International Journal of Plant & Soil Science

Volume 37, Issue 7, Page 322-335, 2025; Article no.IJPSS.139132
ISSN: 2320-7035

Evaluation of Transgenic Pigeon pea [*Cajanus cajan* (L.) Millsp. cv. Manak] Plants Over-Expressing *OsLecRLK* Gene for Salt Stress Tolerance Through Physio-Biochemical Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2025/v37i75577

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/139132

Original Research Article

Received: 02/05/2025
Accepted: 03/07/2025
Published: 10/07/2025

ABSTRACT

Aim: Abiotic stresses especially salinity and drought affect the yield of crops worldwide. The yield achieved on farmer fields and the potential yield differ significantly. Reduced agricultural yields are the result of salt stress's effects on important processes like photosynthesis, protein and lipid metabolism, and more.
Study Design: CRD (Completely Randomized Design) and three replications for each parameter.

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Cite as: *Pratibha, Pushpa Kharb, and Nirmal Singh. 2025. "Evaluation of Transgenic Pigeon Pea [Cajanus Cajan (L.) Millsp. Cv. Manak] Plants Over-Expressing OsLecRLK Gene for Salt Stress Tolerance Through Physio-Biochemical Analysis". International Journal of Plant & Soil Science 37 (7):322-35. https://doi.org/10.9734/ijpss/2025/v37i75577.*

Place and Duration of Study: The studies were carried out in the Department of Molecular Biology, Biotechnology and Bioinformatics CCS HAU, Hisar, Haryana.
Methodology: In the present research, transgenic pigeon pea (cv. Manak) plants were evaluated for the efficacy of *OsLecRLK* gene in salt stress tolerance. Physio-biochemical analysis was done to assess the efficacy of transgene via subjecting wild type and selected T_1 transgenic plants to 75 mM salt stress. Treatments, genotypes and interaction between treatments and genotypes were compared using critical difference (CD) at 5% level of significance with OPSTAT programme.
Results: The transgenic line outperformed the wild type plants in terms of sustaining higher levels of proline, total soluble sugar, chlorophyll, relative water, peroxidase, and catalase. Additionally, transgenic lines showed a considerably lower MDA level and membrane injury index than wild type plants, suggesting that they were less susceptible to salt stress.
Conclusion: L-19 outscored all other transgenic lines in every parameter examined, and it can be used to create transgenic pigeon pea plants that can withstand salt stress. Transgenic lines carrying single copy of gene performed better in comparison to lines carrying two copies of gene followed by wild type.

Keywords: Pigeon pea; transgenic; OsLecRLK gene; salinity stress; physio-biochemical; kinase; peroxydase.

1. INTRODUCTION

Pigeon pea (*Cajanus cajan* [L.] Millsp.) is an important perennial diploid legume crop (2n=2x=22). It belongs to the family Fabaceae and is a member of the tribe Phaseoleae. It is a major pulse crop of the semi-arid tropics that is traditionally cultivated as an annual crop in Africa, Asia, and Australia (Sairam *et al*., 2009). India is the primary centre of origin and diversification for pigeon pea. The crop was domesticated in India 3500 years ago (Kassa *et al*., 2012). It is the sixth most important legume crop cultivated globally in an area of 5 million hectares. India is the world's top producer of pigeon peas, with 4.34 million tons produced on 5.05 million hectares of land and an 859 kg/ha productivity (DES, MoAF&W, 2022). With 674.44 million kg of imports, India holds the top spot (92.65%) of all pigeon pea imports worldwide in 2021. The growth of the pigeon pea is salt sensitive. Excess salt promotes physiological drought in the pigeon pea, and prolonged and excessive exposure is fatal. One such approach is to genetically modify the pigeon pea plant to make it salt resistant. While pigeon pea genetic change is meticulous, tissue culture regeneration proves to be difficult. In the current study the functionality of *OsLec-RLK* in transgenic pigeon pea has been demonstrated using a tissue culture-independent plant transformation technique (https://www.tridge.com/intelligences/pigeon-pea/production).

