

Contributions of three nicotine demethylases to nicotine and nornicotine composition in the plant

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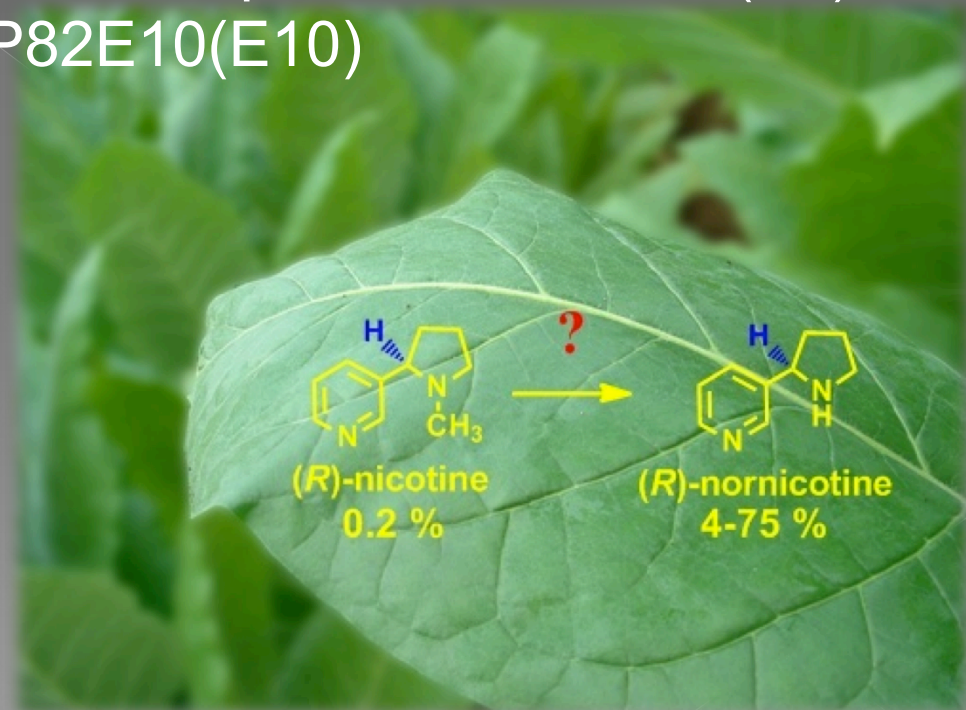
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2011 TSRC

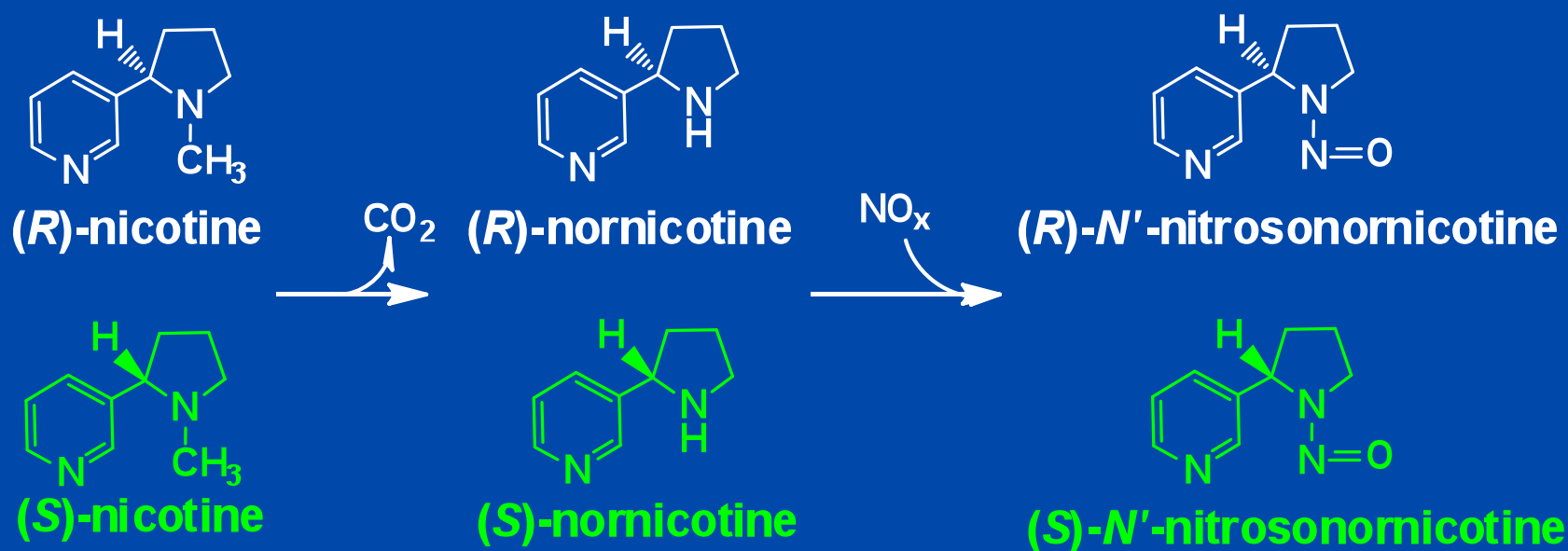
Extensively studied, but...

