

Article

Changes in Root Anatomy of Peanut (*Arachis hypogaea* L.) under Different Durations of Early Season Drought

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Abstract: Changes in the anatomical structure of peanut roots due to early season drought will likely affect the water acquiring capacity of the root system. Yet, as important as these changes are likely to be in conferring drought resistance, they have not been thoroughly investigated. The objective of this study was to investigate the effects of different durations of drought on the root anatomy of peanut in response to early season drought. Plants of peanut genotype ICGV 98305 were grown in rhizoboxes with an internal dimension of 50 cm in width, 10 cm in thickness and 120 cm in height. Fourteen days after emergence, water was withheld for periods of 0, 7, 14 or 21 days. After these drought periods, the first and second order roots from 0–20 cm below soil surface were sampled for anatomical observation. The mean xylem vessel diameter of first- order lateral roots was higher than that of second- order lateral roots. Under early season drought stress root anatomy changes were more pronounced in the longer drought period treatments. Twenty-one days after imposing water stress, the drought treatment and irrigated treatment were clearly different in diameter, number and area of xylem vessels of first- and second-order lateral roots. Plants under drought conditions had a smaller diameter and area of xylem vessels than did the plants under irrigated control. The ability of plants to change root anatomy likely improves water uptake and transport and this may be an important mechanism for drought tolerance. The information will be useful for the selection of drought durations for evaluation of root anatomy related to drought resistance and the selection of key traits for drought resistance.

Keywords: xylem vessel; water stress; root anatomy

1. Introduction

In many areas of the tropics, peanut production is mostly in rain-fed and semi-arid areas with low and unpredictable rainfall and rain distribution. In these areas, drought stress can occur at any growth stage, resulting in yield loss of 22–53% [1]. Drought stress also increases *Aspergillus flavus* infection and aflatoxin contamination by 2–17% [2]. However, drought stress at a pre-flowering growth stage sometimes actually increases yield [3]. Irrigation, planting date selection and drought resistant varieties can improve yield and reduce aflatoxin contamination during periods of drought. However,

management of irrigation requires an available water source and investment in additional equipment. Planting date selection, while less expensive than irrigation is not as effective because rainfall and rain distribution are often unpredictable. The use of drought resistant varieties is a promising and sustainable choice in need of further development. When selecting for drought resistance in peanut, yield and biomass during drought are often used as selection criteria. Yet this selection method is complicated by high genotype by environment interaction. Many physiological and morphological traits have been suggested as surrogate traits for drought resistance to increase selection efficiency, yet measurement for these traits are often quite variable.

Root traits are known to improve drought resistance [4] and are therefore important for plant breeding programs. Improving the water acquiring capacity of crops to extract water from the soil profile during drought is one example. Root traits such as large root systems (root dry weight), root length density and the percentage of root length density that respond to drought have been investigated in peanut [1,3,5–7].

Anatomical parameters, such as xylem vessel number and diameter, have been positively correlated with dry matter production under stress in chili (*Capsicum annum* L.) [8]. Drought resistant varieties of several plant species have been reported to have a higher number of vessel cells and a larger xylem cross-section than susceptible varieties of chili (*Capsicum annum* L.) [8], tomato (*Lycopersicon esculentum*) [9] and grape (*Vitis vinifera* L.) [10]. As in the above studies of other plants, it is likely that studies on the fine root structure of peanut, especially under drought stress, will lead to a better understanding of why some peanut genotypes yield better during a drought than others.

Cell-wall ingrowths or phi-thickening have been reported in loquat (*Eriobotrya japonica* Lindl.) root [11], apple (*Pyrus malus*) [12], geranium (*Pelargonium hortorum*) roots [12] Sibipiruna (*Caesalpinia peltophoroides*) [13] with solute movement (salt stress) [12], water logging [13], and drought stress [11]. Although the effect of early season drought on ingrowths and phi-thickenings has not been investigated in peanut and further investigations are necessary to understand phi-thickenings. The response of phi-thickening might be related to the transport processes in the peanut root.

Root anatomy is interesting, and it might play an important role in plant response to drought. The types of lateral roots during root growth were recognized in peanut [14]. The different types and different structures may be related to different functions. The structure of the first order lateral roots helps determine the efficacy of the axial water transport system, yet the structure within the second order lateral roots helps determine the efficacy of the water uptake process. Unfortunately, this useful information has not been thoroughly investigated in peanut. The objective of this study was to investigate the effects of different durations of drought imposition on the root anatomy of peanut in response to early season drought. The information will be useful for selection of drought durations for evaluation of root anatomy related to drought resistance and selection of key traits for drought resistance.

2. Materials and Methods

2.1. Experimental Design and Plant Material

The experiment was conducted under a rainout shelter at the Field Crop Research Station of Khon Kaen University, Khon Kaen, Thailand (16°28' N, 102°48' E, 200 m. above sea level). The peanut genotype—ICGV 98305—was subjected to four water treatments (0, 7, 14 or 21 days without irrigation), each beginning 14 days after plant emergence (DAE). The experiment used a completely randomized design with three replications and was conducted for two seasons during July–September 2013 and March–May 2014.

ICGV 98305 is a drought resistant line from ICRISAT known for high root length density in the deep sub-soil during periods of drought [1,15].

2.2. Preparation and Irrigation of Rhizobox Experiment

The plants were grown in rhizoboxes with internal dimensions of 50 cm in width, 10 cm in thickness and 120 cm in height (Figure 1a). The rhizoboxes were filled with dry soil to obtain bulk density of 1.57 Mg m^{-3} and height of 115 cm, and water was added to achieve field capacity. Peanut seeds were planted in the center of rhizobox, 5 cm below the soil surface. At 3 days after emergence (DAE), the seedlings were thinned to obtain 1 plant per rhizobox. The front side of the rhizoboxes was transparent and covered with black sheet, and all sides of rhizoboxes were then covered with aluminum foil to reduce light absorption and temperature increase (Figure 1b).

The root needle-board method [16] was used for the observation of root growth and distribution with a minor modification for size and spacing of needles. The root system of the plant in the box was held in place by needles attached to back board of rhizoboxes and projecting out to the transparent front. The needle spacing was $5 \times 5 \text{ cm}^2$. The needle columns started 2.5 cm from left and right margins and the needle rows were started at 12.5 cm from the top of rhizobox and continued at 5 cm intervals to the bottom of the box (Figure 1c).

Soil moisture contents for field capacity and permanent wilting point were determined to be 11.13% and 3.40%, respectively. Water was supplied to the rhizoboxes through horizontal tubes which were installed at 5, 15, 35, 55, 75, 95 and 115 cm below the soil surface. For each rhizobox, water was first supplied at field capacity and all three drought treatments (7, 14 and 21 days without added water) began 14 DAE. The fourth treatment was kept at field capacity for the entire experimental period. The field capacity was maintained uniformly throughout the soil profile by using the six watering tubes. Drainage holes, 1.5 cm in diameter, were placed at the bottom of the rhizobox. Drained water was replenished at the same amount. Crop evapotranspiration was calculated as the sum of water lost through plant transpiration and soil evaporation, as described by Reference [17];

$$\text{ET}_{\text{crop}} = \text{ET}_0 \times K_c \quad (1)$$

where ET_{crop} is crop water requirement (mm/day), ET_0 is evapotranspiration of a reference under specified conditions calculated using the pan evaporation method, and K_c is the peanut water requirement coefficient.

