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ANALYSIS OF GENETIC POLYMORPHISMS IN JOINTED GOATGRASS (*AEGILOPS CYLINDRICA*) AND ANNUAL WILD RYE (*SECALE SYLVESTRE*) POPULATIONS FROM HUNGARY

G. Vörösváry[1], I. Már[1], L. Holly[1] & J. Kissimon[2]
Institute for Agrobotany, Tápiószele[1]
University of Horticulture and Food Industry, Budapest[2]

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From July 1995 to July 1998 the Institute for Agrobotany conducted a series of missions to collect samples of wild relatives of wheat and rye in Hungary. The genetic diversity represented by jointed goatgrass (*Aegilops cylindrica*) and annual wild rye *(Secale sylvestre*) populations in different parts of Hungary was studied to obtain information on the existing variation. These species occur from mountainous regions to the plainis in Hungary.

Seven accessions of *Aegilops cylindrica* and twelve accessions of *Secale sylvestre* were studied. Analysis of variance on morphological characters from collected materials showed significant differences for length of ear, number of grains/ear and seed mass.

Electrophoretic analysis of storage proteins was conducted on extracts from single seeds using polyacrylamid gel systems (A-PAGE and SDS-PAGE). Variation was detected and documented in gliadin and secalin patterns.

The number of accessions of jointed goatgrass and annual wild rye in our study was limited, therefore conclusions related to genetic diversity should be considered as preliminary.

This research will be expanded to other populations in Hungary to characterize the actual genetic variation of these species in order to develop effective *in situ* conservation strategy and monitoring.

Key words: Genetic diversity, *Aegilops cylindrica*, *Secale sylvestre*, storage protein.

Vörösváry, G., Már, I., Holly, L. & Kissimon, J. (2000). Análise de polymorphismos genéticos em algumas populações húngaras de *Aegilops cylindrica* e de centeio selvagem anual (*Secale sylvestre*). *Portugaliae Acta Biol.* **19**: 137-147.

De Julho de 1995 a Julho de 1998 o Instituto de Agrobotanica conduziu uma série de missões no território Húngaro a fim de colher amostras de parentes selvagens de trigo e centeio. A diversidade genética representada por populações de *Aegilops cylindrica* e de centeio selvagem anual (*Secale sylvestre*) em diferentes partes da Hungria foi estudada para obter informações sobre a variação existente. Estas espécies ocorrem, na Hungria, desde as regiões montanhosas até às planícies.

Foram estudadas sete populações de *Aegilops cylindrica* e doze de *Secale sylvestre*. A análise de variância dos caracteres morfológicos mostrou diferenças significativas para o comprimento da espiga, número de grãos/espiga e massa da semente.

A análise electroforética das proteínas de armazenamento foi feita em extractos a partir de sementes únicas usando sistemas de gel de poliacrilamida (A-PAGE e SDS-PAGE). A variação foi detectada e documentada em padrões de gliadina e secalina.

O número de locais de colheita de *Aegilops cylindrica* e *Secale sylvestre* utilizado neste estudo foi limitado, consequentemente as conclusões relativas à diversidade genética devem ser consideradas como preliminares.

Esta pesquisa será ampliada para um maior número de populações na Hungria de modo a caracterizar a variação genética actual destas espécies e com o fim de se desenvolverem estratégias efectivas de conservação *in situ* e de monitorização.

Palavras chave: Diversidade genética, *Aegilops cylindrica*, *Secale sylvestre*, proteína de armazenamento.

INTRODUCTION

During the growing seasons 1995/1998 collecting missions for jointed goatgrass and annual wild rye were conducted in different parts of Hungary (VÖRÖSVÁRY, 1998). Field work was centered on the hilly places of Transdanubia, the middle and south parts of the Great Hungarian Plain where these species are known to occur (SOÓ, 1973).

