Analysis of mainstream cigarette smoke constituents prioritized by the World Health Organization in a core collection of tobacco accessions: variability and correlations.

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1. Introduction

Among the different strategies proposed for tobacco regulation, the WHO study group on Tobacco Product Regulation (TobReg) has proposed mandating ceilings on nine selected smoke constituents. These constituents were selected according to their potential toxicity, their variability among brands and the potential for the constituents to be lowered with apparent existing tools.

To assess the feasibility of using tobacco plant having different genetic material for reducing the proposed constituents, we evaluated the variability of different leaf and smoke constituents in a core collection of tobacco accessions, representative of genetic diversity available before intensive modern breeding. A panel of 148 tobacco varieties from the Imperial Tobacco collection were grown in the field with three repeats and a split–plot design (**Figure 1**).

After curing, cigarettes were made with each variety and were mechanically smoked according to the Canadian intense smoking conditions (**Figure 2**).

A statistical approach, taking into account multiple factors linked to the growing environment and weight of tobacco actively burnt during puffing, and environmental factors, was designed to identify potential differences between varieties (**Figure 3**).

2. Methodology

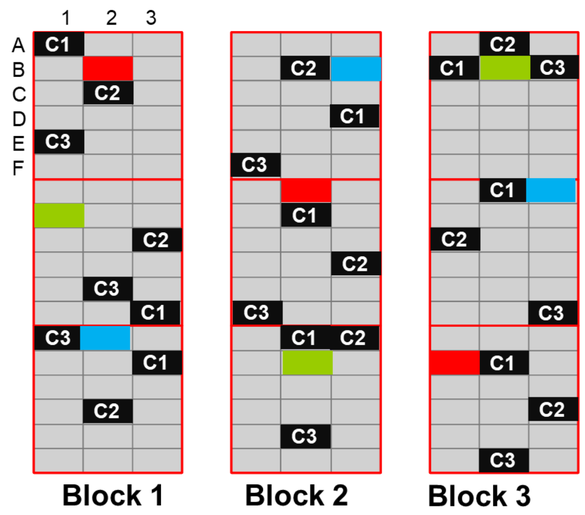


Figure 1: Example of the split–plot design used for the flue-cured tobaccos: 3 blocks / 3 squares per block / 3 bands per square / 6 parcels per square.
To take into account environmental factors, three varieties were used as control in every square C1 C2 C3
Three replicates were used for the other varieties (1 per block, e.g.).

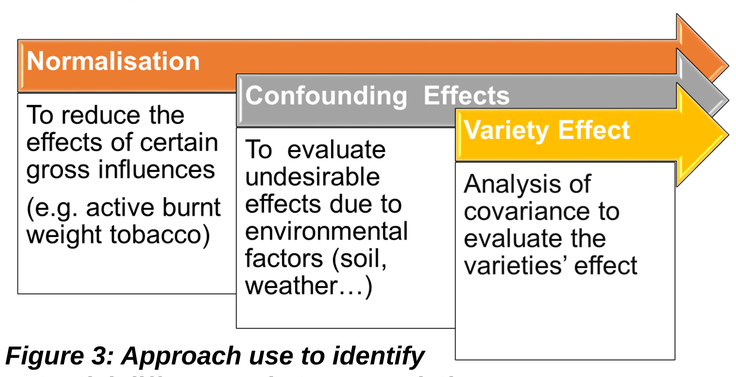


Figure 3: Approach use to identify potential differences between varieties

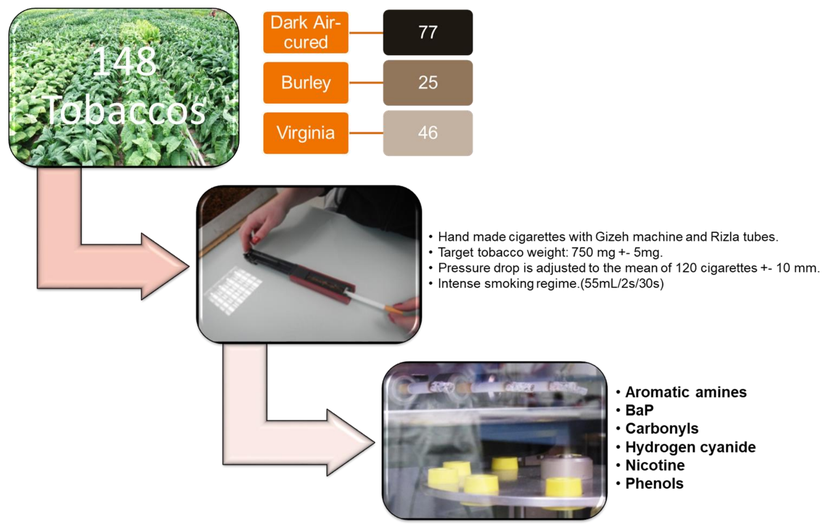


Figure 2: Methodology used for analyzing tobacco varieties
(Internal lab using CORESTA recommended methods)

