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Research article/Araştırma makalesi

The determination of genetic relationships among some*Vicia* L. (Vetch) taxa by using ISSR markers **

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Abstract

The genetic relationship among some naturally growing *Vicia* taxa in Turkey (*V. sativa* subsp. *sativa*, subsp.*nigra* var.*nigra*, subsp*incisa*var.*cordata*, *V. cracca*subsp.*cracca*, subsp*gerardii*, subsp*atroviolacea*, subsps*tenophylla*, *V. hybrida*, *V. peregrina*and*V. palaestina*) was assessed by helping ISSR markers. During PCR assays, although 17 primers were tested, only three primers produced informatics amplification yields for all studied taxa. These ISSR primers had considerably high number of polymorphic locus. According to the dendogram obtained from ISSR profiles, all studied taxa were separated into two main clusters; one of them consists of the section *Vicia* and the other section *Cracca*. While the section *Vicia* comprises the subspecies of *V. sativa*, *V. hybrida*and *V. peregrina*, the section *Cracca* involve in subgroupsof *V. cracca* and *V. palaestina.*

Key words: *Vicia*, vetch, ISSR markers, Turkey

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ISSR markırlarıkullanılarakbazı*Vicia* L. (Fiğ) taksonlarıarasındakigenetikakrabalıklarınbelirlenmesi

Özet

Bu çalışmada, Türkiye'de doğal olarak yetişen bazı *Vicia* taksonları (*V. sativa* subsp. *sativa*, subsp. *nigra* var. *nigra*, subsp *incisa* var. *cordata*, *V. cracca* subsp. *cracca*, *subsp gerardii*, subsp *atroviolacea*, subsp *stenophylla*, *V. hybrida*, *V.peregrina* ve *V. palaestina*) arasındaki genetik akrabalıklar ISSR markırları yardımıyla değerlendirilmiştir. PCR denemeleri sırasında, 17 primer test edilmesine rağmen, sadece üç primer çalışılan tüm taksonlar için bilgi verici amplifikasyon ürünleri vermiştir. Seçilen ISSR primerleri tüm taksonlarda oldukça yüksek sayıda polimorfik lokus oluşturmuştur. ISSR bantlarından elde edilen dendrograma göre, incelenen taksonlardan *Vicia* seksiyonuna ait olanlar ile *Cracca* seksiyonuna ait olanlar iki alt grup olarak ayrılmıştır. *Vicia* seksiyonu alt kümesi, *V. sativa*'nın tür altı taksonları ile *V. hybrida ve V. peregrina* türlerinden; *Cracca* seksiyonu alt kümesi ise *V. cracca*'nın alt türleri ile *V. palaestina* türünden oluşmaktadır.

Anahtar kelimeler: *Vicia*, fiğ, ISSR markır,Türkiye

1. Introduction

The legumes family (Fabaceae / Leguminosae) includes 730 genera and 19400 species (Mabberley, 1997) and is considered as the third largest family of higher plants and is second to grasses in agricultural importance (Young et al., 2003). It has approximately 1128 taxa in 69 genera in Turkey; the number of endemic species and the rate of endemism are 375 and 39.1 % respectively (Davis &Plitmann, 1970; Davis,1988;Seçmen et al., 1989).

The genus *Vicia*, together with genera *Lathyrus* L., *Lens* L. and*Pisum* L.is a member of tribe *Vicieae*, divided into two subgenera, *Vicia* and *Vicilla* (Kupicha, 1976). It includes about 150 annual and perennial herbaceousspecies, widely distributed throughout the temperate zones of both hemispheres. Approximately 40 species, mainly of Eurasian origin, are cultivated (Harlan, 1956).

****Footnote:** This article was produced from master thesis.

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In Flora of Turkey, it is divided into six sections as *Cracca*,*Ervum*, *Trigonellopsis*, *Anatropostylia*, *Vicia*, and *Faba*. It is reported that *Vicia* has got totally21 varieties, 23 subspecies and 64 species in Turkey; five species and three subspecies of them is known to be endemic in that country (Davis &Plitmann, 1970). Vavilov (1951) reported that Turkey is a gene center for *Vicia sativa* species.

