SELECTION AND BREEDING OF GRAIN LEGUMES IN AUSTRALIA FOR ENHANCED NODULATION AND N_2 FIXATION

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Abstract

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During the period 1980-87, the areas sown to grain legumes in Australia increased dramatically, from 0.25 Mha to 1.65 Mha. These increases occurred in the western and southern cereal belts, but not in the north in which N continued to be supplied by the mineralization of soil organic matter. Therefore, there was a need to promote the use of N_2-fixing legumes in the cereal-dominated northern cropping belt.

Certain problems had to be addressed before farmers would accept legumes and change established patterns of cropping. Here we describe our efforts to improve N_2 fixation by soybean, common bean and pigeon pea. Selection and breeding for enhanced N_2 fixation of soybean commenced at Tamworth in 1980 after surveys of commercial crops indicated that nodulation was sometimes inadequate, particularly on new land, and that the levels of fixed-N inputs were variable and often low. Similar programmes were established in 1985 (common bean) and 1988 (pigeon pea). Progress was made in increasing N_2 fixation by these legumes towards obtaining economic yields without fertilizer N and contributing organic N for the benefit of subsequent cereal crops.

1. INTRODUCTION

In Australia, the total area for agriculture is around 470 Mha, with pastures of native species on 90% (420 Mha) and improved grass and legume pastures on 6% (26 Mha). Just under 4% (16 Mha) is used for cropping, most of which occurs in Western Australia (32% of total) and in New South Wales (24%). The other states, in order of importance, are South Australia, Queensland and Victoria.

In all states, most of the cropped area is used for cereal production; legumes are of secondary importance. The cereal:legume ratios are low in WA and Vic. (around 6:1), intermediate in SA and Qld. (around 12:1) and highest in NSW (25:1). The ratio for the whole of Australia is 10:1. In the northern cereal belt of NSW, the area of particular interest to scientists involved in legume N_2- fixation projects, the ratio of cereal:legume is 33:1.

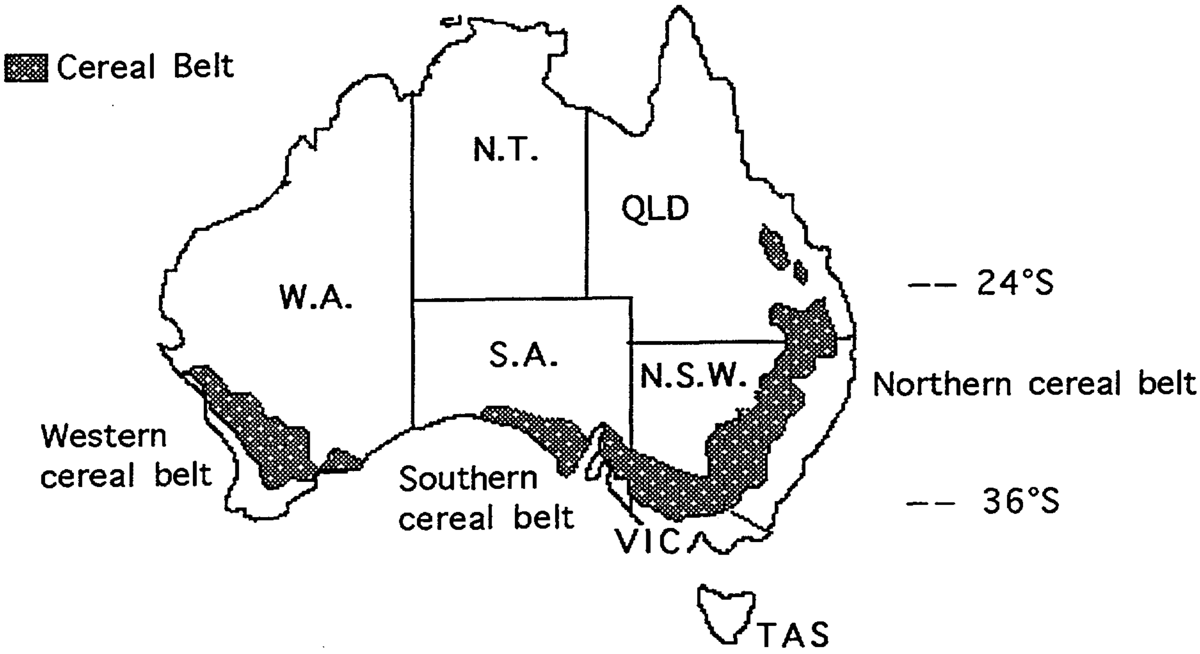

FIG 1. Map of Australia, showing the six states and two territories, and the three major divisions of the cropping belt.

Not included in the above calculations are areas sown to pasture legumes, which are widely used in mixed-farming systems in the western (WA) and southern (SA, Vic. and southern NSW) regions of the cropping belt. Their inclusion would widen the gap between those states and northern NSW and Qld.

2. CROPS GROWN

Cropping in Australia is dominated by wheat (Table I), which accounts for 63% of all cereal production and around 60% of total crop production. Almost all is grown as a rain-fed crop. Barley accounts for a further 20% of total crop production. Average yields for cereals are low (range 1.3 - 1.8 t/ha), except for irrigated rice. Yields of the rain-fed legumes are even lower, ranging from 1.1 to 1.3 t/ha. Soybean, for the most part, is irrigated. The principal legume crops are lupin and field pea, together accounting for almost 80% of legume production.

2.1. Role of legumes

Legumes, both pasture and crop, are important in the western and southern regions of the Australian cereal belt [1]. The pasture legumes have a dual role in these farming systems, sustaining animal production and supplying N to the soil for use by subsequent cereal crops. Such systems are only relatively recent, however. Prior to the early 1950s, plant-available N was conserved in the soil through bare fallowing (Fig. 2), a practice that depleted organic matter and damaged soil structure, and led to large-scale soil erosion. Average wheat yields during this time remained static at around 0.8 t/ha.

TABLE I. AREA AND PRODUCTION FIGURES FOR CEREAL AND PULSE LEGUME CROPS IN AUSTRALIA FOR 1992-93 (CROP REPORT PROJECT, ABARE, 1994)

Crop	Major producer	Total area (Mha)	Production (Mt)	Av. yield (t/ha)
Wheat	WA, NSW	9.10	16.2	1.78
Barley	SA, WA	2.96	5.40	1.82
Oats	NSW, WA	1.15	1.94	1.70
Sorghum	Qld., NSW	0.43	0.55	1.30
Rice	NSW	0.13	0.96	7.38
All cereals	WA, NSW	13.9	25.5	1.83
Lupin	WA	1.03	1.20	1.17
Field pea	SA. Vic.	0.38	0.46	1.21
Chickpea	Qld., NSW	0.15	0.17	1.13
Faba bean	SA, Vic.	0.08	0.10	1.25
Soybean	Qld., NSW	0.03	0.05	1.67
All legumes[a]	WA, SA, Vic.	1.83	2.12	1.16

[a]Includes mung bean, navy bean, cowpea, peanut and pigeon pea.

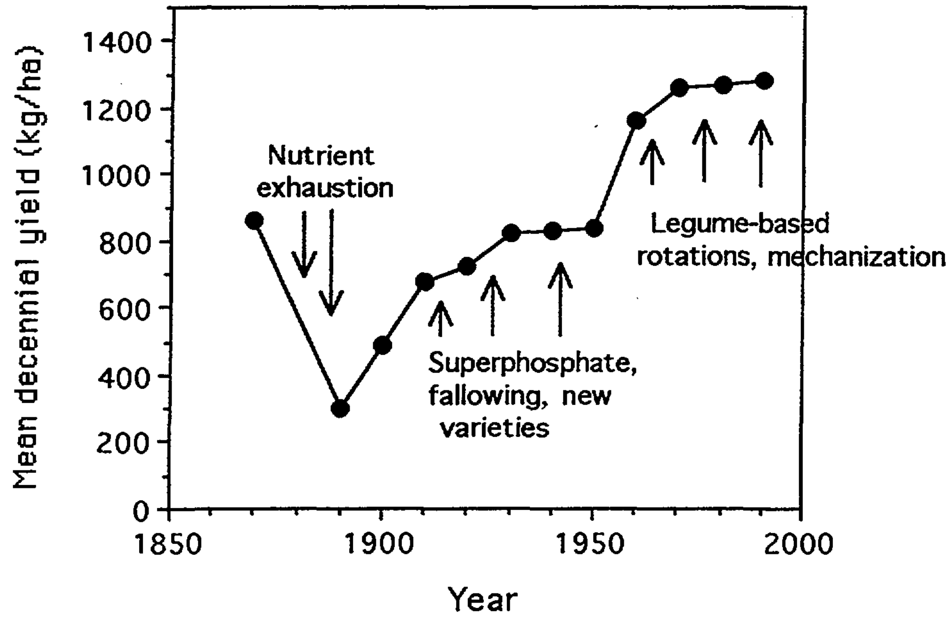

FIG. 2. Average wheat yields for Australia, 1860-1980 (unweighted means in 10-year intervals).

Following the introduction of legume-based pastures in the 1950s, yields increased and stabilized at around 1.3 t/ha, due almost entirely to N benefit from legume N_2-fixation. Net increments in soil N under the pasture commonly ranged from 35 to 100 kg/ha and reflected the productivity of the legume [1]. Soil structure also benefitted from the pasture phase, resulting in enhancements in water infiltration and plant-root penetration.

During the late 1970s (i.e. almost 30 years after the introduction of pasture leys), a number of factors combined to again change the basic cereal-production system, from the pasture ley-cereal to a more flexible combination of pasture ley-cereal and grain legume-cereal. These factors included concern for the decline in thrift of pasture legumes, soil problems (acidity and salinity), increasing cereal disease, particularly root and crown rots, and more favourable returns from cropping compared with livestock production. It is again noteworthy that these legume systems (both pasture and crop) were used only in the western and southern cereal belts. Legumes were not grown in the northern cereal belt, where N was supplied through the mineralization of native organic matter.

During the period 1980-87, the areas sown to grain legumes increased dramatically from 0.25 Mha to 1.65 Mha, an increase of 560%. The initial expansion was entirely because of increased sowing of lupin in WA. During the mid 1980s, the area sown to pea also increased, whereas between 1980 and 1990 cereals actually declined from 15.6 Mha to 13.5 Mha, after reaching a peak of 18.7 M ha in 1983. Thus, the ratio of cereal:legume decreased almost linearly from 60:1 in 1980 to 10:1 in 1990.

2.2. Economic value of legume N_2 fixation

The N_2 fixed by legume crops in Australia has an economic value, in terms of both the N itself and rotational benefits. The value of fixed N in a legume crop can be calculated using an average value for Pfix (the proportion of legume N derived from N_2 fixation) and the average yield data (Table II). The values used for Pfix, harvest index (HI) for total dry matter (DM), and %N of DM in the calculations were derived from the literature. The Pfix value of 80% reflects the low N status of most agricultural soils in the country, but may be excessive for some of the better-quality soils.

