Xylem vessel radii comparison between soybean genotypes differing in tolerance to drought

Marco Nogueira, David Livingston, Tan Tuong & Thomas R. Sinclair

Published online: 03 Feb 2020.

Submit your article to this journal ↗

View related articles ↗

View Crossmark data ↗

Taylor & Francis
Taylor & Francis Group

Check for updates

Xylem vessel radii comparison between soybean genotypes differing in tolerance to drought

Marco Nogueira[a], David Livingston[b], Tan Tuong[b], and Thomas R. Sinclair[c]

[a]Plant Science Research, Embrapa Soja, Londrina, Brazil; [b]US Department of Agriculture, ARS, Raleigh, NC, USA; [c]Department of Crop and Soil Science, North Carolina State University, Raleigh, NC, USA

ABSTRACT

Xylem element radius can be a key factor in determining plant hydraulic conductance and vulnerability to cavitation. Most studies of xylem element radius have been on woody species with a focus on plant survival under severe water-deficit stress. However, xylem element radius, particularly the largest radius elements, can potentially have an influence on hydraulic flow at more moderate water-deficits. Few studies have offered a detailed distribution of xylem element radii, and even fewer on the distribution in crop species. In this study, the xylem element radii of two genotypes of soybean (*Glycine max* L. Merr.) were compared because these two genotypes had been documented to react differently to drying soil. The stems of young plants were harvested from three positions, and in stem cross-sections, the number of xylem elements and the radius of each element were determined. While the number of xylem elements did not differ significantly between the two genotypes, the distribution of the radii was skewed to smaller radii in drought-tolerant PI 4719386 as compared to Hutcheson. This contrast extended to a difference between the genotypes in the radii of the largest elements, which are considered most vulnerable to cavitation.

Hydraulic properties of plants are recognized as important in determining water flow through plants and in influencing plant response to soil water deficits. In particular, the hydraulic conductance for water flow through the xylem, and the possible influence on occurrence of cavitation is of concern. Most studies on xylem anatomy and hydraulic conductance have been with woody species to better understand the traits that enhance plant survival under severe water deficits. Only a few of these studies indicated a distribution in xylem element diameters in the trunks (Lintunen and Kalliokoski 2010; Tombesi, Johnson, Day, and DeJong 2010; Torres-Ruiz et al. 2013).

In relating xylem element diameter to water flow and hydraulic conductance, it is necessary to examine the detailed distribution of the diameters because conductance is not linearly related to diameter. The capillary flow volume, as

---

defined by the Hagen-Poiseulle equation, is dependent on the cell radius to the fourth power. Due to the fourth power dependence on radius, water flow through the xylem is heavily skewed to be through the largest xylem elements. That is, in a collection of xylem elements in a stem, most of the flow volume will be through a few of the largest diameter elements.

While large diameter xylem elements favor high hydraulic conductance and water flow, these larger-diameter elements are the most vulnerable to cavitation (Tyree and Sperry 1989). In the simple model of capillary flow, the hydrostatic pressure differential for cavitation is inversely related to capillary radius. The hypothesis is that the smaller xylem elements are able to withstand greater hydrostatic pressure differentials than larger-diameter xylem elements before cavitation is induced. Based on experimental observations, Lo Gullo et al. (1995) found a relationship between xylem radius and loss of xylem conductance in twigs of Quercus cerris L. when subjected to pressure differentials. In their experiments, loss of conductance occurred with mean xylem radius as low as 10 μm.

Although the relationship between xylem element diameter, cavitation, and survival of woody species is of obvious importance, little attention seems to have been given to the distribution of xylem element radii in herbaceous crop species. In particular, are there differences in the distribution of xylem element radii between genotypes within a crop species? In this study, the radii distribution of stem xylem elements was compared between two soybean (Glycine max Merr. L.) genotypes visually observed to express difference in response to water deficit in the field. Genotype PI 471938 was originally identified as visually expressing delayed wilting as compared with other genotypes when subjected to drought (Hufstetler et al. 2007). Li, Sinclair, and Bagherzadi (2016) tracked changes in hydraulic conductance in PI 471938 with soil drying and found decreasing hydraulic conductance as the soil dried. Sadok et al. (2012) showed that under high vapor-pressure deficits that the hydraulic conductance of PI 471938 was lower than a "standard" cultivar (A 5959) although its transpiration rate was not lower as it also had a lower leaf-water potential. Under lower vapor-pressure deficits, however, no difference in hydraulic conductance was observed between PI 471938 and A 5959 (Bagherzadi et al. 2017).

