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

CROP TYPE	PRODUCTION	LOSSES
CEREALS	433.903	54.349
VEGETABLE	201.691	23.718
FRUIT	66.567	2.462
WINEYARD	50.697	7.909

WORLDWIDE PRODUCTION AND LOSSES DUE TO WEEDS (MILION 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

Types of Herbicides by Chemical Families

Chemical Family	Affected System	Target Protein	Spectrum
Triazines (atrazine, ametryne, cyanazine, prometryn, simazine)	Photosystem II, electron transport from Q_A to Q_B	D-1 protein, product of *psbA* gene	Total
Sulfonylureas, imidazolinones, triazolopyrimidines	Amino acid synthesis	Acetolactate synthetase (ALS)	Selective
Aryloxypenoxypropionates (AOPP), cyclohexanediones	Lipid synthesis	Acetyl coenzyme A carboxylase (ACCase)	Selective
Glyphosate (N-phosphonomethyl)glycine	Amino acid synthesis	5-enoylpyruvyl-shikimate-3-phosphate synthetase (EPSPS)	Total
Bromoxynil	Photosystem II	D-1 protein	Total
Phenoxycarboxylic acids (eg 2,4-D)	Unknown	Unknown	Selective
Glufosinate (Phosphinothricin, PPT)	Amino acid synthesis	Glutamine synthetase (GS)	Total
Biprydiliums, praquats, diquats	Photosystem I	Electron transfer system	Total

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, eg 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

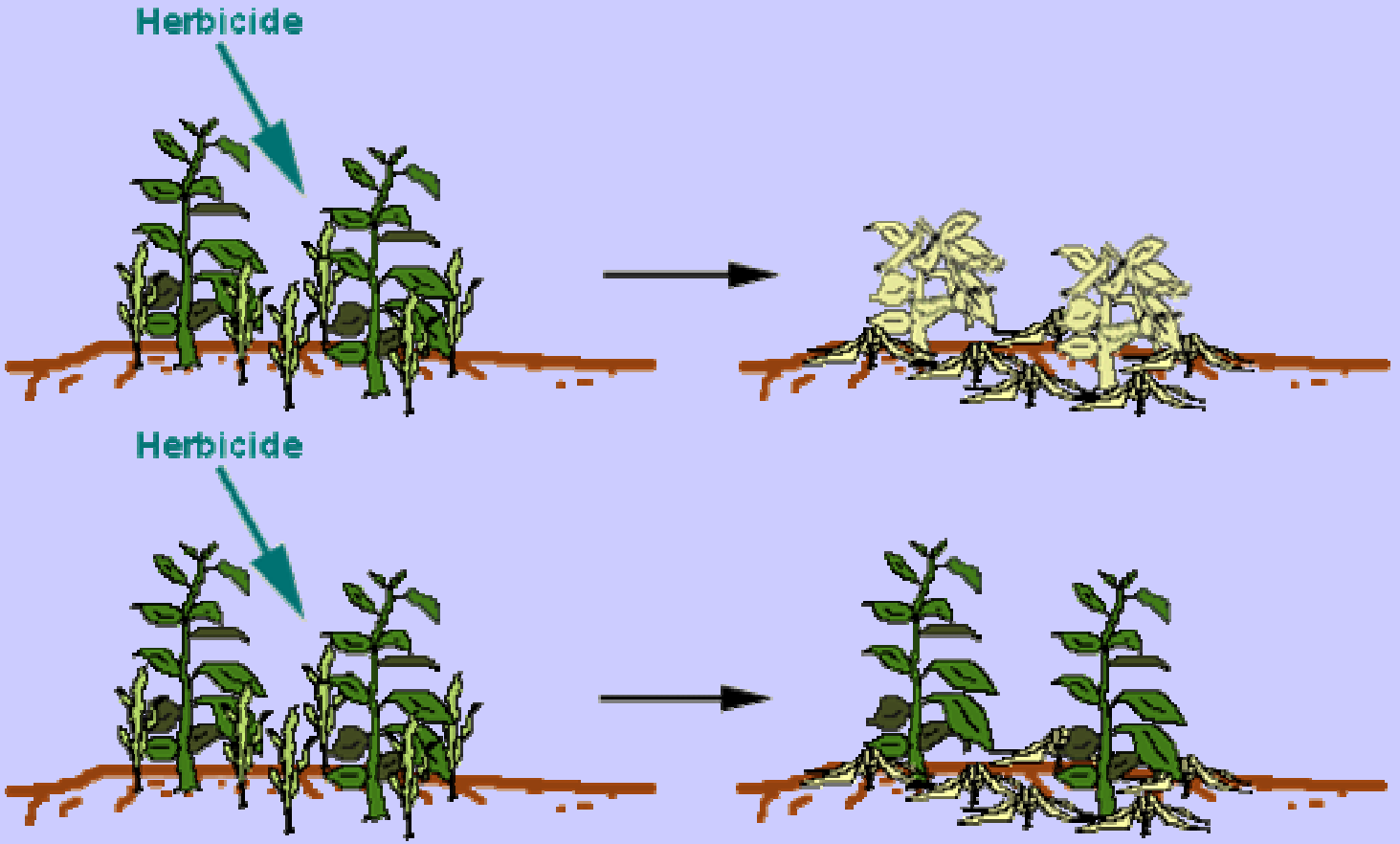
TOTAL HERBICIDES, WHEN APPLIED, KILLS ALL THE PLANTS IN THE FIELD INCLUDING CULTURE PLANT

INCREASE SELECTIVITY

☹ MAXIMUM EFFECT ON HERBS

☺ MINUMUM EFFECT ON CULTURE PLANT

What is the advantage of herbicide resistant plants?

