Comparative Technological Assessment of the Quality Formation of Local and Introduced Varieties of Virginia Flue-cured and Burley Tobaccos

Nikolay Nikolov[1], *Violeta* Nikolova[2], and *Venelina* Popova[3*]

[1]Tobacco and Tobacco Products Institute, Agricultural Academy, 4108 Markovo, Bulgaria
[2]Institute of Food Preservation and Quality, Agricultural Academy, 4000 Plovdiv, Bulgaria
[3]University of Food Technologies, 4002 Plovdiv, Bulgaria

Abstract. The production of "marketable" tobacco is decisive in the preferences of local farmers towards certain varieties of Virginia and Burley tobaccos. The aim of the study was to complete a comparative technological evaluation of the quality potential of introduced and local varieties of Virginia flue-cured and Burley air-cured tobaccos in Bulgaria. The investigation was conducted with tobaccos from 2018 crop year and included 4 varieties of Virginia flue-cured and 2 varieties of Burley tobacco (local and introduced), grown in different regions of Southern and Northern Bulgaria. The complex assessment of tobacco quality was based on leaf and smoke chemical indicators, external leaf quality elements and smoking performance of the studied tobaccos. The final rating was achieved by referring to the value of the calculated "quality indices". Two of the local varieties, V 0454 from region Parvomay and PVH 19 from Central Northern Bulgaria were rated the best in the complex evaluation of the FCV tobaccos. A better complex expression of quality was found in the introduced (trial) variety Burley N compared to the local Burley 1317 in Southern Bulgaria region.

1 Introduction

Despite the unfavorable trends in tobacco production over the past two to three decades, it continues to be one of the most widely grown non-food crops in the world [1, 2]. In terms of revenue and total turnover, tobacco production remains among the leading sectors in the global market arena, showing a progressive shift towards high quality standards and growing concern for consumers and the environment [3, 4]. In this regard, a number of studies have been devoted to the search for solutions to maintain high quality through efficient use of available resources, application of environmentally friendly practices, while adequately covering the high costs of producing this labor-intensive crop [1, 3-11].

In its cultivation, tobacco is often exposed to various forms of abiotic stress, such as drought or floods, heat or cold, low or high light intensity, soil salinity, chlorides, heavy

* Corresponding author: vpopova2000@abv.bg

metals, ultraviolet radiation, etc. In addition, climate changes in terms of higher temperatures and changing precipitation patterns will continue to have a significant effect on its productivity and quality in the future [12]. In this regard, the search for varieties capable of overcoming environmental challenges has increased the importance of tobacco breeding programs [13-15].

It is well-known that tobacco can easily adapt to a wide range of conditions, but, still, it is a species highly sensitive to the environment. The varietal diversity in tobacco predetermines the formation of different leaf quality under deviating environmental, agricultural and processing conditions. In this regard, there are numerous studies, which related the chemical indicators of Virginia and Burley tobacco quality with the influencing factors in leaf production. For example, an investigation on the yield and quality of three cultivars of flue-cured tobacco in Honghe, Yunnan Province, China [16] revealed that climatic factors were the main regulators, but the quality of two of the cultivars, K326 and Yun87, was mostly influenced by moisture, whereas that of the third cultivar, Hongda, was mostly affected by temperature. Depending on the environmental variables, the chemical components and their proportions determining the quality of tobacco leaves in the three cultivars regarded above varied as follows: nicotine, 2.18-3.04%; reducing sugars, 24.87-29.79%; nitrogen – 1.71-2.17%; potassium (K) – 1.37-1.94%; reducing sugars to nicotine ratio – 11.17-16.77; total nitrogen to nicotine ratio – 0.71-0.87 [16]. A study of Virginia flue-cured and Burley tobaccos from Ethiopia confirmed variation in nicotine levels, both between varieties and within the same variety in different growing areas [2]. The nicotine content was 3.26% and 2.20% in Virginia tobacco from Billate and Shewa Robit districts, respectively, and as low as 0.65% in Burley tobacco from Awassa district. The influence of mineral and/or organic fertilizers in flue-cured tobacco production and their correlation with leaf quality indices, such as total and reducing sugars, nicotine, total nitrogen, K, as well as the economic value of the tobacco produced (average price; high, medium and lower grade proportions) was also followed [4, 10, 17, 18].

