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MOLECULAR PHYLOGENETIC RELATIONSHIPS OF SOME *Fabaceae* SPECIES THAT USED AS FORAGE CROPS IN TURKEY

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ABSTRACT

Fabaceae which is called pea family, is the 3rd largest family of Angiosperms. The family consist of over 20.000 species of 700 genera with trees, shrubs, herbs all over the World. Although, many species of the family have economical values, some species especially herbs are used as forage crops like soybeans, peas, alfa alfa, cowpea, clover, etc. in Turkey. Nowadays, molecular regions of DNA were used to clarify genetic relationships, so they were used in taxonomical studies and in breeding studies while hybridizing species to obtain economically and nutritionally valuable plants. Especially ITS (Internal Transcribe Spacer) region was mostly preferred for molecular phylogenetic studies due to its highly repeated in number in plant genomes and large copy numbers that support PCR amplification. In the study, related sequences were obtained from NCBI database and statistics were analyzed in MEGA X software. Phylogenetic tree was constructed via BEAST program package. According to the phylogenetic tree, species were divided into 2 main clusters. One of the clusters composed of *Atriplex* and *Amaranthus* genus which were the members of outgroup family *Amaranthaceae*. Although *Astragalus* and *Onobrychis* genus were located at same sub branch, *Pisum*, *Lathyrus* and *Vicia* genus were positioned close to each other. Therefore, genetic distances among genus and species were clearly identified by checking the phylogenetic tree. Conclusively, both molecular and classical breeding methods could be efficiently applied together for these forage crops in future breeding studies.

Keywords: *Fabaceae*, breeding, phylogeny, ITS, DNA

TÜRKİYE'DE YEM BİTKİSİ OLARAK KULLANILAN BAZI *Fabaceae* TÜRLERİNİN MOLEKÜLER FİLOGENETİK ANALİZLERİ

ÖZ

Baklagiller olarak da bilinen *Fabaceae* ailesi 20.000'den fazla tür ve 700 cins ile *Angiosperm*'lerin 3. büyük familyasıdır. Türkiye'de de yem bitkisi olarak kullanılan soya fasulyesi, bezelye, yonca, korunga gibi familya üyeleri tüm Dünya'da çalılık, ağaç, makilik şeklinde yaklaşık her bölgede yayılış göstermektedirler. Son yıllarda yapılan denemelerde DNA'ya ait bazı bölgeler bitkilerde genetik mesafelerin ölçümü için kullanılmaktadırlar. Ayrıca bu genetik farklılıklar bazı önemli türlerin yaban ve kültür çeşitlerinin melezlenmesi ile daha besleyici daha dirençli yeni bitkiler üretilmesi için kullanılmaktadırlar. Özellikle son yıllarda moleküler filogenetik çalışmalarda kopya sayılarının çokluğu, PZR çalışmalarında kolay elde edilebilmesi sebebiyle ITS (Internal Transcribe Spacer) bölgesi sıklıkla kullanılmaya başlanmıştır. Bu çalışmada, NCBI veri bankasından alınan ve Türkiye'de yem bitkisi olarak kullanılan 33 türün ITS gen bölgelerine ait sekanslar MEGA programı vasıtasıyla analiz edilmiş ve BEAST paket programları kullanılarak da filogenetik ilişki ağacı çizdirilmiştir. Sonuçta 2 ana gruba ayrılan türlerden *Atriplex* ve *Amaranthus* cinslerine ait türler *Amaranthaceae* ailesine ait bir dış grup oluştururken, diğerleri farklı bir grup oluşturmuşlardır. Özellikle *Astragalus* ve *Onobrychis* cinsleri bir alt grup oluştururken, *Pisum*, *Lathyrus* ve *Vicia* cinslerine ait türlerin yakınlığı önemli derecede açıklayıcı ve dikkat çekicidir. Tüm bunlar ele alındığında bu genetik mesafeler gelecekte yapılacak moleküler ıslah çalışmalarında destekleyici ve kolaylaştırıcı bilgiler içermektedir.

Anahtar Kelimeler: *Fabaceae*, ıslah, filogeni, ITS, DNA

INTRODUCTION

Fabaceae is the 3rd largest family of Angiosperm [13] which consists of over 20.000 species of 700 genera with trees, shrubs, vines and herbs all over the World [12]. Legumes identified easily with their remarkable flower, podded fruit and the capacity of 88% of the species checked out

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to date to form nodules with rhizobia [4, 8, 14]. These family includes significant grain, pasture and agroforestry species that used mostly as forage crops like soybeans, peas, alfa alfa, cowpea, clover, etc. These forage plants have significant role on animal and human nutrition directly and indirectly as well as yield and quality improving elements in acquisition of animal brand [16]. Although, these plants have significant roles in animal nutrition with 38% of protein needs, 22% of the vegetable proteins in humans are obtained from these plants worldwide [15, 18, 1].