The ratio of inter-variability on intra-variability was computed for each constituent to identify significant differences between the tobacco varieties. The limit of significance was established using a test of Fisher, for each tobacco type (burley, flue-cured and dark air-cured). The limits for a risk at 99% are ranged from 1.16 to 1.23 depending of the number of varieties per tobacco type. To simplify the illustrations, A common limit was set-up at 1.2, for a risk at 99%.

3. Results

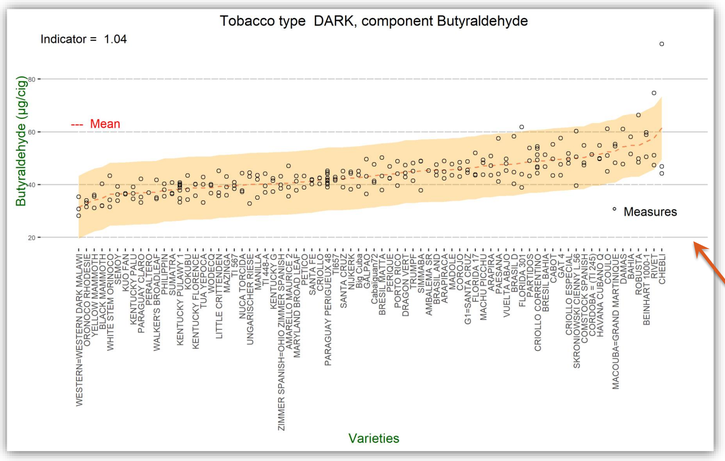
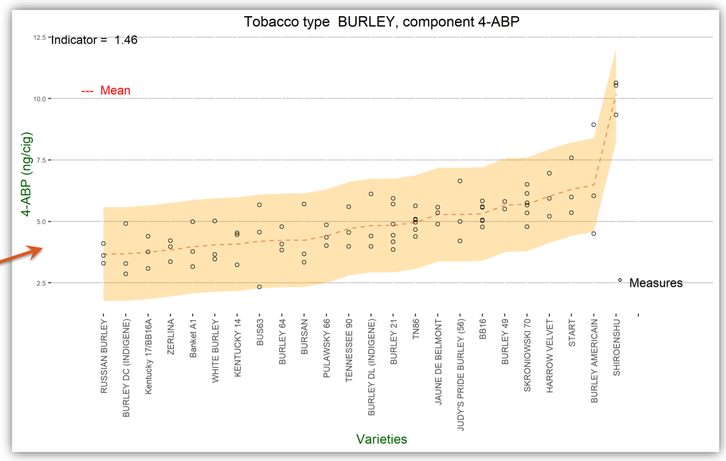
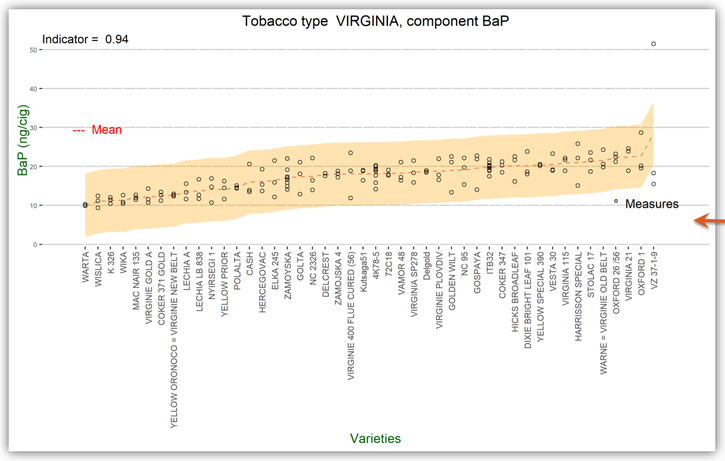


Figure 4 shows the variations of some tobacco smoke constituents within and between tobacco varieties: 77 dark air-cured, 25 burley and 46 flue-cured (Virginia) tobaccos.
A ratio higher than 1.2 for a given smoke constituent, indicates that significant differences exist between varieties.