Aegilops cylindrica is an annual, tetraploid (2n=28) species (Fig.1), widely distributed on the northern coastline of the Eastern Mediterranean and Western Asia, and adventive in Western and Central Europe (TUTIN & HUMPHRIES,1980, VAN SLAGEREN,1994, SOLER et al.,1997). In Hungary

jointed goatgrass populations grow on roadsides and slopes in hilly and mountainous parts. In Transdanubia it is usually found in large populations, in the eastern areas populations tend to be small, with low densities.

Secale sylvestre is an anuual, diploid (2n=14) species, (Fig.2) widely distributed from Central Hungary to southern Russia (EVANS,1974), and in some extend to the Balkan peninsula (HEYWOOD & ZOHARY,1995). In Hungary it occupies habitats in degraded areas and its populations have variable size and density. Its optimum appears to be in the Great Hungarian Plain where populations are very large.

The distribution areas of *Aegilops cylindrica* and *Secale sylvestre* are showed in Fig.3.

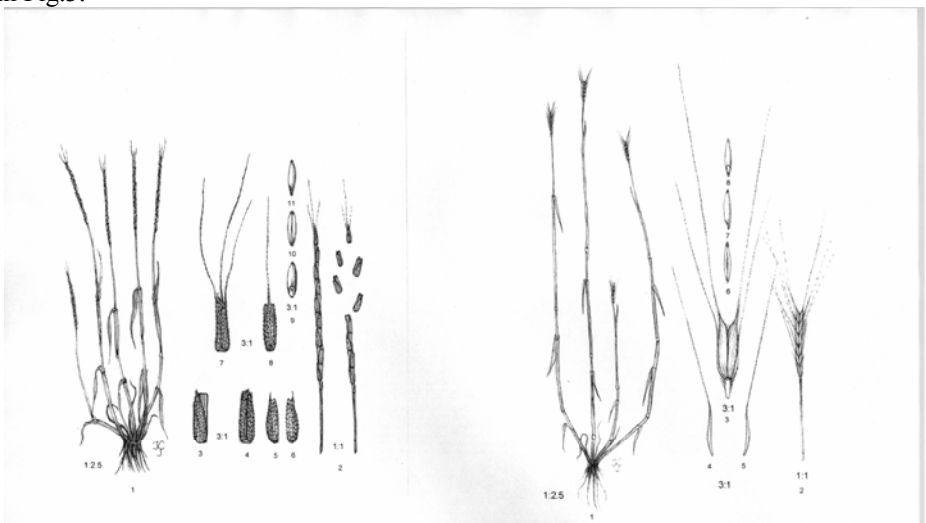

Fig.1. *Aegilops cylindrica* 1. habitus (1:2.5); 2. spike (1:1); 3-4 spikelet (3:1); 5-6. glume (3:1); 7.apical spikelet (3:1); 8. glume of apical spikelet (3:1); 9. dorsal surface of seed (3:1); 10. ventral surface of seed (3:1); 11. lateral surface of seed (3:1). (Drawing by T. Kovács)

Fig.2. *Secale sylvestre* 1. habitus (1:2.5); 2. spike (1:1); 3. spikelet (3:1); 4-5. glume (3:1); 6. ventral surface of seed (3:1); 7. lateral surface of seed (3:1); 8. dorsal surface of seed (3:1)(Drawing by T. Kovács)

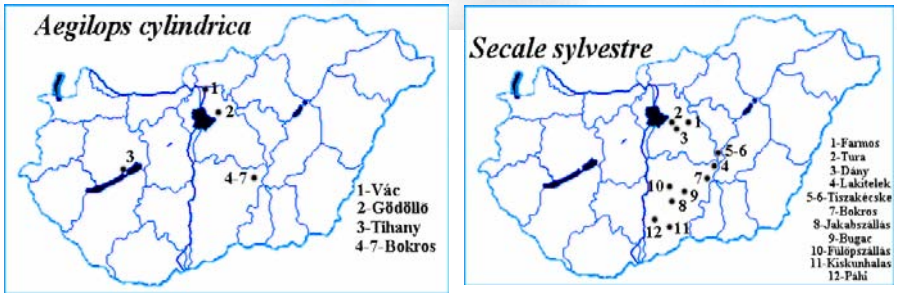

Fig.3. Collection sites of *Aegilops cylindrica* and *Secale sylvestre* accessions in Hungary

These regions are climatically contrasted. The climate in Transdanubia is moderate (annual rainfall 700 mm), and in the Great Plain it is more arid (annual rainfall 450-500 mm).

