Parts from this thesis has been published and presented in lectures and posters above all in securing the priority.

**Publications**


**Lectures**

**KOBAYASHI, N., SCHLIEMANN, W., STRACK, D.**
Betaxanthin biosynthesis in the hairy root culture of *Beta vulgaris* L. subsp. *vulgaris* ‘Golden Beet’ (Garden Beet Group)
Laboratoire de Physiologie végétale, Université de Neuchâtel (Switzerland), May 14, 1998

**SCHLIEMANN, W., KOBAYASHI, N., STEINER, U., STRACK, D.**
Spontaneous reactions in betalain biosynthesis
4th Dutch-German Workshop on Regulation of Secondary Metabolism, Bad Herrenalb, September 27-29, 1998

**KOBAYASHI, N., SCHLIEMANN, W., STRACK, D.**
Betalanthin formation in plants and hairy root cultures of *Beta vulgaris* L.
Chiba University, Faculty of Pharmaceutical Sciences, Laboratory of Molecular Biology and Biotechnology, Research Center of Medicinal Resources, Chiba (Japan), December 18, 1998

**KOBAYASHI, N., SCHMIDT, J., NIMTZ, M., WRAY, V., SCHLIEMANN, W.**
Betalains from Christmas cactus (*Schlumbergera x buckleyi*)
XXth International Conference on Polyphenols, Freising-Weihenstephan, September 11-15, 2000

**Posters**

**SCHLIEMANN, W., KOBAYASHI, N., STEINER, U., STRACK, D.**
Non-enzymic steps in betalain biosynthesis
Botanical Congress, Bremen, August 30 - September 6, 1998
KOBAYASHI, N., SCHLIE Mann, W., STRACK, D.
Is the last step in betalain biosynthesis an enzymic or a spontaneous process?
4th Dutch-German Workshop on Regulation of Secondary Metabolism, Bad Herrenalb, September 27-29, 1998

SCHLIE Mann, W., KOBAYASHI, N., STEINER, U.S., VOGT, T., STRACK, D.
Enzymic and molecular analysis of betalain biosynthesis
75th Annual Meeting of the American Society of Plant Physiologists, Baltimore, MD, USA, July 24-28, 1999

KOBAYASHI, N., SCHMIDT, J., SCHLIE Mann, W.
Metabolic formation and occurrence of dopamine-derived betacyanins

KOBAYASHI, N., SCHMIDT, J., STRACK, D., SCHLIE Mann, W.
A new biosynthetic pathway leading to dopamine-derived betacyanins

KOBAYASHI, N., SCHMIDT, J., NIMTZ, M., WRAY, V., SCHLIE Mann, W.
Betalains from Schlumbergera x buckleyi
Meeting „Biosynthesis and Accumulation of Secondary Products“, Martin-Luther-University Halle-Wittenberg, Halle (Saale), September 24-27, 2000

SCHLIE Mann, W., KOBAYASHI, N., STEINER, U.S., VOGT, T., STRACK, D.
Advances in betalain biosynthesis of higher plants
Meeting „Biosynthesis and Accumulation of Secondary Products“, Martin-Luther-University Halle-Wittenberg, Halle (Saale), September 24-27, 2000
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<th>Abbreviations</th>
<th>Full Form</th>
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<tr>
<td>ABC transporter</td>
<td>ATP-binding cassette transporter</td>
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<tr>
<td>AIP</td>
<td>2-Aminooindan 2-phosphonic acid</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine 5’-triphosphate</td>
</tr>
<tr>
<td>AU</td>
<td>Absorbance unit</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
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<td>2,4-D</td>
<td>2,4-Dichloro-phenoxyacetic acid</td>
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<td>2-D-cyclo-D</td>
<td>2-Desarboxy-cyclo-Dopa</td>
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<td>Dinitrobenzene glutathione</td>
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<tr>
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<td>DL-Dithiothreitol</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediamine tetraacetic acid</td>
</tr>
<tr>
<td>EGTA</td>
<td>Ethylene glycol bis (2-aminoethyl)-tetraacetic acid</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
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<td>GS</td>
<td>Glutathione</td>
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<td>KPi</td>
<td>Potassium phosphate buffer</td>
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<td>PAL</td>
<td>Phenylalanine ammonia-lyase</td>
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<td>PDA</td>
<td>Photodiode array</td>
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<td>pers. commun.</td>
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<td>Prep. HPLC</td>
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<td>rpm</td>
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<td>ThiaPro</td>
<td>(S)-4-Thiaproline</td>
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<tr>
<td>Tris</td>
<td>Tris (hydroxymethyl)-aminomethane</td>
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