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Research Article

The Determination of Sectional and Sub-Generic Relationships among *Vicia* taxa from Algeria Using Plant Morphology and ISSR Markers

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Abstract

The genetic diversity in 46 germplasms of *Vicia* L. from Algeria was analyzed using 78 plant morphological traits and 4 ISSR markers. Cluster analysis based on plant morphology revealed two major groups corresponding to the traditional delimitation of the genus. Classification at sectional level also join traditional placement. The results of principal coordinate analysis supported the Unweighted Pair-Group Method using Arithmetic Averages (UPGMA) clustering. These finding support suggestions that *V. sativa* is an assemblage of taxa presenting an overlap in morphological traits. For the other taxa, the separation is sufficient to warrant distinct sectional status for the taxa studied. Diversity of ISSR profiles has been exploited by UPGMA cluster analysis. Dice and Jaccard indices showed more or less close patterns in the dendrograms. Mantel test gave a strong correlation between the two indices. One hundred and thirteen bands were detected, among these bands, 100% were polymorphic. Primers could be considered suitable for further variability studies in *Vicia* genus. However, they have some disadvantages which make their usefulness questionable.

Keywords: Algeria; Genetic Diversity; ISSR; Morphology; *Vicia*

Introduction

Vetches are herbaceous species known as food and forage legumes and belong to the genus *Vicia* and to one of the largest families of flowering plants (Leguminosae). It comprises a large and certainly not a definite number of species, presently estimated at more than 200 [1]. Taxonomic treatments of the genus have been based on the traditional morphotypological taxonomy, with subgenera and sections delimited variously by differently selected diagnostic characters [2]. Kupicha [3] divided the genus into two subgenera, *Vicia* and *Vicilla* (Schur) Rouy, instead of three or four as used by earlier authors [4-7]. Several researchers have mostly studied the taxonomy of regional species, mainly on the sectional level, on the basis of morphology [8-12]. A thorough overview of the taxonomic history of the genus is presented by Maxted [13]. Linnaeus [14] comments that he examined the flowers of hundreds of plants of *Vicia* species and could not find any characters that differed significantly enough to warrant generic separation. This position seems as justified today as it did two and a half centuries ago [12]. In another side, molecular markers are not prone to environmental influence. Therefore, they are considered as very powerful tools for genotype characterization and genetic diversity estimation. Of the different molecular markers, inter-simple sequence repeat (ISSR) have been widely used to assess species genetic diversity and relationships because of their cost effectiveness, simple operation without requiring sequencing information [15], and requirement of very little starting DNA template [16]. ISSRs proved to be stable and reproducible [17]. By using ISSR markers, some molecular studies including genetic diversity in vetches were reported in recent years [18-21]. There are about 26 *Vicia* species in the Algerian flora [22]. However, there is no report on the application of ISSR markers in genetic diversity assessment of *Vicia* taxa from Algeria. To determine the further genetic affinities between Vicia species at the DNA level, the present work used ISSR markers to investigate their possible use in eleven *Vicia* taxa belonging to four classical sections of the genus. We also search to compare the use of Dice and Jaccard indices to estimate the genetic diversity of taxa. In parallel, 78 morphological plant characters were used to assess the diversity between samples at sectional and sub-generic levels.

Aim of the Study

The aim of this study was also to check the concluded molecular findings how much would be compatible with flora of the country.

Materials and Methods

Plant material and taxa identification

Forty-six populations of *Vicia* L. from various bioclimatic and ecological conditions are used in the current study (Table 1). Plants were collected from different stations of North Algeria as shown in figure 1. In each sampling site, individual plants were randomly collected. Pods were shelled and the dry seeds were poured in to separate paper bags at room temperature and the bags sealed tightly until their utilization. Taxonomic identification of accessions was verified by the morphology of plants grown from seeds, using the key provided by Quézel and Santa [22]. At sub-generic and sectional levels, we follow Kupicha [3] and Maxted [13] classifications. Vouchers are deposited in the laboratory of "génétique biochimie et biotechnologies végétales" at Constantine university.

Figure 1: Geographical origin of the 46 Algerian populations studied.

