Transcriptomic Response of *Arabidopsis* Shoots and Roots after Prolonged Exposure to Trinitrotoluene

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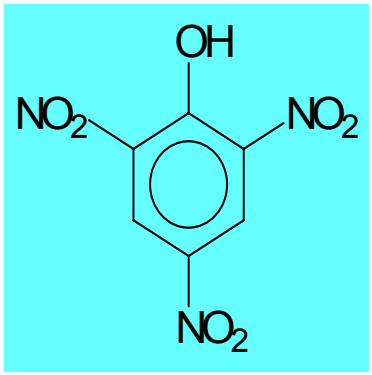
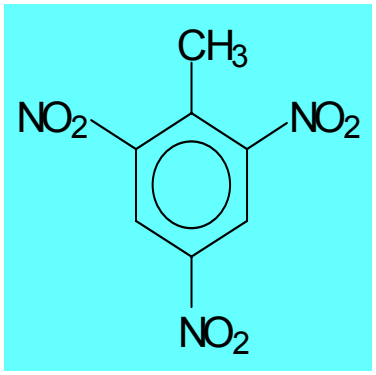
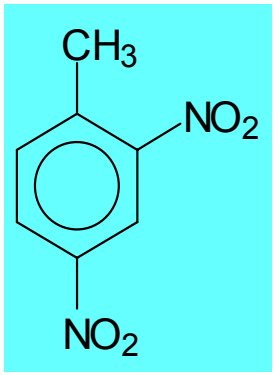
Structure formulas



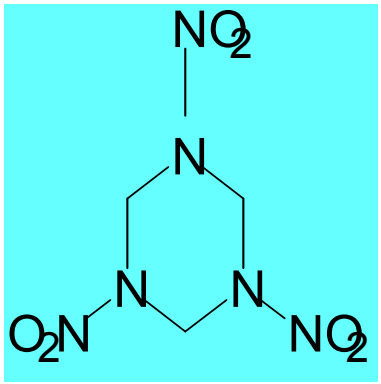
2,4,6-trinitrophenol (picric acid, TNF)



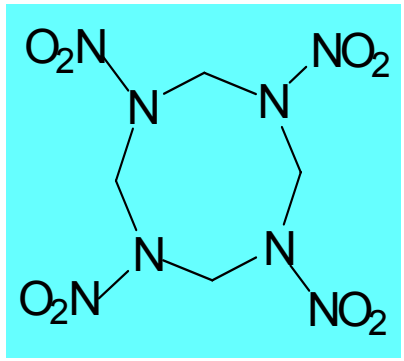
2,4,6-trinitrotoluene (tritol, TNT)



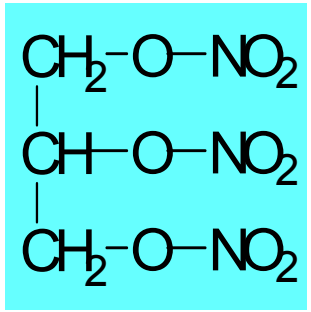
2,4-dinitrotoluene (DNT)



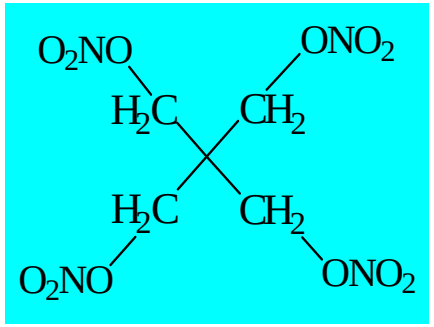
1,3,5-trinitro-1,3,5-triazine (RDX)



1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)



glycerol trinitrate (GTN)



pentaerythritol tetranitrate (pentrit, PETN)

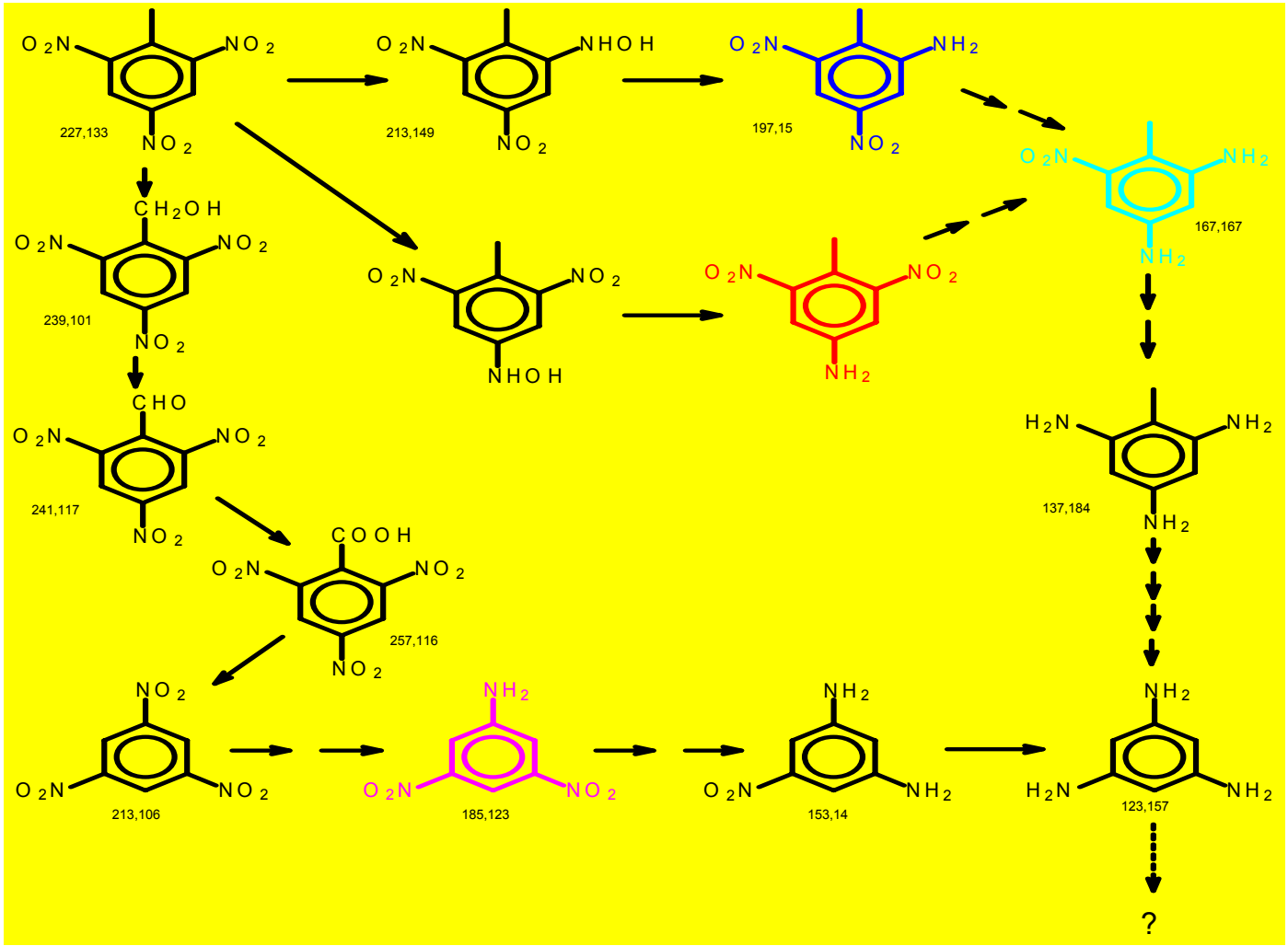
TOXICITY

- **DNT cause metahaemoglobinaemia and aenemia, are hepatotoxic, cause damage to sight, the 2,6-isomer is suspect carcinogen LD_{50} for 2,4- and 2,6-DNTs are 0,268 and 0,177 g/kg, respectively.**
- **TNT is hepatotoxic, causes hypochromaemia, damage to nervous system and sight, induces dystrophic changes in myocardium and kidneys LD_{50}=0,70 g/kg.**
- **RDX causes damage to central neervous system (can induce epileptic seizure-like condition), can cause anaemia LD_{50}=0,20 g/kg.**
- **NG possesses vasodilatory effect, hepatotoxicity, damages kidney function and myocardium, belongs among habit-forming drugs. LD_{50}=0,806 g/kg.**
- **Pentrit has weaker effects than nitroglycerol**

TNT CONTAMINATION

NATURAL ATTENUATION ?

