Changes in root xylem anatomy of peanut genotypes with difference drought resistance levels under early season drought

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Abstract

During the 2014 and 2015 seasons, peanut root anatomy studies were conducted under well-watered and under drought conditions using three peanut genotypes which are known to differ in their physiological responses to early and mid-season drought (ICGV 98305, ICGV 98324 and Tifton-8). Cross sections of the newly formed roots revealed that the average vessel diameter and total vessel area in the first order roots were significantly reduced under drought in ICGV 98305 and ICGV 98324, yet not in Tifton-8, which had the smallest vessel diameters and total area in both well-watered and drought treatments. The xylem vessel structure in newly formed roots of ICGV 98324 was very responsive to changes in soil moisture content. This adaptive capacity of ICGV 98324 to change xylem structure as soil moisture conditions change may provide plant breeders an important trait which will lead to better water use efficiencies in both moist and drought conditions.

KEYWORDS

Arachis hypogaea L., water stress, xylem vessel size, xylem vessel area

1 | INTRODUCTION

In the tropics, peanut is usually grown in the rain-fed and arid areas where drought is a recurring problem and the main cause for poor yields, lower quality, and aflatoxin contamination (Girdthai et al., 2010; Ibáñez and Caiola, 2013; Songsri et al., 2008). Improved peanut cultivars can reduce the effects of drought, yet selection for yield and quality traits is difficult because of high environmental variability. For this reason, identifying traits associated with drought tolerance that can be effectively used to screen many genotypes program should expedite the development of drought tolerant cultivars.

Many morphological, physiological, biochemical and anatomical features related to crop adaptation to drought have been suggested as potential related, and more easily measured, traits that might aid in the development of improved drought tolerant cultivars (Farooq et al., 2012) such as drought tolerance index (Luis et al., 2016; Songsri et al., 2008), harvest index (Ratnakumar & Vadez, 2011), leaf area (Puangbut et al., 2010), specific leaf area (Balota et al., 2010), chlorophyll content (Arunyanark et al., 2008), transpiration efficiency (Arunyanark et al., 2008), plant water status (Painawadee et al., 2009), water use efficiency (Krishnamurthy et al., 2007), stomatal conductance (Thangthong et al., 2018) and root length density (Songsri et al., 2008). Root traits are also important to drought resistance mechanisms (Russell, 1982), and they have long been suggested as a major way of research to improve crop adaptation to water limitations (Vadez, 2014). Root traits have been studied in many crops at different growth stages.

Adaptive strategies to drought are based also on multiple traits which consider both root architecture and anatomy traits. (Micco and Aronne, 2012; Russell, 1982). Morphological traits associated with drought tolerance in peanut include small fine root diameters, root length, and root length density (RLD) in lower soil layer with available water have been reported (Jongrungklang et al., 2012; Junjittakarn et al., 2014; Koolachart et al., 2013; Vadez, 2014; Songsri et al., 2008). In a recent study in peanut, the increase in root length and RLD in the deeper soil layer could explain the root function for water extraction and the traits were reportedly correlated to higher yield under drought (Jongrungklang et al., 2012).

In addition, root water uptake is regulated by several anatomical traits, including xylem vessel diameter, number, and area. In chili (*Capsicum annum* L.), drought resistant cultivars had more and larger xylem vessels than did the susceptible varieties of chili (Kulkarni and Phalke, 2009). This was also true in tomato (*Lycopersicon esculentum*) (Kulkarni and Deshpande, 2006) and grape (*Vitis vinifera* L.) (Kulkarni et al., 2007). In order to improve peanut subsequent resistance to drought, a better understanding of the effects of root anatomy is needed. However, the information on the effect of drought on anatomical structure of peanut roots of different genotypes is still lacking. Fine structure of peanut root might affect water acquiring capacity of peanuts and play an important role in response to drought.

Many studies have documented genotypic differences in peanut root architectural structure at different growth stages and when subjected to various periods of drought (Jongrungklang et al., 2013; Rucker et al., 1995; Songsri et al., 2008; Thangthong et al., 2016). Yet, there have been few studies investigating genotypic differences in peanut root anatomy resulting from changes in the soil environment. This manuscript provides information on whether peanut genotypes with different drought resistance levels change the anatomy of the root in response to early season drought. Changes of root anatomy are related to the efficiency of water uptake under drought conditions. The improvement of root anatomical traits might improve the tissue efficient stretching of crop to extract available water from the soil profile during drought and may be also support to transpiration efficiency. The information obtained in this study will be useful for selection of genotypes with good root anatomical traits related to drought resistance as key traits for drought resistance.

2 | MATERIALS AND METHODS

2.1 | Plant materials and experimental design

Three peanut genotypes (ICGV 98305, ICGV 98324 and Tifton-8) were selected for this study because of differences in their physiological responses to early and mid-season drought (Jongrungklang et al., 2011; 2013). ICGV 98305 and ICGV 98324 are drought resistant genotypes provided by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). ICGV 98305 has been confirmed to have a high RLD, high relative water content (RWC) and high stomatal conductance under early season drought in the field (Jongrungklang et al., 2011; 2013). ICGV 98324 has been confirmed to have a medium RLD, high RWC and high stomatal conductance under early season drought in the field (Jongrungklang et al., 2011; 2013). Tifton-8 is Virginia-type accession with drought resistance developed by the United States Department of Agriculture (USDA; Coffelt et al., 1985). Tifton-8 has been confirmed to have medium RLD, low RWC and low stomatal conductance under early season drought in the field (Jongrungklang et al, 2011; 2013).

The three genotypes were subjected to two water management treatments (well-watered treatment and drought treatment imposed at 14 days after emergence for 21 days). The detailed water management method was as reported by Thangthong et al. (2016), and is covered in the rhizobox section below. Thangthong et al. (2016) reported that the peanut root such as root distribution and root surface subjected to of long duration time water stress (21 days) greater responses than short period of times. The genotypes by water management treatments were arranged in a completely randomized design (CRD) with three replications. The experiment was conducted using rhizoboxes with pin-boards (described below) which were placed under rainout shelters at the Field Crop Research Station of Khon Kaen University, Khon Kaen, Thailand (lat 16º 28′N, long 102º 48′E, 200 m above sea level). This experiment was conducted during two seasons, October–December 2014 and January–March 2015.

