Int.J.Curr.Microbiol.App.Sci (2024) *13*(11): 191-201

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 13 Number 11 (2024)
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2024.1311.023

An Overview on Impact of Salinity Stress in Tomato under *invitro* Conditions

Rupali Seth*

Department of Botany, Fergusson College (Autonomous), Pune, India

**Corresponding author*

Keywords

Salt stress, tomato, seed germination, in vitro, proline, genotypic variation

Article Info

Received:
20 September 2024
Accepted:
26 October 2024
Available Online:
10 November 2024

A B S T R A C T

This review examines the impact of in vitro salinity stress on morphological and biochemical attributes of tomato during early developmental stages.Tomato fruits are endowed with multifaceted properties placing them in high demand for food, pharmaceutical and cosmetic industries. Increasing soil salinity due to changing climatic conditions is a potential threat for tomato cultivation. Tomato is moderately sensitive to salt stress throughout its life cycle. Salinity brings about osmotic, ionic and oxidative stress in tomatoes impairing growth, well-being and productivity. Upon exposure of tomato seeds to salt stress under in vitro conditions the germination period increases and there is decline in germination percentage. The growth and development of tomato seedlings is affected by salt stress resulting in reduction of shoot length, root length, fresh and dry weight. In presence of salinity stress, tomato seedlings show changes in biochemical parameters such as increase in Na^+ and Cl^- ions, free amino acids, protein, proline, antioxidant enzymes and decrease in chlorophyll content. Genotypic variation is exhibited by the tomato cultivars in response to in vitro salinity stress which is helpful in rapid selection of tolerant cultivars for planting in salt stressed environment as well as inclusion in plant breeding studies.

Introduction

Tomato originated in the Andean region of South America and is native to Peru and Ecuador. The Aztecs, are considered to be the first to domesticate, cultivate and consume tomatoes. During the 16th century tomatoes were grown as ornamentals in Spain for their vibrant fruits but were considered poisonous due to their association with night shade family (Solanaceae). The Italians embraced tomato berries in their culinary preparations transforming it from an ornamental to an edible plant (Flores *et al.,* 2024). By mid of 17th century tomatoes spread from Europe to Asia and Africa and became a popular dietary component in a variety of cuisines (Kochar, 2009). Tomatoes are consumed fresh or cooked, they are processed into soups, ketchups, purees, juices, sauces and pickles. Tomato berries contain various phytochemicals essential for health such as minerals, vitamins, carotenoids, glycoalkaloids and polyphenols (Ali *et al.,* 2021).

Tomato fruits are rich in antioxidants such as β-carotene, lycopene and vitamin C which provide protection against cancer, cardiovascular aliments, neurological disorders, gastrointestinal diseases and enhance immunity. The tomato seed oil contains linoleic acid, lecithin, antioxidant, and UV protection qualities making it an ideal natural ingredient in a wide range of cosmetics and personal care products, including sunscreens, anti-aging serums, body butters, and skin lightening lotions (Collins *et al.,* 2022). Owing to its multifaceted properties tomato berries are in high demand for food, pharmaceutical and cosmetic industries. Globally the annual production of tomatoes ranged at 186 million tons in 2022, out of which India contributed 21 million tons being the second largest producer of tomatoes after China followed by Turkey, USA and Egypt. (FAOSTAT). However, changing climatic conditions present a threat to tomato production. Erratic weather patterns causing intense heat wave and drought or excessive rainfall are increasing soil salinity (Ullah, 2021). Globally around 1100 million hectares of soil are salt affected (FAO) and it is estimated that by the end of 2050 more than 50% of cultivable land will become salinized (Wang *et al.,* 2003; Rosca *et al.,* 2023). Continuous increment in soil salinization is preventing the utilization of farmland, reducing the agriculture productivity and threatening food security (FAO). Plants are unable to grow properly in salinized soils because excessive salts prevent the roots from absorbing water triggering water deficit inside the plant system. The plant health is further compromised due to accumulation of Na^+ and Cl^- ions causing ion toxicity and nutritional imbalance (Muhammad *et al.,* 2024). The build-up of salts disturbs physiological and metabolic process such as photosynthesis, respiration and cell division, it also results in synthesis of reactive oxygen species and causes reduction in plant growth and yield (Rodriguez-Ortega *et al.,* 2019). Tomato is moderately sensitive to salt stress and the production begins to decline beyond 2.5 dSm^{-1} (Curatero and Fernandez-Munoz, 1999). Saline soils have negative impact on tomato mainly due to increment in germination period, reduction in germination percentage and poor stand establishment resulting in reduced yield and revenue (Foolad, 2007; Rosca *et al.,* 2023). This review examines the impact of in vitro salinity stress on morphological and biochemical attributes of tomato during early developmental stages.

