

Increasing the Cost-Efficiency By Phosphate Efficient Wheat Cultivars

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Objectives:

The main goal of this proposal is to present a different and new approach to nutrient efficiency and crop productivity that includes plant-microbe interactions and studies the P efficiency of various wheat cultivars. This knowledge is vital to the implementation of conventional and molecular breeding methods to increase yield and genomic 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 of wheat. The main goals are as follows:

- * 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;
- * To test the contribution of different arbuscular mycorrhizal fungi to phosphate uptake of wheat and to determine functional differences in the efficiency and in the response to arbuscular mycorrhizal colonization; and
- * 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 stresses, such as pathogens and drought. It has been estimated that an efficient

use of the symbiosis can substitute phosphate applications of up to 222 kg superphosphate per hectare and this would significantly increase the cost-efficiency of wheat production in the Northern Great Plains. 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.

Procedures:

Differences in P efficiency and mycorrhizal responsiveness of different wheat cultivars. We will test the phosphate efficiency and mycorrhizal responsiveness of 15 different spring wheat cultivars including Briggs, Traverse, Albany, RB07 and Glenn. These modern cultivars will be compared to wheat progenitors, such as durum and Einkorn. The experiments will be carried out under low and high P conditions and with or without inoculation with the arbuscular mycorrhizal fungus *Glomus intraradices*. This fungal strain was isolated from a soil with low P fertility and is highly beneficial in combination with different crops under different agro-climatic conditions. 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, the roots of at least 5 plants will be carefully removed from the soil, washed, and analyzed for root length, area and total volume with image analyzing software. 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.

Contribution of arbuscular mycorrhizal fungi to phosphate uptake by wheat. To get information how much phosphate fertilizers could be reduced through the successful colonization of wheat, we will analyze the contribution of arbuscular mycorrhizal fungi to total phosphate uptake of wheat. For these experiments the cultivars with the highest and the lowest mycorrhizal responsiveness, the highest and the lowest phosphate efficiency, and different arbuscular mycorrhizal fungi will be used. Two compartment pots will be used, 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 uptake by the plant and the fungus. After establishment of the interaction in these pots, phosphate will be provided to the fungus as ^{33}P . After one week the roots will be harvested and analyzed for their colonization rate and the phosphate contents of roots and shoots will be determined.

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.

Deliverables:

1 and 2: It is anticipated that information about the most and least phosphate efficient and responsive wheat cultivars can be given at the end of the funding period. To translate this information into recommendations for farmers will require repeating the proposed greenhouse and laboratory experiments under field conditions. We expect that these experiments could be carried out in 2011. Information on how much phosphate is taken up via the arbuscular mycorrhizal fungus under different phosphate supply conditions, should allow us to draw first conclusions on (1) how significant the arbuscular mycorrhizal fungus is for phosphate uptake, and (2) whether a stimulation of the symbiosis under field conditions, for example through no-tillage, could contribute significantly to the phosphate uptake of different cultivars.

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 preliminary studies in the first year.