TÜBİTAK

Research Article

Turk J Bot
35 (2011) 1-16
© TÜBİTAK
doi:10.3906/bot-1003-39

Morphological, chemical, and molecular analyses of Turkish *Papaver* accessions (Sect. *Oxytona*)

İskender PARMAKSIZ[1,*], Sebahattin ÖZCAN[2]

[1]Department of Biology, Faculty of Arts & Science, Gaziosmanpaşa University, Tokat - TURKEY

[2]Department of Field Crops, Faculty of Agriculture, University of Ankara, Ankara - TURKEY

Received: 16.03.2010
Accepted: 22.07.2010

Abstract: The species of the section *Oxytona* Bernh., classified under the genus *Papaver* L., are of great importance due to their medical alkaloid contents such as morphine, codeine, and thebaine. A clearly established classification system is not available for *Oxytona* species. The aim of this study was to find relationships among 53 accessions belonging to 3 important *Oxytona* species, i.e. *Papaver bracteatum* Lindl., *P. orientale* L., and *P. pseudo-orientale* (Fedde) Medw., using 19 different morphological characters, 1 chemical character, and 15 random amplified polymorphic DNA (RAPD) markers. Nineteen characters, including bract and sepal leaves, petal marks, and capsule shape and size were analysed, and distinct morphological differences among accessions were identified. The thebaine content of the accessions was determined via HPLC analysis. Of the 53 accessions, 21 contained thebaine alkaloid ranging from trace amounts to 2.5% and the rest contained no thebaine. RAPD-PCR markers were used for analysis of accessions at molecular level. A total of 81 of bands generated by 15 successful RAPD-PCR reactions were detected as polymorphic. The polymorphism rate was approximately 85%. The combination of morphological, chemical, and molecular marker analyses resulted in a better classification for the section *Oxytona*.

Key words: *Papaver, Oxytona*, PCR, RAPD, HPLC

Türk *Papaver* Hatlarının (Sect. *Oxytona*) morfolojik, kimyasal ve moleküler analizi

Özet: *Papaver* L. cinsi altında sınıflandırılan *Oxytona* Bernh. seksiyonu türleri morfin, kodein ve tebain gibi tıbbi alkaloit içeriklerinden dolayı büyük öneme sahiptirler. *Oxytona* seksiyonu türleri arasında belirgin bir sınıflandırma sistemi yoktur. Bu çalışmanın amacı *Oxytona* seksiyonundan *Papaver bracteatum* Lindl., *P. orientale* L. ve *P. pseudo-orientale* (Fedde) Medw. türlerine ait 53 materyali arasındaki ilişkiyi belirlemektir. Brakte ve sepal yaprakları, petal izleri, kapsül şekli ve buna benzer 19 karakter analiz edilmiş ve materyaller arasında belirgin morfolojik farklılıklar saptanmıştır. HPLC analizi yolu ile materyallerin tebain içerikleri belirlenmiştir. 53 materyalin 21'i % 0 (iz miktar) ile % 2,5 arasında tebain içerirken, geriye kalan 32 materyalin tebain içermediği saptanmıştır. Materyallerin moleküler düzeyde analiz edilmesi için RAPD-PCR markörleri kullanılmıştır. Başarılı 15 RAPD-PCR reaksiyonu ile üretilen 96 bandın 81'i polimorfiktir. Polimorfizm oranı yaklaşık % 85 olarak bulunmuştur. Morfolojik, kimyasal ve moleküler markörlerin birlikte kullanılması *Oxytona* seksiyonunda daha iyi bir sınıflandırma ile sonuçlanmıştır.

Anahtar sözcükler: *Papaver, Oxytona*, PCR, RAPD, HPLC

* E-mail: iparmaksiz@gmail.com

Introduction

Turkey is one of the ancient 'centres of origin' for thousands of different plant species including the genus *Papaver* L. (Davis, 1988; Atalay, 1994). Of the 110 *Papaver* species around the world, 50 taxa grow naturally in Turkey (Kapoor, 1997; Güner et al., 2000). Some of the species belonging to the section *Oxytona* Bernh., i.e. *P. bracteatum* Lindl., *P. orientale* L., and *P. pseudo-orientale* (Fedde) Medw., are particularly important due to their medicinal alkaloid contents such as morphine, codeine, and thebaine (Carolan et al., 2002; Sarıyar, 2002). The alkaloid content of *P. somniferum* is high in morphine and codeine, whereas thebaine is high in *P. bracteatum*. Morphine and codeine have an addictive effect on the human body, while thebaine is non-addictive. During the 1970s, the United Nations called for extensive studies about utilisation of *P. bracteatum* as a source of thebaine and the possible replacement of *P. somniferum* as the major source of morphine (Kapoor, 1997; Carolan et al., 2002). Molecular techniques offer a potential to accurately differentiate species and bypass the reliance on diagnostic morphological and phytochemical characters. Such techniques involve the generation of characteristic genetic fingerprints (or the detection of species-specific molecular markers), which are then used to differentiate between closely related species, varieties, and cultivars. The species belonging to the section *Oxytona* can be differentiated using morphological, cytological, and phytochemical characters, but the distinction is not always clear (Nyman, 1979). Several species from the section *Oxytona* are polyploid in structure. *P. bracteatum* is dipliod (2n = 14), *P. orientale* (2n = 28) is tetraploid, and *P. pseudo-orientale* (2n = 42) is allo-hexaploid (Goldblatt, 1974). Interspecific hybridisation does occur (Milo et al., 1986; Ojala et al., 1990; Levy & Milo, 1991). For this reason, and because of the importance of their species-specific alkaloids, the correct identification of species is very important.

Recent technical advances in genomics have accelerated the use of molecular markers for varying purposes (Isık et al., 2007). A variety of DNA-based markers have been developed and proved useful in higher plants for germplasm characterisation and polymorphism analysis (Gupta et al., 2002; Andersen, 2003; Gupta & Rustgi, 2004; Gültepe et al., 2010). Random amplified polymorphic DNA (RAPD) markers in combination with polymerase chain reactions (PCR) technology are a well-characterised approach to analyse genetic variation and fingerprints in several crop plants (Williams et al., 1990; Budak et al., 2004; Gupta & Rustgi, 2004). Efficient use of different markers among species of *Papaver* has also been reported (Shoyama et al., 1998; Sangwan et al., 2000; Carolan et al., 2002; Carolan et al., 2006). Application of marker technology along with morphological and chemical characters would be useful for the analysis of genetic variation in the section *Oxytona*.

The aim of the present study was to find relationships among 53 Turkish *Papaver* accessions from the section *Oxytona*. *P. bracteatum*, *P. pseudo-orientale*, and *P. orientale* had been partially studied morphologically and phytochemically (Arslan, 1991) and they were grouped into a set. In addition, 24 accessions of unknown species had never been before studied and they formed the second set. These accessions were analysed in relation to each other in order to correctly identify the species, using morphological, phytochemical (major alkaloids), and genetic characterisations.

Materials and methods

Plant material

A total of 53 accessions were used. These accessions were grouped into 2 sets. The first set consisted of 29 accessions selected among 264 small populations that were collected by a former University of Ankara staff member, Peter Goldblatt, in 1972-73, and supplied by the United Nations. The 53 accessions contained 13 accessions for *P. bracteatum*, 8 for *P. pseudo-orientale*, and 8 for *P. orientale*. The second set consisted of 24 accessions derived from 192 small populations collected from 4 provinces (Ağrı, Erzurum, Kars, and Muş) in Eastern Anatolia (Davis, 1988). All accessions were subjected to morphological, chemical, and molecular analyses. The locations of the accessions are given in Table 1. The plants were grown in the research field of the Agronomy Department, University of Ankara, using fertilisation and irrigation treatments as recommended by Mihalik (2000).

Table 1. Location of the accessions used in the study.

