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The statistical analysis of cytomorphological traits in the distant apple and pear F_1 and F_2 hybrids (*Malus* x *Pyrus*) from artificial and spontaneous outcrosses

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***Abstract*—The complex morphometrical traits of leaf epidermal tissue in species forms *Pyrus communis* L. and *Malus baccata* Borkh. were studies to establish statistically significant differences. These traits can serve as a marker selective indicator of saturation degree estimation in a complex of genes from each parental species, combined in hybrid progeny genotypes by distant hybridization. The stomatal density in leaf epidermal tissue has the most variability among studied morphometric traits of apple and pear wild forms and their hybrids. A mode, kurtosis and skewness of numerical data series are most appropriate to use for a comprehensive statistical analysis of variation curves, obtained from study of different genotypes by the quantitative traits of stomatal guard cells. The differentiation of genotypes into groups, graphically shown in a shape of polymodal variation curves, was noticed among the population of F_2 hybrids. The progeny from free pollination of F_1 hybrids has the largest values of stomatal morphological traits such as the widest data series, the coefficient of variation and the variance. A tendency of partial deviation in stomatal quantitative traits and stomatal index in the studied F_2 hybrids between apple and pear in the direction of a female parent was revealed.**

Keywords—distant hybridization; F_1 and F_2 hybrids; cytomorphological traits; variation curve; stomata; leaf epidermal tissue.

I. INTRODUCTION

A distant hybridization has a significant importance for acclimatization of plants. Several important regularities were revealed as a result of distant crosses research. The more genetical differences obtain crossed plants the more likely is the appearance of artificial new species. F_1 hybrids, obtaining from the cross of crop plants with wild species, represent a new taxonomic unit. However, they are usually temporary because of their sterility. Artificial F_2 hybrids are new temporary forms naturally occurring very rarely, but it is nevertheless possible to breed experimentally a small amount of these genotypes.

Studies were performed at the Centre for Collective Use "Selection of crops and food production technologies, storage and processing of food products for functional and treatment purposes" in the Michurinsk State Agrarian University within the framework of the State Order of the RF Ministry of Agriculture №30 "Breeding winter-hardy dwarf clonal rootstocks using molecular markers" in 2019.

Obtaining F_2 hybrids with improved fruit quality is an important breeding objective for creation of highly adaptive fruit varieties. Creating the second generation of intergeneric hybrids is also a serious problem due to the formation of small amounts of normally developed seeds with low germination. Thus, the formation of new types of highly adaptive crops using distant hybridization should go through the intermediate, transitional forms. Obtaining and study of these breeding stages will allow consciously to direct the formation of new synthetic species and get a hybrid plants with desirable properties.

There is a significant problem of targeted F_2 hybrids breeding due to tissue necrosis of the flower after artificial stamen castration before a free pollination, which subsequently causes the destruction and abscission of flowers without their fertilization. However, the death of flowers at free pollination stage in this hybrid genotypes doesn't happen, and a small amount of morphologically normal and viable seeds are formed [1-3].

As a result of using different ways to improve the effectiveness in obtaining F_2 hybrids, including the use of *in vitro* technologies, it is possible to obtain viable plants. In this case, there is a problem with selection of new plant forms with a genes complex of parental genotypes from specific taxonomic group. In addition, the study of stomata is important to identify the physiological characteristics of plants [4-10].

II. MATERIALS AND METHODS

The biological objects were parental forms (genotypes of *Pyrus communis* L. and *Malus baccata* Borkh.), F_1 hybrids (apple-and-pear hybrid №12 bred by T.A. Gorshkova and pear-and-apple hybrid 01 bred by S.F. Chernenko) and F_2 hybrids, obtained by free pollination of F_1 plants.

The method of preparing cytological specimens for the study of leaf anatomical and morphological traits by light microscopy (patent RF №208106368) was applied to determine a size of stomatal guard cells and a number of stomata per 1 square millimeter of leaf epidermal tissue [11]. Leaves for analysis were prepared using the ultrasonic device UZDN-2T (Fig. 1). The power density of the irradiation was 7,0 W/cm^2, the irradiation frequency – 22 kHz, the exposure time – 30 sec.

