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A review of some cultivation practices affecting the nicotine content of tobacco grown in Bulgaria

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Abstract

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Nicotine is one of the most important constituents in cured tobacco. Genetic and many cultivation factors affected nicotine accumulation in different tobacco types grown in Bulgaria. The highest nicotine concentrations in Oriental, Virginia and Burley tobaccos were obtained with high nitrogen application rates, reduced irrigation, lower plant density and early topping. These practices restricted starch accumulation which could lead to producing chemically imbalanced tobaccos (oriental and flue-cured). Lower nicotine levels were due to lower N fertilizer rates, higher plant density and better water regime. Delaying topping from the early bud stage, through to the full flowering also reduced nicotine levels. These agronomic factors can allow greater starch accumulation, therefore cured oriental and Virginia tobaccos could be imbalanced chemically with high sugar/nicotine ratio. Phosphorus, potassium, and some organic fertilizers had little or inconsistent effect on the nicotine content of tobacco leaves.

Keywords: tobacco; Oriental; Virginia; Burley; nicotine concentration; leaf quality; production practices

Introduction

Alkaloids are plant secondary metabolites that are widely present in *Nicotiana* species and contribute greatly to the quality of tobacco leaves (Sun et al., 2013). Alkaloids in tobacco provide a physiological stimulus to humans, which makes the use of tobacco products pleasurable. Based on the amounts of alkaloid accumulation in the leaves of *N. tabacum*, nicotine, nornicotine, anatabine and anabasine are the major alkaloids present (Bush, 1999). The main alkaloid of tobacco, nicotine, is produced in roots and translocated to the leaves. Nicotine is formed by a pyrrolidine and a pyridine ring in a process involving several enzymes. After synthesis in root cortical cells, a set of transporters is known to translocate nicotine upward to the aerial parts and store it in leaf vacuoles (Zenkner at al., 2019). Nicotine can range in concentration from 0.5% to 8% in the major cultivated tobacco species, *N. tabacum* and *N. rustica* (Leffingwell, 1999). Nicotine levels in tobacco are affected by genetics, environmental conditions and cultivation practices (Bush, 1999). The genotype plays a major role in alkaloid accumulation, indicating a high potential for manipulation of alkaloid content through traditional breeding (Sun et al., 2013). In a given genetic background (variety), certain agronomic practices may be implemented to cause nicotine concentrations to increase or decrease as desired. Production practices such as fertilization, irrigation, planting density, topping, sucker control, and harvesting affect plant growth and nicotine synthesis (Henry et al., 2019; Kurt & Kinay, 2021).

Since nicotine is the most important constituent in tobacco, it is desirable to obtain information on the relationship between nicotine concentration and varying cultivation practices and varieties. The tobacco industry has made efforts to develop low nicotine cigarettes, and therefore it may be desirable to manipulate the nicotine levels up or down (Campbell et al., 1982). Henry et al. (2019) also emphasized that a review of agronomic practices that can impact nicotine levels in tobacco should be useful for developing production strategies to achieve desired nicotine concentrations. Therefore, it is important to determine the effect of cultivation practices on nicotine concentration in different tobacco types (Oriental, Virginia and Burley) grown in Bulgaria.

Variety Selection

Nicotine content in leaves depends on the tobacco type and variety. Two major genes determine the base levels of total alkaloids in tobacco cultivars; however, these levels are further modified by minor genes or quantitative factors (Chaplin, 1975). Regardless of the agronomic practices that can be changed to affect leaf nicotine concentrations, the cultivar effect is the most critical factor for commercial flue-cured tobacco production (Henry et al., 2019). Campbell et al. (1982) also emphasized that the varieties had more effect on nicotine percent than any of the other variables such as nitrogen rates, topping heights and within-row spacing.