Salinity and Morphological Attributes

The impact of in vitro salinity stress during seed germination and seedling growth has been extensively studied in tomato as early developmental phase is more susceptible towards salinity stress compared to flowering and fruiting (Foolad, 2004). The effect of salt stress on seed germination was studied by Amini and Ehsanpour (2006) in four tomato cultivars Isfahani, Shirazy, Khozestani and Khorasani. The seeds were germinated in vitro on Murashige and Skoog's (1962) medium (MS) and water agar medium either with or without sucrose supplemented with different concentrations of salts *viz* 40, 80, 120 and 160 mM. Seed germination declined with increasing levels of salt stress. Maximum germination percentage was observed in cultivar Isfahani while minimum in cultivar Shirazy. There was significant decline in stem-leaf and root dry weight in presence of salt stress. The study revealed that germination response was better in water agar medium in comparison to MS medium. Yokas *et al.,* (2008) studied the effect of MS and half MS medium on seed germination in cultivar Target NF_1 and found 63.5% seed germination in MS and 100% in half MS on control. Higher seed germination was observed on half MS medium in presence of 100 mM salt stress as compared to full MS medium. This study proved that high salt nutrients in MS medium were inhibitory for seed germination. Hassan *et al.,* (2008) studied the effect of salt stress on tomato (Super Strain B) plants under in vivo conditions using sand: clay medium and irrigating plants with Hoagland's nutrient solution supplemented with salt and in vitro selection using cotyledons form 2-day old germinated seeds on MS medium supplemented with salt stress (25, 50, 100 and 150 mM). In vitro tomato plants exhibited better performance and registered lower decline in fresh and dry weight of shoots and roots as compared to in vivo maintained plants. The shoot tip explants obtained from 21 to 25 days old seedlings of 12 tomato cultivars (Omdurman, Allakarim, Strain B, Jazera, Majd, Supermajd, Madona, Amani, Castel Rock, Rio grand, Sudan special and Peto-86) were subjected to different concentrations of salt stress (50, 75 and 100 mM) on MS medium by Osman *et al.,* (2011). There was reduction in shoot and root growth with increment in salt stress, root growth being more vulnerable compared to shoot growth. Maximum root growth and root fresh weight was obtained in cultivar Amani while cultivar Castel Rock exhibited prominent increment in shoot growth and shoot fresh weight. Mohamed *et al.,* (2011) cultured hypocotyl and cotyledon explants of two cultivars Pearl and Beril on MS medium containing 25, 50 and 75 mM NaCl, and reported reduction in fresh and dry weight of regenerated shoots with increment in stress. The cultivar Beril displayed enhanced tolerance potential compared to Pearl. In presence of salt stress, hypocotyl explants exhibited improved shoot length and proved better compared to cotyledon explants for both the cultivars. Abu-Khadejeh *et al.,* (2011) investigated the physiological response of microshoot cultures on salinity stress. The microshoot cultures obtained from seedlings of two tomato genotypes JO112 and JO992 were grown on MS media supplemented with 50, 100, 150 and 200 mM NaCl and $CaCl_2$ in equimolar ratios. Root development was inhibited in the microshoots at 150 mM and higher levels of salt stress. There was reduction in growth parameters such as shoot height, root growth, fresh weight and dry weight of microshoots with increment in salt stress. Sholi (2012) reported that seeds of four tomato cultivars, Jenin 1, Hebron, Ramallah and Maramand when germinated on agar solidified medium supplemented with 50,100 and 150 mM salt stress exhibited delayed germination in presence of salt stress. Hebron cultivar required 7.22 days to achieve 50% germination at 100 mM salt stress, followed by Jenin1 (8.51), Ramallah (9.51) and Maramand (11.50). At 150 mM only Hebron achieved 50% germination while remaining cultivars failed to germinate. Al-Tardeh and Iraki (2013) probed the effect of salinity on morphological and anatomical response during seed germination and early growth stages in Palestinian tomato cultivars J1 and Ram. In vitro germination was performed on water agar medium supplemented with 50, 100 and 150 mM NaCl in cultivars J1 and Ram. At 150 mM stress cv Ram showed 25% while J1 recorded 13.33% germination. The relative seedling height and root length was higher in cv Ram than J1. The development of root system, leaves, vascular system of root and stem was hampered with increment in salt stress. The cortex region of the stem increased in thickness while that of root declined. Based on morphological and anatomical changes cv Ram proved tolerant towards salinity stress than J1. Basha *et al.,* (2015) evaluated tomato cultivars for salt tolerance at seedling stage and reported significant reduction in germination percentage at 0.1M salt stress and complete inhibition of germination at or above 0.2M NaCl in three cultivars Arka Vikas, PKM-OP and YVU-1. Maximum shoot and root length was obtained amongst all the cultivars at 0.02 M and 0.04 M salt stress as compared to control. Seth and Kendurkar (2015) reported that when seeds of five commercial tomato cultivars Abhinav, Rohini, TO1389, N2535 and Naina were germinated on half strength MS medium supplemented with 40, 60, 80 and 100 mM salt stress the days required for germination increased and germination percentage declined. Only two cultivars Abhinav and Rohini germinated at 100 mM salt stress with germination percentage of 33.33% and 15.54% respectively. There was significant reduction in shoot length, root length, fresh weight and dry weight with increasing levels of salt stress. The average reduction in shoot length and root length was 85.04% and 77.64% respectively at 100 mM salt stress within cultivars. High root to shoot ratio indicated that shoot growth was more compromised compared to root growth under NaCl stress. The average fresh weight and dry weight reduced by 91.72% and 84.71% amongst the cultivars at 100 mM salt stress. The study demonstrated that in vitro screening is an effective and rapid method for evaluation of tomato cultivars. In order to screen for salt tolerance, Rashed *et al.,* (2016) performed root growth assay and fresh weight assay with four-day old seedlings of 14 tomato genotypes by culturing them on MS medium appended with 50, 100, 200 and 250 mM NaCl. There was decline in root growth with increment in salinity stress. At 50 mM salt stress maximum root length was achieved in BD-7302. At higher stress levels i.e. 250 mM, BD-7291 recorded maximum root length while BD-7260 achieved minimum root length. Fresh weight declined with increment in salt stress. Least decline in fresh weight was evident in BARI-2 and BD-7292 while