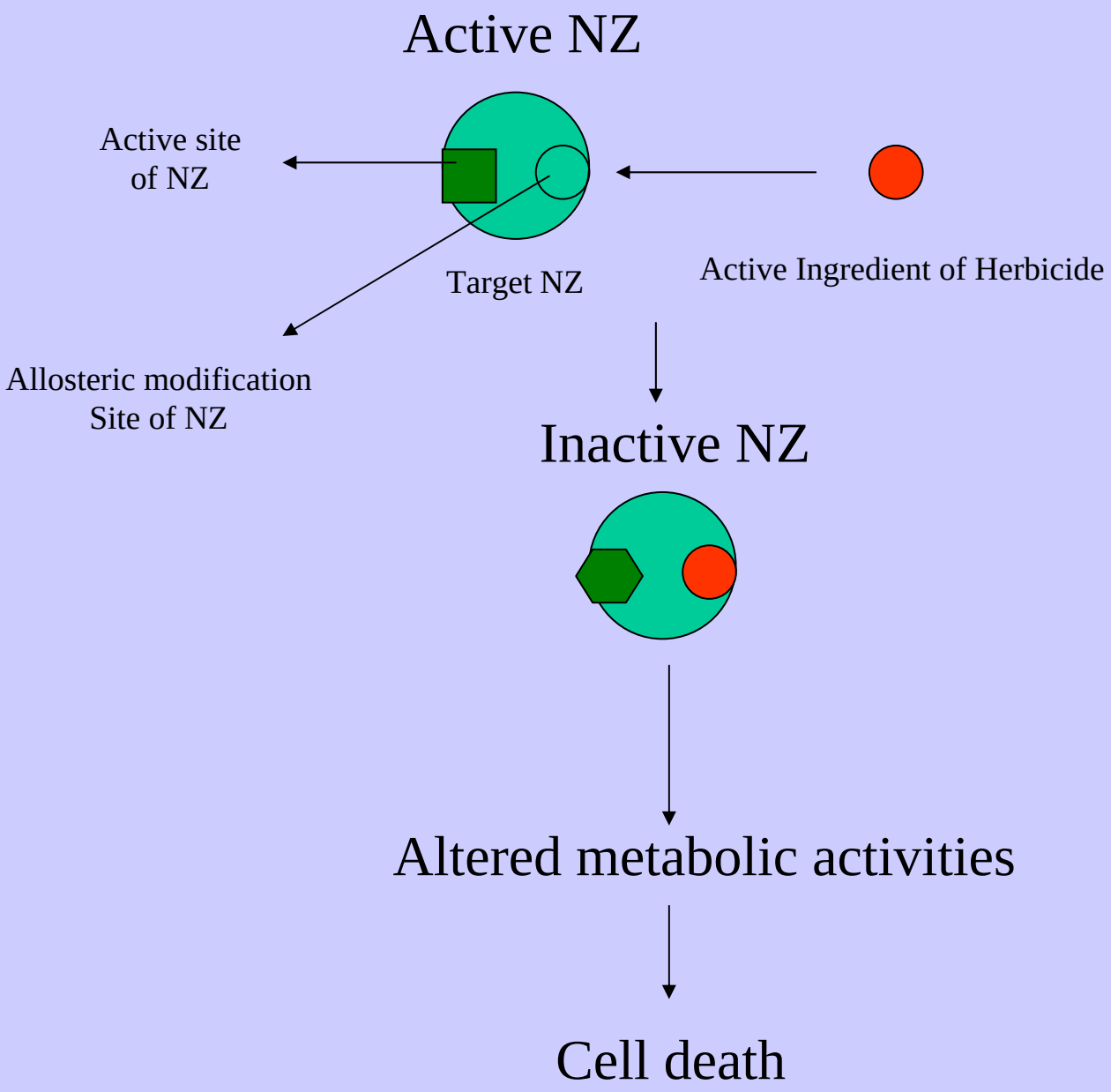


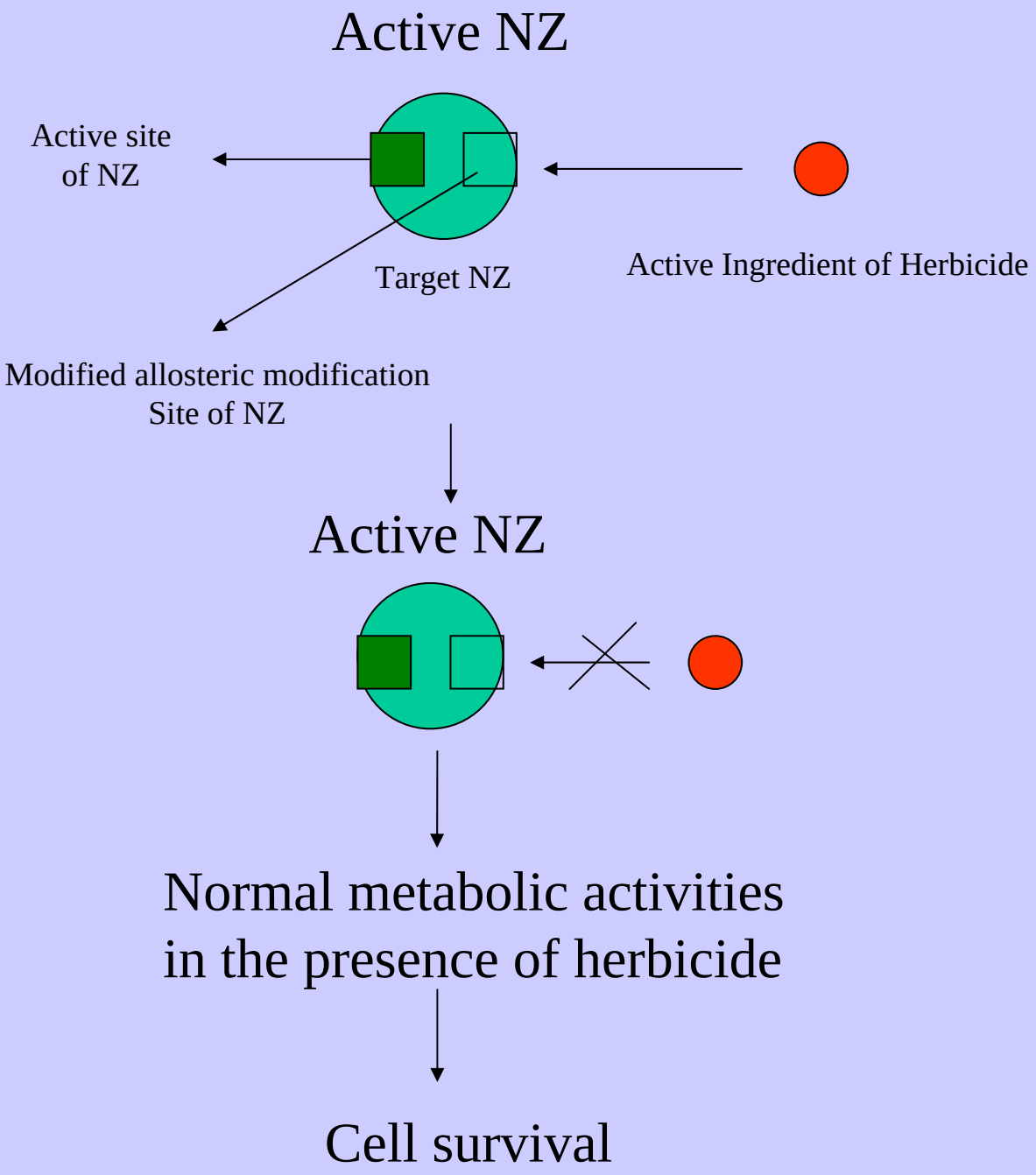
WHAT TYPES OF STRATEGIES TO DEVELOP HERBICIDE RESISTANT PLANTS ?

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
Active Ingredient of Herbicide
Allosteric modification
Site of NZ
Inactive NZ
Altered metabolic activities
Cell death



STRATEGY

Isolate gene of NZ

↓

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

↓

Transfer to target plant

↓

Develop TR plant

↓

Resistant to herbicide

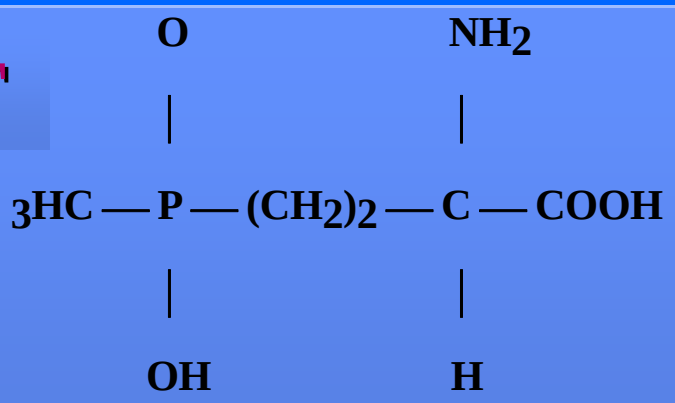
Some strains of *Streptomyces* produces a tripeptide antibiotic

BIALAPHOS

[L-PPT-(L-Alanine)$_2$]

By endopeptidase activity BIALAPHOS is hydrolyzed and produce free L-PPT

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 ?

	Streptomyces hygroscopius		*Streptomyces viridochromogenes*
Gene:	bar (560 bp)	87% homology	pat (560 bp)
Protein Product:	BAR	85% homology	PAT
Structure Info:	183 a.a.		183 a.a.
M.W.(from a.a. sequece)	20.6 kD		20.6
(SDS-PAGE)	22-23 kD		22-23 kD
(Native-PAGE)	41.0 KD	(HOMODIMER)	41.0 kD

GLUTAMINE SYNTHASE :

