RESEARCH ARTICLE Legume Research- An International Journal, Volume 44 Issue 8: 882-887 (August 2021)

Phylogenetic Relationships in *Vicia* Subgenus *Vicilla* (Fabaceae) Based on Combined Evidence from DNA Sequences

Fei-Fei Wu, Weihong Sun, Fang Liu[1], Qiu Gao[1], Meiyan Jin, Bowen Liu, Xian-Guo Wang

10.18805/LR-596

ABSTRACT

Background: *Vicia* L. is rich in protein and can be used as a good forage, green manure, *etc*., but its classification is chaotic, which is not conducive to breeding and utilization. The purpose of the study is sequencing the nrDNA ITS region the chloroplast region (matK, rbcL and trnL-F) of the *Vicia* species of subgenus *Vicilla* of *Vicia* to resolve taxonomic contradictions.

Methods: Collecting seeds from the National Herbage Germplasm Conservation Centre of China (Beijing, China) and the germplasm bank of wild species (Kunming, China). The seedlings were cultivated in greenhouse as experimental materials. The nrDNA ITS region and the chloroplast region (*mat*K, *rbc*L and *trn*L-F) were sequenced in 17 *Vicia* species of subgenus *Vicilla* of *Vicia*. Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) for all the data sets were used to estimate the individual and multilocus phylogenetic trees are used in this study and the result showed a highly-resolved phylogeny of the *Vicia* species.

Result: *V. amurensis*, *V. costata* and *V. tibetica* belong to section *Vicilla*. Sections *Cassubicae*, *Americanae*, *Variegatae* and *Lenticula* were monophyletic groups. The sections *Vicilla*, *Cracca* and *Amurense* appeared polyphyletic. Treatment of sections *Lenticula* and *Ervum*in a separate subgenus *Ervum* was not supported. The research evaluated the phylogenetic position of the subgenus *Vicilla* species and provides a theoretical basis for the precise classification of the subgenus *Vicilla* and the genus *Vicia*.

Key words: Chloroplast DNA, Molecular phylogenetics, nrITS, *Vicia* L.

INTRODUCTION

Vicia L. belongs to the legume tribe Fabaceae, which is widely distributed in the temperate zones in the northern hemisphere (Leht, 2009) and mainly centered in Europe, Caucasus and China (Zhao *et al.* 2001). The number of *Vicia* species has been estimated about 150 by Kupicha (1976), 210 by Hanelt and Mettin (1989) and the different results were mainly attributed to the confused circumscription of species (Maxted, 1993; Shiran *et al*. 2014). There are 140-160 species in subgenus *Vicilla* (Hanelt and Mettin, 1989) and the number is more than the subgenus *Vicia*, which made it difficult to circumscribe species precisely. The phylogenetic relationships of the subgenus *Vicilla* has been studied by morphological characters (Leht, 2005), isozyme (Jaaska, 2005) and ITS sequences (Choi *et al*. 2006). Most of these studies have clarified some confused relationships, *V. amoena* was placed in section *Cassubicae* instead of section *Vicilla*. However, some species were still controversial. For instance, *V. hirsuta* was belonged to section *Cracca* by the studies of Kupicha (1976) and Lersten and Gunn (1982). However, Steele and Wojciechowski (2003) did not support the placement of *V. hirsuta* in section *Cracca*, which was basically branched from other Fabaceae species based on *mat*K dataset. *V. hirsuta* was also branched from other *Vicia* species based on styler types and ITS datasets (Choi *et al*. 2006).

More and more technical methods have been applied to solve better confused circumscription of species. Morphological traits (Abozeid *et al*. 2018; Binzat *et al*. 2014), biochemical methods (Leht and Jaaska, 2002; Pal *et al*. 2010) and molecular characteristics (Choi *et al*. 2006; Schaefer *et al*. 2012; Shiran *et al*. 2014; Katkar *et al*. 2015; Narula *et al*. 2013; Prasanthi *et al*. 2009) have been used to determine the inter specific relationships within the genus *Vicia*. Kupicha (1976) divided the world-wide species of genus *Vicia* into subgenus *Vicia* (5 sections) and subgenus *Vicilla* (17 sections) (Jaaska, 2005) by morphological traits. Previous studies have shown that DNA sequences is good diagnostic characters for distinguishing between species (Choi *et al*. 2006; Schaefer *et al*. 2012; Shiran *et al*. 2014; Wu *et al*. 2020).