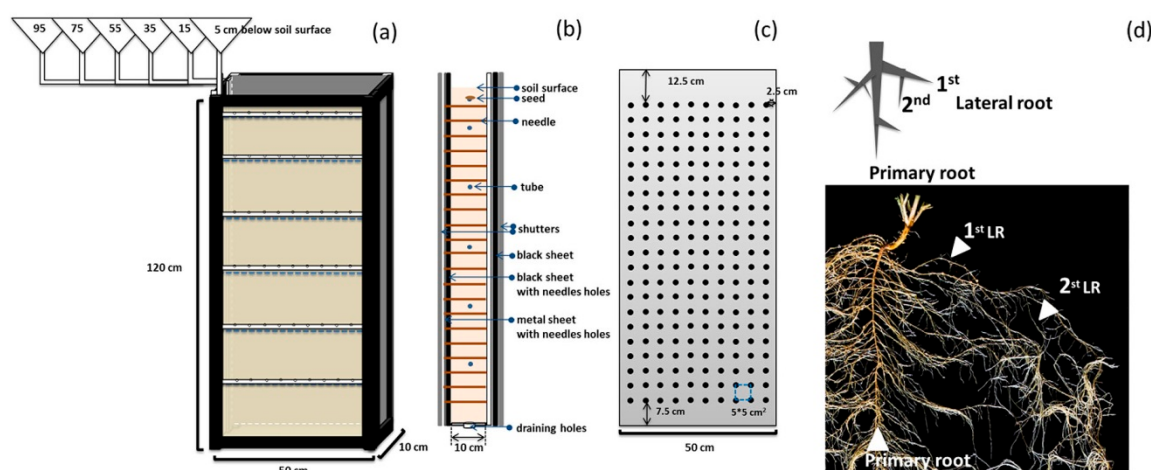


Figure 1. Diagrammatic representation and dimension of rhizobox with six tubes of irrigation (a), cross section showing the different elements of the system (b), spacing of needle at backside of rhizoboxes (c) and taproot system of a rhizobox-grown peanut (d).

2.3. Crop Management

Phosphorus as triple superphosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$) (Chia tai company limited, Phranakhonsiyutthaya, Thailand) at the rate of 122.3 kg ha^{-1} and potassium as potassium chloride (KCl;

60% K₂O) (Chia tai company limited, Phranakhonsiayutthaya, Thailand) at the rate of 62.5 kg ha⁻¹ were applied to the soil before planting. A water-diluted commercial peat-based inoculum of *Bradyrhizobium* (mixture of strains THA 201 and THA 205; Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand) was applied 5 cm below the soil surface through the irrigation tubes. Seeds were treated with captan (3a,4,7,7a-tetrahydro-2-[(trichloromethyl) thio]-1H-isoin-dole-1,3(2H)-dione, Erawan Agricultural Chemical Co., Ltd., Bangkok, Thailand.) at the rate of 5 g kg⁻¹ seeds before planting. Carbosulfan [2-3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate 20% (w/v) water soluble concentrate] (FMC AG Ltd., Bangkok, Thailand) at 2.5 L ha⁻¹ was applied weekly to control thrips, and methomyl [S-methyl-N-((methylcarbamoyl)oxy) thioacetimidate and methomyl [(E,Z)-methyl N-[(methylamino) carbonyl]oxy}ethanimidothioate] 40% soluble powder (Du Pont Co., Ltd., Bangkok, Thailand) at 1.0 kg ha⁻¹ was used to control mites. Weeds were controlled by hand weeding.

2.4. Data Collection

Rainfall, relative humidity, pan evaporation, maximum and minimum temperature and solar radiation were recorded daily from planting to 35 DAE at a weather station located 50 m from the experiment. Soil physical and chemical properties were analyzed before planting. Soil samples for analysis were taken from the mixed pile of soil used for this experiment. The soil's physical properties in the experiment were analyzed for percentage sand, silt and clay. The soil chemical properties were analyzed for pH, organic matter, total N, available P, exchangeable K and exchangeable Ca.

2.5. Soil Moisture Content

Soil moisture content was determined gravimetrically using a micro auger method at 10, 25, 65, and 85 cm soil depths at 14, 21, 28 and 35 DAE. Soil moisture content for each rhizobox was calculated as;

$$\text{Soil moisture content (\%)} = ((\text{wet weight} - \text{dry weight}) / \text{dry weight}) \times 100 \quad (2)$$

2.6. Observation of Root Anatomy

Roots were collected at 7, 14 and 21 days after water withholding began. At the sampling date, the shoot in each box was cut at the soil surface and the roots were carefully washed with a fine spray of tap water to remove soil. Rhizobox needles helped roots maintained the approximate position they were in the soil profile.

Root samples for anatomical observation were taken from 0–20 cm below soil surface. The first- and second-order lateral roots (Figure 1d) were taken at approximately 5 cm from the root tips from each treatment. The root sampling strategy (5 cm from the tip, and 20 cm deep) was as suggested from a previous rhizotron study [18] in which peanut root growth rates of drought and well-watered treatments were 12.6 and 21.9 cm per week, respectively. Therefore, we took the root samples for anatomical study at 5 cm from the root tips, as roots at this position would be expected to be significantly affected by drought. The samples were fixed in a formaldehyde (Sigma-Aldrich; Bangkok, Thailand, 36.5–38% in H₂O)-glacial acetic acid (Fisher Chemical)-40% ethanol-solution (FAA₄₀). Dehydration of the samples was accomplished by adding a series of alcohol concentrations at 10% intervals from 40% to 70%. Free-hand cross sections were stained with Safranin O (Dye content ≥ 85%; Sigma-Aldrich). Anatomical characteristics of the root samples were observed using a Nikon eclipse 50i optical microscope with ocular and stage micrometers. The microscope's digital camera (Nikon DS-Fi1, Shingawa-ku, Tokyo, Japan) was used for photographs. All transverse sections of roots were measured and recorded for diameter and area of the xylem vessels of first-order and second-order lateral roots. Xylem vessel elements consisted of protoxylem and metaxylem. Although the identification of these xylem tissues was difficult, we were able to classify them into two groups by diameter. Smaller xylem vessels were equal to or smaller than the overall mean diameter of xylem vessels and bigger xylem vessels were

larger than the mean diameter of xylem vessels. The cell-wall ingrowths were compared in both the drought and well-watered treatments using the cortical layers of both first-order and second-order lateral roots.

2.7. Data Analysis

The statistical analysis was performed using the statistix-8 program as a completely randomized design. An analysis of variance and least significance difference (LSD) tests were used to compare differences at $p \leq 0.05$.

3. Results

3.1. Meteorological Data and Soil Data

The meteorological details for the two years were collected (data not shown) and are described in Field Crops Research (2016) [19]. Daily air temperatures ranged from 22.7 to 36.8 °C in 2013 and 20.2 to 40.5 °C in 2014. Relative humidity (RH) values ranged between 63–88% in 2013 and 47–87% in 2014. The means of evaporation (E0) were 4.5 mm in 2013 and 5.7 mm in 2014. While rain did not directly fall on the experimental plants, as it was conducted in a rainout shelter; it did affect relative humidity and evapotranspiration.

Differences between years were observed for maximum temperature (T-max) and minimum temperature (T-min) as the trial in 2013 was conducted during the cooler rainy season (May–July) than the 2014 trial conducted from March–May.

3.2. Soil Moisture Content and Relative Water Content

Soil moisture content and relative water content are described in Reference [19]. Soil moisture content measured at field capacity was 11.13% and permanent wilting point was 3.40%. Soil moisture content for non-stress conditions was similar to those at field capacity. However, soil moisture content at field capacity (FC) in the lower soil layers was slightly higher than 11.13% at the initiation of drought stress. Drought and well-irrigated treatments were clearly different at all sampling dates, especially at top soil layers of 10 and 25 cm. The differences between drought and well-irrigated treatments were small in lower soil layers and the treatments became similar at 65 and 85 cm except at 28 and 35 DAE in 2014.

3.3. Observation of Root Anatomy

Peanut has a dicotyledonous root system with a single taproot and branched first-, second-, and higher order lateral roots (Figure 1d). In this study, the anatomy of first- and second- order lateral roots was observed.

3.3.1. First order Lateral Root

Combined analysis of variance for total vessel numbers, bigger vessel numbers, smaller vessel number, total vessel diameter (μm), bigger vessel diameter (μm), smaller vessel diameter (μm), total vessel area (μm^2), bigger vessel area (μm^2), smaller vessel area (μm^2) of the first order lateral root in 2013 and 2014 are shown in Table 1. Significant differences in total vessel numbers, bigger vessel numbers, total vessel area and bigger vessel area were observed in different durations and seasons. The interactions between duration and treatment ($D \times T$) were also significant for total vessel numbers and smaller vessel area traits.

Central cylinders of first order lateral roots had an almost triarch arrangement of the vascular bundles (Figures 2 and 3). Within these bundles, the xylem vessels showed a wide range in size. For ease of discussion, we classified the vessels into two groups (large and small) based on their diameter. Large vessels had a diameter greater than the mean (16.06 μm) of all vessels, and small vessels had a diameter less than the mean.