Key enzyme for ammonium assimilation

- Nitrate reduction
- Amino acid degredation
- Fotorespiration

BIALAPHOS

PPT

(GS)

glutamate + NH_4^+ + ATP ⟷ glutamine + ADP + P_i

Accumulation → LETHAL EFFECT

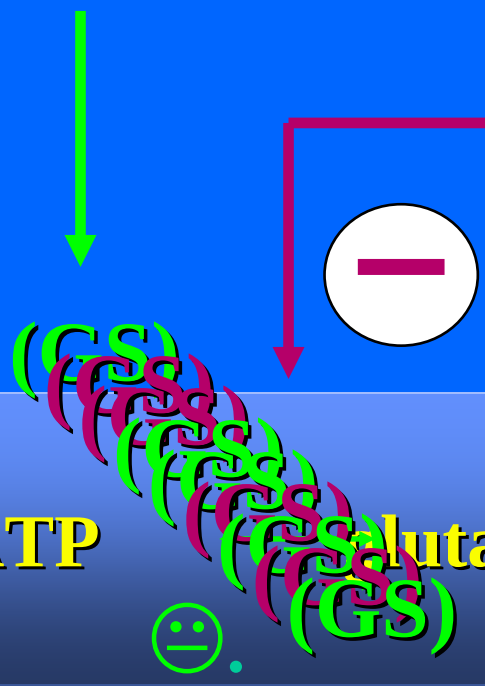
HOW TO DEVELOP PPT RESISTANT TR PLANTS

A. OVERPRODUCTION OF GS

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

BIALAPHOS

PPT



glutamate + NH_4^+ + ATP → glutamine + ADP + Pi

HOW TO DEVELOP PPT RESISTANT TR PLANTS

B. DETOXIFY PPT

Transfer of *bar* gene coding for Phosphinotricin acetyl transferase

BIALAPHOS

(PAT)

PPT → **Acetyl-PPT**

(Inactive on GS)

(GS)

$$\text{glutamate} + NH_4^+ + ATP \longrightarrow \text{glutamine} + ADP + P_i$$

☺.

HOW TO DEVELOP PPT RESISTANT TR PLANTS

B. DETOXIFY PPT

Transfer of *bar* gene coding for Phosphinotricin acetyl transferase

BIALAPHOS

(PAT)

PPT → **Acetyl-PPT**

(Inactive on GS)

(GS)

$$\text{glutamate} + NH_4^+ + ATP \longrightarrow \text{glutamine} + ADP + P_i$$

☺.

ROOT GENERATION IN SELECTIVE MEDIA

LBA:pDHB

MEDIA COMPOSITION

MSC No Selection | MSC with 10 mg/l PPT

CONTROL | TRANSFORMED | CONTROL

17

40

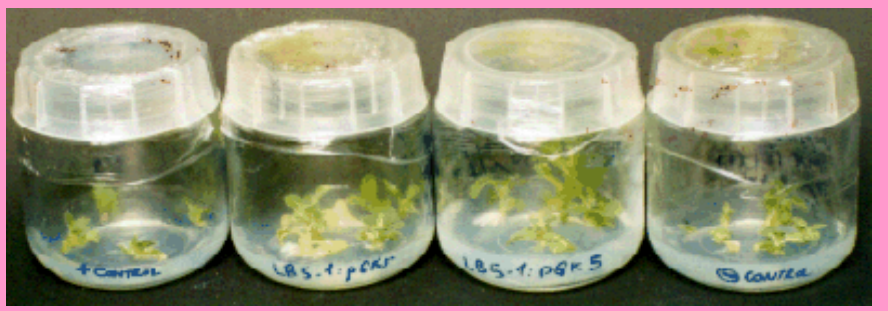
DAYS IN CULTURE

LB5-1:pGKB5

MEDIA COMPOSITION

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

CONTROL | TRANSFORMED | CONTROL



9

21

EFFECT OF 1 % BASTA TREATMEN ON CONTROL PLANTS

WATER

BASTA

2

9

DAYS AFTER TREATMENT

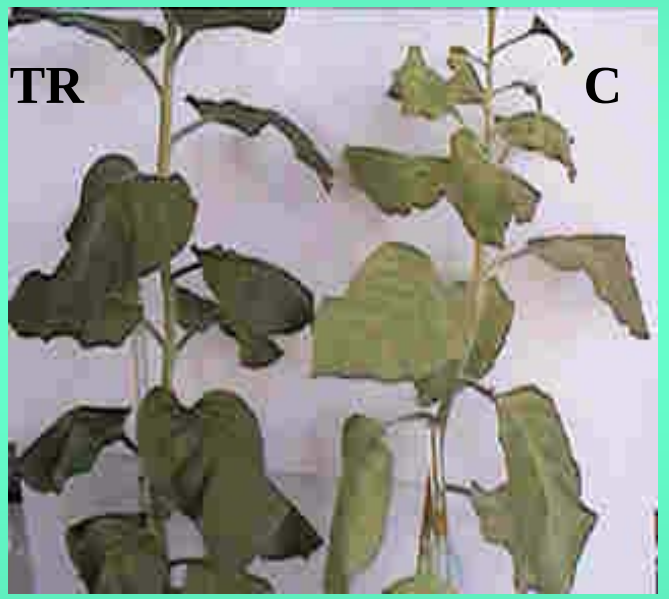
EFFECT OF BASTA TREATMENT ON PLANT DEVELOPMENT