The annual value of N_2 fixed by grain legumes in Australia of US$94 million compares favourably with the estimated annual value of N_2 fixed by pasture legumes of US$1100 million [1]. The total (legume-based) pasture area is 26 Mha, therefore US$1100 million per 26 Mha is equivalent to US$43/ha, similar to the figure of $51/ha derived for grain legumes (Table II). Thus, legume N_2 fixation is a valuable process in Australian agriculture.

The economic value of legume-effects on subsequent cereal yields is not included in Table II, and would add considerably to the totals. An economic analysis of the benefits of chickpea to wheat production in the northern cereal belt, using yields from five rotation experiments, indicated an 85% increase in the annual gross margin, from US$120/ha to US$222/ha (Table III).

For a farmer in the northern region, replacement of every third crop with chickpea, e.g. growing 134 ha wheat and 66 ha chickpea rather than 200 ha wheat, would result in the overall farm gross margin increasing from US$24,000 to US$44,880. On the same basis, increased value of production for the region would be about US$250 million.

TABLE II. ECONOMIC VALUE OF N_2 FIXATION BY GRAIN LEGUMES

Item	Australia
Area sown (ha)	1.83×10^6
Production (t)	2.12×10^6
Average yield (t/ha)	1.16
Total DM yield[a] (t/ha)	3.87
Total N yield[b] (kg/ha)	97
Total N_2 fixed[c] (kg/ha)	77
Value[d] of N_2 fixation (US$/ha)	51
Total value (US$)	$94 x 10^6

[a] Assuming a harvest index of 0.3.
[b] Assuming 2.5% N for DM.
[c] Assuming an average Pfix of 80%.
[d] N valued at US$0.66/kg.

TABLE III. ECONOMIC ANALYSES OF THREE CROPPING SYSTEMS IN AUSTRALIA'S NORTHERN CEREAL BELT. YIELDS ARE AVERAGES OF FIVE EXPERIMENTS, CONDUCTED 1987-92 (W. FELTON, H. MARCELLOS, D. HERRIDGE, UNPUBLISHED)

Item	Wheat (W-W-W)		Chickpea (CP-W-W)		Fallow (F-W-W)	
	Yld (t/ha)	US$	Yld (t/ha)	US$	Yld (t/ha)	US$
Year 1	2.33	252	2.02	465	-	-
2	2.24	242	3.18	343	3.37	364
3	2.47	267	2.51	272	2.59	280
Total		761		1080		644
Less fixed costs[a]		77		77		77
Less variable costs[b]		325		338		217
Gross margin/3 yrs		359		665		350
Gross margin/yr		120		222		117

Wheat yields: 1.43 - 4.34 t/ha, chickpea yields: 1.29 - 2.90 t/ha. On-farm prices: US$110/t for wheat, US$230/t for chickpea.
[a] Calculated at US$26/ha/yr. [b] US$108/ha/yr for wheat; US$121/ha/yr for chickpea.

Although similar wheat yields to those following chickpea could be achieved in a wheat-only system with applications of 60-80 kg N/ha as fertilizer, the net financial return would fall short of the chickpea-wheat rotation, principally because of the high value of chickpea grain and the added cost of the fertilizer N. Furthermore, other rotational benefits of the chickpea phase such as disease and weed management and improved soil structure provide sustainable, long-term benefits.

3. ENHANCING LEGUME N_2 FIXATION THROUGH SELECTION AND BREEDING

We saw a need to promote the use of N_2-fixing legumes in the cereal-dominated agriculture of Australia's northern cropping belt. Summer and winter legumes could be grown, although some species were more adapted and had better market potential (e.g. soybean, mung bean, pigeon pea, chickpea, faba bean) than others. However, there existed certain problems that had to be addressed before farmers would accept the legumes and change established patterns of cropping.

We report three programmes that addressed the N_2-fixation problems of soybean, common bean (also known as navy bean) and pigeon pea. Selection and breeding for enhanced N_2 fixation of soybean commenced at Tamworth in 1980 after surveys of commercial crops indicated that nodulation was sometimes inadequate, particularly on new land, and that the levels of N_2 fixation were variable and often low. Similar programmes were established in 1985 with common bean and in 1988 with pigeon pea (Table IV).

TABLE IV. PROGRAMMES AND COLLABORATING PERSONNEL AT THE NSW AGRICULTURE RESEARCH CENTRE, TAMWORTH

Species	Definition of problem	Research activity	Collaborating personnel
Soybean *(Glycine max)*	• Poor nodulation on new land, low grain yields. • Low and variable N_2 fix'n in commercial crops.	***Selection*** of improved N_2-fixing genotypes; commenced 1980. ***Breeding*** for enhanced nodul'n and fixation; commenced 1986	Dr IA Rose Breeder NSW Ag.
Pigeon pea (*Cajanus cajan)*	• Poor nodulation and N_2 fixation, reduced grain yields on alkaline vertisols.	***Selection*** for high nodulating, high-fixing genotypes with improved grain yields in vertisols. ***Research*** on effects of applied Fe and N.	Mr JF Holland Agronomist NSW Ag.
Common bean (*Phaseolus vulgaris)*	• Commercial crops don't fix sufficient N for high yields. Require fertilizer N	***Selection*** from potentially useful genotypes on basis of nodul'n and N_2 fixation. ***Evaluation*** of rhizobia.	Dr RJ Redden Breeder, QDPI Mr J Brockwell, Dr MB Peoples CSIRO

3.1. Soybean

The rhizobia that nodulate soybean do not occur naturally in Australian soils. Consequently, seed must be inoculated at sowing. Nodulation failures, particularly in land sown for the first time to soybean, resulting in yellowing of foliage, poor crop growth and reduced grain yield, have been a feature of the industry since its inception, and have caused a great deal of concern. Experiments at the NSW Agriculture Field Station on the Liverpool Plains, Breeza, indicated that nodulation failures in first-year crops of soybean resulted from insufficient numbers of rhizobia in the seedling rhizosphere. Suggested strategies to overcome the problem were improved inoculants and inoculation procedures. On the other hand, selection and breeding for more-vigorously-nodulating cultivars would provide a more satisfactory, long-term solution to the problem.

Even when crop yield was not affected by poor nodulation, research indicated that less than 60% of the N required by the average crop was derived from N_2 fixation [2]. The remainder was taken up as nitrate from the soil and the net effect of soybean cropping was loss of soil N fertility. Thus, some of the advantage of growing a legume was lost. We concluded that development of cultivars of soybean capable of fixing large amounts of N_2 even at moderate to high levels of soil nitrate, would provide an economic bonus to the farmer and make soybean a far more valuable and attractive crop. The potential saving of soil and fertilizer N in Australia was 5,000 t/annum, valued at $3 million. Globally, the potential saving was 5 million tonnes of N, valued at $3 billion.

In response to these challenges, we commenced a programme in 1980 with objectives to improve the N_2-fixation capacity of soybean and, at the same time, solve the problem of poor nodulation and low yields of crops sown on 'new' land. The first step was to screen a large number of genotypes for nodulation and symbiotic tolerance of nitrate. Plants were assessed for growth, nodulation and N_2 fixation (relative ureide-N in xylem sap and plant parts; see *3.3.1.*). The first two cycles of screening involved growing plants in sand-filled pots in a glasshouse, supplied with either nitrate-free nutrients or nutrients containing 2.5 m*M* nitrate. A further two cycles were conducted in high-nitrate field soils.

There were large variations in responses to nitrate (Table V; [3]). From the original 489 genotypes, 66 'nitrate-tolerant' lines were identified on the basis of an overall index that combined the three ureide values and the nodulation index. The second screening was similar to the first in identifying variation, and confirmed the consistency of 32 of the original 66 'tolerant' lines. Genotypes of Korean origin displayed higher than average levels of nodulation and N_2 fixation in the presence of nitrate.

Of the original 19 Korean lines, 15 (80%) were included in the second screening, and nine (47%) were selected for the third round of (field) screening. Only 5% of the remaining 470 genotypes were selected as high-fixing after the two glasshouse screenings. It became apparent also that substantial differences in tolerance to nitrate occurred among the commercial cultivars, e.g. Davis and Lee had greater tolerance than Bragg.

In the third year, 40 genotypes were sown into a high-nitrate soil in the field [4]. Genotypes of Korean origin showed the highest levels of nodulation and N_2 fixation (Table VI). They had shoot yields similar to commercial cultivars Bragg and Davis, suggesting that increased N_2 fixation reduced their use of soil N. Post-harvest measurements of soil nitrate confirmed this; immediately after grain harvest, up to 34 kg/ha additional N was recovered from the Korean plots compared with the Bragg plots. Seed yields of the Korean lines were, on average, 30% less than that of Bragg, due to a combination of shattering, early maturity and poor agronomic type. Correlation matrices among the indices of nodulation and N_2 fixation and plant growth and grain yield revealed independence between the symbiotic- and yield-related characters. Therefore, the Korean lines appeared to be suitable for use as high-fixing donor parents in a breeding programme with selection for both grain yield and N_2 fixation.

TABLE V. MEAN VALUES FOR UREIDE INDICES OF N_2 FIXATION AND FOR NODULATION OF GENOTYPES OF SOYBEAN SCREENED FOR NITRATE TOLERANCE (2.5 m*M* NITRATE-N, SUPPLIED WITH NUTRIENTS)

	Relative ureide-N values			
	Xylem sap	Shoots	Roots	Nodulation index[a]
	(%)			(%)
First glasshouse screening				
High N_2-fixing lines (66)[b]	43	39	35	3.3
Low N_2-fixing lines (9)	10	5	3	1.2
Second glasshouse screening				
High N_2-fixing lines (32)	38	20	29	3.7
Low N_2-fixing lines (2)	15	4	10	1.8
Bragg	16	6	8	1.8

[a] (nodule mass/shoot mass) x 100. [b] A total of 489 genotypes in the first screening and 87 in the second. Data show selected groups of high- and low-fixing lines [3].

TABLE VI. ASSESSMENTS OF NODULATION, N_2 FIXATION AND YIELD OF SELECTED 'NITRATE TOLERANT' KOREAN GENOTYPES OF SOYBEAN AND COMMERCIAL CULTIVARS IN A HIGH NITRATE SOIL AT BREEZA, NSW, 1985 [4, 5]

Genotype	Nodulation		Pfix	Shoot DM	Grain yield
	Wt. (mg/plant)	No/plant	(%)[a]	(g/plant)	(t/ha)
Nitrate tolerant					
Korean 466	376	34.5	31	45.9	1.6
Korean 468	254	16.8	18	43.3	1.7
Korean 469	176	19.5	22	41.6	1.4
Korean 464	319	16.5	11	48.1	1.5
Commercial					
Bragg	24	2.0	0	39.7	2.2
Davis	40	1.3	0	48.5	2.2

[a] Proportion of N obtained from N_2 fixation, assessed during mid pod-fill using the xylem ureide technique [6].

