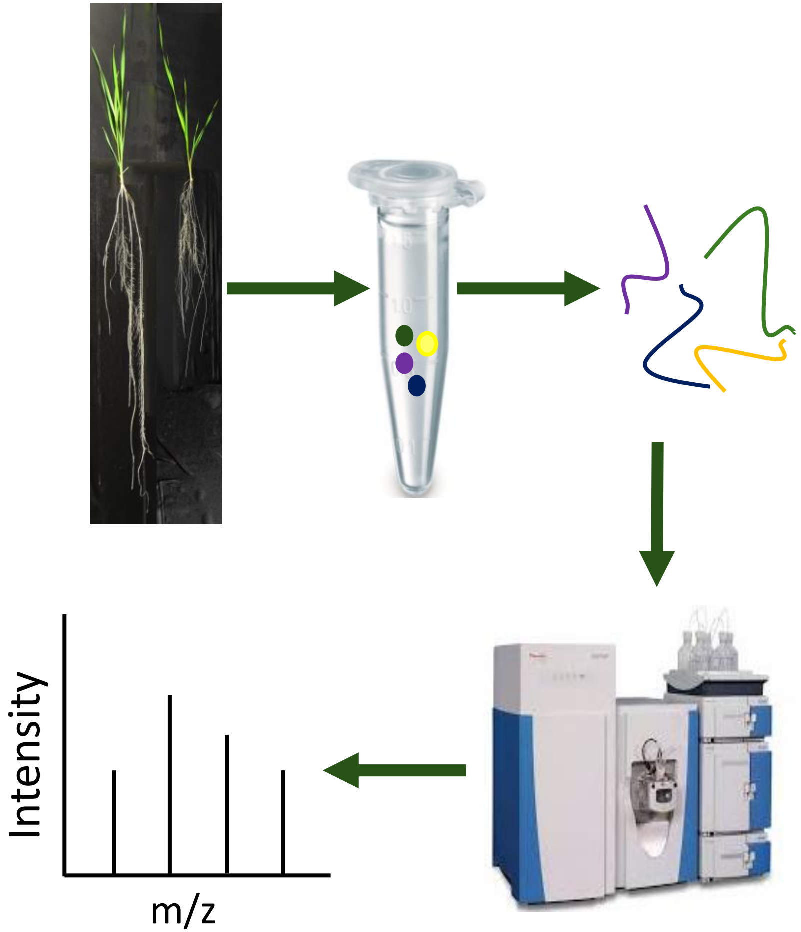
Unravelling protein biomarkers for improving root traits in bread wheat using proteomics



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13/03/2025

Acknowledgments

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Dr **Elke Stroeher**,
Proteomics Facility Manager,
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The University of Western Australia (UWA);

The UWA Institute of Agriculture;

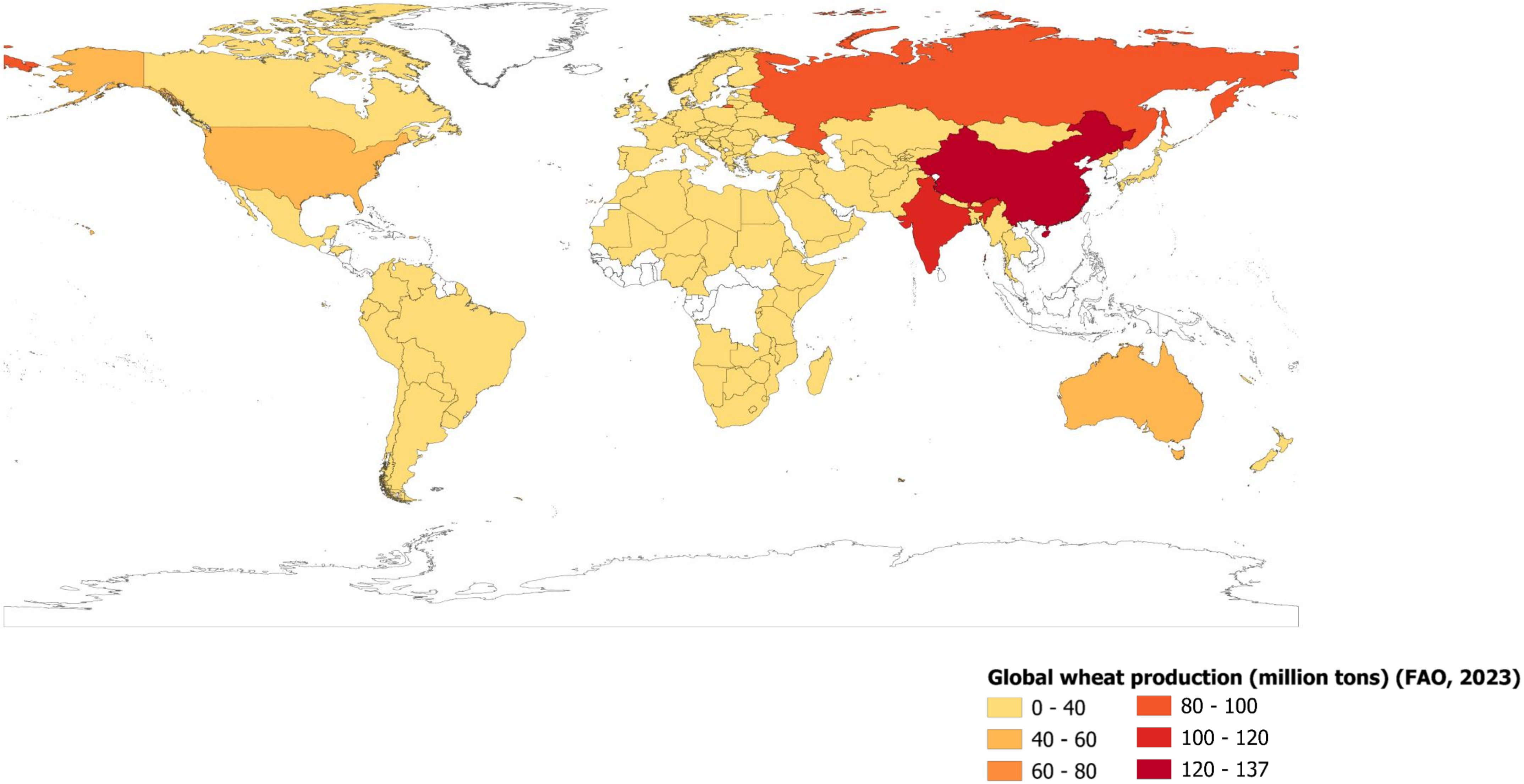
UWA School of Agriculture and Environment

Financial support:

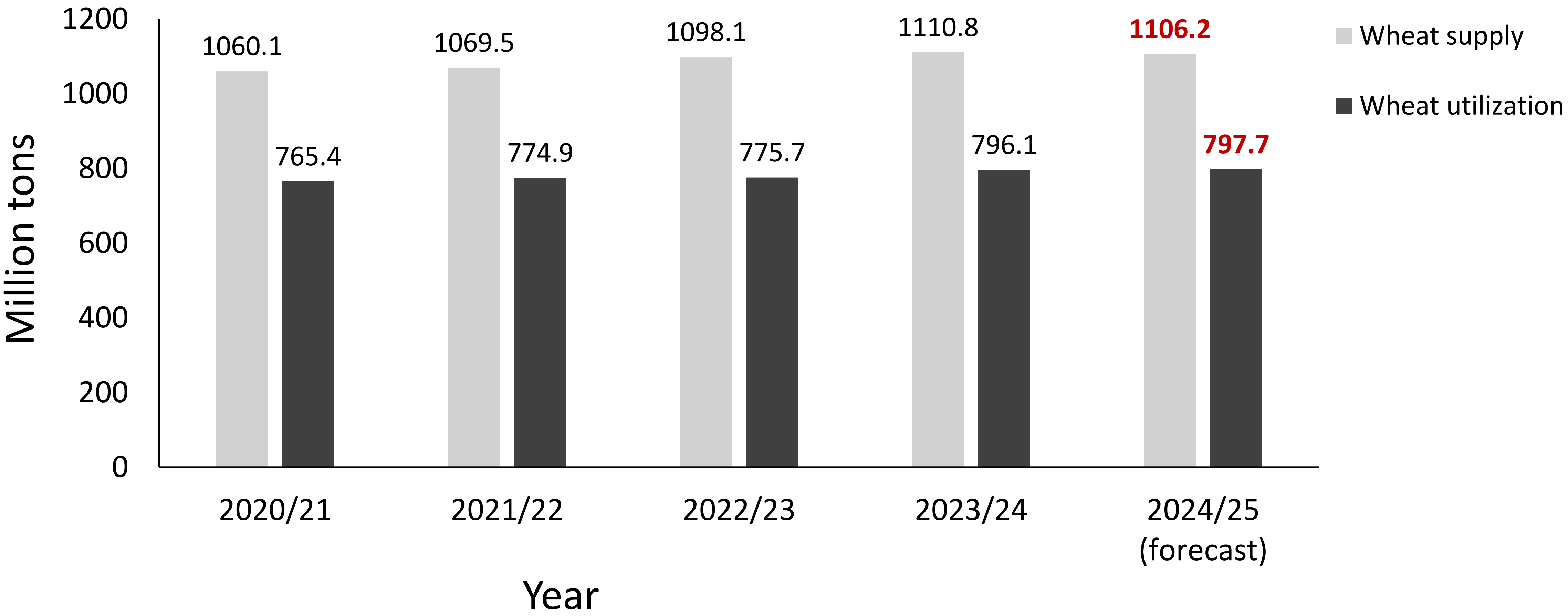
UWA Research Training Scholarship (RTP) and

Underwood PhD Completion Scholarship

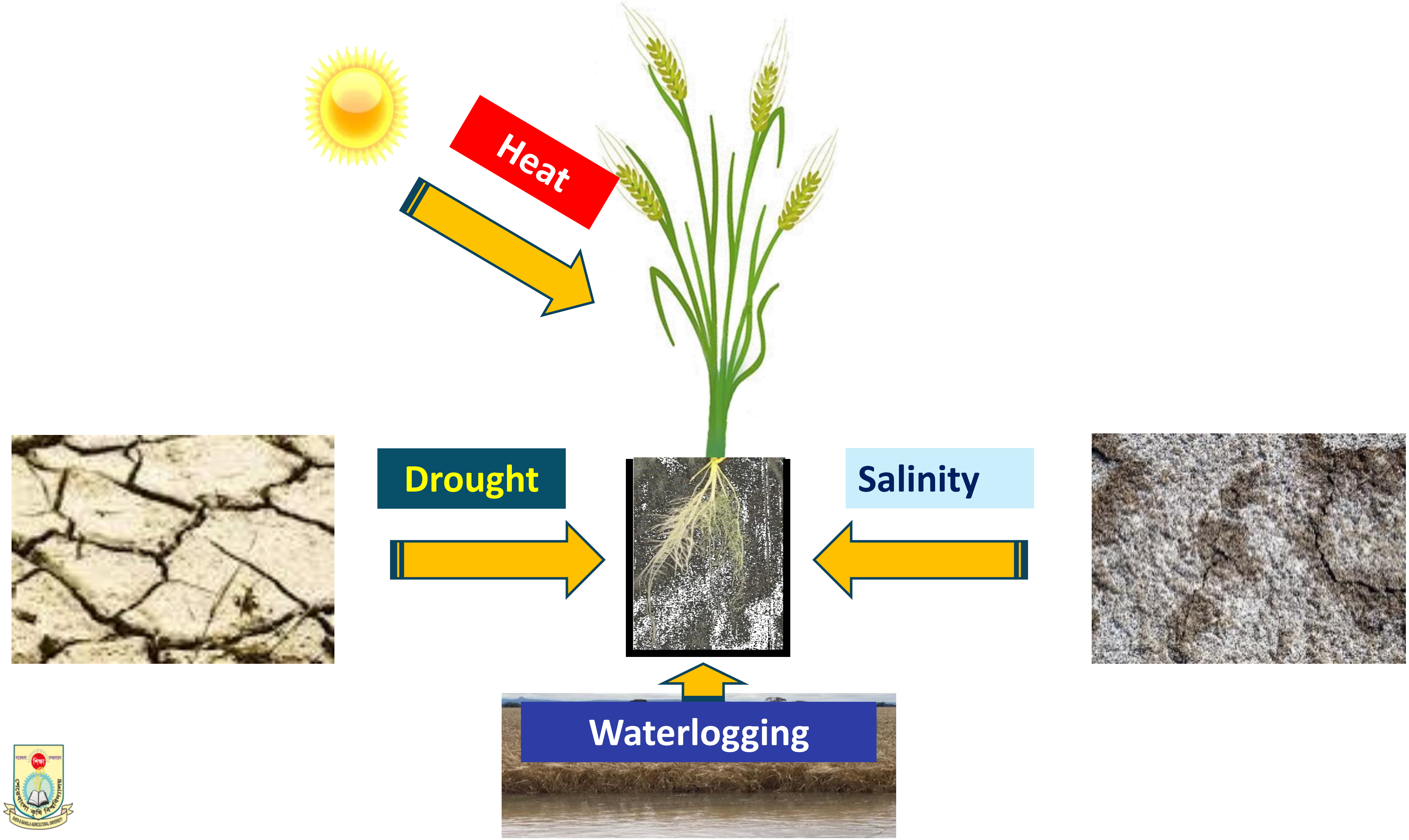
Background


Global wheat production (million tons) (FAO, 2023)
0 - 40
40 - 60
60 - 80
80 - 100
100 - 120
120 - 137

Background



Source: FAO (2025)



