

ISOLATION AND EXPRESSION ANALYSIS OF HEAVY METAL TRANSPORTERS FROM *BRASSICA JUNCEA* BY COUPLING HIGH THROUGHPUT CLONING WITH A MOLECULAR FINGERPRINTING TECHNIQUE

SOUMITA DAS¹, MONALI SEN², CHINMAY SAHA¹, DEBJANI CHAKRABORTY¹
AND ANINDITA SEAL^{1*}

Heavy metal transporters play a key role in regulating metal accumulation and transport in plants. These are important candidate genes to study in metal tolerant and accumulator plants for their potential use in environmental clean up. We coupled a degenerate primer based RT-PCR approach with a molecular fingerprinting technique based on Amplified rDNA Restriction Analysis (ARDRA), to identify novel ESTs corresponding to heavy metal transporters from metal accumulator Brassica juncea. We utilized this technique to clone several family members of Natural Resistance Associated Macrophage Protein (NRAMP) and Yellow Stripe Like protein (YSL) in a high throughput manner and distinguish between closely related isoforms and/or allelic variants from the allopolyploid B. juncea. Partial clones of twenty-three Brassica juncea NRAMPs and twenty seven YSLs were obtained with similarity to known Arabidopsis thaliana and Thlaspi caerulescens NRAMP and YSL genes. Semi quantitative RT-PCR based analysis of chosen members indicated that even closely related isoforms/allelic variants of BjNRAMP and BjYSL have distinct tissue specific and metal dependent expressions which might be essential for adaptive fitness and heavy metal tolerance. Consistent to this, BjYSL1.1 and BjYSL 2.8 were found to show elevated expression specifically in cadmium treated shoots and lead treated roots of B. juncea respectively.
