ACTA BIOLOGICA CRACOVIENSIA Series Botanica 51/1: 91–96, 2009

MORPHOLOGICAL VARIABILITY OF SEEDS AND FRUITS OF *RUSCUS HYPOGLOSSUM* IN CROATIA

DARIO KREMER[1*], KSENIJA KARLOVIĆ[2], AND RENATA JURIŠIĆ GRUBEŠIĆ[1]

[1]*Faculty of Pharmacy and Biochemistry, University of Zagreb,*
1A. Kovačića St, HR-10000 Zagreb, Croatia,
[2]*Faculty of Agriculture, University of Zagreb,*
25 Svetošimunska St, HR-10000 Zagreb, Croatia

Received November 27, 2008; revision accepted April 24, 2009

The paper reports a morphological study of fruits and seeds of *Ruscus hypoglossum* L., a species interesting as an ornamental plant. Initially, the length, width and weight of fruits as well as seeds were measured in 14 natural populations growing in Croatia. The length/width ratios of fruits and seeds were calculated, as well as the fruit/seed ratios of length, width and weight. Interannual differences in the analyzed traits over three years were compared in two populations. The most promising populations for further selection for ornamental use were the Bilogora population with the largest fruits (length 12.30 mm, width 11.56 mm, weight 0.75 g) and the Strahinščica population with the roundest fruits (length/width 1.04). The average number of seeds per fruit for the populations was 1.40. Variability between the three years was minor for fruit traits but significant for seed traits.

Key words: Ornamental plant, *Ruscus hypoglossum*, fruit and seed traits, natural populations, interannual variability.

INTRODUCTION

Ruscus hypoglossum is an evergreen, dioecious and rhizomatous perennial with a simple, suberect or slightly spreading stem, growing up to 40 cm high. It is a species of southeastern and eastern Central Europe, found in northwestern Italy, Austria, Bulgaria, former Czechoslovakia, Greece, Hungary, former Yugoslavia, Romania, Crimea and the European part of Turkey (Yeo, 1980). The species reaches the northern limit of its geographical range in southwestern Slovakia in the Malé Karpaty Mts. (Halada and Erdelska, 2005). Of the countries of former Czechoslovakia and former Yugoslavia, *Ruscus hypoglossum* inhabits Slovakia, Slovenia, Croatia, Bosnia and Herzegovina, Serbia and Macedonia.

It is a vulnerable species and protected by law in many countries in its area of natural distribution (Niklfeld and Schratt-Ehrendorfer, 1999; Rakonzcaj, 1990; Regula-Bevilaqua, 1994; Vangjeli et al., 1995; Shelyag-Sosonko, 1996; Halada and Feráková, 1999). In Croatia it was first listed as vulnerable (Regula-Bevilaqua, 1994), but the more recent Red List of threatened plants and animals of Croatia classifies it as a near-threatened taxa (Nikolić and Topić, 2004).

R. hypoglossum is naturally distributed in the continental and Mediterranean region of Croatia. It grows mainly in mesophytic mountain forests of common beech (*Fagus sylvatica* L.) and less in thermophilic forests (Regula-Bevilaqua, 1994). It is a very attractive but very rare ornamental species in parks and gardens in Croatia. There is no commercial production of *R. hypoglossum* in Croatia, and it is not imported either, so the demand is satisfied by collecting the species in the wild.

The aim of this research was to examine the morphological variability of fruits and seeds of *R. hypoglossum* in Croatia as a first step for selection for ornamental purposes.

