BACCHUS
Cardiovascular benefits from food bioactives

Dalla Lunasina al nsLTP2: identificazione e suo contenuto nei frumenti

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KAMUT® KHORASAN

Bologna, 28.05.2016

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Available literature about lunasin in wheat and other species

(after lunasin from soybean identification)

Review

Lunasin, a novel seed peptide for cancer prevention

Blanca Hernández-Ledesma [1], Chia-Chien Hsieh [1], Ben O. de Lumen *

Peptides 30 (2009) 426–430

J. Agric. Food Chem. 2007, 55, 10707–10713

Cancer-Preventive Peptide Lunasin from *Solanum nigrum* L. Inhibits Acetylation of Core Histones H3 and H4 and Phosphorylation of Retinoblastoma Protein (Rb)

Jin Boo Jeong,[†,‡] Hyung Jin Jeong,[†,‡] Jae Ho Park,[†,‡] Sun Hee Lee,[‡] Jeong Rak Lee,[‡] Hee Kyeong Lee,[‡] Gyu Young Chung,[‡] Jeong Doo Choi,[§] and Ben O. de Lumen*[,∥]

J. Agric. Food Chem. 2002, 50, 5903–5908

Barley Lunasin Suppresses *ras*-Induced Colony Formation and Inhibits Core Histone Acetylation in Mammalian Cells

Hyung J. Jeong,[†] Yi Lam,[‡] and Ben O. de Lumen*[,§]

Journal of Cereal Science xxx (2012) 1–5

Discovery of lunasin peptide in triticale (X *Triticosecale* Wittmack)

Ilva Nakurte [a], Kristaps Klavins [b], Inga Kirhnere [c], Jana Namniece [d], Liene Adlere [d], Jaroslavs Matvejevs, Arta Kronberga [c], Aina Kokare [c], Vija Strazdina [e], Linda Legzdina [c], Ruta Muceniece [d,*]

Nutrition and Cancer, 61(5), 680–686

ISSN: 0163-5581 print / 1532-7914 online
DOI: 10.1080/01635580902850082

The Cancer Preventive Seed Peptide Lunasin From Rye Is Bioavailable and Bioactive

Hyung Jin Jeong, Jeong Rak Lee, Jin Boo Jeong, and Jae Ho Park
Young-keun Cheong
Ben O. de Lumen

J. Agric. Food Chem. 2008, 56, 1233–1240

Bioactive Peptides in Amaranth (*Amaranthus hypochondriacus*) Seed

C. Silva-Sánchez,[†,§] A. P. Barba de la Rosa,[†] M. F. León-Galván,[†] B. O. de Lumen,[‡] A. de León-Rodríguez,[†] and E. González de Mejía*[,§]

Nutrition and Cancer, 62(8), 1113–1119

ISSN: 0163-5581 print / 1532-7914 online
DOI: 10.1080/01635581.2010.515529

Lunasin Is Prevalent in Barley and Is Bioavailable and Bioactive in In Vivo and In Vitro Studies

Hyung Jin Jeong [a], Jin Boo Jeong [a], Chia Chien Hsieh [b], Blanca Hernández-Ledesma [b] & Ben O. de Lumen [b]

Cancer Letters 255 (2007) 42–48

The cancer preventive peptide lunasin from wheat inhibits core histone acetylation

Hyung Jin Jeong [1,a], Jin Boo Jeong [1,a], Dae Seop Kim [1,a], Jae Ho Park [a], Jung Bok Lee [a], Dae-Hyuk Kweon [b], Gyu Young Chung [a], Eul Won Seo [a], Ben O. de Lumen [c,*]

