Quantitative and qualitative investigation of *Duboisia myoporoides* R.BR.

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Introduction

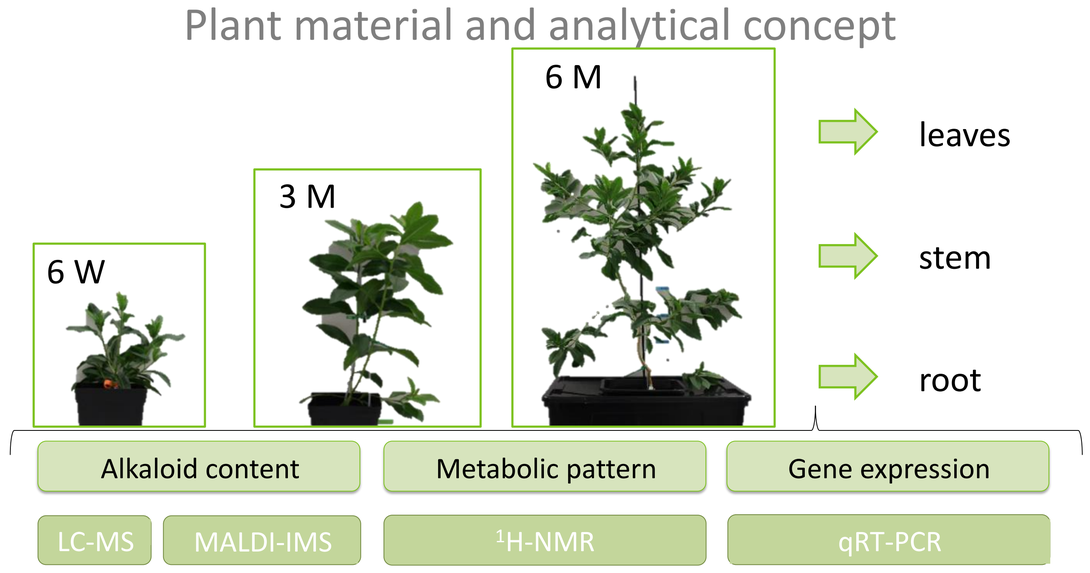
Tropane alkaloids

- Tropane alkaloids (TA), including scopolamine and hyoscyamine, are secondary plant components mainly occurring in the family of Solanaceae
- Scopolamine is an important bulk compound in the semi-synthesis of drugs for clinical medicines like Buscopan® or Spiriva®
- TA are mainly obtained via extraction from field-grown *Duboisia* hybrids
- Demand for scopolamine based drugs is expected to increase in the future

Objectives

- Elucidation and understanding of the TA pathway in *Duboisia myoporoides*
- Improve the breeding approaches by better comprehension
- Ensure sustainable scopolamine supply

Plant material and analytical concept



Biosynthesis

Biosynthetic pathway of TA

- Biosynthesis of TA is located in the roots[1]
- TA are transported to the aerial parts of the plants
- Storage and accumulation of hyoscyamine, 6-OH-hyoscyamine and scopolamine in the leaves (cf. HPLC-MS based quantitation, Fig. 2)

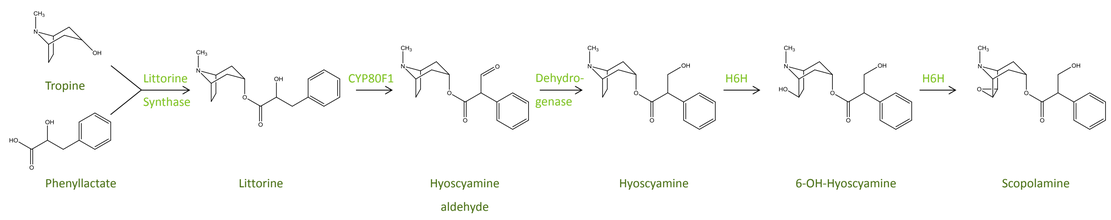


Fig. 1: Section of late tropane alkaloid pathway

Alkaloid content [%]

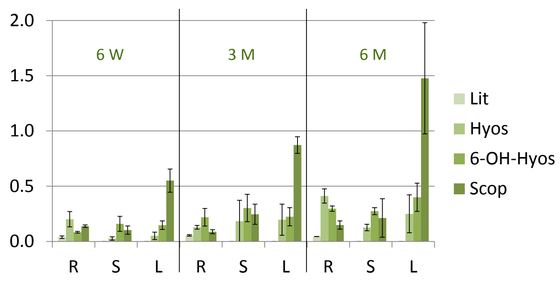


Fig. 2: Alkaloid content in three different tissues: R = root, S = stem, L = leaves at three different time points: 6 W = 6 weeks, 3 M = 3 months, and 6 M = 6 months

