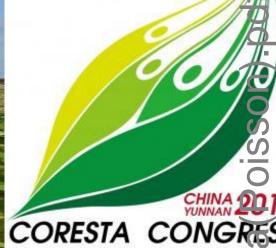
# Field trials with low nicotine tobacco varieties developed by conventional breeding technique: First results



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### INTRODUCTION

In 2015, The WHO Study Group on Tobacco Product Regulation (TobReg) proposed to work on the reduction of nicotine content of cigarettes with the objective to potentially decrease the smoking prevalence. An advisory note was released recommending a strategy of reducing nicotine in tobacco "as low as is technically feasible".

In 2017, the U.S. Food and Drug Administration announced a multi-year plan for nicotine and tobacco regulation. The policy more formally embraces the notion that nicotine is delivered through products that represent a "continuum of risk," and is most harmful when delivered through smoke particles in combustible cigarettes.

In 2018, the FDA announced in an advanced notice of proposed rulemaking to be "particularly interested in comments about the merits of nicotine, levels 0.3, 0.4 and 0.5mg nicotine/g of tobacco filler [0.03, 0.04, 0,05 %] as well as others levels of nicotine". The alkaloid content in dry leaves ranges usually from 0.1% to 5%.

Such nicotine levels in tobacco products can be reached only with the creation of new cultivars using Genetic Engineering. However, new cultivars created by transgenesis or gene editing are classified or could be classified like GMOs in many countries particularly in EU. The recent judgement of the European Court of Justice on plant varieties created by Genetic Engineering (July 25, 2018) and the agreement of the coalition of government in Germany seem to exclude the use of gene editing techniques to speed up the breeding process. The principle of precaution and the freedom of choice of consumers could be applied in some countries in EU.

In that context, low nicotine levels can be hoped only through the development and availability of relevant new cultivars obtained by conventional breeding, associated to massive changes in agronomic practices. An efficient and professional collaboration has been established with the aim to assess the limit of feasibility in nicotine decrease using conventional cultivars. Here are presented the first results of this collaboration.

#### MATERIAL & METHODS

Six conventional Burley and Flue-cured cultivars, listed below, have been selected in 2017 within BSB breeding programs and IMB collection for their specific low nicotine behavior. Two countries have been selected: Zimbabwe as a reference center for Flue-cured production with the support of Kutsaga and Malawi as a reference for Burley production with the support of ARET center. In each country a local reference variety was used as control. Two crop management were compared: Local Best Practices (LBP) and Low Nicotine Management (LNM). Trial design was based on a 2 blocs scheme with 2 replications within each bloc by variety and crop management.

	MALAWI	ZIMBABWE
Tested varieties	Flue-cured: K326, ITB 697 and ITB 6184 Burley: TN90, ITB 5101 and ITB 5120	
Control varieties	Flue-cured: AFH4 Burley: BRK4	Flue-cured: KRK26 Burley: BEY21
Plantation density	LBP: 1.20m x 0.60m LNM: 1.20m x 0.42m (40% increase in plant density)	
Fertilization (LNM with N reduced of 50%)	LBP: 720kg/ha basal fertilization (10-24-20) on plantation day 72-172-144  LNM: 360kg/ha basal fertilization (10-24-20) on plantation day; 140kg/ha of SS Phosphate 16% and 36kg/ha of SOP K50%) 36-108-90	LBP: 400kg/ha of basal fertilization before plantation and 75kg/ha of AN fertilization 1 month after plantation  LNM: 400kg/ha of basal fertilization before plantation and 50kg/ha of AN fertilization 1 month after plantation
Topping	Flue-cured: 18 leaves Burley: 20 leaves	