BD-7762 experienced maximum decline in fresh weight in presence of salt stress. Zaki and Yokoi (2016) compared the variation in salt tolerance response in cultivated tomato (*Solanum lycopersicum* L.) and its wild relatives (*Solanum peruvianum* L. and *Solanum pimpinellifolium* L.) in vitro. The study was undertaken on 12 tomato cultivars, six of *Solanum lycopersicum* (Moneymaker, Aichi-first, Ailsa Craig, M82, Rutgers and Ponderosa), two of *Solanum peruvianum* (lines 0043-1 and 0046) and four of *Solanum pimpinellifolium* (lines 00041 w 1, 0043, 0048 and 0049-w 1). To compare the genotypic variation in salinity response, the shoot apices obtained form in vitro germinated seedlings of 12 tomato cultivars were cultured on half strength MS medium supplemented with 100, 200 and 300 m mol L^{-1} salt stress. The leaves obtained from shoot apex cultures were used for callus induction in presence of NaCl. The fresh weight declined with increment in salt stress however, wild cultivars maintained higher fresh weight compared to cultivated cultivars. Shoot apices of cultivated tomato failed to develop roots upon exposure to low salt stress while the wild cultivars developed roots on all levels of salt stress. With increasing salt stress, the root fresh weight reduced in all cultivars of *S.lycopersicum* and enhanced in all the cultivars of *S. peruvianum* and *S. pimpinellifolium*. The wild tomato cultivars developed longer roots compared to cultivated cultivars under high salinity levels. Salt tolerance index of root fresh weight, total root length and root surface area showed better tolerance potential in the wild cultivars as compared to cultivated cultivars. The callus growth was better in cultivars of *S. peruvianum* and *S. pimpinellifolium* as compared to *S. lycopersicum* at higher levels of salt stress. Al-Daej (2018) investigated the effect of salinity stress (20, 40, 60, 80 and 100 mM) in tomato seedlings in cultivar Rams and C10 and reported that the germination percentage, fresh weight, dry weight, plant height, number of leaves declined with increasing levels of salt stress in both the cultivars. However, cultivar Rams showed better germination percentage and seedling growth as compared to C10. At 100 mM salt stress Rams achieved 10% germination while C10 failed to germinate. Sultana *et al.,* (2019) reported that the germination percentage declined with increment in salt stress in cultivar Marglobe with maximum decline at 150 mM salt stress. The average seedling height and number of leaves also showed a declining trend with increasing salt stress. Sane *et al.,* (2021) studied the effect of salt stress on five tomato varieties by germinating seeds on half strength MS medium supplemented with NaCl (35, 70 and 105 mM). The results revealed that the performance of variety Lady Nema and Mongal was best at 105 mM salt stress showing 35% germination followed by Ganila and Xewel and least in Rodeo. With increasing concentration of salt stress, the survival rate of in vitro plants declined. The variety Xewel recorded the highest average number of leaves at all levels of salinity stress while variety Rodeo recorded lowest average number of leaves at 105 mM salt stress. Maximum taproot length was seen in Lady Nema at 105 mM while Ganila showed highest number of secondary roots at all concentrations of NaCl. Maximum fresh weight of the aerial parts and roots was obtained in Lady Nema. Based on the growth performance, Lady Nema and Mongal were rated as most tolerant, Ganila and Xewel moderately tolerant and Rodeo sensitive. Aazami *et al.,* (2021) studied the effect of salinity stress on four tomato cultivars germinated on half MS medium containing 25, 50, 75 and 100mM salt and reported maximum germination percentage in cultivar PS-10 (41.67%), followed by Peto (15%), Nora (10%) and Roma (8.33%) at 100 mM salt stress. There was decline in fresh and dry weight with increment in salinity stress. At 100 mM NaCl, Peto showed highest fresh (160.5 mg) and dry weight (55.73 mg). Shoot length declined with increase in salt stress. PS-10 showed maximum shoot length (9.30 cm) at 100 mM salt stress. The fresh and dry weight of root also lowered with increment in salt stress. Maximum fresh and dry root weight was seen in PS-10 at 100 mM salt stress. Khaliluev *et al.,* (2021) excised the shoot apex (1.5 - 2 cm in length) from 8 to 10 days old seedlings (at first true leaf stage) of two tomato cultivars, YaLF line and Rekordsmen germinated aseptically on MS medium and transferred it to root induction medium containing NaCl (25 – 300 mM). After 8 days of culture the rooted seedlings were subjected to morphological, physiological, biochemical and cytological analysis. Rhizogenesis frequency declined and time required for root initiation increased at higher levels of salt stress in YaLF as compared to Rekordsmen. Root dry weight declined significantly at low salt stress (25 mM) in YaLF while Rekordsmen tolerated up to 150 mM salinity stress. Shoot fresh weight and shoot dry weight declined with increment in salinity however cv Rekordsmen showed better tolerance potential. Sootahar *et al.,* (2024) studied the early vegetative growth of tomato cultivars under salt stress. Seeds of 14 tomato cultivars were germinated in vitro on MS medium supplemented with 1.5 mg L^{-1} salinity stress. The seed germination of Yellow Milk increased, Pink Jade and Red Jade declined while that of other 11 cultivars remained uninhibited in presence of salinity stress as compared to control. The mean germination time was maximum for Yellow Milk (8.42 days) and minimum for Saint (5.20 days) in presence of salt stress. Maximum shoot length was observed in Saint while Pink cooperative 908 recorded minimum shoot length as compared to control. The cultivar Red Jade recorded highest root length while Yellow milk had lowest root length. Bogoutdinova *et al.,* (2024) examined the effect of sodium chloride salinity on growth of eight tomato genotypes Yurevskij, Paradigma, Byche Serdce, Astrakhanskij, Recordsmen, YaLF line, Belyij Naliv and Geya procured from different geographic regions using half MS medium supplemented with or without 150 mM NaCl. The 8 days old seedlings of cultivars Belyij Naliv, Geya, and YaLF line showed leaf chlorosis and registered lowering in seedling height while the other cultivars did not show any changes as compared to control. The cv. Astrakhanskij recorded maximum shoot (53.1%) and root biomass (72.4%) in presence of salt stress. The studies conducted on the effect of salt stress in tomato during early developmental stages substantiated that salinity significantly lowered the germination percentage and vegetative growth in tomato seedlings. The response towards salinity stress is largely cultivar or genotype dependent paving way for rapid selection of tolerant cultivars using in vitro methods.

