The role of sterol binding and surface charge in elicitin-induced resistance

Jan Lochman, Michal Obořil, Veronika Plešková, Kateřina Dadáková, Nikola Ptáčková, Zbyněk Zdráhal, Tomáš Kašparovský

Faculty of Science, Masaryk University

IPM 2.0 Towards future-proof crop protection in Europe

esf european social fund in the czech republic

EUROPEAN UNION

MINISTRY OF EDUCATION, YOUTH AND SPORTS

OP Education for Competitiveness

INVESTMENTS IN EDUCATION DEVELOPMENT

EFPP

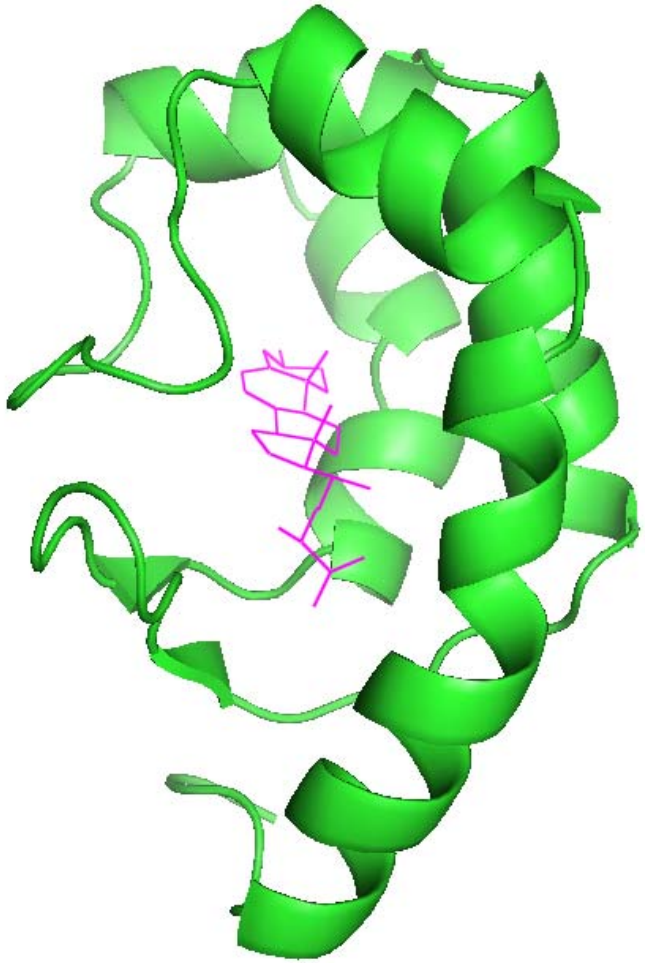
Elicitines history

FEBS 16180

FEBS Letters 374 (1995) 203–207

Evidence for : ceous elicitor

David Wendel Alain Pugin[a,*]



