The role of diamine oxidase (DAO) enzyme with respect to tobacco alkaloids and tobacco-specific nitrosamines (TSNA) formation

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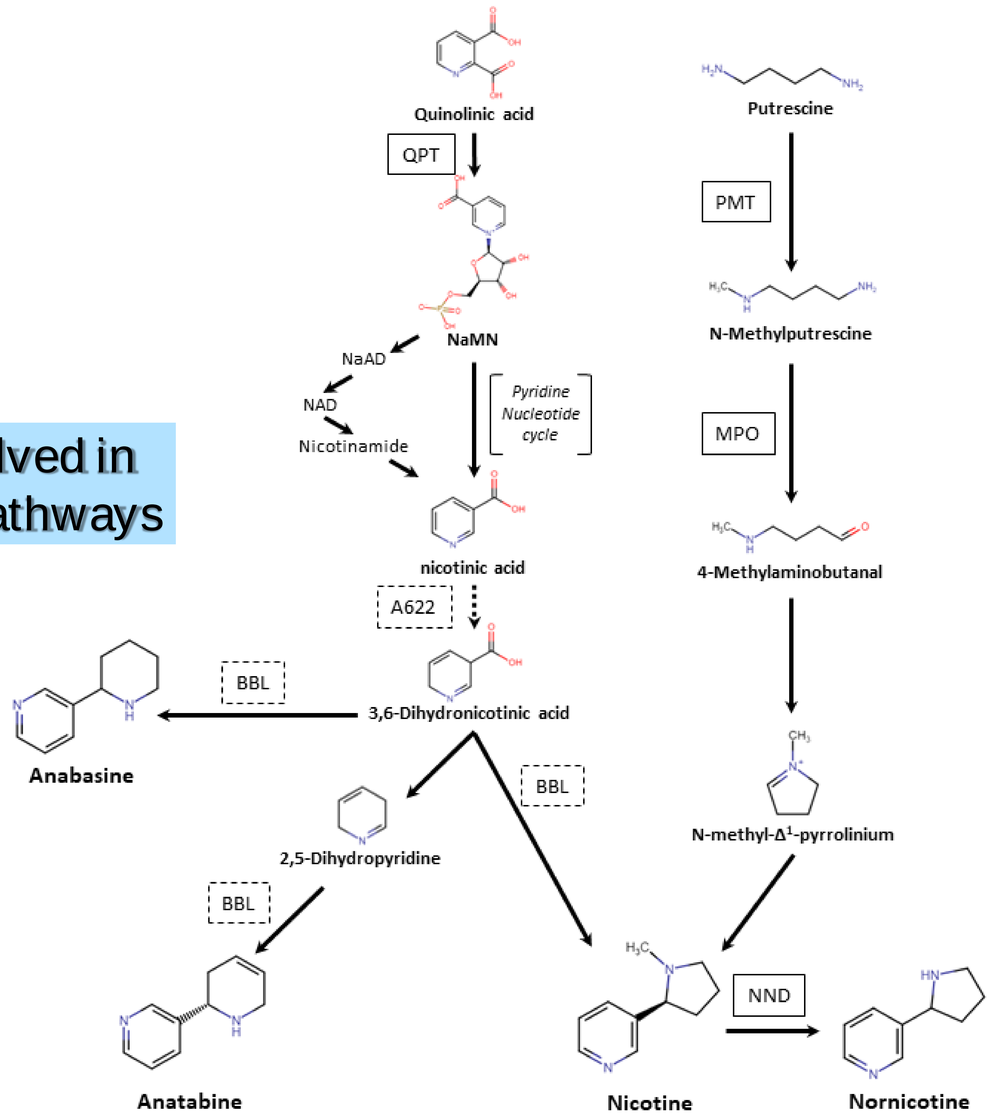
Objective statement

Explore Diamine Oxidase (DAO) enzyme as a new target in tobacco alkaloids pathway for further reduction of nornicotine/NNN

Enzymes in tobacco alkaloids pathways

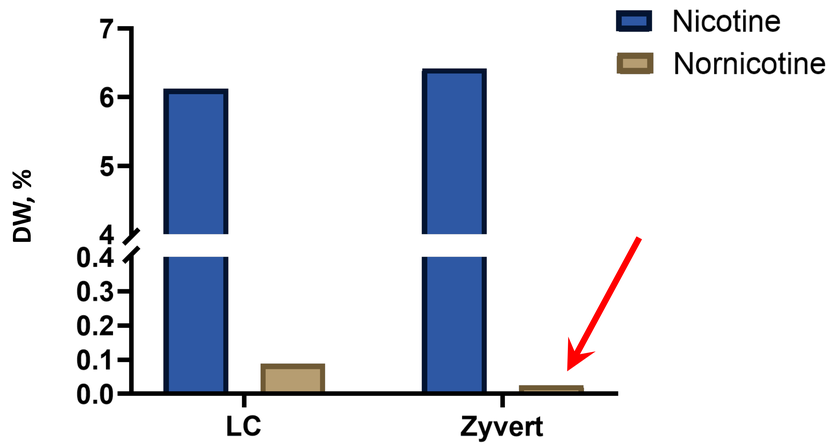
Key Enzymes involved in tobacco alkaloids pathways