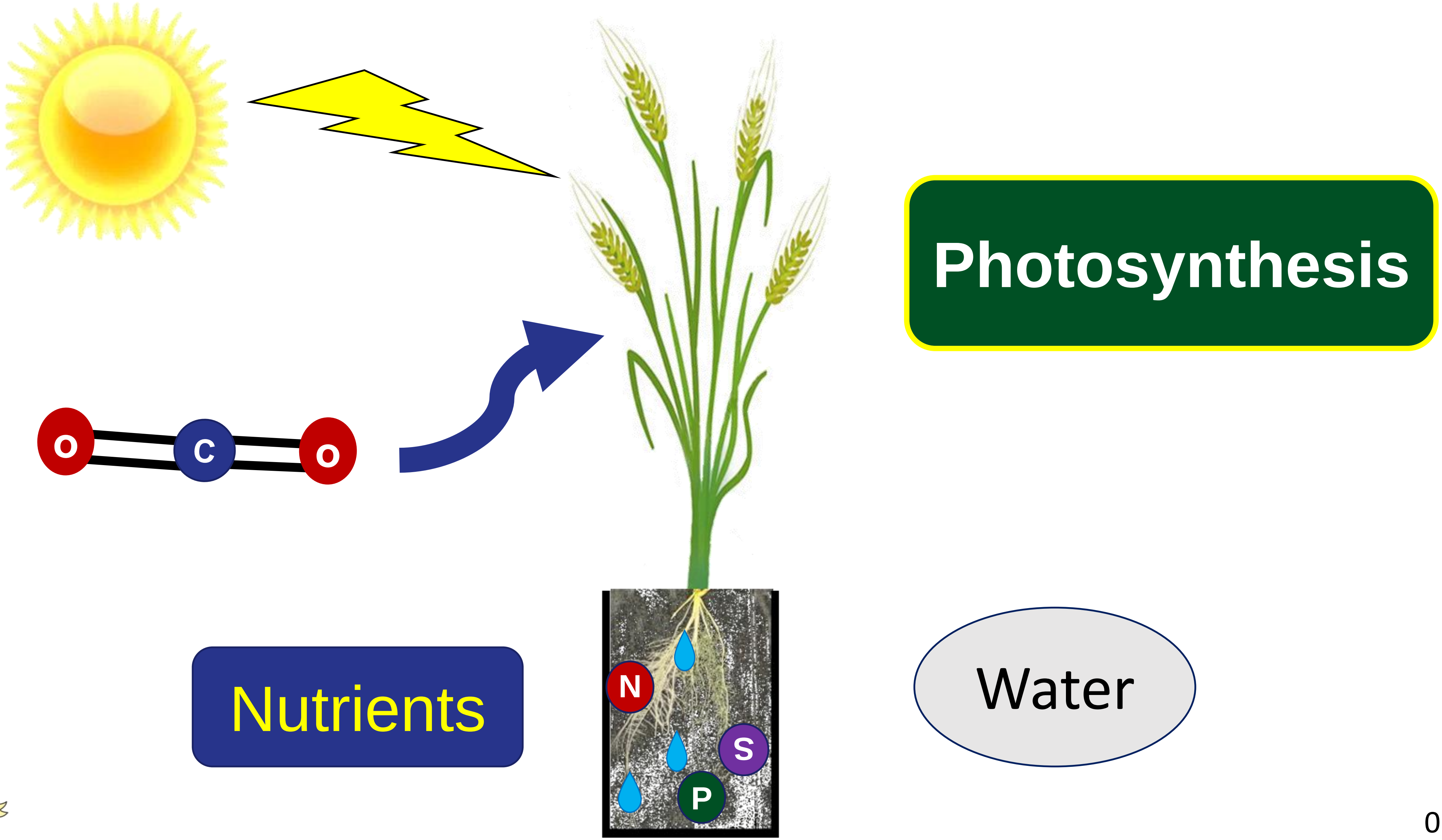
Heat

Drought

Salinity

Waterlogging

Importance of root system architecture (RSA)



Photosynthesis

Nutrients

Water

THE UNIVERSITY OF WESTERN AUSTRALIA

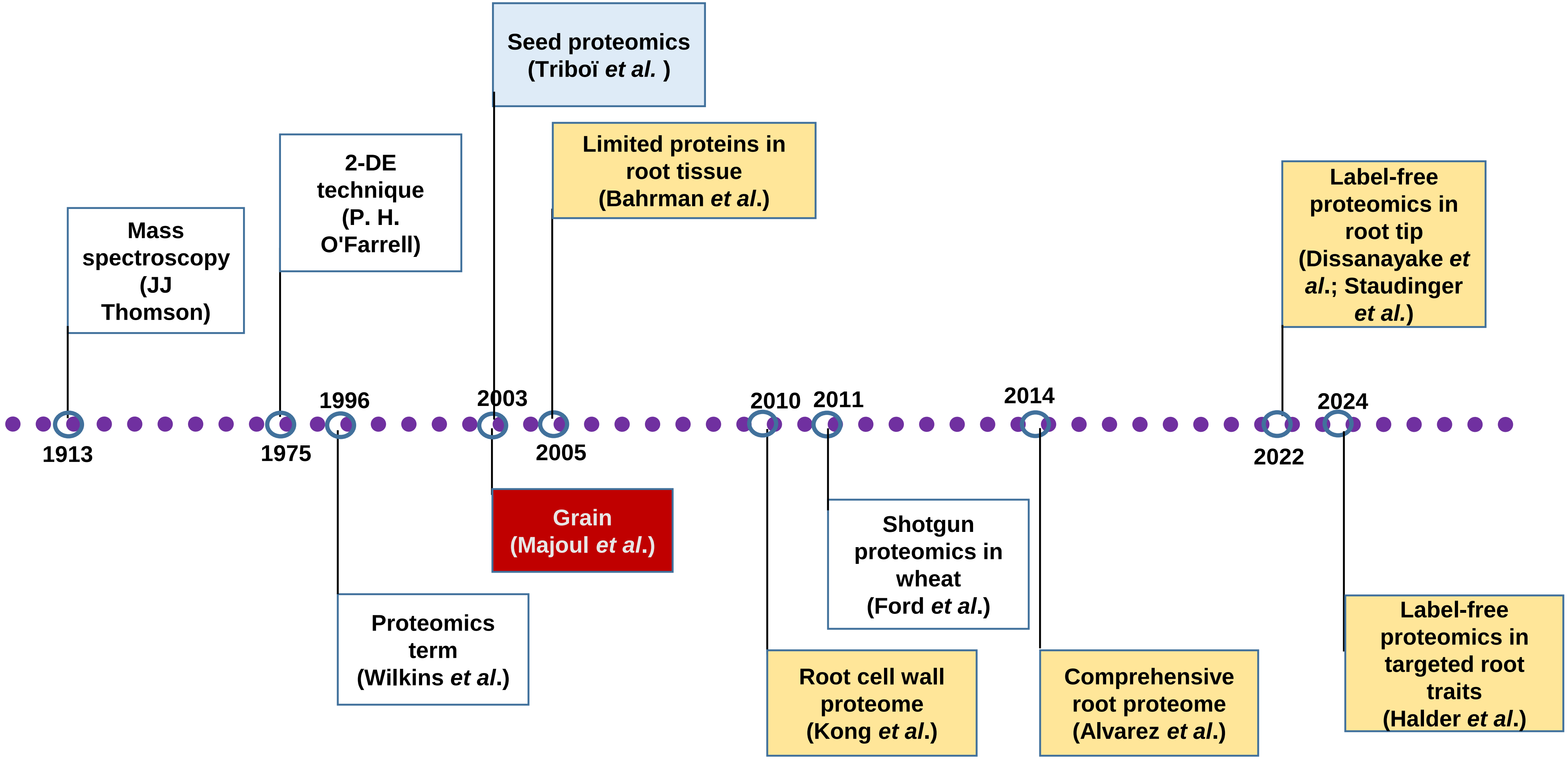
Challenges in studying RSA in wheat

- ❑Below ground plant part
- ❑Soil heterogeneity
- ❑Challenge to get reliable data
- ❑Quantitative nature of root traits
- ❑Large and complex genome of wheat

Strategies for root trait improvement

☐Conventional breeding →	Less precise and time consuming
☐Genomics →	Actual trait controlling protein information is missing
☐Transcriptomics →	Lack of correlation between transcription and translation
☐Proteomics →	Precise genetic data for trait expression

Progress of proteomics on wheat root traits



1913

Mass spectroscopy (JJ Thomson)

1975

2-DE technique (P. H. O'Farrell)

1996

Proteomics term (Wilkins *et al.*)

2003

Seed proteomics (Triboï *et al.*)

Grain (Majoul *et al.*)

2005

Limited proteins in root tissue (Bahrman *et al.*)

2010

Root cell wall proteome (Kong *et al.*)

2011

Shotgun proteomics in wheat (Ford *et al.*)

2014

Comprehensive root proteome (Alvarez *et al.*)

2022

Label-free proteomics in root tip (Dissanayake *et al.*; Staudinger *et al.*)

2024

Label-free proteomics in targeted root traits (Halder *et al.*)

🟥 **Heat** 🟦 **Drought** 🟨 **Root system architecture (RSA)**

Halder *et al*. (2022), *Proteomes.* 10(2), 71.

Progress of proteomics on wheat root traits

proteomes

MDPI

Review

Wheat Proteomics for Abiotic Stress Tolerance and Root System Architecture: Current Status and Future Prospects

Tanushree Halder [1,2,3,*], Mukesh Choudhary [1,2,4,*], Hui Liu [1,2], Yinglong Chen [1,2], Guijun Yan [1,2]
and Kadambot H. M. Siddique [1,2,*]

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Abstract: Wheat is an important staple cereal for global food security. However, climate change is hampering wheat production due to abiotic stresses, such as heat, salinity, and drought. Besides shoot architectural traits, improving root system architecture (RSA) traits have the potential to improve yields under normal and stressed environments. RSA growth and development and other stress responses involve the expression of proteins encoded by the trait controlling gene/genes. Hence, mining the key proteins associated with abiotic stress responses and RSA is important for improving sustainable yields in wheat. Proteomic studies in wheat started in the early 21st century using the two-dimensional (2-DE) gel technique and have extensively improved over time with advancements in mass spectrometry. The availability of the wheat reference genome has allowed the exploration of proteomics to identify differentially expressed or abundant proteins (DEPs or DAPs) for abiotic stress tolerance and RSA improvement. Proteomics contributed significantly to identifying key proteins imparting abiotic stress tolerance, primarily related to photosynthesis, protein synthesis, carbon metabolism, redox homeostasis, defense response, energy metabolism and signal transduction. However, the use of proteomics to improve RSA traits in wheat is in its infancy. Proteins related to cell wall biogenesis, carbohydrate metabolism, brassinosteroid biosynthesis, and transportation are involved in the growth and development of several RSA traits. This review covers advances in quantification techniques of proteomics, progress in identifying DEPs and/or DAPs for heat, salinity, and drought stresses, and RSA traits, and the limitations and future directions for harnessing proteomics in wheat improvement.

check for updates

Citation: Halder, T.; Choudhary, M.; Liu, H.; Chen, Y.; Yan, G.; Siddique, K.H.M. Wheat Proteomics for Abiotic Stress Tolerance and Root System Architecture: Current Status and Future Prospects. *Proteomes* 2022, *10*, 17. https://doi.org/10.3390/proteomes10020017

Academic Editor: Rainer Cramer

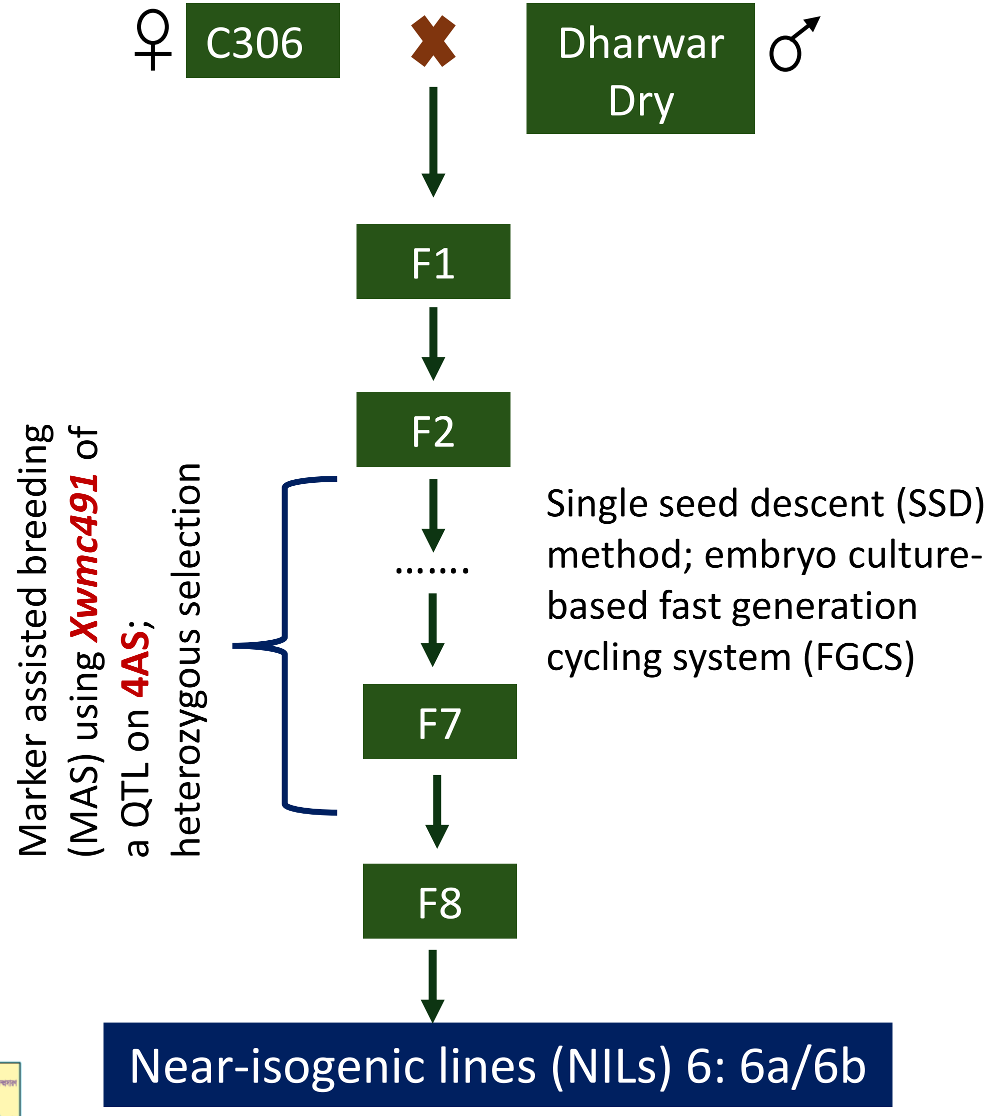
Received: 5 April 2022
Accepted: 11 May 2022
Published: 22 May 2022

DOI: 10.3390/proteomes10020017

Objectives

- To identify the **differentially abundant proteins (DAPs)** controlling total root length (RL) and root dry mass (RM) in near-isogenic lines (NILs) of wheat using a label-free proteomics approach.
- To identify the **molecular pathways** of the DAPs of the NIL pairs.
- To identify the **candidate protein biomarkers** for the target root traits by comparing mRNA and protein expressions.

Materials



♀ C306 ✖ Dharwar Dry ♂

F1

F2

…….

