Intrapopulation variability of fruit quantitative traits *Diospyros lotus* L. in the Inner Mountain Dagestan

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Abstract. The cenopopulation of Gimry village in Untsukulsky district of Inner Mountain Dagestan was employed for the first time to analyze intrapopulation variability of *Diospyros lotus* quantitative traits of fruit. In view of this study were selected 10 random trees in a mature generative stage. It has been made measurements with 30 fruits by 14 morphological and 6 index traits. The multidimensional scaling and cluster analysis have revealed two clusters on the large-fruited (large-fruited and small-fruited) significantly differed in the t-test. The interindividual variability was higher than intraindividual to most studied traits, which may be associated with high genotypic heterogeneity of this population. The analysis of variability by the coefficient of variation (CV, %) and dispersion components (h2,%) has shown the most stable and variable traits. The greatest constancy has found for traits of seed thickness and the calyx index, the smallest – for weighted traits in all degree of variation. The traits of small-fruited trees were strongly correlated and for large-fruited trees – weakly. It has been identified main regularities for the indices variability. The fruit mass, flesh mass and seed mass has same character of correlations with other traits on intraindividual and interindividual levels.

1 Introduction

Diospyros lotus L. (Caucasian persimmon) is a rare protected species, a Tertiary relict, which in the wild has a disjunctive area in China, India, Iran, Asia Minor, Transcaucasus and the North Caucasus [1, 2, 3]. In Russia, it is located in the Krasnodar Territory on the Black Sea coast of the Caucasus. This is a dioecious tree of the Ebenaceae family, which in nature reaches up to 30 m in height, sometimes takes a bush-like shape. It blooms in May-June and fruits in October-November. Fruits are large berries, almost spherical, 10-25 mm in diameter. The fruit contains from 2 to 8 seeds. Coloring fruits from dark red to yellow, with a strong waxy coating and yellow astringency flesh. Life cycle is about 100 years. It is propagated by seeds and shoots. Various botanical gardens are widely cultivated it. The fruits of this species are also used in folk medicine for vitamin deficiencies, gastrointestinal

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diseases [4, 5, 6]. In addition, this species is often used as a rootstock for persimmons, especially in places with a high content of chloride salinization and on limestones [7, 8]. The use of it as a rootstock, show both noticeable resistance to low winter temperatures and drought, and the elevating of the scion growth [9]. In Dagestan, *D. lotus* natural populations are widespread on gravelly and stony slopes in Foothills and Inner Mountain Dagestan up to 1300 m above sea level. The vulnerability of this species is determined with the increased anthropogenic load, small area, low population size and stenotopicity [10, 11].

The study of the variability of morphological traits is one of the ways to identify the adaptive potential of any species in natural conditions. The study of the variability of the morphological characteristics of the fruit and seeds is an indirect method of such assessment for plants.

The identification of genetically heterogeneous plants within populations in nature for a number of agronomic characters and an assessment of their reaction rate has a great practical importance for the purposes of ecological breeding.

In this regard, the studying of the intra- and interindividual variability of the *D. lotus*, is of interest to the extent that it is rare and promising for mountain gardening in Dagestan

This paper presents the results in studying of the regularities of *D. lotus* intrapopulation variability for fruit quantitative traits in conditions of the Inner Mountain Dagestan.

2 Methods

The largest population of *D. lotus* growing in the Inner Mountain Dagestan in the river valley of the Avar Koisu river at the vicinity of the Gimry village of Untsuculsky district (440 m above sea level) were used to study the intrapopulation variability of morphological traits of the fruit (Figure 1).

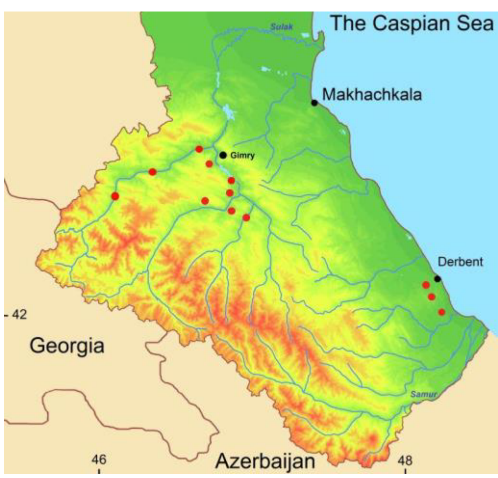

Fig. 1. Spread of *Diospyros lotus* in Dagestan.