The broadleaf tobaccos (Virginia flue-cured and Burley) grown in Bulgaria differ to a certain extent in comparison with the typical renowned tobaccos, such as those produced in the USA, Zimbabwe and some other countries; an observation that also applies to the tobaccos in the dominating tobacco producing countries in the European Union (Italy, Poland, Hungary, Greece, etc.) and beyond EU [10, 19]. In a study on the effect of different levels of nitrogen and foliar fertilization on the chemical characteristics of Burley tobacco in Bulgaria the following contents were reported: nicotine – 2.18-3.17%, reducing sugars – 0.72-2.21%, total nitrogen – 2.26-4.08% [20]. A three-year investigation of the economic and chemical indicators of introduced Burley tobacco lines showed the following variations: nicotine – from 1.96% to 2.24%; soluble sugars – from 0.34% to 0.63%; total nitrogen – from 3.18% to 3.46% [21]. Another study on the quality indicators of domestically produced Virginia tobaccos also revealed significant differences in the formation of tobacco quality, both on a regional and varietal basis, in terms of chemical composition, leaf and smoke evaluation [22]. A comparative analysis of imported and locally produced Burley tobaccos [23] reported a good level of quality in terms of the investigated chemical indicators: high nicotine (2.50-4.52%) and total nitrogen (2.96-3.31%) contents, very low reducing sugars (about 1%), and relatively high levels of ash (between 16.32% and 18.69%).

The production of “marketable” raw material is decisive in the preferences of local farmers towards certain varieties of Virginia and Burley tobaccos. Given the change of climatic factors and their critical influence on the formation of tobacco quality, as well as the limited number of specific technological studies regarding the existing varietal structure, annual investigations of the quality level of the tobaccos produced in the various regions of Bulgaria with a view to their market realization is strongly justified. In this regard, a

comparative technological study was carried out on the quality potential of introduced and local varieties of Virginia flue-cured and Burley air-cured tobaccos in Bulgaria.

2 Materials and methods

2.1 Plant material

The investigation was conducted with 2018 crop year tobaccos and included 4 varieties of Virginia flue-cured and 2 varieties of Burley tobacco, grown in different regions of Southern and Northern Bulgaria. A key to the production regions, which supplied the tobacco material is presented in Table 1.

Table 1. Key to the production regions, which supplied the samples of Virginia flue-cured and Burley tobaccos

Tobacco type	Region	Variety	Variety origin
Virginia flue-cured	Plovdiv	PVH 19	Local
	Parvomay	V 0454	Local
	Central Northern Bulgaria	PVH 19	Local
		VS	Introduced (trial), Poland
		V	Introduced (trial), Greece
Burley	Southern Bulgaria	Burley 1317	Local
		Burley N	Introduced (trial), Spain

Tobacco cultivation followed the farmer practices established in the respective region; leaf harvesting and curing was in accordance with the standardized technologies [24, 25]. The analytical samples were based on middle leaves (cutters stalk position) [26, 27], and the farmer-supplied bulk material was additionally graded, in order to eliminate highly damaged or other non-compliant leaves and to secure uniform samples for the analysis of the regarded local and introduced varieties.

2.2 Methods

The following analyses related to tobacco quality were carried out:

2.2.1 Chemical composition of tobacco leaves

The respective indices (%) were determined according to the standardized methods at the accredited laboratory of the Tobacco and Tobacco Products Institute – Agricultural Academy (TTPI-AA), Markovo, Bulgaria: nicotine (ISO 15152:2003), reducing sugars (ISO 15154:2003), total nitrogen (BDS 15836:1988), ash (ISO 2817:1999), and ammonia (accredited method of TTPI, 1994).

The ratios „total nitrogen/nicotine“ and „reducing sugars/nicotine“ were calculated, as important indicators of Virginia flue-cured tobacco quality [16].

