

EVALUATION OF NEW VIRGINIA TOBACCO GENOTYPES FOR YIELD, MORPHOLOGICAL AND CHEMICAL TRAITS

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Abstract

To evaluate the yield, morphological traits and chemical indicators of Virginia tobacco genotypes, under agroecological conditions of Plovdiv, a study was conducted during 2017-2019 in an experimental field at Tobacco and Tobacco Products Institute, Markovo. Three new genotypes of Virginia tobacco - L 36, H 126 F₁, H 135 F₁ and standard Virginia 0514 were planted in four replications and evaluated by the following traits: plant height; number of leaves; twelfth leaf length, twelfth leaf width; leaf area, cured leaf yield, chemical components - nicotine, sugars, total nitrogen, balance ratio total nitrogen/nicotine and sugars/nicotine. It was found that statistically significant differences existed among the studied Virginia tobacco genotypes only in the indicators cured leaf yield, twelfth leaf length and in the adaptability of plant height trait. Genotypes H 126 and L 36 are distinguished by a proven greater twelfth leaf length (64; 62 cm) and cured leaf yield (3.20; 3.14 t/ha). The presence of statistically significant differences between genotypes in terms of plant height trait proved H 126 to be the most resistant to environmental factors, followed by L 36. The standard V 0514 demonstrated superiority for the sugars/nicotine ratio, but for all genotypes the value was within the reference range. In L 36 the total nitrogen/nicotine ratio was below 1.

Key words: Virginia tobacco, morphological traits, yield, ecological stability.

INTRODUCTION

Nicotiana tabacum L. tobacco is cultivated worldwide (Kishore, 2014). The plant is cultivated as a source of leaves, primarily for use in the manufacture of nicotine-containing products such as cigarettes, cigars and smokeless tobacco products (Lewis, 2020). It can generate a large amount of biomass (Andrianov et al., 2010; Srbinska, 2017), serves as a model system for tissue culture and genetic engineering (Ganapathi, 2004), as an energy crop for biofuel production (Barla & Kumar, 2019), is considered as a promising plant material with strong antioxidant potential and for the production of purified natural extracts with potential application in biopharmaceutics (Docheva et al., 2024).

Despite the decline in production, tobacco is still an important crop for Bulgaria's economy (Drumeva-Yoncheva, 2020). The country produces tobacco from the variety groups - Basma and Kaba Kulak - oriental tobaccos and Virginia and Burley, which are large-leaf tobaccos (Bozukov, 2014). In 2023, Virginia

tobacco accounts for approximately 19% of total tobacco production in Bulgaria (<https://www.mzh.government.bg/>). Currently, Bulgarian selected varieties - Virginia 0514 and Hibrid (Virginia) 0454 - are used in the country. From 2023, the Official Variety List includes new tobacco variety Virginia 0842 (<https://iasas.government.bg/>).

The main goal of any breeding program is to create varieties combining high productive potential (Dražić, 2012; Risteski & Kocoska, 2012; Dyulgerski & Docheva, 2017) and other basic agronomic characteristics (Becker & Leon, 1988), which is the result of the impact of genotype and environment (Ayalneh et al., 2013).

The geographical environment can significantly impact the growth and quality of tobacco leaves Zhao et al. (2024).

The key factor influencing the yield and quality of flue-cured tobacco are: the climate factors (including temperature, illumination and moisture) and cultivation factors (incl. transplant, fertilization, maturity and harvest) (Yang et al., 2015)

The morphological traits of tobacco plants usually depend on the genetic structure of the species and climate characteristics (Dražić, 2012; Mitreski, 2018; Kurt, 2020) and the effects resulting from the genotype x environment interaction (Butorac et al., 2004). The interaction between genotype and environment is a universal problem that applies to all living organisms, from humans to plants to bacteria (Kang, 1998). Tobacco can adapt to a wide range of conditions but is very sensitive to the environment (Tang et al., 2020).

Dynamically changing conditions in recent years has been an additional factor for the creation of new tobacco varieties with the typical characteristics (Spasova-Apostolova et al., 2023).

The different response of genotypes to the environment is mainly related to ecology, climate and soil factors (Wu et al. 2013; Kinay, 2020). Environmental changes have a significant impact on the characteristics of tobacco genotypes (Dimanov & Zapranova, 2002) - on chemical indicators (Kalamanda & Pelivanoska, 2009; Tang et al., 2020; Kurt, 2020; Spasova-Apostolova, 2024), morphological indicators (Spasova-Apostolova, 2024; Kurt et al., 2020) and tobacco yield (Kurt et al., 2020; Tang et al., 2020).

The quality of the tobacco raw material is determined by the variety, location, environmental conditions and cultivation method (Kalamanda & Pelivanoska, 2009; Kasheva et al., 2021).

Temperature and rainfall have been found to be major factors influencing the total sugar and nicotine contents required to produce high quality Flue-cured tobacco during the tobacco growth period (Yanyan et al., 2015).

Studying the potential of tobacco genotypes in different locations and their response to environmental factors is essential to identify genotypes that are tolerant to environmental conditions.

Yield stability is an important trait of crop cultivars (Piepho, 1994). The concept of ecological stability occupies a prominent place in both fundamental and applied ecological research (Kefi et al., 2019).