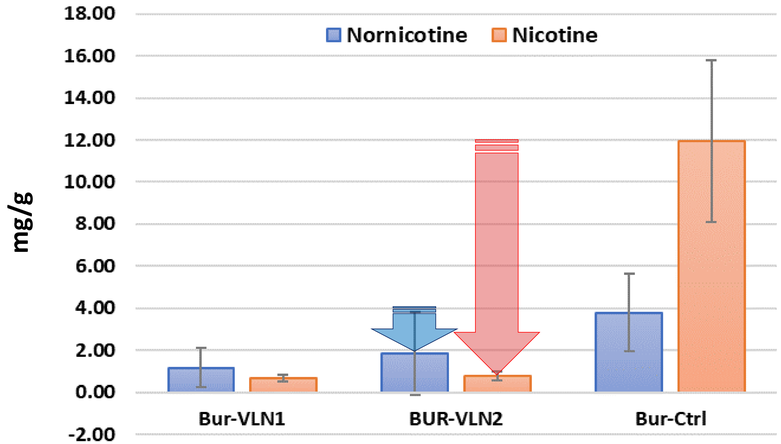
Ralph E. Dewey at al., 2013

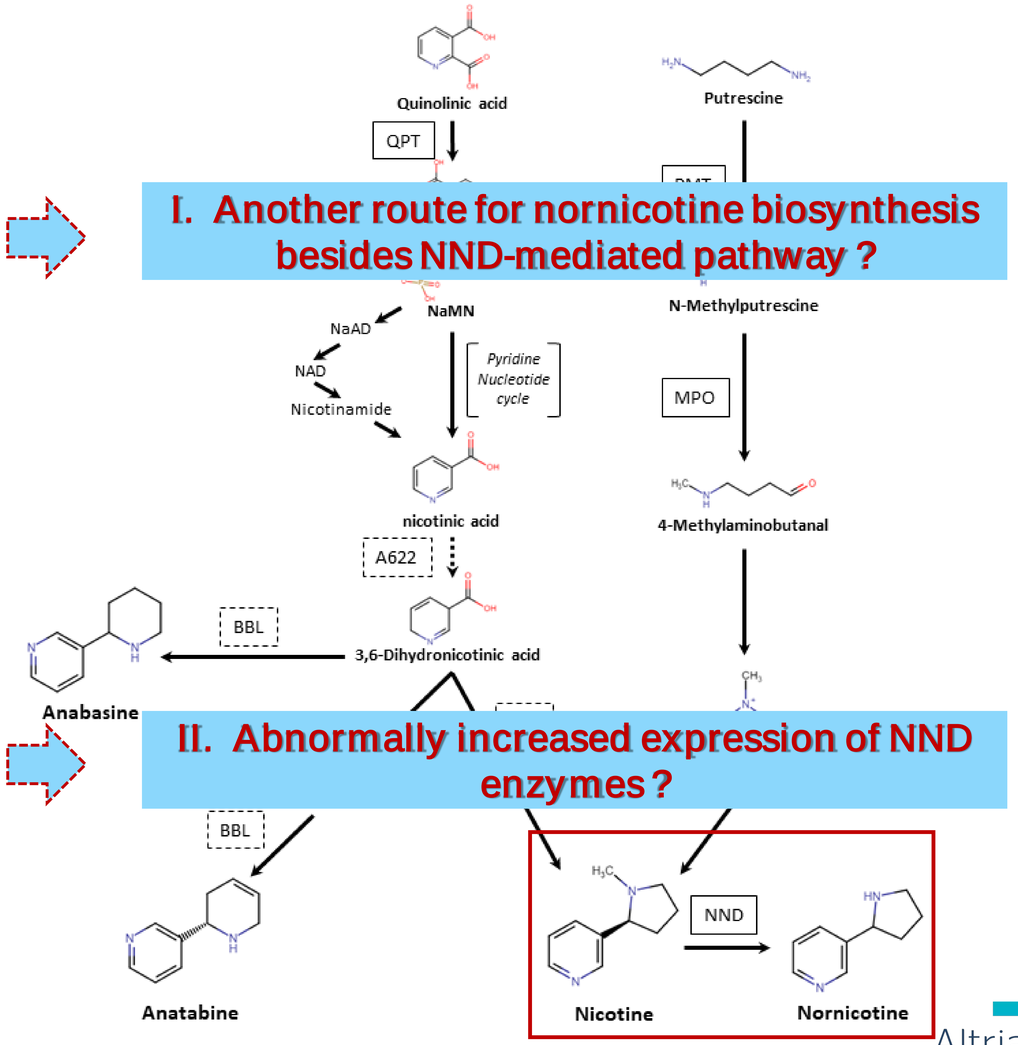
Two Questions from our previous study

Nicotine & nornicotine level in LC vs. Zyvert Dark Tobacco



Nicotine & nornicotine level in normal vs. VLN Burley Tobacco (mg/g)