Laboratory measurements were carried out with 30 fruits from 10 random trees of a mature generative stage. For this aim, it was used 14 continual and 6 index traits of the fruit and seed. The comparison of trees was carried out by such quantitative characteristics as: fruits – length (LF), width (WF), thickness (TF), total weight (MW), flesh mass (MF); seeds – number (N), length (LS), width (WS), thickness (TS), total weight (MS), weight of hundred seeds (MHS), calyx - length (LC), width (WC), mass (MC) ; index – reproductive effort of fruit (Ke = MS/MW), fruit shape (Kf = WF/ LF), fruit roundness (Ko = TF/ LF), calyx shape (Kc = WC /LC), seed shape (Ks = WS/LS), seed flattening (Ku = TS/LS) were used for. The linear dimensions of the fruits are measured in mm, the weight characteristics are presented in grams, the index ones - in percentages. It was used statistical methods of materials processing. The arithmetic mean (X) and the error of the mean (Sx) was analysed for each trait. The assessment of the degree of variation of the characteristics was carried out using the coefficient of variation (CV,%). The statistical analysis by Statistica v.13.3 processing system was carried out using the methods of variance (h^2,%), correlation analyzes (Pearson's correlation coefficient) and graphing for the assessment of the significance of differences between individuals. The differentiation of individuals according to the quantitative characteristics of the fruit was carried out using cluster analysis by the Ward's method.

3 Results

A preliminary multidimensional scaling and cluster analysis of 14 morphological parameters of the fruit showed that the measured trees are divided into two clusters. The results of clustering indicated a significant morphological differentiation of the study sample. The classification of trees according to the quantitative characteristics of the fruit for the *D. lotus* Gimry population is shown in Figure 2.

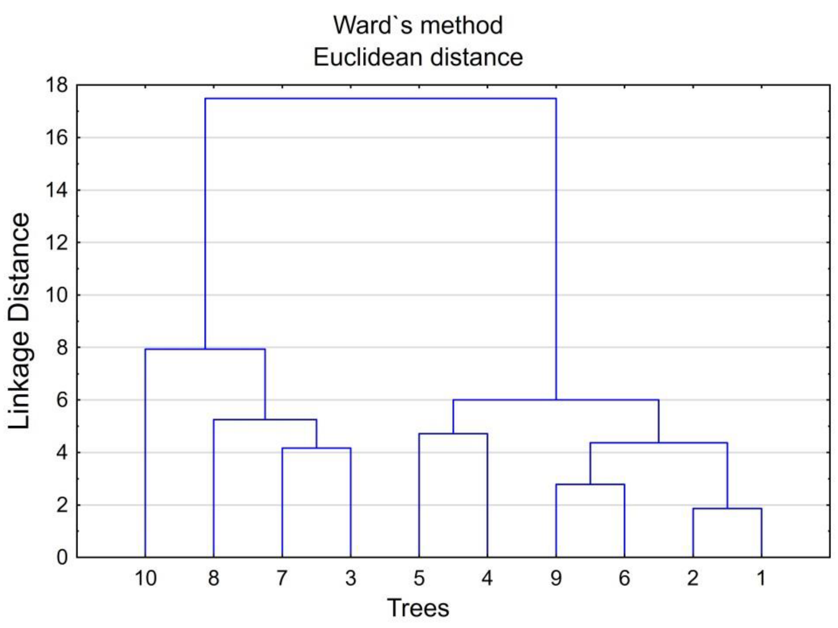

Fig. 2. Diagram of cluster analysis of trees for quantitative characteristics of the fruit.