2.2 | Rhizobox preparation, irrigation and crop management

The rhizoboxes have an internal dimension of 10×50×120 cm and were filled with dry soil at 5 cm intervals from the bottom to 5 cm from the top (115 cm deep). The bulk density of the soil in the rhizoboxes was 1.57 Mg m^{-3}. Three seeds were planted 5 cm deep at the center of the rhizoboxes, and the seedlings were thinned to one plant per rhizobox shortly after emergence.

Each rhizobox had a root pin-board which projected out from the back surface. These pins enabled the roots to remain in-place during the later washing process, aiding root location observations and measurements (Kano-Nakata et al., 2011). Bamboo sticks 3 mm in diameter and 10 cm length projected out from the back of the box arranged in a 5 cm by 5 cm grid with the first row starting 12.5 cm from the top of the rhizobox (Figure 1).

A transparent window was installed at the front of the rhizobox for visual observation of the rhizobox. The window was removable to facilitate washing processes. The rhizobox was further covered with black plastic sheet to make sure that the roots were not exposed to the light, and, finally, the rhizobox was enclosed with thick aluminium foil to reduce heat. Three drainage holes of approximately 1.5 cm in diameter were mounted at 15 cm intervals at the bottom of the rhizobox.

Soil for field capacity (FC) and permanent wilting point (PWP) calculations were collected and soil moisture contents at FC and PWP were calculated as 11.13% for FC and 3.40% for PWP by pressure plate method. Soil water content was controlled as FC for the first water supply for all rhizoboxes until 14 days after emergence (DAE) and then the water management treatments were imposed for 21 days. Well-watered treatment was maintained uniformly at FC from planting until harvest. Water was supplied to the rhizoboxes through six horizontal tubes, each connected to a vertical tube rising 5 cm above the soil surface and attached to the left frame member of the box. The horizontal tubes were placed at 5, 15, 35, 55, 75 and 95 cm below the soil surface and centered 5 cm from both front and back of the box. Crop water requirement including transpiration and soil evaporation was calculated daily for replenishment of water using the methods described by Doorenbos and Pruitt (1992);

$$ETcrop = ETo \times Kc,$$

where ETcrop is crop water requirement (mm day^{-1}), ETo is transpiration of a reference plant and soil evaporation under specific condition calculated by pan evaporation method, and Kc is the crop water requirement coefficient, which the values varied depending on growth stage.

The fertilizers, phosphorus fertilizer as triple superphosphate at the rate of 122.3 kg ha^{-1} and potassium fertilizer as potassium chloride at a rate of 62.5 kg ha^{-1}, were mixed together and applied into the top of soil during soil preparation. A fungicide captan (3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1, 3(2H)-dione) at the rate of 5 g kg^{-1} was applied to the seeds. *Rhizobium* of *Bradyrhizobium* (mixture of strains THA 201 and THA 205) from the Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand was diluted with water and applied to the soil through irrigation tube placed 5 m below the soil surface.

2.3 | Data collection

2.3.1 | Observation of root anatomy

Roots of all three genotypes were collected at 21 days after drought imposition. Shoots were cut at the top soil surface and the root samples were carefully separated from the soil by removing the front panel of the rhizobox and carefully washing the soil away from the roots using a fine spray of tap water. After the soil was removed, the roots remained in their approximate original positions because of the grid of bamboo needles projecting from the back of the rhizoboxes. Needles were then removed from the black sheet and root distribution and position was recorded.

Anatomical studies were conducted on root samples collected from the first- and second-lateral roots that were 0-20 cm below soil surface (Figure 1) and 5 cm from the root tip. The anatomical characters of the vessels were determined in primary growth stage of the roots. Root samples were stabilized using FAA_{40} solution (formaldehyde: acetic acid: 40% alcohol). The roots were further dehydrated in ethyl alcohol at series concentrations 40, 50, 60 and 70%. All observations of roots were performed on transverse sections. Roots samples were cut in 12 µm by plant microtome (NK system Medel: MT-3), stained with Safranin O, mounted with distilled water, and observed with an optical microscope (Nikon eclipse 50i) equipped with ocular and stage micrometers and a digital camera (Nikon DS-Fi1). All root cross-sections were measured for xylem vessels diameter, xylem area and xylem vessel number.

2.3.2 | Stomatal conductance measurement

Stomatal conductance was measured at 21 days after water withholding began. Stomatal conductance was taken at 10.00-12.00 AM under clear sky weather. The samples were taken from one leaflet of the second fully expanded leaf of the top of the main stem. The stomatal measurement was collected using a porometer (model SC1, Decagon Devices, Inc.).

2.3.3 | Data analysis

Analysis of variance was carried out using Statistix-8 program based on a 2×3 factorial experiment in a completely randomized design. Means were separated by least significance difference at 0.05 probability levels. Means and standard deviations of all parameters under different water regimes and genotypes were presented for comparison of the treatments.

Simple correlation coefficients between xylem vessel area of the first order lateral root and stomatal conductance, and coefficients between the xylem vessel area of the second order lateral root and stomatal conductance were analyzed to determine the relationship between traits of each genotype separately.

3 | RESULTS

3.1 | Observation of root anatomy

Peanut is a dicotyledonous plant with a root system consisting of a single taproot and lateral roots (Figure 1). Pith tissue was found in taproot (data not shown) but was absent in central part of first-, second- and higher order lateral roots. The first-, second-, and higher order lateral root branches were shown in Figure 1b. The characteristics of root xylem vessels at 0-20 cm soil depth varied depending on water treatment, genotypes and lateral root orders. The results presented genotypes and order lateral roots under different water regimes. The root xylem vessels of first- and second- order lateral roots of different genotypes were affected by different water regimes.

3.1.1 | First order lateral roots

Combined analysis of variance for total vessel area (μm^2), total vessel diameter (μm), and total vessel number of the first order lateral root in October–December 2014 and from

January–March 2015 were show in the Table 1. Differences between genotypes, seasons and treatments were statistically significant for total vessel area, total vessel diameter. Significant differences in total vessel number were observed in different genotypes and seasons.

The interactions between genotypes and treatments (G×T) were significant for total vessel area and total vessel diameter. The interactions between seasons and treatment (S×T) were also significant for total vessel number. The interactions between genotypes, seasons, and treatments (G×S×T) were also significant difference in the total vessel number.

