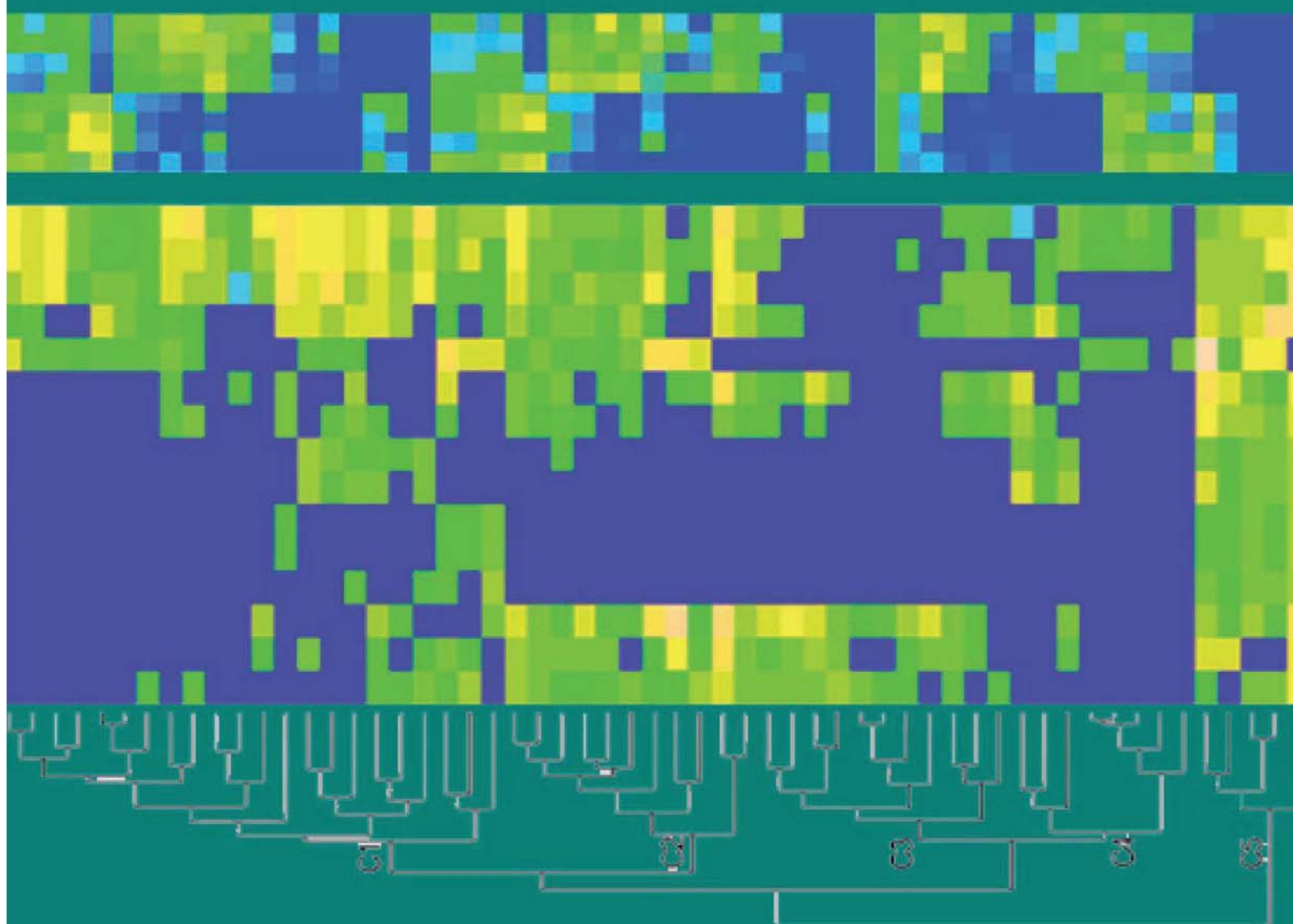


Hua Bai

Transcription patterns of *NRT* family genes and ecophysiological response to nitrate fertilization and drought in poplar