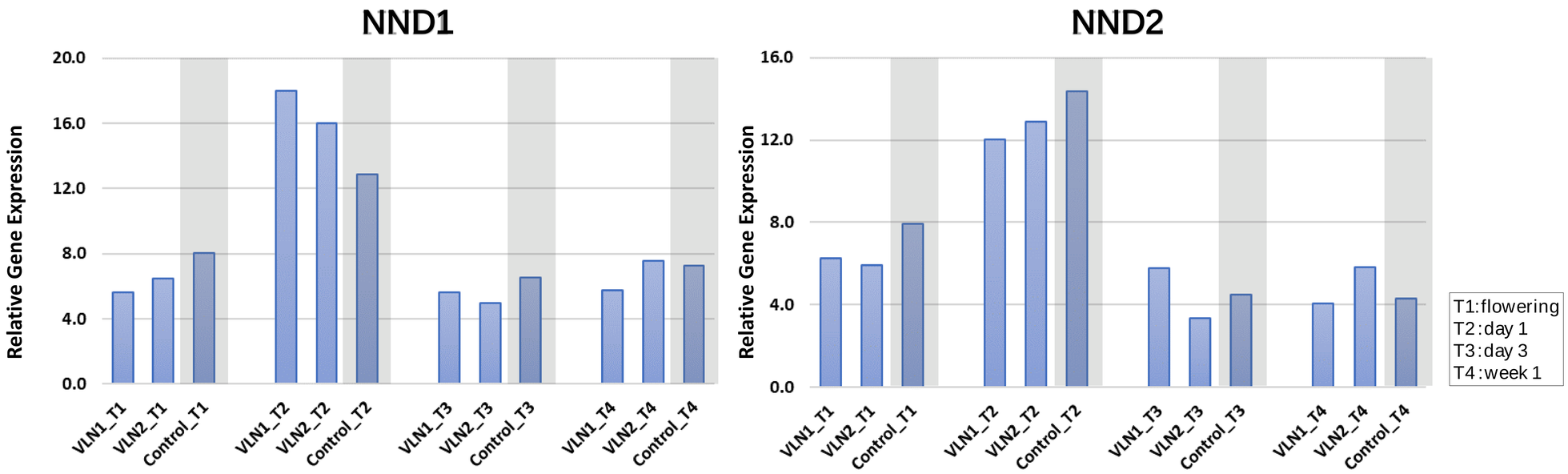




Abnormally increased expression of Nicotine N-Demethylase (NND) ?

- NND gene expression measurement by RT-qPCR in VLN Burley & control tobacco at four time points

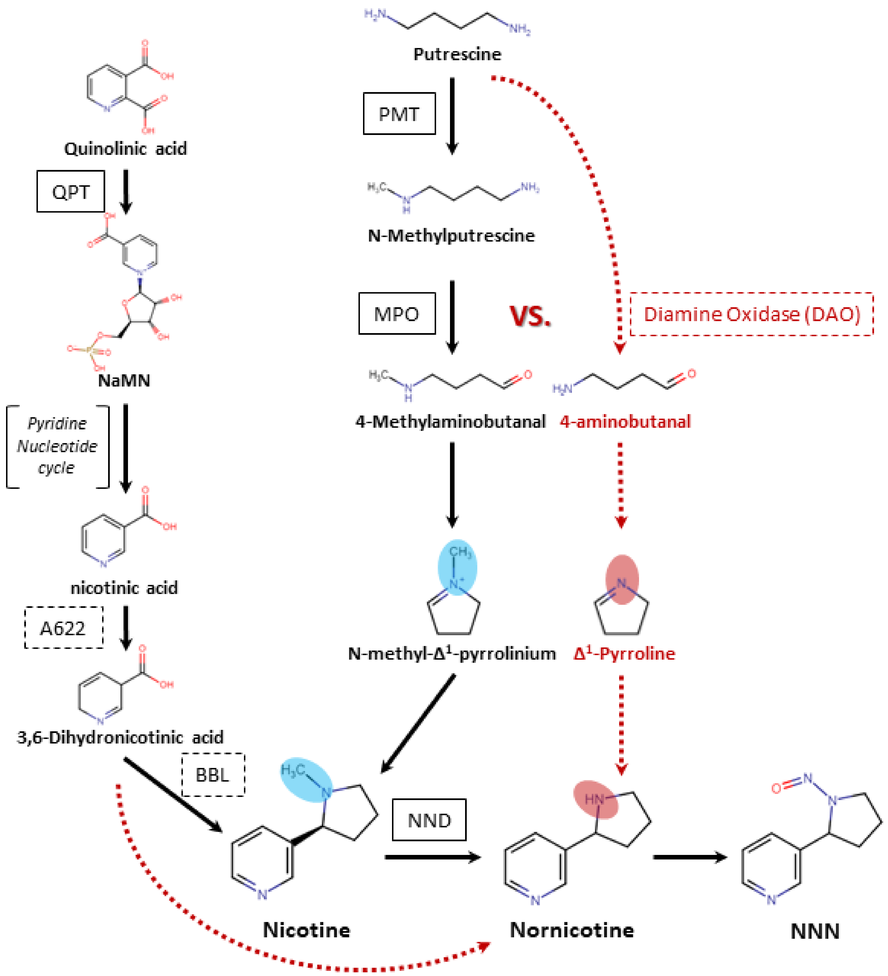
Relative expression of NND genes in normal and VLN tobacco



No clear increase of NND gene expression across varieties at any given timepoint

Another route for nornicotine biosynthesis besides NND pathway ?

