CROP GENETIC VARIABILITY FOR NUTRIENT USE EFFICIENCY: BIOLOGICAL FOUNDATION, AND CASE OF SPRING WHEAT

Roger Nkoa Ondoua and Maral Etesami
Montana State University, Western Triangle Agricultural Research, Conrad, Montana
Roger.ondoua@montana.edu; maral.etesami@montana.edu

ABSTRACT

Recent advances in plant molecular biology have elucidated the mechanisms by which plant species adapt to low-nitrogen and low-phosphorus environments. This has paved the way to new perspectives and strategies for breeding and genetic engineering: the design of low-nitrogen and low-phosphorus cultivars in order to optimize nutrients use efficiency and enhance economic and environmental sustainability. In this paper, we review the enzymatic activity of root hair-embedded transmembrane proteins, nitrate (*NRT1.1*) and phosphate (*Pht1,2,3,4*) transporters. As functional proteins encoded by specific genes, theses transporters constitute the basis of the potential genetic variability for nitrogen and phosphorus acquisition and utilization among and within crop species. Here, we demonstrate the existence of such a variability, at the field level, among five spring wheat varieties grown in the Montana dryland.

INTRODUCTION

To acquire nutrients from the soil solution, root hair, epidermal, and cortical cells of plant roots produce proteins that create passages in the plasma membrane through which essential nutrients can pass. These functional proteins behave as transporters, pumps, and channels. However, unlike channels, pumps and transporters must undergo conformational changes in order to transfer nutrients across cell membranes. Recent progress in plant molecular biology and physiology have elucidated the mechanisms by which transporters transfer nutrients from the soil solution into the cytoplasm. Transporters act like enzymes by displaying affinity for specific substrates. Their behavior can therefore be described by the Michaelis-Menten kinetics where by the Michaelis constant *Km* would determine the degree of affinity of a given transporter to a given substrate. Soils vary, sometimes considerably, among fields, landscapes, and ecosystems and so does the availability of plant nutrients. To ensure that plant cells are not deprived of essential nutrients when their concentration is low in the soil solution, they have evolved high and low affinity transport systems which guarantee that a given nutrient is taken up over a wide range of concentrations. Typically, the two transport systems are mediated by distinct transporters, but in the case of nitrate transporters, a single protein switches affinity through conformational change. In this section, we focus on nitrate and phosphate transporters, and their potential links to N and P acquisition efficiencies, N and P utilization efficiencies, and low-N and low-P tolerance in crops.

Nitrate acquisition

In plants, root system architecture and nitrate and ammonium transporters are essential components of the N-uptake-system. The mode of action of plant nitrate transporters *NRT1.1* (also known as *CHL1 or NPF6.3*) has been elucidated lately (Parker and Newstead, 2014; Yi-

Fang Tsay, 2014). *NRT1.1* nitrate transporters are able to adapt to changing levels of nitrate in the soil. They function as a 'dual affinity transporters', that is, they can switch their ability to recognize nitrates depending on soil availability. In conditions of high concentration of nitrates in the soil solution, *NRT1.1* displays low affinity for nitrates; when nitrate levels fall, intracellular phosphorylation switches it to a high affinity state, able to scavenge the last remains of nitrates for growth. There are therefore two distinct kinetics (with two affinity constants, K_{mHA} and K_{mLA}) for the transport of nitrates from the soil solution into the cytosol of root hairs cells depending on the availability of nitrates in the soil.

Since nitrate transporters are functional proteins, that is, they are gene products, it can be hypothesized that there would be genetic variability for the affinity for nitrate among genetically distant populations within the same species or between species. Genotypes with higher affinity for nitrates (lower K_{mHA}) would be more efficient in extracting nitrates from the soil solution when they are at suboptimal levels, and could be more efficient in utilizing nitrogen for the synthesis of dry biomass and other products. In the following sections, we will illustrate the case of genetic variability for nitrogen use efficiency among five spring wheat varieties grown in the dryland of Montana.

