AIMING LOW

Producing tobaccos with 'minimally addictive' levels of nicotine presents significant challenges for breeders and growers.

By Stefanie Rossel

In recent years, several countries have been considering mandates for manufacturers to reduce nicotine in combustible tobacco to "minimally addictive levels"—that is, under 0.5 mg per gram. By decreasing the habit-forming ingredients, the thinking goes, cigarettes will become less attractive and consumption will decrease.

A webinar organized by Coresta on Dec. 5, 2023, highlighted the current understanding of low-nicotine tobacco (LNT) production capabilities as well as the successes and failures of applied research in the areas of genetics and agronomy.

Ramsey Lewis, a university faculty scholar at North Carolina State University in Raleigh, North Carolina, USA, spoke about the opportunities offered by modified plant genetics to reduce tobacco nicotine levels. A standard cigarette filler contains 15 mg to 25 mg of nicotine per gram or 1.5 percent to 2.5 percent, he noted; the World Health Organization recommends a 35-fold reduction, which corresponds to 0.4 mg per gram or 0.04 percent of nicotine.

Nicotine is the most abundant alkaloid in tobacco, accounting for 90 percent to 95 percent of the alkaloid content. As alkaloids are natural plant products, nicotine levels in a tobacco variety depend on genetics and the environment. In the U.S. *Nicotiana tabacum* collection, there are several lower nicotine species. A decrease in nicotine, however, means an increase in nornicotine, a carcinogenic tobacco-specific nitrosamine.

Lewis described various low-nicotine genetics experiments his team had conducted in 2023, where different known nicotine-expressing genes had been inactivated. While a transgenic approach got them close to a 0 mg nicotine content, the plants in the field were dramatically reduced. In addition, the LNT was high in nornicotine, and its cured leaf quality was altered—both side effects that regulatory authorities wouldn't accept, Lewis explained. His experiments, which also included a greenhouse trial, showed altered gene expression and altered physiology in low-nicotine plants.

Plants are living things and complex, Lewis pointed out, which results in several issues for tobacco growers and buyers of low-nicotine tobacco. At present, there are only a small number of commercially available varieties, which means that if LNT was suddenly mandated in a market, the regulation could practically not be translated into action.

Nicotine is also the natural defense mechanism of tobacco plants. The lower its content, the more the plant is susceptible to disease and insect pests. Lower nicotine also results in lower yields and higher production costs. In addition, Lewis predicted global issues with the "new breeding methodology" and problems with co-mingling. He also raised the question of what should be done with tobacco containing more than 0.4 mg per gram of nicotine.

An Ambitious Target

Anna Malpica, breeder and R&D manager of Bergerac Seed and Breeding (BSB), a seed company owned by the French growers' association, introduced her organization's breeding programs for low-nicotine and ultra-low-nicotine tobacco varieties and evaluated the impact of lower alkaloid lines on plant defense. BSB focuses entirely on practical breeding

projects. Being based in Europe, the company uses non-GMO and non-gene-editing techniques in its programs. For LNT, the breeder target is to achieve reduced and stable nicotine levels with an industrial variety.

Nicotine is synthesized exclusively in tobacco roots, transported to leaves and stored in the leaf cell vacuole to protect the plant when it is stressed. The biggest stress for the plant occurs during topping, which is done to reduce the dry matter production in the leaves and improve the plant's quality. The genome sequence of tobacco provides a large inventory of structural and regulatory genes involved in the now well-known and described nicotine pathway, Malpica related. Researchers benefit from ever more precise studies that quantify the influence of the production environment and the crop management on the nicotine rates in tobacco leaves.

In its plant breeding strategies for low-nicotine and ultra-low-nicotine flue-cured (FC) tobaccos, BSB resorts to three key raw matter sources: a germplasm collection including wild tobaccos with nicotine levels of 0.01 mg per gram to 3.97 mg per gram; U.S. FC historical lines; and short-cycle, ripening breeding lines originating from North Europe. The shorter the plant cycle, the less nicotine accumulation is observed in the plant, she noted.

