

**Nicotine content of wide tobacco germplasm: The search for high and low nicotine content accumulating genotypes**

*Magama F, Shamudzarira M*  
*& Garwe D*



**TOBACCO RESEARCH BOARD**

**KUTSAGA**

# PRESENTATION OUTLINE

- Background
- Materials and Methods
- Results
- Discussion
- Conclusion
- Further work
- References
- Acknowledgements



# BACKGROUND

- Increasing regulation for nic . products
- Need for tobacco with different characteristics for combustible and electronic products
- Need for new varieties & cultural practices propitious



# BACKGROUND

- Renewed research impetus to lower or increase the accumulation of nic. in cured leaves
- Screening of wide germplasm (old cultivars, wild species, landraces, old breeding lines etc) for allelic variability on nic content an important first step
- Nic content affected by genetics, environmental conditions & cultural practices (Henry *et al.*, 2019), therefore, important to test these factors as well

# OBJECTIVES

- To determine the nicotine content of 55 accessions of wide tobacco germplasm
- To assess the effect of fertilisation levels, leaf position and topping practices on the nicotine content of accessions designated as high nicotine content (**HNC**) or low nicotine content (**LNC**) lines

# MATERIALS AND METHODS

# Germplasm source

- Entries selected from Kutsaga Variety Database
- Accessions annotated as high or low nic/alkaloids but with no determined nic. content
- HNC lines (36) & LNC (13)





# Experimental Designs

## Experiment 1

- **Design:** Split Plot
- Main plot – Topdressing Fertiliser level
- Sub plot- Vars (HNC)
- Number of blocks 2

## Experiment 2

- **Design:** Split Plot
- Main plot – Topdressing Fertiliser level
- Sub plot- Vars (LNC)
- Number of blocks 2

Both experiments conducted for two seasons

# Entries-HNC

1.	K RK26R –commercial var.	6.	V875
2.	BAE 150-high nic. var.	7.	V293
3.	BAE 170-high nic .var.	8.	V384
4.	V873	9.	W149
5.	V874	39.	Landrace 1 to 30

# Entries -LNC

1.	B RK2 - medium –high nic.	9.	V882
2.	LI BURLEY 21- intermediate low nic.	10.	V883
3.	LA BURLEY 21-Ultra-low nic.	11.	V884
4.	V184	12.	V885
5.	V600	13.	V886
6.	V773	14.	V887
7.	V880	15.	V879
8.	V881	16.	V1060

# Seedling production & planting

- Seedlings produced using the Float tray system
- Standard field agronomy **BUT**
  - top dressing fert app
  - topping procedures
- Trials planted as dryland crops



# Fertiliser treatments (top dressing)

## Experiment 1

**Fert1** – Standard AN  
(34.5% N) application  
(5 g/plant)

**Fert2** - plus 50% of  
Standard (7.5 g/plant)

## Experiment 2

**Fert1**– Standard AN  
(34.5% N) application  
(5 g/plant)

**Fert2** - minus 50% of  
Standard (2.5 g/plant)

In both experiments, fertiliser was applied 3 WAP

# Topping practices-HNC lines

- Topping done to 14 leaves
- Excess branches and flowers removed from entries with *N rustica* type growth habit
- All entries hand suckered



# Topping practices-LNC lines

- All entries not topped and consequently no de-suckering



# Reaping and curing

- Reaping started when leaves showed maturity signs
- Lower third of the plant was discarded
- Remaining two third reaped in two cycles as middle leaf fraction (**MLF**) & upper leaf fraction (**ULF**)
- All reaps from both experiments were Air-cured



# Nicotine analysis

- Nicotine was extracted from grounded air-cured leaves and nic. content analysed on LC MS/MS
- Nic content measured on % dry weight basis (**dwb**)



