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Genetic variability among populations of *Allium carinatum* subsp. *carinatum*

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ABSTRACT: The genetic diversity and genetic differentiation of ten picked native populations of endangered wild garlic *Allium carinatum* L. from the former Czechoslovakia area were studied by means of isozyme variation. The variability of six isozyme systems was tested (G-6-PDH, AAT, PGM, EST, ACP, PGI) by discontinuous polyacrylamide gel electrophoresis. The chosen species, A. *carinatum* subsp. *carinatum*, is triploid (2n = 24) with obligatory vegetative reproduction by bulbils. Tests of heredity by crossing-over are impossible and genetic variability is difficult to derive from isozyme patterns. For this reason the method of mixed phenotype analysis was applied. The dendrograms of dissimiliarity were constructed and low among-population variability reported. The low variability was disscused in terms of clonality, geographic shaping, distance, weak dispersion, fixed genotype and the action of somatic mutations.

KEYWORDS: isozymes, *Allium carinatum*, genetic diversity, mixed phenotypes

Introduction

The genus garlic (*Allium*) includes more then 500 species widely distributed in the northern hemisphere and extremely diverse in their morphology, anatomy caryology, seasonal development and ecological preference. Garlic is often a sterile plant which does not form seeds. It is therefore propagated almost exclusively asexually by means of underground cloves or vegetative topsets in the inflorescence (HANELT 1991). In the genus *Allium* forms with different

chromosome numbers occasionally occur in nature within the same species or within a group of closely related species (FIALOVÁ 1995). In the majority of cases these then form a polyploid series, the most common case being the occurence of diploids and tetraploids. However, triploids also occur in nature and can acquire a wide geographical distribution by means of vegetative propagation. *Allium carinatum* belongs with *A. paniculatum*, *A. pulchellum* and *A. flavum* in the section Scorodon (TUTIN 1991) of the paniculatum group (LEVAN 1937). *A. carinatum* subsp. *carinatum* is the only triploid (2n=24) of this group, the other species of the paniculatum group are diploids (2n=16). *A. carinatum* subsp. *carinatum* is the only subspecies of *A. carinatum* in the Czech Republic and has been placed in the Red list of endangered species of the Czech Republic. In recent years new facts have emerged regarding its extension, mainly in the Slovak republic (KRAHULEC 1977). Nevertheless its occurence today is sporadic compared with the reports of DOMIN (1928) and PODPĚRA (1911) on its ocurrence in the Moravian area. The exclusive asexuality, generally uncommon occurrence and the relatedeness of a particular population (rescue intervention in the Turček locality) were the reason for this study of genetic variability of this species.

Isozyme markers were applied to species of the genus *Allium* by (ETOH 1981), TASHIRO (1995a,b) and SHIGYO (1994, 1995a,b). But these works concern agriculturally used species of *Alliums*, moreover in other latitudes than the Czech republic. The isozyme technique has not yet been used for wild species of *Allium*, which is why new procedures were developed.

Material and methods

Study sites - The endangered species *Allium carinatum* L. was tested in the area of the former Czechoslovakia. The sites shown in Tab. 1 belong mainly to the largest known populations in this area. To compare genetic variation among populations ten populations were tested. The population at Turček was sampled in detail to measure the within-population variability. Complete uniformity of isozyme patterns was found throughout the whole population consequently similar results from all other tested localities can be expected and uniformity was extended over all these sites. The other species, *Allium oleraceum*, was included only as material for comparison. *A. carinatum* and *A. oleraceum* are morphologically closely related, both were put into the paniculatum group by LEVAN (1937).

Sampling regime - Five plants of each population planted from bulbils was grown in standardized conditions under 16 h light (22°C) / 8 h dark (18°C). Very young leaves were collected because they yielded the best results in exploratory studies of enzyme electrophoresis (SIPES & WOLF 1997, WEEDEN 1989).

