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Silver and zinc inhibitors influence transpiration rate and aquaporin transcript abundance in intact soybean plants

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ABSTRACT

Soybean genotype PI 416937 has previously been found to have low leaf hydraulic conductivity, which was hypothesized to be the basis for limited transpiration (TR_{lim}) rates under high vapor pressure deficit. Previous studies with de-rooted shoots showed there was a consistency in the expression of TR_{lim} and plant response to exposure to silver, an aquaporin (AQP) inhibitor. However, it is not clear what confounding influence treatments with metal AQP inhibitors may have on AQP transcript expression. This study was undertaken to extend the observations of response to silver and zinc using intact plants. In a comparison of four genotypes, intact plants of PI 416937 were uniquely insensitive to exposure to silver and of PI 471938 were uniquely insensitive to zinc. RNA abundance of eight AQPs was measured after treatment with the AQP inhibitors. There were differences in the abundance of RNA among genotypes. There was a general trend of less change in abundance in PI 416937 following silver treatment in contrast to increased abundance in the other genotypes. This result would be consistent with little or no response in transpiration rate in the intact plants of PI 416937 following silver treatment. Following zinc treatment, there was a rapid increase in RNA abundance in PI 471938 in comparison to the other genotypes. However, the changes in AQP abundance following treatment with metal inhibitors indicates the likely interaction of direct response of AQP to metals and alternation of expression of AQP transcripts. These results indicate that the results from treatment with metal inhibitors should likely be considered only as preliminary screens from which genotypes need to be tested directly for the TR_{lim} trait.

1. Introduction

Transpiration is a natural consequence of stomata opening to allow CO_2 to diffuse into the interior of leaves to be assimilated in photosynthesis. Hence, transpirational water loss is unavoidable and quantitatively linked to plant growth (Tanner and Sinclair, 1983). It has been suggested, however, that there may be a greater photosynthetic return per unit of transpiration if stomatal conductance is restricted under high atmospheric vapor pressure deficits (VPD) (Sinclair et al., 2005). In addition to an increased water-use-efficiency, limited transpiration rate (TR_{lim}) under high VPD in the field would result in soil water conservation. Under rainfed conditions, TR_{lim} early in the growing season would result in increased soil water availability later in the growing season to allow sustained physiological activity during critical periods of reproductive development. The visual consequence of water conservation would be delayed plant wilting under late-season drought conditions. Simulation studies with soybean (*Glycine max* (L.) Merr.) have indicated that the TR_{lim} trait would result in increased crop yield in a large fraction of growing seasons in most production areas of the US and Africa (Sinclair et al., 2014; Sinclair et al., 2010).

In fact, soybean genotype PI 416937 has been found to express the TR_{lim} trait above a VPD of about 2 kPa (Fletcher et al., 2007; Sinclair et al., 2008). The visual manifestation of this response in the field for this genotype was delayed plant wilting under drought (Sloane et al., 1990). It was found that the hydraulic conductance in the leaves of PI 416937 was low compared to other soybean genotypes (Sinclair et al., 2008). Genotypes derived from PI 416937 have now been developed that express the TR_{lim} trait and have been selected for increased yields under water-limited conditions (Devi et al., 2014).

Sadok and Sinclair (2010a, 2012) suggested that the low hydraulic conductance in the leaves of PI 416937 might be related to a unique population of aquaporins (AQPs) in its leaves. This speculation was consistent with the observation that de-rooted

Abbreviations: AQP, aquaporin; DTR, decrease in transpiration; TR, transpiration rate; TR_{lim}, limited transpiration rate; VPD, vapor pressure deficit.

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shoots of soybean genotypes differed greatly in their sensitivity of transpiration rate to exposure to silver ions, which can inhibit AQP function. Silver inhibition has been linked to the sulfhydryl group of cysteine residue of AQPs resulting in blockage of the pore for water passage (Niemietz and Tyerman, 2002). The transpiration rate of de-rooted shoots of PI 416937 were essentially insensitive to silver treatment while nearly all other genotypes were quite sensitive to treatment with silver (Sadok and Sinclair, 2010a). An hypothesis to explain these results from exposure to silver is that PI 416937 does not have a population of AQP that is selectively sensitive to silver, and hence, without these AQPs also has a lower hydraulic conductance.

In contrast to the insensitivity to silver, when de-rooted shoots of PI 416937 were exposed to the AQP inhibitor zinc, transpiration rate of PI 416937 was greatly decreased (Sadok and Sinclair, 2012). Tazawa et al. (1996) found that zinc inhibited the hydraulic conductivity of the plasma membranes of *Chara corallina* by acting on the thiol group of the water channel proteins. However, zinc may also interaction with sulfhydryl groups of cysteine residues in AQPs (Yukutake et al., 2009).