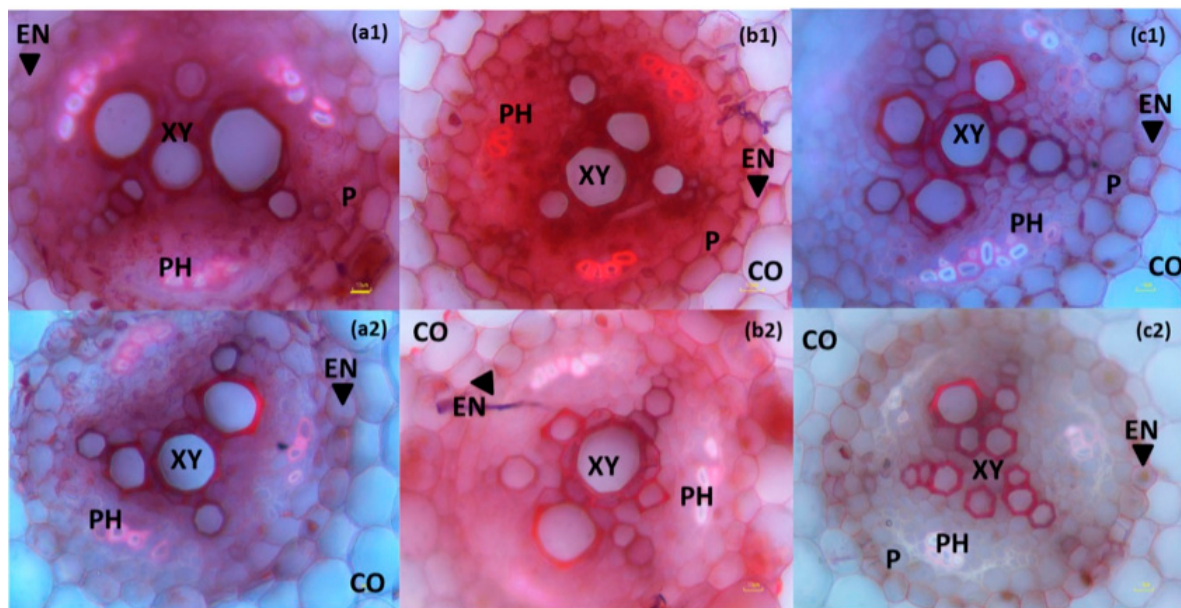


Figure 2. Freehand cross sections of first order lateral roots of peanut under well-irrigated conditions (a1, b1 and c1) and drought stress conditions (a2, b2 and c2) at 21, 28 and 35 DAE, respectively. CO, cortex; EN, endodermis; P, pericycle; PH, phloem; XY, xylem; Scale bar = 10 μ m; 40 $\times$.

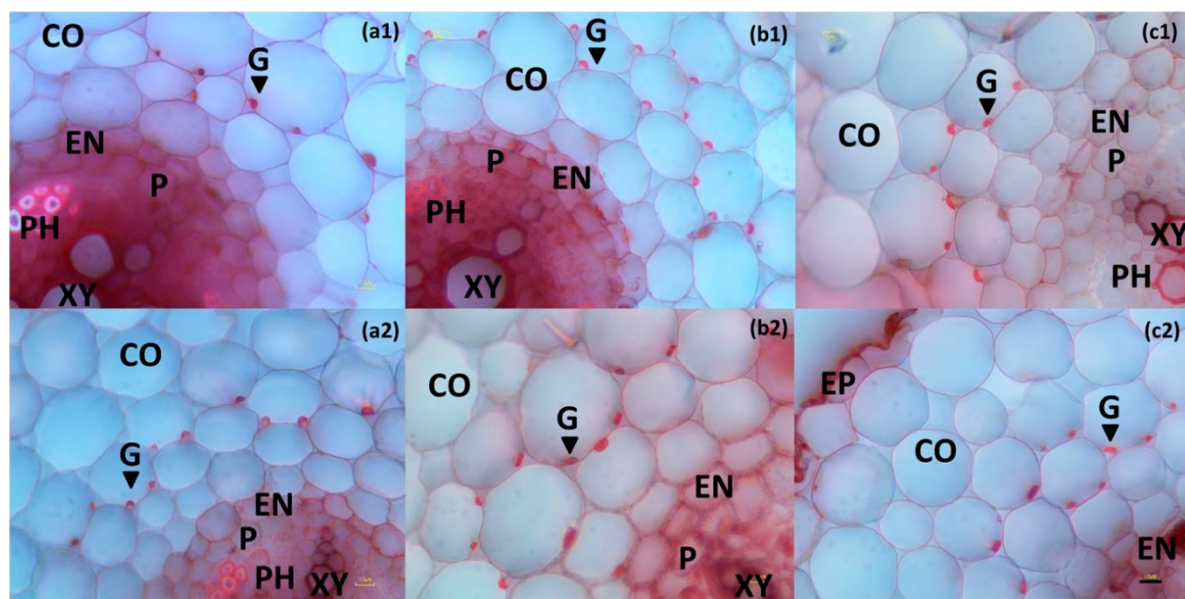


Figure 3. Freehand cross sections of first-order lateral roots under well-irrigated conditions (a1, b1 and c1) and drought stress conditions (a2, b2 and c2) at 21, 28 and 35 days after plant emergence (DAE). CO, cortex; EN, endodermis; G, phi-thickening or cell wall ingrowth; P, pericycle; PH, phloem; XY, xylem; Scale bar = 10 μ m; 40 $\times$.

Total xylem numbers per cross-section of first order lateral roots (Figure 4) in the first and second seasons were not significantly different between drought and well-irrigated treatments at 21, 28 and 35 DAE with one exception at 35 DAE in 2014. At 35 DAE in 2014, the drought treatments had higher vessel numbers, in the small diameter vessels, than did well-irrigated treatments. At 35 DAE in 2013, stress and non-stress treatments were not significantly different for the total number of vessels, yet, like in 2014, stress tended to reduce the number of bigger vessels and increase the number of smaller vessel.

Table 1. Mean square from the combined analysis of variance for total vessel numbers, bigger vessel numbers, smaller vessel number, total vessel diameter (μm), bigger vessel diameter (μm), smaller vessel diameter (μm), total vessel area (μm^2), bigger vessel area (μm^2), smaller vessel area (μm^2) of the first order lateral root in 2013 and 2014.

Source	DF	Total Vessel Numbers	Bigger Vessel Numbers	Smaller Vessel Numbers	Total Vessel Diameter (μm)	Bigger Vessel Diameter (μm)	Smaller Vessel Diameter (μm)	Total Vessel Area (μm^2)	Bigger Vessel Area (μm^2)	Smaller Vessel Area (μm^2)
Duration (D)	2	32.028 **	6.19 *	6.91 ns	14.87 ns	8.81 ns	2.04 ns	6,586,518 **	5185627 **	39262 ns
Season (S)	1	42.25 **	11.11 **	1.01 ns	0.79 ns	5.52 ns	0.29 ns	2,160,885 ns	1,053,634 ns	214,114 *
Treatment (T)	1	0.03 ns	4.00 ns	0.01 ns	3.16 ns	30.24 ns	0.07 ns	2,012,582 ns	2,117,714 ns	110,969 ns
D $\times$ S	2	3.25 ns	4.30 *	33.47 ns	6.97 ns	43.05 ns	1.32 ns	1,576,123 ns	1,723,383 ns	33,482 ns
D $\times$ T	2	8.36 *	0.75 ns	2.40 **	42.04 *	1.39 ns	5.29 ns	1,801,293 ns	3,447,407 *	358,589 **
S $\times$ T	1	0.30 ns	1.78 ns	7.65 ns	0.65 ns	2.24 ns	0.97 ns	56,394 ns	11,259 ns	28,413 ns
D $\times$ S $\times$ T	2	1.36 ns	1.03 ns	5.21 ns	6.77 ns	6.52 ns	2.90 ns	66,096 ns	106,452 ns	91,367 ns
Pooled error	24	1.80	1.13	5.21	10.98	13.75	2.10	566,481	792,286	40,728
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), treatments (well-watered and water stress) and seasons (2013 and 2014).

