UDC 634.04:634.01
Original scientific paper

REPEATED FRUCTIFICATION OF AMORPHA (*Amorpha fruticosa* L.), SEEDLING SURVIVAL AND CHARACTERISTICS

Aleksadar TUCOVIĆ* and Dragica VILOTIĆ
Faculty of Forestry, Belgrade, Yugoslavia

Tucović Aleksandar, Dragica Vilotić (2001): *Repeated fructification of amorpha (Amorpha fruticosa L.), seedling survival and characteristics*, Acta herbologica, Vol. 10, No. 1, 49–58.

A second fructification was observed on amorpha trees in addition to the first one. Seedlings of the second fructification are distinguished by several characteristics, and by formation in different conditions. The second fructification is an ancestral property. Repeated fructification has not yet been used in amorpha improvement because it is very rarely met in natural conditions.

Key words: Amorpha fruticosa L. – amorpha, second fructification, lethality, atavistic property, gene recombination, selection

INTRODUCTION

Variability and repetition of phenophases of reproductive organs have a high significance in the interpretation of both ontogenetic and phylogenetic development of phanerophytes (trees and shrubs). There are two hypotheses in the interpretation of new properties or anomalies occurring in the ontogenetic development of trees and shrubs. The advocates of evolution-morphological interpretation often claim that they are atavistic, ancestral properties (JANKOVIĆ, M., 1995, etc.). A group of authors (SOKOLOV, 1969; HARPER, J.L. etc) treats them as ontogenetic adaptations i.e. only as a variation in the development of individuals, which is predominantly controlled by external factors. Consequently, the rare deviations in tree ontogenesis are significant and contribute to a study of the

* Corresponding author: Aleksandar Tucović, Faculty of Forestry, Kneza Višeslava 1, Belgrade

evolution of tree and shrub species. This paper describes effects of the rare phenomenon of repeated amorpha fructification in the sub-spontaneous coenoses of amorpha at Ada Ciganlija near Belgrade.

MATERIAL AND METHOD

Amorpha (*Amorpha fruticosa* L.) is a meliorative, nectariferous and ornamental species, but it is also a dangerous forest weed (ANIĆ, M., 1943; PETRAČIĆ, A., 1938; SALISBURY, E., 1952; STEBBINS, G. L., 1965; TUCOVIĆ and ISAJEV, 2000 etc). At flooded sites, amorpha is a species with weed characteristics: it is readily reproduced because its one-seed pod ("seed") disperses easily. Its damage is the greater as its natural regeneration is very dense and inaccessible, which disables the development of seedlings of economically significant tree species (pedunculate oak, narrow-leaved ash, spreading elm, etc.).

Seeds for a comparative analysis of seedlings reared from seeds of the first and second fructifications were collected from three trees reared at Ada Ciganlija (trees 1, 11 and 12 - the first fructification, and I, XI and XII - the second fructification). We applied the method of individual selection and the method of genetic analysis of half-sib lines. Three trees (1, 11 and 12) were selected as test trees for individual selection, on which the second, repeated fructification was recorded only in 1999. The "seed" was collected separately for the first and the second fructifications. There is a high and statistically justified difference in the sizes and morphology of inflorescences and one-seed pods of the first and the repeated fructification (TUCOVIĆ, A. and ISAJEV, V., 2000). For inflorescence length and width, number of fruits per inflorescence, length and width of one-seed pods, Student's t-test ranged from 5.00 to 66.3.

When the seedlings were 30 days old, in the lines of 1500 seedlings each, the survival percentage of seedlings was determined for both the first and the repeated fructifications. Several characters were analysed on 30 seedlings of both the first and the repeated fructifications in each half-sib line: length of hypocotyl, length of epicotyl, length and width of cotyledons, number of simple leaves, length and width of the simple leaf in the middle of the epicotyl, length of the first internode, i.e. above-cotyledon internode, i.e. cotyledon length to the first simple leaf, length of root axis, total length of lateral roots (Figure 1). Form coefficient of simple leaflets was calculated by: length/width x 100, and given in percentage. The following data were calculated for each of the measured characters: mean values ($\bar{X}$), standard deviation (S), variation coefficient (V), error of mean value (S_{ξ}), error of standard deviation (Ss) and error of variation coefficient (Sv). Statistical justification of the differences between mean values for the analysed properties was calculated by Student's t-test (TUCOVIĆ and ISAJEV, 1988).