❖ Proposed diamine oxidase (DAO)-mediated nornicotine biosynthesis pathway



1. Recently discovered/Similar to MPO sequence, but different enzyme

Molecular Cloning of *N*-methylputrescine Oxidase from Tobacco

Akira Kato

Nara Institute

Molecular Evolution of *N*-Methylputrescine Oxidase in Tobacco

MPO vs. DAO : 88% identity, 96% homology

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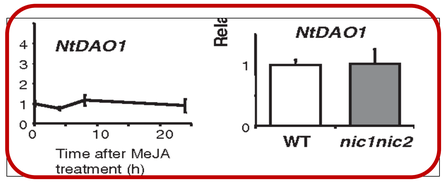
2. DAO has higher affinity with putrescine than N-methylputrescine

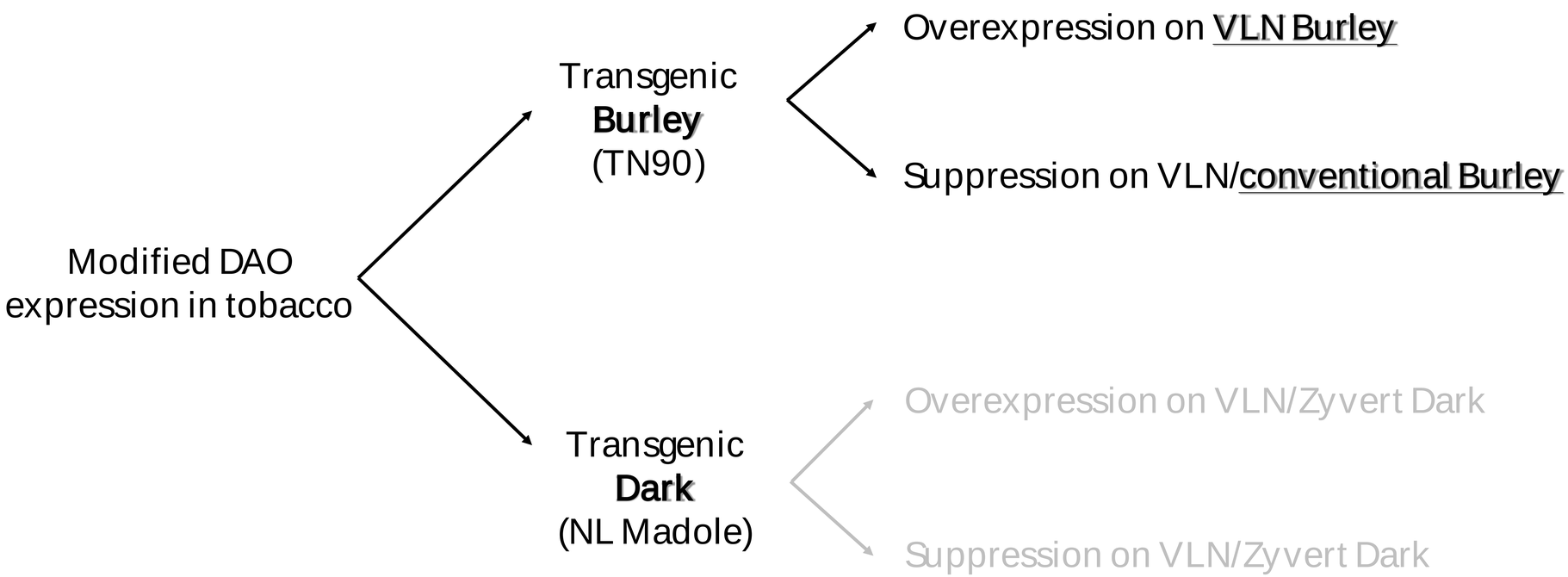
Amine substrate	NtDAO1		
	V_{max}	K_m	rel. K_m
Putrescine	561 ± 34	163 ± 15	0.3
N-Methylputrescine	928 ± 50	478 ± 18	
Cadaverine	840 ± 29	492 ± 28	NA

