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Characterization of *Consolida* S.F. Gray (Ranunculaceae) taxa in Turkey by seed storage protein electrophoresis

[Tohum Depo Protein Elektroforezi ile Türkiye'deki *Consolida* S.F. Gray (Ranunculaceae) taksonlarının Karakterizasyonu]

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ABSTRACT

Objective: This research aimed to investigate the genetic relations of *Consolida* S. F. Gray species collected in Turkey using seed storage proteins. This study has become significant as the taxonomic status of genus *Consolida* changes often.
Methods: Mature seeds of 22 *Consolida* species were collected. Total protein were isolated from seeds collected for each species. Sodium Dodecyl Sulphate-Polycarylamide Gel Electrophoresis was performed by a standard method on a vertical slab gel. After electrophoresis, the protein bands were visualized by staining with Coomassie Brillant Blue G-250. The polymorphic bands were scored visually as present (1) or absent (0). Genetic similarity among taxa was estimated basing on Nei's homology.
Results: In all, 74 polypeptide bands of different sizes ranging from 25 to 140 kDa were observed in the 22 taxa of the genus *Consolida*. Cluster analysis was performed using the unweighted pair-group method and arithmetic averages in Bio1D++ computer program. According to similarity coefficients, the genus *Consolida* was separated into the main two groups. It was discovered that the first group consisted of the species of *Consolida* with 5-lobed corolla, and in the second main group were the species with 3-lobed corolla.
Conclusion: It is concluded that seed storage protein profiles could be useful markers in the studies of genetic relations of *Consolida* species. The grouping as a result of the dendogram has also supported the relationship in Flora of Turkey.
Key Words: *Consolida*, SDS-PAGE, genetic relation, total proteins

ÖZET

Amaç: Bu çalışmada tohum depo proteinleri kullanılarak Türkiye'den toplanan *Consolida* S. F. Gray türlerinin genetik akrabalıklarının incelenmesi amaçlanmıştır. *Consolida* cinsinin taksonomik durumu sık sık değiştiği için, bu çalışma önemli hale gelmiştir.
Metot: Yirmi iki *Consolida* türünün olgun tohumları toplandı. Her bir tür için toplanan tohumlardan total protein izole edildi. Sodyum Dodesil Sülfat-Poliakrilamid Jel Elektroforezi, dikey jelde standart bir metot ile uygulandı. Elektroforezden sonra, protein bantları Coomassie Brillant Blue G-250 ile boyanarak görünür hale getirildi. Polimorfik bantlar görsel bir şekilde değerlendirilerek var (1) veya yok (0) olarak skorlandı. Taksonlar arasındaki genetik benzerlik Nei'nin homolojisine dayanarak tahmin edildi.
Bulgular: *Consolida* cinsinin 22 taksonunda, toplamda 25'den 140 kDa'a kadar değişen farklı büyüklüklerde 74 polipeptit bant gözlenmiştir. Bio1D++ bilgisayar programında ağırlıksız çift-grup yöntemi ve aritmetik ortalamalar kullanılarak kümeleme analizi yapılmıştır. Benzerlik katsayılarına göre, *Consolida* cinsi iki ana gruba ayrılmıştır. Birinci grubun 5 loblu korollaya sahip *Consolida* türlerini ve ikinci grubun 3 loblu korollaya sahip türleri içerdiği bulunmuştur.
Sonuçlar: Tohum depo protein profillerinin *Consolida* türlerinin genetik akrabalık çalışmalarında faydalı belirteçler olarak kullanılabileceği sonucuna varılmıştır. Dendrogram sonucundaki gruplaşma Türkiye Florasındaki akrabalığı da desteklemiştir.
Anahtar Kelimeler: *Consolida*, SDS-PAGE, genetik akrabalık, total proteinler

Introduction

Ranunculaceae family, which is distributed almost all over the world, is represented by 59 genera and 2500 species. While the family is represented the most densely in the Far East by 44 genera, it is known from Europe by 24 genera and North America by 24 genera. Although the geographical distribution of the family is very old, the distribution of some genera having advanced characters is quite new. Since many genera of Ranunculaceae family have beautiful flowers, they are cultivated as indoor plants. Moreover, several genera are used as medical plants as they contain substances with pharmacological activities (1).