Chromosome counting - The youngest roots were collected, immediately washed and fixed in ethanol with acetic acid (3:1). After 24 hour fixation, roots were transfered to 96% ethanol (15 minutes) and stored under 70% ethanol at 4°C before staining. The roots were washed in distilled water and placed in 5M HCl (35 min) and stained by Shiff solution (fuchsinsulfite, 30-45 min). Pieces of

Tab. 1. List of localities

population code	republic	locality	altitude (m a.s.l.)	map square
A	Slovakia	Malá Fatra Mts.; mountain chalet Grúň	980 m	6780
B	Slovakia	Nízke Tatry Mts.; Moštenica - yellow tourist mark by Uhliarský brook	560 m	7281
C	Slovakia	Biele Karpaty Mts.; Bošácká dolina valley - settlement of Ondrášovec	420 m	7172
D	Czech	Bílé Karpaty Mts.; Bošacká dolina valley - north slope	510 m	7072
E	Czech	Bílé Karpaty Mts.; valley of Kazivec brook	420 m	7071
F	Czech	Bílé Karpaty Mts.; Zahrady pod hájem near village of Velká nad Veličkou	360 m	7071
G	Czech	Bílé Karpaty Mts.; Čertoryje - north part by green tourist mark	380 m	7170
H	Czech	Bílé Karpaty Mts.; Machová - up railway station Machová	410 m	7171
I	Czech	Drahanská vrchovina Mts.; Vranovice - 400m toward crossing Vincencov - Otaslavice	240 m	6568
J	Slovakia	Kremnické vrchy Mts.; Turček - 3 km up the village, the orifice Ružová dolina valley	660 m	7279

root tips smaller than 1 mm were placed in a of drop 45% acetic acid (about 2 min.) and mounted in a drop of acetocarmine solution (PAZOUREK & PAZOURKOVÁ 1960).

Electrophoresis and analysis - Fresh leaves were picked just before homogenization. Leaves were ground in liquid nitrogen (WENDEL & WEEDEN 1989) and extraction buffer was added:

0,075 M Tris- H_3PO_3 pH 7,5	100 ml
L-ascorbic acid sodium salt	0,10g
PVP	4,00g
sucrose	2,00g
2-mercaptoethanol	0,1% (added just before use)

The appropriate storage temperature for samples before analysis is -80°C. Proteins were separated in 10% polyacrylamide gels. The non-dissociating discontinuous polyacrylamide gel electrophoresis with 5% stacking gel with Tris-HCl buffer was used (HAMRICK & RICKWOOD 1990). From a preliminary survey of 10 enzymatic systems six were shown to stain and resolve sufficiently for an accurate interpretation of the patterns. Glucose-6-phosphate dehydrogenase (G-6-PDH, 1. 1. 1. 49), alcohol-ammino transferase (AAT, 2. 6. 1. 1), phosphogluco-mutase (PGM, 2. 7. 5. 1), esterase (EST, 3. 1. 1. -), acid phosphatase (ACP, 3. 1. 3. 1) and phosphoglucoisomerase (PGI, 5. 3. 1. 9) were resolved on Tris-glycine electrode buffer. Staining methods generally followed AQUAAH (1992), WENDEL & WEEDEN (1989) and VALLEJOS (1983) with slight modifications.

Data analysis - The variability among-populations was tested by mixed phenotype analysis in each enzymatic system. Relative migration, thicknes of bands and the number of bands in the various samples were used to characterise each particular mixed phenotype and afforded sufficient information for this study. Each mixed phenotype was marked by a small letter (a, b, c, ...) and the faster zone of enzyme activity joined with roman number I, and the slower with roman numeral II (NOYES 1995). The zymogram results were presented in terms of electrophoretic banding patterns and not in terms of individual isozyme loci and alleles (LEHMAN 1997, ETOH 1981). The binary matrix made up for presence/absence of a particular phenotype. Then a dissimilarity dendrogram was performed by UPGMA (Unweighted Pair Group Method using Averages) with the Euclidean distance determined using the NCSS PC program.

Results and discussion

All populations were determined to be triploids without exception, which corresponds with DOSTÁL (1989) and MĚSÍČEK & JAROLÍMOVÁ (1992). Six isoenzyme systems were applied to assess the variability. Both *A. oleraceum* species (diploid) show different phenotypes in all enzyme systems, between each other and among *A. carinatum* species. There are supposed null alleles, intergenetic heteromers and post-translation processes acting (WENDEL & WEEDEN 1989, MAY 1992), that is why mixed phenotype method was apply. Of course, method of mixed phenotypes does not produce as much information as allelic explanation. On the other hand, there was the possibility to describe enzyme patterns only by the presence/absence of particular band(s) and consequently to submit them to cluster analysis. But the results do not reflect the thickness or intensity of bands and thus means loss of information. The AAT enzyme system is appropriate to distinguish particular populations of *Allium carinatum*. AAT patterns showed two zones of enzyme activity (Tab 2). There were two mixed phenotypes in the zone I (Fig. 1). Both phenotypes determined in zone I of AAT resembled the final division in the total dendrogram. The phenotype Ia contained three bands, and Ib showed five bands. There were also two phenotypes in zone II (Fig. 2). The phenotype IIa had two bands, and the phenotype IIb three bands. On the other hand the ACP system (Fig. 3) was able distinctly to separate *Allium oleraceum* from *Allium carinatum*. There was only one mixed phenotype for *A. carinatum* in one zone of enzyme activity with four bands (Tab. 2). The tested species showed no interspecific variability in this system but were distinguished from each other. The G-6-PDH system had distinct bands without a colour background, the PGI and PGM systems were similar and seemed to be very useful for analysis of variability. One mixed phenotype *(c*) had the majority in the G-6-PDH system. Two zones of enzyme activity comprised the G-6-PDH pattern, however there were two bands with low mobility in each track without any variability. There were three mixed G-6-PDH phenotypes in total (Fig. 4).The phenotype a - two bands, the phenotype b - two bands, the phenotype c - three bands (Tab. 2). The most complicated was the