Taxa/section/subgenus	Code	Date of collection	Province/Locality/Origin	Latitude	Longitude	Altitude (m)
	14	1st.6.14	Annaba El bouni	N36°49.777'	E007°38.290'	28
	52	4.6.14	Blida National parc of Chréa	N36°24.538'	E002°45.519'	249
	59	30.5.14	Jijel	N36°35.082'	E006°16.728'	141
V. sativa subsp. *consobrina* (Pomel) Maire. Section *Vicia*. Subgenus *Vicia*	65	9.6.14	Tipaza El Beldj Chenoua mountain	N36°37.667'	E002°21.150'	345
	86	13.6.14	Constantine Djbel El Ouehch	N36°23.690'	E006°39.011'	880
	6	22.5.14	Constantine Chaab ersas	N36°20.628'	E006°37.485'	563
	7	30.5.14	Mila Messaoud Boudjriou	N36°29.743'	E006°25.527'	325
V. sativa subsp. *obovata* Gaudin. Section *Vicia*. Subgenus *Vicia*	10	27.5.14	Constantine Didouche Mourad	N36°28.409'	E006°38.239'	468
	20	3.6.14	Sétif Ain arnat	N36°07.394'	E005°12.172'	866
	22	2.6.14	Oum El Bouaghi Sigus	N36°04.485'	E006°48.867'	822
	32	22.5.14	Constantine Chaab Ersas	N36°20.634'	E006°37.486'	562
	57	6.6.14	Tlemcen Ain fezza	N34°52.732'	W001°13.726'	867
	70	28.5.15	Guelma	N36°14.816'	E007°03.045'	757
	80	28.5.14	Constantine Ain abid	N36°13.543'	E006°55.782'	847
	83	26.5.14	Batna Ain Touta	N35°17.632'	E005°49.035'	683
V. sativa subsp. *angustifolia* (L.) Gaudin. Section *Vicia*. Subgenus *Vicia*	19	18.5.14	Constantine Chaab Ersas	N36°20.634'	E006°37.486'	562
	13	26.5.14	Biskra El Kantra Ain Skhoun	N35°16.087'	E005°44.174'	584
	33	29.5.14	Skikda El hadaik	N36°49.894'	E006°53.079'	26
V. sativa subsp. *cordata* (Will) Batt. Section *Vicia*. Subgenus *Vicia*	35	1st.6.14	Annaba El bouni	N36°49.777'	E007°38.290'	28
	37	28.5.14	Guelma	N36°28.361'	E007°21.280'	223

	38	10.5.14	Jijel	N36°49.348'	E005°56.706'	14
	42	28.5.14	Constantine Ain Abid	N36°13.543'	E006°55.782'	847
	47	22.5.14	Constantine University	N36°20.387'	E006°37.177'	604
	71	30.5.14	Jijel	N36°47.625'	E005°39.746'	17
V. lutea subsp. *vestita* (Boiss.) Rouy. Section Hypechusa. Subgenus *Vicia*	1	28.5.14	Skikda Ramdane Djamel	N36°45.977'	E006°53.432'	42
V. lutea subsp. *eu-lutea* Maire. Section Hypechusa. Subgenus *Vicia*	79	1st.6.14	El Tarf Ben M'hidi	N36°46.402'	E007°53.600'	11
	90	1st.6.14	Annaba	N36°49.980'	E007°34.092'	24
	18	3.6.14	Bordj Bou Areridj Ain taghrout	N36°07.741'	E005°03.364'	934
	26	28.5.15	Guelma	N36°14.816'	E007°03.045'	757
	27	2.6.14	Oum El Bouaghi	N35°51.459'	E007°06.377'	887
	29	2.6.14	Oum El Bouaghi Sigus	N36°04.485'	E006°48.867'	822
	40	28.5.14	Guelma	N36°16.276'	E007°05.751'	711
	43	4.6.14	Médéa Oued Harbil	N36°13.633'	E002°37.643'	464
V. monantha subsp. *calcarata* (Desf.) Maire. Section Cracca. Subgenus *Vicilla*	45	3.6.14	Bordj Bou Areridj	N36°04.070'	E004°41.899'	923
	49	28.5.14	Constantine Ain Abid	N36°13.543'	E006°55.782'	847
	60	2.6.14	Khenchla	N35°33.685'	E007°02.177'	860
	74	14.6.14	Tébessa	N35°15.936'	E007°30.306'	1078
	77	26.5.14	Batna Ain Touta	N35°17.632'	E005°49.035'	683
	102		Constantine University	N36°20.387'	E006°37.177'	604
	46	3.6.14	Bordj Bou Areridj El Achir	N36°04.017'	E004°40.525'	944
V. monantha subsp. *cinerea* (M.B.) Maire. Section Cracca. Subgenus *Vicilla*	91	14.6.14	Khenchla	N35°15.704'	E007°20.957'	1222
	41	28.5.14	Guelma	N36°16.276'	E007°05.751'	711
V. narbonensis L. section Narbonensis. Subgenus *Vicia*	55	27.5.14	Constantine Didouche Mourad	N36°30.023'	E006°40.051'	443
	81	22.5.14	Constantine University	N36°20.387'	E006°37.177'	604
V. tenuifolia Roth. Section Cracca. Subgenus *Vicilla*	89	6.6.14	Sidi Bel Abbes Sidi Khaled	N35°06.59'	W000°44.238'	543
V. leucantha Biv. Section Cracca. Subgenus *Vicilla*	100	10.6.14	Constantine INATAA	N36°19.002'	E006°34.626'	586