The Genus *Consolida* was represented by 26 species in Turkey before, but are now represented by 29 species with the new arrangements (2). Fourteen of these are endemic. Some *Consolida* species are grown not only as fresh and dried but also as seasonal outdoor flowers (particularly *Consolida orientalis* (Gay) Schröd. and *Consolida ambigua* L.) (3,4) and some *Consolida* species are used as medical plants as they contain chemical compounds such as alkaloids (5).

The genus *Consolida* was treated as a group in genus *Delphinium* over years. But in 1821 Gray, who worked on Flora British, raised *Consolida* to species level based on only *C. regalis* (L.) S.F. Gray species. In Flora Europe all species with single petal and single follicule of *Delphinium* were transferred to *Consolida* (6). In Flora Europae (12 species) (6), Flora of Balkan (12 species) (7), Flora Iranica (28 species) (8), Flora Hellenica (10 species) (9) and Flora of Turkey (28 species, 1 subsp) (10), genus *Consolida* have been reported as a different genus from genus *Delphinium.* But many researchers (11,12,13) have reported *Consolida* as a subgenus or a section in genus *Delphinium* in Flora of USSR (14), Lebanon and Syria Flora (15). In Flora Iranica *Consolida* is regarded as a different genus and has two subgenera *Aconitella* and *Consolida*. Five species belonging to *Aconitella, and* 23 species belonging to subgenus *Consolida* were listed (8).

Morphological markers are not sufficient to solve taxonomic problems (16). Thus, a new method has been required for the discrimination of highly morphologically similar species. Electrophoretical methods are indirect analytical tools as they analyse proteins reflecting structural variations of enzymes and other protein genomes (17,18,19). Electrophoretical markers are more advantageous than morphological methods in identification of species with some properties such as being rapid, cheap and not being affected by the growth environment. Therefore, by using preserved proteins such as seed storage proteins as electrophoretical markers, some disadvantages like morphological characters being affected by environmental conditions could be overcome (20).

Although a considerable number of 29 taxa in Turkey are endemic and some of the taxa are rare and economically valuable plants, there is not any detailed study done by molecular and biochemical methods until now. The aim of this study is to determine the genetic relationship of 22 species belonging to genus *Consolida* by using SDS-PAGE method.

Materials and methods

Plant Material

For seed protein profiles, specimens of 22 species belonging to genus *Consolida* were examined. The studied species and their locations were given in Table 1.

Protein extraction

Protein extraction was performed according to Saraswati et al. (21). Seeds were ground to fine powder with mortar and pestle. Sample buffer was added to 0.04 g of seed flour as extraction liquid and mixed thoroughly in Eppendorf tube with vortex. The extraction buffer contained the following final concentration: 0.5M Tris-HCl (pH 6.8), 10 % sodium dodecyl sulphate (SDS), urea and 5% 2-merkaptoethanol. Before centrifugation at 10.000g for 5 min (4˚C), the sample buffer was boiled for 5 min.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed by a standard method on a vertical slab gel. Bromophenol blue was added to the supernatant as tracking dye to watch the movement of protein in the gel. Seed protein was analysed through slab type SDS-PAGE using 10% polyacrylamide gel (22). After electrophoresis, the protein bands were visualized by staining with Coomassie Brillant Blue G-250. Marker proteins (Fermentas) were used as references. Molecular weights of protein bands were estimated by their relative mobilities.

Data analysis

The polymorphic bands were scored visually as present [1] or absent [0]. Genetic similarity among species was estimated based on Nei homology using Bio1D++ computer programme. Cluster analysis was performed using the unweighted pair-group method and arithmetic averages (UPGMA) (23).

