J. Fac. Pharm. Istanbul 29, 1 (1993)

Istanbul Ecz. Fak. Mec. **29,** 1 (1993)

COMPARATIVE MORPHOLOGICAL, KARYOLOGICAL AND CHEMICAL STUDIES ON Allium scorodoprasum COMPLEX IN EUROPEAN TURKEY

N. ÖZHATAY*, L. ÜSTÜN* and A. H. MERİÇLİ**

SUMMARY

A close examination of the outer and inner morphological characters, of A. scorodoprasum subsp. scorodoprasum and subsp. rotundum grow in European Turkey show that they should be treated as different species and their names as had, till 1978, been A. scorodoprasum L. and A. rotundum L.. According to the results of the present work their main differences are summarized as follows: A. scorodoprasum; outer and inner tunics membranous, dark grey or reddish violet; bulblets ovoid-oblong, stalked; spathe yellowish; umbel with bulbils and 0-20 flowered; pedicels almost equal. Cuticle of foliage leaves without micropapilla. Simple pits of parenchymatic cells absent, crystals cubic or prizmatic, simple or twin rarely compound, monohydrate or trihydrate; no sclerenchymatic fibres in bulb scales. Chromosome number 2n=16. A. rotundum; outer tunics coriaceaus, yellowish-brown, inner tunic membranous white or dark purple; bulblets in different shaped; spathe greenish-pink; umbel without bulbils, many flowered; pedicels unequal with bracteol. Cuticle of foliage leaves with micropapilla. Simple

^(*) Faculty of Pharmacy, Department of Pharmaceutical Botany, University of Istanbul, 34452, Istanbul, Turkey.

^(**) Faculty of Pharmacy, Department of Pharmacognosy, University of Istanbul, 34452, Istanbul, Turkey

INTRODUCTION

A. scorodoprasum L. is a complex species, W. T. Stearn (1) logically treated A. rotundum L. and the related A. jajlae Vved. and A. waldsteinii G. Don as subspecies of A. scorodoprasum L.. A. scorodoprasum L. subsp. jajlae (Vved.) Stearn and subsp. waldsteinii (G. Don) Stearn have been distinguished from subsp. rotundum (L.) Stearn largely on account of their flower colour (uniformly pinkish in subsp. jajlae, uniformly deep purple in subsp. waldsteinii) but bicoloured in subsp. rotundum with the outer segments deep purple and the inner whitish or very pale pink. As mentioned before, Stearn treats these as subspecies of A. scorodoprasum L. which is itself easily recognised of the presence of bulbils in the umbel. All subspecies are found in Turkey (2) and 2 of them, subsp. scorodoprasum and subsp. rotundum occur in European Turkey.

The present study deals with morphological anatomical, karyological and chemical studies carried out on A. scorodoprasum L. subsp. scorodoprasum and A. scorodoprasum L. subsp. rotundum (L.) Stearn in European Turkey.

RESULT and DISCUSSION

Detailed examination of two subspecies show that they should be accepted as different species.

I. Outer morphological characteristics

The main diagnostic characteristics of two species based on the examined specimens are summarized below:

A. scorodoprasum L. Fig. 1 and 3A, 3B.

Outer tunics membranous, dark grey or reddish violet. Bulblets with long stipitate on the scape, blackish purple, oblong-ovoid. Scape 60-112 cm. Leaves 0.8-1.5 cm x 20-40 cm. Spathe yellowish. Umbel lax with dark violet bulbils and few flower (0-20); pedicels almost equal without bracteols. Outer perianth segments dark purple, brownish; inner pale pinkish violet; stamens equal or slightly shorter.

Flowering time: May-June

Habitat: Grassy and wet places, scrubs, among Juncus, sandy shore.

Examined specimens: A1 (E) EDIRNE: Sarapyiçi, 19.IV.1967, A. and T. Baytop, ISTE 6563; Tavuk ormanı, 21.V.1975, N. and E. Özhatay, ISTE 31716; Tavuk ormanı, Sarayiçi, 6.VII.1985, L. Üstün, ISTE 56129; KIRKLARELİ: İğneada, near Kocagöl, sandy shore, among Juncus, 27.VI.1974, N. and E. Özhatay, ISTE 30031;

Distribution in Turkey: Northern-West Turkey. Euro-Siberian element.