Numerous reports published in Bulgaria have described the influence of the tobacco type and variety on the accumulation of nicotine in the leaves. Shabanov & Kostanev (1971) reported nicotine concentration differed between two cultivars of oriental tobacco. Average nicotine level ranged from 0.27% in low nicotine variety Nevrokope 1638a to 1.03% in Nevrokope 5. Dyulgerski (2016) demonstrated a strong genotype effect on nicotine accumulation in Burley tobacco lines. According to Dyulgerski & Docheva (2017) the number of genes determining the expression of the nicotine content in Virginia tobacco is lower than in Burley tobacco. Stoilova et al. (2006) found that five Burley genotypes (Burley 21, Burley 1000, Burley 1317, Burley lines 849 and 857) produce nicotine levels between 1.10% and 3.3%. The authors suggested that the large differences observed were not due to environmental variation, as the cultivars were grown under the same conditions. The nicotine content of Bulgarian oriental tobacco varieties (Dzhebel basma 1 and Elenski 817) and introduced ones (Prilep 23 and Prilep 79-94) ranged from 1.22% to 1.98% (Zaprjanova & Hristozova, 2018). No significant differences in the nicotine levels of the three varieties tested (Elenski 817, Prilep 23 and Prilep 79-94) were obtained. According to Campbell et al. (1982) this suggests they have similar genetic potentials for nicotine production. Yancheva et al. (2008) reported that the nicotine concentration in the leaves of Krumovgrad oriental tobacco varieties varied within 0.46%-0.90% for Krumovgrad 988, 0.72%-1.01% for Zlatna Arda, 0.86%-1.11% for Akhrida, 0.70%-0.76% for Krumovgrad 58 and 0.83%-1.06% for Krumovgrad 90. The differences found were most likely due to varietal characteristics, nitrogen and phosphorus fertilization.

Studies carried out in our country indicate that nicotine concentrations varied among tobacco types, cultivars and breeding lines. The results confirm Chaplin's (1975) conclusion that plant breeders are capable of developing cultivars with different levels of nicotine. An important step towards successful tobacco production and achieving the desired nicotine concentrations is choosing the proper variety.

Mineral Fertilization

Mineral nutrition is essential for the growth and development of tobacco, as well as the synthesis and accumulation of nicotine (Henry et al., 2019). Nitrogen is a component of the nicotine molecule and is important in the synthesis of this constituent of tobacco (Flower, 1999). The accumulation of nicotine in the plant is regulated more by the nitrogen supply than any other plant nutrient (McCants & Woltz, 1967). When N fertilization has been precisely controlled and soil moisture kept adequate for the timely uptake and reduction of nitrogen, cured tobaccos obtained were compositionally balanced (Weybrew et al., 1983). Insufficient nitrogen limits nicotine production and allows greater starch accumulation. Excess N delays the transition from N reduction to starch accumulation, allowing for more nicotine synthesis (Peedin, 1999).

A number of researchers studied the effects of mineral fertilization on the chemical properties of tobacco grown in our country. Several authors found a positive relationship between increasing N levels and nicotine content in tobacco (Yancheva, 1998; Bozhinova et al., 2010; Bozhinova & Mutafchieva, 2014; Bozhinova, 2017). The concentrations of nicotine in cured leaves were 1.05%, 1.50%, 1.59%, and 1.83% respectively for 0, 25, 50, and 100 kg N ha^{-1} treatments, but the relationship between increasing N levels and sugar content in oriental tobacco was negative (Bozhinova et al., 2010). The results of Jordanov & Yancheva (1990) also showed a clearly defined trend toward increased nicotine content and decreased sugar concentration with increase in the nitrogen supply. The highest nicotine percentages in varieties Krumovgrad 988 (1.74%) and Harmanli 11 (2.11%) were from treatments that had the highest nitrogen rate. Angelova & Popova (2020) summarized that the Schmuck's number (ratio of soluble carbohydrates and proteins) can objectively characterize the main quality categories of oriental tobaccos. In this regard, Jordanov & Yancheva (1990) concluded that excessive nitrogen fertilizer rates (60 kg ha^{-1}) produced chemically imbalanced oriental tobacco, with a soluble carbohydrate/protein ratio decreasing below 1.0. The ratio of these components was better in tobacco from 20 and 40 kg N ha^{-1} treatments. Bozhinova & Mutafchieva (2014) found that the relationship between the nicotine content in Burley 420 variety and the nitrogen rates (70, 100, and 130 kg N ha^{-1}) fits the regression equation:

$$Y = 2.85 + 0.009x, \quad (1)$$

where Y = percent nicotine in cured leaves and x = kg ha^{-1} applied N.

