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Recessive Inheritance of the Length of Growing Period in Some Oriental Tobaccos by Monohybrid and Backcross Methods

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Abstract: *Inheritance of length of growing period (days from transplanting to the beginning of flowering) was investigated by crossing Basma tobacco variety with shorter growing period and VelikiHercegovac variety with longer growing period. It could be stated that all plants in F_1 generation began their flowering in the same time with parental variety with shorter growing period. From the self-pollinated hybrid individuals of F_1 generation, 3/4 of plants in F_2 generation had short growing period (under the influence of dominant allele) and 1/4 of plants had long growing period under the influence of recessive allele. Split ratio was 3:1 and frequencies distribution was assessed by X^2 (chi-square) test.*

Knowing that breeding work often imposes the use of characters inherited in a monohybrid recessive mode, we made efforts to use this type of inheritance in creation of new, more productive tobacco genotypes with longer growing period compared to the early maturing variety, preserving in the same time its quality features. Transmission of this recessive trait by the backcross method will be also presented.

Keywords: *oriental tobacco, growing period, recessive inheritance, monohybrid – F_1, F_2 and F_3 generation, backcross BC_1, BC_2 and BC_3*

1. INTRODUCTION

In hybridization process, genetically stable parental components, according to Mendel, always have allelomorphic, i.e. allelic characters, which are homozygous and have AA or aa alleles (Borojevic S., Borojevic K., 1976; Stojkovski C., Ivanoska S., 2002, etc). These organisms produce one type of gametes. By merging the gametes with different alleles from both parents, the first hybrid generation (F_1) is obtained, the progeny of which is heterozygous (Aa). Hybrid units of this progeny are equal with regard to the character investigated, in accordance with the First Mendel's Law, i.e. the principle of uniformity of F_1individuals. F_1 hybrid self-pollinate to create F_2 generation, in which segregation of characters in certain phenotypic and genotypic ratio is made, in accordance to the Second Mendel's Law on segregation in F_2 generation(Genchev1980, Marinkovic1982,Gershenson1983, Ayala F. J., Kiger J.A.1984, Dimitrieski, Miceska 2014 etc.). In modern selection, hybridization is the most appropriate method for creation of the necessary diversity of initial breeding material from which new varieties of plants can be created and stabilized.

In selection work and hybridization, sometimes it is necessary to preserve some properties that have a recessive trait. The goal of our investigations was to show practically the model of monohybrid recessive inheritance and stabilization of the trait length of the growing period, in hybridization of the oriental varieties Basma (with short growing period) and VelikiHerzegovac (with long growing period), as well as transmission of this trait via the backcross method.

2. MATERIAL AND METHODS

The trials were set up on the Experimental field of Tobacco Institute Prilep in 2008. Basma tobacco (with short growing period) and Veliki Herzegovac (with long growing period) were used as starting material for hybridization. In Basma variety, the length of growing period from planting to flowering was about 55 days and from planting to the end of maturation of top leaves 95-110 days. In Veliki Herzegovac, it was about 120 days from planting to flowering and over 180 days

from planting to the end of maturation of top leaves (Uzunoski, 1985). The selection of parental varieties was made in accordance with the results of previous research. The crossing process was performed in 2008, with varieties Basma as a mother and Veliki Herzegovacas a father, by the method of interspecific hybridization and according to the Mendel's laws of heredity, using the pattern of monohybrid recessive inheritance of the observed trait (Scheme 1). F_1progeny was obtained in 2009, F_1 and F_2in 2010 and F_3in 2011. The F_2 generation was monitored for cleavage (segregation) of the trait and distribution of frequencies was determined by the chi-square ($\chi^{2)}$ test (Ayala F.J., Kiger J.A., 1984).Statistical analysis of cleavage in F_2 generation was obtained from the ratio between the actual (empirical) and theoretical values, according to which the probability P is estimated in case when deviation is regular, using the Fisher's table. In agricultural science, the *p-value* is arbitrarily taken as equal to 0.05.

Transmission of the recessive trait long growing periodin Basma variety (with short growing period) was performed by backcrossing of the hybrid progenies of F_1, BC_1, BC_2 and BC_3 generations with the dominant parent, applying a suitable scheme for transmission of the observed trait (Scheme 2). Hybrid progenies of F_1, F_2 and F_3 generations, as well as the progenies of backcross generations BC_1, BC_2 and BC_3 were grown on suitable area and with sufficient number of individuals, in accordance with requirements of the breeding program.

