

## INVESTIGATION ON THE NUMBER AND SIZE OF THE LEAVES IN SOME OLD DOMESTIC AND NEW TOBACCO VARIETIES IN THE REPUBLIC OF MACEDONIA

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### Abstract

Investigations were carried out with five old domestic oriental tobaccos of the types: Prilep (P 10–3/2 and P 12–2/1), Djebel (Dj №1) and Yaka (YK 7–4/2 and KY) and five commercial oriental varieties of Prilep tobacco (P–23, P–84, NS–72, P–66–9/7 and P–79–94), to study the number, length, width and area of the middle belt leaves per stalk. The trial was set up in the Experimental field of Tobacco Institute–Prilep in 2013 and 2014, in randomized block design with three replications, using traditional agricultural practices. The aim of the investigation is to study some autochthonous varieties and new commercial varieties for the stated quantitative traits, and with analysis of variance to estimate the significance of differences by varieties and years, which will improve our knowledge on stability of the traits, genotypical homogeneity and progress in selection of oriental tobacco in the Republic of Macedonia. Differences in leaf the number and size among genotypes in the two-year investigations are highly significant, which is genetic indicator of their mutual differences. The error of the mean value is low, indicating stability and homozygosity of the genotypes. The variety P–66–9/7 is characterized with the highest number of leaves ( $\bar{x} = 60$ ) and it has 33 leaves more than YK 7–4/2 and Dj №1. The largest leaf size was measured in P–79–94 ( $\bar{x} = 23,3\text{cm}$  – length, 12,1 cm – width, 179 cm<sup>2</sup>–area). It has 4,8 cm longer, 3,2 cm wider and 74,5 cm<sup>2</sup> larger leaves than YK 7–4/2, which is characterized by the smallest leaves. These data point out to a successful breeding activity of the Tobacco Institute in the selection of oriental aromatic tobaccos. Through evaluation of stability of varieties, the breeder improves its knowledge on their homozygosity and higher security in the choice of parental pairs for implementing selection programs.

**Keywords:** tobacco (*Nicotiana tabacum L.*), old varieties, commercial varieties, quantitative traits, analysis of variance.

### Introduction

Tobacco is a crop that is grown for its leaf and all breeding activities are directed to increasing its yield and quality. Scientific Tobacco Institute - Prilep dates from 1924 and its main activity is the selection of oriental, small-leaf, aromatic tobaccos.

The aim of the study is to investigate and compare the number of leaves per stalk and

size of the middle belt leaves in some old domestic varieties and new commercial varieties, in order to get a better knowledge and more successful selection of oriental tobacco in Macedonia.

### Material and methods

Two-year investigations were made on the number of number, length, width and area of the middle belt leaves in five old domestic

varieties of the types: Prilep (P 10–3/2 and P 12–2/1), Djebel (Dj № 1) and Yaka (YK 7–4/2 and KY –Kishinska Yaka), and five new commercial varieties of the type Prilep: P–23, P–84, NS–72, P–66–9/7 and P–79–94. The traits were measured during tobacco growth in the field. The leaf area (relative area) was calculated by multiplying the length by the width, using the coefficient  $k = 0,6354$  (Gornik, 1973). Each amount represents the arithmetic average for the examined property that is accompanied by error of the mean value (Najceska, 2002).

During tobacco vegetation in field (May - September) in 2013, mean monthly temperature was 19,4°C, number of rainy days 34 and total precipitation amount 153 mm. In the same period in 2014 mean monthly temperature was 18,3°C, number of rainy days 33 and total precipitation amount 223 mm.

#### **General characteristics of the old domestic tobacco varieties**

The cultivation of old tobacco varieties in this region began long ago, during the Ottoman Empire. The centuries–long presence led to their adaptation to the present agro–ecological conditions. Through successive natural selection they have acquired resistance to drought and diseases and can rightly be called autochthonous. Today, the old varieties make a valuable material for breeding activity in the Institute.

**Prilep P 10–3/2**– characterized by cup-like habitus, average stalk height 50cm, with 30-36 sessile leaves, dry mass yield averages 1200 kg/ha.

**Prilep P 12–2/1**– characterized by cup-like habitus, average stalk height 55cm, with 34-38 sessile leaves, dry mass yield averages 1500 kg/ha.

P 10-3/2 and P12-2/1 are put into production in the 30-ies of the last century; phenotypic and genotypic are very similar; originating from the local tobacco variety Djumaj–bale from Gorna Djumaja – Bulgaria.

**Djebel Dj.№ 1**–released in the first half of the last century; Originated from the local variety

Xanthian Yaka grown in the Djebel tobacco producing region in Bulgaria; characterized by a cylindrical habitus, average stalk height 80cm, 26–30 sessile leaves erected toward the stalk, dry mass yield averages 1000 kg/ha.