A prediction from the above interpretation of hydraulic flow in PI 471938 is that it may have a population of small-diameter xylem elements in its stem that may impose a hydraulic restriction at high flow rates. Smaller xylem vessels in PI 471938 might also explain the delayed wilting observed in the field in this genotype since the smaller radius xylem elements could have delayed cavitation allowing sustained water flow in the plant. Given these hypotheses, the objective of this study was to determine if the distribution of xylem element radii in PI 471938 is different from that in a "standard" soybean cultivar.

Materials and methods

Plant material

Unfortunately, seeds were no longer available for the commercial cultivar A5959 so the common variety "Hutcheson" was used for comparison. Riar et al. (2018) showed that the breakpoint for the decrease in transpiration rate with soil drying was lower in PI 471938 than many other genotypes, including Hutcheson. However, a difference between PI 471938 and Hutcheson in the breakpoint of their transpiration rate with soil drying had not been previously reported by Hufstetler et al. (2007) and by Devi and Sinclair (2013). The study by Hufstetler et al. (2007) was done using very sandy soil (80% sand), which has been shown to give skewed values for the breakpoint (Sinclair, Hammond, and Harrison 1998).

Soybean plants were grown in a greenhouse at Raleigh, NC, USA. The greenhouse was maintained at 31°C day/26°C night air temperature. On 28 December 2015, individual 2 L-polyethylene pots were sown with five seeds each of either genotype PI 471938 or Hutcheson (seeds available from Soybean Breeding Project of T.E. Carter). The pots had been filled with a mixture of steam-sterilized substrate consisting of 50% peat-moss growing mix (Redi Earth, W.R. Grace Co., Columbia, MD, USA) and 50% cement sand. Five days after emergence, plantlets were thinned to one per pot. The pots were irrigated every day with distilled water and once a week with a complete nutrient solution.

Twenty-five days after seedling emergence, five plants of each genotype were harvested for anatomical analysis of the stem xylem. At this time, the plants had developed to the V4-V5 developmental stage. This development stage matched the previously cited studies on water use and hydraulic conductance by these soybean genotypes. Stem segments about 1.5-cm long were harvested at three internodal positions from the main stem of each plant. Harvests from three positions at the base of the stem afforded an opportunity to observe xylem elements in fully mature tissue that may impact hydraulic flow in the plant. The three positions were identified as (A) below the cotyledonal node, (B) between the cotyledonal node and the unifoliate leaf node, and (C) between the unifoliate and the first trifoliolate node.

Microscopic observation

The cut stem sections were immediately placed in formalin-acetic acid-ethanol (FAA) fixative at room temperature (Livingston et al. 2009). The samples were then dehydrated in a sequence of increasing concentrations of ethanol up to absolute ethanol (Livingston and Tuong 2014). Dehydrated samples were then embedded with Paraplast Plus using a microwave-processing technique (Livingston and Tuong 2014). The embedded tissues were sectioned transversally at a thickness of 20 μm to obtain at least 50

sequential stem cross sections. The sequential sections were stained with safranin O and fast green FCF. Images of the sequential sections were captured with a Canon Rebel T5i camera (Canon, Tokyo, Japan) attached to a Nikon Elipse 50i microscope (Nikon, Tokyo, Japan) at 400X.

Images were imported into After Effects (Adobe Systems, San Jose, CA, USA) as described in Livingston and Tuong (2014). The images indicated that the vessel sizes were observed to be fairly conserved in the sequence of cross-sections, so that only one cross section from the sequence of sections needed to be analyzed for each position of each plant and genotype. The lumen area (reported as μm^2) in each image was measured with WinCell software (Reagent Instruments Inc., Canada) that had been calibrated with a 10-micron eyepiece reticle.

