Effect of NaCl treatment in *Solanum lycopersicum* L. cultivars from Jharkhand, India

A P Sahu\(^a\), S Kumaria\(^a\), R K Jha\(^a\), R Verma\(^a\), S K Jha\(^a\) & V K Yadav\(^a\)

University Department of Botany, Ranchi University, Ranchi Jharkhand 834001, India

Received 26 August 2021; Revised & accepted 30 September 2021

Tomato (*Solanum lycopersicum*) L. is an economically valuable vegetable. For the agriculture sector, many farmers grow this vegetable, marginal land with different concentrations of salt and pH end up in below average production. Therefore, the effect of salinity on secondary metabolite and protein expression with different levels of salt (NaCl) treatment in tomato varieties were determined. Two varieties were taken, namely, TM-7328 and NP-5005. These two plants were treated against four different concentrations, i.e. 50, 100, 150 and 200 mM of NaCl and distilled water as a control. For secondary metabolite estimation, plant varieties were grown in a pot with irrigated with NaCl of different concentration. After 25 days of treatment of NaCl and control plants, the phytochemical and protein analysis were done. Further, we used the MALDI-TOF mass spectrometry to determine the different molecular bands. The protein expression analysis after MALDI-TOF analysis showed that the purity of protein expression in two varieties. Such efficient analysis determined that the best variety for cultivation under stress condition.

Keywords: Tomato, Secondary metabolite, Variety, NaCl

**Introduction**

Tomatoes are an economically valuable vegetable in India. The nutrient benefit of tomato is very strong because of the different vitamins and minerals and bioactive compounds. Lycopene is one of the most important precursors in the growth of vitamin A biosynthesis because it is the most critical precursor molecules in producing bioactive vitamin A. Salinity has a severe effect on the development of plants like tomato. It's also a standard procedure during plant development to expose the different abiotic environments to determine their impact on the plant's growth and output. Salinity is a significant abiotic element that is incredibly detrimental to plant development and production. Salinity influences plant growth and development by adversely influencing cellular homeostasis, physiology, metabolism and biochemistry. Osmotic stress is the primary outcome of a highly salinated plant. When present, this condition induces stomatal closure, decreases leaf area and suppresses photosynthesis and growth. Due to salinity stress, plants absorb significant sodium ions (Na\(^+\)) and release large amounts of potassium ions (K\(^+\)). As a consequence, plants are prone to ionic stress. Mature leaf chlorosis and necrosis and premature senescence develop due to enzyme production and protein synthesis being impaired. Plants alter cellular metabolism and defence processes in reaction to salinity. The oxidative stress-induced chemical imbalance results in cellular organelles, such as lipids, nucleic acids and proteins, damaged, which induces impaired cellular metabolism and disrupted membrane functions.

This facilitates the production of lipid peroxidation, which may lead to cell death due to apoptosis. Therefore, it is crucial to ensure the correct execution of their signaling roles to prevent injurious reactive oxygen species (ROS) effects. Plants have established various defense mechanisms and signaling behavior to regulate both the development and removal of ROS to avoid oxidative damage. There is a lot of value to learning how plant life interacts with salt. Thus, large regions of salty soils are produced. It's likely that the two genetic groupings that have a role in salinity adaptation may be those that solely ward off fear and those that regulate gene expression under saline conditions. The analysis of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry revealed that the gene's expression varied between two varieties and this study showed that the varieties that are tolerant and susceptible to varying concentrations of salt. The enzyme expressed in the plant help to survive in an adverse condition either

---

\(^a\)Author for correspondence: vinodbhu89@gmail.com
over expressed or less represented in different salt concentration. So, it is beneficial to understand between two variety of tomato and which is better for farming for the farmer.

**Material and Methods**

**Plant Material**

Two variety of tomato were selected. The first variety is TM7328 and another is NP5005, these two varieties are also famous for their fruit types collected from Birsa Agriculture College (BAU), Ranchi, Jharkhand.