Salinity and Biochemical Attributes

To determine the effect of in vitro salinity stress on biochemical characteristics of tomato numerous investigations were carried out which are being discussed here and presented in Table 1. In order to examine the biochemical changes under salt stress, Amini and Ehsanpour (2005) germinated seeds of two tomato cultivars Isfahani and Shirazy on water agar medium and further cultured seedlings on MS medium containing graded series of salt stress (40, 80, 120 and 160 mM) for 21 days. With increment in salinity stress the soluble protein content enhanced in the stem and leaf of Isfahani but declined in Shirazy. The protein content was higher in the roots of Shirazy as compared to Isfahani.

Proline content increased in the stem, leaf and roots as the concentration of salt stress increased in both the cultivars. Stem and leaf accumulated more proline compared to roots. The cultivar Shirazy maintained higher levels of proline. Total carbohydrates increased in the stem and leaf of Shirazy but declined in Isfahani, however carbohydrate content increased in the roots of both the cultivars under salinity stress. Reducing sugars declined in the root, stem and leaves, Na^+ increased and K^+ decreased in presence of salt stress. Amini and Ehsanpour (2006) further reported that chlorophyll content declined in cultivars Isfahani and Shirazy under salinity stress and acid phosphatase activity decreased in stem-leaf and increased in roots in cultivar Shirazy. Hassan *et al.,* (2008) compared the effect of salt stress on tomato under in vitro and in vivo conditions and observed that there was decline in Na^+/K^+ ratio in the in vitro plants but sodium levels increased for in vivo plants under influence of salt stress. Accumulation of proline was more pronounced for in vitro plants while anthocyanin content and enzyme activities of phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), chalcone isomerase (CI) were relatively less, demonstrating better tolerance following in vitro selection. Abu-Khadejeh *et al.,* (2011) reported that the concentration of Na^+, Cl^- and Ca^{+2} ions increased while that of K^+ declined under salinity (50, 100, 150 mM NaCl and $CaCl_2$ in equimolar ratio) in microshoot cultures of tomato genotypes JO112 and JO992. With increment in salt stress, proline content enhanced while cell sap osmotic potential and protein content declined in the microshoots. The genotype JO992 performed better towards salt stress as compared to JO112. Mohamed *et al.,* (2011) estimated the chlorophyll content from leaves of in vitro raised plantlets of two cultivars Beril and Pearl and observed that cultivar Beril was superior in chlorophyll a, b and total chlorophyl compared to Pearl in presence of salt stress. The shoots regenerated from cotyledon explants showed more chlorophyll content compared to hypocotyl explants in both the cultivars. Roy and Sengupta (2014) studied the effect of salt stress in tomato cultivars for short duration. Fifteen days old tomato seedlings of three cultivars Pusa Ruby, Punjab Keshari, Ailsa Craig were cultured in glass bottles containing 0.25x MS liquid basal medium supplemented with graded series of salt stress (50, 100, 150, 200 mM) for 24 days. The seedlings exhibited decline in root/shoot growth and chlorophyll content. Punjab Keshari was least affected wherein the leaves demonstrated gradual increment in chlorophyll content (20%). However, the chlorophyll content declined in Pusa Ruby and Ailsa Craig with increasing salt stress. The study demonstrated that short term exposure to salt stress can bring about changes at the biochemical level which can be helpful in screening tomato genotypes for better tolerance towards salinity. Srinieng *et al.,* (2015) studied the effect of salinity on antioxidant enzymes in tomato. Seeds of six tomato cultivars Pethlanna, Puangphaka, Beefeater, Seeda, Seeda Chompoo and TE VF 1-3-4 were cultured on MS medium supplemented with 5, 10, 25, 50, 100 mM salt stress. At 50 mM salt stress, Puangphaka, Seeda, TEVF 1-3-4 and Seeda Chompoo cultivars exhibited germination percentage of 80%, 50%, 30% and 5% respectively. Based on seed germination Puangphaka proved to be most tolerant. The in vitro maintained tomato seedlings (21 days old) of cultivar Puangphaka were analysed for antioxidant enzymes. The seedlings recorded an increase in enzyme activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in control, 5 mM, 50 mM and 100 mM salt stress while the activity of these enzymes declined at 10 and 25 mM stress.

