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Short note

MOLECULAR AUTOGRAPH OF MATURASE-K GENE IN *ISODON RUGOSUS* (LAMIACEAE)

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Abstract

Srivastava D.K., Bansal P., Singh P.K., Saggoo M.I.S., 2020: Molecular autograph of maturase-K gene in *Isodon rugosus* (Lamiaceae). – Botanica, 26(1): 95–100.

Molecular autograph of *trn/mat-K* gene was used as one of the important candidate marker in addressing the questions of systematics and barcoding of medicinal plants. Features of *trn/mat-K* gene in *Isodon rugosus* (Lamiaceae) were assessed for the first time (NCBI GenBank Accession Number: MH939199.1). Sequence of 756 bp length was amplified by the universal *matK* primers (*matK472F* and *matK1248R*) in the cpDNA of the plant. It was reported to contain *trnK* gene (>1…..50; >746…..756), partial sequence; and *matK* gene (>51…..745), partial coding DNA sequence. Alignment search and analysis showed that only nine different *Isodon* species are currently available for *matK* sequences with repeated copy of submissions in GenBank. The *matK* sequences of *I. rugosus* was reported with 34.2 (G + C)% and 17 variable sites (VS), out of which seven were singleton (ST) and 10 sites were species-specific parsimoniously-informative (PI) that could be used to differentiate *I. rugosus* from other species as well as to authenticate the taxon. Phylogenetic analysis resulted into monophyletic clustering of *I. rugosus* near to the clade having *I. coetsa* by both maximum likelihood (ML) and maximum parsimony (MP) methods. Clades obtained in ML tree were more informative as compared to MP tree.

Keywords: *Isodon rugosus*, Lamiaceae, *matK* gene.

The *matK* (formerly known as *orfK*) gene has first been identified in the chloroplast genome of *Nicotiana tabacum* L. (Sugita et al., 1985). It is located within the intron region of *trnK* gene and flanked by the two exons, leaving the gene intact in the event of splicing (Kar et al., 2015). It codes for maturase like protein, which involved in Group-II intron splicing. The gene contains high substitution rates within species; hence it is emerging as one of the important gene with potential contributions to plant molecular systematics and evolution (Kar et al., 2015; Hilu & Liang, 1997). Plant taxonomists are now advocating the use of multiple marker genes for barcoding the seed plants. Along with other taxonomic markers such as *ITS*, *rbcL*, etc., *matK* is now used as an important candidate in molecular taxonomy.

The molecular autograph of *matK* gene was assessed in the chloroplast genome of *Isodon rugosus* (Wall. ex Benth.) Codd (Lamiaceae) collected from the valley of Baru-Sahib area of Himachal Pradesh, India (Herbarium Acc. No.: IRBSA-01). Species has been studied previously in detail for its morpho-anatomical features by Sharma (2018). It is an important Indian species distributed throughout the North-West Himalayas and common on the steep stony and dry slopes between altitudes of nearly 1500 m to 2500 m (Verma et al., 2015; Sharma, 2018). *I. rugosus* has been reported with wide range of medicinal and pharmacological properties as recognized by many researchers (Abrol et al., 2013; Janbaz et al., 2014; Zeb et al., 2016; Hussain et al., 2017), but plant still lacks its molecular identity or barcode from the world as well as Indian flora.

Plant (IRBSA-01) was identified in a field population in the Baru-Sahib Valley (Himachal Pradesh, India) by using floras of the western Himalayan regions (Hooker, 1884; Chowdhery & Wadhwa, 1984; Polunin & Stainton, 1984) and confirmed with authentic specimens lodged in the Herbarium of Botanical Survey of India (BSI) at Dehradun, India. Genomic DNA was extracted from the previously stored leaves of specimen (IRBSA-01) following the method of Chase & Hills (1991) by using Nucleo-Spin Plant II Kit with PL1 lysis buffer (Macherey-Nagel, Duren, Germany). The universal *matK* primers (*matK472F* and *matK1248R*) were used to amplify the regions in cpDNA of the plant. The amplification was performed with 25 µl reaction cocktail in a Bio-Rad thermal cycler [Initial denaturation at 94ºC (5 min), followed by 34 cycles at 94ºC (45 s), annealing at 55ºC (40 s), initial extension at 72ºC (1 min) and final extension at 72ºC for 5 min]. PCR products were purified with DNA Clean-Up Purification Kit (Promega USA). Purified PCR products were then sequenced at Acme ProGen Biotech (India) Private Limited (Tamil Nadu, India). For editing and assembly, software programme DNAMAN version 7.0 (Lynnon Biosoft Corporation, USA) was used. Basic Local Alignment Search Tool (BLAST; for nucleotide sequence BLASTn) of the National Centre for Biotechnology Information (NCBI); MUSCLE (for multiple sequence alignment), Jukes and Cantor's (JC) model (1969), Kimura's 2 (K2) parameter (1980), maximum likelihood (ML) and maximum parsimony (MP) phylogenetic methods as implemented in Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 (Kumar et al., 2016) were used for sequence analysis.

