 DOI:10.2298/ABS1202573M

GENETIC VARIABILITY OF BOTTLE GOURD *LAGENARIA SICERARIA* (MOL.) STANDLEY AND ITS MORPHOLOGICAL CHARACTERIZATION BY MULTIVARIATE ANALYSIS

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Abstract - A wide range of bottle gourd *Lagenaria siceraria* (Mol.) Standley germplasm was collected from different parts of the world. Genetic resource preservation and determination of genetic variability was carried out as a foundation for future breeding work. The germplasm diversity collected in Serbia is a result of its adaptation to diverse ecological conditions and farmers' selection in accordance with their preference and ethnobotanical utilization. The broad intraspecific variation of the plant, fruit and seed morphology is a direct result of the research carried out. Principal component analysis (PCA) of *L. siceraria* with 13 quantitative traits showed continuous variation among accessions, primarily due to fruit and seed size and shape. The evident reduction in trait variation is a direct result of the preference for ornamental use that favored certain shapes and sizes of the fruit, which has not significantly changed over the centuries.

Key words: Ethnobotanical value, *Lagenaria siceraria*, Principal Component Analysis, variability

INTRODUCTION

The bottle gourd can be easily distinguished from other pumpkin varieties by its white flowers and characteristic fruit, seed and leaf shapes (Cutler and Whitaker, 1967). Tropical Africa is the primary gene centre of the bottle gourd (Sing, 1990) which is the only species that has been used worldwide since prehistoric times. It was widely spread across the world before Columbus discovered America, due to spontaneous dispersion by ocean currents (Whitaker et al., 1961).

The bottle gourd is characterized by minimal agrotechnical requirements and it is well adapted to extremely divergent agroecosystems. Bottle gourd variability has been studied by many authors, including Heiser, 1979; Decker Walters et al., 2001; Marimoto and Mvere, 2004; Marimoto et al., 2005; Achigan Dako et al., 2008, etc. Studies in India demonstrated the significant regional variability (Sivaraj and Pandravada, 2005), whereas Yetisir (2008), who studied a collection from Turkey concluded that morphological variation was most apparent in fruit shape and size. These findings were in line with the results reported by Şakar (2004), who reported fruit size and shape as the most apparently distinguishable morphological traits in the Mediterranean region.

Historically, throughout the Balkan Peninsula the bottle gourd has had significant, primarily practical household and agricultural uses, as well as in rural art. Colloquial names for the bottle gourd in the region are "sudovnjača", "nategača" and "ajduk"

(Berenji, 1982, 1988). Due to is broad fruit-shape variability, bottle gourds can be used as culturally specific utensils and ornaments, whilst the younger juicy fruits are edible and nutritious (Berenji, 1992, 1999, 2000; Morimoto and Mvere, 2004; Schippers, 2004). Bottle gourd seeds are a good source of lipids and proteins (Achu et al., 2005; Loukou et al., 2007). Prasad and Prasad (1979) have created unique bottle gourd varieties in India, primarily for human consumption.

The objective of the present germplasm preservation work was to collect, characterize and conserve genotypes of *Lagenaria siceraria* (Mol.) Standley. The main goal was to study the diversity, identify the most useful variables for discrimination among genotypes, the genetic variations among and within collected samples using progeny test, and correlation among the traits.

MATERIALS AND METHODS

Landraces from a wide range of countries from Balkan Peninsula were collected and compared with material sourced from Africa, Asia and America.

For progeny tests, bottle gourd seeds were germinated at 25°C, between two wet sheets of paper. Outdoor planting was performed during 2009 and 2010 in a field of the Institute of Field and Vegetable Crops in Novi Sad (Serbia). The planting distance between individual plants was 160 × 60 cm. Forty genotypes, each represented by five plants, were grown to maturity. Fruits were harvested for each individual plant and were subsequently used for the fruit and seed variation survey of the progeny.

Thirteen traits were measured in forty genotypes and their abbreviations are: plant height (PH), fruit weight (FW), length (FL) and circumference (FC), handle length (HL), leaf blade width (LW) and length (LL), rind thickness (RT), seed length (SL), width (SW) and thickness (TOS), 100-seed mass (100 SM), and number of seeds per plant (NSP). The chosen quantitative and qualitative fruit characteristics were assessed and evaluated using standard ECPGR (2008) (European Cooperative Programme for Plant Genetic Resources) descriptors. The plant height, leaf blade length and width were expressed in metric units, whereas fruit weight and 100-seed mass were measured using technical scale of 0.01 g precision.

