JOURNAL OF PLANT NUTRITION, 24(3), 549–560 (2001)

NODULATION, NITROGENASE ACTIVITY, AND DRY WEIGHT OF CHICKPEA AND PIGEON PEA CULTIVARS USING DIFFERENT *BRADYRHIZOBIUM* STRAINS

J.E. Bidlack,[1] S.C. Rao,[2] and D.H. Demezas[3]

[1]Deparment of Biology, University of Central Oklahoma, Edmond, OK 73034
[2]USDA-ARS Grazinglands Research Laboratory, 7207 W. Cheyenne Street, El Reno, OK 73036
[3]Deparment of Microbiology & Molecular Genetics, Oklahoma State University, Stillwater, OK 74078

ABSTRACT

Chickpea [*Cicer arietinum* (L.)] and pigeon pea [*Cajanus cajan* (L.) Millsp.] were grown outside in large clay pots from 1992 to 1995 in Edmond, Oklahoma. Plants were studied to evaluate nodulation, nitrogenase activity, and shoot dry weight (DW) of 'ICCV-2' and 'Sarah' chickpea inoculated with multistrain, TAL 1148, and TAL 480 *Bradyrhizobium,* as well as 'Georgia-1' and 'ICPL-87', pigeon pea inoculated with multistrain TAL 1127, and TAL 1132 *Bradyrhizobium*. Following wheat [*Triticum aestivum* (L.) emend. Thell.] harvests in the spring, legumes were planted in the summer and harvested at three successive dates during the following months. Leaves and stems from remaining plants were incorporated into the soil after the last harvest. Across year, chickpea measurements were sensitive to temperature and precipitation whereas pigeon pea measurements

 www.dekker.com

were sensitive to length of growing season as well as climate. Pigeon pea consistently demonstrated higher nitrogen-fixing capacity and shoot DW compared with chickpea. Nodule and shoot DW of both species increased with plant age whereas nodule count and nitrogenase activity generally increased with plant age and leveled off or decreased at flowering. Sarah chickpea demonstrated higher nodule count and nodule DW than ICCV-2, as did the Georgia-1 pigeon pea compared with ICPL-87. Shoot DW of Georgia-1 pigeon pea was generally higher than that of ICPL-87. Multistrain inoculum improved nodulation and shoot DW of chickpea, and TAL 1127 improved nodulation of pigeon pea compared with other treatments. These results indicate that specific chickpea and pigeon pea cultivars, along with appropriate *Bradyrhizobium* strains, may improve nitrogen fixation and DW of these species.

INTRODUCTION

Limited information is available on the nitrogen fixing-capacity and yield potential of chickpea and pigeon pea in the southern Great Plains. Previous investigations in other parts of the world have shown that use of different chickpea and pigeon pea cultivars (1, 2), in combination with specific *(Brady) Rhizobium* strains (1, 3–5), have a significant effect on nodulation and yield of these two legumes. Before agronomists in the United States can effectively include chickpea and pigeon pea as part of cereal-legume rotations, it is necessary to evaluate cultivars and rhizobial populations to achieve sustainable production systems. In this investigation, cultivar and inoculum treatment combinations were used to determine the effect(s) of plant age, cultivar, *Bradyrhizobium* strain, and interactions thereof, on nodulation, nitrogenase activity, and shoot DW of summer grain legumes in a wheat-legume rotation.

MATERIALS AND METHODS

Experiments were conducted from 1992 to 1995 in clay pots on the roof of the science building at the University of Central Oklahoma in Edmond, OK. Legumes were grown during summer and harvested in late summer/early fall of 1992, 1993, and 1994. Wheat was planted after legumes and harvested prior to legume planting during the following spring. All plants were maintained in

36 cm diam. clay pots with a capacity of 20 L containing a 50:50 mixture of Farfard Soil Mix No. 2 (Farfard Soil Company, Quebec, Canada) and Dale silt loam (fine-silty, mixed, thermic Pachic Haplustoll) with a pH of 6.6. The pots were placed on top of wooden pallets to allow for drainage and cooling of the plants. Two early-maturing chickpea cultivars, 'ICCV-2' and 'Sarah', were obtained from germplasm collections of the International Crop Research Institute for Semi-Arid Tropics (ICRISAT) at Patancheru, India, and Washington State University, Pullman, WA, respectively; whereas an early-maturing pigeon pea cultivar, 'ICPL 87', and a mid-maturing cultivar, 'Georgia-1', were obtained from ICRISAT and the Horticulture Department at the University of Georgia, Tifton, GA, respectively.

