**Abstract**

*Picrorrhiza kurroa* Royle ex. Benth. is an endangered medicinal herb of the alpine Himalayas having hepato-protective, anti-oxidative, anti-allergic and anti-asthmatic, liver anti-carcinogenic, and immuno-modulatory activities. Its pharmacological efficacy is attributed to a class of monoterpene iridoid glycosides collectively known as picrosides that include picroside-I-IV, apocynin, feruloylcatalpol, catalpol, kutkoside etc. Although *P. kurroa* has been studied extensively in terms of chemical profile and pharmacological activities but limited attempts have been made to decipher the key regulatory genes involved in picroside biosynthesis. The endangered status of *P. kurroa* coupled with lack of information pertaining to biosynthesis of picrosides necessitates deciphering the biosynthetic pathway of picrosides. Additionally, metabolic engineering is an increasingly powerful method for scaling up the natural product yields and generate novel compounds in biological systems. However, an important prerequisite for any attempt at metabolic engineering demands detailed knowledge of the underlying biosynthetic and regulatory pathways in plants. Therefore, in an endeavour towards metabolic engineering in *P. kurroa*, comprehensive studies on a panel of key pathway genes of picroside biosynthesis namely geraniol 10-hydroxylase (*PkG10H*, Acc. HM187585), cytochrome P450 reductase (*PkCPR*, JN968968) and two divergent isoforms of UDP-glycosyltransferases (UGT86C4 and UGT94F2, Acc. Nos. JQ996408, JQ996409) was carried out. Since the skeleton of picrosides is completed by a final addition of phenolic moieties like cinnamic acid, ferulic acid, or vanillic acid, a full length cDNA of phenylalanine ammonia-lyase (*PkPAL1*, Acc. JQ996410) was also isolated. Open reading frames of *PkG10H*, *PkCPR*, *PkPAL1*, UGT86C4 and UGT94F2 were 1488, 2133, 2142, 1422 and 1455 bp long encoding 494, 710, 713, 473 and 484 amino acids respectively. *PkG10H* was cloned into pYeDP60 vector and expressed in an engineered strain of *Saccharomyces cerevisiae*, WAT11. The functional expression of *PkG10H* cDNA in yeast showed that the microsomal fractions prepared from yeast cell expressing recombinant *PkG10H* protein exhibited the catalytic activity of geraniol 10-hydroxylase as confirmed by GC-MS and NMR analysis. *PkCPR* was heterologously expressed in *Escherichia coli* and the kinetic parameters of the recombinant enzyme were determined. Specific activity, $V_{\text{max}}$ and $K_m$ of *PkCPR* were found to be 5.8±0.05 μmol min$^{-1}$ mg$^{-1}$, 8.1±0.12 μmol min$^{-1}$ mg$^{-1}$ and 7.8 μM respectively. Tissue-specific expression analyses showed that *PkG10H*,
*PkCPR, PkPAL1, UGT86C4 and UGT94F2* expressed ubiquitously in roots, inflorescence and leaves with different transcript accumulation levels. *PkG10H, PkCPR, PkPAL1* and *UGT94F2* expressed copiously in leaves while as *UGT86C4* showed highest expression in inflorescence. Higher transcript levels of *PkG10H, PkCPR, PkPAL1* and *UGT94F2* corroborated positively with higher picroside accumulation in leaves. Furthermore, to elucidate whether the differential expression pattern of the cloned pathway genes correlates with transcriptional regulation via their promoters and to identify elements that could be recognized by known iridoid-specific transcription factors, upstream regions of all the genes (*PkG10H, PkCPR, PkPAL1, UGT86C4* and *UGT94F2*) were isolated and scanned for putative cis-regulatory elements. Interestingly, the presence of cis-regulatory elements within the promoter regions of these genes corroborated well with their expression profiles in response to different elicitors. Exogenous application of elicitors like methyl jasmonate, and salicylic acid up-regulated the expression of cloned genes as well as the picroside content. Further, altitude was found to have a positive effect on the picroside content and also on expression of *PkG10H, PkCPR* and *PkPAL1* transcript levels in samples collected from different ecological niches. Using homology modeling and molecular docking studies, an insight into the donor and acceptor specificities of both UGTs was also elucidated. *UGT94F2* was predicted to be an iridoid-specific glucosyltransferase having maximum binding affinity towards 7-deoxyloganetin while as *UGT86C4* was predicted to be a kaempferol-specific glucosyltransferase. Further, docking analysis revealed eight residues as potentially essential for substrate binding in *PkPAL1*. Efficient plant regeneration via direct organogenesis and *Agrobacterium tumefaciens*-mediated genetic transformation was also developed. Multiple shoot bud induction was achieved from leaf explants cultured in Gamborg’s B5 minimal organics medium containing 3 % (w/v) sucrose supplemented with 3mg L\(^{-1}\) kinetin and 1mg L\(^{-1}\) indole-3-butyric acid. *Agrobacterium*-mediated genetic transformation protocol was also developed using *A. tumefaciens* strain GV3101 harbouring binary vector pCAMBIA1302 with gfp and hpt genes. Transformation system established yielded an efficiency rate of 56% with an average of 3.4 ± 0.4 transgenic plantlets per explant. The present work provides one of the basic foundations for creating designer circuitries both in planta as well as in engineered microbial systems. It is also intended to understand the regulatory role by co-expression of cloned *PkCPR* with P450 monooxygenase, geraniol 10-hydroxylase (*PkG10H*).