2.2.2 Chemical composition of tobacco smoke

The indicators of tobacco smoke composition – nicotine and tar content (mg/cig), were calculated using the regression correlations established previously for the two types of tobacco in the study [28]. Beside leaf nicotine content in relation to smoke nicotine, those regression models combined three other chemical indicators of tobacco leaves to obtain the

respective tar levels in the smoke – potassium, as K_2O (determined according to BDS 17365:1994), hexane extract (using Tecator Soxtec extraction system HT6, Tecator, Sweden) for Virginia flue-cured tobacco; potassium and ether extract (Soxtec HT6) for Burley tobacco [28].

2.2.3 Expert assessment of tobacco leaf quality

The task was completed by a five-member expert panel on coded (blind) samples. The method of direct comparison of samples was applied, in which each expert evaluated the complex manifestation of the external leaf quality elements. The statistical processing of individual results for reliability depended on the number of samples compared. In the case of a paired-comparison (two samples) the unanimity of results was tested by the critical ratio number (*CRN*), and in the case of comparison of three or more samples – by Kendall's coefficient of concordance (*W*) and the *F*-test. The limiting value of the coefficients were 1.96 and 0.50, respectively, at a probability level of 95%, i.e. expert opinions were considered unanimous if $CRN>1.96$ and $W>0.50$ [29].

2.2.4 Expert assessment of tobacco smoking quality

The task was completed by a five-member smoking panel on coded samples. In the smoking test direct comparison was applied when only two tobacco varieties were assessed, while in the case of three or more samples the method of indirect rating was applied, at a full combination of the samples in the series. The evaluation of the statistical significance of the data thus obtained was done in the same way as described above.

2.2.5 Complex evaluation of tobacco quality

The complex evaluation of tobacco quality and the final rating of the compared tobacco varieties was based on the results achieved in the previous steps of the evaluation process, according to the procedure already described [22, 23]. It included the most important quality-defining indicators – selected chemical indices, depending on the tobacco type, smoke tar and nicotine, leaf and smoking quality assessment results. In brief, the samples in a series were awarded ranks based on the value of the respective indicator, accounting for its positive or negative relation to tobacco quality. Each indicator was associated with a respective coefficient of importance (coefficient of relative weight), which was further used to obtain the value of the quality index. The final rating of the compared tobacco samples in the complex evaluation procedure reflected the achieved sum of the quality indices. In the order thus obtained, the sample with the smallest sum of quality indices receives rank 1 (i.e. reveals the highest complex quality).

3 Results and discussion

3.1 Chemical composition of tobacco leaves and smoke

3.1.1 Virginia flue-cured tobacco

The results from the chemical analysis of the Virginia flue-cured (FCV) tobacco varieties are presented in Table 2.

In the discussion of results, the emphasis in the assessment of the chemical indicators of the varieties was mainly on those known as characteristic for FCV tobacco: nicotine and reducing sugars content, and total nitrogen-to-nicotine and reducing sugars-to-nicotine ratios. In general, the ranges characteristic for the high-grade ("typical") FCV tobacco, indicative of a more balanced leaf and smoke composition, are: nicotine – above 1.8%, with a total nitrogen/nicotine ratio between 0.6 and 1.0; medium reducing sugars content (15-20%) at a relatively lower reducing sugars/nicotine ratio values (between 7 and 12).

Table 2. Chemical indices of Virginia flue-cured tobacco

Region	Variety	Index									
		Tobacco composition								Smoke composition	
		NC[a] *(%)*	*RS*[b] *(%)*	*TN*[c] *(%)*	*Ash (%)*	*RS/ NC*	*TN/ NC*	*K_2O (%)*	*HE*[d] *(%)*	*NC (mg/cig)*	*Tar (mg/cig)*
Plovdiv	**PVH 19**	0.91	32.40	1.32	7.50	35.60	1.45	1.20	4.67	0.74	19.01
Parvomay	**V 0454**	2.07	23.60	1.50	8.33	11.40	0.72	1.13	5.68	1.73	18.38
Central Northern Bulgaria	**PVH 19**	0.81	25.80	1.58	9.86	31.85	1.95	1.83	4.27	0.68	18.77
	V	1.60	27.30	1.02	10.30	17.06	0.64	0.70	5.22	1.28	18.96
	VS	0.48	29.60	1.11	8.55	61.67	2.31	1.85	4.34	0.39	18.69

[a] NC – Nicotine
[b] RS – Reducing sugars
[c] TN – Total nitrogen
[d] HE – Hexane extract