Tab. 2. Enzyme systems, its phenotype and presence in populations

enz. system	phenotype	whole percentage [%]	population
AAT	Ia	50	A, B, C, D, J
	Ib	50	E, F, G, H, I
	IIa	40	A, B, I, J
	IIb	60	C, D, E, F, G, H
ACP	a	100	A, B, C, D, E, F, G, H, I, J
G-6-PDH	a	10	J
	b	20	H,I
	c	70	A,B,C,D,E,F,G
EST	a	80	A, B
	b	20	C, D, E, F, G, H, I, J
PGI	a	90	A, B, C, D, E, F, H, I, J
	b	10	G
PGM	a	60	A, E, F, G, H, I
	b	40	B, C, D, J

EST system (Fig. 5), the patterns are formed by a larger number of bands, nevertheless there were only two phenotypes. The main part of the variability is presented in the high mobility area; the phenotypes differentiate by mobility only. There were no differences in the intensities of the bands. The mixed phenotype a - seven bands, the fenotype b - seven bands. The phenotype a predominated in the PGI system (Tab. 2, Fig. 6). There were two phenotypes, a - three bands and one population has the phenotype b - four bands. There was also a difference in the intensity of bands. There are two mixed phenotypes for *A. carinatum* in the PGM system (Fig. 7). The phenotype a - three bands and the fenotype b - three bands. The difference was in mobility, the most intensive band was common for all tracks.

The presence of a higher percentage of mixed phenotypes can be bound together with a trait which brings an advantage for the population or show original phenotypes which are slowly replaced by new phenotypes in some populations. In this case it is only possible by somatic mutation, by virus or coincidetal pollination (KONVIČKA 1972). The overall dendrogram (Fig. 8) of variability divided populations of *A. carinatum* into two basic groups on dissimilarity level 0.51. Generally the populations of the first group belong to the Czech republic area and the second one to the Slovak republic area. The border between the population groups roughly follows the state border or the river Váh. Both populations from the Bošacká dolina valley have the same phenotype and form one population. Populations from Bílé Karpaty mountains are very close and moreover, the relatively separate population in Vranovice belongs to the Bílé Karpaty group and its origin remains obscure.

