Primed cereals against environmental stresses

V. Terzi¹, C. Morcia¹, R. Ghizzoni¹, F. Rizza¹, F. Badeck¹, L. Bernardo², L. Lucini²

¹Council for Agricultural Research and Economics – Research Centre for Genomics and Bioinformatics (CREA-GB), Fiorenzuola d’Arda (PC), Italy

²Department for Sustainable Food process, Research Centre for Nutrigenomics and Proteomics, Università Cattolica del Sacro Cuore, Piacenza, Italy
Research Centre for Genomics & Bioinformatics

Mission: plant physiology, genetics and genomics, molecular traceability

Breeding (with privates)
Barley, oat, triticale, asparagus
Priming is an adaptive strategy that increases the plant resistance to environmental stresses.

AM fungi establish a symbiosis with most plants living in wild and agroecosystems. AM fungi colonize the root cortex, supplying mineral nutrients to plant in exchange for carbon compounds.

Even though wheat is a major global crop, its response to AM symbiosis has been poorly investigated.

Key words
AM fungi, wheat, priming, biotic and abiotic stresses

- Is wheat an AM responsive plant?
- Which are the molecular responses triggered?
- Can AM symbiosis enhance pathogen resistance in wheat?
- Can AM symbiosis enhance drought tolerance in wheat?
<table>
<thead>
<tr>
<th>Phenotype</th>
<th>control</th>
<th>myc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tillers/plant</td>
<td>9.95</td>
<td>11.25</td>
</tr>
<tr>
<td>Vegetative tissue DW (g/pot)</td>
<td>12.9</td>
<td>17.6</td>
</tr>
<tr>
<td>Grain yield (g/pot)</td>
<td>5.5</td>
<td>8.4</td>
</tr>
<tr>
<td>Thousand kernel weight (g)</td>
<td>30.15</td>
<td>35.7</td>
</tr>
<tr>
<td>Kernel area (mm)</td>
<td>15.06</td>
<td>16</td>
</tr>
<tr>
<td>Kernel major ellipse (mm)</td>
<td>5.7</td>
<td>5.8</td>
</tr>
<tr>
<td>Kernel minor ellipse (mm)</td>
<td>2.7</td>
<td>2.9</td>
</tr>
</tbody>
</table>
These experiments demonstrate that AM symbiosis exerts a positive effect on wheat growth.
AMF field inoculation increased wheat dry weight, P, N and Zn uptake.
AMF inoculation increased wheat grain yield by 20% and harvest index by 25%.
Grain yield of wheat was positively related to root colonization (RC) by AMF.
P and Zn concentration in grain were positively correlated to RC by indigenous AMF.
Soil organic matter and soil pH were the most important parameters affecting wheat response to AMF.
Omics characterizations

By integrating whole-transcriptome sequencing with shotgun nanoflow overview we provide a comprehensive functional overview of both local and systemic transcriptomic and proteomic changes in roots and leaves during the mycorrhizal combination.

**Nutrient uptake**: strong activation of nutrient transporter genes and proteins in Myc wheat plants, leading to an increased mineral content in both roots and leaves.

**Primary metabolism and AA content**: AMF enhance primary metabolism in wheat, leading to corresponding changes in sugar, lipid and AA pathways. These dynamic changes in roots are mirrored by increased photosynthetic activity in leaves.

**Phytohormone regulation**: auxin metabolism and transport, modulation of genes involved in ET, SA, JA, and ABA metabolism.

**Defense genes and proteins**: activation of many defence-related genes not only in roots, but also in leaves. Taken together these genetic determinants could predispose wheat to priming.
AMFs have a positive impact on wheat growth in controlled experimental conditions.

The growth effect traditionally demonstrated for mycorrhizal plants has a molecular explanation on the strong systemic effect elicited by the mycorrhizal fungus on plant organs.
**Biotic stress**

Bacterial leaf streak (BLS) is the most important bacterial seed-borne disease of wheat and other small grains grown in warm and humid climates.

