



ACRYRED



FOUR YEARS OF ACRYRED WG2 “AGRONOMY AND PLANT BREEDING”: PROGRESS ON KNOWLEDGE ABOUT FREE ASPARAGINE ACCUMULATION IN CEREALS

Elena Baldoni

ACRYRED Conference

Palermo, 4th June 2026

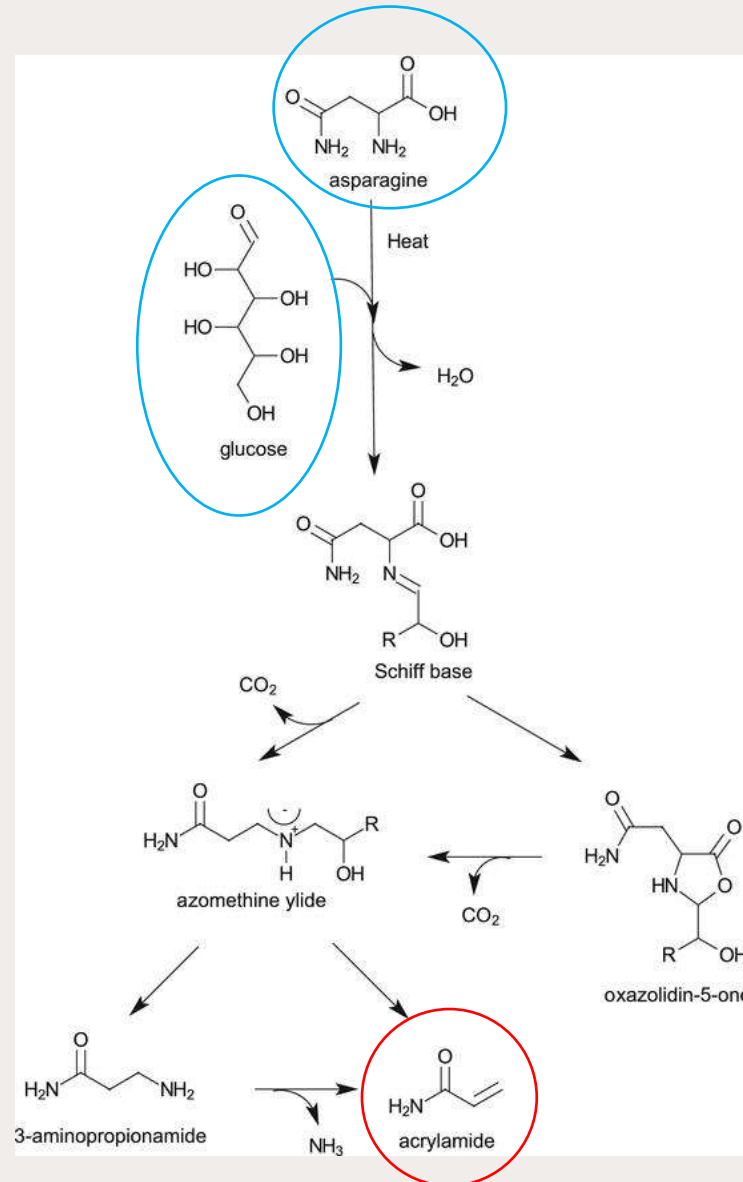
ACRYRED • COST Action CA21149 • Reducing Acrylamide exposure of consumers by a Cereals supply-chain approach targeting Asparagine

This article/publication is based upon work from COST Action ACRYRED, CA21149, supported by COST. COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.



Acrylamide formation

Maillard reaction cascade



Temperature
> 120°C





In cereal-based food, the quantity of **free asparagine** has been identified as a key factor in acrylamide formation.

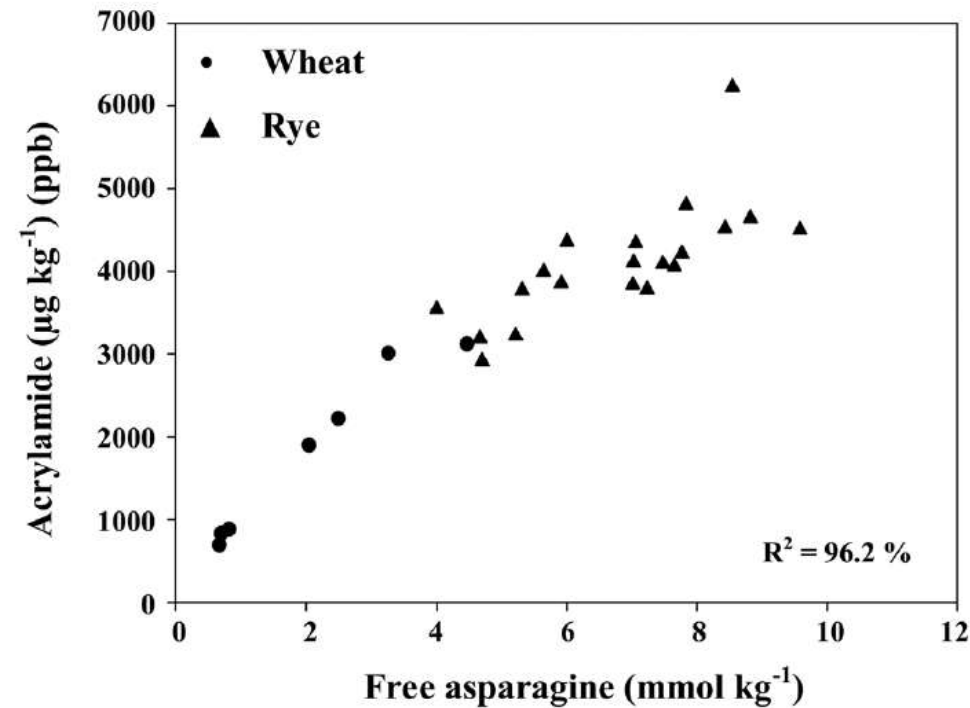


FIGURE 8 Relationship between free asparagine concentration and acrylamide formation in wheat and rye flour heated for 20 min at 180°C. Reproduced with modification from Curtis, Postles, and Halford (2014)



From: Raffan and Halford 2019



In cereal-based food, the quantity of **free asparagine** has been identified as a key factor in acrylamide formation.



Food industry recommends the use of grain with low free asparagine content to reduce acrylamide levels in cereal-based products.

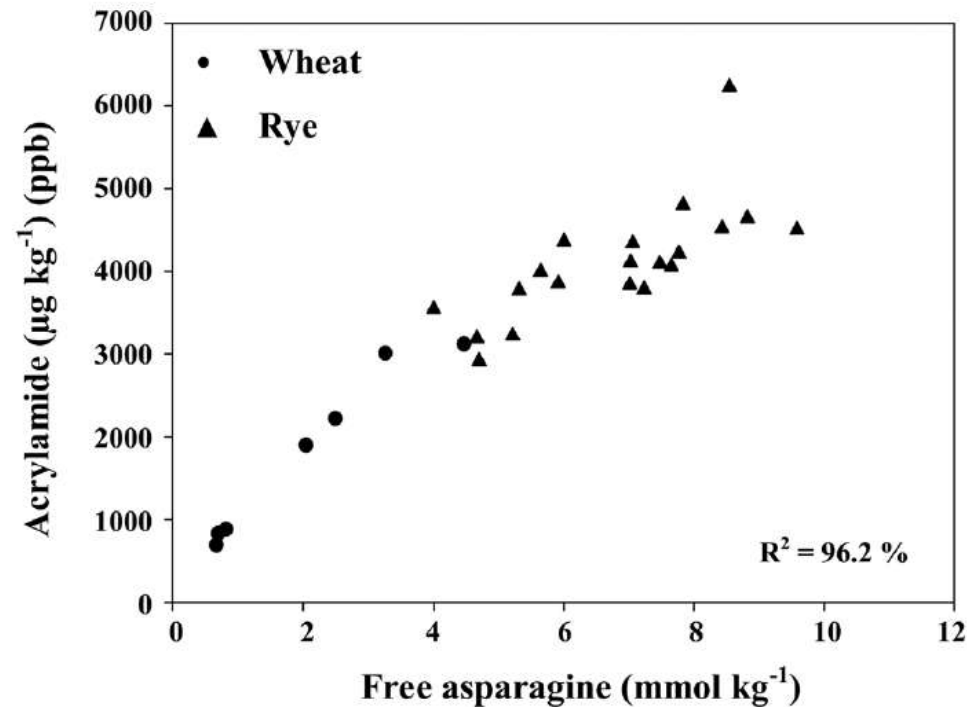


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WG2

Agronomy and Plant Breeding

Objectives

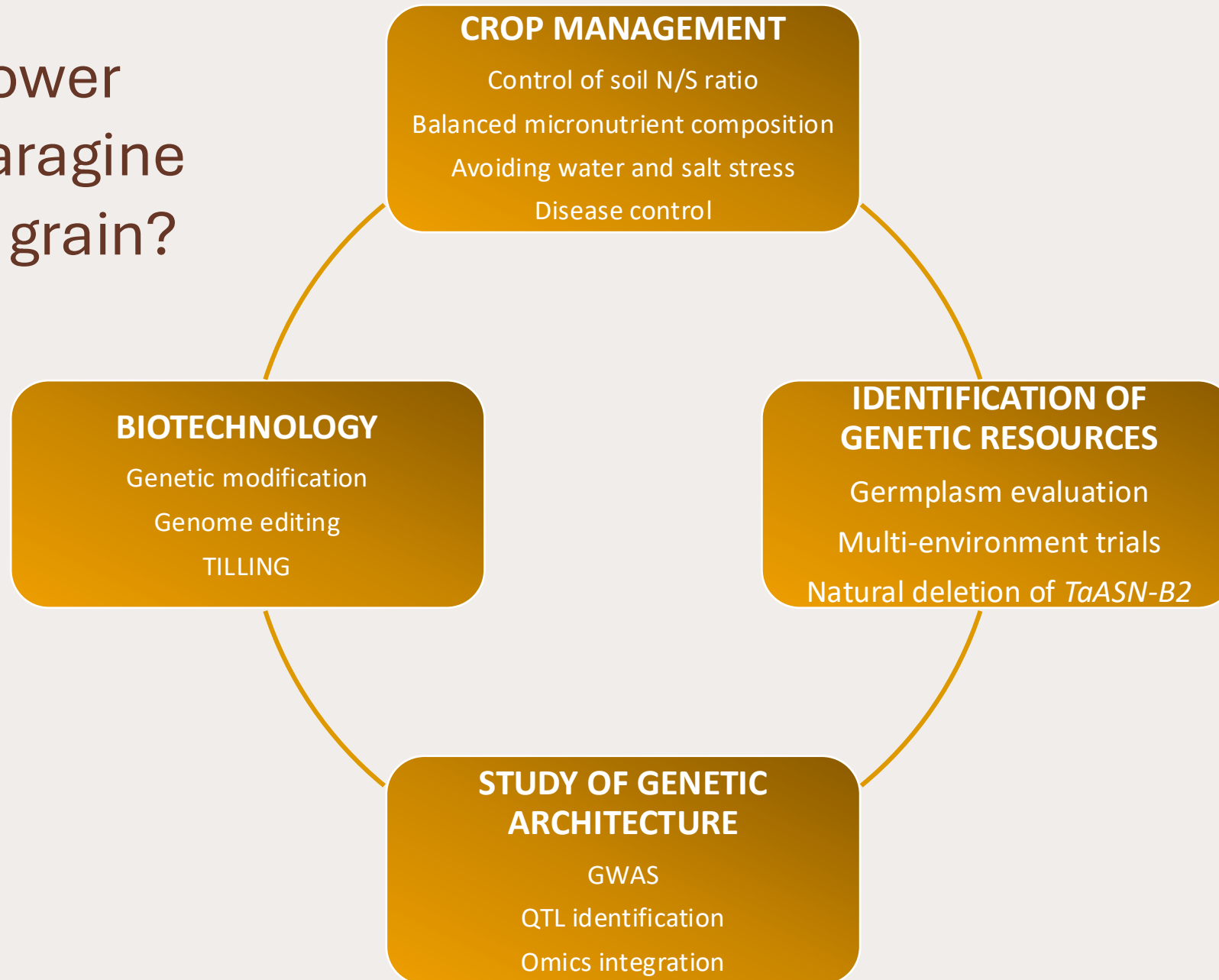
To define research efforts across Europe to 1) understand the effects of farming practices, including conventional – organic farming, on asparagine formation in grain, based on an overview of recent and/or planned field research with wheat, oats, maize, barley, and rye, 2) identify pathways for the reduction of free asparagine levels in cereal cultivars through plant breeding, and 3) include asparagine levels in standard information on cultivars.

Tasks

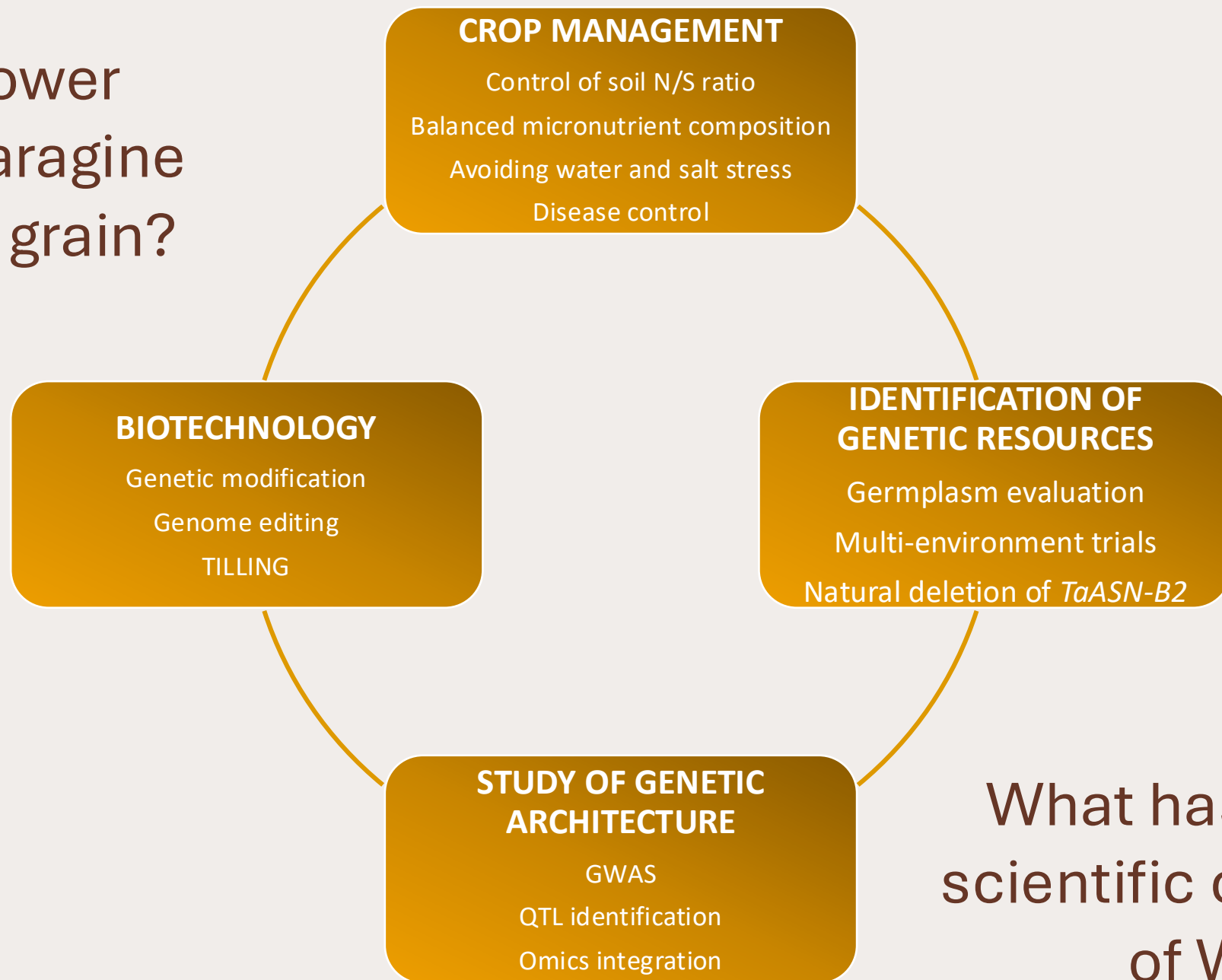
- 🕒 To make a detailed overview of the relevant research in agronomy and plant breeding.
- 🕒 To draft a strategic research agenda for the field.
- 🕒 To develop content for the ACRYRED training school on agronomy and plant breeding, and to identify candidates for participation in this training school.