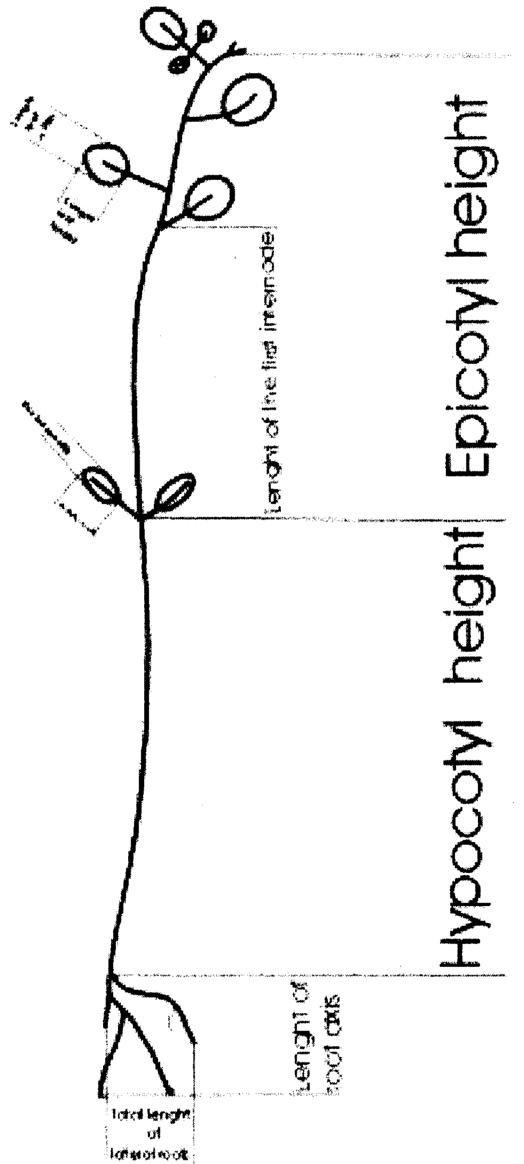

Fig. 1. Seedling properties for variation - statistical processing.
Explanation in the text

RESULTS AND DISCUSSION

A comparative morpho-physiological analysis of the reared half-sib lines of the first and repeated fructifications produced several data on the character of survival, general characteristics of seedlings and genetic potential of seedlings of the first and repeated fructifications. High values of technical germination percentage and absolute germination percentage were determined both for the first and for the second fructifications. The differences in parameters are low and not statistically justified. The first notable differences were analysed 30 days after germination. There were numerous deviations of the main organs of seedlings of the second fructification: lodging, bending, growth inhibition in hypocotyl, epicotyl, root axis, absence of lateral roots, etc. which notably decreases the vitality of seedlings of the second fructification, and their successive dying (Table 1). On the other hand, the seedlings of the first fructification had high vitality and a high degree of survival 95 % (tree 12), 98 % (tree 11) and 100 % (tree 1). Survival percentages of the second fructification seedlings were 18 % for seedlings of the tree line number 12, 36 % for tree 11 and 45 % for the mother tree number 1. The lethality of seedlings from 55 % (tree 1) to 82 % (tree 12) decreases the genetic variability of seedlings within the reared lines compared to the seedlings reared from the first fructification. This phenomenon is essential in the analyses of seedlings of the second fructification, as it reveals the potential endogenous mechanisms of transformation of the genetic potential of this ± weed species (SALISBURY, E., 1952; BAKER, A. G., 1965). Lethality favours (or discriminates) the phenotypes and not the genes or genotypes. The phenotype never reveals the total potential of the genotype. Actually, a continuous increase in genetic variability during generative reproduction is prevented by the factors that, from generation to generation, eliminate a part of the accumulated genetic variability. The factors that eliminate the too high variability are natural selection and random sampling, e.g. lethality of 55 % for tree 1 and 82 % for tree 12. The greatest part of phenotype variability affected by survival - natural selection, results from recombination and not from new mutations. The fact that phenotype determines the adaptive value of seedlings is important for development processes forming the phenotype. Selection pressure is not equal at all times and places. It changes very much, especially in natural conditions, and it is most effective during crisis. Severe drought, severe winter, disease, these events can eliminate genotypes that could "somehow survive" in average conditions and vice versa.