Two distinct strains of chickpea inoculant, TAL 1148 (Nitragin 27A8; USDA 3100) and TAL 480 (USAB 67); and two distinct strains of pigeon pea inoculant, TAL 1127 (IHP 38) and TAL 1132 (IHP 195), recommended by the Nitrogen Fixation by Tropical Agricultural Legumes Project, University of Hawaii at Paia, Hawaii, USA, along with commercial multistrain and no inoculum, were the inoculum treatments. Application of inoculum to seed was performed by mixing 100 mg of peat inoculum (10^9 cells g^{-1} peat) with 10 legume seeds and allowing it to adhere with 'Plantation' Blackstrap Molasses. Seeds treated with only molasses served as controls. Thus, a total of 16 treatments, including two legume species, two cultivars of each species, and three inoculum applications plus no inoculum as control, of each cultivar were evaluated. Pots were randomly arranged on top of the wooden pallets in a complete block design with four replications.

Ten inoculated or uninoculated legume seeds were planted in each designated pot on 30 July 1992, 11 July 1993, and 16 June 1994. Soil was fertilized with phosphorus each year at a rate of 40 kg ha^{-1} and watered as needed throughout the growing season. Temperature and rainfall were recorded at the Oklahoma Climatological Survey Station nearest to the study site in Oklahoma City, OK. Planting of legumes coincided with high temperatures during the early summer whereas harvests usually occurred during cooler and wetter climates in late summer/early fall (data not shown). Maximum temperatures during June through August in 1992 were relatively lower compared with maximum temperatures in 1993 and 1994. However, temperatures remained warmer from August through October in 1992 compared with other years. More precipitation occurred early in the 1992 growing season compared with that of 1993 and 1994, although a large amount of precipitation occurred in September 1993 and November 1994.

Seedlings were thinned to five plants per pot within two weeks of emergence. One whole legume plant (roots and shoots) was harvested at 55, 72, and 85 days after planting (DAP) in 1992; 43, 71, and 92 DAP in 1993, and 63, 87, and 101 DAP in 1994. Harvest dates were scheduled to assess nitrogenase dynamics and growth progression of the legumes. Entire plants were removed by excavating roots and soil 10 cm around the stem to the complete depth of the pot. Roots and attached soil were immediately encased in a 450 mL Mason jar (with serum cap) for acetylene reduction assays while shoots were dried for 96 h at 40°C and weighed. At the last harvest, existing legume pods were removed by hand and the remaining two plants were chopped into pieces no longer than 5.0 cm and incorporated into the top 10 cm of soil.

Legume root samples encased in Mason jars were subjected to nitrogenase assays by acetylene reduction as described by Hardy et al. (6) and modified by Tesfai and Mallik (7). A 40 mL sample of acetylene gas was injected into each sealed Mason jar and incubated for 90 min at 25°C. Five mL of gas from the Mason jar were transferred to a 25 mL bottle with a serum cap. Exactly 0.50 mL of each of these diluted samples were injected into a Gas Chromatograph (Hewlett-Packard Model 5890, fitted with a stainless steel column, 1.8 m × 3.2 mm, packed with Porapak N 80/200 mesh, and flame ionization detector) and quantified for ethylene. Nitrogen gas flow was at 15 psi. Temperatures of injection port, column, and detector were 110, 70, and 220°C, respectively. After the nitrogenase assay, nodules were removed from roots to obtain nodule count and dried for 96 h at 40°C to obtain nodule DW.

Since initial analyses of variance (ANOVA) revealed significant differences ($P < 0.05$) for most measurements as affected by species, there were separate ANOVAs for chickpea and pigeon pea data. Chickpea and pigeon pea ANOVAs analyzed differences in measurements as affected by year, plant age, cultivar, inoculum, and all possible interactions. In many cases, year to year variation justified separate ANOVAs for each year and tables were constructed showing results for each year within a species. Occasional experimental error required the use of SAS PROC GLM (8) to process data sets containing missing values. Least significant differences were evaluated at the 0.05 level of probability.