3. RESULTS AND DISCUSSION

3.1. Monohybrid Recessive in heritance of the Trait Length of the Growing Period

The process of hybridization started by crossing the oriental shorter-growing variety Basma (AA) as maternal component and semi oriental longer-growing variety VelikiHerzegovac (aa) as paternal component. In hybrid progeny of F_1 generation heterozygous, phenotypic ally uniform individuals (Aa) with respect to the investigated character were obtained. All hybrid individuals had a short growing period, i.e. they flowered almost simultaneously with the parent with shorter growing period, which indicates that this alternative character (allele) is dominant, and the longer growing period is recessive. Gornik (1973) reported that the period required for flowering sometimes appears as a dominant and sometimes as recessive character, depending on the varieties taken for hybridization. Such mode of inheritance of the length of growing period was also stated by other authors. Thus, Chinchev (1979), using the top-cross method in his analysis of F_1 hybrids, reported the lowest GCA value for the character days to flowering in oriental varieties Krumovgrad 988 and Plovdiv 7, as well as in Virginia varieties NC 2326 and Mc Nair 20, i.e. they had a shorter growing period, while longer growing period was observed in Virginia varieties 1349 and Coker 254. Stankev (1987) reported the highest GCA value in varieties Krumovgrad 90, Rila 544 and line 202-1a, i.e. those varieties showed the best GCA, i.e. longer growing period, regardless of the change of varieties that served as a tester. He also suggested that varieties Sandanski 321, N^o 888, Rila 9, Plovdiv 7 and Struma 75 can be used in cases where shorter growing period is required. Bogdanceski (1984), in his three-year in vestigations of various hybrid combinations, reported differences in the inheritance of the character length of the growing period until 50% of flowering. Thus, Prilep x Nevrokop 261 and Prilep x Pazardzik 17 and their reciprocal crossings showed dominant inheritance of this character, i.e. all plants from F_1 flowered simultaneously with the variety Prilep (with shorter growing period), and other hybrid combinations showed intermediate inheritance.

Table1. *Ratio between the plants with short and long growing period in hybrid combination Basma x VelikiHerzegovac.*

Variants	Number of plants in F^2 generation		χ^2	**P**
	with short growing period	with long growing period		
Investigated individuals experimental figures**(e)**	219	87		
Expected individuals theoretical figures**(t)**	229,5	76,5	$\chi^2=\Sigma\mathbf{d^2/t}$	
Deviation (**d**)	10,5	10,5		0,05=3,84
$\mathbf{d^2}$	110,25	110,25	$\chi^2=1,92$	
$\mathbf{d^2/t}$	0,4803	1,4411		

From self-pollinated hybrid individuals of F_1 generation, phenol typically different plants were derived in a ratio 3: 1, i.e. 3 plants (75%) are with shorter growing period and 1 plant (25%) is with

longer growing period. The results of statistical analysis (Table 1) of plants with short / long growing period ratio in F_2 progeny showed that the oretically expected segregation (3: 1) was obtained. Since in our case the calculated value ofχ^2 =1.92 does not exceed the Table value for P = 0, 05 (3, 84), it means that actually obtained segregation is in compliance with theoretically expected3: 1ratio.

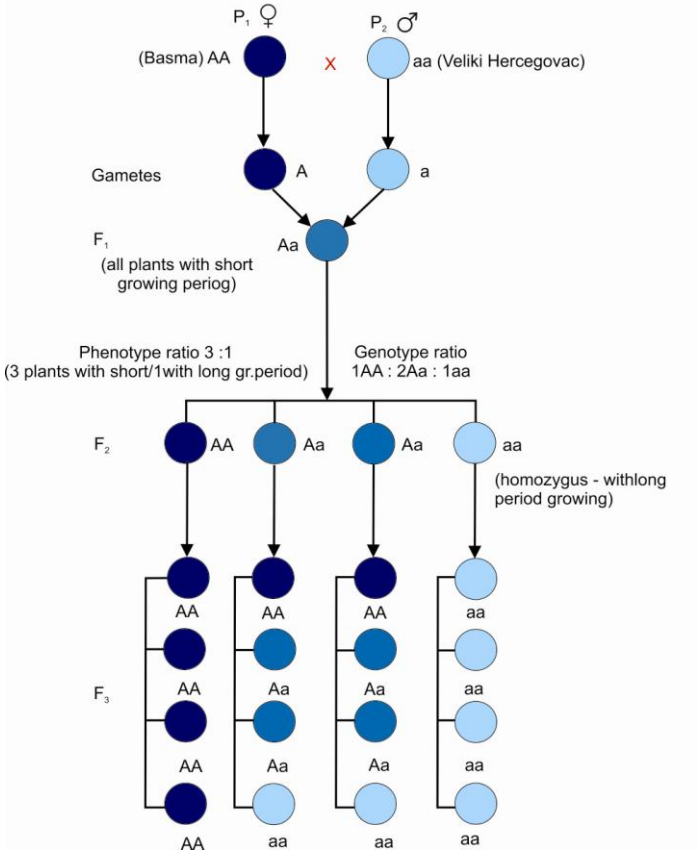
Accordingly, it may be concluded that division (segregation) of the investigated character was observed in F_2 generation, where the theoretical ratio of the genotypes (presented on Scheme 1) was: 1AA: 2Aa: 1aa, i.e. 75% of the plants in this hybrid progeny had short growing period (Aa and AA) and 25% were homozygous, with long growing period (aa). Most of the plants (75%) of Aa and AA genotype with short growing period were not grouped by their time of flowering, because the subject of our investigations was the recessive character long growing period (aa).