Order	Accessions*	Location (Region or City)
1	PB1	University of Ankara GenBank
2	PB2	University of Ankara GenBank
3	PB3	University of Ankara GenBank
4	PB4	University of Ankara GenBank
5	PB5	University of Ankara GenBank
6	PB7	University of Ankara GenBank
7	PB9	University of Ankara GenBank
8	PB10	University of Ankara GenBank
9	PB99	University of Ankara GenBank
10	PB100	University of Ankara GenBank
11	PB101	University of Ankara GenBank
12	PB246	University of Ankara GenBank
13	PB248	University of Ankara GenBank
14	PP60	University of Ankara GenBank
15	PP70	University of Ankara GenBank
16	PP121	University of Ankara GenBank
17	PP122	University of Ankara GenBank
18	PP141	University of Ankara GenBank
19	PP147	University of Ankara GenBank
20	PP249	University of Ankara GenBank
21	PP251	University of Ankara GenBank
22	PO73	University of Ankara GenBank
23	PO74	University of Ankara GenBank
24	PO169	University of Ankara GenBank
25	PO170	University of Ankara GenBank
26	PO180	University of Ankara GenBank
27	PO181	University of Ankara GenBank
28	PO240	University of Ankara GenBank
29	PO244	University of Ankara GenBank
30	U1	A9 Kars: Karsçayı, Akyaka, Kayaköprü-Demirkent village, 1568 m
31	U3	A9 Kars: Arpaçay, Taşdere village, Çirişli 1, 1951 m
32	U12	A9 Kars: Sarıkamış, Yağbasan 1, 2044 m
33	U19	A9 Kars: Sarıkamış-Horasan, Soğanlı 10, 2220 m
34	U22	A9 Kars Merkez, Melik village, 1739 m
35	U47	B9 Ağrı: Tutak, Hamur 7, 1887 m
36	U59	A9 Kars: Sarıkamış, Yağbasan 3, 2044 m
37	U83	A8 Erzurum: Tortum-Narman, Kireçli 6, 2229 m
38	U84	A8 Erzurum: Tortum-Narman, Kireçli 7, 2230 m
39	U121	B8 Erzurum: Merkez, Palandöken, 2177 m
40	U124	B8 Erzurum: Merkez, Palandöken, 2175 m
41	U136	A8 Erzurum: Pasinler, Hamam Deresi 1, 1849 m
42	U139	A8 Erzurum: Pasinler, Hamam Deresi 4, 1851 m
43	U144	A8 Erzurum: Pasinler, Hamam Deresi 9, 1855 m
44	U147	A8 Erzurum: Pasinler, Hamam Deresi 12, 1870 m
45	U148	A8 Erzurum: Pasinler, Hamam Deresi 13, 1870 m
46	U149	B8 Erzurum: Horasan-Eleşkirt, Sarıcan 1, 2216 m
47	U155	B8 Erzurum: Horasan-Eleşkirt, Sarıcan 7, 2220 m
48	U157	B8 Erzurum:Horasan-Eleşkirt, Sarıcan 8, 2221 m
49	U159	B9 Ağrı: Tutak-Hamur, Hamur 1, 1817 m
50	U163	B9 Ağrı: Tutak-Hamur, Hamur 4, 1822 m
51	U169	B8 Muş: Bulanık, Hayat Plateau 2, 1746 m
52	U178	B8 Muş: Bulanık, Hayat Plateau 4, 1748 m
53	U185	B8 Muş: Bulanık, Hayat Plateau 11, 1750 m

*PB: *Papaver bracteatum*, PP: *Papaver pseudo-orientale*, PO: *Papaver orientale* U: *Unknown* (not known which species accession belongs to)

Morphological analysis

A total of 19 morphological characteristics were measured and used for analysis: plant height, leaf size, flowering node, bud shape, bract, number of sepal leaves, flowering time, number of petals, petal colour, petal size (width and length), marked petals, mark size (width and length), capsule size (width and length), number of stigmatic rays, latex on capsules, and shape of capsule top. The morphological characters were mainly identified from the *Flora of Turkey* (Davis, 1965; Davis et al., 1988) and other related sources (Goldblatt, 1974; Mihalik, 2000). The measurements for parameters were taken in 2 consecutive years over a period of 4 months from March to the end of July. Statistical analyses were performed to generate a phylogenetic tree, using SPSS 11.0 (Tables 2-3).

Phytochemical analysis

After the capsules were dried and the seeds removed, the capsules were ground to powder. For each sample, 200 mg of powder was heated, treated with 5% acetic acid, and kept overnight at room temperature. The final volume was completed to 50 mL using double distilled water. The pH was adjusted to 9 and 10 mL of the extract was treated with an equal (1:1) volume of chloroform. A 20 µL sample of this solution was spotted to thin-layer chromatography (TLC). After washing with morphine (methylene chloride 84; CH_2Cl_2, methanol 14; CH_3OH and 10% ammonia; NH_3), the samples were dried, and then washed with an alkaloid solution (Benzene 80: ethanol 20: ammonia 0.3). Following treatment with 60% sulphuric acid, the yellow spots on TLC were heated to 105 °C for 15 min. The samples were taken out of the autoclave and the light purple spots known to contain thebaine alkaloids were analysed by HPLC (HPLC 1050) in order to determine the amount.

HPLC 1050

For the analysis, 8.2 g of NaAc was dissolved in 2 L of distilled water and then filtered using common filter paper. It was adjusted to pH 3.6 with glacial acetic acid. Then 180 mL of acetonitrile, 60 mL of ethanol, and 60 mL of THF were added to 1760 mL of this solution. This mixture was used to determine the amount of thebaine. The column was 300 mm × 3.9 mm Bundapak C18, the flow rate was 1.5 mL/min, and the standard was 0.1 mg/mL.

DNA extraction

A modified cetyl 3 methyl amonium bromide (CTAB) method was used for DNA extraction (Sangwan et al., 2000). The fresh tissues were ground to a fine powder and were suspended in 2× CTAB (3:1) solution. The suspension was heated to 65 °C for 30 min and treated with a chloroform-isopropanol (24:1) mixture for 10 min. After removal of the cell debris by centrifugation at 4000 rpm for 10 min, the DNA pellet was washed with 90% ethanol and re-suspended in Tris EDTA (TE) containing 10 mM Tris-HCl and 1 mM EDTA. DNA purification (Sangwan et al., 2000), quantification, and optimisation steps were also done with some modifications following the standard protocols (Hillis & Moritz, 1990).

PCR amplification

The PCR reactions were performed in a total of 25 µL volume containing 2.5 µM of each dNTP, 50 µM of $MgCl_2$, 0.3 µL (1.5 Unit) of *Taq* DNA polymerase (Promega), 50 µM of primer, 100 ng of the template DNA, and 2.5 µL of 10× PCR reaction buffer (Promega). The PCR conditions were as follows: 60 cycles of 15 s at 94 °C, 30 s at 32 °C, and 1 min at 72 °C, followed by 8 min at 72 °C. A total of 45 RAPD primers were used for the analysis. The names and sequences of all primers are given in Table 4. The amplification products were run and size-separated on a 1.8% agarose gel at 90 V for about 2-3 h following a standard protocol (21). The amplified DNA was treated with ethidium bromide to make the products visible under UV light (UVP Laboratory Products). The 'Directload Wide Range DNA size marker' (Promega) was used to estimate the band sizes.

Data analysis

The data obtained from morphological characters were analysed with *Hierarchical Cluster Analysis Method* using SPSS 11.0. For the measurement of genetic relation and distance among accessions, the data generated from RAPD primers were analysed by using POPGENE32 version 1.32 (Population Genetic Analysis) and MEGA 3.0 (Molecular Evolutionary Genetic Analysis) as described previously (Nei, 1972; Nei & Li, 1979; Kumar et al., 2004).

Table 2. Data for morphological and chemical characters among *Papaver*.