Result

Total seed proteins belonging to *Consolida* taxa were analysed through the slab type SDS-PAGE using 10% polyacrylamide gel. According to the results of the SDS-PAGE, the overall patterns of seed storage proteins show high degree of heterogeneity in inter-species. Similarity coefficient of 22 *Consolida* taxa ranged between 33% and 86%. In all, 74 polypeptide bands of different sizes ranging from 25 to 140 kDa were observed in the 22 taxa of the genus *Consolida* (Figure 1). In all *Consolida* species the protein bands, which are almost common, were determined in sizes ~130kDa, ~100kDa and ~32kDa.

In the dendogram obtained according to Nei's homology (Figure 2), two main groups occurred far from each other by 77%. It was discovered that the first group (inclu-

Table 1. Different taxa of *Consolida* and their localities. (E: Endemic to Turkey, LC: Least concern, CR: Critically endangered, EN: Endangered, VU: Vulnerable).

Taxa	Plant numbers	Locality
C. stenocarpa (P. H. Davis&Hossain) P. H. Davis (E) (LC)	Ertuğrul 2459	C4 Konya: Karaman Ayrancı Kayaönü plateau, steppe 1550m. 23.06.2001
C. scleroclada var. *rigida* (Boiss.) Schröd.	Ertuğrul 2893 Tugay	C8 Diyarbakır: Silvan to Diyarbakır, 10th km, field sides, 690m , 03.07.2003
C. thirkeana (Boiss.) Schröd. (E) (LC)	Ertugrul 2929a Tugay	A4 Çankırı: 5 km N of Çankırı, road sides steppe, 770 m,, 19.07.2003.
C. hohenackeri (Boiss.) Grossh.	Ertuğrul 2529	A8 Erzurum: Uzundere-Erzurum road 2510th km, road side, 1000m. 23.07.2001
C. saccata (Huth) P. H.Davis (CR)	Ertuğrul 2902 Tugay	C8 Mardin: Bakırkırı, road side, 1450m. 04.07. 2003
C. orientalis (Gay) Schröd.	Tugay 1587	C4 Konya; Hadim, Korualan village, field side, 15800 m, 09.06.2001.
C. phrygia (Boiss.) Soó (E) (CR)	Uysal 615	B2 Balıkesir: Dursunbey, Güğü Village road, slopes, on the rock,404 m. 27.05.2004
C. thessalonica (Soó) Ertugrul &Tugay	İlarslan	A1 Edirne: Paşaçayı location, field sides. 35m. 17.05.2001
C. regalis S.F.Gray subsp. *paniculata* (Host.) Soó	Ertugrul 2755	C4 Karaman: Karaman to Kılbasan village, 10th km, fields,1000 m, 21.08.2002
C. divaricata (Ledeb.) Schröd.	Ertuğrul 2599 Tugay	A8 Iğdır: Aralık, fields of Kazım Karabekir DÜÇ, 1100m, 12.06.2002
C. stapfiana P. H.Davis & Sorger (E) (EN)	Tugay 4184 Uysal	C3 Antalya: Korkuteli to Elmalı, 15th km. 1340m. 23.07.2006
C. glandulosa (Boiss.&Huet) Bornm. (E) (LC)	Ertuğrul 2499	B7 Erzincan: Girlevik Waterfall road, Molla Village exit road, 1200m. 21.07.2001
C. oliveriana (DC.) Schröd.	Ertuğrul 2591 Tugay	B7 Elazığ: İçme small town, Şeyh Hacı Village, Yukarubağ location, garden sides, 1150m. 09.06.2002
C. axilliflora (DC.) Schröd.	Ertuğrul 2704 Tugay	C8 Diyarbakır: Mardin road, Kırmasırt entry, crop field side, 700m. 16.06.2002
C. cruciata (Davis&Hossain) Davis (E) (CR)	Ertuğrul 2853 Tugay	C4 İçel: Silifke, Uzuncaburç, Delikılıç neighborhood, field side, 1150m. 29.06.2006
C. raveyi (Boiss.) Schröd. (E) (LC)	Ertuğrul 2458	C4 Konya: between Karaman and Ayrancı, Kayaönü Village, 1500m, 23.06.2001
C. persica (Boiss.) Schröd.	Ertuğrul 2891 Tugay	C7 Şanlıurfa: Ceylanpınar D.Ü.Ç., Şıhhani Göçeri location, 454m. 03.07.2003
C. sulphurea (Boiss. & Hausskn.) Davis	Ertuğrul 2879 Tugay	C6 Adıyaman: Besni to Gölbaşı, 10th km, Kale , Körpınar location, *Vitis* opennesses, 845m. 02.07.2003
C. hellespontica (Boiss.) Chater	Ertuğrul 2834 Tugay	B3 Eskişehir: Eskişehir to Kütahya, 50th km, steppe, 956m. 19.06.2003.
C. staminosa Davis & Sorger (E) (EN)	Ertuğrul 2466	C5 Niğde: The Çaykavak pass, 1500 m, 14.07.2001
C. armeniaca (Stapf ex Huth) Schröd. (E) (VU)	Ertuğrul 2504	B7 Erzincan: Kelkit-Erzincan road, Ağıl Village around, 1700m. 21.07.2001
C. lineolata Hub.-Mor. (E) (EN)	Ertuğrul 2753 Tugay	C4 İçel: between Uzuncaburç and Silifke, 3th km, *Vitis* garden side, 1000m. 22.07.2002