TNT DEGRADATION PATHWAY



Plant selection

Plant selection

GM – Plants?


Worth1000.com


1000.com

GM *Arabidopsis* selection

GM – Plants

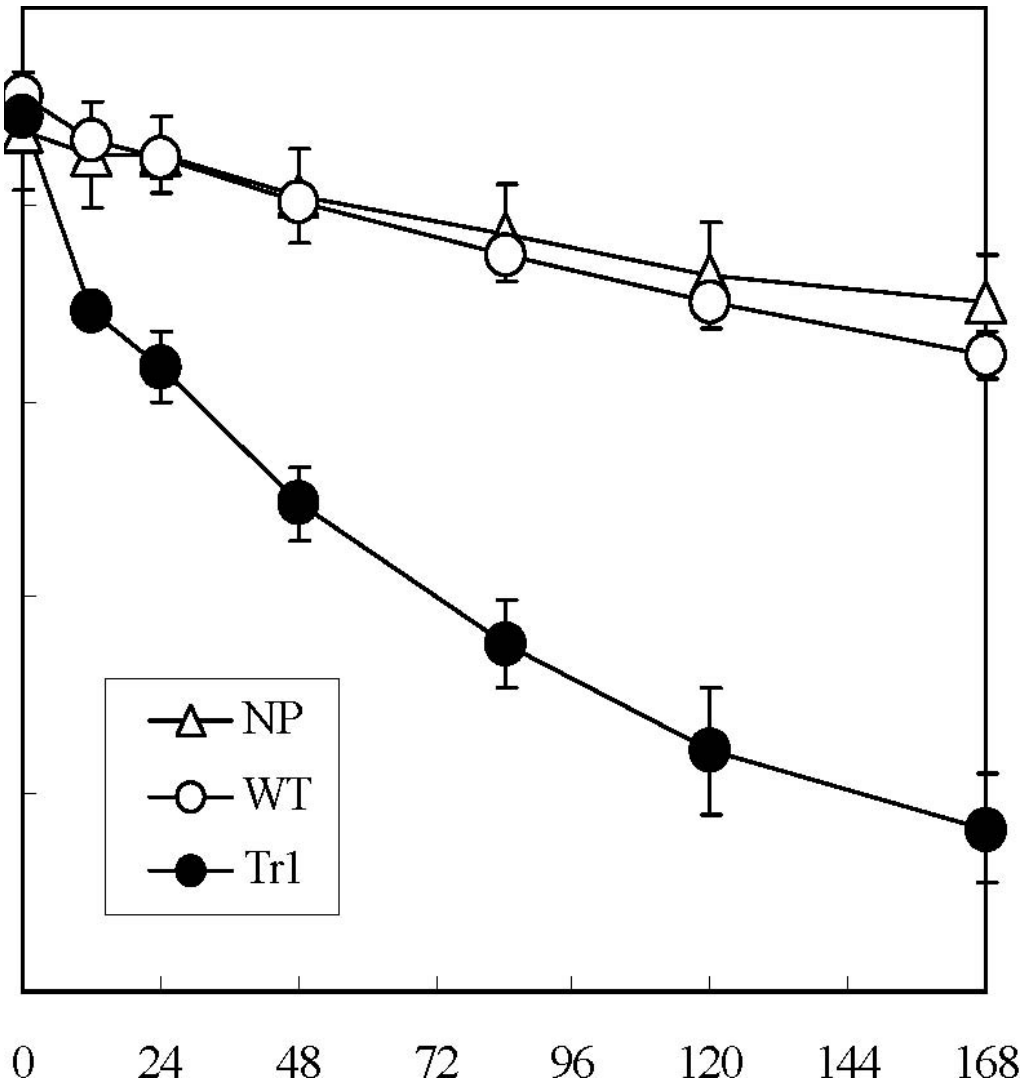
Arabidopsis thaliana was transformed with a chimeric gene of nitroreductase (NTR, E.C.1.6.99.7) from *Escherichia coli* by *Agrobacterium*-mediated *in planta* method

Tolerance to, and uptake and degradation of 2,4,6-trinitrotoluene (TNT) are enhanced by the expression of a bacterial nitroreductase gene in *Arabidpsis thaliana,* Mami Kurumataa*, Misa Takahashia,b, Atsushi Sakamotoa,b, Juan L. Ramosc, Ales Nepovim d, Tomas Vanek d, Toshifumi Hirataa and Hiromichi Morikawaa, Z. Naturforsch. 60c, 272-278 (2005)

The degradation of TNT in the medium in the presence of the wild-type (WT) or transgenic (Tr1) plants

25mg/l

0.25

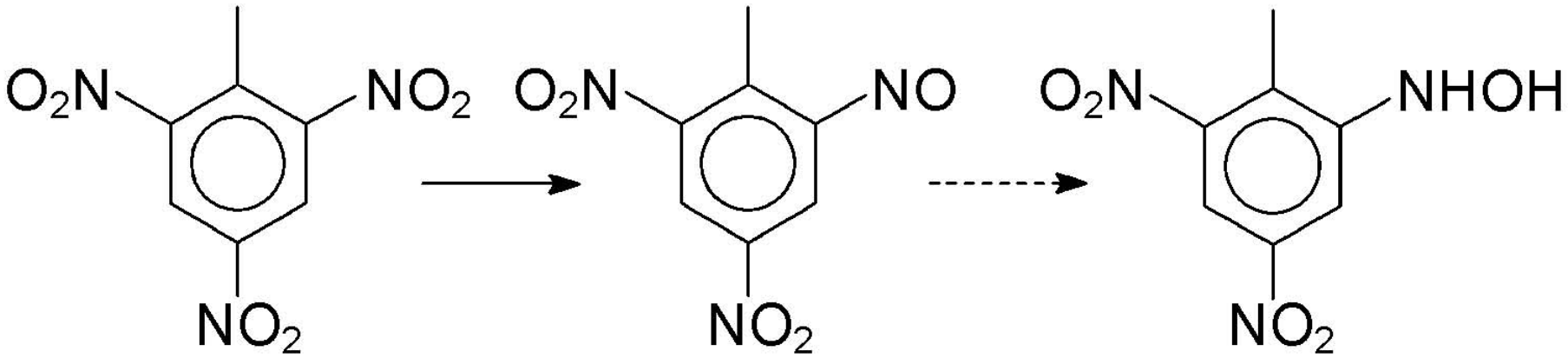


Comparison of tolerance to TNT between the wild-type (WT) and transgenic line (Tr1).