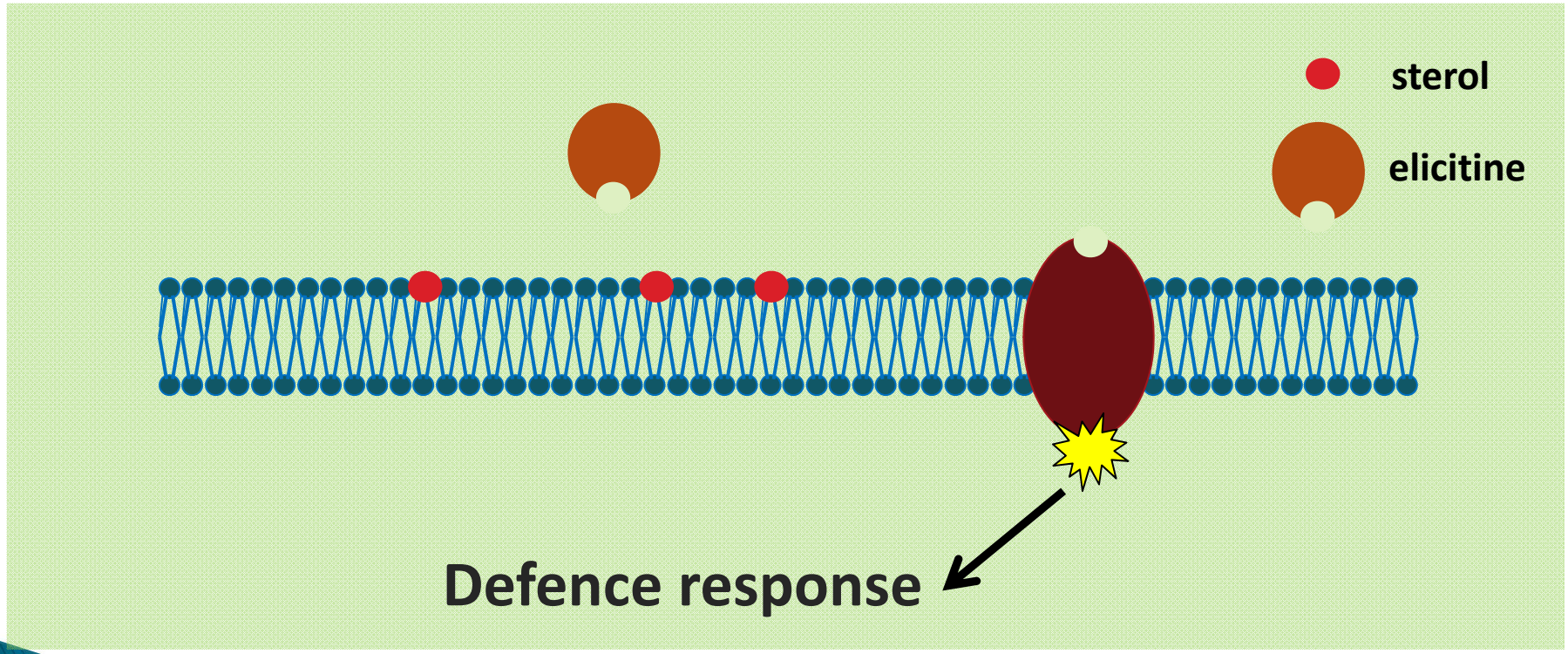
- small prot *ra* and some *Pythuim* s
- classified , pI>7)
- β-elicitins ce on plants
- common s β-sheet
- ability to t
- identificat acco

Elicitines action

Molecular Biology of the Cell
Vol. 12, 2825–2834, September 2001

Mediation of Elicitin Activity on Tobacco Is Assumed by Elicitin-Sterol Complexes

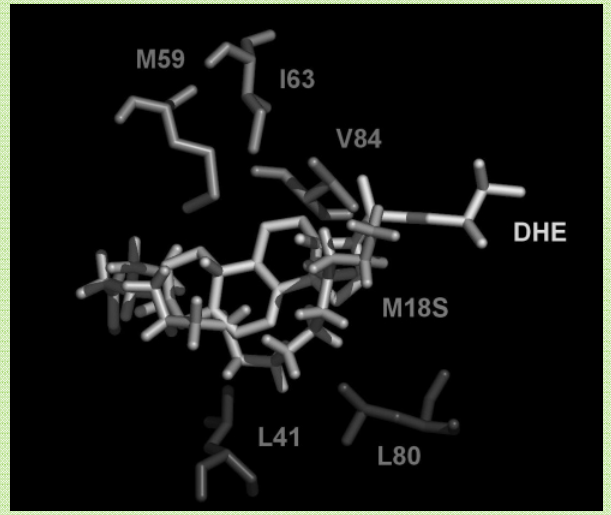
Hanan Osman,*† Sébastien Vauthrin,*† Vladimir Mikes,‡ Marie-Louise Milat,* Franck Panabières,§ Antoine Marais,§ Simone Brunie,‖ Bernard Maume,* Michel Ponchet,§¶ and Jean-Pierre Blein*