As seen from the data in Table 2, nicotine content varied in a wide range between the varieties – from 0.48% in the introduced VS (Poland) variety to 2.07% in the local V 0454 variety. The second of the introduced varieties (V, Greece) had a nicotine content closer to the highest value (1.60%). The highest concentration of reducing sugars was found in PVH 19 variety from Plovdiv (32.40%), and the lowest – in the local variety V 0454 from Parvomay (23.60%). Total nitrogen content varied in a relatively narrow range, from 1.02% to 1.58%; the two introduced varieties, V and VS, had similar and low total nitrogen levels, 1.02% and 1.11%, respectively, while the local varieties were grouped closer to the maximal value. The mineral matter content (expressed as ash content) did not differentiate between the analyzed local and introduced FCV varieties; the biggest ash content was found in V variety (10.30%), and the minimal – in PVH 19 variety from Plovdiv region (7.50%). Much more balanced (i.e. favourable) ratios of reducing sugars/nicotine and total nitrogen/nicotine were registered for the local V 0454 variety (Parvomay) and the introduced V variety. The highest smoke nicotine level was found for the local V 0454 variety (region Parvomay), and the lowest – for the trial variety VS. The tar content did not suggest significant differences between the compared tobaccos, with a variation range between 18.38 mg/cig and 19.01 mg/cig. It could be summarized that in the region of Central Southern Bulgaria the trial variety V (origin: Greece) formed a more balanced chemical composition compared with the local PVH 19 variety and the second introduced variety VS (Poland). In turn, the latter VS variety deviated substantially from the rest of the varieties in the study, with a minimal nicotine content (as low as 0.48%) and maximal reducing sugars/nicotine ratio (61.67). The other characteristic ratio, total nitrogen/nicotine, also took maximal value (2.31), thus determining insufficient balance in the smoking performance of the variety.

Those results complied with the data for the reducing sugars content in FCV tobacco reported in [16], but differed with regard to the other chemical indicators – showing lower nicotine and total nitrogen contents, and higher reducing sugars/nicotine ratio. On the other hand, our results were close to the nicotine content seen in [10], but deviated from the

reported reducing sugars, potassium and total nitrogen levels. The current results agreed well with previous data for the regarded chemical indices for FCV tobacco in Bulgaria [22], with the exception of the lower nicotine content in the studied varieties. Those deviations from previous data once again confirmed the strong impact of variety, soil and climatic factors on the formation of the profile of raw tobacco.

3.1.2 Burley tobacco

Table 3 presents the results from the determination of the chemical indicators for the Burley tobacco varieties in the study.

Table 3. Chemical indices of Burley tobacco

Region	Variety	Index								
		Tobacco composition							Smoke composition	
		NC[a] *(%)*	*RS*[b] *(%)*	*TN*[c] *(%)*	*Ash (%)*	*Ammonia (%)*	*K_2O (%)*	*EE*[d] *(%)*	*NC (mg/cig)*	*Tar (mg/cig)*
Southern Bulgaria	**Burley 1317**	2.74	0.57	4.29	15.97	0.34	2.51	4.40	2.00	27.16
	Burley N	3.89	0.41	3.72	20.10	0.48	4.26	7.07	2.93	18.20

[a.] NC – Nicotine
[b.] RS – Reducing sugars
[c.] TN – Total nitrogen
[d.] EE – Ether extract

The nicotine content in the introduced tobacco variety (Burley N, Spain; 3.89%) was substantially higher than that in the local variety (Burley 1317; 2.74%). Both values were close to the characteristic nicotine content in Burley tobacco from typical producing countries. As it is known, Burley tobacco is associated with minimal (trace) reducing sugars content, thus requiring intensive casing in the manufacturing of American blend cigarettes. The reducing sugars contents was typically low and nearly identical in both varieties studied, about 0.50%. The numerical differences in the total nitrogen content were moderate and the values fall within the range typical for the tobacco type; 4.29% and 3.72% in Burley 1317 and Burley N variety, respectively. The ash content and leaf structure are strongly and positively related to tobacco combustibility. The results showed relatively higher ash content in the trial variety (20.10%), which is close to the typical burley tobaccos. Ammonia in tobacco leaves is considered to adversely affect smoking properties, i.e. its content is negatively correlated with tobacco quality. Our data revealed a type-specific ammonia content in the studied varieties, varying from 0.34% for Burley 1317 to 0.48% for Burley N. Nicotine in tobacco smoke in both samples corresponded to the variation of nicotine content in the leaves. Tar content was much lower in Burley N smoke (18.20 mg/cig) compared to Burley 1317 (27.16 mg/cig).