Enzymes

Genes

First step



Saponaria officinalis

→ seeds

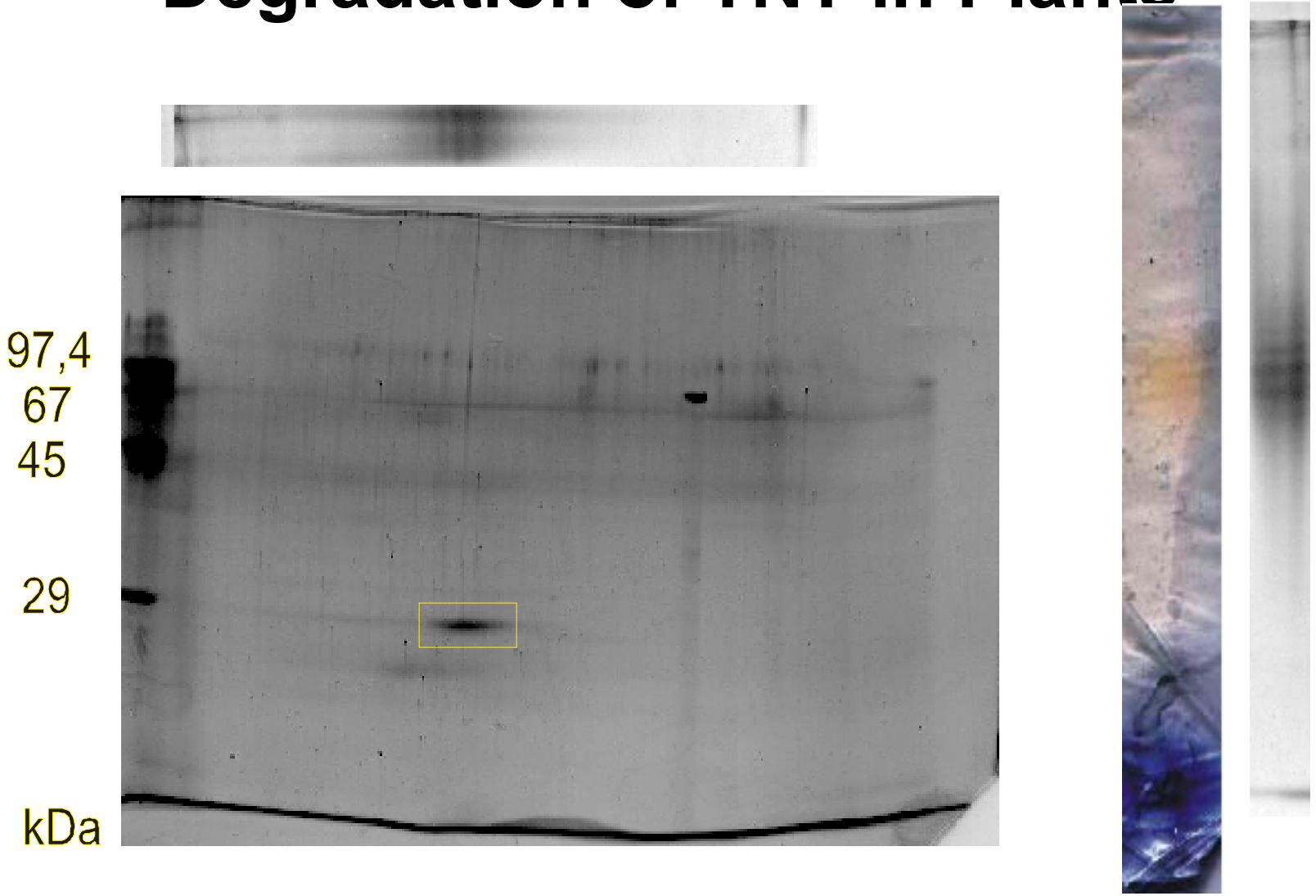
↓ sterilization

callus initiation

↓

undifferentiated
cell culture →

The Enzyme Participating Degradation of TNT in Plants



Enzyme Identification and Characterization

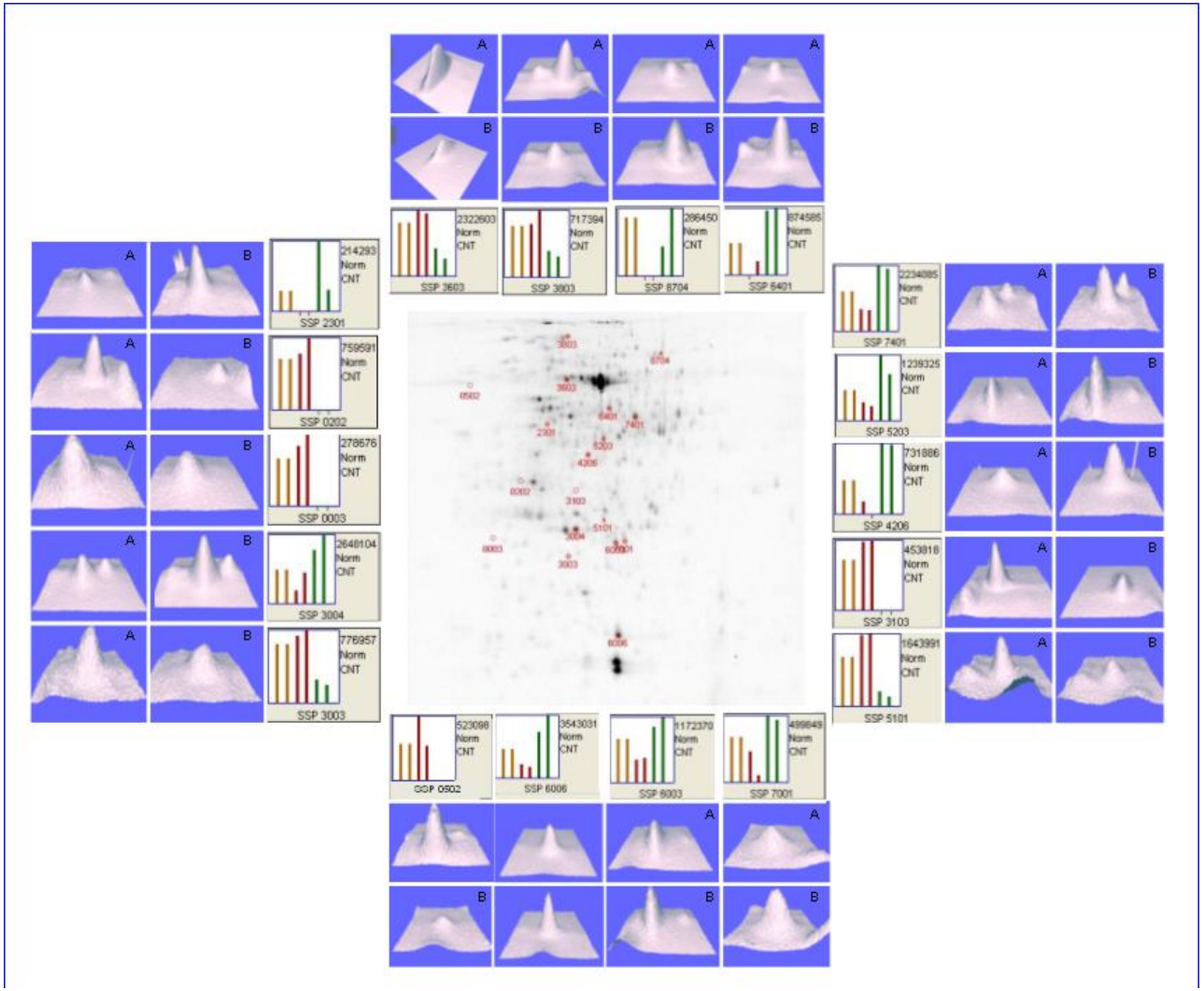
- **SDS electrophoresis – 29 kDa**
- **N-terminal AA sequence: SSGVDVAEFSPPRRLLT**
- **66.7% homology to Arabidopsis thaliana protein**
- **60% homology to tomato (Lycopersicon esculentum).**
- **BLAST Database corresponds to the N-terminus of 12-oxophytodienoate reductase.**
- **FMN containing protein => flavoprotein**

