

# Taxonomic Relationships in Turkish *Verbascum* L. Group A (Scrophulariaceae): Evidence from SDS-PAGE of Seed Proteins and a Numerical Taxonomic Study

[Türkiye'deki *Verbascum* L. Cinsi Grup A (Scrophulariaceae) İçindeki Türlerin Taksonomik İlişkileri: Kanıtlar Tohum Proteinlerinin SDS-PAGE Özellikleri ve Nümerik Taksonomik Bir Çalışma]

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## ABSTRACT

**Objective:** The aim of this study is to clarify the taxonomic relationships of Turkish *Verbascum* species in Group A by means of seed protein profiles and numerical taxonomic study.

**Methods:** Cluster analysis was carried out by UPGMA on the Nei genetic similarity coefficient using seed protein profiles. A numerical taxonomic study has been conducted on taxonomically problematic species such as *V. brachysepalum*, *V. orientale*, *V. flabellifolium*, *V. trapifolium*, *V. dudleyanum* and *V. pyroliforme* in order to elucidate their taxonomic relationships. For this reason, 14 OTUs (Operational Taxonomic Units) were investigated and scored on the basis of 20 character states by means of using MVSP software.

**Results:** In this study, all *Verbascum* species in the Group A in Turkey were investigated by means of morphological, SDS-PAGE and a numerical taxonomic method. The total number of protein band positions observed for each species between 5 and 13. The most similar band pattern was observed in the region of 18 kD – 14.4 kD. One band around 14 kD observed among all species studied and an 18 kD were found most of the species. Genetic distances among species were ranged between 0% and 58%. The results of the numerical taxonomic study are supported both morphologic and SDS-PAGE analysis.

**Conclusion:** Depending on the seed protein profiles, numerical taxonomy and morphological characters, our results clarified the taxonomy of Turkish *Verbascum* (Group A) species. The results of the studies were in broad agreement to the arrangement of these species in Revision of the Genus *Verbascum* L. (Group A) in Turkey. As well as, their expanded and amplified morphological characters were given for the first time.

**Key Words:** *Verbascum*, Group A, seed protein profiles, numerical taxonomy, Turkey

## ÖZET

**Amaç:** *Verbascum* cinsi taksonomik açıdan problemli ve Türkiye'nin en büyük ikinci bitki cinsidir. Bu çalışmanın amacı tohum protein profillerini ve nümerik taksonomik metodları kullanarak Türkiye'deki *Verbascum* Grup A türlerinin taksonomik ilişkilerini aydınlatmaya çalışmaktır.

**Metod:** Tohum protein profilleri kullanılarak UPGMA algoritması altında ve Nei genetik benzerlik katsayısı kullanılarak kümeleme analizi yapılmıştır. Ayrıca, taksonomik açıdan problemli olan türler üzerine, örneğin *V. brachysepalum*, *V. orientale*, *V. flabellifolium*, *V. trapifolium*, *V. dudleyanum* and *V. pyroliforme*, onların taksonomik ilişkilerini aydınlatmak için nümerik taksonomik bir çalışma uygulanmıştır. Bu sebeple bu türleri temsilen 14 örnek (OTUs) seçilmiş ve onların taksonomik öneme sahip 20 karakteri ölçüldükten sonra MVSP yazılımı kullanılarak kümeleme analizi yapılmıştır.

**Bulgular:** Bu çalışmada, morfolojik, SDS-PAGE ve nümerik taksonomik metodlar kullanılarak Grup A'daki bütün *Verbascum* türleri incelenmiştir. Her bir türün sergilediği toplam protein bantı sayısının 5-13 arasında olduğu gözlenmiştir. En benzer bant profili 18kD-14.4 kD arasındaki bölgede gözlenmiştir. Türler arasındaki genetik uzaklıklar %0-58 arasında değişmiştir. Nümerik taksonomik çalışmaların sonuçları morfolojik ve SDS-PAGE analizlerini desteklemiştir.

**Sonuçlar:** Tohum protein profilleri, nümerik taksonomi ve morfolojik karakterlere dayanarak Türkiye'deki *Verbascum* Grup A türlerinin taksonomisi aydınlatılmıştır. Bu çalışmalar en son yapılan Türkiye'deki *Verbascum* Cinsi Grup A'nın Revizyonu başlıklı çalışmalarda büyük uyum sağlamıştır. Ayrıca çalışılan tüm türlerin genişletilmiş ve düzeltilmiş morfolojik karakterleri verilmiştir.