Working Group 2 - Lowering asparagine levels through better breeding activities and farming practices, for reducing downstream acrylamide formation in cereals-based products.

How to lower free asparagine in cereal grain?

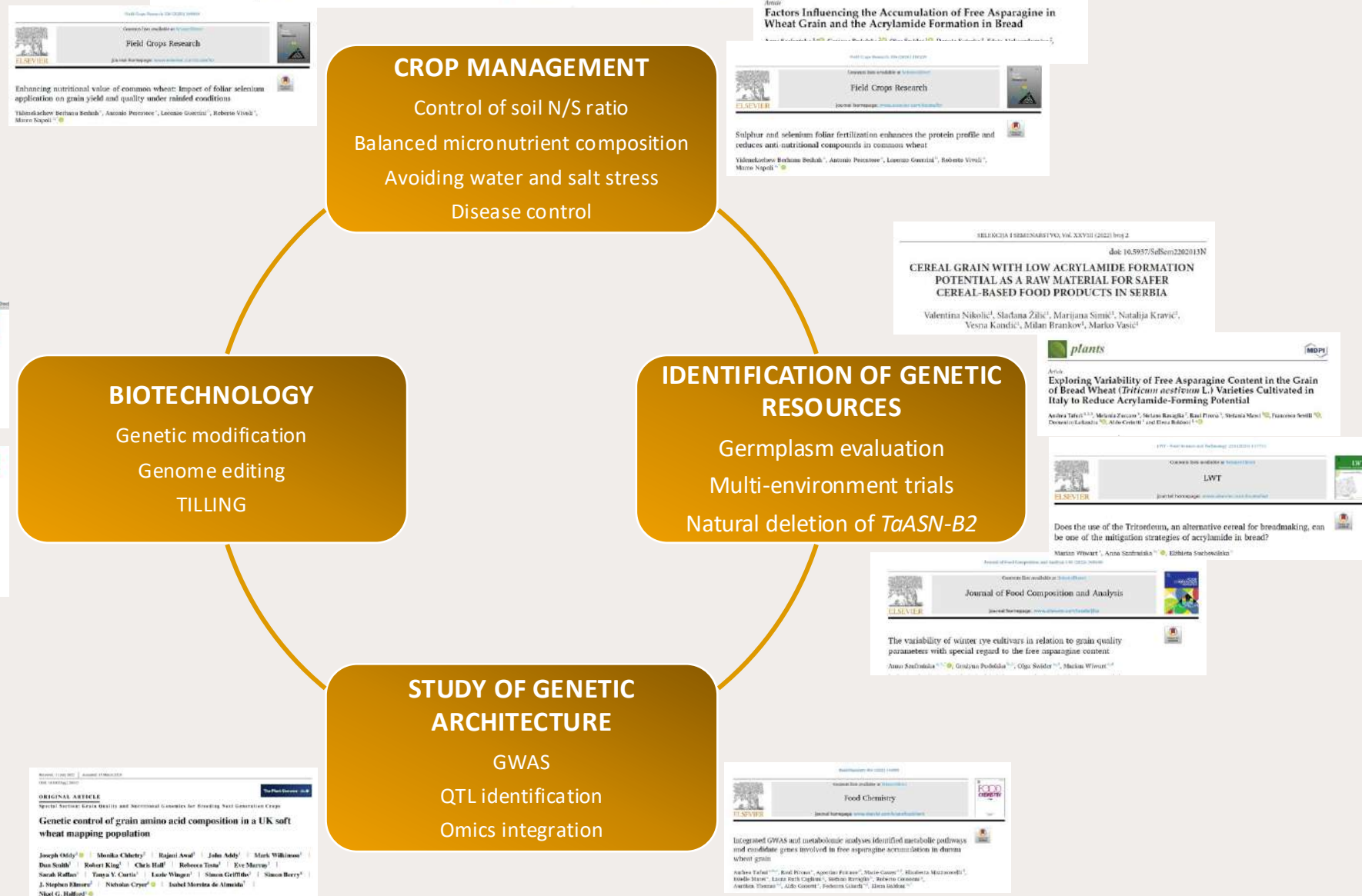


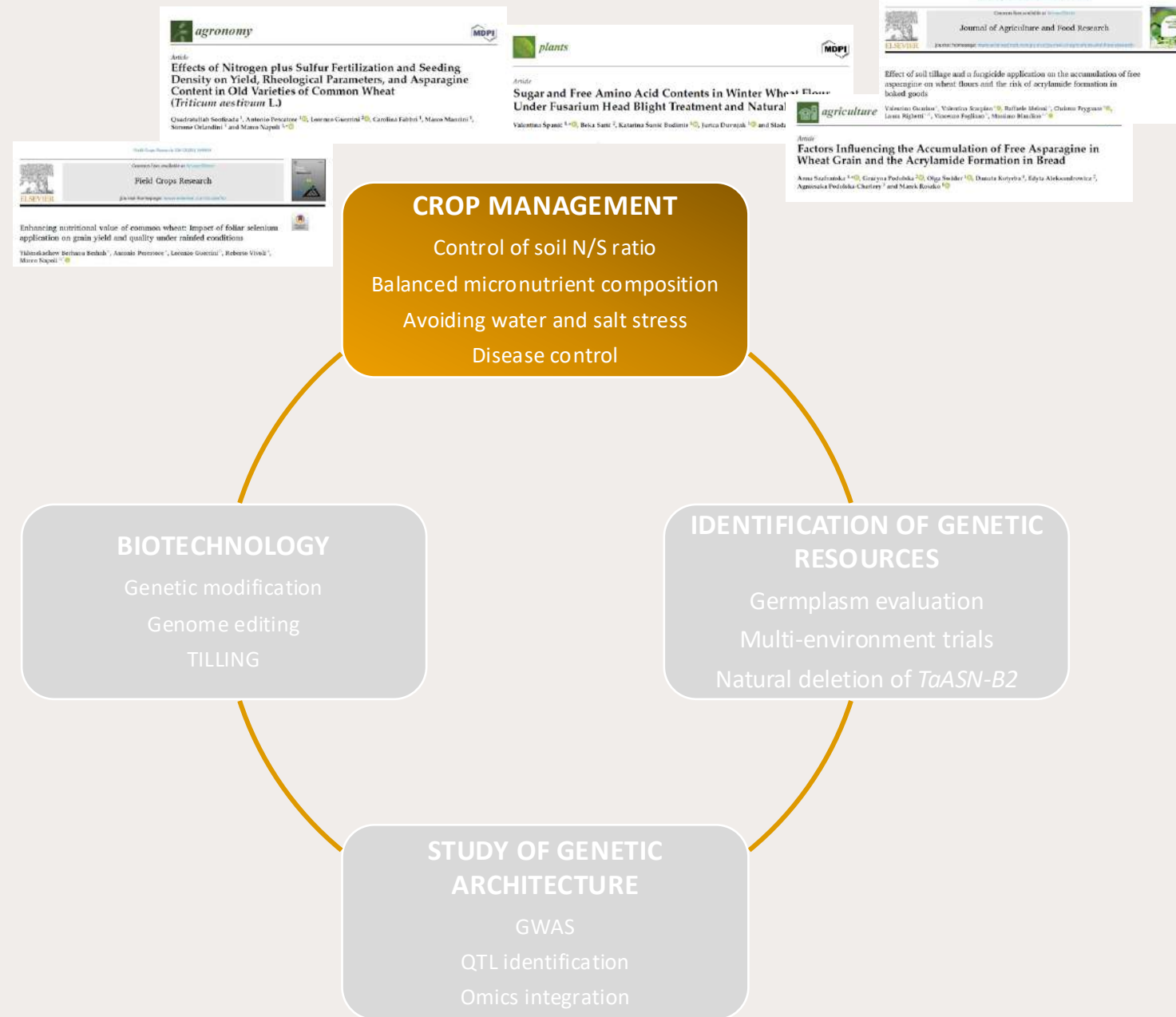
How to lower free asparagine in cereal grain?



What has been the
scientific contribution
of WG2?

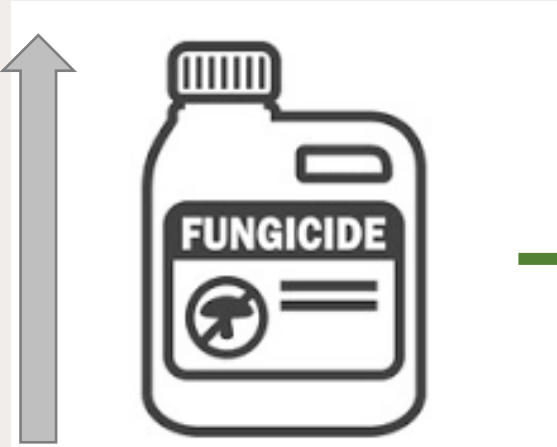
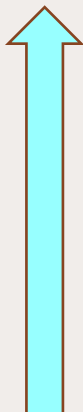
2022-2026







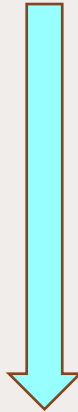
High levels of nitrogen supply



Fungicide application



Sulphur deficiency



Level of precipitations and
temperatures during grain development



changes of free
asparagine levels



agronomy



Article

Effects of Nitrogen plus Sulfur Fertilization and Seeding Density on Yield, Rheological Parameters, and Asparagine Content in Old Varieties of Common Wheat (*Triticum aestivum* L.)

Quadratullah Soofizada ¹, Antonio Pescatore ¹ , Lorenzo Guerrini ² , Carolina Fabbri ¹, Marco Mancini ¹, Simone Orlandini ¹ and Marco Napoli ^{1,*} 

Soofizada et al. 2022. <https://doi.org/10.3390/agronomy12020351>

14 old Italian bread **wheat** varieties

Evaluated Parameters:

- two seeding density levels
 - three nitrogen levels (NL)
 - two sulphur levels (SL)
- effect on
- grain yield
 - rheological characteristics
 - **free asparagine content**



agronomy



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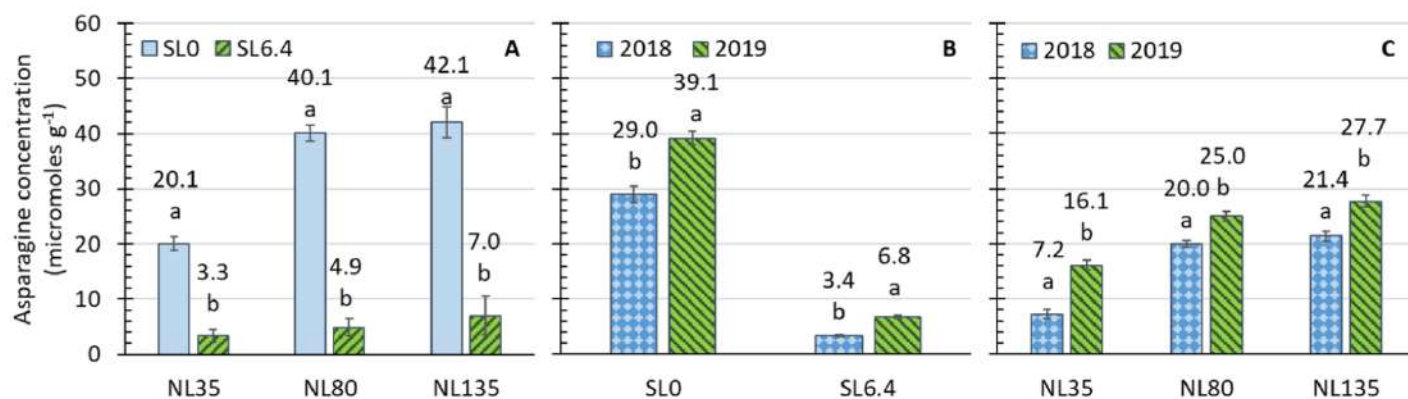


Figure 5. Interactions between years and agronomic treatments on asparagine content in grain; (A) Effect of nitrogen fertilization level (NL) and sulfur fertilization level (SL); (B) effect of year (Y) and sulfur fertilization level (SL); (C) effect of the year (Y) and nitrogen fertilization (NL). Lowercase letters represent the Tukey HSD post hoc test results.

- increased by 111% as NL increased;
- decreased by 85.1% with the SL treatment.



agronomy



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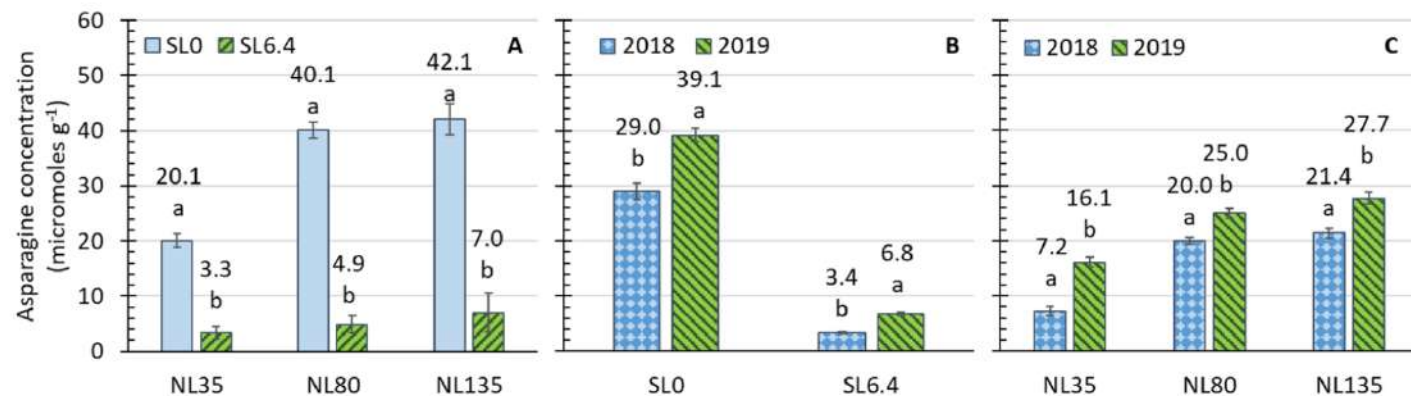


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- increased by 111% as NL increased;
- decreased by 85.1% with the SL treatment.

“135 kg N ha⁻¹ combined with 6.4 kg S ha⁻¹ can improve the technical performance of ‘old’ wheat wholegrain flours while maintaining free asparagine as low as possible.”



agriculture



Article

Factors Influencing the Accumulation of Free Asparagine in Wheat Grain and the Acrylamide Formation in Bread

Anna Szafrńska ^{1,*}, Grażyna Podolska ², Olga Świder ¹, Danuta Kotyrba ¹, Edyta Aleksandrowicz ², Agnieszka Podolska-Charlery ³ and Marek Roszko ¹

Szafranska et al. 2024. <https://doi.org/10.3390/agriculture14020207>

5 bread **wheat** varieties

Evaluated Parameters:

- genotype
- location
- two levels of sulphur fertilization

effect on

- technological properties
- **free asparagine content**
- **acrylamide level in bread**





agriculture



Article

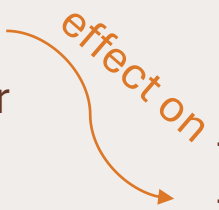
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- technological properties
- **free asparagine content**
- **acrylamide level in bread**

Parameter	Sources of Variation						
	Localization (A)	S Fertilization (B)	Cultivar (C)	A × B	A × C	B × C	A × B × C
Test weight	205.30 **	0.22	28.85 **	2.33	12.03 **	0.85	2.82
Protein content	2695.88 **	1.02	78.34 **	2.61	162.79**	3.09 *	6.92 **
Gluten content	2160.08 **	12.51 **	92.71 **	2.52	84.61 **	0.60	1.56
Zeleny index	84.70 **	0.42	9.97 **	0.58	14.52 **	0.91	0.74
Ash content	15.80 **	0.00	1.54	0.10	1.71	0.20	0.42
Asparagine	3418.61 **	1.50	43.33 **	27.35 **	17.04 **	2.82	4.88 **

Significant at: * $p < 0.05$, ** $p < 0.01$.

The **localization** had the biggest influence on the total variance in the measured technological parameters of wheat grain, followed by **cultivar**, and then by the **interaction between the localization x cultivar (localization x sulphur for free asparagine)**.