The chlorophyll content increased in cultivar Puangphaka with plant age but declined with increasing concentration of sodium chloride. Seth and Kendurkar (2016) examined the biochemical changes in seedlings of five tomato cultivars Abhinav, Rohini, TO1385, N2535 and Naina in presence of in vitro salinity stress. Proline increased in the shoots by 15 folds in Abhinav at 100 mM NaCl and 2.6 folds in Naina at 60 mM salt stress. Increment in free amino acids and total proteins was more pronounced in shoots as compared to roots. Chlorophyll content declined with increasing stress levels however tolerant cultivars Abhinav and Rohini showed better chlorophyll stability. Zaki and Yoki (2016) studied the effect of salt stress on three tomato species (*S. lycopersicum, S. peruvianum* and *S. pimpinellifolium*) and observed that the accumulation of Na^+ and Cl^- ions increased in shoot tissues with increment in salinity stress.

Table.1 Impact of salinity stress on biochemical parameters in tomato in vitro.

Tomato Cultivar/ Species	Range of NaCl Stress	Biochemical Parameters					References
		Chlorophyll	**Protein**	**Proline**	**Ions**	**Enzyme activity & markers***	
Isfahani, Shirazy	40, 80, 120, 160 mM	<	Isfahani>stem, leaf; Shirazy> root	Shirazy >stem,leaf,roots	Na^+> K^+<	acid phosphatase <stem, leaf; >roots-Shirazy	Amini & Ehsanpour (2005, 2006)
Super Strain B	25, 50, 100, 150 mM			>	Na/K ratio<	PAL, TAL, CI<	Hassan et al., (2008)
JO112, JO992	50, 100, 150, 200 mM NaCl and $CaCl_2$ in equimolar ratios		<	>	Na^+, Cl^-, Ca^{+2}>, K^+<		Abu-Khadejeh et al., (2011)
Beril, Pearl	25, 50, 75 mM	<<Pearl <Beril					Mohamed et al.,(2011)
Pusa Ruby, Punjab Keshari, Ailsa Craig	50, 100, 150, 200 mM	Pusa Ruby, Ailsa Craig <<Punjab Keshari >					Roy & Sengupta (2014)
Puangphaka	5, 10, 25, 50, 100 mM	plant age> increment in NaCl<				SOD, CAT,GPx> 0, 5, 50, 100 mM;< 100, 25 mM	Srinieng et al., (2015)
Abhinav, Rohini, TO1385, N2535, Naina	40, 60, 80,100 mM	< Abhinav ++ Rohini ++	shoots>> roots>	> Abhinav>>			Seth & Kendurkar (2016)
S.lycopersicum*, *S.peruvianum*, *S.pimpinellifolium	100, 200, 300 m mol L^{-1}				Na^+, Cl^->> *S. peruvianum*; K^+< *S.lycopersicum*; Na^+/K^+ ratio <<*S. peruvianum*		Zaki & Yoki (2016)

Rams, C10	20, 40, 60, 80, 100 mM				Na, K, Ca < Ram; N > Ram; Na^+/K^+ratio > Ram Na^+/Ca^+<ratio Ram		Al-Daej (2018)
PS-10, Peto, Nora, Roma	25, 50, 75, 100 mM	< PS-10 ++		>	Na >; K, Ca<; Na^+/K^+ ratio <<PS-10	MDA, H_2O_2<<PS-10; CAT, SOD,APX, GPX, GR >PS-10; AsA, GSH <; DHA, GSSH >	Aazami et al., (2021)
YaLF line, Rekordsmen	75, 150 mM	Rekordsmen -No change YaLF< (75 mM)		Rekordsmen> YaLF< (75 mM)			Khaliluev et al., (2021)
Yurevskij, Paradigma, Byche Serdce, Astrakhanskij, Recordsmen, YaLF line, Belyij Naliv,Geya	150 mM	< Astrakhanskij >			Na^+ and Cl^- >Belyij Naliv, Geya, YaLF; K^+/Na^+ ratio >Paradigma, Bych'e Serdce, Recordmen, Astrakhanskij		Bogoutdinova et al., (2024)

< reduced, > increased, >> maximum increment, << maximum decline, ++ better stability

*phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), chalcone isomerase (CI),superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR), ascorbate (AsA), reduced glutathione (GSH), dehydroascorbate (DHA), oxidized glutathione (GSSG),malondialdehyde (MDA)

Amongst the three species, pronounced accumulation of Na^+ and Cl^- ions was evident in *S. peruvianum*. There was low build-up of K^+ ions in *S. lycopersicum* compared with other wild species. *S. peruvianum* exhibited lowest Na^+/K^+ ratio at high concentrations of salt stress and exhibited better tolerance potential towards salinity stress. Al-Daej (2018) on investigating the effect of salt stress reported the accumulation of minerals such as Na, K, Ca and N in both the cultivar's Rams and C10. At 100 mM salt stress the leaves of cultivar Ram showed less accumulation of Na, K, Ca ions as compared to cultivar C10.

