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Inhibitor screen for limited-transpiration trait among maize hybrids

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ABSTRACT

A plant trait to minimize the impact of drought on crop yield is limited-transpiration rate (TR) under high ambient vapor pressure deficit (VPD) so that soil water is conserved to sustain grain fill. Variation among maize (*Zea mays* L.) hybrids has been identified for the existence of the limited-TR trait at high atmospheric VPD, and the VPD at which TR becomes limited. Further, it has been shown that the TR limitation at high VPD is related to plant hydraulic conductance, which may be due to differences in aquaporin expression. This paper reports studies to relate the TR response of 21 maize hybrids to treatment of leaves and intact plants with cycloheximide (CHX) and four aquaporin inhibitors: silver ($AgNO_3$), gold ($HAuCl_4$), zinc ($ZnCl_2$), and mercury ($HgCl_2$). There was no discrimination among hybrids based on treatment with Hg or CHX. Segregation between hybrids for response to increasing VPD corresponded with differences in leaf response to Ag and Au treatment and intact plant response to Zn. The highest correlation ($r = 0.90$) between VPD breakpoint and TR response to inhibitor was with Ag treatment of leaves. These results indicate that Ag may be an effective initial screen for expression of the limited-TR trait under high VPD.

1. Introduction

Maize is an important component of global food security, which is often challenged by regional episodes of drought (Heisey and Edmeades, 1999). The magnitude of the impact of drought on maize yields is dependent upon the adequacy of the cropping system to the production environment. Agronomic practices as well as morphological and physiological characteristics of the genotype affect the crop water balance, with consequences on yield depending upon the environment. Limiting pre-anthesis water use while maintaining high plant stands can contribute to increased crop productivity in regions where late-season drought is common (Passioura and Angus, 2010; Sinclair et al., 2005, 2010). Therefore, one approach to developing cultivars for drought conditions is having expression of limited transpiration rate (TR) under high atmospheric vapor pressure deficit (VPD).

Expression of limited TR under high VPD has now been documented in selected genotypes of several crop species. Species in which genotypes showing the limited-TR trait under high VPD have been identified include soybean (*Glycine max* (L.) Merr.; Fletcher et al., 2007; Gilbert et al., 2011), peanut (*Arachis hypogaea* L.; Devi et al., 2010), pearl millet (*Pennisetum glaucum* L.; Kholova et al., 2010), sorghum (*Sorghum bicolor* L.; Gholipoor et al., 2010), chickpea (*Cicer arietinum* L.; Zaman-Allah et al., 2011), and recently in maize (*Zea mays* L.; Yang et al., 2012; Gholipoor et al., 2013). Among 35 single-cross maize hybrids evaluated for their TR response to vapor pressure deficit (VPD), 12 hybrids expressed limited TR above VPD breakpoints ranging between 1.74 and 2.52 kPa (Gholipoor et al., 2013). In a study of these hybrids, it was found that the VPD breakpoint was positively correlated with indices of hydraulic conductance in both the leaves and roots (Choudhary et al., 2014).

Since direct determination of the TR response at high VPD is laborious and only a few genotypes can be characterized at one time, it would be quite useful to develop an initial screen for the limited-TR trait under high VPD to narrow the number of lines to be directly tested for the trait. One approach would be to explore the possibility that an aquaporin (AQP) inhibitor might relate to the transpiration response to high VPD. Aquaporins are protein channels that facilitate water transport across membranes, and have been implicated in the regulation of root water uptake and TR (Maurel et al., 2008). A number of studies have demonstrated that root and shoot hydraulic conductance can be controlled by the up-regulation of plasma-membrane AQP abundance and/or activity (Javot and Maurel, 2002; Tyerman et al., 2002; Cochard et al., 2007; Parent et al., 2009). Under high evaporative demand, transcellular AQP-controlled water flow may become a significant

Abbreviations: AQP, aquaporin; CHX, cycloheximide; DTR, decreased transpiration rate; TR, transpiration rate; VPD, vapor pressure deficit.

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Table 1
The ionic diameter, hypothesized mode of action and reversibility action with β-mercaptoethanol of four metallic AQP inhibitor mercury, silver, gold, zinc and a non-metallic metabolic AQP inhibitor cycloheximide.

AQP inhibitors	Ionic diameter (Brady and Holum, 1988)	Hypothesized mode of action	Reversibility by β-mercaptoethanol
Mercury (Hg^{2+})	2.2 Å	Non-specific interaction with sulfhydryl group of cysteine residues at various positions (Preston et al., 1993; Shi and Verkman, 1996; Savage and Stroud, 2007)	Yes (Voicu et al., 2008, 2009; Voicu and Zwiazek, 2010)
Silver (Ag^{+})	2.5 Å	Interacts with histidine (Wells et al., 1995) and sulfhydryl group of cysteine residue (Scozzafava et al., 2001) in vicinity of conserved NPA motif (Niemietz and Tyerman, 2002)	No (Niemietz and Tyerman, 2002)
Gold ($Au3^{+}$)	2.8 Å	Interacts with sulfhydryl group of aquaporins (Niemietz and Tyerman, 2002)	No information
Zinc (Zn^{2+})	1.6 Å	Interacts with sulfhydryl groups of cysteine or histidine residues (Cakmak, 2000), but has weaker affinity for sulfhydryl groups than mercury	Yes (Henzler and Steudle, 1995; Tazawa et al., 1996; Watanable et al., 2009)
Cycloheximide (CHX)		A non-metallic metabolic inhibitor that blocks de novo protein synthesis, by inhibiting peptide initiation and extension (Obrig et al., 1971)	No

