

2013-08

# 2013 REU Poster: Modulation of Indolic Plant Defense compound Synthesis by Tryptophan Analogs

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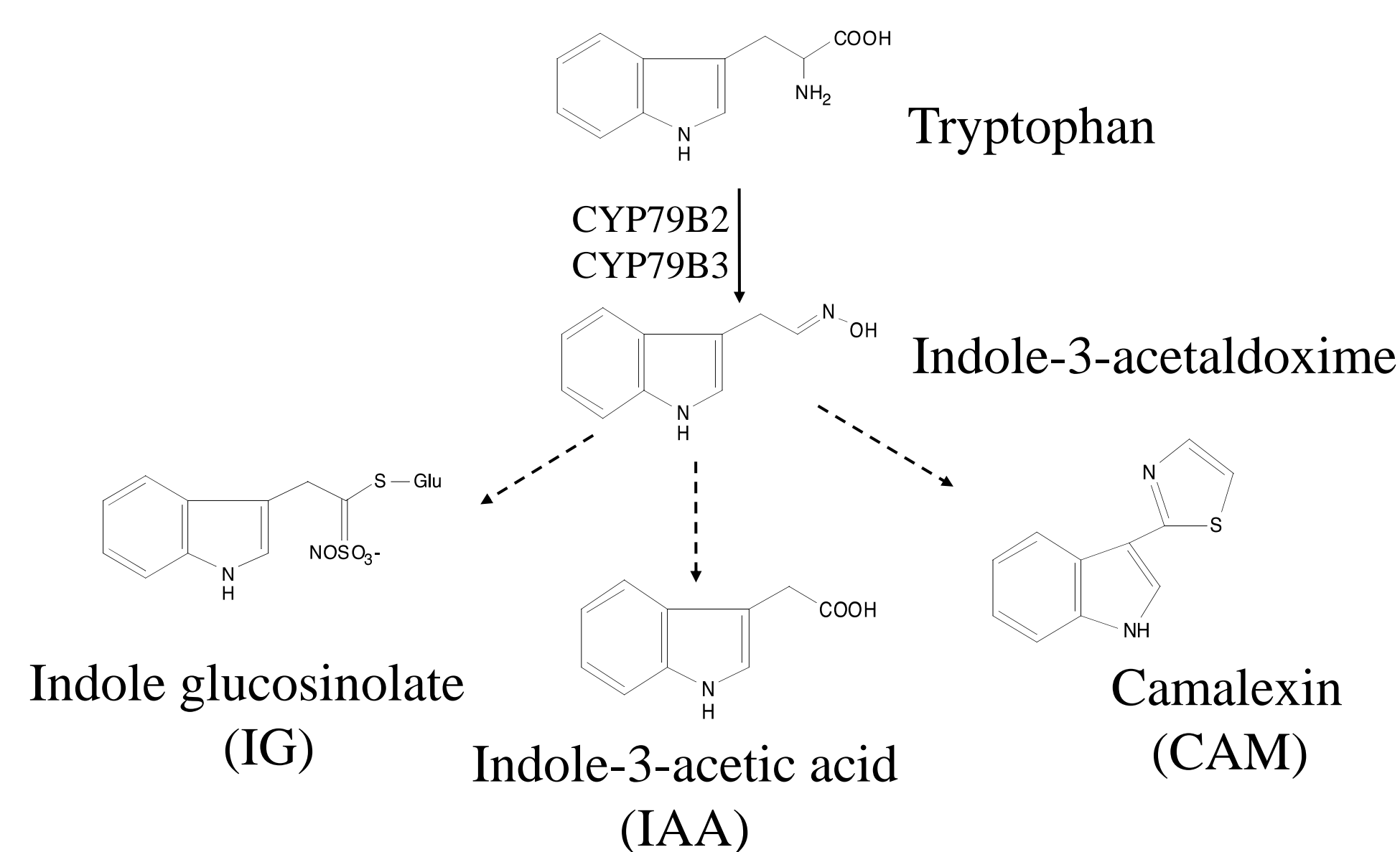
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*Boston University*