- ❖ Nicotine is biosynthesized in root, and translocated to leaf
- ❖ Nicotine demethylation can occur in both leaf and root, but mainly in senescent leaf.
- ❖ Three nicotine demethylases reported: CYP82E4(E4), CYP82E5(E5) and CYP82E10(E10)



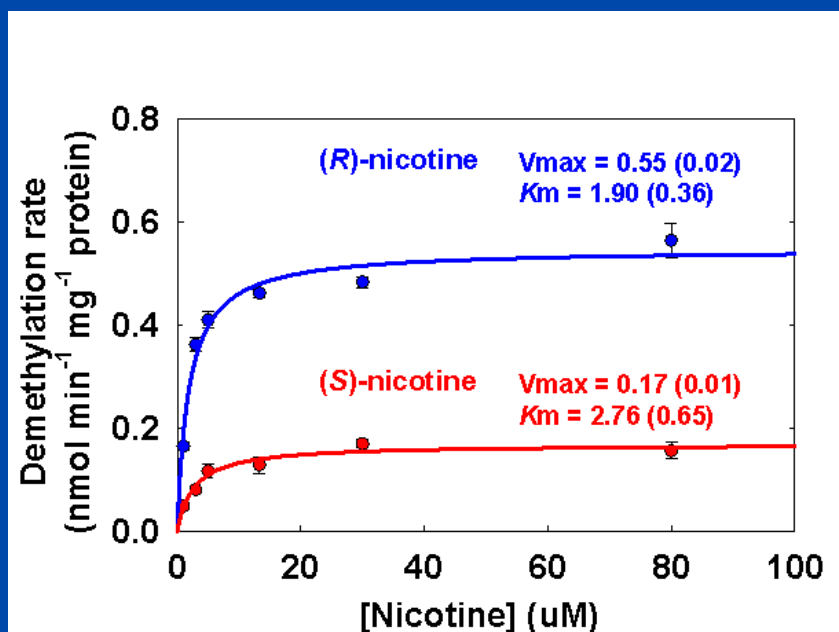
The puzzle of nicotine and nornicotine metabolism

Alkaloids and TSNAs are all chiral compounds

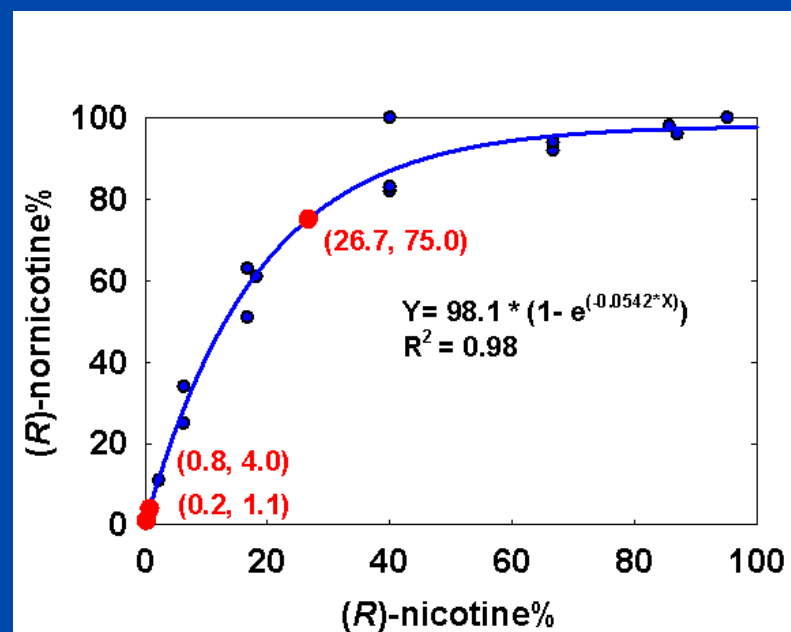


(S)-nicotine is more physiologically active than (R)-nicotine, and (S)-NNN is more carcinogenic.