Table 1: Passport information and taxonomic identification of accessions investigated.

Germination and growing

Dry seeds from each packet were scarified then imbibed overnight in distilled water. They were germinated in the dark at 28°C on Whatman paper in Petri dishes until the appearance of the radicle. Three germinated seeds from each accession were planted in a moist mixture of garden soil, sand and compost in 5 L capacity plastic pots and plants were grown under unheated greenhouse of the laboratory of "génétique biochimie et biotechnologies végétales", at Constantine University.

Morphological analyses

The variation of 78 morphological characters with two to five states was analyzed in 40 *Vicia* L. taxa as described by Bechkri and Khelifi [23]. The observations on growth form were taken on three individual plants per population. In total, 120 individuals were examined. These accessions were already examined using these morphological characters in previous works (Bechkri and Khelifi 2016, a second paper in review). Thus, in the present manuscript, we combine plant morphology and ISSR markers of the same samples with the aim to compare them.

Molecular analyses

Genomic DNA was isolated from approximately 0.3g of fresh leaflets from every accession. Total DNA was extracted using CTAB method [24]. DNA concentration was determined spectrophotometrically by a nanodrop 2000-c (Thermoscientific) when DNA quality was estimated by visualization on 0.8% agarose gels in 1xTBE buffer.

Polymerase Chain Reaction and electrophoresis

Four ISSR markers (Table 2) that proved to give clear polymorphic bands in literature [18,20,21] were used in the present investigation. ISSR amplification was undertaken according to Han and Wang [18] with some modifications: ISSR PCRs were carried out in a 10 µl reaction volume containing 10 ng DNA, 1,5 mM $MgCl_2$, 0.2 µM primers $(AG)_8T$ and $(AC)_8T$ or 2 µM primers $(GACA)_4$ and $(GA)_8T$, 0,2 mM dNTPs, 1 U Taq DNA polymerase and 1x PCR reaction buffer (Mg^{++} free). All amplifications were performed using a Techne-prime PCR thermocycler programmed for 1 cycle of 3 minutes at 94°C, followed by 35 cycles of 92°C for 30s, 50°C or 55.2°C (primer 3) for 30s, and 72°C for 1 minute, followed by a final extension at 72°C for 7 minutes. The PCR products of ISSR markers were resolved by electrophoresis on 1.8% agarose gels with a Tris-Boric acid EDTA buffer system. Fragment size was estimated by using a 100 bp molecular size DNA ladder. BET was used to make bands visible under UV light.

Data analyses

Cluster analysis of morphological characters was performed with Euclidean Distances Matrix based on the Unweighted Pair-Group Method using Arithmetic Averages (UPGMA). In the other side, Principal Component Analysis (PCA) was undertaken to distinguish the samples by the sum of the squared cosinus ≥ 0.00. These analyses were carried out with data analysis software (STATISTICA version 6.1 program). Concerning molecular data, gels were scanned by Bio-Rad Gel-Doc and bands were scored using image-lab 5.0 software. The presence or absence of bands was scored as 1 or 0 and a data matrix was created for the four ISSR primers. NTSYS software (NTSYSpc version 2.21q) was used to elaborate distances matrices using Dice and Jaccard indices, which, in turn, have been used in dendrograms construction using UPGMA. The same software was used to display correlation between Dice and Jaccard distance matrices by Mantel test [25] where 10000 random permutations were performed. Finally, the rate of polymorphism was calculated using the following formula: p=1-(3/N) where N= number of accessions. A band is considered polymorphic when its frequency is lower than the p value.