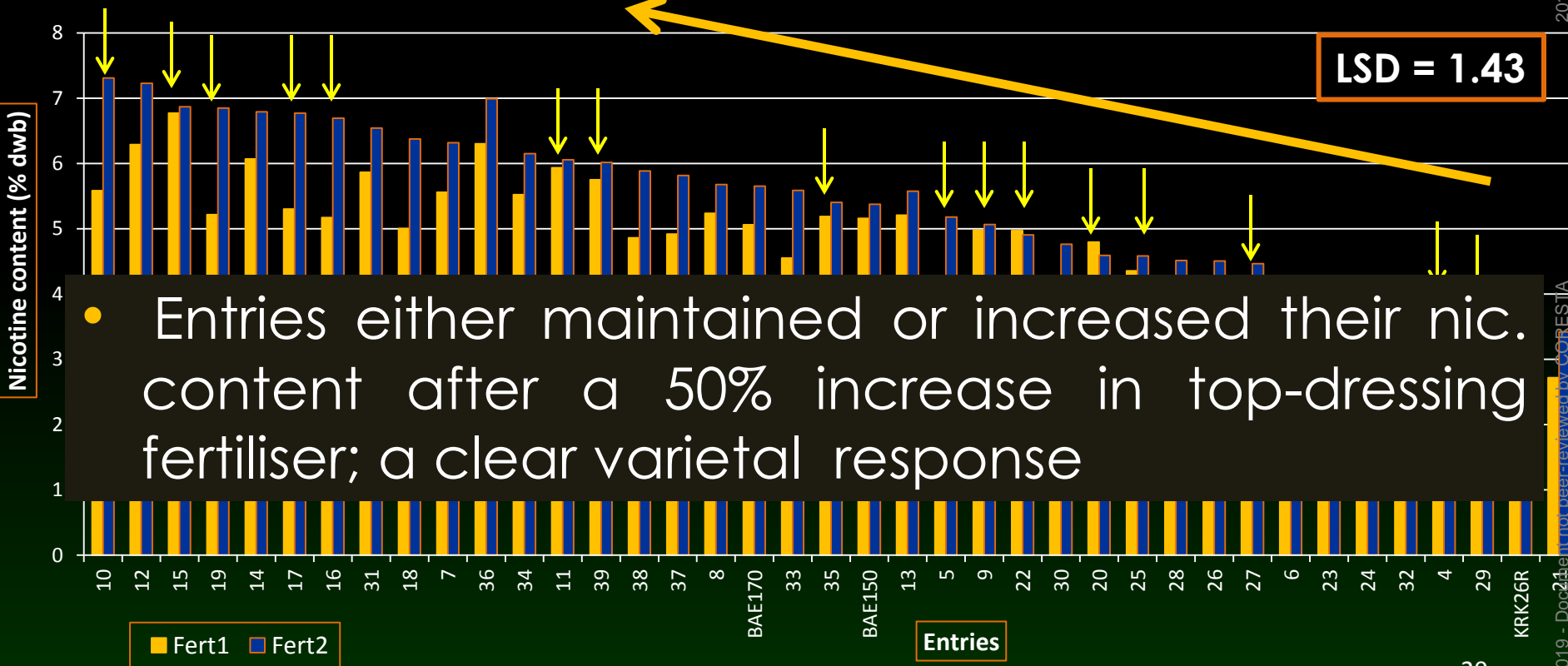
# RESULTS

The search for high nicotine content lines

# Nic content of HNC lines

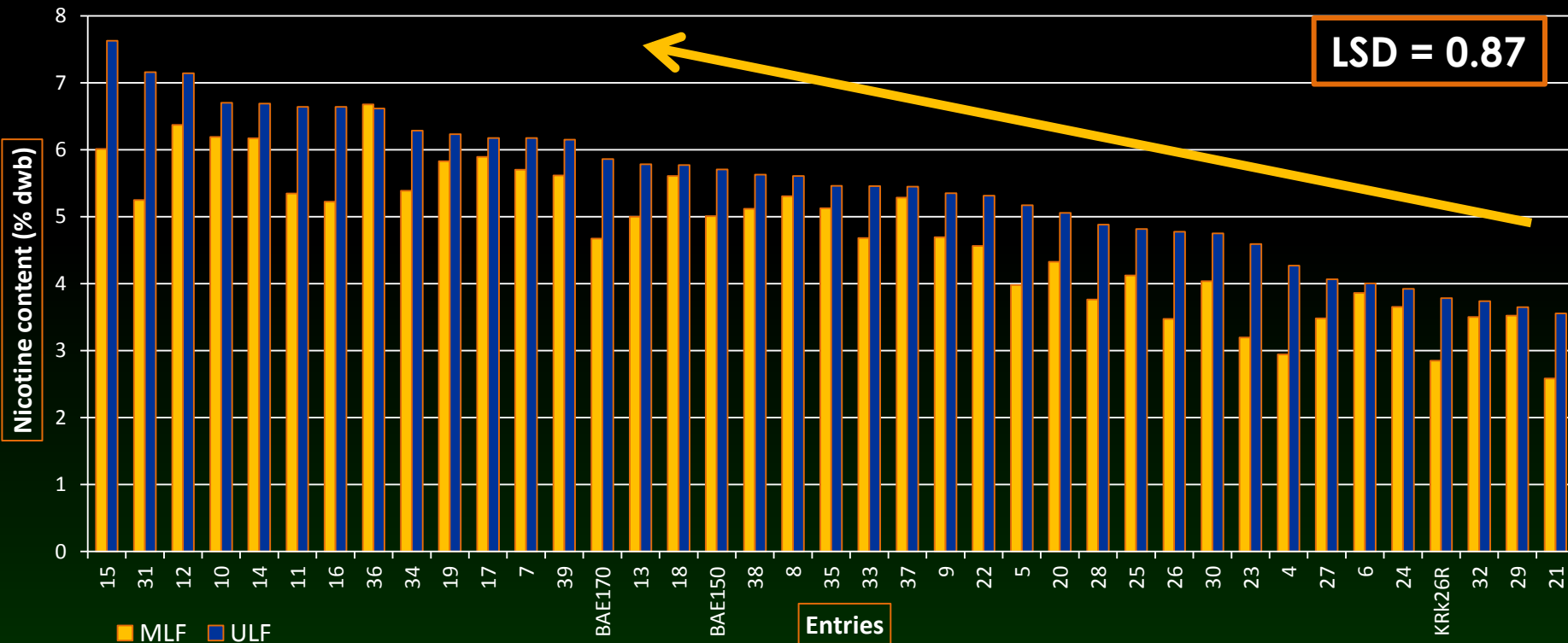
- Of the 36 accessions undetermined for nic content, only 8 were below the 4 % dwb threshold
- Check varieties (BAE150 & BAE170) were above the set threshold
- Nine accessions had highest nic content of between 6-7 % of dwb under standard agronomic practices

# Nic content of HNC lines at 2 fert levels

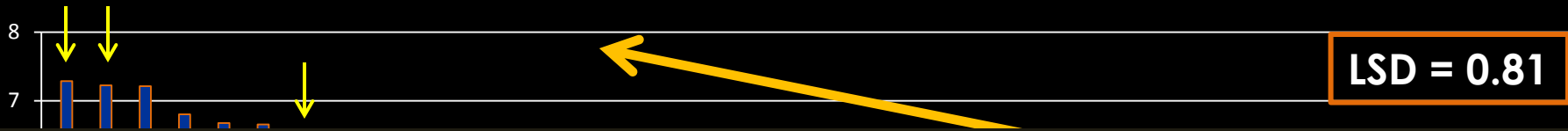