Using Heiser's (1979) criteria for subspecies identification based on seed characters, the seed type for each accession was classified as *siceraria*-type (S), *asiatica*-type (A) or as an intermediate (I). The *siceraria*-type characteristics are dark seed coat color, seed length-to-width ratio less than 2, reduced or absent 'ears', and a lack of pubescent longitudinal lines along the face of the seed. In contrast, the *asiatica*-type is characterized by light color, seed length-to-width ratio exceeding 2, prominent ears, and pubescent lines. If a seed exhibited two *siceraria*-type states and two *asiatica*-type states, it was classified as intermediate.

The collected data were processed using one-way analysis of variance (ANOVA) using the statistical program STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA).

Investigation of multi-character variation was conducted by Cluster Analysis and Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

The geographical variation of *Lagenaria siceraria* (abbreviation for genotype is LAG), fruit shape and seed type are presented in Table 1. The collected germplasm showed immense variation in fruit shape and seed morphological values, irrespective of the origin. Owing to the ease of dispersion of bottle gourd species, both by human and natural means, its geographical origin as well as domestication and migration paths remain uncertain. As a result, the bottle gourd has been the most widely dispersed and common plant organism, both to the Old and New Worlds since ancient times.

Bottle gourd fruit and seed type of different origin support the argument stated by Montes-Hernan-

Table 1. Origin, fruit and seed type of bottle gourd germplasm

Accession code	Landrace or local name[a]	Geographic origin	Fruit type[b]	Seed type[c]
		AFRICA		
LAG 30	African Gourd	na	P	A
LAG 41	Assorted African Gourds African Gourds	na	P	A
LAG 46	African Rattle	na	D	I
LAG 47	Mini Nigerian Bottle	na	S	S
LAG 84		Nigeria	P	S
		ASIA		
LAG 10	Chinese Bottle	China	P	A
LAG 33	Indonesian Bottle	Indonesia	P	A
LAG 61	Japanese Siphon	Japan	E	S
LAG 81	Mini Chinese Bottle	China	P	A
		NEW WORLD		
LAG 14	Penguin	Ohio	E	S
LAG 32	Birdhouse Gourd	na	P	A
LAG 36	Sennary	Collert	P	A
LAG 38	Large Birdhouse	NY	P	A
LAG 42	Large Ornamental Gourd Canteen	Pennsylvania	P	S
LAG 45	213 Lyons 1997	Ohio	D	I
LAG 63	Big Apple	Canada	E	I
LAG 67		na	C	S
		EUROPE		
LAG 01	MS 32	Serbia	E	S
LAG 04	Nategača	Serbia	E	I
LAG 05	MS 34	Serbia	E	A
LAG 09	PI No 379365	Serbia	P	I
LAG 16	No 05	Serbia	E	I
LAG 18	PI No 287533	Italy	E	A
LAG 29	Lagenaria	Serbia	P	A
LAG 50	Nategača	Serbia	E	A
LAG 56	Zierkürbis Kalabassen	Hungary	E	I
LAG 62	Krupnoplodna Lagenaria	Serbia	P	I
LAG 64	NS 3	Serbia	P	A
LAG 65	Lagenaria	Serbia	P	S
LAG 66	Nategača 210	Serbia	Cw	I
LAG 70	Verruqueuse de Maurice	Croatia	Cw	S
LAG 71	Speckled Apple	Hungary	C	S
LAG 72	Emphasi	Hungary	P	I
LAG 73	Macis	Hungary	P	A
LAG 74	Lagenaria	Serbia	P	A
LAG 76	Nategača for food	Serbia	P	A
LAG 77	Lagenaria	Serbia	C	S
LAG 78		Serbia	E	A
LAG 80		Serbia	E	A
LAG 85		Serbia	Cw	A

[a]na = information not available.
[b]C = Cylindrical, Cw = Warted cylindrical, D = Disk, E = Elongated, P = Pear-shaped, S = Spherical
[c]A = asiatica-type, I = intermediate, S = siceraria-type.

dez et al. (2002) that regionally bound human activities significantly buffer genetic variability between plants. Moreover, the fruit and seeds of plants with favourable characteristics constitute planting material for the next season; often sold or exchanged, they contribute to the movement of variability between regions.