Primer	Primer sequence	Annealing temperature °C	Total bands	Polymorphic bands	Polymorphism %	Fragments size (bp)
$(GACA)_4$	GACA GACA GACA GACA	50	18	18	100	205.96 - 956.6
$(AG)_8T$	AG AG AG AG AG AG AG AG T	50	31	31	100	222.6 - 1162.1
$(AC)_8T$	AC AC AC AC AC AC AC AC T	55	39	39	100	406.1 - 1987.9
$(GA)_8T$	GA GA GA GA GA GA GA GA T	50	25	25	100	145.65 - 1723

Table 2: Passport data of ISSR primers used for testing DNA polymorphism within Vicia taxa.

Results

Cluster analysis based on morphological traits

The relationships between these taxa, based on the variation in morphological characters are shown by the dendrogram illustrated in figure 2. The cluster analysis of the tested accessions shows two main groups at a distance of 8.24. The first cluster comprises two sub-clusters. I1 (d = 5.91) is formed by samples 55 and 81, while I2 (d = 7.33) contains 21 samples (Figure 2). The cluster II is further divided into two groups. II1 contains accessions 100 and 89 (d = 5.19) which joined 12 other accessions (Figure 2). II2 (d = 7.14) is composed of 3 populations. The variability between species is examined by the similarity matrix of all pairs of studied populations. The low distance value indicates high level of homology in the variation pattern of the morphological characters. The higher distance (d = 10) is observed between samples 20 and 90 which differ by 37 morphological traits. The same distance is also observed between 55 and 57 which differ by 38 characters. A distance of 9.49 is obtained between 81 and 91 and between 6 - 45, 29 - 33.

Figure 2: Dendrogram generated using UPGMA cluster analysis based on morphological diversity of Vicia accessions.

Principal Component Analysis

Nine characters (Leaflet Abaxial Color, Leaflet Apex Dentate, Leaflet Margin Hairiness, Tendril Branching, Stem Pubescence, Wing Length, Keel Length, Legume Suture Curvature, Legume Beak Shape) were excluded from PCA as they are 100% identical between all accessions. The 69 remaining characters were used for the analysis. Two-dimensional plot from a PCA among the samples of vetches using plant morphology was obtained (Figure 3).

Figure 3: Principal Component Analysis of studied populations based on plant morphology.

ISSR-PCR results

DNA profiles of some accessions generated by primers 1 and 3 are presented in figure 4. A total of 113 bands were scored using the 4 primers screened across 46 *Vicia* L. samples (Table 2), ranging from 1 to 11 (primer 3) per profile. Primer 1 did not amplify 15 samples. Primer 2 did not amplify 6 accessions. 11 samples were not amplified by primer 3, when primer 4 did not amplify 3 samples. Considering the four primers together, the sample which generated the most bands is the accession 18 with 19 bands. The accession 86 was not amplified by any primer (0 bands). Thus, it was removed from the matrix. Samples 6 and 33 have generated 3 bands. The most common band is 976,24 bp generated by the primer 3, obtained in 26 samples. 30 bands are unique and are observed in specific populations. The band 412,59 bp is the most common one generated by the primer 1and is observed in 11 samples. 5 bands are unique. The band 652,2 bp (primer 2) is observed in 17 samples. 13 bands are unique concerning the primer 2. Seven unique bands were obtained by the primer 3. The primer 4 have generated 6 unique bands. The band 594,58 is observed in 21 samples. The p value is equal to 0.93 and all the bands have a frequency lower than this value. Thus, the four primers showed 100% polymorphism.

Figure 4: Electrophoresis patterns of ISSR products amplified with primers 1 and 3.

Cluster analysis based on ISSR markers

Results of UPGMA based on ISSR markers using Dice index one side and Jaccard index another side are illustrated on figure 5. Mantel test gave a correlation coefficient (r) equal to 0.99558 between distance matrices of the two indices. The dendrogram generated by Dice index (Figure 5a) showed that all the 46 germplasms were separated into 2 distinct major clusters. The first one (I) is composed of sample 13 joined to samples 10 and 33. The sec-