A. rotundum (L.) Stearn Fig. 2 and Fig. 3C, 3D.

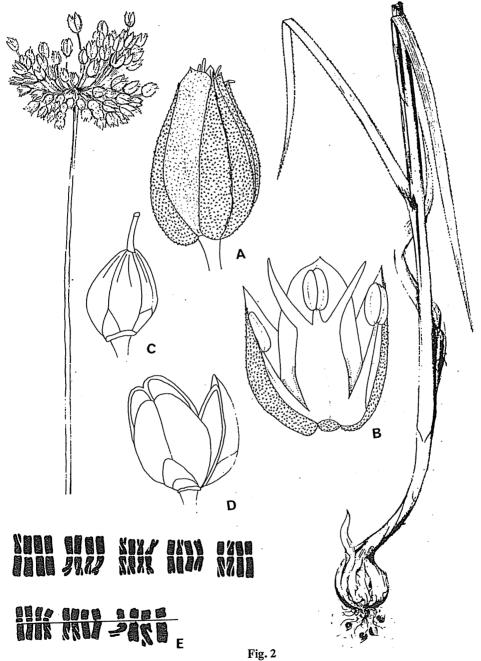
Outer tunics coriaceous, brown or splitting into fibres at the apex then yellowish-orange. Bulblets produced beneath the bulb tunics, blackish-purple, irregular shaped. Scape 22-94 cm. Leaves 0.2-1 cm x 13-35 cm. Spathe greenish-pink. Umbel dense, many flowered (25-100); pedicels unequal with white membranous bracteol. Outer perianth segments dark purple inner pinkish. Stamens clearly shorter than perianth segments.

Flowering time: May-July

Habitat: Rocky and stony places, roadsides, field, fieldsides, meadows, scrub, dry slopes.

Examined specimens: A1 (E) EDIRNE: Keşan-Enez, 17.V.1970, A. Baytop and F. Öktem, ISTE 17857; 5 km from Edirne to Lalapaşa, fieldside 5.VI.1974, N. and E. Özhatay, ISTE 31717; Istanbul to 9 km to Edirne, 21.V.1978, N. and E. Özhatay, ISTE 38478; TEKIRDAĞ: 17 km from Saray-İstanbul, under Quercus, 28.VI.1973, A. Baytop, ISTE 26208, Mürefte 3 km to Şarköy, 23.V.1974, N. and E. Özhatay, ISTE 28432; Saray 3 km to Şarköy, 23.V.1974, N. and E. Özhatay, ISTE 32131; KIRKLARELİ: Vize, 1. VIII.1974, A. Baytop, ISTE 29890; Kapaklıköy-Dereköy, Istirga, stony slopes, 29.VI.1974, N. and E. Özhatay, ISTE 30072; Dereköy-Armutveren, around Manastir Tepe, rocky slopes, 29.VI.1974, N. and E. Özhatay, ISTE 30077; Lüleburgaz-Corlu, near Kepirtepe, pine plantation hill, 21.VI.1975, N. and E. Özhatay, ISTE 31723; Babaeski 33 km to Kırklareli, 25.V.1975, N. and E. Özhatay, ISTE 31731; Kömürköy-Vize, follow fields, fieldsides, 16.VI.1975, A. Baytop, ISTE 31964; 4 km from Saray-Vize, 25.VI.1975, N. and E. Özhatay, ISTE 32125; 3 km from Poyralı Demirköy, 25.VI.1975, N. and E. Özhatay, ISTE 32142; Demirköy-Dereköy, 500 km from Armutveren Military Tower, among Ouercus scrubs, 27.VI.1975, N. and E. Özhatay, ISTE 32198; Demirköy-Dereköy, 5 km from Armutveren, 27.VI.1974, N. and E. Özhatay, ISTE 32206; Demirköy-Dereköy, 5 km to Karadere, 27.VI.1975, N. and E. Özhatay, ISTE 32212; A2 (E) KIRKLARELI: Iğneada-Limanköy, slopes near sea, 26.VI.1975, N. and E. Özhatay, ISTE 32154; A2 (E) ISTANBUL: Halkalı, dry slopes opposide of railway station, 5.VI.1974, N. and E. Özhatay, ISTE 29874; Büyükçekmece, Anarşa, slopes, 16.VI.1974, F. Serin, ISTE 29877; Tekirdağ, 2 km to Gümüşyaka, apple cultivation area, 16.VII. 1974. N. and E. Özhatay, ISTE 30429; 4 km to Çatalca, follow fields, 18.V.1975, N. and E. Özhaay, ISTE 31633; ibid., 21.V.1975, N. and E. Özhatay, ISTE 31729; Above Terkos in fields, 29.V.1975, A. Baytop, ISTE 31833; ibid., 5.IV.1985, L. Üstün, ISTE 56130;