In vitro kinetics study of E4 demethylation

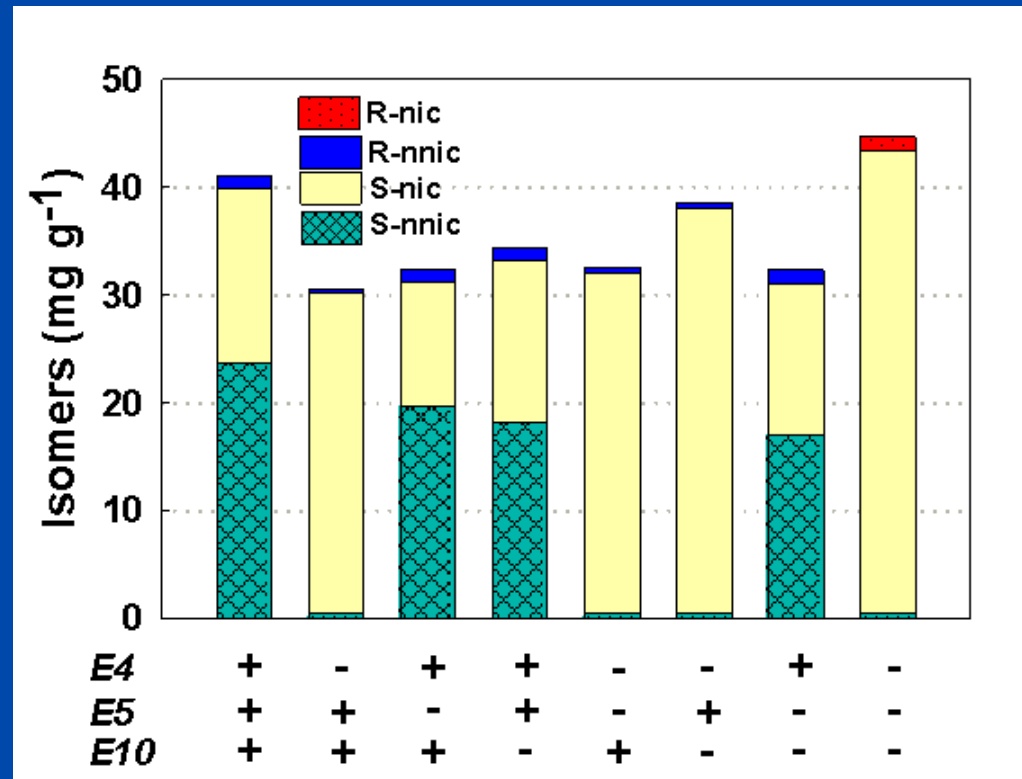


E4 shows preference for (R)-nicotine *in vitro*.



E4 alone can not produce 4-75% (R)-nornicotine percentage from 0.2% (R)-nicotine.

Nicotine and nornicotine composition in mutant cured lamina



- ❖ E5 and E10 are (*R*)-nicotine demethylases
- ❖ E4 can use both (*R*)-nicotine and (*S*)-nicotine
- ❖ Initially synthesized nicotine is 3% of R

Questions

- ❖ What are compositions of nicotine and nornicotine translocated from root to the leaf?
- ❖ What are the functionalities of three demethylases in leaf and their contributions to the final leaf nicotine and nornicotine composition?