## Abstract

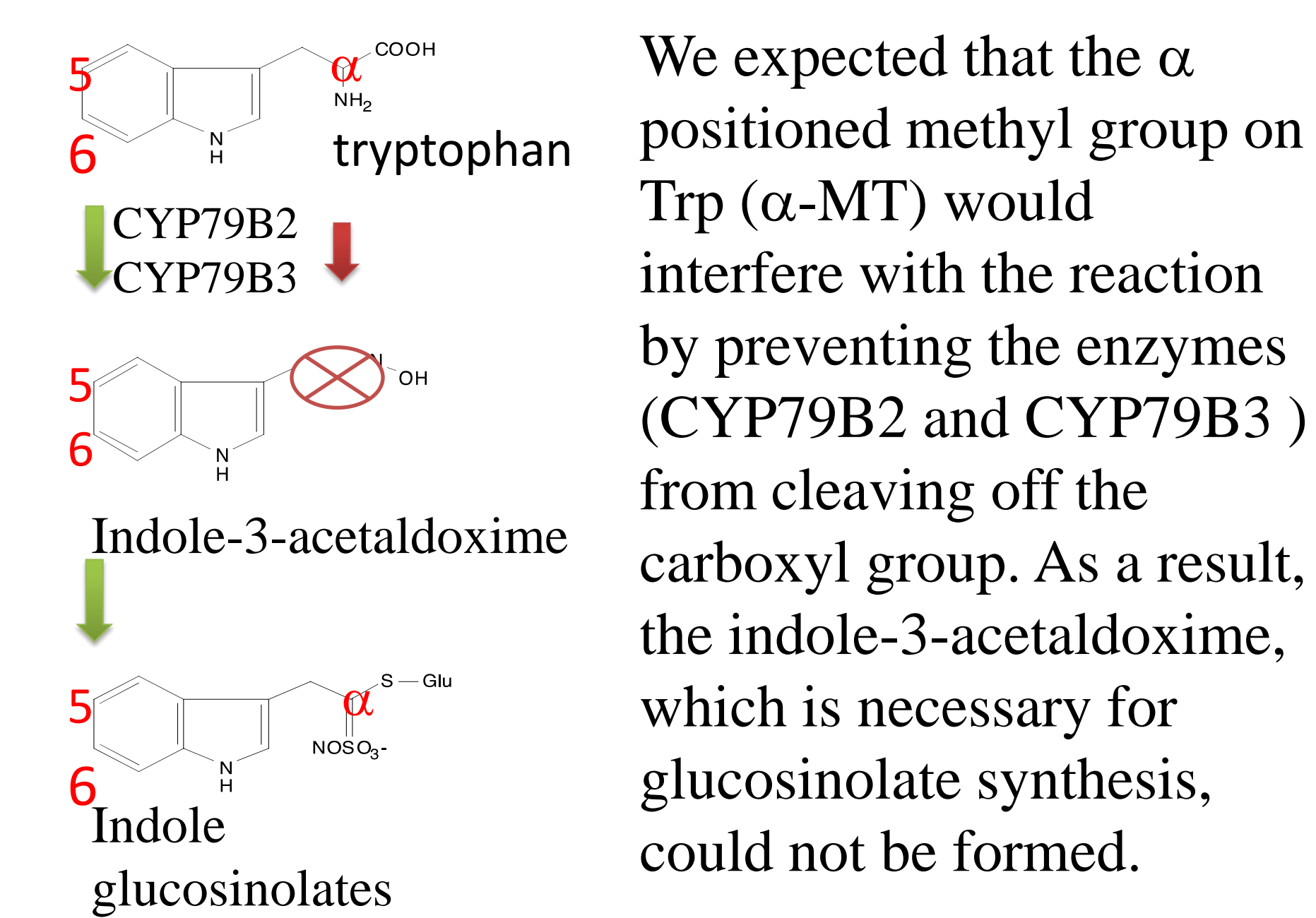
Like bacteria and fungi, plants are able to synthesize aromatic amino acids Tyrosine (Tyr), Phenylalanine (Phe) and Tryptophan (Trp). Those amino acids are used in plants not only for protein synthesis, but also for a variety of compounds that control development and defense. *Arabidopsis thaliana* uses Trp to produce distinct secondary metabolites that function as deterrents to herbivory (indole glucosinolates), as defense against microbial pathogens (camalexin) and as growth regulators (indole-3-acetic acid). To better understand the relationship between Trp biosynthesis and indole glucosinolate (IGs) production, we have tested different analogs of Trp on Columbia, a wild-type *Arabidopsis* accession. We have found that  $\alpha$ -methyl tryptophan cannot be incorporated into IGs and in fact inhibits IG synthesis.