Distribution in Turkey: Widespread, sea level to 2800 m. Mediterranean element.



II. Main anatomical differences of two species are summarized in Table 1.

Tablo 1: Main anatomical characteristics of A. scorodoprasum and A. rotundum

		scorodoprasum	rotundum	
Foliage leaf	Mean thickness of midvein region	340	750	
	Mean thickness of intervein region	280	350	
	Micropapilla on cuticle		+	
	Stomata number per mm² in lower epidermis	82	64	
	Stomata number per mm² in upper epidermis	84	82	
	Location of laticifer	Under 1. row palisade cells	Under 2-3. row palisade cell	
	Xylem fibres	absent or few	many	
	Phloem fibres	absent or few (1-2)	Few (3-5)	
Scape	Diameter	5.73 mm	4.63 mm	
	Number of vascular bundles	28	35	
Bulb	CaC_2O_4 crystals in membranous leaves $(S_1 - S_4)$	Monohydrate and Trihydrate		
•	CaC_2O_4 crystals in membranous leaves (S ₁ - S ₄)	Simple, twin rarely compound	Compound (Druse)	
	Sclerenchymatous fibres in membranous leaf $(S_1 - S_4)$		+	
	Simple pits on parenchymatic cells		+	

III. Karyotypes

A. scorodoprasum L. Fig. 1E.

2n=16; diploid (ISTE 56129).

Karyotype consists of 8 pairs of metacentric to submetacentric chromosomes. Secondary constrictions were observed on the sixth, seventh and eighth choromosome pairs. Chromosomes No 6, 7 have Sativum type secondary constriction on the other hand No 8 has Scorodoprasum type secondary constriction on the short arms. No satellite were observed.

Confirms several previous counts and karyotypes (3) 2n=16 (4-12).

A. rotundum (L.) Stearn Fig. 2E.

2n=32; tetraploid (ISTE 56139).

Karyotype consists of 8 sets of metacentric-submetacentric chromosomes. No satellite were seen but Sativum type secondary constriction was observed in all chromosomes of one set (No 6). The karyotype exhibits a degree of heteromorphy in two sets (No 4 and 8).

Confirms several previous reports, 2n=32 (4, 13-17).

IV. The chemical work has so far, been limited to preliminary investigations, flavonoids and sapogenins analysis by TLC and PC methods.

Preliminary investigations: Infusion (5 %) prepared from leaves, bulbs and scapes were examined for flavonoids, alkaloids, saponins, tannins and anthrasen derivatives (18). The results obtained have been shown on table 2.

Table 2: The results of preliminary chemical investigations of *A. scorodoprasum* s. l. in European Turkey.