Nikolov et al. (2021) concluded that the best complex quality was achieved by Burley 1317 variety, which had a nicotine concentration of 3.56%. Our equation (1) (Bozhinova & Mutafchieva, 2014) can be used to predict the rate of nitrogen fertilizer needed to achieve the desired level of nicotine in Burley tobacco. If y = 3.56% nicotine, the corresponding N rate is 78.9 kg ha^{-1}. This N level is in the 60 to 100 kg ha^{-1} rate range recommended for Burley tobacco in Bulgaria (Tanov et al., 1978).

In oriental tobacco, applied phosphorus had no effect on the nicotine of the cured leaf (Petrov et al., 1971). Other studies have also found that phosphorus and potassium fertilization had no measurable effects on the nicotine content in oriental tobacco (Jordanov & Yancheva, 1990; Yancheva, 1990). Flower (1999) summarized that the application of phosphorus does not have a consistent effect on the concentrations of nicotine in the cured leafs and the discrepancies reported in the literature probably reflect the conditions under which the experiments were done.

Different potassium rates (0, 75 and 450 kg K_2O ha^{-1}) applied over 42 years resulted in a small differentiation in nicotine content in the leaves of oriental tobacco variety Plovdiv 7 (Bozhinova, 2012). Increasing levels of K fertilization had no pronounced effect on nicotine content in Burley tobacco in the experiment we conducted in 2014 (Bozhinova, 2014). A study by Vann et al. (2012) also noted that there was no correlation between increased rates of potassium and total alkaloid content, while Mylonas et al. (1981) found a tendency of total alkaloids in the cured Samsun tobacco to decrease as applied potassium increased.

Field experiments were also conducted to determine the effects of varying levels of compound N-P-K fertilizer on the chemical composition of Oriental and Virginia tobacco. Stamatov & Bozhinova (2016) noted that the highest rate of compound fertilizer (200 kg ha^{-1}) increased nicotine content in leaves of oriental tobacco varieties Krumovgrad 944 and Krumovgrad 17 by 15.3% and 14.8%, respectively, compared to the control. Bozhinova (2021) found a positive correlation between the nicotine content in Virginia tobacco and the levels (0, 200, 400, and 600 kg ha^{-1}) of compound fertilizer NPK (15:15:15). The dependence of the reducing sugars content on the applied rates was negative. A balance in chemical constituents is a better expression of the chemical quality of tobacco than levels of individual chemical constituents (Edmundo & Long, 1988). The cured Virginia tobaccos are compositionally balanced with respect to sugars and nicotine (sugar/nicotine ratio between 6 and 8) (Weybrew et al., 1983). Bozhinova (2021) concluded that flue-cured tobacco variety 0514 was chemically balanced and the ratio between reducing sugars and nicotine was in the optimal range when fertilized with 400 kg ha^{-1} compound fertilizer. The effect of foliar fertility products Master (N:P:K 20:20:20 with high concentration of micro elements) (Valagro, Italy) and Sazolene SC (28% N) (Sadepan Chimica, Italy) on the nicotine content of Burley tobacco was inconsistent in our experiments and varied among years (Bozhinova, 2017). In 2011 nicotine concentration was lower in the unfertilized control compared to the foliar products, while in 2012 nicotine was not positively affected by foliar treatments.

Nitrogen fertilization had a stronger effect on nicotine concentration than other nutrients (phosphorus and potassium) applied. Leaf nicotine concentration was increased in different tobacco types by increasing rates of nitrogen fertilizers, but higher nitrogen rates and excess N limited reducing sugars accumulation. Weybrew et al. (1983) emphasized that excessive N fertilization can lower the sugars/nicotine ratio (S/N < 5) and such flue-cured tobaccos are chemically imbalanced.

Organic Amendments and Biostimulant Applications

Several studies have documented the effects of organic fertilizers and biostimulant applications on chemical properties of cured leaves. Kurt & Ayan (2014) noted that 9.5-10 t ha^{-1} of organic fertilizer (compost, chicken manure and livestock manure) resulted in meeting the required nicotine range for Xanthi/2A cultivar of 1.8-2.1%. On the other hand, Tabaxi et al. (2021) reported that organic fertilizers (compost and cattle manure) did not affect nicotine content in three oriental tobacco varieties.

