

# Genetic variation for nutrient use efficiency in maize under different tillage and fertilization regimes with special emphasis to plant microbe interaction

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## Introduction

Conservation tillage (no-till and reduced tillage) brings many benefits with respect to soil fertility and energy use, but it also has drawbacks regarding the need for synthetic fertilizers and herbicides. To promote conservation tillage in organic farming systems, crop rotation, fertilization and weed control have to be optimized. In addition, crop varieties are needed with improved nutrient use efficiency (NUE) and high weed competitiveness or tolerance. As soil temperature rises slowly in reduced tillage systems in early spring, nitrogen mineralisation is often delayed resulting in temporary shortage of nitrogen (N) supply. Symbiosis with arbuscular mycorrhizal fungi (AMF) play an important role for nutrient acquisition of many crop plants, especially for insoluble elements like phosphorus (P) and zinc (Zn). Crop management that favours native AMF populations or inoculation of selected AMF strains might thus increase crop nutrient uptake. In the scope of an European project for improving nutrient use efficiency in major European crops (NUE-Crops) we studied the effect of different agronomic practices on the NUE and plant microbe interaction of maize (*Zea mays* L.) as well as the effect of AMF inoculations on maize yield.

The objectives of the first study were (i) to quantify the NUE of maize genotypes under different tillage regimes and fertilization levels, (ii) to compare the effect of slow releasing organic *versus* easily soluble mineral fertilizer on dry matter yield and weed coverage, and (iii) to assess the correlation of root colonization by arbuscular mycorrhizal fungi (AMF-RC) and NUE. The main objective of the second study was (iv) to quantify the effect of AMF seed inoculation on grain yield and nitrogen use efficiency of maize elite lines under different fertilization levels.

## Material and Methods

In order to determine the genotype x crop management interaction, we tested in 2009 six maize varieties with different NUE under conventional (CT) and reduced tillage (RT), applying five different fertilization regimes (unfertilized, slurry with either 85 or 170 kg/ha total N, mineral NPK fertilizer with either 85 or 170 kg/ha total N) with four replications. The field trial was carried out on an organically managed arable field with loamy soil in Muri, canton Aargau, Switzerland. Weed pressure, male and female flowering time, plant height, chlorophyll content and corn borer infestation was assessed during the vegetation period. At silage harvest, dry matter content and dry matter biomass of maize was determined as well as silage quality parameters including netto energy lactation (NEL) and N and P concentration for all 240 plots. In addition, root capacitance measurements, shoot and root biomass, N and P concentration in the shoots as well as AMF symbiosis was determined at flowering stage for selected plots. The percentage of AMF-RC was assessed on fine roots derived from two different soil layers (0-10 cm and 10-20 cm) by grid intersect method after bleaching and

staining the mycorrhizal structures with Trypan-blue. The presence and quantity of different AMF species (*Glomus mosseae*, *Glomus claroideum*, *Glomus intraradices*, *Gigaspora margarita*, *Scutellospora pellucida*, and *Diversispora celata*) was determined after DNA extraction by real-time quantitative polymerase chain reaction (RT-PCR).

In order to assess the effect of mycorrhizal seed inoculation on nutrient uptake and grain yield of maize, two different sets of maize genotypes were tested. Set 1 consisted of 100 testcrosses of doubled-haploid lines derived from old flint landraces including elite checks, while set 2 consisted of 100 testcrosses of elite maize lines. Seed inoculation was performed by the company AMYKOR, Germany, using *in-vitro* produced AMF spores. The 200 testcrosses were examined in 2009 with and without artificial inoculation at two locations in Germany (Bernburg, Grucking) applying mineral N at two levels (0 and 160 kg N/ha).