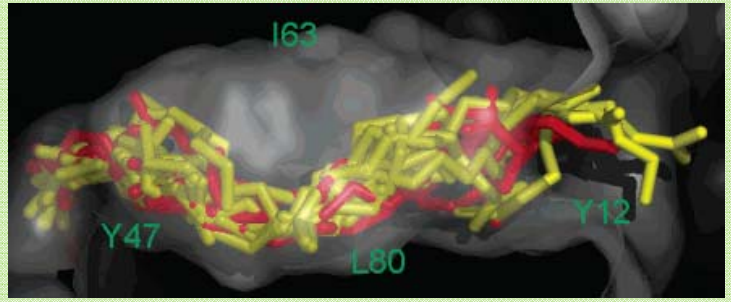


IPM 2.0
Towards future-proof crop protection in Europe

How important is sterol binding activity ?

- on the basis of molecular modelling (Dobeš et al. 2004) were expressed new cryptogein mutants altered in sterol and fatty acid binding.





L41F, L80F
V84F, L41F/V84F, L80F/V84F

- Substitution of the residue Val 84 by phenylalanine = reduced binding of sterol to the cavity.
- Substitutions of the amino acid residues Leu 41 or Leu 80 by phenylalanine = decreased binding of FAs to the cavity but not sterols