Subsequent comparisons of Korean genotypes 466 and 468 with commercial cultivars, Bragg and Davis, and mutants of Bragg, nts1007 and nts1116 [7], at five field sites showed that the Korean genotypes nodulated better than did Bragg, Davis and nts1116, and were about equal to nts1007 [8]. Values for Pfix, estimated using xylem ureide and natural ^{15}N abundance methods, were similar for the two Korean genotypes, nts1007 and Davis. Bragg had the lowest values for Pfix, with nts1116 intermediate between Bragg and the other four. These high levels of symbiotic activity of the Korean genotypes were in spite of low seed yields and early maturity.

Results from the four years of screening indicated that differences in nodulation between the Korean genotypes and commercial cultivars occurred only when symbiosis was stressed, i.e. with moderate to high nitrate supply in glasshouse sand-culture or in the field, or with low numbers of soybean rhizobia in the field. In the absence of stress, nodulation of the two groups was similar. Thus, enhanced nodulation of the Korean genotypes was not mediated through a loss of the autoregulatory processes that limit nodulation, as with nts mutants [9, 10], or through an altered ability to assimilate and metabolize nitrate [3], but likely resulted from more-efficient rhizobial infection and/or nodule initiation.

The four Korean genotypes, 464, 466, 468 and 469 were used as high-fixing parents in crosses with commercial cultivars, Valder (maturity group [MG] IV), Reynolds (MG VI), Forrest (MG VI) and Bossier (MG VIII) [11]. The breeding protocol differed in a number of ways from that used in a successful programme with common bean (F. Bliss and co-workers, University of Wisconsin, USA): material was screened for the most part in high-nitrate rather than low-nitrate soils; the xylem-ureide method was used to assess N_2 fixation; initial assessments of N_2 fixation were with individual F_2 plants, although later assessments (F_6 and F_7) involved populations of plants. A summary of activities is presented in Table VII.

Nitrogen fixation was assessed on individual F_2 plants using the xylem-ureide method. Sap was extracted from the top half of each plant leaving the lower half to continue growth and to produce seed for harvest [11, 12]. The relative abundance of ureide-N of the F_2 plants varied between 2 and 55%, indicating segregation for N_2-fixation activity (Fig. 3). There was no evidence of heterosis, in contrast to results reported before for alfalfa [13], pea [14] and soybean [15]. Average relative ureide-N values for the 11 F_2 populations were surprisingly constant (24 - 29%, equivalent to Pfix values of 13 - 20%) and were between the lower values of the commercial parents (17 - 28%; Pfix values 2 - 19%) and the higher values of three of the four Korean parents (39 - 42%; Pfix values 36 - 40%). The relative ureide-N value for the fourth Korean parent, 464, was 27%, about the same as for Reynolds, the best commercial parent. Although average N_2 fixation activities of the F_2 populations were below the best Korean lines, 35 individual F_2 plants had equally high N_2 fixation, i.e. relative ureide-N >40%.

The F_2 populations were culled on the basis of N_2 fixation (xylem relative ureide-N value >31%) (Fig. 3), plant type (agronomic rating >2 on a scale of 1 to 6) and seed colour (yellow, green or yellow-green). Evidence of linkages between N_2 fixation and other more easily determined plant characters was also sought. Correlation matrices of these characters showed no such linkages. Problems could have occurred if N_2 fixation was found to be linked to certain traits of the Korean genotypes, e.g. black, brown seeds, poor agronomic type. On the other hand, linkage to other more benign traits could have led to simpler procedures for selecting material.

At the commencement of this study, the genetic control of enhanced N_2 fixation in the Korean genotypes was unknown. Major genes had been identified that influence *Bradyrhizobium* compatibility [16, 17, 18] and hypernodulation [19] of soybean. Frequency distributions of relative ureide-N values in each of the 11 F_2 populations were normal, with no evidence of discontinuities to suggest a major gene segregation.

TABLE VII. PEDIGREES OF 11 POPULATIONS OF SOYBEAN AND NUMBERS OF SINGLE PLANTS OR LINES ASSESSED AT EACH GENERATION FOR EITHER PLANT AND SEED TRAITS (F_2-F_7 GENERATIONS), YIELD (F_4-F_7 GENERATIONS) OR N_2 FIXATION (F_2, F_6 AND F_7 GENERATIONS)

Population/ pedigree	F_2 single plants 1986-87	F_2-derived in F_3 gener'n 1987	F_3-derived in F_4 gener'n 1987-88	F_3-derived in F_5 gener'n 1988-89	F_3-der'd in F_6, F_7 1989-91
	(number)				
A. 464 x Valder	161	22	144	36	3
B. Valder x 464	61	6	45	5	0
C. Valder x 466	87	16	89	26	1
D. Valder x 468	49	8	58	14	1
E. Reynolds x 466	72	9	73	25	8
F. Reynolds x 464	14	1	11	1	0
G. 464 x Forrest	38	5	49	10	3
H. Forrest x 469	119	14	112	37	6
J. Bossier x 464	121	6	46	10	0
K. 468 x Bossier	93	12	109	29	10
L. Bossier x 469	34	5	47	7	1
Total	849	104	783	200	33

Culling of F_3-derived lines in the F_4 and F_5 generations was based on yield and agronomic traits. Nitrogen fixation was again assessed in the F_6 and F_7 generations. A number of the F_3-derived lines were clearly superior and, importantly, stable in N_2 fixation (Fig. 4). In both the F_6 and F_7 generations, these lines had relative ureide-N values of around 40%, compared with consistently lower or more variable values for other lines. A number of the consistently high-fixing lines (A82-3, D22-8, K78-1 and E68-5) were subsequently used as parents in a backcrossing programme (I.A. Rose and D.F. Herridge, unpublished data).

The values for yield and N_2 fixation presented in Table VIII summarize the progress made in the first cycle of selection. At the high-nitrate sites in the F_6 and F_7 trials, Forrest was obtaining 27 and 33% of its N from N_2 fixation at the time of sampling. By contrast, N_2 fixation by Korean 468 accounted for 47 and 52% of current inputs of N. Lines D22-8, A82-3, A46-4, and E72-3 were outstanding in terms of N_2 fixation, with Pfix values of around 50%. The Pfix value for commercial cultivar Reynolds was around 41%, suggesting that it already had the desired symbiotic characteristics, i.e. tolerance of the suppressive effects of soil nitrate on nodulation and N_2 fixation.

Grain yields of the F_3-derived lines in the F_6 and F_7 generations, although substantially larger than yields of Korean 468, could not match those of the highest-yielding commercial cultivar, Forrest. Some lines gave yields comparable to older commercial cultivars such as Bossier. In particular, lines A46-4, K78-1 and D22-8 had average yields across the six trials of >2.0 t/ha.

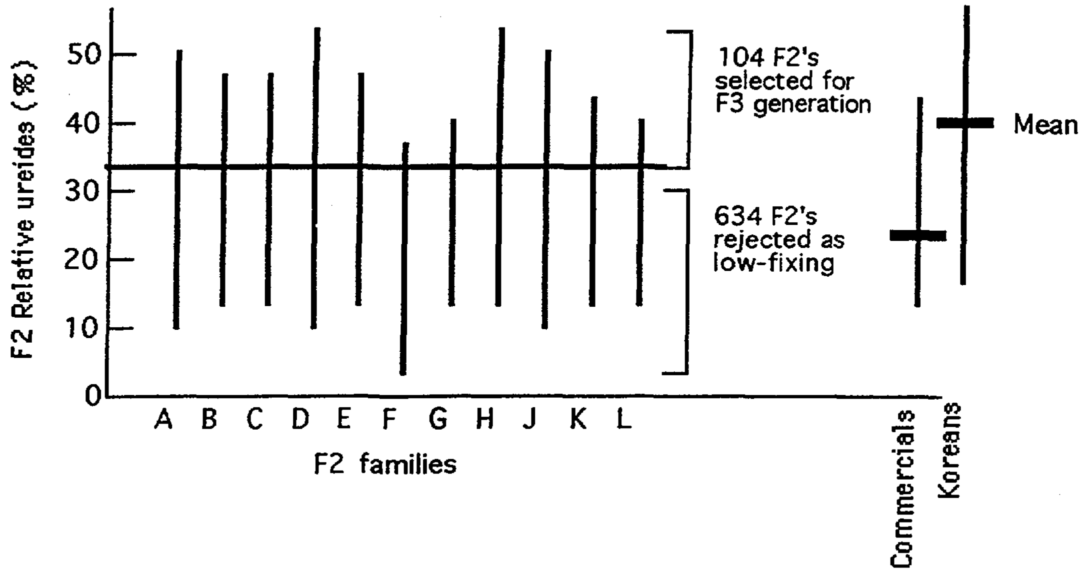

FIG. 3. Ranges of N_2 fixation (relative ureide-N of xylem sap) for the eleven F_2 families and for the commercial and Korean parents. The horizontal line through the families indicates the cut-off point for selection for F_3 generation.

We concluded that the field site chosen for assessing N_2 fixation was vital for discriminating the high-fixing, nitrate-tolerant lines. The enhanced capacity for N_2 fixation of these lines and of the Korean parents was expressed only when N_2 fixation of the commercial cultivars, e.g. Forrest, was suppressed by high-nitrate soil. Data from the F_6 and F_7 trials support this by showing that correlations across sites and/or seasons were improved when the soils were high in nitrate.

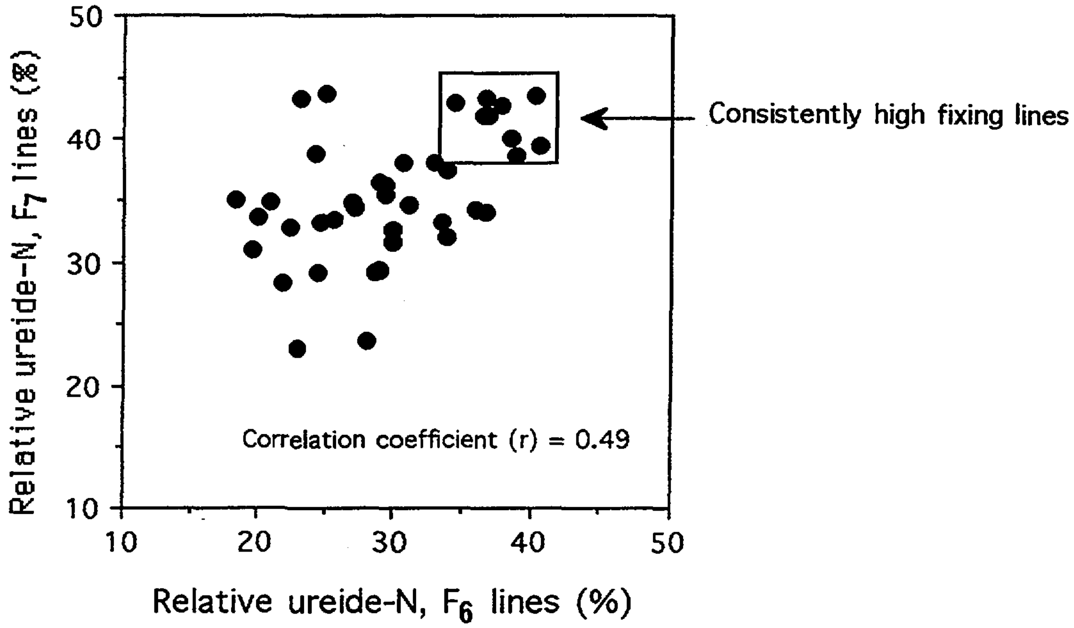

FIG. 4. Relative abundance of ureide-N for F_3-derived F_6 and F_7 lines of soybean, grown in successive years on high nitrate soils at Breeza and Narrabri, Australia.