The results of chloroplast *trn/mat-K* sequences of medicinal plant *I. rugosus* were deposited at NCBI-GenBank (Accession number MH939199.1). The sequence obtained shows the overall length of amplified *trn/mat-K* region with 756 nucleotides. The *trn/mat-K* sequences of nine different *Isodon* species (excluding the copy of same species with similar sequences) from the BLASTn result were retrieved from the NCBI GenBank and analysed with sequence of *I. rugosus* (MH939199.1). Sequence alignment in the region was significant (SA) at 91.93% of nt sites. It was reported to contain tRNA-Lys (*trnK*) gene (>1…..50; >746……756), partial sequence; and maturase K (*matK*) gene (>51…..745), partial coding DNA sequence. The information regarding *matK* sequence in *I. rugosus* (MH931390) and different species, their GenBank accession numbers, nucleotide composition, G + C content (i.e. G + C%), length and span (or position) is provided in Table 1. The *matK* region of *I. rugosus* was poor for GC composition with 34.2%. Nucleotide bases counts in seven *Isodon* species viz. *I. rugosus* (MH939199.1), *I. bulleyanus* (Diels) Kudô (JF954194.1), *I. coetsa* (Buch.-Ham. ex D. Don) Kudô (KM877377.1), *I. flabelliformis* (C.Y. Wu) H. Hara (JF957199.1), *I. megathyrsus* (Diels) H. Hara (MH116681.1), *I. nilgherricus* (Benth.) H. Hara (KM877388.1), *Rabdosia serra* (Maxim.) H. Hara [synonym of *Isodon serra* (Maxim.) Kudô; AF477763.1], were observed with similar trends in their respective regions of *matK* as T > A > C > G; while it was following different trend T > A > G > C in two species: *I. melissoides* (Benth.) H. Hara (JF954203.1) and *I. wightii* (Benth.) H. Hara (KM877392.1), species *I. sculponiatus* (Vaniot) Kudô (JF954207.1) was reported with equal amount of G and C thus reported to follow T > A > G = C. In *I. rugosus*, it was recorded with 97.55% of conserved sites and 17 variable sites (VS), out of which seven were singleton (ST) and 10 sites were species-specific parsimoniously-informative (PI), which were positioned at 63 (T), 92 (C), 217 (C), 308 (C), 313 (C), 316 (C), 552 (A), 582 (C), 652 (A) and 736 (C).

The rate of different substitution patterns for transition and trans-version in the *matK* region was recorded using both the Jukes and Cantor (JC) model (Jukes & Cantor, 1969) and Kimura's two parameter (K2P) model (1980) in MEGA version 7.0 (Kumar et al., 2016). Transitional substitution rate was 19.14 under K2, while 8.33 under JC parameter. Transversion rate was reported as 2.93 under K2, while 8.33 under JC parameter. All the species analysed for their *matK* sequences were used to check the phylogenetic relation of *I. rugosus*. The species *Verbascum thapsus* L. (HQ593484.1) of the family Scrophulariaceae with its *matK* sequence was used to confirm the out grouping in the tree. Average evolutionary divergence for *matK* region of *I. rugosus* with overall sequence pairs was observed as d ± S.E. = 0.009 ± 0.002 by both K2 and JC parameters, and no invalid distance was detected.