IPM 2.0
Towards future-proof crop protection in Europe

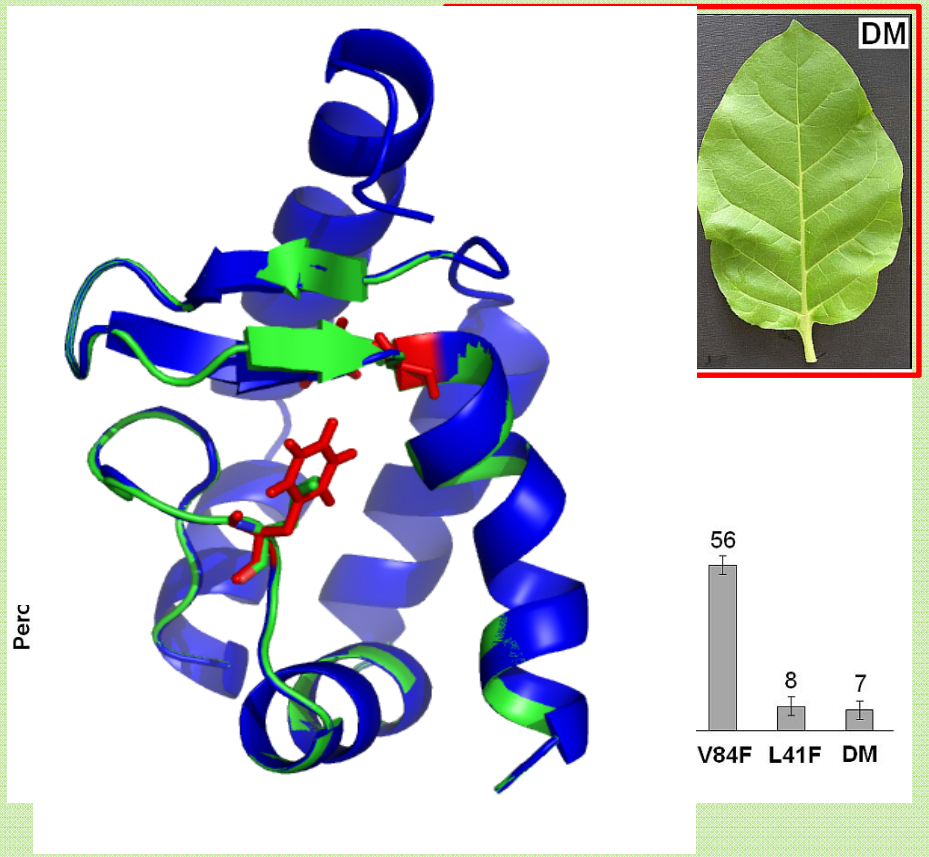
Sterol binding properties of mutans

Protein	K_d [µM]	DHE transfer	NBD-PC transfer
wt cryptogein	0.56 ± 0.04	2,51±0,06	0,51±0,05
L41F	0.85 ± 0.05	1,88±0,08	0,32±0,01
V84F	No binding	0,45±0,02	0,74±0,08
L41F/V84F	No binding	0,38±0,01	0,76±0,04
L80F/V84F	No binding	0.33±0.01	0,75±0,06
Aprotinin	No binding	0,19±0,01	0,15±0,01

- Very good correlation with predicted data

- Mutation V84F causes relaxing of cavity resulting in higher fatty acid binding compared to cryptogein

Correlation of sterol-binding activity with necrosis, A ROS synthesis and capsidiol accumulation



There was no big change in CD spectra

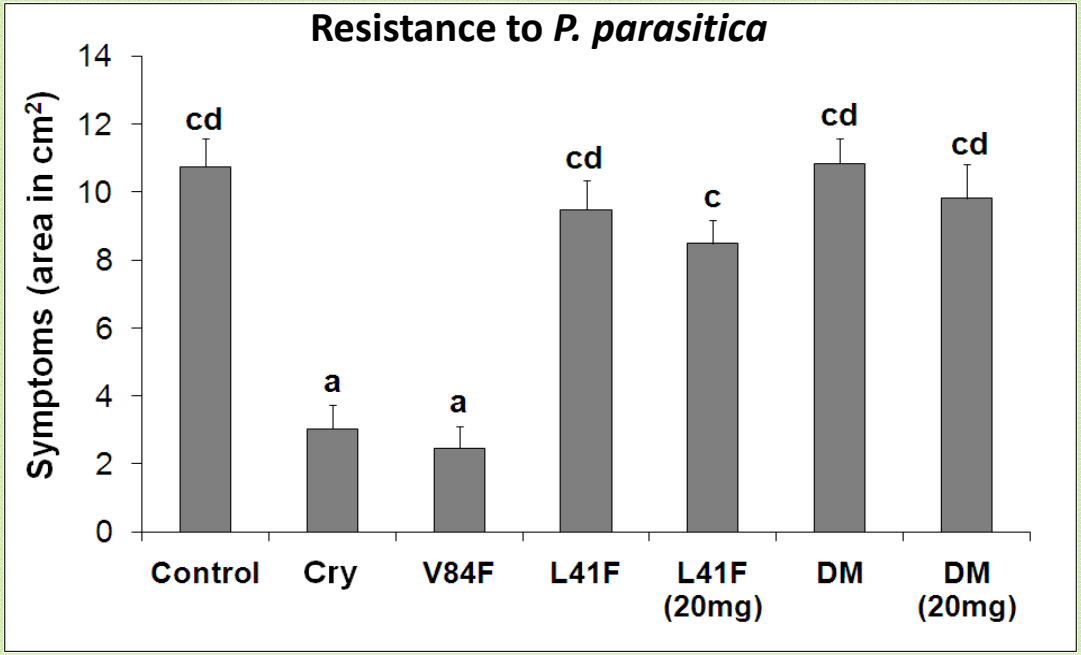
Structure-prediction of the Leu41Phe and Leu41Phe/Val84Phe mutants revealed that the large phenylalanine(s) are easily accommodated by the surrounding residues without any significant changes in the ω-loop