**Anahtar Kelimeler:** *Verbascum*, Grup A, tohum protein profilleri, nümerik taksonomi, Türkiye

## Introduction

The genus *Verbascum* L. (Scrophulariaceae) comprises about 360 species in the world (1). The first revision of *Verbascum* in Turkey was made by Huber-Morath in the *Flora of Turkey and the East Aegean Islands* who recognized 232 species into 13 groups (group A to M), and 126 hybrids (2). In the *Flora of Turkey*, partly artificial groups are used. Since then, ten species and three hybrids have been described from Turkey (3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15).

Turkey seems to be a major centre for *Verbascum* with 242 species and 129 hybrids. The distribution in neighboring countries or flora areas is as follows: 51 species in Russia (16), 49 in the *Flora Iranica* (17), 99 in Europe (18) and 20 in the *Flora Palaestina* (19a, 19b).

The genus *Verbascum* has two sections (*Bohtrospermae* Murb. and *Aulacospermae* Murb.) in the world. However, all Turkish *Verbascum* belong to sect. *Bohtrospermae*. In the *Flora of Turkey*, 29 *Verbascum* taxa were placed into "Group A" by Huber-Morath because they have 4 stamens and stalked bifid placentation (2). After recent publications, the total taxa in the Group A have reached 30. Although the taxa are distributed in all geographic regions of Turkey, they are concentrated in the Mediterranean and in the East Anatolia.

In the *Flora of Turkey*, many taxonomical problems in some genera and sections were mentioned, but not solved, because of the limited time and material. The editors suggested that further revision should be carried out to solve these problems. *Verbascum* is an example with many unresolved taxonomical problems.

The main objective of this study is to clarify the taxonomic status of the Group A (*Verbascum*) in Turkey by means of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and numerical taxonomic methods and to update their morphological properties based on recent taxonomic revision and the available data. SDS-PAGE has proven its reliability for identification of characteristic differences in band patterns of seed storage proteins (20). Seed protein patterns obtained by electrophoresis have been used to resolve the taxonomic problems in several plants (21, 22, 23, 24).

## Material and Methods

### Plant material

Between May 2001 and September 2005, as a part of a revisional study of the genus *Verbascum* Group A in Turkey, a large number of specimens (Table 1) were collected. Dr. Karavelioğulları and deposited at GAZI herbarium. Additional specimens and type photos of *Verbascum* Group A kept at various European (G (Geneve), GB (Göteborg), P (Paris), K (Kew), B (Berlin) and Turkish herbaria (AEF Ankara University, Faculty of Pharmacy), ANK (Ankara University, Faculty of Science), EGE (Ege University, Faculty of Science), GAZI (Gazi

University, Faculty of Science), HUB (Hacettepe University, Faculty of Science), ISTF (Istanbul University, Faculty of Science), ISTE (Istanbul University, Faculty of Pharmacy), KNYA (Selçuk University, Faculty of Science) and VANF (Yüzüncüyıl University, Faculty of Science and Art) were also examined. Morphologic characters were measured from as many as possible specimens. For SDS-PAGE, 36 specimens were selected and studied. The specimens represent all taxa of the Group A. For numerical taxonomic study, 14 specimens were selected. The specimens represent *V. orientale*, *V. brachysepalum*, *V. pyroliforme*, *V. dudleyanum*, *V. trapifolium* and *V. flabellifolium* (Table 1).

### Seed protein analysis

Protein extraction was performed according to Saraswati et al. (25). Electrophoresis was carried out following the Laemmli method (26). The amount of seed taken from samples was weighed equally (0.04 gr) and equal amount of extraction was loaded on the gel. Each run included marker proteins of known molecular weights (Fermentas SM0431). Proteins on the gel (Figure 1) were fixed and stained overnight with Coomassie Brilliant Blue G-250 according to Demiralp et al. (27).