Order	Accession	Plant height (cm)	Leaf size (cm)	Flowering nodes	Bud shape	Bract leaves	Number of sepal leaves
1	PB1	100	65	8	Erected	4-6	3
2	PB2	100	65	8	Erected	4-6	3
3	PB3	85	50	8-9	Erected	4-6	3
4	PB4	100	63	7-8	Erected	4-6	3
5	PB5	95	65	7-8	Erected	4-6	3
6	PB7	100	55	7-8	Erected	4-5	3
7	PB9	95	55	6-7	Erected	5	3
8	PB10	100	63	7	Erected	6-8	3
9	PB99	85	65	7	Erected	4-6	3
10	PB100	85	65	7	Erected	4-6	3
11	PB101	101	60	6-7	Erected	4-5	3
12	PB246	100	63	6-7	Erected	4-5	3
13	PB248	102	65	6-7	Erected	2-4	3
14	PP60	50	25	4-5	Semi-erected	None	2
15	PP70	65	60	6	Erected	3	3
16	PP121	95	60	7-8	Erected	3-4	2
17	PP122	85	52	7-8	Erected	3	2
18	PP141	75	65	6-7	Erected	3-4	3
19	PP147	55	42	5-6	Erected	3-4	2
20	PP249	60	30	4-6	Erected	3	2
21	PP251	92	55	5	Erected	2-3	3
22	PO73	30	35	6	Erected	None	2
23	PO74	50	35	4-6	Erected	None	2-3
24	PO169	55	45	7	Erected	None	2
25	PO170	55	40	4-6	Bent over	None	2-3
26	PO180	62	40	4-6	Bent over	None	2-3
27	PO181	55	45	4-6	Bent over	None	2
28	PO240	75	35	4-6	Bent over	None	2-3
29	PO244	70	40	4-6	Erected	None	2-3
30	U1	48	32	3	Bent over	None	2
31	U3	80	35	5-6	Bent over	None	2
32	U12	45	25	4-6	Semi-erected	None	2
33	U19	55	28	2-6	Erected	None	2
34	U22	55	17	4-6	Bent over	None	3
35	U47	65	27	4	Erected	None	2
36	U59	42	18	5-7	Erected	None	2
37	U83	12	15	—	—	—	—
38	U84	13	10	3-4	Erected	None	2-3
39	U121	50	32	3	Semi-erected	None	3
40	U124	55	30	3-4	Semi-erected	None	2
41	U136	45	23	4	Semi-erected	None	2
42	U139	25	20	5	Bent over	None	2
43	U144	50	26	4-5	Bent over	None	2
44	U147	55	33	3	Erected	None	2-3
45	U148	65	31	5-6	Erected	Rare	2-3
46	U149	65	31	5-6	Erected	Rare	2-3
47	U155	65	35	5-7	Erected	Rare	3
48	U157	60	22	4-6	Semi-erected	None	2-3
49	U159	40	18	4	Bent over	None	2
50	U163	55	30	3-5	Erected	None	2
51	U169	55	30	6	Erected	None	2
52	U178	60	30	5	Bent over	None	2
53	U185	15	12	—	—	—	—

Table 2. (*continued*).

Order	Accession	Flowering time	Number of petals	Petal colour	Petal width (cm)	Petal length (cm)	Marked petals	Petal mark width (cm)
1	PB1	17/05/03	6	Dark Red	7	8.4	To the Bottom	2-3
2	PB2	17/05/03	6	Dark Red	7	8.5	To the Bottom	2-3
3	PB3	17/05/03	6	Dark Red	6-7	8.5	Centre	0.3
4	PB4	17/05/03	6	Dark Red	6-7	5-6	Centre and Small	0.5
5	PB5	17/05/03	6	Dark Red	7	5-6	Centre and Small	0.5
6	PB7	16/05/03	6	Dark Red	6.5-7.5	7.2	Centre	0.6-0.9
7	PB9	16/05/03	6	Dark Red	6	5-7	To the Bottom	1.7-2.3
8	PB10	17/05/03	6	Dark Red	6.5	5.5-6.7	To the Bottom	2-2.2
9	PB99	16/05/03	6	Dark Red	6-6.5	6	To the Bottom	1-1.5
10	PB100	16/05/03	6	Dark Red	6-6.5	6	To the Bottom	1-1.5
11	PB101	16/05/03	6	Light Red	6	6	Centre	1.5-1.7
12	PB246	16/05/03	6	Dark Red	6-7	6-7	Centre	1.6-2
13	PB248	16/05/03	6	Dark Red	6-7	6	To the Bottom	2-2.4
14	PP60	18/05/03	4	Red	5-6	5.5	Centre	1-1.5
15	PP70	17/05/03	4	Red	10-11	7.5-8	Centre	2-2.4
16	PP121	15/05/03	4	Red	6-7	6	Centre	0.7-1.4
17	PP122	18/05/03	4	Red	5.5-6	7	Centre	0.8-1.2
18	PP141	16/05/03	6	Red	6-6.5	6.5	Centre	1-1.1
19	PP147	15/05/03	6	Red	6-7.5	7	Centre	3-3.2
20	PP249	24/05/03	4–6	Red	6.5-8	7	Centre	0.4-0.5
21	PP251	24/05/03	6	Red	7.6-8.8	8.5-9	To the Bottom	3-3.4
22	PO73	30/05/03	6	Light Red	6.5-7	5.5-6	None	—
23	PO74	30/05/03	6	Light Red	6-7	5.8-6.5	None	—
24	PO169	29/05/03	6	Light Red	6.5-7	6.5-7	To the Bottom	1.2-1.5
25	PO170	29/05/03	4–6	Light Red	6-8	5-6	None	—
26	PO180	28/05/03	4–6	Light Red	6.5-7.8	5.4-6.2	None	—
27	PO181	26/05/03	6	Red	6-8	5-6	Centre	3-3.5
28	PO240	27/05/03	4–6	Light Red	5-7	4-6	None	—
29	PO244	26/05/03	4–6	Light Red	5-7	4-6	None	—
30	U1	18/05/03	4	Red	12	6-8	Centre	3.7
31	U3	15/05/03	4	Light Red	8	4-6.5	Centre	1.7
32	U12	16/05/03	4	Red	8-8.4	4.5-6	Centre	3.4
33	U19	16/05/03	4	Red	9.5	5-7	Centre	3.2
34	U22	14/05/03	4	Light Red	6.5-10	5.3	Centre	1-1.4
35	U47	18/05/03	4	Light Red	6.5-8	4-6.5	Centre	1-2
36	U59	19/05/03	4	Light Red	7-8.5	7	Centre	1.2-1.8
37	U83	No flower	—	—	—	—	—	—
38	U84	16/05/03	4	Light Red	4-5	2.8-3.5	Centre	0.8-1
39	U121	16/05/03	4	Light Red	6-7	5.5-6	Centre	1.2
40	U124	17/05/03	4	Light Red	6-8	7	Centre	1.1-1.4
41	U136	15/05/03	4	Light Red	8	6	Centre	1.5-1.8
42	U139	15/05/03	4	Light Red	6.4-7.2	5.7-6.5	Centre	0.6-1
43	U144	19/05/03	4	Light Red	7-8	6	Centre	0.1-0.3
44	U147	21/05/03	4	Light Red	10-11	7.5	Centre	1.4-1.6
45	U148	19/05/03	—	Light Red	8-10	7	Centre	2
46	U149	19/05/03	4-5	Light Red	8-11	7.5	Centre	2-2.3
47	U155	15/05/03	4-6	Light Red	7-7.5	6.4-6.8	Centre	1.8-2.2
48	U157	21/05/03	4-6	Light Red	7-9	6-8	Centre	1.8-2.2
49	U159	25/05/03	4	Light Red	8-10	6-7.5	Centre	2-2.2
50	U163	19/05/03	4	Light Red	9-11	6	Centre	2-2.2
51	U169	17/05/03	4	Light Red	10-12	7.5	Centre	1.5-2
52	U178	20/05/03	4	Light Red	8-10	7-8	None	—
53	U185	No flower	—	—	—	—	—	—

Table 2. (*continued*).