## Tryptophan Metabolism in *A.T.*



The enzymes CYP79B2 and CYP79B3 decarboxylate Trp and then *N*-hydroxylate to form indole-3-acetaldoxime from which derive IG, IAA and CAM.

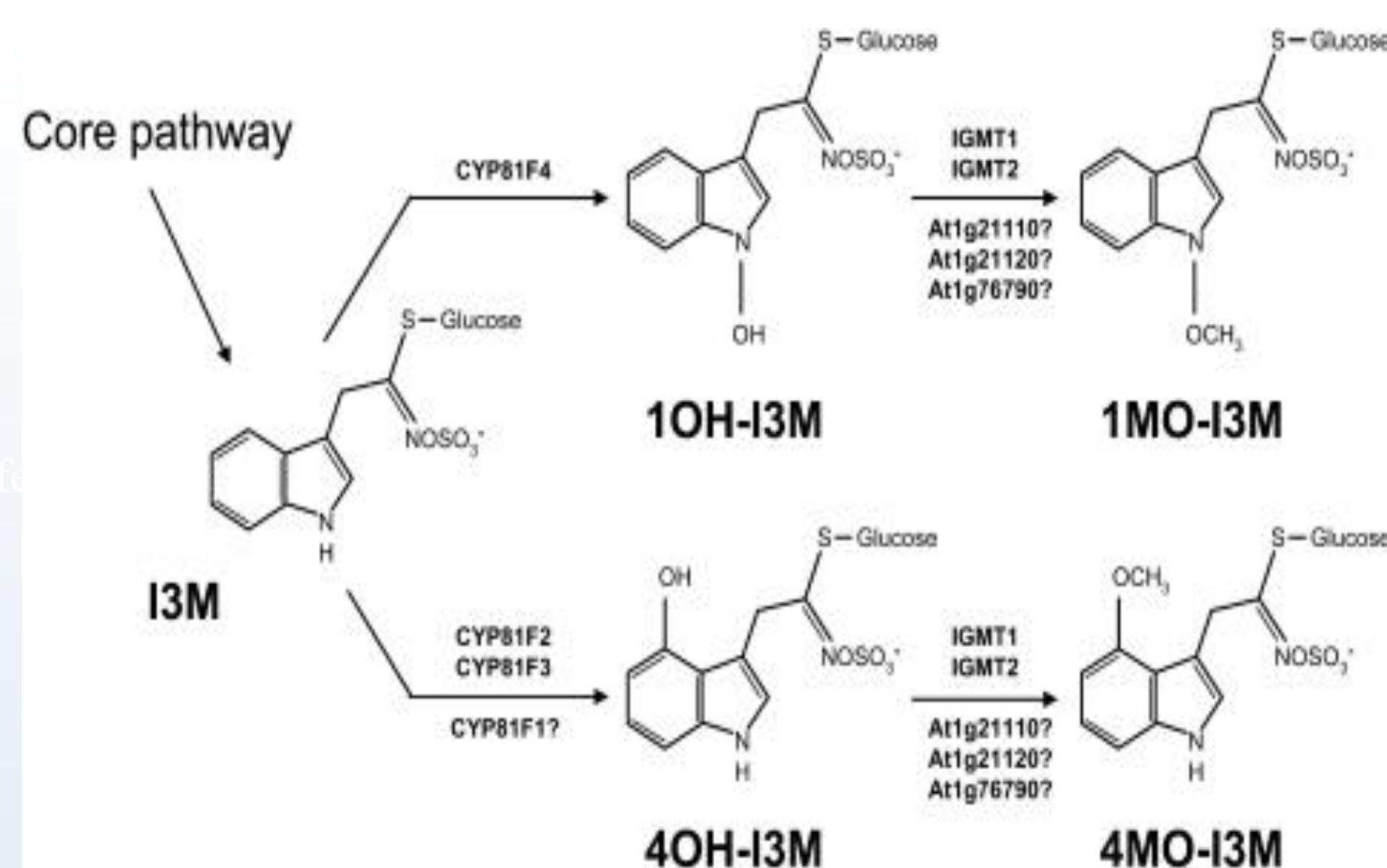
## Hypothesis



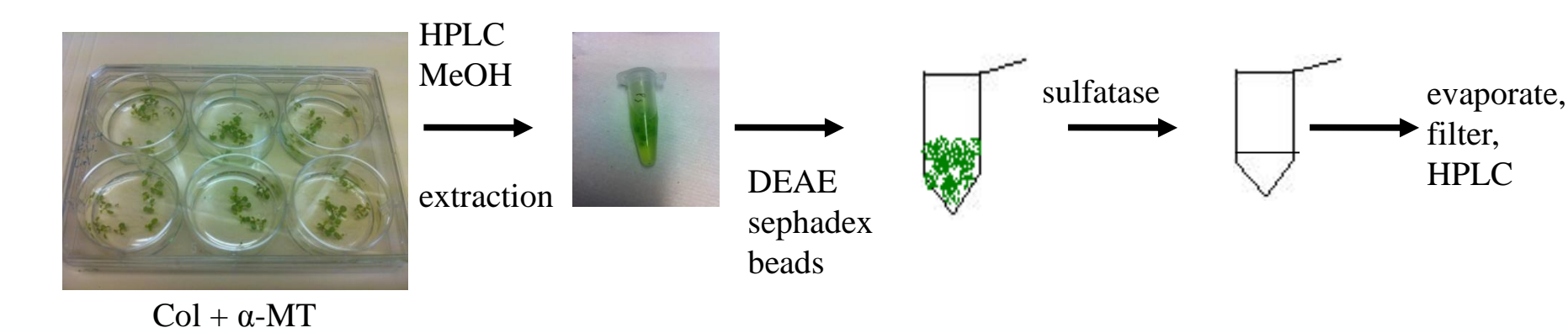
We expected that the  $\alpha$  positioned methyl group on Trp ( $\alpha$ -MT) would interfere with the reaction by preventing the enzymes (CYP79B2 and CYP79B3) from cleaving off the carboxyl group. As a result, the indole-3-acetaldoxime, which is necessary for glucosinolate synthesis, could not be formed.