The plants with long growing period obtained in F_2 (Figure1) progeny undoubtedly indicate that even in this generation we derived homozygous plants in relation to this recessive character. In order to confirm with certainty the resulting genotype with long growing period in the hybrid population, 6 of the plants were selected and isolated (for self-pollination) in accordance with the intended aim of investigations. These plants were used to create specific progenies in F_3(Figure 2) generation and after inspection it was determined that all hybrid individuals of the investigated progenies had a long growing period, which indicates that they are homozygous with respect to this character. Hence, it can be concluded that this character was permanently incorporated into their genome. Our next goal in selection will be to consolidate these progenies with other desired morphological, productional and qualitative characters

Fig1. *(Plot 169)*

Fig2. *(Plot 170)*

Scheme 1- Monohybrid recessive inheritance of the character long growing period in F_1, F_2 and F_3 generation



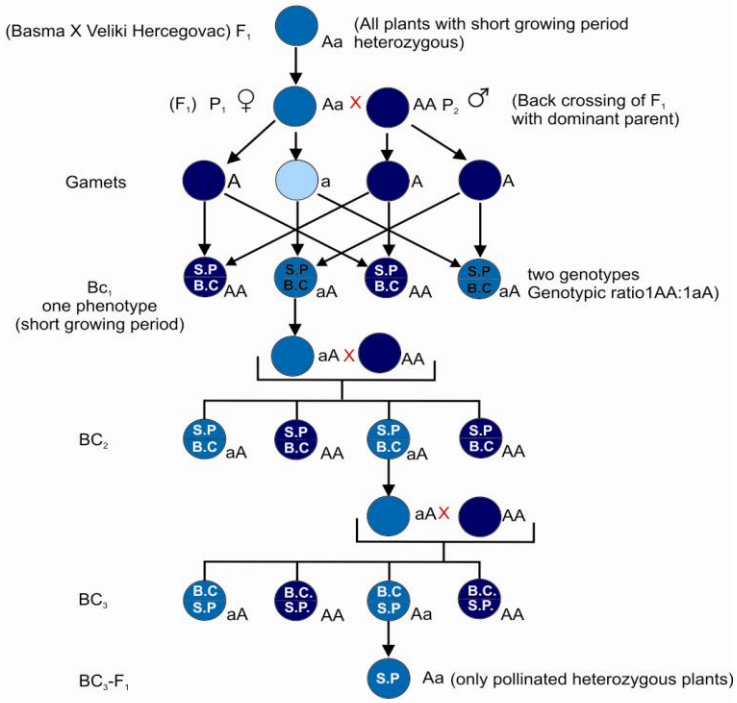
3.2. Inheritance of the Length of Growing Period of Tobacco by Backcross Method