F7

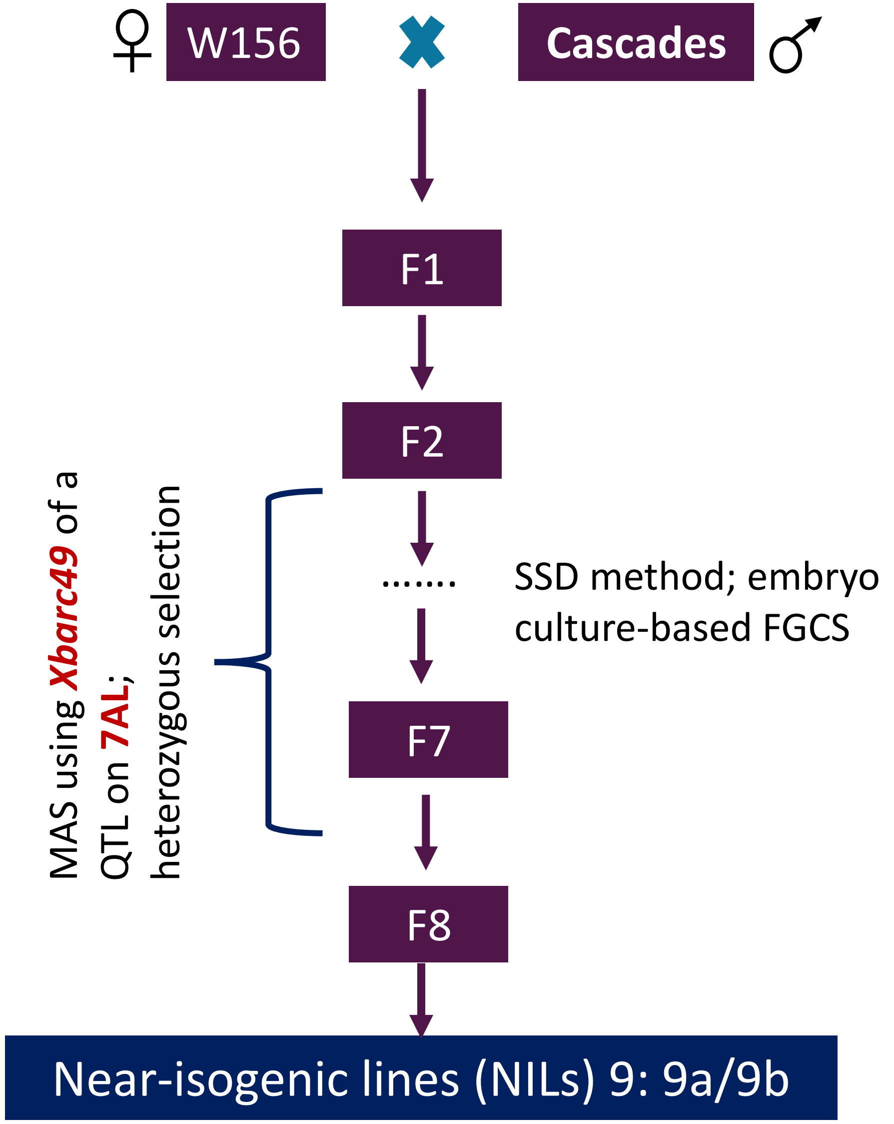
F8

Marker assisted breeding (MAS) using ***Xwmc491*** of a QTL on **4AS**; heterozygous selection

Single seed descent (SSD) method; embryo culture-based fast generation cycling system (FGCS)

Near-isogenic lines (NILs) 6: 6a/6b

Mia *et al*. (2019), *Front. Plant Sci*. 10, 271.



♀ W156 ✖ **Cascades** ♂

F1

F2

…….

F7

F8

MAS using ***Xbarc49*** of a QTL on **7AL**; heterozygous selection

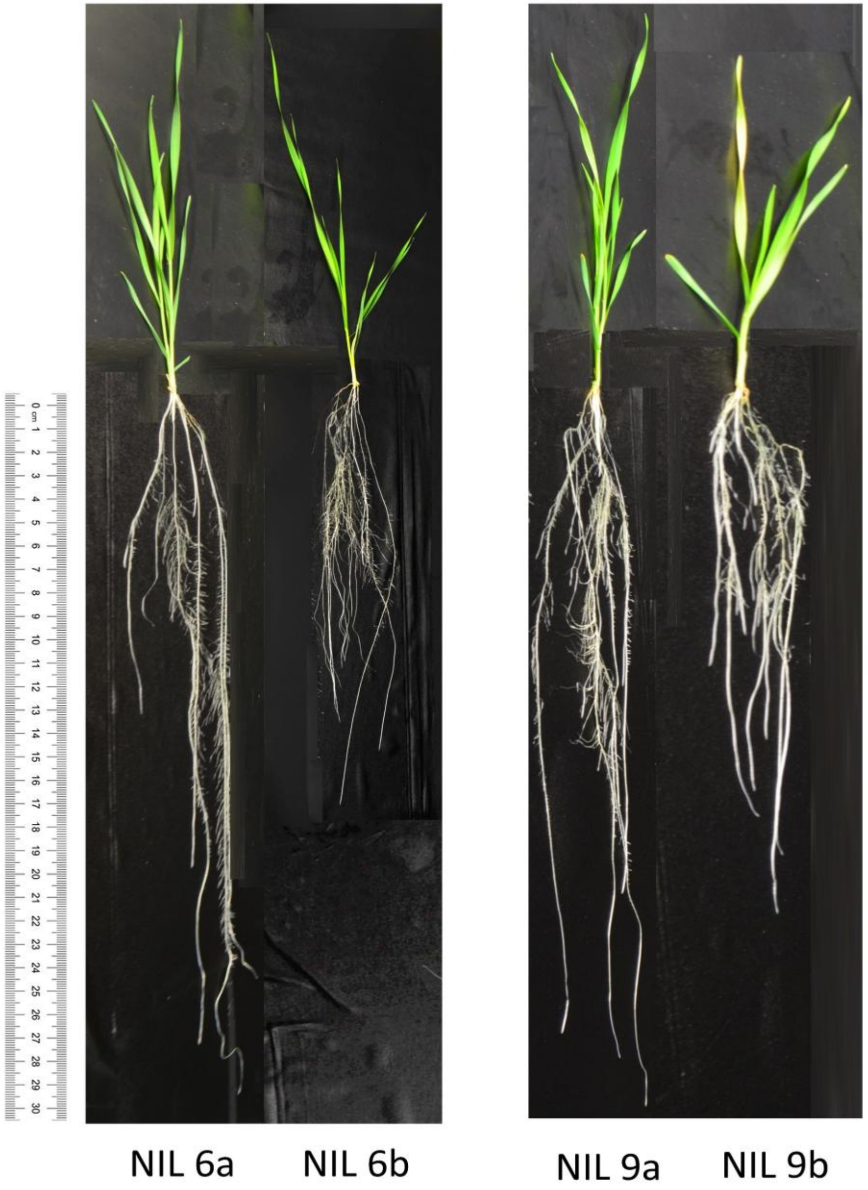
SSD method; embryo culture-based FGCS

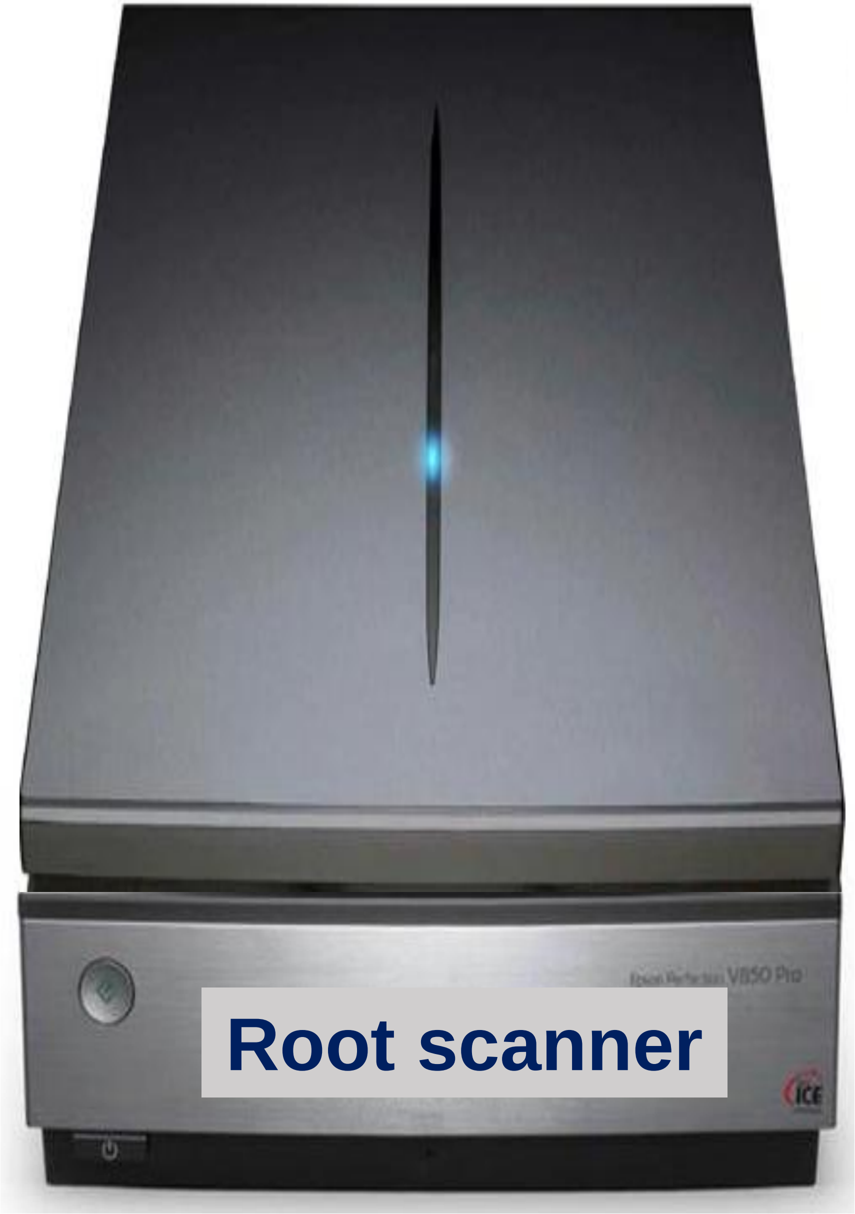
Near-isogenic lines (NILs) 9: 9a/9b

Lu *et al*. (2020), *Front. Plant Sci*. 11, 1316.

Methods

Methods





Root scanner

WinRHIZO software

Methods

Root tissue

Protein extraction:

Chloroform/methanol precipitation method

Protein quantification:

Bradford assay

Protein digestion:

Trypsin

Label-free quantitation:

MaxQuant software

DAPs:

Perseus (version 1.1.16.0)

IWGSC RefSeq v1.0

Pathway enrichment analyses:

GO: agriGO v2.0

KEGG: BLASTKoala

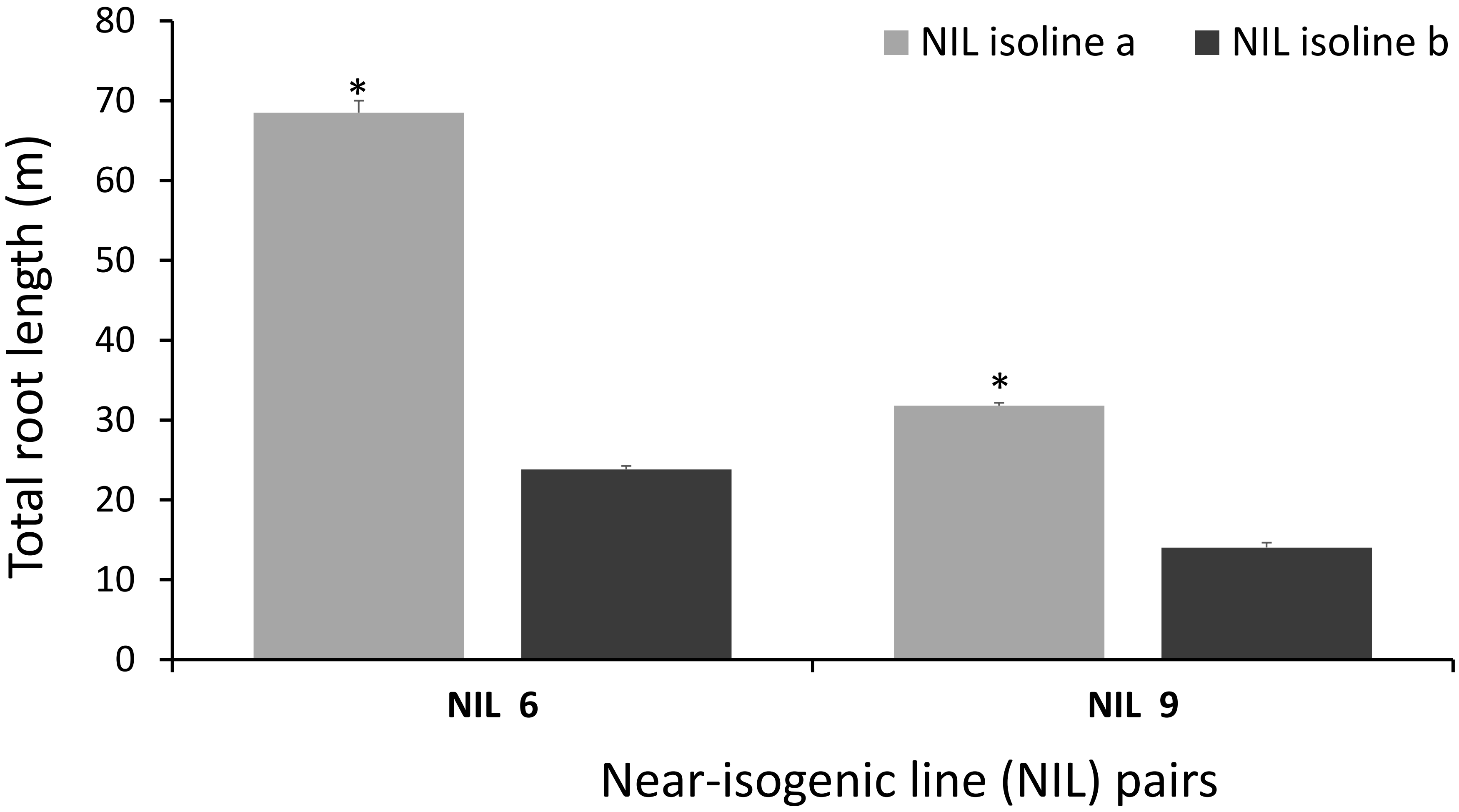
mRNA expression analysis:

qRT-PCR

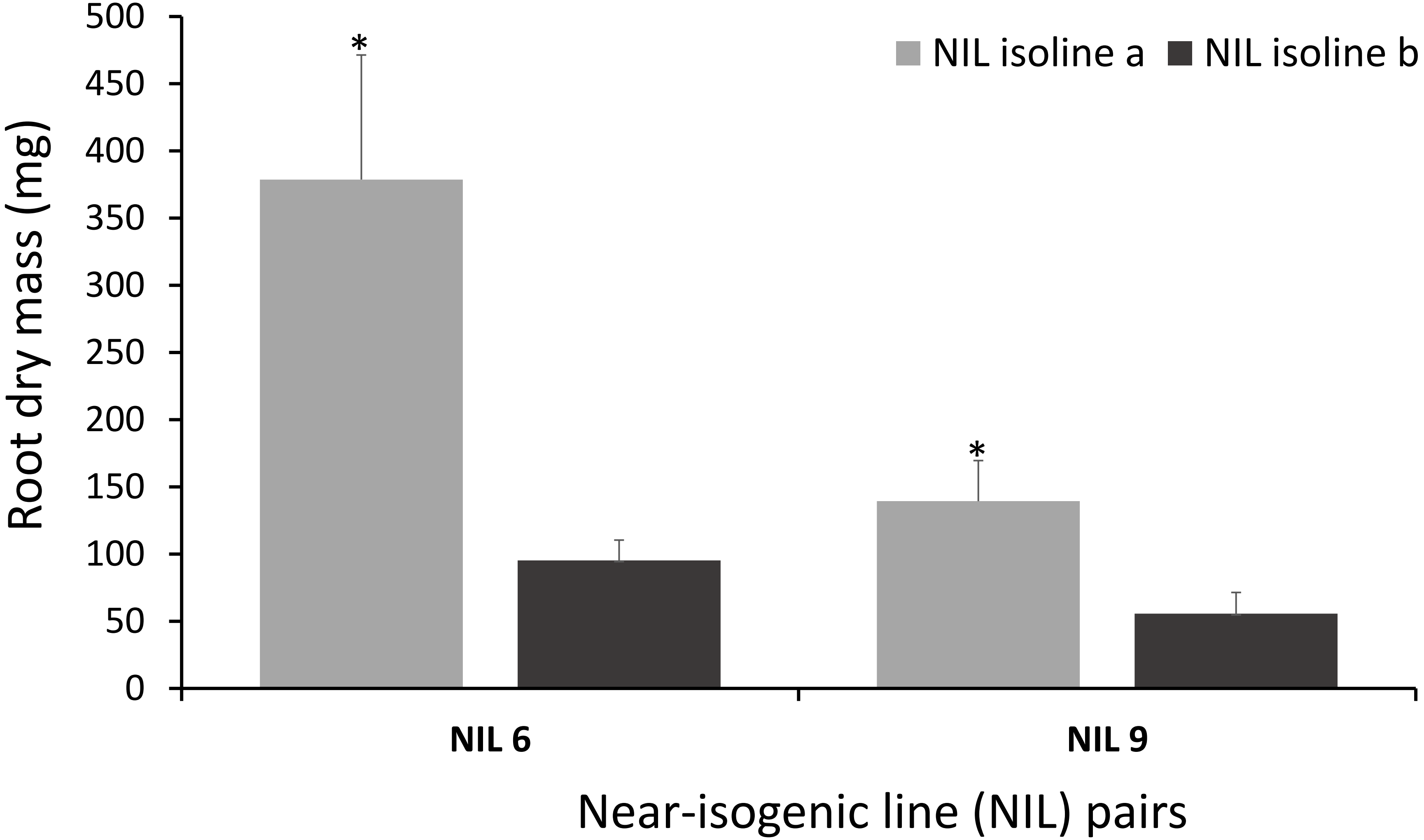
Candidate protein biomarkers:

DAPs showing similar expression at protein and mRNA levels

Phenotypic performance of the NIL pairs



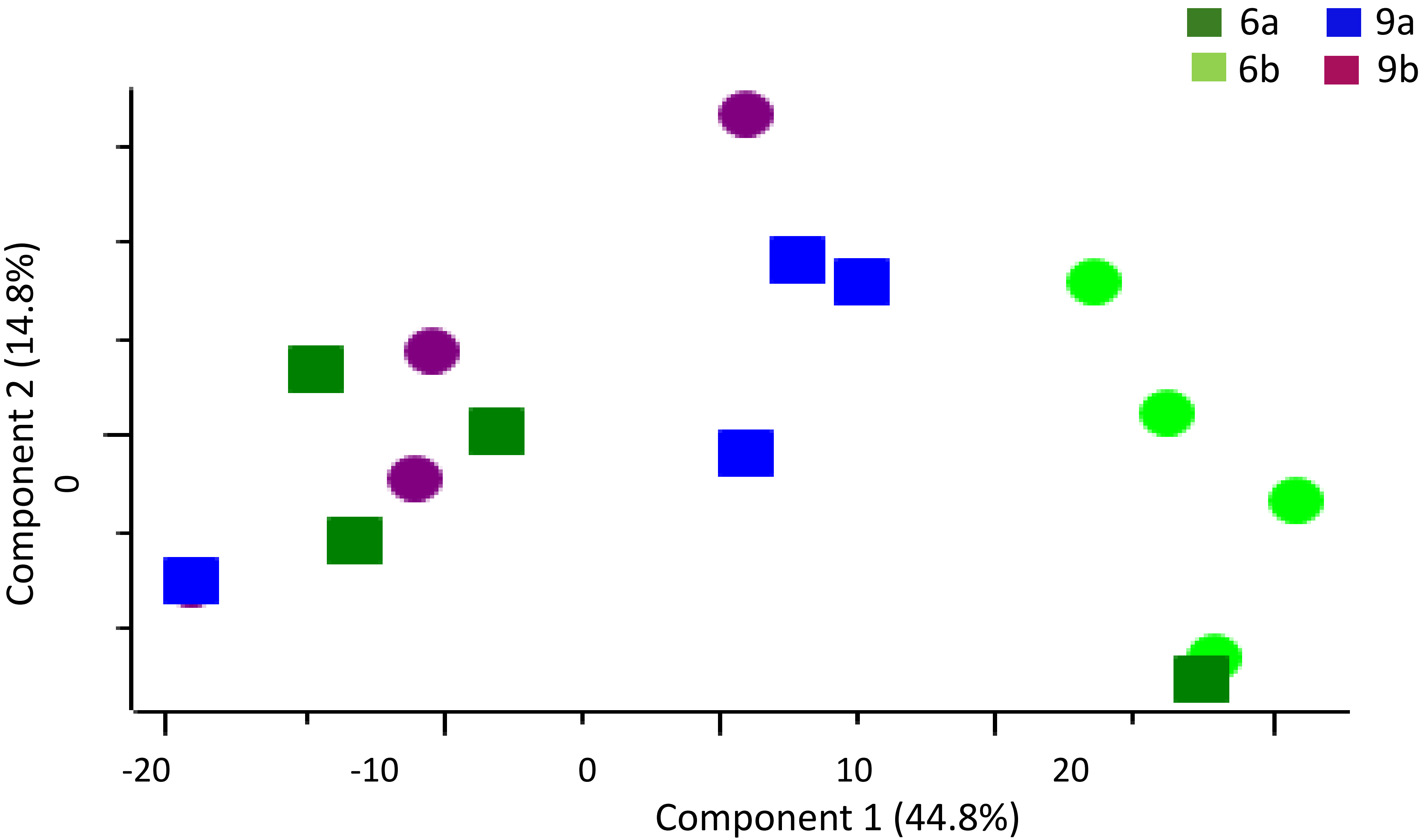
Phenotypic performance of the NIL pairs



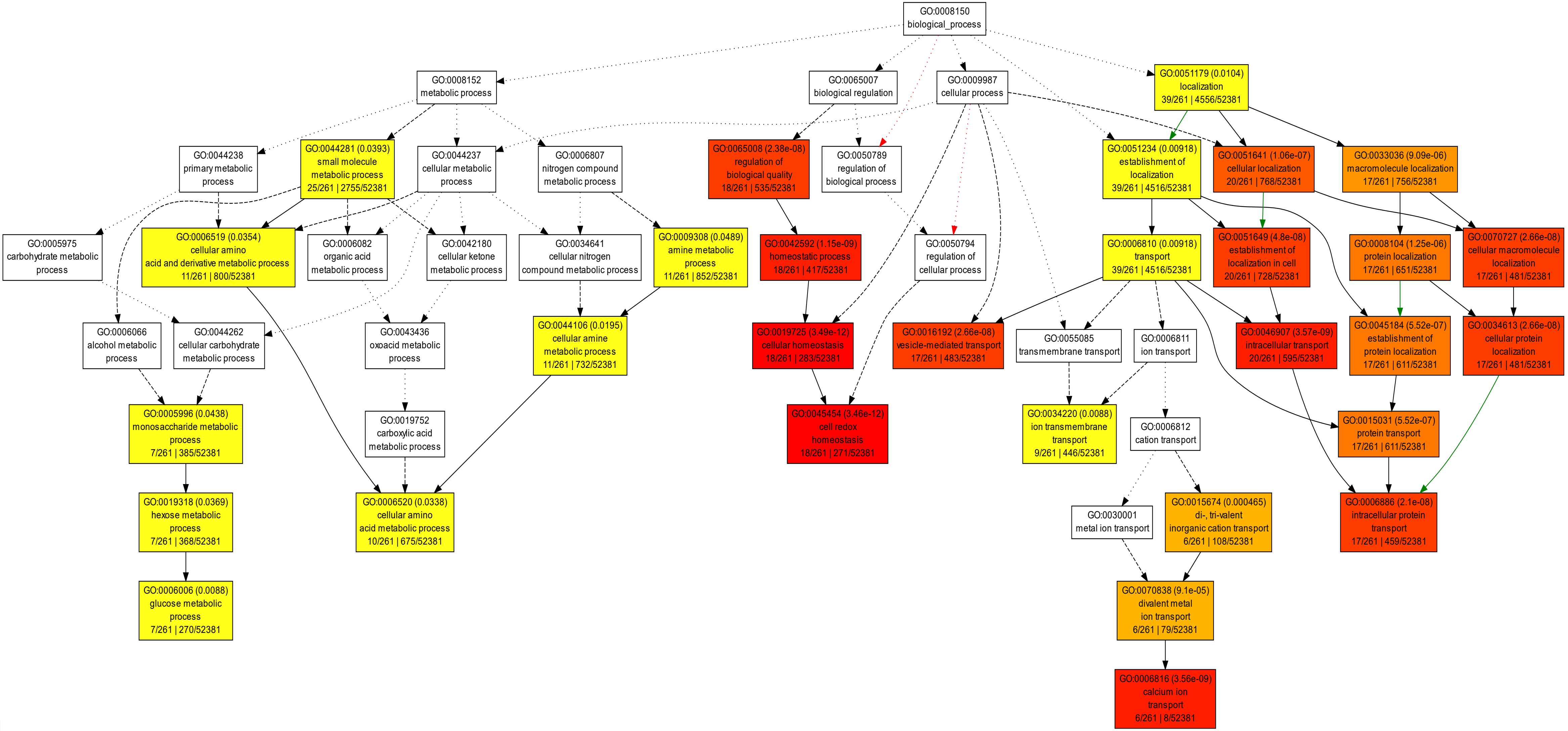
Proteins controlling root traits in wheat

- Total proteins from NIL pairs 6 and 9: **6,721**
- Total proteins with minimum two peptides: 5,882
- Total proteins in NIL pair 6: 4,101
- Total proteins in NIL pair 9: 4,932
- DAPs in NIL pair 6: **113** (high abundant: 99 and low abundant: 14)
- DAPs in NIL pair 9: **30** (high abundant: 09 and low abundant: 21)

Principle component analysis of the DAPs



Biological process of the DAPs in NIL pair 6



GO:0008150
biological_process

GO:0008152
metabolic process

GO:0065007
biological regulation

GO:0009987
cellular process

GO:0051179 (0.0104)
localization
39/261 | 4556/52381

GO:0044238
primary metabolic
process

GO:0044281 (0.0393)
small molecule
metabolic process
25/261 | 2755/52381

GO:0044237
cellular metabolic
process

GO:0006807
nitrogen compound
metabolic process

GO:0065008 (2.38e-08)
regulation of
biological quality
18/261 | 535/52381

GO:0050789
regulation of
biological process

GO:0051234 (0.00918)
establishment of
localization
39/261 | 4516/52381

GO:0051641 (1.06e-07)
cellular localization
20/261 | 768/52381

GO:0033036 (9.09e-06)
macromolecule localization
17/261 | 756/52381

GO:0005975
carbohydrate metabolic
process

GO:0006519 (0.0354)
cellular amino
acid and derivative metabolic process
11/261 | 800/52381

GO:0006082
organic acid
metabolic process

GO:0042180
cellular ketone
metabolic process

GO:0034641
cellular nitrogen
compound metabolic process

GO:0009308 (0.0489)
amine metabolic
process
11/261 | 852/52381

GO:0042592 (1.15e-09)
homeostatic process
18/261 | 417/52381

GO:0050794
regulation of
cellular process

GO:0006810 (0.00918)
transport
39/261 | 4516/52381

GO:0051649 (4.8e-08)
establishment of
localization in cell
20/261 | 728/52381

GO:0008104 (1.25e-06)
protein localization
17/261 | 651/52381

GO:0070727 (2.66e-08)
cellular macromolecule
localization
17/261 | 481/52381

GO:0006066
alcohol metabolic
process

GO:0044262
cellular carbohydrate
metabolic process

GO:0043436
oxoacid metabolic
process

GO:0044106 (0.0195)
cellular amine
metabolic process
11/261 | 732/52381

GO:0019725 (3.49e-12)
cellular homeostasis
18/261 | 283/52381

GO:0016192 (2.66e-08)
vesicle-mediated transport
17/261 | 483/52381

GO:0055085
transmembrane transport

GO:0006811
ion transport

GO:0046907 (3.57e-09)
intracellular transport
20/261 | 595/52381

GO:0045184 (5.52e-07)
establishment of
protein localization
17/261 | 611/52381

GO:0034613 (2.66e-08)
cellular protein
localization
17/261 | 481/52381

GO:0005996 (0.0438)
monosaccharide metabolic
process
7/261 | 385/52381

GO:0019752
carboxylic acid
metabolic process

GO:0045454 (3.46e-12)
cell redox
homeostasis
18/261 | 271/52381

GO:0034220 (0.0088)
ion transmembrane
transport
9/261 | 446/52381

GO:0006812
cation transport

GO:0015031 (5.52e-07)
protein transport
17/261 | 611/52381

GO:0019318 (0.0369)
hexose metabolic
process
7/261 | 368/52381

GO:0006520 (0.0338)
cellular amino
acid metabolic process
10/261 | 675/52381

GO:0030001
metal ion transport

GO:0015674 (0.000465)
di-, tri-valent
inorganic cation transport
6/261 | 108/52381

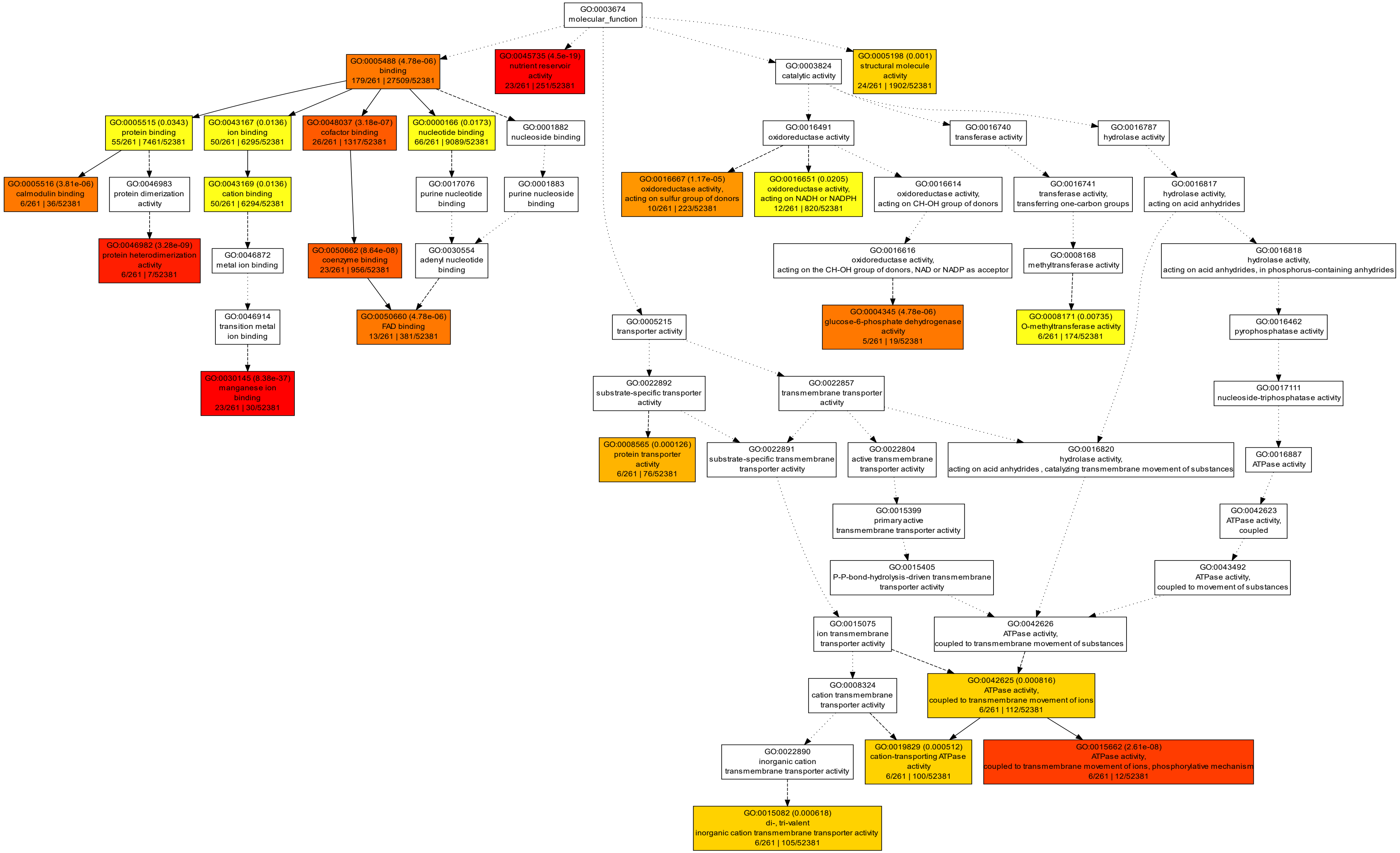
GO:0006886 (2.1e-08)
intracellular protein
transport
17/261 | 459/52381

