Communications in Soil Science and Plant Analysis, 42:2334–2343, 2011

ISSN: 0010-3624 print / 1532-2416 online
DOI: 10.1080/00103624.2011.605491

Morphology, Biomass, and Vessel Diameter of Pigeon Pea Subjected to Water Stress

MONTY A. PORTER[1] AND JAMES E. BIDLACK[2]

[1]Oklahoma Water Resources Board, Oklahoma City, Oklahoma, USA
[2]Department of Biology, University of Central Oklahoma, Edmond, Oklahoma, USA

This research was designed to study the effects of drought on pigeon pea [Cajanus cajan *(L.) Millsp.*] *morphology, biomass, and vessel diameter. Cultivated seeds of pigeon pea (cv. Georgia-II) were germinated, maintained in an environmental chamber, and arranged as a split-plot design with four replications; harvest was the main effect and watering regimens were tested against residual error. Plants were watered every 2, 4, 8, 16, or 32 days. Number of stems and leaves, as well as total plant height, were measured weekly. Dry weight (DW) of roots, stems, and leaves were recorded at each harvest, and root cross sections were viewed to determine vessel diameter. Results indicated that plant morphology, biomass, and vessel diameter were significantly affected by harvest and watering regimen. Plants watered more frequently had more stems and leaves, grew taller, accumulated greater DW, and had larger diameter vessels within root tissue.*

Keywords Biomass, *Cajanus cajan*, morphology, pigeon pea, vessel diameter, water stress

Introduction

Pigeon pea [*Cajanus cajan* (L.) Millsp.] is a member of the family Fabaceae and is one of the major legume crops of the tropics and subtropics. Pigeon pea has several characteristics that make it valuable as either a production or rotation crop. Some of the benefits of incorporating pigeon pea into cropping systems include its role as a soil ameliorant, ability to fix nitrogen and extract phosphorous, and high drought tolerance. The uses of pigeon pea are widely varied and include being a protein source for humans and livestock, windbreaks or shade for smaller crops, a fuel source, a food for commercial insects, and a versatile intercropping and rotational plant (Nene and Sheila 1990).

The effect of water stress on pigeon pea has not been widely studied outside of certain physiological processes (Lopez, Setter, and McDavid 1988), and additional studies are needed to better understand morphological and anatomical changes that take place in this species when water is limited. This research project focused on the effects of simulated drought on pigeon pea morphology, biomass, and vessel diameter. As such, the objectives of this investigation were to (1) detect variations in pigeon pea morphology as a result of cyclical water treatments, (2) determine changes that may occur in pigeon pea biomass

Received 29 August 2010; accepted 15 May 2011.
Address correspondence to James E. Bidlack, Department of Biology, University of Central Oklahoma, 100 N. University Drive, Edmond, OK 73034. E-mail: jbidlack@uco.edu

through use of cyclical water treatments, and (3) evaluate changes that may occur in vessel diameter of pigeon pea roots during water stress.

Materials and Methods

The experiment employed a split-plot design with harvest date as the main effect and watering regimen tested against residual error. Cultivated seeds of pigeon pea (cv. Georgia II) with a germination rate of >70% were sown in conical planting cells with a diameter of 7.62 cm and length of 25.4 cm. Seeds were planted at a depth of 2–3 mm in Berger soil mix (American Plant Products and Services) and maintained in an environmental chamber on a cycle of 14-h days and 10-h nights. Daytime parameters included temperatures ranging from 28 to 30°C, average humidity of 50%, and a light intensity of 260 foot-candles provided by Phillips F30T125ou Gro-Light bulbs. Nighttime parameters included temperatures ranging from 23 to 25°C, humidity at a maximum of 50%, and no light. The plants were watered as needed until acquisition of primary leaves, at which point the population of each cell was thinned to one plant. Fertilization with 18% superphosphate at 112 kg ha^{-1} was applied immediately following hypocotyl emergence. At the initiation of treatments, each plant was watered until the soil was saturated. Thereafter, each plant received 100 mL of tap water according to a schedule of every 2, 4, 8, 16, or 32 days. Leaf number, stem number, and height measurements were made at approximately 1-week intervals.