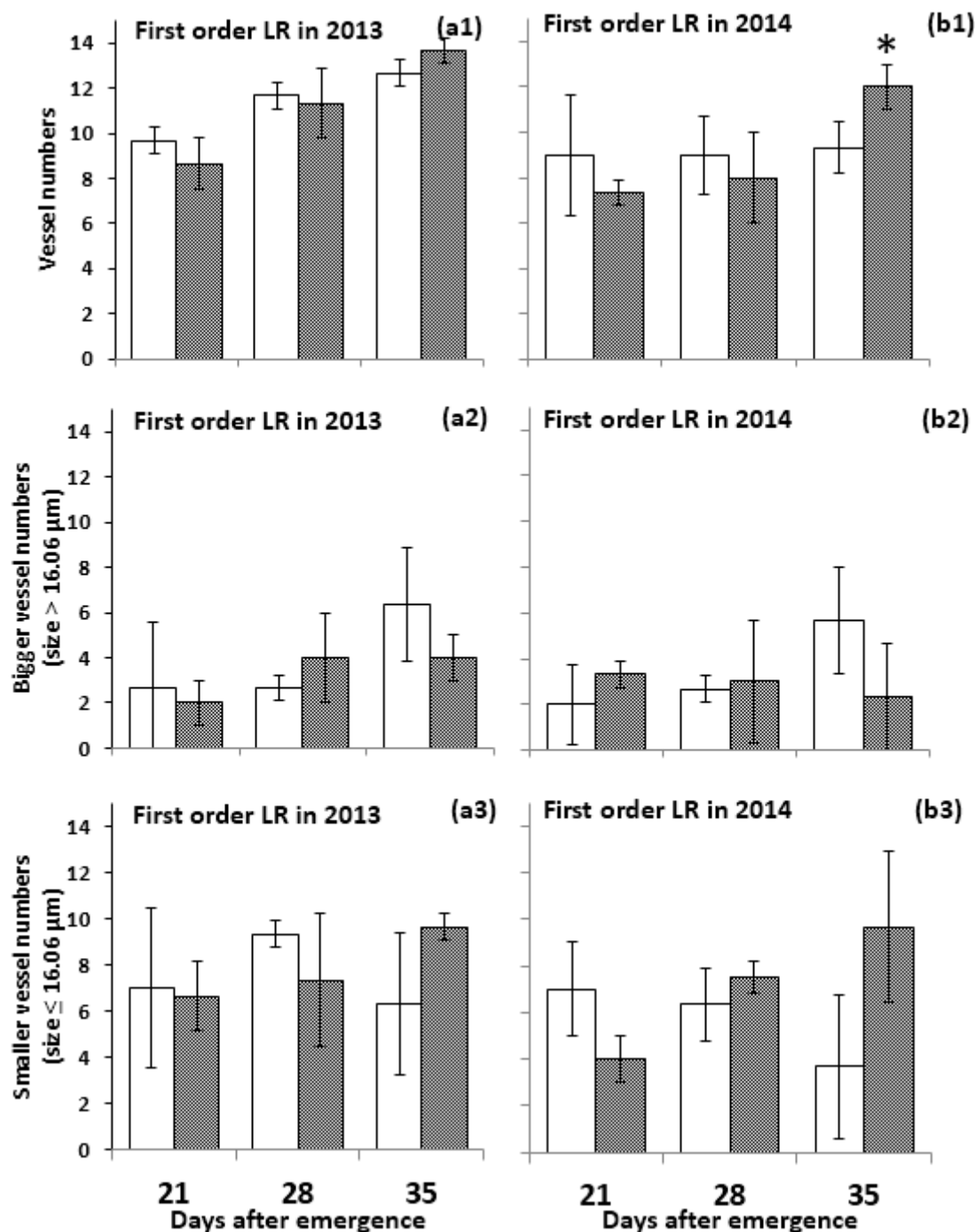


Figure 4. Vessel numbers of first order lateral roots (a1, b1), bigger vessel number (a2, b2) and smaller vessel number (a3, b3) of peanut at 21, 28 and 35 DAE in 2013 (a) and in 2014 (b); Significant at * $p \leq 0.05$, non-stress treatments (□) and stress treatments (■).

Vessel diameters in first order lateral roots (Figure 5) under non-stress and drought stress treatments varied between 4.03 to 41.09 μm (data not shown, unpublished data). Yet, the total vessel area in smaller vessels increased in both 2013 and 2014 and the total vessel area in the large vessels decreased in 2013 and slightly reduced in 2014 when the stress treatments, were compared to the well-watered control (Figure 6) in both 2013 and 2014. Stress and non-stress treatments were not significantly different for vessel diameter at all durations of drought stress. However, the average vessel diameter of long duration stress at 35 DAE and 21 days after irrigation withholding in each season tended to reduce. Figure 5 showed that the diameter of bigger xylem vessels in each season

and the diameter of smaller xylem vessels were not significantly different except for the diameter of smaller xylem vessels at 35 DAE in 2014. The diameters of smaller xylem vessels were smaller in size under long duration stress at 35 DAE and 21 days after irrigation withholding compared to under well-watered treatment in 2014.

A significant reduction was observed in the diameter of the smaller xylem vessels and the diameter of the bigger xylem vessels tended to reduce, ultimately reducing total xylem area per root cross section.

The area of total xylem vessel elements in roots grown under stress conditions was significantly lower than those grown under non-stress conditions and these differences in area increased as the length of stress increased. Non-stress and stress treatments were significantly different for the area of total xylem vessels and the area of bigger vessels at 35 DAE. Stress treatment reduced the area of total vessels in 2013 and to a smaller extent the area tended to reduce in 2014.