The present study was started in order to evaluate the genetic diversity of these species and to save genetic resources which are increasingly threatened by erosion due to many different agro-social factors. *In situ* conservation attempts to protect these species under their natural conditions.

MATERIAL AND METHODS

Seven accessions of jointed goatgrass and twelve accessions of annual wild rye were used in this study. Two accessions of *Aegilops cylindrica* (Vác, Gödöllő) were obtained from the Hungarian botanical garden (Table 1).

Table 1. Collection sites of *Aegilops cylindrica* and *Secale sylvestre* accessions in Hungary

Species	Accession number	Collection site	Latitude	Longitude
Aegilops cylindrica HOST	RCAT042080	Vác	47° 57' N	19° 12' E
	RCAT061813	Gödöllő	47° 37' N	19° 21' E
	RCAT069132	Tihany	46° 55' N	17° 53' E
	RCAT073122	Bokros 1	46° 45' 34" N	20° 04' 02" E
	RCAT073123	Bokros 2	45° 46' 29" N	20° 04' 00" E
	RCAT073124	Bokros 3	46° 45' 25" N	20° 03' 51" E
	RCAT073125	Bokros 4	46° 45' 27" N	20° 03' 51" E
Secale sylvestre HOST	RCAT069231	Farmos	47° 22' N	19° 51' E
	RCAT067232	Tura	47° 38' N	19° 36' E
	RCAT063230	Dány	47° 31' N	19° 33' E
	RCAT073118	Lakitelek	46° 51' N	19° 58' E
	RCAT073116	Tiszakécske 1	46° 57' N	20° 05' E
	RCAT073117	Tiszakécske 2	46° 53' N	20° 01' E
	RCAT073119	Bokros	46° 46' N	20° 00' E
	RCAT067229	Jakabszállás	46° 45' N	19° 36' E
	RCAT067228	Bugac	46° 41' N	19° 41' E
	RCAT041010	Fülöpháza	46° 53' N	19° 27' E
	RCAT073120	Kiskunhalas	46° 29' N	19° 27' E
	RCAT073121	Páhi	46° 42' N	19° 20' E

The distance between the collection sites varied between 1 and 150 km and preference was given to sites where the number of plants was expected to be high. All specimens collected were stored and maintained in the active collection at the Institute for Agrobotany.

The accessions were characterized for a range of morphological characters: length of ear, number of grains/ear, thousand seed mass.

The seed storage protein have been studied in several *Aegilops* species (ESER et al., 1998, PIGNONE et al.,1994), but in *Aegilops cylindrica* they have been little examined.

In the present study, single grain samples (20-50 populations) were analysed to compare their storage protein composition. The electrophoretic patterns of the alcoholsoluble storage proteins (gliadins) were visualized by the method of A-PAGE (acidic polyacrylamid gel electrophoresis) published by WAGNER & MAIER (1982), the water- or buffersoluble proteins were analysed with the method of SDS-PAGE (LAEMMLI, 1970).

RESULTS AND DISCUSSION

Morphological variations

In order to evaluate natural populations, specimens were collected at random. The measurements were carried out on 12-15 ears from each population. The pattern of variation in *Aegilops cylindrica* and *Secale sylvestre* populations is summarized in Table 2.

Table 2. Morphological variation in populations of *Secale sylvestre* and *Aegilops cylindrica* collected in different sites in Hungary.