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Article

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Parameter	Sources of Variation						
	Localization (A)	S Fertilization (B)	Cultivar (C)	A × B	A × C	B × C	A × B × C
Loaf volume	12.22 **	18.03 **	68.75 **	1.47	20.02 **	1.74	1.51
Bread crumb hardness	119.35 **	25.87 **	4.33 **	3.08	6.25 **	29.19 **	37.75 **
Acrylamide content	1518.09 **	359.17 **	92.03 **	9.21 **	8.15 *	65.03 *	37.92 *
Lcrumb	404.92 **	0.61	85.12 **	1.59	11.63 **	0.84	0.81
a*crumb	3059.32 **	12.69 **	62.99 **	0.61	25.49 **	4.68 **	12.79 **
b*crumb	248.16 **	4.36 *	187.01 **	0.00	51.10 **	4.91 **	12.92 **
Lcrust	36.33 **	24.89 **	2.08	0.41	13.96 **	2.03	1.17
a*crust	0.60	2.85	4.03 *	6.67 *	9.71 **	1.43	0.40
b*crust	39.13 **	33.20 **	3.48 *	0.03	7.25 **	1.96	2.14

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5 bread **wheat** varieties

Evaluated Parameters:

- genotype
- location
- two levels of sulphur fertilization



effect on

- technological properties
- **free asparagine content**
- **acrylamide level in bread**



The **localization** had the greatest share in the total variance of the measured acrylamide content in bread, followed by **S Fertilization** and then by **cultivar**.





agriculture



Article

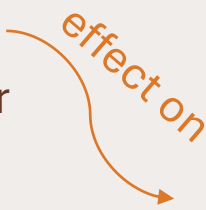
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“Asparagine content in wheat and acrylamide in bread varies mostly depending on cultivar and environment.”

The **localization** had the greatest share in the total variance of the measured acrylamide content in bread, followed by **S Fertilization** and then by **cultivar**.



plants



Article

Sugar and Free Amino Acid Contents in Winter Wheat Flour Under Fusarium Head Blight Treatment and Natural Infection

Valentina Španić ^{1,*}, Beka Sarić ², Katarina Šunić Budimir ¹, Jurica Duvnjak ¹ and Slađana Žilić ²Spanic et al. 2025. <https://doi.org/10.3390/plants14101504>

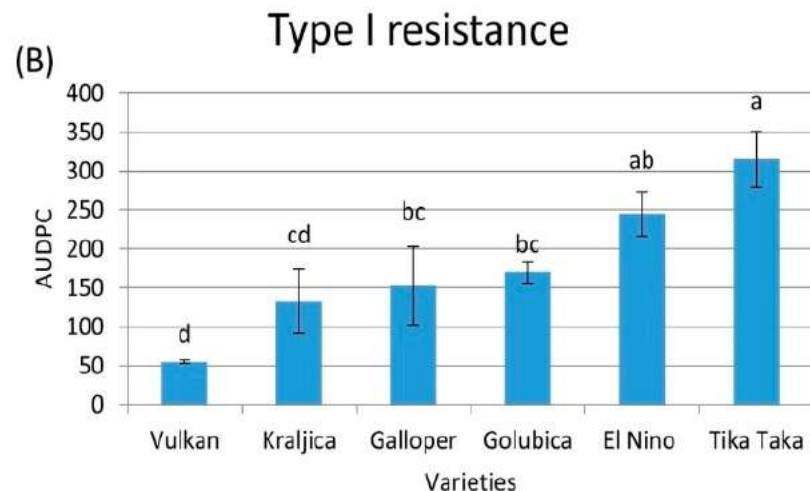
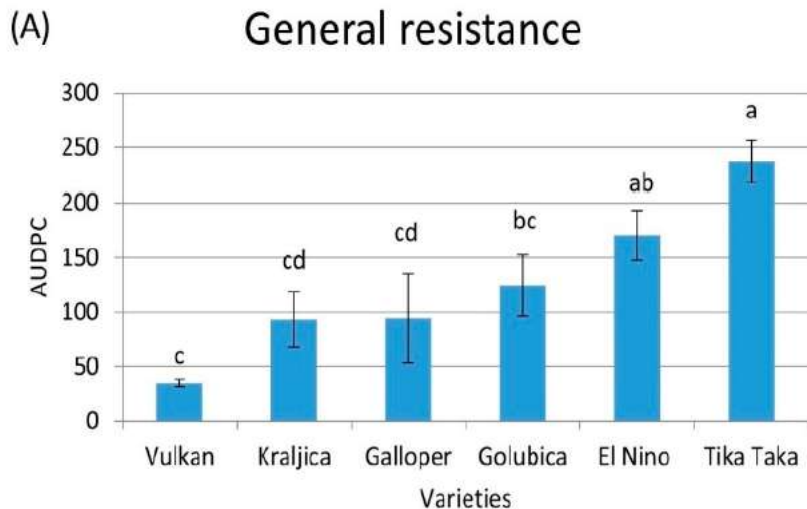
6 bread **wheat** varieties, differing in Fusarium head blight (FHB) resistance.

Evaluated material:

- flour from *Fusarium* infected grains
- flour from *Fusarium* non-infected grains

effect on

- sugar content
- **free amino acid content**



AUDPC: area under disease progress curve



plants



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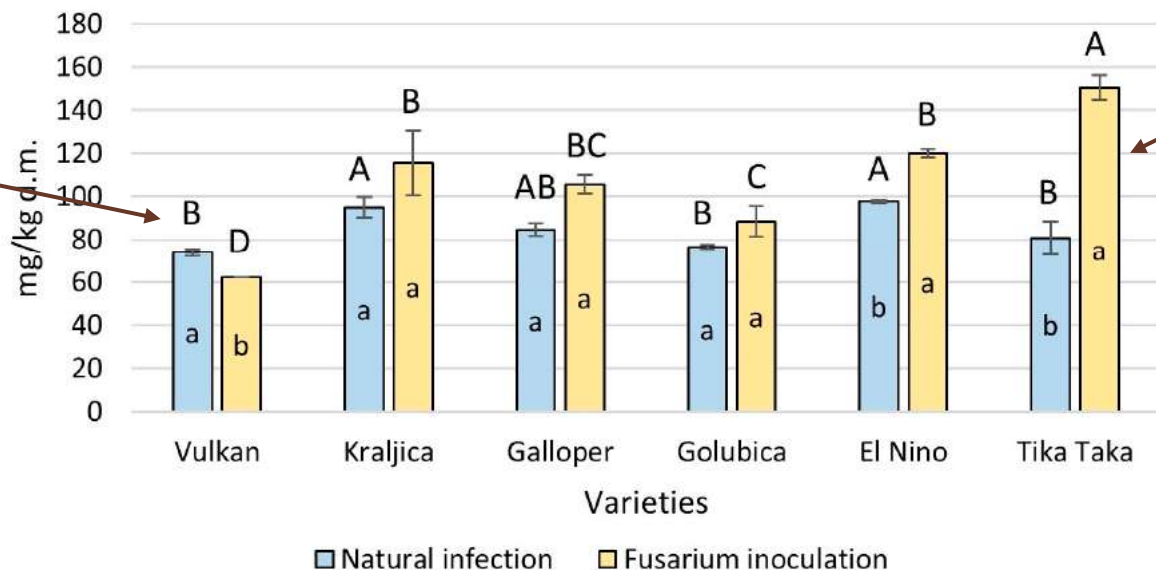
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effect on

- sugar content
- **free amino acid content**

Total amino acid content



-16%

+46%

The total content of free amino acids in flour from FHB-infected varieties increased **in proportion to their susceptibility**.



plants



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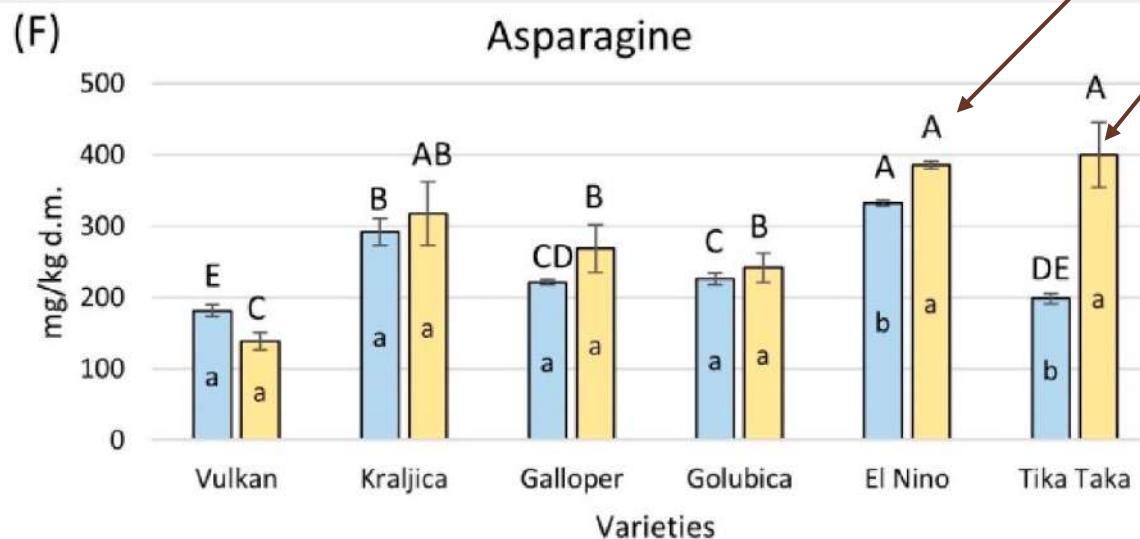
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effect on

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The most FHB susceptible varieties accumulated the highest level of free asparagine.



plants



Article

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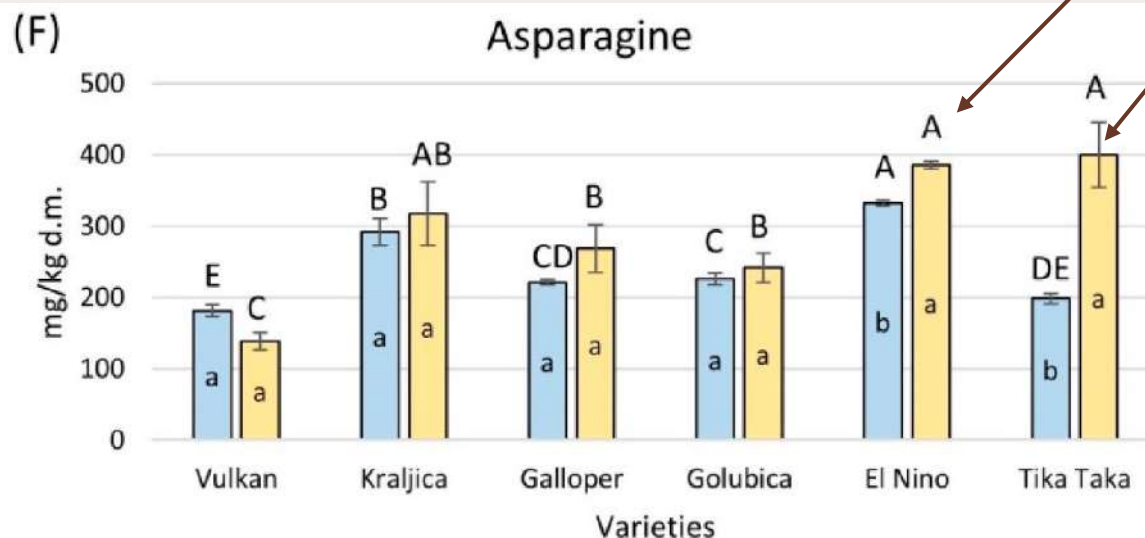
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Evaluated material:

- flour from *Fusarium* infected grains
- flour from *Fusarium* non-infected grains

- effect on
- sugar content
 - **free amino acid content**



+51% The most FHB susceptible varieties accumulated the highest level of free asparagine.

"Winter wheat breeders may have an opportunity to reduce free asparagine concentration in the grain by identifying and selecting genotypes carrying FHB resistance."

2 bread **wheat** varieties (Bologna, modern variety, and Sieve, old variety)

Foliar **Se** application

effect on

- grain yield
- quality:
 - protein content
 - starch concentration
 - **free asparagine levels**
 - phytic acid content



Beshah et al. 2025. <https://doi.org/10.1016/j.fcr.2025.109969>



Beshah et al. 2026. <https://doi.org/10.1016/j.fcr.2025.110229>

Foliar **S** and **Se** application

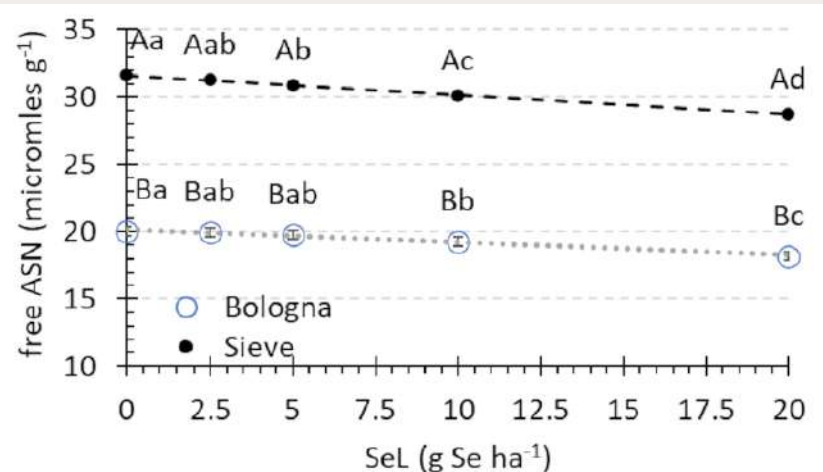
effect on

- grain yield
- protein composition
- mineral bioavailability
- dough properties



Enhancing nutritional value of common wheat: Impact of foliar selenium application on grain yield and quality under rainfed conditions

Yidenekachew Berhanu Beshah¹, Antonio Pescatore², Lorenzo Guerrini³, Roberto Vircoli⁴, Marco Napoli^{1,2}



Grain yield was not significantly affected by either Se or S treatments

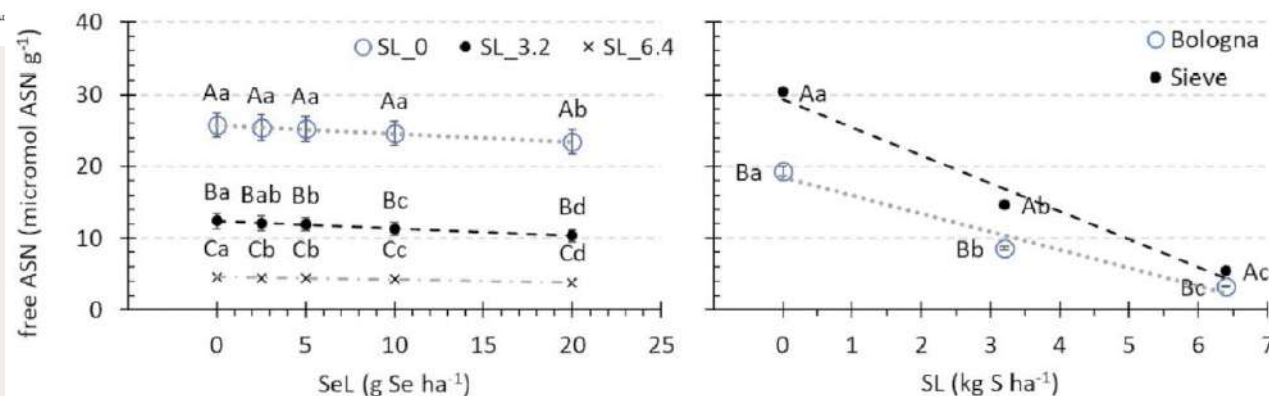
S treatment mostly affected free asparagine (82.5 % variation).

Se further reduced free asparagine, particularly under adequate S conditions.