The polymerizable mixture of organic compounds (butyl acetate, ethyl acetate, nitrocellulose, isopropyl alcohol) was used to make the replicas of leaf plates for epidermal tissue morphoanatomical analysis [12, 13].

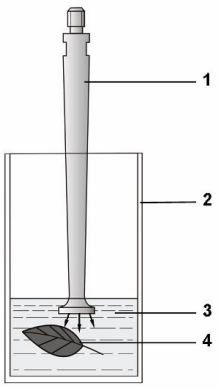


Fig. 1. The scheme of the device unit for making preparations of plant leaves: 1 – ultrasonic emitter; 2 – beaker; 3 – water; 4 – leaf

The preparations were viewed under a Leica DM2500 microscope, pictures were taken with digital camera DCM-500 with Scope Photo software. The data was statistically processed in Microsoft Office Excel 2016.

More than 200 measurements were carried out in triplicate for each studied quantitative trait in every genotype. The mean value with its standard error, coefficient of variation, variance, median, mode, kurtosis and skewness were calculated. The variation curves of the stomatas distribution in the epidermal leaf plates tissue were analyzed.

The variation curves of length guard cells of F_1 and F_2 hybrid generations were built on the cumulative distribution of this trait in all plants produced in this crossing combination. Theoretically, the distribution of characteristic values in hybrids depends on the statistical sample size and may be graphically represented in the shape of curved line. This graph looks like a continuous polymodal curve for the significant number of plants and large diversity of genotypes (Fig. 2). In case of the limited statistical sample of hybrid genotypes, discrete character of the variation curve changes, and it becomes broken with several local maxima and minima.

Statistical functions «median» and «mode» are a measure of the mutual values arrangement and can be used to describe the overall quantitative indicators for each particular trait. The median shows the data set middle in the value array study. Mode is the most frequent value. None of these numbers fully characterize the degree centrality of data. In the case of discrete numeric array of studied traits, graphically expressed in the form of a fragmented multimodal curve with the data grouped in the high and low values, the median can return a value from a relatively empty middle area with non-existent values, as a function of «mode» indicates the dominant value of the real numerical data set [14].

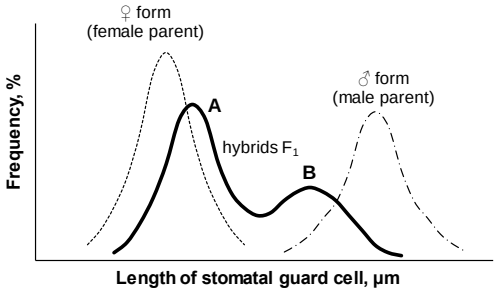


Fig. 2. Examples of the variation curves of stomata sizes in the parental forms and all the F1 hybrid genotypes at a large statistical sample. Partial deviations of this trait in the direction of the female parent (A) or the male parent (B) are shown

Skewness characterizes the asymmetry degree of the distribution relative to its average. Positive skewness indicates the deviation of the distribution in the direction of positive values – more on average; negative asymmetry – a deviation values to lower from the average [14].

Kurtosis characterizes the relative peakedness or flatness of the distribution compared with the normal distribution. Positive kurtosis indicates a peaked distribution, negative – a flattened distribution [14].

III. RESULT AND DISCUSSION

The studies have revealed statistically significant differences in the species *Pyrus communis* L. and *Malus baccata* Borkh. according to morphoanatomical traits of leaf plates, such as the linear dimensions of stomatal guard cells and the stomatal density – a quantity of stomata per unit area of epidermal tissue (Fig. 3).

