Effect of Salinity and Heavy Metal Toxicity on Gene Expression, and Morphological Characteristics in Stevia rebaudiana Plants

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Abstract : Background: Stevia rebaudiana, a member of Asteraceae family is an important medicinal plant and produces a commercially used non-caloric natural sweetener, which is also an alternate herbal cure for diabetes. Steviol glycosides are the main sweetening compounds present in these plants. Secondary metabolites are crucial to the adaption of plants to the environment and its overcoming stress conditions. In agricultural procedures, the abiotic stresses like salinity, high metal toxicity and drought, in particular, are responsible for the majority of the reduction that differentiates yield potential from harvestable yield. Salt stress and heavy metal toxicity lead to increased production of reactive oxygen species (ROS). To avoid oxidative damage due to ROS and osmotic stress, plants have a system of anti-oxidant enzymes along with several stress induced enzymes. This helps in scavenging the ROS and relieve the osmotic stress in different cell compartments. However, whether stress induced toxicity modulates the activity of these enzymes in Stevia rebaudiana is poorly understood. Aim: The present study focussed on the effect of salinity, heavy metal toxicity (lead and mercury) on physiological traits and transcriptional profiling of Stevia rebaudiana. Method: Stevia rebaudiana plants were collected from the Central Institute of Medicinal and Aromatic plants (CIMAP), Patnagar, India and maintained under controlled conditions in a greenhouse at Hamdard University, Delhi, India. The plants were subjected to different concentrations of salt (0, 25, 50 and 75 mM respectively) and heavy metals, lead and mercury (0, 100, 200 and 300 μM respectively). The physiological traits such as shoot length, root numbers, leaf growth were evaluated. The samples were collected at different developmental stages and analysed for transcription profiling by RT-PCR. Transcriptional studies in stevia rebaudiana involves important antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), cytochrome P450 monooxygenase (CYP) and stress induced aquaporin (AQU), auxin repressed protein (ARP-1), Ndhc gene. The data was analysed using GraphPad Prism and expressed as mean ± SD. Result: Low salinity and lower metal toxicity did not affect the fresh weight of the plant. However, this was substantially decreased by 55% at high salinity and heavy metal treatment. With increasing salinity and heavy metal toxicity, the values of all studied physiological traits were significantly decreased. Chlorosis in treated plants was also observed which could be due to changes in Fe:Zn ratio. At low concentrations (upto 25 mM) of NaCl and heavy metals, we did not observe any significant difference in the gene expressions of treated plants compared to control plants. Interestingly, at high salt concentration and high metal toxicity, a significant increase in the expression profile of stress induced genes was observed in treated plants compared to control ($p < 0.005$). Conclusion: Stevia rebaudiana is tolerant to lower salt and heavy metal concentration. This study also suggests that with the increase in concentrations of salt and heavy metals, harvest yield of S. rebaudiana was hampered.