Sulphur and selenium foliar fertilization enhances the protein profile and reduces anti-nutritional com

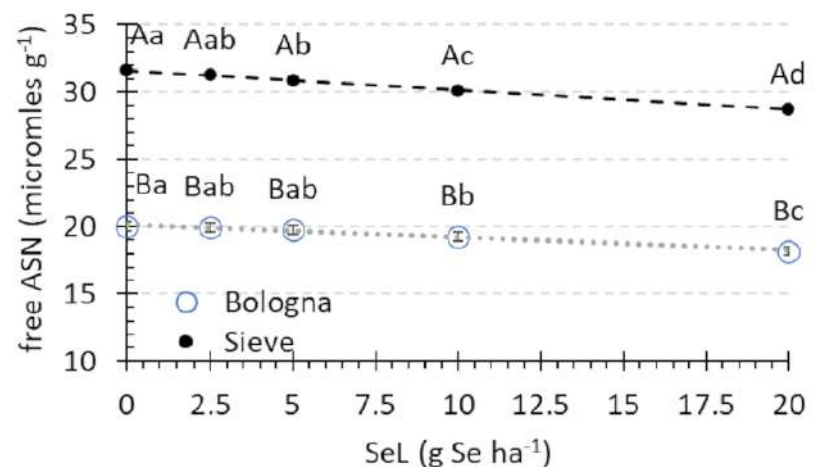
Yidenekachew Berhanu Beshah¹, At Marco Napoli^{1,2}





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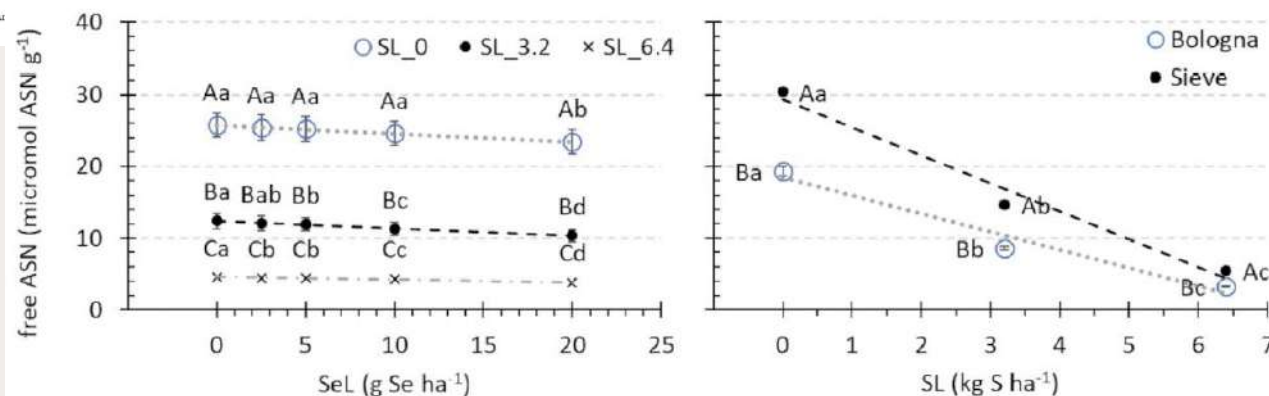
Se further reduced free asparagine, particularly under adequate S conditions.

“These findings support the adoption of targeted foliar fertilization strategies combining Se and S to improve wheat quality.”



Sulphur and selenium foliar fertilization enhances the protein profile and reduces anti-nutritional com

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Journal of Agriculture and Food Research 28 (2026) 102916

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Valentina Guarino^a, Valentina Scarpino^a, Raffaele Meloni^a, Christos Fryganas^b,
Laura Righetti^{c,d}, Vincenzo Fogliano^b, Massimo Blandino^{a,*}

Guarino et al. 2026. <https://doi.org/10.1016/j.jafr.2026.102916>

Evaluated agronomic conditions: combined effect of soil tillage + fungicide application

- MT, minimum tillage,
- CT, conventional tillage,
- NF, untreated control,
- F, fungicide application at flowering

effect on

- **Free asparagine** in wholegrain and refined flour
- **Acrylamide levels** in biscuits
- deoxynivalenol (DON) contamination in wholemeal flours and biscuits



Journal of Agriculture and Food Research 26 (2026) 102916

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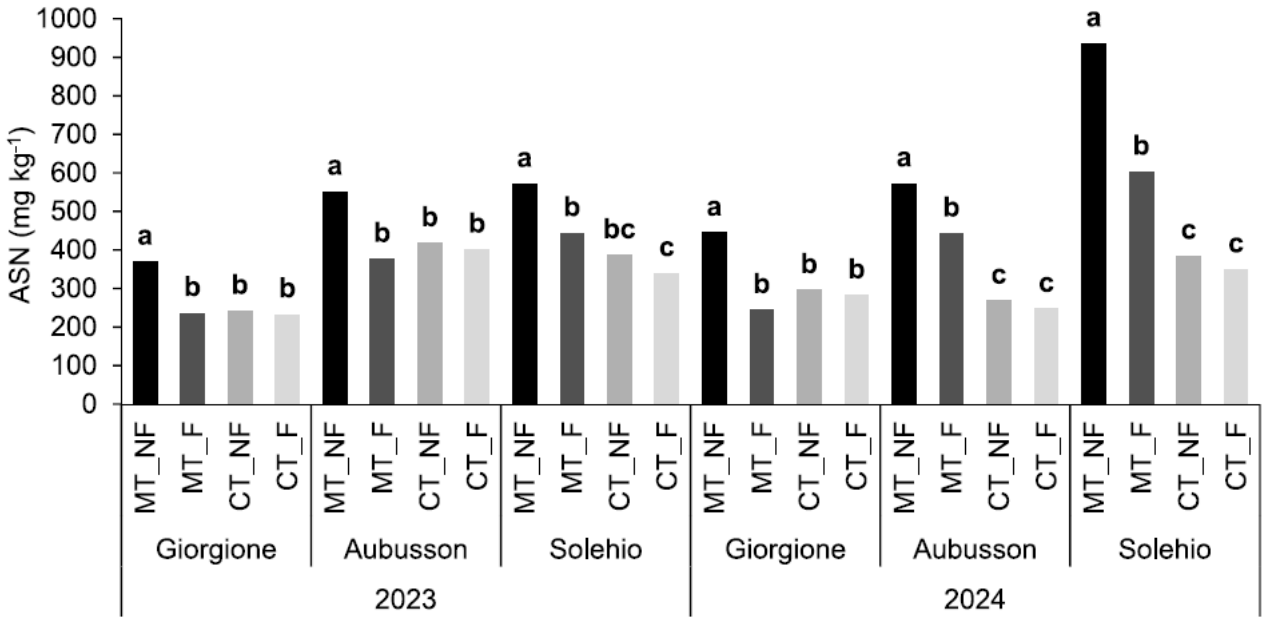
Journal of Agriculture and Food Research

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Laura Righetti^a, Vincenzo Fogliano^b, Massimo Blandino^a



ASN in wholemeal flour:

MT_NF

CT_NF -22%

MT_F -37%

CT_F -48%

AA in biscuits:

MT_NF

CT_NF -14%

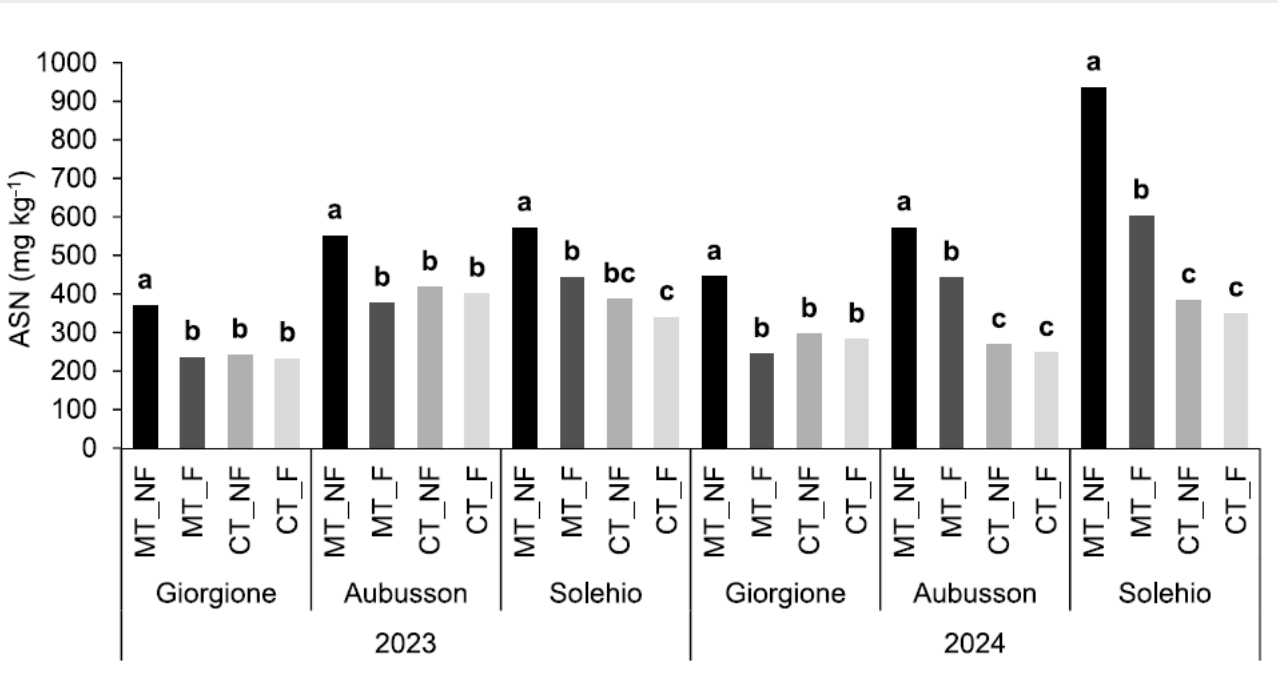
MT_F -18%

CT_F -22%

CT_F (conventional tillage + fungicide application) showed the lowest levels of free asparagine (ASN) in wholegrain flour and acrylamide (AA) in biscuits

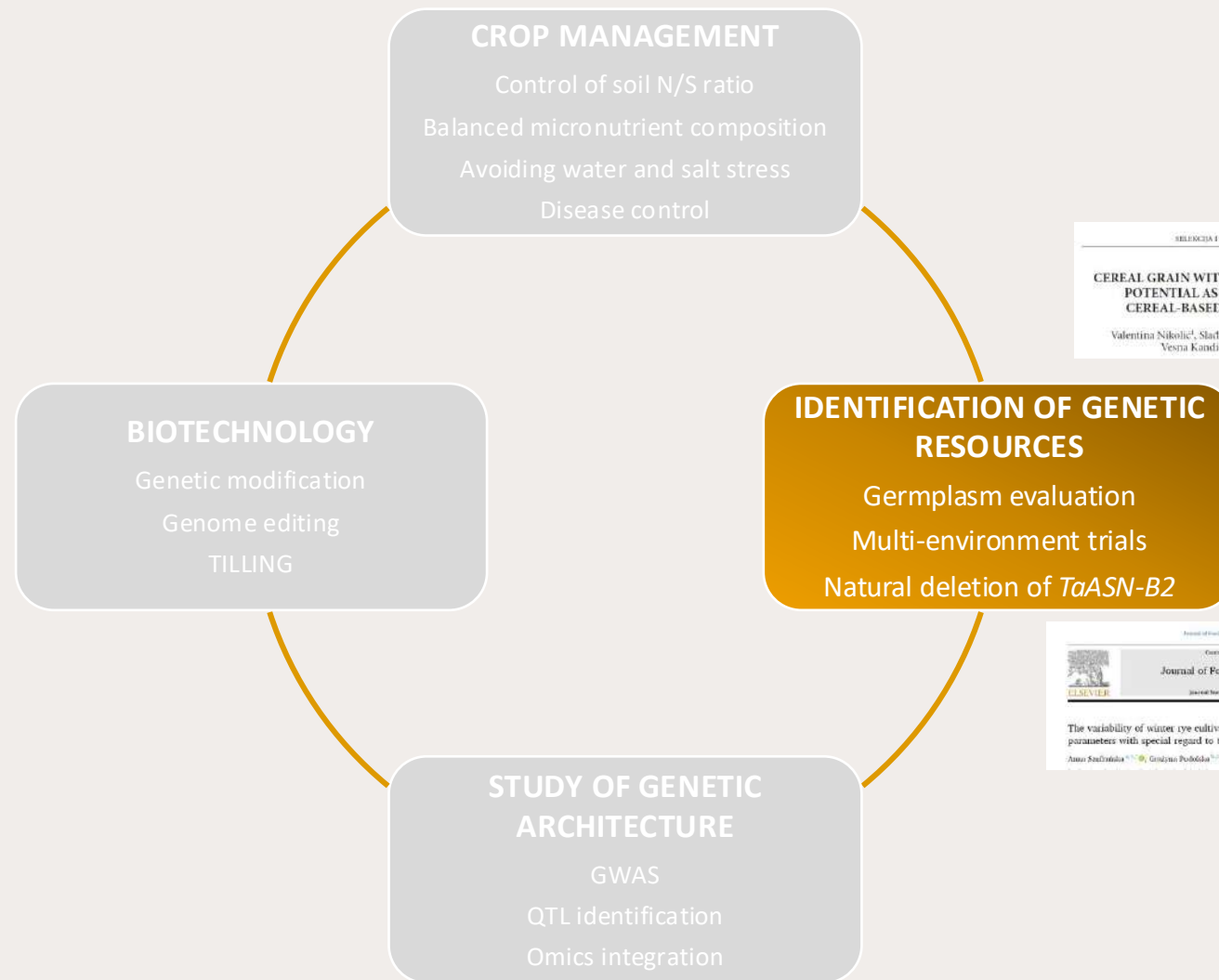


“Agronomic practices that ensure an appropriate grain filling can dilute free asparagine in whole kernels and reduce the acrylamide content of wheat-based products.”



ASN in wholemeal flour:		AA in biscuits:
MT_NF	→	MT_NF
CT_NF -22%	→	CT_NF -14%
MT_F -37%	→	MT_F -18%
CT_F -48%	→	CT_F -22%

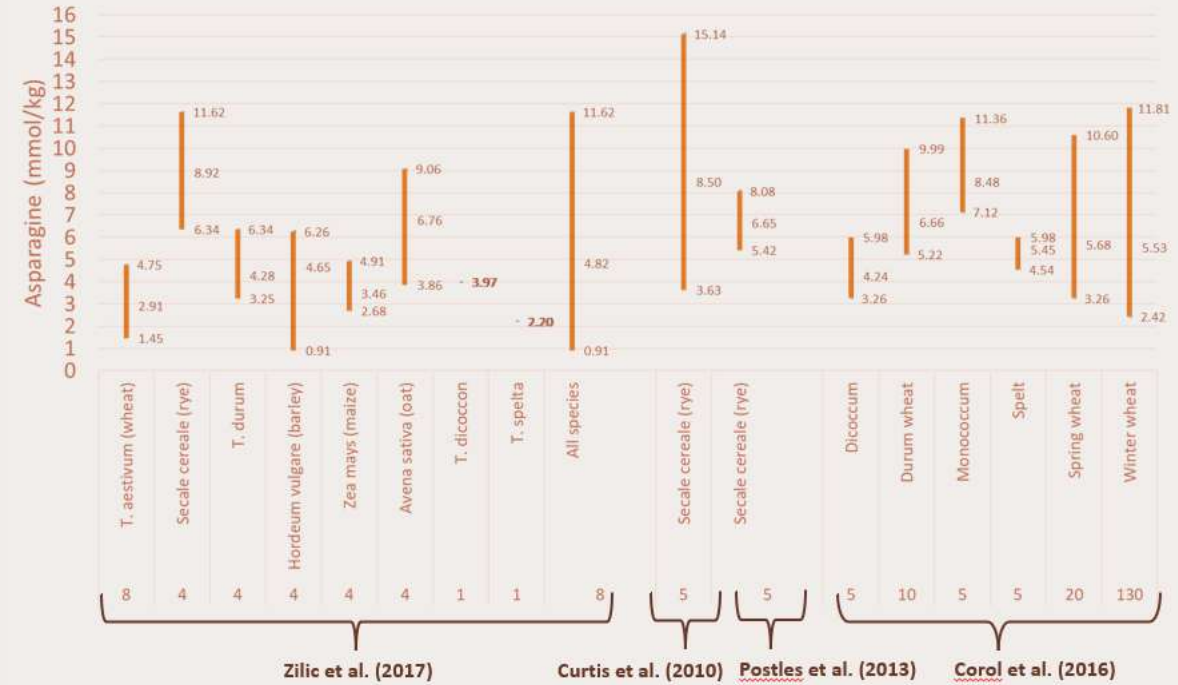
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Several studies have reported data on the differing propensities of cereals genotypes to accumulate free asparagine in the grain.

cereal genotypes



wheat genotypes



Certain genotypes maintain low and stable levels of free asparagine in the grain.