The first cluster included four individuals with large fruit size. The second cluster was divided into two subclusters, of which the first consisted of two trees with small fruits, and the second included four trees with medium fruits. The contrast values of the fruit traits from all the studied trees were distinguished for two trees (No. 5 and No. 10) which accordingly have minimum and maximum values.

Comparative analysis of the identified groups is confirmed by the results of cluster analysis. The differences between the groups are most significant in terms of linear and weight characteristics of the fruit, and are significant according to Student's t-test (Table 1). An exception has the calyx index mean values for which there were no significant differences, and the identified groups were similar and had approximately 81–82%.

Table 1. Comparative characteristics of the morphological traits of the *Diospyros lotus* fruit in the Gimry population.

Traits	Groups				Total n=300		t-test
	small-fruited n=180		large-fruited n=120				
	X±Sx	CV,%	X±Sx	CV,%	X±Sx	CV,%	
LF	16,8±0,14	10,9	18,9±0,13	7,8	17,6±0,11	11,3	10,56***
WF	13,9±0,17	16,0	17,3±0,21	13,0	15,3±0,16	18,3	12,97***
TF	12,6±0,15	15,8	15,6±0,21	14,7	13,8±0,15	18,7	12,15***
LS	11,5±0,06	7,3	12,7±0,09	7,5	12,0±0,06	9,0	12,08***
WS	6,1±0,04	9,5	7,1±0,05	8,1	6,5±0,04	11,6	14,40***
TS	3,2±0,03	11,3	3,1±0,04	13,7	3,2±0,02	12,3	2,10*
LC	11,2±0,09	11,3	12,0±0,13	11,7	11,5±0,08	12,0	5,07***
WC	9,0±0,09	13,0	9,8±0,18	19,6	9,3±0,09	16,8	4,48***
N	3,4±0,13	51,4	4,8±0,17	39,2	4,0±0,11	48,9	6,86***
MW	1,9±0,05	36,2	3,3±0,09	29,5	2,5±0,06	42,5	14,04***
MS	0,50±0,020	53,8	0,91±0,034	40,8	0,66±0,021	55,9	11,00***
MF	1,4±0,04	32,9	2,4±0,06	29,6	1,8±0,04	40,8	13,95***
MHS	14,9±0,16	14,4	16,5±0,17	18,2	16,5±0,17	18,2	14,59***
MC	0,10±0,002	27,0	0,14±0,003	26,6	0,12±0,002	32,9	11,74***
Ke	24,6±0,54	29,6	27,0±0,71	28,9	25,6±0,44	29,6	2,66**
Kf	82,8±0,72	11,6	91,8±0,96	11,4	86,4±0,63	12,6	7,65***
Ko	75,0±0,64	11,4	82,7±1,01	13,4	78,1±0,60	13,2	6,80***
Kc	80,9±0,59	9,8	82,2±1,19	15,8	81,4±0,59	12,6	1,06
Ks	53,1±0,28	7,1	55,7±0,42	8,2	54,2±0,25	7,9	5,30***
Ku	28,2±0,32	15,1	24,7±0,44	19,3	26,8±0,28	17,8	6,56***

Note: Confidence levels * - p <0.05; ** - p <0.01; *** - p <0.001.

The analysis of variability by the coefficient of variation (CV) at the intraindividual level showed that the most stable were linear traits of the fruit and seed and index traits calculated on their basis (Table 2). The greatest variability has the weight traits (excluding the mass of one hundred seeds), number of seeds, and the effectiveness of reproductive effort of fruit. It is important to note that the mass of one hundred seeds is an integrated feature and showed the qualitative characteristic of the seed, the variability of which is comparable to the mass of one seed. The *D. lotus* seed mass is genetically determined at the intraindividual level.

Table 2. Intrapopulation variability of the fruit characteristics at the *D. lotus* Gimry population by the coefficient of variation (CV,%) and components of dispersion (h2,%).