Compost affects the indicators defining the quality of oriental tobacco Krumovgrad 90 (Angelova et al., 2016). The introduction of the compost to the soil leads to an increase of the total nitrogen and nicotine, and reduces the amount of reducing sugars, thus resulting in quality deterioration (particularly in the third and fourth harvest) of tobacco leaves. A study by Angelova & Popova (2020) demonstrated that the nicotine level of oriental tobacco leaves increased in treatments with addition of 20 t da^{-1} compost and 20 t da^{-1} vermicompost and decreased when 40 t da^{-1} compost and 40 t

da^{-1} vermicompost were applied. They concluded that adding 40 t da^{-1} compost and 40 t da^{-1} vermicompost to soils leads to improved tobacco quality compared to the control. However, the incorporation of 20 t da^{-1} of compost and 20 t da^{-1} of vermicompost into the soil results in lower quality tobacco (Schmuck number below 0.5).

Disrupted spores homogenates and exudates from germinating spores of *Glomus etunicatum* caused marked alterations in the content of the tobacco alkaloids and the expression of genes involved in their biosynthesis (De Andrade et al., 2013). The data of Subhashini (2013) and Begum et al. (2020) also showed that nicotine accumulation in tobacco leaves increased due to arbuscular mycorrhizal fungi inoculation. Our experiments confirmed that the addition of microbial inoculant containing *Glomus* spp. and *Bacillus* spp. increased nicotine content of oriental tobacco by 9.0% compared to non-treated control (Bozhinova, 2023).

The above results show that the effect of organic fertilizers on nicotine content of tobacco leaves was somewhat inconsistent. In most cases, nicotine levels depend on the quantity of the organic amendments applied.

Irrigation

Water supply is a major factor for efficient nitrogen use. The inversed relationship of low nicotine levels and high sugars in well-watered tobaccos and, conversely, a high nicotine and low sugars level in drought-stressed tobaccos is well established. Under water deficit conditions, root system gets deeper and greater quantities of nicotine are synthesized (Salehzade et al., 2009). Constant maintenance of soil moisture at a higher level resulted in better quality and a reduced nicotine concentration in flue-cured tobacco (Čavlek et al., 2006).

In our country a study by Ivanov & Slavova (1987) demonstrated that with an increase of irrigation level the nicotine percentage in leaves of oriental tobacco decreased. The highest nicotine percentage was observed in no irrigation treatment. According to Henry et al. (2019) soils with high moisture levels limit N and nicotine accumulation, largely due to N leaching from the soil. Regardless of the N level, the nicotine content of oriental tobacco was affected by the amount of irrigation water (Yordanov, 1979). Nicotine content decreased by 0.20% to 0.40% when the irrigation level increased from 50% of field capacity (FC) to 60%-70% of FC. The author noted that the best leaf quality (with optimal balance between nicotine, sugars and protein nitrogen) was produced by $N_2P_6K_6$ treatment at an irrigation level of 60% of the field capacity. Leaf quality was also acceptable in the $N_4P_6K_6$ treatment at an irrigation level of 70% of the FC. In different set of trials, Yancheva (1990) and Yancheva (1998) evaluated effects of irrigation on chemical characteristics of oriental tobacco. The results indicate that irrigation lowered the nicotine content in all primings of Krumovgrad 58 variety (Yancheva, 1998), as well as in the cured leaves of Krumovgrad 90 from all treatments with different rates of mineral fertilizers applied (Yancheva, 1990). These results were in agreement with the findings of Salehzade et al. (2009) and Kurt & Kinay (2021).

The field studies carried out in our country show that irrigation can be used as an effective tool for manipulating nicotine levels in tobacco leaves in accordance with the requirements of the cigarette industry.

Plant Spacing

The optimum plant density in a population is often determined by leaf usability, or quality, rather than yield (Flower, 1999). Narrow row spacing resulted to a reduction of nicotine content in three cultivars of the Greek Basmas, probably because of a high competition among roots where alkaloids are synthesized (Bilalis et al., 2015). The authors concluded that narrower row spacing may be used to produce tobacco with lower nicotine content, whenever this is desirable for blending purposes.