Nutrition and Cancer, 64(1), 111–120

ISSN: 0163-5581 print / 1532-7914 online
DOI: 10.1080/01635581.2012.630159

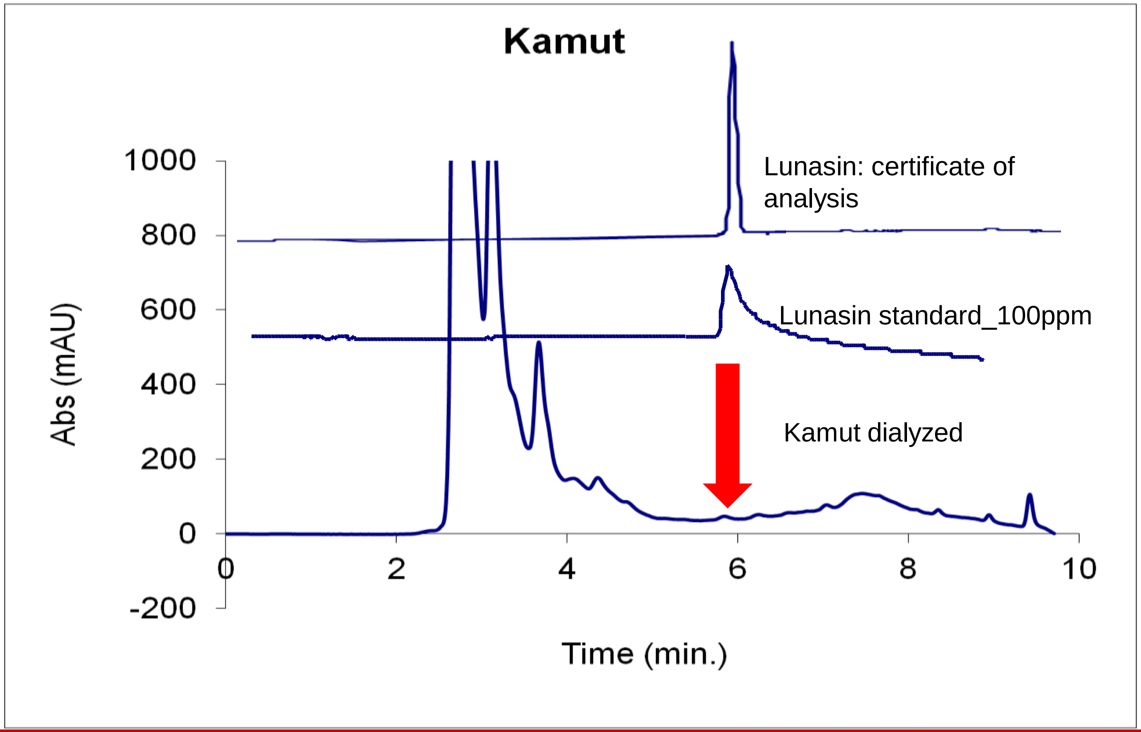
Synthesis of the Cancer Preventive Peptide Lunasin by Lactic Acid Bacteria During Sourdough Fermentation

Carlo G. Rizzello, Luana Nionelli, Rossana Coda, and Marco Gobbetti

The UNIBO work: the beginning of the doubt!!

December 2012

HPLC analysis: our attempts in separation of lunasin standard and its identification in Kamut dialyzed sample.



BAD RESOLUTION

DIFFICULT IDENTIFICATION OF LUNASIN IN WHEAT SAMPLE

LUNASIN is not present in wheat

Jan-Dec 2013: we demonstrated the lacking of lunasin in wheat

- Lunasin gene not identified in wheat (Dinelli *et al. J. Food Chem*, 2014)

Food Chemistry 151 (2014) 520–525

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

ELSEVIER

FOOD CHEMISTRY

Rapid Communication

Lunasin in wheat: A chemical and molecular study on its presence or absence

CrossMark

Giovanni Dinelli [a,*], Valeria Bregola [a], Sara Bosi [a], Jessica Fiori [b], Roberto Gotti [b], Emanuela Simonetti [c], Caterina Trozzi [c], Emanuela Leoncini [d], Cecilia Prata [b], Luca Massaccesi [d], Marco Malaguti [d], Robert Quinn [e], Silvana Hrelia [d]

[a] Department of Agricultural Sciences, Alma Mater Studiorum University of Bologna, Viale Fanin 44, 40127 Bologna, Italy
[b] Department of Pharmacy and Biotechnology, Alma Mater Studiorum University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy
[c] Bioaesis, Via Rocco Scotellaro 1/A, 60035 Jesi (AN), Italy
[d] Department for Life Quality Studies, Alma Mater Studiorum University of Bologna, Corso D'Augusto 237, 47921 Rimini, Italy
[e] Kamut International, 333 Kamut Lane, Big Sandy, MT 59520, USA