## IG Biosynthetic Pathways

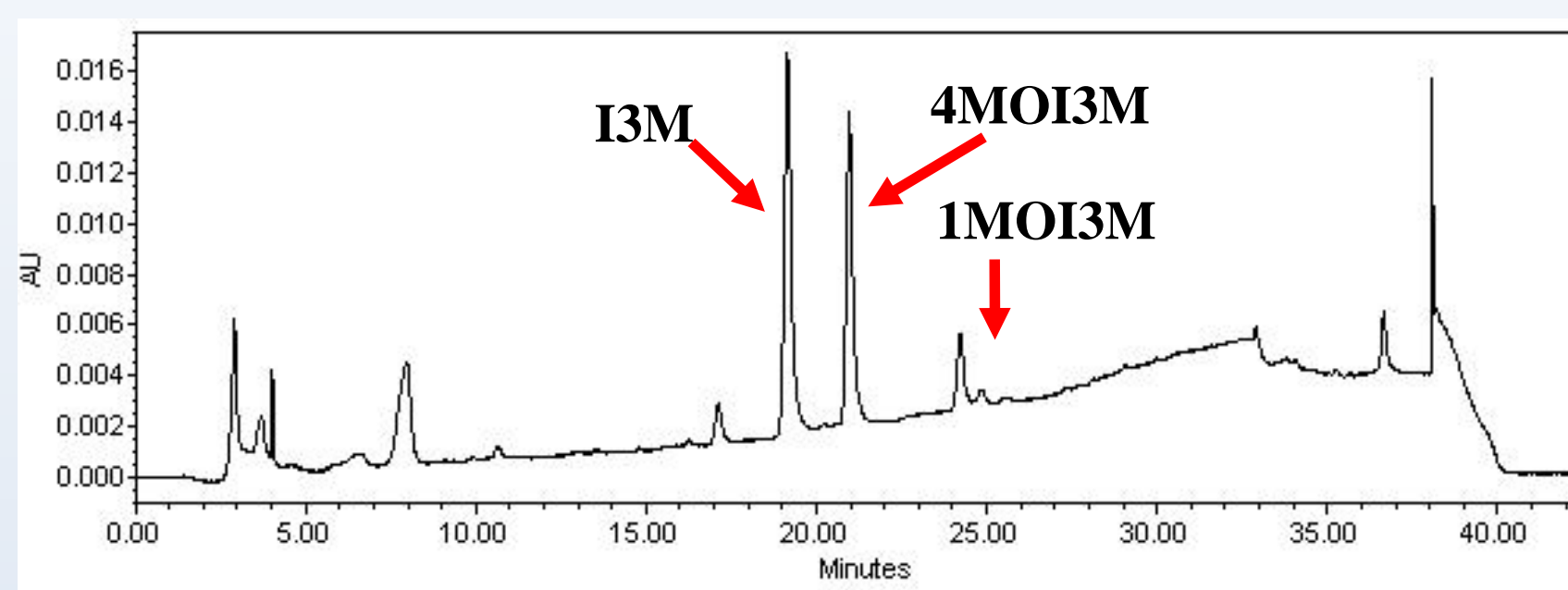
## IG Biosynthetic Pathways



Indole-3-methylglucosinolate (I3M) is the “core” IG and is then converted by hydroxylation and methylation to either 1-methoxy I3M (1MO-I3M) or 4-methoxy I3M (4MO-I3M).