RESULTS

Nodule count per plant for cultivars ranged from 26 to 55 in chickpea and from 22 to 41 in pigeon pea from 1992 through 1994 (Table 1). The Sarah chickpea cultivar produced more nodules than did the ICCV-2 cultivar in 1992 and 1993. The effect of inoculum on nodules per plant did not differ between the two chickpea cultivars. Between pigeon pea cultivars, Georgia-1 produced more nodules than ICPL-87 all years and when averaged across years. The uninoculated pigeon pea control produced the lowest number of nodules in 1992 and 1993. When averaged across years, the uninoculated control had the lowest number of nodules and the TAL 1127 inoculum resulted in the highest number of nodules in pigeon pea.

Table 1. Nodule Count of Chickpea and Pigeon Pea, Averaged Across Harvest, as Affected by Cultivar and *Bradyrhizobium* Treatments

	Year			
Cultivar/Inoculum	1992	1993	1994	Mean
		nodules plant^{-1}		
Chickpea Cultivar				
ICCV-2	39.2	25.6	45.5	36.5
Sarah	55.2	52.2	38.2	48.6
LSD (0.05)	9.4	15.6	NS[a]	7.6
Chickpea Inoculum				
Control	33.9	32.8	41.3	36.0
Multistrain	46.6	44.4	48.6	46.5
TAL 1148	51.6	31.2	45.1	42.6
TAL 480	56.7	47.0	32.3	45.4
LSD (0.05)	NS	NS	NS	NS
Pigeon Pea Cultivar				
Georgia-1	31.4	39.8	41.2	37.4
ICPL-87	21.9	28.9	24.9	25.2
LSD (0.05)	6.9	9.2	8.9	4.4
Pigeon Pea Inoculum				
Control	9.8	30.3	31.2	23.8
Multistrain	27.0	33.4	41.3	33.9
TAL 1127	42.6	34.6	32.8	36.7
TAL 1132	27.0	39.0	27.0	31.0
LSD (0.05)	8.0	NS	NS	6.0

[a]NS = not significant at the 0.05 probability level.

Chickpea nodule DW increased during the first two harvests for all inoculum treatments (Table 2). Thereafter, nodule DW increased, leveled off, or occasionally decreased. Separate ANOVAs for the year*age*cultivar interaction of chickpea (data not shown) revealed that nodule DW of Sarah was higher compared with that of ICCV-2 at 72 DAP in 1992 and at 43 DAP in 1993. In pigeon pea, nodule DW at 85 DAP in 1992 was only about one-half of what was observed at 92 DAP in 1993 and at 101 DAP in 1994. Nodule DW of Georgia-1 pigeon pea was consistently higher than that of ICPL-87 for all plant ages of all years, although this difference was significant only at 101 DAP in 1994, as well as averaged across years.

Nitrogenase activity of both legumes either increased or stayed the same from 1992 to 1994 (Table 3). During 1992 and 1993, nitrogenase activity increased in chickpea and pigeon pea during early growth (between first and

Table 2. Nodule Dry Weight of Chickpea and Pigeon Pea as Affected by Cultivar and *Bradyrhizobium* Treatments

	Year									
	1992			1993			1994			
	Plant Age (d)			Plant Age (d)			Plant age (d)			
Cultivar/Inoculum	55	72	85	43	71	92	63	87	101	Mean
	mg plant^{-1}									
Chickpea Cultivar										
ICCV-2	33	81	135	19	76	32	45	59	109	65
Sarah	85	152	133	54	96	88	82	40	49	87
LSD (0.05)	NS[a]	68	NS	27	NS	NS	NS	NS	NS	17
Chickpea Inoculum										
Control	38	146	85	38	73	51	48	60	94	70
Multistrain	76	91	170	51	108	46	68	29	87	81
TAL 1148	58	115	116	28	70	49	90	53	94	75
TAL 480	63	113	165	30	95	96	46	55	42	78
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pigeon Pea Cultivar										
Georgia-1	160	434	290	74	370	572	242	349	710	358
ICPL-87	118	133	206	44	313	389	138	239	495	233
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	204	60
Pigeon Pea Inoculum										
Control	102	112	128	48	253	368	172	282	607	230
Multistrain	205	347	302	43	320	537	256	377	470	318
TAL 1127	129	158	324	54	400	558	215	259	668	307
TAL 1132	119	517	238	92	394	459	117	258	664	318
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]NS = not significant at the 0.05 probability level.

