ABSTRACT

Indian saga has a long heritage of using numerous medicinal and aromatic plants (MAPs) for human health care. It has been reported that above 2000 species of ethnobotanical plants are being used in various herbal composition in Northeast India. Increased demand of medicinal plants globally has provided an opportunity and opened up a new sector for Indian trade and commerce to cultivate more medicinal plants. Apocynaceae is one of the ten largest Angiosperm families (including Asclepiadaceae), and comprise of several important medicinal plants. More than 200 alkaloids have been derived through scientific investigation from Apocynaceae. Hence, there is an urgent need of precise "DNA passport" of important and vulnerable medicinal plants for correct species-level identification as well as their inventorying. A large number of molecular techniques have been used to authenticate medicinal plants based on species-specific variations in the DNA sequences. Above all, DNA barcoding is a powerful technique for species identification that has been exemplified through its wide application in monitoring and documentation of bioresources. DNA barcode based molecular characterization is in practice for plants but yet lacks total agreement considering the selection of marker. So, the goal of this study was to test the efficiency of *matK and trnH-psbA* in species delineation like DNA barcoding in important medicinal plants.

In this study, DNA from young leaves of several authentically identified plant species as well as a few ethnomedicines were isolated and *matK* gene (~800bp) and *trnH-psbA* spacer (~450bp) of Chloroplast DNA was amplified for species level identification. We tested identification through Basic Local Alignment Search Tool. Phylogenetic study was performed using the molecular evolutionary genetics analysis (MEGA/Phylip) software in accordance with the Kimura 2-Parameter (K2P) model. Phylogenetic tree was constructed using the neighbor joining (NJ) method.

The partial *matK* sequences in comparison to the *trnH-psbA* showed easy amplification (except *Citrus*), alignment and high level of discrimination value among the medicinal plant species. Intergenic spacer *trnH-psbA* exhibited persistent problem in

obtaining constant bidirectional sequences. The *denovo* sequences of *matK* showed high similarity (99%-100%) with the conspecific sequences in the database. Therefore, different plant species were readily identified, except *Hibiscus rosa-sinensis, Crotalaria trichotoma* and *Citrus* species. About one-third of the important medicinal plant species lacked GenBank records for *matK, trnH-psbA* at the time of the study. In comparison to *matK,* very few *trnH-psbA* sequences were found in the database.

In case Apocynaceae family, Partial *matK* sequences exhibited three indels in multiple of three at 5´ end. Evidently, generated *matK* sequences clustered cohesively with their conspecific Genbank sequences. However, repeat structures with AT-rich regions possessing indels in multiple of three could be utilized as qualitative molecular markers in further studies both at the intraspecific and shallow interspecific levels like the intergenic spacers of CpDNA. The study showed the efficiency of *matK* in species delineation like DNA barocding in important medicinal plants. Therefore, DNA barcoding, using *matK* gene as a potential marker, can be adopted for studying and identifying medicinal plant products that are unidentifiable by morphology alone and for detecting fraudulence that would help in developing ethnobotany research.

Standard DNA barcode for plants did not suitably work in complex plant groups like *Citrus* due to the occurrence of natural hybridization. A 54 bp inverted repeat or palindrome sequence (27-80 regions) and 5 multi residues indel coding regions were identified from *trnH-psbA* dataset in the genus *Citrus.* Inverted repeats in cpDNA provide an authentication at the higher taxonomic levels. This study demonstrated that the indel polymorphism is an alternative to the distance-based approach that easily characterizes the *Citrus* species.