Several countries are facing high soil salinity as a major problem, with more severity in arid, semi-arid and coastal rice-producing areas of the tropics. Rice is the staple food of over 65% population of India, besides being a model monocot system. The rice production in the state of Maharashtra is greatly affected by soil salinity, which covers more than 0.6 million ha land of the state. Conventional breeding programmes have given limited success to produce salt tolerant crops across the globe. Therefore, using transgenic approaches towards producing salt tolerant crops are advocated worldwide, and it has given fruitful results in comparably shorter period. For this purpose, we had proposed first to select a high yielding, high quality but salt sensitive, on the basis of our findings, local rice genotype, and to produce transgenic rice plants of this cultivar (cv) using a proline biosynthetic pathway gene followed by testing their performance under NaCl-driven salt stress. This study is of great importance, as the Task Force on Agricultural Biotechnology in India, chaired by Dr. M. S. Swaminathan have recommended to the government of India that genetic engineering of rice should be confined to non-basmati type rice varieties.

In the present research, promising rice cvs, widely cultivated in the Maharashtra state, such as Bhurarata (BR), Kararata (KR), Kajrat-3 (KJT-3), Panvel-1 (PNL-1), Panvel-2 (PNL-2) and Panvel-3 (PNL-3) were selected and screened for their salt (NaCl) stress tolerance during seedling and vegetative growth levels under varying (0-300 mM NaCl) levels of salt stress. On the basis of germination percentage, seedling growth, root-shoot length ratio, biomass production and relative water content, three rice cvs were selected, one as salt tolerant (PNL-3), one moderately salt tolerant (KR) and one salt sensitive (KJT-3) and were further characterised for their physiological and biochemical responses under NaCl stress.

Differential responses to salt stress were noticed between genotypes under NaCl stress. Chlorophyll content was markedly reduced under progressive salt stress with highest rate of reduction in KJT-3 and lowest in PNL-3. Comparably higher protein contents were observed in PNL-3 at 300 mM NaCl followed by KR and KJT-3. A steep increase in free proline content with increasing salinity was observed in all the rice cvs. However, the rate of salt stress-induced proline accumulation was significantly higher, both in the shoots and in roots of PNL-3 than KJT-3 and KR. When exposed to 300 mM NaCl, malondialdehyde (MDA)
level in KJT-3 was about 5 times higher than the control, in KJT-3, it was 3.5 times more, against about 2.5 times more in PNL-3, indicating that the ability of PNL-3 to tolerate salinity-induced oxidative damage better than other two cvs. Likewise, total phenols were enhanced significantly with progression in NaCl level in KJT-3 and KR cvs while, no much effect was seen on PNL-3. Starch content was decreased rapidly under the influence of salinity stress in shoots and roots of KJT-3, while, it increase up to 150 mM NaCl concentration in PNL-3, whereas, no significant difference was seen in KR.

The magnitude of ions accumulation with increasing salinity varied between cvs, with lesser accumulation of Na and Cl in PNL-3, whereas, Ca content was more in PNL-3 than KJT-3 and KR, at all salinity levels. The magnitude of increase in Na content did not vary greatly amongst the shoots of cvs, however markedly higher levels of Na were measured in roots of KJT-3 compared to PNL-3. Under increasing salt stress, PNL-3 maintained significantly lower Na/K ratio than KJT-3, both in shoots and roots, with more differential response in roots.

Constitutive as well as NaCl stress induced-activity of superoxide dismutase (SOD) was significantly higher in PNL-3 than KR and KJT-3. SOD activity was increased with increasing salinity stress in roots of PNL-3, whereas, SOD activity remained either unchanged or decreased sharply in roots of KJT-3 and KR respectively. Similarly, at 300 mM NaCl, about 244% increase was recorded in activity of catalase (CAT) in shoots of PNL-3 followed by 222% increase in KJT-3 shoots and 217% in KR. Salt stress-induced increase in peroxidase (POX) activity was noticeably higher in PNL-3 than KR and KJT-3.