GO:0006006 (0.0088)
glucose metabolic
process
7/261 | 270/52381

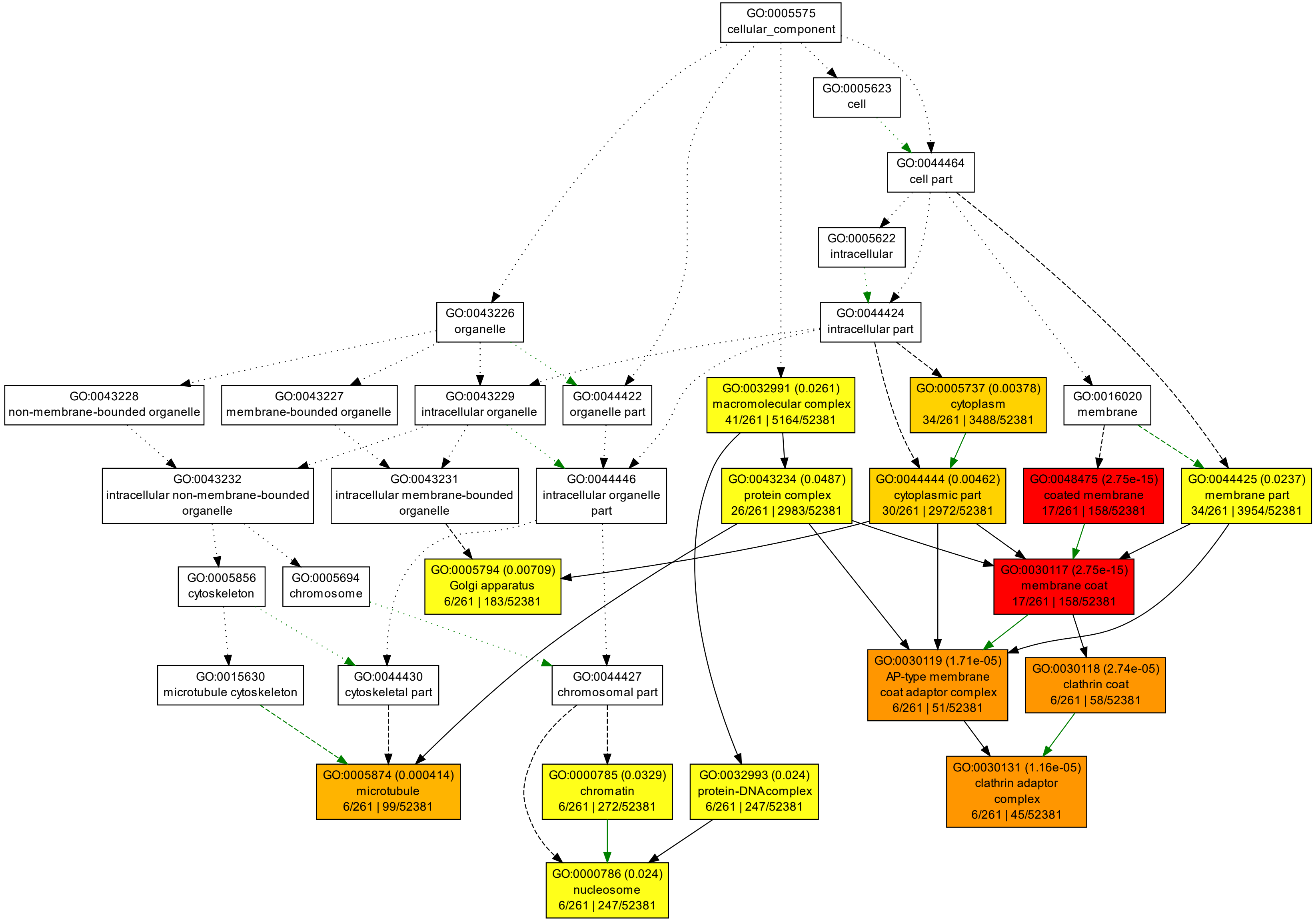
GO:0070838 (9.1e-05)
divalent metal
ion transport
6/261 | 79/52381

GO:0006816 (3.56e-09)
calcium ion
transport
6/261 | 8/52381

Molecular function of the DAPs in NIL pair 6



Cellular component of the DAPs in NIL pair 6



GO:0005575
cellular_component

GO:0005623
cell

GO:0044464
cell part

GO:0005622
intracellular

GO:0043226
organelle

GO:0044424
intracellular part

GO:0043228
non-membrane-bounded organelle

GO:0043227
membrane-bounded organelle

GO:0043229
intracellular organelle

GO:0044422
organelle part

GO:0032991 (0.0261)
macromolecular complex
41/261 | 5164/52381

GO:0005737 (0.00378)
cytoplasm
34/261 | 3488/52381

GO:0016020
membrane

GO:0043232
intracellular non-membrane-bounded organelle

GO:0043231
intracellular membrane-bounded organelle

GO:0044446
intracellular organelle part

GO:0043234 (0.0487)
protein complex
26/261 | 2983/52381

GO:0044444 (0.00462)
cytoplasmic part
30/261 | 2972/52381

GO:0048475 (2.75e-15)
coated membrane
17/261 | 158/52381

GO:0044425 (0.0237)
membrane part
34/261 | 3954/52381

GO:0005856
cytoskeleton

GO:0005694
chromosome

GO:0005794 (0.00709)
Golgi apparatus
6/261 | 183/52381

GO:0030117 (2.75e-15)
membrane coat
17/261 | 158/52381

GO:0015630
microtubule cytoskeleton

GO:0044430
cytoskeletal part

GO:0044427
chromosomal part

GO:0030119 (1.71e-05)
AP-type membrane coat adaptor complex
6/261 | 51/52381

GO:0030118 (2.74e-05)
clathrin coat
6/261 | 58/52381

GO:0005874 (0.000414)
microtubule
6/261 | 99/52381

GO:0000785 (0.0329)
chromatin
6/261 | 272/52381

GO:0032993 (0.024)
protein-DNAcomplex
6/261 | 247/52381

GO:0030131 (1.16e-05)
clathrin adaptor complex
6/261 | 45/52381

GO:0000786 (0.024)
nucleosome
6/261 | 247/52381

Significantly enriched pathways of the DAPs in NIL pair 6

Biological processes

- ❑Cell redox homeostasis
- ❑Calcium ion transport
- ❑Protein localization
- ❑Protein transport
- ❑Metabolic processes of proteins

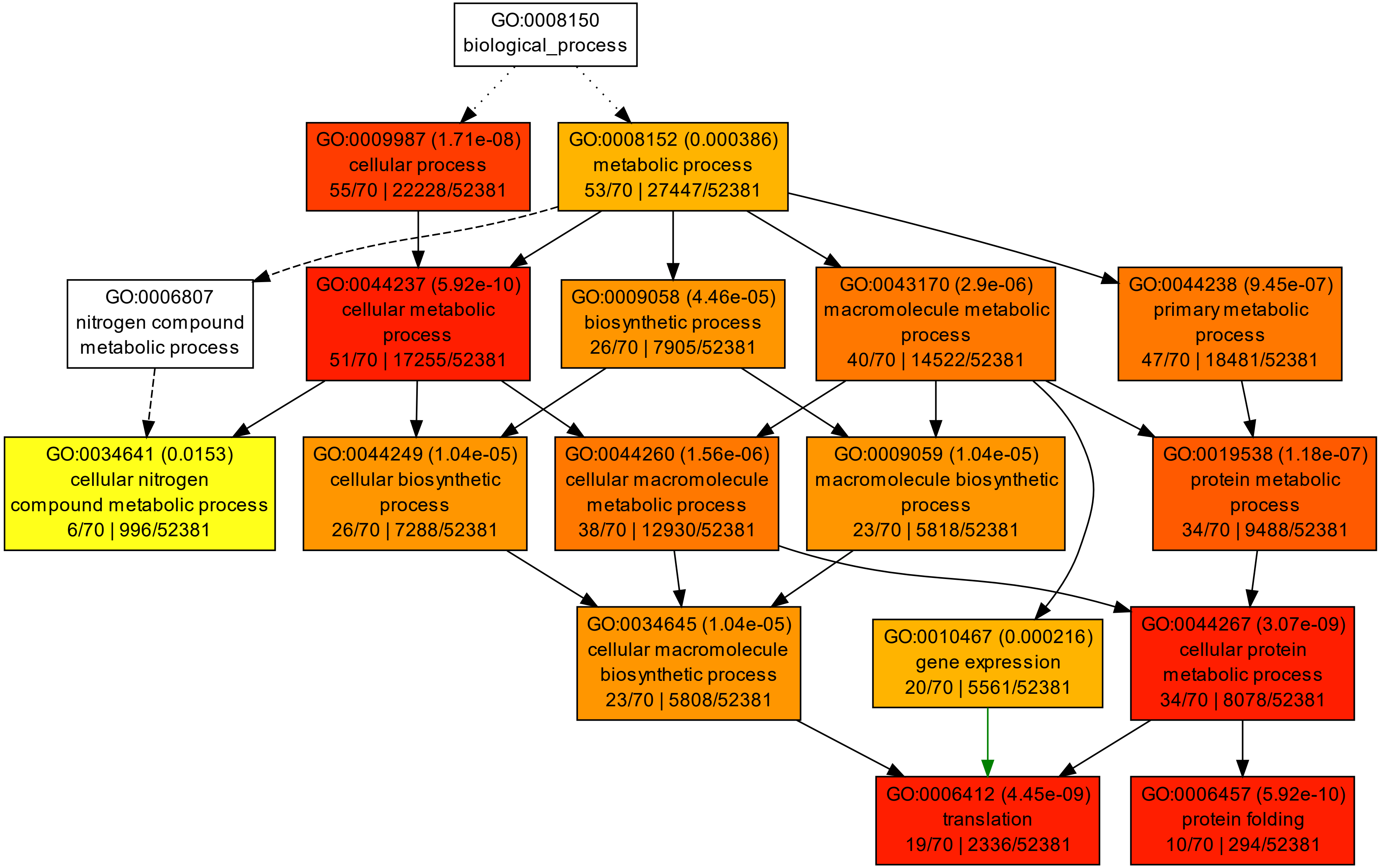
Molecular function

- ❖Structural molecule activity
- ❖Glucose-6-phosphate dehydrogenase activity
- ❖Oxidoreductase activity
- ❖Binding molecules, including, protein, nucleotide and manganese ions