Table 3. Nitrogenase Activity of Chickpea and Pigeon Pea as Affected by Cultivar and *Bradyrhizobium* Treatments

	Year									
	1992			1993			1994			
	Plant Age (d)			Plant Age (d)			Plant Age (d)			
Cultivar/Inoculum	55	72	85	43	71	92	63	87	101	Mean
	μmol plant^{-1} hr^{-1}									
Chickpea Cultivar										
ICCV-2	1.27	3.62	3.75	–	5.30	0.50	2.00	1.52	3.35	2.56
Sarah	0.17	1.98	2.30	0.41	6.19	0.34	1.70	0.60	0.75	1.65
LSD (0.05)	NS[a]	NS	NS	NS	NS	NS	NS	NS	NS	0.68
Chickpea Inoculum										
Control	0.73	3.04	2.43	0.63	4.83	0.22	1.06	2.40	2.76	2.01
Multistrain	0.51	2.42	3.59	0.60	9.83	0.44	2.89	0.37	2.34	2.55
TAL 1148	0.87	3.37	4.09	0.28	4.29	0.50	2.55	1.03	1.63	2.07
TAL 480	0.76	2.38	1.98	0.14	4.05	0.52	0.89	0.46	1.47	1.40
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pigeon Pea Cultivar										
Georgia-1	3.01	6.00	5.63	4.53	29.1	0.58	12.3	6.76	38.6	11.9
ICPL-87	3.14	3.65	4.73	7.58	20.9	0.91	9.59	12.4	21.5	9.64
LSD (0.05)	NS	1.58	NS	NS	NS	NS	NS	NS	11.6	NS
Pigeon Pea Inoculum										
Control	2.89	3.29	2.73	6.36	17.8	0.16	10.1	6.26	35.9	9.50
Multlstrain	3.32	4.68	4.38	5.31	14.1	0.99	13.6	17.2	25.0	9.85
TAL 1127	3.02	6.60	7.68	7.97	32.7	1.13	13.2	5.69	35.0	12.6
TAL 1132	3.08	4.73	5.92	4.57	35.3	0.71	6.8	9.17	24.3	10.5
LSD (0.05)	NS	NS	NS	NS	13.9	NS	NS	NS	NS	NS

[a]NS = not significant at the 0.05 probability level.

second harvests) and then decreased or leveled off at maturity (last harvest). Nitrogenase activity was highest for both species during the second harvest of 1993 or the last harvest of 1994. Nitrogenase activity of ICCV-2 was generally higher than that of Sarah, and this difference was significant when averaged across years. Nitrogenase activity of Georgia-1 was significantly higher than that of ICPL-87 at 72 DAP in 1992 and at 101 DAP in 1994. Inoculum treatment had no significant effect on nitrogenase activity of chickpea. However, the TAL 1127 and TAL 1132 treatments significantly increased nitrogenase activity of pigeon pea at 71 DAP in 1993, compared with control and multistrain treatments. The effect of inoculum on nitrogenase activity in 1992 and 1994 was minimal.

Table 4. Shoot Dry Weight of Chickpea and Pigeon Pea as Affected by Cultivar and *Bradyrhizobium* Treatments