The aim of the study was to evaluate the yield, morphological traits and chemical indicators of Virginia tobacco genotypes, under agroecological conditions of Plovdiv.

MATERIALS AND METHODS

The field experiment was carried out during the period 2017-2019 on meadow-cinnamon soil (Cleyic-Chromic Luvisol) (Bozinova, 2019) at experimental field (location 1) (2017; 2019) and experimental field (location 2) (2018) of the Tobacco and Tobacco Products Institute, Markovo. Three variants with parental components selected by morphological characteristics (Drumeva-Yoncheva, 2020) and resistance to TMV or PVY (Yonchev et al., 2014; Yonchev, 2014) were studied - two hybrid combinations - Hybrid 126 F₁ (Virginia 385xL 0543), Hybrid 135 F₁ (L 0543xL 0842), line L 36 F7-9 (K 326xLechia) and standard Virginia 0514. Transplanting was carried out on 29 May 2017; 22 May 2018 and 17 May 2019. The experiment was laid out in 4 replications with a harvest plot size of 27 m² in a 110-40/45 cm transplanting scheme. The main agrotechnical measures were carried out in accordance with the technology adopted for the cultivation of Virginia tobacco in Bulgaria.

Each genotype was analyzed for the following traits: Plant height (cm) (without the inflorescence); Number of leaves (commercial leaves); Twelfth leaf length (cm) and Twelfth leaf width (cm); Leaf area cm². Leaf area cm² calculated according to the formula: leaf length × width × 0.6345 (Suggs et al., 1960), Cured leaf yield (t/ha); chemical components - Nicotine (%), Sugars (%), Total nitrogen (%), Balance ratio total nitrogen/nicotine and sugars/nicotine. Chemical parameters were determined using AutoAnalyzer II C, Technicon in flow analyzer with methods ISO 15152:2003, ISO 15154:2003, BDS 15836:1988, respectively, under laboratory conditions.

Table 1 shows the data on meteorological conditions (values of average air temperature (°C) and precipitation amount (l/m²) for the study period.

The main climatic indicators affecting Virginia tobacco production in the country are the average monthly temperature and the amount of rainfall. Over the three-year period of the field experiment, for the period of active tobacco growth (July and August), the average monthly temperature was higher than the norm for the region, with higher values recorded in 2017. The

three years of the study (2017, 2018, 2019) differ significantly in terms of rainfall amount. The variability of rainfall amounts across years is significant. The distribution by month during the growing season is uneven. The experimental year 2017 had the least amount of rainfall during the tobacco vegetation season compared to the following two vegetation years (Table 1).

Table 1. Meteorological data for the period 2017-2019, Plovdiv

Month	Rate	2017 (location 1)	2018 (location 2)	2019 (location 1)
Temperature, °C				
June	20.9	23.8	23.0	23.5
July	23.2	25.0	24.7	24.2
August	22.7	25.3	24.8	25.3
Rainfall, l/m ²				
June	63	15	139.9	142.3
July	49	65	82.7	38.5
August	31	9	45.6	8.7

Table 3 presents information on the reported values of the studied indicators during the three years of the experiment for the respective location.

Table 3. Mean values for morphological and chemical indicators and the yield of Virginia flue-cured tobacco genotypes during 2017-2019

Genotype	Year	Location	Plant height (cm)	Number of leaves	Length twelfth leaf (cm)	Width twelfth leaf (cm)	Leaf area (cm ²)	Cured leaf yield (t/ha)	Nicotine (%)	Sugars (%)	Total nitrogen (%)
L 36	2017	location 1	174	28	64.0	34.1	1383	3.16	3.15	6.4	2.82
	2018	location 2	169	25	59.3	34.9	1310	3.06	1.07	30.1	1.25
	2019	location 1	172	21	62.6	36.7	1459	3.19	3	6.42	2.94
H 126	2017	location 1	158	27	66.6	35.1	1483	3.3	2.05	3.24	2.83
	2018	location 2	156	22	60.2	33.5	1281	3.03	0.96	23.1	1.8
	2019	location 1	158	23	66.5	37.0	1560	3.26	2.77	11.9	1.99
H 135	2017	location 1	153	25	57.1	31.7	1150	2.92	2.11	3.15	2.93
	2018	location 2	159	24	52.0	31.0	1021	2.53	0.90	29.4	1.45
	2019	location 1	154	21	60.0	35.6	1353	2.99	2.44	9.93	3.15
V 0514	2017	location 1	158	27	61.5	33.3	1300	3.12	2.17	4.52	2.61
	2018	location 2	143	21	53.0	30.3	1019	2.69	1.41	22.6	2.1
	2019	location 1	176	23	57.6	34.5	1259	2.98	1.67	15.6	2.12

RESULTS AND DISCUSSIONS

The stability of a plant variety is related to its degree of adaptability to changes in environmental factors. This enables its cultivation in areas with dynamic climatic and soil characteristics. Plant height is an important morphological trait in tobacco. It is an indication of the uniformity of the variety. Average plant height values ranged from 143 to 176 cm over the three years of the study. Maximum mean values were measured in 2017 and 2019 at L 36 (174 cm; 172 cm) and in 2019 at standard V 0514 (176 cm) in

Table 2. Soil characteristics

Year	Physical clay (%)	pH (H ₂ O)	Humus (%)	Total N (%)
2017, 2019 (location 1)	21	5.82	1.46	0.095
2018 (location 2)	23	6.76	1.51	0.084

The Wricke method (1962, 1966) was applied to investigate genotype-environment interaction and establish resistant tobacco genotypes:

$$W_i^2 = \sum_{j=1}^n (Y_{ij} - \bar{Y}_i - \bar{Y}_j + Y)^2$$

where Y_{ij} is the mean performance of genotype i in the j -th environment and $\bar{Y}_i$ is the marginal mean of the i -th genotype and $\bar{Y}_j$ is the marginal mean of the j -th genotype and Y is the overall mean.