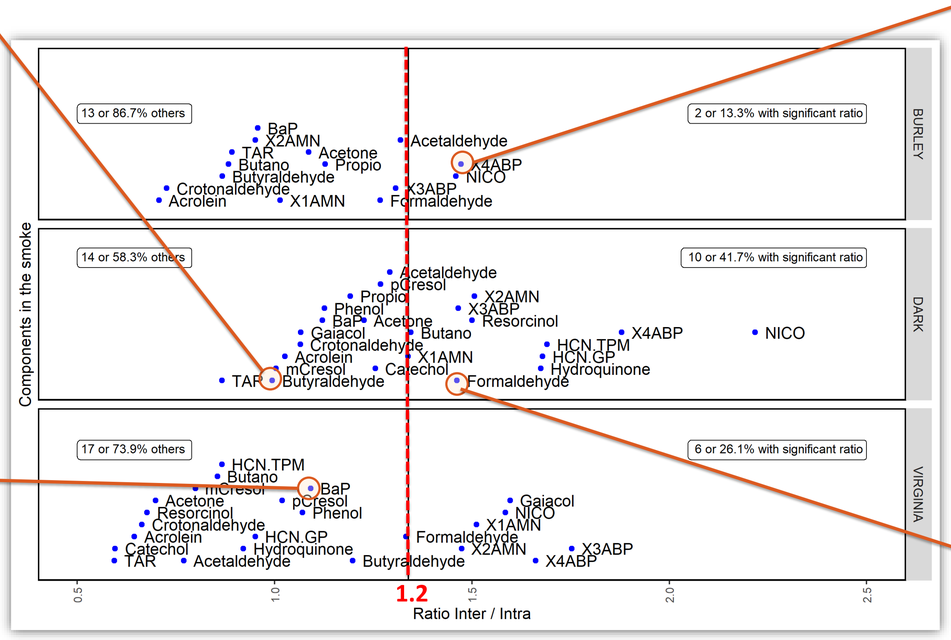
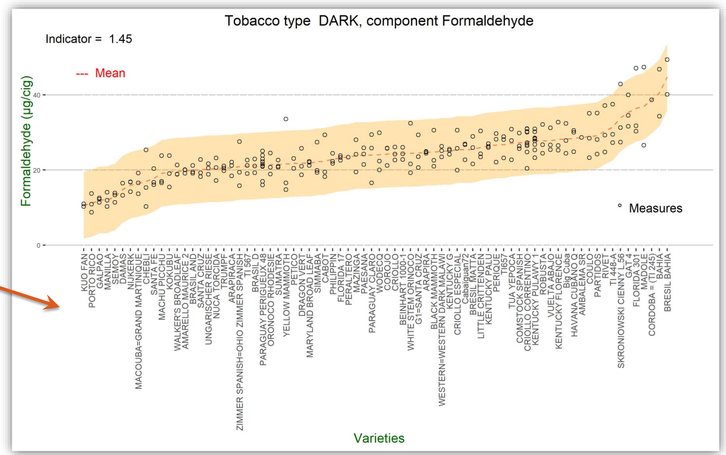


Figure 4: Smoke constituent variations within and between tobacco varieties



4. Discussion

WHO-TobReg proposed a list of toxicants selected on the basis of an assessment that included consideration of data on animal and human toxicity, toxicity indices, variation in toxicants across brands, the potential for the toxicant to be lowered and the inclusion of constituents from both the particulate and the gas phases of smoke and from different chemical classes in cigarette smoke (1). Technologies for decreasing selected tobacco smoke constituent yields generated during combustion can be divided into two very broad categories:

- Using technologies to selectively reduce the yields of smoke constituents
- Developing novel tobacco varieties generating lower yields of smoke constituents

The chemical variations of some tobacco constituents between tobacco varieties has not been studied extensively. Therefore, the aim of this study was to identify if some varieties generate naturally less smoke constituents than some others. We quantified smoke constituents from 77 dark air-cured, 25 burley and 46 flue-cured tobaccos from our germplasm collection. A ratio of inter-variability on intra-variability was computed for each constituent to set up a threshold (1.2) of a significant difference between varieties. Significant differences between the tobacco varieties, given by a ratio higher than 1.2, point out the interest to investigate the genetic variability for these smoke constituents. For example, primary aromatic amines for flue-cured tobacco or formaldehyde for dark air-cured tobacco. However, this is limited by negative correlation existing between some constituents as already demonstrated (2-4). On the other hand, a ratio of less than 1.2 indicates no real difference between tobacco varieties. This is the case for B(a)P or some carbonyls, whatever the tobacco type. A global breeding approach to reduce the toxicants pointed out by the WHO-TobReg is impossible, and it would be pointless to try to develop tobacco varieties in this goal, except for particular constituents for which precursors and major genes have been identified like NNN.

Reference
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