Experiments with mutant L80F/V84F showed the similar behaviour to V84F mutant

IPM 2.0
Towards future-proof crop protection in Europe

Proteomic analysis

The changes of intercellular fluid proteome were studied



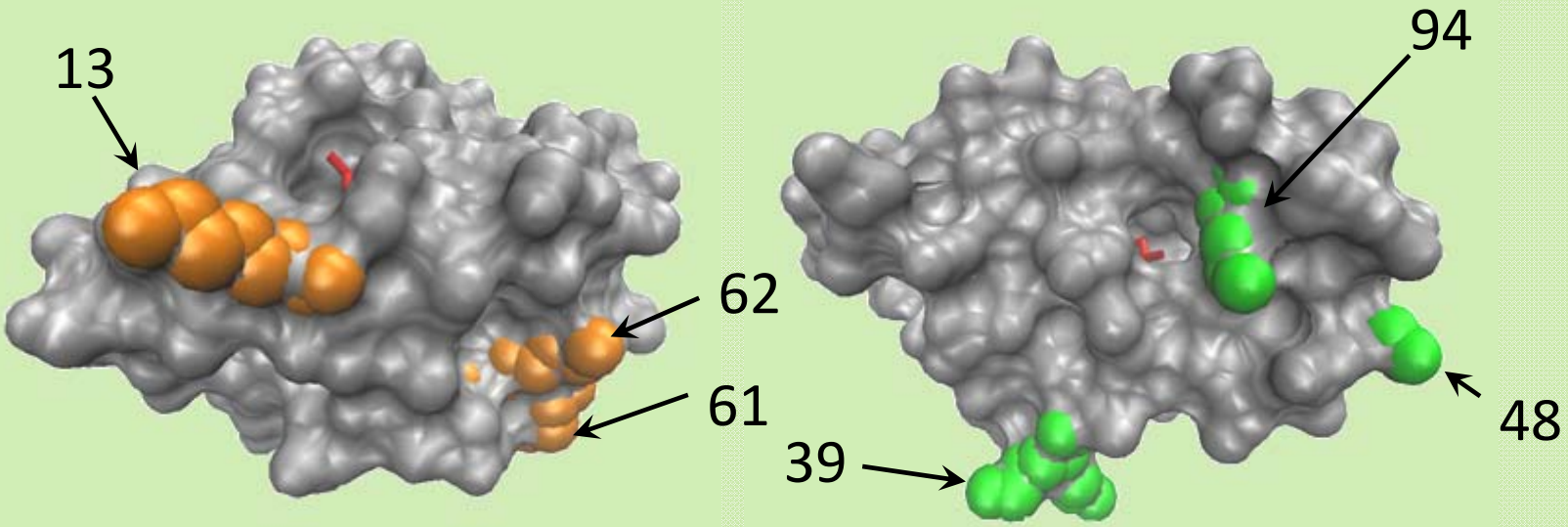
Mutant V84F exhibited similar resistance induction to wt cryptogein. On the other side mutants carrying L41F mutations did not exhibit any resistance to *P. parasitica*.

Germin-like protein	222051768	6	21.4	5.8	+		+	+
NtPRp27	5360263	25	27.4	9.3	+			+
Thaumatin-like protein E22	131015	22	24.7	5.4	+	+		+
Tumor-related protein	1762933	7	23.4	8.5	+			+

IPM 2.0
Towards future-proof crop protection in Europe

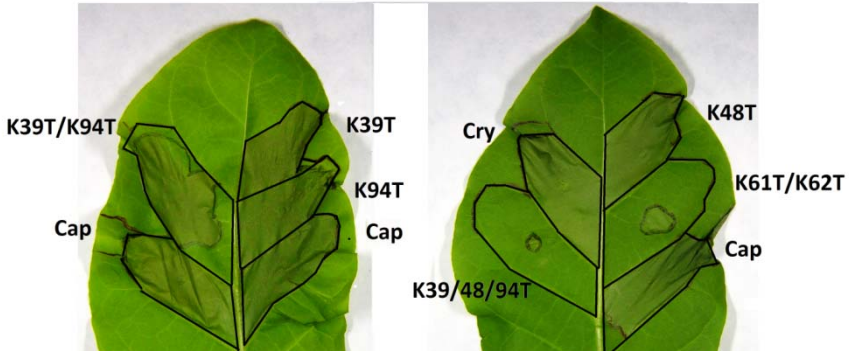
How important is surface charge ?