In recent years, molecular regions of DNA were properly used to clarify genetic relationships among plant species and belong families. These genetic similarities or differences were used both taxonomic studies and in breeding studies to obtain economically and nutritionally valuable plants. The genetic diversification of germplasm collections plays a significant role in conservation and preservation of wild and cultivated plants; therefore, these are especially helpful for the improvement of new potential crops [3]. Especially ITS (Internal Transcribe Spacer) region from genomic DNA was mostly preferred for molecular phylogenetic studies due to its highly repeated in number in plant genomes and large copy numbers that support amplification [17, 9].

In the current study, available sequences of ITS (ITS1 + 5.8S + ITS2) regions of some important forage crops in Turkey were studied to understand phylogenetic relationships of these species which could be helpful for examining future breeding studies both wild and cultivated crops.

MATERIALS AND METHODS

Materials

Totally 33 individuals of species were chosen which 29 of them belong to *Fabaceae* family and 4 of them belong to *Amaranthaceae* family which used as outgroup (close family) were chosen due to accessibility of DNA sequences from NCBI (National Center of Biotechnology Information) databank (Table 1).

Methods

ITS sequences were obtained from NCBI and were aligned MEGA (Molecular Evolutionary Genetics Analysis) X software [11]. All related statistics (total nucleotide length (base pairs), GC contents (%), conserved and variable sites, parsimony informative sites, and overall genetic diversity) were analyzed by MUSCLE (Multiple Sequence Comparison by Log Expectation) tool [7] of MEGA X software. Phylogenetic tree (Figure1) was constructed by using BEAST (Bayesian Evolutionary Analysis by Sampling Trees) program package [2] with maximum likelihood (ML) and Bayesian inference (BI). Completing Markov chain Monte Carlo (MCMC) algorithms is intended and the maximum clade credibility (MCC) tree was burned under 10.000 times and these trees were summarized in Tree Annotator v1.8.0 with a posterior probability limit of 1 [5].

Table 1. NCBI accession numbers of studied species

Çizelge 1. Çalışılan türlerin NCBI veri bankası erişim kodları

Samples *Örnekler*	Accession number *Erişim kodlar*
Amaranthus hybridus	KP318857.1
Amaranthus palmeri	KF385438.1
Astragalus cicer	JQ685631.1
Astragalus gummifer	JX284079.1
Astragalus nitens	JQ685690.1
Atriplex centralasiatica	DQ086481.1
Atriplex lentiformis subsp. *lentiformis*	AY873929.1
Glycine max	EF517917.1
Lathyrus sativus	AY839389.1
Lupinus albus	DQ524191.1
Lupinus angustifolius	DQ524193.1
Medicago constricta	KX027592.1
Medicago rigiduloides	KX027591.1
Medicago sativa	AF053142.1
Onobrychis arenaria	LC137056.1
Onobrychis meshhedensis	LC137022.1
Onobrychis transcaucasica	HM542538.1
Onobrychis viciifolia	KY366168.1
Pisum sativum var. *arvense*	AY143465.1
Trifolium buckwestiorum	AF053148.1
Trifolium gordejevii	KX254380.1
Trifolium incarnatum	AF053160.1
Trifolium kingie	KX254379.1
Trifolium lupinaster	KX254386.1
Trifolium pacificum	KX254388.1
Trifolium pretense	EU348780.1
Vicia faba	JX506216.1
Vicia narbonensis	HM470591.1
Vicia pannonica	JX506268.1
Vicia sativa	JX506281.1
Vicia villosa	JX506308.1
Vigna sinensis	AY195581.1
Vigna unguiculata subsp. *Unguiculata*	AY195581.1

RESULTS

Total length of the regions was 744 bp (with gaps) and 291 of them were conserved. 300 of the variables were parsimony informative and overall genetic divergence among studied species were calculated as 0,179. The results of computing genetic diversity among genus was indicated that although the nearest species were *Lathyrius*, *Vicia* and *Pisum* species, the most different species were Vigna and Glycine (Table 2). Moreover, constructed phylogenetic trees was drawn by using BEAST program via Jukes Cantor (JC) parameters.