determinant of the leaf water balance and stomata conductance (Kaldenhoff et al., 2008). Thus, it has been hypothesized that AQP population activity may underpin the TR response to VPD (Sadok and Sinclair, 2010a). This leads to the idea that genotypic variation in hydraulic conductance and expression of the VPD breakpoint may result from genotypic differences in abundance/activity of AQP populations. Hence, there may be a possibility to do an initial screening of maize populations for the limited-TR trait at high VPD by determining transpiration response to plant treatment with AQP inhibitors. There are several candidate inhibitors that might be appropriate for such a screen (Table 1). The most frequently used inhibitors in AQP studies are $HgCl_2$ (Javot and Maurel, 2002) and physiological concentrations of cycloheximide (CHX, Cochard et al., 2007). Silver and gold are also potent inhibitors of AQP, which in some cases may be insensitive to more common inhibitors such as mercury (Niemietz and Tyerman, 2002; Sadok and Sinclair, 2010a,b). Transpiration rate has also been found to be inhibited by excess Zn (Patel et al., 1980; Kastori et al., 1992; Sagardoy et al., 2009).

The objectives of this study were (i) quantify genotypic variation for expression of the limited-TR trait under high VPD by testing for response of detached leaves and intact plants to AQP inhibitors and (ii) to explore an initial screening protocol using an inhibitor to narrow the number of lines to be directly tested for TR response to VPD. Experiments were done using both detached leaves and roots of intact plants in hydroponic solution. The five inhibitors used were silver ($AgNO_3$), gold ($HAuCl_4$), zinc ($ZnCl_2$), mercury ($HgCl_2$) and cycloheximide (CHX). Decrease in TR after inhibitor treatment was considered as a potential initial screen of the TR-limited trait under high VPD.

2. Materials and methods

2.1. Plant material

The influence of the inhibitors was tested on 21 single-cross maize hybrids selected based on their transpiration response to VPD (Gholipoor et al., 2013). Twelve of the selected hybrids exhibited breakpoints in TR with increasing VPD (PHYBTS004, 07, 10, 13, 17, 19, 21, 24, 27, 29, 30 and 31) and nine hybrids exhibited linear increases in TR over the entire range of tested VPD (PHYBTS002, 05, 08, 22, 23, 26, 28, 32, and 37).

2.2. Inhibitor concentration

Preliminary experiments were conducted to determine concentrations of inhibitor that decrease leaf and whole plant transpiration by approximately one third of that measured prior to the application of the inhibitor. Four concentrations of $HAuCl_4$, CHX, $HgCl_2$ and $AgNO_3$ (50, 100, 200, and 500 μM) and six concentrations for $ZnCl_2$ (200, 500, 750, 1000 μM) were evaluated. The solutions were prepared 1–5 d before the experiments and stored in darkness at 4 °C. The CHX solutions were obtained by dissolving in a 0.05% (v/v) aqueous dimethyl sulphoxide solution, according to the protocol of Cochard et al. (2007). Gold chloride and silver nitrate solutions were placed in opaque, dark brown 60-ml glass bottles to prevent precipitation by light. Decrease in TR in response to AQP inhibitor concentrations was determined for whole plants (hybrid PHYBTS030 and PHYBTS037) and leaves (hybrid PHYBTS019, PHYBTS030, PHYBTS005, PHYBTS008 and PHYBTS037) to include a subset of hybrids that expressed breakpoint and linear TR responses to VPD.

2.3. Leaf transpiration inhibition

Seeds were sown in pots filled with 1.5–3 kg of composted garden soil containing slow-release fertilizer. Three to four seeds were sown in each pot. The plants were grown in a greenhouse (Phytotron, North Carolina State University, Raleigh, NC) with temperature regulated for a minimum temperature of 26 °C and maximum temperature of 32 °C. Pots were watered every 1–2 d. Seven to 15 d after sowing, each pot was thinned to one plant.

Plants were grown for approximately 4–5 weeks until 4–6 fully expanded leaves had developed on the plants. At that time, pots were heavily watered for 2–3 d to ensure dripping of excess water from the pots. On the evening of the day prior to the experiment, fully developed leaves from four replicate plants were gently cut from the leaf sheath below the collar. The cut leaves were immediately placed in a flat tray containing de-ionized water and a second cut was made for each leaf underwater above the collar. The base of the leaves were placed in de-ionized water in a flask and moved to a dark room overnight (approximately 14 h) under a temperature maintained at 22.3 °C (±0.18 se). The following morning, the leaves were moved from the dark room to a walk-in growth chamber. Temperature in the growth chamber was maintained