Abiotic and biotic stresses affect the yield of crops worldwide. There is a large gap between potential yield (2500 kg/ha) and yield obtained on farmer fields (736.2 kg/ha in Africa and 866.2 kg/ha in Asia) due to the biotic and abiotic stresses (Mula & Saxena, 2010). Salt stress affects major processes including photosynthesis, protein and lipid metabolism, and more, resulting in reduced agricultural yields (Rasool *et al*., 2013). Reduced CO_2 assimilation primarily due to stomatal closure, membrane damage, reduced activity of key enzymes of necessary cellular mechanisms such as CO_2 fixation, ATP synthesis and increased ROS due to increased metabolite flux through the photo-respiratory pathway are some of the major effects of salinity on plants. For proper growth and development an optimum concentration of salt is required in soil, but when it is in excess (especially NaCl), becomes toxic to the plants and physiological drought condition can occur. Under high salt concentrations, there is increase in sodium absorption in cells wherein the potassium (K^+) to sodium (Na^+) ratio gets disturbed. It leads to degradation of essential enzymes and ultimately cell death (Horie *et al*., 2009; Adams & Shin, 2014). Increased sodium uptake also disturbs the calcium (Ca^{2+}) uptake as well as disturbance in the osmotic potential, thus leading to the change in water potential of the cells (Kumar *et al*., 2025; Kumar *et al*., 2023). SOS (salt overly sensitive) pathway is a well-known salinity response pathway in plants. It plays an important role in cellular Na^+ detoxification by effluxing the excess Na^+ from root epidermal cells to the external environment (Liu *et al*. 2000; Shi and Zhu, 2002). Gene expression machinery also affected by salt stress and this leads to the expression of some genes which are induced by salt stress and helps in providing tolerance to the plants during unfavourable conditions.

Plants respond to salt stress on a cellular and organism-wide level. Reduced leaf size altered plant water relations, and lower water-use efficiency are some of the physiological and biochemical effects that might be detected. Plants adapt several mechanisms to cope with salinity stress, including the intensification of compatible osmolyte molecules (eg. proline), increased water uptake through water channels in membranes, enhanced root architecture, modified leaves and stems, and stomatal closure to mitigate water loss through transpiration. Keeping in mind the above said point, the present study was demonstrated to evaluate the efficacy of transgenic pigeon pea plants were developed through *in-planta* transformation method (for which patent has been granted) and to carry out physio-biochemical analysis of selected transgenic plants for abiotic stress tolerance.

2. MATERIALS AND METHODS

2.1 Plant Material

Pigeon pea (cv. Manak) was used for genetic transformation. Seeds of this variety were procured from Pulses Section, Department of Genetics & Plant Breeding, CCS HAU, Hisar.

2.2 *OsLec-RLK* gene, plasmid and Agrobacterium tumefaciens strain

Agrobacterium tumefaciens strain LBA4404 harbouring pCAMBIA 1301-*OsLecRLK* gene was procured from Dr. Narender K. Tuteja, ICGEB, New Delhi.

2.3 Development and Characterization of Transgenic Plants

The genetically transformed pigeon pea plants (cv. Manak) carrying *OsLec-RLK* transgene were developed by using the *in-planta* transformation protocol developed by Kharb *et al*. (Patent Application No 201811012099) the putative plants from T_0 were screened by using gene specific primers, and PCR amplicon of size 2.1 kb, assure the presence of transgene. The PCR positive plants were selfed and their seeds were harvested for generating T_1.

2.4 Selection of Lines for T_1 Generation Based on Seed Weight (g/plant)

Seed weight of the wild type and 16 T_0 pigeon pea lines was recorded and analysed statistically. The OPSTAT tool was used to compare the seed yield (g/plant) of several lines using critical difference (CD) at the 5% level of significance.

2.5 Screening of Transgenic Plants T_1 Generation

T_1 generation transgenic plants were screened as the T_0 lines by PCR-based amplification of genomic DNA using gene-specific primers.

2.6 Evaluation of Salt Stress Tolerance in Transgenic Plants

In order to assess the effectiveness of the *OsLec-RLK* transgene in promoting salt tolerance, 15 days following germination, wild type (WT) and transgenic (T) pigeon pea lines chosen from the T_1 generation were exposed to a 75 mM NaCl shock. Numerous physio-biochemical parameters were examined 4th and 8th days following treatment with salt stress. Four different treatments were given (in triplicate). T_1: Wild type (WT) without salt stress (control condition), T_2: WT with 75 mM NaCl stress, T_3: Transgenic without salt stress (control condition) and T_4: Transgenic with 75 mM NaCl stress. For each parameter three replications were used. At the fourth and eighth days following the salt treatment, fresh, healthy leaves were collected.