Half of the plants were harvested at 32 days, and the other half at 64 days. Harvesting occurred in the following sequence: (1) plants were completely removed from the cells; (2) stems were separated at the root–shoot junction of each plant; (3) 2 cm of each root were cut from topmost portion, weighed, and placed in a fixation solution; (4) the remaining root sample was washed thoroughly with tap water to remove all remaining soil; and (5) leaves were separated from stems at the point of petiole attachment.

At harvest, biomass of the whole plant and plant components (root, stem, and leaf) were determined after drying for 72 h at 55°C and weighing each plant component within 30 min after tissues were dried. The dry weight (DW) for the portion of the root that was removed for anatomical study was calculated by applying the percentage of weight lost after drying of the root from which it was removed. Preparation of root tissue was performed according to the method of Berlyn and Miksche (1976), and average vessel diameters were determined though use of a micron slide and photographed at the same magnification for each sample.

Stem number, leaf number, height, total and component biomass, and vessel diameter were analyzed using type III analysis of variance (Zar 2010) with F probability calculated at 0.05 and 0.01 α levels.

Results

Weekly morphological measurements revealed significant changes in number of stems (Table 1, Figure 1) and leaves (Table 1, Figure 2), as well as plant height (Table 1, Figure 3), as affected by watering regimens for weeks 2 through 9.

Significance of water treatments on number of stems varied among weeks (Figure 1). After week 4, the 2-day treatment had more stems than the other treatments through all weeks except week 8. After week 2, the 4- and 8-day treatments had more stems than the other treatments watered less frequently. After week 2, the 32-day treatment had fewer stems than all the other treatments throughout the experiment.

Table 1
Significance among measurements of total stems, leaves, and plant height during each week of the experiment

	Week of the experiment								
Measurement	1	2	3	4	5	6	7	8	9
Total stems	NS	**	**	**	**	**	**	**	**
Total leaves	NS	**	**	**	**	**	**	**	**
Plant height	*	**	**	**	**	**	**	**	**

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability levels.
NS, not significant at the 0.05 probability level.

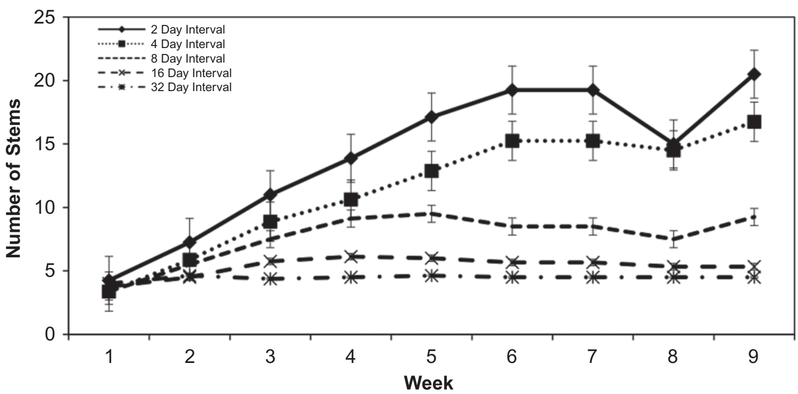

Figure 1. Time series plot of total plant stems by treatment. Each point shows the mean and standard error of four replications.

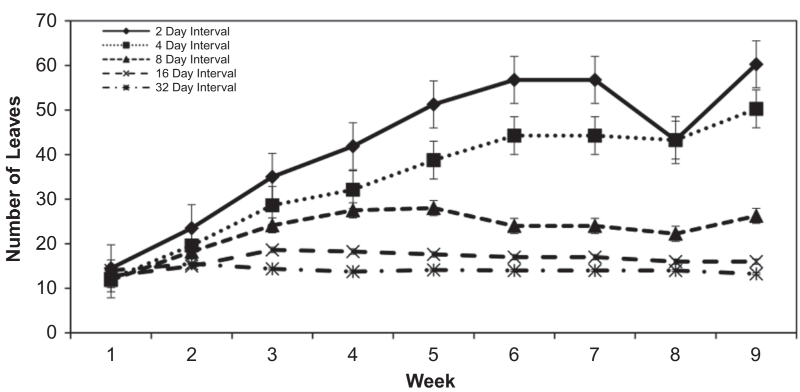

Figure 2. Time series plot of total plant leaves by treatment. Each point shows the mean and standard error of four replications.