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fertilization and drought in poplar





GEORG-AUGUST UNIVERSITY OF GÖTTINGEN

**Transcription patterns of *NRT* family genes and
ecophysiological response to nitrate fertilization and
drought in poplar**

Dissertation

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by

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IV. List of abbreviations

APO	:	Aberrant panicle organization
AlaAT	:	Alanine aminotransferase
AAP	:	Amino acid permease
AMT	:	Ammonium transporter
ANOVA	:	Analysis of variance
At	:	<i>Arabidopsis thaliana</i>
ASN	:	Asparagine synthetase
AspAT	:	Aspartate aminotransferase
Asp	:	Aspartate
BLAST	:	Basic local alignment search tool
C	:	Carbon
CAT	:	Cationic amino acid transporter
CLC	:	Chloride channels
CREs	:	<i>Cis</i> -regulatory elements
CR	:	Coarse roots
CK	:	Cytokinin
DX	:	Developing xylem
Dof	:	DNA-binding with one finger
DW	:	Dry weight
ENOD	:	Early nodulin
FL	:	Average fiber length
FR	:	Fine roots
FW	:	Fresh weight
Glu	:	Glutamate
GS	:	Glutamine synthetase
Gln	:	Glutamine
G lignin	:	Guaiacyl lignin
HA	:	High ammonium concentration
HND	:	High N concentration and drought
HN	:	High N concentration
HATs	:	High-affinity nitrate uptake systems



Hv	:	<i>Hordeum vulgare</i>
INUE	:	Instantaneous nitrate use efficiency
Ls	:	<i>Lactuca sativa</i>
LA	:	Leaf area
LATs	:	Low-affinity nitrate uptake systems
LHT	:	Lysine histidine transporter
W	:	Mature xylem
Ms	:	<i>Medicago sativa</i>
NADH-GOGAT	:	NADH-dependent glutamate synthase
NCBI		National center for biotechnology information
Nia	:	Nitrate reductase
Np	:	<i>Nicotiana plumbaginifolia</i>
NP	:	Net photosynthesis
NAR	:	Nitrate assimilation related protein
NO	:	Nitrogen oxide
NRT	:	Nitrate transporter
NiR	:	Nitrite reductase
N	:	Nitrogen
NR	:	Nitrate reductase
Os	:	<i>Oryza sativa</i>
Pm	:	<i>Panicum miliaceum</i>
PTR	:	Peptide transporter
Pv	:	<i>Phaseolus vulgaris</i>
Pg	:	<i>Populus alba</i> × <i>Populus glandulosa</i>
Pp	:	<i>Populus popularis</i>
Ptre	:	<i>Populus tremula</i>
Pt	:	<i>Populus trichocarpa</i>
PCA	:	Principle component analysis
PPDK	:	Pyruvate orthophosphate dikinase
qRT-PCR	:	Quantitative real-time polymerase chain reaction
R	:	Transpiration rate
RNAi	:	RNA interference
SLAC1/SLAH	:	Slow anion channel- associated 1 homolog 3

IV. List of abbreviations

STA	:	Specific target amplification
T	:	Stem elongation segments
TFW	:	Average thickness of double fiber wall
TP	:	Monosaccharide transporter
TAA	:	Total amino acids
TC	:	Total carbon content
TN	:	Total nitrogen content
TMS	:	Trans-membrane segments
Ts	:	<i>Triticum dicoccoides</i>
Vf	:	<i>Vicia faba</i>
VLA	:	Average vessel lumen area
Zm	:	<i>Zea mays</i>

V. Summary

Nitrate is one of the major sources of nitrogen taken up by plants. A large proportion of the nitrate acquired by plants is actively transported through nitrate transporters (NRT). To cope with low and high nitrate concentration in the soil, plants have developed high-affinity and low-affinity nitrate uptake systems, respectively. Genes of the NRT1 family are mainly low affinity nitrate transporters, and genes of the NRT2 family are high affinity transporters, whose functioning requires the participation of NRT3. Up to now, the molecular features and the regulation of nitrate transporters have been studied mostly in the model plant *Arabidopsis thaliana*. The molecular characterization of all NRT family genes has not yet been conducted in poplar, which is a model plant for genetic analysis in tree species.