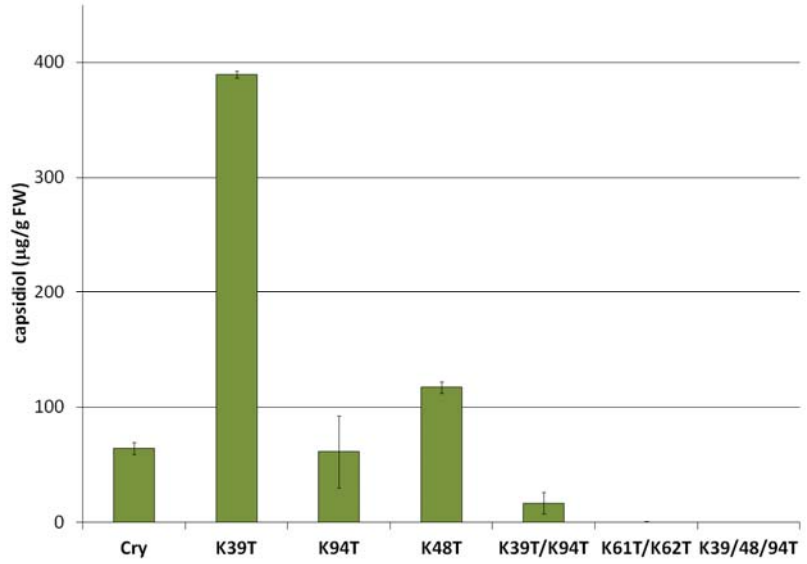
- There are six lysine residues on the surface of cryptogein molecule



- On the basis of previous results mutants modified in surface charge were prepared: K13V, K39T, K48T, K94T, K61/62T, K39T/K94T and K13V/K48T/K94T

Correlation of surface charge with necrosis, ROS synthesis and capsidiol accumulation





Single mutants K39T, K94T, K48T had comparable necrosis and ROS production as wt Cryptogein