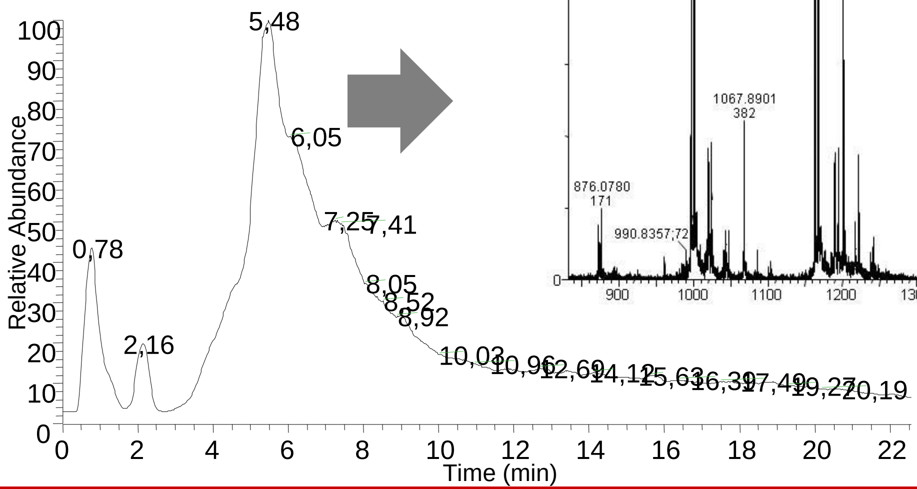
-NEWLY ISOLATED WHEAT PEPTIDE

- Sequenced and identified according to the following database

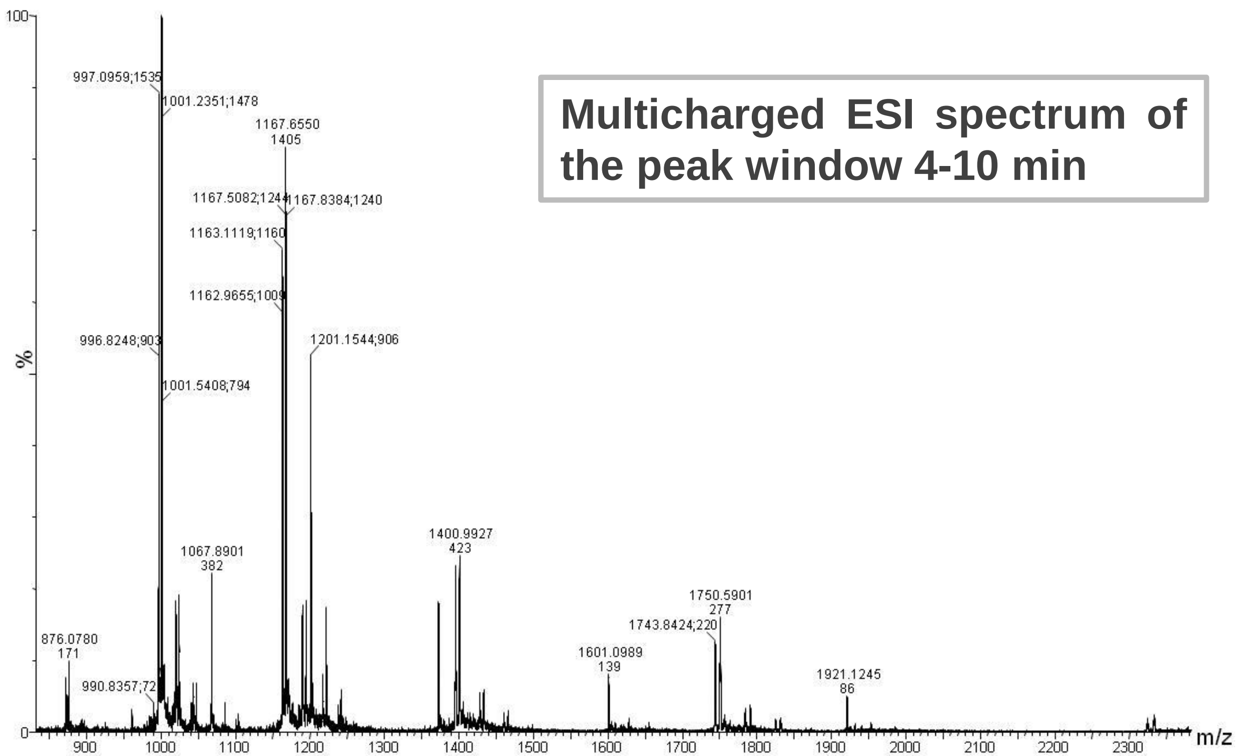
Where did the mistake occur?