A comparative analysis of nine study properties of half-sib seedlings reared from the "seed" of the first and repeated fructifications is presented in Table 2. Statistically justified differences were determined for four out of nine characters: hypocotyl length, epicotyl length and cotyledon length and width. The determined differences were not statistically justified for five properties (number of simple leaflets, length of the first internode, length of root axis, total length of lateral roots and form coefficient of simple leaflets). The depression in hypocotyl and epicotyl growth is evidently the result of forced autogamy or ± sibbing, considering the small number of trees with repeated fructification in 1999 at Ada Ciganlija. Larger cotyledon sizes in seedlings of repeated fructification were measured for cotyledon length (tree number 11) and for cotyledon width (all three lines of seedlings reared from repeated fructification). There was also a statistically justified increase of cotyledon width in the seedlings of the second fructification. The recorded larger sizes of cotyledons of a part of seedlings in the conditions of previous sibbing or autogamy, points to more frequent tetraploid seedlings, or tetraploids, which were reported by several authors in populations species (ČEHOV, V. P., 1935; WANSCHER, J. H., 1934; BERGER, C. A. *et al.*, 1958; etc.).

The recorded variability of seedlings of the first and second fructifications enables the effect of disruption, stabilization or directed selection in amorpha progeny depending on the diversity of ecological conditions in flooded forest sites. Seedling distribution per classes in variation series for hypocotyl length (Table 3) and cotyledon width (Table 4) illustrates the nature of interdependence of phenotype variability and the diversity of flooded forest plant communities in Serbia (TOMIĆ, Z., 1992), which are at the bases of fast and ± successful adaptation of amorpha to diverse flooded forest sites. These are the reasons of efforts being made to analyse these interdependencies in experimental, i.e. laboratory, nursery and field conditions.

Repeated, i.e. second fructification was recorded for the first time in winter 1999 on three amorpha trees grown at Ada Ciganlija. A great and statistically justified difference was found in the sizes and morphology of inflorescences and one-seed pods of the first and repeated fructifications (TUCOVIĆ, A. and ISAJEV, V., 2000). For the length and width of inflorescence, number of fruits in inflorescence, length and width of one-seed pods, Student's t-test varied from 5.00 to 66.3. The analysis of amorpha seedlings reared from the "seed" of the second fructification has a scientific and practical significance. A high difference in survival was also recorded. Phenotype similarity of seedlings of the first and second fructifications is explained by elimination of a great number, i.e. up to 82% of seedlings of the second fructification during the first 30 days. The notably changed conditions during the repeated flowering affect the morphology of inflorescence, one-seed pods and the inheritance basis of the reared seedlings. The comparative morpho-physiological analysis of seedlings confirms this conclusion. Despite the phenotype similarity of seedlings, the lines of repeated fructification are characterised by a far narrower genetic base, resulting from natural selection and random sampling. Still, the second amorpha fructification has not yet been utilised in the improvement of species because it has been recorded for the first time in natural conditions.

Table 1. Seedling survival from the first and the repeated (second) fructification of amorpha (Amorpha fruticosa L.) in 1999

Fruit	Survival %		
	Tree 1	Tree 11	Tree 12
First fructification	100	98	95
Second fructification	45	36	18

Table 2. Comparative statistical parameters for nine analysed properties of half-sib seedlings of three trees 1, 11 and 12 (first fruit) and I, XI and XII (second fruit)