3. DAO is independent from nicotine synthesis pathway



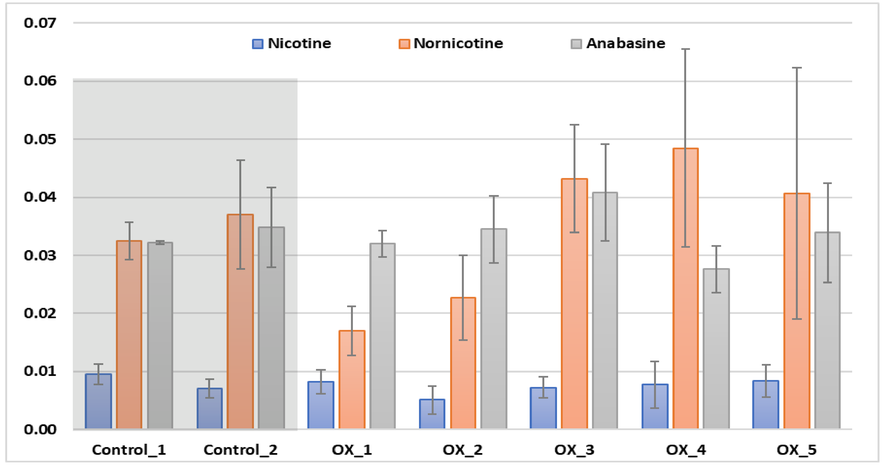
4. Bypassing nicotine formation/NND reaction to generate nornicotine

Approaches to study DAO function – Burley tobacco

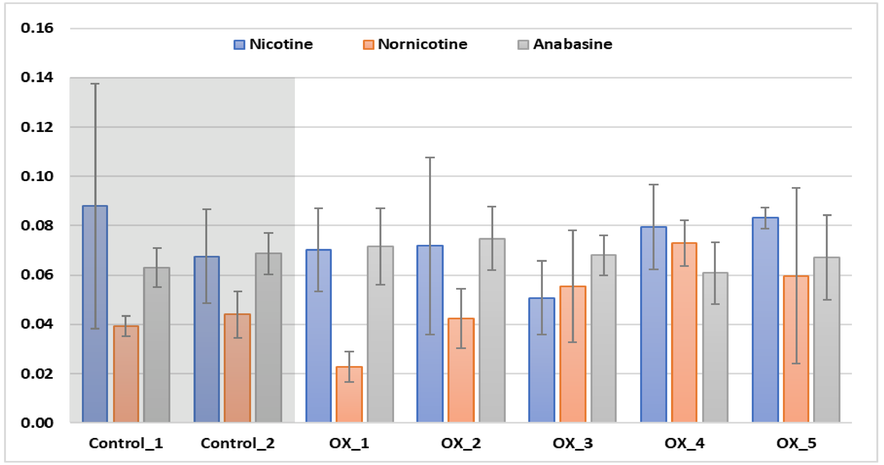


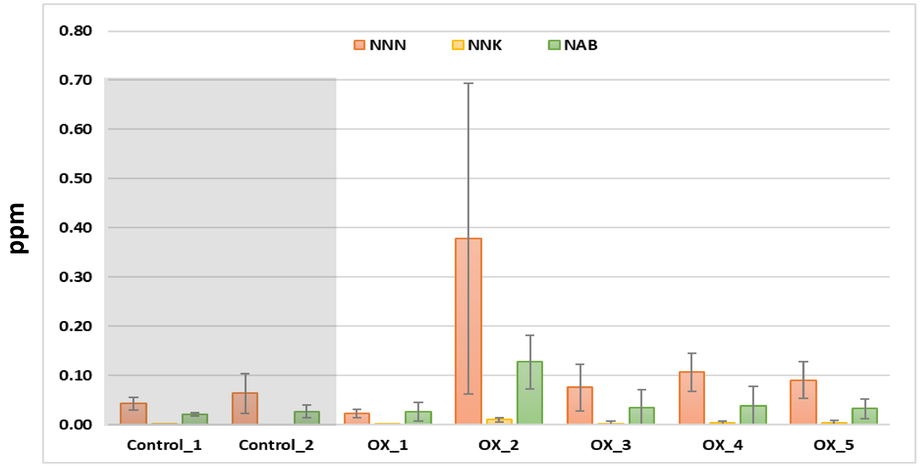
Alkaloids & TSNA in DAO-overexpressed VLN Burley