February 2014: we identified a new wheat peptide

TIC MS chromatogram

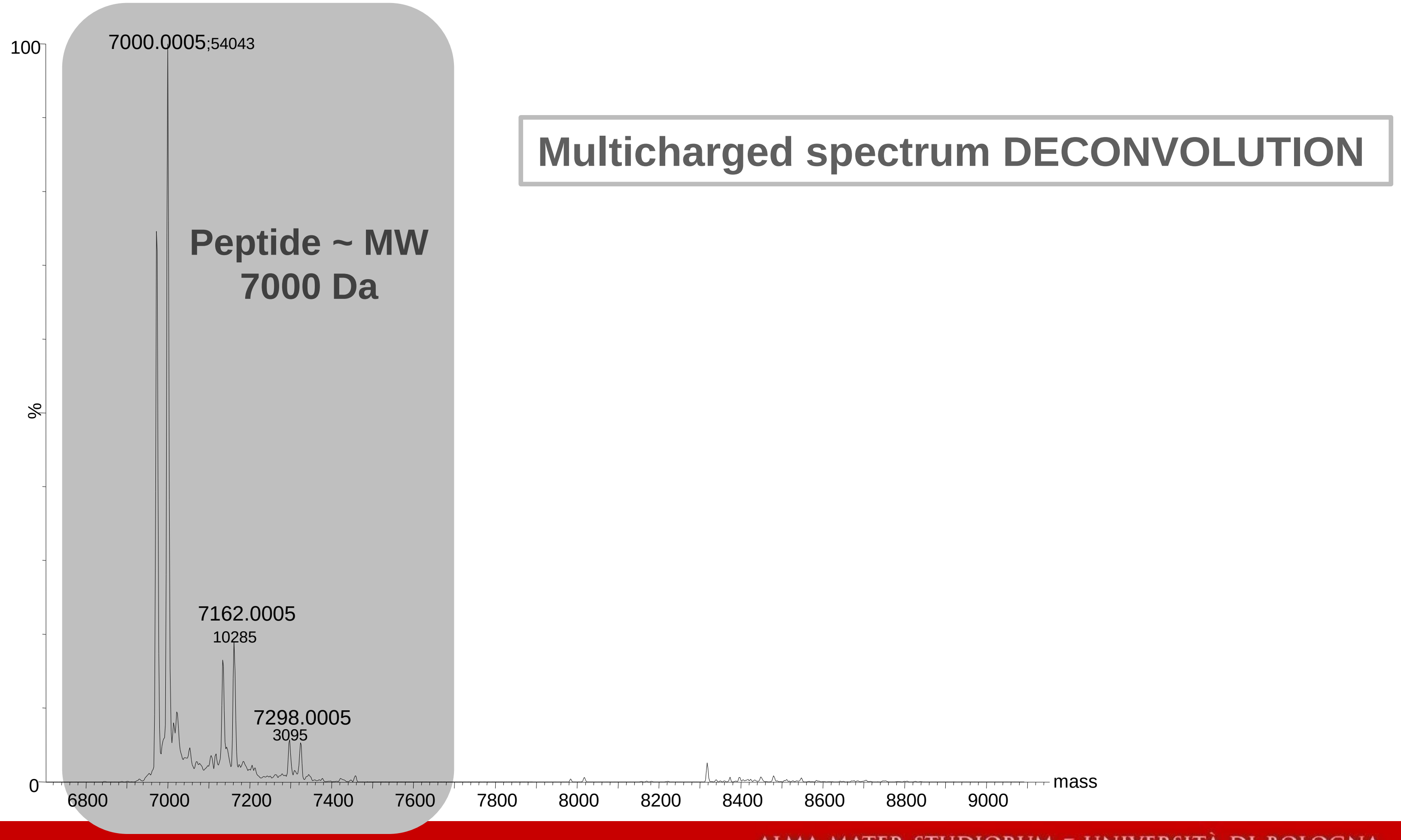


Multicharged ESI spectrum of the peak window 4-10 min



WHERE did the mistake occur?

The multicharged spectrum deconvolution showed the presence of one main pepetide



NEW WHEAT PEPTIDE IDENTIFICATION

MASCOT

Peptide MW around 7000 Da

SwissProt, NCBI

NLT2P Wheat; PM 7046

Non-specific lipid-transfer protein 2P OS=Triticum aestivum

Protein sequence coverage: 76%

1 **ACQASQLAVC** **ASAILSGAKP** **SGECCGNLR**A QQPCFCQYAK **DPTYGQYIR**S

51 PHAR**DTLQSC** **GLAVPHC**

NCBI

Chain A, Solution Structure Of A Liganded Type 2 Wheat Non-Specific Lipid Transfer Protein; PM 6979

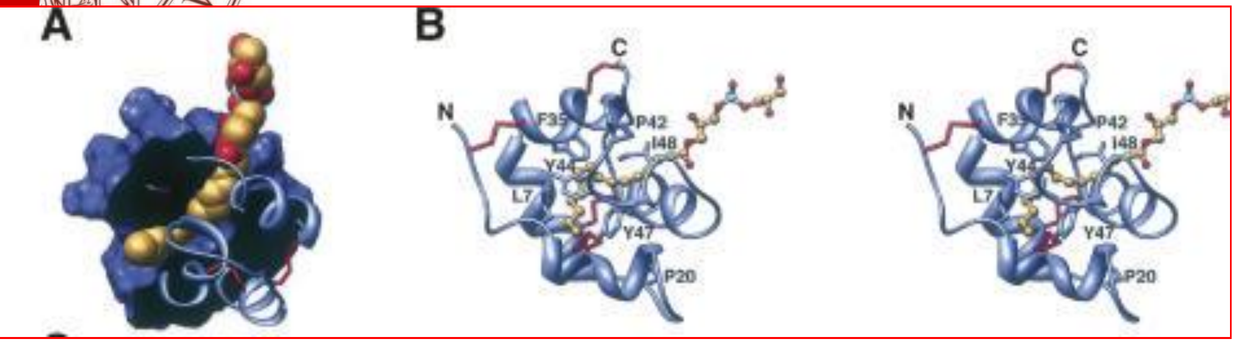
Protein sequence coverage: 92%

1 **ACQASQLAVC** **ASAILSGAKP** **SGECCGNLRA** **QQGCFCQYAK** **DPTYGQYIR**S

51 PHAR**DTLTSC** **GLAVPHC**

→ **NsLtP type 2**

NEW WHEAT PEPTIDE IDENTIFICATION



A) Cutaway image showing wheat LTP2 bound to L-α-palmitoyl-phosphatidyl glycerol .