These experiments demonstrate that AM symbiosis exerts a positive effect on wheat growth and provides protection against *X. translucens*.

Phenotypic evaluation of disease symptoms caused by the bacterial pathogen *Xanthomonas translucens* in control (C) and mycorrhizal (M) plants. (A) Disease area (cm) was assessed on leaves from control (LC) and mycorrhizal (LM) plants 24 h post inoculation (hpi) and 14 days post inoculation (dpi). (B) The pictures show lesions provoked by *X. translucens* on LC and LM 14 dpi. Data (means ± SD, n ≥ 6) were subjected to one-way analysis of variance (ANOVA). The asterisks indicated significant differences at the 5% level using Tukey’s test.
By integrating whole-transcriptome sequencing with shotgun nanoflow overview we provide a comprehensive functional overview of both local and systemic transcriptomic and proteomic changes in roots and leaves during the tripartite interaction (plant-AMF-pathogen). We identified DEGs and DEPs in the contrasts LMX vs LCX and RMX vs RM.

AMF alleviates the symptoms caused by the pathogen through a broad down-regulation of transcripts and proteins levels, particularly evident in the leaves. Here a change in the level of protein oxidation and a specific activation of genes involved in disease resistance and nutrient transport suggests a specific MIR reaction.
Local and systemic changes overview

SM = myc+ seeds
SC = myc- seeds

LM = myc+ leaves
LC = myc- leaves
LMX = myc+X+leaves
LCX = myc-X-leaves

RM = myc+ roots
RC = myc- roots
RMX = myc+X roots

Pathways (omics):
- Photosynthesis
- Phytohormones
- Broad spectrum defence response

Pathways (omics):
- Pathogen specific defence response
- ROS scavenging activity

Pathways (omics):
- Nutrient uptake
- Carbohydrate and lipid metabolism
- Phytohormones
- Broad spectrum defence response

Pathways (omics):
- Response to stress
- Redox process
- Protein folding and ATP synthesis

Minerals: Mg, Zn, P
AA: Asp, Lys, Met, Orn, Tyr, Trp

Minerals: K, Cu, Mg, Fe
AA: Phe, Tyr

Minerals: decrease in K content
AA: Ala, Asp, Phe, GABA, Gly, Glu, His, Leu, Orn, Ser, Tyr, Val

Minerals: K, Cu
AMFs have a positive impact on wheat growth in controlled experimental conditions.

The growth effect traditionally demonstrated for mycorrhizal plants has a molecular explanation on the strong systemic effect elicited by the mycorrhizal fungus on plant organs.

AMFs surely protect wheat from Xantho, not only thanks to their priming effect (specific Myc genes), but creating a new balance (for example phytohormones).
Abiotic stress

Drought is considered a major threat in many regions of the world

- Pot experiment
- Mychorrized bread and durum wheat
- Moderate drought stress
Proteomic and metabolomic insight into the mitigation of wheat root drought stress by AMF

Protein (and metabolite) profiles of roots grown under drought with beneficial interaction with AMF clustered with roots grown under normal water regime
The interactome highlighted a relationships in the protein modulation involved in cell wall metabolisms and carbohydrate biosynthesis, as well as proteins related to cell wall rearrangements. All together supports the idea that myc roots underpin maintainance of cell integrity and limit the need for root cell expansion, improving the water uptake from the soil and increasing water content in above ground biomass.
Broad down regulation of proteome
- Sugars and cell wall metabolism
- Cytoskeleton rearrangement
- AA metabolism
- Stress response

Reprogramming of root secondary metabolome
- Sugars
- Lipids
- Alkaloids and flavonoids
- Lignans
- Glutathione
- Phytohormones

AMF colonization of wheat roots under drought stress correlates with:
- Better wheat performance (morpho-physiological traits)
- Decreased sufferance (reduction of stress-related response)
AMFs have a positive impact on wheat growth in controlled experimental conditions.

The growth effect traditionally demonstrated for mycorrhizal plants has a molecular explanation on the strong systemic effect elicited by the mycorrhizal fungus on plant organs.