The first order lateral root structure had almost triarch vascular system (Figure 3). At 5 cm from the root tip, the endodermis cells were in the primary stage of their development, and cell division of vascular parenchyma cells of vascular cambium tissue did not appear at this growth stage. The xylem vessels in the mature zone of the apical root 5 cm from the tip, were arranged centrally. The xylem vessels of the first order lateral roots were generally larger and greater in number than those in the second order lateral roots.

Both average vessel diameter and total vessel area in the first order roots were significantly reduced under drought in ICGV 98305 and ICGV 98324, yet not in Tifton-8, which had the smallest vessel diameters and total area in both well-watered and drought treatments (Figure 2 and 3). The ICGV 98324 had the biggest xylem vessels and the largest vessel area under well-watered treatment in both seasons and the vessel number was also highest under drought in second season, whereas the highest xylem vessel number was found under well-watered condition in the first season.

The vessel diameters of roots varied considerably among genotypes as shown for the first order lateral root. Mean of xylem vessel diameters of first order lateral roots averaged from three genotypes was 17.40 µm (data not shown). Xylem vessels in the first order roots that were formed during the experimental period averaged 17.36 µm, 18.70 and 15.90 µm for ICGV 98305, ICGV 98324 and Tifton-8, respectively.

Drought reduced xylem vessel diameter of first order lateral root in all peanut genotypes in both years although Tifton-8 did not show a clear reduction in vessel diameter in 2015 (Figure 2a1 and b1). The biggest xylem size was observed in ICGV 98324 in the well-watered treatment - with mean of 22.30±1.56 µm in first season and 21.29±0.57 µm in second season. Yet, the mean vessel diameter of ICGV 98324 in the drought treatments were 16.16±0.91 µm first season and 15.22±1.21 µm in second season.

Figure 2 shows the mean xylem vessel numbers for each peanut genotype. ICGV 98324 had highest numbers of xylem vessel in both seasons. Interestingly, the vessel numbers of ICGV 98305 in both seasons and ICGV 98324 in the second season were higher in the drought treatment than in the well-watered treatment (Figure 2a1 and a2). Tifton-8, however, did not have a significant difference in vessel numbers between the drought and well-watered treatments.

Drought significantly reduced vessel area per cross-section in both ICGV 98305 and ICGV 98324. The xylem vessel area per cross-section in roots grown under well-watered condition was significantly higher than those grown under drought condition. Well-watered and drought treatments were significantly different for the total xylem vessels area in ICGV 98305 and ICGV 98324 in both seasons, but the difference was not found in Tifton-8. The highest reduction in the area of total vessels as affected by drought was found in ICGV 98324. However, the xylem vessel areas of total vessels per cross section of peanut genotypes under drought were significantly reduced except for Tifton-8. Therefore, Tifton-8 generally had small diameter and area of vessels per cross section in both well-watered and drought conditions.

3.1.2 | Second order lateral roots

Combined analysis of variance for variance for total vessel area (μm^2), total vessel diameter (μm) and total vessel number of the second order lateral root in October–December 2014 and from January–March 2015 were show in the Table 2. The significant differences in genotypes were significant for total vessel area and total vessel number traits. The season was significant for total vessel numbers. The difference in treatment and the interactions between genotypes, seasons and treatments were not found in any traits.

The central cylinders in of vascular bundles of second order lateral roots were mostly classified into diarch type and triarch type (Figure 5). The second order lateral roots of all peanut genotypes were thinner, and the stele and vascular bundle tissues were narrower than in the first order lateral roots. ICGV 98324 had the largest vessel diameter in second order lateral roots (16.28 μm), ICGV 98305 had vessels with intermediate diameters (15.59 μm), and Tifton-8 the smallest diameter (14.14 μm). The size and area of total xylem per cross-section of second order lateral roots of ICGV 98324 grown under drought were significantly different from the irrigated treatment (Figure 4) in both years. ICGV 98324 was also the most responsive and sensitive to water stress. Drought significantly reduced the total vessel diameter of second order lateral roots in ICGV 98324, but not in ICGV 98305 and Tifton-8. The second order lateral roots of ICGV 98305 and Tifton-8 were smaller than in ICGV 98324 in both well-watered and drought conditions.

3.1.2 | Relationship between the xylem vessel area and stomatal conductance

The correlation coefficients of the three genotypes between xylem vessel area of the first order lateral root and stomatal conductance were 0.16 to 0.77 and the correlation coefficients of the three genotypes between xylem vessel area of the second order lateral root and stomatal conductance were 0.03 to 0.44. Most correlation coefficients between the xylem vessel area of the first order lateral root and stomatal conductance (Figure 6 a1, a2, and a3) were positive and significant difference in ICGV98305 genotype (0.68*, $P \leq 0.05$) and ICGV 98324 genotype (0.77**, $P \leq 0.01$) except in Tifton-8 genotype (0.16, $P \leq 0.05$). ICGV 98305 and ICG V98324 genotypes with bigger xylem vessel area had higher stomatal conductance ICGV 98300, although Tifton-8 genotype did not respond.

The correlation coefficients of the three genotypes between xylem vessel area of the second order lateral root and stomatal conductance were 0.03 to 0.44. The relationship coefficients between the xylem vessel area (μm^2) of the second order lateral root and stomatal conductance (Figure 6 b1, b2 and b) were positive but no statistical differences in any genotypes.

4 | DISCUSSION

The strategies for adaptation to drought are based on many traits which are considered in both root architecture and anatomy (Micco and Aronne, 2012). The adaptation to drought on morpho-anatomical traits in different organs at different levels in various ecosystems have been reported (Micco and Aronne, 2012). In some agroecosystems, the soil moisture available in the subsoil is sufficient for crop growth and therefore, improving the axial conductivity of crop plants will improve water acquisition (Lynch et al., 2014). Yet, anatomical, and physiological adaptions of plants to better survive during periods of drought may also reduce growth and development during periods of no stress, unless the plant is resilient in its response to environmental changes. The modification of the root/shoot ratio to drought is a well-established response that determines an increasing of the root density over the shoot (Sanchez-Blanco et al., 2014).