Grafts

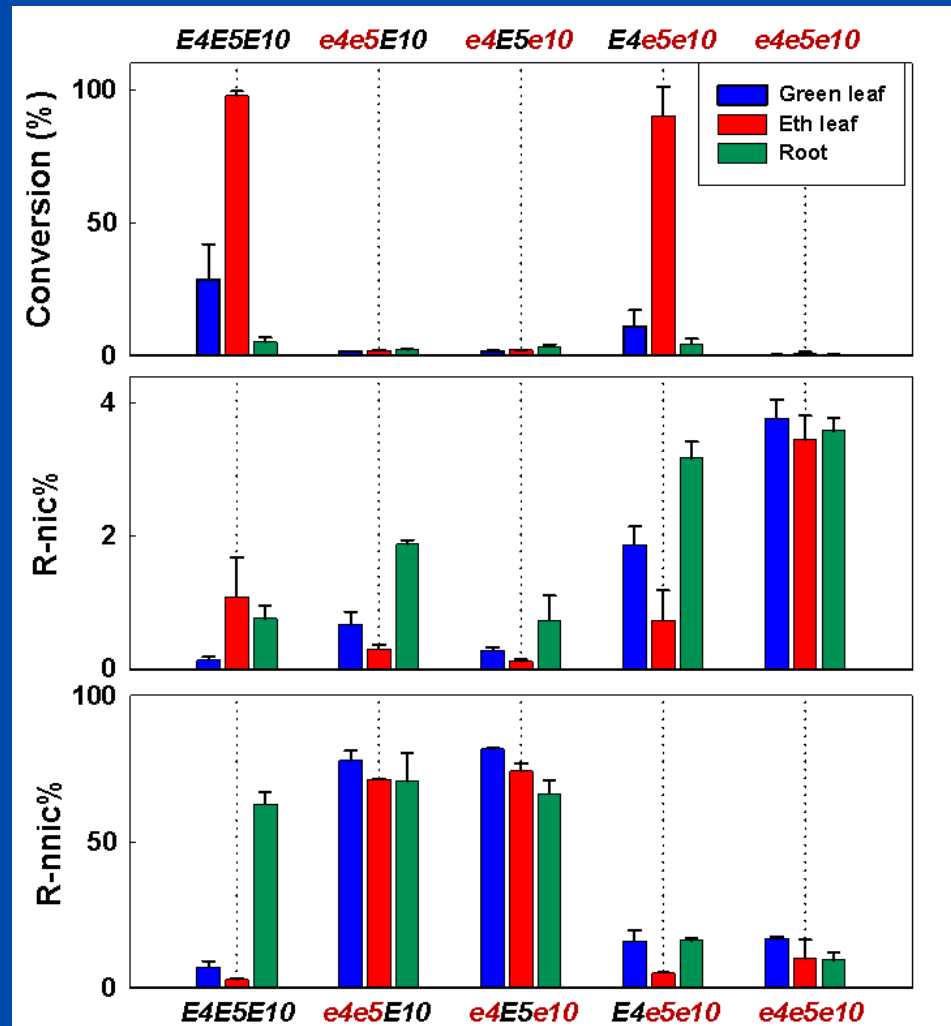


Tomato/ Tobacco

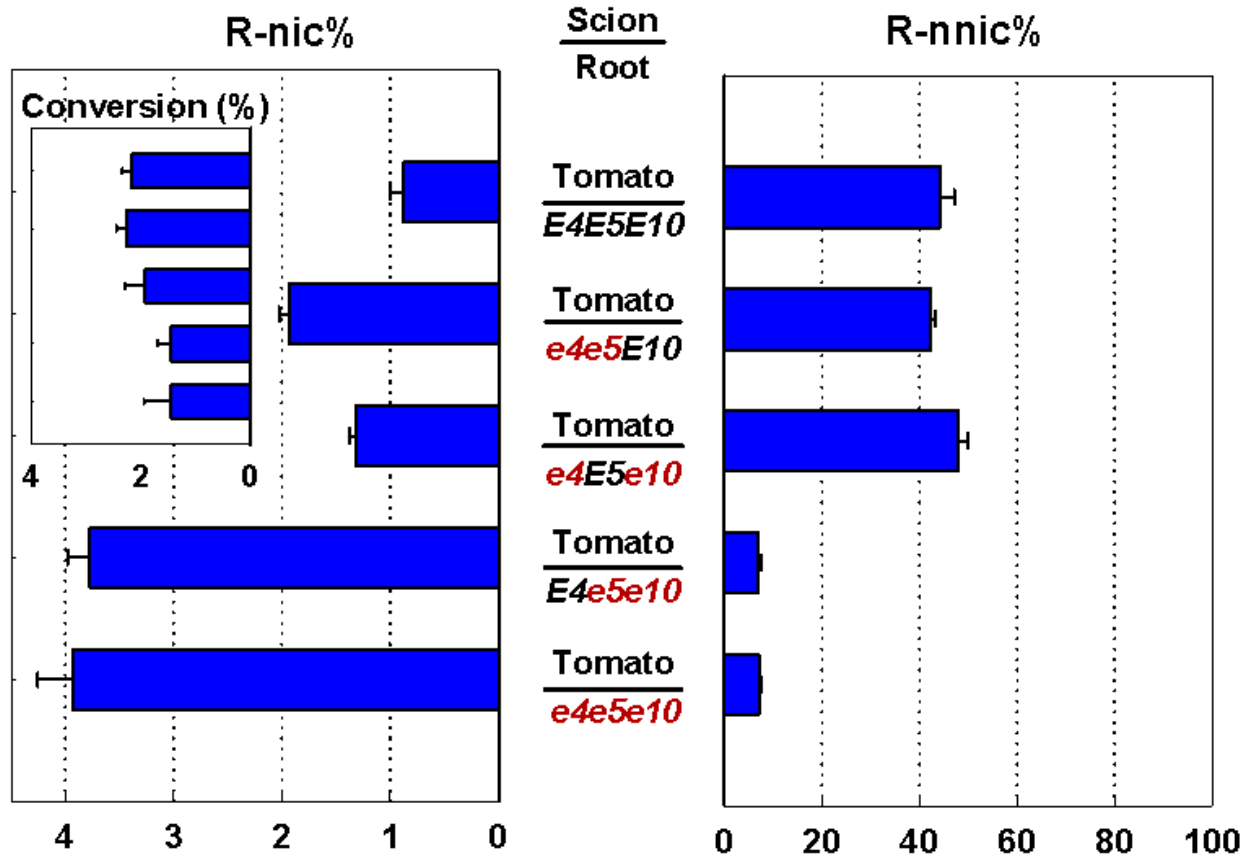