The concentration of N was higher in cultivar Rams compared to cultivar C10. The cultivar Ram showed high Na^+/K^+ ratio and low Na^+/Ca^{+2} ratio compared to C10 and demonstrated better salt tolerance capacity. Aazami *et al.,* (2021) studied the biochemical changes in four tomato cultivars PS-10, Peto, Nora and Roma in presence of salinity stress (25 to 100 mM) and detected declining trend in chlorophyll and carotenoid content with increasing levels of salinity stress. Roma showed maximum decline in chlorophyll and carotenoid content while PS-10 maintained better chlorophyll and carotenoid stability as compared to other cultivars. The sodium content increased and potassium as well as calcium declined in all the cultivars with increasing salinity levels. At 100 mM salt stress Roma showed maximum while PS-10 recorded minimum Na^+/K^+ ratio. At 100 mM salt stress Roma exhibited highest while PS-10 showed lowest malondialdehyde (MDA) content, electrolyte leakage and hydrogen peroxide level. As the salinity stress increased, proline content also enhanced while total soluble protein declined in all the cultivars. The activity of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR) increased in PS-10 and declined in Roma. With increasing salt stress, the activity of ascorbate (AsA) and reduced glutathione (GSH) declined while that of dehydroascorbate (DHA) and oxidized glutathione (GSSG) enhanced. Khaliluev *et al.,* (2021) studied biochemical changes in two tomato genotypes YaLF line and Rekordsmen under salinity stress at early developmental stage. The study showed significant differences in the respiratory and photosynthetic CO_2 gas exchange. At 75 mM salt stress seedlings of YaLF showed a reduction in proline (1.4 folds) and chlorophyll a (1.9 folds) compared to control. However, in Rekordsmen, the proline content enhanced and chlorophyll a content did not change compared to control. The epidermal and mesophyll cotyledonary leaf cells, chloroplast and nuclear compartments in Rekordsmen were more tolerant to salinity stress compared to YaLF. Thus, shoot apex from in vitro germinated seedlings at early true leaf stage can sever as a tool for rapid bioassay of tomato genotypes. Bogoutdinova *et al.,* (2024) examined the biochemical characteristics in tomato genotypes at seedling stage for salt tolerance and demonstrated increased shoot and root water content in cultivars Astrakhanskij, Byche Serdce, Yurevskij and YaLF. Easily soluble salts showed maximum enhancement in cultivars Belyij Naliv, Geya, YaLF line, Yurevskij and Paradigma, The Na^+ and Cl^- ions increased in Belyij Naliv, Geya, YaLF line as compared to control.

The K^+/Na^+ ratio increased in tolerant cultivars Paradigma, Bych'e Serdce, Recordmen and Astrakhanskij indicating reduced decline of K^+ and less permeability of cell membranes. In Astrakhanskij, the chlorophyll a and b content increased by 1.6 and 2.1 folds but there was no significant change in the carotenoid content as compared to control. Chlorophyll declined in the sensitive cultivars. As compared to control, flavonoid content enhanced only in salt tolerant cultivars Bych'e Serdce and Astrakhanski by 1.1 and 1.3 folds respectively while it declined in the remaining cultivars. Phenolic compounds showed a declining trend amongst all the cultivars. On the basis of growth and biochemical parameters, the cultivars Belyij Naliv, Geya, YaLF, Paradigma were rated as salt sensitive and Records men, Yuryevskij, Bych'e Serdce, Astrakhanskij as tolerant. These studies suggest that tomato plants undergo several changes in biochemical attributes in presence of salt stress. Salinity results in the accumulation of Na^+ and Cl^- ions and prevents the uptake of K^+ and Ca^{2+} causing ionic imbalance within plant tissues (Arif *et al.,* 2020; Guo *et al.,* 2022). Tomato seedlings suffer from osmotic stress in saline environment and accumulate osmolytes which help in replacing water in biochemical reactions, maintain osmotic balance as well as preserve enzymatic functions (Parida and Das, 2005). Salt stress increases reactive oxygen species (ROS) and creates oxidative stress; therefore, tomato plants show increment in antioxidant enzymes to detoxify ROS (Gupta and Hung, 2014).

Further, there is decline in chlorophyll content under salt stress because the photosynthetic machinery gets hampered resulting in chlorophyll degradation (Arif *et al.,* 2020). In presence of in vitro salinity stress, the tomato cultivars exhibited genotypic variation in their biochemical attributes, which is helpful in understanding the mechanisms adopted by different cultivars for acclimatization in salt stressed environments.

In conclusion, continuous increment in soil salinization is preventing the utilization of farmland, reducing the agriculture productivity and threatening food security. Saline soils have negative impact on tomato as it moderately sensitive to salinity and the production begins to decline beyond 2.5 dS m^{-1}. The early development phases such as seed germination and seedling growth are more vulnerable to salt stress.

The impact of in vitro salinity stress on tomato resulted in increment in germination period and reduction in germination percentage due to salt induced osmotic stress. There was decline in seedling length, fresh and dry weight impacting the overall growth and well-being of tomato seedlings under salt stress. The Na^+ and Cl^- ions increased while K^+ ions declined in presence of salt stress in tomato seedlings. There was increment in free amino acids, protein, proline while chlorophyll content declined in tomato genotypes in response to salt stress. The activity of antioxidant enzymes increased for detoxification of reactive oxygen in tomato cultivars. The response of tomato towards in vitro salinity stress is genotype specific being helpful in rapid selection of tolerant cultivars for planting in salt stressed environment as well as inclusion in plant breeding studies. Furthermore, the genotypic variation is beneficial in understanding the physiological and biochemical mechanisms operating in tolerant cultivars for successful adaptation in saline ecosystems.

Acknowledgements

The author is thankful to The Principal, Fergusson College (Autonomous), Pune for providing the necessary infrastructure, laboratory facilities and continuous support during the research work.

Author Contributions

Dr. Rupali Seth: Conceptualization, Formal Analysis, Investigation, Writing - Original Draft

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Aazami, M.A., Rasouli, F. and Ebrahimzadeh, A. Oxidative damage, antioxidant mechanism and gene expression in tomato responding to salinity stress under *in vitro* conditions and application of iron and zinc oxide nanoparticles on callus induction and plant regeneration. BMC Plant Biology, 2021, 21: 597. https://doi.org/10.1186/s12870-021-03379-7

Abu-Khadejeh, A., Makhadmeh, I., Shibli, R. A., and Mohammad, M. J. Physiological responses of tomato microshoot cultures to in vitro induced salinity stress. Jordan Journal of Agricultural Sciences, 2011, 7(2): 260- 272.