TA quantitation

- Ratio of TA in the roots and in the stem remains equal
- Scopolamine accumulates over time within the leaves

qPCR

- Highest expression of gene transcripts in the roots
- Intermediate plants show highest levels of transcripts

qPCR results

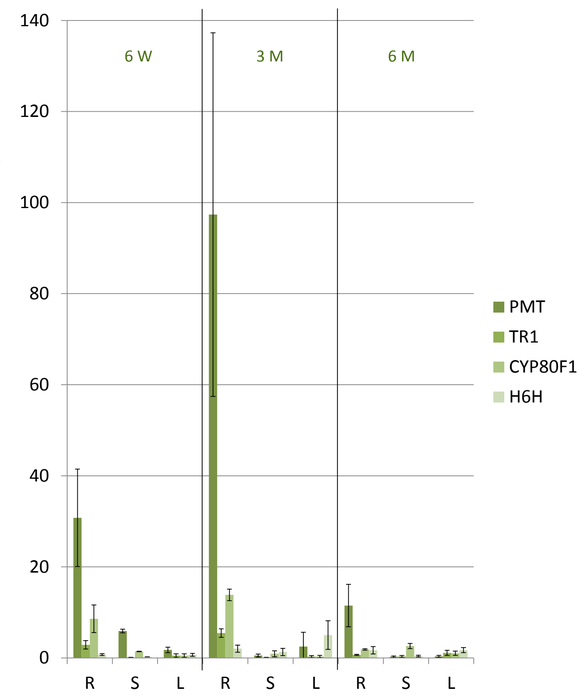


Fig. 3: Relative gene expression measured via quantitative RT-PCR, normalized using GAPDH for endogenous control. R = root, S = stem, L = leaves at three different time points: 6 W = 6 weeks, 3 M = 3 months, and 6 M = 6 months

Quantitative measurement

Spatial distribution

MALDI imaging-MS of *Duboisia myoporoides*

- Investigation of root, stem, and leaves at different time points
- Images showing the spatial distribution of the TA hyoscyamine aldehyde, hyoscyamine, 6-OH-hyoscyamine and scopolamine

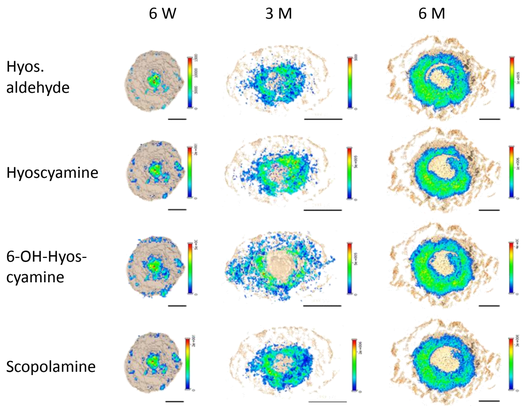


Fig. 4: Ion images showing the spatial distribution of tropane alkaloids in *Duboisia myoporoides* roots. Localization of hyoscyamine aldehyde ([M+H]$^+$; *m/z* 288.16), hyoscyamine ([M+H]$^+$; *m/z* 290.18), 6-OH-hyoscyamine ([M+H]$^+$; *m/z* 306.17), scopolamine ([M+H]$^+$; *m/z* 304.15). Scale bar 1 mm.

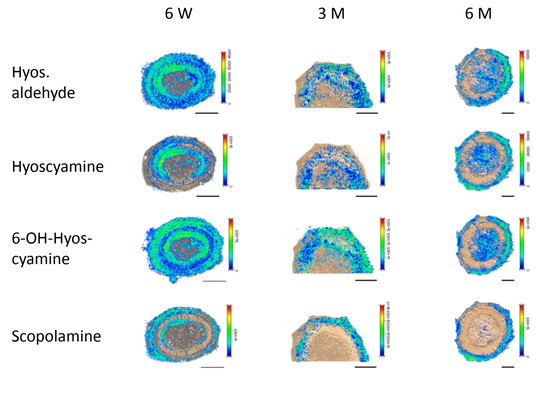


Fig. 5: Ion images showing the spatial distribution of tropane alkaloids in *Duboisia myoporoides* stem, cross section. Localization of hyoscyamine aldehyde ([M+H]$^+$; *m/z* 288.16), hyoscyamine ([M+H]$^+$; *m/z* 290.18), 6-OH-hyoscyamine ([M+H]$^+$; *m/z* 306.17), scopolamine ([M+H]$^+$; *m/z* 304.15). Scale bar 1 mm.