Tobacco/Tobacco

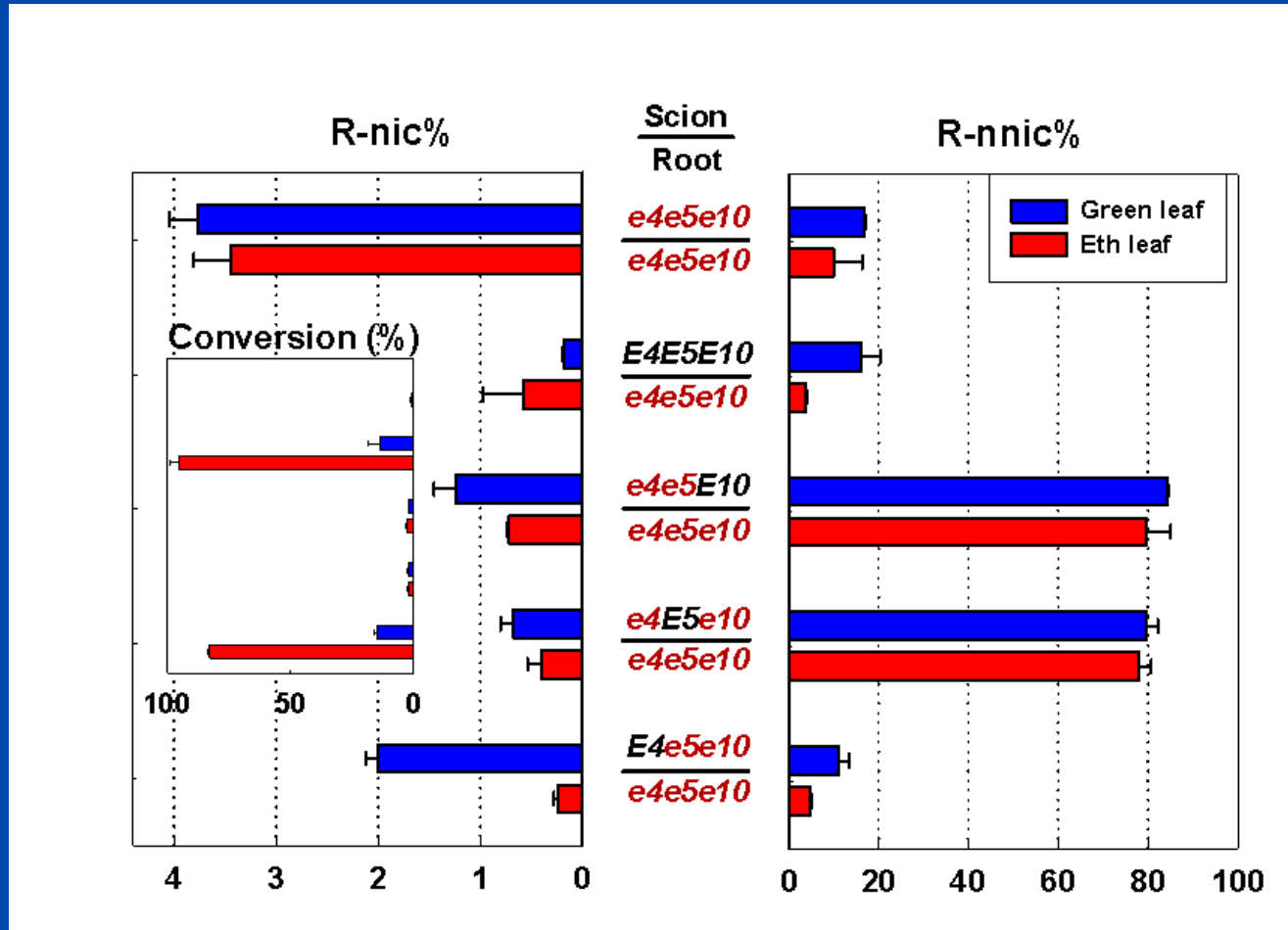
Nicotine and nornicotine composition in self-grafted tissues