The diversity in fruit and seed morphology of 40 accessions was further investigated in detail (Tables 2 and 3). The most important characteristics of the bottle gourd are related to fruit, seed and leaf. In relation to fruit weight, LAG 47 (4.555 kg) and LAG 84 (0.102 kg) are distinct. Genotypes differ in fruit length, from very long LAG 63 (137.70 cm) to very short LAG 84 (9.40 cm). A wide range of variability was also recorded in the quantitative traits for other fruit, leaf and seed characteristics: plant height (281-322 cm), fruit circumference (10.66-84 cm), handle length (5-17 cm), leaf blade width (17.89-30.13 cm), leaf blade length (14.49-23.01 cm), seed length (8.50-20.52 mm), seed width (5.20-12.34 mm), seed thickness (2.12-3.80 mm), 100-seed mass (9.82-30.32 g), rind thickness (1.43-1.87 mm) and number of seeds per plant (66-647.4). The genotypes were predominantly pear-shaped or elongated, most likely because these were the key characteristics that determined usability. Fruit color ranged from light green to the dark green with a few spots, while the fruit surface was smooth, rarely with warts (genotypes LAG 66, LAG 70 and LAG 85). In the collected material, only one genotype (LAG 76) was not bitter and suitable for human consumption. Such a small number of edible bottle gourd genotypes can be explained by the large number of more nutritious crops of the same family. Consequently, the focus of bottle gourd selection was to obtain specific fruit shapes, sizes and colors.

Many authors have confirmed genetic diversity within *Lagenaria siceraria* (Sivaraj and Pandravada, 2005; Morimoto et al., 2005; Morimoto et al., 2006; Decker-Walters et al., 2001). The observed variation in the qualitative and quantitative traits of the germplasm analyzed as a part of the present work in many cases exceeded the values reported by Sivaraj and Pandravada (2005) for India as a secondary centre of diversity. The deliberate and sustained human selection of desirable traits was evident, irrespective of the geographical origin. As a result, it is difficult to correlate unique fruit characteristics with specific localities and customs.

Although wild species of *L. siceraria* are found exclusively in Africa, the oldest known Asian remains of bottle gourd are Japanese seeds (6000-80000 B.P.) (Crawford, 1992). The lack of comparable ancient remains from western and southern Asia during that period (Decker-Walters et al. 1999) indicates that the earliest dispersal from Africa to eastern Asia was probably via the Indian Ocean. Thus, the only possible connection between Asian germplasm and Balkan Peninsula landraces can be established via the S*ilk Road* network of trade routes across the Asian continent that connected Eastern, Southern, and Western Asia, as well as parts of North and East Africa, with the Mediterranean. It is most likely that the Tea Road was much less important. The bottle gourd's history as a small-scale commercial crop in Europe, as well as on the Balkan Peninsula, is unclear due to undocumented hybridization and selection practices over the past 100-200 years. Genetic identities are maintained by inbreeding, probably resulting from frequent successive self-pollination. Using morphological diversity and RAPD segregation in half-sib progeny within-accession, testing 9 accessions collected in Kenya, Morimoto et al. (2005, 2006) detected relatively small variation compared to those of the among-accessions. The percentage of within-accession variance to total variance in the cultivated species *L. siceraria* was 1.1%. Similar results were obtained from morphological analysis (Morimoto et al., 2005).

Selection based on fruit shape and size has resulted in subdividing the species into several groups according to specific uses. In most rural world communities, such as in Cote d'Ivoire as well as Serbia, common names were designated to different accessions of *Lagenaria siceraria* according to these morphological differences (Koffi et al., 2009; Berenji, 2000).

Table 2. Mean values and coefficients of variation for fruit traits in 40 *Lagenaria siceraria* accessions