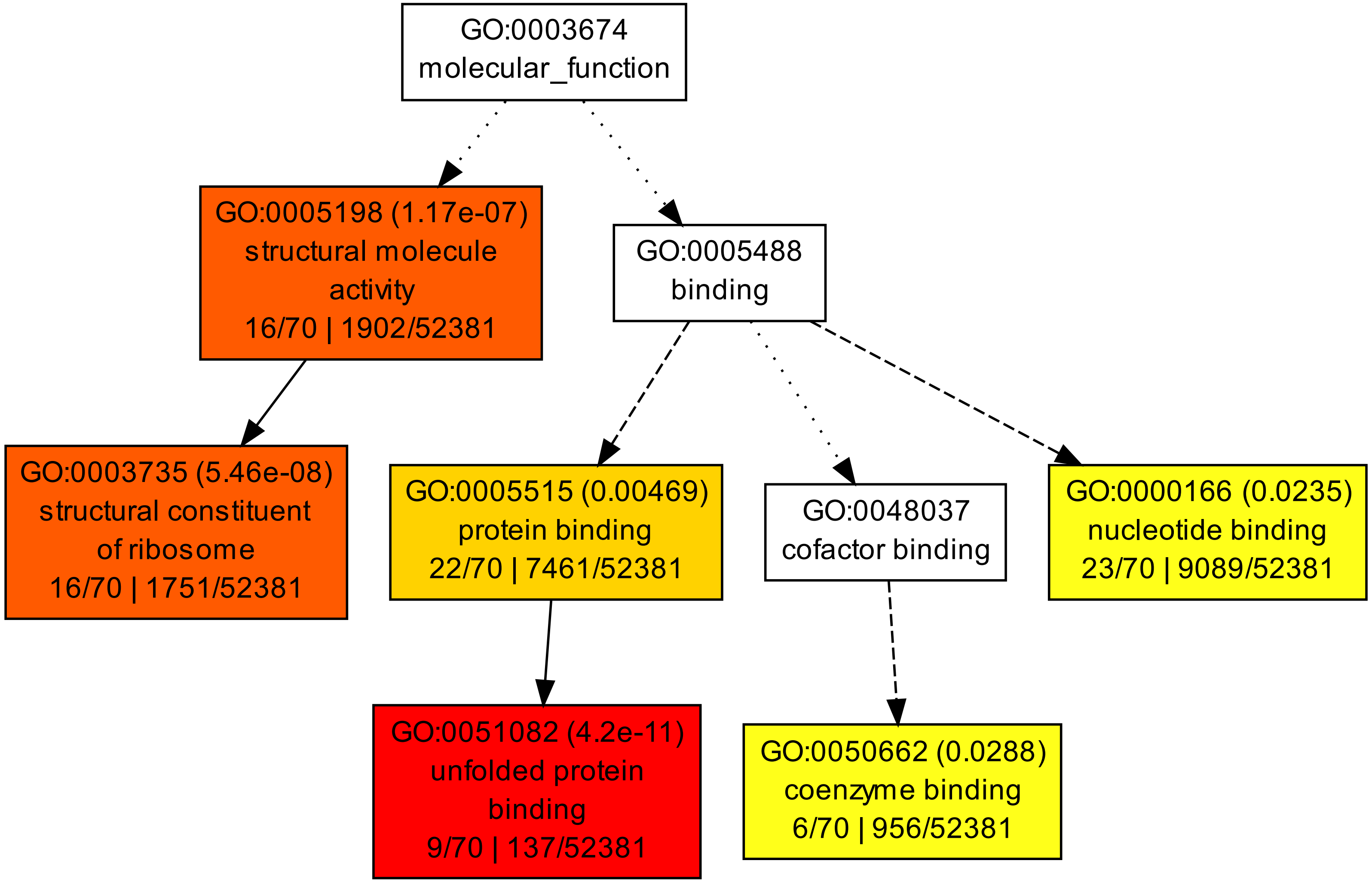
Cellular component

- ➢Membrane coat
- ➢Clathrin coat
- ➢Microtubule
- ➢Cytoplasm

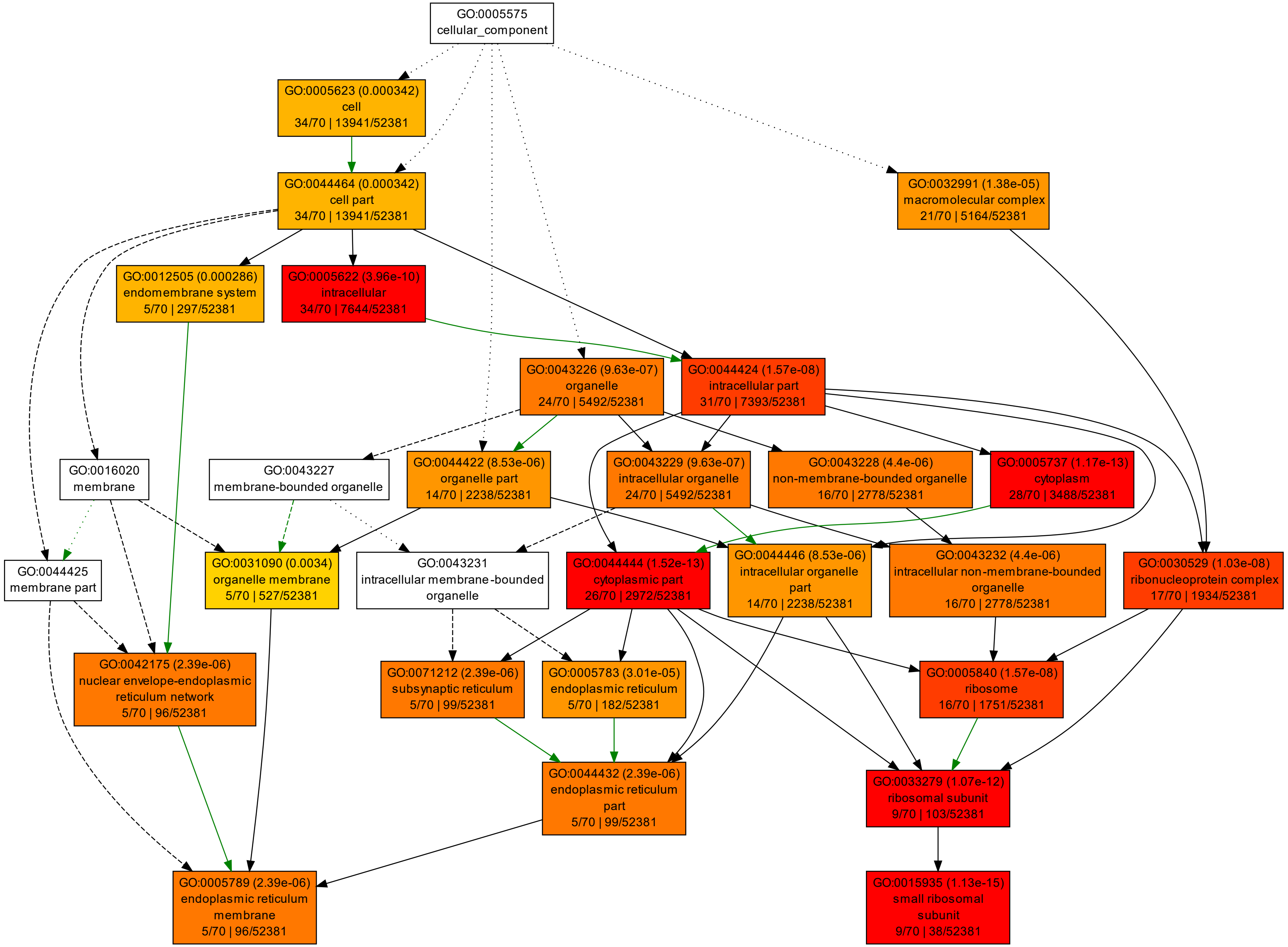
Biological process of the DAPs in NIL pair 9



Molecular function of the DAPs in NIL pair 9



Cellular component of the DAPs in NIL pair 9



Significantly enriched pathways of the DAPs in NIL pair 9

Biological processes

- Metabolic processes of cell, protein, macromolecules and other compounds
- Cellular processes and biosynthesis

Molecular function

- Unfolded protein binding
- Structural molecule formation
- Molecule binding activities

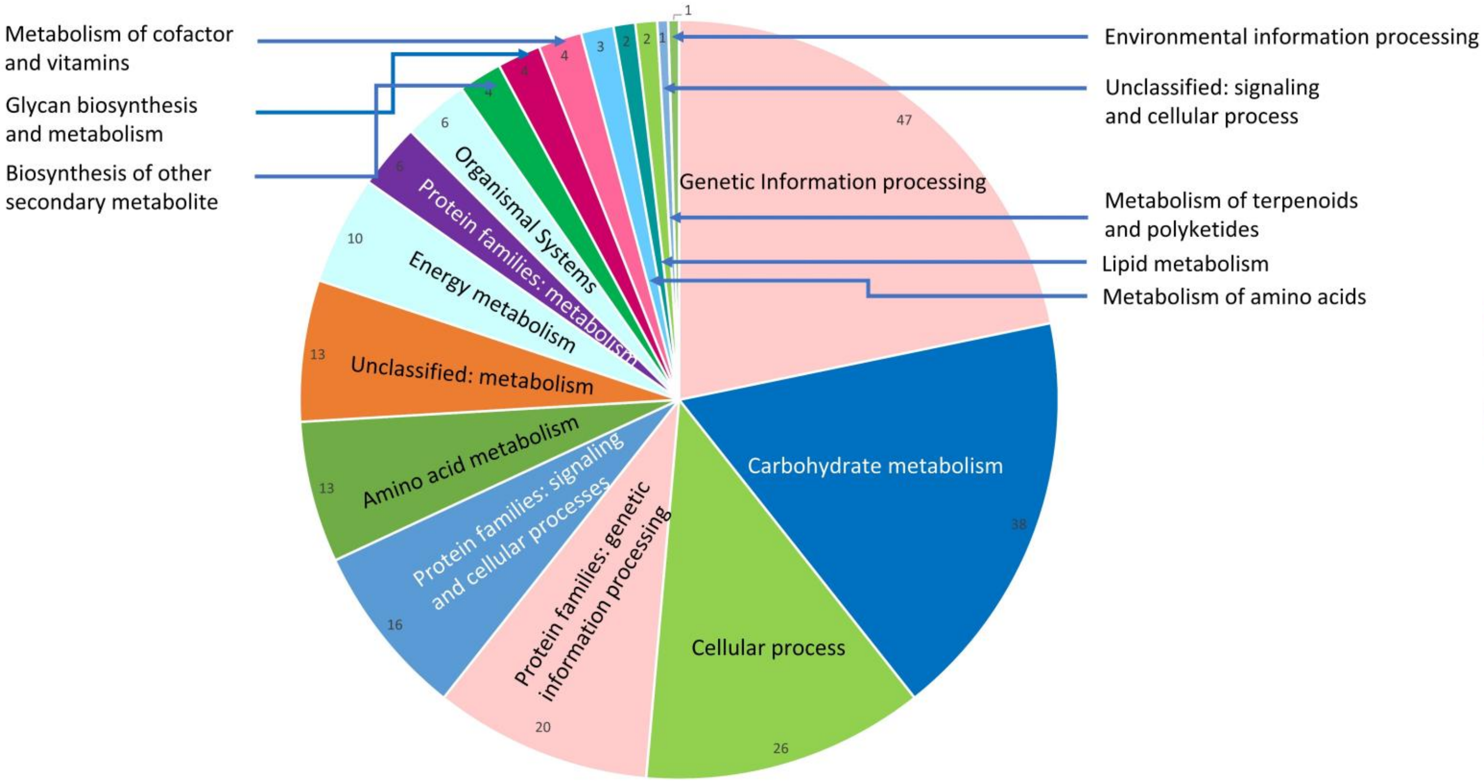
Cellular component

- Ribosomal subunits
- Cytoplasm
- Endoplasmic reticulum
- Other membranes

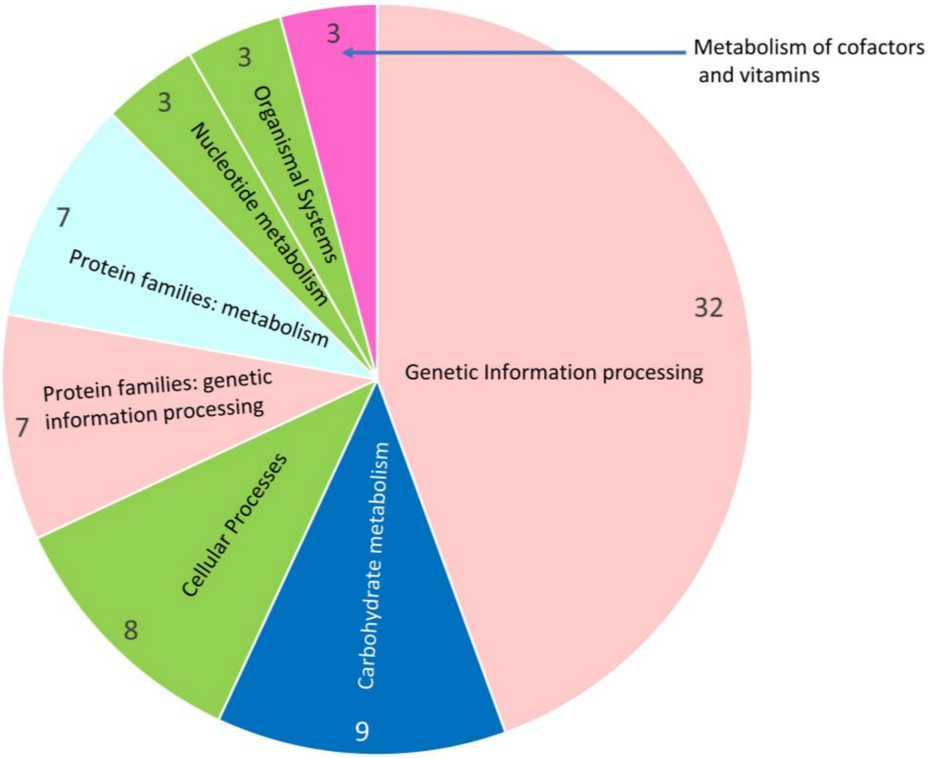
Common biological processes and molecular functions of DAPs in NIL pairs 6 and 9

Gene Ontology (GO) terms	Description
GO:0034641	Cellular nitrogen compound metabolic process
GO:0044281	Small molecule metabolic process
GO:0005198	Structural molecule activity
GO:0005515	Protein binding
GO:0050662	Coenzyme binding
GO:0048037	Cofactor binding
GO:0000166	Nucleotide binding
GO:0017076	Purine nucleotide binding

