Abstract

The present thesis was carried out in shikonins yielding plant *Arnebia euchroma* (Royle) Johnston, wherein no molecular work was reported so far. Red coloured shikonin and its derivatives (collectively called shikonins) are used as active ingredients in several pharmaceutical and cosmetic preparations, and as dye for fabrics and food items. Shikonins are composed of isoprenoid (geranyl pyrophosphate) and \( p \)-hydroxybenzoate moieties. Plants have single phenylpropanoid pathway for the synthesis of \( p \)-hydroxybenzoate, whereas geranyl pyrophosphate is synthesised through mevalonate (MVA) and 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways. Supply of geranyl pyrophosphate has been shown to be rate-limiting in several terpenoids biosynthesis. Therefore, the genes associated with geranyl pyrophosphate biosynthesis pathway assume central importance. Further, phenylpropanoid pathway is equally important due to its role in supply of \( p \)-hydroxybenzoate.

The objective of the present thesis was (1) cloning and characterization of various cDNAs involved in shikonin biosynthesis, and (2) understanding regulation of the pathway.

Studies with mevinolin and fosmidomycin, inhibitors of MVA and MEP pathway, respectively suggested that biosynthesis of shikonins predominantly uses MVA pathway for geranyl pyrophosphate supply. To clone various genes of the pathway, suppression subtractive hybridization (SSH) was performed using a high shikonins producing system (HSPS) and low shikonins producing system (LSPS). HSPS was presumed to have up-regulation of transcripts for shikonins biosynthesis as compared to LSPS. Forward SSH library and reverse SSH library yielded, 407 and 334 ESTs, respectively.

Library screening yielded only two genes of shikonin biosynthesis pathway, *AeHMGR* and *AePGT*. Cloning of rest of the genes namely, *AeACTH, AeHMGS, AeMVK, AePMVK, AeMVDD, AeIPPI, AeGDPS, AePAL, AeCAH,* and *Ae4-CL* was achieved by degenerate primers. Ten full-length cDNAs namely, *AeACTH, AeHMGR, AePMVK, AeMVDD, AeIPPI, AeGDPS, AePGT, AePAL, AeCAH* and *Ae4-CL* were obtained by rapid amplification of cDNA ends. Bioinformatics analyses suggested these genes to be functional based on the putative functional/conserved domains and predicted secondary structures.

Expression analysis showed that mevinolin down-regulated expression of all the twelve genes of shikonins biosynthesis pathway. Data suggested a substrate/product
mediated feed-back and feed-forward regulation of the genes under study. *AeHMGR*, and *AePGT* exhibited evident up-regulation in HSPS as compared to LSPS and a positive correlation (r) 0.95 and 0.91, respectively was obtained between their expression and the shikonins content suggesting these genes to be regulatory. Also, all the genes of PP pathway exhibited up-regulation in HSPS as compared to LSPS and suggested a requirement of higher PP pathway activity for shikonins biosynthesis. Higher gene expression in mature as compared to younger leaf tissues was suggestive of the former tissue in supplying substrates possibly to the roots for shikonins biosynthesis.

The role of *cytochrome P450 (CYP)* family genes could be important in discovery of terminal steps of shikonins biosynthesis, thirty six putative *CYP*s were cloned. Ten *CYP*s exhibited expression in accordance with the expression of regulatory genes of the pathway and these might be target for identification of the terminal genes.

Various cues namely ABA, MJ, SA, PHB, GPP, MVA and H$_2$O$_2$ were examined in relation to shikonins accumulation, ABA led to pronounced increase in shikonins production with concomitant change in expression of *AeHMGR* and *AePGT* and genes of PP pathway.

Concomitantly, the upstream sequences of *AeACTH, AeHMGR, AeMVDD, AeIPPI, AeGDPS*, and *AePGT* were cloned and analysed. These showed the prevalence of light, drought, and LT responsive elements in accordance with the ecological niche of *A. euchroma*.

This is the first report on molecular regulation of shikonin biosynthesis that would lay basis for synthetic biology of this important moiety.