GERMPLASM EVALUATION / MULTI-ENVIRONMENT TRIALS

SELEKCIJA I SEMENARSTVO, Vol. XXVIII (2022) broj 2

doi: 10.5937/SelSem2202013N

CEREAL GRAIN WITH LOW ACRYLAMIDE FORMATION POTENTIAL AS A RAW MATERIAL FOR SAFER CEREAL-BASED FOOD PRODUCTS IN SERBIA

Valentina Nikolić¹, Slađana Žilić¹, Marijana Simić¹, Natalija Kravić¹,
Vesna Kandić¹, Milan Brankov¹, Marko Vasić¹

Nikolic et al. 2022. doi: 10.5937/SelSem2202013N

“In Serbia, there are no guidelines for the cultivation of cereals with a reduced potential for acrylamide formation.”

Review in “Selekcija i Semenarstvo” (“Plant Breed and Seed Production”)



Figure 1. The mitigation measures for safer low acrylamide cereal-based food production
Grafikon 1. Preventivne mere za bezbedniju proizvodnju hrane na bazi žitarica sa niskim sadržajem akrilamida



Article

Exploring Variability of Free Asparagine Content in the Grain of Bread Wheat (*Triticum aestivum* L.) Varieties Cultivated in Italy to Reduce Acrylamide-Forming Potential

Andrea Tafuri ^{1,2,3}, Melania Zuccaro ¹, Stefano Ravaglia ², Raul Pirona ¹, Stefania Masci ³, Francesco Sestili ³, Domenico Lafiandra ³, Aldo Ceriotti ¹ and Elena Baldoni ^{1,*}

Tafuri et al. 2023. <https://doi.org/10.3390/plants12061349>

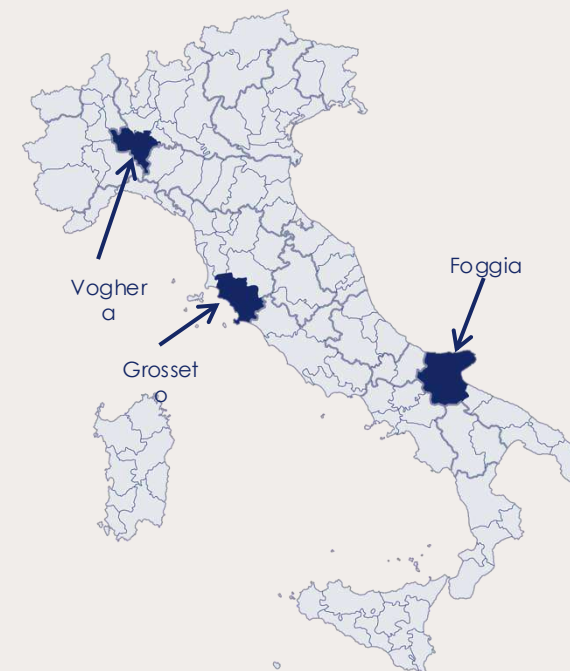
36 varieties in 2018–2019
3 field trials: 3 locations

36 varieties in 2019–2020
3 field trials: 3 locations

18 varieties in both years
6 field trials: 3 locations for 2 years

Analysis of 54 **bread wheat** cultivars - used in the Italian market

- 3 growth locations
- 2 years








Article

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First year of field trials (2018–2019)

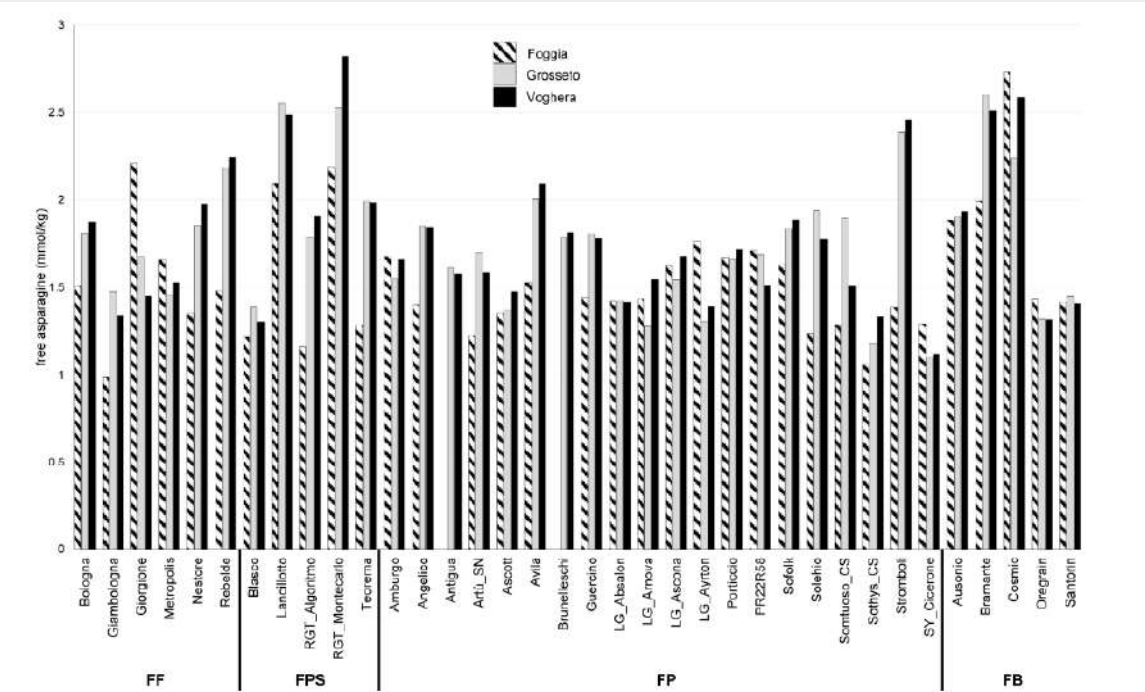


Figure 1. Concentration of fAsn in the grain of 36 bread wheat varieties grown in 2018–2019 in the three locations Foggia, Grosseto, and Voghera. FF: improver wheat; FPS: superior bread making wheat; FP: ordinary bread making wheat; FB: wheat for biscuits.

Second year of field trials (2019–2020)

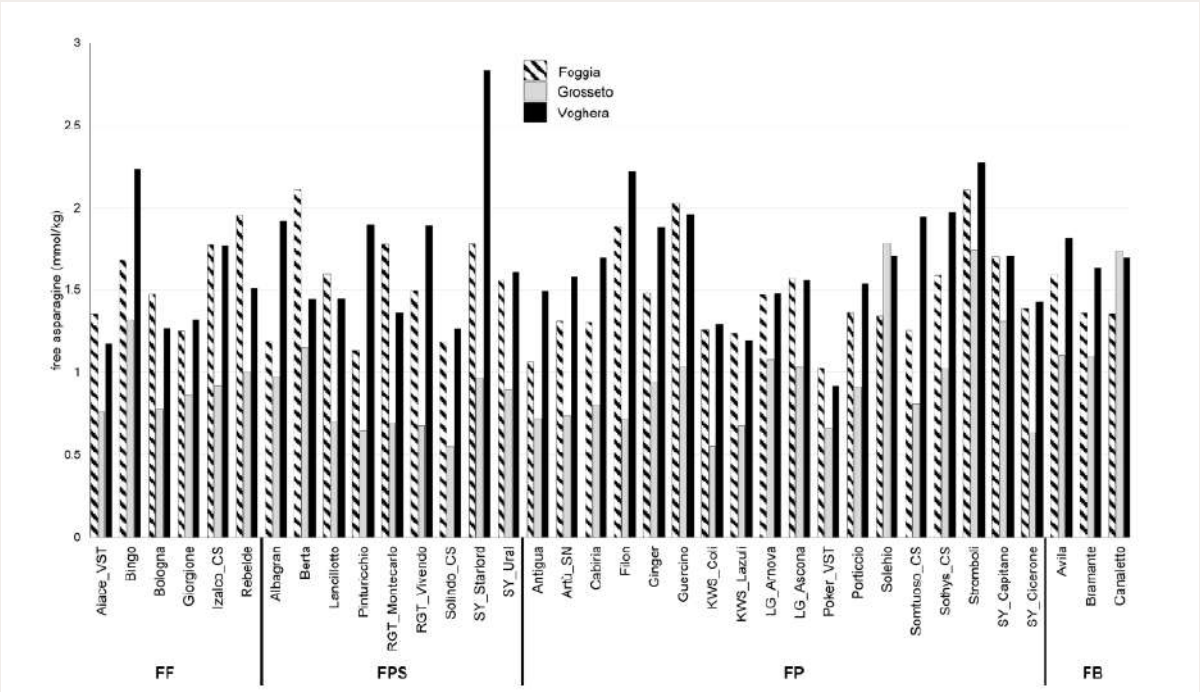


Figure 2. Concentration of fAsn in the grain of 36 bread wheat varieties grown in 2019–2020 in the three locations Foggia, Grosseto, and Voghera. FF: improver wheat; FPS: superior bread making wheat; FP: ordinary bread making wheat; FB: wheat for biscuits.

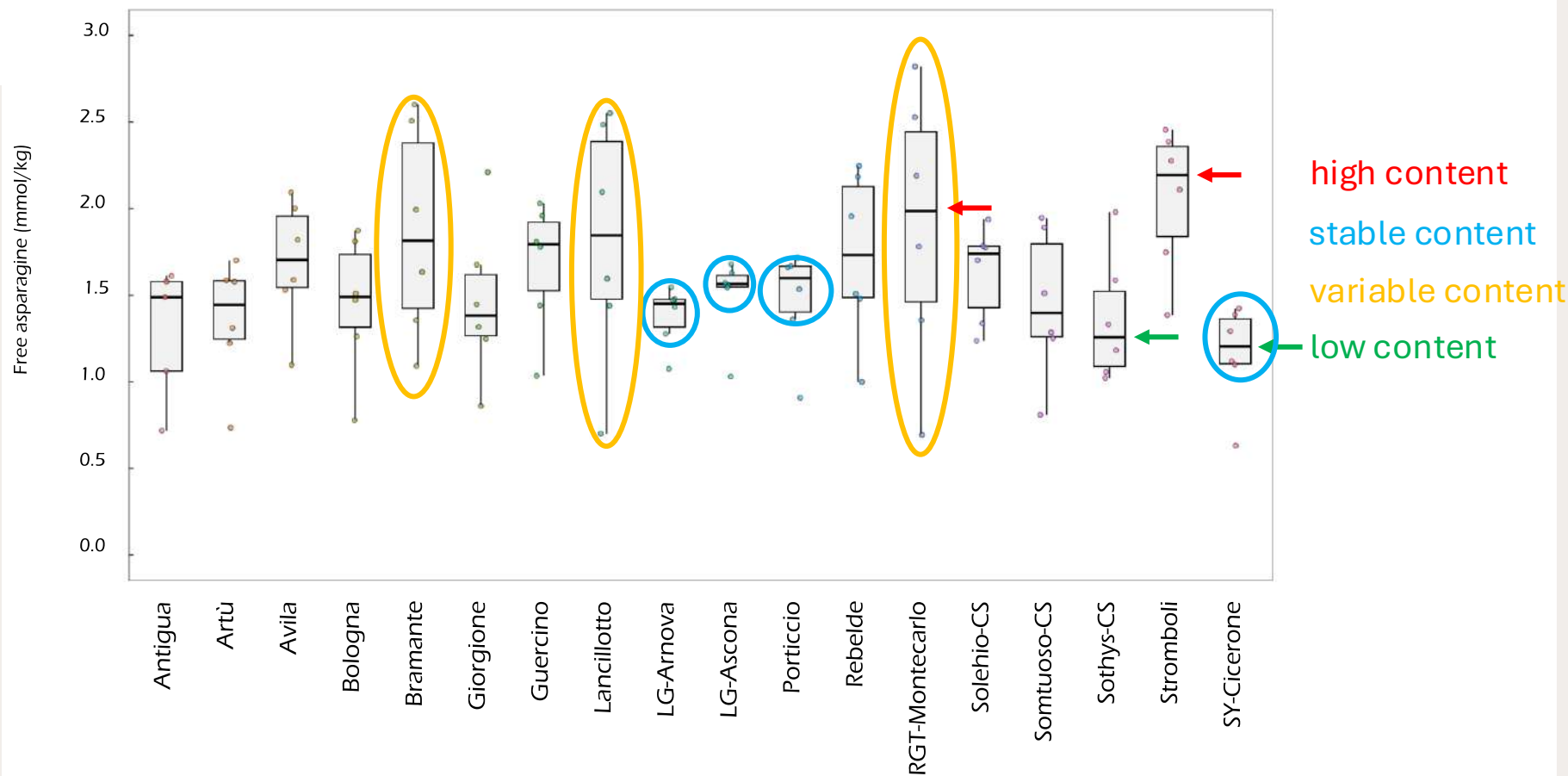


Article

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Identification of cultivars with low and stable free asparagine content



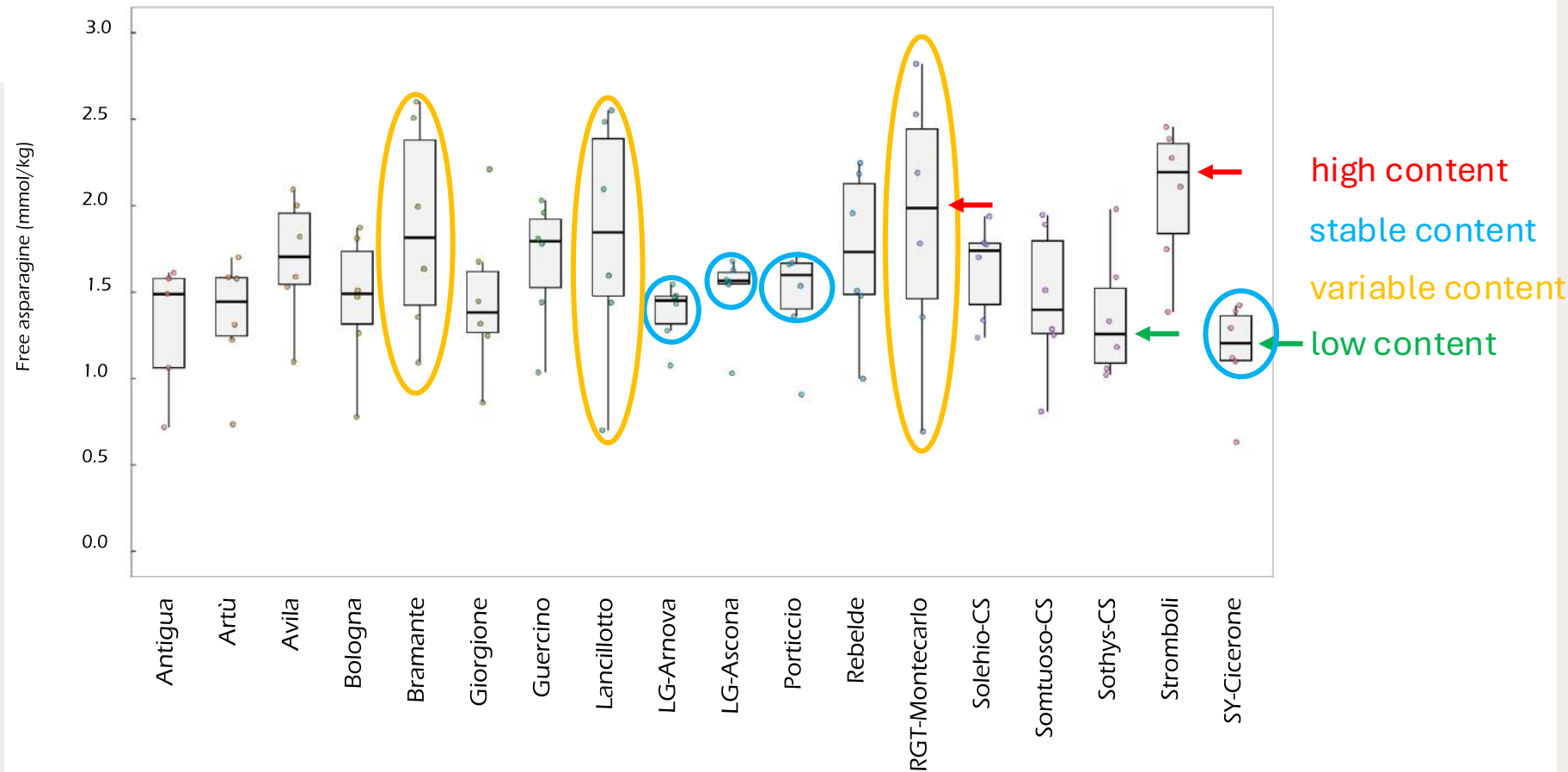


Article
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Andrea Tafuri ^{1,2,3}, Melania Zuccaro ¹, Stefano Ravaglia ², Raul Pirone ¹, Stefania Masci ³, Francesco Sestili ³, Domenico Lafiandra ³, Aldo Ceriotti ¹ and Elena Baldoni ^{1,*}

“These materials can be useful to dissect the mechanisms involved in free asparagine accumulation in wheat grain, and in breeding programs to reduce the acrylamide-forming potential of wheat varieties.”