Genotype	PH	FW	FL	HL	LW	LL	FC	RT
				AFRICA				
LAG 30	318	1.572	40.20	16.4	26.58	22.15	66.7	1.71
LAG 41	316	0.898	19.50	7.6	23.32	16.92	50.5	1.54
LAG 46	315	0.521	11.06	12.4	19.42	14.49	51	1.63
LAG 47	301	4.555	25.76	15	25.51	20.10	84	1.87
LAG 84	282	0.102	9.40	9.4	21.18	14.55	17.3	1.54
				ASIA				
LAG 10	313	0.835	20.12	6.4	23.89	20.09	46.8	1.53
LAG 33	312	2.112	47.40	8.6	27.93	22.17	75	1.74
LAG 61	305.4	1.898	60.98	9.4	24.67	18.71	46.8	1.59
LAG 81	287.5	0.290	14.45	11.1	22.99	17.43	22.7	1.55
				NEW WORLD				
LAG 14	322	1.454	38.34	6.4	22.52	16.46	32.2	1.73
LAG 32	313	1.396	45.06	7.2	22.99	18.87	58.6	1.73
LAG 36	317	0.248	15.16	11.2	24.67	20.01	25.6	1.63
LAG 38	315	0.191	14.74	9	23.17	15.95	23.1	1.43
LAG 42	311	1.170	57.90	9.8	24.54	18.80	57.9	1.64
LAG 45	313	0.707	10.20	15	24.20	20.05	66.5	1.64
LAG 63	314	2.002	137.70	9.6	27.86	22.92	26.6	1.64
LAG 67	283	1.972	23.40	8.2	17.89	15.94	42.7	1.83
				EUROPE				
LAG 01	314	1.321	71.06	15.2	24.75	18.95	48.7	1.64
LAG 04	314	1.204	80.88	8.2	23.71	18.13	45.4	1.54
LAG 05	314	1.266	83.88	9.6	23.81	18.01	53.7	1.54
LAG 09	315	1.282	50.66	15.2	30.13	23.01	57.3	1.76
LAG 16	312	2.409	60.48	12.6	26.36	20.92	35.4	1.63
LAG 18	321	2.416	84.94	18.4	25.50	20.82	25.7	1.57
LAG 29	321	1.215	32.34	9	20.94	16.17	57.8	1.63
LAG 50	312	1.195	48.46	8.4	18.02	15.38	38.5	1.54
LAG 56	303	0.830	32.60	9.2	25.46	20.78	27.6	1.64
LAG 62	290	1.001	23.70	7.3	19.72	18.20	10.7	1.83
LAG 64	303	1.509	26.72	7	23.33	17.12	59.9	1.74
LAG 65	310	0.503	15.61	5.6	22.99	19.88	31.5	1.51
LAG 66	304	1.208	33.22	13.8	25.53	20.02	51.3	1.74
LAG 70	312.5	1.980	36.78	15.6	24.95	17.95	49.3	1.86
LAG 71	281	1.965	21.10	7.4	24.60	18.75	38.2	1.84
LAG 72	282	1.009	36.86	9	23.03	17.06	63.9	1.87
LAG 73	300	1.112	40.20	17	24.98	20	67.8	1.77
LAG 74	311	1.005	29.68	14.2	28.33	18.85	52.9	1.72
LAG 76	313	1.854	33.52	12.2	20.23	18.89	64.1	1.73
LAG 77	281	2.133	23.48	7.8	21.02	16.96	38.7	1.84
LAG 78	306	0.994	70.40	5	21.22	18.22	38.4	1.65
LAG 80	311	1.436	91.64	7.2	19.38	18.14	46.5	1.64
LAG 85	280	1.992	33.94	11.9	26.49	18.22	43.7	1.86
Mean	3.06	1.370	41.53	10.5	23.69	18.64	46.0	1.67
CV	4.19	57.62	65.05	35.5	11.73	11.35	35.77	7.36
χ^2	94.12	99.78	99.37	83.55	98.73	99.51	98.55	99.26

Plant height (PH) in cm, fruit weight (FW) in kg, fruit length (FL) in cm, handle length (HL) in cm, leaf blade width (LW) in cm, leaf blade length (LL) in cm, fruit circumference (FC) in cm, rind thickness (RT) in mm.

Table 3. Mean values and coefficients of variation for seed traits in 40 *Lagenaria siceraria* accessions