- Podlipná, R., Nepovím, A., Vágner, M., Vaněk, T.: A novel oxidoreductase participating TNT detoxification in plant. Biologia Plantarum , 51, 367-371, 2007

Search of Enzymes Participating the Degradation of Nitrocompounds

- Comparison of protein maps
- Change of protein expression
- Characterization of inducible proteins
- MALDI-TOF, N-terminal AA sequence
- Match of data with protein library

DIGE approach…



Enzymes identified

UniProtKB/Swiss-Prot database

Proteins with presumable function in TNT degradation:

3004 – Glutathione S-transferase

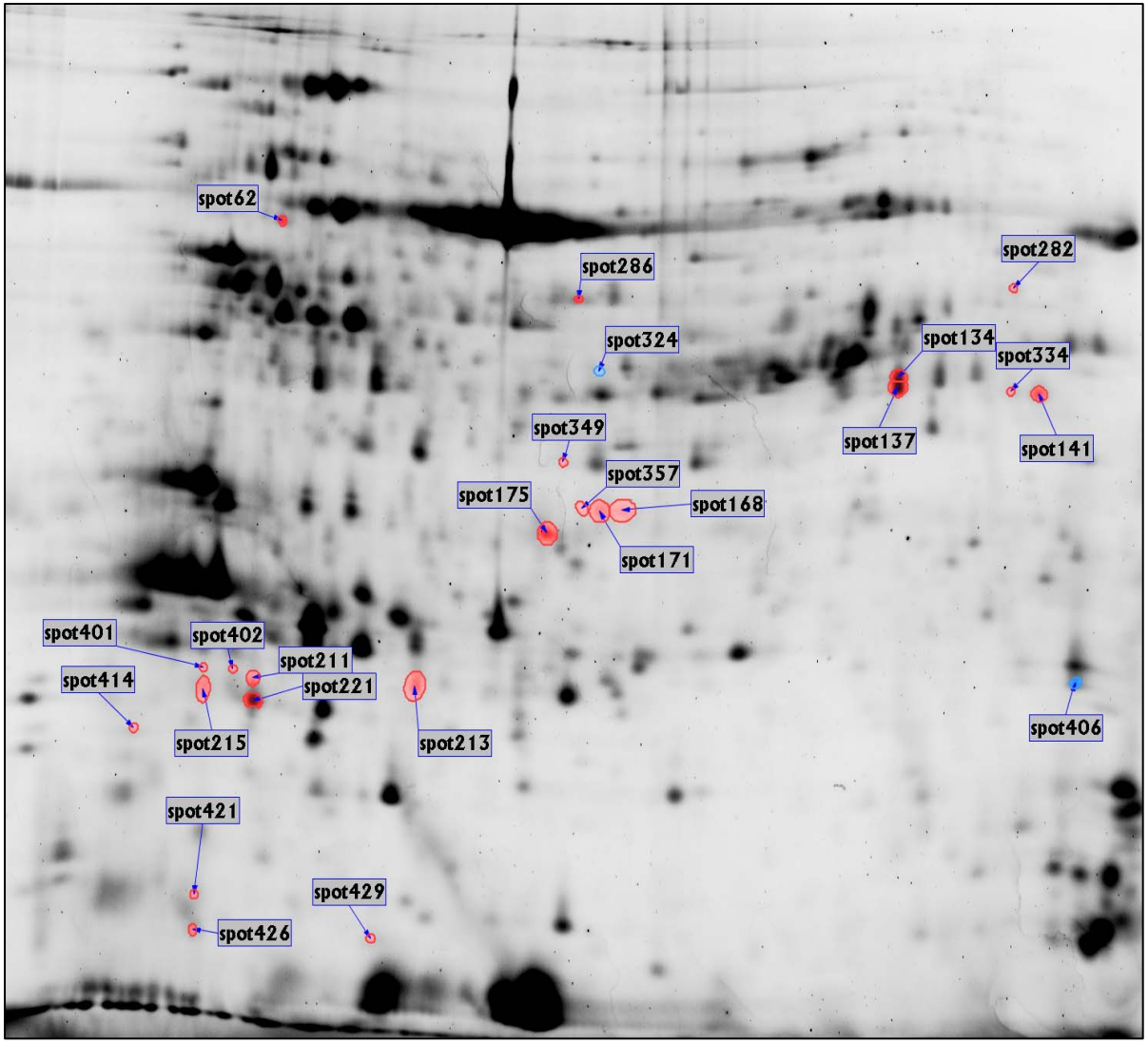
3103 – UDP-glucuronosyl/UDP-glucosyltransferase

5101 – Glutathione S-transferase

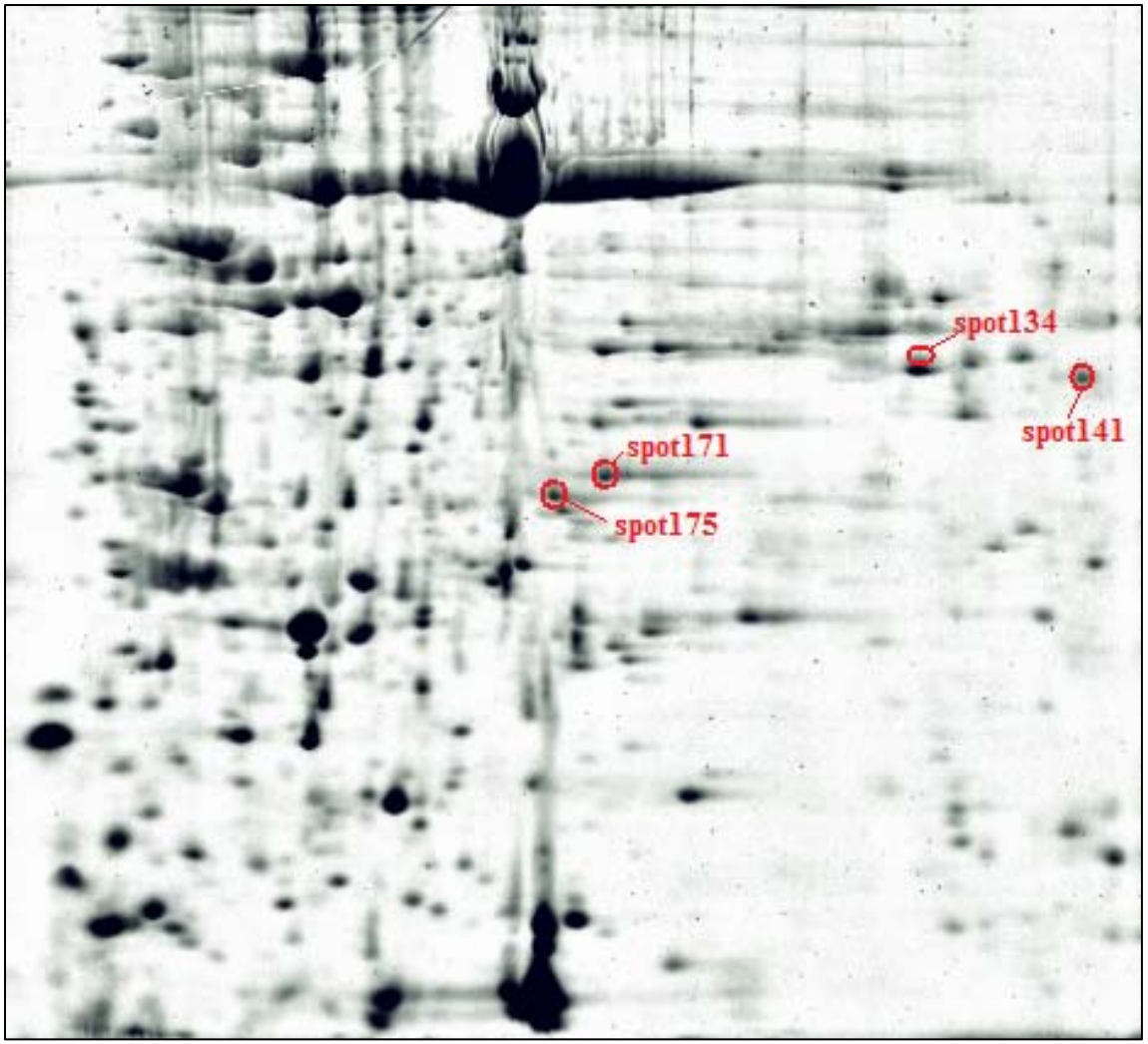
6006 – Glutathione S-transferase

8704 – Cytochrome P450

Up-regulated (blue) and down-regulated (red) proteins

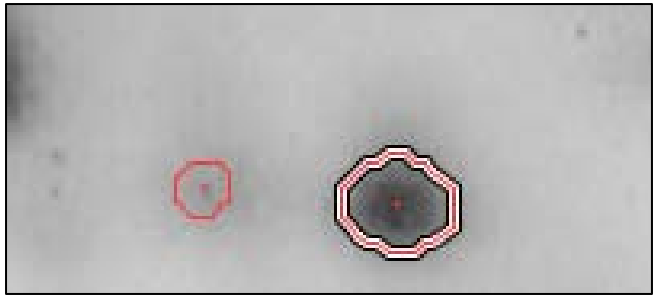


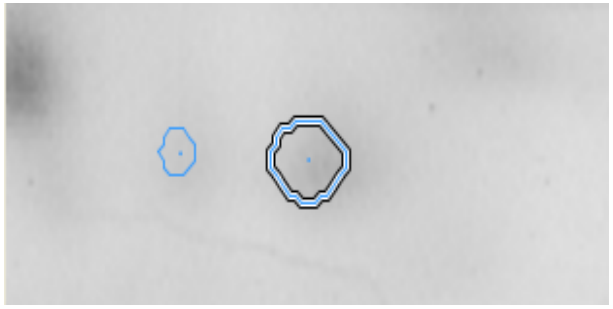
Preparative gel



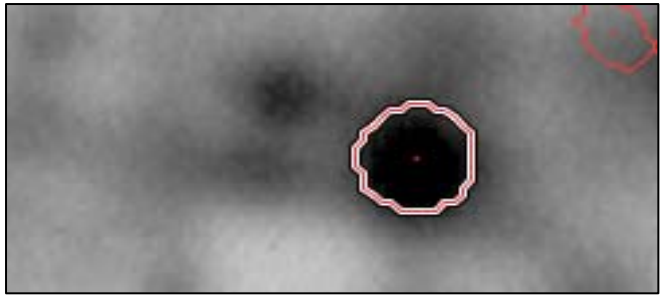
Spot analysys

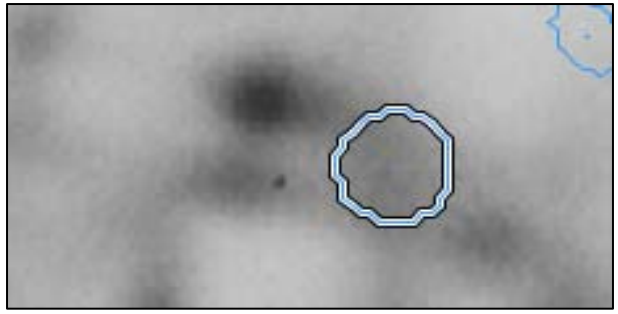
141





175





control

TNT

Spot identification

Maldi-Tof

- **175**
- **B3H5S2_ARATH**
- **Q0WVH4_ARAT**

- **ribulose bisfosfat carboxylase**

- **141**

- **NADPH:protochlorofylide oxidoreductase A**

- **Q8LAV9_ARATH**
- **Protochlorofylid reductase**

Transcriptomic analysis

- Roots and leaves……

Distribution of radioactivity in *Buphthalmum*

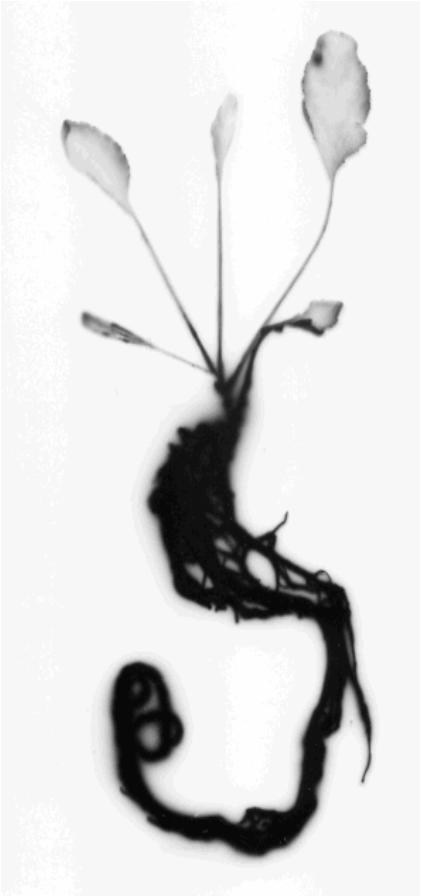
Whole plant

Autoradiogram

Distribution of radioactivity in *Senecio*

Whole plant

Autoradiogram



Plant material: *Arabidopsis thaliana* (WT, cv. Columbia)