**Yaka YK 7–4/2**–released in mass production in 1932. Originated from Xanthian Yaka originating from Xanthi – Greece; a plant with narrow, spindle shaped–elliptic habitus; average stalk height 100cm, with 26–32 sessile leaves, dry mass yield averages 1000 kg/ha.

These four domestic varieties were created in the Tobacco Institute–Prilep by Rudolf Gornik (Gornik, 1973) by individual selection (Borojević, 1981).

**KY (Kishinska Yaka)**, it is believed to be transmitted from Moldova (Uzunoski, 1985). Environmental conditions had a great influence on the morphology and chemistry of this genotype and with multi–decades selection a uniform and stable variety was formed. It is characterized by elongated–elliptic habitus with about 40 sessile leaves.

#### **General characteristics of the new commercial tobacco varieties**

The new commercial oriental sun–cured varieties created in the Scientific Tobacco Institute–Prilep present a high quality raw material for the domestic and world market. Due to their pleasant aroma and harmonious chemical composition they enter in the mixtures of the highest–quality cigarette brands.

**Prilep P–23** – created by Kostadin Nikoloski and Milan Mitreski, through hybridization and selection in Tobacco Institute – Prilep; recognized by the Ministry of Agriculture, Forestry and Water Management of the Republic of Macedonia in 1995 (Korubin – Aleksoska A., 2004). It has elliptical–conical habitus, average stalk height 65 cm, with about 55 densely arranged leaves, dry mass yield 2000–2500 kg/ha.

**Prilep P–84** – created by Kiril Naumovski and Ana Korubin–Aleksoska, through hybridization and selection; recognized in 1988 in former Yugoslavia, as one of the first

varieties of the type Prilep. Characterized by cylindrical – elliptical habitus, average stalk height 65 cm, with approximately 40 – 42 sessile leaves, elliptical in shape, dry mass yield 2500–3200 kg/ha.

**Prilep NS–72** –created by Dushko Boceski and Simeon Karayankov; recognized in 1984 in former Yugoslavia as one of the first varieties of the type Prilep obtained by crossing (Korubin – Aleksoska A. et al., 2012); characterized by cylindrical–elliptical habitus, average stalk height 75 cm, with approximately 50 sessile leaves, elliptical in shape, dry mass yield 2800–3300 kg/ha.

**Prilep P–66–9/7** –created in Tobacco Institute–Prilep by Miroslav Dimitrieski and Gordana Miceska; recognized by the Ministry of Agriculture, Forestry and Water Management of R. Macedonia in 2004 (Korubin – Aleksoska A. et al., 2012); characterized by elliptical–conical habitus, with 54–60 ovate leaves, sessile and evenly distributed on the stem, dry mass yield 3000–3600 kg/ha. It has been the most represented tobacco variety in our country in recent years.

**Prilep P–79–94** – created in Tobacco Institute–Prilep by Milan Bogdanceski; recognized by the Ministry of Agriculture, Forestry and Water Management of R. Macedonia in 2001 (Korubin – Aleksoska A., 2004); characterized by cylindrical–elliptical habitus, average stalk height 75 cm, with about 55 sessile leaves densely distributed, especially in the upper part of stem, dry mass yield 2500–3000 kg/ha.

### Results and discussion

The highest leaf number among the old varieties was found in Kishinska Yaka – KY (36–2013, 38–2014, i.e.  $\bar{x} = 37$ ) and the lowest in YK 7–4/2 (26–2013, 28–2014, i.e.  $\bar{x} = 27$ ) and Dj № 1 (26–2013, 29–2014, i.e.  $\bar{x} = 27.5$ ). Among the new commercial varieties, the highest leaf number was found in P–66–9/7 (58–2013, 62–2014, i.e.  $\bar{x} = 60$ ) and the

lowest in P–84 (40–2013, 42–2014, i.e.  $\bar{x} = 41$ ). The variety P–66–9/7 has 33 leaves more than YK 7–4/2 and Dj № 1 (Table 1).

Analysis of variance for the number of leaves per stalk showed highly significant differences among the varieties. Only in the combination Dj № 1 – YK7–4/2 in 2013 the difference was not significant, and in 2014 its significance was 0,05. Differences in leaf number per years showed no significance. This is an indication of different genotypes and proves that this is a highly heritable trait and varietal characteristic.

The highest leaf length among the investigated varieties were measured in P–79–94 (23,2 cm –2013, 23,4 cm – 2014, i.e.  $\bar{x} = 23,3$  cm) and the lowest in YK 7–4/2 (18,2 cm –2013, 18,7 cm – 2014, i.e.  $\bar{x} = 18,45$  cm). The difference in length of the middle belt leaves between the two varieties is 5 cm (Table 1). The comparison of the middle belt leaf length of the semi–oriental variety Otlja O 9–18/2 (32,5 cm – 2013, 33 cm – 2014, i.e.  $\bar{x} = 32,75$  cm), with that of the variety P–79–94 shows that leaves of O 9–18/2 are 9,5 cm longer compared to P–79–94 and 14,3 cm longer compared to YK 7–4/2.