Genotype	SL	SW	TOS	100 SM	NSP
			AFRICA		
LAG 30	17.60	7.30	2.20	21.80	474.4
LAG 41	16.90	7.40	3.18	24.92	185.6
LAG 46	18.20	8.10	2.56	22.66	322.2
LAG 47	19.30	12.34	3.76	29.19	647.4
LAG 84	8.50	5.30	2.20	12.78	79.2
			ASIA		
LAG 10	14.40	6.40	3.16	18.56	487.8
LAG 33	16.90	7.40	3.10	15.80	577.8
LAG 61	10.50	8.30	3.66	28.12	129.4
LAG 81	12.30	6.20	2.12	15.98	89.4
			NEW WORLD		
LAG 14	18.38	8.30	3.80	29.12	364.8
LAG 32	18.12	7.20	3.10	22.72	222.8
LAG 36	12.50	5.30	2.16	9.82	188
LAG 38	13.10	6.30	2.20	11.40	66
LAG 42	17.18	9.20	2.80	25.08	109.2
LAG 45	19.40	8.38	2.22	22.12	346.2
LAG 63	17	6.36	2.30	25.90	333.4
LAG 67	15.70	9.10	2.22	24.80	415.4
			EUROPE		
LAG 01	16.20	6.30	2.16	20.76	617.6
LAG 04	18.36	8.48	3.26	26.50	294
LAG 05	19.10	7.40	3.16	23.82	297
LAG 09	20.52	8.40	3.14	29.66	427.2
LAG 16	17.50	6.20	2.20	21.80	592.2
LAG 18	18.40	7.40	3.34	26.88	431.6
LAG 29	17.48	7.70	3.18	25.58	175
LAG 50	18.40	7.50	3.22	22.45	222
LAG 56	16.90	7.90	3.10	28.40	175.4
LAG 62	9.30	7.50	2.30	19.08	104.4
LAG 64	15.50	7.28	3.30	21.04	244.6
LAG 65	10.30	5.30	2.22	13.16	131.4
LAG 66	14.30	6.24	3.28	17.58	119.8
LAG 70	15.70	9.50	3.16	29.18	185.6
LAG 71	17.50	8.24	2.64	25.66	145.2
LAG 72	17.20	6.24	3.36	18.67	191.2
LAG 73	16.40	7.28	3.26	30.32	69.4
LAG 74	15.80	7.06	2.30	23.50	95
LAG 76	17.30	5.20	3.10	20.84	363.8
LAG 77	15.40	10.30	3.30	23.66	274.8
LAG 78	18.40	7.14	3.80	21.50	292.4
LAG 80	19.50	7.32	3.14	19.87	265.6
LAG 85	19.40	8.90	3.28	25.86	267.4
Mean	16.27	7.49	2.88	22.41	275.54
CV	18.09	19.75	19.68	22.97	58.01
χ^2	86.43	95.92	90.12	78.29	99.91

Seed length (SL) in mm, seed width (SW) in mm, thickness of seed (TOS) in mm,100-seed mass (100 SM) in g, number of seed per plant (NSP)

Table 4. Correlation matrix among characteristics studied

Variable	FW	FL	HL	WL	LL	FC	RT	SL	SW	TOS	100 SM	NSP
PH	-0.06 ns	0.33*	0.18*	0.21*	0.27*	0.18*	-0.44*	0.29*	-0.16*	0.05 ns	0.08 ns	0.27*
FW		0.30*	0.24*	0.22*	0.32*	0.40*	0.50 *	0.40*	0.59 *	0.39*	0.53*	0.59*
FL			0.05 ns	0.24*	0.38*	-0.01 ns	-0.13ns	0.38*	-0.02 ns	0.17*	0.33*	0.27*
HL				0.45*	0.36*	0.29*	0.16*	0.20*	0.11 ns	-0.11 ns	0.26*	0.24*
LW					0.76*	0.24*	0.11 ns	0.18*	0.03 ns	0.001 ns	0.21*	0.23*
LL						0.22*	0.12 ns	0.20*	-0.06 ns	-0.03 ns	0.14*	0.41*
FC							0.34*	0.52*	0.34*	0.36*	0.32*	0.37*
RT								0.21*	0.42*	0.22*	0.36*	0.14 ns
SL									0.40*	0.37*	0.56*	0.48*
SW										0.42*	0.65*	0.22*
TOS											0.49*	0.08 ns
100 SM												0.18*

* significant correlation at p=0.05
ns - not significant correlation

Coefficients of variation were the highest for fruit weight and length, and for number of seeds per plant (CV = 57.62-65.05 %), while they were the lowest for rind thickness (CV= 7.36 %).

The heritability estimates were high (>90%) for plant height, fruit weight, fruit length, fruit circumference, leaf blade length, leaf blade width, seed width, thickness of seed, rind thickness and number of seeds per plant. Other characteristics, such as handle length and seed length, showed heritability values ranging between 83.55% and 86.43%, whereas a somewhat lower heritability was observed for 100-seed mass (78.29%).