40

DAYS IN SOIL

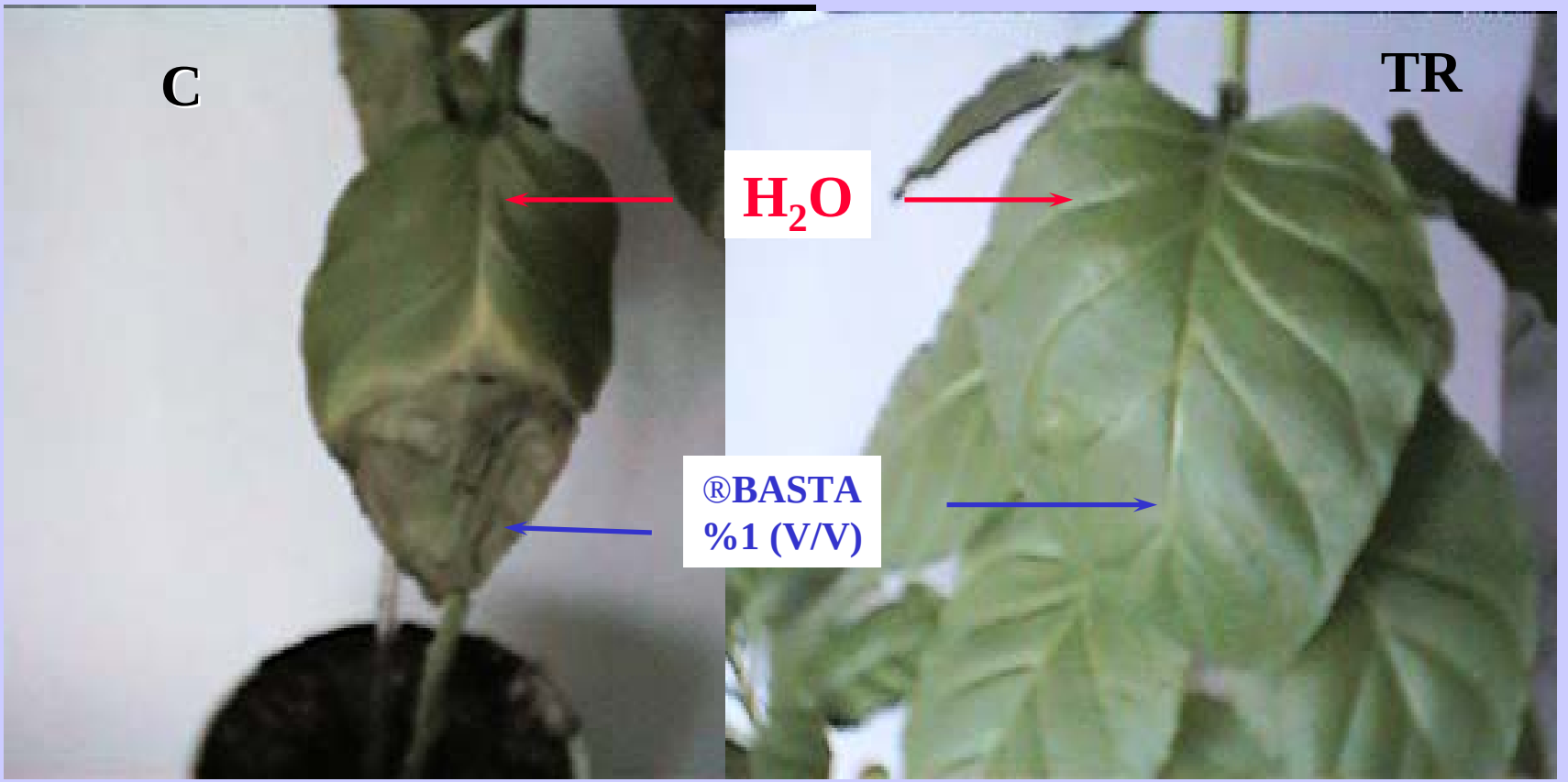
96

®BASTA 1% (V/V) TREAMENT



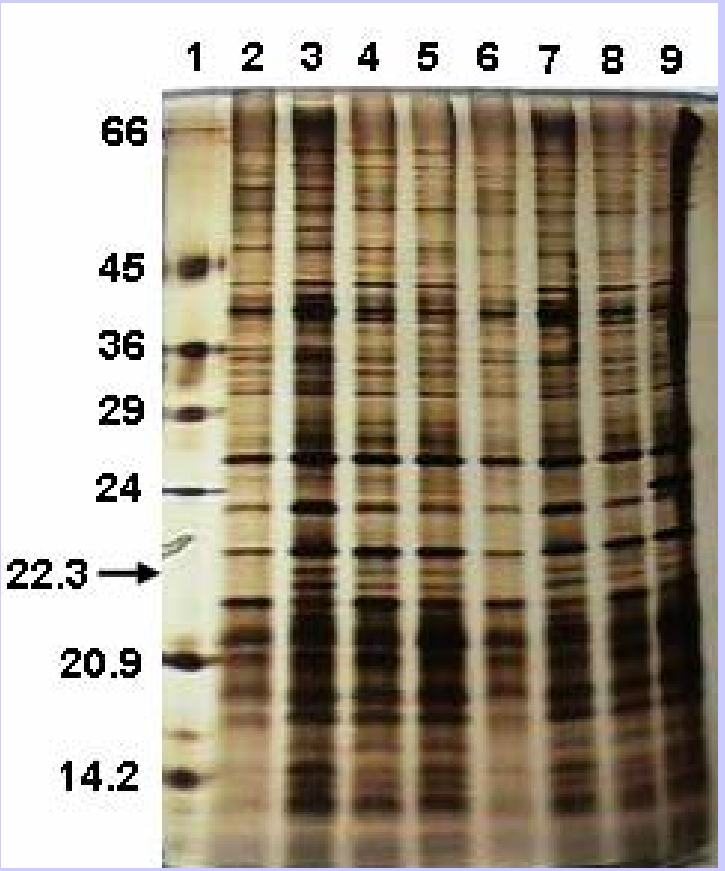
2 days a.t.

