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## **DIRECTIONS AND GOALS IN THE SELECTION OF LARGE-LEAF TOBACCOS IN THE SCIENTIFIC INSTITUTE OF TOBACCO PRILEP - REPUBLIC OF MACEDONIA**

### **SUMMARY**

Large-leaf tobacco varieties, mostly of the types Virginia and Burley, account for over 80% in the composition of blend cigarettes. These varieties should meet the requirements of primary producers, processors, manufacturers and, finally, the consumers - smokers.

In practice, however, some variety is better in one trait and other variety in another. For this reason, there is a need to create new varieties, with a capacity to meet new requirements on permanent basis. That was the challenge to the breeders of Tobacco Institute - Prilep. Working hard in this field, they achieved significant results. From the available genetic base (starting materials), by interspecific hybridization and properly defined objectives, they obtained varieties and lines which in many traits surpassed their predecessors. For example, the yield of Burley tobacco has increased from 3000 kg/ha in variety B-96/85 CMS F1 to 4350 kg/ha in variety Pelagonec CMS F1, without negative impact on the quality of raw material.

**Keywords:** selection, tobacco, varieties, Virginia, Burley, yield, quality, resistance, tasting.

### **INTRODUCTION**

Large-leaf tobaccos, mostly of the types Virginia and Burley, account for over 80% in the composition of blend cigarettes. These two types are leading in the world's total production of tobacco. Regionalization of types and varieties is important condition for obtaining stable yields and good quality of the raw material, but the factor which has decisive impact on the most important parameters in tobacco and other agricultural crops is variety.

Therefore, the first requirement for improving the traits is creation of tobacco varieties which have the desired properties in their hereditary base. The genetic structure of tobacco varieties controls almost all properties, and the assignment of the breeder is to obtain new varieties with desirable traits. Besides on genetic constitution, all properties depend on external factors (climate, soil, applied agro-techniques, etc.).

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The basic requirement for obtaining a new variety is to have a collection of tobaccos (starting material) that will contain the traits which we want to transmit to the new variety. Previous results in the selection of these types of tobacco in our country and in the world, however impressive in terms of certain properties (yield, quality, resistance, etc.), are still not satisfactory.

Producers, processors and consumers continuously put new requirements before the selectioners, mostly related to the increase of yield and quality, resistance to deceases and reduction of harmful substances (tars). In many of the new creations only some of these requirements are met, because it is practically impossible to simultaneously incorporate all desired traits. For example, main nerve and veins in larger leaves are expressed more intensely, which prolongs the period of curing and impairs the physical properties of tobacco.

Objectives of the Scientific Tobacco Institute - Prilep in selection of these two types in the future will be directed towards obtaining varieties that will be closer to the ideal.

### **MATERIAL AND METHODS**

The collection that served as a material for creation of new varieties is composed of wild species, local varieties and commercial varieties and breeding lines. The latter two have been mostly used in the process of hybridization in Tobacco Institute-Prilep.

Several breeding methods can be applied in creation of new tobacco varieties (genotypes), such as: mass and individual selection from natural populations, selection from hybrid populations and hybridization, which can be of intervarietal and interspecific type.

The most common method used in TI-Prilep is intervarietal hybridization, with previous selection of parental pairs based on the knowledge of the characteristics and goals that should be achieved. Depending on the choice of parents and their role in hybridization (mother or father), the obtained progeny can be in fertile or male sterile form.

### **RESULTS AND DISCUSSION**

Until late 80s of the last century, the work of the Scientific Tobacco Institute-Prilep was focused on selection of oriental tobaccos. From that time on, the breeders of this Institute have paid much more attention to the large-leaf tobaccos Virginia and Burley and to creation of new varieties of these two types. As a result of their activities, the first Virginian variety MV-1 was recognized in 1987 by Federal Variety Commission of former Yugoslavia. Due mainly to its high quality, this variety partially or completely pressed out the previous Virginia varieties produced in Macedonia, Serbia, Montenegro, Bosnia, Albania etc. The need to preserve tobacco genetic diversity as a part of biodiversity in the Republic of Macedonia has been recognized by the Scientific Tobacco Institute-Prilep (Srbinoska et al., 2010). In the period that followed, the Institute has established or intensified the cooperation with other institutes in the world, from

United States, Zimbabwe, India, France, Italy, Australia, Croatia, Bulgaria, and a rich collection of seeds of Virginia and Burley varieties was created on the exchange basis.

