Response of pigeon pea to iron, nitrogen and two rhizobium strains

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Summary. We examined the effects of iron (Fe), N and strains of *Bradyrhizobium* sp. on nodulation, N_2 fixation and yields of dry matter and grain of pigeon pea on an alkaline, black-earth soil. Iron was supplied as FeEDDHA, N as nitram and the two strains of rhizobia were CB756 and CB 1024, the former and current inoculant strains for pigeon pea. Nodulation at flowering increased with increasing rates of Fe. By early pod-fill effects had disappeared. There was no effect of Fe on Pfix, the proportion of plant N derived from N_2 fixation. In the presence of fertiliser-N, grain yields were increased with increasing rates of Fe (1.67-2.43 t/ha); in the absence of fertiliser-N, effects of Fe were not significant (average of 1.39 t/ha). Strain CB 1024 outyielded CB756 by >200% (-Fe) and 83% (+Fe).

Introduction

In 1969 a program was initiated by the Faculty of Agriculture at the University of Queensland to develop and release cultivars of pigeon pea, *Cajanus cajun,* that were high yielding, short statured, thy-length insensitive and adapted to the summer rainfall regions of the cropping belt of eastern Australia (12). Selection and evaluation of material in the program was done largely in moderate to high rainfall environments on neutral to acid soils, such as those found at the University's experimental station at Redland Bay (5, 12). When grown under these environmental and edaphic conditions, the plants produced high yields (11), were nodulated and appeared to be sufficient in N (R. Troedson, pers. comm.). Cultivars Royes, Hunt, Quantum and Quest were released from the program in 1979, 1983, 1985 and 1988, respectively.

Pigeon pea is an attractive crop for northern NSW because of the high value of the grain ($350$400/t) and because of its potential for N_2 fixation. Research at the NSW Agriculture Research Centre, Tam worth, during the past decade indicated that the cultivars released by the University of Queensland could yield around 2 t/ha on the alkaline, black-earth soils of moderate to high N fertility, but produced low yields, for example, <1 t/ha, when the soils were low in mineral N (2). In experiments on alkaline soils near Tamworth, low yields of inoculated pigeon pea were shown to be associated with insufficient supply of N. At two sites, grain yields were increased by 87 and 98% with the additions of 200 and 100 kg fertiliser-N/ha, respectively. In each case, plants were unnodulated and showed no evidence of N_2 fixation, irrespective of rhizobial inoculation or fertiliser-N treatment. However, at a third Tamworth site on an acidic red-earth soil (pH 5.5), yields were unaffected by fertiliser-N. In additional experiments on alkaline soils, where comparisons with other summer grain legumes could be made, pigeon pea was invariably inferior in nodulation and N_2 fixation activity (2). Farmer experience has been disappointing also with reports of poor nodulation and foliar symptoms of N deficiency (J.F. Holland, unpublished observations). Thus, it is unlikely that the current small area sown to pigeon pea (2,500 ha in 1988) will expand until farmers are confident that their crops will nodulate, fix N2 and yield reliably over a range of soil types and N fertilities.

In 1989, we screened almost 500 lines from the University of Queensland germplasm on an alkaline, black earth at Tamworth, New South Wales, for N_2 fixation, grain yield and maturity. In the following year, 100 of the most promising lines were further evaluated at two nearby sites. Results from the two cycles of selection and associated research suggested that there was little variation in N fixation in unamended soils (J.F. Holland and D.F. Herridge, unpublished data) and that insufficiency of iron (Fe) may be a factor restricting nodulation, N_2 fixation and grain yield on the alkaline soils. In an experiment at Breeza in 1989, 5 kg Fe/ha applied to the soil at sowing as FeEDDHA increased plant dry matter by 113% and grain yield by 315% (A.S. Hodgson, J.F. Holland and E.F. Rodgers, unpublished data). There were no responses to two rates of Fe applied as foliar sprays. Reports of M.J. Dilworth and colleagues at Murdoch University and the University of Western Australia had already shown that insufficient Fe specifically depressed nodule development and N fixation in peanut, *Arachis hypogaea (7) ,* and narrow-leafed lupin, *Lupinus* *angustifolius (10),* and that the success of foliar applied Fe in correcting the lack of nodulation was species specific.

We report an experiment to examine the effects of Fe, applied to the soil or as a foliar spray, and fertiliser-N on nodulation, N fixation and yields of dry matter and grain of pigeon pea. Two strains of rhizobia, *Bradyrhizobium* sp., were included as treatments: CB1024, the current inoculant strain and CB756, the strain used in pigeon pea inoculants for approximately 20 years until 1990.