Yields of the high-fixing, nitrate-tolerant material need to be improved by about 20% before commercial release. A second cycle of selection was commenced in 1991 with crossing of lines D22-8, A82-3, A46-4 and K78-1 with high-yielding genotypes. Single-seed descent lines were formed as F_4 single plant progeny and more than 1400 lines from six populations were field-tested in F_5 and F_6 trials for phenology, growth habit, lodging, disease resistance, yield, shattering and seed oil and protein. The best lines from those assessments were then evaluated for N_2 fixation in the F_7 generation during the 1994-95 summer season.

TABLE VIII. DAYS TO FLOWERING, SEED YIELD AND N_2 FIXATION AT F_6 AND F_7 FOR LINES SELECTED FOR BACKCROSSING AND FOR HIGH (KOREAN 468) AND LOW N_2-FIXING (FORREST) PARENTS

Line	Original cross	Flowering	Seed yield mean 3 sites		Pfix high nitrate sites	
			F_6	F_7	F_6	F_7
		(days)	(t/ha)		(%)	
Forrest		50	2.67	2.66	27	33
D22-8	Valder x Korean 468	46	2.08	2.29	47	55
A82-3	Korean 464 x Valder	52	2.00	1.70	49	49
K78-1	Korean 468 x Bossier	57	1.91	2.09	46	54
A46-4	Korean 464 x Valder	58	2.24	2.20	51	56
Korean 468		43	0.95	0.58	47	52

3.2. Pigeon pea

Pigeon pea has potential as a summer-crop alternative to winter legumes in Australia's northern wheat belt. There are problems, however. Research during the 1980s showed that pigeon pea nodulates poorly, fixes little N_2 and produces low yields when grown on the alkaline, black-earth soils that are common to the area. Table IX shows a typical set of results from Duri, near Tamworth - no effect of inoculation with rhizobia, virtually no N_2 fixation activity and a doubling of grain yield with applied fertilizer N. Such poor performance of unfertilized pigeon pea on these soils represents a barrier to adoption by farmers, even though the selling price of the grain is high ($350 - $400 per tonne) compared with other crops.

We believed that genotypes of pigeon pea better adapted to nodulating and growing on alkaline soils might be present in the Australian pigeon-pea germplasm collection at the University of Queensland. In 1988, we assembled some 500 genotypes from the collection and commenced screening for N_2 fixation, grain yield and maturity. Our aim was to identify genetic variation in nodulation and yield capacity on the alkaline, black earths, and to select superior genotype(s) for commercial release or for use as parent(s) in a breeding program.

In the first season (1988-89), the genotypes were grown on a black earth at Tamworth. In the following year (1989-90), 105 of the most promising lines were further evaluated at two nearby sites. The check cultivars, Quest, Quantum and Campea yielded consistently (range 1.42 - 1.49 t/ha), but showed no evidence of N_2 fixation in five of the six combinations of cultivar x site (Table X). Eleven of the 105 test genotypes showed potential for either N_2 fixation (Pfix range 10 to 19%, after ignoring a low value for E137 at Currabubula) or yield (range 1.47 to 1.65 t/ha). The relationship between Pfix and yield was not significant ($P = 0.05$) and none of the 11 genotypes gave high values for both characters. Nitrogen fixation activity could not be detected for 86 of the 100 genotypes at the TARC site, nor for 51 of the 62 genotypes at Currabubula.

At that time, it was found that low iron (Fe) availability depressed yields of pigeon pea on an alkaline black earth on the Liverpool Plains (Breeza) of northern New South Wales [20]. Seed yields were increased by more than 400% with additions of 20 kg Fe/ha to the soil as the iron chelate, FeEDDHA. Plant nodulation and N_2 fixation were not assessed in that study. It has also be shown that Fe deficiency specifically limits nodule initiation and development in peanut [21] and lupin [22], and that strains of rhizobia producing siderophores could form functioning nodules under conditions of low Fe availability. Similarly, plant genotypes with improved resistance to iron chlorosis had been identified for a range of agricultural species, including legumes [23]. Together, these results suggested that variation in tolerance to alkalinity/low Fe may exist for genotypes of pigeon pea [23] and of rhizobial strains [21], and that symbiotic combinations should be sought to replace the currently-recommended cultivar (cvv. Quest, Quantum)-*Bradyrhizobium* strain CB756 combinations. Although previous results [24] indicated that depressed symbiotic activity and yield of pigeon pea were associated with the host plant and not with CB756, there was no evidence that the latter was a siderophore-producer or was adapted to alkaline soils.

TABLE IX. EFFECTS OF INOCULATION AND FERTILIZER N (100 kg N/ha AS UREA) ON YIELD AND NITROGEN FIXATION OF PIGEON PEA GROWN ON AN ALKALINE, BLACK EARTH SOIL AT DURI (1987)

Cultivar/Treatment	Grain yield		Pfix	
	-Fertilizer N	+Fertilizer N	-Fertilizer N	+Fertilizer N
	(t/ha)		(%)	
Quantum				
Uninoculated	0.72	1.32	0	0
Inoculated	0.74	1.37	0	3

In the following season (1990-91), we evaluated effects of Fe, fertilizer N and strain of rhizobia on nodulation, N_2 fixation and yield. Results were encouraging (Table XI). We increased grain yields from a low of 0.5 t/ha (old strain of rhizobia, no fertilizer) to 2.4 t/ha with a new strain of rhizobia and Fe and N fertilizers. Curiously, the highest yields were obtained only when fertilizer Fe and N were applied together. In the absence of fertilizer N, when the pigeon pea was more dependent on N_2 fixation, yields

TABLE X. YIELDS AND N_2 FIXATION OF SELECTED GENOTYPES OF PIGEON PEA IN 1989-90. A TOTAL OF 108 GENOTYPES, INCLUDING THREE CHECKS, WERE SOWN INTO ALKALINE BLACK EARTHS AT TWO SITES NEAR TAMWORTH. EACH VALUE IS THE MEAN OF EITHER TWO (TARC) OR THREE (CURRABUBULA) REPLICATES

Genotype TARC [a]	Genotype UQ [b]	Days to flowering	Seed yield TARC (t/ha)	Pfix TARC (%)	Pfix Currabubula (%)
Lines with N_2-fixation potential					
E25	4	71	1.30	10	11
E32	994	72	1.31	19	-[c]
E42	622	70	-	-	12
E93	1039	70	1.07	14	-
E137	1017	69	0.97	14	0
M63	356	71	1.08	11	-
Lines with yield potential					
E51	990	69	1.55	0	-
E111	116	74	1.54	0	-
M61	885	74	1.47	0	1
M77	748	72	1.65	0	-
M144	T30	71	1.50	0	6
Check lines					
Quest		72	1.44	0	7
Quantum		72	1.42	0	0
Campea		79	1.49	0	0

[a] Tamworth Agricultural Research Centre. [b] University of Queensland. [c] Not determined.

flattened out at 1.5 t/ha. With N applied but no Fe, yields were only marginally better at 1.7 t/ha. These results confirmed the importance of Fe to nodulation, N_2 fixation, and grain yield, but challenged us to bring the yields up to 2.4 t/ha without relying on fertilizer N.

To answer that challenge, we continued to screen for genetic variation in capacity of pigeon pea to nodulate, fix N_2 and yield on these high-pH soils. Thus, between 1991 and 1994, we evaluated 22 of the most promising genotypes from the original screenings at a number of sites in northern NSW.

In the summer of 1990-91, 14 genotypes were evaluated at Breeza in the presence and absence of Fe. The soil is a deep, black earth, pH 8.2, low in plant-available N following 15 years of cereals. In the unamended soil (-Fe), we found significant differences among genotypes in nodulation, N_2 fixation and yields of dry matter and N of shoots and grain. During during vegetative growth, Fe increased nodulation, N_2 fixation and dry matter of shoots but reduced shoot %N. Differences in the responsiveness of the genotypes to applied Fe were significant also. Four genotypes (E76, M48, M144 and Quest) showed a low level of response to Fe, whereas others (E33, M96, L133 and Campea) were highly responsive (Table XII). In the unfertilized plots, nodulation, N_2 fixation and shoot dry matter were generally higher for the non-responsive genotypes than for the responsive group. By late pod-fill, Fe increased shoot dry matter by an average of 28% for the non-responsive group and by 50% for the responsive group. Iron increased grain yields by an average of 0.32 t/ha (25%) (non-responsive group) and 0.70 t/ha (108%) (responsive

TABLE XI. EFFECTS OF STRAIN OF RHIZOBIA AND FERTILIZER FE AND N ON SYMBIOTIC ACTIVITY (NODULATION AND N_2 FIXATION) AND GRAIN YIELD OF PIGEON PEA, GROWN ON THE ALKALINE, BLACK EARTH SOILS OF NORTHERN NSW

Treatment	Nodulation (mg/plant)	Pfix (%)	Grain yield (t/ha)
Rhizobia			
Old strain (CB756)	12	22	0.47
New strain (CB1024)	81 (+575)[a]	50 (+127)	1.47 (+212)
Fertilizer			
None	81	50	1.47
+Fe	107 (+32)	46 (-8)	1.46 (0)
+N	13 (-84)	17 (-66)	1.67 (+14)
+Fe +N	40 (-50)	14 (-72)	2.43 (+65)

group). Genotype E76 was particularly promising, out-yielding the commercial check, Quest, by 0.20 t/ha (17%) in the absence of Fe and by 0.39 t/ha (26%) when Fe was applied. Campea nodulated poorly and produced the lowest yield of all 14 genotypes (0.51 t/ha). It was also the most responsive genotype to Fe (170% increase). Effects of Fe and genotype on %N of shoot dry matter and grain were less than during early growth (data not shown).

In the following season, 18 genotypes were grown under irrigation at Breeza. The experiment was sampled three times for nodulation, N_2 fixation (xylem-sap ureides) and yield parameters: at flowering/early pod-fill, late pod-fill and maturity.