Our peanut root studies were conducted over two consecutive years in 2014 and 2015. The data set of the weather condition was referred to Thangthong et al. (2018). The air temperature and evaporation rate in 2015 were higher than 2014. The higher evaporation rapidly reduced soil moisture content, especially in the upper soil layers and the high temperatures likely resulted in higher transpiration rate in some peanut genotype. Based on physiological and root morphological traits, three peanut genotypes responded differently to pre- flowering drought. Under drought condition, the shoot growth had negative effect especially in Tifton-8 but it is more likely be beneficial to water use restriction (Thangthong et al., 2018). The differential responses for RLD and distribution patterns (Jongrungklang et al., 2011; 2012) associated with drought tolerance were found. The ICGV 98305 genotype responded to drought by maintaining or stimulating root growth which maintains root dry weight or increased root length in deep soil layers when a drought is imposed (Thangthong et al., 2018). The increasing of root length in lower soil layers or maintaining of root dry weight might contribute water uptake under drought, and it can mine sufficient water for normal transpiration. ICGV 98305 was identified as the drought avoidance mechanism with genotype having morphological responding (Thangthong et al., 2018). The transpiration was also greatly reduced in ICGV 98324 and Tifton-8 under drought (Thangthong et al., 2018). The reduction of transpiration may increase ability of crop water use, also enabling the plant to better tolerate drought.

The adaptive function of inner structure in root on presence of traits linked to the regulation water uptake and avoidance of water loss (Osakabe et al., 2014). Under reduced water availability, the small diameter roots is considered as a maximizing water surface absorptive aim, consequently, increasing rates of water and nutrient uptake (Eissenstat, 1992). The control of water loss is also exerted by specific tissues, for example, thickened outer cell walls of rhizodermis, a well-developed exodermis with suberin, or a produced many layer of thin- or thick-walled suberised cells (Makbul et al., 2011). The reduction of cortical layers is also considered of adaptive advantage under drought and it is shorter distance from soil to stele supporting a quicker radial water transport (Kadam et al., 2015).

Once the water has entranced the stele then it will be transported to leaves efficiently. The morpho-functional traits are involved that the water transport efficiency and the effective hydraulic conductivity of vascular system are underlying for the survival of plants in arid environments. In fact, in the context of climate changes, considering as an increasing in the drought frequency and higher temperatures, plants in arid and semi-arid environments have to cope increased xylem vessel cavitation (Micco and Aronne, 2012; Willsona and Jackson, 2006. In these environments, the adaptation capacity is linked to their root xylem characteristics which should optimize water movement according to changing water availability (De Micco and Aronne 2010; Kadam et al., 2015).

The effect on crop yield and quality to a crop's adaptation to the environment will depend on many parameters including temperature, timing, duration, and intensity of the stress (Nageswara Rao et al., 1989; Songsri et al., 2008). In general, plant cell development is very sensitive to drought, and changes in photosynthesis and stomatal conductance is often reported.

Yet it is likely that the physiological changes measured in the above ground portions of the plant were, in part, resultant from changes in root water transport (Boyer, 1970; Myburg and Sederoff, 2001; Songsri et al., 2008). The ability to uptake water in plant is highly influenced by number and size of the vessel elements (McMichael et al., 1999; Vasellati et al., 2001) and vascular tissue area (Ristic and Cass 1991). Yet, Kulkarni and Plake, (2009) noted that under drought, the root diameter and root area of tomato were sharply reduced but the number of vessels were not significantly affected and therefore vessel number alone was not a good selection criterion.

Henry et al. (2012) reported reduced vessel diameter of rice roots in response to drought, with the reduction proportional to the severity of the drought and greater reduction was observed under more severe drought. Interestingly, the drought resistant genotypes showed smaller xylem vessel diameters than did drought-susceptible ones when grown under the severe drought treatment (Henry et al., 2012). While smaller diameter vessels have a reduced the risk of xylem vessel cavitation, they also have an increased water flow resistance. (Atkinson and Taylor, 1996; Tyree and Sperry, 1989; Vasellati et al., 2001). Root vessel diameter was not only feature that affected to drought. Makbul et al. (2011) also found that the rate of cortex width per vascular bundle width belonging to the root varies between the stress and unstressed plants.

Thus Comas et al. (2013) noted that large xylem diameters in deep roots may improve root uptake of water when water in deep soil layers is sufficient. Kondo et al. (2000) suggested that breeding for rice genotypes with larger xylem vessels and deep roots would improve water uptake and yield. Likewise, Kulkarni and Phalke, (2009) proposed that large xylem vessel diameter could be used as a selection criterion for hot pepper breeding, where the target is improved water acquisition and flow rather than conservation.

The xylem vessel structure in newly formed roots of peanut, ICGV 98324 was very responsive to changes in soil moisture content. Yet, Tifton-8's vessel size, which is already small, did not change as dramatically as the other genotypes during drought. Tifton-8 is known to be drought tolerant, yet not high yielding under well-watered conditions. The adaptive capacity of ICGV 98324 to change xylem structure as soil moisture conditions change may provide plant breeders an important trait which will lead to better water use efficiencies in both moist and drought conditions.

Reduced xylem vessel size in low soil moisture conditions would aid water uptake and lower the risk of cavitation, yet it will reduce water movement through the root system during non-stress periods by increasing the hydraulic resistance as noted in Poiseuille's law (Lovisolo and Schubert, 1998; Steudle and Peterson, 1998). Therefore, genotypes which are resilient in plant structure responses to the environment would be valuable to plant breeding programs.

An understanding of root anatomical adaptation mechanisms to drought remains an important goal because root anatomy can be considered as sensor to detect the changing of water availability in soil. The plant with combinations of various root architecture (Thangthong et al. 2018) and different anatomical traits may coexist to respond to drought. Moreover, the various traits of root morphological and anatomical responses were triggered by drought and it may be adjusted with different intensities and in different genotypes within species (Thangthong et al., 2016; 2018). A comprehensive analysis of 3 difference genotypes, one (ICGV 98324) adapted to drought in both the first and the second order lateral roots, one (ICGV 98305) responded only the first order lateral root and other one (Tifton-8) rarely changed, allowed us to explain the functional role of cell or tissue plasticity for adapting to drought. Plasticity in xylem diameter, xylem number and xylem area along the root length and distribution patterns (Thangthong et al., 2018) in peanut genotypes may facilitate the efficient use of available water under drought.

The correlation between some anatomical traits stomatal conductance may be exhibiting the potential for drought avoidance mediated mechanism by stomatal closure (Pirasteh-Anosheh et al., 2016), deeper root systems (Songsri et al., 2009) and/or root anatomical traits (Thangthong et al, 2016). The two peanut genotypes (ICGV 98305 and ICGV 98324) showed positive correlation between xylem vessel area of the first order lateral root and stomatal conductance and could explain pathways and mechanisms driving plant water loss minimizing and water uptake maximizing under different conditions. Anatomical traits might contribute water uptake condition for normal transpiration under drought and greatly reduced transpiration might increase the plant ability to save water when in drought conditions, also enabling the plant to better tolerate a drought. However, the future studies should aim on the relationship between morpho-anatomical traits and yield or yield components under drought.