AMFs surely protect wheat from Xantho, not only thanks to their priming effect (specific Myc genes), but creating a new balance (for example phytohormones).

AMFs significantly improve wheat drought stress tolerance, with the ability to cope with ROS-mediated oxidative stress and the regulation of hormone crosstalk playing a pivotal role.
The metabolomic approach demonstrated a broad chemical diversity, with more than 2900 compounds annotated in the root exudates. Different inoculants and different means of application can modulate the exudate profiles. Most of the differences could be ascribed to lipids (sterols and membrane lipids), phenolic compounds and terpenoids, siderophores and chelating acids, derivatives of amino acids and phytohormones.
AMFs have a positive impact on wheat growth in controlled experimental conditions.

The growth effect traditionally demonstrated for mycorrhizal plants has a molecular explanation on the strong systemic effect elicited by the mycorrhizal fungus on plant organs.

AMFs surely protect wheat from Xantho, not only thanks to their priming effect (specific Myc genes), but creating a new balance (for example phytohormones).

AMFs significantly improve wheat drought stress tolerance, with the ability to cope with ROS-mediated oxidative stress and the regulation of hormone crosstalk playing a pivotal role.

The modulation of root exudate composition is among the processes underlying plant performance induced by microbial biostimulants.
Data exploitation for breeding

Thank you for attention!

MIC-CERES
Project
Materials and methods

- **Green-house trials:**
  - two years,
  - six wheat genotypes (bread and durum),
  - three N levels,
  - two AMF strains,
  - two levels of soil treatment (natural and sterilized)

- **Field trial**
  - one year,
  - six wheat genotypes (bread and durum),
  - one N level,
  - four AMF preparations (two AMF strains, two commercial inocula)
• N fertilization significantly impacts the root colonization by AMF
• Variability for AMF root colonization level has been observed among different wheat genotypes

• In natural – not sterilized- soils (pots and open field) the effect of AMF inoculation on yield and yield component is not significant
• However, the interaction between AMF treatment and genotypes is significant

• In sterilized soil it can be observed a significant increase for above-ground biomass and yield in all the mycorrhized plants
• Variability among genotypes has been observed even in this experimental condition
## 2° EXPERIMENT

**Everton, H3402, Pearson after stress**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>$F_v/F_m1$</th>
<th>EL%1</th>
<th>$F_v/F_m2$</th>
<th>$F_v/F_m3$</th>
<th>SPAD3</th>
<th>CHL3</th>
<th>FLAV3</th>
<th>ANTH3</th>
<th>NBI3</th>
<th>VS3 (val. 0-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL</td>
<td>0.572 b</td>
<td>55.48 a</td>
<td>0.528 c</td>
<td>0.544 c</td>
<td>31.57 c</td>
<td>26.48 b</td>
<td>0.953 c</td>
<td>0.435 a</td>
<td>28.77 b</td>
<td>2.49 c</td>
</tr>
<tr>
<td>G</td>
<td>0.728 a</td>
<td>36.21 b</td>
<td>0.776 a</td>
<td>0.787 ab</td>
<td>50.13 a</td>
<td>37.51 a</td>
<td>1.250 a</td>
<td>0.402 a</td>
<td>31.14 b</td>
<td>4.39 a</td>
</tr>
<tr>
<td>B</td>
<td>0.718 a</td>
<td>38.89 b</td>
<td>0.719 ab</td>
<td>0.768 b</td>
<td>49.61 a</td>
<td>35.15 a</td>
<td>1.090 b</td>
<td>0.381 ab</td>
<td>32.96 b</td>
<td>3.87 b</td>
</tr>
<tr>
<td>G+B</td>
<td>0.662 a</td>
<td>42.26 b</td>
<td>0.707 b</td>
<td>0.802 a</td>
<td>45.32 c</td>
<td>34.34 a</td>
<td>0.872 c</td>
<td>0.323 a</td>
<td>40.03 a</td>
<td>3.85 b</td>
</tr>
</tbody>
</table>