Nicotine and nornicotine composition in tom/tob grafts lamina



Nicotine and nornicotine composition in tob/e4e5e10 grafts lamina

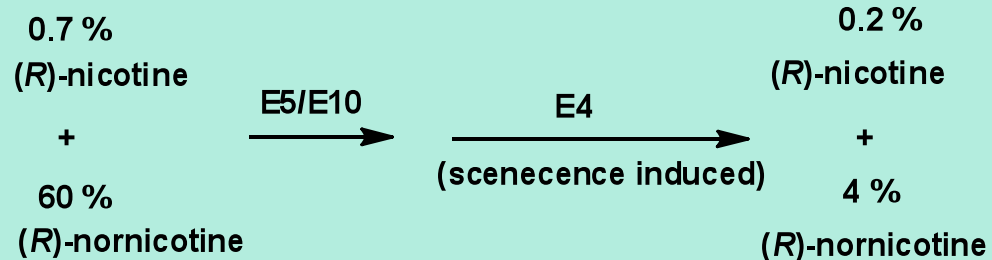


E10 is active in leaf?

Summary

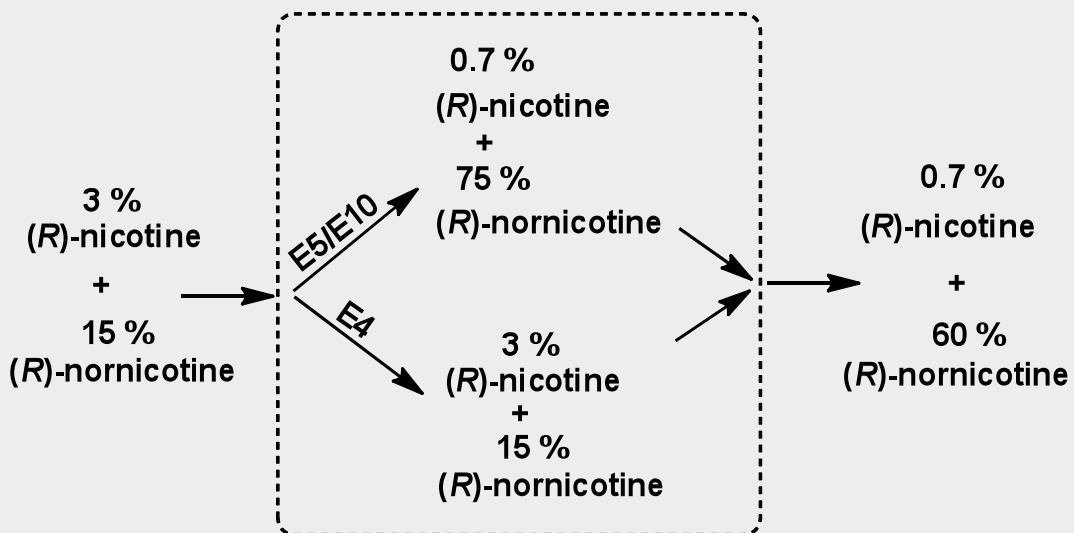
- ❖ E5 and E10 are active in both root and leaf; E5 and E10 have high enantioselectivity for (*R*)-nicotine.
- ❖ E4 is active in both root and leaf, especially in the senescent leaf; E4 has lower selectivity for (*R*)-nicotine and is responsible for the (*S*)-nornicotine production

Leaf



↑
nicotine
nornicotine

Root



**Proposed roles of three nicotine demethylases
in the composition of nicotine and nornicotine.**

Nicotine composition change...



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