In most cases, the lower plant density levels increased percent of nicotine in tobacco grown in our country. The optimum plant population for conventional Djebel-basma tobacco production was 330,000 plants per hectare (Perfanov, 1969). If fertilizers were applied, plant populations can be increased to 400,000 plants ha^{-1}. Higher plant density (500,000 and 600,000 plants ha^{-1}) resulted in decreased leaf area. Nicotine levels also decreased whereas reducing sugars increased. Increased plant spacing from 15 to 20 cm within the row resulted in higher nicotine content in oriental tobacco (Petrov et al., 1971). In Greece higher nicotine content was also obtained from tobacco grown at wider spacing, since probably growth was higher in that case because of a lower plant density (Bilalis et al., 2015). Chifoudov (1979) reported that increased plant populations resulted in lower nicotine concentration in the leaves of oriental tobacco grown in Northeast Bulgaria. Dimitrova & Dragiev (1982) investigated the effect of plant spacing on four Burley tobacco varieties. Plant spacing of 80.0, 106.0 and 115.0 cm between rows and 45.0 and 50.0 cm within a row were studied. Lower plant populations (106 x 45 cm and 115 x 45 cm) resulted in a higher percentage of nicotine in cultivars studied. Closer spacing of plants (80 x 50 cm) resulted in increased reducing sugars and protein content.

Results of these studies indicate that variation in plant population could affect cured leaf quality and increasing of planting density generally produces cured leaves with decreased nicotine concentration and increased sugar content.

Topping

Eliminating the inflorescence via topping and controlling suckers lead to higher nicotine concentrations (Henry et al., 2019). The height and time of topping and their effects vary according to the type of tobacco. It is generally accepted that topping increases root growth (Flower, 1999). Nicotine is produced in root tips and early topping enlarged root system which ultimately raised nicotine content in leaves (Karim et al., 1999).

The general response to early topping was increased nicotine concentration of cured tobacco leaves. We also noted (Bozhinova & Dulgerski, 2010) that when tobacco plants were topped in the button stage, concentrations of nicotine in three burley tobacco genotypes were increased by 25.8%-68.7% compared to topping during late flowering. Time of topping had little effect on reducing sugars content. In a second field experiment we have studied the effects of different N rates and time of topping on leaf quality of tobacco variety Burley 420 (Bozhinova & Mutafchieva, 2014). Topping at early flowering stage produced higher nicotine concentration in leaves by 37.1%-44.7% than removal of the inflorescences at full flowering. Similar results were obtained by Donev et al. (1970) who found a trend for a decrease in nicotine in Burley tobacco with delay in stage of topping. The lowest concentration occurred in the untopped treatment (2.35%). The increase in nicotine concentration was largest in the early topped tobacco treated with maleic hydrazide (4.33%). The late topped plants (at full flowering) had a lower concentration of nicotine (2.84%). It was also found that the reducing sugars values for the topped Burley tobacco were higher than for the untopped tobacco. Petrov et al. (1971) reported that the maximum nicotine concentration in leaves of untopped oriental tobacco was 1.25%. A noticeable increase in nicotine (over 3%) was obtained in the topped plants (when removing the inflorescence and uppermost 12 leaves). Topping stage affected nicotine content of Virginia tobacco but had little effect on reducing sugars (Donev & Pamukov, 1970). The authors concluded that topping at button stage or 50% flowering produced the best quality and yield. Therefore, time and height of topping can also be used to modify the nicotine concentrations according to the market demands.

Conclusions

The objective of this review was to summarize the potential of some production practices to affect nicotine levels in three tobacco types (Oriental, Virginia and Burley) grown in Bulgaria. The results demonstrated that nicotine concentration was influenced by genetic and cultivation factors. Choosing the proper variety is the key step to successful tobacco production and achieving desired nicotine concentrations. Numerous studies have shown that the highest nicotine concentrations were obtained with high nitrogen application rates, reduced irrigation, lower plant density and early topping. These practices restricted starch accumulation which could lead to producing chemically imbalanced tobaccos (oriental and flue-cured). Lower nicotine levels result from lower N fertilizer rates, higher plant density and better water regime. Delaying topping from the early bud stage, through to the full flowering also reduced nicotine levels. These agronomic practices can allow greater starch accumulation, therefore oriental and Virginia tobaccos produced could be imbalanced chemically with high sugar/nicotine ratio. Phosphorus, potassium, and some organic fertilizers had little or inconsistent effect on the nicotine content of tobacco leaves. Proper N fertilization rate, optimal plant density, time and height of topping, moderate irrigations, applied when needed, produced optimal leaf quality, with balance in chemical constituents.

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