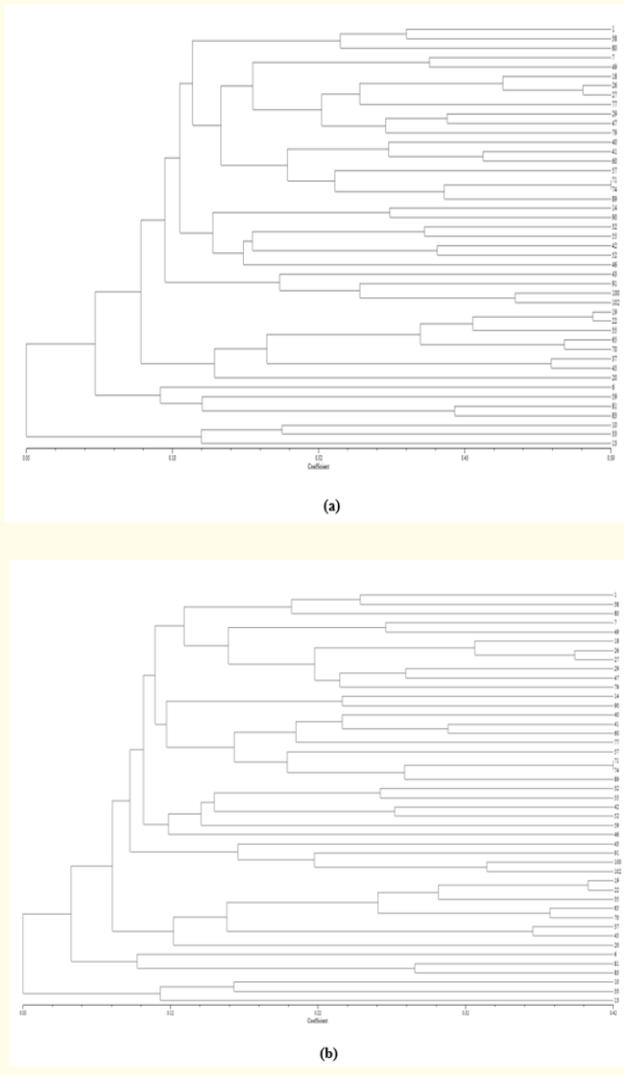

Figure 5: Dendrograms generated using UPGMA cluster analysis based on ISSR polymorphism of 45 Vicia accessions a) Dice index b) Jaccard index.

ond cluster (II) which is the most important in term of accessions number, can be divided into several sub clusters : a) samples 83 and 81 joined sample 59 which are all clustered with sample 6, b) sample 20 join samples 37 and 45, which clustered with 70, 65, 35, the all clustered with samples 19 and 22, c) 102 - 100 join sample 91 which clustered with sample 43, d) sample 46 is clustered with 52 - 42, 55 - 32 and 90 - 14, e) sample 89 join 74 - 71 and 57, the all join 60 - 41 and 40, f) 79 clustered with 47 - 29 which join 77. The latter is regrouped with 27 - 26 and 18. The all join 49 - 7, g) sample 80 join samples 38-1. The distance matrix (Figure 6a) showed that big similarities (0.59) are observed between accessions 71 and 74, between samples 19 and 22 (0.57) and also between samples 26 and 27 (0.56), 65 and 70 (0.55). The high dissimilarity (0.00) is observed between several accessions as it is also shown in figure 6a. The dendrogram generated by Jaccard index (Figure 5b) gave more or less similar picture of the dendrogram generated by Dice index, except for some populations in some groups. The same observations are obtained concerning the highest and lowest distances (Figure 6b). Differences concern the following samples: a) absence of sample 6, d) absence of samples 90 and 14, e) absence of 60, 41 and 40, a new sub-cluster is appeared and regrouped samples 77, 60 - 41, 40, 90 - 14, f) absence of sample 77.