From this collection, a good quality starting material composed of fertile and male sterile varieties and lines was created gradually, which served as a solid base in selection of Virginia and Burley tobaccos.

Literature data (Hawks, 1978; Gornik, 1973) suggest that F1 hybrids in male sterile form have some advantages in terms of their resistance to diseases, early maturation, yields, etc. These findings were confirmed by the researchers from Tobacco Institute –Prilep, whose selection work was focused on obtaining male sterile hybrid varieties of these two types. They do not form seeds, which definitely solves the problem of varietal purity. Male sterility is inherited from one parent – mother, and through hybridization it must be preserved at least until F7 generation. According to Cikov & Cikova (1981) the male sterility in the plants was first noticed in 1687 among the forms of genus *Huacynthus* and *Raninculus*.

Presently, disease resistance in many crops, including tobacco, is improved by genetic engineering. According to Zlatanova (1986), this is achieved by incorporating fragments of foreign DNA. However, this way of acquisition of resistance is categorically rejected in Tobacco Institute – Prilep. According to Djulgovski (2009) process of tobacco selection should move towards the newly created varieties which have a wide adaptive ability and are able to grow in a wide range.

Differences between Virginia and Burley tobaccos require different directions and goals in the way of obtaining new varieties. The final appearance of tobacco and quality of the raw material are affected by genetic constitution (hereditary component), the activities of external factors and applied agrotechniques. In implementation of certain traits, specific variations occur as a result of the influence of external factors on the genotype.

One of the aims in selection of these two types of tobacco is to reduce such variations as much as possible. In general, directions and objectives in selection of Virginia and Burley tobaccos are as follows:

**Stalk** - strong, around 180-200 cm high, with 32 to 36 leaves, which in our agro-ecological conditions allows the plant to be fully matured before harvest and properly dried thereafter. According to Srbinoska et al. (2012), the dried stalks of Burley tobacco type (B 98/N variety) were characterized with 179 cm length, diameter of 8.5 cm and 210 g weigh.

**Middle belt leaves** - not less than 55-65 cm long and 32-35 cm wide. Kalamanda (2009) points out that in Posavina in 2006 the middle belt leaves from the variety DH-17 had reached a width of 25.4 cm and the variety Hewessi - 17 indicated 23.0 cm. Uzunovski (1985) suggested that the leaves of Virginia and Burley tobacco should not be less than 40 cm. It is very important that several leaves on the stalk should mature simultaneously before harvest. This will result

in the same leaf maturity during harvest and uniform color and quality later, after curing.

**Length of the growing period** in Burley tobacco, which is harvested by the end of August, should not exceed 115-120 days. For Virginia tobacco this period can be somewhat longer, because the way of curing (flue curing) allows longer persistence of stalks in the field.

**Resistance to diseases and pests** of newly created Virginia and Burley tobacco varieties is of major importance. Diseases, particularly viral ones (TMV, PVY etc.), can negatively affect the yield and quality, but there are varieties with high resistance that can be transferred to the new variety. These varieties, however, often yield poor quality raw material which is partly or entirely transferred to their progenies. Delač et al (1994) concluded that diseased leaves from PVY have different chemical composition. The level of soluble sugars and the nicotine and nitrogen materials has reduced, but the ashes and the proteins have increased. Thus, the aim of selectioners is to create resistant varieties that will provide high quality raw material. Such are our variety B-2/93 CMS F1 (Burley), line V-53 CMS F1 (Virginia) and others, obtained from parental pairs which also showed resistance to most of the diseases.

**Yield** is a very important segment in production of Virginia and Burley tobacco which directly affects the productional costs and net profit of the farmers. According to Dražić (1986), tobacco yield as a quantitative character depends on genotype and on interaction between genotype and external conditions. Jovović (1957) states that according to the results of tests performed on the experimental field of Bar (Montenegro) in the fifties of the last century, the yield per hectare in of the type Burley varied on average of 1276 kg and the type Virginia with 1707 kg. Also Budin (1988) reports that in the period from 1980 to 1985 in Zimbabwe the type Burley had average yields per hectare which ranged from 1202 to 1760 year.

Our experience in improving the genetic potential for yield has shown that it can be achieved only through gradual increase. Risteski & Kočoska (2012) presented the upward yield movement of Virginia and Burley tobacco varieties created in TI - Prilep. The average yield ranges from 3000 kg/ha in the first recognized Macedonian Burley variety B-96/85 CMS F1 to 4350 kg/ha in the last recognized variety Pelagonec CMS F1. The authors give similar example for yield in the newly created varieties and lines of Virginia tobacco, ranging from 2500 kg/ha in the first recognized Macedonian Virginia MV-1 to 3590 kg/ha in the last created line V-88/09 CMS F1. Kočoska et al. (2004) report that the American variety SP.G-58 produced in the Prilep region achieved a yield of 2458 kg/ha.