MATERIAL AND METHODS

STUDY SITE

According to Köppen's (1936) classification, the climate of the western part of the study area (populations VJ and VGD) is of climatic type Cfsb"x": mod-

*e-mail: dkremer@pharma.hr

PL ISSN 0001-5296

TABLE 1. Location and altitude of studied *Ruscus hypoglossum* L. populations*

Population (Abbreviation)	Latitude; Longitude	Altitude (m a.s.l.)	Number of analyzed fruits	Number of analyzed seeds
Vrbovsko – Japlenica (VJ)	45°20' N; 15°01' E	706	10	14
Vrbovsko – Gluhe Drage (VGD)	45°20' N; 15°01' E	726	100	100
Skupica (S)	45°29' N; 15°32' E	110	10	16
Žumberak (Z)	45°47' N; 15°40' E	479	100	100
Vrhovčak (V)	45°48' N; 15°42' E	190	100	100
Falščak (F)	45°40' N; 15°45' E	151	100	100
Strahinščica – Gorjak (SG)	46°11' N; 15°54' E	382	100	100
Medvednica (M)	45°54' N; 15°55' E	261	100	100
Gornji Dragonožec (GD)	45°38' N; 15°56' E	206	100	100
Peršinovec (Pe)	45°39' N; 15°58' E	107	100	100
Kalnik (K)	46°07' N; 16°35' E	255	100	100
Bilogora (B)	45°57' N; 17°00' E	224	100	100
Papuk (Pa)	45°30' N; 17°47' E	790	19	25
Oriovac (O)	45°12' N; 17°52' E	135	100	100

*Fruits were collected by permission of the Ministry of Culture of the Republic of Croatia.

erately warm, rainy and without a dry period. Precipitation is marked by two maximums, in early spring and late autumn. The climate of the northwestern part of the study area (populations S, Z, V, F, SG, M, GD, Pe, K, and B) is of climatic type Cfwb"x": moderately warm, rainy, without a dry period, and with precipitation uniformly distributed throughout the year. The least rain falls in the cold part of the year. The climate of the eastern part of the studied area (populations Pa and O) is of climatic type Cfwb"x": temperate, rainy, and with one pronounced precipitation maximum. The summers are warm, without a dry period, and precipitation gradually decreases towards the east. There is a significant influence of cold easterly winds in the winter period.

Mesozoic limestones and dolomites with outcrops of silicate and silicate-carbonate clastites dominate in habitats of *R. hypoglossum* in the western part of the researched area. Cambisol on limestone and luvisol prevails on these substrates. Easily weathered substrates predominate in the soils of the north and northwest regions. They are mostly various unconsolidated clastites, marls, sandstones and metamorphic rocks. Eutric or dystric cambisol and luvisol prevail on these substrates.

PLANT MATERIAL

Fruits were collected in 2005 from 14 randomly selected populations within the natural distribution area of *R. hypoglossum* in Croatia (Tab. 1). Length, width and weight of fruits and seeds were measured. The length/width ratios were calculated for fruits and seeds, as well as the fruit/seed ratios for length, width and weight. A higher ratio indicates narrower fruits (or seeds), while lower ratios indicate rounder fruits (or seeds). The fruits/seeds used for analysis of interannual variability were collected from populations V and F. The number of analyzed fruits from population F was 100 (2005), 57 (2006) and 100 (2007), while from V population the number was 100 (2005), 40 (2006) and 100 (2007).

DATA ANALYSIS

Descriptive statistics were done for all traits. The results were evaluated using the unweighted pair-group method with arithmetic means (UPGMA) with Euclidean distance (D_E). UPGMA generally yields the most accurate results for classification purposes. Before cluster analysis each variable was standardized (Sneath and Sokal, 1973; Hartigan, 1975; Mook et al., 1992; Miller and Miller, 2000). Differences between populations were analyzed by the general linear models (GLM) procedure and Scheffé's post-hoc test at $p \leq 0.05$. Between-year differences in yield were analyzed by GLM using a nested design (years nested in populations) of ANOVA and Scheffé's post-hoc test at $p \leq 0.05$. The statistical analyses employed Statistica 7 (StatSoft Inc., Tulsa, OK, USA).

RESULTS

Figure 1 gives descriptive statistics for the analyzed fruit and seed traits of *R. hypoglossum*, which are the most important characters for further selection for ornamental purposes.