Traits	Level of variability (CV,%)													F
	E (tree number)										I	S	L	
	1	2	3	4	5	6	7	8	9	10				h^2,%
1. LF	10,5	7,0	6,9	6,6	10,8	5,8	4,5	7,3	5,8	6,9	11,3	10,9	7,8	58,1***
2. WF	16,9	11,5	11,9	11,4	16,7	12,2	9,1	13,2	14,4	15,1	18,3	16,0	13,0	48,0***
3. TF	17,5	11,9	14,2	11,4	15,5	9,9	11,7	14,3	14,0	16,3	18,7	15,8	14,7	45,0***
4. LC	12,3	8,7	7,8	6,6	11,7	12,6	5,4	12,3	10,0	13,8	12,0	11,3	11,7	24,6***
5. WC	14,2	8,5	18,7	7,4	13,1	11,9	12,2	13,3	10,7	16,6	16,8	13,0	19,6	37,7***
6. MW	37,4	25,4	26,8	25,8	40,0	23,0	19,7	27,9	29,1	33,8	42,5	36,2	29,5	51,2***
7. MC	29,6	19,3	17,6	17,0	27,2	21,4	15,5	26,7	19,7	22,9	32,9	27,0	26,6	54,2***
8. MS	50,3	42,9	32,3	44,0	62,5	45,0	33,7	42,6	53,4	47,7	55,9	53,8	40,8	38,0***
9. MHS	14,7	9,4	12,6	11,8	14,8	12,0	9,3	7,2	8,6	13,5	18,2	14,4	18,2	59,8***
10. MF	35,1	20,6	25,8	20,6	33,6	20,6	15,0	25,9	22,7	29,8	40,8	32,9	29,6	57,5***

11. N	51,7	43,7	30,2	43,3	60,3	42,2	34,0	41,8	52,0	46,2	48,9	51,4	39,2	23,0***
12. LS	7,0	5,1	6,1	4,6	9,2	4,6	4,8	3,8	4,4	6,5	9,0	7,3	7,5	60,6***
13. WS	6,7	6,2	8,0	7,1	9,4	7,5	6,0	6,6	6,8	8,6	11,6	9,5	8,1	60,8***
14. TS	13,0	8,7	9,5	11,9	9,9	9,7	10,9	13,1	12,5	13,3	12,3	11,3	13,7	17,2***
15. Ke	26,0	27,3	13,6	22,9	30,5	30,8	19,6	32,0	33,7	28,8	29,6	29,6	28,9	24,6***
16. Kf	13,3	11,1	10,2	8,6	9,8	10,2	9,7	11,3	11,5	11,5	12,6	11,6	11,4	27,9***
17. Ko	12,7	11,6	12,7	9,3	9,9	7,9	12,4	13,1	11,4	12,5	13,2	11,4	13,4	25,6***
18. Kc	7,5	9,6	18,5	6,1	11,1	8,2	11,4	14,8	11,4	9,4	12,6	9,8	15,8	20,9***
19. Ks	5,0	6,3	5,6	5,0	5,5	6,9	4,8	6,0	4,6	5,9	7,9	7,1	8,2	51,6***
20. Ku	16,0	10,6	12,9	14,0	16,8	12,8	12,7	14,9	14,9	18,1	17,8	15,1	19,3	33,6***

Note: Level of variability: E - intraindividual; I - interindividual; F - factorial between trees; Groups of trees: S - small-fruited; L - large-fruited; h^2,% - dispersion components.

A visual representation of the variability of fruit traits is given on Figure 3, in which groups of traits are distinguished according to the type of variability.