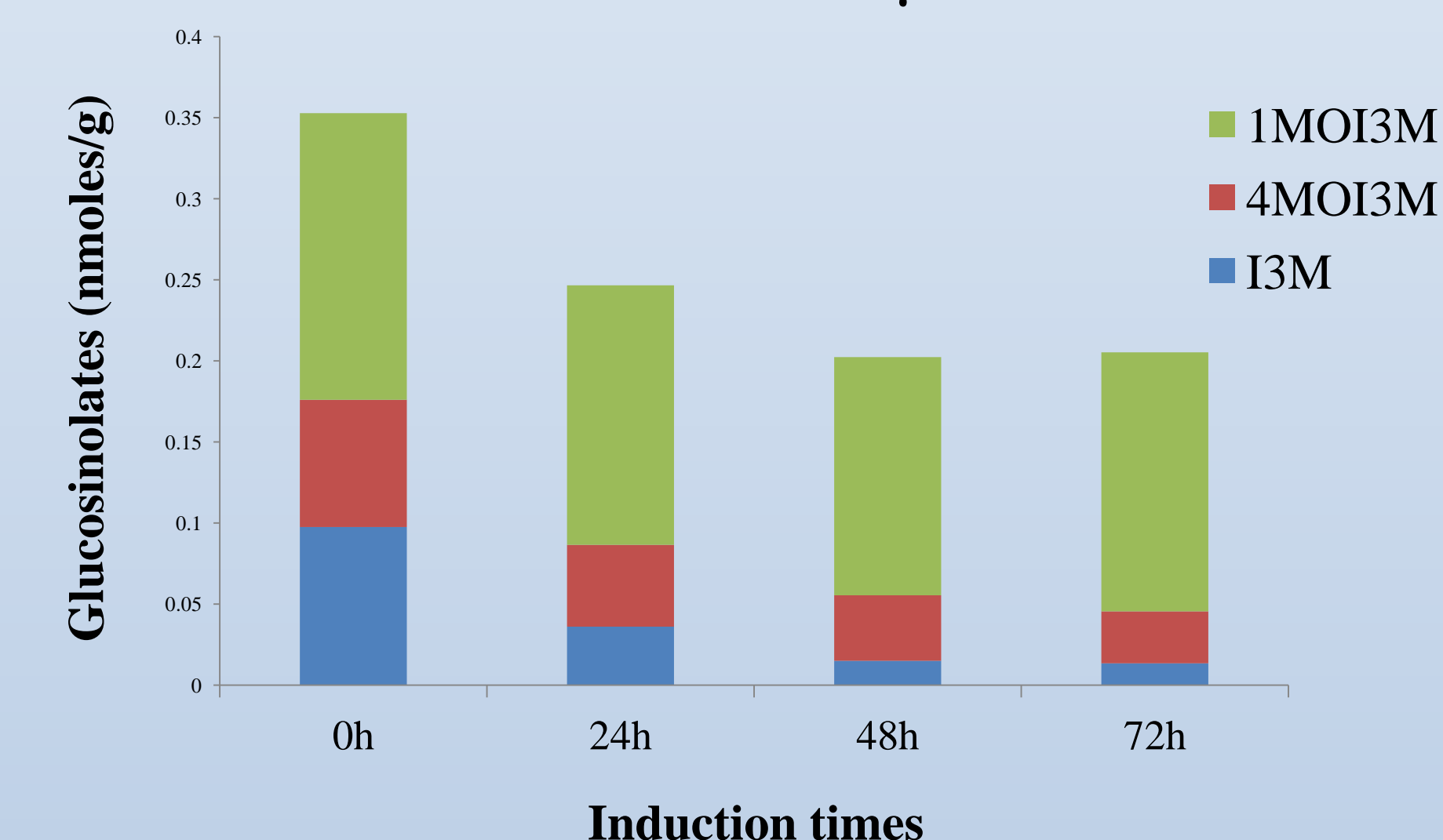


- HPLC run is an increasing acetonitrile gradient
- Different desulfoglucosinolates were detected by their absorbance at 229nm.
- For quantification, peak areas were normalized to plant tissue weight and a known weight standard