2.7 Observations Recorded

2.7.1 Relative water content (RWC)

Relative water content was analysed using the procedure given in Smart & Bingham (1974). RWC% was calculated by the formula:

$$RWC\ (\%) = \frac{(Fresh\ weight - \ Dry\ weight)}{(Turgid\ weight\ -\ Dry\ weight)} \times 100$$

2.7.2 Electrolyte leakage or membrane injury index (MII)

Membrane injury was analysed using the method given in Sullivan *et al*., (1979).

$$Electrolyte\ leakage\ (\%) = \frac{\mathrm{ECa}}{\mathrm{ECb}} \times 100$$

where, ECa and ECb are electric conductivity after and before boiling, respectively.

2.7.3 Malondialdehyde (MDA) content

The quantity of MDA, a byproduct of lipid peroxidation, was evaluated and calculated by detecting the amount of thiobarbituric acid reactive substances (TBARS) in the reaction. The amount of MDA was measured by minor modifications in the method given by Heath & Packer (1968).

2.7.4 Chlorophyll content

Leaf discs weighing 0.03 g were cleaned, blotted dry, and then dipped in test tubes with 3 ml of dimethyl sulfoxide (DMSO) throughout the whole night as explained by Sawhney and Singh (2002). The absorbance of the extracted chlorophyll in DMSO was measured at 663 nm (A663) and 645 nm (A645), respectively, and its concentration was computed using the following formula:

$$Total\ chlorophyll\ (mg/g)\ tissue\ = \frac{20.2\ A645\ +\ 8.02\ A663}{1000\ \times W} \times V$$

Where, V - Volume of DMSO, A - Absorbance at specific wavelengths, W - Weight of tissue taken.

2.7.5 Proline content

The technique described by Bates *et al*. (1973) was used to assess the proline concentration of cell free extract.

2.7.6 Total soluble sugar content (TSS)

The technique described by Yemm & Willis (1954) utilizing the anthrone reagent was used for estimating the total soluble sugar content.

2.7.7 Catalase activity

The process outlined by Aebi (1984) was used to estimate the catalase activity. The final volume of the reaction mixture was 3 ml, and it comprised 100 µl of cell free extract, 0.1 mM H_2O_2, and 0.1 M phosphate buffer (pH 7.0). The reaction was started with the addition of H_2O_2 and enzyme activity was assessed by monitoring the degradation of H_2O_2 at 240 nm for two minutes. The extinction coefficient value of 39.4 mM^{-1} cm^{-1} for H_2O_2 was used to compute the enzyme activity. One mMol of H_2O_2 consumed per minute during the reaction corresponded to one unit of enzyme activity.

2.7.8 Peroxidase activity

Peroxidase activity was estimated by following the method given by Siegel (1993). Three ml of reaction mixture contained 0.1 M phosphate buffer (pH 7.0), 0.1 mM guaiacol, 0.1 mM H_2O_2 and 100 µl cell free extract. Reaction was started with the addition of H_2O_2 and increase in absorbance at 470 nm was recorded for 2 min. The activity was calculated using the extinction coefficient value of 26.6 mM^{-1} cm^{-1} for guaiacol.

2.8 Statistical Analysis

The pigeon pea plants' physio-biochemical data was analysed utilizing a two factorial CRD (Completely Randomized Design) test. The OPSTAT tool was used to evaluate treatments, genotypes, and interactions between treatments and genotypes using critical difference (CD) at a 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Raising of T_1 Generation

Seed yield of all the sixteen T_0 transgenic and non-transgenic plants was recorded. The average seed yield in non-transgenic plants was 4.04 g/plant. In case of transgenic plants, the seed yield ranged between 2.26g - 8.30g/plant (Table 1). A total of six transgenic lines L-9 (4.89 g); L-17 (5.76 g); L-18 (8.30 g); L-19 (6.43 g); L-48 (7.34 g) and L-89 (5.60 g) were selected out of 16 T_0 transgenic pigeon pea lines based on their higher seed yield.

3.2 Screening of T_1 transgenic pigeon pea lines carrying *OsLecRLK*

T_1 generation was raised in the transgenic green house, from seeds collected from T_0 plants. Genomic DNA was isolated from the selected T_1 generation plants and subjected to PCR amplification using the *OsLecRLK*-specific primers. The PCR product was run on 1.7% agarose (w/v) gel electrophoresis. An amplicon size of 2.1 kb in T_1 generation pigeon pea plants and the positive control was observed (Fig. 2A, B). No amplification was observed in the wild type plants. This confirmed the stable integration and inheritance of the transgene in the transgenic lines. Ten plants each from twelve T_1

lines were screened for the presence of the transgene. Out of a total 120 plants screened, 54 plants were found to carry *OsLecRLK* gene in T_1 generation (Table 2).