## Results

The six different maize varieties tested in Muri under different tillage and fertilization regimes had a significant effect on almost all traits except for weed coverage in early vegetation period. Dry matter yield (DMY) of silage averaged across management techniques ranged from 15.4 to 18.6 t/ha for the tested genotypes. The produced energy for animal feed (NEL) per acreage ranged from 9.5 to 11.3 MJ/m<sup>2</sup>. Although no significant genotype x tillage interaction were detected for these traits, the differentiation of the 6 varieties was much more pronounced under RT. Significant genotypic and fertilization effects were found for nitrogen use efficiency (Nit\_UE) measured as dry matter yield per kg N<sub>available</sub> (fertilizer + N<sub>min</sub> in soil). Nit\_UE ranged from 188 up to 232 kg DMY/kg N for the tested varieties. Mineral fertilizer resulted in significantly higher DMY (18.4 t/ha for both 85 and 170 kg N input/ha) than realized with slurry fertilizer (16.3 and 16.9 t/ha for 85 and 170 kg total N input/ha, respectively), which was significantly higher than the unfertilized control (14.0 t/ha). Tillage regime had no significant effect on DMY (16.5 t/ha for RT vs. 17.1 t/ha for CT), but strongly affected weed pressure, ripening, chlorophyll and N contents. Reduced tillage resulted in 50% increase in weed scores and higher dry matter content and reduced chlorophyll and protein contents. Increasing fertilization level resulted in shorter silking anthesis interval, higher chlorophyll content, increased plant height, delayed ripening, higher N and reduced P content, and reduced Nit\_UE. Significant genotype x tillage x fertilization interaction were found for plant height, weed scoring and Nit\_UE.

Tillage and fertilization regime had also a significant effect on AMF root colonisation. Higher AMF-RC was found under RT compared to CT and also in the unfertilized control plots compared to the mineral fertilizer treatment (Fig. 1). In addition, significant genotype x tillage interaction was detected for AMF-RC. Similar results were obtained by the DNA quantification of the different AMF species by real-time quantitative PCR. Focusing on single AMF species, significant genotypic effects were identified. While *G. mosseae* and *G. claroideum* were more frequently found on roots of variety S4 than on roots of S3, the opposite was true for *S. pellucida* and *D. celata*. AMF-RC was negatively correlated with K and N content and DMY. A weak positive correlation was found between AMF-RC and N uptake and Nit\_UE, but not with the P content. Instead a strong positive correlation was found for AMF-RC and to the sugar content of the plant at flowering suggesting a more parasitic plant microbe interaction.

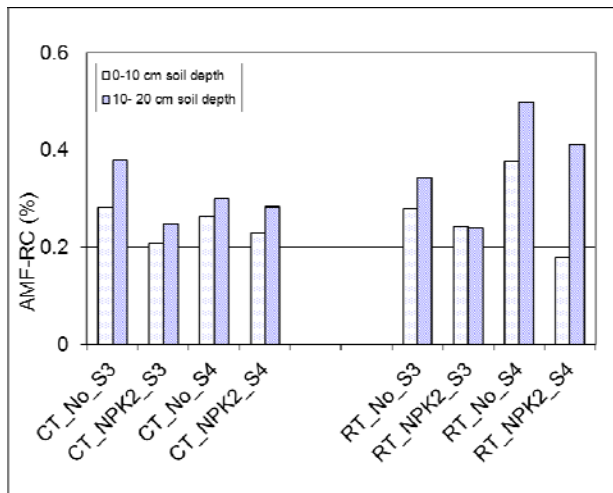


Fig. 1: AMF-RC of two different maize varieties (S3 and S4) cultivated under different tillage regime (CT and RT) and fertilization levels (No fertilizer and NPK fertilizer) in Muri during flowering time (21.07.2009)

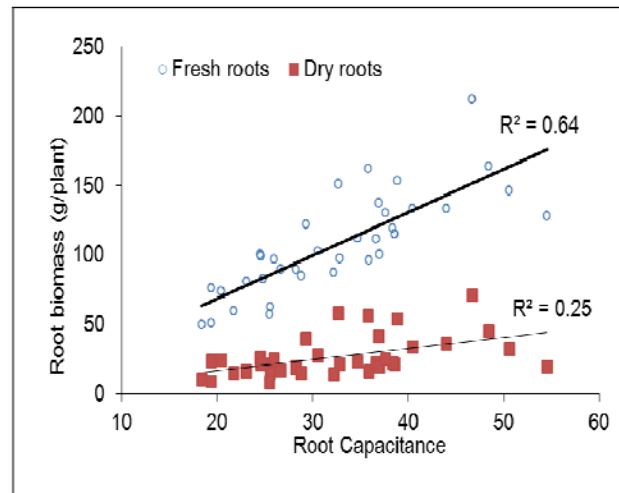


Fig. 2: Correlation of root capacitance measure with root fresh weight and dry weight during flowering in Muri 2009

The measurement of root capacitance in water saturated soils was proposed as a non-destructive method indicative for the active root surface. In order to calibrate this method, the plant-soil system was measured for its capacitance in the field first and afterwards plants were uprooted to determine root fresh and dry weight at flowering stage. Significant correlations were found between the measured root capacitance and root fresh weight per plant, but only weak correlation with root dry weight (Fig. 2).