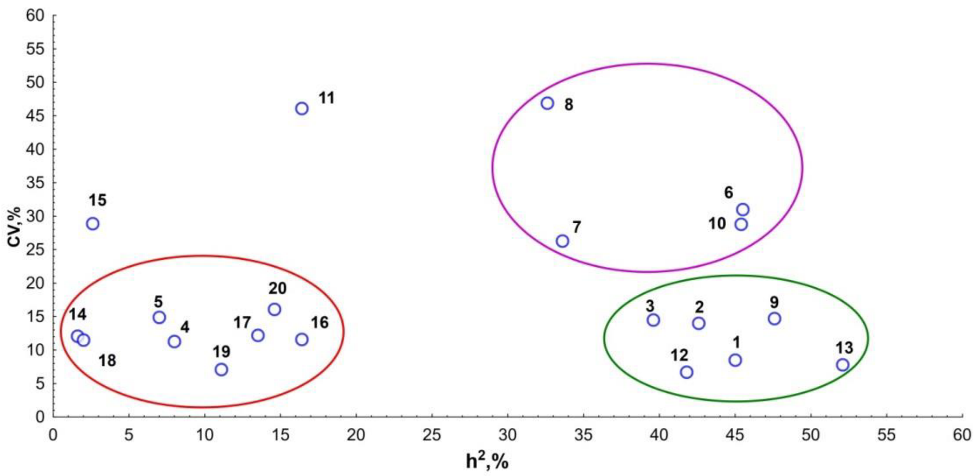

Fig. 3. Diagram of the ratio interindividual and factorial variability on quantitative traits of the *D. lotus* fruit.

Note: the numbers of traits go according to the numbering given in table 2.

Correlation analysis between the traits used in the calculation of the indices showed that their correlation is different (table 3). The highest correlation was observed among the traits of seed weight and fruit weight (Ke index). The least correlation (negatively) was found between the seed thickness and seed length (Ku index). The variability of the first pair of traits turned out to be higher than for the index calculated on their basis. On the other hand, the variability of the second pair of traits, on the whole, turned out to be lower than the index calculated on their basis. The other trait pairs were characterized by intermediate results. The variability of the indices occupied an intermediate position or was closed to one of the components.

Table 3. Correlations of morphological characteristics of the fruit in the index indicators of *D. lotus* Gimry population.

Index / traits	**E (tree number)**										**I**	**S**	**L**	**T**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**				
Ke / MS&MW	92*	94*	94*	93*	96*	81*	93*	89*	95*	96*	90*	92*	81*	90*
Kf /	67*	41*	57*	66*	83*	55*	13	54*	65*	73*	70*	59*	51*	74*

WF&LF														
Ko / TF&LF	74*	36	50*	58*	78*	60*	07	45*	59*	77*	70*	62*	43*	71*
Kc / WC&LC	86*	33	09	62*	61*	77*	33	20	39*	82*	69*	70*	53*	62*
Ks / WS&LS	72*	36*	73*	72*	82*	46*	61*	63*	75*	72*	67*	59*	46*	73*
Ku / TS&LS	-16	-11	-29	-20	-58*	-51*	-22	-55*	-44*	-54*	-24*	-09	-52*	-37*

Note: Level of variability: E - intraindividual; I – interindividual; Groups of trees: S - small-fruited; L - large-fruited; T – all trees; correlations are presented without null and comma.

Individuals in the population of any kind of plant differ, first of all, in biomass, which acts as an integrated indicator of the vital state. The total biomass (or total weight) consists of many other parameters that are correlatively related to it to one degree or another [9]. One of the integrated indicators of the vital state of woody plants with their long-term form of existence can be a mass of reproductive organs (fruits, seeds), as a result of complex morphogenetic and ecological-physiological processes.

The main components of the fruit mass of *D. lotus* L. are the flesh mass and the seed mass. The correlation analysis was revealed to identify the specific traits of intra-linear and intergroup correlation of quantitative fruit traits (Table 4).

Table 4. Correlation of weight traits of the fruit of the Gimry population *D. lotus.*