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AUTHOR CONTRIBUTIONS

Study concept and design: email to S. Jogloy, N. Thangthong, N. Jongrungklang and N. Vorasoot. Analysis and interpretation of data, statistical analysis and drafting of the manuscript: S. Jogloy and N. Thangthong. Critical revision of the manuscript for important intellectual content: email to S. Jogloy, N. Thangthong and C.K. Kvien. Obtained funding: S. Jogloy. Study supervision: S. Jogloy, N. Jongrungklang, C.K. Kvien, I.C. Dodd and N. Vorasoot.

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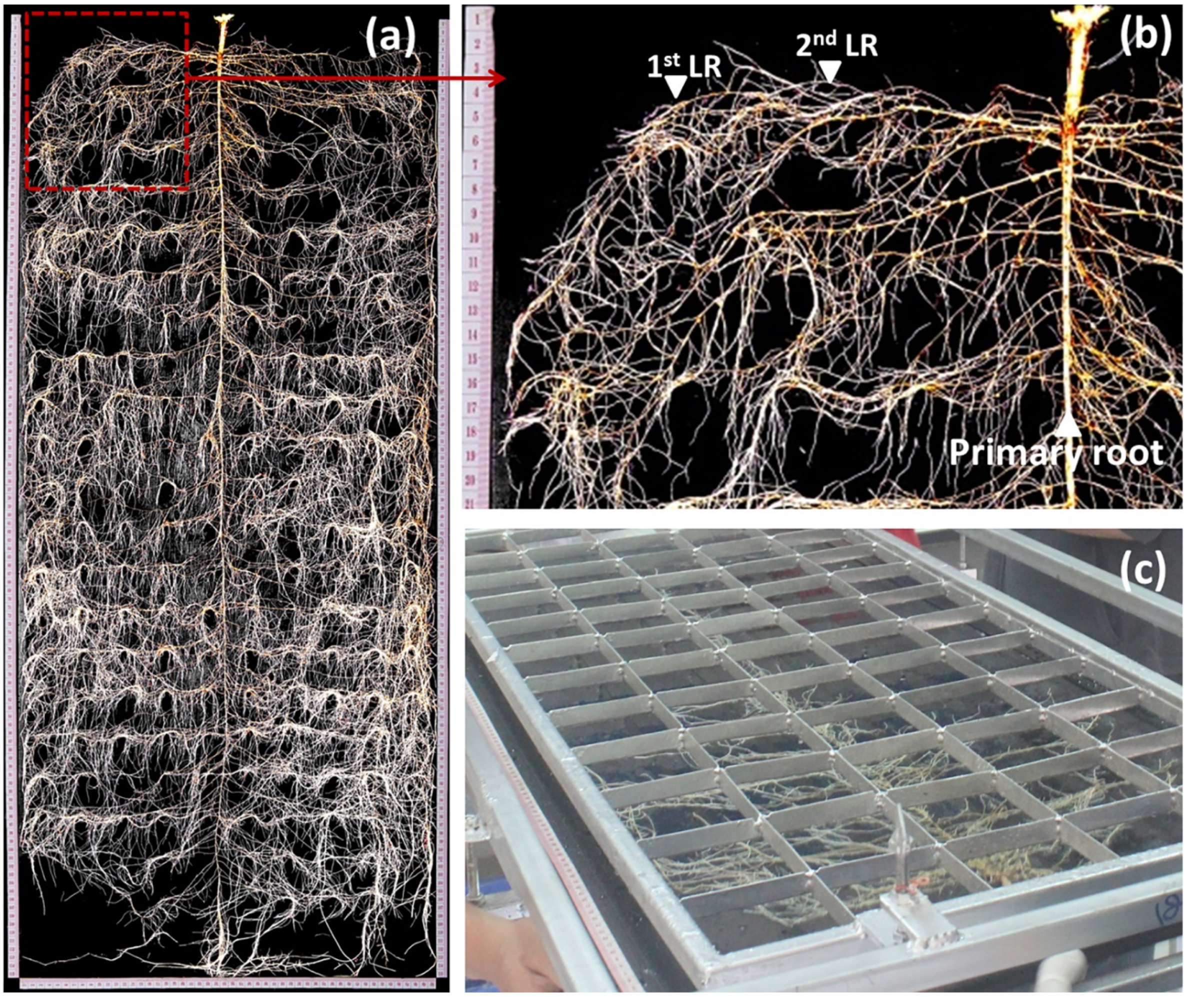

FIGURE 1 Peanut root system with the scale bar (a), root samples from taproot root system for the anatomical studies were taken from 0-20 cm below soil surface at 35 DAE (b) and peanut root system with 10×10 cm^2 basic units (c).