This study described DNA variations of 17 species belonging to 8 traditional sections of the subgenus *Vicilla*. The nrDNA ITS region and the chloroplast region (*mat*K, *rbc*L and *trn*L-F) were sequenced by DNA sequences,

College of Grassland Science and Technology, China Agricultural University, Beijing, China.
[1]National Herbage Germplasm Conservation Centre of China, Beijing 100125, China.

Corresponding Author: Xian-Guo Wang, College of Grassland Science and Technology, China Agricultural University, Beijing, China. Email: grasschina@126.com

How to cite this article: Wu, F.F., Sun, W., Liu, F., Gao, Q., Jin, M., Liu, B. and Wang, X.G. (2021). Phylogenetic Relationships in *Vicia* Subgenus *Vicilla* (Fabaceae) Based on Combined Evidence from DNA Sequences. Legume Research. 44(8): 882-887. DOI: 10.18805/LR-596.

Submitted: 04-11-2020 **Accepted:** 29-03-2021 **Online:** 06-05-2021

meanwhile the phylogenetic trees (MP, ML and Bayesian trees) were constructed based on individual and multi locus. Our goal was to evaluate the phylogenetic position of the subgenus *Vicilla* species and the results will contribute to the precise classification of the subgenus *Vicilla* and the genus *Vicia.*

MATERIALS AND METHODS

Materials

Seeds were collected from the National Herbage Germplasm Conservation Centre of China (Beijing, China) and the germplasm bank of wild species (Kunming, China) (Table 1). The seedlings were raise in greenhouse as experimental materials.

DNA extraction and PCR amplification

Total genomic DNA was extracted from fresh or silica-gel-derived leaves using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China) according to the manufacturer's instructions.

The ITS region was amplified using the primers ITS-p5 and ITS-u4 (Cheng *et al*., 2016), the *mat*K region was amplified using the primers *mat*K472F and *mat*K1248R (Yu *et al.*, 2011), the *rbc*L region was amplified using the primer s*cp063*F and*cp063*R (Dong *et al.*, 2013) and the *trn*L-*trn*F region was amplified using the primers *trn*L-c (Taberlet *et al*., 1991) and *cp054*R (Dong *et al*. 2013). Amplification reactions were performed in a total volume of 20 µL, containing 2.0 µL of 10× Taq buffer, 2.0 µL of dNTPs (2 mmol/L), 0.2 µL of Taq polymerase (2.5 U/µL), 1 µL of each primer (5 µmol/L), 1 µL of genomic DNA (30-40 ng) and water to adjust the volume. The PCR program consisted of 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min and a final extension at 72°C for 5 min. The PCR products were examined via 1% agarose gel electrophoresis with ethidium bromide and visualized using an ultraviolet trans illuminator.

Phylogenetic analysis

Phylogenetic trees were estimated using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) for all the datasets. Two species of the sister genus *Lathyrus* L., *Lathyrus latifolius* L. and *Lathyrus sylvestris* L. were used as out groups (Jaaska, 2005; Kenicer *et al*., 2005).

Maximum parsimony analyses were performed using PAUP* v4.0 b 10 (Swofford, 2003). Heuristic search was conducted with 1000 bootstrap replicates using random addition, with all characters unordered and equal weighted. Gaps were treated as missing and multistate taxa was interpreted as uncertainty. Branch-swapping algorithm, tree-bisection-reconnection (TBR) was conducted with zero-length branches collapsed and all minimal-length trees (MulTrees) saved on different datasets. To evaluate node support, bootstrap analysis (BS) (Felsenstein, 1985) was performed using 1000 pseudo replicates, each with 10 random taxon addition replicates and TBR branch swapping. The combined dataset was tested for incongruence using the partition homogeneity test (PHT) (Farris *et al.*, 1994), this test was performed as implemented with 10,000 bootstrap replicates. The overall degree of homoplasy was estimated using consistency and retention indices (CI and RI). Descriptive statistics reflecting the amount of phylogenetic signal in the parsimony analysis were given by the consistency index (CI) (Kluge and Farris, 1969), retention index (RI) (Farris, 1989), and the resulting rescaled consistency index (RC) (Farris, 1989).We considered nodes with bootstrap support values of ≥85% as strongly supported, 75%-84% as moderately and 50-74% as weakly supported.