Phylogenetic tree of *matK* sequences generated by both maximum likelihood (ML) and maximum

Table 1. Nucleotide base composition and distribution of parsimonious informative sites in the *matK* region of *Isodon rugosus* and related species

No.	Taxon (Accession No)	Nucleotide base composition (%)					Length (Region)	Parsimonious-informative sites and nucleotide base positions									
								matK (>51…….745)									
		A	T	G	C	G + C		63	92	217	308	313	316	552	582	652	736
1.	*I. rugosus* (MH939199.1)	28.6	37.1	16.7	17.6	34.2	695 (>51.....745)	T	C	C	C	C	C	A	C	A	C
2.	*I. bulleyanus* (JF954194.1)	28.5	37.2	16.7	17.6	34.3	695 (>08.....702)	–	–	–	–	–	–	–	–	–	–
3.	*I. coetsa* (KM877377.1)	28.8	37.1	16.5	17.6	34.1	695 (>108.....802)	C	T	G	–	T	T	G	T	C	T
4.	*I. flabelliformis* (JF957199.1)	28.5	37.2	16.6	17.7	34.3	695 (>08.....702)	C	T	G	–	T	T	G	T	C	T
5.	*I. megathyrsus* (MH116681.1)	28.5	37.2	16.7	17.6	34.3	695 (>01.....695)	–	–	–	–	–	–	–	–	C	C
6.	*I. melissoides* (JF954203.1)	28.3	37.8	17.0	16.9	33.9	695 (>08.....702)	–	–	–	T	–	–	–	–	C	C
7.	*I. nilgherricus* (KM877388.1)	28.7	37.3	16.4	17.6	34.0	695 (>108.....802)	–	–	–	T	–	–	–	–	C	C
8.	*I. sculponiatus* (JF954207.1)	28.3	37.7	17.0	17.0	34.0	695 (>08.....702)	–	–	–	T	–	–	–	–	C	C
9.	*I. wightii* (KM877392.1)	28.6	37.3	17.2	16.9	34.1	695 (>108.....802)	–	–	–	T	–	–	–	–	C	C
10.	*Rabdosia serra** (AF477763.1)	28.5	37.4	16.7	17.4	34.1	695 (>528.....1222)	C	T	G	–	T	T	G	T	C	T

*Synonym of *Isodon serra*; A = Adenine; T = Thymine; G = Guanine; C = Cytosine; '–': Bars represent same nucleotides to that of *Isodon rugosus* in their respectively aligned sequence sites.

parsimony (MP) method placed the *I. rugosus* in the same clade having sister species *I. coesta* (Fig. 1). Cladogram based on MP method was more informative for this clade as compared to ML tree. However, most of the clades obtained in ML tree were partially more informative as compared to MP tree, as bootstrap closeness value for it was above 50. The highest bootstrap closeness value was observed in the clade having sister species *I. nilgherricus* by both the ML and MP methods, respectively. Similar attempt has been made by other researchers independently studying different *Isodon* species using *matK* and other taxonomic markers. Some of the noticeable attempts include molecular markers like internal transcribed spacers, e.g. *ITS-1* and *ITS-2* (Zhong et al., 2010; Harris et al., 2012; Xia et al., 2013; Yu et al., 2014; Tan et al., 2018; Li et al., 2011), maturase K (*matK*) gene (Tan et al., 2018; Li et al., 2011), *psbA-trnH* intergenic spacers (Xia et al., 2013; Yu et al., 2014; Tan et al., 2018; Li et al., 2011), *rbc-L* (Tan et al., 2018; Li et al., 2011; Ogishima et al., 2019), *trnL-trnF* intergenic spacer and *rps16* gene (Zhong et al., 2010; Paton et al., 2018).

Species *I. rugosus* was studied for the first time for its molecular *matK* region. It is a further addition to the taxonomic knowledge of the plant, and the study also confirms the presence of *I. rugosus* in the local flora. Analysis of *matK* autograph in *I. rugosus* revealed the absence or extremely low inter-specific variability and barcode gap. Low and insignificant inter-specific variation with the use of independent marker sequence in the plants may restrict the limit of marker up to the identification or classification of the genus at family level. It may be due to partial or incomplete genic regions. However, the use of the entire region of *matK* gene might not be as informative or necessary as the use of sections of the gene. In fact, some regions of the gene might be phylogenetically more noise depending on the score of variability and informative sites in those regions (HILU & LIANG, 1997; PATWARDHAN et al., 2014).