AI-Tardeh, S. and Iraki, N. Morphological and anatomical responses of two Palestinian tomato (*Solanum lycopersicon* L.) cultivars to salinity during seed germination and early growth stages. African Journal of Biotechnology, 2013, 12(30): 4788-4797. https://doi.org/10.5897/AJB12.2707

Al-Daej, M. I. Salt tolerance of some tomato (*Solanum lycopersicum* L.) cultivars for salinity under controlled conditions. American Journal of Plant Physiology, 2018, 13: 58-64. https://doi.org/10.3923/ajpp.2018.58.64

Ali, M. Y., Sina, A. A. I., Khandker, S. S., Neesa, L., Tanvier, E.M., Kabir, A., Khalil, M. I. and Gan, S. H. Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A review. Foods, 2021, 10, 45. https://dx.doi.org/10.3390/foods10010045

Amini, F. and Ehsanpour, A. A. Response of tomato (*Lycopercicon esculentum* Mill.) cultivars to MS, water agar and salt stress in *in vitro* culture. Pakistan Journal of Biological Science, 2006, 9 (1): 170-175. https://doi.org/10.3923/pjbs.2006.170.175

Amini, F. and Ehsanpour, A.A. Soluble proteins, proline, carbohydrates and Na+/K+ changes in two tomato (*Lycopercicon esculentum* Mill.) cultivars under in vitro salt stress. American Journal of Biochemistry and Biotechnology, 2005,1(4): 212-216. https://doi.org/10.3844/ajbbsp.2005.204.208

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A. and Hayat, S. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiology and Biochemistry, 2020, 156, 64-77. https://doi.org/10.1016/j.plaphy.2020.08.042

Basha, P. O., Reddy, M. S. M., Riazunnisa, K. and Reddy, M. S. In vitro evaluation of tomato genotypes for salt tolerance at seedling stage, International Journal of Plant, Animal and Environmental Sciences, 2015, 5(1): 102-106.

Bogoutdinova, L. R., Khaliluev, M. R., Chaban, I. A., Gulevich, A. A., Shelelpova, O.V. and Baranova, E. N. Salt tolerance assessment of different tomato varieties at the seedling stage. Horticulturae, 2024, 10, 598. https://doi.org/10.3390/horticulturae10060598

Collins, E. J., Bowyer, C., Tsouza, A. and Chopra, M. Tomatoes: An extensive review of the associated health impacts of tomatoes and factors that can affect their cultivation. Biology, 2022, 11:239. https://doi.org/10.3390/biology11020239

Cuartero, J. and Fernandez-Munoz, R. Tomato and salinity. Scientia Horticulture, 1999, 78 (1-4): 83-125. https://doi.org/10.1016/S0304-4238(98)00191-5

Flores, S. S., Cordovez, V., Oyserman, B., Stopnisek, N., Raaijmakers, J. M. and van't Hof, P. The tomato's tale: Exploring taxonomy, biogeography, domestication, and microbiome for enhanced resilience. Phytobiomes Journal, 2024, 8: 5-20. https://doi.org/10.1094/PBIOMES-09-23-0091-MF

Food and Agriculture Organization of the United Nations Statistics Division FAOSTAT, 2023. https://www.fao.org/faostat/en/#data

Food and Agriculture Organization of the United Nations, https://www.fao.org/global-soil-partnership/resources/highlights/detail/en/c/1412475/

Foolad, M. R. Genome Mapping and Molecular Breeding of Tomato. International Journal of Plant Genomics, 2007: Article ID 64358, 52 pages, Hindawi Publishing Corporation. https://doi.org/10.1155/2007/64358

Foolad, M. R.Recent advances in genetics of salt tolerance in tomato. Plant Cell Tissue Organ Culture, 2004, 76:101-119. https://doi.org/10.1023/B:TICU.0000007308.47608.88

Guo, M., Wang, X. S., Guo, H. D., Bai, S. Y., Khan, A., Wang, X. M., Gao, Y. M. and Li, J. S. Tomato salt tolerance mechanisms and their potential applications for fighting salinity: A review. Frontiers in Plant Science, 13: 949541. https://doi.org/10.3389/fpls.2022.949541

Gupta, B. and Hung, B. Mechanism of salinity tolerance in plants: physiological, biochemical and molecular characterization. International Journal of Genomics, 2014, Article ID 701596, 18 pages, Hindawi Publishing Corporation. https://doi.org/10.1155/2014/701596

Hassan, N. M., Serag, M. S., El-Feky, F. M. and Nemat Alla, M. M. In vitro selection of mung bean and tomato for improving tolerance to NaCl. Annals of Applied Biology, 2008, 152: 319-330. https://doi.org/10.1111/j.1744-7348.2008.00221.x

Khaliluev, M. R., Bogoutdinova, L. R., Ralugina, G. N. and Baranova, E. N.A simple and effective bioassay method suitable to comparative in vitro study of tomato salt tolerance at early development stages. Methods and Protocols, 2021, 5, 11. https://doi.org/10.3390/mps5010011

Kochhar S.L. (ed): *Economic Botany in the Tropics*, 3rd ed. MacMillian Publishers India Ltd. 2009; pp. 265-267.