Classification of most of the *Vicia* taxa is not easy by using conventional taxonomical techniques.Molecular biology and gene technology are creating promising possibilities for a rapid and accurate determination of phenotypic and genetic variation among plant species (Agar et al., 2006; Uysal et al., 2012).

Many species of *Vicia* are highly variable in point of both genetically and response to environmental differences. Homologous variation is widespread (Davis &Plitmann, 1970).

V. cracca is a very polymorphic species, therefore, it is particularly difficult to distinguish between inherited variation and phenotypic plasticity in this complex. The complex is most variable in the Balkans, Anatolia and Caucasia. The five subspecies recognized for Turkey form a partly discontinuous series.. Although the *V. cracca*complex were studied biosystematicly in Europe, almost nothing is known about the cytogenetic of this group in Anatolia and Caucasia.

The cosmopolitan species, *V. sativa* is one of the most variable in point of genetic and phenotypic features within the genus. Five main taxa, at subspecific rank,can be distinguished in the complex. The variability in all taxa, or populations, of*V. sativa* is homologous, parallel and consequently overlapping. Many of thesubdivisionsare known to interbreed with each other. The species show considerable variations in almost every trait, but particularlyin leaflet morphology and in basic chromosome number (Davis &Plitmann, 1970).

By using ISSR markers, some molecular studies including genetic diversity in *Vicia*were reported in recent years (Terzopoulos&Bebeli, 2008; Noorozi et al., 2009; Han & Wang, 2010; Wang et al., 2012). In this paper, some problematic and complicated *Vicia* taxa fromTurkey were investigated in view of their genetic distance and intraspecificinteractions byhelping ISSR markers.The aim of this study was also to evaluate the genetic relationship of the some Turkish *Vicia*, particularly for sectional level, to check the concluded genetic findings how much would be compatible with Flora of Turkey.

2. Materials and methods

2.1. Plant Material

Ten *Vicia* taxa used in this study were collected mainly from different locality of Mediterranean region in Turkey.Voucher specimens were deposited at KNYA; the Herbarium of Biology Department at Selçuk University, (Table 1).

2.2. DNA extraction

Total genomic DNA was extracted following the 2xCTAB method of Doyle and Doyle (1987) as modified by Soltiset al. (Soltis et al., 1991) and Cullings (1992) from silica gel-dried leaves collected in the field.

Tablo 1. ISSR analizleriiçinkullanılan*Vicia*taksonları
Table 1.*Vicia* taxa used for ISSR analyses

Sample Number	Taxa	Locality	Collector(s) and collector's number
1	*V. sativa* subsp. *sativa*	Antalya, aroundAlanya road junction, fields, 10m.	Y.Bağcı 3240&T.Uysal
2	*V. peregrina*	Hatay, İskenderun-to Antakya, above Belen, 560m.	K.Ertuğrul 3476 &O.Tugay
3	*V. sativa* subsp.*nigra* var.*nigra*	Antalya, above Kepez, 281m.	Y.Bağcı-3255-T.Uysal
4	*V. sativa* subsp*incisa*var.*cordata*	Antalya, above Kepez, 281m.	Y.Bağcı 3250 &T.Uysal
5	*V. cracca*subsp.*cracca*	Niğde, Aladağlar, Mazmılı Mountain, 1568m.	Y.Bağcı 3382 &H.Demirelma
6	*V.cracca*subsp*gerardii*	Antalya, aroundAlanya road junction, 0-10 m.	Y.Bağcı 3242 &T.Uysal
7	*V. cracca*subsp*atroviolacea*	Yozgat, National park of Yozgat Pine Grove,1400m.	Y.Bağcı 3437 &HDural
8	*V. cracca*subsp*stenophylla*	Hatay, road of Belen, 1550m.	Y.Bağcı 3260 &T.Uysal
9	*V. hybrida*	Hatay,Antakya toYayladağ, Çabala village, 630m.	K.Ertuğrul 3525&O.Tugay
10	*V. palaestina*	Gaziantep, Fevzipaşa toOsmaniye, 5.km, 1000m.	K.Ertuğrul 3547 &O.Tugay

2.3. Inter Simple Sequence Repeats(ISSR)-PCR

Our modified ISSR-PCR analyses were basically performed according toZietkiewicz et al. (Zietkiewicz et al., 1994). During PCR-optimization reactions, some modifications were quantitativelycarried out particularly for Mg and primer amounts as well as Tm selection. The designedISSR primers by British Colombia Universitywerepreferredfor PCR-amplifications. Amplification products were separated by electrophoresis in 1.2 % agarose gel run in TAE buffer at 100 V. Fragment size was estimated by using a 100-1500 bp molecular size DNA ladder.

