Herbicide Resistant Transgenic Plants

BTCH704
Advances in TR Plant Utilization in Agriculture
Prof. ÖKTEM
**WHAT ARE WEEDS?**

All plants in a cultivated field giving harm rather than benefit.

<table>
<thead>
<tr>
<th>CROP TYPE</th>
<th>PRODUCTION</th>
<th>LOSSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEREALS</td>
<td>433.903</td>
<td>54.349</td>
</tr>
<tr>
<td>VEGETABLE</td>
<td>201.691</td>
<td>23.718</td>
</tr>
<tr>
<td>FRUIT</td>
<td>66.567</td>
<td>2.462</td>
</tr>
<tr>
<td>WINEYARD</td>
<td>50.697</td>
<td>7.909</td>
</tr>
</tbody>
</table>

Worldwide production and losses due to weeds (million tons)
HOW TO CONTROL?
DIFFERENT WAYS TO FIGHT WITH WEEDS

- MECHANICAL: HOING, HAND PLUCKING
- PHYSICAL: HEAT and LIGHT
- BIOLOGICAL: USE OF LIVING ORGANISM
- CHEMICAL: USE OF HERBICIDES
<table>
<thead>
<tr>
<th>Chemical Family</th>
<th>Affected System</th>
<th>Target Protein</th>
<th>Spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triazines (atrazine, ametryne, cyanazine, prometryn, simazine)</td>
<td>Photosystem II, electron transport from Q_A to Q_B</td>
<td>D-1 protein, product of $psbA$ gene</td>
<td>Total</td>
</tr>
<tr>
<td>Sulfonylureas, imidazolinones, triazolopyrimidines</td>
<td>Amino acid synthesis</td>
<td>Acetolactate synthetase (ALS)</td>
<td>Selective</td>
</tr>
<tr>
<td>Aryloxybenzoxypipiones (AOPP), cyclohexanediones</td>
<td>Lipid synthesis</td>
<td>Acetyl coenzyme A carboxylase (ACCase)</td>
<td>Selective</td>
</tr>
<tr>
<td>Glyphosate (N-phosphonomethyl)glycine</td>
<td>Amino acid synthesis</td>
<td>5-enoylpyruvyl-shikimate-3-phosphate synthetase (EPSPS)</td>
<td>Total</td>
</tr>
<tr>
<td>Bromoxynil</td>
<td>Photosystem II</td>
<td>D-1 protein</td>
<td>Total</td>
</tr>
<tr>
<td>Phenoxyacetic acids (eg 2,4-D)</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Selective</td>
</tr>
<tr>
<td>Glufosinate (Phosphinothricin, PPT)</td>
<td>Amino acid synthesis</td>
<td>Glutamine synthetase (GS)</td>
<td>Total</td>
</tr>
<tr>
<td>Biprydiliums, praquats, diquats</td>
<td>Photosystem I</td>
<td>Electron transfer system</td>
<td>Total</td>
</tr>
</tbody>
</table>

Dekker and Duke, 1995
Problems in the application of herbicides

- Lack of tolerance to the chemical by one or more of the major world crops, e.g., rice, maize, soybean, wheat, rapeseed.
- Use of multiple types of herbicides to broaden the spectrum of the affected weeds, which in turn increases the possibility that the crop is injured also.
- Lack of high toxicity to weeds while crops are not affected
What is herbicide resistance in plants?

Herbicide resistance is the ability, trait, or quality of a population of plants within a species or larger taxon, or of plant cells in culture, to withstand a particular herbicide at a dosage that is substantially greater than the wild type of that plant is able to withstand, with a near normal life cycle.
Types of herbicide resistance

- Exclusionary resistance mechanisms
  - Herbicide uptake
  - Translocation
  - Compartmentation
  - Metabolic detoxification
- Altered molecular/cellular target of herbicide action
- Site of action overproduction
WHY HERBICIDE RESISTANT PLANTS?

SELECTIVITY OF A HERBICIDE IS AN IMPORTANT CRITERIA

TOTAL HERBICIDES, WHEN APPLIED, KILLS ALL THE PLANTS IN THE FIELD INCLUDING CULTURE PLANT

INCREASE SELECTIVITY

😊 MAXIMUM EFFECT ON HERBS
😊 MINIMUM EFFECT ON CULTURE PLANT
What is the advantage of herbicide resistant plants?
WHAT TYPES OF STRATEGIES TO DEVELOP HERBICIDE RESISTANT PLANTS?