	A. scorodoprasum (ISTE 56129)			A. rotundum (ISTE 56130)		
	Leaf	Scape	Bulb	Leaf	Scape	Bulb
Flavonoids	++	+ (Trace)	+	+++	+	+
Antrasen		·				
Derivatives		********				
Tannins	++			+	<u></u>	
	(Catechic)			(Catechic)		
Alkaloids	++	++	+	++	,++	+
Saponins	+	+ (Trace)	+++	+	+ (Trace)	+++

Identification of Sapogenins: It has been reported *Allium* species contain sapogenins like Diosgenin, Diosgenin acetate, Ruscogenin, Alligenin, Hecogenin, Gitogenin, Tigogenin (19-26). The plant material (bulbs, leaves and scapes) were each extracted according to the previously used methods (27-28). Crude extracts were each examined by TLC using authentic samples of Diosgenin, Diosgenin acetate, Hecogenin, Botogenin, Cammogenin and Kristogenin.

TLC: Silica gel G.

Solvent systems: a) Chloroform: Methanol (100:3)

b) Hexan: n-Butanol (100-10)

The spots were detected with a reagent consisting of Vanilin + conc. H₂SO₄.

The presence of Diosgenin was shown in both subspecies as a major compound while Cammogenin was found as a minor compound also in both subspecies.

Identification of Flavonoids: It has been reported *Allium* species contain flavonoids like Apigenin, Quercitrin, Kaempferol (29). For the detection of flavonoids by TLC and PC the ethanolic extracts, prepared from the same organs, were each worked up in the conventional manner (30-32) using authentic samples Apigenin, Luteolin, Kaempferol, Quercetin, Apigenin 7-glucosite, Luteolin 7-glucoside, Kaempferol 3-glucoside and Quercetin 3-glucoside.

Solvent sytems for aglicons:

TLC: Silica gel G

Toluol: Ethylacetate: Formic acid (5:4:1)

PC: Whatman Nr. 1

Acetic acid Water (60:40)

Solvent system for glucosides:

TLC: Silica gel G

Ethyl acetate: Ethyl methyl Keton: Formic acid: Water (5:3:3:1)

PC: Whatman Nr. 1

Asetic acid: Water (15:85) Reagent: 5 % KOH in MeOH

As a result of TLC and PC, the presence of Apigenin, Luteolin and Luteolin 7-glucoside were found in both subspecies.

MATERIAL and METHODS

All the plants studied are from natural populations, as shown in Pages 33 and 35 as examined specimens. For morphological studies dried and living material were used. For karyological studies (ISTE 56129 and 56130), root tip squashes were prepared using

the method previously described (33). For chemical studies, plant material (ISTE 56129 and 56130) were collected at flowering stage then leaves, scapes and bulbs were dried separetely at the room temparature and powdered. Voucher specimens have been placed in the ISTE.

Acknowledgements: The S.E.M. pictures of seeds were taken in Anatomy section of Jodrell Laboratory (Kew, England) with kind permission of Prof. K. Jones and Dr. D. Cutler. We would like to express our sincere thanks to them, also to Prof. Dr. S. Kurucu (Ankara) for providing the authentic samples of sapogenins.