As stated before, the progeny of the hybrid Basma x VelikiHerzegovac in F_1 generation gives heterozygous (Aa) phenotypic ally uniform individuals with short growing period which bloomed almost simultaneously with the variety Basma. From each F_1 progeny (Aa), three well developed uniform plants were selected and then backcrossed with a pollen from the dominant parent - recipient variety (AA). In the progeny of the first backcross generation BC_1 (second year) only one phenotype was obtained (with short growing period), and two genotypes (AA and aA) in a ratio of 1:1 (Scheme 2). Since the recessive trait is not manifested in backcross with dominant parent, for identification and monitoring of heterozygous individuals (Aa) of the BC_1 generation 20 plants were selected randomly, of which 10 flowers were pollinated with the parent with shorter growing period (AA). The other flowers of the cluster in each plant were left to self-pollinate in special isolator. Seeds of the self-pollinated flowers and of the flowers pollinated with dominant parent (AA) from each plant were kept in separate bags and labeled with plant's ordinal number. In BC_2 generation (third year) we monitored the trait long growing period of the progenies obtained from the self-pollinated flowers of individual plants. Identification of the long growing period in these progenies suggests that the plant from which the seed was taken is heterozygous (Aa). This convinced us that the progenies obtained from pollinated flowers (Aa x AA) will also give heterozygous plants (Aa) which contain the trait long growing period in their genome. Therefore, only from this hybrid combination, 20 plants were selected randomly once again and they were backcrossed (10 flowers) with the dominant parent. In the fourth year, the selection procedure was performed in the same manner as in the third year, first determining the heterozygous progenies (Aa) obtained from self-pollinated flowers of hybrid individuals in which cleavage with plants with long growing period was present. In the same manner, the progenies of the pre-determined heterozygous hybrid individuals received from the pollinated flowers backcrossed with the dominant parent (AA) were in BC_3 generation and they were considered as F_1 generation. For these progenies appropriate selection procedure was applied, following the model of monohybrid recessive inheritance according to Scheme 1. From the most typical progenies (BC_3 generation - F_1), which were phenotypically uniform and most similar to the recipient variety (Basma), seed was taken from 25-30 self-pollinated progenies in the current year. The next (fifth) year, 25-30 progenies in BC_3 generation - F_2 were grown from the seed of these plants. They were

used for selection of progenies typical for the recipient variety, which consisted only of plants with long growing period (aa), showing absolute homozygosity with respect to the studied trait.

According to Gornik (1973), the receiver variety can be renewed with less than 6 backcross generations, which depends not only on the plants selected for backcrossing but also on the parents used in investigation. It should be also mentioned that the resistance to diseases to some varieties is more easily transmitted than to others. The same author (quote from Lukas, 1965), reported that only two back crossings will be enough for transmission of the resistance to blue mold, by further use of the method of individual selection, with obligatory testing of the progenies obtained from the selected resistant plants.

Scheme 2- Inheritance of the length of growing period of tobacco by the backcross method



Appropriate breeding procedure was applied on BC_3-F_1 и BC_3-F_2 generation, analogous to F_1 and F_2 generation as in monohybrid recessive inheritance according to Scheme 1

S.P.-self pollinated flowers
B.C.- backcrossed flowers on a same hybrid individual

4. CONCLUSION

Research was made on the recessive inheritance of the trait length of the growing period by the methods of monohybrid and backcross hybridization, performed with varieties Basma (with short growing period) and VelikiHerzegovac (with long growing period). The obtained results lead to the following conclusions:

- The trait long growing period has a monohybrid recessive mode of inheritance.
- The recessive nature of the investigated trait makes the selection process more complicated because it doesn't appear in F_1 generation. Therefore, it is necessary to transplant higher number of plants (over 150) in F_2 generation, to achieve higher probability for determination of plants that are carriers of this trait.
- Monohybrid recessive inheritance of the trait from the parent with long growing period will allow plant breeders to obtain homozygous plants (aa) even in F_2 generation, and the trait can be easily controlled because flowering is a visible biological characteristic.
- The applied Scheme 1 is simple and, along with monitoring the growing period, it allows parallel selection of hybrid individuals with respect to other quantitative and qualitative traits.

- The backcross method, accompanied by appropriate breeding procedure, allows completely controlled and successful transmission of this recessive trait in oriental tobacco varieties.
- Scheme 2 applied in our research is a very practical and effective model for transmission of the recessive trait long growing period and it can be used for transmission of this trait in other varieties without negative impact on other quality traits. It can be also used for transmission of some other traits inherited in a monohybrid recessive mode.

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- Physiology of tobacco growth and development and Tobacco culture,
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- Methodology of research work,
- Optional courses

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- Author and co-author of 88 scientific papers in the field of genetics, breeding and physiology of tobacco, co-author of the Practicum of Genetics, breeding and seed control, and of the Monograph on the 35-th jubilee of the foundation of Society of Sciences and Arts – Prilep
- Participation in a number of national and international scientific-research and development projects.
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