It could be summarized that the chemical composition data above showed very good results for the quality level of both tobacco varieties. Tobacco assessment by the objective chemical indicators spoke of an advantage for the introduced variety Burley N (origin: Spain) compared to the local Burley 1317 variety.

3.2 Expert assessment of tobacco leaf quality

The summarized results from the expert assessment of leaf quality for the studied varieties of Virginia flue-cured and Burley tobaccos in Bulgaria are presented in Table 4.

Table 4. Rating by leaf quality of Virginia flue-cured and Burley tobaccos

Expert No	Variety / Region						
	Flue-cured Virginia tobacco					Burley tobacco	
	PVH 19	V 0454	PVH 19	V	VS	Burley 1317	Burley N
	Plovdiv	Parvomay	Central Northern Bulgaria			Southern Bulgaria	
1	4	3	1	2	5	2	1
2	3	2	1	5	4	2	1
3	5	3	1	2	4	2	1
4	3	2	1	5	4	2	1
5	5	2	1	3	4	2	1
Rating	**4.5**	**2**	**1**	**3**	**4.5**	**2**	**1**

3.2.1 Virginia flue-cured tobacco

The results proved statistically reliable difference between the compared varieties (with W=0.70), which gave reason to accept the rating order indicated in Table 4, namely: the first place according to the complex assessment of external leaf quality elements occupied PVH 19 variety (from Central Northern Bulgaria region), followed by another local variety, V 0454 (Parvomay), while the last two positions took PVH 19 (from Plovdiv region) and VS varieties.

3.2.2 Burley tobacco

Burley N (trial variety, Spain) achieved a better evaluation in terms of external leaf quality characteristics compared to the local Burley 1317 variety, with a proven reliable difference between the samples (CRN=2.24).

3.3 Expert assessment of tobacco smoking quality

The results from the assessment of the smoking profile of the studied varieties of Virginia flue-cured and Burley tobaccos are presented in Table 5.

Table 5. Rating by smoking properties of Virginia lue-cured and Burley tobaccos

Expert No	Variety / Region						
	Flue-cured Virginia tobacco					Burley tobacco	
	PVH 19	*V 0454*	*PVH 19*	*V*	*VS*	*Burley 1317*	*Burley N*
	Plovdiv	*Parvomay*	*Central Northern Bulgaria*			*Southern Bulgaria*	
1	2	1	3	5	4	1	2
2	2	1	3	5	4	1	2
3	4	1	2	5	3	1	2
4	3	1	2	5	4	1	2
5	2	1	3	5	4	1	2
Rating	**2.5**	**1**	**2.5**	**5**	**4**	**1**	**2**

3.3.1 Virginia flue-cured tobacco

The comparison of the smoking properties of FCV varieties proved significance and unanimity in the individual rankings by the panelists (*W*=0.70), and, correspondingly, validated the resultant rating order shown in Table 5. The variety with the best smoking profile was the local V 0454 from Parvomay region, followed by PVH 19 variety from the regions of Plovdiv and Central Northern Bulgaria with the same rank, and the two trial, introduced varieties, VS (Poland) and V (Greece).

3.3.2 Burley tobacco

Better score in the smoking assessment tests was achieved by the local Burley 1317 compared to the introduced Spanish Burley N variety, with a proven reliable difference between the compared tobaccos (*CRN*=2.24).

3.4 Complex evaluation of tobacco quality

3.4.1 Virginia flue-cured tobacco

The complex rating of the studied FCV tobaccos based on the score of quality indices is presented in Table 6.