KEGG pathways of NIL pairs

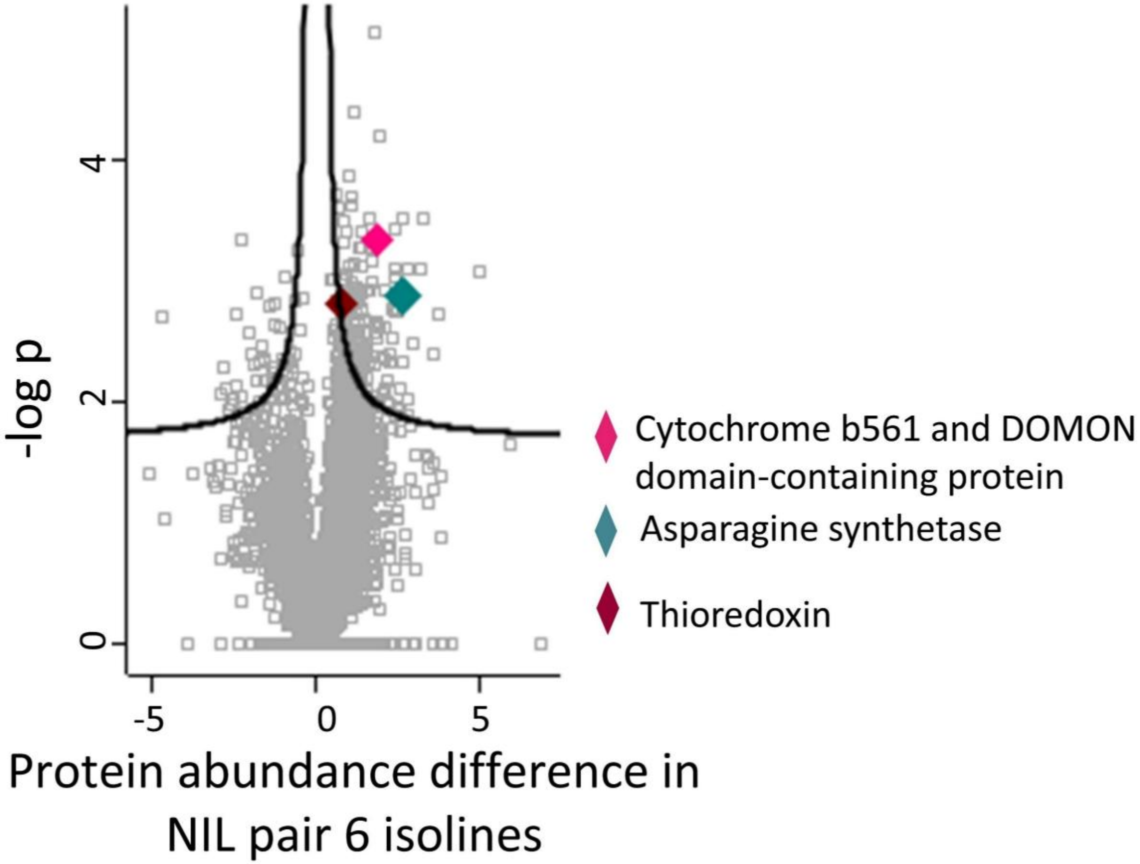


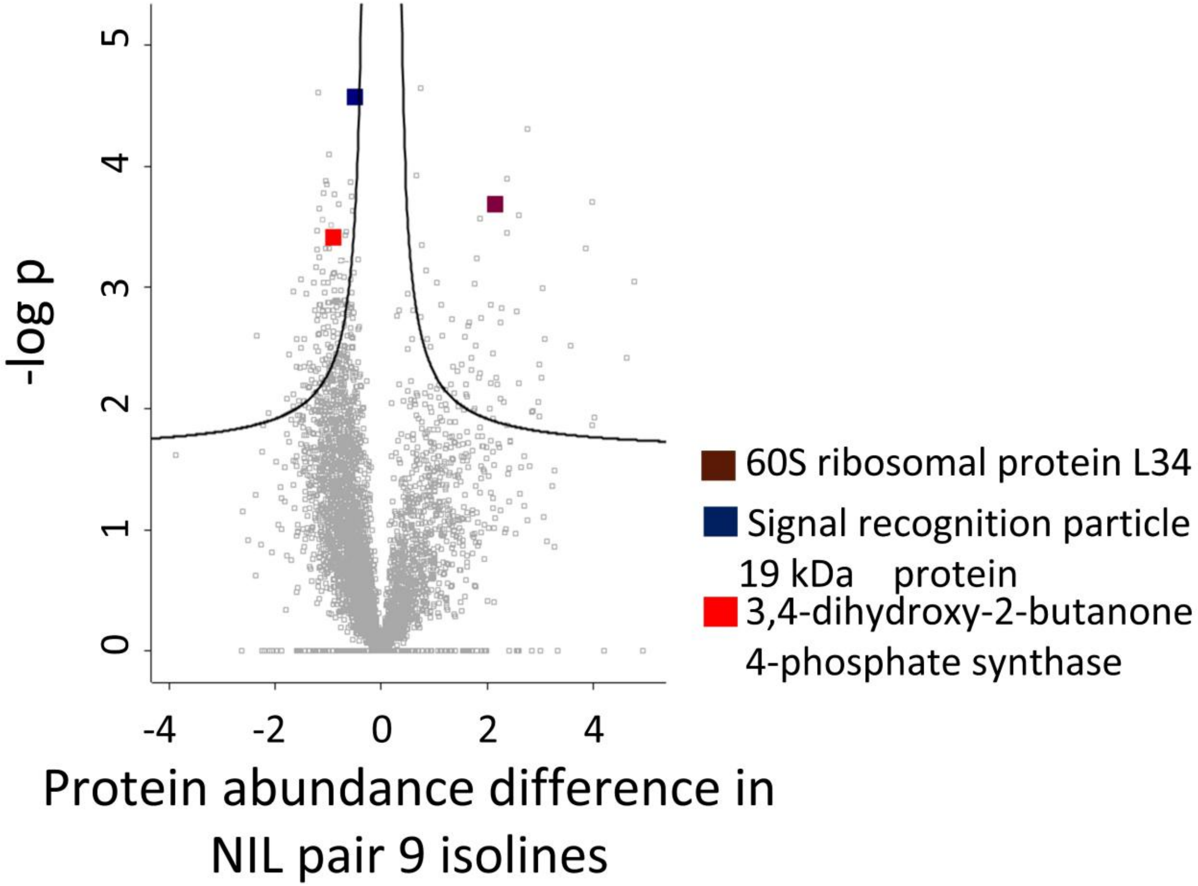
NIL pair 6



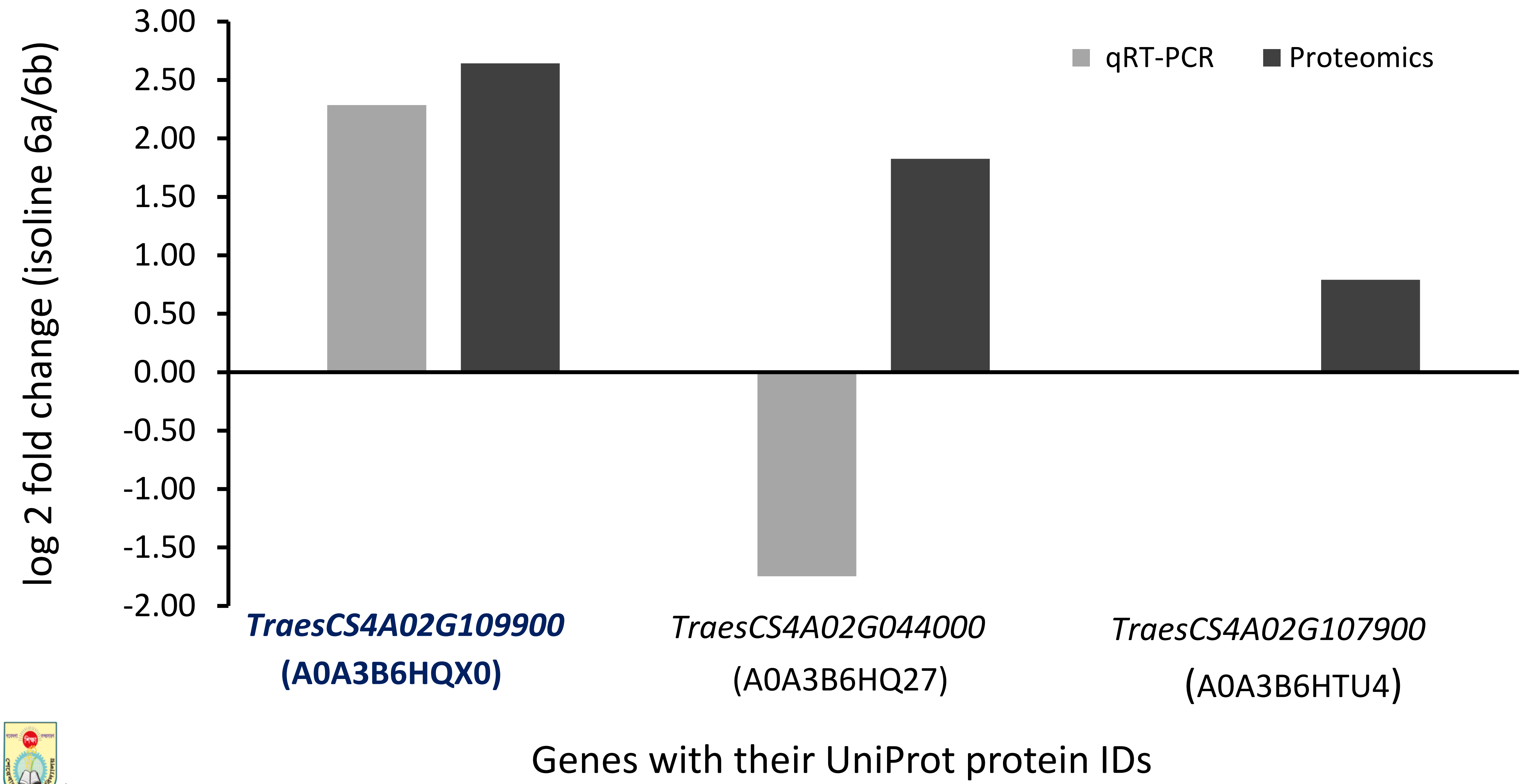
NIL pair 9

Proteins within the genomic regions (GRs) of the NIL pairs

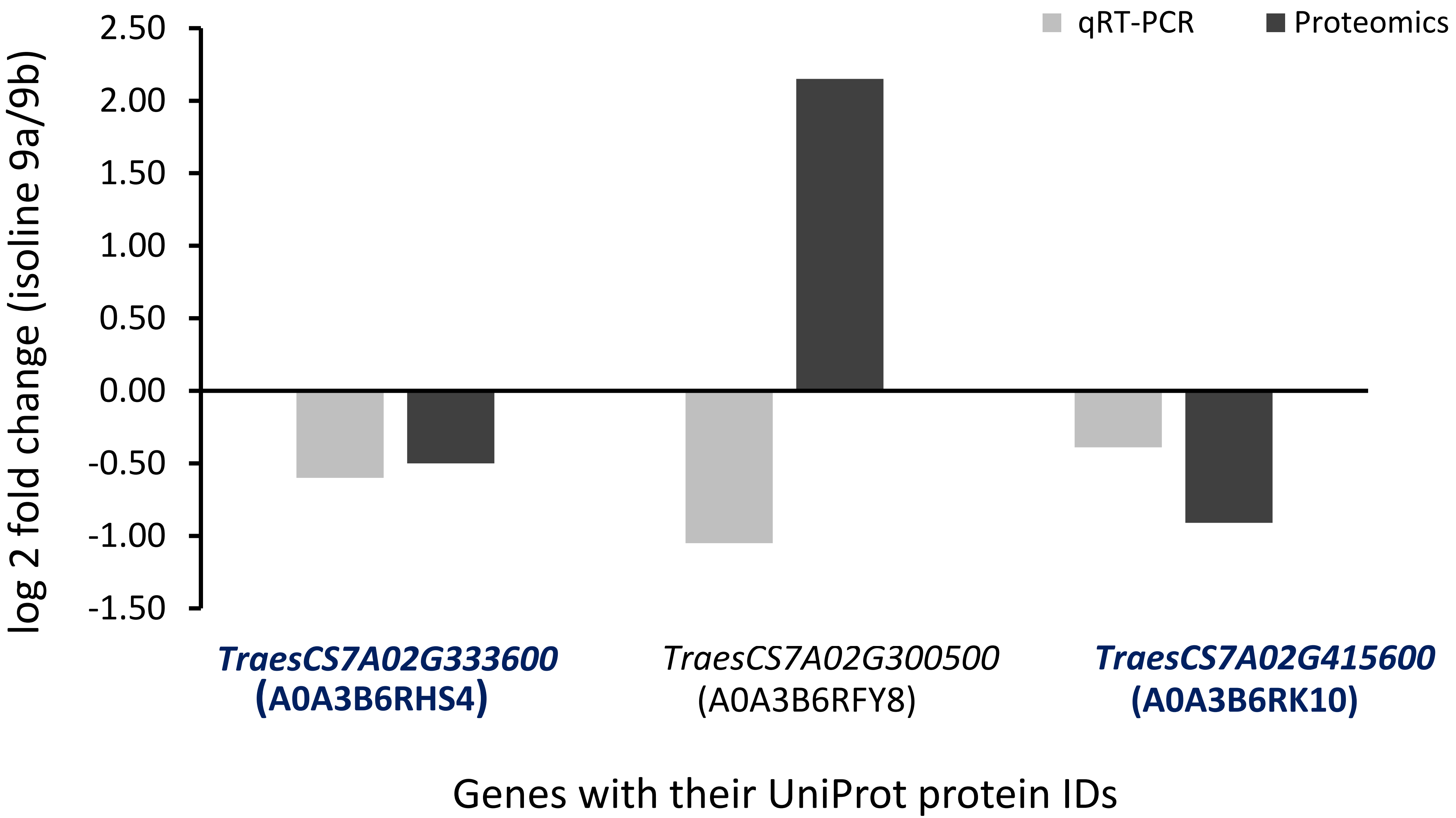




m-RNA expression analysis

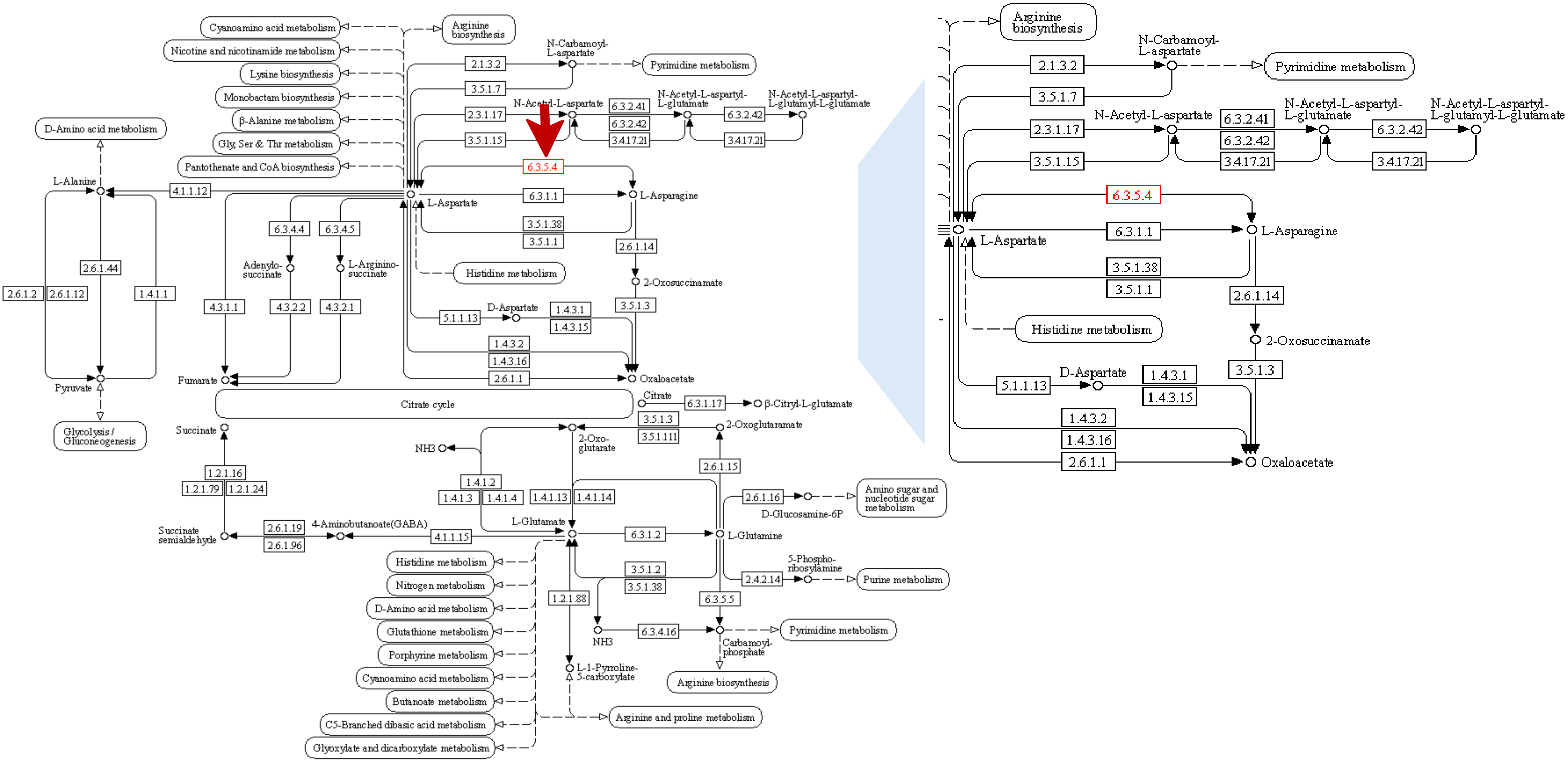


m-RNA expression analysis



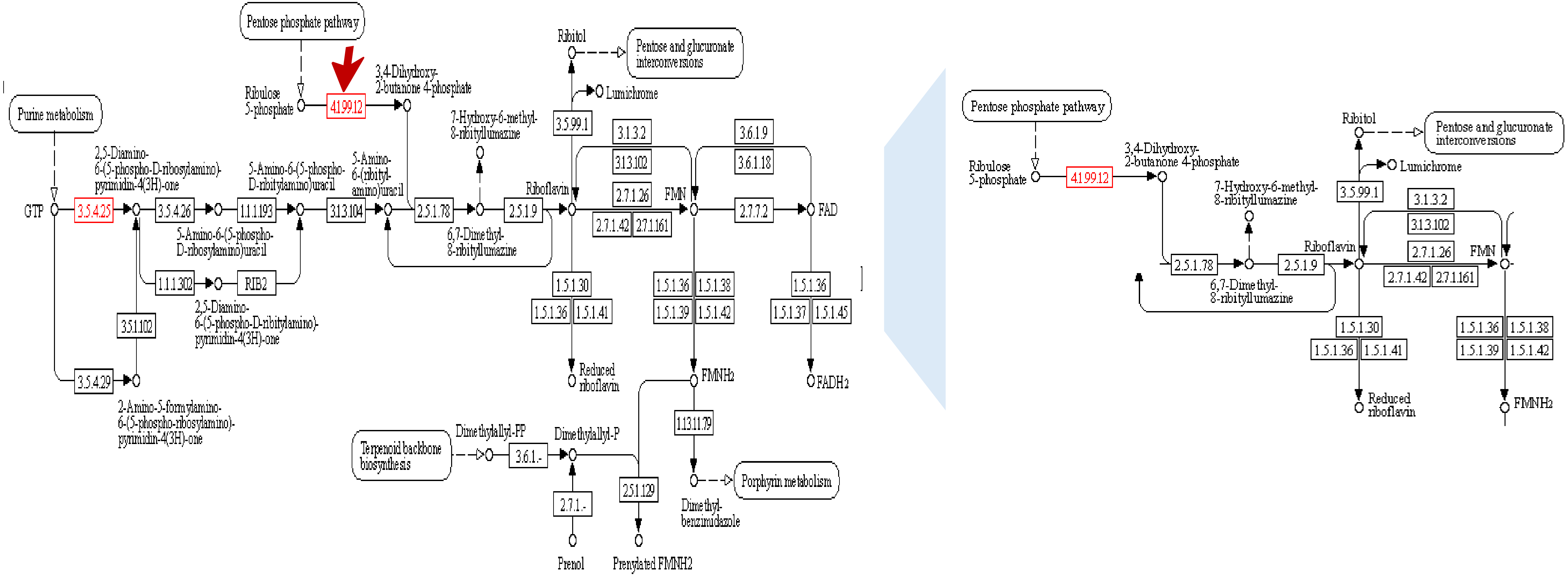
Molecular pathways of the candidate proteins