N	Limiting values	ξ ± Sξ	S ± Ss	V ± Sv	t - between first and second fruit
		Hypocotyl length in cm			
1	4 - 8	5.63 ± 0.14	0.76 ± 0.10	13.50 ± 1.74	-
11	5 - 8	6.27 ± 0.10	0.57 ± 0.07	9.09 ± 1.17	-
12	4 - 8	6.10 ± 0.15	0.84 ± 0.11	13.77 ± 1.80	-
I	3 - 7	4.53 ± 0.18	0.98 ± 0.13	21.63 ± 2.79	1.96
XI	3 - 7	4.77 ± 0.10	0.57 ± 0.07	13.35 ± 1.72	2.22
XII	2 - 6	4.40 ± 0.14	0.79 ± 0.10	17.95 ± 2.32	3.15
		Epicotyl length in cm			
1	3 - 8	4.93 ± 0.14	0.77 ± 0.10	15.62 ± 2.02	-
11	4 - 7	5.70 ± 0.13	0.70 ± 0.09	12.28 ± 1.59	-
12	3 - 8	5.63 ± 0.15	0.84 ± 0.11	14.92 ± 1.93	-
I	3 - 6	4.47 ± 0.10	0.57 ± 0.07	12.75 ± 1.65	0.94
XI	3 - 6	4.63 ± 0.10	0.53 ± 0.07	11.45 ± 1.48	2.23
XII	2 - 6	4.43 ± 0.13	0.73 ± 0.09	16.48 ± 2.12	2.27
		Cotyledon length in mm			
1	7 - 13	10.17 ± 0.22	1.22 ± 0.16	11.99 ± 1.55	-
11	9 - 14	11.17 ± 0.15	0.83 ± 0.11	7.43 ± 0.96	-
12	9 - 13	11.57 ± 0.14	0.77 ± 0.09	6.65 ± 0.86	-
I	8 - 13	10.10 ± 0.17	0.95 ± 0.12	13.05 ± 1.68	0.11
XI	10 - 16	12.50 ± 0.20	1.13 ± 0.14	9.04 ± 1.17	2.25
XII	10 - 14	12.00 ± 0.15	0.81 ± 0.11	6.75 ± 0.87	0.80
		Cotyledon width in mm			
1	3.5 - 6.0	3.69 ± 0.09	0.48 ± 0.06	13.01 ± 1.68	-
11	4.5 - 7.0	5.15 ± 0.08	0.45 ± 0.06	8.74 ± 1.13	-
12	4.0 - 6.5	5.03 ± 0.07	0.40 ± 0.05	7.95 ± 1.03	-
I	3.0 - 6.0	4.60 ± 0.09	0.47 ± 0.06	10.22 ± 1.32	2.17
XI	4.0 - 6.5	5.50 ± 0.09	0.50 ± 0.06	9.09 ± 1.17	2.06
XII	4.5 - 7.0	5.72 ± 0.10	0.53 ± 0.07	9.26 ± 1.20	4.06
		Number of simple leaves			
1	2 - 5	4.10 ± 0.12	0.66 ± 0.08	16.24 ± 2.09	-
11	2 - 5	4.07 ± 0.10	0.57 ± 0.10	14.00 ± 1.81	-
12	2 - 5	3.67 ± 0.08	0.41 ± 0.05	11.17 ± 1.44	-
I	2 - 5	3.77 ± 0.11	0.61 ± 0.08	16.38 ± 2.12	0.69
XI	2 - 5	3.93 ± 0.10	0.57 ± 0.07	14.50 ± 1.87	0.31
XII	3 - 7	4.78 ± 0.13	0.70 ± 0.09	14.64 ± 1.89	4.33
		Length of the first internode (cm)			
1	1.5 - 4.0	3.06 ± 0.09	0.49 ± 0.06	16.01 ± 2.07	-
11	2.0 - 4.5	2.97 ± 0.10	0.95 ± 0.07	18.52 ± 2.39	-
12	2.0 - 4.0	3.05 ± 0.07	0.38 ± 0.05	12.46 ± 1.61	-
I	1.5 - 3.5	2.32 ± 0.08	0.44 ± 0.06	19.10 ± 2.47	1.80
XI	1.5 - 3.5	2.63 ± 0.08	0.46 ± 0.06	17.49 ± 2.26	0.81
XII	1.0 - 3.5	2.48 ± 0.07	0.41 ± 0.05	33.33 ± 4.31	1.54

