Increasing the Cost-Efficiency of Wheat Production by Phosphate Efficient Wheat Cultivars
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Principal Investigator: Heike Bücking, Biology and Microbiology Department, SDSU
Collaborators: Jose Gonzalez, Applied Crop Genetics/Genomics, SDSU
Karl Glover, Spring Wheat Breeder, Plant Science Department, SDSU
Prashant Ambilwade, Biology and Microbiology Department, SDSU

Objectives:
Long term goal of this project is to increase the efficiency with which wheat absorbs and utilizes phosphate and nitrogen through the colonization with beneficial arbuscular mycorrhizal (AM) fungi to lower the required fertilizer inputs and thereby reduce the wheat production costs and improve the environmental sustainability of wheat production. To achieve this goal, we propose (1) to study the effect of different AM fungi on nutrient efficiency and mycorrhizal responsiveness (growth benefit by AM fungi) of different wheat genotypes, (2) to determine the contribution of AM fungi to phosphate and nitrogen uptake in wheat, (3) to identify characteristics in the root architecture of various genotypes, that may be responsible for differences in the nutrient efficiency and mycorrhizal responsiveness, and (4) to identify candidate genes for these agricultural important traits that can be used in molecular breeding programs to identify genotypes that are productive under low nutrient availabilities and that can make the best use of AM fungi for growth benefit.

Importance to South Dakota:
Wheat is one of the major crops in South Dakota and the productivity of wheat is highly dependent on fertilizer additions, particularly of phosphate (P) and nitrogen (N). In 2008, 1.65 million tons of N fertilizer and 0.73 million tons of P fertilizer were used in U.S. wheat production alone (National Agricultural Statistics Service - NASS, 2010). The costs for these fertilizers increased dramatically in the last couple of years and this has doubled the expenditures of farmers for fertilizer input in the Great Plains (from 2,880 million dollars in 2005 to 5,470 million dollars in 2008). Therefore, research with the goal to improve the nutrient efficiency of wheat should represent an urgent priority to increase productivity and cost efficiency of wheat agriculture in South Dakota and the Northern Great Plains.

Outcomes and Deliverables:
Significant progress has been made in the evaluation of genotypic differences in the nutrient efficiency and mycorrhizal responsiveness of wheat. We are currently starting the first crosses of these genotypes and will examine their productivity under low input conditions with and without AM colonization. At the end of the next funding period we anticipate that we will be able to complete the studies on the mycorrhizal contribution to nutrient uptake and efficiency and will be able to provide more data on root architectural traits of different wheat genotypes. This is important to evaluate whether the observed differences are based on differences in the nutrient acquisition or utilization efficiency. These genotypes will be used to identify potential candidate genes of these important traits for marker production.

Objectives:
The main goal of this continuing project is to present a different and new approach to nutrient efficiency and crop productivity that includes beneficial plant-microbe interactions and studies the impact of these associations on the phosphate efficiency of various wheat cultivars. This knowledge is vital to the implementation of conventional and molecular breeding methods to increase yield and agronomic nutrient efficiency. The main focus of this proposal is on genotypic differences in the phosphate efficiency, but it can be expected that the produced data will also have implications for the nitrogen efficiency or the drought resistance of wheat. The main goals are as follows:

- **Objective 1:** To analyze genotypic differences in phosphate efficiency and mycorrhizal responsiveness of different wheat cultivars to determine which cultivar would be best suited for low input-agriculture and in soils with low P fertility. A first set of these experiments is completed. We will start with the first crosses and will evaluate the nutrient efficiency of these genotypes also in combination with different AM fungi that are known to colonize wheat. We will also evaluate whether differences in the root architecture are involved in the observed differences in nutrient efficiency and mycorrhizal responsiveness.