Statistical processing of data was performed using the statistical software product IBM Statistics SPSS 24 (Field, 2013; Weinberg & Abramowitz, 2016; Landau & Everitt, 2004) и MS Excel (Mokreva et al., 2001; Pashova et al., 2025).

location 1. Mitreski et al. (2018) indicates that tall-growing varieties are over 130 cm tall (Virginia and Burley). The lowest value for this trait is shown by standard V 0514 (143 cm) in location 2. Genotypes H 126 and H 135 formed shorter plants and similar height over the three-year period in both locations (Table 3). The results of the variance analysis of plant height are presented in Table 4 and Figure 1. The presence of statistically significant differences among genotypes in terms of plant height trait proved H 126 as the most resistant to environmental factors, followed by L 36.

Table 4. Comparative assessment of tobacco genotypes according to height (cm)

Genotype	Plant height (cm)	Plant height - Eco
L 36	172	8.1 ^b
H 126	157	7 ^b
H 135	155	32 ^{ab}
V 0514	159	103 ^a
Average	161	37.4
Std. Dev.	10.0	57.2
Sign.	0.110	0.048



Figure 1. Stability of tobacco genotypes according to plant height (cm)

Leaf number is an essential trait determining plant productivity and is an important varietal trait (Shah et al., 2008). The number of technically suitable leaves in the studied genotypes during the three years was in the range of 21-28. In location 1, genotypes formed 21-28 number of leaves and in location 2 the number of leaves ranged from 21 to 25 (Table 3). The largest number of leaves (28) in flue-cured tobacco was reported by Zeb et al. (2023) and Nghiem et al. (2024).

In H126 and standard V 0514 the number of leaves ranged from 21 to 27, in H135 from 21 to 25 (Table 3). Shah et al. (2008) and Ahmed et al. (2014) reported maximum leaf number (25) for flue-cured tobacco genotypes.

There was no proven difference in the adaptability of genotypes for the leaf number trait over the three-year period (Table 5, Figure 2).

Table 5. Comparative assessment of tobacco genotypes according to number of leaves

Genotype	Number of leaves	Number of leaves - Eco
L 36	25	7.4
H 126	24	0.2
H 135	23	5.2
V 0514	24	0.0
Average	24	3.2
Std. Dev.	3	4.7
Sign.	0.916	0.114

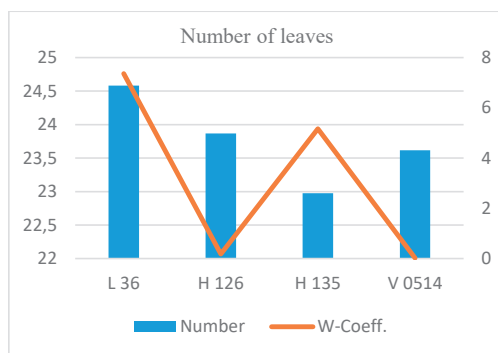


Figure 2. Stability of tobacco genotypes according to number of leaves

Leaf length and width are important morphological traits characterizing tobacco type and are the main factor influencing plant productivity. A strong positive correlation between twelfth leaf dimensions - length and width and dry tobacco yield has been demonstrated (Drumeva-Yoncheva, 2020). The relationship of leaf length and width with yield in flue-cured tobacco has also been reported by Maleki et al. (2011).

Table 3 presents the experimental data for the variants studied with respect to the twelfth leaf length trait. In the Virginia tobacco genotypes, the twelfth leaf length trait ranged from 52.0 cm to 66.6 cm over the three-year period. Mean values in location 1 ranged from 57.1 to 66.6 cm and in location 2 ranged from 52.0 to 60.2 cm. Maximum leaf length values were recorded for H 126 (66.6 cm) and L 36 (64.0 cm) in location 1 in 2017 and 2019 and lower values in location 2. The minimum twelfth leaf lengths were H 135 and standard V 0514 in location 2.

There are statistically proven differences among genotypes for the twelfth leaf length trait. There is no proven difference with respect to the stability of the twelfth leaf length trait (Table 6, Figure 3).