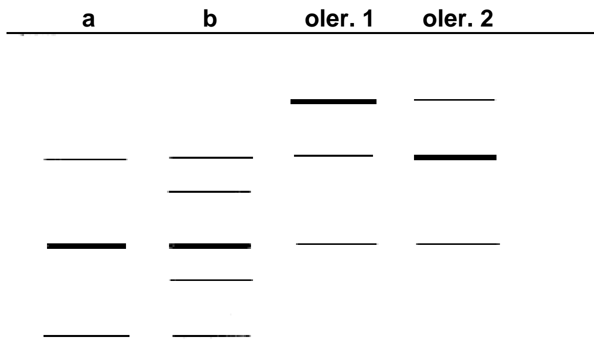

Fig. 1 Isozyme pattern of AAT I

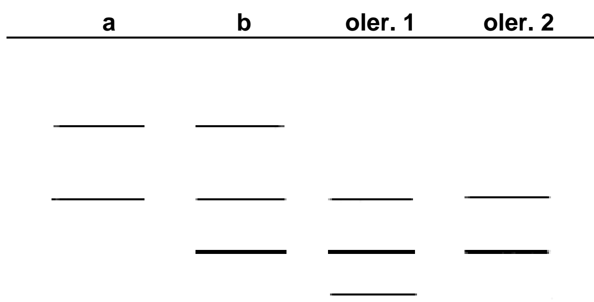

Fig. 2 Isozyme pattern of AAT II

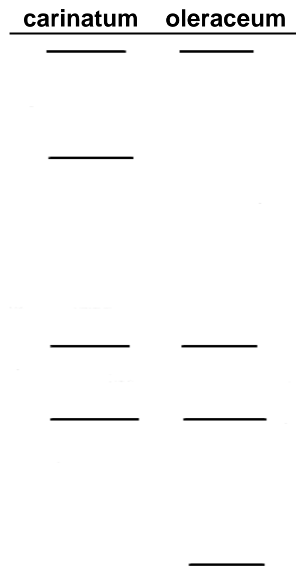

Fig. 3 Isozyme pattern of ACP

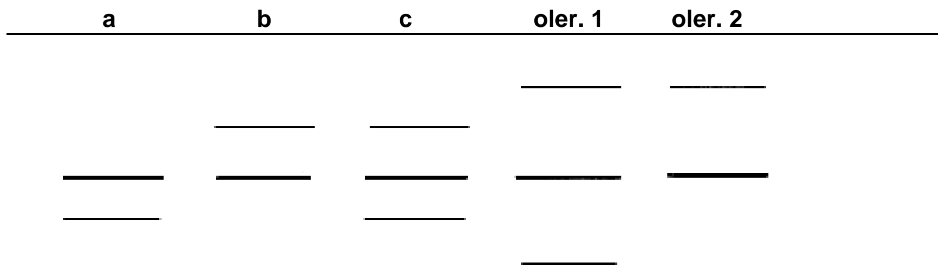

Fig. 4 Isozyme pattern of G6PDH

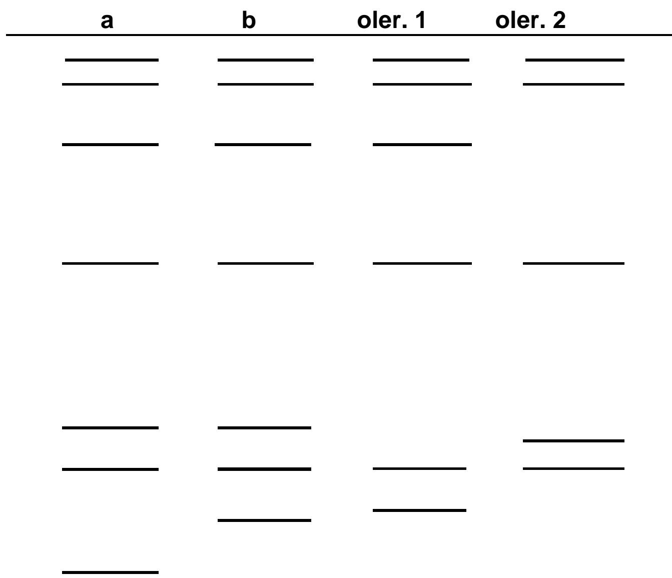

Fig. 5 Isoenzyme pattern of EST

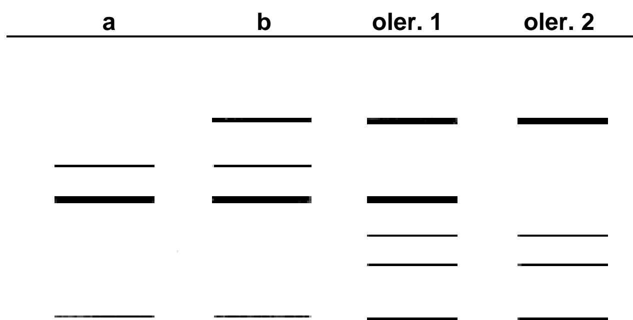

Fig. 6 Isoenzyme pattern of PGI

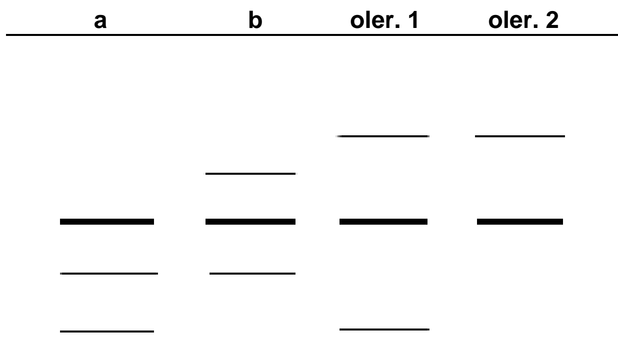

Fig. 7 Isoenzyme pattern of PGM

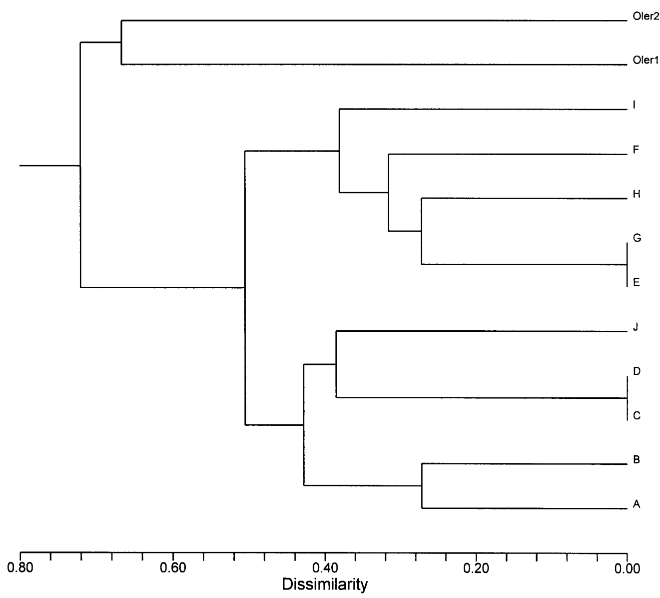

Fig. 8 Particular localities dissimilarity dendrogram

The similarity of tested populations often depends on distance between each other. This technique seems to be very powerful to distinguish both tested species (*A. carinatum*, *A. oleraceum*) and promises good results in the determination of sterile species. This fact is useful because there is possibility of finding the two mentioned species in similar habitats. Isozyme variability of *A. carinatum* is generally low compared with *A. oleraceum*. It corresponds with obligatory asexual reproduction. Mating systems affect genotype structure by this process and a genotype storage function (KLIMEŠ & al. 1997) is apparent in this case. The variability of a population can appear during population life mainly as a consequence of somatic mutation. Sterile hybrids and polyploids are often found to reproduce through vegetative means. Correlation is seen between sterility and the presence of vegetative propagation in *Allium* (VAN DER PIJL 1982). *A. carinatum* moreover follows a reversed pattern, flowering more frequently in wet seasons with few bulbils in the inflorescence and producing smaller but more numerous bulbils in dry seasons (SALISBURY 1942). This fact implies a preference for dry habitats because of obligate the vegetative propagation of this species. The hypothesis of KLIMEŠOVÁ et KLIMEŠ (1997) that each population becomes established separately in a particular place seems doubtful in this case. The presumption is the influence of natural isolation mechanisms and human action. The level of isolation rises (STANĚK & al. 1996) because populations get smaller (or become split into smaller populations) than were present at the begining of the 20th century (PODPĚRA 1911, DOMIN & PODPĚRA 1928) consequently a comparison and the development of variability testing will be important for future work. Habitats of *A. oleraceum* get smaller and fewer, which is why the observation of changes becomes necessary for the efficient management and conservation of habitats.

References

ACQUAAH G. (1992): Practical protein electrophoresis for genetic research. – Dioscorides Press, Portland.

DOMIN K. & PODPĚRA J. (1928): Klíč k úplné květeně republiky Československé. – Olomouc.

DOSTÁL J. (1989): Nová květena ČSSR 1,2. – Academia, Praha.

ETOH E. & OGURA H. (1981): Peroxidase isozymes in the leaves of various clones of garlic, Allium sativum L. – Mem. Fac. Agric. Kagoshima Univ. 17: 71-77.

FIALOVÁ R. (1995): Karyological study of Allium vineale L. – Acta Univ. Palack. Olomuc. Fac. Rerum Nat., Ser. 2, Biol. 33: 35-41.

HAMRICK B. D. & RICKWOOD D. (1990): Gel electrophoresis of proteins - a practical approach. 2nd ed. – IRL Press at Oxford University Press, Oxford.

HANELT P. & al. (1991): Infrageneric grouping of Allium - the Gatersleben approach. – In: HANNELT P. & al. (ed.): The genus Allium - taxonomic problems and genetic resources. – Proceedings of International Symposium, Gatersleben, Germany, June 11-13, 1991.

KLIMEŠ L., KLIMEŠOVÁ J., HENDRIKS R. & GROENENDAEL J. V. (1997): Clonal plant architecture: a comparative analysis of form and function. – In: DE KROON H. & van GROENENDAEL J. (ed.): The ecology and evolution of clonal plants – Backhuys Publishers, Leiden, p. 1-29.