Xylem element radius

To obtain a radius for each xylem element, lumen area was converted to radius by assuming each element was approximately circular (Figure 1). That is, radius was calculated as equal to the square root of the lumen area divided by π. The radii of individual xylem elements in a cross section were put in rank order from smallest radius to largest radius. To allow comparison among plants, the number of elements was normalized for the total number of xylem elements in each stem cross-section so a fractional number was assigned to each xylem element from 0 to 1.0.

The fractional xylem number was plotted versus the radius of each element. That is, the fractional number for each element was plotted for its fractional number 0 to 1.0 on the ordinate, versus the radius of that element on the abscissa. Hence, the resultant graphs showed an ascending pattern reflecting the increasing radius with increasing fractional number. Plants with more xylem elements with smaller radii will have a steeper increase in the slope of the plot than plants with larger xylem radii. To focus on the larger xylem elements expected to account for most of the hydraulic flow and vulnerability to cavitation, the radius at which the fractional element number was 0.95 was identified. The 0.95 value was taken as a threshold of the radius above which exists the largest 5% of the xylem elements for each stem segment. The xylem element radius for each plant at the 0.95 threshold was used to calculate a mean threshold for each genotype. The probability of difference between genotypes at each of the three stem positions for number of xylem elements and the radius at the 0.95 threshold was calculated using the t-test (Prism, GraphPad, San Diego, CA).

Results

The average number of xylem elements per stem cross section for the three stem positions ranged from 154 to 185 for PI 471938 and from 128 to 175 for
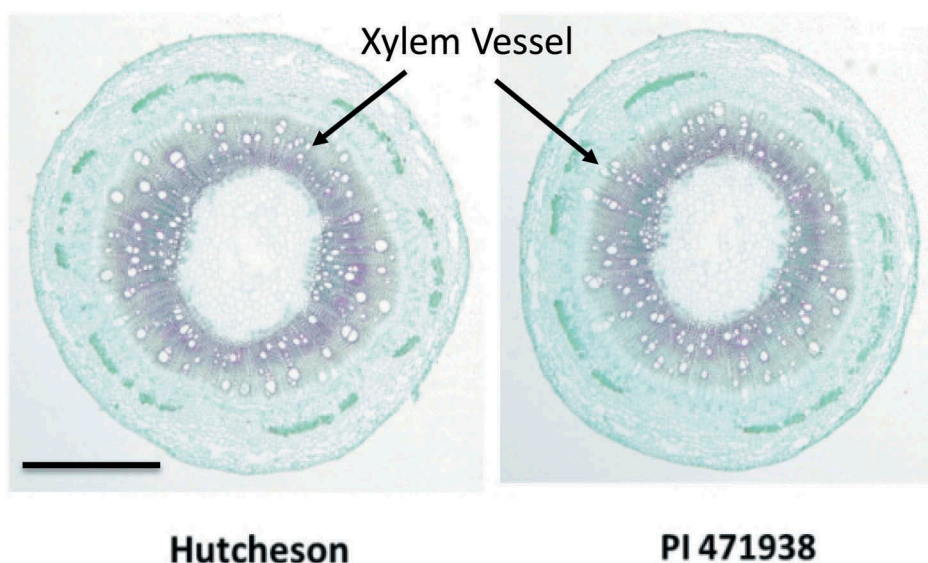
Figure 1. Stem cross-section of soybean Hutcheson (left panel) and PI 471,938 (right panel). The solid bar in the figure represents 1 mm.

Hutcheson (Table 1). The probability of difference between genotypes in total element number ranged from 0.13 to 0.32 among the three sampled positions of the stem.

A cursory examination of the stem cross-sections appeared to indicate the existence of more large-xylem elements in Hutcheson than in PI 471938 (Figure 1). The distribution graphs for position B and C (Figures 2 and 3) of xylem element radius supported this impression (position A is not shown due to redundancy). The initial steeper rise in the graph for PI 471938 as compared with Hutcheson indicated that a higher fraction of the xylem elements had smaller radii in PI 471938 than in Hutcheson.