The creation of higher-yielding varieties remains the main goal of Scientific Tobacco Institute – Prilep, up to the moment when the increase of yield will have severe adverse effects on tobacco quality.

**Quality** of raw material obtained from these two tobacco types, as well as the yield, has a strong impact on the ultimate economic effects. Due to the

different ways of growing, harvesting and drying, there are substantial differences in the elements for assessment of quality of Virginia and Burley and, consequently, different criteria for their classification. To achieve the maximum leaf quality in some variety, there is a set of measures that should be properly applied (fertilization in due time and with adequate formulation, irrigation, harvest, yellowing, adequate curing method, etc.). Thus, the variety is not the only carrier of this trait. Yet, only parental pairs with desired qualitative properties have been selected during hybridization (leaves larger than 40 cm, with characteristic chemical composition, uniform and fast decomposition of chlorophyll, etc.).

In organoleptic assessment of leaves, most attention is paid to color. Light brown to brown, without presence of other shades, is considered to be the best **color** of high quality Burley tobacco. Such material was observed in our varieties B-2/93 CMS F1 and Pelagonec CMS F1 (middle belt) and it was inherited from their parents. The color of the high quality Virginia tobacco is orange, which is typical characteristic of the raw material originating from the US, Brazil, Zimbabwe etc. Unfortunately, in our agro-ecological conditions such color could not be obtained even in varieties introduced from the above countries. The Virginia varieties and lines created in Tobacco Institute-Prilep are golden yellow (V-53 CMS F1, V-78/07 CMS F1, V-82/02 CMS F1, V-63/04 CMS F1, V- 88/09 CMS F1) and lemonish yellow (MV-1). According to the Measures for quality assessment, the varieties with these colors also belong to Class I.

Only the Virginia and Burley varieties where the share of upper classes is 75-80% are interesting and profitable to the producers and breeding of such varieties remains our priority. According to Ostojić (1958) the quality of raw material in bright Virginia which was produced in the fifties in Montenegro had a far worse quality than the others. In this way, the first class was represented with 5% the second with 10 % and the third class with 30% or the total of these three classes was 45 %.

**Physical and chemical characteristics** of Virginia and Burley are directly dependent on the mode of production, maturity of leaves during harvesting, yellowing and curing. The midrib content in leaf is essential for usability value of tobacco in fabrication. From the available literature data it can be seen that the midrib percentage in Burley tobacco leaf ranges from 20% to over 35%. Despite the aim to reduce the midrib percentage to a minimum, in B-2/93 CMS F1 and Pelagonec CMS F1 it ranges between 29% and 31%, which can be considered as moderate value.

The share of midrib content in Virginia tobacco is slightly smaller compared to Burley and ranges about 24-25% (Uzunoski 1985). Jankuloska (1991) reports that the average percentage of midrib in Macedonian variety MV-1 is 24.81%. This indicates that, with respect to this trait, the variety MV-1 is in compliance with the international standards.

**Thickness and substantiality** of Virginia and Burley tobacco leaves achieve high values. In Burley B-2/93 CMS F1 leaf thickness is about 63  $\mu\text{m}$  and substantiality 43  $\text{g}/\text{m}^2$ , while in Virginia MV-1 leaf thickness is 81.85  $\mu\text{m}$  and substantiality 56.95  $\text{g}/\text{m}^2$  (Jankuloska, 1991). In future, our goal will be to create varieties with moderate response to environmental conditions in order to minimize their decisive role on these two traits.

In genetic constitution of tobacco there are carriers for certain **chemical components**. To what extent they will be formed and incorporated in the leaf depends on the environmental conditions. The standards that determine whether the share of particular chemical component is high or low are different for each type of tobacco. According to Beljo (1996), the **nicotine** content ranges from 0.5% to over 4% in the type Burley and from 0.3% to over 3.7% in Virginia. Popović (1955) mentions that in the American tobacco type Virginia variety Joyner grown in Titograd-DOS in 1952 the content of nicotine amounted 3.73%.

**The proteins content** in Burley tobacco ranges from 4% to 12% and in Virginia from 3% to 8%. Risteski (2008) found that the protein content in Burley tobacco depends on the method of harvesting and drying. The results of his study show that tobacco primed and dried as whole plant harvest contains less protein than variant primed and stringed.