To date, it is uncertain to conclude for the independent and efficient use of *matK* in obtaining evolutionary relationships between the *Isodon* species, it is because most of the *matK* data of closely related sister groups and taxa are yet not accessed,

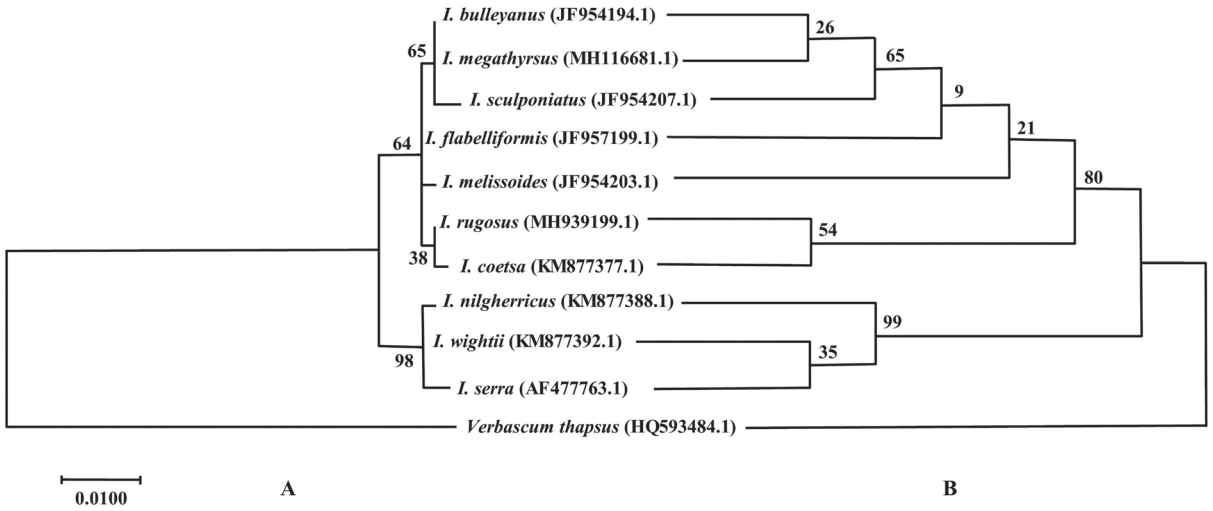

Fig. 1. Molecular phylogenetic tree of the selected *Isodon* species generated using *matK* sequence: A – by Maximum likelihood method, B – by Maximum parsimony criterion. *Verbascum thapsus* (HQ593484.1) is used as an outgroup. Numbers above/near lines are the bootstrap values in 1000 replicates

and those available in databases are incomplete and partial. Possibly, together with *matK*, the use of other taxonomic markers such as *ITS*, *rbcL* or *psbA-trnH* will probably strengthen the identity code of *Isodon rugosus* and reveal the full molecular barcode of the plant.

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MOLEKULINIS *ISODON RUGOSUS* (LAMIACEAE) MATURAZĖS K GENO ŽYMUO

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Santrauka

Molekulinis *trn/mat-K* geno žymuo buvo naudojamas vaistinių augalų sistematikos ir barkodavimo klausimų sprendimui. Pirmą kartą buvo nustatyti *Isodon rugosus trn/mat-K* geno požymiai. Naudojant universalius *matK* pradmenis (matK472F ir matK1248R) buvo amplifikuota cpDNR 756 bp grandinės seka. Geno *trnK* seka (> 1… ..50; > 746… ..756) ir *matK* genas (> 51… .. 745) iš dalies koduoja DNR seką. Surinktų duomenų analizė parodė, kad šiuo metu Genų banke yra tik devynios skirtingos *Isodon* spp. rūšys su kartotinėmis *matK* geno sekomis. Žinomos *I. rugosus* geno *matK* segmento 34,24 (G + C) % 17 kintamos sritys, iš kurių septynios yra singletoninės, o dešimt – paraimoniškai informatyvios ir gali būti naudojamas rūšies identifikavimui bei autentiškumui nustatyti. Filogenetinė analizė atlikta tiek maksimalios tikimybės, tiek maksimalios parsimonijos metodais sujungė *I. rugosus* ir *I. coetsa* rūšis į vieną kladą. Maksimalios tikimybės medžio klados buvo informatyvesnės, palyginus su maksimalios parsimonijos medžiu.