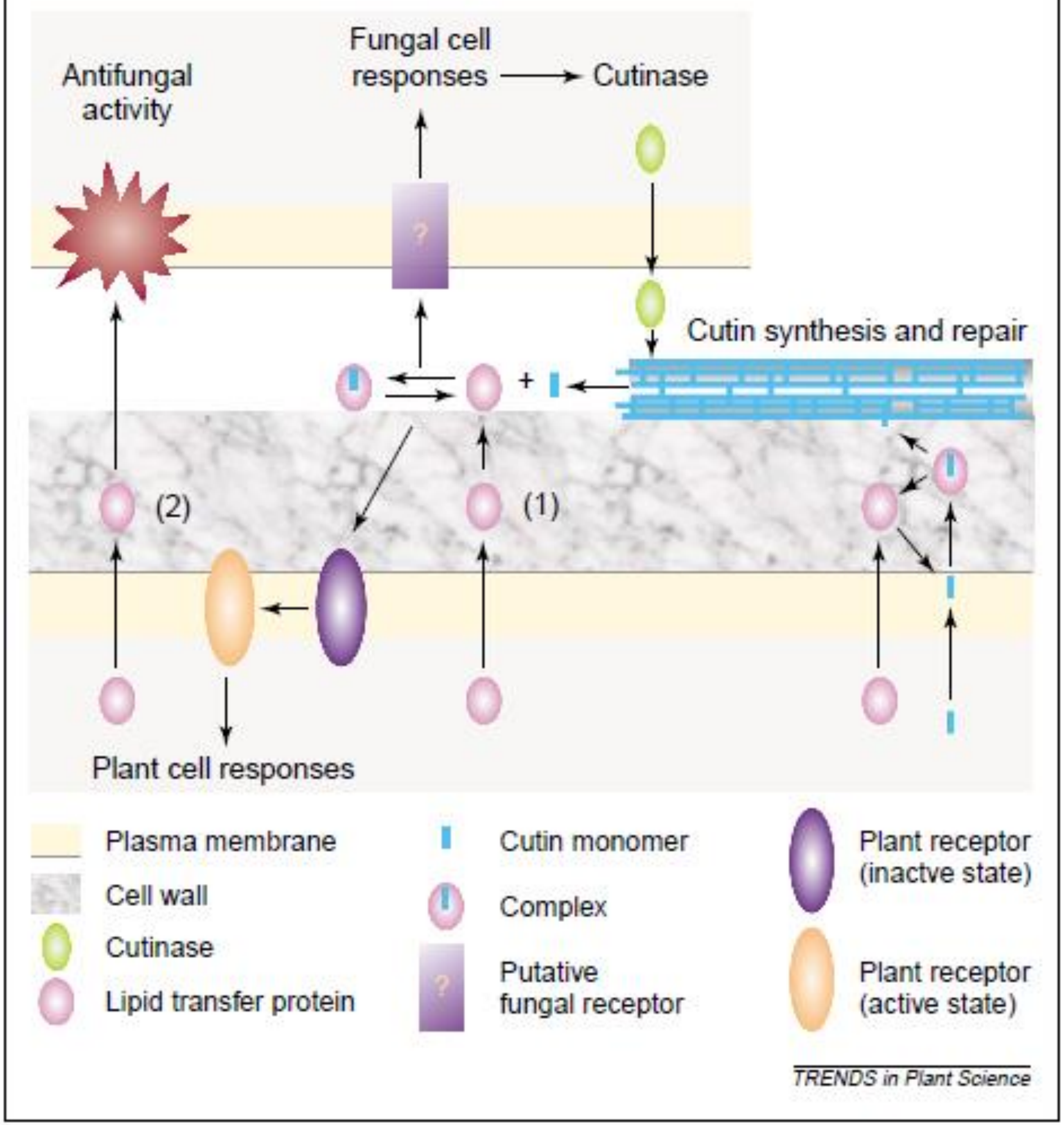
(B) Stereoview of the same model highlighting the four helix structure of family 2 LTPs with disulfide bridges in red.

✓ The peptide appears to belong to the family of lipid-transfer protein 2 (LTP).

✓ LTP fold is characterized by a compact domain composed of 4 α-helices bound by four disulfide bonds enclosing a hydrophobic lipid binding cavity.

✓A number of biological roles (antimicrobial defense, signaling, assembly of cutin) have been proposed, but conclusive evidence is generally lacking [Yeats TH, Rose JK. *Protein Sci*, 2008].