Fertilizer Fe and N increased yields of shoot dry matter and grain (Table XIII). Although responses were negligible at flowering, by late pod-fill the fertilized plants were, on average, 23% larger than the unfertilized plants. At maturity, the fertilized plants contained 35% more dry matter and produced 27% more grain. We recorded an opposite effect of the fertilizers on symbiotic characters. The applied Fe and N depressed nodule weight and number by 85-90% and the N_2 fixation index (xylem sap relative ureide-N) by about 30%. It is probable that both Fe and N contributed to the growth and yield increases and that the N alone depressed nodulation and N_2 fixation.

A number of the genotypes performed reasonably well, although none was outstanding. The data in Table XIV are from the unfertilized (-Fe -N) plots; 11 of the 18 genotypes are included. Results were generally consistent with the two genotype-evaluation trials of the previous (1990-91) season.

In 1991-92 the highest-yielding group of genotypes, Quest, E76, M48 and M144 were all high yielders in the previous season, and showed low responsiveness to fertilizer Fe (Table XII). Genotypes E31 and E42 were intermediate in yield and responsiveness in that trial. The low-yielding genotypes, Campea and E33 were similarly identified in 1990-91 in the absence of fertilizer Fe and highly responsive to Fe.

TABLE XII. EFFECTS OF FE ON NODULATION, N_2 FIXATION AND GRAIN YIELD OF GENOTYPES OF PIGEON PEA, GROWN AT BREEZA, NSW, ON AN ALKALINE, BLACK EARTH (1990-91)

Genotype[a]	Nodulation		Pfix				Grain yield	
			Flowering		Late pod-fill			
	-Fe	+Fe[b]	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
	(mg/plant)		(%)				(t/ha)	
Low response to Fe								
E76	14.2	30.2	25	24	55	48	1.40	1.89
E88	4.2	22.6	17	19	42	38	1.25	1.29
M48	16.3	28.6	25	30	55	40	1.27	1.50
M144	10.6	30.8	24	21	58	68	1.18	1.42
Quest	19.0	31.0	17	20	63	50	1.20	1.50
Highly Responsive to Fe								
E33	3.2	17.3	12	16	28	36	0.90	1.41
M96	1.7	27.2	13	18	35	50	0.68	1.18
M132	3.4	39.8	16	19	58	60	1.02	1.68
L133	1.8	28.3	13	22	38	44	0.70	1.61
Campea	1.2	43.3	16	21	49	70	0.51	1.38

[a] Fourteen genotypes were in this experiment; E31, E42, E51 and E61 showed intermediate responsiveness to Fe.

[b] Fe applied at 16 kg/ha at sowing as FeEDDHA.

Only one genotype performed inconsistently: M132 was in the highest-yielding group in 1991-92, but was classified as low yielding and responsive to applied Fe in 1990-91. Nodulation and relative ureide-N values were inconsistent within the two groups of genotypes (Table XIV). The largest effects were due to fertilizer (N) (data not shown).

A summary of the yield data from the trials to evaluate potentially adapted genotypes is presented as Table XV. In 1990-91, 22 genotypes were sown at Narrabri, a number of which yielded as well as the standard cultivar Quest. Campea again yielded poorly. Grain %N values were low (range 2.77 - 3.45, equivalent to about 20% protein) and there was a tendency for the low yielders to have higher grain %N values.

In 1991-92, eighteen genotypes were included in the Premer and Moree trials. Yields were higher at Premer (range 1.37-2.38 t/ha) than at Moree (0.28-0.73 t/ha) (Table XV). All of the genotypes flowered within a few days of Quest. Genotypes E42 and E51 had the earliest anthesis of 72 days (mean of the two sites) compared with 74 days for Quest and 78 days for Quantum. Genotype E76 was the highest-yielding line overall.

TABLE XIII. EFFECTS OF FERTILIZER FE (4 kg/ha) AND N (200 kg/ha) AS UREA) APPLIED AT SOWING ON YIELD AND SYMBIOTIC COMPONENTS OF PIGEON PEA, GROWN ON AN ALKALINE VERTISOL AT BREEZA, NSW (1991-92)

Component	-Fe -N	+Fe+N	Response to +Fe+N
Yields (t/ha)			
Shoot DM - flowering	2.11[a]	2.13	+1%
- pod-fill	3.11	3.82	+23%
- final harvest	2.42	3.28	+35%
Grain yield	0.77	0.98	+27%
Symbiosis			
Nodule DM (mg/plant)	88	9	-90%
Nodule no./plant	19	3	-85%
Rel. ureides - flower (%)	46	34	-26%
- pod-fill (%)	35	24	-32%

[a] Values presented are the means of the 18 genotypes.

TABLE XIV. YIELD AND SYMBIOTIC TRAITS OF SELECTED GENOTYPES, GROWN WITHOUT FERTILIZER IN AN ALKALINE, BLACK EARTH AT BREEZA, NSW (1991-92)

Genotype[a]	Plant pop. (no/m^2)	Shoot dry wt. late pod-fill (t/ha)	Grain yield (t/ha)	Nodulation No.	Nodulation Wt. (mg)	Rel. ureides S1[b] (%)	Rel. ureides S2[c] (%)
Highest yielding							
Quest	16	3.22	0.93	22	137	45	41
E31	14	3.50	0.98	34	135	48	34
E42	23	3.23	0.85	16	60	47	38
E76	10	3.17	0.86	14	82	46	32
M48	19	3.38	0.87	39	132	54	38
M132	13	2.98	0.84	14	63	42	32
M144	20	3.38	0.94	15	90	50	33
Lowest yielding							
Campea	18	3.70	0.69	36	184	55	33
E28	16	2.75	0.70	18	79	27	34
E33	11	2.74	0.71	16	63	41	31
E61	16	3.20	0.55	17	80	57	36

[a] Data are not presented for genotypes Quantum, E6, E51, M58, M117, M136 and L133.

[b] Flowering/early pod-fill. [c] Late pod-fill.

TABLE XV. GRAIN YIELDS OF SELECTED PIGEON-PEA GENOTYPES, GROWN WITHOUT FERTILIZER ON ALKALINE BLACK EARTHS AT SITES IN NORTHERN NSW IN THE 1990-91 TO 1993-94 SEASONS

	1990-91	1991-92		1992-93	1993-94
Genotype[a]	Narrabri	Premer	Moree	Breeza	Spring Ridge
			(t/ha)		
High-yielding					
Quest	1.55	2.30	0.69	0.22	1.84
Quantum	1.56	*[b]	*	*	1.90
E31	1.61	2.26	0.73	0.21	2.03
E76	1.59	2.38	0.68	0.33	2.19
M48	1.50	2.16	0.61	*	*
M132	1.55	2.20	0.64	0.37	2.28
M144	1.69	*	*	*	2.20
ICPL85014	*	*	*	*	2.18
Low-yielding					
Campea	1.10	1.37	0.28	*	*

[a] Genotypes E28, E33, E51, E54, E61, M58, M114 and L133 were intermediate between the two listed groups.

[b] Designates that the genotype was either not sown or was not high-yielding in that trial.

In 1993-93 seventeen genotypes were grown without irrigation at Breeza. The trial was sown with little sub-soil moisture. Total rainfall was below average for the season, consequently yields were low, ranging from 0.19 to 0.37 t/ha. Genotype E76 was again in the high-yielding group. Quest produced only 0.2 t/ha grain, about 50% below that of the high-yielding genotypes. Relative ureide-N values, indicative of N_2 fixation, were low for all genotypes.

During the 1993-94 season, nineteen genotypes, including two early-maturing lines from ICRISAT, were grown without irrigation at Spring Ridge Yields ranged between 1.7 and 2.3 t/ha, generally higher than in previous years (Table XV). Genotypes M132 and M144 were the highest yielders, superior to the standard cv. Quest by about 0.4 t/ha. Genotype E42 was again the earliest to flower, at 4 days before Quest, 6 days before Quantum and simultaneously with an early-maturing accession from ICRISAT, India, ICPL85014. This genotype was acquired at TARC as two selections with the same maturity, but slightly different seed colour and a yield difference of 0.5 t/ha.

Our original objective in this project was to identify genetic variation in pigeon pea in capacity to nodulate and yield on the alkaline, black earth soils, and to select superior genotype(s) for commercial release or for use as parent(s) in a breeding program. Our strategy was to assemble a large collection of genotypes and to evaluate them on the black soils for maturity, grain yield, and nodulation and N_2 fixation (symbiotic activity). During the course of this project, we were able to increase yields and, to some degree, symbiotic activity, through applications of fertilizer Fe and N, through the use of a new inoculant strain of rhizobia, and through plant-genotype selection.

Results from the four seasons of experiments with pigeon pea indicate that the problem of poor nodulation, little N_2 fixation and low grain yields on the alkaline, black earths is complex. In one experiment, we succeeded in raising yields from 0.5 to 2.4 t/ha through: rhizobial strain, fertilizer Fe and fertilizer N. In other trials, genotypes such as E76 outyielded Quest, the current commercial cultivar, by as much as 20% in the absence of fertilizer Fe and N applications and by an average of 6% over all trials. It was not clear, however, that these increases were large enough to be used in isolation as the solution to the problems of growing pigeon pea on the alkaline, black earths. In the experiments in which fertilizer Fe and N were applied as treatments, yields of Quest were increased by 65% when both Fe and N were applied. Thus, even higher yields than those recorded for E76 may be necessary before pigeon pea can be considered an economic alternative to soybean and mung bean on the alkaline, black earths.

Yields were, at best, adequate on the lighter and better-drained (Spring Ridge and Premer) soils and generally low on the heavier, poorly-drained, Breeza-type soils, suggesting that pigeon pea is better adapted to the lighter black-earth soils of the region rather than to the heavier soils of the plains. It may also have a place on the more acid, red-earth soils of the slopes, on which Fe would not be deficient and its ability to withstand dry conditions would hold it in good stead. Work at ICRISAT revealed low yields of pigeon pea on heavy, black earth (vertisol) soil types [25]; yields on the black earth were about half of those on a red earth (alfisol), and excess soil moisture was believed to contribute to the depressed yields. It is likely that waterlogging played a role also in our experiments.

In conclusion, although we have made progress in better understanding the critical role of Fe and in unravelling other problems linked to low N_2 fixation and poor yields on our alkaline soils, we believe that a major breakthrough in pigeon-pea genotype improvement (i.e. 50% increases in N_2 fixation and yield) may be difficult and will certainly require a larger research programme.

