Moving On Up: H⁺-PPase Mediated Crop Improvement

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Upregulation of H⁺-PPase in diverse crop systems triggers agriculturally beneficial phenotypes including augmented stress tolerance, improved water and nutrient use efficiencies, and increased biomass and yield. We argue that further research is warranted to maximize the full potential of this simple and successful biotechnology.

Since humans began crop domestication, yield increase has been of paramount importance. Increased atmospheric temperatures, altered precipitation patterns, drought, and field salinization all represent major impediments to crop production. Here we emphasize the relevance of a simple agbiotechnological approach that entails the upregulation of the type I plant H⁺-pyrophosphatases (H⁺-PPases).

H⁺-PPases are hydrophobic proteins that function as homodimers in the generation of proton gradients across membranes using the energy of the phosphoanhydride bond of pyrophosphate (PP_i) molecules ([5] and references therein). Plant type I H⁺-PPases were first isolated from vacuoles and were considered to be vacuolar markers. However, later studies with gold-conjugated H⁺-PPase-specific antibodies and proteomic approaches show a dual localization at the tonoplast and the plasma membrane (PM) ([3,9] and references therein) (Figure 1B). Interestingly, there is *in vitro* evidence obtained with

tonoplast fractions of maize coleoptiles and oranges consistent with a reverse PP_i -synthase function of the H^+ -PPase provided a steep trans-tonoplast proton gradient is present ([8] and references therein).

The H^+ -PPase, a single subunit enzyme highly conserved among plant species, is an amenable candidate for enhanced expression in a variety of crops. Overexpression in *Arabidopsis thaliana* results in plants that tolerate high salinity conditions and exhibit enhanced drought resistance. It has been subsequently demonstrated that increased expression of plant type I H^+ -PPase genes can increase both salt and drought tolerance in diverse crops including rice, barley, wheat, tobacco, cotton, alfalfa, maize, and creeping bentgrass ([3] and references therein; [2,6,11]). Originally, the salt-tolerant phenotype was ascribed to increased Na^+ uptake into vacuoles and drought resistance was attributed to enhanced vacuolar osmoregulatory capacity ([3] and references therein).

Upregulation of H^+ -PPases in *Arabidopsis*, rice, corn, barley, wheat, tomato, lettuce, cotton, and finger millet also contribute positively to many energetic plant processes including general shoot and root growth, nutrient acquisition, and CO_2 fixation ([3] and references therein; [1,6,7,11]). For example, cotton plants expressing a H^+ -PPase are salt and drought tolerant. Interestingly, these plants also show increases in photosynthesis, stomatal conductance, and root development. Furthermore, these parameters are significantly lower when H^+ -PPase activity is reduced ([3] and references therein). In rice, shoot and root biomass are enhanced 82% and 109%, respectively, when plants express higher levels of a H^+ -PPase ([3] and references therein). Overexpression in an elite inbred corn line results in 21% higher accumulation of total soluble sugars and enhanced root development. These differences are elevated to approximately 50% after 3

days of osmotic stress treatment and the 1000-grain weight from plants subjected to 6 weeks drought stress is 30% higher ([3] and references therein). These phenotypes, while important for agriculture production, are not explained by the prevailing vacuolar centric model of H^+ -PPase function. Recent work suggests an important additional role for the H^+ -PPase in phloem sucrose (Suc) loading and transport functioning as a PP_i synthase [4,8] (Figure 1A,B). Phloem loading of Suc into the sieve element–companion cell (SE–CC) complex of plants that load from the apoplasm requires the active uptake of Suc from the extracellular space by Suc/proton symporters. This process is energized by the proton motive force established by P-type H^+ -ATPases (Figure 1B). To generate the required ATP, a portion of the loaded Suc is oxidized in companion cells. Particularly in these cells, Suc oxidation via the PP_i -dependent Suc Synthase pathway is essential (Figure 2). The PP_i synthase model suggests that the upregulation of the H^+ -PPase localized to the PM of SE–CC complexes increases the PP_i supply facilitating Suc loading and transport from source to sink tissues ([3] and references therein; [4]) (Figure 1A,B). Physiological, genetic, and ultrastructural evidence in greenhouse and laboratory conditions support this model [4,8,9]. More efficient Suc transport to sink organs in H^+ -PPase overexpressing plants explains the larger and more energized root systems with higher water and nutrient uptake capacities. This PP_i synthase activity is not mutually exclusive with the initially proposed H^+ -PPase mediated vacuolar sodium detoxification model and the improved PP_i scavenging activity potentially associated with seedling vigor ([2,3,11] and references therein). However, in transgenic barley and wheat engineered to have heightened H^+ -PPases expression, salt stress tolerance is achieved without enhanced vacuolar sodium sequestration capacity [6,11]. For example, these

