

PROJECT TITLE

BenzimidazOles to improve Nitrogen use Efficiency in Maize

CONSORTIUM

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SUMMARY OF THE REPORT

The project proposes an integrative approach that couples i) accurate experimental design for maize plant growth under N limitation, and ii) high-throughput phenotyping for dissecting the physiological mechanisms involved in the responses to omeprazole (OP).

Maize plants grown in hydroponics by UNINA unit with 3 levels of nitrate (10, 1 and 0.1 mM) and OP 1 μ M for 15 days were analyzed at HiTMe by UNICAMPANIA personnel in the first visit from 1st June to 31st July. Analyses were performed on 258 samples of roots and shoots of plants harvested at 0, 8h, 1d, 2d, 3d, 7d, 13d, 15d. Total amino acids, glucose, fructose, sucrose, starch and the enzymes glucose 6 phosphate dehydrogenase, glutamate dehydrogenase, glutamine synthetase, PEP carboxylase and pyruvate kinase, that are involved in C4 photosynthetic central C and N metabolism, were analyzed.

Measurements of single amino acids, cations, anions, and transcripts (RNAseq and qRT-PCR) for nitrate uptake genes (*NRT1.1*, *NRT1.2*, *NRT1.5*, *NRT2.1*, *NRT2.2*, *NRT3.1*), nitrate uptake regulatory gene (*NAR2.1*), nitrate assimilation genes (*NR*, *NiR*, and *ASN4*) and the plasma membrane ATPase (*MHA3*) are in progress at UNICAMPANIA and UNINA.

The analysis of data obtained at HiTMe and summarized in a heat map (Figure 2) showed that in the first 15 days there was no significant difference between control and OP treated plants; however, differences could be present in amino acid pool and transcripts (analyses in progress).

Since the preliminary results from HiTMe indicate the previously observed effects of OP on N assimilation are a result of long-term treatment, UNINA is growing new plants for obtaining additional samples (3, 4, 5, 6 and 8 weeks), that will be analysed by HiTMe with UNICAMPANIA personnel in 1 month in January 2019. These samples should more clearly demonstrate the long-term effects of OP on nitrate metabolism in maize.

The preliminary results showing phenotypic differences under OP treatment at 4 weeks treatment (Figure 1) have been enriched with further analyses (Figure 3) and data have been discussed among UNINA, UNICAMPANIA, and HiTMe units. The main results are that in plants treated for four weeks with OP, nitrate uptake and assimilation greatly increased through a direct increase of nitrate reductase's affinity for its substrate, and an increase of expression of the plasma membrane ATPase MHA3 (not shown). We will submit these data in a high impact journal in one month acknowledging EPPN2020.

The long-term treatment results will be analyzed and correlated with the summer results and submission of a second article is planned for spring acknowledging EPPN2020.

A final Workshop with all researchers belonging to the project and external parties will be organized at the end of the project.