Maximum likelihood (ML) trees were inferred using the fast and effective stochastic algorithm implemented in IQ-TREE v1.6.2 software (Nguyen *et al.*, 2015). Best-fit substitution models were selected using the test function in IQ-TREE based on the Bayesian Information Criterion (BIC). We performed 1000 nonparametric bootstrap replicates to investigate nodal support across topologies.

Bayesian inference (BI) was performed on MrBayes 3.2.6 (Ronquist *et al*., 2012). The Akaike information criterion (AIC), which performed with MrModel test 2.3 (Nylander, 2004), was used as a selection criterion to evaluate non-nested models. The Bayesian analyses were run with four Markov Chains, starting from random trees, for ten million generations and sampled every 100th generation. After the first 20% generations were discarded as burn-in, a majority-rule consensus tree with posterior probabilities (PP) was constructed. Posterior probabilities were used to evaluate support for all nodes. We consider that clades with posterior probabilities above 0.95 are strongly supported.

RESULTS AND DISCUSSION

Characteristics of DNA regions

Based on alignment using MAFFT and manual adjustment in MEGA, the ITS sequences comprised 670 positions, among which 592 were invariant and 59 were parsimony-informative characters. The *mat*K alignment comprised 648 positions, among which 577 were invariant and 59 were parsimony-informative characters. The *rbc*L sequences comprised 1268 positions, among which 1215 were invariant and 48 were parsimony-informative characters. The *trn*L-*trn*F alignment comprised 547 positions, among which 494 were invariant and 49 were parsimony-informative characters. The combined sequence of the ITS and chloroplast regions was 3144 bp, among which 2869 were invariant and 216 were parsimony-informative characters (Table 2).

Cladistic analysis of relationships between species

The MP and ML trees were similar to the Bayesian topologies. Most phylogenetic analysis of individual and combined datasets have supported that almost all the species can gather to a highly supported monophyletic

Table 1: List of the taxa and accession investigated.

Taxon name	Reference	Geographical origin (accession numbers)
I Genus Vicia L. subgenus Vicilla (Schur) Rouy 1899 = subgenus Cracca Peterm. 1847		
Section Vicilla (Schur) Ascherson and Graebner		
V. amoena Fisch.	Kupicha, 1976	NGG (G3744/YN2007-129/Zhongxu -551/ JL10-028/JL06-050)
V. amurensis Oett.	Kupicha, 1976	NGG (JL15-072/Zhongxu-2014/XJ06-178)
V. ramuliflora (Maxim.) Ohwi	He and He, 1994	GBW (868710342219)
V. dichroantha Diels	Kupicha, 1976	GBW (868710295080)
V. japonica A.Gray	Kupicha, 1976	HLJ (Z1515) PC (Shanxi24-2) GBW(868710144729)
V. unijuga A.Br.	Kupicha, 1976	NGG (JL09116/GS1210)
V. pseudorobus Fisch. and C.A.Mey.	Kupicha,1976	NGG (JL09066/JL14-011/Zhongxu-1142/)
Section Variegatae Radzhi		
V. megalotropis Ledeb.	Kupicha,1976	GBW (868710342219)
Section Lenticula (Endl.) Aschers. et Gearbn		
V. hirsuta (L.) Gray	Hanelt and Mettin, 1989	NGG (HB2009-126/ HB2012-461/ HB2009-289/ SC2008-261)
Section Ervum (L.) Taub.		
V. tetrasperma (L.) Schreb.	Kupicha, 1976	NGG (E1260/E227/HB2011-045)
Section Cracca S.F. Gray		
V. costata Ledeb.	Kupicha, 1976	GBW (868710021846-1/868710021846-2)
V. cracca L.	Kupicha, 1976	NGG (E1039/SCH014/SCH2003-404/SCH 03-229) PC (Shanxi22-1)
V. villosa Roth	Kupicha, 1976	NGG (2807/CHQ03-160/JS0071/JS0072/ SCH02-177/YN2004-414)
Section Cassubicae Radzhi		
V. multicaulis Ledeb.	Kupicha, 1976	NGG (SCH2005-044)
Amurense Y. Endo and H. Ohashi		
V. nummularia Hand.-Mazz.	Endo and Ohashi, 1996	GBW (868710046506)
V. tibetica C.E.C.Fisch.	Endo and Ohashi, 1996	PC (Linzhi1/Linzhi2/Linzhi3)
Americanae Kupicha		
V. bungei Ohwi	Kupicha, 1976	NGG (GS2076/GS4053/SC2009-172/ Zhongxu-668)
Genus *Lathyrus* L.		
Lathyrus sylvestris	Kenicer *et al.* 2005	Genbank Number (AY839398/ KX676551/ MK526071/ AY839465)
Lathyrus latifolius	Kenicer *et al.* 2005	Genbank Number (KP338193/AF522085/ MK526068/ KP338311)