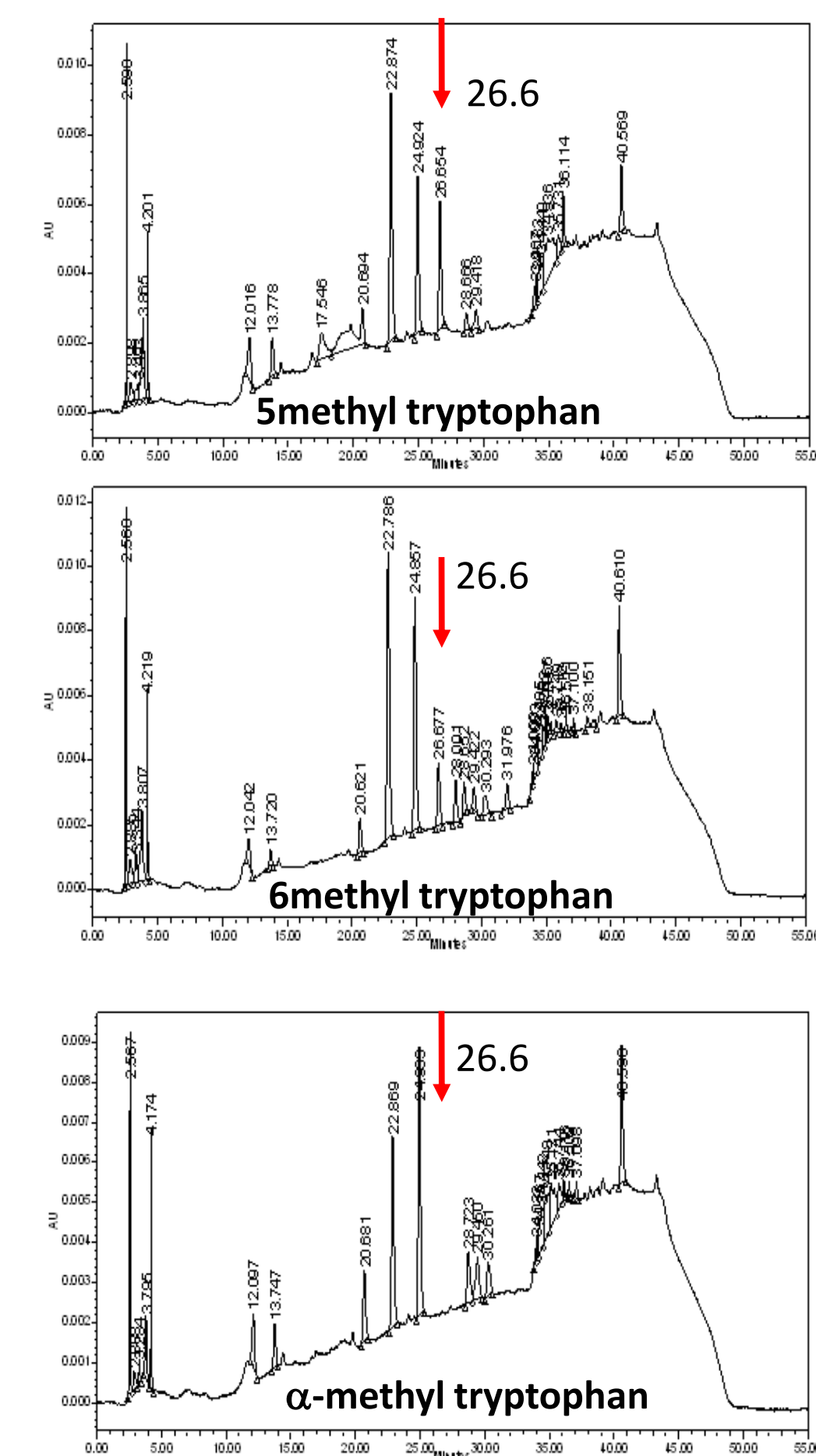


## Results

Indole glucosinolates production at varying induction times with 100  $\mu$ M of  $\alpha$ -MT



The longer the treatment the lesser I3M production.