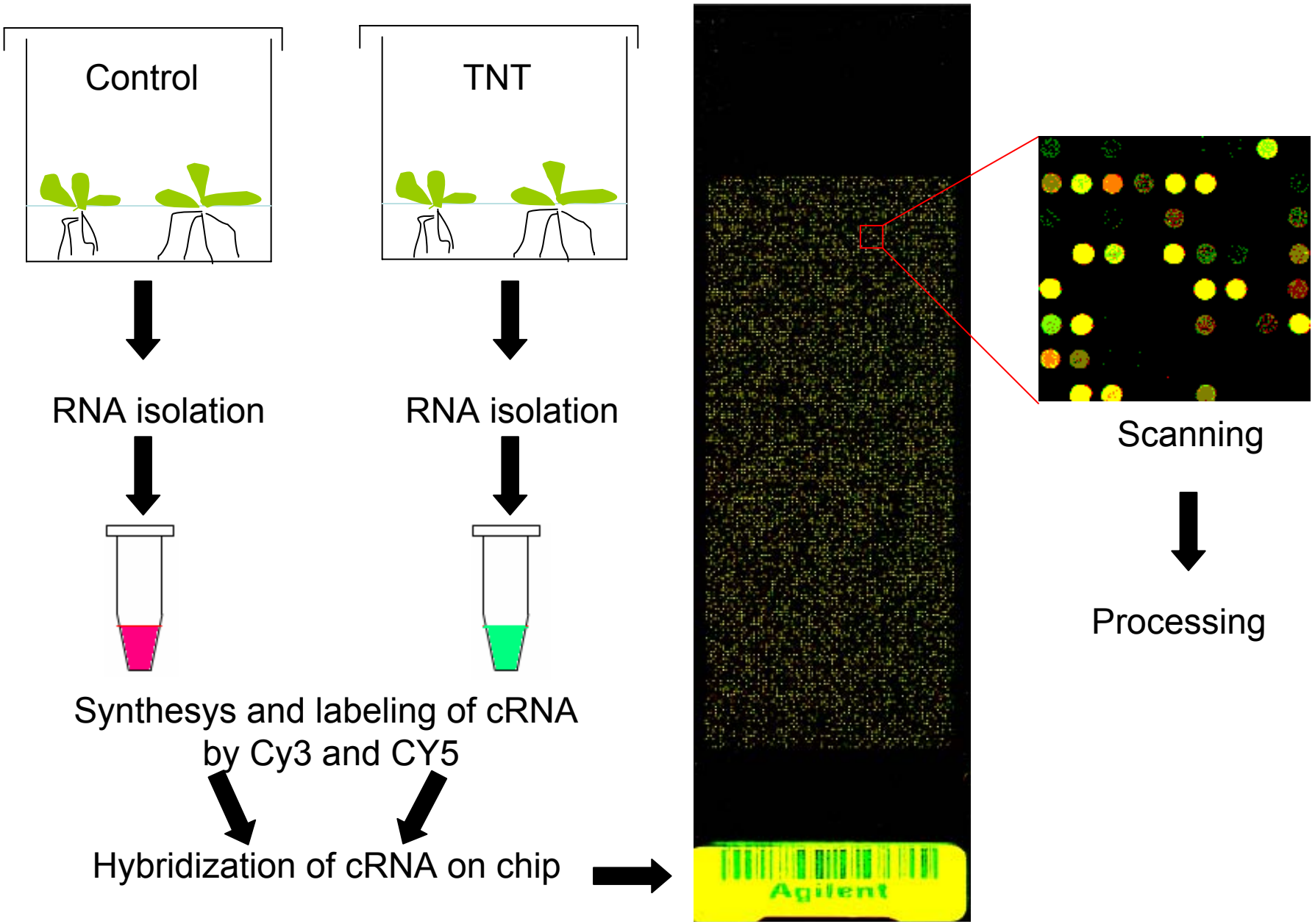
Cultivation: *in vitro* on modified MS medium oncultivated at 23°C with 16/8 h light/dark cycle, at light intensity 7200 $\mu mol.m^{-2}.s^{-1}$

Concentration 5 µg/ml and 7-day treatment stress was chosen for expression analysis

Agilent 60-mer oligo microarray Arabidopsis 2 containing 20,436 unique genes

Two color platform for cRNA synthesis and labeling

Procedure


Control
TNT
RNA isolation
RNA isolation
Synthesys and labeling of cRNA
by Cy3 and CY5
Hybridization of cRNA on chip
Agilent
Scanning
Processing

Data processing

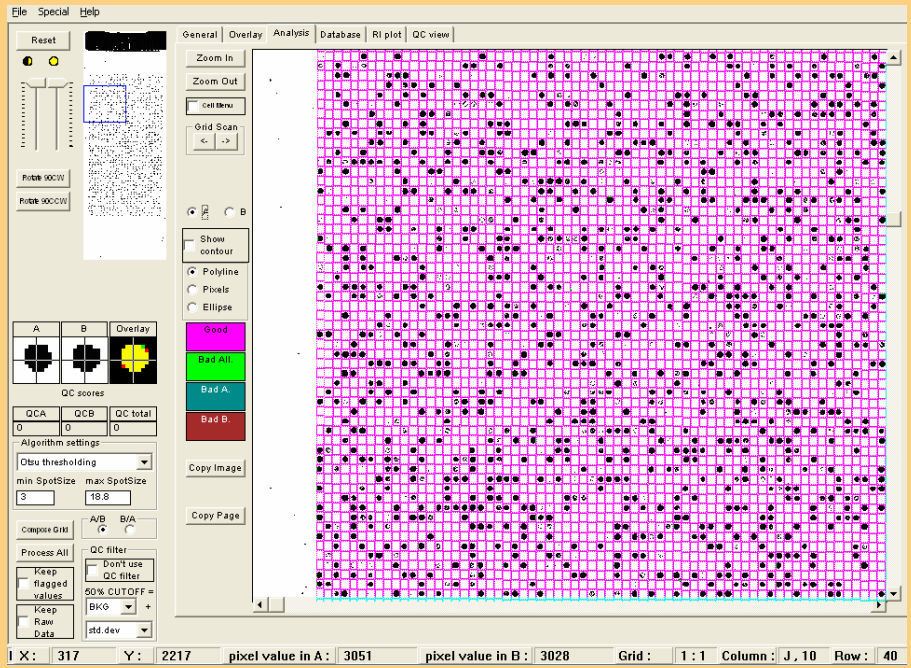
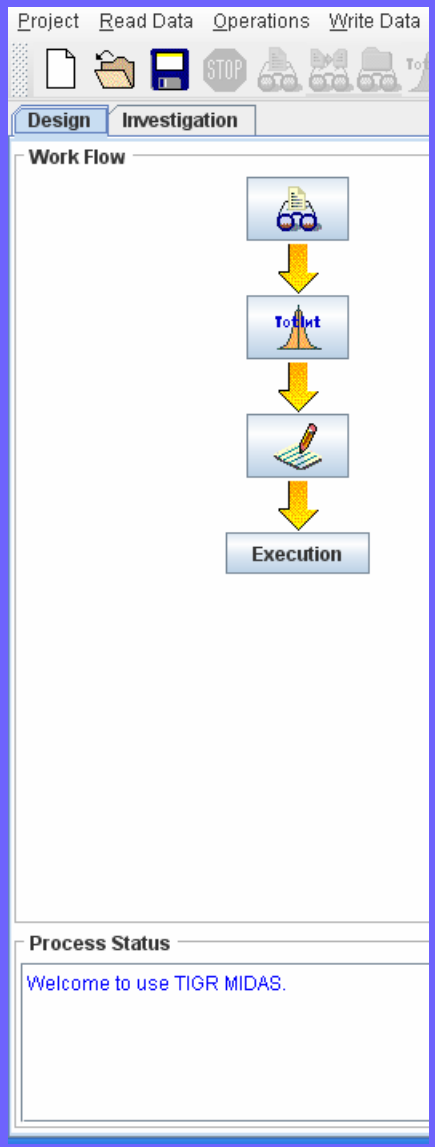


Image processing
(TIGR Spotfinder)

Data cleaning
(Excel)