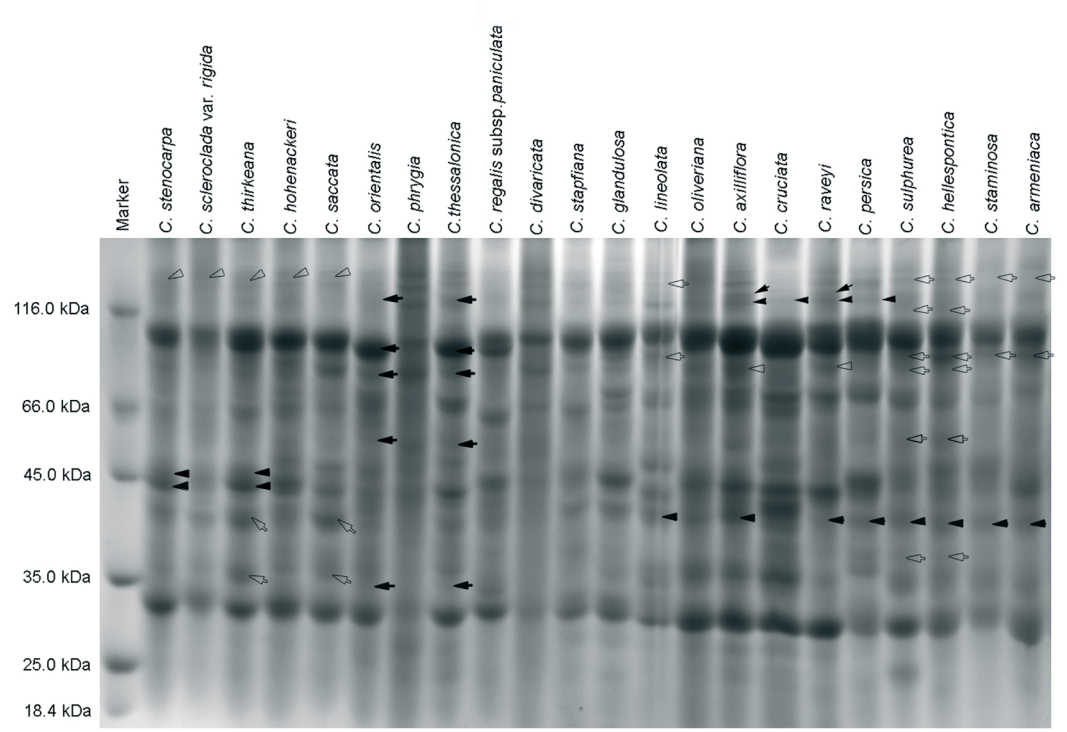

Figure 1. SDS-PAGE protein profiles of different taxa of *Consolida.* The two from common bands of *C. stenocarpa* and *C. thirkeana* species are shown by dark arrow heads, the two from common bands of *C. thirkeana* and *C. saccata* species are shown by pale arrows and the species of *Consolida* with 5-lobed corolla (including the first five lines) are shown by pale arrow heads. Common bands of *C. orientalis* and *C. phrygia subsp. thessalonica* species are shown by dark arrows. Common and non-common bands of *C. lineolata*, *C. sulphurea, C. hellespontica, C. staminosa* and *C. armeniaca* species are shown by pale arrows and dark arrow heads. Common and non-common bands of *C. axilliflora, C. cruciata, C. raveyi* and *C. persica* species are shown by dark arrows and dark arrow heads.

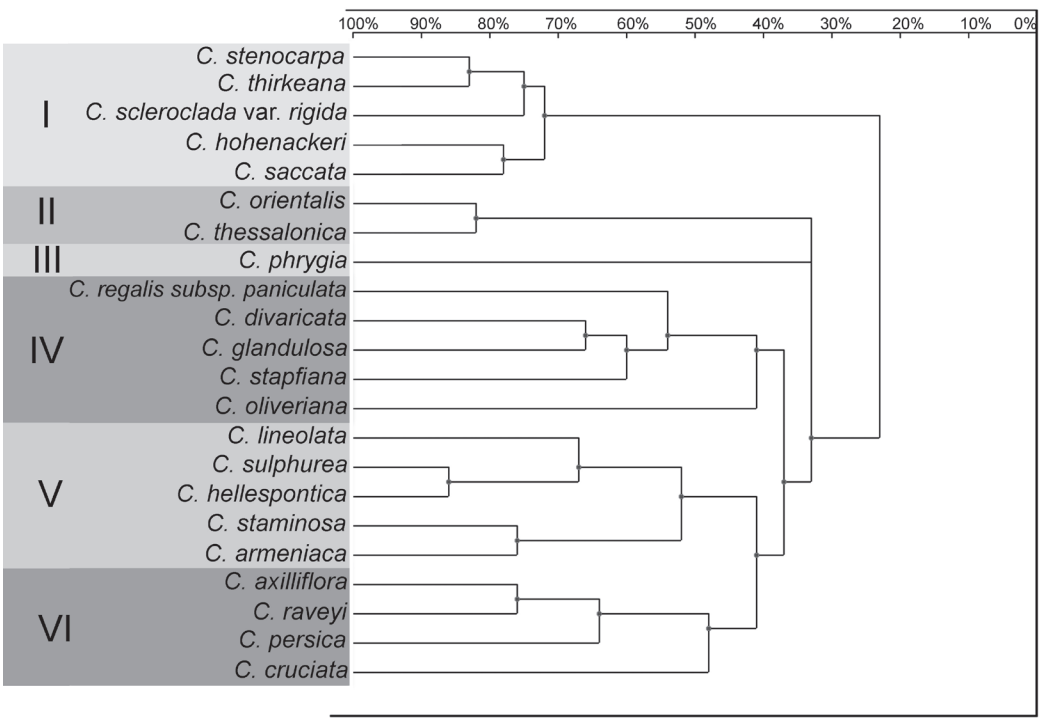

Figure 2. UPGMA dendrogram showing genetic homology among taxa of the genus *Consolida.*

ding group I.) consisted of the species of *Consolida* with 5-lobed corolla, and in the second main group (including groups II. III. IV. V. VI.) were the species with 3-lobed corolla. While the members of the first group showed genetic relationship in different rates ranging between 73% and 84% (only one of the common bands in Figure 1 is shown at 128 kDa by pale arrow heads), between the members of other groups (II. III. IV. V. VI.) high variation was observed. Groups II and III are 34% far from groups IV. V. VI. *C. phrygia subsp. phrygia,* a member of group III, is closer to group II, which contains *C. orientalis* and *C. phrygia subsp. thessalonica* (only one of the common bands of the two species are shown by dark arrows in Figure 1) that are close to each other by 82%.