- **Objective 2:** To test the contribution of arbuscular mycorrhizal fungi to phosphate and nitrogen uptake and to determine functional differences in efficiency and in response to arbuscular mycorrhizal colonization. Originally we planned to conduct these experiments mainly with P, but we will now extend these experiments also to nitrogen, because the results of our first set of experiments indicate that mycorrhizal fungi may contribute to the nitrogen nutrition of wheat.
- **Objective 3:** To identify candidate genes which control (1) phosphate acquisition and utilization efficiency and (2) mycorrhizal dependency of wheat and analyze their regulation to identify diagnostic markers.

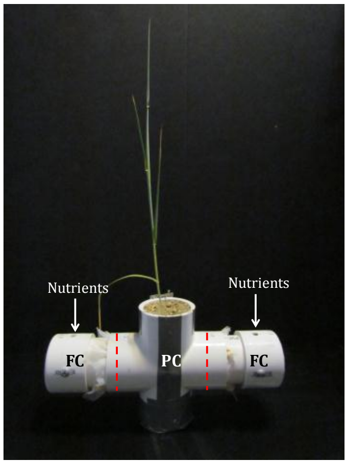
Justification/Importance for SD Wheat Producers:
Phosphate and nitrogen deficiency are the major limiting factors in crop productivity and in the past four decades crop productivity was significantly increased through successes in classical breeding and the use of nitrogen and phosphate fertilizers. However, the extensive use of fossil fuel resources for the production of nitrogen fertilizers has led to rapidly escalating prices. The production of phosphate fertilizer will even become more critical in the long term, because in contrast to nitrogen, phosphate rock that is used for the production of most phosphate fertilizers is a non-renewable resource and the current known reserves are likely to be depleted in 50-100 years. As a consequence, the prices for superphosphate in the Northern Great Plains have increased from $329 per ton in 2006 to $837 per ton in 2008 (2.5 fold increase), and can be expected to increase even further. Therefore, increasing the efficiency with which wheat absorbs or utilizes nutrients represents an urgent priority to ensure cost-effective and sustainable agriculture in the Northern Great Plains. To facilitate breeding of wheat with an increased nutrient efficiency is also important, because cultivars with improved nutrient uptake capability will also have a higher water uptake efficiency and drought resistance.

Wheat is under field conditions normally associated with arbuscular mycorrhizal fungi and these fungi can contribute substantially to the nutrient efficiency of their host. The mycelium of the fungus acts as an extension of the root system and has been shown to increase the uptake of phosphate, nitrogen and other elements. More than 50 percent of the phosphate that is taken up by wheat is supplied by the mycorrhizal fungus. In addition, the fungus increases the resistance of plants against abiotic stresses, such as drought and salinity, and against biotic stresses, such as various pathogens. It has been estimated that an efficient use of the symbiosis can substitute phosphate applications of up to 222 kg superphosphate per ha. However, conventional breeding techniques have reduced the responsiveness (yield increase in response to colonization) of wheat to this beneficial symbiosis. Wheat varieties that were developed before 1900 show a higher responsiveness to mycorrhizal colonization than modern varieties.

Material and methods:
Objective 1: Differences in P efficiency and mycorrhizal responsiveness of different wheat cultivars and the role of root architectural traits.
We have completed the first set of experiments and will continue these efforts by using different AM fungi in combination with our genotypes that were tested with *G. intraradices*. We will also start to evaluate the P efficiency and mycorrhizal responsiveness of crosses that we plan to perform after our first set of experiments has been completed. All experiments will be carried out under low and high P conditions and with or without inoculation with different arbuscular mycorrhizal fungi. The plants will be harvested at maturity, dried, and the dry weight of root and shoot and all parameters for productivity will be determined. The phosphate content in different tissues will be analyzed and will be used to calculate the phosphate efficiency of the different cultivars. To determine whether traits in root architecture are primarily responsible for genotypic differences in phosphate efficiency, we will use root observation chambers. The plants will be grown with substrate between two Plexiglas plates in a root compartment with a depth of 1 cm. This will allow to continuously observe the root growth and to determine the root architectural parameters: root length growth, thickness, length of root hair zones, area and total volume with image analyzing software. These root architectural traits will also be observed under various nutrient

supply conditions. The responsiveness to mycorrhizal inoculation will be calculated in terms of plant growth and of phosphate and nitrogen content, by comparing the dry weights or phosphate and nitrogen content of mycorrhizal plants with the respective means of non-mycorrhizal plants.