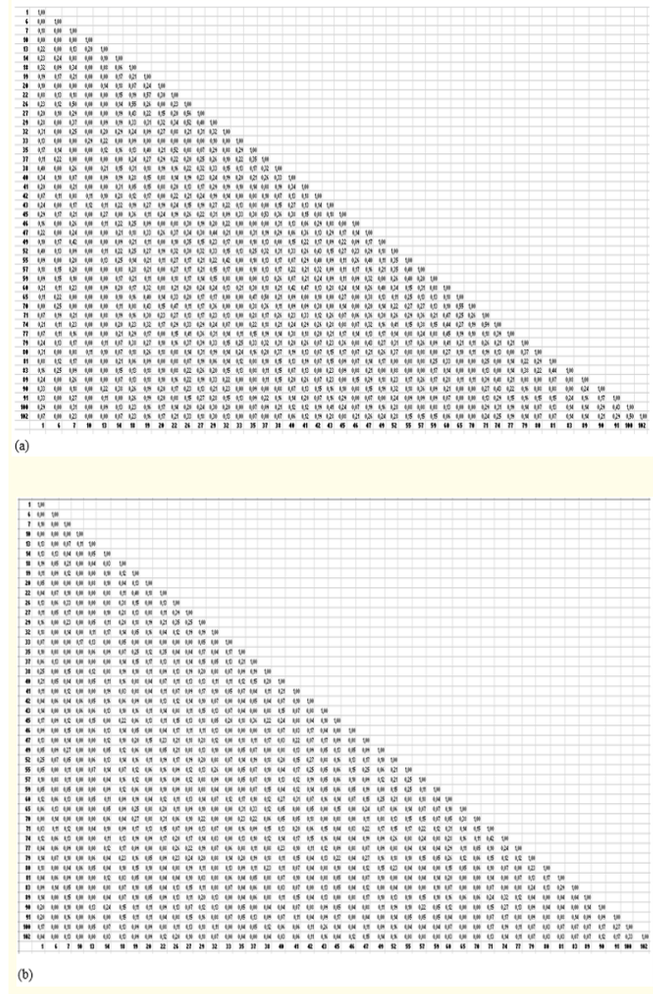

Figure 6: Distance matrices a) Dice index b) Jaccard index.

Discussion

Morphological data

Intraspecific variation within the 4 taxa belonging to the *V. sativa* group one side and within the seven other taxa another side, was previously discussed and will not be resumed again. Thereby, we only focus on the relationship on sectional and sub-generic levels.

Two major clusters are obtained. Within the cluster I (sub-genus *Vicia*), two sub-clusters are evident in the phylogram: the cluster I1 is constituted by two samples of *V. narbonensis* (sect. Narbonensis), while the cluster I2 comprises all taxa of *V. sativa* s.l. Within the cluster II, there are also two major groups. II1 contains all samples of section Cracca (subgenus *Vicilla*): *V. leucantha* and *V. tenuifolia* clustered together with all samples of *V. monantha*. The section *Cracca* is monophyletic based on the present results. II2 comprises the three samples of *V. lutea* (section Hypechusa). Thus, phylogenies derived from morphological traits strongly support the view that section Hypechusa represented by *V. lutea* is distant from the NSC represented by *V. narbonensis* (section Narbonensis). Section Hypechusa is more closely aligned with section Cracca while section Narbonensis is more closely aligned with section *Vicia* represented by samples of *V. sativa* s.l. The UPGMA, therefore, shows two distinct clusters corresponding to the two traditional subgenera (Vicia and *Vicilla*) established by several authors. However, samples of *V. lutea* belonging to section Hypechusa, sub-genus *Vicia* are linked to taxa of section *Cracca* (sub-genus *Vicilla*); whereas, in the absence of samples of *V. sativa*, they clustered with *V. narbonensis* accessions (paper in review). These results are supported by the utilization of the PCA (accessions projection) which regroups samples of *V. lutea* with those of section *Cracca*. The placement of *V. narbonensis* samples with those of *V. sativa* is justified as the two taxa belong to the sub-genus Vicia. In our previous work on the same taxa, without *V. sativa* (paper in review), *V. lutea* is placed near *V. narbonensis* and not with samples belonging to sect. *Cracca*. However, by adding samples of *V. sativa, V. narbonensis* approximates and away from *V. lutea*. Our previous works using morphological and biochemical markers showed a closer relationship between the NSC and section Hypechusa. These findings are supported by several other authors like Jaaska [26] and Shiran., *et al* [27]. *V. lutea* has several morphological characters which suggests a distant link to *V. narbonensis* and so this species is placed on the *Vicia* side of *V. sativa* (section *Vicia*). Results of the present work join those of Potokina., *et al.* [28] who showed a closer relationship between the NSC and section Vicia than between the NSC and section Hypechusa. The results of the analysis consistently indicate a more natural affinity of *V. lutea* with section *Cracca*. In another side, *V. monantha, V. tenuiflolia* and *V. leucantha* form a closely related group. The analysis suggests the forms are closely allied, but the existence of sets of correlated characters that elsewhere in the classification distinguish species suggests that the three section *Cracca* taxa should retain their specific status.

Molecular data

ISSR analyses have been focal point to explore genetic diversity in many plant taxa since last decade. Because of their good discrimination efficiency and high reproducibility, they are particularly suitable to identify the closely related species and varieties [19]. In the present work, the average polymorphism of ISSR primers was very high (100%), confirming results that this type of markers could be useful in species differentiation and diversity studies in *Vicia* sp. as reported by Rajkovic., *et a*l [21]. Despite the high levels of genetic diversity in taxa studied, the diversity changed a lot at the level of populations of the same taxon. The results indicate that the genetic diversity of *Vicia* taxa was distributed mainly within the populations, which could indicate a high level of gene flow among populations as reported by Han and Wang [18].