Methods

Site details and sowing

The experiment was located on the NSW Agriculture Liverpool Plains Field Station, Breeza, NSW. The soil was a deep, black earth (Ug 5.15) (6), pH 8.2 (1:2.5, H_20) and was low in plant-available N following approximately 15 years of cereal cropping. Grain sorghum had been grown at the site in the previous summer. The land was then cultivated and lay fallow during the 1990 winter. Six weeks before sowing, beds were formed to allow for seed rows 0.9 m apart (two rows/bed) and furrow irrigation between beds and a pre-emergent herbicide (trifluralin) applied. Weeds were controlled thereafter by hand.

The experimental design was a randomized complete block with 13 treatments and four replicates. Plots were 1.8x10 m and contained 2 rows plus untreated buffer rows. Treatments were 0, 0.25, 1.0, 4.0 and 16.0 kg Fe/ha applied as FeEDDHA, with and without 200 kg N/ha, applied as nitram. The Fe was drilled 10 cm deep into the soil to the side of the seed row just prior to sowing. The nitram was drilled into the soil to a depth of 5 cm and 15 cm to the side of the seed row, 20 days after sowing. An eleventh treatment involved Fe applied as FeEDDHA at 0.25 kg Fe/ha in a foliar spray (0.71 g Fe/1 H_20) three weeks after sowing (without fertiliser-N). All 11 treatments were inoculated at sowing with the commercial inoculant for pigeon pea containing *Bradyrhizobium* sp. CB1024. Treatments 12 and 13 were the previous inoculant strain for pigeon pea, *Bradyrhizobium* sp. CB756, with 0 and 16 kg Fe/ha applied to the soil as FeEDDHA. The experiment was sown on 20 December 1990. Pigeon pea cv. Quest was used; seeding rate was 50 kg/ha to give an established population of approximately 30 plants/m2.

Sampling

On days 74, 96 and 118 after sowing, when the pigeon pea was commencing to flower, in early pod-fill and in late pod-fill, respectively, 2 m of row (1.8 m^2) were sampled for plant numbers, nodulation (days 74 and 96 only), shoot dry matter and N, and N_2 fixation activity. Fifteen-plant samples were dug at random from within the sampling area. Roots were detached from the shoots to be assessed for nodule number and nodule dry weight. Xylem sap was extracted immediately from 10 of the 15 shoots (see section below). The shoot clippings from the extraction procedure, together with the five unused shoots and all remaining shoots from the sampling area were collected, dried at 80?C to a constant weight and weighed. Samples were finely ground for Kjeldahl analysis of N. Grain yield was estimated from a sampled area of 3.6 m^2. Sampling was done by hand to allow dry matter and N contents of shoots to be estimated also at maturity. The number of pods/plant was assessed on 24 plant samples. Grain was dried at 80?C; yields were adjusted to 12% H_20. Seed weight was determined on 100 seed samples.

Nitrogen fixation

The ureide method (4) was used to assess N_2 fixation activity. On three occasions (74, 96 and 118 days after sowing), xylem sap was collected under mild vacuum (60-70 kPa) from the cut shoots of 10 plant samples (4,8). The sap samples were cooled immediately (on ice) and stored at -15?C within 3 h. Concentrations of ureides (allantoin and allantoic acid) in xylem saps were measured using the method of Young and Conway (14). Nitrate was measured by cadmium reduction (8). The a amino-N contents of saps were determined colorimetrically with ninhydrin (3,13). The relative abundance of ureide-N in xylem sap and the proportion of plant N derived from N_2 fixation, Pfix, were calculated as described in Peoples *et al. (8).*

Results

At flowering, nodulation of the pigeon pea was substantially improved with Fe applied to the soil and *as* a foliar spray (Table 1). Nodule weight was increased by as much as 300% in both the absence and presence of fertiliser N. Effects on nodule number were smaller (100 and 140% increases for -N and +N, respectively [data not shown)). Fertiliser-N depressed nodulation at all rates of soil-applied Fe. Nitrogen fixation activity at flowering was low in all treatments; there were no responses to either Fe or N. Plant densities were unaffected by treatment; average populations were 27 plants/m^2. Increases in shoot weights were small (up to 30%) but significant with increasing rates of Fe.

Table 1. Effects of iron, fertiliser N and strain of rhizobia on nodulation, N fixation and yields of dry matter and grain of pigeon pea, grown at Breeza, NSW, on an alkaline, black- earth soil.