Aegilops cylindrica					
Collection site	Length of ear (mm)		Number of grain/ear		Thousand seed mass(g)
	Min.	Max.	Min.	Max.	
Vác	50	110	5	10	10.4
Gödöllő	50	120	5	12	11.0
Tihany	40	110	4	9	10.6
Bokros 1	50	80	5	8	5.9
Bokros 2	40	60	4	7	6.0
Bokros 3	40	60	4	8	6.1
Bokros 4	40	70	4	6	6.2

Secale sylvestre					
Collection site	Length of ear (mm)		Number of grain/ear		Thousand seed mass(g)
	Min.	Max.	Min.	Max.	
Farmos	30	60	16	28	6.5
Tura	40	70	16	24	7.5
Dány	30	60	16	24	8.9
Lakitelek	40	60	12	24	6.3
Tiszakécske	40	60	16	20	6.9
Tiszakécske	30	60	16	20	6.8
Bokros	30	60	12	20	5.3
Jakabszállás	40	70	12	20	6.6
Bugac	40	60	12	24	6.1
Fülöpháza	40	60	12	20	7.9
Kiskunhalas	40	70	16	24	5.8
Páhi	30	60	16	26	6.9

In the *Aegilops cylindrica* specimens length of ear generally varied from 40 to 120 mm, number of grains per ear varied from 4 to 12 and thousand seed mass (TSM) from 5.9 g to 11 g. (Fig. 4).