2.4. Data analysis

ISSR profiles were scored as present (1) or absent (0). Only reproducible bands were scored as monomorphic or polymorphic. Dendrogram was performed using NTSYS-pc version 2.1 (Rohlf, 1998).

3. Results and discussion

Totally 17 ISSR primers were tested during ISSR amplifications of *Vicia*taxa. Although nine primers previously worked in our experiments during ISSR-PCR amplifications, three primersproduced only clear informatics bandsto evaluate genetic relationship among the studied species.The obtained DNA profiles werethen manually scored for each taxa, 1 for presence and 0 for absence, to generate a data matrix. A total of 80 amplicon in the size range200-1500 bp were produced viathree different primers for ten taxa (Table 2andFig.1).

Tablo 2.*Vicia*taksonlarında ISSR varyasyonunözeti
Table 2.Summary of ISSR variation in *Vicia* taxa

Primers	Total number of bands	Total number of polymorphic bands	Size of fragments (bp)	Sequence of primers (5´- 3´)
UBC820	34	34	280-1500	GTG TGT GTG TGT GTG TC
UBC818	26	26	260-1300	CAC ACA CAC ACA CAC AG
UBC809	20	20	200-1000	AGA GAG AGA GAG AGA GG

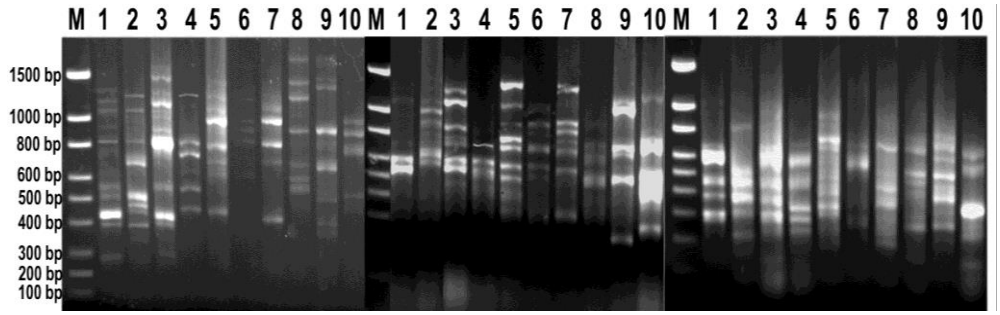

Figure1.Electrophoresis Patterns of ISSR products amplified with primers UBC820,818,809 for *Vicia* taxa
Şekil 1.*Vicia*taksonlarıiçinUBC820, 818, 809 primerleriileçoğaltılan ISSR ürünlerininelektroforezpatternleri

In dendrogram, *Vicia* taxa were clearly distinguished in level of section, species and alsosubdivisons.The dendrogramindicated that there are genetically two main group (Fig.2). The firstgroup consisted of*V. sativa* and itssub-categories, except from*V.peregrina* and *V.hybrida*.In the identification key of Flora of Turkey, these two species are separated basically by flowers color and indumentums characters existing on upper surface of the standard. Unlike *V. hybrida*, the standard of *V. peregrina* is without hair and itscolors are partly variable (violet or sometimes whitish)comparing to *V. hybrida* having sulphure yellow flowers.In spite of several differences among them, they would be evaluated relative species according to common vegetative and generative features and they take place together within the same section in conclusion.According to our molecular analyses, *V.peregrina* and *V. hybrida*were placed in the outer part of *V. sativa* complex by distance ca. 31 %. They are related with 72 % genetically. The secondgroup consists of *V. cracca*'s subspecies and *V.palaestina.V. cracca* is a perennial species and fairly polymorphic species. Itshows many variations in point of leaflets numbers, shape as well as flowers sizes and colors. *V. palestiana*is an annual species that it can be separated easily with smaller calyx than species of *V. cracca* complex. It can be sad clearly that the relationshipof two species is not very much when compared their morphologic features. *V. palaestina*took place genetically alone as compatible with its morphology, and it closely related to sister clade by rate of similarity 78 %. In the sister clade, four taxa belonging to *V. cracca* were placed by distance furthest 24 %. *V.cracca*subsp*cracca*and subsp. *atroviolacea* took place together by distance 22 %, while *V.cracca* subsp. *gerardii* and *stenophylla* were positioned as a twain by distance 18%. The subtaxaof *V. cracca* complex was classified basically