A. CONVENTIONAL: Seed coating etc.

B. MOLECULAR APPROACH

1. Modification of Target Protein
2. Over Production of Target Protein
3. Detoxification of Active Ingredient
4. Production of Antibodies Against Active Ingredient
Active NZ

Active site of NZ

Target NZ

Inactive NZ

AllostERIC modification Site of NZ

Active Ingredient of Herbicide

Inactive NZ

Altered metabolic activities

Cell death
Modified allosteric modification Site of NZ

Active NZ

Active site of NZ

Target NZ

Active Ingredient of Herbicide

Mutate Allosteric modification site of NZ (NZ retains its catalytic activity)

Isolate gene of NZ

Transfer to target plant

Develop TR plant

Resistant to herbicide

STRATEGY

Normal metabolic activities in the presence of herbicide

Cell survival
Some strains of *Streptomyces* produce a tripeptide antibiotic

**BIALAPHOS**

\[ \text{L-PPT-(L-Alanine)}_2 \]

By endopeptidase activity **BIALAPHOS** is hydrolyzed and produces free **L-PPT**

L-PPT is an inhibitor of GLUTAMINE SYNTHASE (GS)
WHY microorganisms produce PAT enzyme?

To protect themselves from the toxic effect of PPT

WHICH microorganisms produce PAT enzyme?

<table>
<thead>
<tr>
<th></th>
<th>Streptomyces hygroscoptus</th>
<th>Streptomyces viridochromogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene:</td>
<td>bar (560 bp) 87% homology</td>
<td>pat (560 bp)</td>
</tr>
<tr>
<td>Protein Product:</td>
<td>BAR 85% homology</td>
<td>PAT</td>
</tr>
<tr>
<td>M.W. (from a.a. sequence)</td>
<td>20.6 kD</td>
<td>20.6</td>
</tr>
<tr>
<td>(SDS-PAGE)</td>
<td>22-23 kD</td>
<td>22-23 kD</td>
</tr>
<tr>
<td>(Native-PAGE)</td>
<td>41.0 kD (HOMODIMER)</td>
<td>41.0 kD</td>
</tr>
</tbody>
</table>
GLUTAMINE SYNTHASE: Key enzyme for ammonium assimilation

- Nitrate reduction
- Amino acid degradation
- Photorespiration

\[
glutamate + NH_4^+ + ATP \rightarrow (GS) \rightarrow glutamine + ADP + P_i
\]

BIALAPHOS

PPT

Accumulation \rightarrow LETHAL EFFECT
A. OVERPRODUCTION OF GS

Transfer a gene coding for extra copies of glutamine synthase (GS)

\[
\text{glutamate} + \text{NH}_4 + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + \text{Pi} + \text{ATP}
\]
B. DETOXIFY PPT

Transfer of \textit{bar} gene coding for phosphinotricin acetyl transferase

Acetyl-PPT $\xrightarrow{(PAT)}$ PPT

(BIALAPHOS)

\[
\text{glutamate} + \text{NH}_4 + \text{ATP} \quad \rightarrow \quad \text{glutamine} + \text{ADP} + P_i
\]

(Inactive on GS)
B. DETOXIFY PPT

Transfer of *bar* gene coding for Phosphinotricin acetyltransferase.

Acetyl-PPT ➔ PPT

(glutamine + ADP + P_i) ➔ glutamate + NH_4 + ATP

(BIALAPHOS)

(PAT)
ROOT GENERATION IN SELECTIVE MEDIA

**LBA:pDHB**

**MEDIA COMPOSITION**

- MSC
  - No Selection
- MSC with
  - 10 mg/l PPT

CONTROL  TRANSFORMED  CONTROL

![Image of control and transformed samples with days in culture: 17 days](image)

**LB5-1:pGKB5**

**MEDIA COMPOSITION**

- MSC
  - No Selection
- MSC with
  - 50 mg/l kan, 5 mg/l PPT

CONTROL  TRANSFORMED  CONTROL

![Image of control and transformed samples with days in culture: 21 days](image)
EFFECT OF 1% BASTA TREATMENT ON CONTROL PLANTS

WATER

BASTA

DAYS AFTER TREATMENT

2

9
EFFECT OF BASTA TREATMENT ON PLANT DEVELOPMENT

Days in soil: 40

Days in soil: 96
®BASTA 1% (V/V) TREATMENT

2 days a.t.

14 days a.t.
SDS-PAGE for detection of PAT

- Lane 1: Molecular weight standards
- Lane 2, 6: Control
- Lane 3, 7: LP₃F₃A
- Lane 4, 8: LP₃F₃B₁
- Lane 5, 9: LP₃F₃B₂

SDS-PAGE analysis of PAT showed a MW of 22-23 kD
Analysis of F1 Progeny
LP3-F1-A  LP3-F1-B

F1 Plantlets  F1 Transgenics

Analysis of F2 Progeny
HERBICIDE RESISTANT TR TOBACCO

1% BASTA - 14 days post application

3% BASTA application
DAY 1

3% BASTA

1% BASTA

30 days post application

pDHB, F3

EmuPAT, Fo
Inducible cross-tolerance to herbicides in transgenic potato plants with the rat CYP1A1 gene

Introduction

- The residues of agrochemicals affect ecosystem and result in the pollution of crops.
- Development of a system of rapid degradation in the agricultural environment after use.
- Cytochrome P450 monooxygenases in higher plants play an important role in the oxidative metabolism of xenobiotics as well as endogenous substrates.
- In several plants these enzymes are found to be important in the metabolism of agrochemicals such as several herbicides.
- However, more information is available about the P450 dependent monooxygenases in the mammalian liver than in the plants.
Introduction

- The xenobiotic metabolizing P450 cDNAs derived from mammals confer on several TR plants a high level of tolerance to many herbicides.