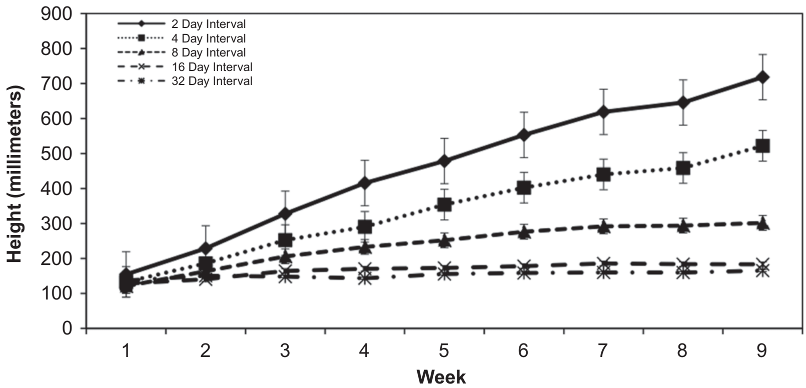

Figure 3. Time series plot of total plant height by treatment. Each point shows the mean and standard error of four replications.

For number of leaves, the significance of water treatment differed among weeks (Figure 2). Number of leaves in the 2-day treatment was greater than all other treatments after week 4, except for week 8. After week 2, leaf numbers of the 4- and 8-day treatments were greater than other treatments watered less frequently. For the 16- and 32-day treatments, numbers of leaves showed a trend similar to numbers of stems. The 16-day plants had more leaves than the 32-day plants after week 2, and the 32-day plants had fewer leaves than all the other treatments throughout the experiment after the second week of treatment.

Significance of water treatments on plant height varied among weeks (Figure 3). Plant height of the 2-day treatment was higher than all other treatments from weeks 4 though 9. After week 2, the 8-day treatment was significantly taller than the 16- and 32-day treatments. Height of the 16- and 32-day treatment plants was not significantly different, but notably after the second week of experimentation, the height of the 32-day treatment was shorter than all the other treatments throughout the experiment.

Harvest, water treatment, and the harvest by water treatment interaction significantly affected root, stem, leaf, and total dry weights (Table 2). For harvest 1, root DW varied

Table 2

Significance among measurements of root, stem, leaf, and total biomass as affected by harvest date (harvest) and watering regimen (treatment)

		Significance among plant biomass measurements			
Source	df	Root	Stem	Leaf	Total
Harvest (H)	1	**	**	**	**
Rep	3	*	NS	NS	NS
Error a	3				
Treatment (T)	4	**	**	**	**
H × T	4	**	**	**	**
Residual	23				

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability levels.
NS, not significant at the 0.05 probability level.

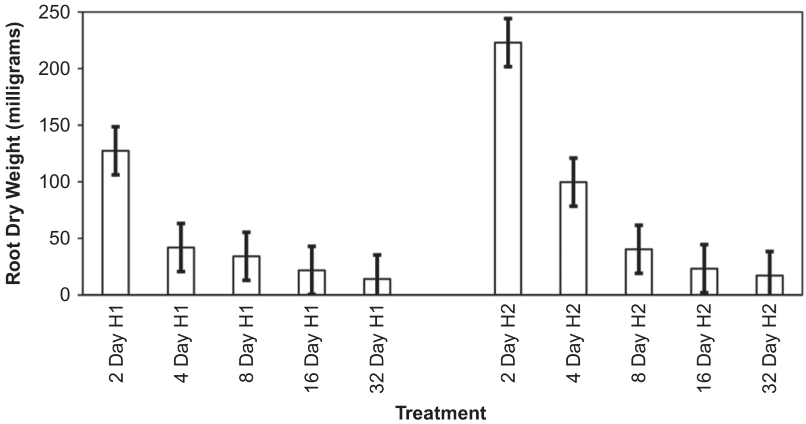

Figure 4. Mean and standard error from four replications of root dry weights from harvests 1 and 2 (2 Day = 2-day treatment interval, 4 Day = 4-day treatment interval, 8 Day = 8-day treatment interval, 16 Day = 16-day treatment interval, and 32 Day = 32-day treatment interval; H1 = harvest 1 and H2 = harvest 2).