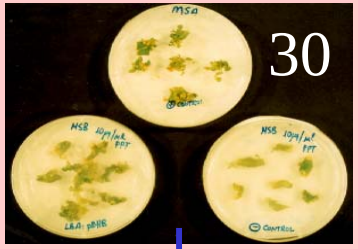
14 days a.t.

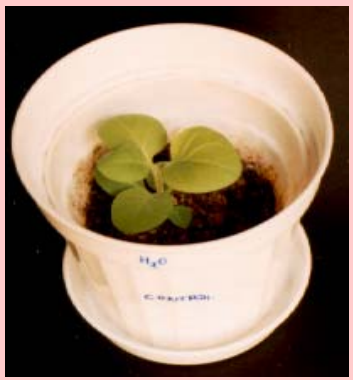

C
TR
H_2O
®BASTA
%1 (V/V)

SDS-PAGE for detection of PAT

- Lane 1: Molecular weight standards
- Lane 2, 6: Control
- Lane 3, 7: LP_3F_3A
- Lane 4, 8: $LP_3F_3B_1$
- Lane 5, 9: $LP_3F_3B_2$
- SDS-PAGE analysis of PAT showed a MW of 22-23 kD

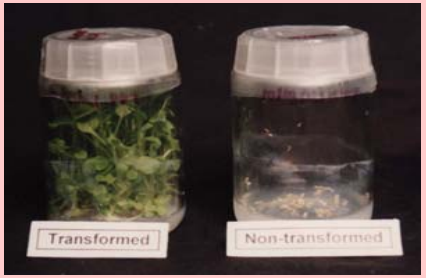






Fo TR PLANTS

Analysis of F1 Progeny



480

520

F1 Plantlets

LP3-F1-A LP3-F1-B

540

605

635

725

F1 Transgenics

810

780

810

Analysis of F2 Progeny

900


LP3F3-A
LP3F3-B1
LP3F3-B2
Control(-)
Control(+)

960


LP3F3-A
LP3F3-B1
LP3F3-B2
Control(-)
Control(+)

1000

1060

HERBICIDE RESISTANT TR TOBACCO

1200

EmuPAT, Fo

1% BASTA-14 days post application

3% BASTA application
DAY 1

30 days post application

pDHB, F3

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

Yamada et al., Theor.Appl.Genet. 2002

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 wel as benzothiadiazole (BTH)

↓

Non-phytotoxic plant protection agent

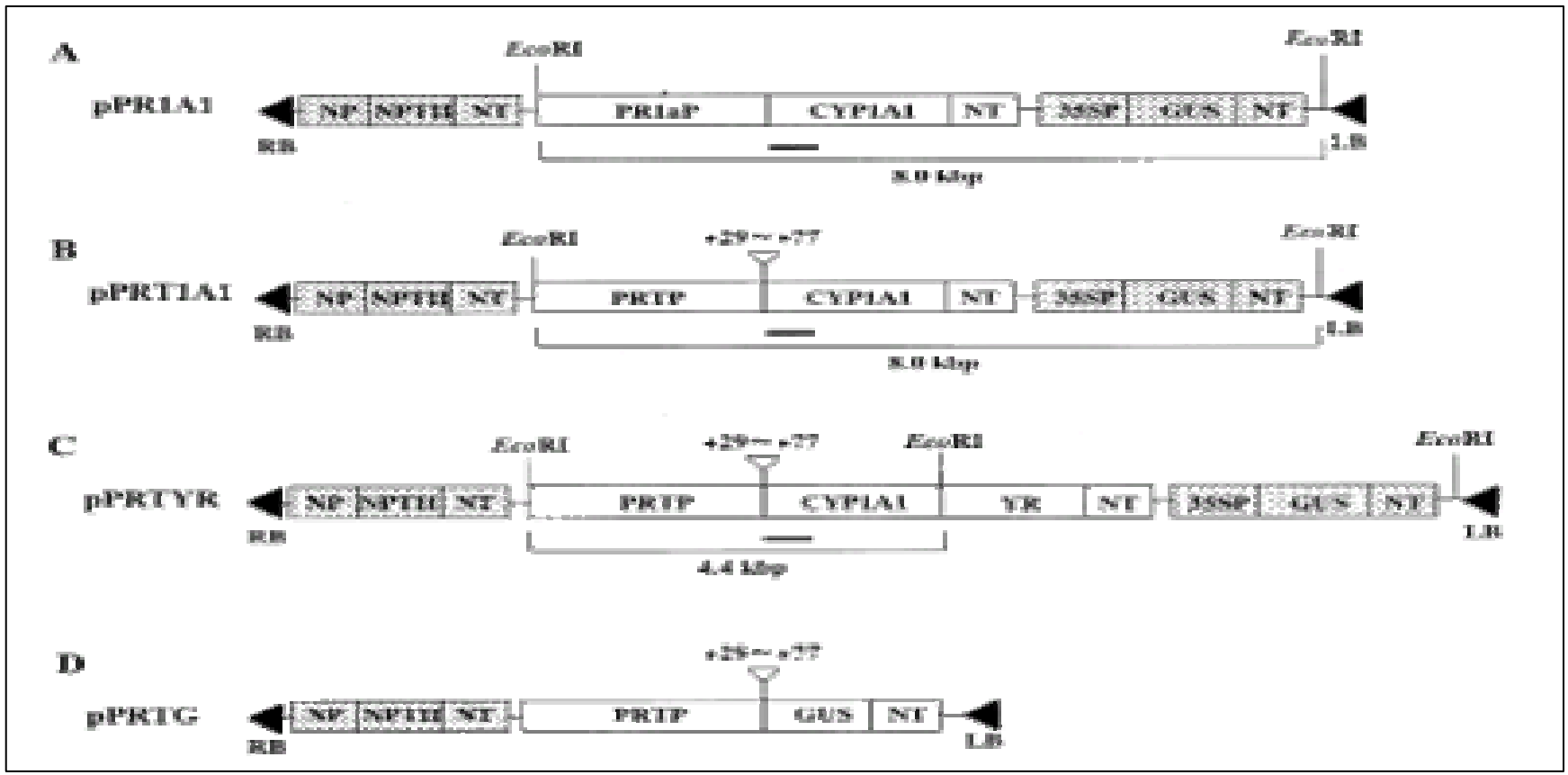
- BTH activates a number of systemic acquired resistance (SAR) genes including PR1A in tobacco, *Arabidopsis* and wheat