**The total nitrogen** in Burley tobacco is in the range from 1.5% to 4.7%, while in Virginia this indicator varies from 1% to 4%.

**The ashes content** in Burley ranges from 12% to 27% and in Virginia from 7% to 21%. According to Ražnatović (1959) the tests performed in 1956 in region of Bijelo Polje (Montenegro) with the tobacco type Virginia variety Joyner had shown that in this variety the ash amounted with 10.97 %. Beside these, there are many other components in tobacco composition that highly depend on external conditions. All these components and their ratios have a strong impact on tasting characteristics – the ultimate indicator of tobacco quality. Therefore, in selection of these two tobacco types we use only the parental pairs which are characteristic for their good chemical properties.

**Smoking characteristics** are distinguishing feature of tobacco type, expressed through the following traits: taste, flavor, strength, irritation, combustibility and ash compactness. In formation of these traits, the impact of the variety is much less pronounced than that of fertilization, irrigation, maturation, curing, etc. but still, only parental pairs with good smoking characteristics, typical for the type, are used in hybridization. According Nuneski & Nuneski (2009) the tobacco will have a pleasant taste, if certain chemical components in it are represented in harmonious proportion.

Due to the good directions in selection and breeding, Tobacco Institute - Prilep has created a number of Virginia and Burley varieties which, in terms of their chemical and tasting characteristics, provide a high quality raw material interesting for the producers.

## CONCLUSIONS

- A rich collection of Virginia and Burley varieties has been created in Scientific Tobacco Institute – Prilep.
- Breeding lines and commercial varieties are used as a starting material from which parental pairs are selected.
- Due to the advantages of male-sterility, our goals in selection of these two types of tobacco will be to continue with creation of new varieties in CMS form.
- By the method of intervarietal hybridization, a number of Virginia and Burley varieties, with characteristics that are specific for these tobaccos, have been created in Tobacco Institute – Prilep.
- The desired morphological traits of the type can be inherited in future progenies only by good selection of parental pairs through various measurements and tests.
- The yield increase in newly created varieties moves gradually, up to the limits that will not impair the quality. The increase in Burley tobacco varies in the range between 3000 kg/ha in B-96/85 CMS F1 and 4350 kg / ha in Pelagonec CMS F1.
- Resistance in newly created varieties is transmitted from parents through intervarietal hybridization. Resistance achieved by incorporation of foreign DNA fragments has been categorically rejected in Tobacco Institute – Prilep. The highest resistance to PTA disease was recorded in varieties V-53 CMS F1 and B-2/93 CMS F1.
- Quality of the obtained tobacco raw materials, as well as chemical and tasting characteristics in the new varieties is specific for the type to which they belong, due to the strictly controlled hybridization and clearly set directions and goals.
- There is evident improvement in cultivation of the types Virginia and Burley in Tobacco Institute – Prilep, which is confirmed by the fact that each newly created line or variety is characterized by better characteristics than the previous one.

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**PRAVCI I CILJEVI U SELEKCIJI KRUPNOLISNIH  
DUVANA U NAUČNOM INSTITUTU ZA DUVAN U  
PRILEPU - REPUBLIKA MAKEDONIJA**

**SAŽETAK**

Krupnolisne duvanske sorte, uglavnom tipova Virdžinija i Berlej, čine preko 80 % mješavina duvana za cigarete. Ove sorte treba da zadovolje zahtjeve proizvođača, prerađivača i konačno potrošača-pušača.

U praksi, međutim, neka sorta je trenutno bolja u jednoj osobini, a druge sorte karakterišu drugačija svojstva. Iz tog razloga, postoji potreba da za stvaranjem novih sorti, koje mogu zadovoljiti nove zahteve na trajnoj osnovi. To je bio izazov selekcionera duvana iz Instituta za duvan u Prilepu. Iz raspoložive genetičke osnove (polaznih materijala), međusobnom hibridizacijom i pravilnim definisanjem ciljeva, dobijene su sorte i linije koje u mnogim osobinama nadmašuju svoje prethodnike. Na primjer, prinos duvana tipa burley povećao se sa 3000 kg / ha u sorte B-96/85 CMS F1 do 4350 kg / ha, sorte Pelagonec CMS F1, bez negativnog uticaja na kvalitetu sirovine.

**Ključne riječi:** selekcija, duvan, sorte, Virdžinija, Berlej, prinos, kvalitet, otpornost, degustacija