The analysis of variance for the length of the middle belt leaf shows highly significant differences among varieties in 82,2% (2013) and 77,8% of the combinations (2014) and 0,05 significance in 6,7% (2013) and 8,9% (2014). No significance was observed in 11,1% (2013) and 13,3% (2014). The significance of differences in about 87% of the combinations indicates that this trait is varietal characteristic.

The highest leaf width among the varieties was measured in P–79–94 (11,9 cm – 2013, 12,3 cm – 2014, i.e.  $\bar{x} = 12,1$  cm), and the lowest width in YK 7–4/2 (8,7 cm – 2013, 9,1 cm – 2014, i.e.  $\bar{x} = 8,9$  cm). The difference in width of the middle belt leaves between these two varieties is 3,2 cm (Table 1). Comparison of width in the middle belt leaves shows that the

semi-oriental variety O 9–18/2 (17,5 cm – 2013, 18 cm – 2014, i.e.  $\bar{x} = 17,75$  cm) has 5,65 cm wider leaves than P–79–94 and 8,85 cm wider compared to YK 7–4/2.

The analysis of variance for the width of the middle belt leaves shows highly significant differences among varieties in 88.9% (2013) and in 82.2% (2014) and 0.05 significance in 4.4% (2013) and 8.9% (2014), while in 6.7% (2013) and in 8.9% (2014) no significance was observed. The significance of differences in about 93% of the combinations indicates that the investigated trait is varietal characteristic. The largest area of the middle belt leaves was measured in variety P–79–94 (175,9 cm<sup>2</sup>– 2013, 183 cm<sup>2</sup> – 2014, i.e.  $\bar{x} = 179$  cm<sup>2</sup>), and the lowest in YK 7–4/2 (101 cm<sup>2</sup> – 2013, 108 cm<sup>2</sup> – 2014, i.e.  $\bar{x} = 104,5$  cm<sup>2</sup>). This means that P–79–94 has about 74,5 cm<sup>2</sup> larger leaves than YK 7–4/2 (Table 1). Still, this difference is within the allowed limits for oriental tobaccos. Comparison of the area of middle

belt leaves in the semi-oriental variety Otlja O 9–18/2 (361 cm<sup>2</sup> – 2013, 377 cm<sup>2</sup> – 2014, i.e.  $\bar{x} = 369$  cm<sup>2</sup>) shows that it has 190 cm<sup>2</sup> larger leaves than P–79–94 and 264 cm<sup>2</sup> larger than YK 7–4/2, which indicates that they are two different categories oftobacco.

The analysis of variance for the area of the middle belt leaves in 2013 shows highly significant differences among varieties in 66,7%, 0,05 significance in 20% and no significance in 13,3% of the combinations, while in 2014 high significance was assessed in 62,2%, 0,05 significance in 20% and no significance in 17,8%. The significance of differences in about 84% of the combinations indicates that the investigated trait is varietal characteristic. The differences in leaf area by years are small but highly significant and they appear as a result of various meteorological factors during the growing season.

Table 1. Number and size of the middle belt leaves in old domestic and new commercial tobacco varieties from the Republic of Macedonia