1	2	3	4	5	6
1	1 - 4	2.87 ± 0.10	0.54 ± 0.07	18.81 ± 2.43	-
11	2 - 5	3.57 ± 0.11	0.63 ± 0.08	17.64 ± 2.28	-
12	2 - 6	3.50 ± 0.17	0.50 ± 0.12	27.43 ± 3.54	-
I	0 - 5	2.77 ± 0.16	0.89 ± 0.11	32.13 ± 4.15	0.20
XI	1 - 5	3.10 ± 0.13	0.71 ± 0.09	22.90 ± 2.96	0.96
XII	1 - 6	3.73 ± 0.14	0.76 ± 0.10	20.37 ± 2.68	0.41
		Length of lateral roots in cm			
1	0 - 3	0.83 ± 0.10	0.54 ± 0.07	65.06 ± 8.40	-
11	0 - 5	1.67 ± 0.15	0.82 ± 0.10	49.10 ± 6.34	-
12	0 - 4	1.57 ± 0.14	0.77 ± 0.10	49.04 ± 6.34	-
I	0 - 5	1.03 ± 0.17	0.94 ± 0.12	91.26 ± 11.79	0.38
XI	0 - 4	1.33 ± 0.16	0.86 ± 0.11	64.66 ± 8.35	0.61
XII	0 - 5	1.63 ± 0.17	0.92 ± 0.12	56.44 ± 7.29	0.11
		Form coefficient of simple leaves			
1	70 - 140	113.30 ± 2.11	11.54 ± 1.49	10.21 ± 1.32	-
11	80 - 160	110.33 ± 2.11	11.75 ± 1.52	10.68 ± 1.38	-
12	80 - 160	109.34 ± 2.41	13.19 ± 1.70	12.10 ± 1.56	-
I	100 - 140	107.67 ± 1.24	6.78 ± 0.87	6.29 ± 0.81	3.08
XI	80 - 140	108.33 ± 1.79	9.79 ± 1.26	9.04 ± 1.17	1.01
XII	80 - 140	106.33 ± 1.74	9.54 ± 1.23	8.97 ± 1.16	1.47

Table 3. Seedling distribution per classes of the variation series for hypocotyl length in cm, in three amorpha half-sib lines from the first fruit (1, 11, and 12) and repeated fruit (I, XI and XII) in 1999

Classes / Trees	2 - 3	3 - 4	4 – 5	5 - 6	6 - 7	7- 8 (cm)
1			6	15	8	1
11				9	19	2
12			2	13	10	5
1, 11, 12			8	37	37	8
I		11	10	6	3	
XI		1	21	7	1	
XII	1	5	20	4		
I, XI, XII	1	17	51	17	4	

Table 4. Seedling distribution per classes of the variation series for cotyledon width in three amorpha half-sib lines from the first fruit (1, 11, and 12) and repeated fruit (I, XI and XII) in 1999

Classes Trees	2,5 - 3,0	3,0 - 3,5	3,5 - 4,0	4,0 - 4,5	4,5 - 5,0	5,0 - 5,5	5,5 - 6,0	6,0 - 6,5	6,5 - 7,0 (mm)
1			2	5	11	11	1		
11					12	15	1	1	1
12				2	12	14	1	1	
1, 11, 12			2	7	35	40	3	2	1
I		2	23	5					
XI				2	1	12	10	5	
XII					1	12	8	6	3
I, XI, XII		2	23	7	2	24	18	11	3

CONCLUSIONS

The morpho-physiological analysis of amorpha (*Amorpha fruticosa* L.) seedlings confirms the hypothesis that the seedlings reared from the "seed" of the first and second fructifications differ notably in the scope of genetic variability. High lethality of seedlings, between 55 % and 82 %, narrows the genetic variability within the half-sib lines of the second fructification, compared to the reared lines of seedlings of the first fructification (lethality up to 5%). The factors which eliminate variability are natural selection and random sampling. Selection pressure is not equal at all times and all places. Actually, it changes very much, especially in natural conditions, and it is the most effective during crisis (severe drought, extremely severe winter, disease, etc.). Differences in the scope of seedling genetic variability point to endogenous mechanisms of regulating amorpha genetic variability in successive generations in natural conditions. The greatest part of variability affected by natural selection results from the recombination in conditions of self-pollination and not from new mutations. Interdependence of genetic variability and site conditions is at the basis of fast and very successful colonisation and adaptation of amorpha to diverse, flooded forest sites in Serbia. This is why efforts are being made to analyse these interdependent relations both in natural and in experimental conditions. The repeated, second fructification is an ancestral property characteristic of ancient species from warmer regions, which are characterised by absence of significant climatic and other changes during the vegetation period.

REFERENCES

ANIĆ, M. (1983): Klijanci, Šumarska enciklopedija. Zagreb, 2, 246-259.

BAKER, H. G. (1965): Characteristics and modes of origin of weeds, The Genetics of Colonizing Species. New York - London, 147-168.

BERGER C. A., WITKUS, E., McMAHON, R. M. (1958): Cytotaxonomic studies in the Leguminosae. Bull. Torrey Bot. Club, 85, 6, 405-414.

