Title: Measurements of maize root plasticity under water stress in hydroponic chambers

Running Title: Maize root plasticity under water stress

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1. Abstract

Under water stress, plants adjust root traits including depth of root system, root diameter, density of root per volume of soil, hydraulic conductance of root. In this experimental study, we present a method to quantify how hydraulic traits of maize roots adapt to drought. The experiments involve a microfluidic flow sensor and a custom-built pressure chamber, made of transparent plastic for visualization purposes. We measured how maize genotypes (PHB47 and PHZ51) grown for a week in deionized (DI) water and one day in hydroponic nutrients solution (called the irrigated condition) respond to one week of water stress. Conditions of water stress (called drought conditions) were created by mixing Polyethylene Glycol with the nutrients solution. Results show that under drought, the roots of both genotypes respond by approximately halving their global hydraulic conductance. This adjustment seems to be achieved mainly by reductions of the total surface area of the roots. Interestingly, the measured hydraulic conductivity of the roots grown under drought was significantly larger. In all, this study sheds light on how plants adapt to water stress in a hydroponic system, by decreasing root area and increasing root permeability.

Keywords: pressure chamber, drought, water relations, hydraulic conductance, microfluidic sensor, root plasticity.

2. Introduction

Climate change and population growth incite both scientists and engineers to investigate methods to increase food production. Climate change increases drought-index (insufficient soil moisture level to meet the plant needs for water as defined in [1]) all over the world, and in the near future we would have to increase yield and decrease water use.

Improved performance of crops under water stress [2], and conservative or efficient water use would have a vast impact on the sustainability of agriculture, especially in the 1.3 billion hectares of marginal agricultural land [3] globally. In arid regions where irrigation water is at a premium, conservative crop
water use would allow the cultivation of a broader range of crops, even with just percentage decreases in water use. In developing countries, such as those in sub-Saharan Africa, conservative water use would be beneficial as it would increase the probability of crops surviving typical dry periods during the growing season [4]. Plant’s root system size, properties, and distribution strongly influence it’s access to water, and plants respond to water stress by adjusting root traits rooting density [5], root length[5, 6], root diameter [7], along with other physiological traits [8]. Therefore, it is imperative to find a way to track and quantify root system attributes in assessing desirable root traits that could improve plant performance under drought condition.

Among various important root traits, root depth, root diameter, rooting density, hydraulic conductance, hydraulic conductivity are found to be regulated by plants under drought [9, 10]. Tracking and quantification of root traits including root depth, root diameter, rooting density are performed by 2D/3D imaging of the root system and subsequent image analysis. On the other hand, several methods are used to quantify hydraulic conductance namely, root pressure probe [11], and high-pressure flow meter [12], pressure chamber [13]. Qing-ming et al. showed that [14] there were no significant differences among hydraulic conductance values of maize seedlings measured by these methods.

In their seminal work, J. Passioura et al. [13] developed a pressure chamber encapsulating the root of a plant, while the stem and the shoots were outside of the chamber. Measurements of the pneumatic pressure applied inside the chamber and of transpiration rates and water flow rates measured on cut leaves allow the determination of the hydraulic conductance of the plant. So-called Passioura chambers are typically metallic and do not allow optical access to the root. Here we develop a Passioura chamber with optical access to the roots, and a microfluidic sensor mounted on the stem for measuring water transport. This optical access could facilitate 3D imaging [15] while the root system is in the chamber.

In this manuscript, we report on the design and manufacturing of a transparent pressure chamber, as well as two main results describing how maize adapts to drought under hydroponic conditions.
3. Materials and methods

Plant cultivation and artificial drought condition

Measurements were performed on 15-day-old seedlings for two maize genotypes, PHB47 and PHZ51. PHB47 and PHZ51 are two elite expired PVP (Plant Variety Protection) inbred lines which belong to Iowa Stiff Stalk and Non-Stiff Stalk heterotic pools, respectively [Brenner et al. 2012]. Seeds were collected from cold storage, and 50 similar sized seeds are selected by visual inspection. Seeds were sterilized with Clorox solution (6% sodium hypochlorite) for 15 min in a beaker with a magnetic stir and subsequently washed twice using DI water. Seeds were then placed on brown germination papers (Anchor Paper, St. Paul, MN, USA), twenty seeds per paper, and rolled up vertically. Before placing the seeds, the papers were moistened with fungicide solution Captan (2.5 g/L). All seed rolls were placed into 2-L glass beakers containing 1.4 L of autoclaved deionized water. The glass beakers were kept in a growth chamber, where growing conditions were maintained as per the protocols in [16] with a 16 h photoperiod, a temperature cycle of 25 °C/22 °C (day/night) and 65% relative humidity, light intensity was 200 μmol photons m⁻² s⁻¹. Every other day the beakers were refilled with water to maintain the water level at 1.4 L.