Phosphates acquisition

In plants, the phosphate acquisition system differs from the nitrate acquisition system in terms of the complexity of the system itself and the type of uptake mechanism involved. Thus and unlike the nitrate acquisition system, there are more components in the phosphate acquisition system: root architecture, transporters, mychorrhizal association, organic acids, and phosphatases production (Lopez-Arredondo et al., 2014). Regarding the type of mechanism involved, in the case of nitrate uptake, a single protein mediates the process by switching its affinity for nitrates from low to high depending upon its availability in the soil solution. Whereas for P uptake, distinct proteins are involved depending upon the level of phosphates in the soil solution. Phosphate uptake is regulated through two distinct mechanisms: an inducible high-affinity and a constitutive low-affinity uptake systems. The high-affinity system operates at low phosphate concentrations and has an apparent *Km* that varies from 3 to 10 μM, whereas the low-affinity system which functions at high phosphate availability has a *Km* that ranges from 50 to 300 μM (Raghothama and Karthikeyan, 2005). Phosphate transporters are encoded by members of the *Pht1* gene family, one of the four gene family that have been characterized in detail in *Arabidopsis.* They are predominantly expressed in epidermal, cortical, and root hairs cells. These transmembrane proteins have been identified as mediators of phosphate uptake from the soil solution when phosphate anions, mainly $H_2PO_4^-$ and HPO_4^{-2}, are limiting (Mudge *et al*., 2002; Nacry *et al.,* 2005). They transfer phosphate anions, against a concentration gradient, from the soil solution (which typically contains 0.1–10 μM Pi) and the cytosol of root cells (which typically contains 5–10 mM Pi) (see review of Raghothama and Karthikeyan, 2005).

Perspectives for breeding and genetic engineering for nutrient acquisition and use efficiencies

Two observations can be made from the above literature review on nitrate and phosphate uptake systems:

i) Nitrate and phosphate transporters are transmembrane proteins, as such, they are gene products and genetic variability ought to be expected among crop species and within crop species;

ii) For each individual plant, and for each uptake system (nitrate or phosphate), there are two distinct kinetics (with two affinity constants, K_{mHA} and K_{mLA}) for the transport of nitrates or phosphates from the soil solution into the cytosol of root cells depending on the availability of nitrates or phosphates in the soil solution: a low-affinity and a high-affinity kinetics. Specifically, the phosphorus uptake system in plants involves two distinct gene regulatory mechanisms, the inducible and the constitutive. In other words, two distinct genes are involved depending upon whether or not the available phosphorus level in the soil solution is optimal or suboptimal.

The above observations pave the way for new perspectives in the fields of breeding and genetic engineering with respect to nitrogen and phosphorus acquisition and utilization efficiencies. Genotypes with higher affinity for nitrates or phosphates (lower K_{mHA}) would be more efficient in extracting nitrates or phosphates from the soil solution when they are at suboptimal levels, and could be more efficient in utilizing nitrogen or phosphorus for the synthesis of dry biomass and other plant products. Since for decades, conventional breeding and selection of crop varieties across the world has been conducted under optimal conditions of nitrogen and phosphates, that is, under the low-affinity systems of nitrate and phosphate transporters, it is therefore within the realm of possibility that parent lines of varieties with high affinity for nitrates or phosphates may have been discarded during the breeding and selection process. It is therefore imperative that breeding programs revisit their procedures and fine-tune their strategies in order to meet the goals of nutrient use efficiency and economic and environmental sustainability.

The following sections describe the methodology and the results of a two-year study on the varietal nitrogen use efficiency differences among spring wheat in two Montana cropping systems. The study demonstrates, at the field level, the existence of genetic variability for nitrogen use efficiency among five spring wheat varieties under dryland conditions. It has to be pointed out that this study is not one on neither nitrogen acquisition efficiency, nor one nitrate uptake systems. It does represent however an example of synergy between the fields of agronomy and breeding aiming at identifying genotypes that would undergo further biochemical and biological assays that would confirm the activity or the presence of genes responsible for low-N or low-P stress tolerance in crops.

MATERIALS AND METHODS

The experiment was conducted in 2015 and 2016 at two locations. At each location, the experimental site consisted of two adjacent fields, one of which was a spring wheat fallow, and the other a recently-cropped field (**Figure 1**). The experimental design was a split-split-plot in a randomized complete block design with four repetitions. The main plot factor was the rotation type with two levels (Fallow-Crop, Continuous Cropping), the sub-plot factor was spring wheat variety with five levels, and the sub-sub-plot factor was available nitrogen rate (at 0-3 ft) with three levels (50; 100; and 150 lbs N/ac). At harvest, aboveground biomass was sampled from each subplot, chopped, and ground using and industrial mill. The samples were shipped to a commercial laboratory for total N analysis.