The dendrogram generated by Dice index showed that the first major cluster contains three samples belonging to the same species: *V. sativa* (sect. *Vicia*, subgenus *Vicia*). The second major cluster shows several pictures. Some sub-clusters agree with subgeneric and sectional classifications of the genus because species belonging to subgenus *Vicia*, sect. *Vicia*, (*V. sativa*), sect. Narbonensis (*V. narbonensis*) and sect. Hypechusa (*V. lutea*) cluster together, except for two sub-clusters where there is no clear discrimination between the two sub-genera and sections. Species belonging to sect. *Cracca*, sub-genus *Vicilla*, also clustered together as in the case of *V. leucantha, V. tenuifolia* and some samples of *V. monantha*. The dendrogram generated by Jaccard index showed a general similar picture, but the cited index gives more groups inside subclusters and is more discriminatory. Except for the instable subclusters, sect. Narbonensis represented by *V. narbonensis* and sect. Hypechusa represented by *V. lutea*, formed a rather cohesive group with sect. *Vicia*, represented by *V. sativa*, whereas the populations of section *Cracca* were more remote from them. It is important to mention that samples presenting lower distances belong to the same species and thus to the same sections and sub-genera. Pattern formation of several clusters might be due to the special climatic conditions. Several studies which have assessed the genetic diversity of plants using molecular markers have established the correlation between geographical distance and genetic similarity

between individuals [29]. The high ecogeographic diversity of the present collection has already be elucidated in our previous works [23,30]. Species with a wide geographic area generally have more genetic diversity [31]. The taxa studied are widely distributed in Algeria (Table 1), which may be one of the reasons for the unexpectedly high level of genetic diversity. Samples 1 and 38 are a good example in this case as they are collected from stations characterized by the same bioclimate (Less Humid with warm and mild winters, respectively). Another example concerns samples 100 and 102 belonging to stations characterized by Higher semi-arid bioclimate and cool winter. Accessions 14 and 90 belong to different sections (*Vicia* and Hypechusa respectively) but have the same bioclimate (sub-humid with mild winter). Thus, cluster analysis showed that groupings of the germplasms can be correlated with their comprehensive environmental conditions and geographic origin. The same findings were reported by Liu., *et al* [20]. Despite the high diversity observed in the molecular data, several accessions were not amplified by each of the four primers after several repetitions. Moreover, the use of the four primers did not produce stable groupings related to taxonomy, which can indicate that their usefulness in further variability studies is questionable.

Conclusion

The separation obtained by the morphological traits is sufficient to warrant distinct sectional status for the taxa studied. The polymorphism of ISSR primers was very high. However, the number of bands obtained and their inability to amplify several samples make their use questionable. We join several author's opinion that in variability studies of *Vicia* taxa, some other approach should be considered, but, before drawing conclusions too quickly, it is preferable to test more ISSR primers on the studied taxa.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Bibliography