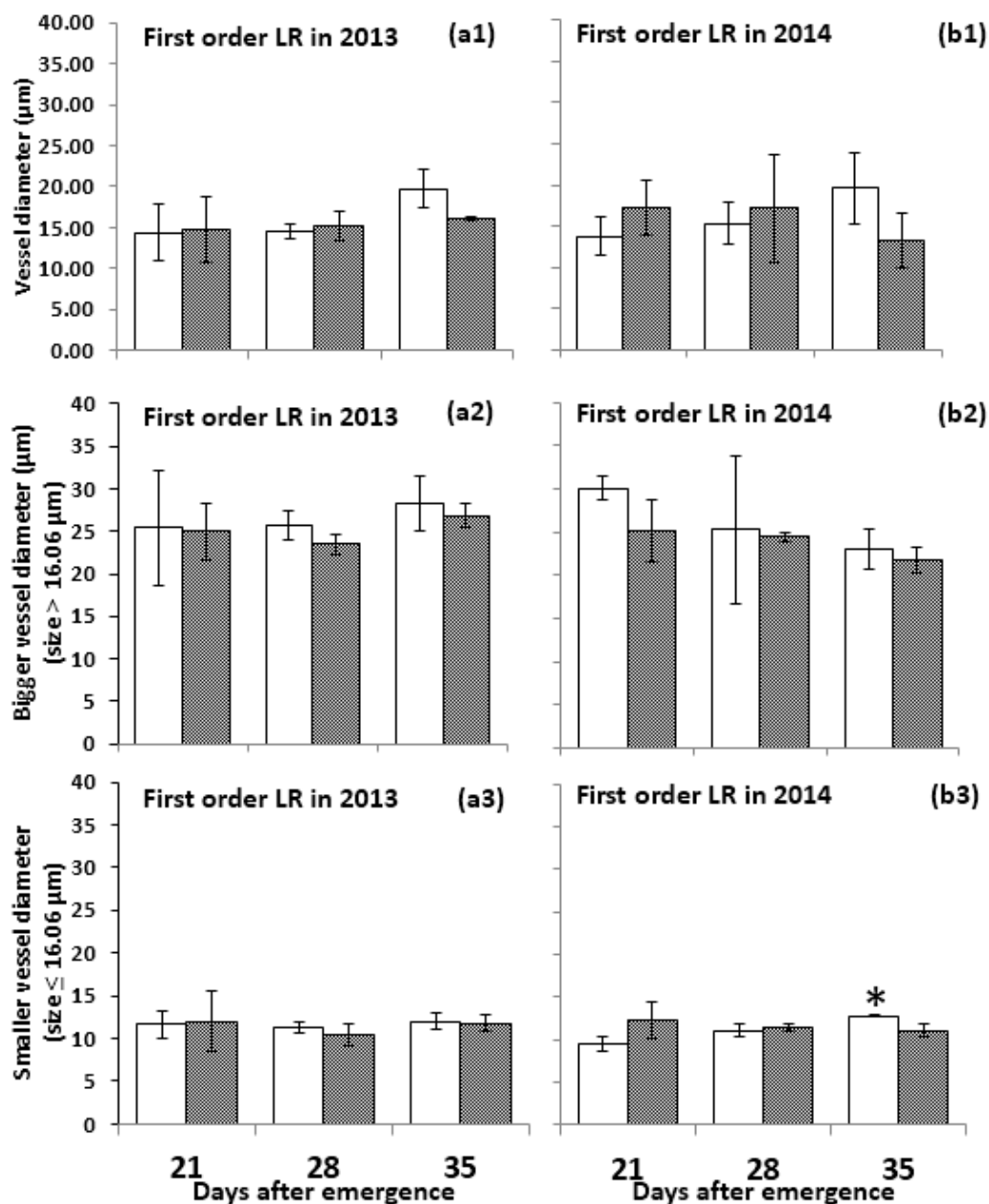


Figure 5. Average vessel diameter of first order lateral roots (a1, b1), bigger vessel diameter (a2, b2) and smaller vessel diameter (a3, b3) of peanut at 21, 28 and 35 DAE under well-irrigate and drought stress in 2013 (a) and in 2014 (b); Significant at * $p \leq 0.05$, non-stress treatments (□) and stress treatments (■).

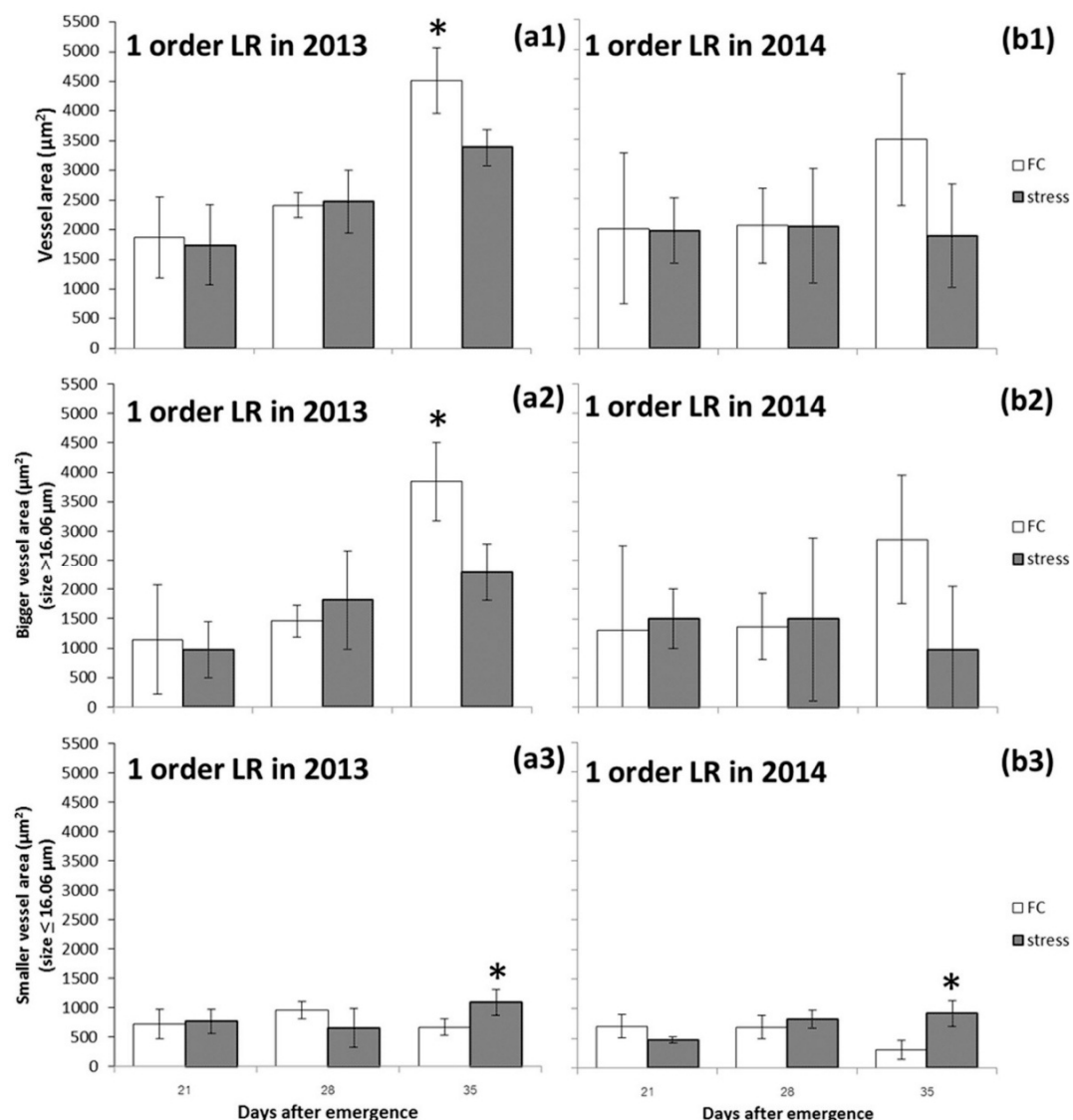


Figure 6. Vessel area (a1, b1), bigger vessel area (a2, b2) and smaller vessel area (a3, b3) of first order lateral roots of peanut at 21, 28 and 35 DAE in 2013 (a) and in 2014 (b); Significant at $* p \leq 0.05$, non-stress treatments ($\square$) and stress treatments ($\blacksquare$).

The cell-wall ingrowths in the first order lateral roots were detected in the cortical cells under both well-watered and drought stress treatments (Figure 3). The cell-wall ingrowths were localized at the opposite side of the intercellular spaces adjacent to the endodermis except in under drought at 28 DAE (Figure 3b2). The cell-wall ingrowths were found in two positions which were on the opposite side of the intercellular spaces and cell-cell conjunction. The 1–2 layers of this cell were found and indicated as the peri-endodermal layer.

3.3.2. Second Order Lateral Root

Combined analysis of variance for total vessel numbers, bigger vessel numbers, smaller vessel number, total vessel diameter (μm), bigger vessel diameter (μm), smaller vessel diameter (μm), total vessel area (μm^2), bigger vessel area (μm^2), smaller vessel area (μm^2) of the second order lateral root in 2013 and 2014 are shown in Table 2. Differences in duration (D) and treatment (T) were significant ($p \leq 0.01$ and $p \leq 0.05$) for most traits. Season (S) was significant for total vessel numbers and bigger vessel numbers. The interactions between duration $\times$ treatment (D $\times$ T) and duration $\times$ season (D $\times$ S) were also significant for some traits.

The structure of second order lateral roots differed from that of the first order lateral roots. First order lateral roots are thicker, and the stele and vascular bundle tissues are more extensive than in the second order lateral roots. Second order lateral roots had an almost diarch and triarch organization of vascular bundles (Figures 7 and 8). Average value of vessel diameter was $14.21\ \mu\text{m}$ (data not shown, unpublished data).

Drought and well-irrigated treatments at all durations were not significantly different for number of total xylem per cross-section of second order lateral roots (Figure 9) in 2013 and 2014. Drought and well-watered treatments were also not significantly different in the number of bigger vessels but the number of bigger vessels tended to reduce at 35 DAE, whereas the number of smaller vessels increased at 35 DAE (21 days after water withholding began).