Table 1. Seed yield (g/plant) in T_0 WT and T plants

Sr. no.	Plant no.	Seed yield (g/plant)
1	L-9	4.89
2	L-13	4.78
3	L-14	3.15
4	L-16	3.56
5	L-17	5.76
6	L-18	8.30
7	L-19	6.43
8	L-26	2.52
9	L-55	3.16
10	L-48	7.34
11	L-62	2.26
12	L-66	3.47
13	L-10	2.86
14	L-89	5.60
15	L-117	4.46
16	L-118	3.88
17	Wild type	4.04
*CD. at 5%		0.005

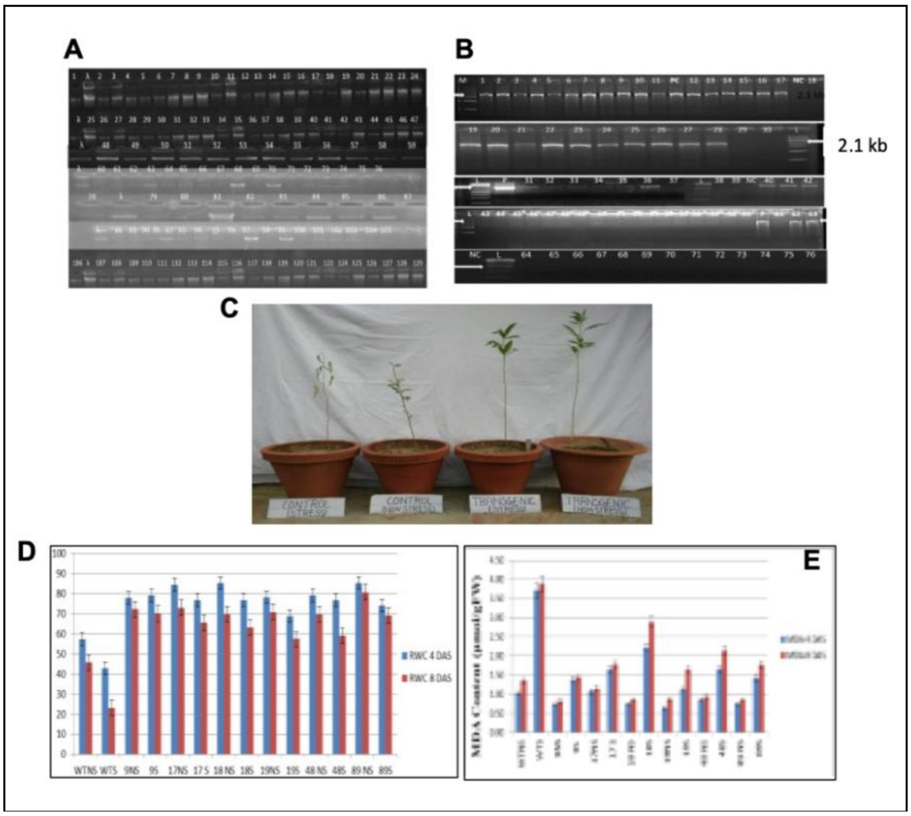

Fig. 1. A & B : Screening of transgenic pigeon pea T_1 generation plants carrying *OsLecRLK*; C: Visual effect of 75mM NaCl salt stress on transgenic and wild type (control) plants; D & E: Bar diagram showing effect of salt stress (75 mM) on relative water content (D) and MDA content (E) in wild type and T_1 transgenic pigeon pea plants

Table 2. Screening of T_1 transgenic pigeon pea lines for presence of *OsLecRLK* gene

S. No.	Transgenic lines	No. of *OsLecRLK* positive plants
1	L-9	6
2	L-13	2
3	L-14	4
4	L-16	2
5	L-17	6
6	L-18	7
7	L-19	8
8	L-48	8
9	L-66	1
10	L-89	6
11	L-117	2
12	L-118	2
Total	**120**	**54**

3.3 Physio-Biochemical Analysis of Selected Lines to Evaluate Transgene Efficacy for Salinity Stress Tolerance

3.3.1 Relative water content (RWC)

RWC dropped in both wild-type (WT) and transgenic (T) plants as the duration of the salt stress increased. However, at 4th and 8th day post salt stress treatment (DAS), greater RWC % was detected in the T lines compared to WT plants. On the fourth day, the RWC percentage was 42.86 % in WT plants but 78.09 %, 76.71 %, 76.53 %, 68.80 %, 76.70 %, and 74.05 % percent in T Lines L-9, L-17, L-18, L-19, L-48, and L-89, respectively. On the eighth day, RWC was found to be 23.08% in WT plants and in T lines a range of 57.49 % to 69.88 %. The percentage reduction in RWC from 4th to 8th day after 75 mM NaCl salt treatment was 46.20 % in wild types while 6.66 % to 23.20 % in T lines.