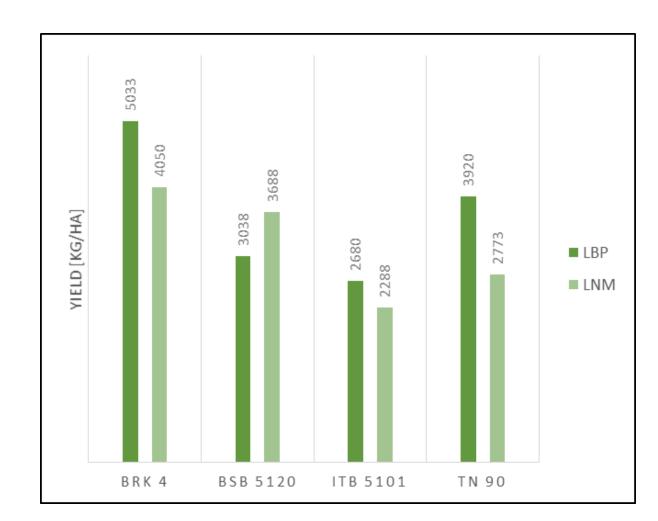


# RESULTS: Burley in Malawi

**YIELD** 

QUALITY

**NICOTINE** 



26% decrease observed as average

between local best practices and low

BRK 4 is evaluated as the best variety for

BRK 4 and BSB 5120 are evaluated as the

best varieties for quality in LNM block.

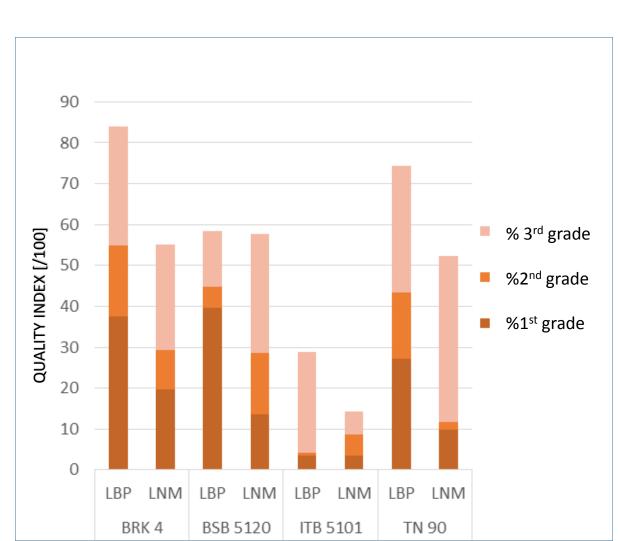
Average decrease of 13% of yield observed

**BRK4** has the best average yield in LBP bloc.

BRK4 and BSB 5120 have the best average

between LBP and LNM blocs.

yield in LNM bloc.



nicotine management.

quality in LBP blocs.

and low stalk position (SPL), except for BSB 5120 showing aligned results between SPH and SPL.

Average decrease of nicotine level, for all varieties between LBP and LNM is 57%.

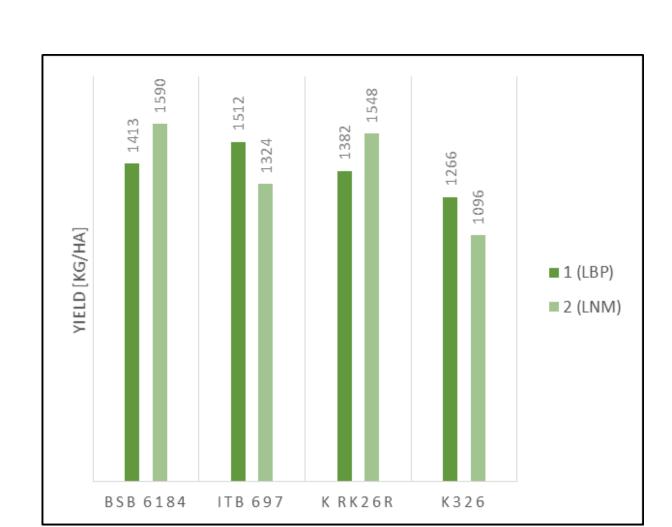
→ strong impact of the crop management.

in LNM, with an average level of 1.27% DM.

→ strong impact of the variety choice.

