Isolation of high quality RNA from plant rich in flavonoids, Melastoma decemfidum Roxb ex. Jack.

ABSTRACT

Chalcone synthase (CHS) is a plant-specific enzyme that synthesises naringenin chalcone, an essential precursor of the flavonoid biosynthetic pathway. Naringenin and kaempferol are two flavonoids that have been demonstrated to inhibit the proliferation of HeLa cells. To study chalcone synthase gene regulation in Melastoma decemfidum, we developed a high-yield total RNA isolation method to assemble a partial putative CHS cDNA sequence. Our results indicated that a modified CTAB method produced the highest total RNA yield (8.26±3.99 µg/gFW) compared to other methods. Thus, we used this method to isolate total RNA from different types of tissues from this plant. Our improved protocol produced high-quality total RNA from different tissues, including the mature leaf (7.02±2.60 µg/gFW), stem (4.27±1.72 µg/gFW), flower bud (37.54±10.61 µg/gFW), flower (21.31±5.20 µg/gFW), and root (3.38±1.89 µg/gFW). The total RNA was then converted into cDNA, and a putative CHS gene product (~1049 bp fragment) was amplified using degenerate primers. A partial CHS gene sequence shared 80% homology with an Anthurium andraeanum CHS gene sequence (AY232492) and 92% homology with the amino acid sequence of the Acer maximowiczianum CHS gene (AEK80412.1), as determined using BlastN and BlastX, respectively. This study shows that our modified CTAB method allows for the isolation of high-quality and high-yield total RNA from various tissues of M. decemfidum. A partial putative CHS gene was amplified,thus confirming that the modified CTAB method is suitable for RT-PCR and gene isolation.

Keyword: Melastoma decemfidum; Chalcone synthase; Flavonoid biosynthesis; RNA extraction; CTAB.