Table 6. Complex Rating of Virginia flue-cured tobacco

Indicator	Variety rank					CI[a]	Variety quality index				
	PVH 19	***V 0454***	***PVH 19***	***V***	***VS***		***PVH 19***	***V 0454***	***PVH 19***	***V***	***VS***
	Plovdiv	***Parvomay***	***Central Northern Bulgaria***				***Plovdiv***	***Parvomay***	***Central Northern Bulgaria***		
Nicotine (%)	3.5	1	3.5	2	5	0.20	0.70	0.20	0.70	0.40	1.00
Total nitrogen/ Nicotine	3	1.5	4	1.5	5	0.18	0.54	0.27	0.72	0.27	0.90
Reducing sugars/ Nicotine	4	1	3	2	5	0.12	0.48	0.12	0.36	0.24	0.60
Tar (mg/cig)	3	3	3	3	3	0.10	0.30	0.30	0.30	0.30	0.30
Leaf quality	4.5	2	1	3	4.5	0.15	0.68	0.30	0.15	0.45	0.68
Smoking quality	2.5	1	2.5	5	4	0.25	0.63	0.25	0.63	1.25	1.00
Sum of quality indices							***3.33***	***1.44***	***2.86***	***2.91***	***4.48***
Complex rating							**4**	**1**	**2**	**3**	**5**

[a.] CI – Coefficient of importance

The results from the final complex assessment showed that the local variety V 0454 from Parvomay region was rated as the best, followed by the other local variety, PVH 19 from Central Northern Bulgaria, the introduced V (Greece) variety, the local PVH 19 variety (from Plovdiv region) and in the last place – the second trial variety VS (origin: Poland, from Central Northern Bulgaria region).

3.4.2 Burley tobacco

The results from the complex rating of the studied Burley varieties based on the score of their quality indices is presented in Table 7.

Better overall quality level was found for the introduced variety Burley N (origin: Spain) compared to the local Burley 1317 variety.

Table 7. Complex rating of Burley tobacco

Indicator	Variety rank		CI[a]	Variety quality index	
	Burley 1317	***Burley N***		***Burley 1317***	***Burley N***
Nicotine (%)	2	1	0.20	0.40	0.20
Reducing sugars (%)	1.5	1.5	0.18	0.27	0.27
Ash (%)	2	1	0.12	0.24	0.12
Ammonia (%)	1	2	0.10	0.10	0.20
Leaf quality	2	1	0.15	0.30	0.15
Smoking quality	1	2	0.25	0.25	0.50
Sum of quality indices				***1.56***	***1.44***
Complex rating				**2**	**1**

[a] CI – Coefficient of importance

4 Conclusion

The complex quality level of local and introduced (trial) varieties of Virginia flue-cured and Burley tobaccos from different growing regions in Bulgaria was assessed, based on the analysis of leaf and smoke chemical indicators, the external leaf quality elements and the smoking performance of the studied tobaccos.

The general assessment of the chemical composition of the investigated Virginia flue-cured varieties showed markedly better indicators – close to those characteristic of the "typical" flue-cured Virginia tobacco – in the local V 0454 variety from Parvomay region and the introduced V variety (Greece) from Central Northern Bulgaria region (e.g. leaf and smoke nicotine content, reducing sugars, total nitrogen/nicotine, reducing sugars/nicotine). Two of the local varieties, V 0454 from region Parvomay and PVH 19 from Central Northern Bulgaria were rated better, with statistically validated difference, with regard to the overall perception of external leaf features and smoking quality. Correspondingly, the same two varieties were also pointed out as the best in the final grading of the FCV tobaccos in the study, based on their complex evaluation.

With regard to their chemical composition, the studied Burley varieties in Southern Bulgaria region indicated very good overall quality – high nicotine (2.74-3.89%) and total nitrogen (3.72-4.29%) content, minimal reducing sugars (about 0.50%), and relatively high ash content (15.97-20.10%). The introduced variety Burley N (Spain) revealed better chemical parameters and external leaf quality features than the local Burley 1317 variety, but was inferior to it in smoking properties. Thus, a better complex expression of quality was found in the introduced (trial) variety Burley N compared to the local Burley 1317.