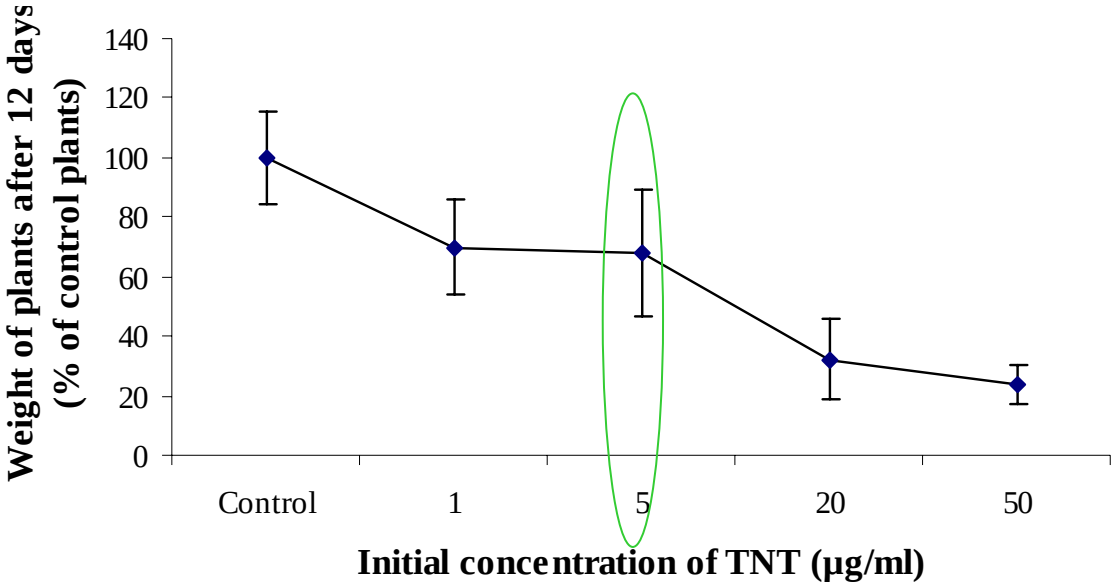
Statistical analysis

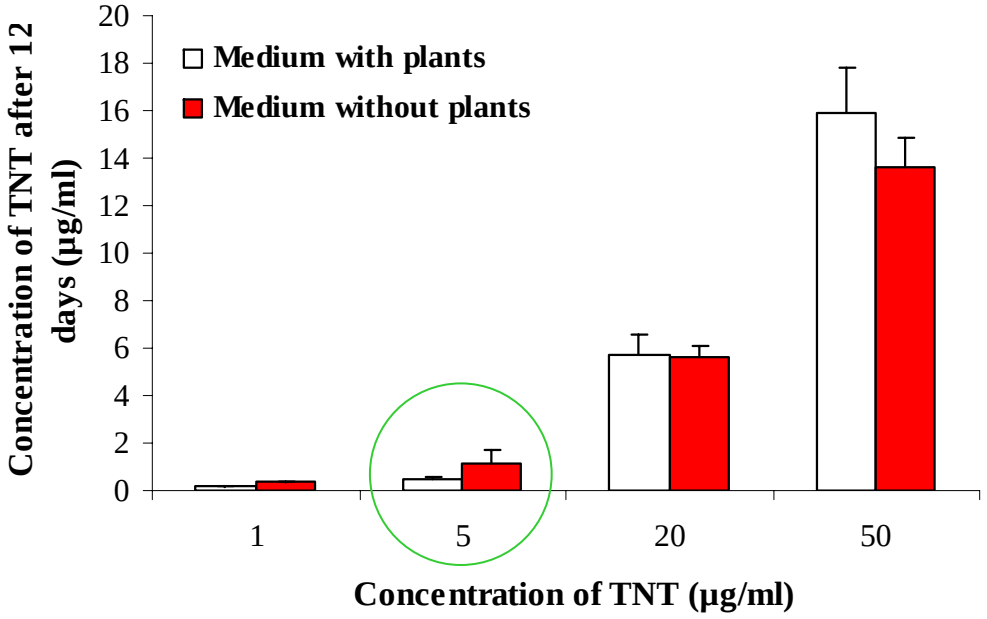
(t-test)



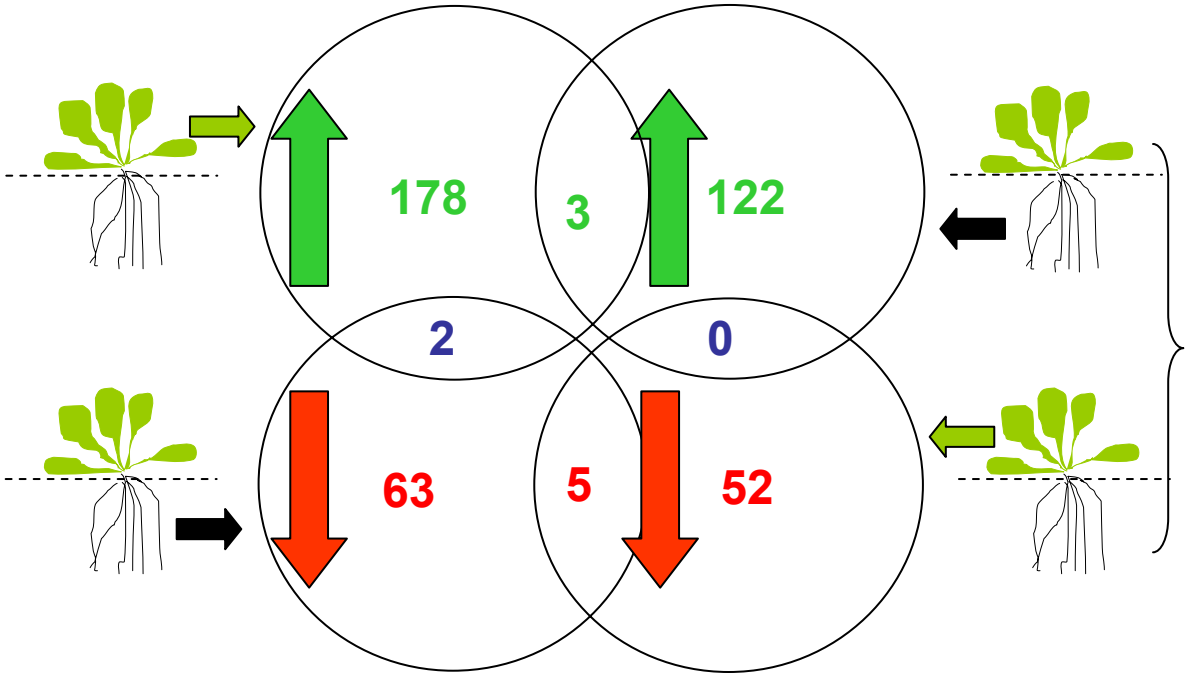
Normalization of Cy3 and Cy5 channel intensities
(TIGR Midas)

Growth of A. thaliana exposed to TNT





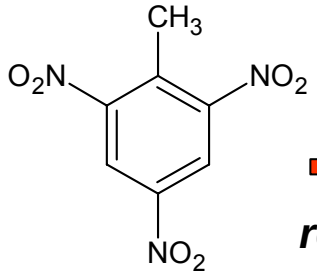
From total amount of 20,436 unique genes 13,908 was identified in shoots



expression difference >1.5 fold; p[t-test] < 0.05

and 15,470 in roots

Phase I



reduction

12-oxophytodienoate reductases (OPRs)
Ekman et al. (2003)
Gandia-Herrero et al. (2008)
Beynon et al. (2009)

After long term stress (7-9) no induction of OPRs
Rao et al. (2009)

Our results

Nitrate reductase 1 (*At1g77760*) ?

Phase II

2-HADNT
2-ADNT
4-HADNT
4-ADNT

conjugation

UDP glycosyltransferases (UGTs)
Gandia-Herrero et al. (2008)