| Tobacco varieties                | Quantitative traits        |           |                                       |             |                                      |             |   |            |
|----------------------------------|----------------------------|-----------|---------------------------------------|-------------|--------------------------------------|-------------|---|------------|
|                                  | Number of leaves per stalk |           | Length of the middle belt leaves (cm) |             | Width of the middle belt leaves (cm) |             | Area of the middle belt leaves (cm <sup>2</sup> ) |            |
|                                  | $\bar{X} \pm s_X$          |           | $\bar{X} \pm s_X$                     |             | $\bar{X} \pm s_X$                    |             | $\bar{X} \pm s_X$                                 |            |
|                                  | 2013                       | 2014      | 2013                                  | 2014        | 2013                                 | 2014        | 2013  | 2014       |
| Old domestic tobacco varieties   |                            |           |                                       |             |                                      |             |   |            |
| 1. P 10-3/2                      | 32 ± 0,09                  | 34 ± 0,08 | 22,1 ± 0,10                           | 22,4 ± 0,11 | 10,2 ± 0,05                          | 10,5 ± 0,04 | 143 ± 1,65  | 149 ± 1,63 |
| 2. P 12-2/1                      | 34 ± 0,10                  | 36 ± 0,11 | 22,5 ± 0,12                           | 23,3 ± 0,11 | 10,4 ± 0,07                          | 11,1 ± 0,06 | 149 ± 1,83  | 164 ± 1,70 |
| 3. Dj № 1                        | 26 ± 0,16                  | 29 ± 0,14 | 19,4 ± 0,11                           | 20,2 ± 0,10 | 11,5 ± 0,04                          | 11,8 ± 0,04 | 142 ± 1,55  | 151 ± 1,49 |
| 4. YK7-3/2                       | 26 ± 0,17                  | 28 ± 0,15 | 18,2 ± 0,08                           | 18,7 ± 0,09 | 8,7 ± 0,08                           | 9,1 ± 0,07  | 101 ± 1,59  | 108 ± 1,52 |
| 5. KY                            | 36 ± 0,15                  | 38 ± 0,15 | 21,8 ± 0,12                           | 22,1 ± 0,11 | 11,6 ± 0,07                          | 11,8 ± 0,05 | 161 ± 1,93  | 166 ± 1,84 |
| New commercial tobacco varieties |                            |           |                                       |             |                                      |             |   |            |
| 6. P-23                          | 45 ± 0,20                  | 48 ± 0,18 | 20,2 ± 0,13                           | 22,1 ± 0,12 | 10,7 ± 0,04                          | 11,1 ± 0,03 | 137 ± 1,85  | 156 ± 1,74 |
| 7. P-84                          | 40 ± 0,14                  | 42 ± 0,15 | 20,4 ± 0,10                           | 21,3 ± 0,09 | 10,5 ± 0,05                          | 10,7 ± 0,03 | 136 ± 1,77  | 145 ± 1,59 |
| 8. NS-72                         | 43 ± 0,21                  | 45 ± 0,17 | 21,8 ± 0,17                           | 22,9 ± 0,15 | 10,8 ± 0,07                          | 10,9 ± 0,06 | 149 ± 2,17  | 158 ± 2,03 |
| 9. P-66-9/7                      | 58 ± 0,15                  | 62 ± 0,15 | 20,9 ± 0,12                           | 21,5 ± 0,14 | 11,2 ± 0,05                          | 11,2 ± 0,04 | 149 ± 1,58  | 153 ± 1,54 |
| 10. P-79-94                      | 52 ± 0,14                  | 54 ± 0,15 | 23,2 ± 0,12                           | 23,4 ± 0,11 | 11,9 ± 0,06                          | 12,3 ± 0,02 | 158 ± 1,69  | 183 ± 1,62 |
| LSD <sub>0,05</sub>              | 0,581                      |           | 0,420                                 |             | 0,163                                |             | 5,655   |            |
| LSD <sub>0,01</sub>              | 1,053                      |           | 0,756                                 |             | 0,293                                |             | 10,179  |            |

## Conclusions

Two-year investigations of some old domestic oriental varieties (P 10–3/2, P 12–2/1, Djebel Dj № 1, Yaka YK 7–4/2, KY – Kishinska Yaka) and new commercial varieties (P–23 P–

84, NS–72, P–66–9/7, P–79–94) showed a small error of the mean value for the traits: number, length, width and area of the middle belt leaves per stalk, which is an indication of correct setting of the experiment, high genetic

stability of the traits and homozygosity of the varieties.

The highest leaf number per stalk was obtained in P-66-9/7 (60), which is 33 leaves more than YK 7-4/2 and Dj № 1 – characterized by the lowest number of leaves. The analysis of variance shows highly significant differences between varieties and no significant differences between the years, which is indication that these are different genotypes and that the investigated trait is highly heritable and varietal characteristic.

The highest leaf length and width was measured in P-79-94 ( $\bar{x} = 23,3$  cm length,  $\bar{x} = 12,1$  cm –width), and the lowest length and width in YK 7-4/2 ( $\bar{x} = 18,45$  cm length,  $\bar{x} = 8,9$  cm - width), showing that the leaves of P-79-94 are 5 cm longer and 3,2 cm wider compared to YK 7-4/2. The significance of 87% for the leaf length and 93% for the width indicates the differences between varieties, suggesting that these traits are varietal characteristics.

The largest area of the middle belt leaves was measured in P-79-94 ( $\bar{x} = 179$  cm<sup>2</sup>), and the lowest in YK 7-4/2 ( $\bar{x} = 104,5$  cm<sup>2</sup>), which means that P-79-94 has 74,5 cm<sup>2</sup> larger leaves than YK 7-4/2. The significance of differences between the varieties in about 84% of the combinations confirms that the investigated trait is a varietal characteristic.

Compared to the semi-oriental variety Otlja O 9-18/2 it can be concluded that differences in leaf size among the ten genotypes is within allowed limits for oriental aromatic tobaccos.

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