### Data analysis

Bands on SDS-PAGE were scored as either absent (0) or present (1) for all species (Table 2). Only reproducible and clear bands were scored for the construction of the data matrix. The data matrix thus prepared was the input file for the calculation of the Nei coefficient. The similarity matrices were used for the construction of dendrograms with unweighted pair-group method on arithmetic averages (UPGMA) (28).

### Numerical Taxonomic Method

Numerical taxonomic study was conducted on *V. orientale*, *V. brachysepalum*, *V. pyroliforme*, *V. dudleyanum*, *V. trapifolium* and *V. flabellifolium*. They are taxonomically problematic and closely related species group. This view was also confirmed by Karavelioğulları and Aytaç (29). A total of 20 morphological characters (Table 3) were selected having taxonomic significance and measured on the individuals of *V. orientale*, *V. brachysepalum*, *V. pyroliforme*, *V. dudleyanum*, *V. trapifolium* and *V. flabellifolium*. 14 specimens (Table 1) were selected as OTUs (Operational Taxonomic Units, the specimens) representing all species of *V. orientale*, *V. brachysepalum*, *V. pyroliforme*, *V. dudleyanum*, *V. trapifolium* and *V. flabellifolium* and scored for the multivariate analysis. The selection was undertaken according to well preserved and dried specimens, well developed flowers, fruits and seeds.

A similarity matrix was created first using Gower's general coefficient similarity (30), which can be used directly with a mixture of character types (binary, alternative, qualitative, quantitative and semi-quantitative