3.3. Common Bean

The many cultivated types of common bean (dry bean, kidney bean, navy bean, culinary bean, etc.) are inefficient N_2 fixers. This has been attributed to insufficient nodulation and to the plant's inherent inability to fix N_2, and, as a result, farmers must grow them either in highly fertile soils or apply fertilizer N to obtain economic yields. Therefore, improvement in N_2 fixation would offset or wholly replace the need for fertilizer N, thereby reducing costs of production. In Australia, the culinary and navy bean industries are currently worth about $8-10 million annually, involving 400-500 growers producing 12,000-15,000 tonnes from about 15,000 ha . Eliminating the need to apply fertilizer N to crops of navy and culinary bean would save growers $1-1.5 million annually.

The first stage of the Queensland Department of Primary Industries programme to introduce high N_2 fixing cultivars to the industry involved screening 1462 genotypes/cultivars of common bean for capacity to nodulate under a range of field conditions, using indigenous and introduced rhizobia [26]. From the initial screening, 92 genotypes were selected for further evaluation at a single site (Inglewood) in 1985, from which nineteen were selected from further assessment, together with four check cultivars, at four sites in 1985 (Rocklea) and 1987 (Hermitage, Applethorpe and Kingaroy). The major findings of those assessments are presented in Table XVI. The best performing genotype, over all management practices, was ICA21573, and others nodulated well under two of the three management treatments, e.g. Campbell

20, Small White 38. The low-nodulating check, Gallaroy, performed poorly. Surprisingly, Puebla 152, the high N_2-fixing parent from the University of Wisconsin breeding programme performed only moderately. No assessments were made of N_2 fixation.

In 1988, an experiment was conducted at Inglewood as a first step in developing and evaluating the ureide method for measuring N_2 fixation by common bean in the field, and to test whether genotypes that are selected for increased nodulation also display improved N_2 fixation [28]. Plant nodulation and relative ureide-N in xylem sap were significantly correlated at each of the two samplings, suggesting that the ureide method had promise as a field assay of N_2 fixation. There were no cultivar effects on relative ureide-N values.

We considered the results of our own programme (1983-88) and of overseas research indicating that nodule function, i.e. N_2 fixation, rather than nodulation *per se* was the most likely cause of low productivity in bean. Thus, a greater emphasis needed to be given to assessing N_2 fixation, rather than nodulation, of bean genotypes. The ureide method had shown potential as an assay of N_2 fixation in preliminary experiments. Further research was required to calibrate the method using ^{15}N, so that it could be used quantitatively, rather than as a qualitative assay for treatment comparisons. The poor performance in Canada and South America of rhizobial strain CC511, then used in Australian commercial inoculant for common bean, raised doubts about the wisdom of its continued use. These issues needed to be addressed.

In 1989, we conducted a field experiment in which the main-plot treatments were (i) fertilizer N (80 kg N/ha) plus rhizobial inoculum, (ii) inoculum only and (iii) untreated control. Subplots were 10 genotypes of bean, chosen from the 1985 and 1987 trials for high nodulation (BAT419, Campbell 20, Selection 46, Small White 38, BAT1198, ICA21573, Epicure and Amarillo 155) and low nodulation (Gallaroy and Kerman). Nitrogen fixation was assessed using the ureide method. Nodulation could not be assessed with confidence because of problems of recovery from the heavy clay soils.

Application of commercial inoculant (strain CC511) resulted in reduced shoot weights in two of the three samplings (Table XVII). Shoot weight for the third sampling and grain yield were reduced also by inoculation, although differences were not significant at $P=0.05$. Fertilizer N increased plant growth and grain yields, although effects were not statistically significant in all cases; time to maturity was extended by 5%. The 10 genotypes varied significantly in the yield and agronomic characteristics examined. The largest plants did not necessarily produce the highest grain yields, although two of the three smallest genotypes, Epicure and Small White, did produce the lowest yields. The majority of the genotypes were bush types (vining score <2.0).

These results confirmed previous observations that bean yields can be increased with fertilizer N, and provided additional evidence that their N requirements are not met entirely from N_2 fixation. They indicated also that the commercial inoculant strain, CC511, may not be highly effective in N_2 fixation.

The largest effects on N_2 fixation, assessed as ureide-N in xylem sap, were from fertilizer N (Table XVII). In the first sampling, inoculation increased relative ureide-N levels above those of the uninoculated controls for nine of the ten genotypes (data not shown), although when main-plot effects were considered the differences were significant only at the 10% level. At the two remaining samplings, values for the plus and minus inoculation treatments were identical. The best performers were BAT419, Selection 46 and BAT1198.

There were no differences between the high- and low-nodulating genotypes in N_2 fixation. Kerman and Gallaroy, the two low nodulators, had average relative ureide-N values (Table XVII). On the other hand, ureide values for the high-nodulating group were both high (BAT419 and Selection 46) and low (Amarillo 155 and Epicure). In research overseas, high levels of N_2 fixation have been associated

TABLE XVI. EFFECTS OF INOCULATION AND FERTILIZER N (100 kg/ha AS UREA) ON NODULATION OF COMMON BEAN CULTIVARS IN SCREENING TRIALS AT KINGAROY, HERMITAGE, APPLETHORPE AND ROCKLEA IN 1985 AND 1987 [27]

Genotype	Nodule mass[a]		
	Uninoculated	+Inoc. +N	+Inoc.
ICA21573	2.06	3.00	3.17
Campbell 20	2.19	*[b]	4.17
Amarillo 155	2.00	2.75	*
BAT1198	1.75	2.60	*
Small White 38	*	2.50	4.33
Selection 46	*	1.50	4.00
Epicure	1.63	3.00	*
Puebla 152	2.06	1.37	*
Gallaroy	1.00	1.00	2.08

[a] Nodule abundance (rating of 1, 2 or 3) x size (rating of 1, 2 or 3). [b] Nodul'n not sig. better than Gallaroy.

with late maturity and a climbing habit [29], implying a direct relationship between leaf-area duration and N_2 fixation. Our results did not support these findings. The most consistent in N_2 fixation, BAT419, was a bush type of intermediate maturity. The two late, vining genotypes, Epicure and Amarillo 155, had the lowest levels for N_2 fixation. All three had been identified previously as high nodulators [27].

The yield reductions with rhizobial inoculation suggested that the native soil rhizobia were more effective at fixing N_2 than was CC511, the strain used in the inoculant. Mr J. Brockwell, CSIRO Plant Industry, subsequently joined the programme to evaluate the effectiveness of CC511 by comparing it with a number of highly effective strains from overseas (Table XVIII) and with strains isolated from field soils in southern Queensland.

To gain a better understanding of the effectiveness of the native soil rhizobia in navy-bean areas, 15 isolates from three field soils were tested for N_2 fixation in a controlled-environment study. There were 80 host cultivar x rhizobial strain combinations, i.e. 5 cultivars of bean x the 15 strains plus an uninoculated control.

Results indicated that the 15 rhizobial isolates differed in symbiotic capacity with the five bean cultivars (Table XIX). The best (6, 3, 7, 13, 8) produced around 30% more growth than did the poorest (14, 9). There was a pattern in their origins: the five best were isolated from Rocklea or Inglewood soils, four or which were from BAT419 or Campbell 20. Three of the four poorest performers were isolated

TABLE XVII. EFFECTS OF MANAGEMENT PRACTICE AND GENOTYPE OF BEAN ON SHOOT WEIGHTS AND RELATIVE UREIDE-N VALUES AT THREE SAMPLINGS, AND AGRONOMIC CHARACTERISTICS (AT HERMITAGE, 1989)

Practice/Genotype	S1[a]	S2	S3	U1[b]	U2	U3	Y[c]	V[d]	M[e]
		(g/plant)			(%)		(t/ha)		(days)
Management practice									
Control	41	92	99	27	28	24	1.65	1.9	91
+Inoc.	36	82	85	33	27	25	1.53	1.8	92
+Inoc. +N	42	98	111	12	15	15	1.99	1.9	97
Genotype									
Gallaroy	43	99	92	22	24	25	1.68	1.0	80
BAT419	50	119	108	26	29	28	1.72	1.3	89
Campbell 20	41	100	98	26	22	18	1.60	2.3	90
Selection 46	40	95	103	29	25	25	2.04	1.0	85
Small White	29	79	78	25	22	21	1.22	2.4	87
Kerman	40	88	92	25	22	21	1.55	1.0	89
BAT1198	42	98	120	22	25	26	2.02	1.0	91
ICA21573	42	92	115	21	23	21	1.69	1.0	102
Epicure	34	57	86	22	17	12	1.39	4.0	106
Amarillo155	37	81	92	21	24	16	2.02	3.4	115

[a] Shoot dry weight. [b] Relative ureide-N from the 46- (1), 60- (2) and 76- (3) day samplings. [c] Grain yield. [d] Vining scale 1 (= 0) to 4 (= 100% viney). [e] Time to 80% maturity.

from Hermitage soil. Overall, genotype ICA21573 was again the highest yielder (mean over the 15 strains: 1.44 g/plant), followed by Selection 46 (0.84 g/plant). The others, in order, were BAT419 (0.74 g/plant), Campbell 20 (0.66) and BAT76 (0.62).

Nine rhizobial strains (designated in Table XVIII), plus CC511 and Isolate 6 from Table XIX (CC507) were used to inoculate the same five cultivars - ICA21573, Campbell 20, Selection 46, BAT419, and BAT76. Plants were grown in 25-cm pots as before, and were harvested at 40 days.

Results (Table XX) indicate three broad groups of rhizobial strains, of low, moderate and high effectiveness. The current Australian inoculant strain (CC511) and the field isolate from Rocklea (Isolate 6) were highly effective on all five cultivars - an unexpected result in light of the previous year's field experiment at Hermitage, needing verification under field conditions. The low effectiveness of strains 127K89, CIAT652 and CIAT7001 was unexpected also, since, presumably, they had been selected for high effectiveness in inoculation trials in the United States and Colombia (Table XVIII).

TABLE XVIII. INFORMATION ON STRAINS OF BEAN RHIZOBIA RECEIVED FROM THREE OVERSEAS LABORATORIES FOR EFFECTIVENESS TESTING AGAINST THE AUSTRALIAN INOCULANT STRAIN, CC511

Source	Country	Contact	Strain	Synonym
NifTAL	USA	Dr HH Keyser	TAL182[a]	
			TAL943[a]	KIM-5, 127K102a
			TAL1382	C-05
			TAL1383	CIAT632, 21
			TAL1797[a]	CIAT899, M188, 127K119
Nitragin Co.	USA	-	127K119	CIAT899
			127K102a	KIM-5, TAL943
			127K89[a]	
			127K105[a]	CIAT161, Z164
CIAT	Colombia	Dr J Kipe-Nolte	CIAT632[a]	TAL1383, 21
			CIAT652[a]	
			CIAT7001[a]	
			CIAT2513[a]	

[a] Strains used to produce the data in Table XX.