BSB creates large segregating populations from the best seeds, applying strong selection pressure oriented on nicotine rates. It also secures and selects yield quality and some target resistance genes. The objective is to obtain low-nicotine varieties with nicotine levels under the company's threshold of 10 mg per gram. For its ultra-low-nicotine breeding programs, BSB adds lines with mutations as initial donor material. The company is capable of breeding very low-nicotine lines with a controlled average nicotine rate of under 1 mg per gram. A look at BSB's low-nicotine breeding activities between 1997 to 2023 revealed that the threshold of 0.4 mg per gram has not been reached yet in BSB's usable lines, though.

In 2023, the company investigated the impact of low-nicotine breeding on yield, quality and plant defense in a trial using burley, dark and FC varieties. On average, no decrease of yield was observed in the low-alkaloid (LA) varieties. BSB says it has been able to compensate for the genetic yield decrease of the LA lines by population breeding. This, however, could not make up for the lower quality, which on average declined by 24 percent. The lowest nicotine level was obtained in an XC stalk position with 1.5 mg per gram in a burley variety. Higher nicotine varieties were less susceptible to budworms whereas there was no significant impact of the nicotine rate on the plants' susceptibility to the tobacco flea beetle. The role of nicotine on the plant defense front, researchers presumed, may vary from one insect species to another.

In conventional breeding, Malpica concluded, low nicotine levels of 5 mg per gram to 15 mg per gram can be obtained with conventional breeding and adapted agronomical practices. They are available from BSB as commercial varieties. Ultra-low-nicotinecontents of under 0.4 mg per gram with acceptable behavior regarding quality and aroma, however, seem difficult to achieve with stability from conventional breeding and are not part of BSB's portfolio yet.

Time of Topping

T. David Reed, Extension agronomist for tobacco at the Virginia Tech Agricultural Research and Extension Centers, provided a closer look at the agronomic practices impacting nicotine concentration of FC tobacco. He referred to a 2019 study by Henry, Vann and Lewis, which suggested that proposed regulations mandating lower nicotine concentrations in tobacco products would likely require changes in tobacco production while maintaining yield and quality.

In standard FC tobacco production practices, Reed pointed out, a population of 13,600 plants per hectare to 16,100 plants per hectare is typical. The topping height is between 18 leaves to 22 leaves per plant, and the total number of leaves amounts to around 296,000 per hectare.

Topping is a standard production practice, and its timing impacts yield and sucker control. When topping is delayed past the early flower stage, a yield loss of 17 kg per hectare per day may occur. With late topping, the cured leaves are thinner and less bodied.

Regarding fertilization, nitrogen is the most responsive nutrient in terms of tobacco yield and quality. The nitrogen fertilization rate is determined based on soil texture and depth to a clay layer as well as field history. The recommended nitrogen rate is 67 kg to 90 kg per hectare but can be as high as 112 kg per hectare.

While tobacco is relatively drought tolerant, it is responsive to rainfall and irrigation, with too much rain being a more common occurrence. Unlike other crops, such as certain grains or soybeans, tobacco is not as dependent on timely rainfall to produce an adequate yield. Dry conditions paired with high temperatures can impact cured leaf quality and leaf chemistry.

According to Reed, the number of harvests or primings has decreased in recent years. For most growers, three harvests are typical; some harvest four times. The time from topping to harvest can range from 8 weeks to 14 weeks or more on a given farm.

Tobacco growers, Reed emphasized, choose their agronomic practices in order to maximize their yield potential of high-quality, marketable tobacco. Leaf chemistry, sugar or nicotine content are not part of their consideration.

Reed quoted three studies by Caleb Hinkle that investigated plant population, topping time and topping height of low-nicotine FC production practices in field trials in 2019 and 2020 as well as a collaborative Coresta study of the low-nicotine tobacco agronomic production practices task force. Modified production practices with low-nicotine FC varieties, he concluded, did not consistently reduce nicotine to the proposed target levels. While plant population, topping height and nitrogen fertilization rate had minimum impact, delayed topping had a significant effect, with no topping having the greatest impact on nicotine. Not topping tobacco, Reed stressed, is not commercially viable in the U.S. The growing season was a major factor on nicotine levels. Both yield and leaf quality, as currently measured, were significantly lower with low-nicotine flue-cured varieties. Leaf texture and body were altered, which impacts the handling of the cured leaf. TR