**Table 1.** Geographic origin of the 36 *Verbascum* included in this study

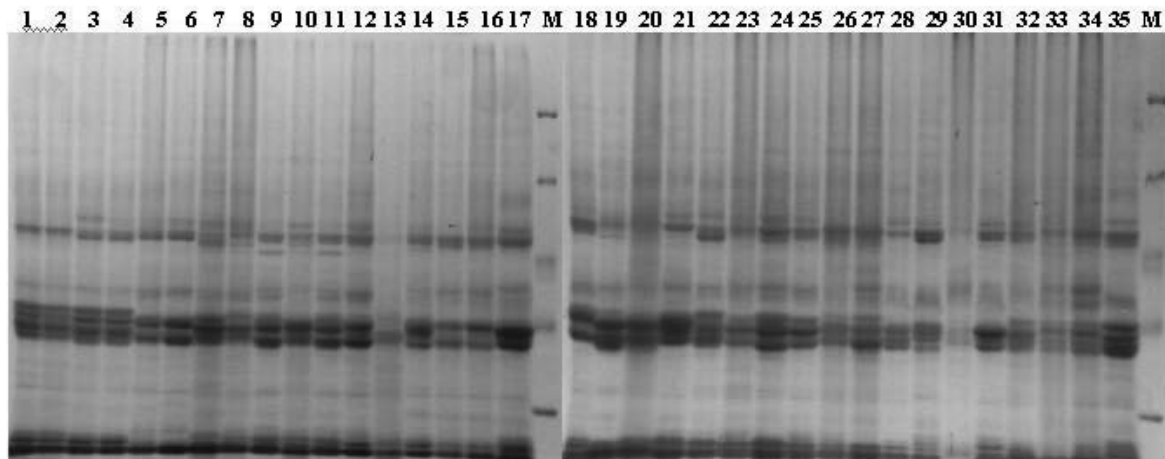
Taxa	Collection	Altitude (m)	Habitat	Collectors
<i>V. natolicum</i>	B6 Malatya	1700	Limestone slopes	FAK 3008, <i>et al.</i>
<i>V. spodiotrichum</i>	C3 Antalya	20	Limestone slopes	FAK 3390, <i>et al.</i>
<i>V. orientale</i>	C3 Antalya	250	Quercus forest	FAK 3197, <i>et al.</i>
<i>V. brachysepalum</i>	C6 Hatay	1185	Quercus forest	FAK 3397, <i>et al.</i>
<i>V. cilicium</i>	C5 Niğde	1100	Rocky place	FAK 3421, <i>et al.</i>
<i>V. flabellifolium</i>	C2 Burdur	1170	Quercus forest	FAK 3322, <i>et al.</i>
<i>V. trapifolium</i>	C2 Burdur	1170	Quercus forest	FAK 3321, <i>et al.</i>
<i>V. pyroliforme</i>	B4 Konya	850	Halophilic place	FAK 3323, <i>et al.</i>
<i>V. dudleyanum</i>	C2 Burdur	850	Damp places	FAK 3431, <i>et al.</i>
<i>V. coronopifolium</i>	B2 Kütahya	1835	Limestone slops	FAK 3388, <i>et al.</i>
<i>V. serratifolium</i>	B4 Ankara	897	Serpentine	FAK 3006, <i>et al.</i>
<i>V. serratifolium</i>	B3 Eskişehir	902	Serpentine	FAK 3382, <i>et al.</i>
<i>V. basivelatum</i>	B3 Eskişehir	902	Serpentine	FAK 3386, <i>et al.</i>
<i>V. bourgeanum</i>	C2 Antalya	2500	Steppe	FAK 3429, <i>et al.</i>
<i>V. serpenticola</i>	C2 Burdur	1600	Serpentine	FAK 3320, <i>et al.</i>
<i>V. sorgerae</i>	C3 Isparta	1650	Cedrus forest	FAK 3316, <i>et al.</i>
<i>V. nudicaule</i>	B9 Van	1915	Serpentine	FAK 3372, <i>et al.</i>
<i>V. suworowianum</i> var. <i>suworowianum</i>	A9 Kars	1281	Steppe	FAK 3362, <i>et al.</i>
<i>V. suworowianum</i> var. <i>papillosum</i>	A9 Kars	1550	Volcanic tufa	FAK 3392, <i>et al.</i>
<i>V. luciliae</i>	B2 Kütahya	1050	Metamorphic place	HD 8757, <i>et al.</i>
<i>V. rupicola</i>	C1 İzmir	200	Rocky	S. Şenol 3025, <i>et al.</i>
<i>V. agrimoniifolium</i> subsp. <i>agrimoniifolium</i>	C9 Şırnak	1500	Road side	FAK 3125, <i>et al.</i>
<i>V. levanticum</i>	C3 Antalya	250	Limestone slopes	FAK 3195, <i>et al.</i>
<i>V. bugulifolium</i>	A2(A)Kırklareli	50	Forest	HA 3524, <i>et al.</i>
<i>V. ponticum</i>	A4 Kastamonu	600	Damp place	FAK 3402, <i>et al.</i>
<i>V. bornmuellerianum</i>	C9 Şırnak	1500	Limestone slopes	FAK 3123, <i>et al.</i>
<i>V. oreophilum</i> var. <i>oreophilum</i>	A4 Kastamonu	650	Meadow	FAK 3397, <i>et al.</i>
<i>V. oreophilum</i> var. <i>oreophilum</i>	B9 Van	1560	Meadow	FAK 3044, <i>et al.</i>
<i>V. oreophilum</i> var. <i>oreophilum</i>	B8 Erzurum	2050	Meadow	FAK 3403, <i>et al.</i>
<i>V. oreophilum</i> var. <i>joannis</i>	A9 Kars	1600	Meadow	FAK 3405, <i>et al.</i>
<i>V. oreophilum</i> var. <i>joannis</i>	B8 Bingöl	1640	Meadow	FAK 3353, <i>et al.</i>
<i>V. oreophilum</i> var. <i>joannis</i>	C10 Hakkari	1180	Meadow	FAK 3036, <i>et al.</i>
<i>V. oreophilum</i> var. <i>joannis</i>	B8 Erzurum	2840	Meadow	FAK 3373, <i>et al.</i>
<i>V. gaillardotii</i>	C6 Hatay	1450	Rocky place	FAK 3144, <i>et al.</i>
<i>V. freynii</i>	A5 Kastamonu	1300	Damp place	FAK 3426, <i>et al.</i>
<i>V. transcaucasicum</i>	A9 Kars	1950	Damp place	FAK 3363, <i>et al.</i>

characters) as well as taking into account missing values (31). This similarity matrix was then clustered by using UPGMA (the unweighted pair-group method using arithmetic averages) and the results are shown in the phenogram (Figure 3). UPGMA is the most frequently used (32) and also appears to produce the best results in terms of following criterias: accurate reflection of the similarity matrix, symmetrical hierarchical structure and congruence with classification derived by traditional methods (33). The characters used in the analysis were assumed to be as important as each others and were unweighted. For this analysis, the MVSP (a multivariate

statistics package for IBM PC and compatibles) program package for clustering analysis was applied.