Note: NGG= National grass germplasm bank of China.
GBW= Germplasm Bank of wild species in southwest China.
HLJ= Heilongjiang Academy of Agricultural Sciences.
PC= Personal collection.

Table 2: Nucleotide sequence characteristics of the amplified DNA regions in *Vicia* species.

DNA regions	Number of constant sites	Number of parsimony-informative characters	Nucleotide diversity (π)	Aligned length
ITS	592	59	0.02638	670bp
*mat*K	577	59	0.02588	648bp
*rbc*L	1215	48	0.00865	1268bp
*trn*L-*trn*F	494	49	0.02646	547bp
ITS+*mat*K+*rbc*L+*trnL-trn*F	2869	216	0.01940	3144bp

(Fig 1). The *Vicia* species which formed a monophyletic clade were sperated in five hierarchical subclades. In both individual trees (ITS, *mat*K and *rbc*L) and combined trees (ITS+*mat*K+*rbc*L+*trn*L-*trn*F), *V. tetrasperma* belonging to section *Ervum* formed a basal sub clade with a high boot strap value (BS_ML=100; BS_MP=100; PP=1.00). The species *V. hirsuta* formed a strongly supported sub clade (BS_ML=100; BS_MP=100; PP=1.00) based on the combined DNA dataset and ITS. The section *Cracca* clade contained two well-supported species, *V. cracca* (BS_ML=100; BS_MP=95; PP=0.99) and *V. villosa* (BS_ML=100; BS_MP=99; PP=1.00). *V. bungei* (Sect. *Americanae*) formed a sub clade with strongly supported (BS_ML=100; BS_MP=100; PP=0.99) in *rbc*L and combined trees and was linked with *V. megalotropis* (BS_ML=81; BS_MP=72; PP=0.99) as sister-species couples with a high support value in ITS-tree. But *V. bungei* was linked with *V. hirsuta* (BS_ML=59; BS_MP=40; PP=0.64) with a low support value in *mat*K-tree. In *trn*L-*trn*F tree, *V. bungei*, *V. hirsuta* and *V. tetrasperma* were linked with a variable support value (BS_ML=69; BS_MP=72; PP=0.98).

The species of section *Vicilla*, *Amurense* and *Cassubicae* formed a well-supported monophyletic sub clade (BS_ML=100; BS_MP=100; PP=1.00). However, the section *Cracca* was supported as monophyletic group, while the section *Vicilla* appeared polyphyletic based on isozyme analysis (Jaaska, 2005). Unexpectedly, *V. costata* of section *Cracca* was intermingled to the sub clade with low bootstrap support (BS_ML=59; BS_MP=51; PP=0.51). The section *Amurense* (*V. amurensis* and *V. tibetica*) (Endo and Ohashi, 1996) and section *Vicilla* (*V. amoena*, *V. pseudorobus*, *V. unijuga*, *V. ramuliflora* and *V. japonica*) (Kupicha, 1976) were formed a subclade with a relative high supported value (BS_ML=81; BS_MP=61; PP=0.95). The *V. multicaulis* (section *Cassibicae*) (Kupicha, 1976) was basically linked to the subclade with significant bootstrap support (BS_ML=100; BS_MP=100; PP=1.00).