✓ Although both LTP1 and LTP2 proteins can be found in plant seeds, thus far, <u>only LTP1 proteins have been characterized as allergens</u>. In fact, the official allergen list of the International Union of Immunological Societies currently contains no entries for allergens belonging to the LTP2 subfamily [Egger M *et al*., *Curr Allergy Asthma Rep*, 2010]



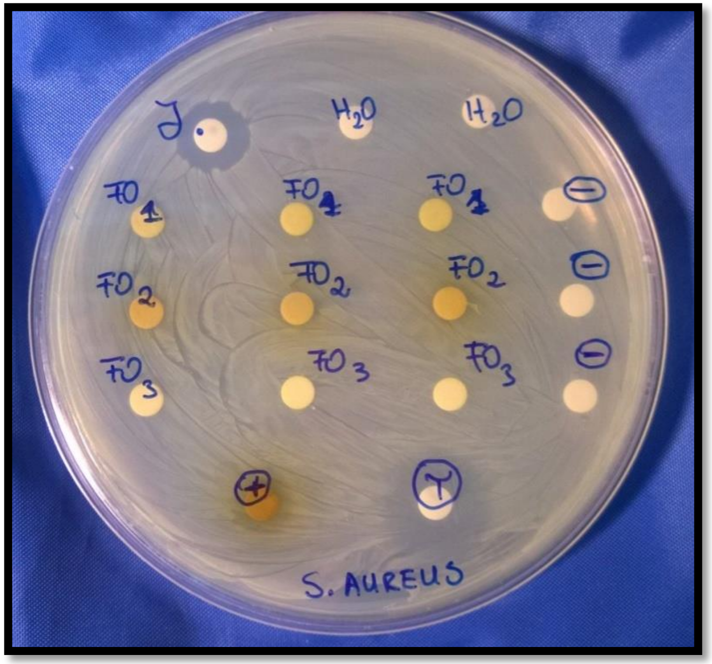
NEW WHEAT PEPTIDE IDENTIFICATION

<u>nsLTP is involved in plant defense mechanism aginst abiotic (ie salinity) and biotic stresses (bacteria, fungi)</u>

<u>Double action: direct (ie antifugal activity) and indirect (ie «SHIELD» effect)</u>

NEW WHEAT PEPTIDE IDENTIFICATION

Since the mentioned **NsLtP type 2** is reported to have antibacterial activity, a specific test carried out with *S. aureus* confirmed the chemical identification.



J = new peptide
T = Tetracycline

NEW WHEAT PEPTIDE IDENTIFICATION

TRENDS IN MICROBIOLOGY 73 VOL. 3 NO. 2 FEBRUARY 1995

The defensive role of nonspecific lipid-transfer proteins in plants

Francisco García-Olmedo, Antonio Molina,
Ana Segura and Manuel Moreno

Questions

- What function(s), other than their defensive role, do nonspecific lipid-transfer proteins (nsLTPs) have in plants?
- What mechanism(s) are responsible for the toxicity of nsLTPs to pathogens?
- What mechanisms are responsible for possible microbial resistance to nsLTPs?
- Are nsLTPs essential for plants?
- Are nsLTPs a lead for drug development?

WP 3 : Extending eBASIS to study habitual intakes of bioactive compounds in the diet