## Results and Discussion

In this study, all *Verbascum* species in the Group A in Turkey were investigated by means of morphological, SDS-PAGE and a numerical taxonomic method. Their diagnostic morphologic characteristics have been amplified and expanded for the first time, such as stem length, indumentum type, basal and cauline leaves shape, inflorescence type, lower and upper bracts shape and length,



**Figure 1.** Electrophoretic pattern of seed proteins of *Verbascum* species

(1) *V. orientale*, (2) *V. orientale*, (3) *V. brachysepalum*, (4) *V. brachysepalum*, (5) *V. flabellifolium*, (6) *V. trapifolium*, (7) *V. cilicium*, (8) *V. coronopifolium*, (9) *V. serratifolium*, (10) *V. basivelatum*, (11) *V. bourgeauanum*, (12) *V. serpenticola*, (13) *V. sorgerae*, (14) *V. nudicaule*, (15) *V. suworowianum* var. *suworowianum*, (16) *V. suworowianum* var. *papillosum*, (17) *V. agrimoniifolium* subsp. *agrimoniifolium*, **M: Marker**, (18) *V. ponticum*, (19) *V. bornmuellerianum*, (20) *V. macrocarpum*, (21) *V. bugulifolium*, (22) *V. oreophyllum* var. *oreophyllum*, (23) *V. oreophyllum* var. *oreophyllum*, (24) *V. oreophyllum* var. *joannis*, (25) *V. oreophyllum* var. *joannis*, (26) *V. oreophyllum* var. *joannis*, (27) *V. trascaucasicum*, (28) *V. natolicum*, (29) *V. spodiotrichum*, (30) *V. levanticum*, (31) *V. luciliae*, (32) *V. gaillardotii*, (33) *V. dudleyanum*, (34) *V. pyroliforme*, (35) *V. freynii*, **M: Marker** (Fermentas SM0431;  $\beta$ -galactosidase 116 kD, Bovine serum albumin 66.2 kD, Ovalbumin 45,000, Lactate dehydrogenase 35 kD, Restriction endonuclease Bsp981 25 kD,  $\beta$ -lactoglobulin 18.4 kD, Lysozyme 14.4 kD).

**Table 2.** The presence and absence of the bands on the SDS-PAGE for each species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35		
1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
2	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	0	1	0	0	0	1	1	0	1	1	0	0	0	1	
3	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	0	0	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
6	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	
7	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
11	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
12	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
13	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Total number of bands	9	9	10	10	6	11	10	11	8	11	9	6	8	8	8	8	8	7	8	5	9	10	9	11	8	8	10	8	11	5	8	6	9	9	13		

**Table 3.** Morphological characters scored for the numerical taxonomy

1	Annual	0
	Biennial or Perennial	1
2	Indumentum of stems (below) glandular puberulent	0
	Long eglandular and papillose	1
	Stalked glandular and eglandular	2
	Glabrous and strigose	3
	Only glabrous	4
3	Xerophytic	0
	Halophytic	1
	Hydrophytic	2
4	Stem simple	0
	Stem branched at base	1
5	Leaves pinnatifid-pinnatisect	0
	Flabellate-semicircular	1
	Oblong, oblong-linear or linear spatulate	2
6	Indumentum of basal leaves glandular puberulent	0
	Eglandular and densely papillose	1
	Short glandular and long eglandular	2
	Glabrous	3
7	Stem leaves pinnatisect	0
	Lanceolate	1
8	Inflorescence type panicle	0
	Raceme	1
	Both raceme and panicle	2
9	Bracts pinnatisect and linear-lanceolate	0
	Only linear-lanceolate	1
	Only lanceolate	2
	Only ovate-lanceolate	3
10	Indumentum of pedicels puberulaent to glabrescent	0
	Glandular and few or numerous eglandular with papillose	1
	Strigose	2
	Glabrous	3
11	Calyx teeth linear-lanceolate to lanceolate	0
	Only lanceolate	1
	Ovate to ovate lanceolate	2
12	Spots on corolla, absent	0
	A few brownish spots	1
	Red spot in central	2
13	Filament colour whitish yellow	0
	White and purple-violet	1
	Only whitish	2
14	Indumentum of filaments, right up to anthers	0
	Two anterior glabrous near apex	1
15	Ovary ovate	0
	Ovate to ovoid	1
	Ovate to lanceolate	2
16	Stilus length up to 4 mm	0
	7-9 mm	1
17	Fruit width (average), up to 3 mm	0
	3 - 4 mm	1
	4 - 6 mm	2
18	Fruit length (average), up to 4 mm	0
	4-5 mm	1
	5-7 mm	2
	7-8 mm	3
19	Fruit shape globose	0
	Globose-ovoid	1
	Ovate	2
	Ovate-ovoid	3
20	Indumentum of fruit, glabrous	0
	Sparsely glandular	1
	Densely glandular	2