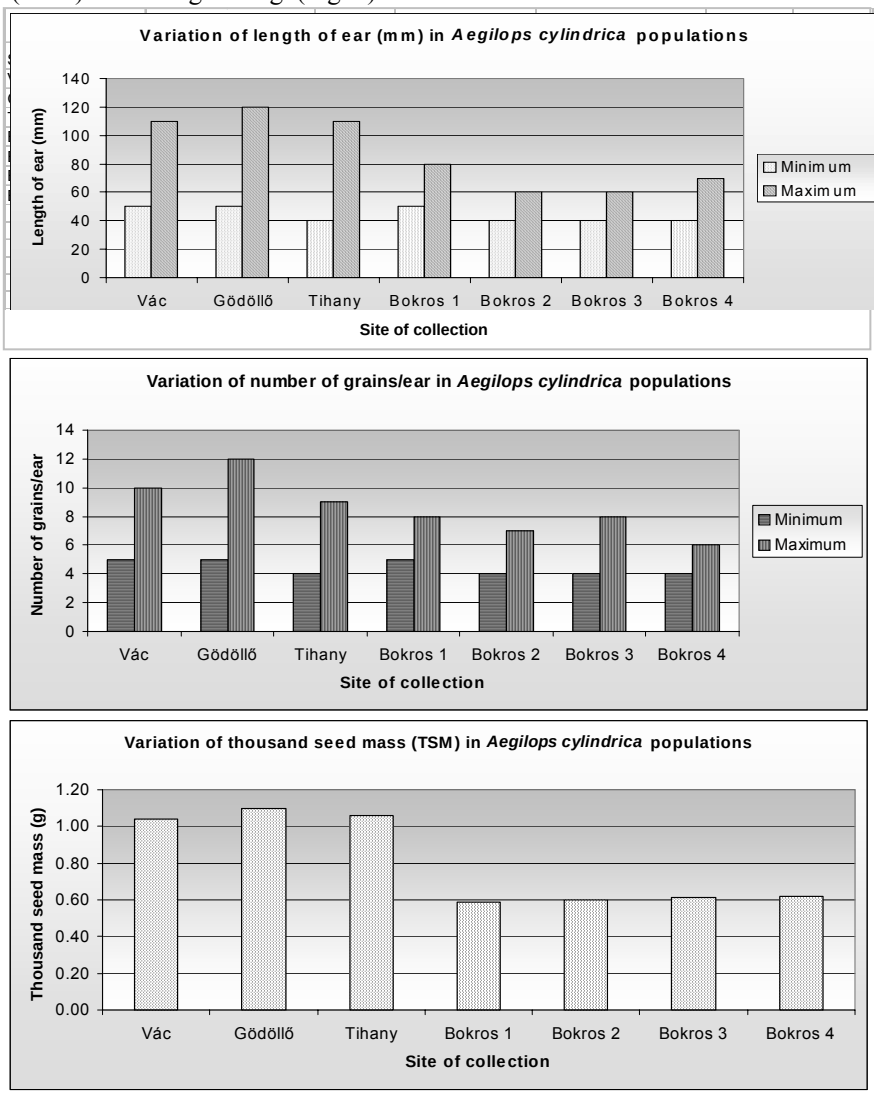

Fig.4. Variation of length of ear,number of grains/ear and thousand seed mass in *Aegilops cylindrica* populations

In the *Secale sylvestre* accessions length of ear varied from 30 to 70 mm, number of grains per ear values varied from 12 to 28, while the thousand seed mass (TSM) was limited from 5.3 to 8.9 g. (Fig. 5).

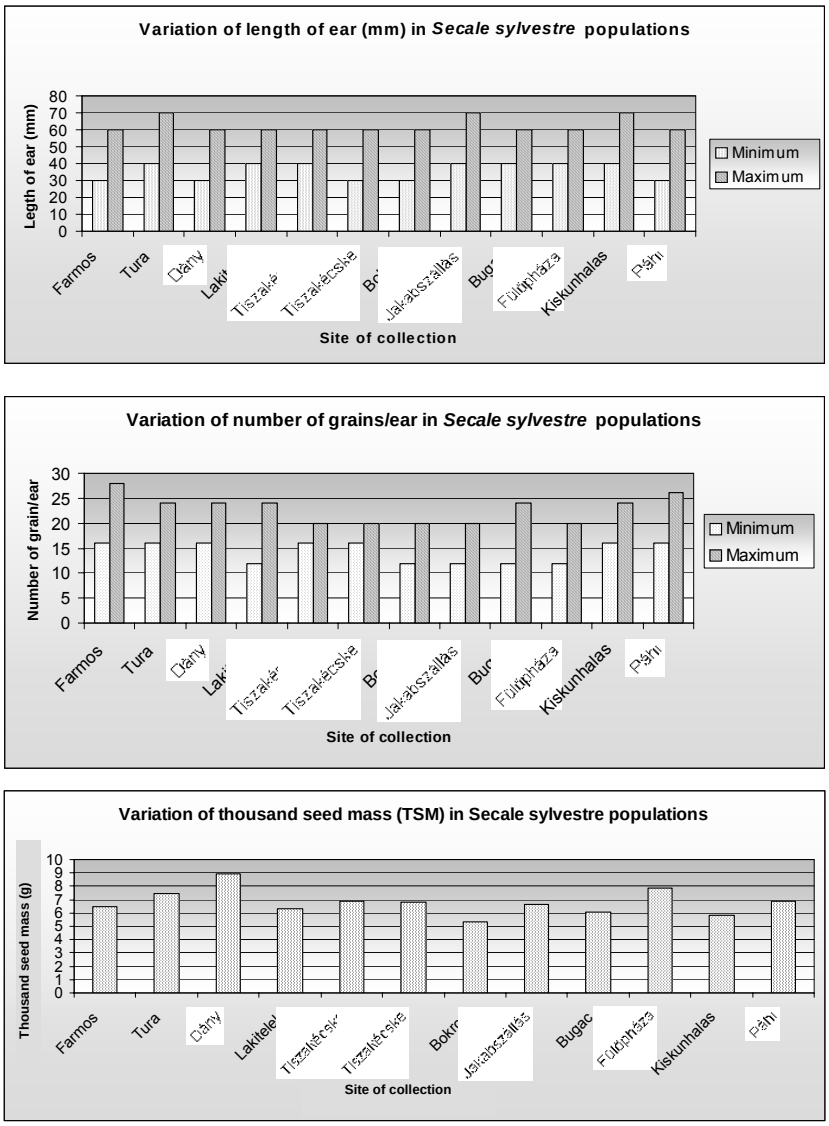

Fig. 5. Variation of length of ear, number of grains/ear and thousand seed mass in *Secale sylvestre* populations.