Table 6. Comparative assessment of tobacco genotypes according to length twelfth leaf (cm)

Genotype	Length twelfth leaf (cm)	Length twelfth leaf - Eco
L 36	62 ^{ab}	0.8
H 126	64 ^a	0.1
H 135	56 ^b	2.1
V 0514	57 ^{ab}	1.9
Average	60.0	1.2
Std. Dev.	4.7	1.4
Sign.	0.050	0.255

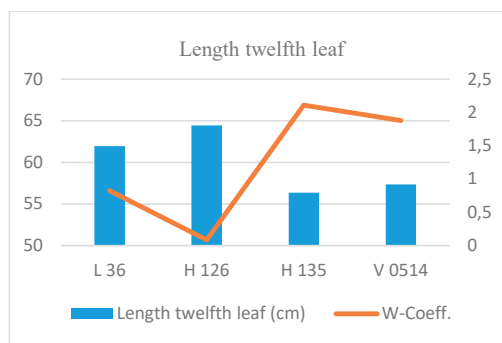


Figure 3. Stability of tobacco genotypes according to length twelfth leaf (cm)

The twelfth leaf width trait in the genotypes studied ranged from 30.3 to 37.0 cm for the three years of study. For this trait, the values obtained correspond to the values typical for the variety group of Virginia tobacco. Maximum twelfth leaf width was recorded for H 126 and for L 36 in location 1 in 2017 and 2019 (Table 3).

For the twelfth leaf width trait, there were no proven differences between the studied Virginia tobacco genotypes, both in terms of leaf size and stability of this trait (Table 7, Figure 4).

Table 7. Comparative assessment of tobacco genotypes according to width twelfth leaf (cm)

Genotype	Width twelfth leaf (cm)	Width twelfth leaf - Eco
L 36	35	0.71
H 126	35	0.04
H 135	33	0.39
V 0514	32,7	0.61
Average	34	0.43
Std. Dev.	2.12	0.44
Sign.	0.268	0.325

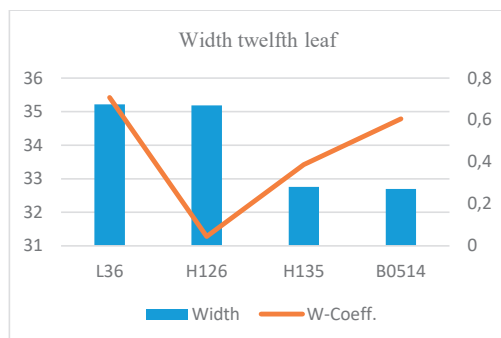


Figure 4. Stability of tobacco genotypes according to width twelfth leaf (cm)

Leaf area is very important component of yield in tobacco (Ahmed et al., 2016).

Data for the three years of the study on the leaf area indicator are presented in Table 3. The leaf area of the genotypes in location 1 ranged from 1150 to 1560 cm² and in location 2 from 1019 to 1310 cm². Maximum mean values for leaf area trait were recorded for H 126 (1483-1560 cm²) and L 36 (1383-1459 cm²) in location 1. Minimum leaf area was observed in location 2 for H 135 and V 0514, respectively (1021-1019 cm²).

Over the three-year period, H 126 (1441 cm²) had the highest values for the leaf area trait of the V 0514 standard and the other genotypes. Similar results were reported by Zeb et al. (2023). There were no proven differences in the leaf area trait and trait stability in the studied Virginia tobacco variants (Table 8, Figure 5).

Table 8. Comparative assessment of tobacco genotypes according to leaf area (cm²)

Genotype	Leaf area (cm ²)	Leaf area - Eco
L 36	1384	2194
H 126	1441	198
H 135	1174	2672
V 0514	1192	2961
Average	1298	2006
Std. Dev.	169	2025
Sign.	0.108	0.371

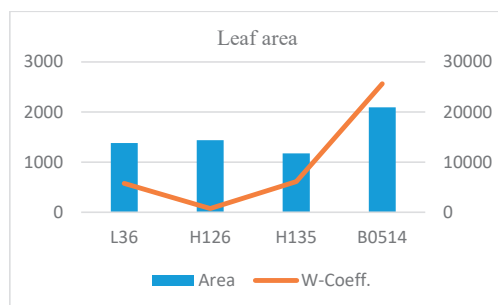


Figure 5. Stability of tobacco genotypes according to leaf area

Table 3 shows that the average values of cured leaf yield in location 1 ranged from 2.92 to 3.3 t/ha. Similar results were reported by Khan et al. (2017). In location 2, yields were in the range from 2.53 to 3.06 t/ha. Yields at L 36 and H 126 were higher and relatively stable over the three years of study. For all genotypes studied, yields were lower in location 2 in 2018. This may be due to environmental (location) conditions. Ahmed et al. (2019) summarized that tobacco genotypes were inconsistent in yield efficiency in different environments.

H 126 and L 36 were shown to form higher yields. H 135 and standard V 0514 were inferior in this indicator.

Given the values of the environmental coefficient, no line should be considered to stand out as more stable than the others (Table 9, Figure 6).

Table 9. Comparative assessment of tobacco genotypes according to cured leaf yield (t/ha)

Genotype	Cured leaf yield (t/ha)	Cured leaf yield - Eco
L 36	3.14 ^{ab}	0.01
H 126	3.20 ^a	0.00
H 135	2.80 ^b	0.01
V 0514	2.93 ^{ab}	0.00
Average	3.0	0.00
Std. Dev.	0.2	0.00
Sign.	0.051	0.305

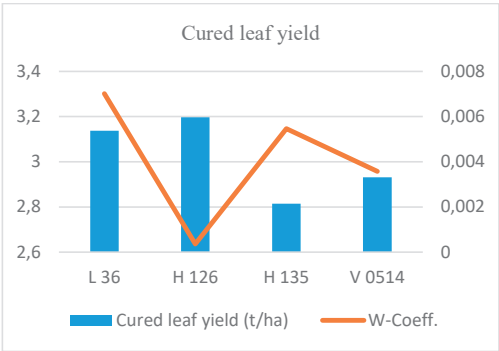


Figure 6. Stability of tobacco genotypes according to cured leaf yield (t/ha)

The content of the main chemical components nicotine %, sugars %, total nitrogen % in the genotypes was studied in 2017-2019.