2 weeks post-topping



End of Curing



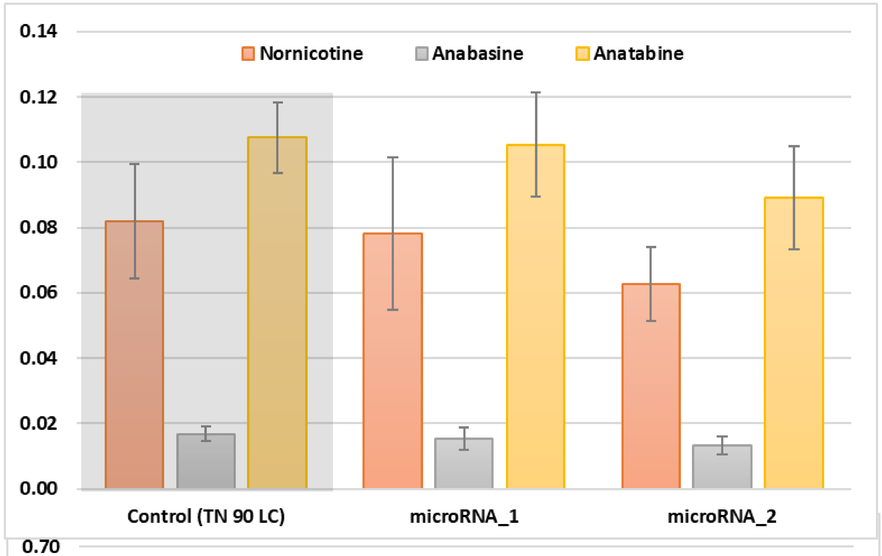


End of Curing

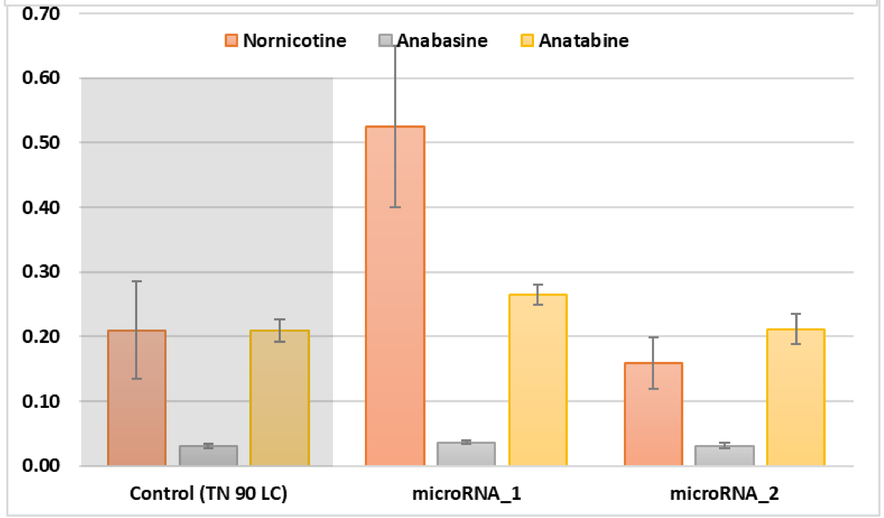
No nornicotine or NNN increase observed from the DAO-overexpressed VLN Burley samples

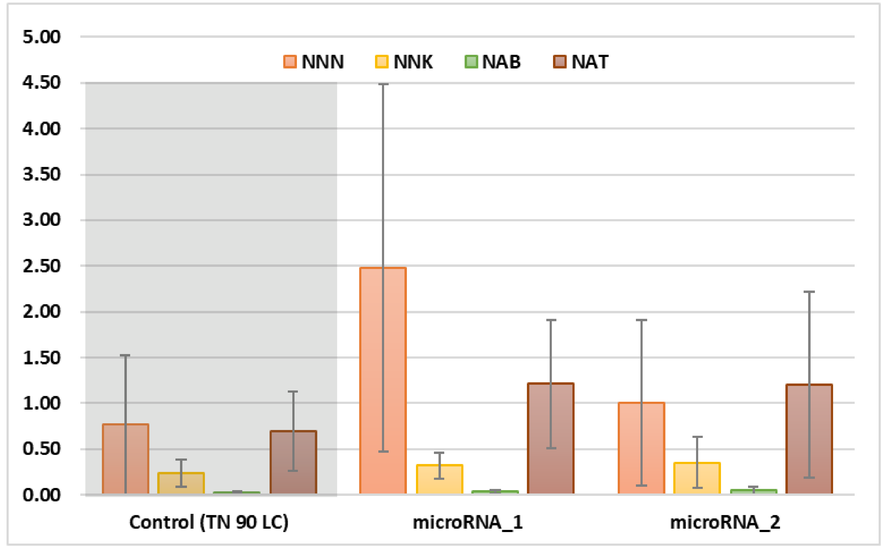
Alkaloids & TSNA in DAO-suppressed conventional Burley