3.3.2 Electrolyte leakage or Membrane injury index (MII)

Both wild-type and transgenic plants showed elevated membrane damage index or electrolyte leakage percentage. However, extent of membrane injury was much higher in Wild Type plants as compared to Transgenic lines under stress conditions after 4th & 8th days of salt treatment. On 4th day MII ranged from 18.77-28.3% in T lines whereas in WT it was recorded to be 40 %. In WT, it rose to 80.95 % on the eighth day, whereas in T lines, it varied from 32.36 % to 37.38 %. Under no stress, MII was comparatively lower in the WT and T lines. Under salt stress, membrane injury increased by 102.4 % for the WT, whereas the maximum membrane injury was seen for L-48, with a 99.36 % rise from the 4th to the 8th DAS. On the other hand, L-17 and L-19 exhibited the least amount of membrane damage, increasing by 14.36 % and 22.70 %, respectively under salt stress conditions.

3.3.3 Malondialdehyde (MDA) content

Malondialdehyde content was found increased in WT and T lines. MDA content ranged between 0.75 µmol/g FW and 2.21 µmol/g FW in T lines and; 3.71 µmol/g FW in WT 4 DAS. However, there was relatively low MDA content recorded in T lines and WT both 4 & 8 DAS, under control conditions. The same trend was recorded in WT and T lines 8 DAS. The MDA content increased to 3.89 µmol/g FW in WT and in T lines ranged from 0.82 - 2.89 µmol/g FW. L-19 had the minimum MDA content of 1.13 µmol/g FW and 1.64 µmol/g FW at 4 and 8 DAS respectively. The percentage increase in MDA content from 4th day to 8th day was found 4.86 % to 30.52 % in T lines, while 33.12 % in WT plants.

3.3.4 Proline content (µmol/g FW)

Under the 75 mM NaCl-induced salt stress, the proline level rose in the WT & T lines. Under control conditions, the proline content in the WT & T lines was found to be quite low. On the other hand, a significant rise in proline content was noted in salt stress situations. On the fourth- and eighth-day following salt treatment, however, proline levels were greater than in the WT plants. Compared to WT (1.74 µmol/g FW), the proline level in T lines ranged from 14.50 - 36.86 % (1.24 to 4.41 µmol/g FW) higher. After eight days of treatment, the T lines showed a similar trend in proline content. A 0.145-fold rise in proline content was seen in WT, but 2.45-to-4.33-fold increases (3.90-6.03 µmol/g) were observed in T lines.

3.3.5 Total soluble sugar (TSS) content (mg/g FW)

TSS increased in both T and WT plants under 75mM NaCl stress. Under no stress, relatively lesser TSS content was observed in both WT and T lines at 4 and 8 days after salt stress. T lines expressed higher TSS content (52.01 - 69.25 mg/g FW) as compared to the WT i.e. 56.03 mg/g FW on 4th day of salt stress. Similar trend was observed after 8 days of salt stress. In transgenic lines 4.13 % to 13.96 % (65.23 - 74.43 mg/g FW) increase in TSS was observed whereas, in wild type 8.21% (60.63 mg/g FW) increase in TSS content was observed. On 8th day, 70.69 mg/g FW TSS was recorded in L-19.

3.3.6 Total chlorophyll content (mg/g FW)

Both the WT and T lines showed declines in the total chlorophyll content. In contrast to T lines, the content was comparatively lower in WT plants. On the fourth day, T lines' chlorophyll content was found to be between 0.3 and 1.6 times greater than that of WT plants. The amount of chlorophyll of T lines was, however, 0.2 to 2.19 times greater than that of WT plants following 8 days of salt stress. WT plants showed a 25.08 % decrease in chlorophyll content from the fourth to the eighth day of salt stress, while T lines showed a range of 2.79 to 6.18 per cent.

3.3.7 Catalase (CAT) activity

The catalase activity increased in both T and WT plants after 75 mM NaCl treatment. WT plants had an increase of 0.27-folds, whereas in T lines 0.8- 2.58-folds increase in catalase activity after 4 days of salt stress was observed. The trend remained same in both the T lines and WT even after 8 days of salt treatment. In WT there was a 0.28-folds increase and in T line 0.5-2.48 folds' increase in catalase activity was observed. L-19 expressed the highest catalase activity with 28.20 enzyme units/g FW and L-9 had the lowest 22.31 enzyme units/g FW.