In the present study, *V. amurensis* and *V. tibetica* formed a well-supported clade, which was consisted with Kupicha (1976). Our results supported the attribution of both two species to section *Vicilla* based on the DNA phylogenetic trees. In terms of other two species, *V. dichroantha* formed a clade with *V. villosa*, and *V. nummularia* formed a clade with *V. tetrasperma*, which were different from the results of Endo and Ohashi (1996) based on floral organ morphological structure and Schaefer *et al*. (2012) based on DNA sequences. This may be attributed to the low number of species used to construct phylogenetic trees.

In this study, *V. tetrasperma* appeared as a separate monophyletic clade apart from the Lathyrus clade and was clustered with *V. nummularia*. Therefore, the results argued against the placement of *V. tetrasperma* in a separate subgenus *Ervum* (Choi *et al*. 2006; Radzhi, 1970) and favored its position in the section *Ervum* of subgenus *Vicilla* (Jaaska, 2005; Kupicha, 1976).

V. costata was basally linked with the section *Vicilla* and formed sister clade with *V. multicaulis* of section *Cassubicae* in this study. The result was different from the view of placing *V. costata* in section *Cracca* (Kupicha, 1976; Leht *et al*. 2002), which was mainly based on the morphological traits. Our results favored to classify *V. costata* in section *Vicilla*, which was consistent with the results of Schaefer *et al*. (2012) based on DNA sequences.

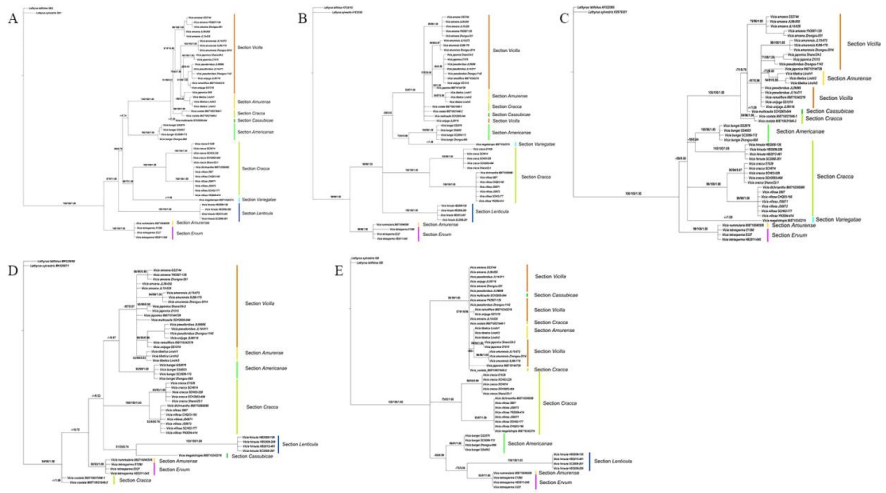

Fig 1: Phylogenetic analysis (MP, ML and Bayesian trees) based on gene sequence of nrDNA ITS region and the chloroplast region (*mat*K, *rbc*L and *trn*L-F). A. Phylogenetic analysis based on the combined datasets. B.Phylogenetic analysis based on the gene sequence of nrDNA ITS. C. Phylogenetic analysis based on the gene sequence of *mat*K. D. Phylogenetic analysis based on the gene sequence of *rbc*L. E. Phylogenetic analysis based on the gene sequence of *rbc*F.

CONCLUSION

In conclusion, the nuclear and chloroplast phylogenetic study yielded a highly-resolved phylogeny in 17 *Vicia* species of the subgenus *Vicilla* in this study. The results showed that sections *Cassubicae*, *Americanae*, *Variegatae* and *Lenticula* were monophyletic groups. The sections *Vicilla*, *Cracca* and *Amurense* appeared polyphyletic, *V. amurensis*, *V. costata* and *V. tibetica* were placed into section *Vicilla*. Treatment of sections *Lenticula* and *Ervum* in a separate subgenus *Ervum* was not supported. We have provided additional evidence to resolve the chaos of subgenus *Vicilla*. Further morphological and phylogenetic studies are needed to determine the precise taxonomic location of *Vicia* species.