A low N-fertility site was prepared at Applethorpe, southern Queensland, to field-test rhizobial strains that had been identified as highly effective on five bean cultivars under glasshouse conditions. However, the experiment was not sown as planned because of drought in early 1991. Another site at Applethorpe was prepared for sowing two experiments in January 1992. Treatments in the first were rhizobial strains CC511, CIAT 161, CIAT 2513, TAL 182 and commercial inoculant (strain CC511), either singly or in various combinations with and without fertilizer N, with cultivars BAT419, Selection 46, Kerman, ICA21573 and Epicure. The second experiment consisted of 80 genotypes of bean, inoculated with a mixture of effective rhizobial strains. Both experiments were destroyed by flooding.

Following the abandoned field experiments of 1991 and 1992, a pot experiment was done in a temperature-controlled glasshouse at Hermitage using essentially the same cultivar x rhizobia combinations. The potting medium was an Applethorpe soil. Rhizobial inoculation treatments were as for the abortive experiments, with cultivars BAT419, Selection 46, Kerman, ICA21573 and BAT1198 (instead of Epicure).

No strain of rhizobia produced greater nodulation or plant growth than did CC511 (pure strain) or the commercial inoculant containing CC511 (Table XXI). Surprisingly, the uninoculated plants had the best nodulation, although it was not reflected in enhanced plant growth. Predictably, nodulation was depressed in the +N treatment. We concluded from these results that CC511 was as good in nodulating and fixing N_2 over a range of cultivars as the most effective rhizobial strains assembled from overseas,

TABLE XIX. ORIGIN AND EFFECTIVENESS WITH COMMON BEAN OF 15 RHIZOBIAL ISOLATES FROM THREE BEAN-FIELD SOILS

Isolate	Origin		Shoot dry wt.[a] (g/plant)
	Soil	Trap host	
1	Inglewood	ICA21573	0.92
2	Inglewood	Selection 46	0.88
3	Inglewood	BAT419	0.99
4	Rocklea	Selection 46	0.88
5	Inglewood	BAT76	0.88
6	Rocklea	BAT419	1.04
7	Inglewood	Campbell 20	0.97
8	Rocklea	BAT76	0.95
9	Hermitage	Selection 46	0.74
10	Hermitage	BAT76	0.90
11	Hermitage	BAT419	0.79
12	Hermitage	Campbell 20	0.82
13	Rocklea	Campbell 20	0.95
14	Rocklea	ICA21573	0.76
15	Hermitage	ICA21573	0.80
Uninoculated control			0.46

[a] Mean growth of cvv. ICA21573, Campbell 20, Selection 46, BAT419, BAT76.

but that inoculation using any of the strains resulted in decreases in nodulation but not in plant growth. These findings must be treated with caution because of the restrictions of pot culture, and need to be confirmed under the more demanding conditions that exist in the field.

Cultivar effects on both nodulation and growth were significant (Table XXI). Cultivars BAT419, ICA21573, Selection 46 and BAT1198 were similar in nodulation and were superior to Kerman. Shoot dry matter and N reflected nodulation, with Kerman again inferior to the other four.

Field comparisons of the most promising strains of rhizobia against the current commercial navy bean inoculant, strain CC511, were made in 1993. The trial was sown at Applethorpe, on a coarse-textured soil and was sampled for nodulation, xylem sap, shoot dry matter and grain. Treatments were eight inoculant strains or strain mixes x five cultivars, arranged as split plot with cultivar as main plots. Results are summarized in Table XXII.

TABLE XX. EFFECTS OF STRAIN OF RHIZOBIA ON SHOOT DRY MATTER AND N ACCUMULATION BY COMMON BEAN

Strain	Dry wt.[a] (g/plant)	Shoot %N[a]	Total N[a] (mg/plant)
Highly effective			
127K105 (CIAT161)	0.88	3.52	30.7
TAL182	0.88	3.46	30.3
Isolate 6 (CC507)	0.84	3.53	29.4
CC511	0.84	3.73	30.7
TAL943 (127K102a)	0.86	3.35	28.2
CIAT2513	0.92	3.45	31.5
Moderately effective			
CIAT632 (TAL1383)	0.79	3.31	25.3
TAL1797 (127K119)	0.84	3.08	25.8
Low effective			
127K89	0.66	2.64	17.3
CIAT652	0.65	2.61	16.2
CIAT7001	0.77	2.83	21.5
Uninoculated	0.58	2.79	15.7

[a] Interactions of genotype x strain were essentially non-significant; therefore, shown are the mean values for the five lines, ICA21573, BAT419, Selection 46, Campbell 20, BAT76.

At flowering, we recorded responses to inoculation in nodulation and N_2 fixation (xylem ureides), but not in shoot dry matter. There were similarities, rather than differences, amongst the strains, with CIAT 2513 having consistently high values for all parameters measured. Fertilizer N suppressed nodulation and N_2 fixation, but had no real effect on shoot dry matter. By pod-fill, the effects of inoculation had disappeared; fertilizer N continued to depress N_2 fixation. At maturity, highest grain yields were recorded for the fertilizer-N treatment followed by strains CIAT 2513 and TAL 182. The uninoculated plants had the lowest yields. Cultivar effects were less significant than strain effects.

Overall, Selection 46 and BAT1198 had the highest values for symbiotic and/or yield traits. Kerman and ICA211485 were the poorest nodulators (Table XXII). The commercial inoculant strain, CC511, was about average for all parameters measured and all three samplings. Within a few months, however, the strain used in the commercial inoculants was changed to RCR3644 (G. Gemmell, personal communication), because of the development of colony variants in CC511, rather than because of questionable effectiveness. In summary, our results and those unpublished from the Australian Inoculants Research and Control Service (AIRCS) indicate that a number of strains, including CC511 and the other three in Table XXII, are effective on navy and culinary bean and would be suitable for use in commercial inoculants.

TABLE XXI. EFFECTS OF RHIZOBIAL STRAIN ON GROWTH AND NODULATION OF *P. VULGARIS*, IN A TEMPERATURE CONTROLLED GLASSHOUSE AT HERMITAGE IN 1992

Rhizobia/cultivar	Nodulation[a]		Shoot[a]		
	No. (per pot)	Wt. (g/pot)	Wt. (g/pot)	%N	N (mg/pot)
Rhizobia					
CIAT 161	204	0.24	21.1	1.85	388
CIAT 2513	162	0.18	20.1	1.85	374
TAL 182	159	0.16	21.3	1.88	398
CC511	157	0.18	20.4	1.79	372
Commercial inoculant	173	0.20	22.2	1.87	412
Comm'l inoc. + N[b]	141	0.16	19.4	1.68	335
Uninoculated	267	0.40	20.5	1.82	372
Cultivar					
BAT419	173	0.22	22.2	1.68	374
ICA21573	219	0.15	23.8	2.00	470
Selection 46	235	0.31	23.2	1.85	430
BAT1198	185	0.34	19.3	1.72	334
Kerman	87	0.07	15.0	1.89	286

[a] Values shown are for the strains/inoculation treatments averaged over the five cultivars, and the cultivar means averaged over the six strains.

[b] 100 kg N/ha.

At the Applethorpe site, three additional trials were sown simultaneously to evaluate early- and late-maturing genotypes of navy and culinary bean for nodulation and N_2 fixation (xylem ureides). The genotypes were selected on the basis of good growth characteristics in previous screenings. A total of 116 genotypes were sown in the three trials - 30 for each maturity group, replicated twice; 56 in the 'Other' group, unreplicated. Plants were sampled twice for nodulation, xylem sap, shoot weight and grain.

Data for 27 of the best performing genotypes from the three trials and for the five check lines (Selection 46, Gallaroy, Spearfelt, Sirius and Rainbird) are presented in Table XXIII. Some showed particular promise, because of high nodulation (699X, 1128, 223, 1059, 220, 843 and 830), high relative ureide-N value (1128, 216, 1301, RIZ36, 830 and 1094), high plant yield (832, RIZ32, ICA153446 and Spearfelt) or high grain yield (96, 1325, RIZ32 and RIZ36). These genotypes will be further evaluated for symbiotic and yield traits in the field.

TABLE XXII. RHIZOBIAL AND CULTIVAR EFFECTS ON SYMBIOTIC AND GROWTH TRAITS OF COMMON BEAN, GROWN IN THE FIELD (AT APPLETHORPE, 1993.

	Flowering (51 DAP)[a]				Pod-fill (72 DAP)[a]		Maturity
Rhizobia/cultivar	Nodulation No.	Nodulation Mass (mg)	RUN[b] (%)	Shoot DM (t/ha)	RUN[b] (%)	Shoot DM (t/ha)	Grain yield (t/ha)
Rhizobia							
-Inoc.	16	22	15	1.45	29	3.43	1.31
+Inoc. +N[c]	11	10	12	1.39	24	3.76	1.70
CIAT161	31	45	18	1.34	31	3.44	1.36
CIAT2513	58	57	21	1.40	30	3.72	1.56
TAL182	34	40	17	1.42	31	3.72	1.56
CIAT161+2513	38	72	20	1.46	31	3.43	1.41
CC511	51	53	20	1.36	29	3.50	1.43
All-strain mix	45	49	19	1.42	28	3.53	1.51
Cultivar							
BAT419	39	48	19	1.22	28	3.36	1.52
ICA211485	33	23	13	1.55	29	3.61	1.35
Selection 46	45	48	17	1.51	31	3.91	1.64
BAT1198	41	64	21	1.41	28	3.45	1.40
Kerman	21	34	18	1.33	29	3.49	1.49

[a] Days after planting. [b] Relative abundance of ureide-N in xylem sap. [c] 100 kg N/ha as urea.

3.3.1. 1991-92 calibration of the xylem ureide method for common bean using ^{15}N

Many crop legumes, e.g. soybean, cowpea, pigeonpea and mung bean, transport the bulk of fixed N from root nodules to shoots as ureide compounds (allantoin and allantoic acid). They also take up mineral N from the soil and transport it as nitrate and amino compounds. Therefore, since the nitrogenous compounds in the transpiration stream represent the current products of N uptake (including N_2 fixation), it has proved possible to distinguish the sources of incoming N as from soil or atmosphere, by analyzing the xylem sap.

The ureide method can be used without calibration as an index of N_2 fixation [28]. After calibration it can be used to quantify N_2 fixation, i.e. to determine Pfix and in combination with N-accumulation data, to determine the amount of fixed N in kg/ha. Preliminary experiments had indicated that the method could be applied in studies of common-bean N_2 fixation [30] and we considered that the method should be properly calibrated for use in this and subsequent programs. Calibration involved growing plants with ^{15}N-labelled nitrate applied in the glasshouse with sampling throughout growth for ^{15}N enrichment of dry matter and for ureides, amino-N and nitrate in xylem sap. At each sampling time, the relative abundance of ureide-N in the xylem sap could be related to Pfix, determined using the isotope-dilution method.