Objective 2: Contribution of arbuscular mycorrhizal fungi to phosphate and nitrogen uptake of wheat.



To get information on how much phosphate and nitrogen fertilizers could be reduced through the successful colonization of wheat, we will analyze the contribution of arbuscular mycorrhizal fungi to total phosphate and nitrogen uptake of wheat. For these experiments the cultivars with the highest (Brick and Grenora) and the lowest mycorrhizal responsiveness (NSG), the highest and the lowest phosphate and nitrogen efficiency, and different arbuscular mycorrhizal fungi will be used. Two compartment pots will be used (see Figure), in which the different compartments are separated by two nylon meshes (30 µm), that will allow fungal hyphae, but not the roots, to pass through and will allow us to distinguish between phosphate and nitrogen uptake by the plant and the fungus. After establishment of the interaction in these pots, phosphate will be added to the fungal compartment (FC) and to the fungus as ^{33}P and nitrogen as ^{15}N. After one week the roots will be harvested and analyzed for their colonization rate and the phosphate and nitrogen contents of roots and shoots will be determined.

Objective 3: Identification of candidate genes for phosphate efficiency and mycorrhizal dependency of wheat. We will use cDNA-AFLP (amplified fragment length polymorphism) profiling to identify candidate genes and their differential expression in roots and shoots of wheat under P stress, and when the plants are colonized with AM fungi. cDNA-AFLP is an efficient, reproducible method to identify differentially expressed transcripts and polymorphisms on a genome-wide scale and a great number of samples can be assayed in a cost-effective manner. The technique will allow us to track gene expression changes in roots and shoots of the most and least phosphate efficient wheat cultivars under low and high phosphate conditions. Seedlings in each treatment will be subjected to inoculation or mock inoculation and we will follow the time course of phosphate stress, or responses to arbuscular mycorrhizal colonization. Validated, differentially expressed sequences will be converted to PCR markers, where possible, and tested for their association with phosphate efficiency for molecular breeding in further experiments.

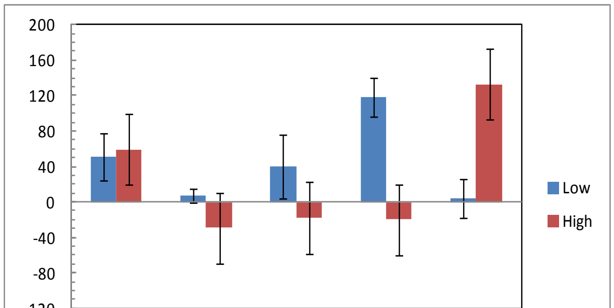
Project Deliverables:

Objective 1 and 2: It is anticipated that information about root architectural differences and information about the contribution of the AM to nutrient uptake of wheat cultivars can be given at the end of the funding period. Information on how much P is taken up via the AM fungus under different P supply conditions, should allow us to draw first conclusions on (1) how significant the AM fungus is for P and N uptake, and (2) whether a stimulation of the symbiosis under field conditions, for example through no-tillage, could contribute significantly to the phosphate and nitrogen uptake of different cultivars.

Objective 3: To identify candidate genes that could potentially be used as diagnostic markers in molecular breeding will take longer than the one year funding period of this proposal. However, we expect that we will be able to establish the required techniques and to run first preliminary studies in the next year. The first conducted experiments provided the first information which genotypes are suitable for these studies.