The existence of more, larger xylem-elements in Hutcheson as compared with PI 471938 was examined based on the average radii at the 0.95 fractional

Table 1. Mean threshold radii where 0.95 of xylem elements have lower radii and number of cells per stem cross section for PI 471938 and Hutcheson. Standard error of the mean presented in parenthesis. Cross-sections of stem were collected at three positions. Section A was below the cotyledonal node. Section B was between the cotyledonal node and the unifoliate leaf node. Section C was between the unifoliate node and the first trifoliolate node.

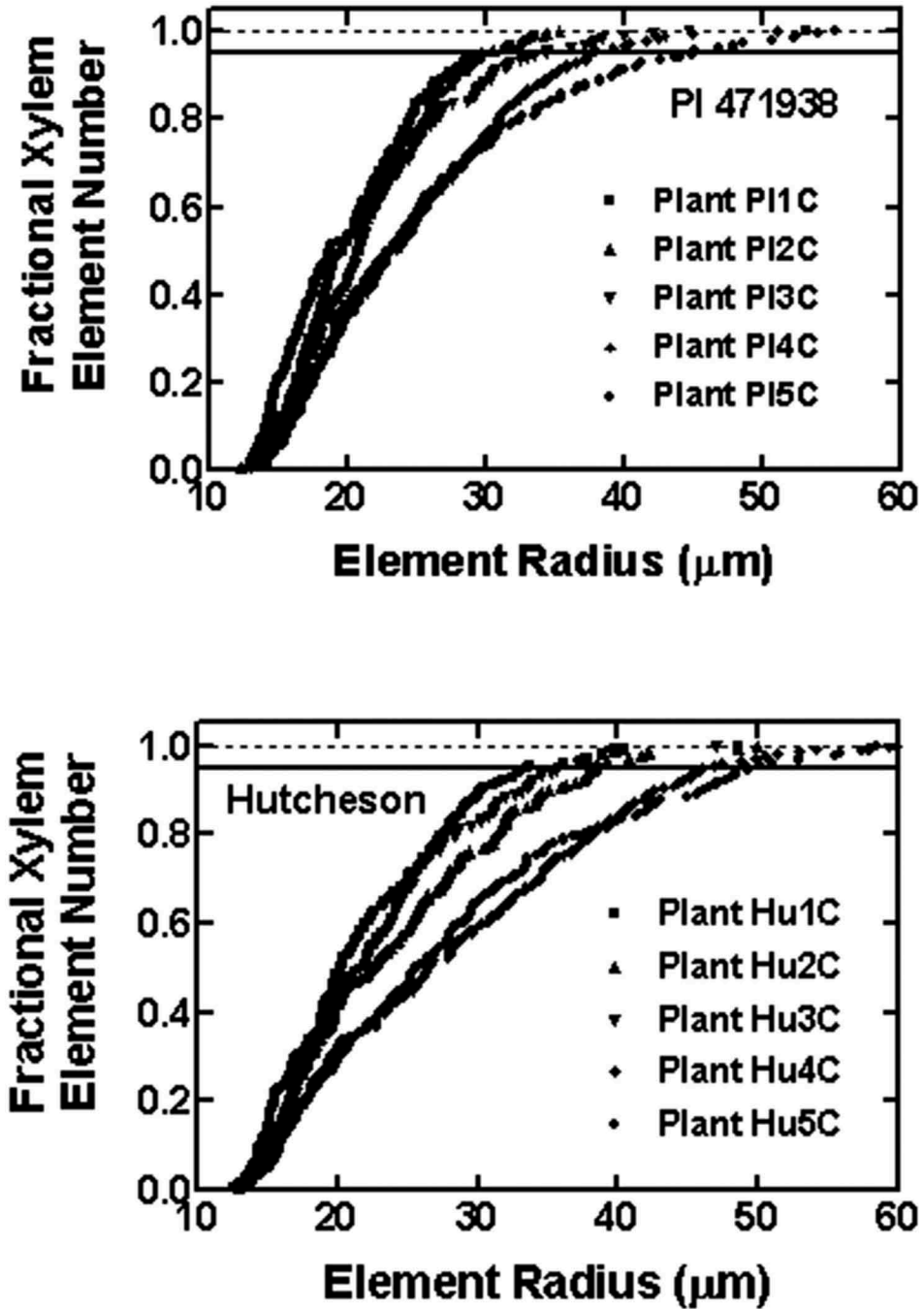| Mean value	Section A	Section B	Section C
PI threshold radii (µm)	38.5 (2.23)	40.3 (2.63)	35.7 (2.71)
Hu threshold radii (µm)	43.9 (2.63)	49.1 (3.23)	41.0 (3.19)
Probability difference	0.07	0.03	0.12
PI number of cells	185 (21.2)	154 (14.4)	184 (14.9)
Hu number of cells	160 (12.6)	128 (16.3)	175 (10.6)
Probability difference	0.16	0.13	0.32