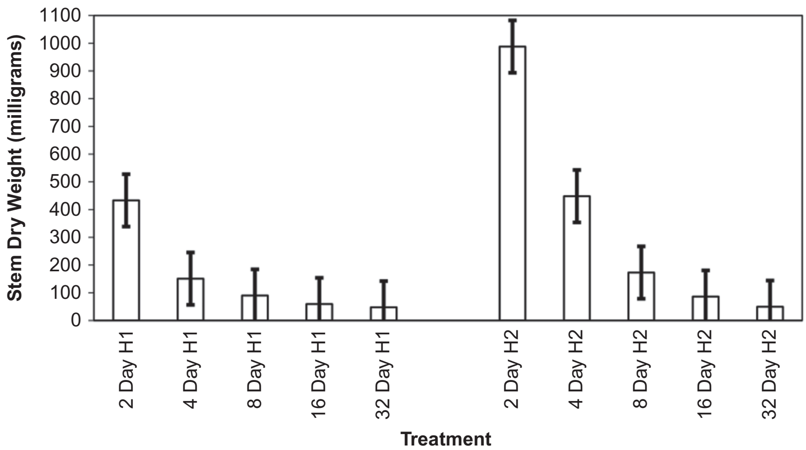

Figure 5. Mean and standard error from four replications of stem dry weights of harvests 1 and 2 (2 Day = 2-day treatment interval, 4 Day = 4-day treatment interval, 8 Day = 8-day treatment interval, 16 Day = 16-day treatment interval, and 32 Day = 32-day treatment interval; H1 = harvest 1 and H2 = harvest 2).

significantly when comparing the 2-day treatment to other treatments (Figure 4). This trend was similar to stem DW (Figure 5), in that, at the first harvest, only the 2-day treatment showed significantly greater stem DW than the other treatments. However, at harvest 2, root (Figure 4) and shoot (Figure 5) DW varied more widely among treatments than they did at the first harvest. Additionally, at harvest 2, root and stem DW for the 2-day and 4-day treatments were significantly greater than the other treatments.

Leaf DW varied among treatments just as profoundly as root and stem DW for both harvests (Figure 6). Decreased leaf DW was observed as watering frequency declined, particularly for the 8-, 16-, and 32-day treatments. No significant differences were observed

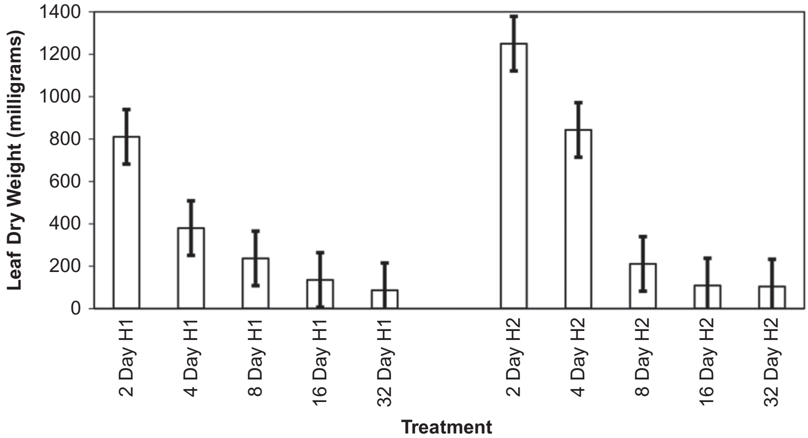

Figure 6. Mean and standard error from four replications of leaf dry weights of harvests 1 and 2 (2 Day = 2-day treatment interval, 4 Day = 4-day treatment interval, 8 Day = 8-day treatment interval, 16 Day = 16-day treatment interval, and 32 Day = 32-day treatment interval; H1 = harvest 1 and H2 = harvest 2).

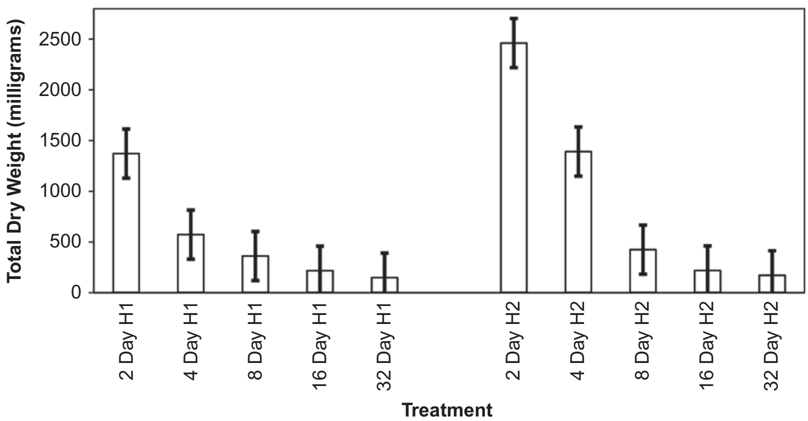

Figure 7. Mean and standard error from four replications of total dry weights of harvests 1 and 2 (2 Day = 2-day treatment interval, 4 Day = 4-day treatment interval, 8 Day = 8-day treatment interval, 16 Day = 16-day treatment interval, and 32 Day = 32-day treatment interval; H1 = harvest 1 and H2 = harvest 2).