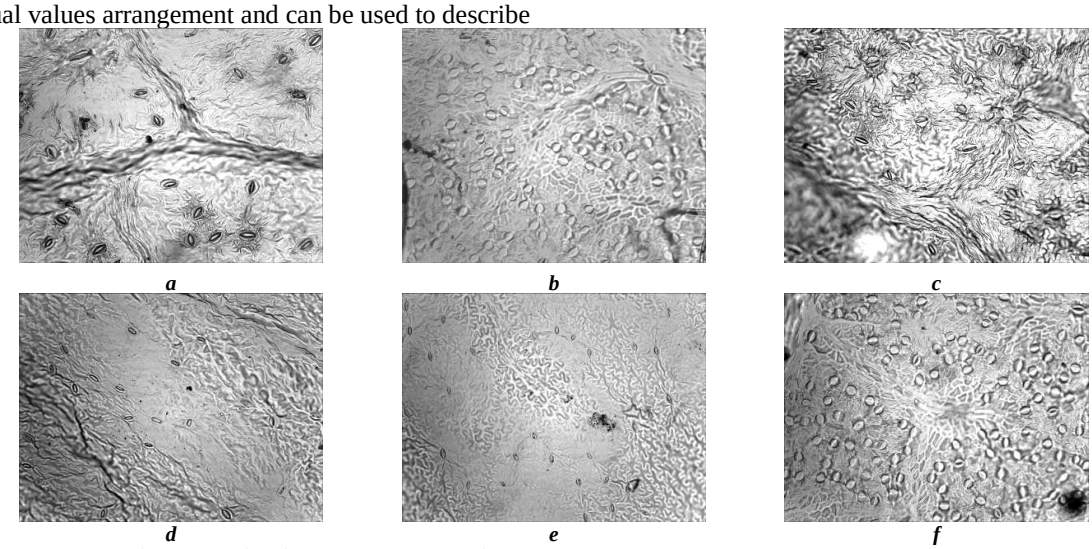
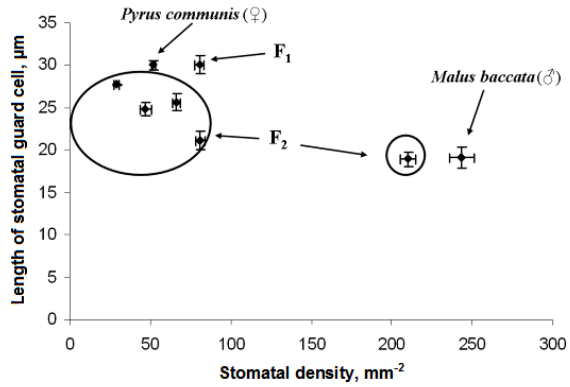


Fig. 3. Epidermal tissue of adaxial leaf surface in studied species forms and distant hybrids: a – Pyrus communis L.; b – Malus baccata Borkh.; c – F1; d – F2-5; e – F2-8; f – F2-7 leaves: 1 – ultrasonic emitter; 2 – beaker; 3 – water; 4 – leaf

The stomata of the studied genotypes are anomocytic type and locate at the same level with the surface of epidermal cells. Significant statistical differences in this morphological traits of the parental forms can be used as a reliable indicator in the selection of their new distant hybrids deviating toward a specific taxonomic direction.

Analysis of the stomatal guard cells of hybrid genotypes in comparison with parental species forms according to their length and the number of stomata per 1 square millimeter of leaf epidermal tissue was made. It should be noted that the stomatal density is the more variable trait (Fig. 4a, 5a).



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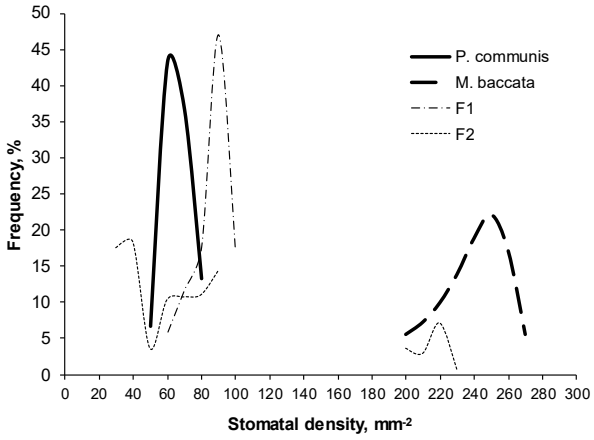