High homozygosity within the collected accessions is a result of successive multiplication on isolated farms and villages, very characteristic to agriculture in 18th and early 19th century. *L. siceraria* is a self-compatible and monoecious species. Female flowers are usually surrounded by 8 to 26 male flowers, depending on the environmental conditions. Farmers usually select one or two fruits from each landrace and remove the seeds from the fruit at the time of seeding. Thus, the probability that half-sibs are sown on the same spots or side-by-side is very high. All these factors seem to explain the high rate of inbreeding. This was confirmed by our findings, whereby progeny plants derived from a common mother showed a low level of segregation regardless of the examined traits.

Results presented in Table 4 show the existence of a positive correlation between plant height and fruit length (r = 0.33). The correlation between fruit weight and all other variables was positive (r = 0.22-0.59). The correlation between fruit weight and 100-seed mass was positive, which is in accordance with the findings of Koffi et al. (2009) and Achigan-Dako et al. (2006). Leaf blade length was correlated with leaf blade width (r = 0.76) characterizing the leaf shape as an important taxonomic feature. An important positive correlation was determined for fruit length and leaf blade length, leaf blade width, seed length, thickness of seed, 100-seed mass and number of seeds per plant (r = 0.17-0.38). Handle length was correlated with the length and weight of leaf blade (r = 0.36-0.45) and fruit circumference

Table 5. Eigenvalues, proportion of total variability and correlation between the original variables and the first three principal components (PCs).

Variable	PC1	PC2	PC3
FW	**0.870**	-0.006	0.492
FL	0.512	-0.804	-0.301
FC	**0.692**	0.603	-0.396
Eigenvalue	1.499	1.010	0.490
% Var.	49.98	33.69	16.32
% Cum.	49.98	83.68	100.00

(r = 0.29). Fruit circumference was correlated with rind thickness and seed characteristics, which were also positively correlated.

Principal component analysis was used to identify the most significant variables in the data set (Table 5).

The results indicated that the first two components explain about 83.68% of the total variability observed, whereby the first accounted for 49.98%. Variables with higher scores on PC1 are related to fruit weight and fruit circumference (FW, FC). The highest contribution on PC2 corresponded to variables related to the fruit length (FL).

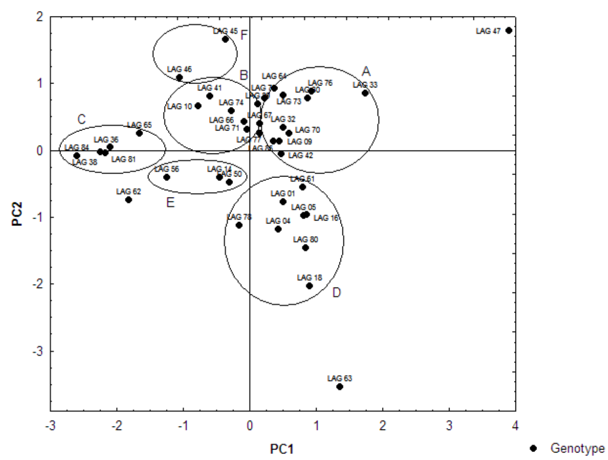

Fig. 1. Factor scores for the first two principle components for 40 bottle gourd genotypes

For PC analysis (Fig. 1), only fruit characteristics were used. Six groups of genotypes were separated based on morphometric fruit characteristics and shape. Group A consisted of genotypes with large pear-shaped fruits with similar fruit weight, circumference and length. Thus, in future selection work it is sufficient to take just a few genotypes from this group. Group B included genotypes with both cylindrical and medium size pear shaped-fruits. Five pear-shaped genotypes were in Group C, characterized by small fruit weight, circumference and length. Such genotypes are ideal for decoration and were typically used for salt keeping. Group D was formed by genotypes with elongated fruits, some of which have wide ends that can be used to extract wine and brandy from barrels. Group E also included elongated genotypes, but with shorter fruit of smaller circumference. Two genotypes with disk-shaped fruit (LAG 45 and LAG 46) comprised Group F. LAG 47 was unique by its characteristics of large fruit weight and circumference, making it suitable for use as a utensil or for seed production, as it produces the largest number of seeds per plant as well as maximum 100-seed mass. LAG 63 is another extreme due to its longest fruit.

