential. They concluded that the tomato seed germination rate was mainly affected by the osmotic rather than ionic effects of the medium. It could be concluded that the stress imposition resulted in higher gains in the biomass owing to the increased cell division and material synthesis such as sugars [34].

The present observations were inhibition of germination, decreased shoot and root length of tomato seedling with the increasing level of NaCl may be related to radicle emergence due to insufficient water absorption, or may be ascribed to toxic effects on the embryo. Osmotic adjustment is another mechanism that plants have developed to tolerate the low soil water potential caused by salinity, as well as by drought. In salt sensitive plants, low water potential stress leads to cell membrane damage [35, 36]. In vitro conditions over 100mM Na+ concentration the enzymatic reactions were inhibited and severely affected at 200mM concentration [3]. In some species more than 200 mM high salt concentration found in leaves but still they function normally, due to sequestering salts in the vacuole they keeping the salt out of the cytoplasm[8].Osmotic adjustment occurs in plants when subjected to saline stress; under these conditions the organic solutes such as proline, glycine betaine, soluble sugars, free amino acids etc. and K^+ will accumulate in the cytoplasm to balance the osmotic pressure of the ions in the vacuole [16].

CONCLUSION

Efforts have been initiated to identify and explore salt tolerance germplasm and mutants at different levels to develop the salt-tolerant crop varieties through breeding programmes. Salt tolerance is not a static feature to crop species or varieties and may vary with the environmental conditions and this degree of tolerance varies at different growth stage of the crops such as seedling, flowering, maturity etc. The results reported in the present study, suggest that Arka Vikas and YVU-1 cultivars showed enhanced growth at 0.02M and 0.04M salt concentrations. But in PKM-OP significant increase in growth has been observed only at 0.04M NaCl concentration. Drastic decrease in seedling growth has been observed at 0.10 M NaCl concentration in all cultivars. The results indicate that PKM-OP is more tolerant than Arka Vikas followed by and YVU-1against salinity. The developed datacan be more useful to screen mutants for salt tolerance or sensitivity.

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