Aspartate and glutamate metabolism: *TraesCS4A02G109900*



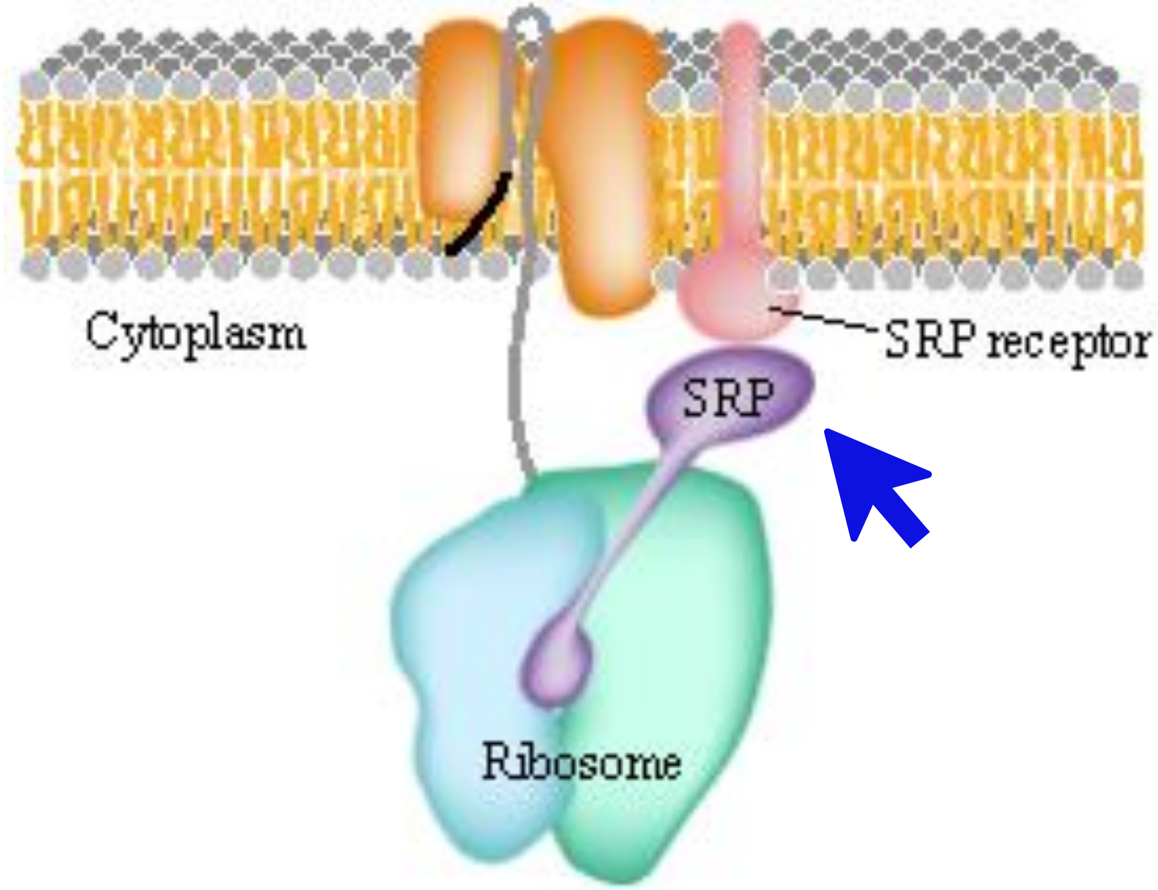
Molecular pathways of the candidate proteins

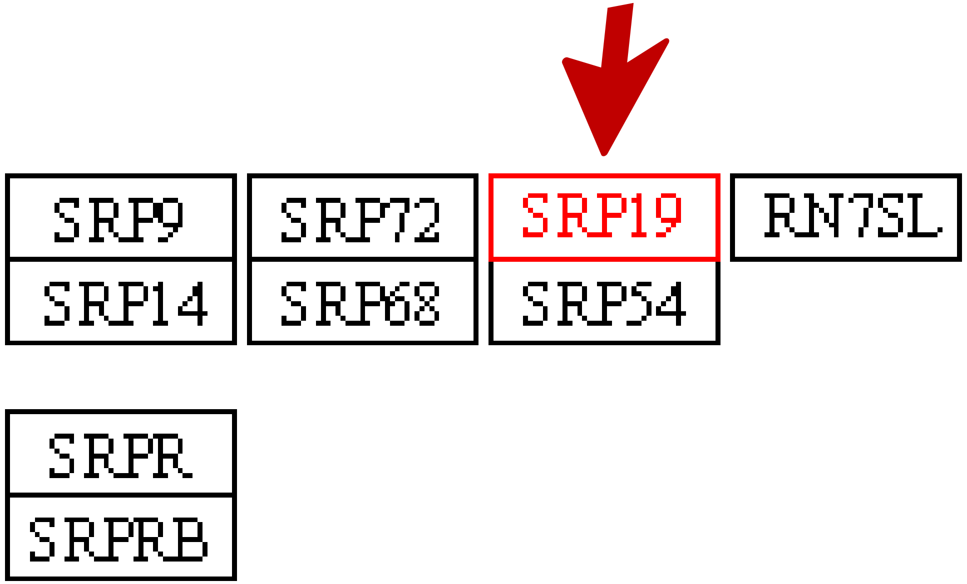
Riboflavin metabolism: *TraesCS7A02G415600*



Molecular pathways of the candidate proteins

Protein export: *TraesCS7A02G333600*





Importance of the candidate proteins

Asparagine synthetase

- Maintains C-N metabolic balances in crop (Miflin and Habash, 2002)
- Contributes to ROS scavenging and abiotic stress, including drought, tolerance in wheat (Curtis et al., 2018; Oddy et al., 2020)

3,4-dihydroxy-2-butanone 4-phosphate synthase

- Indirectly contributes to root growth in *Arabidopsis* (Hedtke et al., 2012) and maize (Tian et al., 2022)

Signal recognition particle subunit 19, SRP19

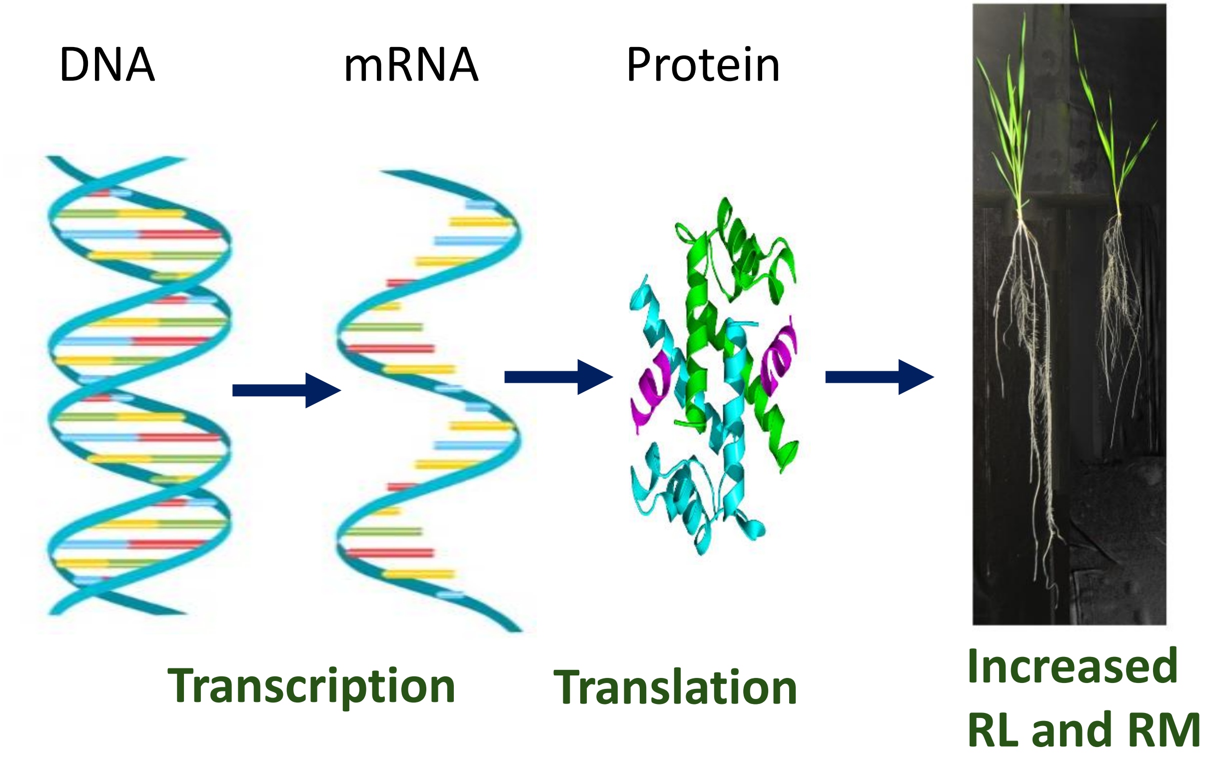
Supports SRP54 binding to RNA for drought tolerance in wheat (Lingelbach et al., 1988; Nouraei et al., 2022)

Strategy to increase protein numbers in the target GRs of NIL pairs

Generations	Heterozygosity (%) in the genetic background (for contrasting loci of the two parental lines)	Homozygosity (%) in the genetic background	Estimated number of genes heterozygous in the genetic background (assuming all genes are heterozygous)
F1	100.00	0.00	105200
F2	50.00	50.00	52600
F3	25.00	75.00	26300
F4	12.50	87.50	13150
F5	6.25	93.75	6575
F6	3.13	96.88	3288
F7	1.56	98.44	1644
F8	0.78	99.22	822
F9	0.39	99.61	411
F10	0.20	99.61	205
F11	0.10	99.61	103
F12	0.05	99.61	51
F13	0.02	99.61	26
F14	0.01	99.61	13
F15	0.01	99.61	6
F16	**0.00**	**99.70**	**3**

Summary

- ☐ The identified DAPs are important for increased total root length and root dry mass in wheat
- ☐ **Three novel candidate protein biomarkers** were identified for the target root traits
- ☐ **Cell redox homeostasis, structural molecule activity and protein binding**, are important molecular mechanisms for wheat root growth
- ☐ **Aspartate and glutamate metabolism, riboflavin metabolism, protein export and carbohydrate metabolism** are important molecular pathways for increasing RL and RM in wheat



- ✓ **Asparagine synthetase** ↑
- ✓ **Signal recognition particle 19 kDa protein** ↓
- ✓ **3,4-dihydroxy-2-butanone 4-phosphate synthase** ↓

Summary

Journal of Proteomics 291 (2024) 105044

Contents lists available at ScienceDirect

Journal of Proteomics

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

Protein biomarkers for root length and root dry mass on chromosomes 4A and 7A in wheat

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ARTICLE INFO

Keywords:
Asparagine synthetase
Label-free proteomics
qRT-PCR
Signal recognition particle
3,4-dihydroxy-2-butanone 4-phosphate synthase

ABSTRACT

Improving the wheat (*Triticum aestivum* L.) root system is important for enhancing grain yield and climate resilience. Total root length (RL) and root dry mass (RM) significantly contribute to water and nutrient acquisition directly impacting grain yield and stress tolerance. This study used label-free quantitative proteomics to identify proteins associated with RL and RM in wheat near-isogenic lines (NILs). NIL pair 6 had 113 and NIL pair 9 had 30 differentially abundant proteins (DAPs). Three of identified DAPs located within the targeted genomic regions (GRs) of NIL pairs 6 (*qDT.4A.1*) and 9 (*QHtscc.ksu-7A*), showed consistent gene expressions at the protein and mRNA transcription (qRT-PCR) levels for asparagine synthetase (*TraesCS4A02G109900*), signal recognition particle 19 kDa protein (*TraesCS7A02G333600*) and 3,4-dihydroxy-2-butanone 4-phosphate synthase (*TraesCS7A02G415600*). This study discovered, for the first time, the involvement of these proteins as candidate biomarkers for increased RL and RM in wheat. However, further functional validation is required to ascertain their practical applicability in wheat root breeding.

Significance of the study: Climate change has impacted global demand for wheat (*Triticum aestivum* L.). Root traits such as total root length (RL) and root dry mass (RM) are crucial for water and nutrient uptake and tolerance to abiotic stresses such as drought, salinity, and nutrient imbalance in wheat. Improving RL and RM could significantly enhance wheat grain yield and climate resilience. However, breeding for these traits has been limited by lack of appropriate root phenotyping methods, advanced genotypes, and the complex nature of the wheat genome. In this study, we used a semi-hydroponic root phenotyping system to collect accurate root data, near-isogenic lines (NILs; isolines with similar genetic backgrounds but contrasting target genomic regions (GRs)) and label-free quantitative proteomics to explore the molecular mechanisms underlying high RL and RM in wheat. We identified differentially abundant proteins (DAPs) and their molecular pathways in NIL pairs 6 (GR: *qDT.4A.1*) and 9 (GR: *QHtscc.ksu-7A*), providing a foundation for further molecular investigations. Furthermore, we identified three DAPs within the target GRs of the NIL pairs with differential expression at the transcript level, as confirmed by qRT-PCR analysis which could serve as candidate protein biomarkers for RL and RM improvement.

DOI: 10.1016/j.jprot.2023.105044

Take home message

- **Three novel protein biomarkers** and the **molecular pathways** identified in this study provide the molecular foundation for improving total root length and root dry mass in wheat
- The candidate proteins with further **functional validation** could be used in wheat root breeding
- Protein-protein interactions and further investigation on the molecular pathways will provide more insights into wheat root trait improvement through proteomics