	Year									
	1992			1993			1994			
	Plant Age (d)			Plant Age (d)			Plant Age (d)			
Cultivar/Inoculum	55	72	85	43	71	92	63	87	101	Mean
	g plant^{-1}									
Chickpea Cultivar										
ICCV-2	1.15	2.67	4.33	1.49	3.19	5.15	1.59	2.71	4.62	2.99
Sarah	1.88	2.78	4.04	0.94	2.22	4.75	2.48	3.36	3.35	2.87
LSD (0.05)	NS[a]	NS	NS	0.23	NS	NS	NS	NS	NS	NS
Chickpea Inoculum										
Control	1.38	2.84	4.14	1.03	2.73	5.41	2.17	2.80	2.55	2.78
Multistrain	1.95	3.40	3.67	1.29	2.59	5.58	1.87	4.16	6.33	3.43
TAL 1148	1.16	2.91	4.20	1.13	3.20	4.52	2.42	2.24	3.48	2.81
TAL 480	1.59	1.74	4.74	1.40	2.29	4.29	1.67	2.97	3.59	2.70
LSD (0.05)	NS	0.84	NS	NS	NS	NS	NS	NS	NS	0.55
Pigeon Pea Cultivar										
Georgia-1	2.69	4.56	4.78	2.48	11.4	13.6	7.65	16.2	41.6	11.7
ICPL-87	2.85	3.37	4.60	2.08	8.84	11.4	6.34	17.6	30.3	9.70
LSD (0.05)	NS	1.08	NS	0.25	1.33	NS	NS	NS	NS	1.30
Pigeon Pea Inoculum										
Control	2.27	3.97	4.19	2.33	10.3	12.3	8.80	17.9	41.3	11.5
Multistrain	2.79	4.10	4.50	1.96	9.56	10.1	8.05	12.6	39.0	10.3
TAL 1127	3.23	3.81	5.44	2.29	10.8	12.8	5.96	16.6	29.9	10.1
TAL 1132	2.78	3.97	4.65	2.55	9.73	14.6	5.16	20.5	33.7	10.8
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]NS = not significant at the 0.05 probability level.

Shoot DW of chickpea did not vary across years even though plants grew for a longer period of time in 1993 and 1994 (Table 4). Within years, chickpea DW generally increased with plant age in 1992 and 1993, and leveled off after the second harvest in 1994. Shoot DW of ICCV-2 was significantly higher than that of Sarah at 43 DAP in 1993. The multistrain inoculum treatment significantly increased shoot DW of chickpea at 72 DAP in 1992 compared with the TAL 480 treatment. The multistrain treatment also increased shoot DW of chickpea averaged across years compared with control and TAL 480 treatments.

Shoot DW of pigeon pea at 85 DAP in 1992 was about half of that observed in 1993 and less than one-fifth of what was obtained in 1994 (Table 4). Pigeon pea shoot DW increased with plant age in all years, especially during the longer growing season of 1994. Shoot DW of Georgia-1 was higher than that of ICPL-87 at 72 DAP in 1992 and at 71 DAP in 1993, as well as averaged across years. Inoculum treatment did not affect shoot DW of pigeon pea.

DISCUSSION

Although the length of growing season increased each year, chickpea nodule count, nitrogenase activity, and shoot DW did not vary across years. This indicated that other factors, such as temperature and precipitation, may influence chickpea measurements more than length of growing season. With determinant species such as chickpea, the timing of stress may be as important as the degree of stress (9). Previous work by Beck (1) has shown that nodule and shoot DW of chickpea are decreased and cultivar differences are diminished during drier seasons. Hot and dry conditions in 1994 may have reduced nodulation and biomass production, even though the growing season was longer, and thus fostered results similar to those reported for 1992 and 1993.

Shoot DW of pigeon pea increased consistently from 1992 to 1993 and from 1993 to 1994, indicating that pigeon pea biomass increased with length of growing season. Previous investigations have suggested that pigeon pea yield is sensitive to delayed planting and can be increased by length of growing season (10). Indeterminant species such as pigeon pea have the potential to flower over a longer period of time and may not be as sensitive to stress as determinant species (9). Limited precipitation may reduce growth rate (11) and decrease yield (23) in pigeon pea. Differences in measurements among pigeon pea cultivars may be diminished by these conditions, as was the case for shoot DW in 1994 compared with other years.