Each type of tobacco within a blend has defining effects on the physical and chemical quality composition (Kurt, 2021).

There are differences in nicotine, sugars and total nitrogen content between the different years of the study (2017-2019), between the two locations and in the different genotypes.

In location 1, nicotine content varied from 1.67% (V 0514) to 3.15% (L 36), with L 36 having the maximum value. Similar results were reported by Zeb et al. (2023). At H 126 and H 135, nicotine is about 2% (Table 3). The values obtained by us are close to those indicated (Gjuzev, 1980; Tabakova & Drachev, 1996) for a typical Virginia flue-cured (about 2-2.5%). In location 2, where the amount of rainfall during the growing season was significant

nicotine ranged from 0.9 to 1.41% (Table 3). The obtained values are close to the values indicated by Gjuzev (1980) for Virginia produced in atypical regions – 0.6-1.2%. Hawks, 1970 assumed that high humidity contributes to lower nicotine content.

The average value of nicotine for a three-year period at L 36 (2.4%) is closest to those indicated by Tabakova & Drachev (1996) meanings of "balanced" Virginia (Table 10).

The results of the comparative assessment and ecological plasticity analysis of the tobacco genotypes showed that there were no statistically significant differences in their chemical composition (Tables 10-12, Figures 7-9).

Table 10. Comparative assessment of tobacco genotypes according to nicotine (%)

Genotype	Nicotine (%)	Nicotine - Eco
L 36	2.4	0.11
H 126	1.9	0.07
H 135	1.8	0.01
V 0514	1.8	0.21
Average	2.0	0.10
Std. Dev.	0.8	0.10
Sign.	0.787	0.209

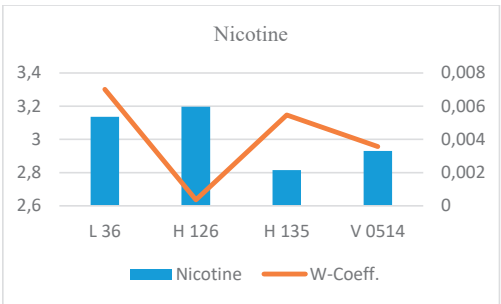


Figure 7. Stability of tobacco genotypes according to nicotine (%)

Soluble carbohydrates are one of the most important components of light tobaccos. They determine the manifestation of the characteristic pleasant taste of these tobaccos (Gjuzev, 1980). They are generally regarded as having a positive influence on tobacco quality.

The sugar content in the 2017 and 2019 harvest samples studied was below 10%, which is atypical for the Virginia variety group tobaccos. Exceptions are H 126 and the standard V 0514 grown in location 1 in 2019, 11.9% and 15.6%, respectively. Our results are consistent with the data of Shah et al. (2008) and Zeb et al. (2023). The sugar content for the 2018 harvest is typical

for Virginia tobacco for H 126 and the V 0514 standard (Table 3).

On average over the three-year period, L 36, H 135 and V 0514 had a sugar content of 14%. In terms of the sugar content indicator, there were no statistically proven differences between the genotypes, although H 126 and H 135 stood out as more resistant to external changes compared to the other two genotypes.

Table 11. Comparative assessment of tobacco genotypes according to sugars (%)

Genotype	Sugars (%)	Sugars - Eco
L 36	14.3	12.93
H 126	12.7	2.85
H 135	14.2	3.93
V 0514	14.2	11.60
Average	13.9	7.83
Std. Dev.	10.1	8.14
Sign.	0.998	0.335

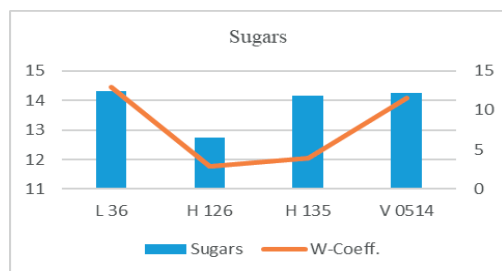


Figure 8. Stability of tobacco genotypes according to sugars

The optimal total nitrogen content for Virginia tobacco is recommended to be 1.7-2/2.3/ (Gjuzelev, 1980).

The total nitrogen content in the studied genotypes of the variety group Virginia varied widely over the three years - from 1.25 to 2.94%, with the highest nitrogen content being the samples from the 2017 and 2019 harvests, location 1, and the genotypes from the 2018 harvest having lower values (Table 3). On average for the three-year period, the genotypes with optimum total nitrogen content were H 126, V 0514 and L 36 (Table 12).