## **RESULTS: Flue-cured in Zimbabwe**

**YIELD** 

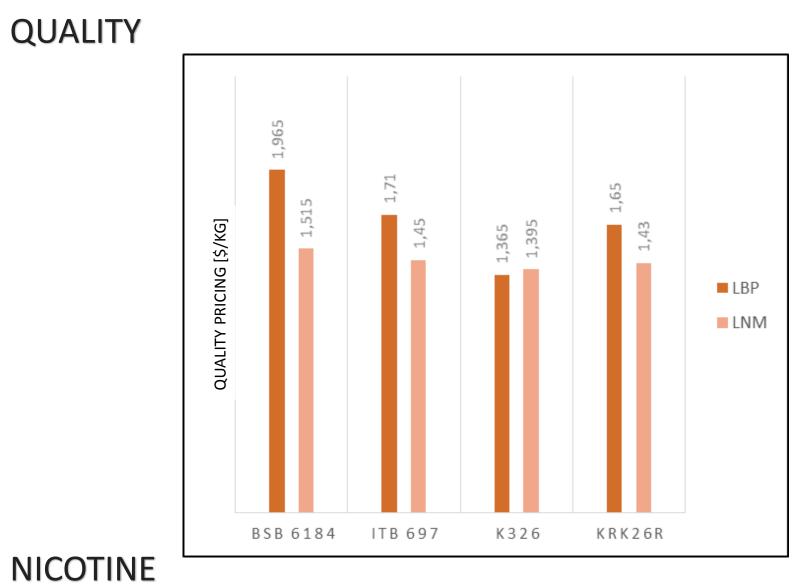


No general trend observed for yield between LBP and LNM blocs.

ITB 697 seems to have the best average yield in LBP bloc (no significant difference in this bloc).

ITB 6184 and KRK 26 have the best average yields in LNM bloc.

QUALITY



Quality target: full flavor tobacco.

24% decrease observed as average between local best practices and low nicotine management.

BSB 6184 is evaluated as the best variety for quality in both blocs.

Global country average price is 2,88\$. Station trials seem far from commercial crops reality even for the local control KRK26R.

A clear difference in nicotine level is observed between high stalk position (SPH) and low stalk position (SPL).

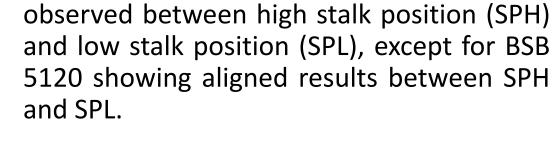
Average decrease of nicotine level, for all varieties between LBP and LNM, is 43%.

→ strong impact of the crop management.

The lowest nicotine levels were obtained with ITB697, low nicotine variety, in LNM, with an average level of **0.5% DM**.

→ strong impact of the variety choice.





The lowest nicotine levels were obtained in Malawi with ITB 5101, low nicotine variety,

# **DISCUSSION & NEXT STEPS**

In the Flue-cured trial, in Zimbabwe, we observed no clear yield loss between low nicotine varieties and local control, but a high decrease in potential quality and value for all varieties cultivated with the low nicotine crop management. In the Burley trial, in Malawi, we observed a significant decrease of potential yield and quality for all varieties cultivated with a low nicotine crop management. On one hand, it seems after this first season of trial, that variety has a stronger impact than crop management on yield and quality performance; on the other, nicotine level is clearly impacted by crop

management and variety. Conventional non converter low nicotine varieties can be 59.8% lower for nicotine level than control in Flue-cured and 86.6% lower in Burley. The objective of this collaborative study was to assess the limit of feasibility with conventional cultivars: we obtained on this first trial average levels of 0.5% in Flue-cured (min 0.41%) and 1.27% in Burley 🚫 (min 0.22%).

As next steps it will be important to validate these first observations but also to quantify more precisely the yield and quality losses observed with low nicotine crop management and low 志 nicotine conventional varieties in order to evaluate the economic impact for an industrial process. New trials will be organized in 2019 in Zimbabwe, Malawi and 💍 Philippines comparing the two same crop managements in order to build a robust dataset.