The effect of artificial mycorrhizal seed inoculation on grain dry matter yield (GDY) relative to the untreated seed varied according to the N level, the tested gene pool and individual genotypes. On average the GDY under low N conditions (no N fertilizer since several years) was reduced by 60% compared to the high N level, indicating severe N deficiency. Testcrosses of set 2 derived from elite breeding lines had under both low and high N level a higher yield potential than testcrosses of set 1 derived from old flint landraces.

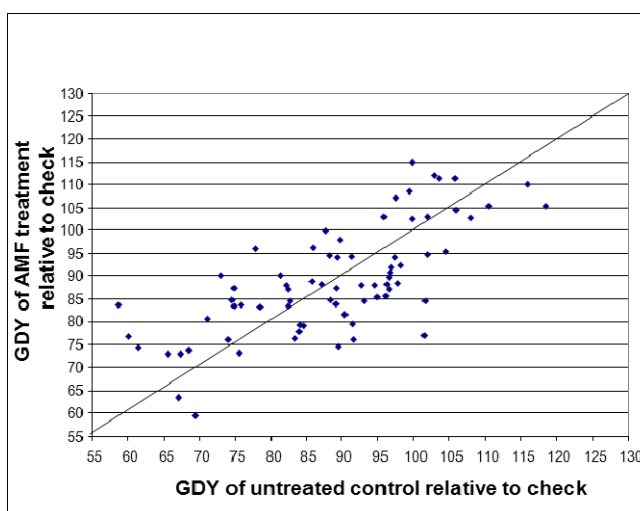


Fig. 3: Relative grain dry matter yield (GDY) of set 1 maize genotypes with (y axis) and without AMF seed inoculation (x axis) tested under low N conditions at two locations in 2009

The AMF seed inoculation of set 1 resulted in an average yield increase (GDY relative to the untreated control) of 2.9% under low N and of 1.1% under high N conditions. In contrast, set 2 showed an average yield increase of 4.9% under low N, but an average yield decrease of 1.8% under high N conditions due to artificial seed inoculation. However, the response to artificial AMF seed inoculation of individual genotypes varied a lot within each material set (Fig. 3), indicating a strong genotype x AMF inoculation interaction.

## **Discussion**

Although reduced tillage resulted in higher weed pressure during the whole vegetation period than conventional tillage, not significant reduction in DMY was observed. The N supply was reduced under RT which was clearly visible by the reduced chlorophyll content of the unfertilized plots. This resulted in a stronger positive response in plant height and DMY with increasing fertilizer input compared to CT. Under CT, no yield increase could be obtained by doubling the amount of mineral fertilizer or doubling the amount of slurry application. Therefore, the dosage of applied fertilizer needs to be adjusted to the respective tillage system to avoid N leaching. For the optimal choice of variety, not only the genotypic effects on DMY needs to be considered but also the significant genotype x tillage x fertilizer interactions for Nit\_UE.

There was significant variation for AMF-maize symbioses, measured by AMF-RC and DNA-based quantification of commonly occurring AMF species. While RT and no fertilization increased AMF-RC, different varieties promoted different AMF species and significant genotype x tillage interaction were encountered. Averaged across all treatments we found a negative correlation of AMF abundance with DMY of maize under the Muri growing conditions. This is in line with the yield reduction observed in the elite breeding material after AMF seed inoculation under high N conditions. However, under stress conditions, like the severe N deficiency at the two locations in Germany, the AMF inoculation with selected strains were on average beneficial to the maize plants resulting in GDY improvement. This agrees well with the hypothesis that the maize-AMF symbiosis is of special benefit for the plant under stress conditions (mutualism), while under optimal growing conditions plant growth might be reduced due to the parasitic behaviour of the fungi.

However, these results are based on one year data and need to be verified in further field experiments that were set up in 2010.

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