Table 12. Comparative assessment of tobacco genotypes according to total nitrogen (%)

Genotype	Total nitrogen (%)	Total nitrogen - Eco
L 36	2.34	0.10
H 126	2.21	0.10
H 135	2.51	0.11
V 0514	2.28	0.14
Average	2.33	0.11
Std. Dev.	0.63	0.08
Sign.	0.961	0.956

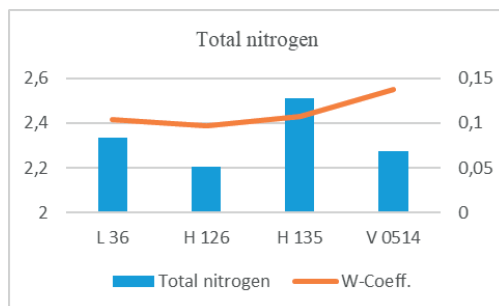


Figure 9. Stability of tobacco genotypes according to total nitrogen

When assessing the quality of tobacco based on chemical properties, the balance /ratio/ of the individual components is more decisive than the specific quantities found (Hawks, 1970). With a quantitative ratio of "total nitrogen/nicotine" < 1, the taste is well balanced (Tso, 1972). In L 36, the total nitrogen/nicotine ratio is below 1, in H 126 it is close to 1, and in the remaining genotypes it is above the permissible values. The "sugars/nicotine" ratio provides information about the fullness and smoothness of the taste. The reference limits of this indicator are from 6 to 10.

In Table 13, the value of the sugars/nicotine ratio in the genotypes is in the range 5.68 to 8.13. The V 0514 standard demonstrates superiority for the sugars/nicotine ratio, and for H 126, H 135 and L 36 the sugars/nicotine ratio is within the reference limits according to Gjuzelev (1980).

Table 13. Balance ratio total nitrogen/nicotine and sugars/nicotine in Virginia tobacco (%)

Genotype	Balance ratio	
	Total nitrogen/ nicotine	Sugars/ nicotine
L 36	0.97	5.68
H 126	1.14	6.59
H 135	1.38	7.82
V 0514	1.29	8.13

CONCLUSIONS

The comparative evaluation carried out proves that on the traits of twelfth leaf length, cured leaf yield and plant height stability there are proven differences between the studied Virginia tobacco genotypes. On average over the three-year period, H 126 and L 36 had higher values for a twelfth leaf length, leaf area and a potential in terms of Cured leaf yield than the V 0514 standard. Based on the results of three growing

seasons, in L 36 the total nitrogen/nicotine ratio is below 1. The genotypes studied can be used in future breeding programmes.