After seven days, the seedlings were taken out of the roll, and roots with similar length were selected for transplantation. The selected seedlings were transplanted individually in two custom-made hydroponic systems as shown in Fig 1 with static nutrient solution and continuous aeration, corresponding to water potential of about 0 MPa (our control case). The solution was prepared by mixing eleven chemical compounds (Calcium nitrate (Ca(NO₃)₂·4H₂O), potassium sulfate (K₂SO₄), Magnesium sulfate (MgSO₄·7H₂O), potassium chloride (KCl), Monopotassium phosphate (KH₂PO₄), Boric acid (H₃BO₃), Manganese II sulfate (MnSO₄·H₂O), Copper sulfate-5 hydrate (CuSO₄·5H₂O), Zinc sulfate-7 hydrate (ZnSO₄·7H₂O), Ammonium molybdate ((NH₄)₆Mo₇O₂₄·4H₂O), Iron chelate(Fe-EDTA) with water as described in [17].

Fig 1. Custom-built hydroponic systems were used to grow transplanted seedlings. The seedlings were individually transplanted via a Styrofoam sheet and pieces of sponge. First through holes were made
on the sheet and then the seedlings were transplanted through the holes and held with a piece of sponge.

In the system, continuous aeration was introduced using an air pump.

Initially, both hydroponic systems contain the only nutrient solution. After 24 hours, water stress was created in one of the hydroponic systems by adding 15% (w/v) Polyethylene Glycol (PEG) 6000M in the nutrient solution [18], corresponding to water potential of -0.3 MPa. Then, after another seven days, seedlings were removed from the growth chamber for measuring the hydraulic conductance with the pressure chamber described below, and extract root traits with the software ARIA [19].

**Design of pressure chamber and flow sensor**

We designed and assembled a pressure chamber to measure the hydraulic conductance of whole root systems (see S1 Appendix for the detail). The device is based on the root transport model described in [13], where the water flow rate \( J = L(\Delta P - \sigma \Delta \Pi) \), and \( L \) is the root conductance \( [m^3/Pas] \), \( \Delta P = P_1 - P_2 \) is the difference of hydrostatic pressure, including gravity, and \( \Delta \Pi \) is a difference of osmotic pressure, with \( \sigma \) is the reflection coefficient. In the device the gradient of water potential \( (\Delta P - \sigma \Delta \Pi) \) is in the natural direction, that is, the potential is higher around the root system than that at the stem.

**Measurement of hydraulic conductance**

For the measurement, a plant was cut at the stem just above the base of the root system and inserted in the pressure chamber as shown in Fig 2. The pressure chamber was filled with ultrapure deionized (DI) water (Milipore, US). The differential pressure \( \Delta P \) across the root system and the end of the cut stem was controlled and measured with a pressure controller (Alicat, PC-Series). A transparent tubing was fitted to the cut stem and another end of the tube was connected to a flow sensor. The flow rate, \( J \), through the tube was measured with the flow sensor or calibrated fluidic resistance. The hydraulic conductance \( L \) was measured by using \( J = L(\Delta P - \sigma \Delta \Pi) \), where \( \Delta \Pi \) was considered zero, as only DI water was used in
our system. A detailed description of the apparatus and of the experimental protocols are included in the supplementary information.

Fig 2. Experimental setup of the flow sensor

The pressure chamber was filled with DI water. A custom-made compression gasket-fitting was used to hold the cut stem and ensure leak proof. The end of the cut stem was connected to a calibrated microfluidic tube (thick black line), the pressure drop across the calibrated tubing was measured via a differential pressure gauge ($P_2 - P_{atm}$). The pressure drop across the root system was measured via another pressure gauge ($P_1 - P_2$).

Measurement of root traits from digital image

After hydraulic conductance measurement, the root systems were imaged using a high-resolution scanner, EPSON Expression 10000 XL scanner system (Copyright 2000–2014 Epson America, Inc). During the imaging the roots were spread out as much as possible to remove overlapping. Using ARIA [20], 27 different visible traits including total surface area, root length, were measured from the image. The surface area was evaluated by considering root system had a circular cross-section where the diameter was the local width of the root segment observed in the image.

4. Results

First we validated the accuracy, repeatability, and fidelity of our measurements. We, then, quantified and compared hydraulic conductance of the root system for the genotypes, PHB47 and PHZ51. Finally, we measured the total surface area, total length of the root systems using ARIA and investigated the adaptation of the hydraulic conductivity between the genotypes.