2 weeks post-topping



End of Curing

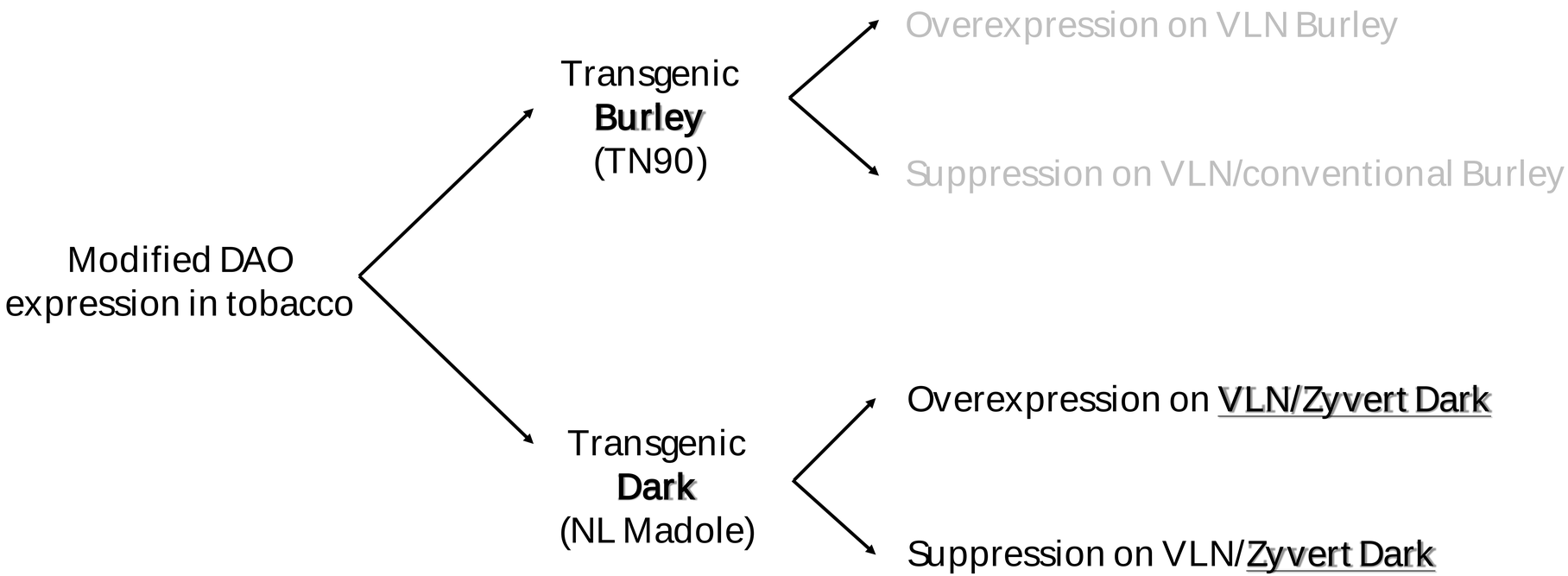




End of Curing

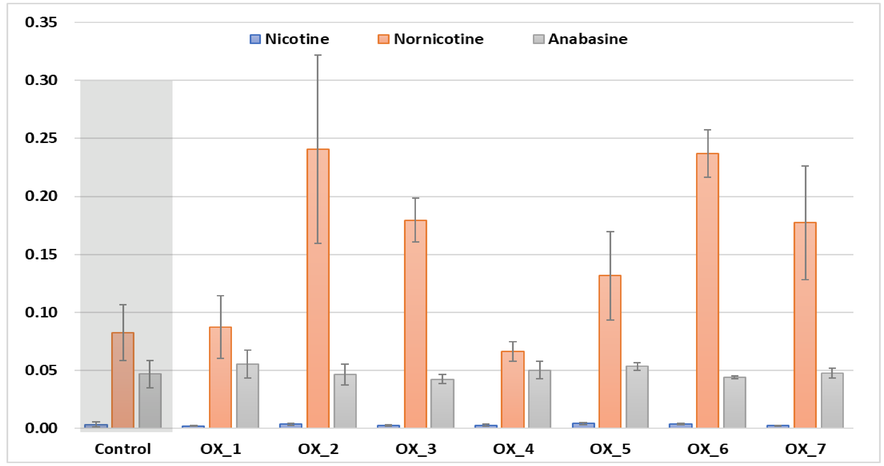
No nornicotine or NNN decrease observed from the tested conventional Burley