pedicel length, calyx and corolla properties, filament length, fruit shape and hairs.

In order to clarify taxonomic relationships in the species, all the specimens of Group A were analyzed by SDS-PAGE. The total number of protein band positions observed for each species between 5 and 13. Figure 1 illustrates a typical gel in which different patterns are shown for 30 taxa. The most similar band pattern was observed in the region of 18 kD for betalactoglobulin and 14.4 kD for lysozyme. One band around 14 kD observed among all species studied and an 18 kD were found most of the species. The three or two bands smaller than 18 kD could be genus specific. The bands bigger than 45 kD were variable among all those species. This band could be species specific. Some species have the same banding patterns (1-4 line in Figure 1).

Genetic distance parameters were calculated for the 36 samples of *Verbascum* (Table 4). In general, genetic distances among species were ranged between 0% and 58%. Figure 2 shows a dendrogram that allows two main groups to be distinguished. The upper cluster contains *V. spodiotrichum*, *V. bugulifolium*, *V. oreophyllum* var. *joannis*, *V. natolicum*, *V. oreophyllum* var. *joannis*, *oreophyllum* var. *joannis*, *V. oreophyllum* var. *joannis*, *V. oreophyllum* var. *oreophyllum*, *V. oreophyllum* var. *oreophyllum*, *V. oreophyllum* var. *oreophyllum*, *V. ponticum*, *V. bornmuellerianum*, *V. oreophyllum* var. *oreophyllum*, *V. agrimoniifolium* subsp. *agrimoniifolium*, *V. rupicola*, *V. levanticum*, *V. nudicaule*, *V. luciliae*, *V. orientale* and *V. brachysepalum*. The lower cluster contains *V. sorgerae*, *V. bourgeauanum*, *V. freynii*, *V. gaillardotii*, *V. trascaucasicum*, *V. pyrolifforme*, *V. flabellifolium*, *V. suworowianum* var. *suworowianum*, *V. basivelatum*, *V. serratifolium*, *V. dudleyanum*, *V. suworowianum* var. *papillosum*, *V. coronopifolium*, *V. serpenticola*, *V. cilicicum* and *V. trapifolium*.

In the upper cluster, *V. spodiotrichum* is distance to remaining species. *V. bugulifolium*, *V. bornmuellerianum*, *V. ponticum* have decurrent anthers and they were grouped in the same cluster (Figure 2). *V. oreophyllum* var. *oreophyllum*, *V. oreophyllum* var. *joannis* and *V. natolicum* were placed in the same group because of their morphological similarity.