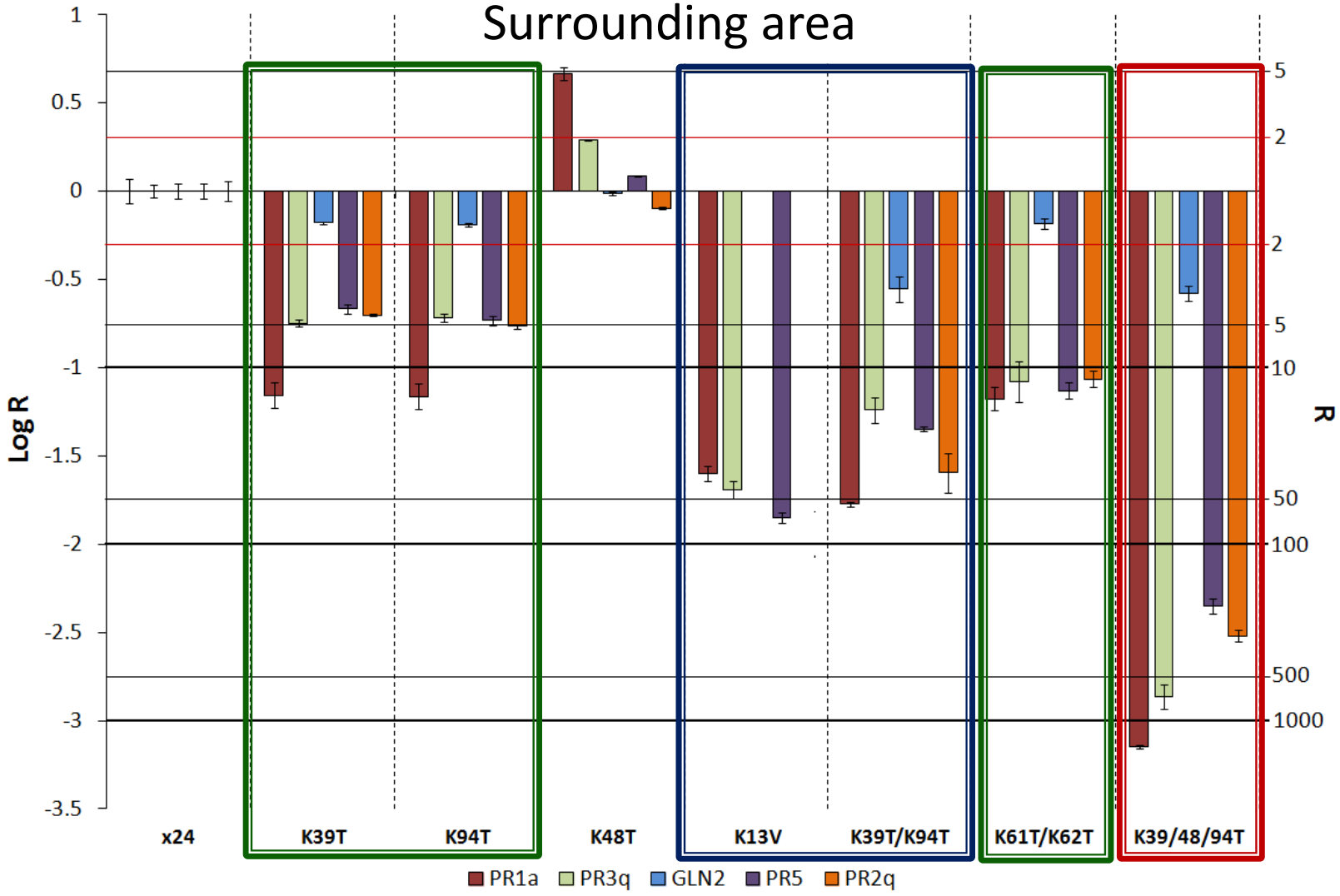
Double mutant K39T/K94T and single mutant K39T had lower necrosis and half ROS production as wt Cryptogein

Double mutant K61T/K62T and triple mutant K39/48/94T showed almost no necrosis and ROS production.

Capsidiol accumulation was in a good relationship with necrosis and ROS production