among leaf DW when plants were watered at the 8-, 16-, and 32-day treatments. Total DW (Figure 7) followed the same trend as root, stem, and leaf DW in that decreased DW was observed as watering frequency declined.

Harvest and water treatment significantly affected root vessel diameters (Table 3). Vessel diameters for harvest 1 plants were larger in the 2-day treatments compared with the 16- and 32-day treatments (Figure 8). The 4- and 8-day treatments were not significantly different from either extreme. For harvest 2 plants, significant differences in vessel diameters were detected in the 2- and 4-day treatments compared with the 8-day treatment.

Table 3
Significance of vessel diameter as affected by harvest date (harvest) and watering regimen (treatment)

	Significance among vessel diameter measurements	
Source	df	Vessel diameter
Harvest (H)	1	*
Rep	3	NS
Error a	3	
Treatment (T)	4	*
H × T	4	NS
Residual	23	

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability levels.
NS, not significant at the 0.05 probability level.

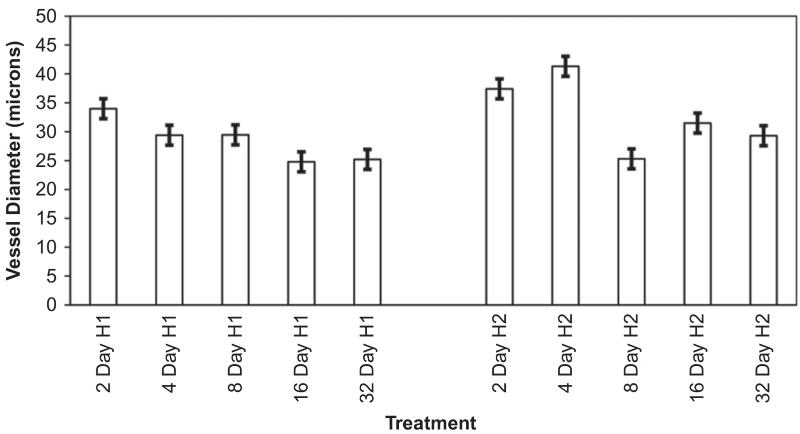

Figure 8. Mean and standard error from four replications of harvests 1 and vessel diameters (2 Day = 2-day treatment interval, 4 Day = 4-day treatment interval, 8 Day = 8-day treatment interval, 16 Day = 16-day treatment interval, and 32 Day = 32-day treatment interval; H1 = harvest 1 and H2 = harvest 2).

In general, vessel diameters were larger during the second harvest, particularly for plants watered more frequently.

Discussion

The general trend of plants that demonstrate alterations in vegetative morphology, as affected by water stress, is not unusual. Chiariello and Gulmon (1991) suggest that perennial plants employ a stress-tolerance strategy that includes short stature and high partitioning to belowground structures in unfavorable conditions. This adaptation may explain the general reduction in number of stems and leaves, as well as decreased plant height, as water limitations were imposed in this experiment. A significant relationship between vegetative morphology and drought stress has been documented in pigeon pea (Nam, Chauhan, and Johansen 2001). Height of soybean has also been significantly linked to drought stress by decreases in internode length and numbers of nodes (Desclaux, Huynh, and Roumet 2000). Under water stress, pine trees have demonstrated a significant decrease in both height and number of branches (Morte et al. 2001). Studies conducted by Byari and Al-Sayed (1999) on tomato cultivars indicated that water deficits decreased plant height, leaf number, and leaf area. Furthermore, maize, when under varying irrigation regimes, had a reduction in plant height and leaf area (Cakir 2004). In contrast, drought did not affect leaf area in several *Brachiaria* species but did affect the length of the leaf blade in grasses (Guenni, Marin, and Baruch 2002). Additionally, studies of pea have concluded that both plant height and leaf area are not significantly reduced by drought stress (Alexieva et al. 2001).