Figure 2. Plot of xylem element fraction number versus xylem element radius for five individual plants of PI 471,938 (top panel) and Hutcheson (bottom panel). The stem cross-sections were collected between the unifoliate leaf node and first trifoliolate leaf node (position C). The solid horizontal line represents the 0.95 fraction of cells with lower radii thereby showing the threshold for the largest cells.

Figure 3. Plot of xylem element fraction number versus xylem element radius for five individual plants of PI 471,938 (top panel) and Hutcheson (bottom panel). The stem cross-sections were collected between the cotyledon nodes and the unifoliate leaf node (position B). The solid horizontal line represents the 0.95 fraction of cells with lower radii thereby showing the threshold for the largest cells.
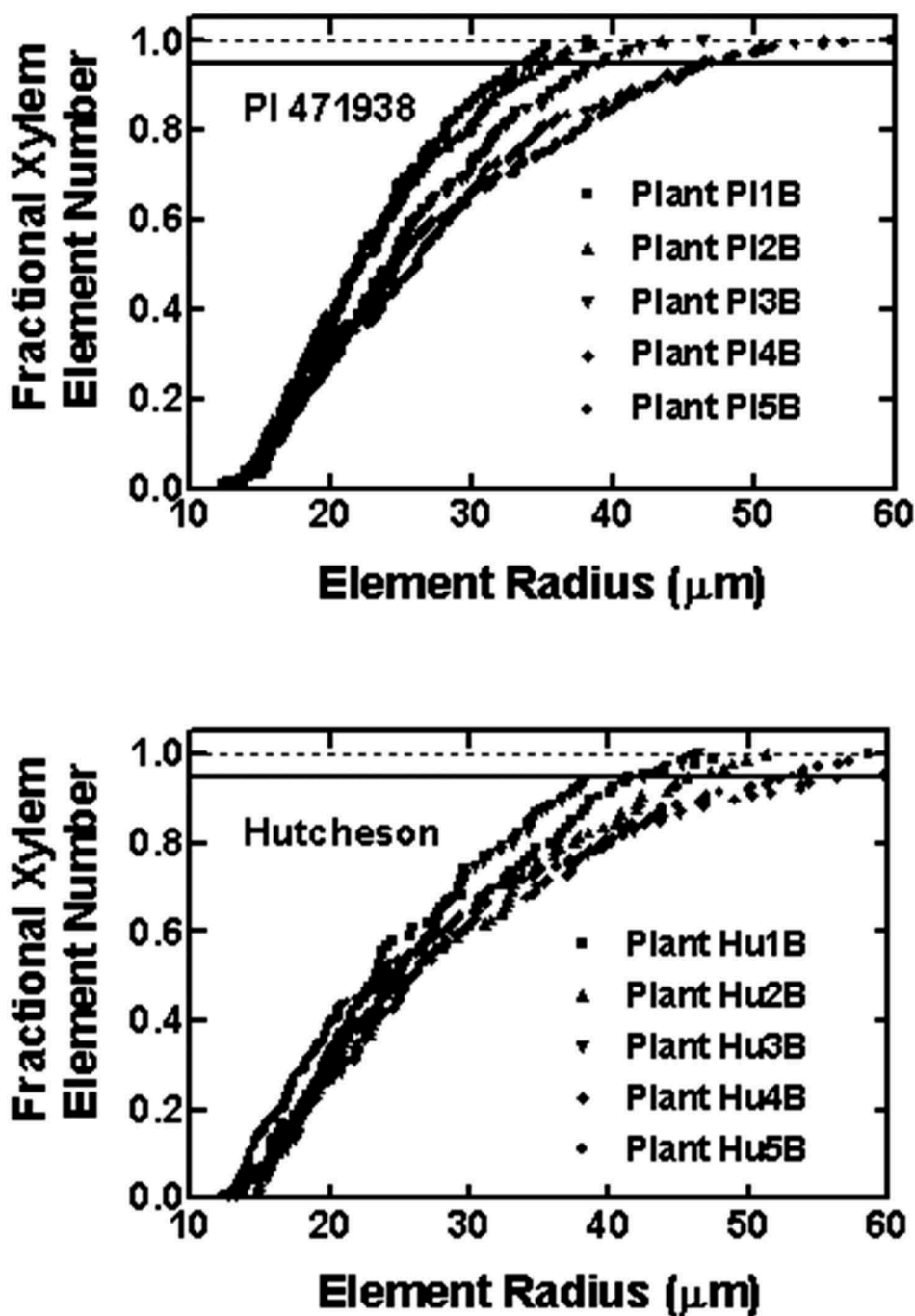
cell number. That is, this statistic gave the radius threshold above which occurred the largest 5% of the cells in each section. The average threshold radius for the 0.95 fractional xylem element across all three stem positions in PI 471938 was 36 to 40 μm, whereas the average for Hutcheson was 41 to 49 μm (Table 1). The probability of difference between the two genotypes was 0.07, 0.03, and 0.12 for postions A, B, and C, respectively.