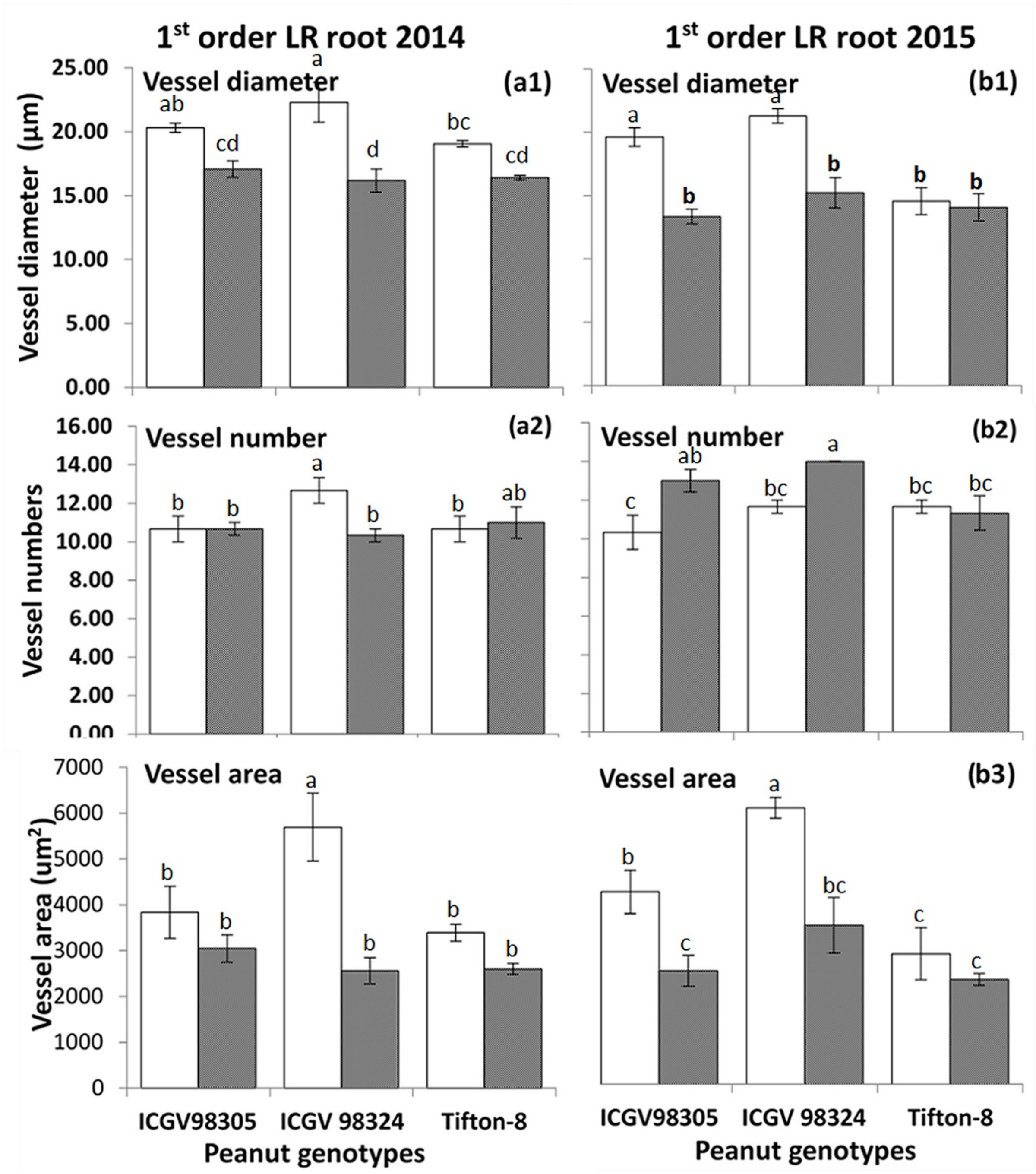

FIGURE 2 Vessel diameter (a1, b1), vessel number (a2, b2) and vessel area (a3, b3) of first order lateral roots of ICGV 98305, ICGV 98324 and Tifton-8 peanut genotypes under well-watered (□) and drought (■) in October–December 2014 (1st year) and from January–March 2015 (2nd year). Significant at $P \leq 0.05$.

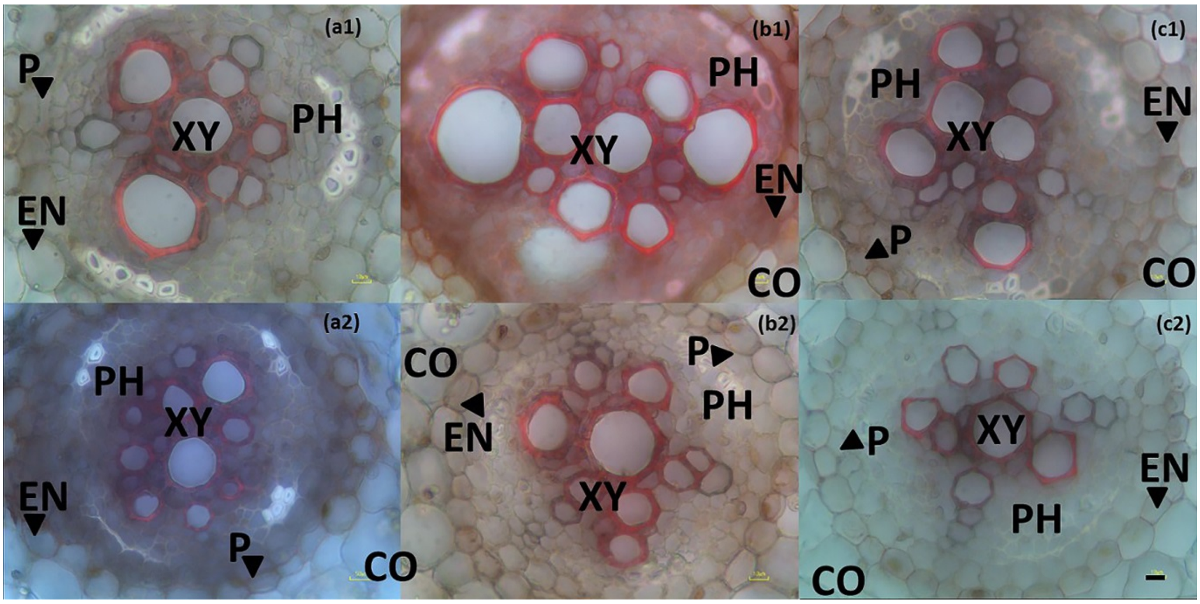

FIGURE 3 Microtome cross sections of first order lateral roots of ICGV 98305, ICGV 98324 and Tifton-8 peanut genotypes under well-watered (a1, b1 and c1) and drought conditions (a2, b2 and c2) in October–December 2014 (1st year) and from January–March 2015 (2nd year). CO, cortex; EN, endodermis; P, pericycle; PH, phloem; XY, xylem; Scale bar=10µm; 40x