KLIMEŠOVÁ J. & KLIMEŠ L. (1997): Klonální rostliny: fylogeneze, ekologie a morfologie. Biol. Listy 4: 214-263.
KONVIČKA O. (1972): Cytotaxonomische studien von vier sterilen arten der gattung Allium. Biol. Pl. 14: 62-70
KRAHULEC F. (1977): Poznámky k určování československých česneků (Allium L.) v nekvetoucím stavu. – Zprávy Českoslov. Bot. Společn. 12: 145-159.
LEHMAN C. (1997): Clonal diversity of populations of Calamagrostis epigejos in relation to environmental stress and habitat heterogenity. – Ecography 20: 483-490
LEVAN A. (1937): Cytological studies in the Allium paniculatum group. – Hereditas (Lund) 23: 319-370.
MĚSÍČEK J. & JAROLÍMOVÁ V. (1992): List of chromosome numbers of the Czech vascular plants. – Academia, Praha.
NOYES R. D., SOLTIS D. E. & SOLTIS P. S. (1995): Genetic and cytological investigation in sexual Erigeron compositus (Asteraceae). – Syst. Bot. 2: 132-146
PAZOURKOVÁ Z. & PAZOUREK J. (1960): Rychlé metody botanické mikrotechniky. – Praha.
VAN DER PIJL (1982): Principles of dispersal in higher plants. – Springer Verlag, New York.
PODPĚRA J. (1911): Květena Hané. – Brno.
SALISBURY E. J.(1942): The reproductive capacity of plants. – London.
SHIGYO M., TASHIRO Y., ISSHIKI S. & MYIAZAKI S. (1994): Chromosomal locations of glutamate oxaloacetate transaminase gene loci in Japanese bunching onion (Allium fistulosum L.) and shallot (A. cepa L. Aggregatum group). – Jap. J. Genet. 69: 417-424.
SHIGYO M., TASHIRO Y., ISSHIKI S. & MYIAZAKI S. (1995a): Chromosomal locations of five isozyme loci (Lap-1, Got-1, 6-Pgdh-2, Adh-1 and Gdh-1) in shallot (Allium cepa L. Aggregatum group). – Jap. J. Genet. 70: 399-407.
SHIGYO M., TASHIRO Y., ISSHIKI S. & MYIAZAKI S. (1995b): Chromosomal locations of isocitrate dehydrogenase and phosphoglucoismerase gene loci in shallot (Allium cepa L. Aggregatum group). – Jap. J. Genet. 70: 627-632.
SIPES S. D. & WOLF P.G. (1997): Clonal structure and patterns of allozyme diversity in the rare endemic Cycladenia humilis var. jonesii (Apocynaceae). – Amer. J. Bot. 84(3): 401-409.
STANĚK S. & al. (1996): Historická květena Bílých Karpat. – Uherské Hradiště.
TASHIRO Y., SHIGYO M., MIZUTANI, T. & MYIAZAKI S. (1995a): Genetic analysis of Glutamate-oxaloacetate transaminase isozymes in wild species in section Cepa of Allium. – J. Jap. Soc. Hort. Sci. 64: 129-133.
TASHIRO Y., SHIGYO, M., MIZUTANI, T. & MYIAZAKI, S. (1995b): Genetic analyses of glutamate dehydrogenase isozymes in cultivated and wild species of section Cepa in Allium. – J. Jap. Soc. Hort. Sci. 64: 121-127.
TUTIN T. G. & al.(eds.)(1991): Flora europaea. Vol. 5. – Cambridge University Press, Cambridge etc.
VALLEJOS E. (1983): Enzyme activity staining. – In: S. D. TANKSLEY & T. J. ORTON (eds.): Isozyme in plant genetic and breeding, p. 469-516. – Elsevier Science Publishers B. V., Amsterdam.
WEEDEN N. F. (1983): Evolution of plant isozymes. – In: S. D. TANKSLEY & T. J. Orton (eds.): Isozymes in plant genetics and breeding, p. 177-208. – Elsevier Science Publishers B. V., Amsterdam.
WENDEL J. F. & WEEDEN N. F. (1989): Visualization and interpretation of plant isozymes. – In: D. E. SOLTIS & P. S. SOLTIS (eds.): Isozymes in plant biology, p. 5-45. – Dioscorides Press, Portland, Oregon.

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