Earlier studies on poplar suggested that the response to N fertilization of poplar growth and wood properties were species-specific traits. We hypothesized that these traits also show intra-specific variation in response to N fertilization, because large differences in plant productivity were found among poplar genotypes. To investigate the role of *NRT* genes in poplar growth, four questions will be addressed in the present thesis.

(1) Which genes are members of the NRT family with functions in poplar?

The first task was to identify the NRT family members in poplar using all NRT family genes in *Arabidopsis* as query sequences. 68 *PtNRT1/PTR*, 6 *PtNRT2*, and 5 *PtNRT3* genes were retrieved from the poplar genome database for *P. trichocarpa*. This distribution shared common patterns with *Arabidopsis*, rice and lotus. In order to further investigate the properties of the *PtNRT* genes, a bioinformatic analysis of the complete gene set of the *PtNRT* family was conducted. A phylogenetic analysis revealed that the NRT proteins in poplar and *Arabidopsis* belonged to two main clades: NRT1/PTR members to clade I and NRT2 and NRT3 members to clade II. In addition, gene multiplication and gene loss were found in the *PtNRT* family of poplar compared to the *AtNRT* family of *Arabidopsis*.

To examine whether *PtNRT* genes in poplar are expressed, we studied the transcript abundance of 79 *PtNRT* genes using the public and unpublished microarray experiments representing five tissues in poplar. The microarray profiles revealed the expression of 52 *PtNRT1/PTR*, 4 *PtNRT2*, and 3 *PtNRT3*. Ten genes were not present on the microarrays

V. Summary

and for ten genes no significant transcript levels were detected. The preferential expression of a number of the *PtNRT2* and *PtNRT3* genes were found in roots and leaves. Genes of the *PtNRT1/PTR* family were expressed in the roots, leaves, developing xylem, stem elongation segments and mature xylem. The distinct expression pattern of *PtNRT1/PTR*, *PtNRT2*, and *PtNRT3* genes pointed to possibly diverse biological functions in specific tissues.

(2) How does N fertilization and drought stress affect the expression of *PtNRT* genes?

As drought may diminish the N uptake by roots, the effects of nitrogen and water availability on the expression of 17 *PtNRT* genes in roots, stem elongation segments, and developing xylem of *P. trichocarpa* were analyzed by qRT-PCR. All members of *PtNRT2* (*PtNRT2.4A*, *PtNRT2.5A/B*, and *PtNRT2.7*) and *PtNRT3.2A* were significantly down regulated by N fertilization and drought stress, which suggested the possible functions as high affinity nitrate transporters. N fertilization caused decreased expression of *PtNRT1.7* in the developing xylem and increased expression of *PtNRT1.9B* in the stem elongation segments. We found that 6 *PtNRT* genes in the developing xylem and of 8 *PtNRT* genes in the stem elongation segments were only regulated by drought, but not by N fertilization. These results revealed that the effects of N fertilization on expression of *PtNRT* genes were mainly in roots. However, many drought regulated *PtNRT* genes were observed in not only roots, but also in the stem elongation segments and the developing xylem. This suggested that drought might diminish the long distance transportation of nitrate.

(3) Does the N-uptake ability influence the responses to N fertilization and drought on plant growth in different poplar demes?

Natural phenotypic variations provide a framework to study the relation between the adaptation traits of plant growth and the genetic variations. Plants of five *Populus tremula* demes (A = Austria, G = Geismar Germany, R = Russia, H = Halle Germany, F = Finland) were used to study the physiological performance of tree growth in response to N fertilization and drought stress. We found strong growth differences among the poplar demes. After one month of N fertilization, the biomass and N contents were highest in the plants of deme A and least in the plants of deme R. A regression analysis showed that plant biomass and N contents in the five demes were significantly correlated in low nitrogen (LN) and high nitrogen (HN) treatments, respectively. A higher intercept of the regression