Identification of cultivars with low and stable free asparagine content





Szafranska et al. 2025. <https://doi.org/10.1016/j.jfca.2025.108605>

- 2 cultivation managements (integrated and intensive), differing in the amount of nitrogen fertilization and fungicide protection.
- 2 years (2018/2019 and 2019/2020)

26 (13 open-pollinated – OPC, and 13 hybrids cultivars - HC) winter rye cultivars

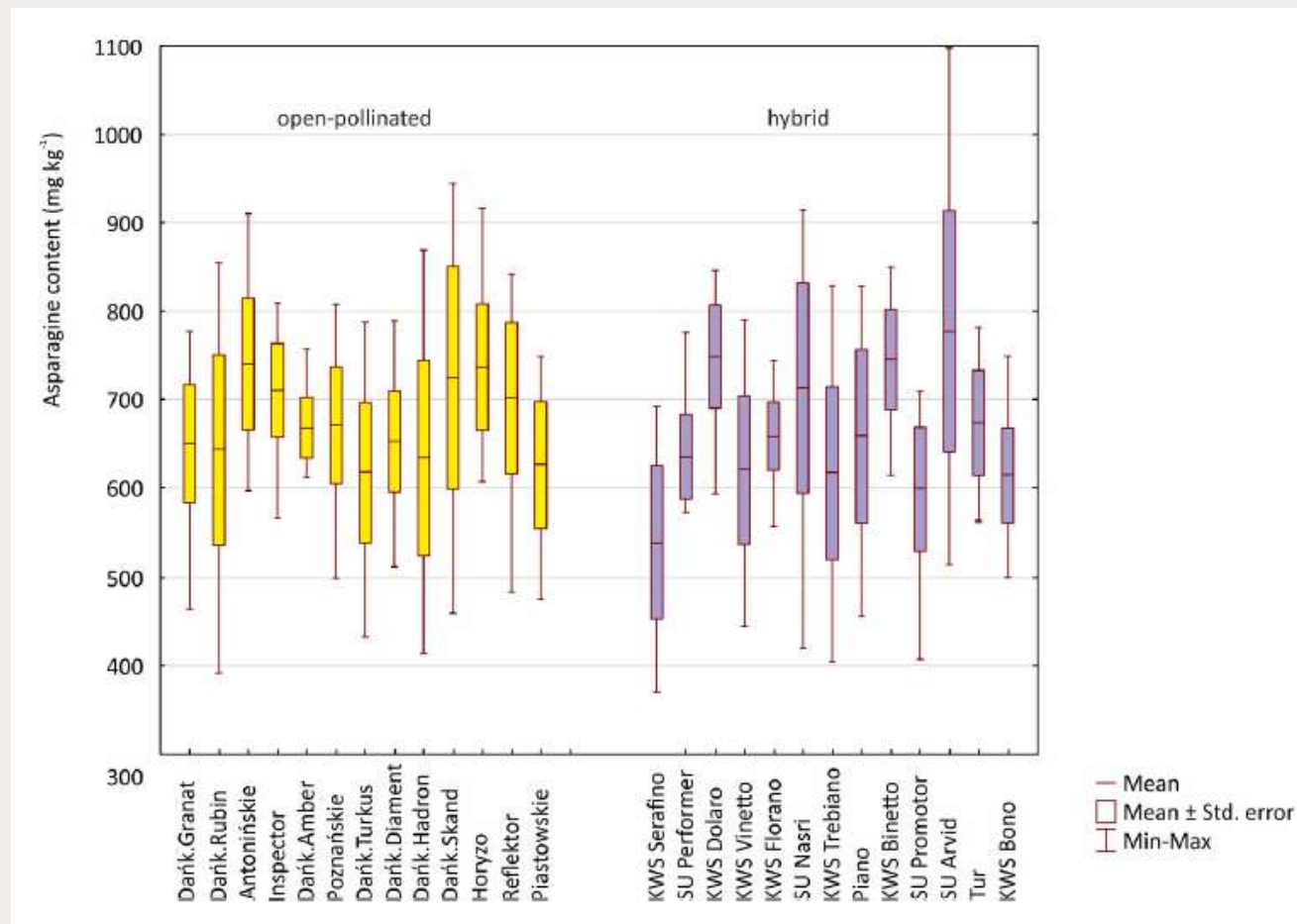
Table 1

Open-pollinated and hybrid winter rye cultivars examined in the crop years 2018/2019 and 2019/2020 on field trials.

Population (open-pollinated) cultivars		Hybrid cultivars	
Cultivar	Origin	Cultivar	Origin
Antonińskie	Poznańska Hodowla Roślin sp. z o.o.	KWS	KWS Lochow
Dańkowskie	DANKO Hodowla Roślin sp. z o.o.	Binntto	Polska sp. z o.o.
Amber	DANKO Hodowla Roślin sp. z o.o.	KWS Bono	KWS Lochow
Dańkowskie	DANKO Hodowla Roślin sp. z o.o.		Polska sp. z o.o.
Diament	DANKO Hodowla Roślin sp. z o.o.	KWS	KWS Lochow
Dańkowskie	DANKO Hodowla Roślin sp. z o.o.	Dolaro	Polska sp. z o.o.
Granat	DANKO Hodowla Roślin sp. z o.o.	KWS	KWS Lochow
Dańkowskie	DANKO Hodowla Roślin sp. z o.o.	Florano	Polska sp. z o.o.
Hadron	DANKO Hodowla Roślin sp. z o.o.	KWS	KWS Lochow
Dańkowskie	DANKO Hodowla Roślin sp. z o.o.	Serafino	Polska sp. z o.o.
Rubin	DANKO Hodowla Roślin sp. z o.o.	KWS	KWS Lochow
Dańkowskie	DANKO Hodowla Roślin sp. z o.o.	Trebiano	Polska sp. z o.o.
Skand	DANKO Hodowla Roślin sp. z o.o.	KWS	KWS Lochow
Dańkowskie	DANKO Hodowla Roślin sp. z o.o.	Vinetto	Polska sp. z o.o.
Turkus	DANKO Hodowla Roślin sp. z o.o.	Piano	Saaten-Union
Horyzo	Hodowla Roślin Smolice sp. z o.o.	SU Arvid	Polska sp. z o.o.
Inspector	Saaten-Union Polska sp. z o.o.	SU Nasri	Saaten-Union
Piastowskie	Poznańska Hodowla Roślin sp. z o.o.		Polska sp. z o.o.
Poznańskie	Poznańska Hodowla Roślin sp. z o.o.	SU	Saaten-Union
Reflektor	Saaten-Union Polska sp. z o.o.	Performer	Polska sp. z o.o.
		SU	Saaten-Union
		Promotor	Polska sp. z o.o.
		Tur	Hodowla Roślin Smolice sp. z o.o.



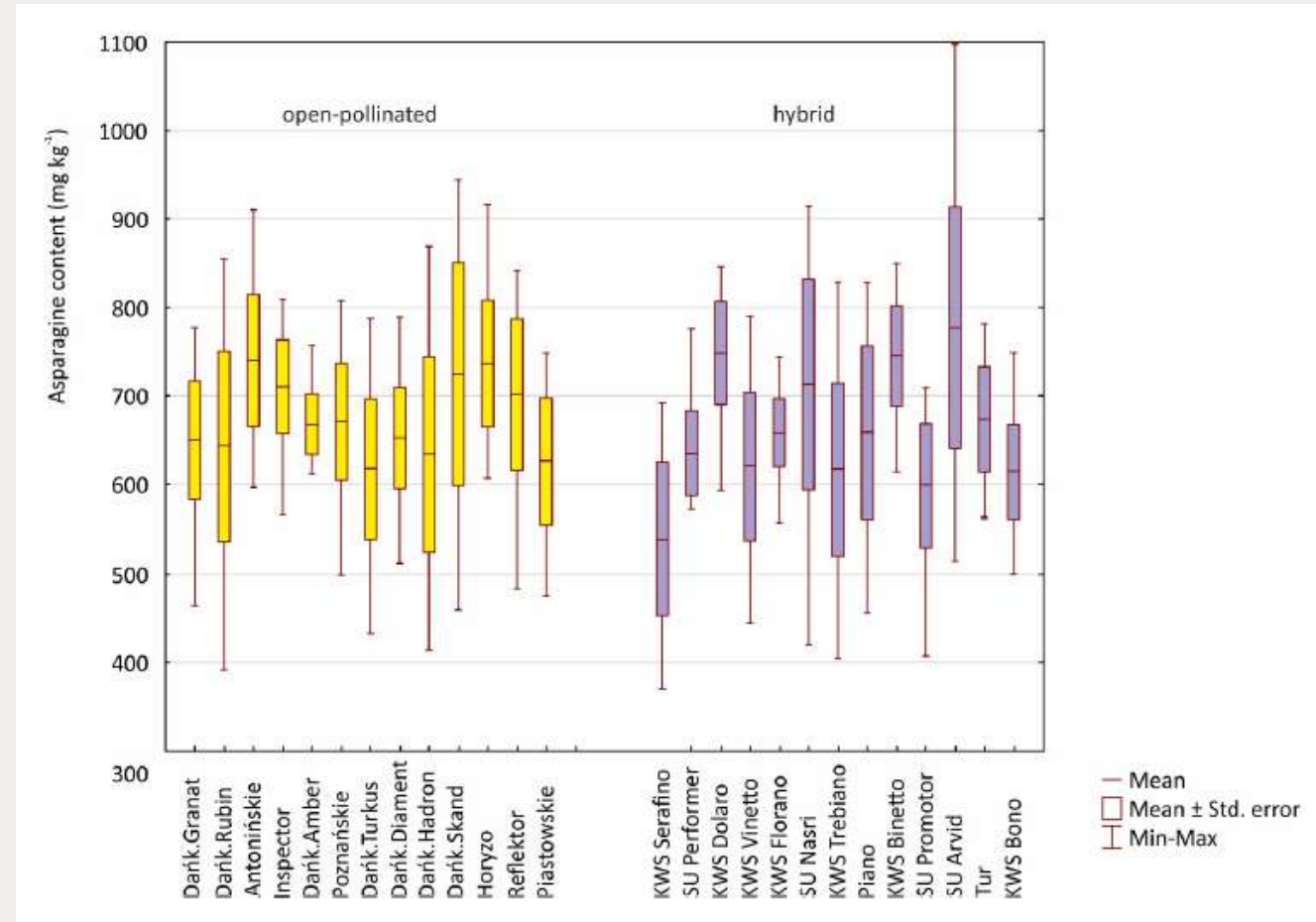
- Free asparagine content varied widely among cultivars (370–1098 mg kg⁻¹);
- Weather conditions strongly affected its levels;
- Nine cultivars were highly affected by crop year and cultivation technology;
- The highest free asparagine content was found in HC SU Arvid;
- The highest free asparagine content was found in HC KWS Serafino had the lowest;
- OPC Dąnkowskie Amber showed the lowest variability.





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- OPC Dąnkowskie Amber showed the lowest variability.

“Overall, harvest year had the greatest effect on Asn content (67 %), followed by cultivar (14 %) and the interaction between harvest year and cultivar (9 %).”





LWT - Food Science and Technology 223 (2025) 117715

Contents lists available at ScienceDirect

LWT

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

Does the use of the Tritordeum, an alternative cereal for breadmaking, can be one of the mitigation strategies of acrylamide in bread?

Marian Wiwart^a, Anna Szafrńska^{b,*}, Elżbieta Suchowilska^a



Wiwart et al. 2025. <https://doi.org/10.1016/j.jfca.2025.108605>

Characterization of 11 **spring *Tritordeum*** breeding lines

Comparison with modern cultivars of durum wheat, bread wheat, and naked barley

Tritordeum (*Tritordeum martinii* A. Pujadas, nothosp. nov.) is a relatively new allohexaploid crop obtained by crossing diploid wild barley (*Hordeum chilense* Roem. et Schultz) (HchHch) with tetraploid durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) Husn.) (AABB)



protein content
ash
wet gluten
gluten index

Tritordeum vs bread wheat

14%	13.3%
0.60%	0.54%
34.3%	31.9%
51%	90%

Free asparagine content in grain
content of acrylamide in bread

185 mg/kg	188 mg/kg
15 µg/kg	21.9 µg/kg



protein content
ash
wet gluten
gluten index

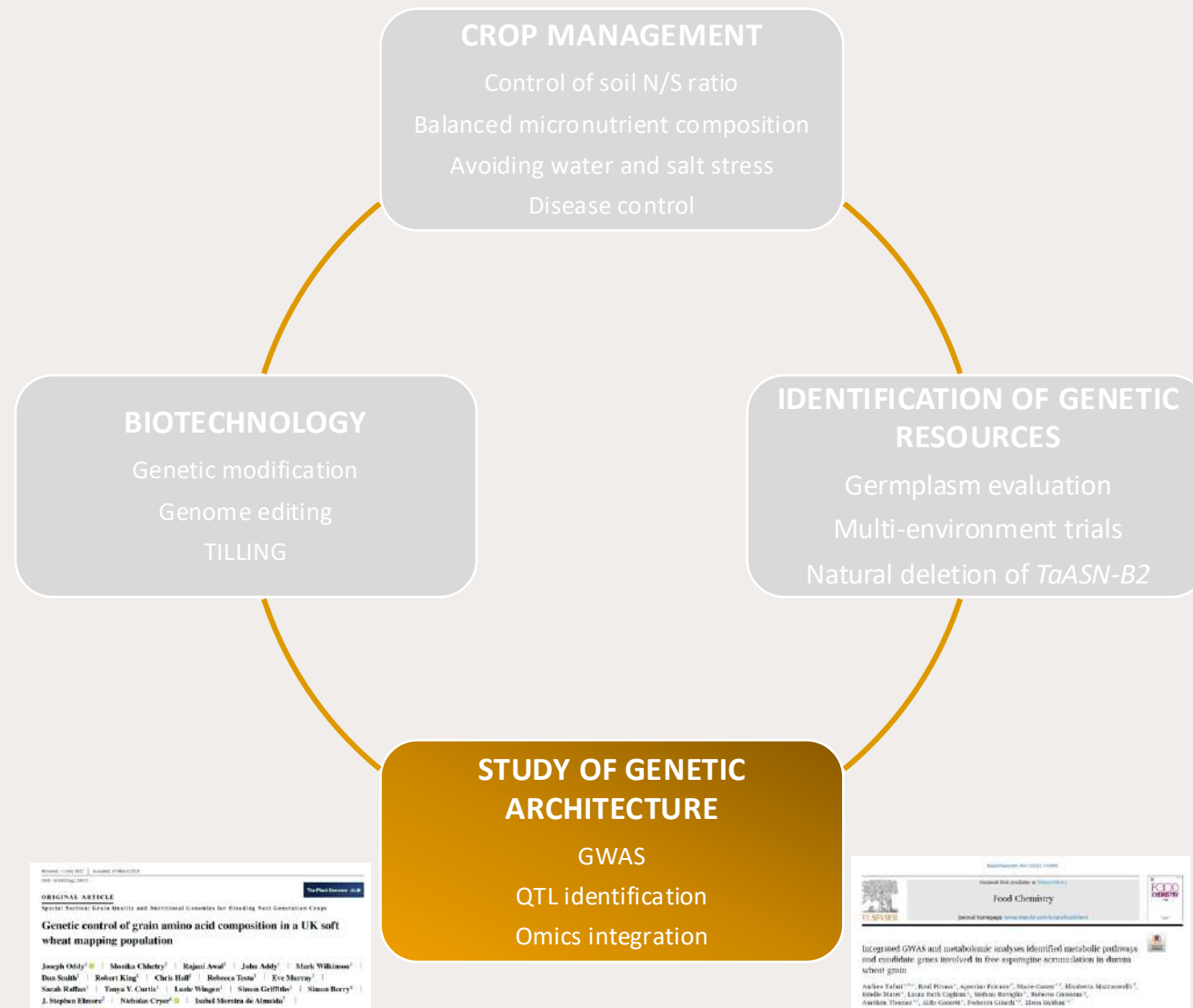
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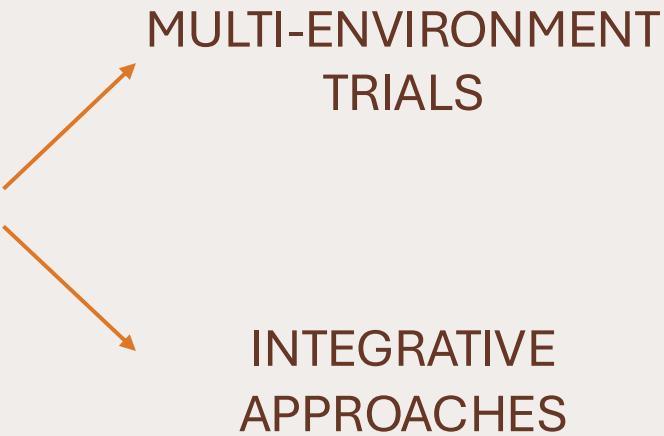
“The lower acrylamide content in Tritordeum bread is likely due to its reduced asparagine content, lower starch damage content and denser bread structure. However, other factors, such as sugar composition and Maillard reaction kinetics, may also play a role in this outcome.”