Order	Accession	Petal mark length (cm)	Latex on capsule	Capsule width (mm)	Capsule length (mm)	Number of capsule rays	Shape of capsule top	Thebaine (%)
1	PB1	3.2	Rarely	19.88	34.74	17	Oval-centred	1.5
2	PB2	3.2	Rarely	20.06	26.19	17	Flat-topped	1.6
3	PB3	0.2	Rarely	17.12	27.48	18	Oval-centred	0.8
4	PB4	0.4	No	22.01	39.81	17	Flat-centred	0.6
5	PB5	0.3	No	17.83	27.38	17	Flat-topped	2.1
6	PB7	1.3-1.5	Rarely	20.89	34.75	18	Flat-topped	0.8
7	PB9	3	No	17.56	31.71	16	Flat-topped	1.3
8	PB10	2.8	No	18.20	32.15	14	Flat-topped	1.4
9	PB99	2.7-3	Rarely	23.21	27.23	15	Flat-centred	2.4
10	PB100	2.7-3	Rarely	20.81	30.05	16	Flat-topped	2.5
11	PB101	1.7-2	Rarely	20.83	25.71	17	Flat-topped	__
12	PB246	1.5-2.3	No	19.40	31.58	13	Flat-topped	1.6
13	PB248	2.6-2.9	No	20.08	27.47	15	Flat-topped	1.6
14	PP60	1.4-1.7	Rarely	10.67	23.70	10	Flat-topped	__
15	PP70	1.5-1.7	Rarely	17.28	31.11	11	Flat-topped	__
16	PP121	1-1.5	Rarely	19.43	31.08	16	Flat-topped	__
17	PP122	1.2-1.5	Rarely	18.74	24.63	14	Flat-topped	__
18	PP141	0.4-0.6	Yes	13.03	22.12	15	Oval-centred	0.09
19	PP147	2-2.5	Yes	21.53	23.99	15	Convex	__
20	PP249	0.5-0.7	No	13.97	26.09	11	Convex	1.5
21	PP251	5-5.4	No	18.80	24.04	14	Flat-topped	0.2
22	PO73	__	No	12.52	23.87	11	Concave	__
23	PO74	__	No	10.69	19.95	11	Flat-topped	__
24	PO169	2.3-2.5	__	13.48	21.50	10	Flat-topped	__
25	PO170	__	No	13.48	21.50	10	Concave	__
26	PO180	__	No	14.20	17.60	12	Flat-topped	Trace
27	PO181	2.5-3	No	19.20	26.92	14	Flat-centred	__
28	PO240	__	No	9.71	16.92	11	Flat-topped	__
29	PO244	__	No	14.94	16.96	14	Concave	__
30	U1	2.2	No	17.86	32.52	14	Flat-topped	__
31	U3	1.2	No	1931	26.96	13	Flat-topped	__
32	U12	1.8	No	13.54	21.77	10	V. Rays-Flat topped	0.02
33	U19	2.2	No	1892	31.76	10	V.-Flat topped	__
34	U22	0.4	No	12.24	19.50	9	V. Rays-Flat topped	0.06
35	U47	0.5	No	18.24	22.64	11	V. Rays-Flat topped	__
36	U59	0.5-0.9	No	15.07	28.88	10	V. Rays-Flat topped	__
37	U83	__	__	__	__	__	__	0.01
38	U84	0.3-0.6	No	__	__	__	V. Rays-Flat topped	0.01
39	U121	0.8-1	No	__	__	__	V. Rays-Flat topped	0.4
40	U124	1	No	17.91	28.38	12	V. Rays-Flat topped	__
41	U136	1.1-1.4	No	14.12	24.20	12	V. Rays-Flat topped	__
42	U139	0.5-0.6	No	11.07	19.50	9	V. Rays-Flat topped	__
43	U144	0.1	No	13.87	28.17	10	V. Rays-Flat topped	__
44	U147	0.8-1.2	Yes	19.91	25.80	13	V. Rays-Flat topped	__
45	U148	1-1.5	Yes	__	__	__	__	__
46	U149	1.3-1.5	Yes	19.95	28.13	11	V. Rays-Flat topped	__
47	U155	0.8-1.2	Yes	20.95	34.68	14	V. Rays-Flat topped	__
48	U157	1.4-1.8	Yes	18.06	24.86	13	V. Rays-Flat topped	__
49	U159	1.4-1.9	No	18.98	25.37	12	V. Rays-Flat topped	__
50	U163	2.2-2.5	No	19.53	28.7	13	V. Rays-Flat topped	__
51	U169	1-1.2	No	18.55	22.69	11	V. Rays-Flat topped	__
52	U178	__	No	18.31	25.0	10	V. Rays-Flat topped	__
53	U185	__	__	__	__	__	__	__

V. = Vertical

Table 3. Statistical values for morphological and chemical characters.

Character	Gap	Statistical values	Character	Gap	Statistical values
Plant height (cm)	90 >	9	Petal mark width (cm)	3 >	8
	80-90	8		2.5-3	7
	70-80	7		2-2.5	6
	60-70	6		1.5-2	5
	50-60	5		1-1.5	4
	40-50	4		0.5-1	3
	30-40	3		0.3-0.5	2
	20-30	2		0.3<	1
	20 <	1			
Leaf length (cm)	60 >	6	Petal mark length (cm)	3 >	8
	50-60	5		2.5-3	7
	40-50	4		2-2.5	6
	30-40	3		1.5-2	5
	20-30	2		1-1.5	4
	20 <	1		0.5-1	3
				0.3-0.5	2
				0.3 <	1
Bud shape	Bent over	3	Latex on capsule	Yes	3
	Semi-erected	2		Rarely	2
	Erected	1		No	1
Flowering node	8 >	6	Bract leaves	6>	6
	7-8	5		4-6	5
	6-7	4		3-4	4
	5-6	3		2-3	3
	4-5	2		Rare	2
	3	1		None	1
Petal width (cm)	10 >	6	Capsule length (mm)	32 >	7
	9-10	5		29-32	6
	8-9	4		26-29	5
	7-8	3		23-26	4
	6-7	2		20-23	3
	6<	1		17-20	2
				17 <	1
Flowering date	24/05/03 and up	4	Petal marks	To the bottom	4
	20-24	3		Centre (>1 cm)	3
	18-20	2		Centre (<1 cm)	2
	17/05/03 and down	1		None	1
Number of petals	6	3	Petal colour	Dark red	3
	4-6	2		Red	2
	4	1		Light red	1
Petal length (cm)	8 >	5	Number of capsule rays	16 >	5
	7-8	4		14-16	4
	6-7	3		12-14	3
	5-6	2		10-12	2
	5 <	1		10 <	1
Number of sepals	4-5	4	Thebaine (%)	2 >	7
	3	3		1.5-2.0	6
	2-3	2		1.0-1.5	5
	2	1		0.5-1.0	4
				0.01-0.5	3
				Trace	2
				None	1
Shape of capsule top	Flat	6	Capsule width (mm)	21 >	5
	Flat-centred	5		21-18	4
	V.rays-Flat topped	4		18-15	3
	Oval centred	3		15-12	2
	Convex	2		12 <	1
	Concave	1			

Table 4. Names and sequences of the RAPD primers.

Order	Primer name	Primer sequence (5' to 3')	Number of bands amplified	Number of Polymorphic bands	Polymorphism rate
1	OPA-03	AGTCAGCCAC	5	3	60.0
2	OPA-04	AATCGGGCTG	5	4	80.0
3	OPA-07	GAAACGGGTG	6	4	66.6
4	OPF-04	AGGGGTCTTG	10	7	70.0
5	OPF-05	CCGAATTCCC	3	2	66.6
6	OPF-06	GGGAATTCGG	9	7	77.7
7	OPF-08	GGGATATCGG	7	7	100
8	P-443	GGCGTGATAG	5	5	100
9	UBC-238	CTCTCCAGCA	7	7	100
10	OPO-02	ACGTAGCGTC	8	7	87.5
11	OPO-04	AAGTCCGCTC	5	4	80.0
12	OPO-19	GGTGCACGTT	8	8	100
13	P-123	GGGATTCGAC	6	6	100
14	P-166	GTGACGGACT	1	_	_
15	P-437	CGGATCGACA	11	10	90
		Total bands	**96**	**81**	**85.4**

Results

Morphological and chemical characters

A total of 19 morphological characters were used to classify the accessions phenotypically. The detailed analysis of the morphological characters is given in Table 2 and the parameters used for the measurements and statistical values are given in Tables 2 and 3. Significant differences were observed among the accessions, and they were classified into groups accordingly. Nine different classes were observed for plant height (Table 2). The shortest accession was U83 (U: unknown) (12 cm) and the tallest one was PB248 (102 cm). The plant height among accessions varied about 8.5-fold. The leaf sizes varied about 6.5-fold. The smallest and largest leaves belonged to the accessions U84 (10 cm) and PP141 accessions (65 cm), respectively. Similarly, number of bract leaves among accessions varied. Several accessions contained no bract leaves (PO, PP60, and several U accessions). The accession PB10, however, contained the highest number of bract leaves (6-8). Dramatic differences were also identified for several petal leaf related characters such as width, length, marks, mark length, and mark width, and for some other characters such as bud shape, capsule length, capsule width, and number of capsule rays. However, some of the characters, such as number of sepals, number of petals, and petal colour, did not significantly differ among the accessions. The number of sepals ranged from 2 to 3 and the number of petals from 4 to 6. The petal colours, such as the tones of red, were very similar among all accessions (Table 2). A sample picture showing some of the morphological stages of a *Papaver* species is given in Figure 1.