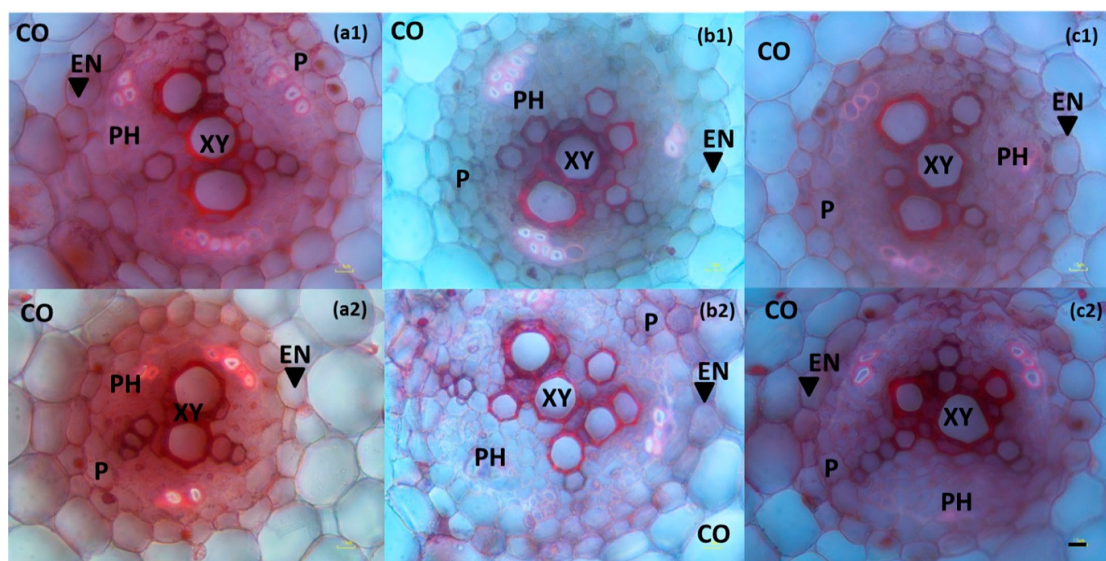


Figure 7. Freehand cross sections of second order lateral roots of peanut under well-irrigated conditions (a1, b1 and c1) and drought stress conditions (a2, b2 and c2) at 21, 28 and 35 DAE. CO, cortex; EN, endodermis; P, pericycle; PH, phloem; XY, xylem; Scale bar = $10\ \mu\text{m}$; 40 $\times$.

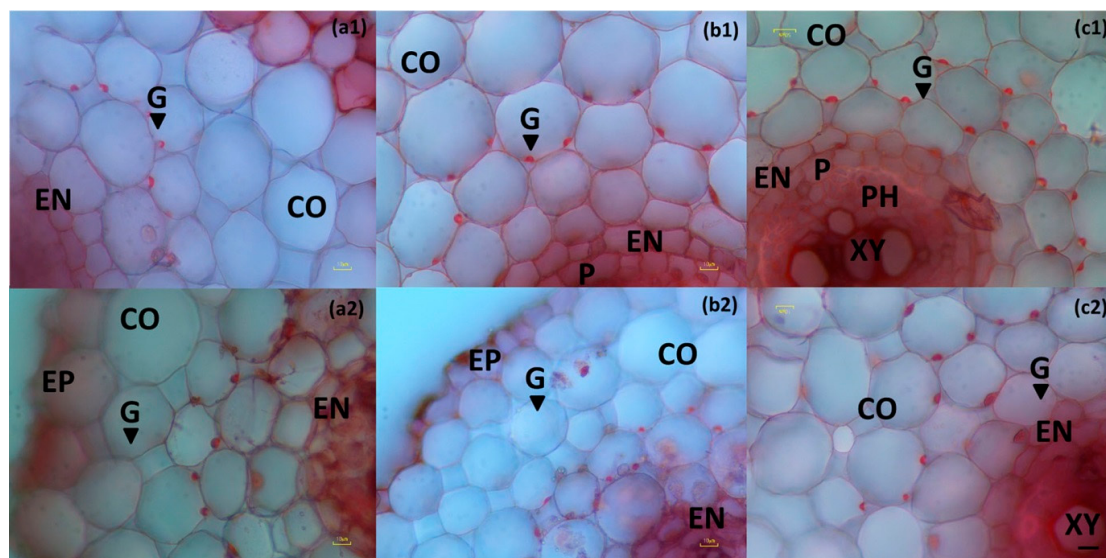


Figure 8. Freehand cross sections of second-order lateral roots under well-irrigated conditions (a1, b1 and c1) and drought stress conditions (a2, b2 and c2) at 21, 28 and 35 DAE. CO, cortex; EN, endodermis; G, phi-thickening or cell wall ingrowth; P, pericycle; PH, phloem; XY, xylem; Scale bar = $10\ \mu\text{m}$; 40 $\times$.

Table 2. Mean square from the combined analysis of variance for total vessel numbers, bigger vessel numbers, smaller vessel number, total vessel diameter (μm), bigger vessel diameter (μm), smaller vessel diameter (μm), total vessel area (μm^2), bigger vessel area (μm^2), smaller vessel area (μm^2) of the second order lateral root in 2013 and 2014.

Source	DF	Total Vessel Numbers	Bigger Vessel Numbers	Smaller Vessel Numbers	Total Vessel Diameter (μm)	Bigger Vessel Diameter (μm)	Smaller Vessel Diameter (μm)	Total Vessel Area (μm^2)	Bigger Vessel Area (μm^2)	Smaller Vessel Area (μm^2)
Duration (D)	2	11.44 **	6.19 *	3.03 ns	26.52 **	4.63 ns	9.02 **	2,437,286 **	1,689,456 **	59,535 *
Season (S)	1	18.78 **	1.11 **	1.00 ns	1.41 ns	13.96 ns	0.23 ns	1,155,729 *	860,956 ns	24,033 ns
Treatment (T)	1	7.11 *	4.00 ns	31.78 **	34.54 **	35.64 *	0.19 ns	1,747,821 **	2,743,513 **	95,334 *
D $\times$ S	2	1.44 ns	4.36 *	2.08 ns	12.83 **	11.36 ns	2.66 *	1,174,800 **	1,167,283 **	1126 ns
D $\times$ T	2	3.11 ns	0.75 ns	5.86 *	3.55 *	31.43 *	0.00 ns	413,242 ns	669,743 ns	22,934 ns
S $\times$ T	1	1.00 ns	1.78 ns	0.11 ns	3.08 ns	0.23 ns	0.04 ns	158,148 ns	138,356 ns	1298 ns
D $\times$ S $\times$ T	2	1.00 ns	1.02 ns	0.36 ns	3.95 ns	3.39 ns	1.85 ns	285,065 ns	199,055 ns	15,256 ns
Pooled error	24	1.17	1.14	1.33	1.84	7.91	0.08	185,616	202,569	13,384
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), treatments (well-watered and water stress) and seasons (2013 and 2014).

Means for the vessel diameter of second-order lateral roots (Figure 10) of all treatments varied between 4.29 to 38.48 μm (data not shown). Stress treatment significantly reduced the vessel diameter of second-order lateral roots at 35 DAE with drought imposition for 21 days in 2013 and slightly reduced the vessel diameter of second-order lateral roots at 35 DAE with drought imposition for 21 days in 2014. Stress treatment significantly reduced the diameter of bigger xylem vessels in 2014 at 35 DAE with drought imposition for 21 days and stress treatment also reduced the diameter of bigger xylem vessels in 2013, although the reduction was not significant. Stress treatment did not significantly affect the diameter of smaller xylem vessel diameter in 2013 and 2014.