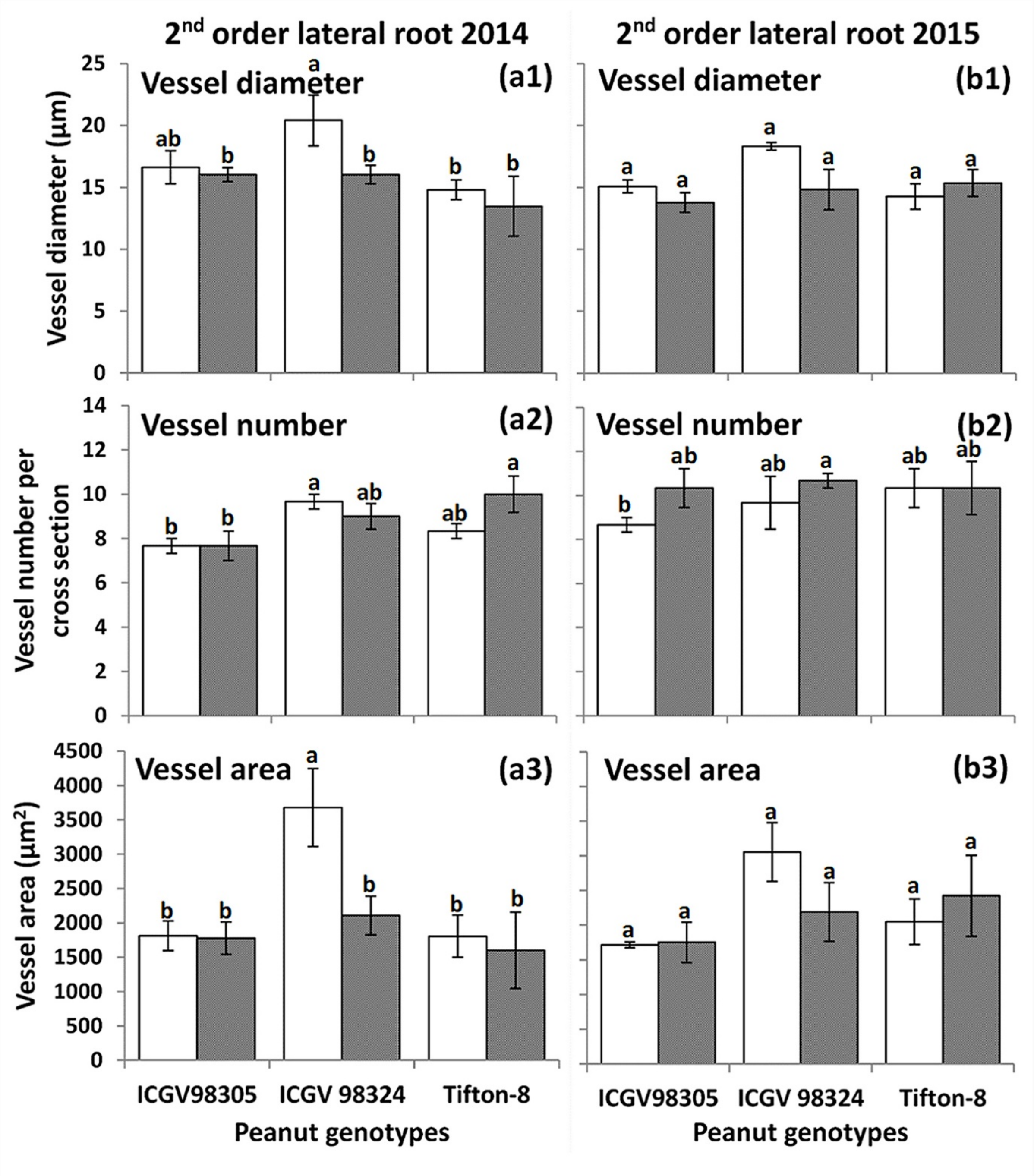

FIGURE 4 Vessel diameter (a1, b1), vessel number (a2, b2) and vessel area (a3, b3) of second order lateral roots of peanut roots of peanut under well-watered (□) and drought (■) of ICGV 98305, ICGV 98324 and Tifton-8 peanut genotypes in October–December 2014 (1st year) and from January–March 2015 (2nd year). Significant at * P ≤ 0.05.

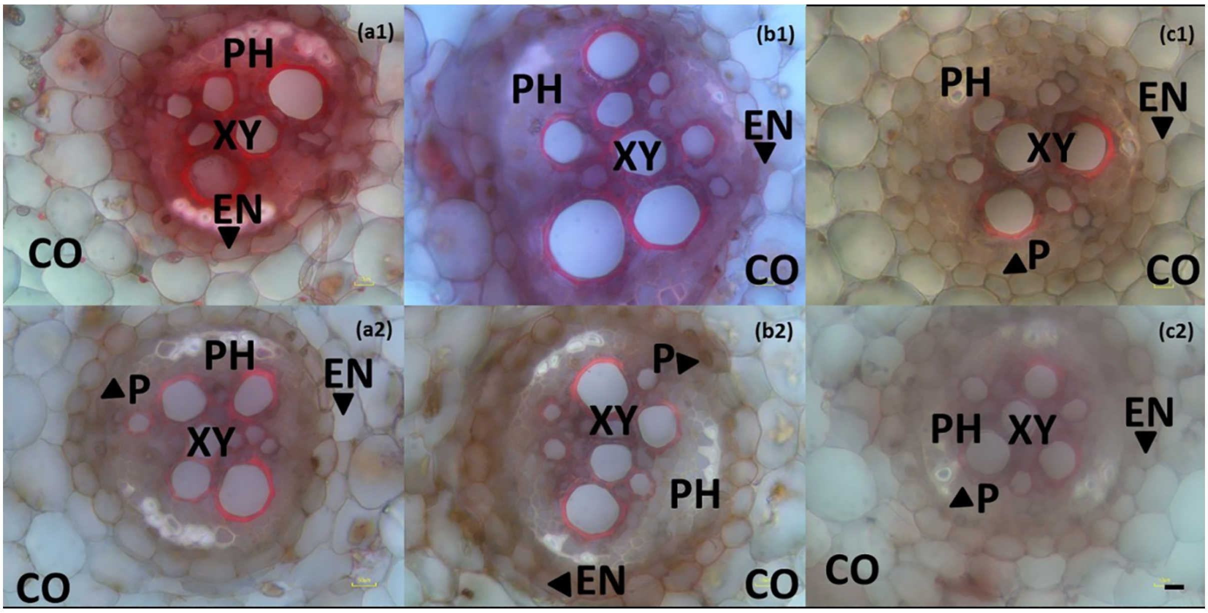

FIGURE 5 Semi-auto microtome cross sections of second order lateral roots of peanut under well-watered (a1, b1 and c1) and drought conditions (a2, b2 and c2) in ICGV 98305, ICGV 98324 and Tifton-8 in October–December 2014 (1st year) and from January–March 2015 (2nd year). CO, cortex; EN, endodermis; P, pericycle; PH, phloem; XY, xylem; Scale bar= 10µm; 40x.