The thebaine alkaloid content analysis of the 53 accessions was carried out via HPLC. The thebaine content (%) in dry capsule samples is given in Table 2. In general, thebaine content ranged between 0% and 2.5% with 15 accessions containing thebaine between 0.1% (more than one accession) and 2.5% (PB100). The remaining 38 accessions contained either trace amounts (<0.1%) or no thebaine. The majority of the *P. bracteatum* accessions contained high thebaine. Among PB accessions, only PB101 contained no thebaine. In contrast, none of the PO accessions in Table 2 contained any thebaine. Among the 24 unknown (U) accessions, however, only U121 contained 0.4% thebaine and the remaining 23 accessions contained either trace amounts (only 3 accessions) or no thebaine (20 accessions).

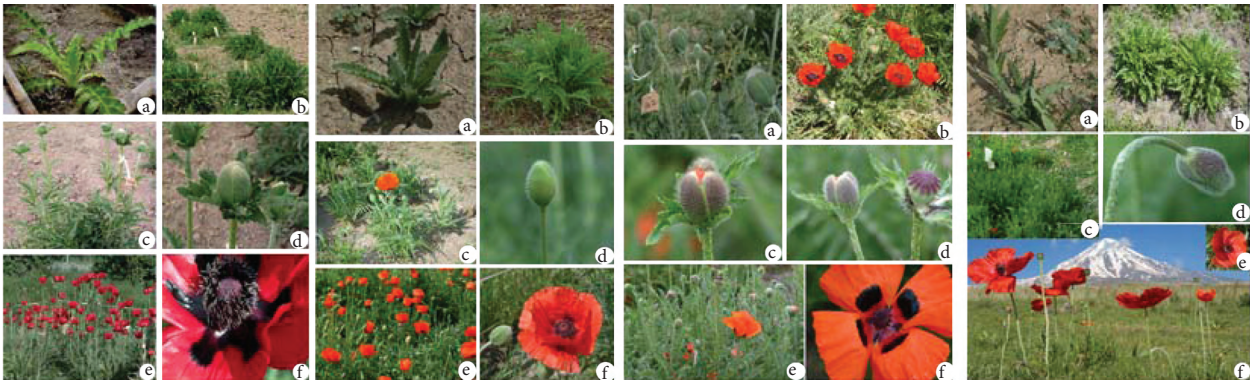

Figure 1. Morphological stages of species I- *Papaver bracteatum,* II- *Papaver pseudo-orientale,* IV- *Papaver orientale* as a- first year rosette, b- second year early stage, c- mature plant, d- bud shape and cauline leaves, e- flower and capsules, and f- petals, anthers and stigma and III- morphological stages for cauline and bract leaves in *Papaver pseudo-orientale* as a- bract leaves, b- general shape of plant, c, d- bract leaves with bud and capsules, e- general bud shape with stem and bract leaves, and f- petal, anther, and stigma.

Using morphological and chemical data (Table 3), the 53 *Papaver* accessions were subjected to phylogenetic analysis (*Hierarchical Cluster Analysis Method*) (SPSS for Windows 11.0) and the resulting dendrogram is given in Figure 2. Except for U83 and U185, all accessions mainly clustered under 4 different branches and each branch also contained sub-clusters. However, 1 of the main branches contained only 1 *P. pseudo-orientale* accession (PP147). Except for PP60 and PP249, accessions clustered under a different branch, all PB and PP accessions clustered in 1 of the 2 large branches. The PO and U accessions showed a diverse phylogenetic relation. Six different PO accessions (PO73, PO74, PO170, PO180, PO240, and PO244) and U accessions were clustered together under the other large branch, where the PO and U accessions were sub-clustered separately.

The morphological characteristics used to identify phylogenetic relations were higher among the *P. bracteatum* accessions compared to the others. The closest relationship was detected between accessions PB99 and PB100 (0.042 – according to matrix data 0-1) and the relationship between the PB3 and PB9 (0.499) accessions was the most distant. In contrast to *P. bracteatum*, the *P. pseudo-orientale* accessions showed a diverse relation. PP147 and PP249 were the most diverse accessions and PP60 was related with U accessions rather than PP. No more than 2 PP accessions clustered under the same sub-clusters. The closest relations among PP accessions were detected between PP121 and PP122 (0.113) and the furthest relation was identified between PP60 and PP141 (0.681). The relation among the PO accessions was less compared to PP accessions. The closest distance was between PO180 and PO240 (0.057) and the furthest between PO169 and PO170 (0.583) accessions, respectively. The U accessions, however, mainly clustered under 2 branches. The closest genetic distance was detected between U19 and U163 (0.093) and the least relation was detected between U59 and U84 (0.929).

Molecular analysis

The 53 accessions were analysed by RAPD-PCR technique at the molecular level using 15 different primers and 96 bands in *Papaver* were successfully amplified. Names and sequences of the primers are given in Table 4. Two sample RAPD-PCR gels for OPF-04 and UBC-238 primers are given in Figure 3. Of the 96 bands amplified, 81 (85%) were polymorphic among the accessions. The remaining 15 bands were, however, monomorphic. These

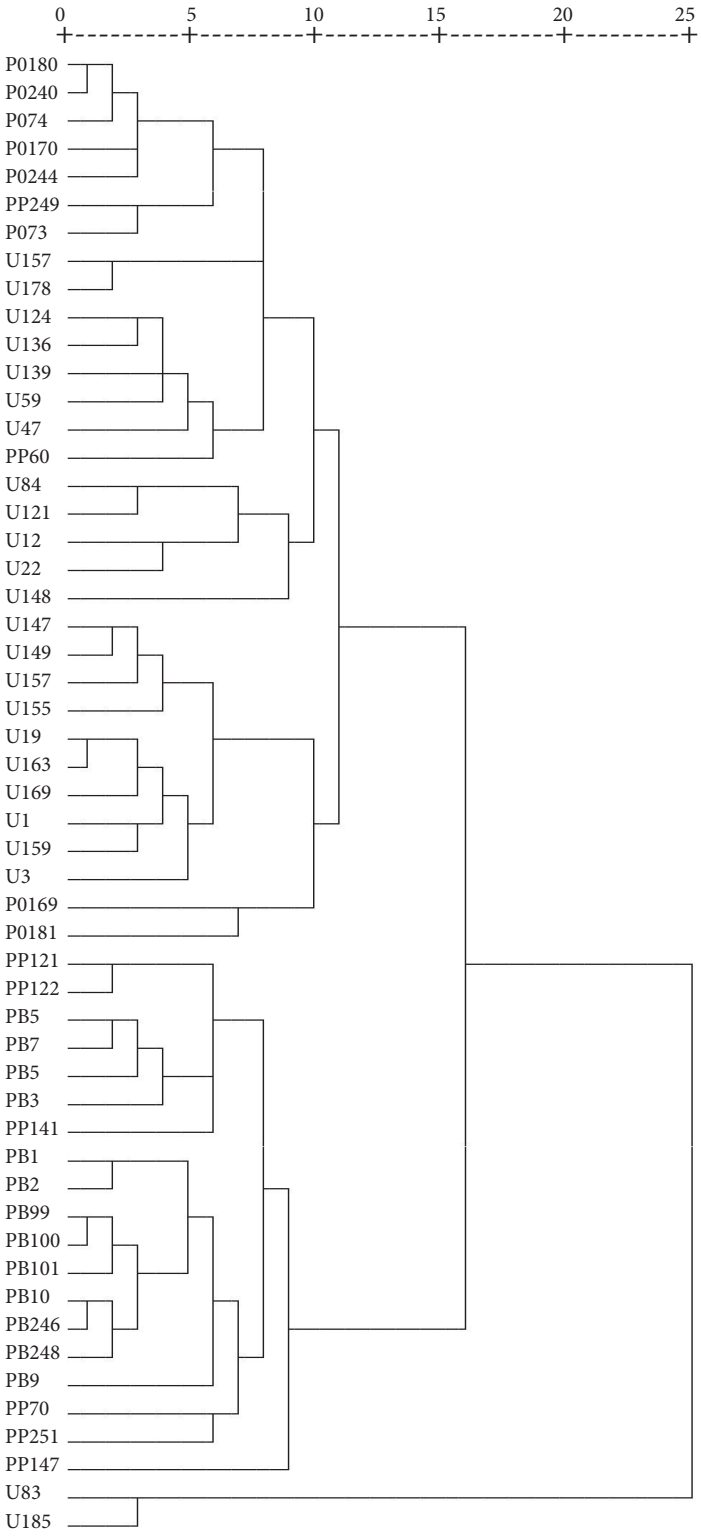

Figure 2. A hierarchical dendrogram of morphological and chemical data obtained from accessions of the section *Oxytona*. Accession names are given as 'PB' for *P. bracteatum*, 'PO' for *P. orientale*, 'PP' for *P. pseudo-orientale*, and 'U' for unknown. SPSS 11.0 was used for analysis.