Validation of the hydraulic conductance measurement
To validate the accuracy, repeatability, and fidelity of our system, we measured the global root conductance of the hydroponically grown seedlings of an arbitrary maize genotype. The seedlings were grown in irrigated condition ((pure water and nutrient solutions). Fig 3 indicates the global conductance measurement of three samples: two two-week-old seedlings (samples 1 and 2), one three-week-old seedling (sample 3). The measured conductance of two-week-old seedlings are $7.4 \times 10^{-15}$ [m$^3$/Pa.s] and $6.6 \times 10^{-15}$ [m$^3$/Pa.s], respectively, and that of the three-week-old is $5.1 \times 10^{-14}$ [m$^3$/Pa.s]. The repeatability and precision of the measurements are very good as shown by the error bars in Fig 3. The measured global root conductance of two-week-old seedlings is consistent with the range of published values [21], from 6.6 to 7.7 $\times 10^{-15}$ [m$^3$/Pa.s].

**Fig 3. Validation of the flow sensor measurement.** Flow rates through the root system of two- and three-week-old seedlings were measured at different applied pressures. Each measurement was repeated ten times. The error bars indicate the standard deviation among the flow rate measurements. The global hydraulic conductance was evaluated from the slope of the best-fitted line passing through the pressure drop vs. mean flow rate measurements. The measured conductance of two-week-old seedlings is consistent with the range of published values [17].

**Adaptation of root traits under drought**

Our measurements indicate that seedlings cultivated for seven days under drought (water stress condition) typically exhibit a lower hydraulic conductance than those cultivated in irrigated condition (pure water and nutrient solutions) (**Fig 4** (A)). The reduction of hydraulic conductance of the genotypes PHB47 and PHZ51 are, respectively, 53% and 60%. Reduction in hydraulic conductance indicates plant become water conservative, uses less water during drought. They had similar total root volume (via eyeballing), after the initial first week in the germination paper, but after the second week in different water stress conditions, the total root volumes were different. Under drought, the total root volumes were smaller than those in the irrigated conditions for both genotypes (**Fig 4** (B)), i.e. the root system has less surface area.
and pathways to uptake water. The overall root surface area under drought was also found around 70% smaller than that in normal/irrigated condition.

**Fig 4.** (A) Overall hydraulic conductance of the genotypes under normal/irrigated and drought conditions, the box plots were generated from three data set, (B) photograph of the genotypes in normal/irrigated and drought conditions.

The overall hydraulic conductivity [22] of the genotypes were estimated by dividing overall hydraulic conductance with overall surface area. The hydraulic conductivity under drought was increased significantly for both genotypes (**Fig 5**), that is 50% for PHB47 and 150% for PHZ51. Similar trends also reported for young maize seedlings grown in pots by Zhang et al. [23]. They found an increased hydraulic conductivity when plants grown under drought conditions were re-watered. The adaptation of the hydraulic conductivity could be due to the change in the structural components in roots and/or increased abundance and conductance of aquaporins [24-28].

**Fig 5. Hydraulic conductivity of plants under normal and drought conditions.**

### 5. Conclusions

In this paper, we present a methodology to quantify the adaptation of maize root hydraulic traits to the water stress/drought. The experiments utilize microfluidic flow sensors and a custom-built pressure chamber. The pressure chamber was made of transparent material for visualization purposes. Two inbred maize genotypes (PHB47 and PHZ51) were used to investigate the root systems adaption to artificial drought condition. The drought condition was created by mixing Polyethylene Glycol (PEG) 6000 with the nutrients solution. In response to drought, roots of both genotypes adapted similarly — the roots grew smaller in size compared to the roots in irrigated condition, i.e. the overall surface area of roots was reduced. The surface area was measured by an in-house quantitative phenotyping software. Interestingly, the measured hydraulic conductivity of the roots grown under drought is significantly larger than that of roots grown under irrigated conditions. Similar observations were reported for several genotypes of maize.
in the responses to daily vapor pressure demand [29] and moderate water stress [30]. In all, this study
sheds light on how plants adapt to water stress in a hydroponic system, by decreasing root area and
increasing root hydraulic conductivity. The developed methodology can be used to investigate plant
responses in other abiotic stresses e.g., salt, heat.

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7. Supporting information

S1 Appendix. Design and fabrication of pressure chamber.
S2 Appendix. Design and calibration of microfluidic pressure sensor.
S3 Appendix. Sample loading for the measurement of hydraulic conductance.
S1 Fig. Photograph and simplified exploded view of the pressure chamber.
S2 Fig. Sequential images of the sample loading procedure in the chamber.

8. References

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$K_{\text{root}} = \frac{Q}{(P_1 - P_2)} = \frac{Q}{\Delta P}$
Sample 1: Two-week-old maize
Sample 2: Two-week-old maize
Sample 3: Three-week-old maize

\[ \frac{Q}{\Delta P} = 5.09 \times 10^{-14} \frac{m^3}{Pa.s} \]

\[ K_{root} = \frac{Q}{\Delta P} \]

\[ \frac{Q}{\Delta P} = 7.36 \times 10^{-15} \frac{m^3}{Pa.s} \]

\[ \frac{Q}{\Delta P} = 6.63 \times 10^{-15} \frac{m^3}{Pa.s} \]