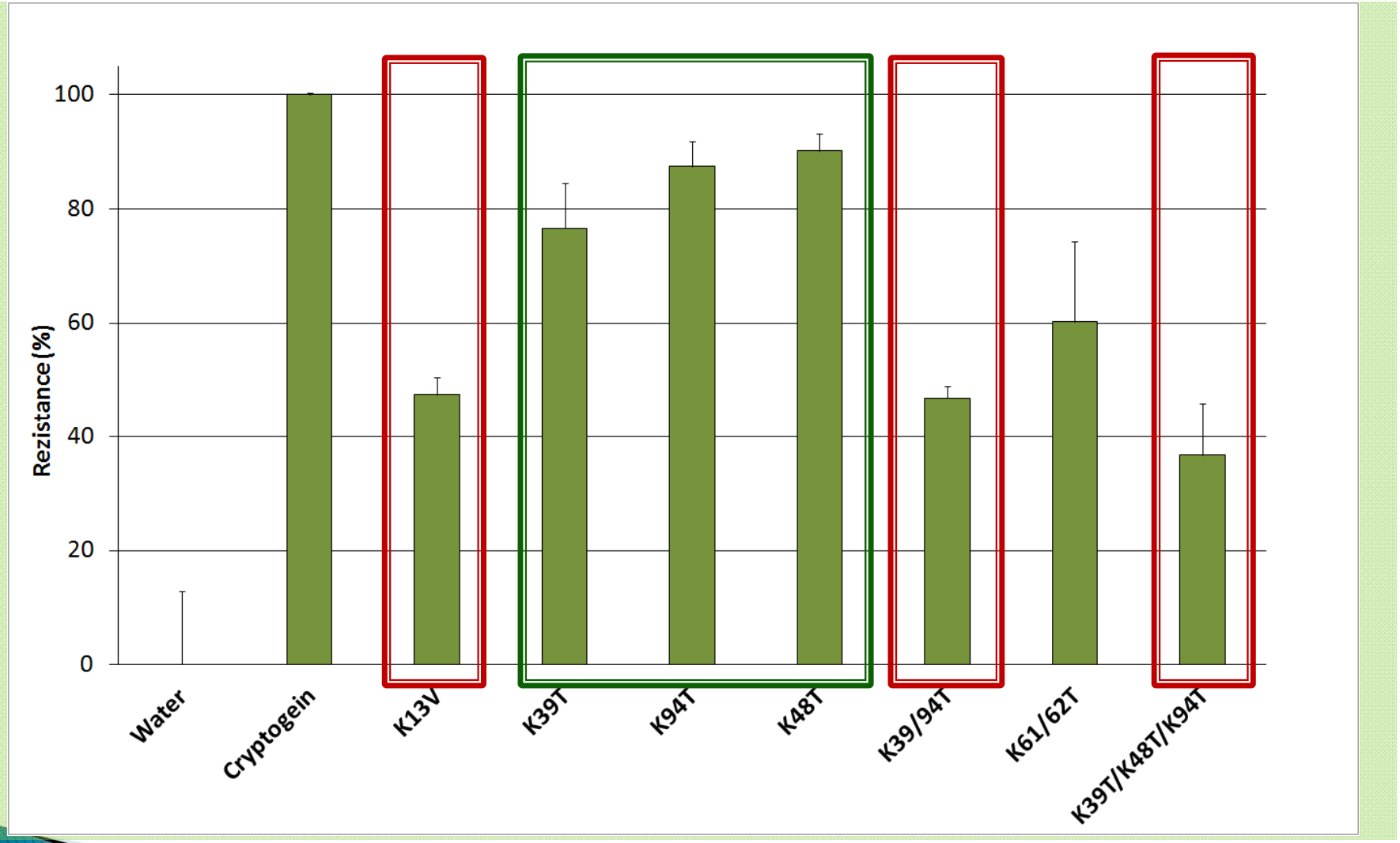
IPM 2.0
Towards future-proof crop protection in Europe

Transcriptomic analysis



IPM 2.0
Towards future-proof crop protection in Europe

Rezistance analysis



IPM 2.0
Towards future-proof crop protection in Europe

Conclusion

- There is n…vity and ROS production
- Distributio…icitins ability to pick-up
- Distributio…icitins ability to trigger c
- Full descri…tion of high affinity bin
- Our findin…otic activity without su

	N fmol/mg protein	K_d nM
	reactive plants	
N. tabacum	101 ± 7	8.8 ± 1.9
B. napus var:		
yudal	190 ± 15	5.7 ± 0.2
liberator	898 ± 107	13.5 ± 2.4
lirabon	251 ± 48	10.3 ± 2.1
cobra	292 ± 47	7.7 ± 1.4
	unreactive plants	
L. esculentum	148 ± 17	7.3 ± 1.3
A. thaliana	210 ± 23	10.2 ± 1.4
A. pseudoplatanus	203 ± 15	6.1 ± 1.5
B. napus var:		
darmor nain	100 ± 3	3.1 ± 0.2
jet 9	845 ± 18	22.5 ± 1.2
bolko	448 ± 29	4.0 ± 1.7
shogun	1012 ± 275	5.3 ± 1.7

IPM 2.0
Towards future-proof crop protection in Europe

Thank You For Attention

Thanks to these funding:

GAČR
GRANTOVÁ AGENTURA ČESKÉ REPUBLIKY
P: 501/11/1003

esf european social fund in the czech republic
EUROPEAN UNION
MINISTRY OF EDUCATION, YOUTH AND SPORTS
OP Education for Competitiveness
INVESTMENTS IN EDUCATION DEVELOPMENT