According to phylogenetic tree based on ITS DNA sequences (Figure1), species divided into 2 major groups. One of the major groups which was outgroup family were consist of *Atriplex* and *Amaranthus* genus. Another major group, *Fabaceae* family was divided into 2 sub-groups and *Vigna* genus was separated from others with high probability value. Other subgroup was consisted of other studied genus (Glycine, *Trifolium*, *Medicago*, *Vicia*, *Pisum*, *Lathyrus*, *Onobrychis*, *Astragalus*). Although all these genera were seemed like very different than each other, especially *Pisum sativum* and *Lathyrus sativus* species were located very close to each other. Moreover, they were positioned very near to *Vicia species* rather than others. Even if *Medicago* genus was seemed different from others, they were closely positioned under same subbranch with *Vicia*, *Pisum* and *Lathyrus* genus.

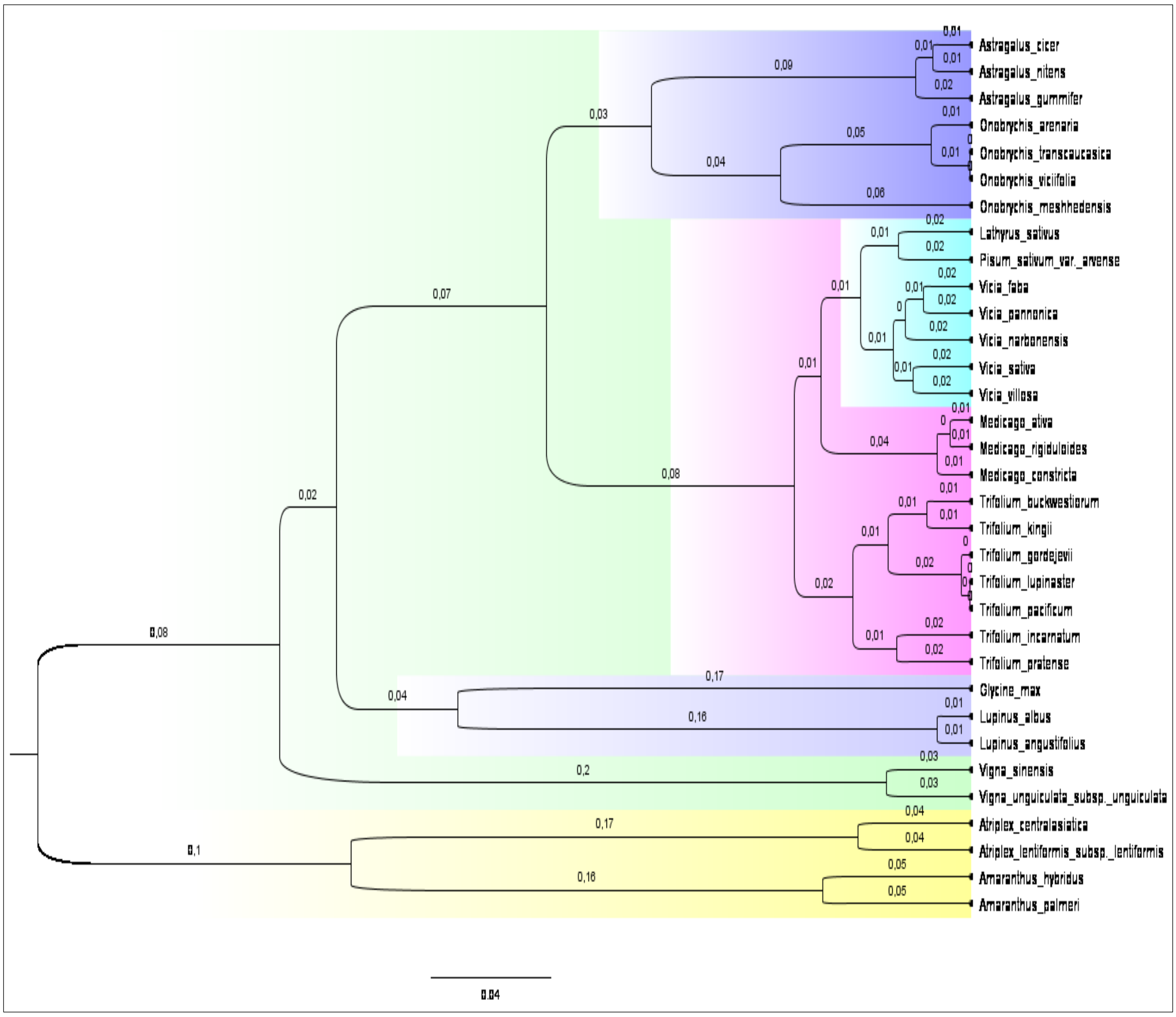

Figure1. Bayesian consensus tree, from ITS gene region of 33 species. Posterior probabilities (PP) are given above each branch. PP values were obtained from 10000 trees, ML values replicates with subsequent maximum likelihood optimization.