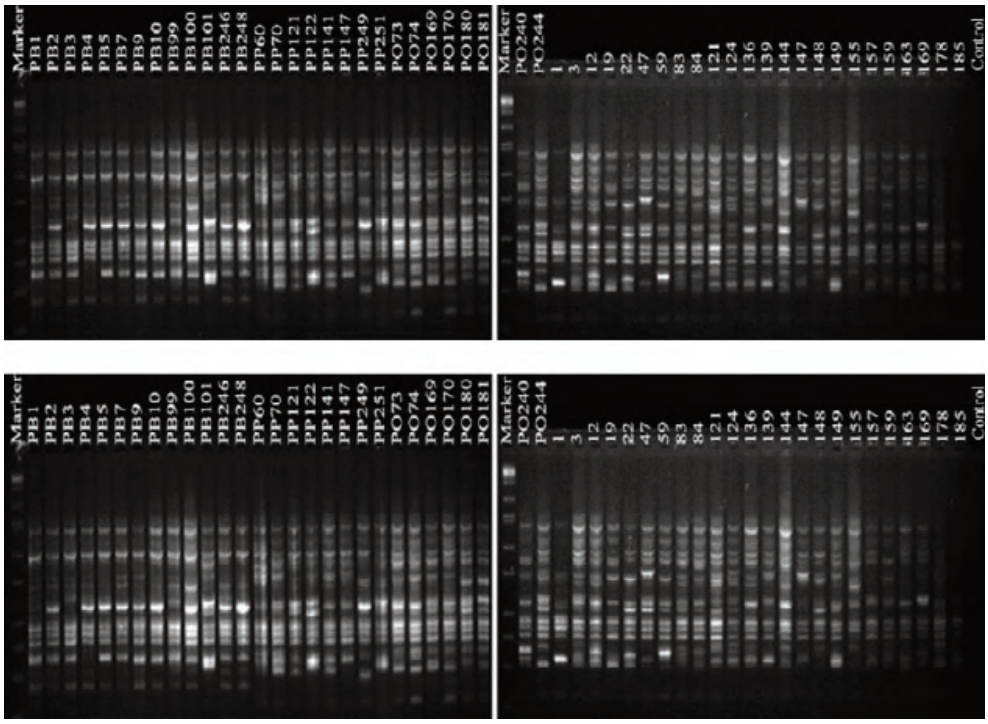

Figure 3. Sample RAPD-PCR analysis for *Papaver* accessions of the section *Oxytona*. A- The amplified PCR products were separated on 1.8% agarose gel with the OPF-04; and B- UBC-238 RAPD primers. The names of the accessions are given at the top of the lanes.

results are given in Table 4. The greatest number of bands was amplified with primer P-437 (11 bands) and the lowest number of bands with P-166 (one monomorphic band). The polymorphism rate among the primers varied greatly. The highest polymorphism (100%) was detected with 5 different primers (OPO-19, P-123, P-443, UBC-238, and OPF-08) and the lowest polymorphism was detected with primer P-166 (0%). Each accession was uniquely identified by the RAPD-PCR data generated by 15 primers, although no single primer discriminated all accessions alone.

A neighbour-joining phylogenetic tree for the accessions is given in Figure 4. Of the 53 accessions, 49 clustered in major branches of the tree consisting of further sub-clusters under the main branches. The first branch contained mainly the PB accessions. The second branch mainly contained the PO and the PP accessions. The third branch contained the U accessions.

Except for the accessions PO240 and PO244, all accessions clustered together. Only the U1 clustered separately among U accessions. With no exception, however, all the PB accessions clustered under the centre branch. The closest genetic distance (0.054 according to matrix data 0-1) was detected between PB5 and PB7 and the furthest genetic distance (0.575) was observed between the PO181 and U185 accessions. The average genetic distance among the 53 accessions was calculated to be about 0.34. Further analysis of the subgroups related to U accessions revealed that the accessions collected from different cities are clustered together. For instance, 8 of the 12 accessions collected from Erzurum province showed a high level of genetic relation compared to other city accessions. The closest genetic relation among unknown accessions was detected between accessions obtained from the towns of Tortum and Narman in Erzurum province and the furthest genetic relation was detected between Kars and Erzurum accessions (0.557).

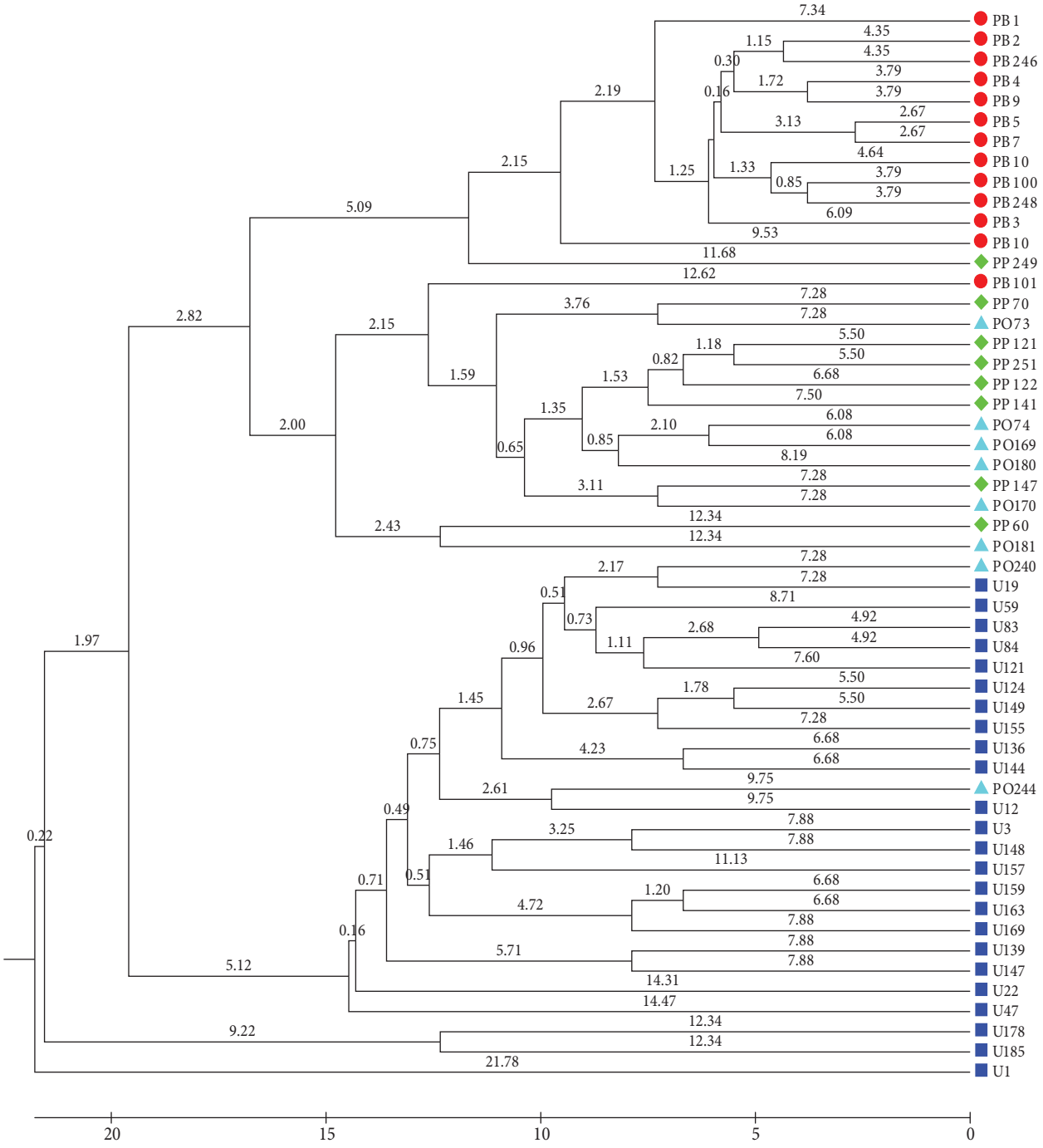

Figure 4. Population genetic analysis (POPGENE 32 version 1.32) and the molecular evolutionary genetic analysis (MEGA 3.0) of phylogenetic relationships of 53 accessions from the section *Oxytona* genotypes based on PCR-RAPD analysis using Nei's genetic distance matrix. The numbers on the branches show the branch lengths.