## 5-MT and 6-MT Vs $\alpha$ -MT

- There is an extra peak at 26.6 min for 5-MT and 6-MT but not for  $\alpha$ -MT.

## Conclusion

- $\alpha$ -MT cannot be efficiently incorporated
- $\alpha$ -MT inhibits IG synthesis

## Future Goals

- Extend the treatment time to confirm a decrease in 1MOI3M and 4MOI3M production
- Incorporate  $\alpha$ -MT directly into leaves
- Expose the treated plants to potential herbivores to determine if differences in indolic glucosinolate production are really significant
- For 5- and 6-MT confirm the structure of the putative methylated IGs that comes off at 26.6 min

## Acknowledgments

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## Reference

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- S nderby IE, Geu-Flores F, and Halkier BA (2010). Biosynthesis of glucosinolates--gene discovery and beyond. *Trends in Plant Science* 15:283-90.

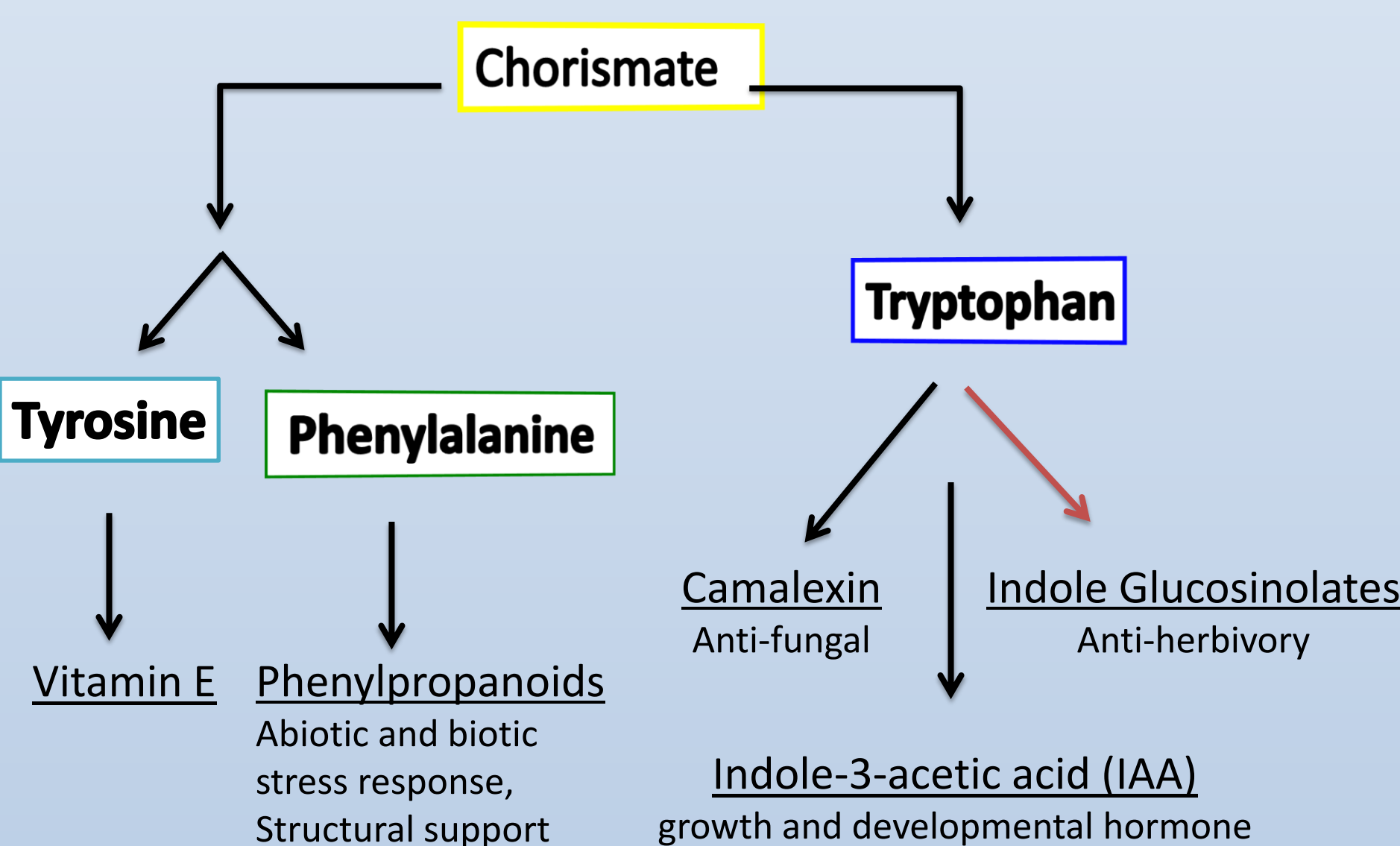
## *Arabidopsis thaliana* (*A.T.*)