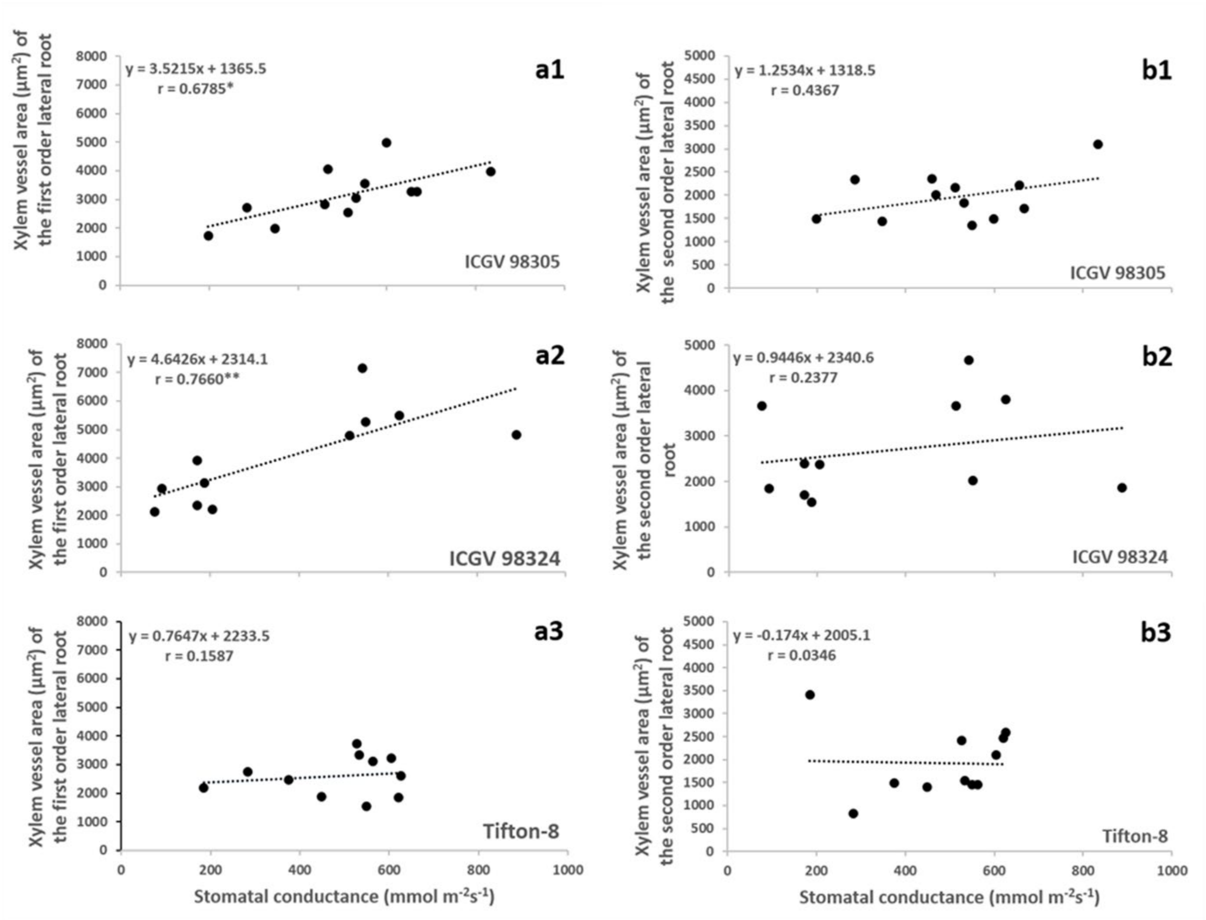

FIGURE 6 Relationship between the xylem vessel area (µm^2) of the first order lateral root and stomatal conductance (mmol m^{-2}s^{-1}) (a1, a2 and a3), and relationship between the xylem vessel area (µm^2) of the second order lateral root and stomatal conductance (mmol m^{-2}s^{-1}) (b1, b2 and b3). Significant at P ≤ 0.05.

TABLE 1 Mean square from the combined analysis of variance for total vessel area (μm^2), total vessel diameter (μm) and total vessel number of the first order lateral root in October–December 2014 and from January–March 2015.

Source	DF	Total vessel area (μm^2)	Total vessel diameter (μm)	Total vessel number
Genotype (G)	2	6617958 **	20.94 **	3.92 *
Season (S)	1	1863752 *	42.19 **	8.64 **
Treatment (T)	1	18930000 **	148.11 **	1.71 ns
G×S	2	426769 ns	4.18 ns	0.31 ns
G×T	2	3237926 **	15.00 **	1.74 ns
S×T	1	71244 ns	0.18 ns	10.67 **
G×S×T	2	503904 ns	4.94 ns	5.08 *
Pooled error	24	492199	2.304	1.04
Total	35			

ns, *, ** = non-significant and significant at $P < 0.05$ and $P < 0.01$ probability levels, respectively, genotypes (ICGV 98305, ICGV 98324 and Tifton-8), seasons (2014 and 2015) and treatments (well-watered and drought treatments).

Abbreviations: G, genotype, S, season, T, treatment, G×S, genotype by environment interaction, G×T, genotype by treatment interaction, S×T, genotype by seasonal interaction, G×S×T genotype by environment by treatment interaction.

TABLE 2 Mean square from the combined analysis of variance for total vessel area (μm^2), total vessel diameter (μm) and total vessel number of the second order lateral root in October–December 2014 and from January–March 2015.

Source	DF	Total vessel area (μm^2)	Total vessel diameter (μm)	Total vessel number
Genotype (G)	2	2061483 *	12.26 ns	9.25 **
Season (S)	1	189459 ns	11.53 ns	30.82 **
Treatment (T)	1	1296900 ns	15.46 ns	0.11 ns
G×S	2	709770 ns	12.80 ns	1.10 ns
G×T	2	639494 ns	3.15 ns	2.77 ns
S×T	1	401382 ns	5.03 ns	1.29 ns
G×S×T	2	841333 ns	13.68 ns	1.29 ns
Pooled error	24	568115	5.62	1.13
Total	35			

^{ns}, *, ** = non-significant and significant at $P < 0.05$ and $P < 0.01$ probability levels, respectively, durations (7, 14 and 21 days without added water), seasons (2014 and 2015) and treatments (well-watered and drought treatments).