Traits	**Flesh mass**					**Seeds mass**					**Total weight**				
	S	**L**	**№5**	**№10**	**T**	**S**	**L**	**№5**	**№10**	**T**	**S**	**L**	**№5**	**№10**	**T**
LF	80*	67*	89*	78*	80*	58*	22*	74*	74*	58*	77*	57*	85*	79*	77*
WF	88*	84*	94*	90*	90*	86*	81*	93*	93*	88*	92*	92*	96*	93*	94*
TF	89*	83*	92*	94*	90*	89*	86*	94*	96*	91*	94*	93*	96*	97*	95*
LC	33*	47*	42*	37*	48*	36*	18	39*	38*	37*	36*	41*	42*	38*	46*
WC	45*	39*	61*	33	47*	50*	20*	64*	39*	39*	50*	36*	64*	36*	47*
MW	97*	95*	98*	99*	97*	90*	81*	96*	96*	90*	-	-	-	-	-
MC	62*	68*	75*	79*	78*	62*	59*	78*	79*	72*	66*	74*	79*	81*	80*
MS	77*	59*	88*	91*	77*	-	-	-			90*	81*	96*	96*	90*
MHS	43*	-05	68*	39*	64*	23*	74*	42*	43*	51*	38*	25*	60*	42*	63*
MF	-	-	-	-		77*	59*	88*	91*	77*	97*	95*	98*	99*	97*
N	66*	47*	77*	89*	63*	96*	95*	97*	98*	94*	81*	70*	88*	94*	77*
LS	67*	40*	95*	75*	69*	52*	12	85*	72*	53*	65*	33*	94*	75*	67*
WS	70*	70*	79*	74*	81*	63*	57*	66*	77*	73*	72*	73*	76*	77*	83*
TS	-24*	-22*	-54*	-57*	-25*	-49*	-35*	-63*	-67*	-41*	-35*	-29*	-60*	-62*	-32*
Ke	24*	-05	66*	70*	17*	77*	74*	92*	91*	71*	46*	25*	79*	79*	37*
Kf	48*	51*	62*	75*	61*	64*	79*	75*	82*	77*	57*	68*	69*	79*	69*
Ko	47*	53*	48*	84*	59*	68*	82*	65*	89*	79*	58*	70*	56*	88*	70*
Kc	24*	14	28	06	17*	25*	09	34	14	16*	25*	14	31	10	18*
Ks	25*	33*	-25	26	40*	30*	44*	-29	33	46*	28*	42*	-28	29	44*
Ku	-51*	-32*	-82*	-70*	-52*	-62*	-32*	-82*	-77*	-56*	-58*	-35*	-84*	-74*	-56*

Note: Groups of trees: S - small-fruited; L - large-fruited; T – all trees; * - The level of reliability $p < 0.05$; correlations are presented without null and comma.

4 Discussion

Since the intraindividual variability is due to the environmental component (internal and external), and the interindividual genotypic and environmental components, the second form of variability can either increase or decrease depending on the ratio of these components. Identifying the genotypic component is the most difficult task in such studies. In our study, the interindividual variability of the Gimry population for most of the studied traits turned out to be higher than the intraindividual one, which may indirectly indicate a high genotypic heterogeneity of this population.

Analysis of variance is the main statistical method for identifying phenotypic differences. ANOVA was used to assess the total intrapopulation variability and differences between the isolated groups. The results of ANOVA showed that the differences between individuals of *D. lotus* fruit traits are significant by all considered parameters (except for thickness of seed, calyx index) at the highest level of significance and generally exceeds intraindividual variability (Table 2, Figure 2). Significant differences between the groups were identified in terms of seed width, weight of one hundred seeds, seed length, fruit length, fruit width, fruit thickness. The most deterministic traits were thickness of seed, length of calyx, width of calyx, and number of seeds. The isolated groups (F) differentiate well the linear and weight characteristics of fruit, length of seed and width of seed.

Based on the results of visual representation of different types of variability (Figure 3), four categories of traits were identified: 1) constant - deterministic for both levels of variability. There are thickness of seed, length and width of calyx, and almost all indices; 2) genotypic - stable intraindividual, but variable at the interindividual level. These are all linear traits of fruit, length and width of seed, width of calyx, and mass of 100 seeds; 3) ecologically dependent - traits with high intraindividual variability, but low interindividual (effectiveness of reproductive effort of fruit and seed number); 4) variable - irregular in both types of gradation, this includes all weight characteristics.

The analysis of index traits represents the interests in the study of intrapopulation variability of *D. lotus* according to the fruit characteristics. The index traits are characterized by the low variability because of a high range of functional correlation between parameters used in the calculating the indices. All the used indices had large differences in mean values with the exception of calyx index.