- A small flowering plant native to Europe, Asia and northwestern Africa
- Member of the Brassicaceae family -informally known as mustards, mustard flowers or cruciferae

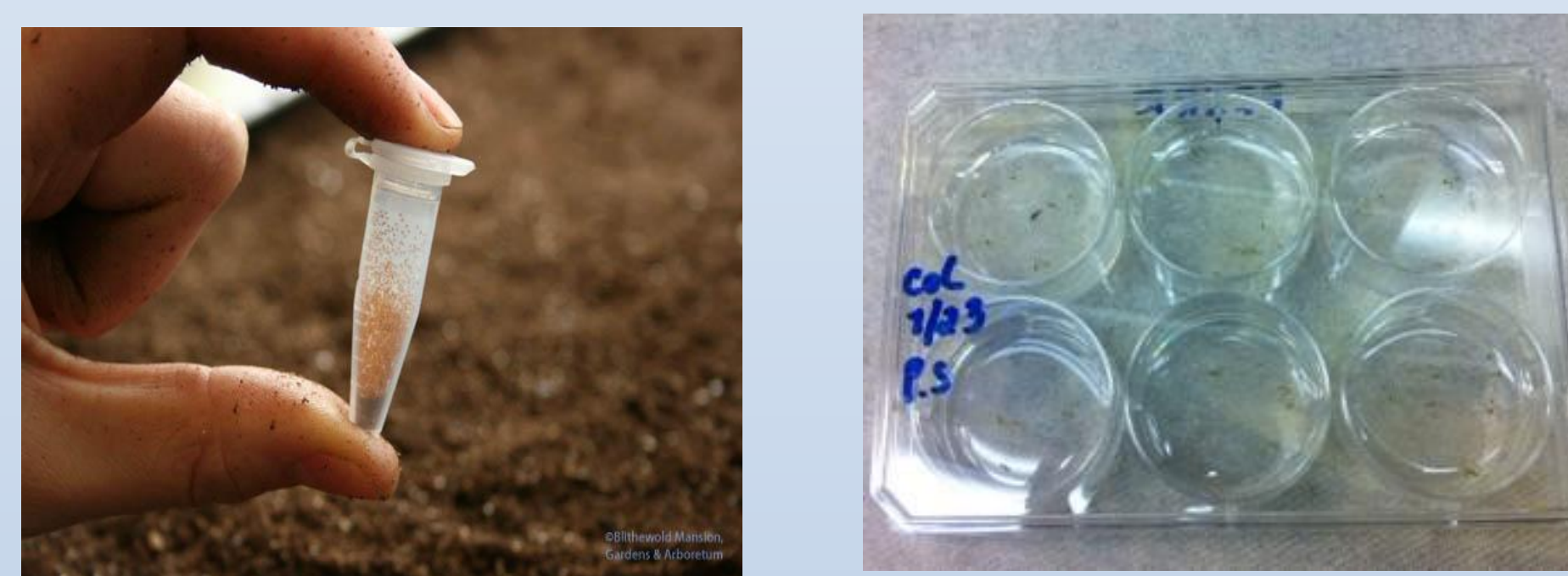


- Fast life cycle
- Cheap/easy to maintain
- Self fertile
- Transformable
- 5 chromosomes (diploid)
- Small genome: approx. 26,000 genes

## Aromatic Amino Acids in *A.T.*



## Experimental Methods



10 days after germination in liquid PNS medium, the plants were fed with 100 $\mu$ M of  $\alpha$  methyl tryptophan ( $\alpha$ -MT) at varying induction time : 24h, 48h, 72h before collection