transgenic barley lines display enhanced biomass production and yield in the field with and without salinity stress [11]. The heightened expression of H^+ -PPases in these barley plants may enhance biomass and yield due to augmented phloem sucrose loading and transport [4,8]. It could be hypothesized that as a consequence of augmented sucrose transport to the roots, these transgenic barley plants should have a constitutive upregulation of sucrose-dependent high affinity root K^+ transporters [8]. Constitutive expression of root high affinity K^+ transporters would be instrumental in selectively discriminating K^+ over Na^+ under saline conditions, reducing net Na^+ influx. Enhanced K^+ uptake capacity is evident when the H^+ -PPase is enhanced in *Arabidopsis* plants [12] and in transgenic wheat [10]. When grown in K^+ -limiting soils (Udic-Argosol), these wheat lines show 40% and 50% increases in yield and K^+ economic utilization index (the relation of shoot K^+ content and yield), respectively. Furthermore, these plants also develop roots that are at least two times larger and have increased organic acid exudation and rhizosphere acidification capacities compared with controls [10]. All these phenotypes in various crops (Figure 1C–E) are consistent with an increase in sucrose fluxes from source to sinks as hypothesized by the PP_i synthase model ([3,4,8] and references therein). However, this model does not directly address some other phenotypes observed in H^+ -PPase transgenic crops. For example, increased antioxidant enzyme activity has also been observed [1], and further studies are required to determine if this beneficial phenotype is as universal as the improved salt and drought responses, augmented plant biomass, and yield observed in a plethora of other agriculturally important crops.

The ability to dramatically improve both monocot and dicot crops through augmented H^+ -PPase expression is now self-evident. However, to fully harness this know-how, and leverage it with other technologies, a mechanistic rationale for these changes in a variety of crops is required. Given the recent advances, it is tempting to now speculate that H^+ -PPase upregulation specific to both phloem and heterotrophic tissues could have additive effects that further enhance crop yield.

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Figure 1. Model of Phloem Sucrose Loading Mediated by Enhanced H⁺-Pyrophosphatase (H⁺-PPase) Expression. (A) Longitudinal section through the sieve element (se)–companion cell (cc) complex in a rice leaf minor vein showing the general route of apoplastic phloem sucrose loading. A portion of the sucrose needs be metabolized at the cc via the Sucrose Synthase (SUS) pathway and the remaining sucrose loaded to the se for transport. (B) Higher magnification micrograph from (A) showing the cc overlaid with the schematic layout of pyrophosphate (PPi)-dependent SUS pathway. (C) Magnified inset of the boxed region in (B) showing plasma membrane (PM) localization of H⁺-PPase-specific immunogold label. We argue that here the H⁺-PPase functions as a PPi synthase. (D) Shoot and root development of H⁺-PPase and wild-type (WT) *Arabidopsis* grown 30 days under limiting nitrate (1.5 mM). (E,F) Enhanced shoot and root biomass in H⁺-PPase rice grown hydroponically 180 days in nutrient-sufficient media, relative to WT. Scale bars: (A) 5 μ m; (B) 1 μ m; (C) 200 nm.

Figure 2. Sucrose Utilization in the Absence of Invertases. Sucrose hydrolysis via the pyrophosphate (PPi)-dependent Sucrose Synthase pathway (modified from [2]).