Fruits were largest (length 12.30 mm, width 11.56 mm) and heaviest (0.75 g) in population B, and smallest (length 9.89 mm, width 8.55 mm) and lightest (0.46 g) in population Pa. Population SG had the roundest fruits (length/width 1.04), while populations M and Pa had the narrowest ones

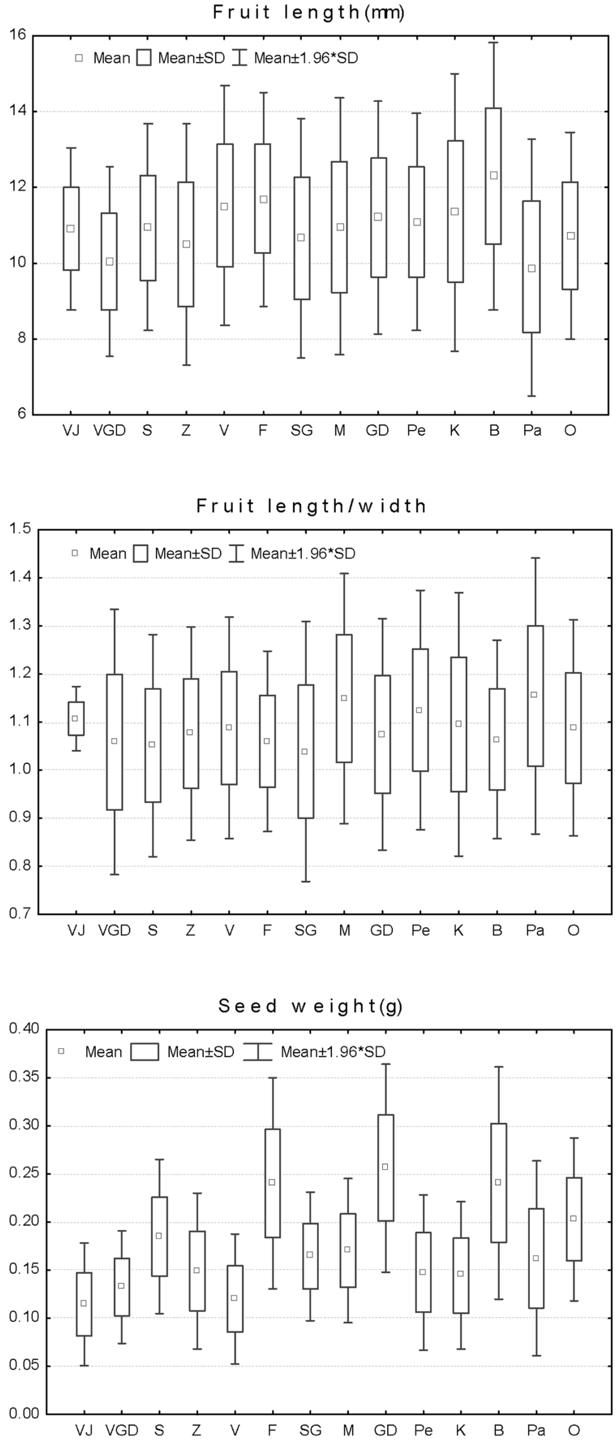

Fig. 1. Descriptive statistics for the most important traits of *Ruscus hypoglossum* fruits and seeds. For abbreviations see Table 1.

(length/width 1.15). The most variable trait was fruit weight (CV = 31.75%), and the least variable was fruit width (CV = 11.65%).

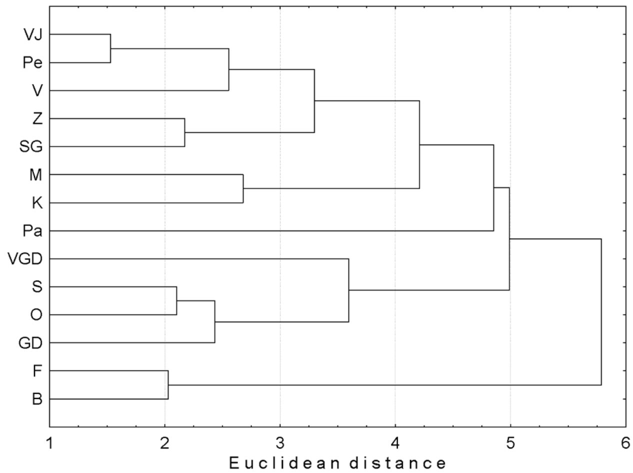

Fig. 2. UPGMA dendrogram of 14 Croatian wild populations of *Ruscus hypoglossum*. For abbreviations see Table 1.