Discussion

Morphological parameters used for characterisation

All 3 species from the section *Oxytona* show perennial growing and phenotypic resemblance. A number of morphological characters were previously used to differentiate phenotypically similar plant selections from the section *Oxytona* (Goldblatt, 1974; Davis, 1988). However, either the use of an insufficient number of morphological characters or mistiming of measurements for parameters resulted in failure to solidly characterise section *Oxytona* species (Oztekin et al., 1985; Ojala & Rousi, 1986; Ojala et al., 1990; Levy & Milo, 1991). For example, 'bract leaves' and some other bract related characteristics as well as 'number of sepals' are distinctive characteristics of the *Oxytona* species (Materials and methods). Due to the above-mentioned reasons, however, these characters failed to clearly distinguish the *Oxytona* species (Goldblatt, 1974; Phillipson et al., 1981; Davis, 1988; Mihalik, 2000; Carolan et al., 2002). To the best of our knowledge, the current study combined the highest number of parameters (19 characters) and involved the best timing for morphological characterisation. Consequently, it resulted in a strong and comprehensive phenotypic analysis among accessions of section *Oxytona* species.

Striking morphological differences among the accessions were observed for characteristics such as bract leaves, bud shape, petal colour, and mark shape on petals. The *P. bracteatum* plants have bract leaves, but *P. orientale* does not. This character is variable as both phenotypes occur among accessions of *P. pseudo-orientale*. Moreover, the bud shape is erect in *P. bracteatum*, bent over in *P. orientale*, but mixed in *P. pseudo-orientale*. Similarly, petal colour is dark-red in *P. bracteatum*, light red in *P. orientale*, and intermediate in *P. pseudo-orientale*. *P. bracteatum* contains a dark and wide mark extending from the centre to the bottom of the petals, but *P. orientale* contains no spot (Goldblatt, 1974; Carolan et al., 2002). The mark on *P. pseudo-orientale* petals, however, shows a gradient from none to a wide dark spot on the bract leaves (Goldblatt, 1974; Davis, 1988; Mihalik, 2000).

Matrix analysis for morphological and chemical characters

Data from 20 different parameters were grouped into classes (Figure 2). Use of a matrix for similarity and difference efficiently differentiated 53 accessions in major clusters and further sub-clusters. This method is rarely used for plant species and has been applied to the genus *Papaver* for the first time in this study. The PB and PP accessions were compiled under separate clusters. Furthermore, clustering of unknown plants with known accessions of *Oxytona* species allowed us to make better assessments. About 50% of the U accessions showed a clear relation to the PO accessions, whereas the remaining 50% were not closely related to any of the known accessions of the section *Oxytona*.

Thebaine analysis

Several discrepancies were observed in thebaine content among accessions (Table 2). The thebaine content of *P. bracteatum* accessions ranged from 0.6% to 2.5%. Interestingly, a PB accession contained no thebaine (PB101) and a *P. orientale* accession (PO180) contained a trace amount, even though thebaine is the main alkaloid in *P. bracteatum* (Sarıyar, 2002; Carolan et al., 2002), and no thebaine is reported in *P. orientale* (Dawson & James, 1956; Stermitz & Rapoport 1961; Milo et al., 1990; Sarıyar, 2002). Furthermore, 5 of the U accessions (U12, U22, U83, U84, and U121) related to PO in the phylogenetic tree contained 0.01% to 0.4% thebaine in dry capsules (Figure 2). The thebaine content of the PP accessions ranged from none to 1.5%; hence, it is difficult to distinguish *P. pseudo-orientale* accessions from *P. bracteatum* based on thebaine content (Shoyama et al., 1998; Sarıyar, 2002). Based on previous reports and our data, therefore, use of thebaine content as a chemical parameter would be misleading for characterisation in the section *Oxytona*.

RAPD-PCR based molecular analysis

With the use of the RAPD-PCR method, a high level of polymorphism was detected among accessions. Fifteen different primers generated 96 bands, of which 81 were polymorphic (85%). Number of bands per primer ranged between 1 and 11 and average band number was 6.4 bands/primer (Table 4). Detection of a high level of polymorphism using

RAPD primers was previously reported for several plants species (Millan et al., 1996; Schontz & Rether, 1999; Carolan et al., 2002; Budak et al., 2004; Erayman et al., 2004; Isık et al., 2007; Alam et al., 2009) and for some *Papaver* species such as *P. setigerum, P. somniferum*, and *P. pseudo-orientele* (Sangwan et al., 2000). Only 15 primers were sufficient for determination of genetic variation. Similar results were obtained with 4 to 12 primers among different plant genomes (Millan et al., 1996; Schontz & Rether, 1999). In general, if the variation among the accessions is very high, the number of primers required may be lower (Li & Midmore, 1999; Andersen, 2003; Budak et al., 2004).

The neighbour-joining phylogenetic tree generated by molecular data revealed dramatic differences in the section *Oxytona*. Genetic variation between the PP and PO accessions was high compared to PB. Except for U1, all U accessions surprisingly showed no genetic relation with PP, PO, or PB accessions (Figure 4). The main reason for this could be that the unknown accessions were collected from nature, where the plants are open to cross pollination (Goldblatt, 1974; Milo et al., 1986; Milo & Levy, 1988), whereas the PP, PB, and PO accessions were grown for several years under controlled conditions (Materials and methods).

Comparison of morphological and molecular phylogenetic trees

Two different phylogenetic trees were generated (Figures 2-4). Clustering of similar accessions in the molecular phylogenetic tree was more prevalent compared to the morphological dendrogram. Even though the highest number of morphological parameters was used in the current study, we failed to achieve a solid characterisation for accessions belonging to the same species. However, the *Oxytona* species were clearly differentiated using molecular analysis (Figure 3). All 4 groups (PB, PP, PO, and U) were clustered separately in the tree except for 4 discrepant accessions (PO240, PO244, PB101, and U1). These 4 accessions may be plants showing interspecific characters or intermediate accessions resulting from natural interspecific hybridisation (Goldblatt, 1974; Shoyama et al., 1998; Carolan et al., 2002; Coşkun et al., 2010; Dirmenci et al., 2010). Hence, the true naming of these accessions remains under debate. In general, even though the molecular analysis is more reliable than morphological analysis, use of parameters from both methods would be highly effective for characterisation of variation in the section *Oxytona*.

In conclusion, the high-thebaine-containing and naturally growing accessions of Turkish section *Oxytona* species were analysed in detail at a multidisciplinary level for the first time. Optimisation of the RAPD-PCR method and identification of polymorphic primers for the section *Oxytona* allowed us to characterise the current *Papaver* germplasm and paved the way for marker assisted selection in *Papaver* breeding programmes. In fact a map and gene mapping would be required for this. Even though the thebaine content of the accessions is not a distinctive parameter, use of molecular techniques along with phenotypic characters will greatly help in the differentiation of accessions within and across the species of the section *Oxytona*.

Acknowledgements

We gratefully thank Prof. Dr. Neşet Arslan (University of Ankara, Turkey) for obtaining the plant accessions, and the Turkish Green Board (TMO) for analysing the thebaine content in dry capsules. This study was supported by the Scientific and Technological Research Council of Turkey (Project No: TOGTAG/TARP-1843) and the State Planning Organisation (DPT) of Turkey (Project No. 98 K120640 and 2001 K 120240).