3.3.8 Peroxidase (POD) activity

A similar trend was observed for peroxidase as in case of catalase activity. Peroxidase activity increased in both T and WT plants. Relatively lower peroxidase activity was observed in T and WT plants under control conditions. After 4 days of salt stress WT had an increase of 1.76-folds while, in T lines peroxidase activity increased from 2.06- to 6.25-folds. The trend remained same after 8 days of salt treatment i.e. in WT plants 1.8-fold increase was observed whereas, in T line 2.50- to 6.04-folds' increase in peroxidase activity in comparison to the control plants was observed.

3.3.9 Visual observation during salt stress

After fifteen days of germination the six transgenic lines and wild type plants were exposed to 75 mM NaCl salt stress. During stress implementation both types of plants transgenic as well as wild type were visually observed for appearance of symptoms of salt stress. The transgenic plants were found greener and healthier than the wild type plants which died after 8 days of stress treatment (Fig. 1 C).

Relative water content is a physiological metric which may be utilize to analyze a plant's ability to retain water as well as its water status and osmotic changes in response to abiotic stress (Ravikumar *et al*., 2014). Increased amounts of salts deteriorate water-plants interactions which results in disrupted osmotic potential and reduced relative water intake of plants. This results in drought-like symptoms, reduced stomatal conductance, and a decrease in cell growth in plants (Rasool *et al*., 2013; Isayenkov and Maathuis, 2019). As a result, plant cells must maintain an adequate water content for essential cellular processes like photosynthesis (Egamberdieva *et al*., 2015). Under salt stress, RWC dropped in both WT and T plants, but it was more pronounced in WT plants. This suggests that the transgenic lines may be able to retain more water in their tissues than WT when subjected to salt stress for longer periods of time. According to related transformation research, the results demonstrated enhanced water retention in transgenic crops, such as tobacco (Yadav *et al*., 2012), pigeon pea (Surekha *et.al*., 2014), and tomato (Rai *et al*. 2013), potato overexpressing Ib MIPS 1 (Zhai *et al*., 2016), chilli expressing PDH45 (Shivakumara *et al*., 2017), rice expressing *OsGS1* gene (James *et al*., 2018), and in tobacco for drought stress (Liu *et al*. 2008). Better water retention suggests improved osmotic stress adaptation, which leads to improved growth in transgenic pigeon pea plant. Additionally, the outcomes suggest that *OsLecRLK* was successful in fostering tolerance to the transgenic plants.