Seed length ranged from 4.72 mm in population K to 7.06 mm in population B, and seed width ranged from 4.74 mm in population VGD to 7.80 mm in population B. Seeds were heaviest (0.26 g) in population GD and lightest (0.10 g) in population VJ. The most variable trait was seed weight (CV = 35.34%), while the least variable was seed length (CV = 16.84%). Seed traits were generally more variable than fruit traits.

The number of seeds per fruit ranged from 1.36 (VGD, S, Z, GD, Pa, O) to 1.52 (M). The calculated ratio ranged from 0.42 (K) to 0.61 (S) for seed/fruit length; from 0.50 (VGD and S) to 0.70 (Z) for seed/fruit width; and from 0.19 (V) to 0.42 (GD) for seed/fruit weight.

The ANOVA results for interpopulational variability of the analyzed traits are shown in Table 2 (fruit) and Table 3 (seeds). Population B differed significantly in fruit length, width and weight from most other populations (Tab. 2). Interpopulational variability was generally greater for seed traits than for fruit traits. The majority of populations showed significant differences for all analyzed seed traits (Tab. 3).

UPGMA of the analyzed fruit and seed traits separated the *R. hypoglossum* populations as shown in Figure 2. The most dissimilar populations are F and B, which form one cluster at Euclidean distance 2.03. Fruits and seeds are the largest in these two populations. At Euclidean distance 5.79 this cluster is connected to the group of the remaining populations. The most similar populations are VJ and Pe (D_E = 1.53).

Analysis of fruit traits over three years showed minor between-year variability in two populations during that period (Tab. 4). Differences for fruit characteristics were significant only between F 2005

TABLE 2. Between-population variability for fruit length (A), width (B), weight (C), and length/width ratio (D). Asterisk next to letter indicates significance at p≤0.05. For abbreviations see Table 1.

Population	VJ	VGD	S	Z	V	F	SG	M	GD	Pe	K	B	Pa	O
VJ														
VGD	ABCD													
S	ABCD	ABCD												
Z	ABCD	ABCD	ABCD											
V	ABCD	A*B*CD	ABC*D	AB*CD										
F	ABC*D	A*B*C*D	ABCD	A*B*C*D	ABC*D									
SG	ABCD	AB*C*D	ABCD	ABCD	ABC*D	AB*C*D								
M	ABCD	ABC*D*	ABCD	ABCD	AB*C*D	AB*C*D*	AB*CD*							
GD	ABC*D	A*B*C*D	ABC*D	AB*C*D	ABC*D	ABCD	ABC*D	AB*C*D						
Pe	ABCD	ABCD	ABCD	ABCD	AB*CD	AB*C*D	ABCD*	ABCD	ABC*D					
K	ABCD	A*B*CD	ABCD	ABCD	ABCD	ABC*D	ABCD	AB*CD	ABC*D					
B	AB*C*D	A*B*C*D	ABCD	A*B*C*D	AB*C*D	ABCD	A*B*C*D	A*B*C*D*	A*B*CD	A*B*C*D	AB*C*D			
Pa	ABCD	ABCD	AB*CD	AB*CD	AB*CD	AB*C*D	AB*CD	ABCD	AB*C*D	AB*CD	AB*CD	A*B*C*D		
O	ABC*D	ABC*D	ABCD	ABC*D	AB*C*D	AB*C*D	ABC*D	ABC*D	ABC*D	ABC*D	ABC*D	A*B*C*D	AB*CD	

TABLE 3. Between-population variability for seed length (A), width (B), weight (C), and length/width ratio (D). Asterisk next to letter indicates significance at p≤0.05. For abbreviations see Table 1.