- Pathogenesis related 1a (PR1a) protein in tobacco is induced upon pathogen attack and chemical treatment with SA and INA, as well as benzothiadiazole (BTH).

  \[ \text{Non-phytotoxic plant protection agent} \]

- BTH activates a number of systemic acquired resistance (SAR) genes including PR1A in tobacco, Arabidopsis and wheat.
Aim of the Study

- To generate TR potato plants harboring rat P4501A1 (CYP1A1) cDNA with a tobacco PR1a promoter and to control the expression of transgenes artificially by BTH treatment if necessary

- To transfer CYP1A1 cDNA fused with yeast P450 reductase (YR)

- To examine the transgenic plants for their ability to detoxify several herbicides
Materials and Methods

- Plasmid Construction
Materials and Methods

Plant Transformation

- Microtubers of *Solanum tuberosum* cv May Queen
- *Agrobacterium tumefaciens* strain LBA4404
- Plant selection → kanamycin resistance
- PCR
- Southern blotting
- Western blotting
- Northern blotting
- In vivo herbicide tolerance test
Results

57 PR1A1 containing TR plant
88 PRT1A1 containing TR plant
85 PRTYR containing TR plants

\[ \text{For each group 4 highly tolerant TR plants were selected with BTH and chlortoluron} \]

<table>
<thead>
<tr>
<th>P450 species</th>
<th>Promoter</th>
<th>Expression plasmid</th>
<th>Selected plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat CYP1A1</td>
<td>PR1a</td>
<td>pPR1A1</td>
<td>P2562, P2576, P2577, P2586</td>
</tr>
<tr>
<td>Rat CYP1A1</td>
<td>PRT</td>
<td>pPRT1A1</td>
<td>T2432, T2434, T2437, T2500</td>
</tr>
<tr>
<td>Rat CYP1A1/YR</td>
<td>PRT</td>
<td>pPRTYR</td>
<td>F2355, F2365, F2371, F2384</td>
</tr>
</tbody>
</table>
Results

Southern-Blotting Analysis

- May Queen
  - PR1A1: 23.1 kbp, 9.4 kbp, 6.6 kbp, 4.4 kbp
  - T2432, T2434, T2437, T2560, T2565, T2571, T2584

- PRT1A1
  - 4.4 kbp

- PRTYR
  - 8.0 kbp
Results

GUS Activity

GUS activity increased 3 days after the BTH treatment and reached to max at 10 days

3.0 µmol/pot BTH
Results

Western-Blotting Analysis

- The microsomal proteins were isolated from the leaves 7 day after the BTH treatment.
- Without BTH treatment no protein band was detected in TR plants.
- With BTH, bands corresponding to rat CYP1A1 or its fused enzyme were observed in all TR plants.
- Low level of accumulation of CYP1A1 fused with YR protein.
Results

Northern-Blotting Analysis

- 15 days after the BTH treatment mRNA levels of CYP1A1 increased markedly
Results

**In vivo Herbicide Tolerance Test**

The herbicides chlortoluron, methabenzthiazuron and norflurozon were sprayed onto leaves in the TR and control plants 7 day after BTH treatment

<table>
<thead>
<tr>
<th>Plant lines</th>
<th>Chlortoluron</th>
<th>Methabenzthiazuron</th>
<th>Norflurozon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-BTH</td>
<td>BTH</td>
<td></td>
</tr>
<tr>
<td>P2577</td>
<td>Plants died</td>
<td>No damage</td>
<td>Chlorosis</td>
</tr>
<tr>
<td>T2432</td>
<td>Plants died</td>
<td>No damage</td>
<td>Chlorosis</td>
</tr>
<tr>
<td>T2355</td>
<td>Plants died</td>
<td>No damage</td>
<td>Chlorosis</td>
</tr>
</tbody>
</table>
Discussion

- The transgenes under the control of PR1a and PRT promoter were easily induced by BTH treatment.
- GUS activity had increased after 3 days of BTH application.
- BTH application efficiently led to the expression of CYP1A1 and its fused genes.
- mRNA of CYP1A1 and its fused gene with YR were induced in the TR plants treated with BTH, and did not reach maximum level even after 15 days, indicating the absence of feedback inhibition.
Discussion

Although having a lower level of accumulation of CYP1A1 and its fused protein, F2355 showed a high tolerance to chlortoluron and methabenzthiazuron in the same way as P2577 and T2432; suggesting that CYP1A1 fused enzyme with YR shows a higher specific activity than CYP1A1 alone.

All TR plants could be efficiently detoxify chlortoluron and methabenzthiazuron, but they developed chlorosis around the leaf vein upon norflorazon application, indicating that rat CYP1A1 could not metabolize this herbicide sufficiently.