**Extraction of Plant Material for Phytochemical Analysis**

After thirty-seven days of the plant in pot condition, the leaf was collected of control as well as four different concentration of salt for phytochemical extraction. The shadow dried grow powdered was blend with methanol (1:10 ratio). The shadow dried grow powdered was blend with methanol (1:10 ratio)\(^5\). For phytochemical screening, the aqueous extract was further used to determine the compositions of constituents, viz. phenol, flavonoid, tannin, and alkaloid\(^6\)\(^7\). A preliminary phytochemical screening was carried out as per the conventional methods described by Harborne and Evans\(^8\)\(^9\).

**Estimation of Total Phenolic Content (TPC)**

Total phenolic content in the methanolic extract was determined with Folin-Ciocalteau reagent using the method of Chen \(^10\).

**Determination of Total Flavonoid Content**

One mL aliquots of quercetin (1, 10, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600 μg/mL) was taken into 10 mL volumetric flask and absorbance was measured at 510 nm using a UV-visible spectrometer following other steps as defined by Sahu & Saxena\(^11\).

**Determination of Total Tannin and Alkaloid Content and Protein Separation**

Tannins were quantified with the help of the Folin-Ciocalteu method. Briefly, in 0.1 g of dry plant sample, 50 mL of water was applied, the water was heated for 30 minutes and the sample was purified. When dissolved, K3[Fe (CN)\(_6\)] solution is pure, colorless and has no discernible odor. The total alkaloid precipitate was calculated in mg/g of the dried plant material based on the procedure defined John \(et\ al\)^12. The separation of proteins polyacrylamide gel in the presence of SDS-PAGE was used\(^14\).

**MALDI Analysis and Protein Database Identification**

Plant extracts were evaluated using Maldi Reflector Analyzer TOF 4800. For MSMS and MS calibration, a 6-peptide ABSciex mixture was used. The 3500 Explorer system automatically acquired MS specific accompanied by MS/MS performance. In MS reflector mode, 20 kV acceleration voltage was used with 8 kV MSMS. The software provides a ParagonTM archive search engine and includes the data-compiling algorithm ProGroupTM. The least acceptance requirement was 99% confidence. Proteins were measured using at least 4 protein peptides, demonstrating ProGroup's algorithm. Proteins dependent on ITAG2.3 database queries at http://solgenomics.net/organism/solanum lycopersicum/genome is measured. The outcome for the National Centre for Biological Information (NCBI) website was tested and is rich in protein.

**Phytochemical Estimation of Secondary Metabolite**

Quantitative estimation of some secondary metabolites like, phenol and tannins were performed by comparing with a standard curve of gallic acid is present in Figure 1 and for flavonoid estimation, standard curve, quercetin is present in Figure 2.

**Results and Discussion**

The total flavonoid was calculated from the regression equation of the standard plot \(y = 0.000 x - 0.002, R^2 = 0.993\) and is expressed as quercetin equivalents (QE, Fig. 2). Total flavonoid recorded in two varieties in different stress condition was shown in Figure 3. To compare total flavonoid content in two varieties, the higher amount was found in variety NP-500 (\(y = 0.008x + 0.018, R^2 = 0.992,\) Figure 1). The values obtained for the concentration of tannin was

---

Fig. 1 — Calibration curve of standard gallic acid for determination of total phenolic and tannin content in *Solanum lycopersicum*
found that the variety NP-5005 have higher amount tannin as shown in Figure 4.). It was also observed that the amount is maximum in 150 mM and 200 mM NaCl irrigated plant. The total phenolic and alkaloids content in two varieties shown (Fig. 5 & 6) in different NaCl condition and it was found that total phenolic content was maximum in TM-7328 and total alkaloids contents was more in NP-5005.

