Identification of *Rhazya stricta* plant using DNA Barcoding

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Abstract

The desert plant ***Rhazya stricta*** Decne from the ***Apocynaceae*** holds a significant status among the indigenous medicines of Saudi Arabia. Its innumerable therapeutic properties specifically, anti-cancer, anti-diabetic and antimicrobial ones are attributed to more than100 alkaloids it possesses. However, these pharmacological benefits are directly linked to authentic identification of this species. And, when this folkloric herb is procured from the market its identification becomes challenging in the absence of distinct phenotypic traits, as there it is found in dried or powdered form. Thus, ensuing a dire need for accurate identification of this plant species at the molecular level. With a molecular tool like DNA barcoding, this species can be accurately identified irrespective of its physical state. However, there are challenges pertaining to this tool concerning the marker locus variation.

The primary objective of this study was to safeguard consumers' health by developing the best DNA barcode markers of *Rhazya stricta.* Nine potential barcode markers from coding (*matK, rbcL, rpoB* and *rpoC1*), and non-coding (*psbA-trnH, atpF-atpH* and *psbK-psbI*) loci from chloroplast and (*nrITS1* and *ITS2*) nuclear genomes were accessed to investigate taxonomic accuracy of *R. stricta.* Another purpose of this study was to rule out the occurrence of any other species belonging to the ditypic genus *Rhazya* here in Saudi Arabia, other than the investigated plant species *R. stricta.* To accomplish these intents sixty fresh and dried samples were collected. DNA sequences from the fresh samples were compared with the database sequences of *R. stricta, R. orientalis,* and eight other related species. Basic Local Alignment Search Tool, nearest distance and tree methods: Maximum Likelihood (ML) and Bayesian tree were utilized to ascertain the results. Two-dimensional DNA barcode (i.e., QR codes) for the proposed barcode regions were created and validated using dried powered market samples. Geneious and MEGA 7.0. were the main Softwares used to investigate sequences and genetic divergence in this study.

The mean intraspecific genetic divergence of most of the loci was zero conforming the monotypic nature of *R. stricta,* while for *ITS1, ITS2* and *psbA-trnH* regions it was marginally higher than zero due to random nucleotide variations. The nrDNA-*ITS1*, *ITS2* and chloroplast markers *psbK-psbI* and *atpF-atpH* showed the highest sequence polymorphism unique to *R. stricta*. The concatenated sequences *ITS1+ITS2* and *psbK-psbI+atpF-atpH* also displayed promising results. *ITS2* followed by *ITS1* could distinguish *R. stricta* from *R. orientalis,* emerging as a suitable DNA barcode for **Genus *Rhazya***. Intergenic spacer *psbA-trnH* though being variable for *R. stricta*, showed

indistinct alignment, discouraging its use as a single locus barcode. Contrary to these cpDNA loci *matK*, *rbcL*, *rpoB* and *rpoC1*exhibited maximum similarity to other taxa, hence are not suitable for barcoding this species. This study recommends *ITS2*, *ITS1* from the nuclear genome and *psbK-psbI*, *atpF-atpH* from the chloroplast genome as potential DNA barcodes for molecular authentication of *R. stricta*. This study will be a major move towards the market supervision of medicinal plants of Saudi Arabia.