Discussion

The objective of this study was to determine if the distribution of stem xylem element radii of drought-tolerant PI 471938 differed from the distribution of Hutcheson. While the number of xylem elements per stem cross section was not found to be different between the two genotypes, the probabilities of radius difference for the 0.95 threshold of fractional xylem element were lower. In comparing xylem element radii at any faction xylem element number (Figures 2 and 3), the xylem elements of PI 471928 had smaller radius than did Hutcheson. Further, PI 471938 had smaller radii for the largest xylem elements (Table 1), which would likely allow delayed cavitation relative to that in Hutcheson.

Since PI 471938 did not have as large xylem-element radii as found in Hutcheson, this meant that the xylem conductance of PI 471938 was likely less than that of Hutcheson. The conductance difference can be amplified over the difference in xylem radius because of the dependence of flow on the fourth power of the radius. This impact of the smaller xylem element radii is consistent with the hydraulic conductance measurements of Sadok et al. (2012) that under high vapor-pressure deficit conditions plant conductance of PI 471938 expressed lower hydraulic conductance than the standard genotype.

The smaller xylem element radii of PI 471938 may, however, impact temporal dynamics of water use by PI 471938 as compared with other standard genotypes of soybean when soil water becomes limiting. In this case, the largest xylem elements of a soybean genotype such as Hutcheson might become vulnerable to cavitation resulting in a decrease in hydraulic conductance and eventually a hydraulic conductance less than that of PI 471938. The visual consequence of such a scenario might be delayed wilting by PI 471938 as reported by Hufstetler et al. (2007).

An important, documented trait of PI 471938 is the greater tolerance of its nitrogen fixation activity to soil drying. This genotype sustains nitrogen fixation to a lower level of soil drying than most soybean genotypes (Devi and Sinclair 2013). The basis for the nitrogen fixation tolerance has not been explained. Perhaps the ability of PI 471938 to sustain xylem flow as the soil dries on account of smaller radii xylem elements might be critical in sustaining the transport in the plant supporting nitrogen fixation activity.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Bagherzadi, L., T. R. Sinclair, M. Zwieniecki, F. Secchi, W. Hoffmann, T. E. Carter Jr, and T. W. Rufty. 2017. "Assessing Water-Related Plant Traits to Explain Slow-Wilting in Soybean PI 471938." *Journal of Crop Improvement* 31: 400–417.

Devi, M. J., and T. R. Sinclair. 2013. "Nitrogen Fixation Drought Tolerance of the Slow-wiltIng Soybean PI 471938." *Crop Science* 53: 2072–2078. doi:10.2135/cropsci2013.02.0095.