Plant age, represented by the three different harvest dates each year, was the most significant factor affecting measurements of both species. Nodule and shoot DW increased, leveled off, or occasionally decreased with plant age in chickpea and pigeon pea each year. Nitrogenase activity reflected normalized growth curves in that, enzyme activity increased during vegetative growth (from first to second harvest) and leveled off or decreased toward the end of the growing season (from second to third harvest). In 1994, however, nitrogenase activity of Georgia-1 pigeon pea increased at the last harvest, indicating that nitrogenase may be stimulated during fruit set to meet the plant's additional nitrogen needs. Other work by La Favre and Focht (13) has suggested that nitrogenase in pigeon pea peaks at floral initiation and fruit set. Studies involving partial removal of pods and other plant parts has shown that pod set in pigeon pea is determined by the capacity of the source to supply assimilates (14, 15), thereby supporting the need for increased nitrogenase activity. It is possible that the prolonged season in 1994 resulted in increased plant mass, thereby increasing the capacity for nitrogen fixation and the requirement for nitrogen.

Significant differences in nodule count, nitrogenase activity, and shoot weight were revealed by cultivar and/or inoculum treatments of chickpea but no cultivar by inoculum interactions were detected. Sarah demonstrated more nodules and higher nodule DW compared with ICCV-2, although ICCV-2 had a higher nitrogenase activity than Sarah when averaged across years. Shoot DW of Sarah did not exceed that of ICCV-2. It is noteworthy, however, that shoot DW of ICCV-2 at maturity was always higher than that of Sarah. Preliminary results indicated that higher shoot DW in ICCV-2 compared with Sarah may be due to early maturity, higher rate of DW accumulation, and a greater number of pods formed in ICCV-2 (16). The benefits of soil nitrogen provided by Sarah, suggested by higher nodule number and nodule DW, may outweigh the benefits of reproductive units produced by ICCV-2. Among chickpea inoculants studied, the multistrain treatment was most consistent in increasing nodule count, nodule DW, and shoot DW. However, nodule count and nodule DW of the control treatment were not different from that of other treatments, indicating that, in some instances, native *(Brady)Rhizobium* in the soil may be just as effective as other introduced strains. Dry and cooler temperatures may have reduced the effectiveness of introduced inoculants. Other studies have shown that inoculation of chickpea with selected *(Brady)Rhizobium* strains may increase yield and nitrogen fixation in some cultivars (1, 3, 5). However, Beck and coworkers (1) found environments when the same strains were ineffective in competing with indigenous rhizobial populations. This suggests that chickpea cultivars may vary in response to different *Rhizobium* stains under different environments (5) *Bradyrhizobium* stains explain why no cultivar by inoculum interactions were detected in this experiment.

Significant differences were noted in three out of four measurements as a result of pigeon pea cultivars and, in one case, as a result of inoculum treatments. However, no cultivar by inoculum interactions were revealed by pigeon pea measurements. Georgia-1 generally had more nodules, higher nodule DW, and greater nitrogenase activity compared with ICPL-87. This resulted in shoot DW of Georgia-1 which was significantly higher than that of ICPL-87, across years and at several harvest dates within years. The pure inoculum treatment, TAL 1127, was the most consistent in increasing nodule count, nodule DW, and nitrogenase activity compared with other treatments. However, inoculum treatments did not consistently affect pigeon pea shoot DW across years. These results support previous studies demonstrating the variable response of pigeon pea to *(Brady)Rhizobium* strains under different environments (4, 7, 13). It is generally accepted in this study and others (18), that nodule formation and the resulting benefits to yield in pigeon pea are affected by soil type, season, and the duration of the cultivar (19).

ACKNOWLEDGMENTS

This project was funded by the United States Department of Agriculture (USDA) National Research Initiative Competitive Grants Program (Grant No. 93-37311-9580), Oklahoma Wheat Commission, University of Central Oklahoma (UCO) Office of Sponsored Research & Grants, and the USDA Agricultural Research Service Grazinglands Research Laboratory. Authors thank members of the UCO Plant Physiology Research Group for assisting with the project. Authors also thank Dr. John Imsande, Iowa State University, for assistance with analyzing the data and review of the manuscript.