Population	VJ	VGD	S	Z	V	F	SG	M	GD	Pe	K	B	Pa	O
VJ														
VGD	ABCD													
S	ABCD	ABCD												
Z	AB*CD	AB*CD*	A*B*CD*											
V	ABCD	AB*CD*	A*BC*D*	AB*CD*										
F	A*B*C*D	A*B*C*D*	AB*CD*	A*B*C*D	A*B*C*D									
SG	ABCD	AB*C*D*	ABCD*	ABCD*	A*BC*D	A*B*C*D								
M	ABCD	A*B*C*D*	A*B*CD*	A*BCD	AB*C*D*	A*B*C*D*	A*BCD*							
GD	A*BC*D	A*B*C*D	ABC*D	A*B*C*D*	A*BC*D*	AB*CD*	A*B*C*D*	A*B*C*D*						
Pe	ABCD	AB*CD*	A*BCD*	AB*CD*	ABCD	A*B*C*D	ABCD	A*B*CD*	A*BC*D*					
K	ABCD*	A*B*CD*	A*B*CD*	A*BCD	A*B*CD*	A*B*C*D*	A*BCD*	ABCD	A*B*C*D*	A*B*CD*				
B	A*B*C*D	A*B*C*D*	AB*CD*	A*B*C*D	A*B*C*D	ABCD	A*B*C*D	A*B*C*D*	A*B*CD*	A*B*C*D	A*B*C*D*			
Pa	ABCD	AB*CD*	A*BCD*	AB*CD	ABCD	A*B*C*D	A*BCD	ABCD	A*BC*D*	ABCD	ABCD	A*B*C*D		
O	ABC*D	A*B*C*D	ABCD	A*B*C*D*	A*BC*D*	A*B*C*D*	AB*C*D*	A*B*C*D*	ABC*D	A*BC*D*	A*B*C*D*	A*B*C*D*	A*BCD*	

and F 2006. Fruit showed stability of traits during the three-year period, but seed traits were more variable. For seed weight, all differences were significant except between F 2005 and F 2007.

DISCUSSION

In general, wild populations of *R. hypoglossum* were shown to vary more in seed traits than in fruit traits. It was the same with interannual variability: fruit traits proved more stable than seed traits over the three-year period. Both between populations and between years, fruit and seed weight varied more than fruit and seed dimensions. Since seed weight is generally considered the least plastic of plant traits (Harper, 1970), the high variability of seed weight (CV = 35.34%) was unexpected. Turnbull et al. (2006) reported much lower (CV = 25%) average variability of seed weight for 63 species reviewed from the literature. Generative organs, including seeds, are organs with relatively constant features (Szkudlarz, 2003), but in our research we found variability in both fruit and seed characteristics, with seed traits demonstrating larger interpopulational and interannual variability. Interpopulational variability of seed and fruit traits has also been reported in different species (Ngulube et al., 1997; Ali Ghars et al., 2006; Goulart et al., 2006). In accord with our results,

TABLE 4. *p* values from Scheffé post-hoc test for interannual differences in fruit and seed traits during three years in two populations (V and F) of *R. hypoglossum*. Asterisks indicate significance at $p \le 0.05$

Population /Year	V 2005	V 2006	F 2005	F 2006
		Fruit length		
V 2005				
V 2006	0.9782			
V 2007	0.8513	0.5029		
F 2006			0.1083	
F 2007			0.9998	0.0632
		Fruit width		
V 2005				
V 2006	0.9948			
V 2007	0.6788	0.4615		
F 2006			0.0370*	
F 2007			0.9262	0.2592
		Fruit weight		
V 2005				
V 2006	0.9996			
V 2007	0.4225	0.8034		
F 2006			0.6992	
F 2007			0.7015	0.1023
		Seed length		
V 2005				
V 2006	0.0000*			
V 2007	0.0000*	0.0000*		
F 2006			0.5747	
F 2007			0.9975	0.8200
		Seed width		
V 2005				
V 2006	1.0000			
V 2007	0.2970	0.1944		
F 2006			0.0000*	
F 2007			0.0000*	1.0000
		Seed weight		
V 2005				
V 2006	0.0000*			
V 2007	0.0000*	0.0000*		
F 2006			0.0473*	
F 2007			1.0000	0.0290*

Bednorz (2007) also noted higher variability of size and shape traits in seeds than in fruits. Seed weight, which in our study varied the most, was probably influenced by the environment of the maternal plants, which together with internal variables affects seed weight (Krannitz, 1997). Other reports on fruit and seed trait variability in *R. hypoglossum* are lacking, but the data we collected here are in accord with data on diameter range (1–2 cm for fruit and 6–8 mm for seed) given by Halada and Erdelska (2005).