The general regularities in using index traits are: 1) the stronger the positive correlation of the index constituting parameters is, the lower its index variability, 2) in the absence of correlation, or its negative value, the index variability is higher than its constituent parameters, and 3) the index variability has an intermediate nature for the average (fluctuating) correlation between the traits.

Two groups identified by cluster analysis by grouping for large-fruitedness are also differed well in the nature of the correlation of the index characteristics. Strong correlation was noted for small-medium-fruited group, and low in large-fruited group.

Correlations of the fruit weight and its two main components with the rest of the traits both at the intraindividual level (trees No. 5, No. 10) and at the group level showed the same nature of correlation with the exception of some weak unreliable correlation (Table 4). However, the nature of their character of their correlation was different. The "small-fruited" trees are generally stronger correlation than correlation in "large-fruited" trees if we compare individual groups according to the degree of contingency. Considering that the correlations within the identified groups of trees are also phenotypic (the result of a combination of ecological and genotypic variability), the main point is the combination of the genotypes that make up these groups, which may not coincide at the intraindividual level and in the all population. In general, intrapopulation correlations were higher than intragroup correlations in terms of the characteristics that these groups linearly differentiate to the greatest extent. These are linear and weight fruit traits. The traits for which these groups differed slightly, on the contrary, had higher correlations than in the pooled population. As an illustration of their correlations at the intraindividual level from all trees, we will give an example for the most contrasting variants, i.e. "the small-fruited" (No. 5) and "the large-fruited" (No. 10) trees. Correlations of given three weight traits with the rest revealed a number of differences. Weighted traits of «the small-fruited» tree correlate more with linear parameters of a calyx (especially with a width), a length of seed and weight of 100 seeds. The "large-fruited" tree has generally stronger correlations with weight traits (mass of fruit, mass of calyx, mass of seeds), thickness of fruit, number of seeds, i.e. the most determine the fruit productivity. The greatest differences are found between these trees are observed by the mass of flesh and the smallest by seeds weight. So it can be assumed that the differences in correlations between these genotypes are the different degree of realization of potential productivity. Since for any species, the formation of full-fledged seeds is the main purpose, and the concomitant elements of the fruit are secondary formation, the final formation of secondary components occurs only after the minimum program is achieved. Accordingly, the implementation of potential productivity at the tree No. 5 possible was not complete. Indirectly, it is indicate by a stronger variation of the traits of the fruit of this tree compared to the tree No. 10 (Table 2).

5 Conclusion

Multidimensional scaling and cluster analysis of 14 morphometric parameters of the fruit showed that the *D. lotus* Gimry population allowed us to select two clusters (small-fruited and large-fruited) and these differences are reliable by t-test, which indicates a significant morphological differentiation of the trees.

The analysis of variability in terms of the coefficient of variation (CV, %) at the intraindividual level showed that the most stable traits were the linear fruit traits and seed traits, mass of a hundred seeds, as well as a number of index traits calculated on their basis. The most variable characteristics were the weight traits, seed number, and effectiveness of reproductive effort of fruit.

Comparative analysis of the variability of fruit traits by the coefficient of variation (CV, %) and dispersion components (h2,%), has identified groups of traits by the degree of variability: 1) constant (seed thickness, calyx length, calyx width, and almost all indices); 2) genotypic (all linear characteristics of the fruit, the length and width of the seed, the width of the calyx, the mass of one hundred seeds); 3) ecologically dependent (the effectiveness of the reproductive effort of the fruit and the seed number; 4) variable (all weight characteristics).

The main regularities in the indices variability were revealed. They are: 1) the stronger the positive correlation of the traits constituting the index is, the weaker its the index variability, 2) the variability of the index is higher than its constituent traits in the absence of correlation or it has a negative value, and 3) the index variability has an intermediate nature with the average strength (fluctuating) of correlation between parameters.

Correlations of fruit weight and its two main components with the another of the traits at the intraindividual and group levels showed the same nature of the correlations. Small-fruited and medium-fruited groups of plants, on the whole, have a higher correlation of traits than large-fruited ones. In general, intrapopulation correlations turned out to be higher than intragroup correlations in terms of the characteristics that differentiate these groups to the greatest extent.

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