Several trends were observed for the data compiled in this investigation from the component and total DW in response to water treatments. Plants watered less frequently demonstrated lower biomass than the 2-day control at both harvests, with plants watered at 8-, 16-, and 32-day intervals demonstrating a greater divergence in biomass reduction than the more frequently watered plants. When comparing DW from harvest 1 and 2, plants watered more frequently (2- and 4-day treatments) demonstrated relatively high gain in component and total biomass; whereas the plants watered less frequently (8-, 16-, and 32-day treatments) revealed lower gain in component and total biomass. For instance, plants in this experiment that were watered at a 4-day interval experienced an 83% gain in biomass between harvests, whereas plants watered less frequently demonstrated increases in biomass of 9–16% between harvests. In response to drought, plants can begin allocating nutrients to nonfoliar tissues (Geiger and Servaites 1991). Past research has shown that lack of precipitation can lead to decreased biomass (Le Favre and Focht 1983).

Plants watered at an 8-day interval had significantly less biomass during the second harvest and were more closely related to the 16-day and 32-day intervals for all component and total DW. This trend indicates that plants watered at the 8-day interval may be at the critical point where stress is initiated. Other studies suggest that plants can postpone dehydration by decreasing productivity or inhibiting protein synthesis (Geiger and Servaites 1991), and it is possible that, under the conditions of this experiment, postponement of dehydration in pigeon pea may be limited to 8 days without water.

This study documents a consistent reduction in DW as the watering interval decreased. This relationship, whether in measurements of total or component DW, has been suggested in pigeon pea and a number of other crop plants. A significant reduction in dry matter, or biomass, as a result of water stress, has been shown in other plants (Chugh, Kuhad, and Sheoran 1988; Bidlack, Rao, and Demezas 2001; Nam et al. 2001). The shoot biomass of common bean, for instance, was significantly reduced under drought conditions (Ramirez-Vallejo and Kelly 1998; Rosales-Serna et al. 2004), and additional studies have shown that this reduction in biomass is correlated with phenological and morphological changes (Ramirez-Vallejo and Kelly 1998). In other species, including pea (Alexieva et al. 2001) and chickpea (Behboudian et al. 2001), a significant decrease in total plant DW was observed in plants under water stress as indicated by decreased stem height and diameter (Desclaux, Huynh, and Roumet 2000). Even in regard to noncrop plants, a significant reduction in total biomass was noted in pine during periods of drought (Morte et al. 2001).

In general, pigeon peas watered less frequently in this experiment demonstrated smaller vessels. Previous investigations have shown that xylem vessels under stress, when compared with control vessels, will decrease in diameter and will be distributed in different patterns about the stele of the plant (Mapfumo, Aspinall, and Hancock 1994). Furthermore, this pigeon pea study revealed that stress became more pronounced as the experiment progressed from 2 to 64 days. This is most clearly seen in the 8-day plants (Figure 8). During the first harvest, vessel diameters in the 8-day plants could not be differentiated from plants watered more frequently. However, at the second harvest, vessel diameters in these plants significantly diverged from the 4-day plants and became nearly identical in size to plants watered less frequently.

Xylem transport of water in plants is accomplished by the cohesion–tension model (Hacke and Sperry 2001). The tradeoff of the mechanism is negative hydrostatic pressure, rendering the plant susceptible to cavitation. The probability of cavitation is enhanced by introduction of stress and, in the case of drought stress, cavitation occurs by the air seeding mechanism. The premise of this mechanism is that, under drought stress, air enters the vessel conduit, causing a change to atmospheric pressure and a subsequent release of water to surrounding tissues (Hacke and Sperry 2001). Perhaps, in pigeon pea, cavitation is circumvented by reduction in vessel diameter, which helps to increase capillary action and continued water flow to aerial portions of the plant.

Acknowledgments

Funding was provided by the University of Central Oklahoma (UCO) Office of Graduate Studies and Research and the National Science Foundation's Division of Undergraduate Education through Grant DUE-9250582.

References

Alexieva, V., I. Sergiev, S. Mapelli, and E. Karanov. 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell, and Environment* 24:1337–1344.