according to flowers, peduncle and raceme size besides flowers color in Turkish Flora.*V. cracca* subsp. *atroviolacea*is specified as having large flower with very dark violet and short peduncle. On the other hand, our molecular results don't support this main diagnostics coming from the past relevant to morphological descriptions.Also, the observations sourced from our field studies indicated that flowers size and color show broadly variation within *V. cracca*complexand these features could not be used certainly to identifyin lower categories of species. Instead of these, we can suggest using the ratio of flowers claw to lamina for *V. cracca* complex. The highest differentiation rate within*V.sativa*was determined as 23 % and therefore the furthest taxon within this cladewas *V. sativa* subsp. *incisa* var. *cordata*. Already, this taxon can be easily separated than other relativeswith cordate leaves. The remainingtaxa of this group were closely related and genetically differences among them were lower than previous by distance 15 %. These two taxa, *V. sativa* subsp. *sativa* and subsp. *nigra* can be recognized by fruit shape, so that the fruit formation depicted as "torulose" is very characteristic for the first taxa. As conclusion, these molecular findings support strongly sectional divisions of *Vicia* genus such in Flora of Turkey, and also coincidedexactly with results of Agar et al.(Agar et al., 2006).

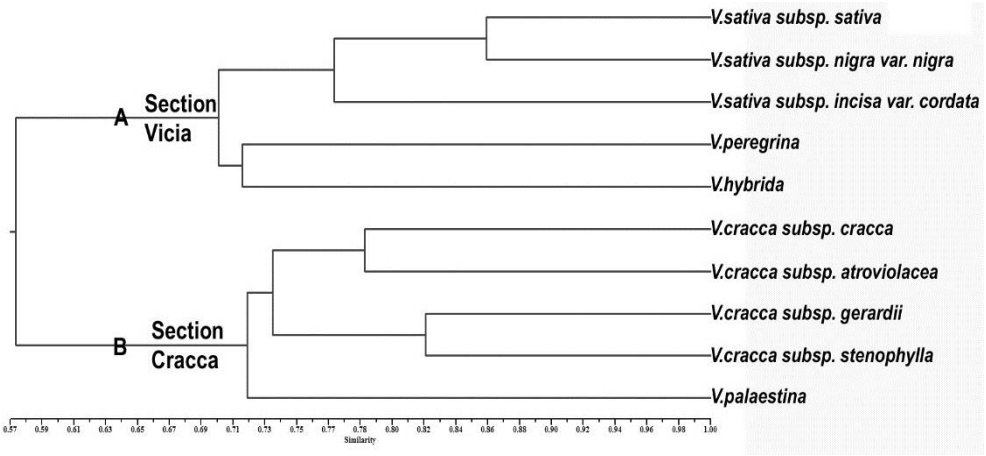

Figure 2.The dendrogram explaining the genetic relationship of the *Vicia*taxa
Şekil 2. *Vicia*taksonlarınıngenetikakrabalıklarınıaçıklayandendogram

ISSR analyses have been focal point to explore genetic diversity in many plant taxa since last decade(Sica et al., 2005; Venkatachalamet al., 2008; Han, 2010; Wang, 2012; Uysal et al.,2012) and they haveused confidentially on the contrary other molecular approaches such as RAPD. They not only assist to systematical and taxonomical studies, but can be used faithfully to develop new agronomical varieties and lines in all legumes.ISSR markers aredominant DNA markers, having high resolution power and hence appear to offer many advantages in establishing genetic distances. Therefore, we suggest that ISSR markers can be preferred firstly to select new genotypes of*V. sativa* havingagronomical importance and economic values.

According to our results, ISSR has been very useful to determine genetic differences within and among of *Vicia*species as well as sectional classifications. As a consequence, ISSRs are also very promising genetic markers for identification of Vetches cultivars. Because of their good discrimination efficiency and high reproducibility, they are particularly suitable to identify the closely related species and varieties.

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