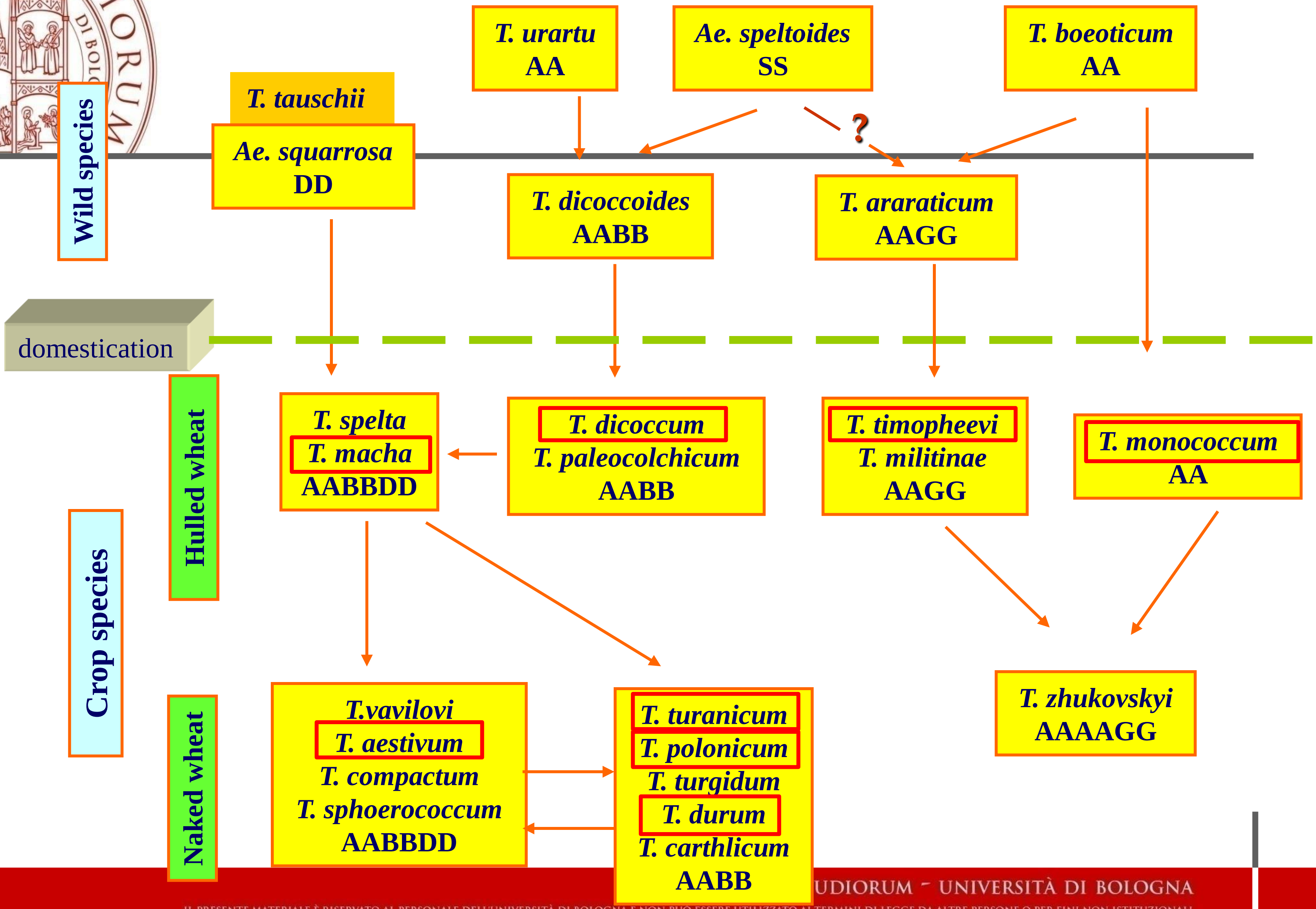
Task 3.1.4.2: nsLPT2 composition of European wheats

A germplasm collection of **60 accessions** of wheat genotypes held at UNIBO, and representative of the main cultivars cultivated in Europe, have been screened for their nsLTP2 content in whole grain.

- **11 durum wheat;**
- **2 *Triticum turgidum* ssp. turanicum,**
- **38 *Triticum aestivum*;**
- **1 *Triticum polonicum*;**
- **1 *Triticum macha*,**
- **1 *Triticum timophevi*,**
- **3 *Triticum monococcum*,**
- **3 *Triticum dicoccum.***

The results produced have been added to the eBASIS database, using a quality assessment system identical to that used for peer reviewed publications.

WHEAT GENEALOGY



ALL HEXAPLOID SPECIES mean 0.208 mg/g *(0.095 ÷ 0.469)*

Species	Variety	nsLTP 2 (mg/g grain)
Tr. aestivum	**Abbondanza**	0.172 ± 0.021
Tr. aestivum	**Amarok**	0.107 ± 0.006
Tr. aestivum	**Andriolo**	0.219 ± 0.017
Tr. aestivum	**Argelato**	0.095 ± 0.008
Tr. aestivum	**Aubusson**	0.118 ± 0.009
Tr. aestivum	**Autonomia A**	0.224 ± 0.034
Tr. aestivum	**Autonomia B**	0.201 ± 0.025
Tr. aestivum	**Benco**	0.232 ± 0.017
Tr. aestivum	**Bianco nostrale**	0.262 ± 0.041
Tr. aestivum	**Bilancia**	0.121 ± 0.012
Tr. aestivum	**Blasco**	0.098 ± 0.002
Tr. aestivum	**Bolero**	0.103 ± 0.005
Tr. aestivum	**Bologna**	0.114 ± 0.010
Tr. aestivum	**Bramante**	0.109 ± 0.007
Tr. aestivum	**Canove**	0.274 ± 0.028
Tr. aestivum	**Carosello**	0.399 ± 0.051
Tr. aestivum	**Fiorello**	0.214 ± 0.022
Tr. aestivum	**Frassineto**	0.175 ± 0.012
Tr. aestivum	**Funo**	0.205 ± 0.016
Tr. aestivum	**Gamba di Ferro**	0.396 ± 0.037
Tr. aestivum	**Gentil Bianco**	0.301 ± 0.022
Tr. aestivum	**Gentil Rosso**	0.205 ± 0.017
Tr. aestivum	**Guà 113**	0.305 ± 0.033
Tr. aestivum	**Inallettabile**	0.224 ± 0.021
Tr. aestivum	**Marzuolo d'Aqui**	0.194 ± 0.011
Tr. aestivum	**Marzuolo Val Pusteria**	0.149 ± 0.010
Tr. aestivum	**Mentana**	0.169 ± 0.013
Tr. aestivum	**Mieti**	0.106 ± 0.008
Tr. aestivum	**Nobel**	0.101 ± 0.006
Tr. aestivum	**Palesio**	0.112 ± 0.011
Tr. aestivum	**Piave**	0.255 ± 0.023
Tr. aestivum	**Rieti**	0.243 ± 0.018
Tr. aestivum	**Serio**	0.119 ± 0.009
Tr. aestivum	**Sieve**	0.469 ± 0.048
Tr. aestivum	**Solina**	0.335 ± 0.026
Tr. aestivum	**Terricchio**	0.170 ± 0.022
Tr. aestivum	**Verna**	0.162 ± 0.016
Tr. aestivum	**Villa Gloria**	0.428 ± 0.037
Tr. macha		0.242 ± 0.023