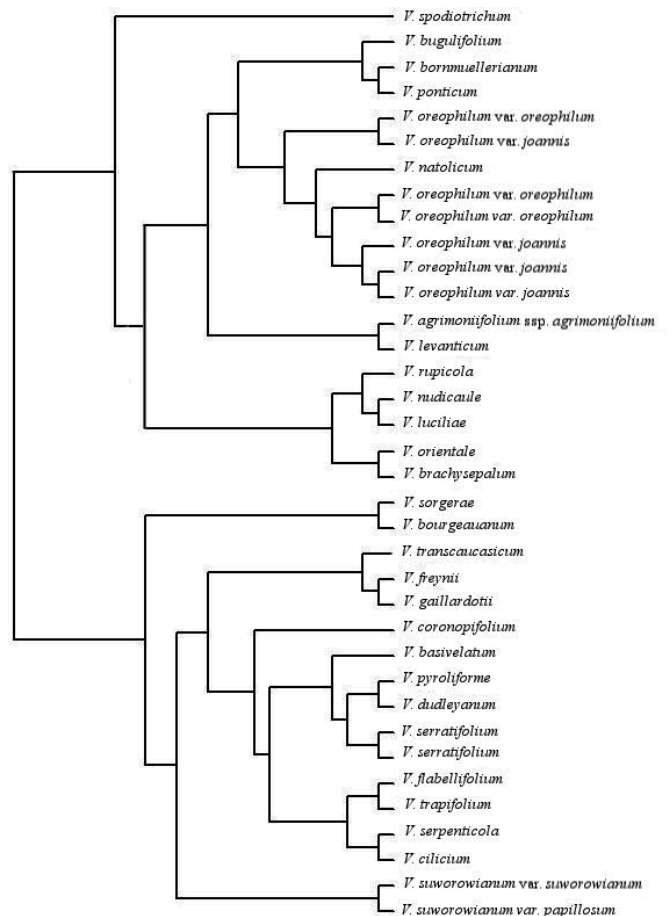
In addition, *V. agrimoniifolium* subsp. *agrimoniifolium*, *V. levanticum* and *V. rupicola*, *V. nudicaule* and *V. luciliae* were also placed in the same cluster. All of them have pinnatisect leaved (14). Taxonomic relationships among the species are also good depicted by lower cluster.

SDS-PAGE analysis was confirmed to morphological treatments of Karavelioğulları & Aytaç (29), *V. orientale* with *V. brachysepalum*, *V. trapifolium* with *V. flabellifolium* and *V. pyrolifforme* with *V. dudleyanum* have the same banding pattern (genetic distance value equal to 11%). Similarly, *V. suworowianum* var. *suworowianum* and *V. suworowianum* var. *papillosum* have the same banding pattern at 11 % genetic distance.

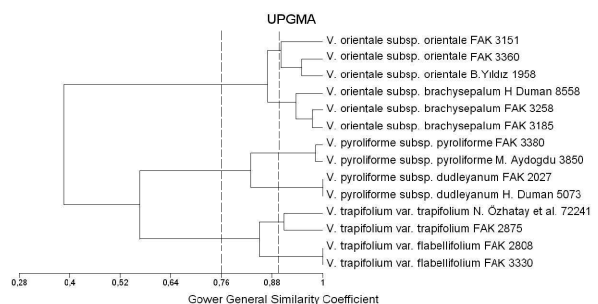
A numerical taxonomic study has been conducted on the

basis of the *V. orientale*, *V. brachysepalum*, *V. pyrolifforme*, *V. dudleyanum*, *V. trapifolium* and *V. flabellifolium* in order to elucidate their taxonomic relationships.

The results of the numerical taxonomic study are supported both morphologic and SDS-PAGE analysis. The phenogram resulting from UPGMA clustering of similarity matrix is presented in Figure 3. As a result of the numerical analysis, a cut-off line across the phenogram at 0.76 similarity level empirically distinguishes the species or phenons from each other, namely *V. orientale*, *V. pyrolifforme* and *V. trapifolium*, and second line across



**Figure 2.** Dendrogram of *Verbascum* species (Group A) based on genetic differences.



**Figure 3.** Dendrogram constructed by means of UPGMA algorithm and Gower General Similarity Coefficient.



the phenogram at 0.90 similarity level results in subspecies or variety of them.

The first phenon representing *V. orientale* and its subspecies which were collected from Antalya in the western Mediterranean region and Hatay in the eastern Mediterranean region of Turkey. The second phenon representing endemic *V. pyroliforme* and its subspecies which were collected from Konya and Burdur. The third phenon representing endemic *V. trapifolium* and its varieties which were collected from Burdur. The results of the studies were in broad agreement to the arrangement of these species in Revision of the Genus *Verbascum* L. (Group A) in Turkey (29).

As mentioned above, depending on the proteins and morphological characters, our results clarified the taxonomy of Turkish *Verbascum* (Group A) species. Although seed protein profiles and numeric taxonomy cannot be used as the sole character for species identification, these characters can be used as additional characters to help resolve species relationships in plant taxonomy.

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