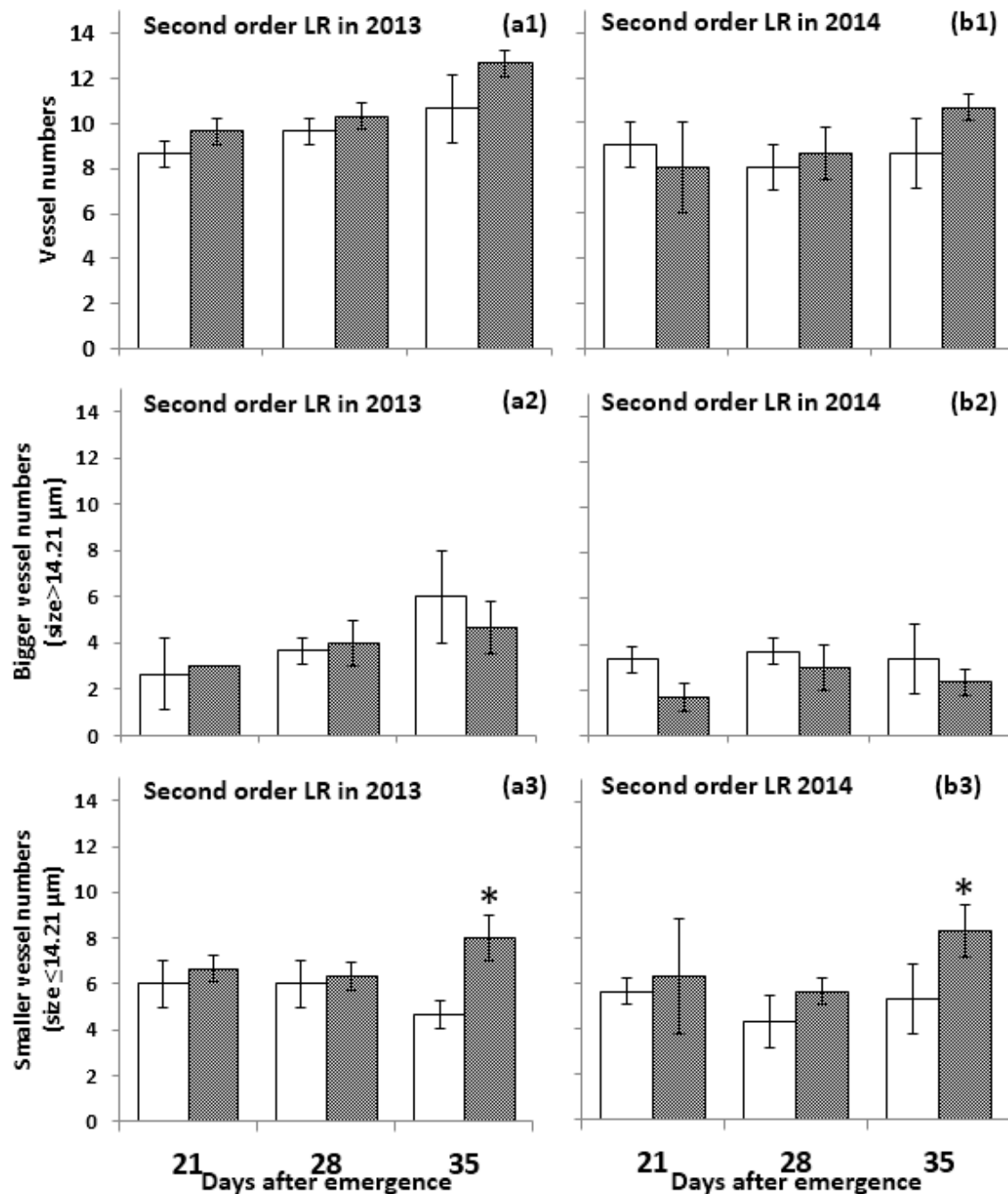


Figure 9. Vessel numbers of second order lateral roots (a1, b1), bigger vessel number (a2, b2) and smaller vessel number (a3, b3) of peanut at 21, 28 and 35 DAE in 2013 (a) and in 2014 (b); Significant at * $p \leq 0.05$, non-stress treatments (□) and stress treatments (■).

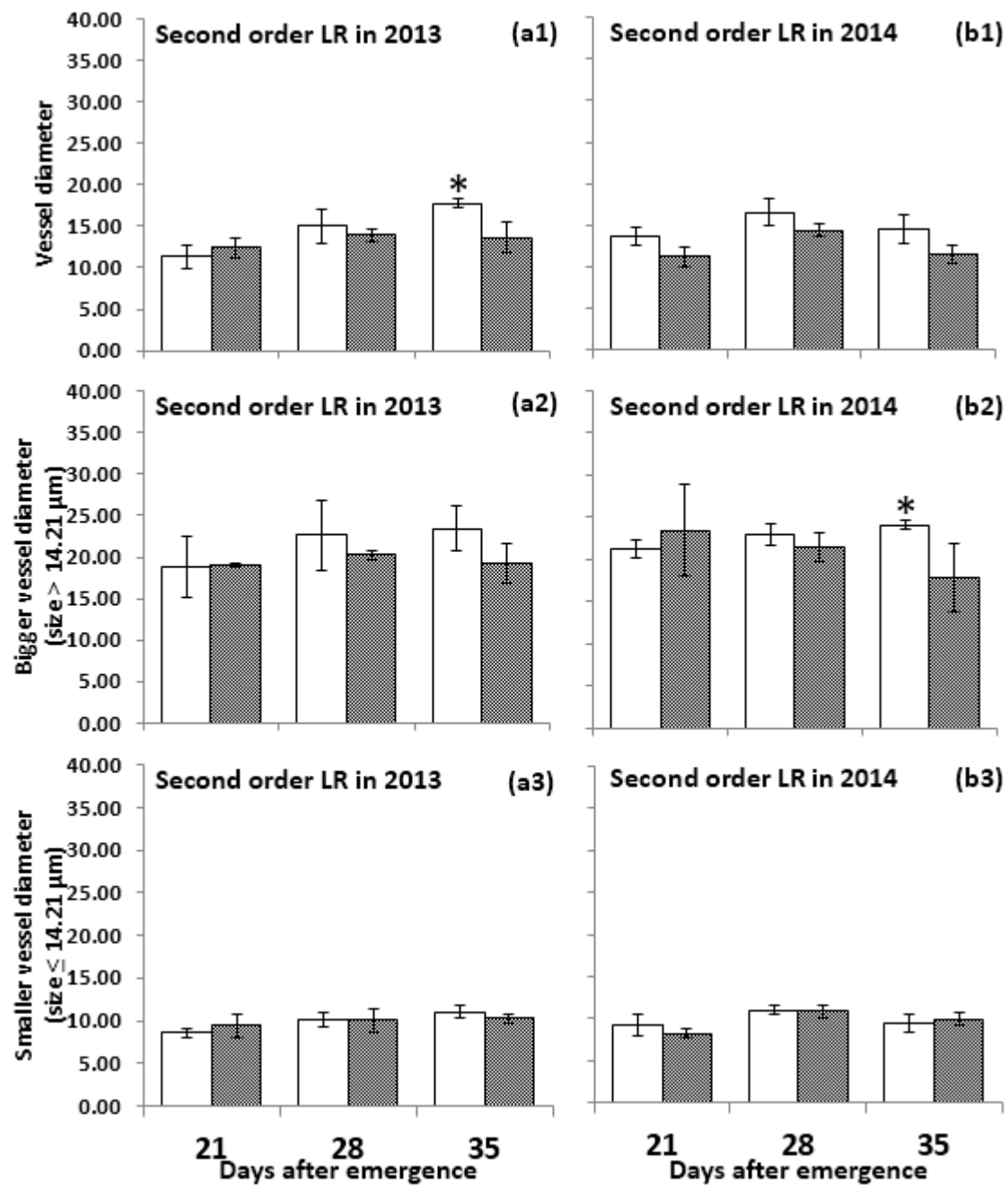


Figure 10. Vessel diameter of second order lateral roots (a1, b1), bigger vessel diameter (a2, b2) and smaller vessel diameter (a3, b3) of peanut at 21, 28 and 35 DAE in 2013 (a) and in 2014 (b); Significant at * $p \leq 0.05$, non-stress treatments (□) and stress treatments (■).

Because stress treatment reduced the diameters of the average xylem vessels and bigger xylem vessels, the area of vessels per cross section of each season and the area of bigger vessels in 2014 was reduced at 35 DAE, although the reduction was not significant and the area of bigger vessels area was significantly reduced at 35 DAE in 2013 (Figure 11). The area of smaller xylem vessels per cross section under stress treatment was increased.