REFERENCES

- Ahmed, S., Mohammad, E., Ahmed, O., & Atiq, M. (2014). Assessing Genetic Variation for morpho-agronomic traits of some native and exotic Fcv tobacco genotypes in Pakistan. *American-Eurasian J. Agric. & Environ. Sci.*, 14(5), 428–433.
- Ahmed, S., Mohammad, F., Ullah Khan, N., Ahmed, O., Gul, S., Ahan, S., Romena, M., Fikere, M., Ali, I., & Din A. (2019). Assessment of flue-cured tobacco recombinant inbred lines under multi-environment yield trials. *International journal of agriculture & biology*. ISSN Print: 1560–8530; ISSN Online: 1814–9596 19–0540/2019/22–3–578–586 DOI: 10.17957/IJAB/15.1102. <http://www.fspublishers.org>. 578-586.
- Ahmed, Q., & Mohammad, F. (2014). Yield attributing traits in parents versus hybrids in FCV tobacco (*Nicotiana tabacum* L.). *Sarhad J. Agric.*, 30(2), 193–201.
- Ahmed, Q., Mohammad, F., Hidayat-ur-Rahman, Sheraz A., & Fakharuddin. (2016). Heterotic Studies in Flue-Cured Tobacco across Environments. *Sarhad Journal of Agriculture*, 32(2), 112–120.
- Andrianov V., Borisjuk, N., Pogrebnyak, N., Brinker, A., Dixon, J., Spitsin, S., Flynn, J., Matyszczyk, P., Andryszak, K., Laurelli, M., Golovkin, M., & Koprowski, H. (2010). Tobacco as a production platform for biofuel: overexpression of Arabidopsis DGAT and LEC2 genes increases accumulation and shifts the composition of lipids in green biomass, *Plant Biotechnol Journal*, 8(3), 277–287.
- Ayalneh, T., & Abinasa, L. (2013). Assessment of stability, adaptability and yield performance of bread wheat (*Triticum aestivum* L.) cultivars in South Eastern Ethiopia. *Plant Breeding and Seed Science*, 67, 1–11.
- Barla, F., & Kumar, S. (2019) Tobacco biomass as a source of advanced biofuels. *Biofuels*, 10(3), 335–346.
- Becker, H. & Leon, J. (1988). Stability analysis in plant breeding. *Plant Breeding*, 101, 1–23.
- Butorac, J., Beljo, J., & Gunjača, J., (2004) Study of inheritance of some agronomic and morphological traits in burley tobacco by graphic analysis of diallel cross. *Plant, Soil and Environment*, 50(4), 162–167.
- Bozhinova, R. (2019). Fertilization of Virginia tobacco with compound fertilizers. II. Mineral and chemical composition of tobacco. *Bulgarian Journal of Soil Science, Agrochemistry and Ecology*, 53(3-4), 17–24.
- Bozukov, H. (2014). History, present and future of oriental tobacco in Bulgaria. *Jubilee International Scientific Conference “70 Years of the Tobacco and Tobacco Products Institute”* ISBN - 978-954-702-103-7.
- Dimanov, D., & Zapryanova, P. (2002). Yield and quality of oriental tobacco genotypes of origin “Nevrokop” depending on soil and climatic conditions. *Second Balkan Scientific Conference “Quality and efficiency of production, processing and processing of tobacco”*, Plovdiv, 214–217.
- Docheva, M., Kirkova, D., Stoyanova, L., Dureva, V., Dimova, R., Dimitrova, D., Bachvarova, M., & Syuleyman, E. (2024). Polyphenol content in tobacco (*N. tabacum* L.) and antioxidant activity. *Bulgarian Chemical Communications*, 56, 178–183
- Dražić, S., Risteski, I., Filiposki, K., & Kocoska, K. (2012). Results of studies on recently developed Virginia Tobacco genotypes under different growing conditions in Serbia. *Tobacco*, 62(1), 3–8.
- Drumeva-Yoncheva, M. (2020). Assessment of basic morphological and economic indicators and tolerance of abiotic stress in Virginia tobacco. *Dissertation*. Sadovo. 52–60.
- Dyulgierski, Y., & Docheva, M. (2017). Production characteristics and chemical indicators of perspective lines of Burley tobacco. *Tobacco*, 67(1-6), 41–47.
- Field, A. (2013). *Discovering Statistics using IBM SPSS Statistics, Mobile Study*, London.
- Ganapathi, T., Suprasanna, P., Rao, P., & Bapat, V. (2004). Tobacco (*Nicotiana tabacum* L.) - A model system for tissue culture interventions and genetic engineering. *Indian Journal of Biotechnology*. 3(2), 171–184.
- Gjuzelev, L. (1980). Stock knowledge. Plovdiv. 71–78
- Hawks, S.N. (1970). Principles of the production of Flue-Kurd tobacco, *DSO Bulgarian Tobaccos*, Plovdiv, 33
- Kalamanda, O., & Pelivanoska, V. (2009). Hemiski sostav na virxiniska surovina proizvedena vo Republika Srpska. *Tobacco*, 59(11-12), 279–290.
- Kang, M. (1998). Using genotype by environmental interaction for crop cultivar development. *Department of Agronomy Louisiana Agricultural Experiment Station Louisiana State University Agncultural Center Baton Rouge, Louisiana* 70803-2 110, 200–252.
- Kasheva, M., Bozukov, H. & Kochev, Y. (2021). Chemical and technological characteristics of large leaf tobacco Virginia 0842. *Bulgarian journal of agriculture sciences*, 27(1), 110–114.
- Kefi., S., Domunguez-Garcia, V., Donohue, I., Forntaine, C., Thebault, E., & Dakos, V. (2019). Advancing our understanding of ecological stability. *Ecology Letters*, 22(9), 1349–1356.
- Khan, M.R., Shah, K., Zahid, M., Noman, M., Khan, Muhammad Zahir Afridi, Sarmad Iqbal, Syed Minhaj Ali Shah, Rashid Ullah & Syed Junaid Ahmad. (2017). Morpho-agronomic and qualitative performance of various FCV tobacco exotic hybrids. *Pure Appl. Biol.*, 6(3): 942–947.
- Kinay, A. (2020). Agronomic and chemical properties of hybrid oriental tobacco (*Nicotiana tabacum*) lines and their stabilities. *Indian Journal of Agricultural Sciences*, 90(5), 874–878.
- Kishore K. (2014). Monograph of Tobacco (*Nicotiana Tabacum*). *Indian Journal of Drugs*, 2(1), 5–23.
- Kurt, D., Yilmaz, G. & Kinay, A. (2020). Effects of environmental variations on yield of oriental tobaccos. *International Journal of Agriculture and Wildlife Science*, 6(2), 310–324.
- Kurt, D. (2021). Impacts of environmental variations on quality and chemical contents of oriental tobacco. *Contributions to Tobacco and Nicotine Research*