- Entries either maintained or increased their nic. content after a 50% increase in top-dressing fertiliser; a clear varietal response

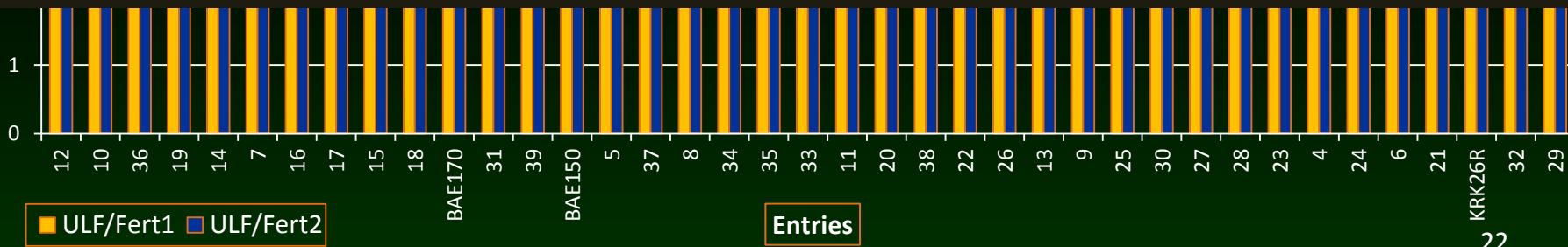
# Nic content of HNC lines by leaf position



# Influence of fert level & leaf position on nic content of HNC lines



- Several entries show highly significant treatments interaction effects to nic. accumulation
- Three entries achieve the highest nic. Content of between 7-8% dwb