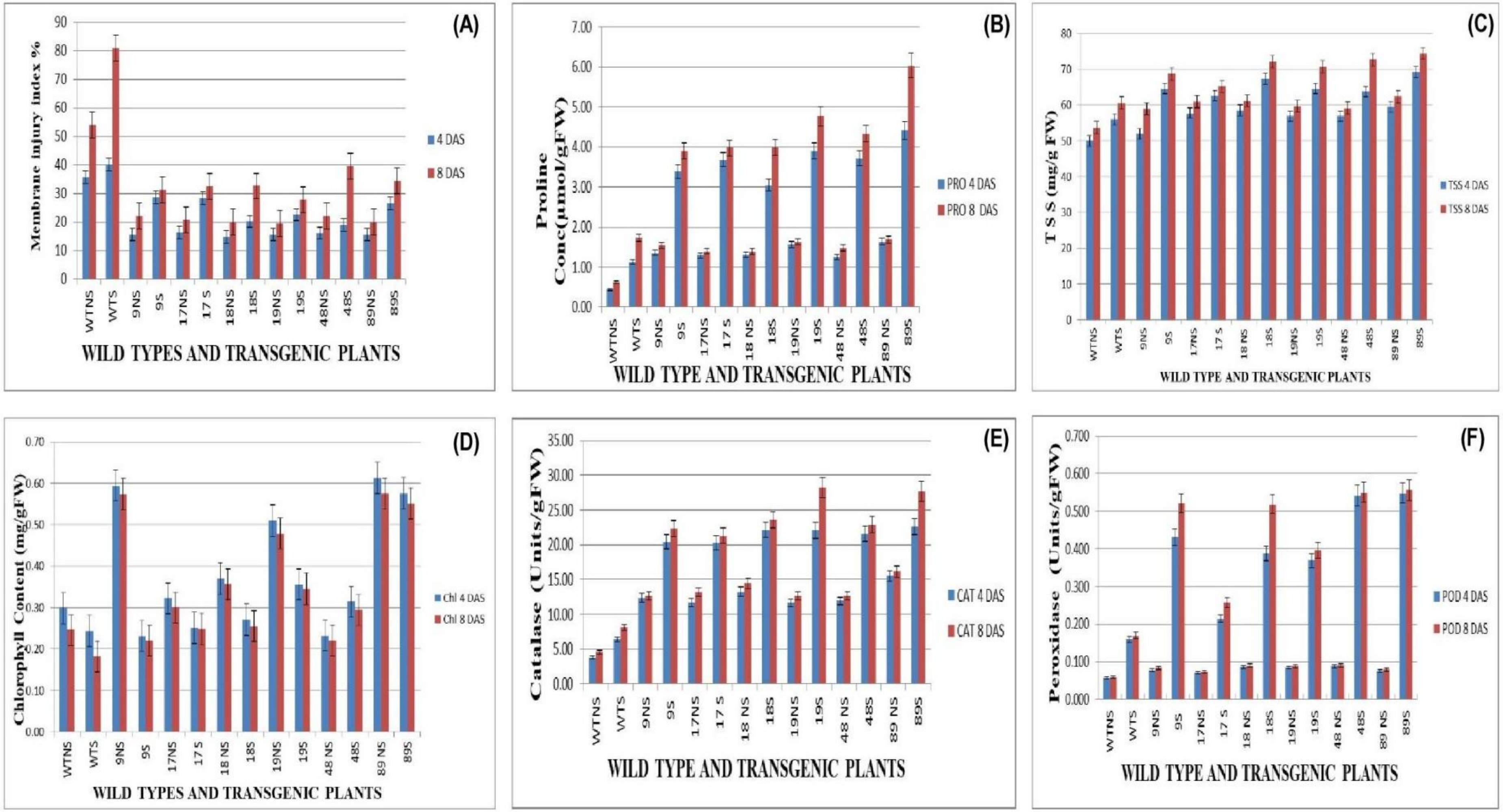

Fig. 2. Bar diagram showing effect of salt stress (75 mM) on MII (A), Proline (B), TSS Total Soluble Sugar content (C), Chlorophyll content (D), Catalase (E) and Peroxidase (F) activity in wild type and T_1 transgenic pigeon pea plants

TSS plays a crucial role in preserving cell homeostasis as an osmolyte. An increase in TSS levels allows root cells to absorb water in salt-stressed environments while simultaneously preserving the cell's osmotic potential. (Benzarti *et al*., 2014; Rosa *et al*., 2009). Accumulation of soluble sugars also boosts proline content under salinity conditions. Proline serves as an essential osmolyte as well which helps in osmoregulation and in scavenging of ROS by enhancing antioxidant activity under stressed conditions (Hayat *et al*., 2012; Ravikumar *et al*., 2014) and is vital for maintaining agricultural plants' osmotic balance and protecting their enzymes and cell organelles (Surekha *et al*. 2014). In the current study, under stressful conditions, both TSS and proline content were observed to be more prevalent in transgenic and wild type plants than in the control wild type plants. These findings suggest that elevated TSS and proline content serves as an osmoprotectant and aids in stress reduction. Nonetheless, T plants under stress showed a much greater increase in proline and TSS. Comparable outcomes were noted in pigeon pea (Singh *et al*. 2020), soybean (Wang *et al*., 2018), chrysanthemum (He *et al*., 2018), peanut (Banavath *et al*., 2018), transgenic tobacco (Tuteja *et al*., 2014), and alfalfa (Zhang *et al*., 2015).

Carbohydrates are essential for osmoprotection, osmotic adjustments and radical species scavenging under salt stress (Nemati *et al*., 2011 and Rasool *et al*., 2013). Increased photochemical activity that results in the production of ROS which in turn, harms cellular components, demolish chlorophyll and causes membrane lipids peroxidation. Chlorophyll synthesis is halted by oxidative stress and its decomposition also starts. Low yields are marked by low photosynthesis, which is caused by less chlorophyll. According to research, a higher chlorophyll content boosts photosynthetic rate (Augustine *et al*., 2015). The transgenic lines also retained a higher chlorophyll content when exposed to salt stress. This demonstrates that photosystem-II's photochemical activity has not been impacted and that the transformation has successfully controlled the oxidative stress. Thus, it may be inferred that *OsLecRLK* gene modification can enhance the pigeon pea crops ability to produce higher yield under salinity stress. Our results also coincide with the study of Soni & Savalia, (2023) where they reported the elevated chlorophyll and proline contents in pigeon pea under salinity stress.

