Cost-effective DNA Extraction Method Suitable for Downstream Applications and Molecular Identification of Salicornia brachiata

Siridewa¹, K., Neththipola¹, T., De Silva¹, W.L., Perera², D. and *Attanayake¹, R.N

¹Department of Plant and Molecular Biology, University of Kelaniya, Sri Lanka ²Department of Bioprocess Technology, Faculty of Technology, Rajarata University of Sri Lanka

*renuka@kln.ac.lk

Sri Lanka is blessed with diverse vegetation types, and salicornia is one of the most frequently reported genera. Some *Salicornia* spp. has a high potential to be introduced as a crop suitable for marginal soils. The first step in such an attempt is accurate species identification. However, due to strong phenotypic plasticity, morphology-based species identification is challenging. The plant tissue rich in secondary metabolites also challenges molecular-based identification. This signifies the requirement for a high-quality DNA extraction technique suitable for PCR and sequencing. Since the affordability of commercial DNA extraction kits is questionable for a developing country like Sri Lanka, this study was done to assess a low-cost DNA extraction protocol suitable for Salicornia spp., to determine the suitability of extracted DNA for downstream applications, and to identify the genus up to the species level. Samples were collected from Karative, Puttalam district, and stored at -80 °C.. Three Cetyltrimethylammonium bromide (CTAB) based DNA extraction methods were used with several modifications, and the DNeasy Plant Pro kit (Qiagen) was used as the standard method. DNA quantification was done using spectrophotometric methods. There was no significant difference in DNA quantities extracted with the commercial kit, and Contreras et al., (2018) method (p<0.01). To determine the species identity, rDNA-ITS region, and chloroplast matK region were PCR amplified with universal primers. PCR was successful only for the above two methods and when Bovine Serum Albumin was added $(50\mu g/$ ml) to the PCR mixture, the rest of the DNA samples also produced clear amplification. Clean PCR products were subjected to Sanger di-deoxy sequencing. The NCBI GenBank database lack reference sequences for ITS and ETS regions and BLASTn searches of matK sequence confirmed that the species was 99% similar to the previously published S. brachiata species. Extracted DNA was suitable for SCoT marker analysis as well.

Keywords: Salicornia, DNA extraction, Species identification