REFERENCES

1. Beck, D.P. Yield and Nitrogen Fixation of Chickpea Cultivars in Response to Inoculation with Selected Rhizobial Strains. Agron. J. **1992**, *84*, 510–516.
2. Cobbina, J. Herbage Yields of Pigeonpea (*Cajanus Cajan*) Accessions in a Humid Tropical Environment as Affected by Pruning Management. J. Agric. Sci. **1995**, *125*, 227–232.
3. El Hadi, E.A. Effect of Rhizobium Inoculation and Nitrogen Fertilization on Yield and Protein Content of Six Chickpea (*Cicer arietinum* L.) Cultivars in Marginal Soils Under Irrigation. Nutr. Cycl. Agroecosys. **1999**, *54*, 57–63.
4. Quilt, P.; Dalal, R.C. Effect of Soil Mineral Nitrogen Levels and Inoculation on Nodulation, Nitrogenase Activity, and Grain Yield of Pigeon Pea. Agron. J. **1979**, *71*, 450–452.
5. Somasegaran, P.; Hoben, H.J.; Gurun, V. Effects of Inoculation Rate, Rhizobial Strain Competition, and Nitrogen Fixation in Chickpea. Agron. J. **1988**, *80*, 68–73.
6. Hardy, R.W.F.; Holsten, R.D.; Jackson, E.K.; Burns, R.C. 1968. The Acetylene-Ethylene Assay for N_2-Fixation: Laboratory and Field Evaluation. Plant Physiol. **1968**, *43*, 1185–1207.
7. Tesfai, K.; Mallik, M.A.B. Effect of Fungicide Application on Soybean-Rhizobia Symbiosis and Isolation of Fungicide-Resistant Strains of *Rhizobia japonicum*. Bull. Environ. Contam. Toxicol. **1986**, *36*, 819–826.
8. SAS Institute. *SAS User's Guide: Statistics*, 5th Ed.; SAS Institute, Cary, NC, 1985.

9. Gardner, F.P.; Pearce, R.B.; Mitchell, R.L. *Physiology of Crop Plants*. Iowa State University Press: Ames, IA, 1985.
10. Nam, N.H., Y.S. Chauhan, and C. Johansen. Comparison of Extra-Short-Duration Pigeonpea with Short-Season Legumes Under Rainfed Conditions on Alfisols. Exp. Agric. **1993**, *29*, 307–316.
11. Chauhan, Y.S.; C. Johansen, C.; Saxena, K. Physiological Basis of Yield Variation in Short-duration Pigeonpea Grown in Different Environments of the Semi-arid Tropics. J. Agron. Crop Sci. **1995**, *174*, 163–171.
12. Lopez, F.B.; Johansen, C.; Chauhan, Y.S. Limitations to Seed Yield in Short-Duration Pigeonpea Under Water Stress. Field Crops Res. **1994**, *36*, 95–102.
13. La Favre, J.S.; Focht, D.D. 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 Physiol. **1983**, *72*, 971–977.
14. Pandey, R.K.; Singh, V.B. Influence of Source and Sink Size on Flower Drop and Seed Yield of Pigeon Pea. Indian J. Agric. Sci. **1981**, *51*, 185–188.
15. Sheldrake, A.R.; Narayanan, A.; Venkataratnam, N. 1979. The Effects of Flower Removal on the Seed Yield of Pigeonpeas (*Cajanus cajan*). Ann. Appl. Biol. **1979**, *91*, 383–390.
16. Rao, R.C.; Bidlack, J.E.;. Demezas, D.H. Chickpea and Pigeonpea in Wheat-Legume Cropping Systems of the Southern Great Plains. Int. Chickpea Pigeonpea Newsl. **1996**, *3*, 25–27.
17. Hernandez, B.S.; Focht, D.D. Effects of Phosphorus, Calcium, and Hup^- and Hup^+ Rhizobia on Pigeon Pea Yields in an Infertile Tropical Soil. Agron. J. **1985**, *77*, 867–871.
18. Thompson, J.A.; Kumar Rao, J.V.D.K.; Dart, P.J. Measurement of Inoculation Response in Pigeonpea. In *Proceedings of the International Workshop on Pigeonpeas*; International Crops Research Institute for the Semi-Arid Tropics: Patancheru, A.P., India, 1981; Vol. 1, 249–253.
19. Reddy, L.J. Pigeonpea: Morphology. In *The Pigeonpea*; Nene, Y.L., Hall, S.D., Sheila, V.K., Eds.; CAB International, Tucson, AZ, 1990; 47–87.