GWAS and QTL identification

Complex genetic architecture of the trait	No major-effect QTL have been identified - multiple loci with moderate additive effects.	—
High influence of the environment	Instability in MTAs - many loci exhibiting environment-specific effects.	—
Strong G×E interactions	Potential pleiotropic effects on agronomic traits , such as yield and grain quality.	—
Heritability of free asparagine content is medium to high .	Possibility to breed for this trait.	+



Received: 11 July 2022 | Accepted: 13 March 2023

DOI: 10.1002/tpg2.20335

ORIGINAL ARTICLE

Special Section: Grain Quality and Nutritional Genomics for Breeding Next Generation Crops

The Plant Genome

Genetic control of grain amino acid composition in a UK soft wheat mapping population

Joseph Oddy¹ | Monika Chhetry² | Rajani Awal² | John Addy¹ | Mark Wilkinson¹ | Dan Smith¹ | Robert King¹ | Chris Hall¹ | Rebecca Testa¹ | Eve Murray¹ | Sarah Raffan¹ | Tanya Y. Curtis³ | Luzie Wingen² | Simon Griffiths² | Simon Berry⁴ | J. Stephen Elmore⁵ | Nicholas Cryer⁶ | Isabel Moreira de Almeida⁷ | Nigel G. Halford¹

Tafari et al. 2023. <https://doi.org/10.3390/plants12061349>

“High free asparagine and low lysine concentrations limit the nutritional value of wheat grain”

- Robigus × Claire doubled haploid population
- 2 years field trial (2017–2018, 2018–2019)

Evaluation of:

amino acids composition

grain related trait:

- grain diameter
- kernel hardness index (KHI),
- Grain weight measurements
- Grain length, width, and area
- Hagberg falling number

✓ Linkage analysis

✓ Identification of quantitative trait loci (QTL) controlling free amino acids and other traits



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TABLE 1 Multi-environment quantitative trait locus (QTL) for measured amino acids.

Multi-environment single trait linkage analysis (H18 and H19)								
Trait	Marker	Chr.	cM	Mbp	−log ₁₀ (p)	H18 (%)	H19 (%)	High val.
Ala	WC.0223839	7B	211.2	719	5.03	7.1	5.7	Robigus
Asn	WC.0221262	4B	114.47	601	5.96	2.6	14.8	Robigus
Asp	WC.0218489	1B	54.4	530	5.4	8	5.9	Claire
	WC.0214359	3A2	2.3	738	7.95	7.3	15.3	Robigus
	WC.0221037	4A	148.8	703	8.08	12.6	9.3	Claire
	WC.0227146	4D	48.8	16	3.7	5.5	4.1	Claire
Gln	WC.0221302	4B	103.7	547	3.5	5.4	4.5	Robigus
	WC.0228471	6B	19.7	25	5.09	8.2	6.7	Claire
Glu	WC.0221329	4B	100.8	518	4.27	3.7	10.1	Robigus
Gly	WC.0226796	4B	155.2	327	4.26	3.2	5.3	Robigus
Iso	WC.0223785	7B	211.2	717	3.6	6.8	3.7	Robigus
Lys	WC.0218011	1A2	27.3	593	4.95	12.1	2.6	Claire
Phe	WC.0220622	3B1	78.1	116	3.83	6.2	5.6	Robigus

Abbreviations: Chr., chromosome; cM, centimorgan; H18/H19 (%), percentage of variation explained by QTL in respective year; High val., parental line carrying the allele responsible for the higher value; Mbp, megabase pair location in Chinese Spring v1.0.



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“Breeding for lower free asparagine and higher lysine using Claire and Robigus diversity is possible but limited.”

“High free asparagine and low lysine concentrations limit the nutritional value of wheat grain”

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- 2 years field trial (2017–2018, 2018–2019)

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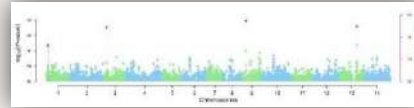
Integrated GWAS and metabolomic analyses identified metabolic pathways and candidate genes involved in free asparagine accumulation in durum wheat grain

Andrea Tafuri^{a,b,c}, Raul Pirona^a, Agostino Fricano^d, Marie Gasser^{e,f}, Elisabetta Mazzucotelli^d, Estelle Maret^e, Laura Ruth Cagliani^g, Stefano Ravaglia^b, Roberto Consonni^g, Aurélien Thomas^{e,f}, Aldo Ceriotti^a, Federica Gilardi^{a,f}, Elena Baldoni^{a,g}



Tafuri et al. 2025. <https://doi.org/10.3390/plants12061349>

- 201 genotypes (180 landraces) of *Triticum turgidum* L. ssp. *durum* (Desf.) originated from 33 different countries from the Global Durum Panel (GDP)
- 3 years field trial



Genome Wide Association
Study (GWAS)

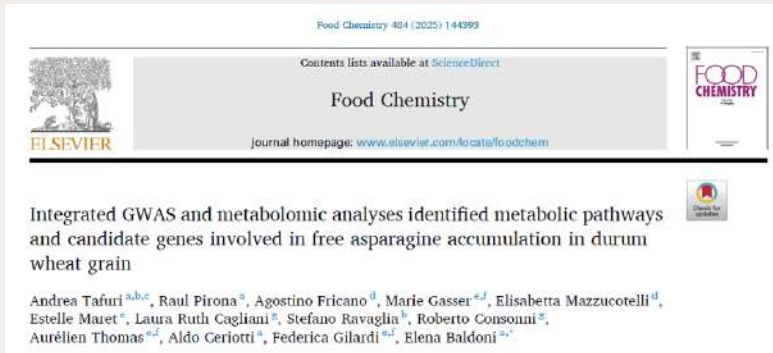


Metabolomic analysis
(+/- UPLC-HRMS) –

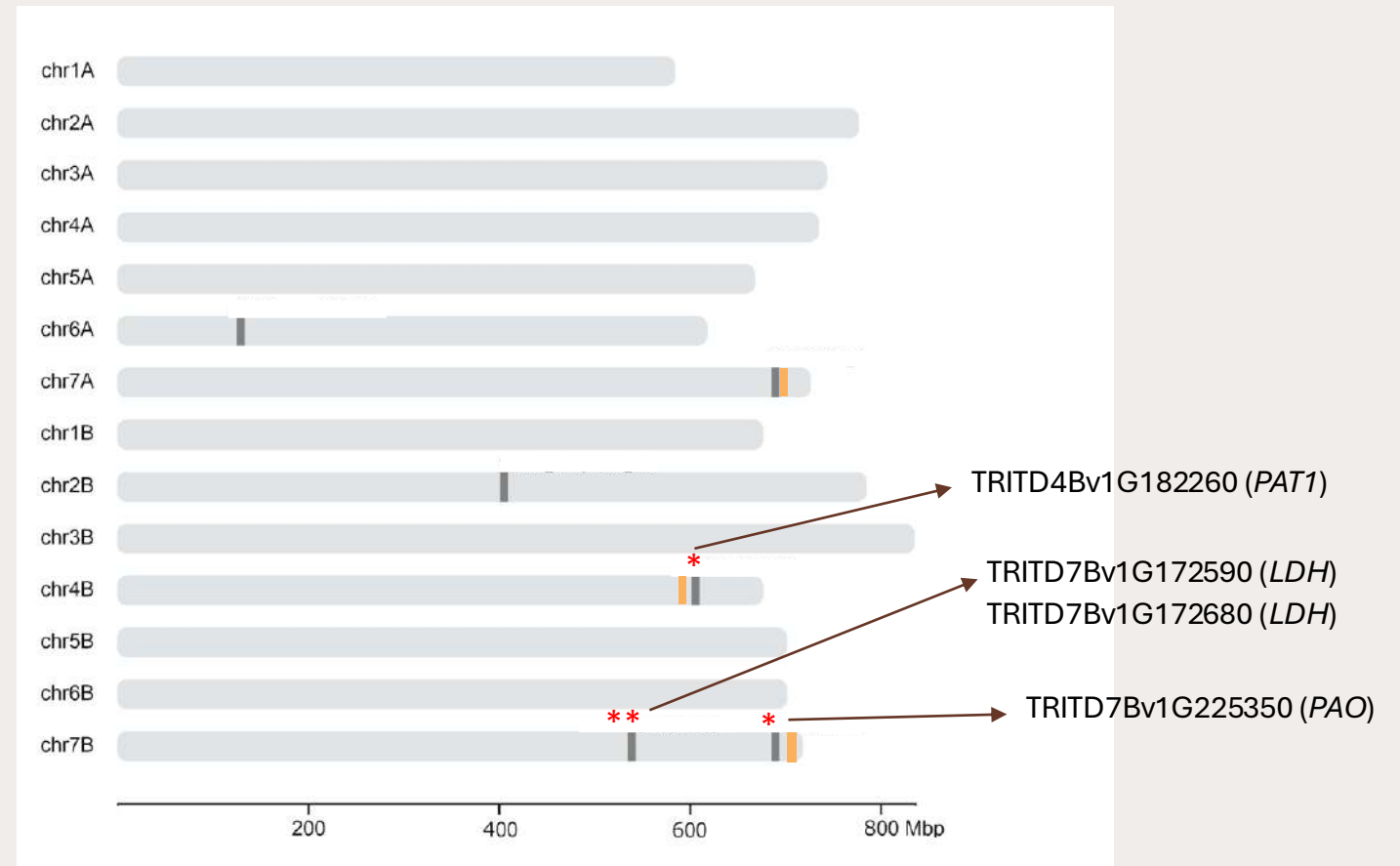


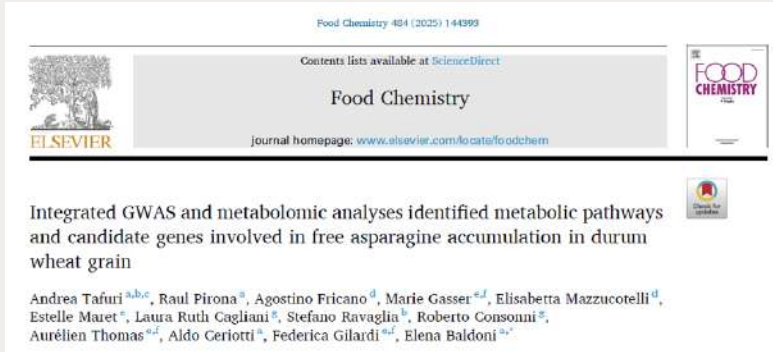
Identification of candidate genes





Identification of six QTNs associated to free asparagine content in the grain and four candidate genes involved in this trait.

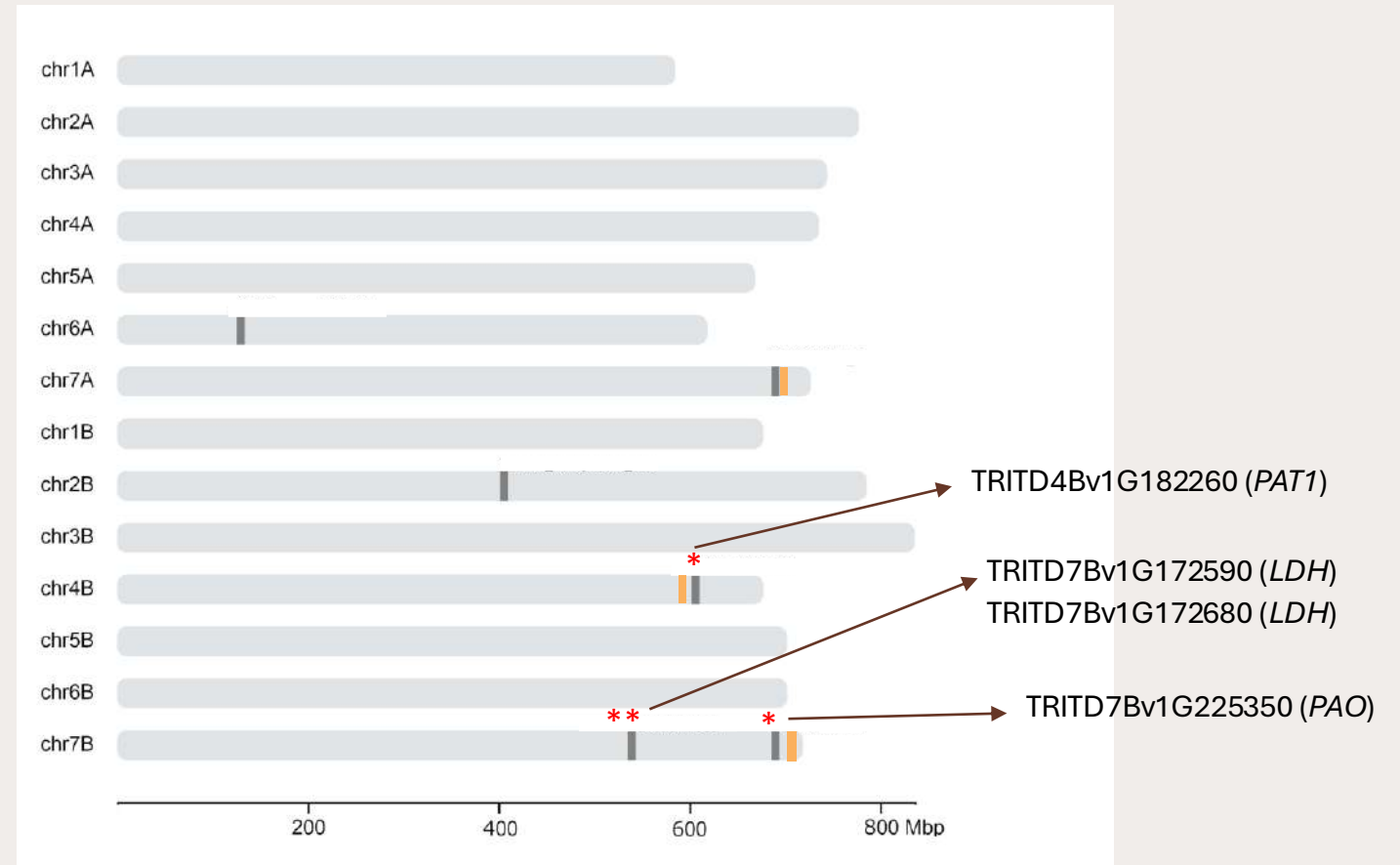




“The six detected QTNs could be of interest for future breeding programs in elite varieties aimed to reduce acrylamide forming potential.”

This study gave new insight in the complex metabolic network that control free asparagine levels in wheat grain.”

Identification of six QTNs associated to free asparagine content in the grain and four candidate genes involved in this trait.