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 Contruction



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
  - Nothern blotting
  - In vivo herbicide tolerance test

Results

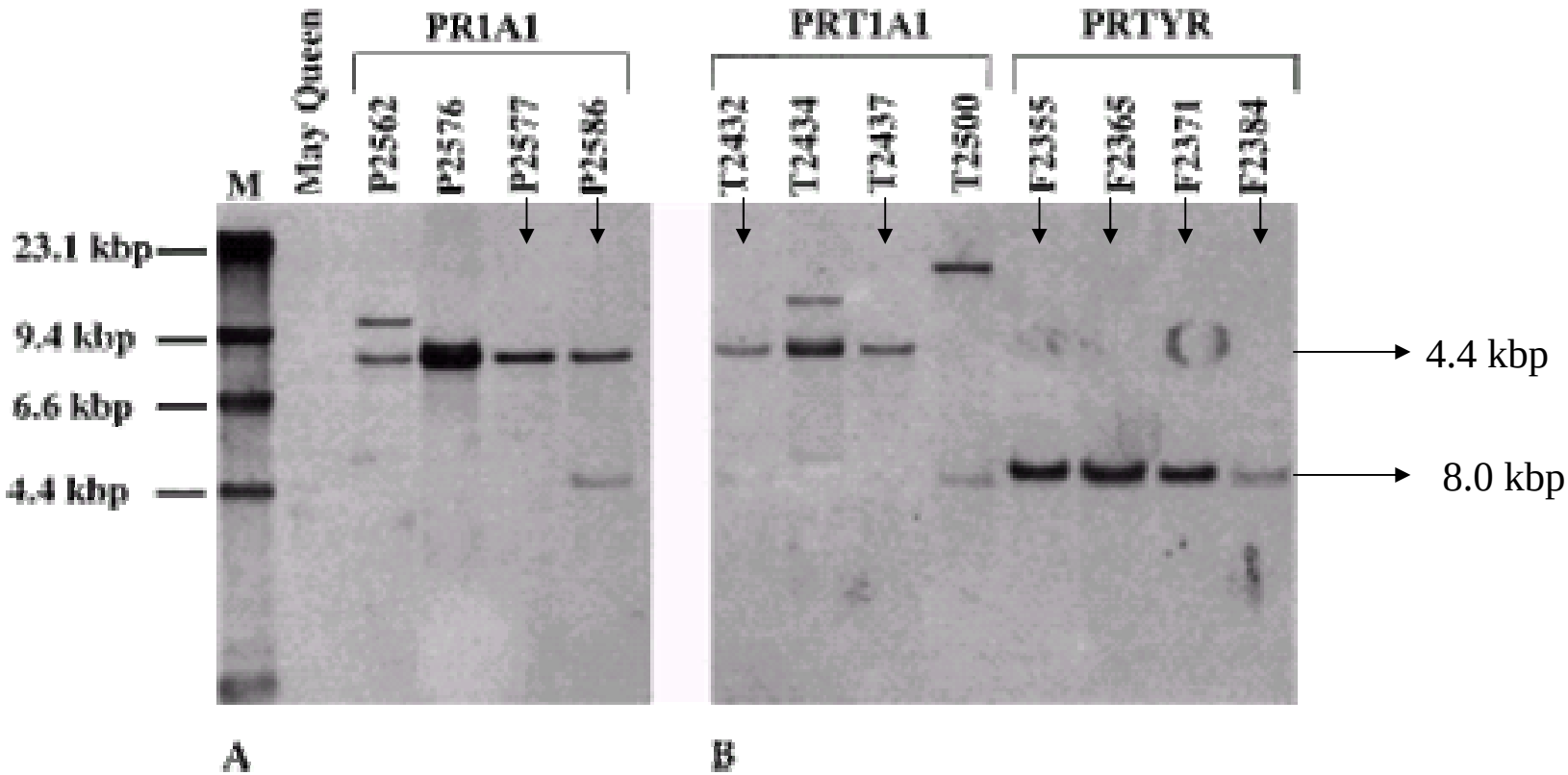
57 PR1A1 containing TR plant
88 PRT1A1 containing TR plant
85 PRTYR containing TR plants
} Selected by PCR

→For each group 4 highly tolerant TR plants were selected with BTH and chlortoluron

P450 species	Promoter	Expression plasmid	Selected plants
Rat CYP1A1	PR1a	pPR1A1	P2562, P2576, P2577, P2586
Rat CYP1A1	PRT	pPRT1A1	T2432, T2434, T2437, T2500
Rat CYP1A1/YR	PRT	pPRTYR	F2355, F2365, F2371, F2384