Approaches to study DAO function – Dark tobacco

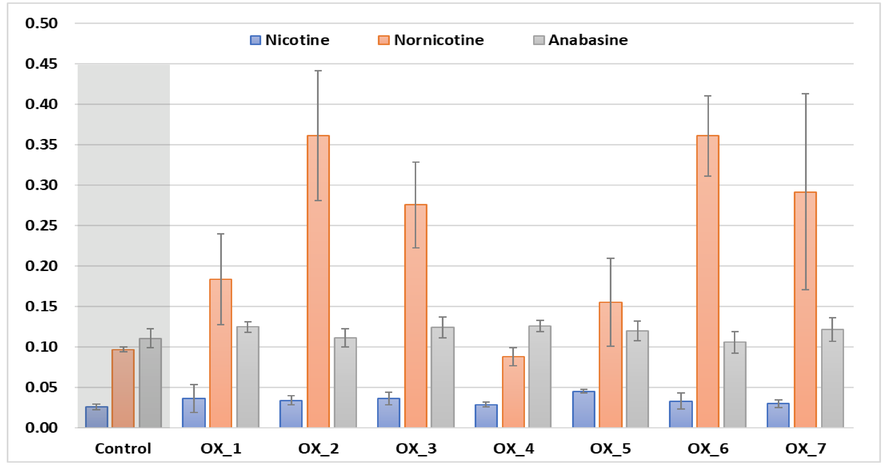


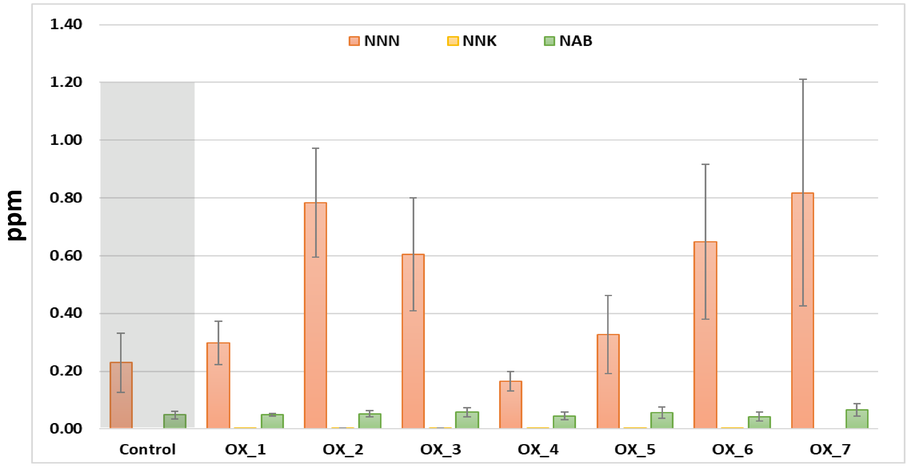
Alkaloids & TSNA in DAO-overexpressed VLN/Zyvert Dark

2 weeks post-topping



End of Curing



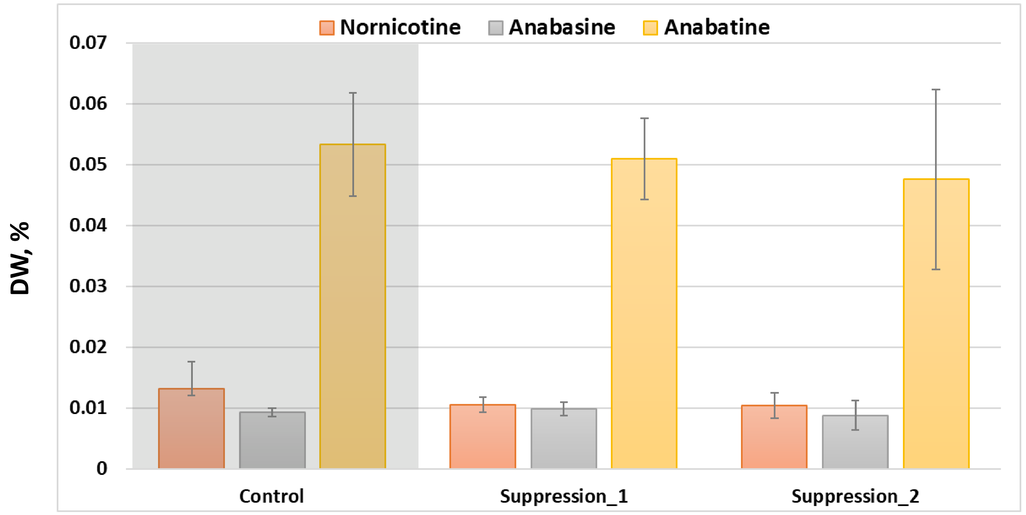


End of Curing

Increased nornicotine(~250%) & NNN(~210%) formation from DAO-overexpressed VLN/Zyvert Dark tobacco samples

Alkaloids in DAO-suppressed Zyvert Dark – Ongoing study

2 weeks post-topping



DAO suppression seems to reduce nornicotine formation in Zyvert Dark tobacco, but need more study to confirm

Conclusions

- The DAO was proposed to be involved in another pathway for nornicotine biosynthesis besides NND pathway in VLN and Zyvert tobacco
- The DAO expression modification effect was examined in Burley and Dark tobacco
  - ➔ In Burley tobacco, no significant change in nornicotine/NNN level was observed from both overexpression and suppression trials
  - ➔ The increase of nornicotine/NNN level was observed from DAO-overexpressed VLN/Zyvert Dark tobacco
  - ➔ The suppression of DAO in Zyvert Dark tobacco showed a small decrease of nornicotine contents in the preliminary test, however, a large-scale field study is necessary to confirm the results.
- In Dark tobacco, DAO could be a new target for further reduction of nornicotine/NNN
- Planning to generate DAO-suppressed Zyvert Dark tobacco to further reduce NNN

References and Acknowledgement

1. Heim, William G., et al. "Cloning and characterization of a Nicotiana tabacum methylputrescine oxidase transcript." Phytochemistry 68.4 (2007): 454-463.
2. Hashimoto, Takashi, Akira Mitani, and Yasuyuki Yamada. "Diamine oxidase from cultured roots of Hyoscyamus niger: its function in tropane alkaloid biosynthesis." Plant Physiology 93.1 (1990): 216-221.
3. Katoh, Akira, Tsubasa Shoji, and Takashi Hashimoto. "Molecular cloning of N-methylputrescine oxidase from tobacco." Plant and Cell Physiology 48.3 (2007): 550-554.
4. Naconsie, Maliwan, et al. "Molecular evolution of N-methylputrescine oxidase in tobacco." Plant and Cell Physiology 55.2 (2014): 436-444.

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Thank you

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