In *Aegilops cylindrica* populations variation in spike shape is pronounced (Fig.6) (Photo by T.Kádas). The same character is more uniform within the populations of *Secale sylvestre* (Fig. 7). (Photo by T. Kádas)

Fig. 6. Variation of spike shape in populations of *Aegilops cylindrica*

Fig.7. Variation of spike shape in populations of *Secale sylvestre*

The results indicate that there are genetic differences in the evaluated characters. The morphological variation can be summarized as follow: considerable variation is present in all examined characteristics; even adjacent populations may differ significantly in many characters.

Electrophoretic analysis

The gliadin and secalin protein separation revealed some variation among populations from the same geographical area (Fig. 8).

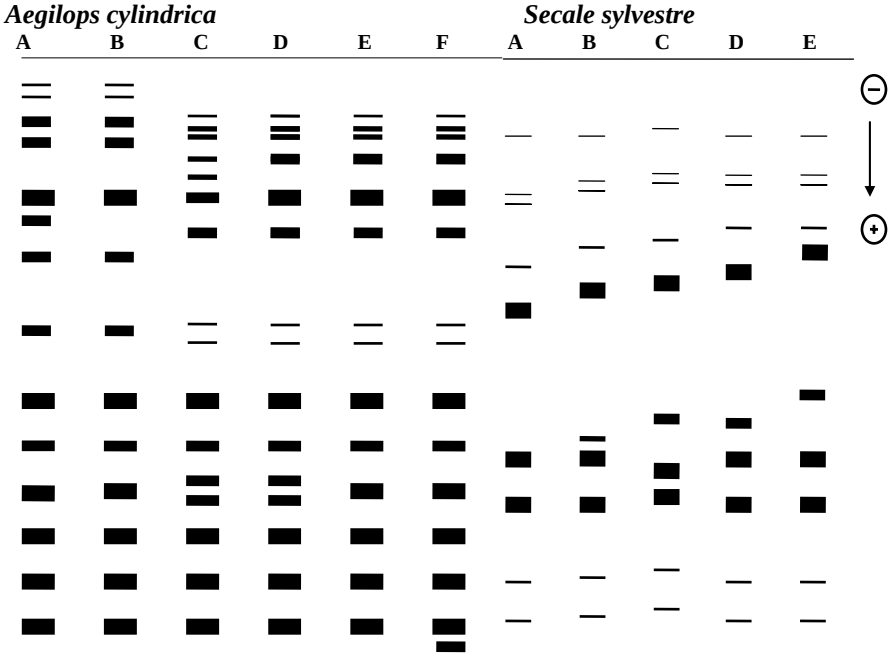

Fig. 8. Electrophoregram of gliadin and secalin proteins in seeds of *Aegilops cylindrica* and *Secale sylvestre.*

The electrophoregrams of the samples of *Aegilops cylindrica* are shown in Figure 8. They revealed six types of gliadin patterns: A, B and C types are characteristic for Bokros sites, and D, E, F patterns are present at Tihany, Vác, Gödöllő sites (Table 3).

Accessions from Tihany, Vác and Gödöllő and the four sites in Bokros represented distinct groups but the similarity of storage proteins within each group is high. Differences might reflect the contrasting ecological conditions of the two groups of habitats.

Secale sylvestre has more heterogeneous populations with variable secalin patterns. The populations have small differences in secalin constitution, but their variability is high. A and B types were frequent, D and E were rare and C type was found only in Páhi site. (Table 3).

There was no variation in glutenin subunits in all of the *Aegilops cylindrica* and *Secale sylvestre* samples.

Table 3. Frequency distribution of gliadins and secalin patterns (%) in populations of *Aegilops cylindrica* and *Secale sylvestre* in different natural habitats in Hungary.