References

1. M. Sifola, L. Carrino, E. Cozzolino, L. del Piano, G. Graziani, A. Ritieni, Potential of preharvest wastes of tobacco (*Nicotiana tabacum* L.) crops, grown for smoke products, as source of bioactive compounds (phenols and flavonoids). Sustainability **13**(4), 2087 (2021). https://doi.org/10.3390/su13042087
2. Z. Tassew, B. Chandravanshi, Levels of nicotine in Ethiopian tobacco leaves. SpringerPlus **4**, 649 (2015). https://doi.org/10.1186/s40064-015-1448-y
3. M. Bortolini, M. Gamberi, C. Mora, A. Regattieri, Greening the tobacco flue-curing process using biomass energy: A feasibility study for the flue-cured Virginia type in Italy. Int. J. Green Energy **16**(14), 1220-1229 (2019). http://dx.doi.org/10.1080/15435075.2019.1671397
4. I. Tabaxi, I. Kakabouki, C. Zisi, A. Folina, S. Karydogianni, A. Kalivas, D. Bilalis, Effect of organic fertilization on soil characteristics, yield and quality of Virginia tobacco in Mediterranean area. Emir. J. Food Agric. **32**(8), 610-616 (2020). http://dx.doi.org/10.9755/ejfa.2020.v32.i8.2138
5. L. Zou, J. Su, T. Xu, X. Ji, T. Wang, Y. Chen, Y. Jiang, B. Hu, Untargeted metabolomics revealing changes in aroma substances in flue-cured tobacco. Open Chem. 21(1), 20220326 (2023). https://doi.org/10.1515/chem-2022-0326
6. C. He, R. Chen, K. Ren, G. Zhao, C. He, B. Hu, C. Zou, Y. Jiang, Y. Chen, A predictive model for the sensory aroma characteristics of flue-cured tobacco based on a back-propagation neural network. SN Appl. Sci. 2, 1-11 (2020). https://doi.org/10.1007/s42452-020-03656-4
7. K. Wei, J. Bin, F. Wang, C. Kang, On-line monitoring of the tobacco leaf composition during flue-curing by near-infrared spectroscopy and deep transfer learning. Anal. Lett. 55(13), 2089-2107 (2022). https://doi.org/10.1080/00032719.2022.2046021
8. A. Upadhyaya, P. Jeet, P. Sundaram, A. Sing, K. Saurabh, M. Deo, Efficacy of drone technology in agriculture: A review. J. AgriSearch 9(3), 189-195 (2022). https://doi.org/10.21921/jas.v9i03.11000
9. N. Diana, A. Jamil, Y. Yogi, S. Nugraheni, L. Verona, Improving the production and quality of Virginia tobacco through topping and suckering: A review. IOP Conf. Ser.: Earth Environ. Sci. 974, 012020 (2022). https://doi.org/10.1088/1755-1315/974/1/012020
10. C. Jiang, C. Zu, H. Wang, Y. Yan, Y. Liu, J. Shen, Straw return with biochar increased soil macro-aggregates and improved Flue-cured tobacco (*Nicotiana tabacum* L.) yield and quality. Appl. Ecol. Environ. Res. **20**(1), 207-219 (2022). http://dx.doi.org/10.15666/aeer/2001_207219
11. P. Yang, P. Drohan, H. Long, M. Yang, Y. Bian, E. Ma, Water use efficiency, yield and quality of tobacco (Nicotiana tabacum L.) using negative pressure irrigation. Ind. Crops Prod. 178, 114552 (2022). http://dx.doi.org/10.1016/j.indcrop.2022.114552
12. K. Sarala, K. Rao, C. Nanda, K. Baghyalakshmi, R. Darvishzadeh, K. Gangadhara, J. Rajappa, Abiotic stress resistance in tobacco: advances and strategies, in Genomic Designing for Abiotic Stress Resistant Technical Crops (Springer International Publishing, Cham, 2022), 329-427. https://doi.org/10.1007/978-3-031-05706-9_10
13. A. Dadras, H. Sabouri, G. Nejad, A. Sabouri, M. Shoai-Deylami, Association analysis, genetic diversity and structure analysis of tobacco based on AFLP markers. Mol. Biol. Rep. **41**, 3317-3329 (2014). http://dx.doi.org/10.1007/s11033-014-3194-6