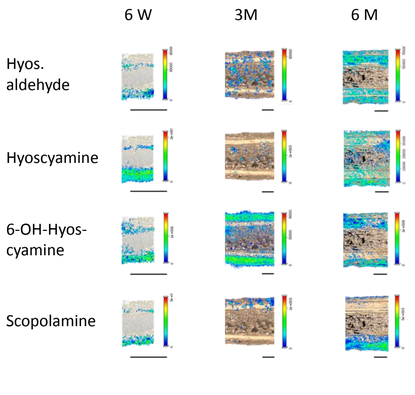


Fig. 6: Ion images showing the spatial distribution of tropane alkaloids in *Duboisia myoporoides* stem, longitudinal section. Localization of hyoscyamine aldehyde ([M+H]$^+$; *m/z* 288.16), hyoscyamine ([M+H]$^+$; *m/z* 290.18), 6-OH-hyoscyamine ([M+H]$^+$; *m/z* 306.17), scopolamine ([M+H]$^+$; *m/z* 304.15). Scale bar 1 mm.

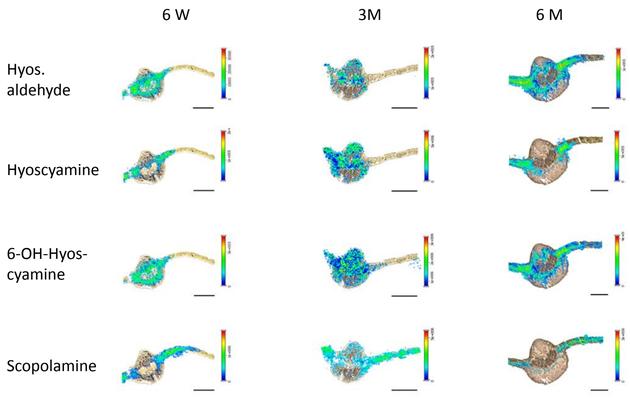


Fig. 7: Ion images showing the spatial distribution of tropane alkaloids in *Duboisia myoporoides* leaves. Localization of hyoscyamine aldehyde ([M+H]$^+$; *m/z* 288.16), hyoscyamine ([M+H]$^+$; *m/z* 290.18), 6-OH-hyoscyamine ([M+H]$^+$; *m/z* 306.17), ([M+H]$^+$; *m/z* 304.15). Scale bar 1 mm.

- Different spatial distribution over time; biosynthesis of TA in young roots is located within the central cylinder, whereas the localization of TA in older plants is found in the inner cortex and outer central cylinder

^{1}H NMR measurement

Metabolic profiling

- Comparison of metabolite composition in different plant organs (Fig. 8 + Table 1) and at different growth stages (Fig. 9 + Table 2) by multivariate analysis using PLS-DA
- Partial least squares (PLS) regression is a statistical method that is related to principal components (PCA) regression. It is used to find the fundamental relations between two matrices (*X* and *Y*). A variant of PLS is PLS-DA. Here the *Y* is categorical.

Metabolite profile in different plant organs

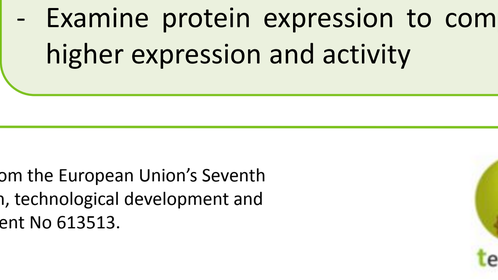


Fig. 8: Partial least squares discriminant analysis, samples coloured according organ

Metabolite profile at different growth stages

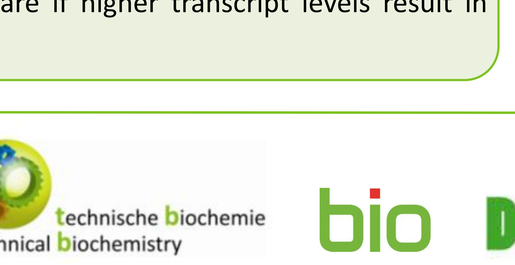


Fig. 9: Partial least squares discriminant analysis, samples coloured according age

- Root extracts consist of metabolites used for TA biosynthesis like the early precursor putrescine and littorine, whereas leaf extracts show a high amount of secondary metabolites like TA and chlorogenic acid
- Furthermore, the metabolic pattern changes over time: young plants show metabolites of the primary metabolism, e.g. myo inositol and glucose while matured plants are described by secondary plant metabolites
- Intermediate plants indicate a high amount of amino acids in particular alanine and threonine

Summary

- The biosynthesis takes place in the roots (Table 1), then the TA are transported via the vascular tissue (Fig. 5 + 6) to the leaves
- Accumulation of TA in the leaves (Table 1 + Fig. 2)
- Spatial metabolite distribution is age dependent
- Highest gene transcript levels in the roots of the intermediate plants (3 M)

Outlook

- Gene encoding for the Littorine Synthase (Fig. 1) is not known yet. To fully elucidate the pathway, this gene needs to be determined
- Examine protein expression to compare if higher transcript levels result in higher expression and activity

References

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[3] Kim, HK, Choi YH, Verpoorte R, (2010) NMR-based metabolomic analysis of plants. Nat. Protoc., pp.536–549.

Acknowledgements

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