Şekil 1. 33 türe ait ITS gen bölgesine dayalı Bayesiyan filogenetik ağacı. Olasılık değerleri (PP) en fazla yakınlığın (ML) ölçülmesi için 10000 tekrarla çizdirilmiş ve her dal için verilmiştir.

Table 2. Genetic distances among species of *Fabaceae* family which was analyzed via MEGA X software

Çizelge 2. MEGA X programı ile analiz edilmiş Fabaceae ailesine ait türler arası genetik mesafeler

Genetic diversity among *Fabaceae* family *Fabaceae ailesi arasındaki genetik mesafeler*	*Astragalus cicer*	*Glycine max*	*Vicia faba*	*Vigna sinensis*	*Onobrychis viciifolia*	*Pisum sativum var. arvense*	*Lupinus angustifolius*	*Lathyrus sativus*	*Medicago rigiduloides*
Astragalus cicer									
Glycine max	0,313								
Vicia_faba	0,191	0,317							
Vigna_sinensis	0,376	0,398	0,321						
Onobrychis viciifolia	0,175	0,319	0,206	0,389					
Pisum sativum var. arvense	0,194	0,317	0,052	0,326	0,212				
Lupinus angustifolius	0,256	0,279	0,268	0,386	0,243	0,279			
Lathyrus sativus	0,198	0,319	0,036	0,325	0,217	0,040	0,272		
Medicago rigiduloides	0,171	0,322	0,067	0,343	0,217	0,075	0,270	0,073	
Trifolium lupinaster	0,167	0,309	0,060	0,328	0,216	0,071	0,244	0,064	0,069

DISCUSSION

In the current study total length of the regions was obtained as 744 bp (with gaps) and 453 of them were variable. This result was looks different but normal because although these 29 species were the member of same family, they were different genus, so, it could be said that this variability was normal. Moreover, the overall genetic diversity was 0,216 means that neither these family members were different than each other but also genetically not so far.

There are many different molecular phylogenetic studies on *Fabaceae* family members in Turkey due to their economically and scientifically importance. The family includes important grain, pasture and agroforestry species that used mostly as forage crops like soybeans, peas, alfa alfa, cowpea, clover, etc. This means that these forage plants have significant role on animal and human nutrition directly and indirectly as well as yield and quality improving elements in acquisition of animal brand [16]. In the phylogenetic tree, *Vicia*, *Pisum* and *Lathyrus* species were seemed to be genetically closer to each other rather than *Lupin species*. In 2016, Koivunen et al. [10] were reported that apparent metabolizable energy (AME) and coefficient of apparent ileal digestibility (CAID) were very important in animal nutrition. They compared *Vicia faba*, *Pisum sativum* and *Lupinus angustifolius* cultivar to check AME and CAID values. As a result, it was found that *Pisum sativum* was a good source of energy than *Lupinus angustifolius*, however lupin species was well digested than others. Moreover, *Vicia species* had moderate on energy source but also well digest at some amino acids. If the results of molecular data in the current study was combined with the Koivunen's study, it could be said that these species features could be combined to solve both AME and CAID problems in animal nutrition. Especially in modern technology there were many new methods for breeding studies like protoplast fusion techniques and there will be found new forage crop which will be the combination of these two or three. Furthermore, in 2014 Tsegay and Gebreslassie [19] were indicated that high salt level effected germination of early seedlings of *Fabaceae* family members. After experiments they found that some crops have low salt tolerant especially *Pisum sativum* than others like *Lathyrus sativus*. As a result, it could be said that these high resistant genes might be used for low tolerant species and new generation will be more resist to salt in early seedling stages. Moreover, there are some studies to increase genetic variability. In 2000, Durieu and Ochatt [6] were tried to fuse protoplasts of wild relatives of both pea and grass pea. They were tried to adapt new cells to produce new valuable and resistant individuals for forage crops. However, they couldn't continue their experiments in field studies. Also, after the phylogenetic tree which was constructed in the current study, some new protoplast fusion techniques and continued field studies will be tried.

CONCLUSION

Consequently, it could be said that, molecular phylogenetic studies will be helpful for determining the genetic distances between related and near species of same family to create new valuable hybrids. Also, wild relatives of these cultivars may solve crucial problems in field studies based on climate stress conditions. Moreover, for animal and human nutrition, improving the quality of amino acids for energy is an important topic at these days. Therefore, knowledge of genetic distances among crucial forage crops will be helpful for molecular breeding studies that creates more digestible and richer in useful amino acids for energy species.

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