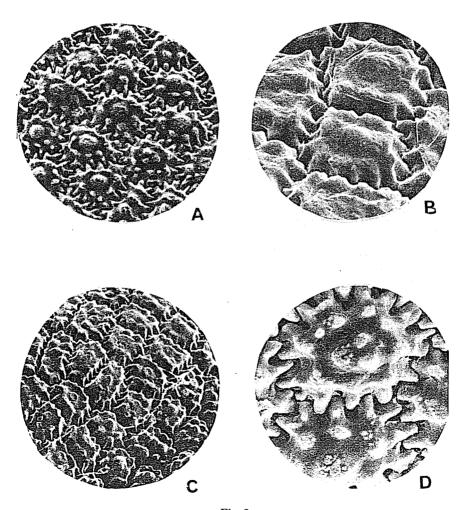


Fig. 3

REFERENCES

- 1. Stearn, W. T., Ann. Mus. Goulandris, 4:178 (1978).
- 2. Kollmann, F., Allium in Davis, P.H., Flora of Turkey and the East Aegean Islands, vol. 8, 172-174, University Press, Edinburgh (1984).
- 3. Chesmedjiyev, I. V., Both. Arch., 24 (1): 165-168 (1979).
- 4. Levan, A., Hereditas, 15 (3): 347-356 (1931).
- 5. Katayama, T., Jour. Coll. Agric. Imp. Univ. Tokyo, 13 (5): 431-441 (1936).
- 6. Hindakova, M., Cincura, F., In Index of Chromosome numbers of Slovakian Flora (Part 1) (1967).
- 7. Sopova, M., God. Zborn. Biol. Fak. Skopje Univ. Prir.-Mat., 20: 147-157 (1968).
- 8. Kim, J.H., Korean J. Bot., 17: 113-117 (1974).
- 9. Love, A., Love, D., Taxon 31: 583-587 (1982 b).
- 10. Speta, F., Linzer Biol. Beitr., 16: 45-81 (1984).
- 11. Halkka, L., Ann. Bot. Fenn., 22: 315-317 (1985).
- 12. Semerenko, L.V., Bot. Zurn. SSSR, 70:130-132 (1985 a).
- 13. Delay, C., Rev. Cytol. et Cytophysiol. Veg., 9 (1-4): 169-222, 10 (1-4): 103-229 (1947).
- 14. Chesmedjiev, J.V., Bot. Zurn. (Moscow and Leningrad), 58: 864-875 (1973).
- 15. Ruiz Rejon, M., IN IOPB Chromosome number report LII. Taxon 25: 341-346 (1976).
- 16. Özhatay, N., Istanbul Univ. Eczacılık Fak. Mecmuası (J. Fac. Pharm. Istanbul), 20:43-65 (1984 a).
- 17. Tzanoudakis, D., Ann. Mus. Goulandris, 7:233-247 (1985).
- Baytop, T., Farmakognozi Ders Kitabı Cilt I. Eczacılık Fakültesi Yayınları No. 29, Fatih Yayınevi Matbaası (1980).
- 19. Azarkova, A.F., et al., Khim Prir Soedin, 3:407 (1974). Ref. C.A. 81:166358 v (1974).
- 20. Azarkova, A.F., et al., Khim Prir Soedin, 5:65 (1983). Ref. C.A. 100:135771 f (1984).
- 21. Ismailov, A.I., Tagiev, S.A., Uch. Zap., 37:60-64 (1974). Ref. C.A. 87:180731 s (1974).
- Ismailov, A.I., Tagiev, S.A., Khromotogr. Medody Farm., 137-143 (1977). Ref. C.A. 90:68651 b (1979).

- 23. Kerestelidze, E.V. etal., Khim Prir Soedin, 6 (3): 372 (1970). Ref. C.A. 73:117181 h (1970).
- Okanishi, T., et al., Chem. Pharm. Bull., 23 (3): 573-579 (1975). Ref. C.A. 82: 167519 f (1975).
- 25. Pkheidze, T.A., et al., Vses Sézda Farm., 215-221 (1967). Ref. C.A. 75:115868 e (1971).
- Pkheidze, T.A., et al., Khim Prir Soedin, 7 (6): 841-842 (1971). Ref. C.A. 76:138210 u (1972).
- 27. Koyuncu, M., Kurucu, S., IV. Bitkisel İlaç Hammeddeleri Toplantısı Bildirileri, Anadolu Üniversitesi Basımevi, Eskişehir (1982).
- 28. Noyanalpan, N., Anadolu'da yetişen bazı bitkilerin, steroidal ilaçların yarı sentezi yönünden değerlendirilmesi hakkında araştırmalar. Doçentlik tezi, Ankara (1973).
- 29. Kaweta, M., et al., Agric. Biol. Chem., 44 (6): 1405-1406 (1980). Ref. C.A. 93: 1105584 w (1980).
- 30. Çubukçu, B., Meriçli, A.H., Plant Med. et Phyt., 11:294 (1977).
- 31. Meriçli, A.H., Istanbul Ecz. Fak. Mec., 16:84 (1980).
- 32. Meriçli, A.H., et al., Fitoterapia, 55:112 (1984).
- 33. Özhatay, N., J. Fac. Pharm. Istanbul, 19: 25-36 (1983).