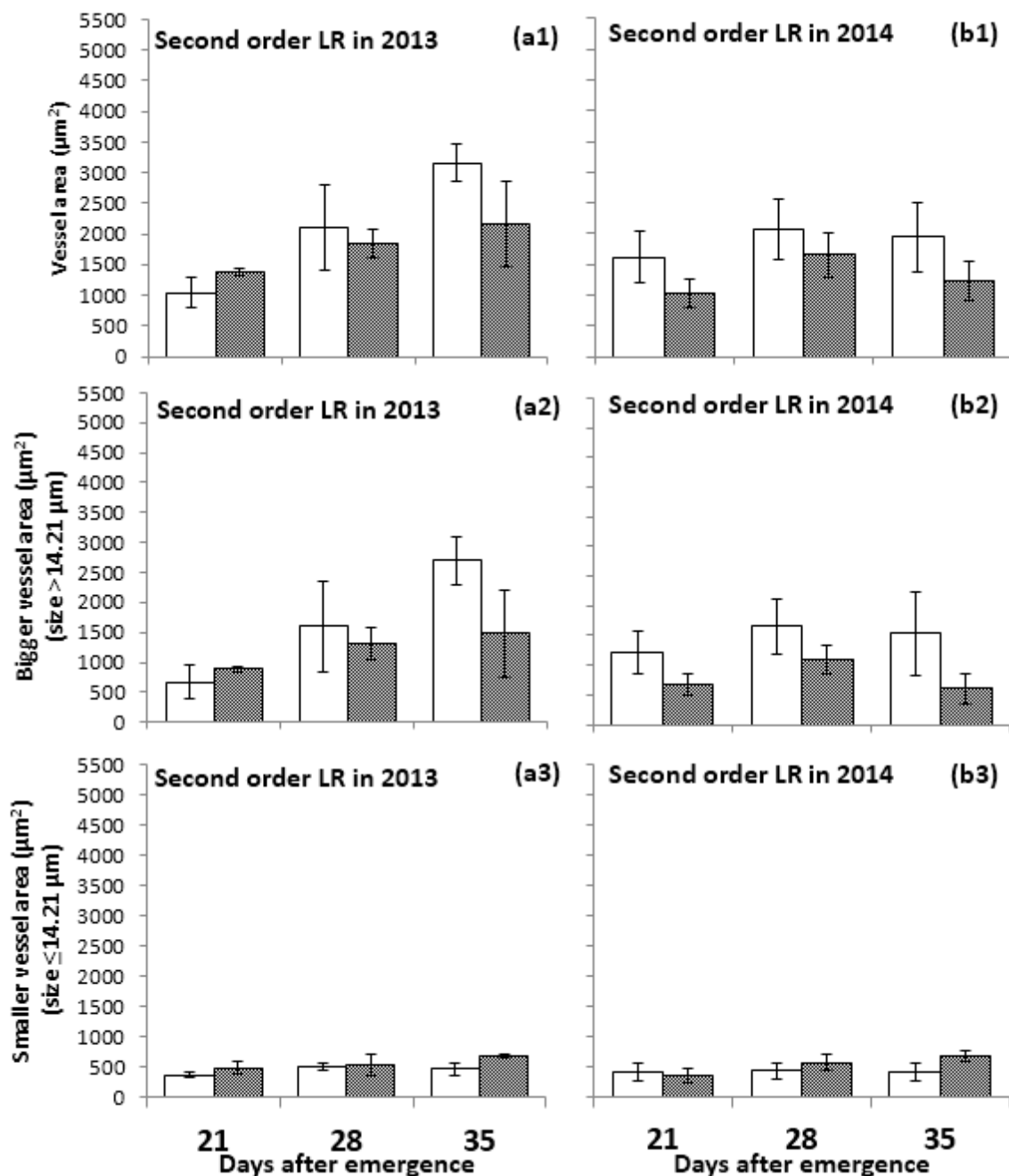


Figure 11. Vessel area (a1, b1), bigger vessel area (a2, b2) and smaller vessel area (a3, b3) of second order lateral roots of peanut at 21, 28 and 35 DAE in 2013 (a) and in 2014 (b); Significant at * $p \leq 0.05$, non-stress treatments (□) and stress treatments (■).

Cell-wall ingrowths appeared in the cortical cells of the second order lateral root under both conditions (Figure 8). The 1–2 layers of cell-wall ingrowths were found in the peri-endodermal layer.

4. Discussion

Weather conditions may be a key factor affecting the root anatomy of peanut. The experiment was conducted for two years. In the rainy season, air temperature and humidity were low, but in the summer to the early rainy season, air temperature and humidity were rather high. Soil moisture in the drought and well-watered treatments were clearly different in the upper soil layers. Soil moisture content for drought stress treatment at 28 and 35 DAE at the 10 cm of soil layer was less than 3.4%

(the permanent wilting point). However, soil moisture content for drought stress treatment at 65 cm and 85 cm of soil levels was higher than the permanent wilting point. The rate of water loss in 2013 was slower than in 2014, and soil moisture content at 21 days after irrigation withholding in 2013 was similar to those at 14 days after irrigation withholding in 2014.

The responses of plants to water stress depend on many things including timing and the intensity and duration of the drought. Root anatomy and root growth, like other plant parts, are sensitive to drought [20]. In this study, the long duration of the early season drought changed the root anatomical traits of peanut. Long periods of stress caused a significant increase in the number of xylem vessels in first and second order lateral roots but a significant decrease in the vessel diameter and the area of these first and second order lateral roots.

In both seasons, the mean xylem vessel diameters of first order lateral roots was higher than that of the second order lateral roots. The reduction in vessel diameter of first order lateral roots was higher than that of the second order lateral roots and these results may explain the differential root functions. The reduction in vessel diameter of first order lateral roots will better support the transport system's hydraulic conductivity according to Poiseuille's law [21]. In hot pepper, drought stress significantly reduced the diameters of xylem vessels in all of cultivars [8]. Vessel diameter is closely and positively correlated with volume of water flow and therefore it is correlated with the 'safety' of the conductive system [22,23]. The large vessel size under water deficit resulted in xylem cavitation [24]. The narrower diameter of metaxylem vessels maintain the water column, lowers the risk of cavitation, increases water flow resistance and saves water columns in narrower capillaries from damage [25]. Formation of narrower vessels occurring in drought-tolerant dicotyledons (including short-lived perennials and annuals with secondary structure) will likewise be advantageous when the plants are grown under drought [26,27].

Morphometric measurements on xylem vessels showed that the vessels of water-stressed plants had lower sectional areas. These results suggested that the reduction in vessel sectional area due to a diminished growth in response to water stress was the main factor affecting conductivity. Under a water deficit environment, roots develop to help extract soil moisture which being held at greater surface tension [28]. Deep root growth and large xylem diameter in deep roots may also increase the ability of roots to mine more water in deep soil when water in deep soil is abundant [29]. However, small and fine roots with greater specific root length enable plants to efficiently increase water uptake and maintain plant productivity under drought by increasing surface area and root length in contact with soil water, especially at deeper soil with available water [19,29].

The ability of plant to take up water is highly influenced by the number and size of the water conductive elements [25]. The change in number and size of the vessel xylem could help maintain water uptake under water stress [8].

In Ferná'ndez-García, Lo'pez-Berenguer, and Olmos book chapter on the role of phi Cells under abiotic stress the authors noted that phi thickening is not the exception in the root anatomy [30]. They noted that the literature has described 16 different families, covering more than 100 species, which present the phi thickening in the roots. The phi thickening is classified into three types based on their root cell location: Type I, the most frequently found phi cell layer, is located in contact with the endodermis. Type II phi cell layer is located in contact with the epidermis and Type III phi cell layers are located in the inner cortical cells but not in contact with either the epidermis or the endodermis. In this study, cell-wall ingrowths were detected in the cortical cells of all first and second order lateral roots under well-watered and drought stress treatments. The 1–2 layers of these cells were localized at the opposite side of the intercellular spaces adjacent to the endodermis. The cell-wall ingrowths layers were indicated as the peri-endodermal layer and also called phi-thickening [31]. In previous studies, phi-thickening was induced under salt stress [30,31] and drought stress [11]. Phi-thickening of loquat roots grown under drought stress developed dramatically compared to normal conditions and the formation of phi-thickening was thought to be a defense mechanism against water stress. As the functions of these cells are difficult to determine precisely, phi thickening would play a role

in controlling the water and solute rate of transportation through cell walls [32]. In peanut, cell wall ingrowth development in cortical cells might be a drought resistance mechanism for peanut roots as well. In this study, the 1–2 layers of cell-wall ingrowths were detected in both well-watered and drought stress treatments which were not significantly different for number of cell-wall ingrowth layers. However, the cells could be seen at higher magnification and using an electron microscope.

5. Conclusions

Under early season drought stress, root anatomy changes were more pronounced in the longer drought period treatments. At 21 days after imposing water stress, the drought treatment and irrigated treatment were clearly different in diameter, number and area of xylem vessels of first- and second-order lateral roots. Plants under drought conditions had smaller diameter and area of xylem vessels than did the plants under irrigated control. The ability of plant to change root anatomy likely improves water uptake and transport, and this may be an important mechanism for drought avoidance.

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