Since treatment of plants to metal inhibitors potentially allows screening of fairly large plant populations, inhibitors has been proposed as an intermediate screen in breeding programs to develop drought-tolerant genotypes. Screens using metal inhibitors have already been explored for soybean (Sadok and Sinclair, 2010a), peanut (Devi et al., 2012), sorghum (Choudhary and Sinclair, 2014), and maize (Choudhary et al., 2014). In these trials, de-rooted shoots or individual leaves were exposed to solutions containing metal inhibitors and the transpiration response was measured. However, there are two concerns in regards to these previous experiments. First, tests were not done with intact plants so the overall responses of plant transpiration to the metal inhibitors were not tested. Second, it is unknown how the metal inhibitors may themselves alter AQP populations in the plant allowing the possibility that the response to the inhibitors is confounded by altered expression of individual AQP. Therefore, this study was undertaken to examine also the change in AQP transcripts for eight AQP genes in roots and leaves of intact plants as a result of exposure to silver and zinc ions.

To allow a greater scope in interpreting the experimental results, PI 416937 plus three additional genotypes (PI 471938, Benning and A5959) were included in the experiment. PI 471938 has also been observed to have delayed wilting in response to drought in the field but it does not express TR_{lim} at high VPD (Fletcher et al., 2007; Sadok et al., 2012). Consistent with the lack of the TR_{lim} trait, transpiration in PI 471938 is sensitive to exposure to silver (Sadok and Sinclair, 2010a). Genotypes Benning and A5959 are normal-wilting commercial genotypes for which transpiration rate was found to be sensitive to feeding of silver to de-rooted shoots (Sadok and Sinclair, 2010a) and moderately sensitive to feeding of zinc (Sadok and Sinclair, 2012).

2. Materials and methods

2.1. Plant material and growing conditions

Seeds (obtained from T.E. Carter, Jr., USDA-ARS, Raleigh, NC) of the four genotypes to be tested were placed in sterile germination paper and rolled loosely to form a cylinder. A rubber band was used around the rolled paper to hold the seeds in position. The paper cylinder was placed on its end in a sterile 2-L flask and 250 mL of 100 μM $CaSO_4$ solution water was poured over the paper cylinder and allowed to collect in the flask. Finally, a polyethylene bag was placed over the mouth of the flask and the upper end of the paper cylinder, and the flask was placed in a germination chamber for about 7 days.

Germinated seedlings were removed from the germination paper and transferred to 1-L glass flasks containing hydroponic solution. The flasks had been wrapped with aluminum foil to prevent exposure of the nutrient solution to light. In addition to preventing algal growth, the blockage of light avoided photochemical alterations of the inhibitors when they were fed to the plants. The hydroponic solution during the growth of the plants was the standard solution used in the Phytotron, North Carolina State University (Saravitz et al., 2009): $MgSO_4$ (0.3 mM), K_2NO_3

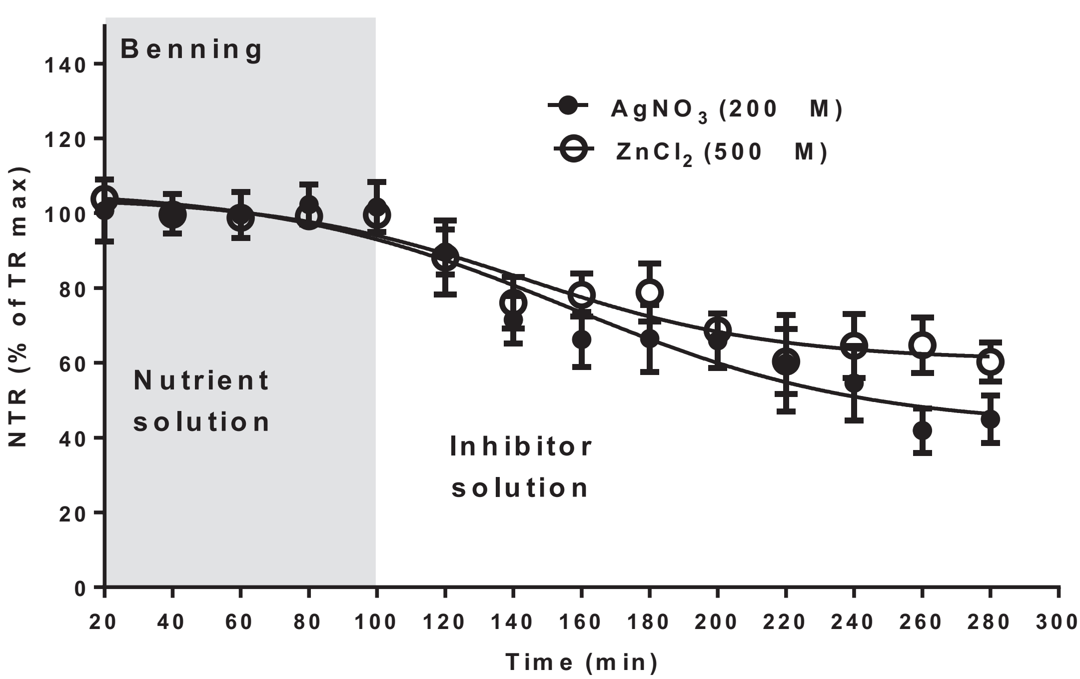

Fig. 1. Normalized transpiration rate ± S.E. before (gray area) and after treatment (clear area) of intact Benning plants with solutions containing 200 and 500 μM of $AgNO_3$ and $ZnCl_2$, respectively.