ANOVA showed less interpopulational variability for the fruit length/width ratio than for fruit length, width and weight (Tab. 2). This is to be expected, since the environment has a greater effect on plant organ dimensions than on organ shape. Shape characters give a much better representation of the phylogenetic and genetic relations between living organisms (Reyment, 1985).

Cluster analysis showed that the geographically nearest populations, VJ and VGD, had somewhat different morphological fruit and seed characteristics. Possible explanations for these differences could be the small number of analyzed fruits in VJ population and/or micro-habitat characteristics. VJ is a very small population (a few dozen plants) situated on a small plain. Population VGD is situated in a depression several kilometers from the VJ population. The seeds of *R. hypoglossum* from population VJ were the lightest, in accord with findings from Buide (2004), who found that seeds of *Silene acutifolia* from the smallest and more isolated population were the lightest. Both population size and the level of genetic relatedness among individuals influence maternal reproductive success (Elam et al., 2006). In the same way as small population size probably influenced the fruit/seed characteristics in population VJ, the poor health of population Pe (a relatively large population) presumably influenced its analyzed traits, resulting in high similarity between these two otherwise rather distant populations.

Annual precipitation decreases towards the east of Croatia, indicating a certain connection between morphological traits and the geographical distribution of *R. hypoglossum*. However, the UPGMA dendrogram showed no connection between geographic position and cluster formation. For example, VGD (annual precipitation ~1470 mm) and O (annual precipitation ~700 mm) are less separated than the westernmost (VJ and VGD) or easternmost (Pa and O) populations. Nor is there any evident connection between the analyzed traits and altitude. For example, populations VJ and Pa grow at the highest altitude (Tab. 1) but are rather distant according to UPGMA.

Our results showed considerable variability of the analyzed morphological traits among wild populations of *R. hypoglossum* in Croatia. The high variability and interannual stability of fruit traits displayed by the populations will enable the most interesting populations to be selected for breeding of ornamental plants. Supporting that effort, the recorded variability of seed traits will provide the basis for future research on the influence of seed size on germination success in this endangered ornamental species. Popularization of cultivated varieties of the plant in Croatia should help conserve it in the wild.

ACKNOWLEDGEMENTS

The research was supported by the Ministry of Sciences, Education and Sport of the Republic of Croatia (project no. TP-178/85, "Protection of *Ruscus hypoglossum* L. through introduction into cultivation," and project "Micromorphological and chemotaxonomic researches on some species of family Lamiaceae"), and by the City Council of Samobor, Zagreb County, and the City Office for Agriculture and Forestry.

REFERENCES

ALI GHARS M, DEBEZ A, SMAOUI A, ZARROUK M, GRIGNON C, and ABDELLY C. 2006. Variability of fruit and seed-oil characteristics in Tunisian accessions of the halophyte *Cakile maritima* (Brassicaceae). In: Khan MA, and Weber DJ [eds.], *Ecophysiology of High Salinity Tolerant Plants*, 55–67. Springer, Dordrecht.

BEDNORZ L. 2007. Morphological variability of fruits and seeds of *Sorbus torminalis* in Poland. *Dendrobiology* 57: 3–14.

BUIDE ML. 2004. Intra-inflorescence variation in floral traits and reproductive success of the hermaphrodite *Silene acutifolia. Annals of Botany* 94: 441–448.

ELAM DR, RIDLEY CE, GOODELL K, and ELLSTRAND NC. 2006. Population size and relatedness affect fitness of a self-incompatible invasive plant. Proceedings of the *National Academy of Sciences of the United States of America* 104: 549–552.

GOULART MF, PIRES LEMOS FILHO J, and LOVATO MB. 2006. Variability in fruit and seed morphology among and within populations of *Plathymenia* (*Leguminosae-Mimosoideae*) in areas of the Cerrado, the Atlantic forest, and transitional sites. *Plant Biology* 8: 112–9.