Nitrogen use efficiency (NUE) was calculated as:

$$NUE = \text{Total aboveground tissue N (kg. acre}^{-1}\text{)/Total soil available N (kg. acre}^{-1}\text{)/}$$

RESULTS AND DISCUSSION

The results show that there were significant differences among the five spring wheat cultivars with respect to nitrogen use efficiency (**Figure 2**). Of the three factors evaluated, cultivar and N rate affected NUE. Cultivars Duclair and Corbin displayed the highest NUE. It should be pointed out that this study doesn't not demonstrate that cultivars Duclair and Corbin possess a greater nitrate uptake system or genes for low-N stress tolerance because several biological and environmental factors can interfere with the agronomic evaluation of nitrogen use efficiency. Examples of such factors are tolerance to leaf diseases and water stress. However, such a study represents an important first step towards toward the identification of candidate cultivars that possess a nitrate high-affinity uptake system.

SUMMARY

Recent advances in the area of nitrate and phosphate uptake systems have demonstrated that both systems use distinct kinetics, and in the case of phosphates, distinct genes to acquire nitrates or phosphates from the soil solution depending upon the availability level of these elements. In plants, low affinity kinetics or low affinity genes operates when nitrates or phosphates are present in the growing medium at optimal levels. Whereas high-affinity uptake systems or genes are induced when these elements are present in the soil solution at suboptimal levels. Since most breeding programs typically select their elite varieties under optimal N and P conditions, it is conceivable that parent lines which were more efficient in N and P acquisition, and possibly in N and P use efficiencies, had been discarded all along. We have therefore recommended a paradigm shift in the breeding and selection of varieties for characters such as, N or P acquisition efficiency, N or P use efficiency, low-N stress tolerance, and low-P stress tolerance. These traits should be evaluated under suboptimal conditions.

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Figure 1. *Layout of the nitrogen use efficiency study in Valier (top) and Ledger(bottom) in August and July 2016, respectively.*

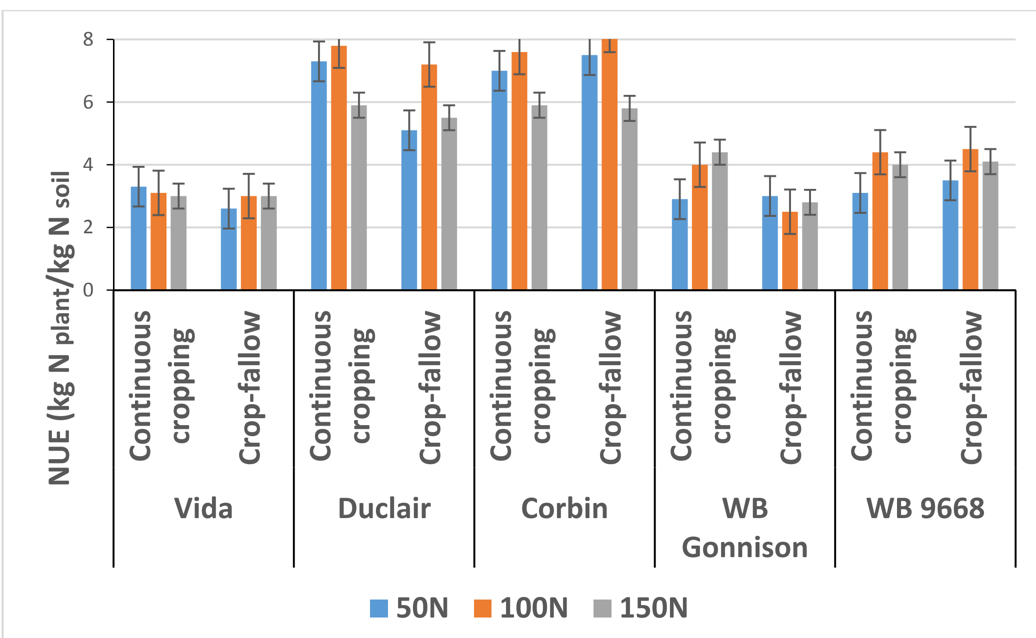

Figure 2. *Effects of cultivar, rotation type, and N rate on the nitrogen use efficiency of five spring wheat grown the Montana dryland.*