Hufstetler, E. V., H. R. Boerma, T. E. Carter Jr., and H. J. Earl. 2007. "Genotypic Variation for Three Physiological Traits Affecting Drought Tolerance in Soybean." *Crop Science* 47: 25–35. doi:10.2135/cropsci2006.04.0243.

Li, X., T. R. Sinclair, and L. Bagherzadi. 2016. "Hydraulic Conductivity Changes in Soybean Plant-soil System with Decreasing Soil Volumetric Water Content." *Journal of Crop Improvement* 30: 713–723. doi:10.1080/15427528.2016.1231729.

Lintunen, A., and T. Kalliokoski. 2010. "The Effect of Tree Architecture on Conduit Diameter and Frequency from Small Distal Roots to Branch Tips in *Betula Pendula, Picea Abies* and *Pinus Sylvestris*." *Tree Physiology* 30: 1433–1447. doi:10.1093/treephys/tpq085.

Livingston, D. P., III, and T. D. Tuong. 2014. "Three-dimensional Reconstruction of Frozen and Thawed Plant Tissues from Microscopic Images." In *Methods in Molecular Biology, Plant Cold Acclimation, Methods and Protocols*, edited by D. K. Hincha and E. Zuther, 117–137. Vol. 1166. NY, USA: Springer.

Livingston, D. P., III, T. D. Tuong, C. A. Haigler, U. Avci, and S. P. Tallury. 2009. "Rapid Microwave Tissue Processing of Winter Cereals for Histology Allows Identification of Separate Zones of Freezing Injury in the Crown." *Crop Science* 49: 1837–1842. doi:10.2135/cropsci2009.02.0077.

Lo Gullo, M. A., S. Salleo, E. C. Piaceri, and R. Rosso. 1995. "Relations between Vulnerability to Xylem Embolism and Xylem Conduit Dimensions in Young Trees of *Quercus Cerris*." *Plant, Cell & Environment* 18:: 661–669. doi:10.1111/j.1365-3040.1995.tb00567.x.

Riar, M. K., P. Cerezini, A. Manandhar, T. R. Sinclair, Z. Li, and T. E. Carter. 2018. "Expression of Drought Tolerant N_2 Fixation in Heterogeneous Inbred Families Derived from PI 471938 and Hutcheson Soybean." *Crop Science* 58: 364–369. doi:10.2135/cropsci2017.02.0089.

Sadok, W., M. E. Gilbert, M. Aown, S. Raza, and T. R. Sinclair. 2012. "Basis of Slow-Wilting Phenotype in Soybean PI 471938." *Crop Science* 52: 1261–1269. doi:10.2135/cropsci2011.11.0622.

Sinclair, T. R., L. C. Hammond, and J. Harrison. 1998. "Extractable Soil Water and Transpiration Rate of Soybean on Sandy Soils." *Agronomy Journal* 90: 363–368. doi:10.2134/agronj1998.00021962009000030008x.

Sinclair, T. R., and M. A. Nogueira. 2018. "Selection of Host-Plant Genotype: The Next Step to Increase Grain Legume N2 Fixation Activity." *Journal of Experimental Botany* 69: 3523–3530. doi:10.1093/jxb/ery115.

Tombesi, S., R. S. Johnson, K. R. Day, and T. M. DeJong. 2010. "Relationships between Xylem Vessel Characteristics, Calculated Axial Hydraulic Conductance and Size-controlling Capacity of Peach Rootstocks." *Annals of Botany* 105: 327–341. doi:10.1093/aob/mcp281.

Torres-Ruiz, J. M., A. Diaz-Espejo, A. Morales-Sillero, M. J. Martin-Palomo, M. Mayr, B. Beikircher, and J. E. Fernandez. 2013. "Shoot Hydraulic Characteristics, Plant Water Status and Stomatal Response in Olive Trees under Different Soil Water Conditions." *Plant and Soil* 173: 77–87. doi:10.1007/s11104-013-1774-1.

Tyree, M., and J. Sperry. 1989. "Vulnerability of Xylem to Cavitation and Embolism." *Annual review of plant biology* 40: 19–36. doi:10.1146/annurev.pp.40.060189.000315.