# Summary results of HNC

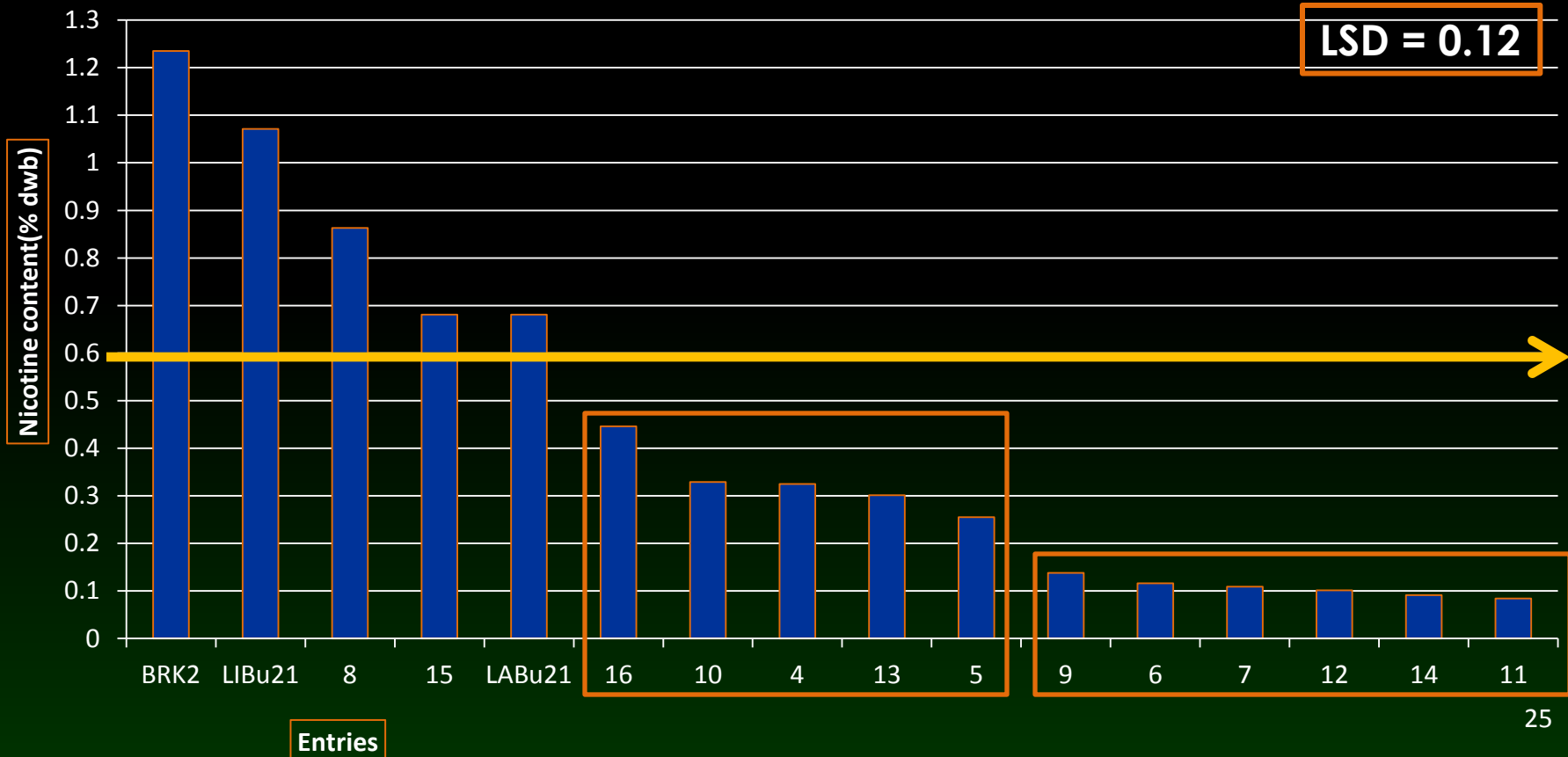
- The majority of entries significantly responded to individual or interaction between treatments
- The modal nicotine content class was 5-5.99 % dwb across treatments
- The highest nic content achieved was 7.29% dwb