1. ILDIS. "ILDIS World Database of Legumes". International Legume Database and Information Service (ILDIS) (2005).
2. Jaaska V. "Isozyme Variation and Phylogenetic Relationships in Vicia subgenus Cracca (Fabaceae)". *Annals of Botany* 96.6 (2005): 1085-1096.
3. Kupicha FK. "The infrageneric structure of Vicia". NotesRoy Bot Gard Edinb 34 (1976): 287-326.
4. Fedchenko BA. "Vicia L". In: Shishkin BK, Bobrov EG. (eds.) Flora of the USSR 13. Moscow (1948): 406-475.
5. Ball PW. "Vicia L". In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA. (eds.) Flora Europaea, volume 2, Rosaceae to Umbelliferae. Cambridge University Press, Cambridge (1968): 129-136.
6. Davis PH and Plitmann U. "Vicia L". In: Davis PH. (ed.) Flora of Turkey and the East Aegean Islands. University Press, Edinburgh (1970): 274-321.
7. Radzhi AD. "Conspectus systematis specierum Caucasicarum Generis Vicia L". In: Tzvelev NN. (ed.), Novit. Syst. Plant. Vascul., volume 7. Nauka, Leningrad (1970): 228-240.
8. Tzvelev N. "Systema specierum generis Vicia L. in parte Europaea URSS". *Novitates Systematicae Plantarum Vascularium* 17 (1980): 200-208.
9. Nikiforova OD. "The system of the genus Vicia (Fabaceae) in Siberia". *Botanicheskii Zhurnal (Leningrad)* 70.5 (1985): 604-611.
10. Nikiforova OD. "Wild vetches of Siberia". Nauka, Novosibirsk (1988).
11. Roti-Michelotti G and Serrato-Valentini G. "Seed characteristics in Italian species of genus Vicia section Ervum and their diagnostic value". *Seed Science Technology* 14 (1989): 391-402.
12. Endo Y and Ohashi H. "The infrageneric position of East Asian species of Vicia (Leguminosae)". *Journal of Japanese Botany* 71 (1996): 254-262.
13. Maxted N. "A phenetic investigation of Vicia L. subgenus Vicia (Leguminosae, Vicieae)". *Botanical Journal of the Linnean Society* 111.2 (1993a): 155-182.
14. Linnaeus C. "Species Plantarum". Volume 2. Salvius, Stockholm (1753).
15. Williams JGK., *et al*. "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers". *Nucleic Acids Research* 18.22 (1990): 6531-6535.
16. Zietkiewicz E., *et al*. "Genome fingerprinting by simple sequence repeats (SSR)-anchored PCR amplification". *Genomics* 20.2 (1994): 176-183.
17. Bornet B and Branchard M. "Nonanchored Inter Simple Sequence Repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting". *Plant Molecular Biology Reporter* 19.3 (2001): 209-215.

18. Han Y and Wang HW. "Genetic Diversity and Phylogenetic Relationships of Two Closely Related Northeast China Vicia Species Revealed with RAPD and ISSR Markers". *Biochemical Genetics* 48.5-6 (2010): 385-401.

19. Bozkurt M., *et al*. "The determination of genetic relationships among some Vicia L. (Vetch) taxa by using ISSR markers". *Biological Diversity and Conservation* 6.3 (2013): 135-139.

20. Liu Y., *et al*. "Genetic diversity in Vicia amoena (Fabaceae) germplasm resource in China using SRAP and ISSR markers". *Biochemical Systematics and Ecology* 51 (2013): 86-93.

21. Rajković D., *et al*. "Potential Use of SSR and ISSR Markers in Estimation of DNA polymorphism within genus Vicia". *Ratarstvo i Povrtarstvo* 52.2 (2015): 85-89.

22. Quézel P and Santa S. «Nouvelle flore de l'Algérie et des régions désertiques méridionales». Tomes 1 et 2. Paris: CNRS (1962).

23. Bechkri S and Khelifi D. "Variation in Vicia sativa s.l. from Algeria based on morphological characters and ecogeographic parameters". *Genetic Resources and Crop Evolution* 64.4 (2016): 815-832.

24. Doyle JJ and Doyle JL. "A rapid DNA isolation procedure for small quantities of fresh leaf tissue". *Phytochemical Bulletin* 19 (1987): 11-15.

25. Mantel N. "Adaptation of Karber's method for estimating the exponential parameter from quantal data, and its relationship to birth, death, and branching processes". *Biometrics* 23.4 (1967): 739-746.

26. Jaaska V. "Isoenzyme diversity and phylogenetic affinities in Vicia subgenus Vicia (Fabaceae)". *Genetic Resources and Crop Evolution* 44.6 (1997): 557-574.

27. Shiran B., *et al*. "Internal transcribed spacer sequences of nuclear ribosomal DNA resolving complex taxonomic history in the genus Vicia L". *Genetic Resources and Crop Evolution* 61 (2014): 909-925.

28. Potokina E., *et al*. "Phylogeny of Vicia subgenus Vicia (Fabaceae) based on analysis of RAPDs and RFLP of PCR-amplified chloroplast genes". *Genetic Resources and Crop Evolution* 46.2 (1999): 149-161.

29. Islam A. "Genetic diversity of the genus Curcuma in Bangladesh and Biotechnological Approaches for in vitro regeneration and long-term conservation of C. longa Germplasm". PhD thesis. University of Hannover (2004).

30. Bechkri S., *et al*. "Ecogeographic variability and genetic diversity associated with seed albumins, globulins and prolamins patterns in Vicia taxa from Algeria". *Botanical Studies* 58 (2017): 27.

31. Hamrick JL and Godt MJW. "Allozyme diversity in plant species". In AHD Brown, MT Clegg, AL Kahler, and BS Weir (eds.), Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, MA (1989): 43-63.

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