Abstract of the Thesis

Folates (vitamin B₉) are important cofactors as one-carbon donors, with a much faster turn-over rate than ATPs. Folates are needed for DNA methylation, synthesis of thymine, amino acids (methionine, glycine and serine), purine, pantothenate and formyl methionine-transfer RNAs. Since animals cannot synthesize folates de novo, they solely depend on plants and bacteria. While most developed countries adopted fortification programs to alleviate folate deficiency, this approach is seldom successful in developing countries where fortified foods are un-popular. Green leafy vegetables are rich inexpensive sources of folates and other nutrients, of which coriander is vastly consumed on daily basis in tropics. Coriander foliage has high folate content of 196 µg/100 g FW. Therefore, the present study analysed folates in different commercial cultivars, characterized elicited folates and developed a method that enhance stability during post-harvest storage. The study has also elucidated sub-cellular mechanisms involved in folate enhancement and identified folate-stabilizing proteins.

The concentration of total folates in f oliages of ten commercially cultivated varieties (cv.) of coriander was evaluated by microtestplate assisted-microbiological assay using Lactobacillus rhamnosus (ATCC 7469). Certified seeds grown under identical greenhouse conditions displayed marked variations in total folates as well as biomass yield, the highest folates (1577 µg/100 g DW) being in cv. GS4 Multicut (45–50 days, marketable stage). Coriander foliage showed high sensitivity to the light intensity where 2650 lux resulted in the highest foliar-folate, whereas in the dark, the content of folate reduced to 1257 µg/100 g DW in cv. GS4 Multicut.

Folate enhancement was analysed using coriander callus cultures (to rule out other effects). Growth regulators (6-benzylaminopurine, kinetin and abscisic acid) marginally enhanced folates after 6 h whereas elicitor (salicylic acid - SA) doubled folates. In vivo foliar applications of SA (250 µM, 24 h) doubled folates (3112 µg/100 g DW), the content being sensitive to diurnal rhythms. Postharvest stability in treated foliage was 10% higher than in control when stored at 25 °C and 4 °C. The elicitation of folates (5-CH₃-H₄folate, 5- and 10-CHO-H₄folates) was differential, where the first two compounds nearly doubled and the third increased six fold post-elicitation, with all forms showing concomitant increase in in vitro bio-accessibilities, particularly after microwave drying.

Elucidation of biochemical and molecular mechanisms involved in elicitor-mediated folate enhancement was done using coriander as well as the model plant Arabidopsis, due to the availability of complete genomic data of the latter. In both plants, elicitation decreased reactive oxygen species (ROS) with concomitant increase in folates and higher
activities of antioxidant enzymes (CAT, POX and SOD). Based on the screening of the microarray data (GENEVESTIGATOR) of SA-response of Arabidopsis, expression profiles of 19 genes were analysed by qPCR, which showed the down regulation of folate biosynthetic genes and the up-regulation of folate-stabilizing genes, particularly that of a putative folate binding protein (FBP) (AtFBP1, At5G27830) as well as a cytoplasmic folate polyglutamate synthase (AtFPGS3, At3G55630). The accumulation of FBP was confirmed by affinity column purification of the foliar proteins. Computational analysis of FBPs revealed that the predicted model of one of the proteins - the AtFBP1 protein showed a strong binding affinity with folic acid, with a free energy change (ΔG) of −9.3 kcal/mol at the predicted binding site. Heterologous expression of AtFBP1 in a yeast model, tagged with an N-terminal 6His, showed its over-expression upon induction, confirmed by western blot analysis using specific antibody. Transformed-yeast cells showed significant uptake and accumulation of the fluorescent-tagged folate, but not the wild type. The gene coding for this FBP appears to be an interesting target for metabolic engineering in plants for enhancing the concentration of folates and their post-harvest stability.