Multivariate Cluster Analysis based on morphological characteristics resulted in the dendrogram shown in Fig. 2. The genotypes are mainly separated according to the fruit weight, circumference and fruit length. The Cluster Analysis results are in agreement with those obtained by PC analysis.

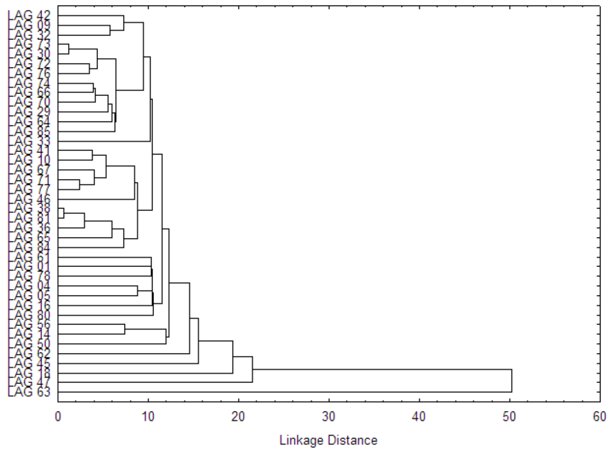

Fig. 2. Dendrogram for complete linkage of investigated selections, expressed in Euclidean distances

A desirable trait is defined by its ethnobotanical value. These findings are important for understanding the evolutionary processes that have shaped this species and the influence that humans have had through domestication. It was possible to trace back the use of bottle gourd in this region for at least 200 years. The first records of bottle gourd usage in Serbia can be found in 17th century icons in the monastery Mikolja. According to archival records, it was also used as a float for traditional fishing on the Danube during the same period.

Museum exhibits from the Ethnographic Museums in Belgrade, Senta and Kikinda also suggest that, throughout history, the bottle gourd was used as a utensil, instrument, toy, food and ornament, depending on its shape and size. For example, pear-shaped fruits were used mostly for dishes, while elongated fruits were used for musical instruments. Historically, the main value of bottle gourd was its domestic use as kitchenware, until it was replaced by pottery. Elongated forms with a wide end have the colloquial name "nategača" and are used for extracting wine and brandy from barrels. Such forms (LAG 05, LAG 50, LAG 78 and LAG 80) have been maintained in the same households for at least one hundred years, restoring the seeds each year. In contrast, pear-shaped gourds have much wider applicability, as bottles, bowls, milk-pots, spoons, kitchen spoons, funnels and strainers for pouring liquids (water, milk). The genotype from Žabalj is pear-shaped with a wide neck and it was used as a pitcher for water (LAG 74). In the early 19th century, soldiers carried water in pear-shaped bottle gourds, which were tied around their belts (Rodić, 2004).

Today, the bottle gourd is mostly displayed as an ornament or used as art material that can be cut and carved. A wide range of skillfully crafted items is still present at individual farms as well as in museums. Museum exhibits from Kikinda suggest that in 20th century, gourds with an elongated shape and round end were also used as musical instruments, one of which was "dvonjci" (LAG 04). "Dvonjci" was a shepherd flute made from two cane pipes. The genotype from Čurug is a pear-shaped, ideally suitable as a material for fine art (LAG 09). Genotypes exhibited in the City Museum in Senta present brandy glasses due to their widened top (LAG 29), the oldest of which dates back to 1863. The gourd was also widely used as a smoking pipe, manufactured in many sizes and shapes.

CONCLUSION

The present study shows that *Lagenaria siceraria* in Serbia is very divergent and endowed with rich variability. With the aim to maintain and increase the genetic resources of bottle gourd, we developed a promotional strategy of germplasm preservation through the media, specifically targeting inventors and individual producers. We recognize that these groups play a very important role in the preservation of genetic resources, especially in relation to older forms that could be carriers of special genes. The most important results of this effort are genotypes with a unique fruit length of 137.70 cm (LAG 63).

However, even though a wide range of samples from different regions throughout the world was analyzed, no diversity patterns could be associated with geographical origin. There is a possible connection between the germplasm of Turkey, the Mediterranean region and the Balkan Peninsula, founded in the ancient Silk Road, and due to similar morphological variations of fruit size and shape. In addition, these findings suggest that variability is predominantly driven by the types of use. A valuable core collection was established as a foundation for future breeding work and inheritance studies (research in progress).

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