Behboudian, M. H., Q. Ma, N. C. Turner, and J. A. Palta. 2001. Reactions of chickpea to water stress: Yield and seed composition. *Journal of the Science of Food and Agriculture* 81:1288–1291.

Berlyn, G. P., and J. P. Miksche. 1976. *Botanical microtechnique and cytochemistry*. Ames: Iowa State University Press.

Bidlack, J. E., S. C. Rao, and D. H. Demezas. 2001. Nodulation, nitrogenase activity, and dry weight of chickpea and pigeonpea cultivars using different *Bradyrhizobium* strains. *Journal of Plant Nutrition* 24:549–560.

Byari, S. H., and A. S. Al-Sayed. 1999. The influence of differential irrigation regimes on five green house tomato cultivars, I: The influence of differential irrigation regimes on vegetative growth. *Egyptian Journal of Horticulture* 26:109–125.

Cakir, R. 2004. Effect of water stress at different development stages on vegetative and reproductive growth of corn. *Field Crops Research* 89:1–16.

Chiariello, N. R., and S. L. Gulmon. 1991. Stress effects on plant reproduction. In *Response of plants to multiple stresses*, ed. H. A. Mooney, W. E. Winner, and E. J. Pell, 161–188. New York: Academic Press.

Chugh, L. K., M. S. Kuhad, and I. S. Sheoran. 1988. Responses of pigeonpea genotypes to salt and water stresses at iso-osmotic level during germination and early growth. *Annals of Biology* 4:20–24.

Desclaux, D., T. T. Huynh, and P. Roumet. 2000. Identification of soybean plant characteristics that indicate the timing of drought stress. *Crop Science* 40:716–722.

Geiger, D. R., and J. C. Servaites. 1991. Carbon allocation and response to stress. In *Response of plants to multiple stresses*, ed. H. A. Mooney, W. E. Winner, and E. J. Pell, 103–127. New York: Academic Press.

Guenni, O., D. Marin, and Z. Baruch. 2002. Responses to drought of five *Brachiaria* species, I: Biomass production, leaf growth, root distribution, water use, and forage quality. *Plant and Soil* 243:229–241.

Hacke, U. G., and J. S. Sperry. 2001. Functional and ecological xylem anatomy. *Perspectives in Plant Ecology, Evolution and Systematics* 4:97–115.

La Favre, J. S., and D. D. Focht. 1983. Comparison of N_2 fixation and yields in *Cajanus cajan* between hydrogenase-positive and hydrogenase-negative rhizobia by in situ acetylene reduction assays and direct ^{15}N partitioning. *Plant Physiology* 72:971–977.

Lopez, F. B., T. L. Setter, and C. R. McDavid. 1988. Photosynthesis and water vapor exchange of pigeonpea leaves in response to water deficit and recovery. *Crop Science* 28:141–145.

Mapfumo, E., D. Aspinall, and T. W. Hancock. 1994. Vessel-diameter distribution in roots of grapevines (*Vitis vinifera* L. cv. Shiraz). *Plant and Soil* 160:49–55.

Morte, A., G. Diaz, P. Rodriguez, J. J. Alarcon, and M. J. Sanchez-Blanco. 2001. Growth and water relations in mychorrhizal and nonmycorrhizal *Pinus halepensis* plants in response to drought. *Biologia Plantarum* 44:263–267.

Nam, N. H., Y. S. Chauhan, and C. Johansen. 2001. Effect of timing of drought stress on growth and grain yield of extra-short-duration pigeonpea lines. *Journal of Agricultural Science* 136:179–189.

Nene, Y. L., and V. K. Sheila. 1990. Pigeonpea: Geography and importance. In *The pigeonpea*, ed. Y. L. Nene, S. D. Hall, and V. K. Sheila, 1–14. Wellingford, Oxon, UK: CAB International.

Ramirez-Vallejo, P., and J. D. Kelly. 1998. Traits related to drought resistance in common bean. *Euphytica* 99:127–136.

Rosales-Serna, R., J. Kohashi-Shibata, J. A. Acosta-Gallejos, C. Trejo-Lopez, J. Ortiz-Cereceres, and J. D. Kelly. 2004. Biomass distribution, maturity acceleration, and yield in drought-stressed common bean cultivars. *Field Crops Research* 85:203–211.

Zar, J. H. 2010. *Biostatistical analysis*, 5th ed. Upper Saddle River, N.J.: Pearson Prentice-Hall.