# RESULTS

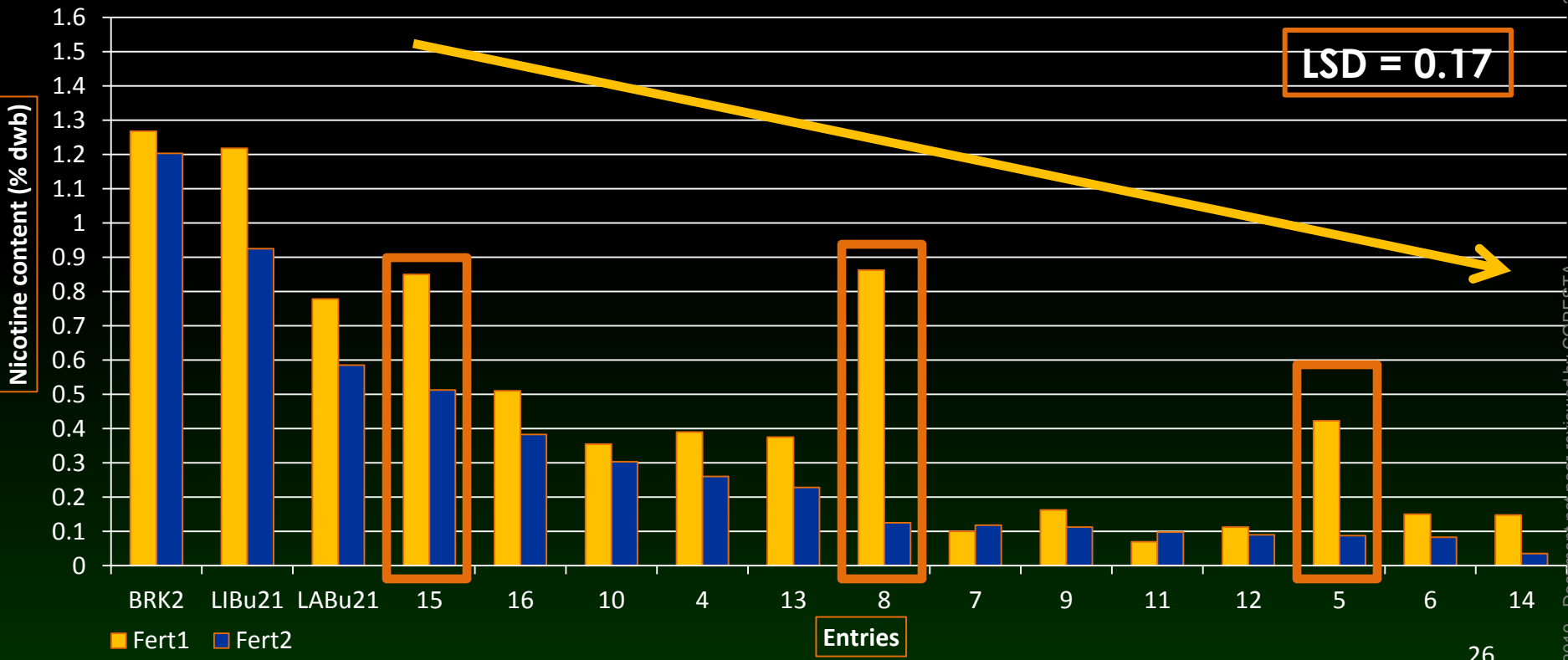
The search for low nicotine content lines



