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Vitamin D₃ in plants – effect of UVB exposure

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Introduction

As a surprise for many not only vitamin D₂, but also vitamin D₃ can be found in plants. Vitamin D₃ is formed in the skin of vertebrates by exposure to UVB light (Fig. 1). The synthesis of vitamin D₃ in plants is on the other hand unresolved and contradicting results regarding the dependence of UVB-light has been presented (1,2,3). The aim of this study was, therefore, to investigate vitamin D₃ synthesis and metabolism in plants and how it changes upon UVB-exposure. Most work on vitamin D₃ in plants has been done with non-selective methods such as bioassays, but this study utilizes LC-MS/MS with derivatization to improve sensitivity and selectivity.

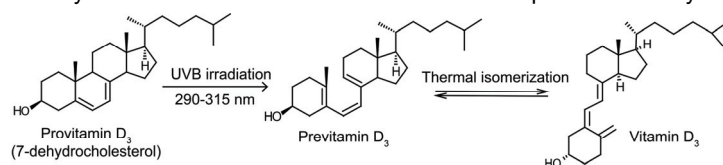


Fig. 1. Biosynthesis of vitamin D₃ from 7-dehydrocholesterol. UVB exposure of 7-dehydrocholesterol breaks the B-ring to form previtamin D₃, which undergoes thermally induced rearrangement to vitamin D₃.

Material

Plants were grown in growth chambers with or without UVB light. Three *Solanaceous* species were used:



Solanum glaucophyllum Desf.
(waxy leaf nightshade)



Solanum lycopersicum L.
(tomato)



Capsicum annuum L.
(pepper)

Method

The leaves were harvested, freeze-dried and saponified overnight. The vitamin D₃ metabolites were extracted from the non-saponified matter followed by solid phase clean-up. Further clean-up was performed with semi-preparative HPLC. Fractions of vitamin D₃, 25-hydroxy vitamin D₃ and 1,25-dihydroxy vitamin D₃ were collected separately and derivatized with 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD) to increase sensitivity (Fig. 2). The derivatized extracts were subsequently analyzed by LC-ESI-MS/MS. The vitamin D₃ metabolites were quantified using their deuterated form as internal standard.

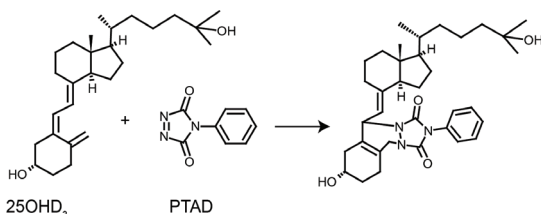


Fig. 2. The vitamin D₃ metabolites, as here 25-hydroxy vitamin D₃, were derivatized with 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD) to enhance sensitivity in ESI-MS/MS.

Results

Vitamin D₃ was identified in *S. glaucophyllum*, *S. lycopersicum* and *C. annuum* (Table 1). The vitamin D₃ content in the UVB-exposed plants was 18-64 times higher than for the not UVB-exposed plants. 25-hydroxy vitamin D₃ was only identified in the UVB-exposed plants, whereas 1,25-dihydroxy vitamin D₃ only was found in UVB-exposed *S. glaucophyllum* (Table 1).

Table 1. Content of vitamin D₃, 25-hydroxy vitamin D₃ and 1,25-dihydroxy vitamin D₃ in plants grown with (+UVB) or without (-UVB) UVB light

Plant	ng per gram dry weight		
	D ₃	25OHD ₃	1,25(OH) ₂ D ₃
<i>S. glaucophyllum</i> (+UVB)	200	31	32
<i>S. glaucophyllum</i> (-UVB)	3.2	0.8	<0.1
<i>S. lycopersicum</i> (+UVB)	100	4.3	<0.1
<i>S. lycopersicum</i> (-UVB)	1.7	<0.02	<0.1
<i>C. annuum</i> (+UVB)	2.9	0.5	<0.1
<i>C. annuum</i> (-UVB)	<0.02	<0.02	<0.1

Conclusion

It is remarkable that the leaves of the *Solanaceous* family contain high amounts of vitamin D₃ bearing in mind that the fruits from, e.g. tomato is an important food for humans. Thus, the potential of plants as a vitamin D₃ source exists. This study demonstrates that both UVB-dependent and independent pathways for biosynthesis of vitamin D₃ exist in plants.

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