The present study highlighted high variability among the investigated ***Triticum aestivum*** genotypes (**AABBDD**); in fact the observed values ranged between 0.095 (cv. Argelato) and 0.469 mg/g whole flour (cv. Sieve).

Main source of variation: **plant *habitus*** and the **breeding year**

In the 11 post "green revolution" dwarf genotypes, the nsLTP-2 content was approximately halve than that found in the 27 not dwarf pre "green revolution" varieties (0.110 $mg.g^{-1} \pm 0.008$ whole flour *vs* 0.247 $mg.g^{-1} \pm 0.091$ whole flour respectively).

DIPLOID SPECIES mean 0.318 mg/g *(0.311 ÷ 0.325)*

Species	Variety	nsLTP 2 (mg/g grain)
Tr. monococcum	white-type	0.319 ± 0.018
Tr. monococcum	black-type	0.325 ± 0.021
Tr. monococcum	white-type	0.311 ± 0.016

Among the eight different species analyzed, the diploid species ***Triticum monococcum*** (**AA genome**) was characterized by the highest mean value content of nsLTP2 (0.318 $mg.g^{-1} \pm 0.007$ whole flour), with concentration range between 0.311 $mg.g^{-1}$ and 0.325 $mg.g^{-1}$ whole flour.

ALL TETRAPLOID SPECIES mean 0.181 mg/g *(0.059 ÷ 0.312)*

Species	Variety	nsLTP 2 (mg/g grain)
Tr. turgidum ssp. turanicum	Etrusco	0.297 ± 0.035
Tr. turgidum ssp. turanicum	KAMUT® khorasan wheat	0.304 ± 0.053
Tr. turgidum ssp. durum	Alemanno	0.220 ± 0.018
Tr. turgidum ssp. durum	Anco Marzio	0.171 ± 0.075
Tr. turgidum ssp. durum	Ciccio	0.224 ± 0.027
Tr. turgidum ssp. durum	Claudio	0.187 ± 0.062
Tr. turgidum ssp. durum	Iride	0.167 ± 0.027
Tr. turgidum ssp. durum	Levante	0.162 ± 0.023
Tr. turgidum ssp. durum	Orobel	0.180 ± 0.043
Tr. turgidum ssp. durum	Senatore Cappelli	0.205 ± 0.032
Tr. turgidum ssp. durum	Solex	0.175 ± 0.045
Tr. turgidum ssp. durum	Svevo	0.170 ± 0.051
Tr. turgidum ssp. durum	Urria 12	0.198 ± 0.017
Tr. turgidum ssp. dicoccum	white-type	0.059 ± 0.006
Tr. turgidum ssp. dicoccum	white-type	0.067 ± 0.003
Tr. turgidum ssp. dicoccum	white-type	0.063 ± 0.004
Tr. turgidum ssp. *polonicum*		0.312 ± 0.049
Tr. timophevi		0.098 ± 0.011

mean 0.300 mg/g

mean 0.187 mg/g

mean 0.063 mg/g

The lowest nsLTP-2 concentration was observed in the three **Emmer genotypes** (concentration range between 0.059 $mg.g^{-1}$ and 0.063 $mg.g^{-1}$ whole flour) and in the ***Triticum timophevi*** genotype (0.098 $mg.g^{-1}$ ± 0.011 $mg.g^{-1}$).

The ***Tr. turgidum* ssp. durum** showed intermediate mean value content with a limited variability among the eleven investigated varieties (0.162 $mg.g^{-1}$ and 0.224 $mg.g^{-1}$ whole flour in the varieties Levante and Ciccio, respectively).

The highest concentration of the peptide was observed for ***Triticum turanicum*** and ***Triticum polonicum*** with a concentration ranging between 0.297 $mg.g^{-1}$ and 0.312 $mg.g^{-1}$ whole flour.

WHEAT GENEALOGY