**GENOTYPE**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>$F_v/F_m1$</th>
<th>EL%1</th>
<th>$F_v/F_m2$</th>
<th>$F_v/F_m3$</th>
<th>SPAD3</th>
<th>CHL3</th>
<th>FLAV3</th>
<th>ANTH3</th>
<th>NBI3</th>
<th>VS3 (val. 0-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVERTON</td>
<td>0.640 n.s.</td>
<td>45.32 n.s</td>
<td>0.701 a</td>
<td>0.750 a</td>
<td>42.20 b</td>
<td>31.33 b</td>
<td>1.040 n.s</td>
<td>0.373 n.s</td>
<td>31.83 n.s</td>
<td>3.71 b</td>
</tr>
<tr>
<td>H3402</td>
<td>0.689 n.s.</td>
<td>39.84 n.s</td>
<td>0.645 b</td>
<td>0.725 ab</td>
<td>44.02 ab</td>
<td>34.67 a</td>
<td>1.000 n.s</td>
<td>0.369 n.s</td>
<td>35.43 n.s</td>
<td>3.91 a</td>
</tr>
<tr>
<td>PEARSON</td>
<td>0.681 n.s.</td>
<td>44.47 n.s</td>
<td>0.702 a</td>
<td>0.701 b</td>
<td>46.25 a</td>
<td>34.11 a</td>
<td>1.080 n.s</td>
<td>0.413 n.s</td>
<td>32.41 n.s</td>
<td>3.33 c</td>
</tr>
</tbody>
</table>

**TREAT*GENO**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>$F_v/F_m1$</th>
<th>EL%1</th>
<th>$F_v/F_m2$</th>
<th>$F_v/F_m3$</th>
<th>SPAD3</th>
<th>CHL3</th>
<th>FLAV3</th>
<th>ANTH3</th>
<th>NBI3</th>
<th>VS3 (val. 0-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

~0.8 confirms the ability of G e B to help the plants to resume growth quickly after chilling stress.

Modern genotypes showed highest $F_v/F_m$ after 15d of regrowth.

Seedlings at the end of chilling stress

A) Everton
B) H3402
C) Pearson
Genetic markers associated to arbuscular mycorrhizal colonization in durum wheat

Pasquale De Vita¹, Luciano Avio², Cristiana Sbrana³, Giovanni Laidò¹, Daniela Marone¹, Anna M. Mastrangelo¹, Luigi Cattivelli⁴ & Manuela Giovannetti²

In this work we investigated the variability and the genetic basis of susceptibility to arbuscular mycorrhizal (AM) colonization of wheat roots. The mycorrhizal status of wild, domesticated and cultivated tetraploid wheat accessions, inoculated with the AM species Funneliformis mosseae, was evaluated. In addition, to detect genetic markers in linkage with chromosome regions involved in AM root colonization, a genome wide association analysis was carried out on 108 durum wheat varieties and two AM fungal species (F. mosseae and Rhizogomus irregulare). Our findings showed that a century of breeding on durum wheat and the introgression of Reduced height (Rht) genes associated with increased grain yields did not select against AM symbiosis in durum wheat. Seven putative Quantitative Trait Loci (QTLs) linked with durum wheat mycorrhizal susceptibility in both experiments, located on chromosomes 1A, 2B, 5A, 6A, 7A and 7B, were detected. The individual QTL effects ($r^2$) ranged from 7 to 16%, suggesting a genetic basis for this trait. Marker functional analysis identified predicted proteins with potential roles in host-parasite interactions, degradation of cellular proteins, homeostasis regulation, plant growth and disease/defence. The results of this work emphasize the potential for further enhancement of root colonization exploiting the genetic variability present in wheat.
Figure 4: Histograms of fresh shoot weight of three wheat genotypes (two common wheat Terminillo and Apache, and durum wheat Cappelli) at four weeks of growth, inoculated with 27 strains. Asterisk above histograms indicate significant differences observed compared to uninoculated control (at 5%; t-student test). Numbers indicate ABIP collection numbers as listed in Supplementary Table S5.