	Electrophoretic patterns					
Collection site	**A**	**B**	**C**	**D**	**E**	**F**
Aegilops cylindrica						
Vác	-	-	-	-	100	-
Gödöllô	-	-	-	-	50	50
Tihany	-	-	-	-	40	60
Bokros 1	100	-	-	-	-	-
Bokros 2	-	-	100	-	-	-
Bokros 3	-	-	-	100	-	-
Bokros 4	20	80	-	-	-	-
Secale sylvestre						
Farmos	-	100	-	-	-	-
Tura	-	-	-	30	70	-
Dány	20	80	-	-	-	-
Lakitelek	10	10		20	60	
Tiszakécske 1	50	50	-	-	-	-
Tiszakécske 2	90	10	-	-	-	-
Bokros	80	20	-	-	-	-
Jakabszállás	-	100	-	-	-	-
Bugac	80	20	-	-	-	-
Fülöpháza	90	10	-	-	-	-
Kiskunhalas	30	30	-	-	40	-
Páhi	20	-	80	-	-	-

CONCLUSIONS

Results obtained by morphological and biochemical methods exhibit genetic variation within and between populations of jointed goatgrass and annual wild rye. Research on genetic polymorphism of these wild relatives of cereal crops in Hungary may be useful to obtain information on the genetic structure of the natural populations. Recent studies have revealed genetic diversity in populations of *Aegilops cylindrica* and *Secale sylvestre* in their natural habitats, and it can be used in the development of effective *in situ* methods for conservation of the genetic variability of these species.

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REFERENCES

ESER, V., GÖCMEN, B., ERISEN, S., BARAN, I., DÖNMEZ, E., BARUT, A. & A., KARAGÖZ. 1998. Determination of biochemical variation in an *Aegilops tauschii*

population collected from Ceylanpinar. The Proceedings of International Symposium on *in situ* Conservation of Plant Diversity: 93-97.

EVANS, G. M. 1974. Rye (*Secale cereale*) In: Simmonds, N.W. Evolution of CropPlants: 108- 111. Longman, London and New York.

HEYWOOD, V. H., ZOHARY, D. 1995. A Catalogue of the Wild Relatives of Cultivated Plants Native to Europe: 413. Council of Europe.

LAEMMLI, U.K. 1970. Cleavege of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

PIGNONE, D., GALASSO, I., HAMMER, K., PERRINO, P. 1994. Cytogenetic and genetic relationships of *Aegilops ventricosa* Tausch, Euphytica 79(1-2): 81-85.

SOLER, C., RUIZ-FERNANDEZ, J., VICENTE MONTE, J., DE BUSTOS, A., JOUVE, N. 1997. The assessment of variability in Spanish populations of wild relatives of cereals. Bocconea 7: 107 - 119 , Proceedings of 3rd Workshop on the "Conservation of the Wild Relatives of European Cultivated Plants", Palermo.

SOÓ, R., 1973. Synopsis systematico-geobotanica florae vegetationisque Hungariae Vol. V: 354-355; 359. Budapest.

VAN SLAGEREN, M.W. 1994. Wild Wheats: a Monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig: 206-209. ICARDA/ Wageningen Agric. Univ. Wageningen.

TUTIN, T.G., HUMPHRIES, C.J. 1980. *Aegilops* L. – In: Tutin,T.G., Heywood, V.H., Burges, N. A., Moore, D. M.,Valentine, D. H.,Walters, S. M.,Webb, D.A. (ed.) Flora Europaea 5: 200-201. Cambridge University Press, Cambridge.

VÖRÖSVÁRY, G. 1998. The geographic distribution of some wild relatives of crops in Hungary. Book of abstracts: 87. VIIth IOPB Symposium 1998 Amsterdam, The Netherlands.

WAGNER von K., MAIER, G., 1982. Weizensortenidentifizierung durch Polya-cryalmidgel-Electrophorese. Das österreichische Sortiment an Weich- und Hartweizensorten. Die Bodenkulture, 33: 322-332.