ЧЕХОВ, В. П. (1955): Кариосистематическиј очерк трибы Galegeae Bronn. Тр. Биолог. Институт Томск, 1, 143-196.

ISAJEV, V. i MANČIĆ, A. (2001): Testovi za utvrđivanje kvaliteta semena, Šumsko semenarstvo. Izdanje Univerziteta Banja Luka - Beograd, 130-177.

HERPER, J. L. (1977): Populatin biology of plants. London - New York, 895.

JOVANOVIĆ, B. (1991): Rod *Amorpha* L., Dendrologija. Izdanje Naučna knjiga, Beograd, 314-315.

JANKOVIĆ, M. (1995): Biodiverzitet i ontogenetsko razviće. Biodiverzitet: suština i značaj. Izdanje Zavoda za zaštitu prirode Srbije, Beograd, 55-67.

СОКОЛОВ, С. JА. (1969): К теорији интродукции растении. Дати и методи обогашчении дендрофлори. Новосибирск, 4-23.

PETRAČIĆ, A. (1938): *Amorpha fruticosa* L. kao nov i opasan korov u posavskim šumama. Šumarski list, Zagreb, 623-626.

SALISBURY, E. (1952): Natural selection and plant invasion. S. Afr. J. Sci., vol. 49, 115-119.

STEBBINS, G. L. (1965): Colonizing species of the nature California flora. The genetics of colonizing species. New York - London, 173-191.

TOMIĆ, Z. (1992): Šumske fitocenoze Srbije. Šumarski fakultet, Beograd, 11-86.

TUCOVIĆ, A. i ISAJEV, V. (1988): Individualna promenjivost biljaka i metode njenog proučavanja. Praktikum iz genetike sa oplemenjivanjem biljaka. Naučna knjiga, beograd, 28-36.

TUCOVIĆ, A. i ISAJEV, V. (2000): Kolonizacija šumskih staništa žbunovima *Amorpha fruticosa* L. u Srbiji i njene biološke osobine. Acta herbologica, vol. 8, No. 1, 43-53.

WANSCHER, J. H. (1934): The basic chromosome number of the higher plants. New Phytol., 33, 2, 101-126.

Received June 2, 2001
Accepted October 2, 2001

PONOVLJENI UROD BAGRENCA (*Amorpha fruticosa* L.), PREŽIVLJAVANJE I OSOBINE KLIJAVACA

Aleksandar TUCOVIĆ i Dragica VILOTIĆ
Šumarski fakultet, Beograd
Jugoslavija

Izvod

Morfološko-fiziološka analiza klijavaca bagrenca (*Amorpha fruticosa* L.) potvrdila je hipotezu da se klijavci iz »semena« prvog i drugog uroda značajno razlikuju u pogledu genetske varijabilnosti. Visoka smrtnost klijavaca od 55% do 82% značajno ograničava genetsku varijabilnost linija polusrodnika iz drugog uroda u odnosu na klijavce iz prvog uroda (smrtnost 5%). Faktori koji onemogućavaju varijabilnost su prirodna selekcija i nasumično uzorkovanje. Selekcioni pritisak nije isti u svako vreme i na svim mestima. On se značajno menja u prirodnim uslovima, a najsnažniji je u vreme stresa (jaka suša, izrazito hladna zima, bolest i dr.) Razlika u opsegu genetske varijabilnosti klijavaca ukazuje na endogene mehanizme regulacije varijabilnosti bagrenca iz narednih generacija u prirodnim uslovima. Varijabilnost, na koju utiče i prirodna selekcija, najvećim delom je rezultat rekombinacije, a ne novih mutacija. Međuzavisnost genetske varijabilnosti i uslova sredine predstavlja osnovu brze i veoma uspešne kolonizacije i adaptacije bagrenca na različitim poplavnim šumskim staništima u Srbiji. Iz tog razloga je i obavljena analiza ovih odnosa međuzavisnosti kako u prirodnim tako i u eksperimentalnim uslovima. Ponovljeni, odnosno drugi urod je ancestralno svojstvo karakteristično za stare vrste poreklom iz toplijih područja gde nije dolazilo do klimatskih i drugih neujednačenosti tokom vegetacije.

Primljeno 2. juna 2001.
Odobreno 2. oktobra 2001.