GWAS and QTL identification

Reference	n. of analysed genotypes	n. of QTLs	Chromosome
Emebiri, 2014	92 varieties	1 QTL (9 SNPs)	5A
Rapp et al. 2018	149 varieties	8 QTL/SNPs	1A, 4A, 7A, 1B, 6B, 7B, 2D, 6D
Lavoignat et al. 2024	481 varieties	16 QTL (146 SNPs)	1A, 3A (2), 6A, 7A, 1B, 2B, 3B (2), 5B, 6B (2), 7B (2), 2D
Rączka et al. 2025	174 elite winter wheat lines	15 QTL (43 SNPs)	1B, 1D, 2B, 2D, 3A, 3B, 3D, 4A, 5B, 5D, 6B, 6D, 7A, 7B, 7D
Oddy et al. 2023	171 doubled haploid lines of Robigus × Claire	1 QTL/SNP	4B
Tafari et al. 2025	201 landraces and varieties of durum wheat	6 QTNs	6A, 7A, 2B, 4B, 7B



Candidate genes and associated markers

Species	Candidate gene	Gene name	Metabolic pathway	Chr	Associated QTL	Peak marker position (Mbp)	Reference
<i>T. aestivum</i>	<i>TraesCS3A02G077100; TraesCS3D02G077300</i>	asparagine synthetase 2 (<i>TaASN2</i>)	asparagine synthesis in seed	3A, 3D	-	-	Raffan et al. 2021
	-	asparagine synthetase 2B (<i>TaASN-B2</i>)	asparagine synthesis in seed	3B	-	-	Oddy et al. 2021
	<i>TraesCS1B03G0736700</i>	H-subunit of mitochondrial NAD(P)H dehydrogenase	mitochondrial electron transport chain	1B	QGfac.rut.1B.1	468.9	Rączka et al. 2025
<i>T. turgidum ssp. durum</i>	<i>TRITD4Bv1G182260</i>	anthranilate phosphoribosyl transferase (<i>PAT1</i>)	tryptophan biosynthesis pathway	4B	GENE-4008_418	609.4	Tafari et al. 2025
	<i>TRITD7Bv1G172590</i>	L-lactate dehydrogenase (<i>LDH</i>)	glucose metabolism / asparaginase II pathway	7B	BS00108573_51	545.2	
	<i>TRITD7Bv1G172680</i>	L-lactate dehydrogenase (<i>LDH</i>)	glucose metabolism / asparaginase II pathway	7B	BS00108573_51	545.2	
	<i>TRITD7Bv1G225350</i>	polyamine oxidase (<i>PAO</i>)	polyamine metabolism /asparagine-methionine bicycle	7B	BS00062611_51	697.6	



CROP MANAGEMENT

Control of soil N/S ratio
Balanced micronutrient composition
Avoiding water and salt stress
Disease control

IDENTIFICATION OF GENETIC RESOURCES

Germplasm evaluation
Multi-environment trials
Natural deletion of *TaASN-B2*

STUDY OF GENETIC ARCHITECTURE

GWAS
QTL identification
Omics integration

BIOTECHNOLOGY

Genetic modification
Genome editing
TILLING





Plant Biotechnology Journal (2021) 19, pp. 1602–1613

doi: 10.1111/pbi.13573

Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene *TaASN2*

Sarah Raffan¹, Caroline Sparks¹, Alison Huttly¹, Lucy Hyde^{1,a}, Damiano Martignago^{1,b}, Andrew Mead², Steven J. Hanley², Paul A. Wilkinson³, Gary Barker³, Keith J. Edwards³, Tanya Y. Curtis⁴, Sarah Usher⁴, Ondrej Kosik⁴ and Nigel G. Halford^{1,*}

Raffan et al. 2021. doi: 10.1111/pbi.13573



Field Trials and Baking Studies of Ultra-Low Asparagine, Genome Edited (CRISPR/Cas9) and Mutant (TILLING) Wheat

Navneet Kaur¹ | Sarah Raffan¹ | Suzanne J. Clark¹ | Shpresa Musa² | Katharina Scherf^{3,4} | J. Stephen Elmore⁵ | Tanya Y. Curtis⁶ | Emma Honan⁶ | Nigel G. Halford¹

Kaur et al. 2026. <https://doi.org/10.1111/pbi.70661>



Plant Biotechnology Journal (2023) 21, pp. 1097–1099

doi: 10.1111/pbi.14026

Brief Communication

Field assessment of genome-edited, low asparagine wheat: Europe's first CRISPR wheat field trial

Sarah Raffan¹, Joseph Oddy¹, Andrew Mead¹, Gary Barker², Tanya Curtis³, Sarah Usher³, Christopher Burt⁴ and Nigel G. Halford^{1,*}

Raffan et al. 2023. doi: 10.1111/pbi.14026



What we have learned...

- ✓ A **comprehensive and balanced approach** to soil management and fertilization, coupled with **innovations in soil biology and digital agriculture**, is essential.
- ✓ **Genetic diversity** for free asparagine content can be used in breeding programs.
- ✓ The trait is complex and multigenic, but it is possible to **find specific genetic determinants** that control free asparagine content.
- ✓ Using **integrated -omic approaches** to provide novel information about this complex trait.



COLLABORATIVE FIELD TRIALS

SHARING OF PHENOTYPIC AND GENETIC DATA

COLLABORATION AMONG PUBLIC INSTITUTIONS AND PRIVATE COMPANIES



SIS/CNR: a successful example of public-private collaboration

Public Research Institution



Private Seed Company

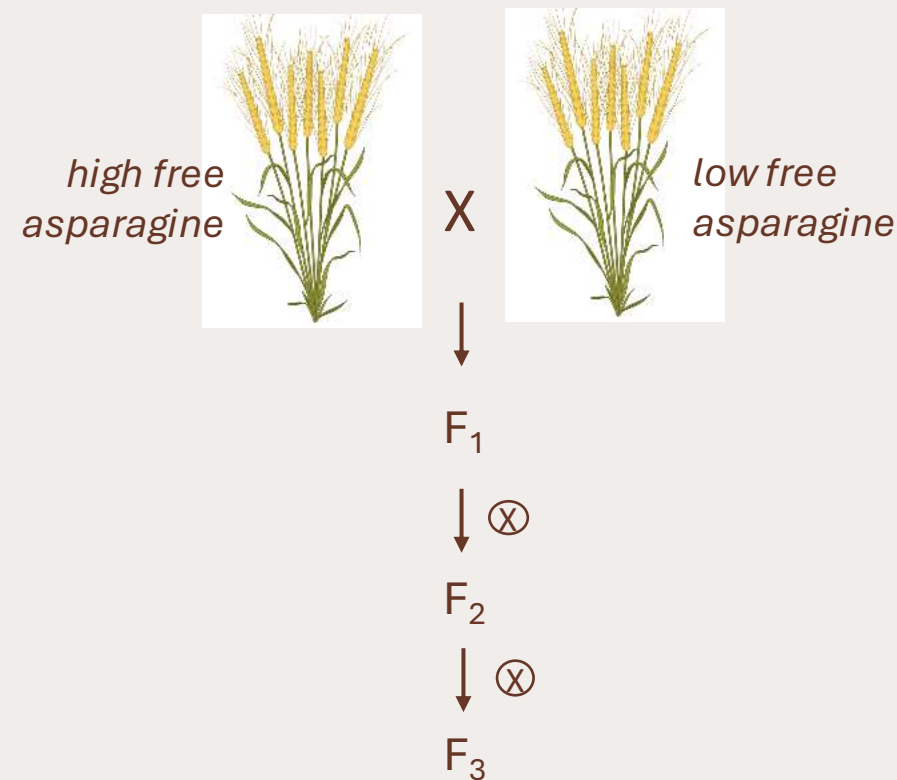


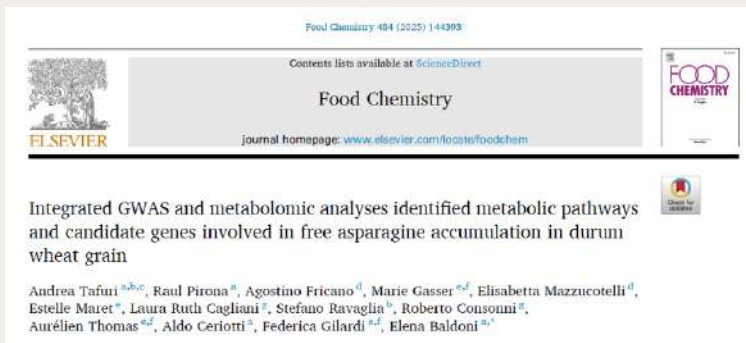
Exploring Variability of Free Asparagine Content in the Grain of Bread Wheat (*Triticum aestivum* L.) Varieties Cultivated in Italy to Reduce Acrylamide-Forming Potential

Andrea Tafuri ^{1,2,3}, Melania Zuccaro ¹, Stefano Ravaglia ², Raul Pirona ¹, Stefania Masci ³, Francesco Sestili ³, Domenico Lafiandra ³, Aldo Ceriotti ¹ and Elena Baldoni ^{1,*}

Production of recombinant lines for fine mapping

bread wheat





✓ Validation of QTLs on bread wheat

Phenotyping (free asparagine) and genotyping (Illumina 25K Wheat Infinium Array) of 326 bread wheat (*Triticum aestivum* L.) varieties representing the SIS germplasm

single-locus and multi-locus GWAS

Eleven QTNs on eight chromosomes were found as associated to free asparagine content by both GWAS
- two of them close to the QTNs reported in durum wheat



FRUMENTO TENERO
SY PASSION

CICLO MEDIO - TARDIVO

Elevate e stabili produzioni
 e P/L sempre basso

Potenziale produttivo al top in tutti gli ambienti.

Bassissimo contenuto di asparagina.

"Base" ideale per le farine sane: P/L basso ed equilibrato.

L'asparagina è un amminoacido componente fondamentale nella sintesi delle proteine, che rimane presente nella farina in misura diversa a seconda delle caratteristiche genetiche della varietà (Sy Passion ha i valori più bassi riscontrati) e delle condizioni ambientali e di coltivazione. È il precursore dell'acrilammide, un composto pericoloso per la salute, che si forma durante la cottura ad alta temperatura attraverso la reazione di Maillard. La scelta di Sy Passion è pertanto il primo passo per una produzione ad "Alta Sanità".

CARATTERISTICHE	
Alternatività	Invernale
Taglia	Media
Tipo di spiga	Aristata
Colore della granella	Rossa

QUALITÀ	BASSO	MEDIO	ALTO
Peso attecchito			
Peso mille semi			
Durezza			
W		180-200	
P/L		0,3-0,4	
Classificazione	Frumento Panificabile		

TOLLERANZE	BASSA	MEDIA	BUONA	ELEVATA
Allettamento				
Freddo invernale				
Cidio				
Ruggine bruna				
Ruggine gialla				
Fusarium spp.				

SIS

very low free asparagine

FRUMENTO TENERO

SY PASSION
 FRUMENTO AD ALTA SANITÀ

ACRILAMIDE

◆ Rese elevate in tutti gli ambienti
 ◆ Base per le farine: valori di P/L sempre bassi
 ◆ Il frumento sul mercato con i più bassi valori di asparagina

IL PROGETTO «FRUMENTO ALTA SANITÀ SY PASSION»

Per venire incontro alla richiesta di prodotti sani e controllati da parte del consumatore e alla esigenza dell'industria molitoria di garantire bassi contenuti di asparagina, SIS propone un percorso agronomico e di filiera che si basa su queste azioni:

- ◆ Fornire ai cerealicoltori un frumento con rese elevate e stabili: SY PASSION.
- ◆ Favorire con il coinvolgimento delle proprie reti di distributori/stoccatori l'approvvigionamento da parte dell'industria molitoria del frumento con il più basso contenuto di asparagina presente sul mercato e con eccellenti e stabili caratteristiche molitorie SY PASSION.
- ◆ Esaltare le caratteristiche qualitative e di salubrità di SY PASSION attraverso un protocollo di produzione messo a punto dal servizio tecnico SIS, che prevede:
 - Piano di concimazione equilibrato con le corrette somministrazioni di N e S per l'ulteriore riduzione del contenuto di asparagina nelle farine e per valorizzare le caratteristiche molitorie di SY PASSION;
 - Programma di difesa da patogeni fungini per il contenimento delle micotossine e per evitare fattori di stress alla coltura che possono alterare la sintesi proteica e favorire la formazione di asparagina;
 - Monitoraggio satellitare dello «stato di salute» delle colture.

CHE COSA È L'ACRILAMIDE?

L'acrilammide è un composto organico, altamente solubile in acqua, pericoloso per la salute. La sua presenza negli alimenti può aumentare il rischio di sviluppare un cancro in consumatori di tutte le fasce di età e i bambini costituiscono la fascia di età più esposta. Si forma negli alimenti che subiscono un trattamento ad alte temperature, attraverso la reazione tra gli zuccheri e le proteine nota come reazione di Maillard, ed è dovuta principalmente all'aminoacido asparagina. Al fine di ridurre la presenza di acrilammide negli alimenti, il REGOLAMENTO (UE) 2017/2158 DELLA COMMISSIONE del 20 novembre 2017 istituisce misure di attenuazione e livelli di riferimento e prevede una serie di azioni e misure da parte degli «OSA» (operatori del settore alimentare) che a ricaduta interessano i mulini tenuti a tenere sotto controllo il contenuto del precursore dell'acrilammide presente nelle farine: l'asparagina.

SIS

healthy wheat

What is still to be done...



- ✓ Better understanding of **agronomic practices beyond fertilization**, as well as the **impact of abiotic stresses** on free asparagine accumulation in cereals.

What is still to be done...

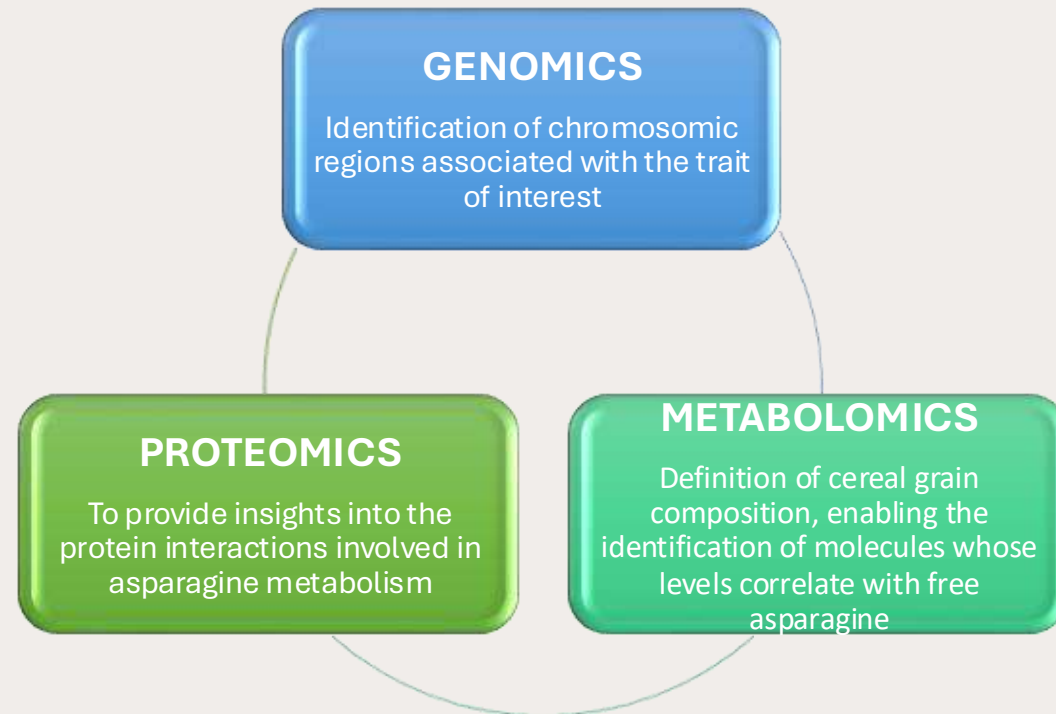


- ✓ Better understanding of **agronomic practices beyond fertilization**, as well as the **impact of abiotic stresses** on free asparagine accumulation in cereals.
- ✓ Identification of **genes and molecular pathways** involved in free asparagine accumulation.



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- ✓ Exploitation of **genome editing** for the creation of cereal genotypes accumulating low free asparagine.

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- ✓ Set up of **molecular markers** for free asparagine accumulation in the grain.

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- ✓ Better understanding of **agronomic practices beyond fertilization**, as well as the **impact of abiotic stresses** on free asparagine accumulation in cereals.
- ✓ Identification of **genes and molecular pathways** involved in free asparagine accumulation.
- ✓ Exploitation of **genome editing** for the creation of cereal genotypes accumulating low free asparagine.
- ✓ Set up of **molecular markers** for free asparagine accumulation in the grain.
- ✓ Creation of a shared **database about cereal genotypes** accumulating low and stable free asparagine levels, for breeding programs.



July 2024



Deliverable 9 – Draft agenda for the next 5 years for agronomy and plant breeding research (M20)

Project Name	ACRYRED
Contractual Delivery Date	18.06.2024
Actual Delivery Date	15.07.2024
Working Group	WG2
Authors	Elena Beldoni, CNR-IBBA, and WG 2 of ACRYRED consortium
Grant Period	GP2



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July 2024



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D2.3 Agenda for the next 5 years for agronomy and plant breeding research – M42



ACRYRED



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