Members of groups IV, which contain species *C. regalis* subsp. *paniculata*, *C. divaricata*, *C. glandulosa*, *C. staphiana* and *C. oliveriana*, show morphological similarity as spur is longer than corolla and follicules are semi-compressed. *C. divaricata* and *C. glandulosa* were found to be the closest species to each other by 67%. *C. staphiana* is close to them by 61%, *C. regalis* subsp. *paniculata* is close to those three by 54%, and *C. oliveriana* is the farthest species of group IV with 41% similarity rate.

Groups V and VI are scarcely close to each other by 41%. In group V, formed by species with corolla that are slightly 3-lobed *C. lineolata*, *C. sulphurea*, *C. hellespontica*, *C. staminosa* and *C. armeniaca* take place (In Figure 1, two of the common bands are shown by pale arrows). While *C. sulphurea* and *C. hellespontica* are observed as the closest relative by 86% in this group (In Figure 1, some of the common bands are shown by pale arrows), C. *lineolata* was discovered to be similar to them by 66%. The three species are also morphologically similar with characteristics of their fruits with recurved pedicels. *C. staminosa* and *C. armeniaca,* which are relative to each other by 76%, are close to them by 52%. The two species differ in only with fruits having recurved pedicels, although they are morphologically quite similar.

And group VI, which contains species with common characteristics such as their flowers without pedicel or with short pedicel, is figured in the dendogram as a relative to group V instead of forming a clad except members of groups II, III, IV and V, where the flowers have pedicel (In Figure 1, common bands are shown at 42 kDa by dark arrow heads). We can explain this with the fact that proteins reflecting morphological characteristics do not form remarkable bands. *C. persica*, *C. raveyi*, *C. axilliflora* and *C. cruciata* take part in group VI. These species were discovered as similar with rates ranging between 75% and 48%. *C. axilliflora* and *C. raveyi* are figured with the highest resemblance rate (75%), (In Figure 1, only one of the common bands is shown in dark arrow at 120 kDa). *C. cruciata* with 52% is the farthest species.

Discussion

Although no biochemical study has ever been encountered about genus *Consolida,* in the studies conducted good markers have been observed in calculating at different levels taxonomic and phylogenetic relationship of seed storage proteins. It was advocated that seed storage proteins in individual accession, species or genera, which show morphologically major difference, were useful to determine phylogenetic positions or incorrect taxonomic assessment (24). Therefore, seed proteins have been used to solve various systematic problems in angiosperms.

Some researchers have reported that they solved the relationship of species in plant taxonomy by using protein profiles, which have been valuable in recent years, with DNA based methods (25, 26). Celebi et al (27) evaluated seed proteins with RAPD markers in order to clarify the taxonomic relationship of species belonging to genus *Ebenus*. The result shows that the seed protein profiles are distinct between the species. Also some researchers used protein profiles and numerical taxonomy study or agronomic characters both in interspecies relationship and in selection of desirable genotypes to be used in breeding programmes and thus it were determined that both methods correlated with each other (28, 29, 30).

Those methods have been evaluated mostly as trustable, fast and easy biochemical methods by molecular biologists since 1970. This study has become significant as the taxonomic status of genus *Consolida* changes often. The grouping as a result of the dendogram has also supported the relationship in Flora of Turkey. Consequently, SDS-PAGE has given quite convenient results in the classification of species belonging to genus *Consolida* but, the phylogeny of species of *Consolida* will be clarified provided that this kind of study is also supported by molecular studies based on DNA.

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