Mohamed, A. N., Ismail, M. R., Kadir, M. A. and Saud, H. M. In vitro performances of hypocotyl and cotyledon explants of tomato cultivars under sodium chloride stress. African Journal of Biotechnology, 2011, 10 (44): 8757-8764. https://doi.org/10.5897/AJB10.2222

Muhammad, M., Waheed, A., Wahab, A., Majeed, M., Muhammad, N., Liu, Y. H., Li, L. and Li, W. J. Soil salinity and drought tolerance: An evaluation of plant growth, productivity, microbial diversity, and amelioration strategies. Plant Stress, 2024, 11: 100319. https://doi.org/10.1016/j.stress.2023.100319

Murashige, T. and Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 1962, 15 (3): 473 - 497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Osman, M. G., Elhadi, E. A. and Khalafalla, M. M. *In vitro* screening of some tomato commercial cultivars for salinity tolerant. International Journal of Biotechnology and Biochemistry, 2011, 7(5): 543-552.

Parida, A. K. and Das, A. B. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and

Environmental Safety, 2005, 60(3): 324-349. https://doi.org/10.1016/j.ecoenv.2004.06.010

Rashed, M. D. U., Roy, M. R., Paul, S. K. and Haque, M. M. In vitro screening of salt tolerant genotypes in tomato (*Solanum lycopersicum* L.). Journal of Horticulture, 2016, 3: 186. https://doi:10.4172/2376-0354.1000186.

Rodriguez-ortegal, W. M., Martinez, V., Nieves, M., Simon, I., Lidon, V., Fernandez-Zapata, J. C., Martinez-Nicolas, J. I., Camara-Zapata, J. M. and Garcia-Sanchez, F. Agricultural and physiological responses of tomato plants grown in different soilless culture systems with saline water under greenhouse conditions. Scientific Reports, 2019, 9: 6733. https://doi.org/10.1038/s41598-019-42805-7

Rosca, M., Mihalache, G. and Stoleru, V. (2023). Tomato responses to salinity stress: from morphological traits to genetic changes. Frontiers in Plant Science, 14: 1118383. https://doi.org/10.3389/fpls.2023.1118383

Roy, C. and Sengupta, D. N. Effect of short term NaCl stress on cultivars of *S.lycopersicum*: A comparative biochemical approach. Journal of Stress Physiology and Biochemistry, 2014, 10 (1): 59-81.

Sane, A. K., Diallo, B., Kane, A., Sagna, M., Sane, D. and Sy, M. O. In vitro germination and early vegetative growth of five tomato (*Solanum lycopersicum* L.) varieties under salt stress conditions. American Journal of Plant Sciences, 2021, 12:796-817. https://doi.org/10.4236/AJPS.2021.125055

Seth, R. and Kendurkar, S. V. Biochemical changes in commercial cultivars of tomto under in vitro salinity stress. Advances in Plant Sciences, 2016, 29(1):119-122.

Seth, R. and Kendurkar, S. V. In vitro screening: An effective method for evaluation of commercial cultivars of tomato towards salinity stress. International Journal of Current Microbiology and Applied Science, 2015, 4: 725-730.

Sholi, N. J. Y. Effect of salt stress on seed germination, plant growth, photosynthesis and ion accumulation of four tomato cultivars. American Journal of Plant Physiology, 2012, 7(6): 269-275. https://doi.org/10.3923/ajpp.2012.269.275

Sootahar, R.K., Sootahar, S.L., Lin, M., Rais, N., Jamro, G.M., Rais, M., Iqbal, R., Ditta, A., Eladin, S.M., Ali, I., Alwahibi, M. S., Elshikh, M.S. and Kumarasamy, V. In vitro early vegetative growth of tomato (*Solanum lycopersicum* L.) cultivars under salt stress. Polish Journal of Environmental Studies, 2024, 33(5): 5879-5885. https://doi.org/10.15244/pjoes/183451

Srinieng, K., Saisavoey, T. and Karnchanatat, A. Effect of salinity stress on antioxidative enzyme activities in tomato cultured in vitro. Pakistan Journal of Botany, 2015, 47 (1) :1-10.

Sultana, N., Sultana, I. and Sultana, S. Effect of salinity on in vitro performance of tomato (*Lycopersicon esculentum* L.), Bangladesh Journal Environment Science, 2019, 36: 43-46.

Ullah, A., Bano, A. and Khan, N. Climate change and salinity effects on crops and chemical communication between plants and plant growth microorganisms under stress. Frontiers in Sustainable Food Systems, 2021, 5: 618092 2021. https://doi.org/10.3389/fsufs.2021.618092

Wang, W., Vinocur, B. and Altman, A. Plant responses to drought, salinity and extreme temperature: towards genetic engineering for stress tolerance. Planta, 2003, 218:1-14. https://doi.org/10.1007/s00425-003-1105-5

Yokas, I., Tuna, L. A, Burun, B., Altunlu, H., Altan, F. and Kaya, C. Responses of the Tomato (*Lycopersicon esculentum* Mill.) Plant to Exposure to Different Salt Forms and Rates. Turkish Journal of Agriculture Forestry, 2008, 32: 319-329.

Zaki, H. E. M. and Yokoi, S. A comparative *in vitro* study of salt tolerance in cultivated tomato and related wild species. Plant Biotechnology, 2016, 33: 361-372. https://doi.org/10.5511/plantbiotechnology.16.1006a

How to cite this article:

Rupali Seth. 2024. An Overview on Impact of Salinity Stress in Tomato under *invitro* Conditions. *Int.J.Curr.Microbiol.App.Sci.* 13(11): 191-201. **doi:** https://doi.org/10.20546/ijcmas.2024.1311.023