- formerly. *Beiträge zur Tabakforschung International*, 30(1), 50–62.
- Landau, S., & Everitt, B. (2004). *A Handbook of Statistical analyses using SPSS*, Charman and Hall/CRC, London.
- Lewis, R. (2020). *Nicotiana tabacum* L.: Tobacco. In: Novak J., Blüthner WD. (eds) *Medicinal, Aromatic and Stimulant Plants. Handbook of Plant Breeding*, 12. Springer, Cham. Chapter 9 *Nicotiana tabacum* L.: Tobacco. https://doi.org/10.1007/978-3-030-38792-1_9. 345-375.
- Maleki, H., Karimzadeh, G., Darvishzadeh, R & Sarrafi, A. (2011). Correlation and sequential path analysis of some agronomic traits in tobacco (*Nicotiana tabacum* L.) to improve dry leaf yield. *Australian Journal of Crop Science*, 5(12), 1644–1648.
- Mitreski, M., Aleksoski, J., Korubin-Aleksoska, A., Trajkoski, M., & Trajkoski, J. (2018). Variation of morphological properties in Virginia tobacco types. *Journal of Agriculture and Plant Sciences*, 16(1), 83–87.
- Mokreva, T., Rojtchev, V., Dimova, D. (2001). Possibilities in MS Excel for analysis of genotype-environment interaction in agricultural crops. *Agricultural University-Plovdiv, Scientific papers, XLVI*(1), 79–84.
- Nghiem Dung Tien, Hang Thi Thuy Vu, Ninh Van Nguyened, Cham Thi Tuyet Le. (2024). Growth, yield and quality variability of flue-cured tobacco in response to soil and climatic factors in Northern Vietnam. *Italian Journal of Agronomy*, 19, 1–16.
- Pashova, T., Tabakov, S., & Mihaylova, E. (2025). Effect of different photo-sensitive nets on the colour of apple cv. Florina. *Agricultural Science Digest*, 0, 1–7.
- Piepho, H. (2019). A Comparison of the ecovalence and the variance of relative yield as measures of stability. *Journal of Agronomy and Crop Science*, 173(1), 1–4.
- Risteski, I., & Kocoska, K. (2012). Results of broadleaf tobacco breeding in Scientific Tobacco Institute – Prilep. *Genetics and plant breeding*, 643-648.
- Shah Syed Mehar Ali, Ashfaq Shfaq Ahmad, Fida Mohammad, Hidayat-Ur-Rahman, Gul Woras, Mohammad Yasir Khan & Dawood Jan. (2008). Genotypic evaluation of some flue-cured Virginia tobacco genotypes for yield and quality traits. *Sarhad J. Agric.*, 24(4), 607–611.
- Spasova-Apostolova, V., Kasheva, M., Masheva, V., & Radev, Z. (2023). Study of main chemical indicators nicotine, total sugar and total nitrogen in tobacco lines of Krumovgrad ecotype. *Bulgarian Journal of Crop Science*, 60(6), 27–36.
- Spasova-Apostolova, V. (2024). Comparative study of oriental tobacco varieties from four ecotypes in the conditions of an experimental field – Markovo. *Bulgarian Journal of Crop Science*, 61(2), 61–69.
- Srbinoska, M., Risteski, I., & Kocoska, K. (2017). Harvesting and curing Burley and Virginia Tobacco Stalks for Biomass. *Tobacco*, 67(1), 56–64.
- Suggs, C., Beeman, J., & Splinter, W. (1960). Physical properties of green Virginia-type tobacco leaves. *Tobacco Sciences*, 71-77.
- Tabacova, E., & Drachev, D. (1996). Growing high-quality Virginia tobacco in the Pazardzhik region. *Bulgarian Tobacco*, 2, 10–13.
- Tang Zuoxin, Lulu Chen, Zebin Chen, Yali Fu, Xiaolu Sun, BinbinWang, Tiyyuan Xia. (2020). Climatic factors determine the yield and quality of Honghe flue cured tobacco. *Scientific Reports*, 10, 19868.
- Tso, T. (1972). *Physiology and biochemistry of tobacco plants*. ISBN: 978-0879330002, Dowden, Hutchinson & Ross; First Edition, 305-311.
- Weinberg, S., & Abramowitz, S. (2016). *Statistics using IBM SPSS, An Integrative Approach*, Cambridge University Press.
- Wricke, G. (1962). Über eine Methode zur Erfassung der ökologische Streubreite in Feldversuchen, *Z. Pflanzenzüchtung*, 47, 1, 92–96.
- Wricke, G. (1966). Über eine Biometrische Methode zur Erfassung der ökologischen Anpassung. *Acta Agric. Scand. Suppl.*, 16(1), 98–101.
- Wu Wei, Xiao-Ping Tang, Chao Yang, Hong-Bin Liu, Nai-Jia Guo. (2013). Investigation of ecological factors controlling quality of flue-cured tobacco (*Nicotiana tabacum* L.) using classification methods. *Ecological Informatics*, 16, 53–61.
- Yanyan, Li, Wang Lin, Li Xihong, Li Jianping, Zhang Ting, & Chen Zhengu. (2015). The climatic characteristics and the influence of climate factors on flue-cured tobacco quality in Jinshennong Tobacco-producing Area, *Chinese Tobacco Science*, 36(3), 13–18.
- Yang Jing, Jie Chen, Rusong Yang, JunTang, Hao Huang. (2015). Research Progress of Factors Influencing the Yield and Quality of Flue-cured Tobacco. *Agricultural Science & Technology*, 16(4), 820–825.
- Yonchev, Y. (2014). Study of the distribution of some viral diseases und the respective resistance in Virginia and Burley tobacco in South Bulgaria. *Thesis*, TTPI – Markovo, 97–98.
- Yonchev, Y. Drumeva-Yoncheva M., & Stoimenova E., (2014). Selection material of tobacco from the Virginia variety group with complex resistance to tobacco and potyviruses. *Jubilee international scientific conference. "70 years of the Tobacco and Tobacco Products Institute"*, ISBN - 978-954-702-103-7.
- Zhao, X., Q. Cheng, M. Luan and Y. Zhang. (2024). Effects of Environmental Factors on the Growth and Quality of the Tobacco Variety Zhusha 2: A Case Study in Yunan, China. *Pakistan Journal of Agricultural Sciences*, 61, 733–741.
- Zeb, A., Rauf, A., Bano, N., Qayash, M., Yasin, M., Khan, I., Abidullah, S., Khan, W., Ullah, M., Khan, A., & Gul, S. (2023). Morphogenetic analysis of different flue cured virginia (FCV) exotic hybrid varieties. *Pakistan Journal of Weed Science Research*, 29(4), 221–228.