TABLE XXIII. EVALUATION OF EARLY- AND LATE-MATURING GENOTYPES OF COMMON BEAN FOR SYMBIOTIC AND YIELD TRAITS (AT APPLETHORPE, 1993)

Genotype	Nodulation		Rel. ureide-N	Shoot DM	Grain
	No. (per plt)	Mass (mg)	(%)	(t/ha)	(t/ha)
Early-maturing					
699X	37	130	30	3.22	1.04
1128	33	124	37	3.17	1.73
96	18	97	35	3.32	1.84
1261	31	85	30	3.12	1.18
1115	37	83	31	3.29	1.15
832	22	58	32	3.60	1.60
RIZ53W	30	50	35	3.20	1.41
1325	21	41	35	3.51	1.62
216	18	17	39	2.62	0.79
Selection 46	42	59	25	3.05	1.36
Gallaroy	21	41	30	3.54	1.48
Late-maturing					
223	89	332	33	3.33	0.80
1059	61	185	19	3.99	1.69
220	42	160	35	3.12	1.27
1341	55	147	31	2.85	1.06
Purple Pod	37	147	28	3.52	1.39
1301	53	129	36	3.39	0.59
RIZ32	29	110	31	4.07	1.83
ICA153446	27	99	35	4.51	1.50
RIZ36	43	93	40	3.85	1.80
Spearfelt	34	101	30	4.05	1.38
Sirius	31	73	31	3.31	1.41
Rainbird	29	71	20	4.00	1.66
Other					
843	39	184	26	-[a]	-
830	50	161	41	-	-
RIZ103	69	148	25	-	-
1294	35	146	24	-	-
RIZ104	74	126	27	-	-
1094	35	79	38	-	-
907	18	75	44	-	-
391	37	63	36	-	-
Mutiki 2	18	30	34	-	-

[a] Not determined

Navy bean cv. Gallaroy plants were grown in a mixture of sand and vermiculite in large pots in a temperature-controlled glasshouse. Once seedlings had emerged, pots were watered on alternate days during the first six weeks and daily thereafter with 2 to 3 L of a nutrient solution supplemented $K^{15}NO_3+Ca(NO_3)_2$ to give concentrations of 0, 1, 2, 5, or 10 m*M*. Enrichments (% atom excess) in ^{15}N were 0.52 (10 m*M* nitrate), 1.03 (5 m*M*) and 2.05 (1 and 2 m*M*). Plants were harvested on four occasions: during vegetative growth, and at the flowering, pod-fill and seed-filling stages, for dry matter, %N, ^{15}N, and xylem sap as root-bleeding sap (RBS) and vacuum-extracted sap (VES). Experimental details were similar to those previously reported for soybean [6].

Nitrate levels had a consistent effect on Pfix and relative ureide-N in RBS and VES (Fig. 5). The Pfix values ranged from 100% for plants supplied with the N-free nutrient solution to zero for uninoculated plants supplied with 10 m*M* nitrate. Values for Pfix for intermediate treatments were 88-91% (1 m*M* nitrate), 75-80% (2 m*M*), 40-53% (5m*M*) and 12-31% (10 m*M*) (Fig. 5A). Values for relative ureide-N were in the range 5 to 82% for RBS and 5 to 70% for VES (Figs. 5B, C). The higher values for RBS are consistent with those obtained with soybean [6].

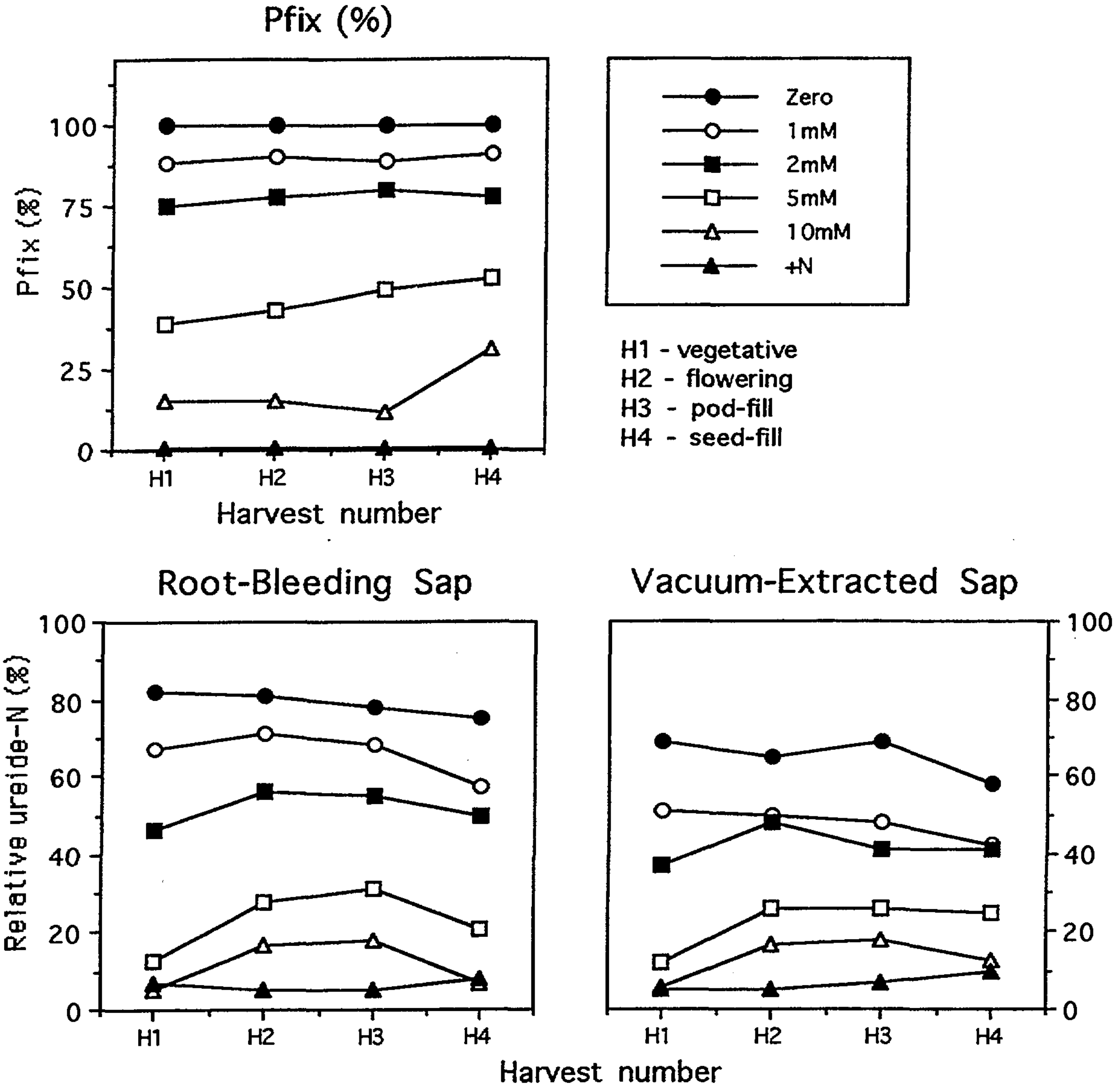

FIG. 5. Effects of nitrate supply on Pfix, relative ureide-N (%) of root-bleeding sap, and relative ureide-N (%) of vacuum-extracted sap of common bean over four harvest times: H1 - vegetative; H2 - flowering; H3 - pod-fill; H4 - seed filling.

The relative abundance values for ureide-N in RBS and VES were plotted against Pfix and subjected to regression analysis. Relationships were very strong with 97 (RBS) and 95% (VES) of the variation in relative ureides explained by variations in Pfix (Fig. 6). These regressions are very similar to those for pigeon pea and soybean [6, 31], and provide a means for calculating Pfix from solute analysis of xylem sap of common bean.

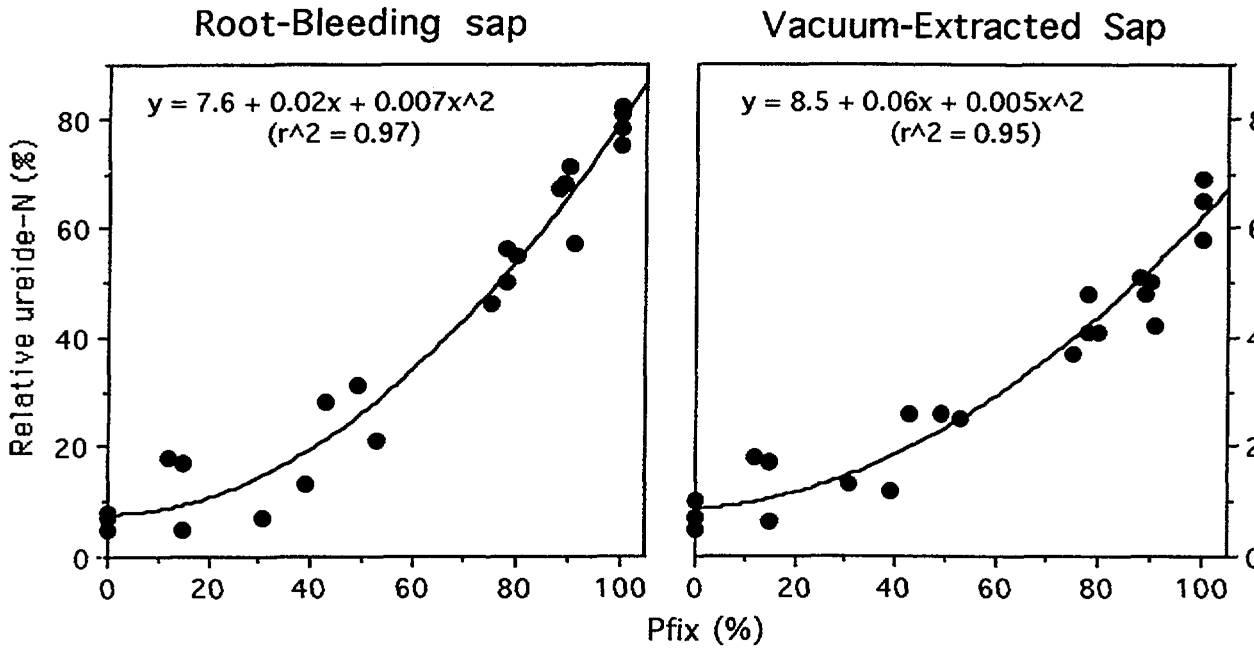

FIG. 6. Relationships between Pfix and relative abundance of ureide-N in root-bleeding sap and vacuum-extracted sap of common bean.

In conclusion, release of cultivars of navy and culinary bean with enhanced capacity for N_2 fixation could save Australian growers $1-1.5 million annually in fertilizer costs. Since 1988, we have made substantial progress towards this goal, having shown quantitative differences in nodulation and N_2 fixation capacity of bean genotypes and rhizobial-strain effects on symbiotic and yield traits of bean. With calibration, the xylem ureide method with ^{15}N, can be used as a field assay of N_2 fixation. Future research will continue these assessments until genotype(s) are identified with superior symbiotic and yield traits.

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