ACKNOWLEDGEMENT

The present work was financially supported by the National Natural Science Foundation of China (No. 31772657).

REFERENCES

Abozeid,A., Liu, Y., Liu, J. and Tang, Z. (2018). Taxonomic implication of embryo micromorphology in the genus *Vicia* L. (Fabaceae). Plant Systematics and Evolution. 304: 33-42.

Binzat, O.K., Kahraman, A. and Doğan, M. (2014). Pollen morphology of some taxa of *Vicia* L. subgenus *Vicilla* (Schur) Rouy (Fabaceae) from Turkey. Plant Systematics and Evolution. 300: 1867-1876.

Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y. and Zhou, S. (2016). Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. Mol. Ecol Resour. 16: 138-149.

Choi, B.H., Seok, D.I., Endo, Y. and Ohashi, H. (2006). Phylogenetic significance of stylar features in genus *Vicia* (Leguminosae): an analysis with molecular phylogeny. Journal of Plant Research. 119: 513-523.

Dong, W.P., Xu, C., Cheng, T., Lin, K. and Zhou, S.L. (2013). Sequencing angiosperm plastid genomes made easy: a complete set of universal primers and a case study on the phylogeny of Saxifragales. Genome Biol. Evol. 5: 989-997.

Endo, Y. and Ohashi, H. (1996). The Infragenetic Position of East Asian Species of *Vicia* (Leguminosae). Journal of Japanese Botany. 71: 254-262.

Farris, J.S. (1989). The retention index and the rescaled consistency index. Cladistics. 5: 417-419.

Farris, J.S., Källersjö, M., Kluge, A.G. and Bult, C. (1994). Testing significance of incongruence. Cladistics. 10: 315-319.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 39: 783-791.

Fennell, S.R., Powell, W. and Wright, F. (1998). Phylogenetic relationships between *Vicia* faba (Fabaceae) and related species inferred from chloroplasttrnL sequences. Plant Systematics and Evolution. 212: 247-259.

Hanelt, P. and Mettin, D. (1989). Biosystematics of the genus *Vicia* L. (Leguminosae). Annual Review of Ecology and Systematics. 20: 199-223.

Jaaska, V. (1997). Isoenzyme diversity and phylogenetic affinities in *Vicia* subgenus *Vicia* (Fabaceae). Genetic Resources and Crop Evolution. 44: 557-574.

Jaaska,V. (2005). Isozyme variation and phylogenetic relationships in *Vicia* subgenus *Cracca* (Fabaceae). Annals of Botany. 96: 1085-1096.

Katkar, M., Mane, S.S. and Kadam, N. (2015). Molecular characterization races of Fusarium oxysporumf.sp.Ciceri using RAPD and ISSR markers. Legume Research. 38: 246-252.

Kenicer, G.J., Kajita, T., Pennington, R.T. and Murata, J. (2005). Systematics and biogeography of *Lathyrus* (Leguminosae) based on internal transcribed spacer and cpDNA sequence data. Am J Bot. 92: 1199-1209.

Khalik, K.N.A. and Algohary, I.H. (2013). Taxonomic relationships in some *Vicia* species from Egypt, based on seed morphology and SDS-PAGE of seed proteins. Acta Scientiarum Biological Sciences. 35: 603-611.

Kluge, A.G. and Farris, J.S. (1969). Quantitative phyletics and the evolution of anurans. Systematic Biology. 18: 1-32.

Kupicha, F.K. (1976). The infrageneric structure of *Vicia*. Notes from the Royal Botanic Garden Edinburgh. 34: 287-326.

Leht, M. (2005). Cladistic and phenetic analyses of relationships in *Vicia* subgenus Cracca (Fabaceae) based on morphological data. Taxon. 54: 1023-1032.

