**SUMMARY**

Potato (*Solanum tuberosum*) is the world’s fourth important crop after wheat, rice and maize and constitutes almost half of the world’s annual output of all the root and tuber crops. Productivity of the potato crop is severely limited by different biotic and abiotic stresses, which mainly include microbial pathogens, pests, drought, salt and cold (chilling and freezing) stress. All the developmental stages are susceptible to these stresses. Breeding for stress tolerance in potato is difficult due to tetraploidy, tetrasomic inheritance, high heterozygosity, low flowering and seed setting habit and high floral sterility. Therefore, an alternative strategy for deploying useful genes is through their direct introduction using a transgenic approach.

In plants during a pathogen attack, the defense strategy is classified either as hypersensitive response (HR) or systemic acquired resistance (SAR). SAR provides protection to infected tissues by activating different signaling pathways leading to the *de novo* synthesis of a variety of pathogenesis related (PR) proteins and antimicrobial compounds leading to tolerance. From amongst the various classes of PR protein genes, we validated the deployment of thaumatin-like protein (*TLP*) and polyphenol oxidase (*PPO*) genes both derived from tea (*Camellia sinensis*) and expressed them in potato cultivar Kufri Giriraj (KG) for conferring tolerance to charcoal rot and late blight disease. We also assessed the potential of *CsTLP* gene for drought tolerance.

In achieving an efficient transgenic protocol for cultivar Kufri Giriraj, we observed that the inclusion of silver thiosulphate (STS), an ethylene inhibitor, into the MS basal medium was necessary to raise healthy cultures and explants suitable for transformation experiments. Our experiments show that STS make the plant insensitive to ethylene by blocking the ethylene perception sites. At receptor level, we elucidated that, STS increased the transcript levels of ethylene receptor gene, *StERS1*. For efficiently regenerating shoots from cut ends of internodal explants, the use of low concentration of TIBA (anti-auxin; 0.5 mg l⁻¹) in combination with relatively low levels of zeatin (0.25 mg l⁻¹) lead to the formation of shoot primordia. However, for further shoot growth a low concentration of NAA (0.01 mg l⁻¹) and higher levels of zeatin (1.0 mg l⁻¹) was important. Using this protocol, four transgenic lines of *CsTLP* and one transgenic line of *CsPPO* were obtained by *Agrobacterium tumefaciens* mediated genetic transformation. Molecular analysis
confirmed the presence and expression of transgenes introduced. *CsTLP* transgenic lines TL1 and TL2 were chosen for stress analysis based on the single copy insertion (Southern analysis) and higher expression of *CsTLP* by semi-quantitative PCR and quantitative real-time PCR analysis in these transgenic lines.

Morphologically both WT and transgenic plants were apparently similar during their vegetative growth. However, at the time of tuber harvesting, greater proportions (>30%) of WT tubers were infected and rotten with an unknown fungus. Pure culture of the unknown fungus was raised from the infected tubers and was characterized and identified by Internal Transcribed Spacer (ITS) region sequencing followed by BLASTn analysis as *Macrophomina phaseolina*. This fungus was responsible for causing charcoal rot in potato. A protocol to screen the tubers against the pathogen *M. phaseolina* was developed and WT tubers were severely infected from the periderm to the tuber pith. Contrastingly, TL1 tubers showed a tolerant phenotype and TL2 tubers exhibited partial tolerance to charcoal rot. This difference in tolerance among lines TL1 and TL2 was due to different expression levels of *CsTLP*. On further screening young leaves of WT, TL1, TL2 and those of highly susceptible late blight potato cultivar Kufri Chandramukhi (KCM) against the late blight disease caused by *Phytophthora infestans*, *P. infestans* induced disease symptoms appeared early in KCM followed by WT and were delayed in the *CsTLP* transgenics. Hence the present study elucidated the role of *CsTLP* gene in imparting tolerance to two fungal pathogens, *M. phaseolina* (necrotrophic) and *P. infestans* (hemibiotrophic), which differ in their developmental patterns. To verify, if fungal tolerance conferred by *CsTLP* overexpression in potato is due to induction of defense response genes, we studied the expression of endogenous defense response pathway genes of potato *viz. Solanum tuberosum TLP* (*StTLP*), phenylalanine ammonia lyase (*StPAL*) and lipoxygenase (*StLOX*) in response to *M. phaseolina* infection using quantitative real-time PCR. Transgenic tubers of TL1 plants with higher tolerance to *M. phaseolina* relative to WT, showed a concomitant and significant increase in transcripts of *StPAL*, *StLOX*, and *StTLP* genes involved in the phenylpropanoid, lipoxygenase, and general defense response pathways, respectively, after infection.

The potential of *CsTLP* overexpression was also assessed under water stress induced by withholding water for 3-15 days. The transgenic plants exhibited higher RWC than the WT which led to the delay in stress response and stress related damage. Higher accumulation of soluble sugars in transgenic plants under water stress led to osmotic adjustments and could be a part of the strategy adopted by the transgenic plants to counter
moisture stress. *CsTLP* transgenic plants showed an early response in terms of proline production which is known to provide an enduring protection against water stress. *CsTLP* transgenic plants maintained a higher stomatal conductance (gₛ) and net photosynthetic rate (P₆) under water stress in comparison to WT plants. Further, higher chlorophyll fluorescence (Fv/Fm) for *CsTLP* plants clearly reflected that only small proportion of PSII reaction centers were damaged in transgenic plants causing minimum photoinhibition and better adaptability.

Desirably, reduced color change in tuber slices during storage was observed for TL1 tubers. After cooking, low values of texture profile analysis (TPA) parameters in TL1 tubers decreased the mealiness of potato and reflected that transgenic tubers require a relatively higher cooking time than the WT tubers. For storage attributes, TL1 tubers exhibited delayed sprouting and reduced shriveling in comparison to WT tubers. The overall keeping quality of TL1 tubers was better than that of the WT.

We also showed that *CsPPO* transgenic plants (TP) evinced higher PPO activity in the stem and leaves in comparison to WT. In future, these transgenic plants need to be validated for stress tolerance against bacterial pathogens and insect pest tolerance. Enhanced PPO activity can also be looked at from the perspective of pharmaceutical uses.

The present thesis in particular provides an evidence of improving potato cultivar KG and in general indicates a basis of approach to improve potato cultivar for charcoal rot and late blight tolerance. In addition, the gene *CsTLP* also imparts a degree of tolerance to drought and can be considered as a valid candidate in potato improvement program for the future. In addition the work clearly illustrates protocols for raising healthy potato plants *in vitro* and also for inducing shoot morphogenesis on internodal explants in potato cultivars. This work can serve as a valuable reference to researchers working on potato improvement using biotechnological tools.