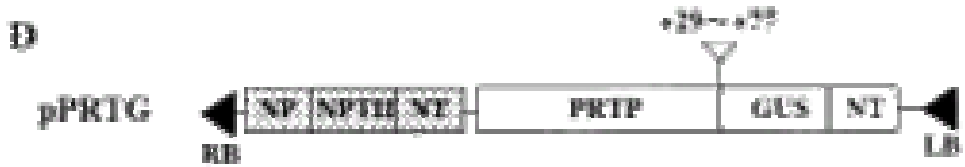
Results

- Southern-Blotting Analysis



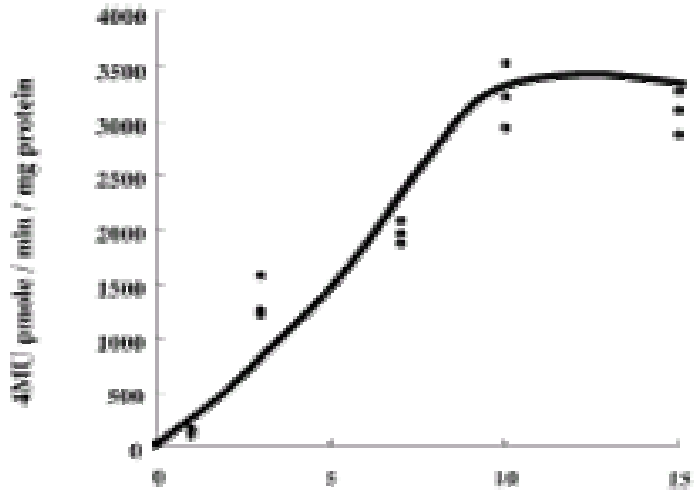
Results

- GUS Activity



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

3.0 μmol/pot BTH



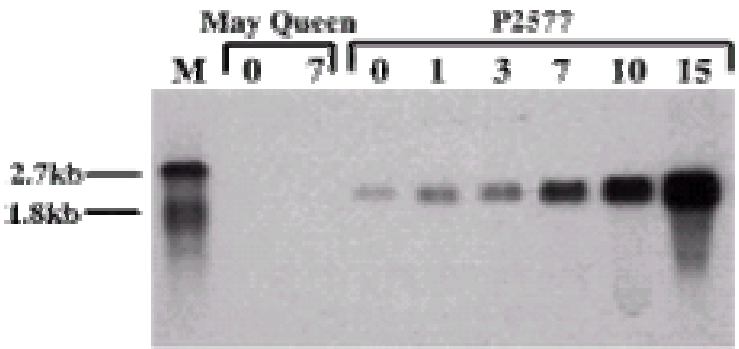
Results

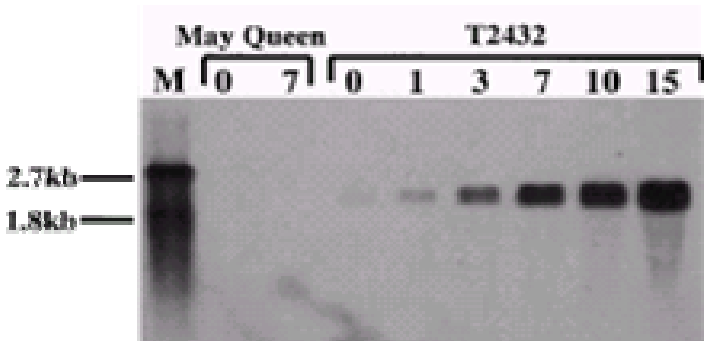
- Western-Blotting Analysis

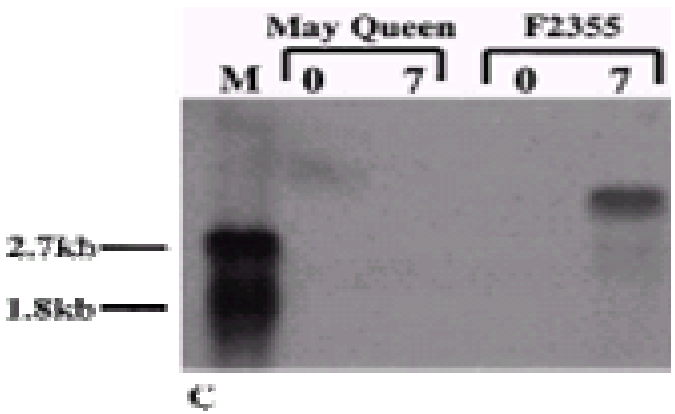
- The microsomal proteins were isolated from the leaves 7 day after the BTH treatment
- Without BTH treatment no protein band was detected in TR plants
- With BTH, bands corresponding to rat CYP1A1 or its fused enzyme were observed in all TR plants
- Low level of accumulation of CYP1A1 fused with YR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

Plant lines	Chlortoluron		Methabenzthiazuron		Norflurazon	
	Non-BTH	BTH	Non-BTH	BTH	Non-BTH	BTH
F2577	Plants died	No damage	Slightly damage	No damage	Chlorosis	Chlorosis
T2432	Plants died	No damage	Plants died	No damage	Chlorosis	Chlorosis
F2355	Plants died	No damage	Plants died	No damage	Chlorosis	Chlorosis

Discussion

- The transgenes under the control of PR1a and PRT promoter were easiliy 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 reached max 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.