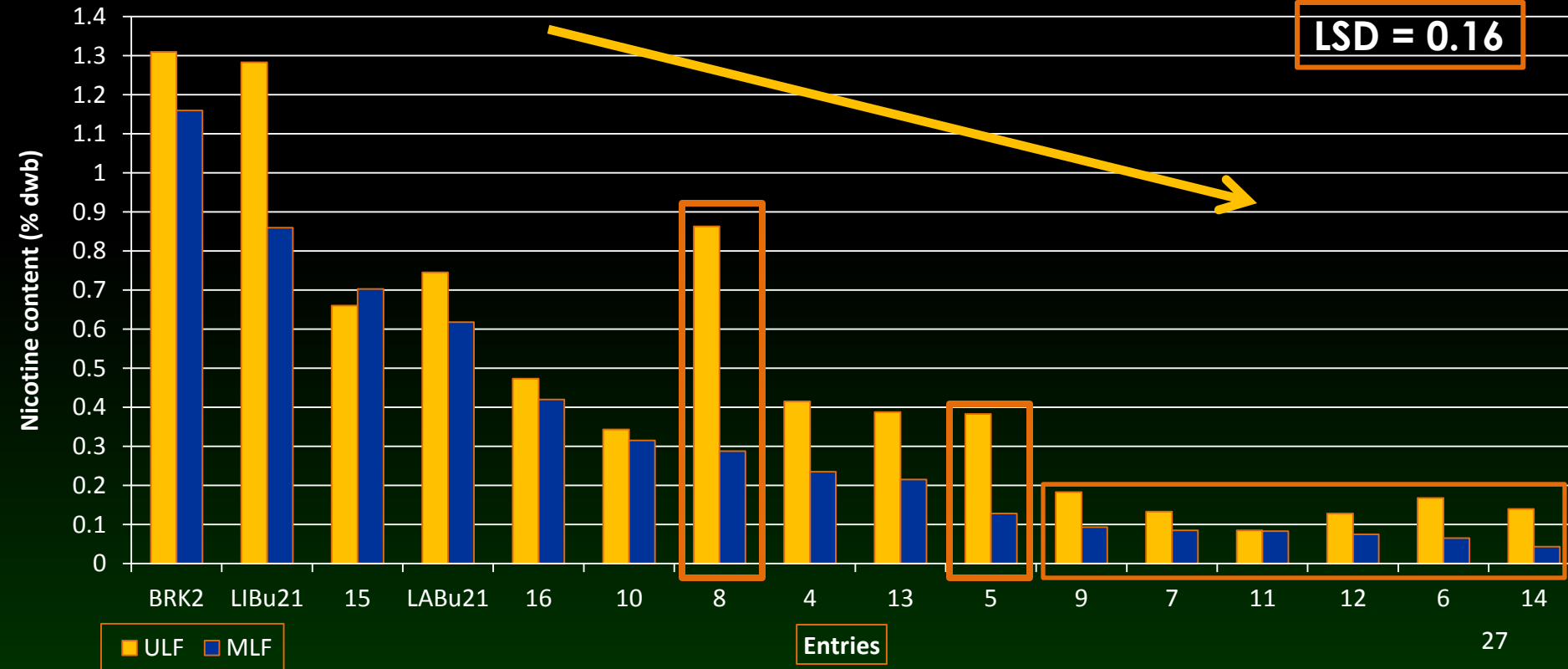
# Nic content of LNC lines



# Nic content of LNC lines at 2 fert levels



# Nic content of LNC lines by leaf position



# Influence of fert level & leaf position on nic content of LNC lines

- Strong influence of cultural practices to reduction in nic. content
- For all entries nic. content reduced to ultra low levels
- Lowest nic. content recorded was 0.1% dwb with entry 14

## Summary results of LNC

- The majority of entries significantly responded to individual or interaction between treatments
- The modal nicotine content class was 0.0 - 0.099 % dwb across treatments
- The lowest nic content was with interaction between treatments

# DISCUSSION

- Efforts to manipulate nic. content of cultivars for the purpose of facilitating compliance with expected tobacco product regulations is a current research thrust world wide
- Current cultivars are bred for specific limits of nicotine ,thus, there is motivation to search for germplasm that can be manipulated to meet new regulations

# DISCUSSION

- Nicotine free, non –transgenic tobacco edited by CRISPR –Cas 9 (Schachtsiek and Stehle,2019) now exists, but the technology is not permitted in a number of places.
- The search for natural variation in nic. content remains a pragmatic approach for developing varieties that will be acceptable and also comply with regulations

# CONCLUSION

- Nic. content was determined for accessions in the Kutsaga Variety Database under standard conditions, varying fertiliser levels & leaf fractions
- HNC entries, 12, 10, 16, 33 & 27 showed the greatest potential for high nic. accumulation (7.3 % dwb)
- LNC entries, 5, 8, 9, 7, 11 & 14 show the lowest ability to accumulate nic (0.1% dwb)



# Further work

- Determine the extremes of nic. possible for some identified lines through further manipulation of cultural practices
- Determination of composition of other alkaloids
- Understanding of the genetics involved

# REFERENCES

- Lewis RS, Lopez HO, Bowen SW, Andres KR, Steede WT, Dewey RE (2015) Transgenic and Mutation-Based Suppression of a *Berberine Bridge Enzyme-Like (BBL)* Gene Family Reduces Alkaloid Content in Field-Grown Tobacco. PLoS ONE 10(2)
- Henry, J. B., M. C. Vann, and R. S. Lewis. 2019. Agronomic Practices Affecting Nicotine Concentration in Flue-Cured Tobacco: A Review. Agron. J. . doi:10.2134/agronj2019.04.0268
- Schachtsiek , J. and Stehle , F. (2019) Nicotine free, non – transgenic tobacco edited by CRISPR –Cas 9), Plant Biotechnology Journal ,pp 1-3

# ACKNOWLEDGEMENTS



**TOBACCO RESEARCH BOARD**  
KUTSAGA



**Universal**  
ZIMBABWE LEAF TOBACCO COMPANY



**CORESTA AP 2019**

Victoria Falls - Zimbabwe