Isozymatic pattern of antioxidant enzymes and their expression analysis using native PAGE (polyacrylamide gel electrophoresis) showed maximum increase in SOD activity in PNL-3 under salt stress than other cvs. CAT isozymes activated under progressive salt stress in shoots of comparably salt tolerant cvs, where enhancement in expression of CAT II was significantly higher in PNL-3 as compared to KR. The POX activity staining on native PAGE revealed at least five isoforms (POX I-V) in KR and PNL-3; however, POX IV and POX V were not detected in KJT-3, neither in non-saline conditions nor under salt stress conditions.

Electrophoretic banding patterns of protein showed that shoots of KJT-3 did not show any new band under salinity stress, whereas, a new band of 32 kDa was observed in the shoots vi
and two new bands of 37 and 116.7 kDa were induced by salinity stress in the roots of PNL-3. In KR, new bands of 34, 37 and 43 kDa were appeared under salinity stress, in addition to this, the expression levels of two bands of 54.5 and 75 kDa was increased under NaCl stress.

From these results, we concluded that the higher biomass, proline accumulation, lowest lipid peroxidation level, least reduction in chlorophyll content and proteins, maintenance of ionic balance and efficient antioxidant enzymatic machinery under salt stress may be correlated with salt tolerance ability of PNL-3. More polypeptides either might be induced de novo or their expression level was increased under salt stress in comparably salt tolerant rice cvs than the sensitive one, which may play a protective role in combating higher salt stress.

Therefore, KJT-3, a recent promising, high yielding (5-6 t/ha), early maturity (110-115 days) and insect resistant, high quality non-basmati type cv, widely cultivated in the state but found highly salt sensitive, as evident from our results, was selected for Agrobacterium-mediated transformation using *P5CS-F129A* gene under the control of CaMV 35S promoter for the first time.

Δ1-Pyrorline-5-carboxylase synthase (*P5CS*) is a proline biosynthetic pathway gene that catalyses the first two steps of proline biosynthesis in plants. A mutated version of *P5CS* gene: *P5CS-F129A* produced using site directed mutagenesis, by which the expression of this gene remains no more subjected to feedback control. It was proposed to introduce *P5CS-F129A* gene in a local high yielding salt sensitive rice cv.

MS medium supplemented with 2 mg l\(^{-1}\) 2,4-D with proline and casein hydrolysate (500 mg l\(^{-1}\) each) resulted in optimal production of mature-embryo-derived hard, compact, embryogenic-like callus from KJT-3 cv. Indirect organogenesis was achieved from this callus on MS media fortified with 4 mg l\(^{-1}\) Kn and 1 mg l\(^{-1}\) NAA. Rooting of shoots was achieved in vitro on MS media. MS media containing 6 mg l\(^{-1}\) TDZ resulted in high rate of multiple shoot formation from apical shoot meristems (up to 20 shoots/ explant) and these shoots were rooted on MS medium. Both these explants (callus and shoot apex) were used successfully as targeting material for co-cultivation.

Acetosyringone (AS) proved to be essential for any transformation and 100 μM AS was optimal concentration. The optimal concentration of *Agrobacterium* culture for transformation of rice tissues was noticed to be 0.6, whereas, the most appropriate period for
co-cultivation was 3 days. Based on these results a reproducible and efficient method for Agrobacterium-mediated transformation of KJT-3 with P5CS-F129A gene was standardised.

We have achieved successfully the transformation of indica rice cv KJT-3 using P5CS-F129A gene using calluses (1.07% transformation efficiency) and shoot apices (1.78% transformation efficiency) as targeting materials for co-cultivation and later gave putative transgenics in a shorter time period. Total 8 putative transformed plants were obtained after antibiotic selection, however, only 5 showed insertion of gene as revealed by PCR products using gene specific primers. Southern blot analyses further confirmed the introduction and establishment of P5CS-F129A gene into the genome of KJT-3 in all five PCR positive T0 lines. T1 plants were selected on hygromycin containing MS media and the transgenic plants showed hygromycin resistant and sensitivity ratio of 3:1 in plants of four lines and 1:1 in plants of one line.

The transgenic hygromycin-resistant plants of T1 progeny were tested for their salt stress tolerance under 150 mM NaCl stress. Transgenic plants showed better plant growth performance associated with higher proline accumulation and lower lipid peroxidation level under 150 mM NaCl, which shows the stable insertion and functional expression of this gene in KJT-3.