Report of progress:

Goal of the first project period was to identify genotypic differences in the nutrient efficiency and mycorrhizal responsiveness (growth benefit by mycorrhizal fungi) of different spring wheat varieties. We used for our experiments a collection of diploid, tetraploid, and hexaploid wheat varieties that represented a wide range of genetic backgrounds and a spectrum from progenitors, such as *Aegilops tauschii*, to modern wheat cultivars, such as Brick, Lebsock and Granger. The plants were cultured in a randomized block design in the SDSU greenhouse under controlled conditions. The plants were grown under different nutrient supply conditions that ranged from extremely low (no additional supply of N and P, nutrient supply only from the organic soil component of the substrate) to relatively high nutrient supply conditions (100 percent of the P and N concentration detected in marginal lands in SD) with or without inoculum of the AM fungus *Glomus intraradices*. This fungus was selected for the experiments, because it is the most commercially used AM fungus. The plants were harvested at maturity and analyzed for their biomass (fresh and dry weight of root and shoot), grain yield, phosphate and nitrogen contents, and mycorrhizal colonization. Fig. 1 shows the changes in the fresh weight of the spikelets of the different wheat varieties after colonization with the AM fungus *Glomus intraradices* and under low or high nutrient supply conditions.

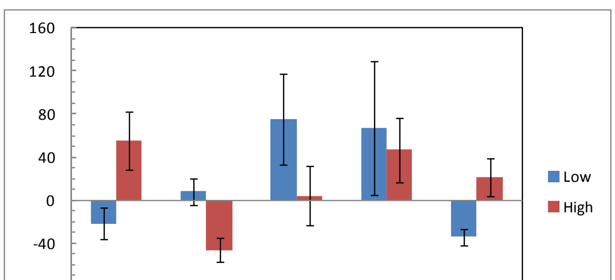


	Granger	NSG	Lebsock	Grenora	Brick
Low	51	7	40	118	3
High	59	-29	-18	-20	133

Fig. 1: Mycorrhizal responsiveness (change in percent compared to non-mycorrhizal controls) of the growth parameter spikelet fresh weight.

There were large genotypic differences in the response of the various wheat varieties to mycorrhizal colonization. The response varied from largely positive under all nutrient supply conditions (Granger) to positive under low nutrient supply conditions (Lebsock, Grenora), to positive under high nutrient supply conditions (Brick) to no effect (NSG). Some reductions in the weight of the spikelet were also observed, but these were in the range of variability. Genotypic differences in the response of wheat cultivars to AM fungi have also been described in the literature.

Most of the genotypic differences described above correspond well to increases in the P content of the shoots. For example, the hexaploid wheat cultivar NSG that did not show any positive growth response (Fig. 1) also showed no increase in the P content in the shoot (Fig. 2).



	Granger	NSG	Lebsock	Grenora	Brick
Low	-21.81	8.19	74.77	67.08	-34.10
High	55.77	-46.62	4.15	46.93	21.67

Fig. 2: Mycorrhizal responsiveness (change in percent compared to non-mycorrhizal controls) of the parameter P content in the shoot.

By contrast, the increase of 118 percent or 40 percent in the spikelet fresh weight of the tetraploid wheat cultivars Grenora and Lebsock under low nutrient supply conditions, respectively, seems to be primarily due to an increase in P supply. The P contents in the shoot under these conditions increased by 74.7 percent in the cultivar Lebsock, and by 67.1 percent in the cultivar Grenora. The wheat varieties Granger and Brick showed an increase in the P contents in the shoot of 55 percent or 21 percent, respectively, after colonization with the arbuscular mycorrhizal fungus G. intraradices but only under high nutrient supply conditions.

The differences in nutrient efficiency are not correlated to the primary genomic background of the wheat cultivar that was tested. Tetraploid (AA,BB; Grenora, Lebsock) as well as hexaploid wheat varieties with an AABBDD background are able to benefit from the mycorrhizal colonization. Particularly under low nutrient supply conditions an increase in biomass and an increase in the P content can be observed. We currently analyze the samples for their N content to get an information, whether the AM fungus was able to provide N to the plant.