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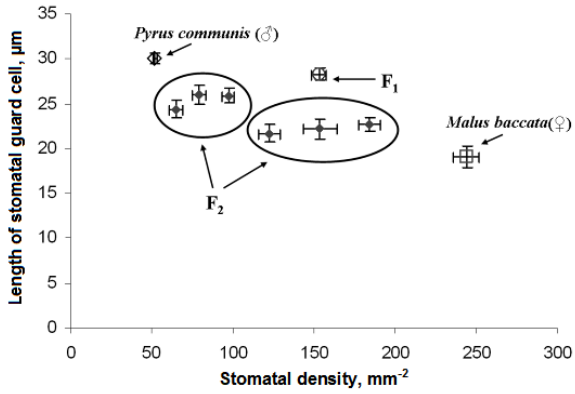
Fig. 4. Cytomorphological traits of the stomata in hybrids (pear-and-apple F_1 hybrid 01 and F_2 population from his free pollination) in comparison with the parental forms: *a* – correlation «the length of stomatal guard cell – the stomatal density»; *b* – variability of the stomatal density in leaf epidermal tissue leaves: 1 – ultrasonic emitter; 2 – beaker; 3 – water; 4 – leaf

The length of stomatal guard cells ranges significantly from 18,9±0,8 µm in the form F_2-8 (obtained by free pollination of the apple-and-pear hybrid 01) and 19,1±1,2 µm in *Malus baccata* Borkh. to 30,0±0,6 µm in *Pyrus communis* L. The stomatal density varies very considerably from 27,7±0,1 mm^{-2} in the form F_2-5 (obtained by free pollination of the apple-pear hybrid 01) to 243,8±19,1 mm^{-2} in *Malus baccata* Borkh. In both cases, the parental forms take extreme values – *Pyrus communis* L. in minimum and *Malus baccata* Borkh. in maximum, while the values of the stomatal morphological traits are intermediate in the hybrids. It is clearly fixed on the diagrams and proves the intermediate nature of traits inheritance complex in hybrid genotypes in comparison with their parental forms (Fig. 4b, 5b).



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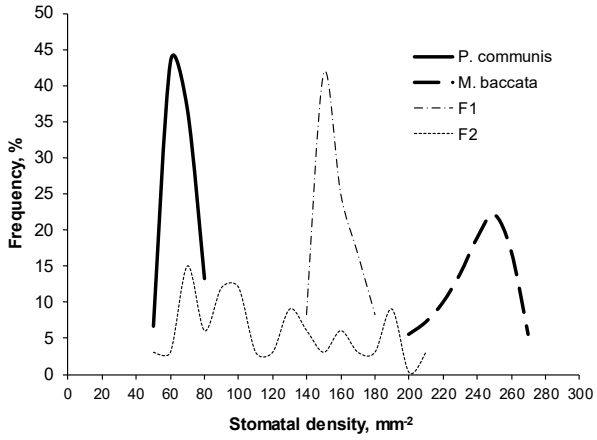

b

Fig. 5. The cytomorphological traits of the stomata in hybrids (apple-and-pear F_1 hybrid №12 and F_2 population from his free pollination) in comparison with the parental forms: *a* – correlation relationship «the length of stomatal guard cell – the stomatal density»; *b* – variability of the stomatal density in leaf epidermal tissue leaves: 1 – ultrasonic emitter; 2 – beaker; 3 – water; 4 – leaf

Variation curves of cytomorphometrical traits in the parental forms and the F_1 hybrids are unimodal, continuous, with similar median and mode values these traits (Table 1). Statistical authentic differences were found among the F_2 hybrids – both among them and according to the F_1 generation, associated with the peculiarities of their variation curves. Thus, a variation curve of this stomatal traits in the progeny (F_2), obtained by a free pollination of the apple-and-pear hybrid 01, is polymodal with intermittent area and, as a consequence, with a median in the absence values of intermediate sector. Among the statistics in F_1 hybrids, a mode and a median have similar values as the parental forms.

At the kurtosis magnitude, a peaked value distribution was observed in apple-and-pear F_1 hybrid 01 and its F_2 progeny, while a flattened distribution was observed for both hybrid generations in form №12. The deviation of values in the data array to lower of the arithmetic average, determined by a skewness magnitude, was revealed in *Malus baccata* Borkh. and F_1 hybrid 01.

The stomatal density analysis of the hybrids showed some differences between F_1 and F_2 genotypes. The coefficient of variation and the dispersion of a number of

stomata per square millimeter leaf area significantly increase in the second generation obtained by free pollination of F_1 hybrids. The coefficient of variation stomatal density in hybrid 01 exceeds in F_2 genotypes 5,9 times the similar parameter of F_1, the ratio of the dispersions F_2/F_1 is 31,9. These statistical parameters in apple-and-pear F_2 hybrid №12 respectively 3,7 and 7,8 times higher than the appropriate values of F_1. At the same time, the coefficient of variation and the dispersion of stomatal density in F_1 hybrids are intermediate values relatively of their parental forms (Table 1).