Leht, M. (2009). Phylogenetics of *Vicia* (Fabaceae) based on morphological data. Feddes Repertorium. 120: 379-393.

Leht, M. and Jaaska, V. (2002). Cladistic and phenetic analysis of relationships in *Vicia* subgenus *Vicia* (Fabaceae) by morphology and isozymes. Plant Systematics and Evolution. 232: 237-260.

Lersten, N.R. and Gunn, C.R. (1982). Testa characters in tribe Vicieae, with notes about tribes *Abreae*, *Cicereae* and *Trifolieae* (Fabaceae). Technical Bulletins. 118: 274-285.

Maxted, N. (1993). A phenetic investigation of *Vicia* L. subgenus *Vicia* (Leguminosae, Vicieae). Botanical Journal of the Linnean Society. 111: 155-182.

Narula, S., Anand, R.C., Dudeja, S.S. and Pathak, D.V. (2013). Molecular diversity of root and nodule endophytic bacteria from field pea (*Pisum sativum* L.). Legume Research. 36: 344-350.

Nylander, J. (2004). MrModeltest ver. 2. Evolutionary Biology Centre.

Pal, M., Brahmachary, R. L. and Ghosh, M. (2010). Comparative studies on physicochemical and biochemical characteristics of scented and non-scented strains of mung beans (*Vigna radiata*) of Indian origin. Legume Research. 33: 1-9.

Prasanthi, L. Reddy, B.V., Rani, K.R. and Naidu, P.H. (2009). Molecular marker for screening fusarium wilt resistance in pigeonpea [*Cajanus cajan* (L.) Millspaugh]. Legume Research. 32: 212-216.

Radzhi, A. (1970).Konspekt sistemy kavkazskikh vidov roda *Vicia* L. (Conspectus systematis specierum caucasicarum generis *Vicia* L.). Novosti Sist. Vyssh. Rast. 7: 228-240.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology. 61: 539-542.

Schaefer, H., Hechenleitner, P., Santos-Guerra, A., Sequeira, M.M.D., Pennington, R.T., Kenicer, G. and Carine, M.A. (2012). Systematics, biogeography and character evolution of the legume tribe Fabeae with special focus on the middle-Atlantic island lineages. BMC Evolutionary Biology.12: 250-268.

Shiran, B., Kiani, S., Sehgal, D., Hafizi, A., ul-Hassan, T., Chaudhary, M. and Raina, S.N. (2014). Internal transcribed spacer sequences of nuclear ribosomal DNA resolving complex taxonomic history in the genus *Vicia* L. Genetic Resources and Crop Evolution. 61: 909-925.

Steele, K.P. and Wojciechowski, M.F. (2003). Phylogenetic Analyses of Tribes Trifolieae and Vicieae, based on Sequences of the Plastid Gene matK (Papilionoideae: Leguminosae), In: Advances in Legume Systematics. [Klitgaard, B.B., Bruneau, A. (Eds.)]. Royal Botanic Gardens, Kew. pp. 355-370.

Swofford, D. (2003). PAUP: Phylogenetic Analysis Using Parsimony, version 4.0 b10.

Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology. 17: 1105-1109.

Van de Wouw, M., Maxted, N., Chabane, K. and Ford-Lloyd, B. (2001). Molecular taxonomy of *Vicia* ser. *Vicia* based on amplified fragment length polymorphisms. Plant Systematics and Evolution. 229: 91-105.

Wu, F.-F., Gao, Q., Liu, F., Wang, Z., Wang, J. L. and Wang and X. G. (2020). DNA barcoding evaluation of *Vicia* (Fabaceae): Comparative efficacy of six universal barcode loci on abundant species. Journal of Systematics and Evolution. 58(1): 77-88

Yu, J., Xue, J. and Zhou, S. (2011). New universal matK primers for DNA barcoding angiosperms. Journal of Systematics and Evolution. 49: 176-181.

Zhao, Y. and Liu, Y. (2001). Study on Floristic and Ecological Geogrophical Distribution of the Genus *Vicia* L. in the Mongolian Plateau. Acta Scientiarum Naturalium Universitatis Neimongol. 32: 207-210.