14. T. Radoukova, Y. Dyulgerski, Biological indicators of Bulgarian and introduced Burley tobacco varieties. Bulg. J. Agric. Sci. **24**(6), 1059-1064 (2018). https://www.agrojournal.org/24/06-17.pdf
15. S. Kirkova, Y. Dyulgerski, Physical, technological, chemical and smoking properties of new lines and hybrids Burley tobacco. Tobacco **65**(7-12), 65-71 (2015).
16. Z. Tang, L. Chen, Z. Chen, Y. Fu, X. Sun, B. Wang, T. Xia, Climatic factors determine the yield and quality of Honghe flue-cured tobacco. Sci. Rep. **10**, 19868 (2020). https://doi.org/10.1038/s41598-020-76919-0
17. Y. Chen, K. Ren, X. He, Y. Chen, B. Hu, X. Hu, J. Li, Y. Jin, Z. Zhao, C. Zou, The response of flue-cured tobacco cultivar K326 to nitrogen fertilizer rate in China. J. Agric. Sci. **158**(5), 371-382 (2020). https://doi.org/10.1017/S0021859620000738
18. M. Sifola, I. Di Mola, E. Cozzolino, L. Ottaiano, G. Piccirillo, L. Del Piano, M. Mori, Yield response, quality traits, and nitrogen-use efficiency of a Burley tobacco crop grown in Mediterranean areas (Southern Italy) as affected by intensive N management. Agronomy **11**(9), 1837 (2021). https://doi.org/10.3390/agronomy11091837
19. L. Verona, Supriyadi, N. Diana, G. Fatah, Virginia tobacco sustainable production in Indonesia. IOP Conf. Ser.: Earth Environ. Sci. **974**, 012116 (2022). https://doi.org/10.1088/1755-1315/974/1/012116
20. R. Bozhinova, Effect of nitrogen rate and foliar fertilization on yield, quality leaf chemistry in Burley tobacco. Bulg. J. Crop Sci. 54(2), 33-39 (2017). https://cropscience-bg.org/page/en/details.php?article_id=472
21. M. Mutafchieva, N. Tahsin, Economic and chemical indicators of introduced male sterile lines of Burley tobacco. J. Mt. Agric. Balk. 18(4), 774-784 (2015). https://jmabonline.com/en/journal/1311-0489/issue/2015-18-4/
22. N. Nikolov, V. Nikolova, V. Popova, Complex evaluation of the quality of introduced and local varieties of Virginia flue-cured tobacco in Bulgaria, BIO Web Conf. **45**, 01013 (2022). https://doi.org/10.1051/bioconf/20224501013
23. N. Nikolov, V. Nikolova, V. Popova, D. Drachev, Comparative analysis and complex technological evaluation of Burley tobaccos, imported and produced in South Bulgaria tobacco region. Bulg. J. Agric. Sci. 27(6), 1147-1152 (2021). https://journal.agrojournal.org/page/en/details.php?article_id=3657
24. Tobacco and Tobacco Products Institute, Instruction for the Harvesting and Curing of Tobacco from Virginia Variety Group (Ministry of Agriculture and Food, Sofia, Bulgaria, 2016). https://www.mzh.government.bg/media/filer_public/2018/02/13/su6-vir-2016.pdf
25. Tobacco and Tobacco Products Institute, Instruction for the Harvesting and Curing of Tobacco from Burley Variety Group (Ministry of Agriculture and Food, Sofia, Bulgaria, 2016). https://www.mzh.government.bg/media/filer_public/2018/02/13/su6-byrl-2016.pdf
26. North Carolina State University (NCSU), 2021 Flue-cured Tobacco Guide (NCSU Cooperative Extension Service, NC, USA, 2021).
27. A. Bailey, B. Pearce, Stripping and preparation of tobacco for market, in 2023-2024 Burley and Dark Tobacco Production Guide (University of Kentucky, Lexington, KY, USA, 2023), 61-64. https://www2.ca.uky.edu/agcomm/pubs/ID/ID160/ID160.pdf
28. S. Gueorgiev, V. Popova, Developing a system for prognosis of tar and nicotine in cigarette smoke. Biotechnol. Biotechnol. Eq. **1**, 61-65 (1999). https://doi.org/10.1080/13102818.1999.10819020

29. V. P. Borovikov and I. P. Borovikov, STATISTICA. Statistical Analysis and Data Processing in the Windows Environment (Filin, Moscow, 1998).