TABLE I. STATISTICAL ANALYSIS OF THE STOMATAL DENSITY IN STUDIED APPLE AND PEAR SPECIES FORMS AND THEIR HYBRIDS

Genotypes, generation		**C_V**	**σ^2**	**Median**	**Mode**	**Kurtosis**	**Skewness**
Parental forms							
Pyrus communis L.		14,1	53,8	51	52	-0,2	0,3
Malus baccata Borkh.		8,9	472,5	249	258	-0,2	-0,5
Hybrids							
01	F_1	13,0	109,3	85	88	0,8	-1,2
	F_2	**77,2**	**3484,6**	**61**	**86**	1,8	1,7
№12	F_1	10,3	246,2	146	140	-1,4	0,6
	F_2	**38,4**	**1931,0**	**100**	**86**	-0,8	0,5

In addition to determining stomatal quantitative traits for identifying a correlative relationship of received data pairs at all studied genotypes, we have proposed a synthetic parameter «the stomatal index» (SI), expressed as a relation of the average number of stomata per 1 square millimeter of leaf epidermal tissue to the average length of stomatal guard cell. Analysis of study results has shown the following values SI for all genotypes (Table 2).

TABLE II. A STOMATAL INDEX IN SPECIES FORMS PYRUS COMMUNIS L. AND MALUS BACCATA BORKH. AND THEIR F1 HYBRIDS

Genotypes, generation			**Stomatal index (SI)**		
			for genotype	***generally mean for each generation***	
Parental forms	*Pyrus communis* L.		1,7	7,3±5,6	
	Malus baccata Borkh.		12,8		
Hybrids	01	F_1	2,6	2,9±0,3	4,0±0,7
		F_2	3,2		
	№12	F_1	5,4	5,1±0,3	
		F_2	4,8		

Due to the extreme values of stomatal guard cell length and stomatal density in the parental forms, as a consequence, the stomatal index accepts polar values among all studied genotypes – at minimum in *Pyrus communis* L. and maximum in *Malus baccata* Borkh. The stomatal index was the same at each of two hybrid generations with small difference only standard error of the average: 4,0±1,4 for all F_1 generation (progeny from hybrids 01 and №12); 4,0±0,8 in the whole F_2 generation and 4,0±0,7 generally for all F_1 and F_2 hybrids.

However, the average value SI in all hybrid progeny (F_1+F_2) differed 1,8 times – from 2,9±0,3 by the hybrid 01 to 5,1±0,3 in №12. Thus, hybrids 01 and №12 are differentiated into two groups according to SI values, correlating with their phenotypic characteristics. Similarly, there is a trend of partial deviation of synthetic indicator SI towards the female parent – pear-and-apple hybrid 01 in the direction of *Pyrus communis* L., while the apple-and-pear hybrid №12 was similar to *Malus baccata* Borkh. This trend has been maintained in hybrids F_2 (obtained by spontaneous outcrosses), despite much more extended variational series of data and the increased coefficient variation and dispersion of the stomatal morphoanatomical parameters.

Thus, the combination of cytological research by microscopy technique and a method of statistical analysis is effective for study of the cytomorphological traits of leaf epidermal tissue in F_1 and F_2 hybrids.

IV. CONCLUSIONS

1. The density of stomatal distribution in leaf epidermal tissue has the highest variability among the stomatal morphometric traits of apple and pear species forms and hybrids. This feature can be used as a diagnostic indicator for determining the existence and degree of genes inheritance in the hybrid generation parental forms.

2. The F_2 hybrids from free pollination have the largest values of stomatal morphological characteristics such as the variational data series, the coefficient of variation and the variance.

3. The tendency of partial deviation in stomatal quantitative traits and stomatal index in the studied F_2 hybrids between apple and pear in the direction of a female parent was revealed.

4. A mode, kurtosis and skewness of numerical data series are the most appropriate to use for a comprehensive statistical analysis of variation curves at the study of the quantitative traits of stomatal guard cells.

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