Treatment	Flowering			Early pod-fill		Maturity	
	Nodule wt (mg)	*P*fix (%)	Shoot DM (t/ha)	Nodule wt (mg)	*P*fix (%)	Shoot DM (t/ha)	Grain yield (t/ha)
N0							
0 kg Fe/ha	7.0	15	1.28	81	50	4.25	1.47
.25 "	13.3	16	1.15	98	64	3.72	1.18
1.0 "	20.0	11	1.46	54	41	3.87	1.27
4.0 "	24.2	27	1.68	73	53	4.69	1.56
16.0 "	28.8	16	1.38	107	46	4.32	1.46
N200							
0 kg Fe/ha	4.7	17	1.33	13	17	5.06	1.67
.25 "	4.7	17	1.50	51	23	5.19	1.80
1.0 "	6.5	15	1.49	35	26	5.13	1.76
4.0 "	8.5	13	1.70	34	17	6.07	2.33
16.0 "	16.1	18	1.71	40	14	6.00	2.43
N0-Foliar Fe	21.5	16	0.87	95	52	3.31	1.13
N0							
CB756, -Fe	5.1	10	1.10	12	22	2.13	0.47
CB756, +Fe	4.0	12	1.51	30	38	2.97	0.80
F test	***	n.s.	***	***	***	***	***
l.s.d.(P=0.05)	11.8	-	0.36	26	21	0.65	0.30

n.s. not significant at P=0.05; *** P<0.001.
N0 - nil fertiliser N; N200 - 200 kg N/ha.

In the period between flowering (74 days after sowing) and early pod-fill (96 days after sowing), the effects of Fe on nodulation had almost disappeared. As with the first sampling, Fe did not affect Pfix. Fertiliser-N continued to depress nodulation and N_2 fixation activity. It became apparent at this sampling that the new inoculant strain for pigeon pea, CB1024, was more effective in nodulation and N_2 fixation than CB756, the previous strain. Nodulation by CB 1024 in the absence of Fe was six-fold greater than that of CB756; Pfix of CB 1024 was approximately double that of CB756.

At maturity, effects of Fe on yield of shoot dry matter were insignificant in the absence of fertiliser-N. Shoot yields were increased, however, by Fe when N was applied. The response to fertiliser-N, averaged over all rates of Fe, was 32%. Strain CB1024 produced 45 (+Fe) and 100% (-Fe) more shoot dry matter than CB756. Grain yields were similarly affected. In the absence of fertiliser-N, grain yields did not respond to Fe. In the presence of fertiliser-N, yields were increased with increasing rates of Fe. The highest yield of 2.43 t/haa (Fe at 16 kg/ha) was 46% greater than the yield at nil Fe. Strain CB 1024 outyielded CB756 by >200% in the absence of Fe and by 83% in the presence of Fe. Correlation matrices of grain yield, shoot dry matter at maturity, pods/plant, seed size and harvest index indicated that yield was positively correlated ($P<0.001$) with plant size (shoot dry matter), pod load (pods/plant) and harvest index.

Discussion

Our results confirmed earlier work (the late A. Hodgson and co-workers; 2) that yields of pigeon pea on alkaline, black-earth soils can be depressed by insufficient Fe and N supply. Our results showed also that nodulation and N_2 fixation by pigeon pea on these soils were insufficient to meet the N requirements of the crop, and that fertiliser-Fe increased yields substantially but only when the N requirements of the crop were optimized with fertiliser-N.The early responses in nodulation to Fe were not sustained beyond flowering. Thus, the lack of response of N_2 fixation activity to Fe, particularly after flowering, may have resulted from a lack of late nodulation because of low numbers of *Bradyrhizobium* sp. in the root infection zones. Future research should examine effects of Fe in soils that boast a well-distributed population of effective *Bradyrhizobium* sp. The clear superiority of CB1024 over CB756 vindicates the replacement of the latter strain *as* the commercial inoculant for pigeon pea.

Whilst the use of fertiliser-N on pigeon pea at rates of 100-200 kg/ha may be economic, supplying Fe to the crop as FeEDDHA is not. Alternative sources of available Fe have been suggested, for example, farmyard manure, organic mulches (1), but these would appear to be inappropriate for broadacre farming in Australia. A more worthwhile strategy may be to select and breed plant genotypes that are tolerant of low Fe supply. Cultivars and genotypes with improved tolerance of low Fe have been released for a number of the agricultural crops, including soybean, *Glycine max (9).* It is likely that similiar tolerance can be found in the germplasm for pigeon pea.

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