Our UGTs

At3g50740
At1g60140
At3g62660
At3g62720
At2g41640

Phase III

HADNT and ADNT glycosides

sequestration to vacuole

incorporation to the cell wal

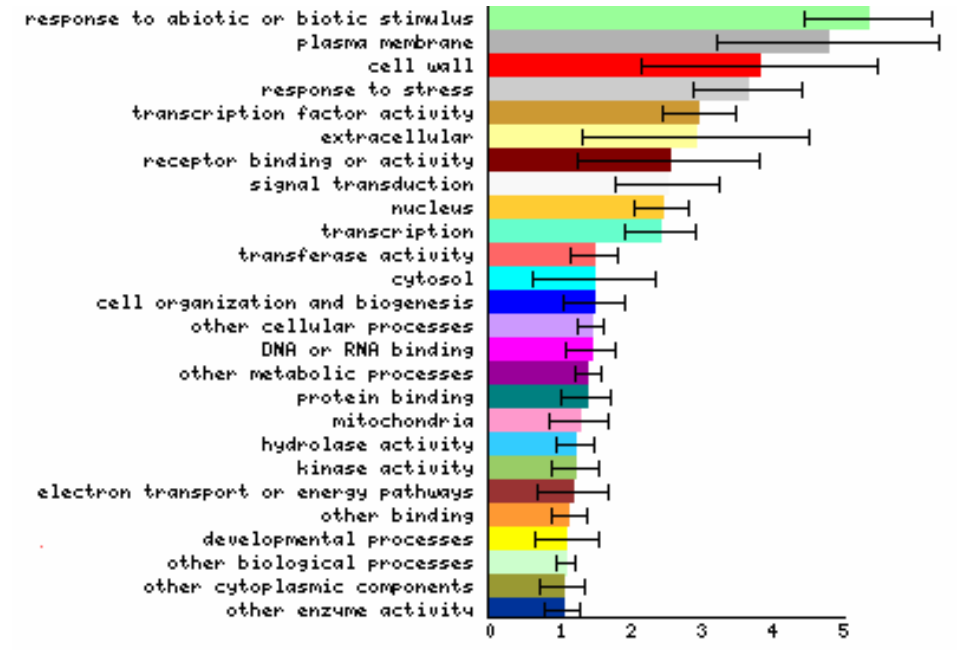
export from the cell

Transporters

ABC transporter members
P-glycoprotein 21 (*At3g62150*)
P-glycoprotein 19 (*At3g28860*)
ATABC1 (*At4g04770*)

Cell wall modifying
expansin 1 (*At1g69530*),
α-xylosidase 1 (*At1g68560*),
alpha-L-arabinofuranosidase (*At1g68560*)
touch 4 (*At5g57560*)

Proportion of up-regulated genes in a functional category

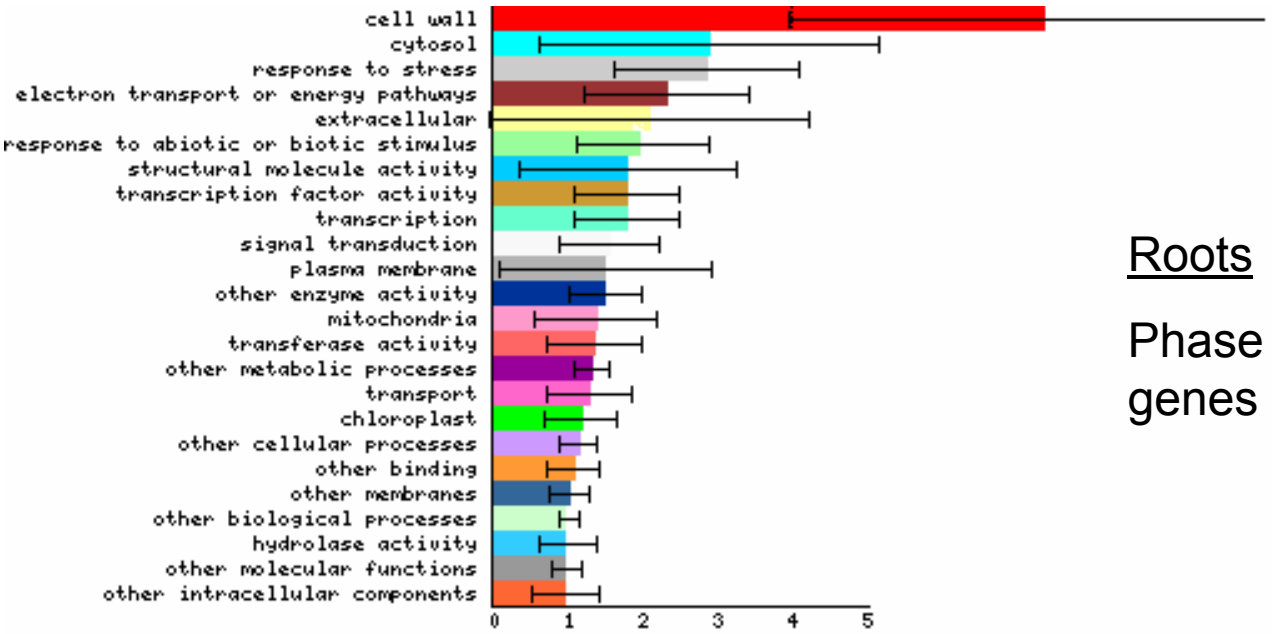


Shoots

Phase I

Phase II

Phase III



Roots

Phase III (cell wall modifying genes and hydrolases)

Oxidative stress

thioredoxin-dependent peroxidase (*At1g65970*)

γ-glutamyltransferase (*At4g39640*)

} Enzymes with antioxidant activity

3 peroxidases (*At2g37130, At4g36430, At5g05340*)
5 cytochromes P450 members (*At5g45340*, *At3g26220*, *At3g26280*, *At5g05690*, *At2g26710*)

} Oxidative metabolism - general stress response to the pollutants

4 glutathion transferases (*At1g02930, At1g02920, At4g02520, At2g02930*)

} Oxidative stress response rather than in direct TNT conjugation (no TNT-glutathione conjugates observed, Mezzari et al. (2005))

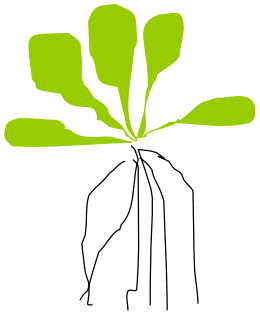
Induced after seven days

Potentially directly involved in TNT detoxification pathway

Nitrate reductase 1
Glucoyltransferases
ABC transporters
Cell wall modifying genes

Not directly involved in TNT detoxification pathway

Brassinosteroide and auxin signaling (cell expansion and elongation or/and stress)
Antioxidative activity
Cytochromes P 450
Glutathione transferases
Stress response -ethylen, jasmonic and salicylic acid signaling



Potentially directly involved in TNT detoxification pathway

Transport - sucrose-proton symporter 2 AtSUC2
Cell wall modifying genes and hydrolases

Not directly involved in TNT detoxification pathway

Peroxidases
Various stress response genes

Decreased after seven days

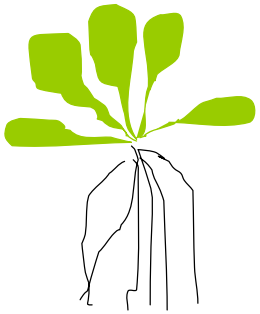
Members of basic/helix-loop-helix superfamily (*At2g41240, At5g04150, At3g47640*)

Ammonium transmembrane transporter, ATAMT1;2 (*At1g64780*)

Lipid transporters (*At1g55260, At5g64080*)

Lipid metabolism (three GDSL-motif lipase/hydrolase family proteins; fatty acid desaturase 2; lipid-associated family protein *At2g22170*)

} **Lipid metabolism**



Light responsive genes (ELIP1, ELIP2, sigma factor E)

Six members engaged in flavonol biosynthesis

Two genes from strictosidine synthase family (*At5g22020, At3g57020*) engaged in alkaloid biosynthesis

Summary 1

- Analysis confirmed induction of several genes known are being concerned with toxin metabolism such as UDP glycosyltransferases and ABC family transporters.
- We also identified nitrate reductase 1 as potential candidate for reduction of nitro groups on TNT ring.

Summary 2

- **Surprisingly, these transcripts were induced in shoots but not in roots where genes coding enzymes involved in cell wall modifications were relatively most abundantly up-regulated indicating that TNT metabolism proceeded mainly in aerial parts after seven day treatment.**
- **Results obtained by microarray hybridization were validated by quantitative real-time PCR**
- **Paper submitted…..**

Thank you