HALADA L, and FERÁKOVÁ V. 1999. *Ruscus hypoglossum* L. In: Čeřovský J, Feráková V, Holub J, Maglocký Š, and Procházka F [eds.], *The Red Book of Threatened and Rare Plant Species – Vascular Plants*. Priroda, Bratislava.

HALADA L, and ERDELSKA O. 2005. Reproductive biology of *Ruscus hypoglossum* L. in Slovakia. *Acta Biologica Cracoviensia Series Botanica* 47: 213–217.

HARPER JL. 1970. The shapes and sizes of seeds. *Annual Review of Ecology and Systematics* 1: 327–356.

HARTIGAN JA. 1975. *Clustering Algorithms*. Wiley, New York.

KÖPPEN W. 1936. *Das Geographische System der Klimate*. Gebrüder Borntraeger, Berlin.

KRANNITZ PG. 1997. Seed weight variability of antelope bitterbrush (*Purschia tridentatata*: Rosaceae). *The American Midland Naturalist* 138: 306–321.

MILLER JN, and MILLER JC. 2000. *Statistics and Chemometrics for Analytical Chemistry*. Pearson Education Limited, Essex.

MOOK JH, HAECK J, VAN DER TOORN J, and VAN TIENDEREN PH. 1992. Ecology of *Plantago* populations. In: Kuiper PJC and Bos M [eds.], *Plantago*: A Multidisciplinary Study, 69–87. Springer-Verlag, Berlin.

NGULUBE MR, HALL JB, and MAGHEMBE JA. 1997. Fruit, seed and seedling variation in *Uapaca kirkiana* from natural populations in Malawi. *Forest Ecology and Management* 98: 209–219.

NIKLFELD H, and SCHRATT-EHRENDORFER L. 1999. Rote Liste gefährdeter Fern- und Blütenpflanzen (Pteridophyta und Spermatophyta) Österreichs. In: Niklfeld H [ed.], *Rote Listen Gefährdeter Pflanzen Österreichs. Grüne Reihe des Bundesministeriums für Umwelt, Jugend und Familie*, Band 10. Wien.

NIKOLIĆ T, and TOPIĆ J. 2004. Vascular flora. In: *Red List of Threatened Plants and Animals of Croatia*. State Institute for Nature Protection. Zagreb.

RAKONZCAJ Z. 1990. *Hungarian Red Data Book*. Akademiai Kiado, Budapest.

REGULA-BEVILAQUA LJ. 1994. *Ruscus hypoglossum* L. In: Šugar I [ed.], *Red book of Croatian plant species*, 446–447. Department of environment protection, Zagreb.

REYMENT RA. 1985. Multivariate morphometrics and analysis of shape. *Mathematical Geology* 17: 591–609.

SHELYAG-SOSONKO YR. 1996. *Red Data Book of the Ukraine*, vol. 1: *Vegetable Kingdom*. Ukrainskaya Enciklopedia, Kiev.

SNEATH PHA, and SOKAL RR. 1973. *Numerical Taxonomy – The Principles and Practice of Numerical Classification*. W. H. Freeman and Company, San Francisco.

SZKUDLARZ P. 2003. *Loiseleuria procumbens*: differentiation of the seed size of some chosen European populations. *Dendrobiology* 50: 33–36.

TURNBULL LA, SANTAMARIA L, MARTORELL T, RALLO J, and HECTOR A. 2006. Seed size variability: from carob to carats. *Biology Letters* 2: 397–400.

VANGJELI J, RUCI B, and MULLAY A. 1995. *Red Book of Threatened and Rare Plant Species of Albania*. Akademia e Shkencave e Republikës Së Shqipërisë & Instituti I Kërkimeve Biologjike Komiteti I Mbrojtjes Së Mjedist, Tirana.

YEO, PF. 1980. *Ruscus* L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, and Webb DA. [eds.], *Flora Europaea*, vol. 5, 73. Cambridge Univ. Press, Cambridge.