**Protein Profiling and Identification**

Gel analysis proved a minimum of 4 bands differing in expression degree between the 2 tomato varieties at exactly the four different concentrations, i.e. 50, 100, 150 and 200 mM of NaCl and distilled water as a control. These proteins’ expression amount usually increased in TM 7328 and was also constant or even decreased in the salt-tolerant NP 5005. These rings, labelled A, B, C and D, separated at 65, 42, 25 and 20 kDa, respectively. The proteins in these 4 bands have been diagnosed by MALDI TOF mass...
spectrometry. Each differentially expressed band offered 2 - 3 hits when examined utilizing MALDI TOF LC/MS/MS spectrometry (Table 1 and Fig. 8 & 9). B and A includes a large ribulose-1, 5-bisphosphate, a high-protein structural chromosome (SMC) and a theory protein, carboxylase/oxygenase subunit. In addition to a myosin-like heavy chain-like protein, B and B produces a hypothetical protein-rich, a basic helix loop helix (bHLH) domain-containing protein. B and C has uncharacterized proteins, but everyone is locating the plasma membrane. B and D contained a 40S ribosomal-rich S28 protein and a repeat-containing hypothetical protein pentatricopeptide.

The criterion to calculate the salinity impact at various NaCl concentrations\(^{15}\) is the secondary
The flavonoid estimate result indicates that the material was similar in the control and assay, validated with the in the control state. In contrast with TM-7328, the flavonoid content of NP-5005 is improved by the higher NaCl concentration. The tannin level was minimal and the 50 mM NaCl treatment was somewhat different in both sets, but the NP-5005 levels were substantially increasing for 100 mM and 200 mM NaCl. Similarly, the alkaloid content is large at NaCl concentrations at both 150 and 200 mM. In TM-7328, the phenol content was functional and even the NaCl concentration was between 50 and 200 mM. Salt stress is a constant functional and even the NaCl concentration was differed in the reaction to salinity stress of the tomato variety in the aggregation of soluble protein. This work was mainly conducted to compare two tomato cultivars' output with soluble protein accumulations, which vary in salt stress tolerances.

Rubisco degrades under stress through ATP-dependent ubiquitisation and is typically present in ribosomal protein vesicles as fragments rather than as intact proteins. Three proteins have been found in band C that has no specific molecular role. This has not been extensively investigated, yet. This 28 S ribosomal subunit was identified in-band D. Marker-assisted selection (MAS) is regularly applied through morphological, biochemical or DNA markers as oblique range conditions for choosing agriculturally vital features in crop breeding. This method is utilized to increase the performance of desire in breeding programs. Using MAS for manipulating simple/qualitative elements is easy and it has been perfectly claimed. Having said that, MAS with the advancement of complex/polygenic features, together with plant tolerance/resistance to abiotic stresses, is a lot more sophisticated, whilst its usefulness has long been regarded. While using the latest innovations in marker technological know-how, together with high throughput genotyping of crops, along with the event of nested affiliation mapping populations, it is envisioned which the utility of MAS for breeding for worry tolerance features will maximize.

**Conclusion**

The tomato plant is widely used in all over the world as vegetable and other purposes. In this study, we studied the effect of different stress condition like NaCl on two varieties of tomato which was popularly cultivated in Jharkhand areas. The method discussed in this paper provides insights about the impact of salt on these cultivars. India has large areas of sodic soils and before farming it is important to know which variety show the highest level of stress tolerant. Indian farmers in general and seed developing company in particular may find these varieties suitable to grow in salty soil. It is also finding in study the number of flowers; fruiting time and yield of fruit are highly affected by increasing the salt level. It may be realized the secondary metabolite production in various focus has a completely different amount of phenol, alkaloid, tannin are existing. That's, by an increased salt focus, these indices are quickly reduced.

**Acknowledgement**

The corresponding authors extend their appreciation to the Head of University Department of Botany, Ranchi University Ranchi, Jharkhand, India.

**References**