References

Alam MA, Naik PK & Mishra GP (2009). Congruence of RAPD and ISSR markers for evaluation of genomic relationship among 28 populations of *Podophyllum hexandrum* Royle from Himachal Pradesh, India. *Turk J Bot* 33: 1-12.

Andersen JR (2003). Functional markers in plants. *Trends Plant Science* 8: 554-559.

Arslan N (1991). *Papaver bracteatum* Lindl. Hatlarının Kapsüllerinde Tebain oranının varyasyonu üzerine araştırmalar. 9. Bitkisel Ilaç Hammaddeleri Toplantisi. Bildiriler. 16-19 Mayıs, Eskişehir.

Atalay I (1994). Turkey Vegetation Geography. Ege University Press, İzmir.

Bernath J (2000). Poppy the genus papaver. Harwood Academic Publishers Budapest, pp. 279-289, Hungary.

Budak H, Shearman RC, Parmaksız İ & Dweikat I (2004). Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. *Theor Appl Genet* 109: 280-288.

Carolan JC, Hook ILI, Chase MW, Kadereit JW & Hodkinson TR (2006). Phylogenetics of *Papaver* and related genera based on DNA sequences from ITS nuclear ribosomal DNA and plastid *trnL* intron and *trnL–F* intergenic spacers. *Annals of Botany* 98: 141-155.

Carolan JC, Hook ILI, Walsh JJ & Hodkinson TR (2002). Using AFLP markers for species differentiation and assessment of genetic variability of *in vitro*-cultured *Papaver bracteatum* (Section *Oxytona*). *In vitro Cell Dev Biol-Plant* 38: 300-307.

Coşkun F, Selvi S & Satıl F (2010). Phylogenetic relationships of some Turkish Crocus (Iridaceae) taxa based on morphological and anatomical characters. *Turk J Bot* 34: 171-178.

Cullen J (1965). *Papaver* L. In: Davis PH (ed.), *Flora of Turkey and the East Aegean Islands*. Edinburgh: Edinburgh Univ Press, Vol.1, pp 219-236.

Davis PH, Mill RR & Tan K (1988). *Flora of Turkey and the East Aegean Islands* (Suppl. 1), Vol. 10, Edinburgh: Edinburgh Univ Press.

Dawson RF & James C (1956). Alkaloids of *Papaver orientale* L. I. Qualitative detection and occurrence. *Lioydia* 19: 59-64.

Dirmenci T, Dündar E, Deniz G, Arabacı T, Martın E & Jamzad Z (2010). Morphological, karyological and phylogenetic evaluation of Cyclotrichium: a piece in the tribe Mentheae puzzle. *Turk J Bot* 34: 159-170.

Erayman M, Sandhu D, Sidhu D, Dilbirligi M, Baenziger PS & Gill KS (2004). Demarcating the gene-rich regions of the wheat genome. *Nucleic Acids Res* 32: 3546-3565.

Goldblatt P (1974). Biosystematic studies in *Papaver* section *Oxytona*. *Annals Miss Bot Garden* 61: 264-296.

Gültepe M, Uzuner U, Coşkunçelebi K, Beldüz AO & Salih Terzioğlu (2010). Internal transcribed spacer (ITS) polymorphism in the wild Primula (Primulaceae) taxa of Turkey. *Turk J Bot* 34: 147-157.

Güner A, Özhatay N, Ekim T & Başer KHC (2000). Flora of Turkey and the East Aegean Islands. Vol: 11. Edinburgh: Edinburgh Univ Press.

Gupta PK & Rustgi S (2004). Molecular markers from the transcribed/expressed region of the genome in higher plants. Funct Integr *Genomics* 4: 139-162.

Gupta PK, Varshney RK & Prasad M (2002). Molecular markers: principles and methodology. In: Jain SM, Brar DS, Ahloowalia BS (eds.). Molecular techniques in crop improvement. Kluwer, Dordrecht.

Hillis DM & Moritz C (1990). Molecular systematics, Sinauer Associates, Inc. Publishers, pp. 217-220, Sunderland.

Isık Z, Parmaksiz I, Coruh C, Geylan-Su YS, Cebeci O, Beecher B & Budak H (2007). Organellar genome analysis of rye (*Secale cereale*) representing diverse geographic regions. *Genome* 50: 724-734.

Kapoor LD (1997). Opium Poppy: botany, chemistry and pharmacology. Food Products Press, New York.

Kumar S, Tamura T & Nei M (2004). MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief in Bioinformatics* 5: 2-10.

Levy A & Milo J (1991). Inheritance of morphological and chemical characters in interspecific hybrids between *P. bracteatum* and *Papaver pseudo-orientale*. *Theor Appl Genet* 81: 537-540.

Li M & Midmore DJ (1999). Estimating the genetic relationships of Chinese water chestnut [*Eleocharis dulcis* (Burm. f.) Hensch] cultivated in Australia using random amplified polymorphic DNAs (RAPDs). *J Hort Sci Biotech* 74: 224-231.

Millan T, Osuna F, Cobos S, Torres AM & Cubero AM (1996). Using RAPDs to study phylogenetic relationships in rosa. *Theor Appl Genet* 92: 273-277.

Milo J & Levy A (1988). Phylogenetic and genetic studies in *Papaver* section *Oxytona*: cytogenetics, isozyme analysis and chloroplast DNA variation. *Theor Appl Genet* 75: 795-802.

Milo J, Levy A, Ladizinsky G & Palevitch D (1986). Phylogenetic studies in *Papaver* section *Oxytona*: cytogenetics of the species and interspecific hybrids. *Theor Appl Genet* 72: 524-529.

Milo J, Levy A, Palevitch D & Ladizinsky G (1990). Genetic evidence for the conversion of the morphinan alkoloid thebaine to oripavine in interspecific hybrids between *Papaver bracteatum* and *Papaver orientale*. *Heredity* 64: 367-370.

Nei M (1972). Genetic distance between populations. *The American Naturalist* 106: 283-292.

Nei N & Li W (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci* (USA) 76: 5269-5273.

Nyman U & Bruhn JG (1979). *Papaver bracteatum*: a summary of current knowledge. *Planta Medica* 35: 97-117.

Ojala A & Rousi A (1986). Interspecific hybridization in *Papaver* F1 hybrids of *Papaver somniferum* with perennial species of section Oxytona. *Ann Bot Fennici* 23: 289-303.

Ojala A, Rousi A, Lewing E, Pyysalo H & Widen CJ (1990). Interspecific hybridization in *Papaver* F1 hybrids between species of section Oxytona. *Hereditas* 112: 221-230.

Oztekin A, Baytop A, Hutin M, Foucher JP, Hocquemiller R & Cave A (1985). Comparison of chemical and botanical studies of Turkish *Papaver* belonging to the section *Pilosa*. *Planta Medica* 5: 431-434.

Phillipson JD, Baytop A, Scutt A, Sarıyar G & Ozahatay N (1981). Alkaloids from Turkish samples of *Papaver bracteatum* and *Papaver pseudo-orientale*. *Planta Medica* 43: 261-271.

Sangwan RS, Yadav U & Sangwan NS (2000). Isolation of genomic DNA from defatted oil seed residue of opium poppy (*Papaver somniferum*). *Plant Mol Biol Rep* 18: 265-270.

Sarıyar G (2002). Biodiversity in the alkaloids of Turkish *Papaver* species. *Pure Appl Chem* 74: 557-574.

Schontz D & Rether B (1999). Genetic variability in Foxtau Millet (*Setaria italicar* L.) P.Beauv: identification and classification of lines with RAPD Markers. *Plant Breed* 118: 190-192.

Shoyama Y, Kawachi F, Tanaka H, Nakai R, Shibata T & Nishi K (1998). Genetic and alkaloid analysis of *Papaver* species and their F1 hybrids by RAPD, HPLC and ELISA. *Foren Sci Int* 91: 207-217.

Stermitz FR & Rapoport H (1961). Biosynthesis of opium alkaloids. Alkaloid interconversions in *Papaver somniferum* and *Papaver orientale*. *J American Chem Soc* 83: 4045.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA & Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl Acids Res* 18: 6531-6535.