Higher levels of ROS cause cellular component dysfunction and subsequently cell destruction. The ROS concentration is controlled by plants through the synthesis of antioxidant enzymes. Under salt stress it’s difficult to control ROS, and a failure to scavenge could lead to subpar yields. T lines showed considerably increased catalase and peroxidase activity, demonstrating their capacity to scavenge ROS. Enhanced catalase and peroxidase activity has been documented under salt stress in Arabidopsis (Zhao *et al*., 2017), peanut (Singh *et al*., 2014), *Sorghum bicolor* (Anjaneyulu *et al*., 2014), pigeon pea (Neha, 2019), and chick pea (Kharb, 2022), where the enhanced productivity resulted in efficient ROS control and scavenging.

The H_2O_2 accumulation causes oxidative damage to the plants and induces activation of ROS scavenging enzymes such as peroxidase and catalase. Catalase and peroxidise both are heme-containing enzymes that help in preventing oxidative damage by removal of H_2O_2. Under stressful situations, peroxidase eliminates excess peroxide from the cell by oxidizing specific substrates at the expense of H_2O_2 (Kaymakanova and Stoeva, 2008). In the current investigation, both T and WT plants showed a rise in peroxidase activity when exposed to salt stress. Increased POD activity under saline stress conditions suggests that It serves as a natural defensive mechanism against oxidative damage brought on by stress in plants (Krishnamurthy & Rathinasabapathi, 2013). Similar findings of elevated POD activity were documented in transgenic peanuts (Singh *et al*., 2014), transgenic canola (Ashraf & Ali, 2008), and transgenic alfalfa (Zhang *et al*., 2016), transgenic Physiolus (Taibi *et al*., 2016), transgenic mungbean (Patel *et al*., 2017) transgenic pigeon pea (Singh, 2018) and transgenic rice (Passricha *et al*., 2019a). Similarly, catalase prevents oxidative damage by catalysing breakdown of H_2O_2 into H_2O and O_2. Similar trends of increased catalase activity under stressed conditions were observed in poplar (Yang *et al*., 2015), Arabidopsis (Tamirisa *et al*., 2017), rice (Passricha *et al*., 2019). Catalase aids in the energy-efficient elimination of H_2O_2 when cells are under stress, which causes them to waste more energy and generate more H_2O_2 through catabolic processes (Das & Roychoudhury, 2014). In the current investigation, it was discovered that both WT and T plants had elevated catalase activity when exposed to salt stress. Compared to WT plants, T lines extinguished more catalase activity.

Reduced oxidative damage was further supported by the transgenic plants' lower membrane injury index and MDA levels as compared to WT pigeon pea plants. MDA is a lipid peroxidation indicator for membranes and is therefore regarded as a reliable sign of cellular damage brought on by oxidative stress. Better oxidative stress control and a decreased membrane damage index are achieved by lowering the MDA concentration. Thus, it may be concluded that the transgene *OsLecRLK* is essential for preserving a lower ROS content and minimizing plant membrane damage in instances of salt stress. According to comparable transformation research on pigeon pea, there was less membrane damage and MDA content (Surekha *et al*., 2014). The outcomes were found to be comparable to similar research on some other genetically modified plants eg. Pigeon pea (Singh *et al*., 2020), Tobacco (Yadav *et al*., 2012), Alfalfa (Tang *et al*., 2014;), apple (Li *et al*., 2015), cotton (Zhang *et al*., 2014) under salinity stress.

4. CONCLUSION

The present study showed the function of *OsLecRLK* in lessening the strain of salt as T lines exhibited improved performance in terms of different physio-biochemical matrics under 75 mM salt stress over WT. T lines carrying single copy of gene performed better in comparison to lines carrying two copies of gene. While, T line with two copies of transgene performed better than WT but lower than T lines with single copy of transgene. Relative to earlier transformation research, these transgenic plants exhibit greater economic promise for sustainable agriculture than previous transformation studies using pigeon pea. Line L-19 performed best in terms of various physio-biochemical parameters. Therefore, L-19 was found promising for salt tolerance and can be taken to next generation for further studies. However, further investigation is needed to pinpoint the precise mechanism by which *OsLecRLK* improves physio-biochemical responses during salt stress. The role of different osmolytes in fostering tolerance in pigeon peas has to be clarified.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENTS

Author expresses sincere gratitude to CCS Haryana Agricultural University's Director of Research for providing the necessary financial support for the completion of this research work and also thankful to Department of Botany and Plant Physiology and Department of Seed Science and Technology for proper guidance and conducting the experiments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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