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Evaluation of genotypes of navy and culinary bean (*Phaseolus vulgaris* L.) selected for superior growth and nitrogen fixation

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Summary. Levels of nitrogen fixation by navy and culinary beans (*Phaseolus vulgaris* L.) in Australia are low and contribute little to the N economies of the crops. As a consequence, they must be grown in highly fertile soils or fertilised with N to obtain economic yields. Eliminating the need for fertiliser nitrogen would save growers A$1 million annually. Following a 10-year program in which almost 1500 genotypes of *P. vulgaris* were screened for superior nodulation and nitrogen fixation, we conducted experiments at the Southedge Research Station, Mareeba, during 1995–97 to identify elite genotype(s), which could either be released as cultivar(s) or used as donor parent(s) in a breeding program. Selection criteria were plant biomass, nitrogen fixation activity assessed using the ureide method and grain yield.

The best-performing genotypes were ICA20667 and ICA21573. They produced about 20% more shoot biomass than the commercial check cultivars, Spearfelt, Gallaroy and Rainbird, and had Pfix (percentage of plant nitrogen derived from nitrogen fixation) values that were consistently about 30% higher. However, both genotypes responded strongly to fertiliser nitrogen (>200% increase in shoot nitrogen and >100% increase in grain yield at rate of 150 kg nitrogen/ha), suggesting that their nitrogen fixation capacity was inadequate. This study reinforced current recommendations that commercial crops of *P. vulgaris* be fertilised with nitrogen and indicated a low likelihood of release of high nitrogen-fixing cultivars to growers in the immediate future.

Introduction

Levels of nitrogen (N) fixation of navy and culinary bean crops in Australia are low and contribute little to their N requirements (Adams *et al.* 1985). Thus, farmers must either grow them in highly fertile soils or apply fertiliser N to obtain economic yields. The navy and culinary bean industries are currently worth A$6–8 million annually, and involve production of about 10 000 t from 10 000 ha. Eliminating the need to apply fertiliser N to these crops would save growers $1 million annually.

Genetic variation for N_2 fixation has been identified within *P. vulgaris* (Graham 1981; Westerman *et al.* 1981; Rennie and Kemp 1983*b*) and breeding programs for enhancing N_2 fixation initiated (Bliss *et al*. 1986; CIAT 1987). Variation in N_2 fixation was shown to be related to growth habit and maturity (Graham 1981; Duque *et al.* 1985; Attewell and Bliss 1985) but other factors, such as nodule efficiency and earliness and duration of nodulation, may be involved (Pacovsky *et al*. 1984; Piha and Munns 1987*a*; Chaverra and Graham 1992; Kipe-Nolt *et al*. 1993; Kipe-Nolt and Giller 1993). Bliss and co-workers released five high N_2-fixing cultivars of *P. vulgaris* for Central and South America in the late 1980s (Bliss *et al.* 1989), although Bliss later cautioned that the cultivars' commercial acceptance and use depended just as much on other traits such as disease resistance and seed type (Bliss 1993). Thus, current breeding programs in South America aim to combine traits like disease resistance with enhanced N_2 fixation in adapted breeding lines.

The first stage (1983–88) of the Queensland Department of Primary Industries program to identify high N_2-fixing genotypes of *P. vulgaris* involved screening of the available germplasm (1462 accessions) for capacity to nodulate under a range of field

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conditions, using both indigenous and introduced rhizobia (Redden *et al*. 1985). Lines that showed superior nodulation, compared with the check cv. Gallaroy, were identified although no assessments were made of their capacity to fix N_2 (Redden *et al*. 1990).

After 1988, greater emphasis was placed on evaluating N_2 fixation, rather than nodulation. This meant developing techniques for measuring N_2 fixation by *P. vulgaris* under field conditions. The xylem ureide method had been used successfully in a similar program to improve N_2 fixation of soybean (*Glycine max*) (Herridge and Rose 1994) and showed potential in one of the early *P. vulgaris* screening experiments (Diatloff *et al*. 1991). The ureide method was subsequently calibrated with ^{15}N isotope dilution for *P. vulgaris* and used to quantify *P*fix, i.e. percentage of plant N derived from N_2 fixation, in subsequent field experiments (Herridge *et al*. 1998).

The poor performance in Canada and South America of rhizobial strain CC511, the commercial inoculant strain for *P. vulgaris* in Australia during the 1980s and early 1990s, raised doubts about the wisdom of its continued use (Rennie and Kemp 1983*a*). Laboratory, glasshouse and field research at Hermitage and Applethorpe, Queensland, during 1989–93, involving 15 isolates from south-eastern Queensland field soils and 10 strains of rhizobia, assembled from local and overseas collections, in combination with 9 *P. vulgaris* cultivars indicated that: strain CC511 was as effective as the best strains from the USA and Colombia; the native soil rhizobia were generally effective and not likely to cause reductions in growth; cultivar x rhizobial strain interactions were negligible, and inoculation responses of higher nodule numbers (15–40/plant at flowering) did not translate into increased N_2 fixation or yield (Herridge *et al.* 1998). The results overall reinforced the notion that nodulation *per se* was not the limiting factor. Fertiliser N depressed N_2 fixation and increased grain yield by 20–30%.

By the end of 1994, 56 genotypes, from the almost 1500 at the start of the study, had been identified with either superior growth and/or nodulation and N_2 fixation. We report experiments, conducted in the field at the QDPI Southedge Research Station, Mareeba, in far-north Queensland during 1995–97, with the overall objective of screening the selected genotypes for yield and N_2 fixation under low soil nitrate conditions, and in the presence of fertiliser N. Our hypothesis was that 1 or a number of those genotypes had sufficient N_2 fixation capacity to eliminate the need for fertiliser N when growing the crop. This would be determined by monitoring N_2 fixation and yield of the genotypes under a range of soil nitrate supply conditions. The genotype(s) could then be released as a cultivar(s) if also high-yielding and well-adapted, or used as a donor parent(s) in a breeding program.

Materials and methods

Site details

The 3 years of experiments were located on the QDPI Southedge Research Station, Mareeba, far-north Queensland (17°00'S, 145°28'E). Soil type was a near-neutral (pH 6.6, in a 1:5 soil:H_2O slurry) Kandosol (Isbell 1996) of low fertility containing less than 5 mg nitrate-N/kg soil in the surface 15-cm layer. Each experiment followed a previous sorghum crop with a 2–3 month fallow in between.

Treatments, experimental design and sowing

In 1995, 60 genotypes of *P. vulgaris* were sown on 27 July in single-row plots, 8-m long with 1 m between the rows. There were 2 replicates. Commercial checks were cvv. Rainbird and Spearfelt; low N_2 fixing checks were cvv. Gallaroy and Actolac. Seed of all genotypes was inoculated with commercial, peat-based rhizobial inoculant immediately prior to sowing. Plots had previously been fertilised with P (10 kg/ha), K (40 kg/ha), Mg (10 kg/ha) and trace amounts of Zn, Cu and Bo.

The 1996 experiment consisted of 13 genotypes, selected in the previous experiment either for high N_2 fixation (Acc. 1240, ICA20667, ICA21573) or high yield (RIZ53, RIZ53W, Multiki 2, ICA153446, UNS171, Acc. 391, RIZ103, Acc. 1413). Check cultivars were Spearfelt and Rainbird. Plots consisted of 4 rows, 10-m long and 1 m apart. There were 4 replicates. Seed was inoculated with commercial inoculant as above and sown on 2 August. Basal fertiliser was as follows: P (15 kg/ha), K (40 kg/ha), Mg (15 kg/ha) and trace amounts of Zn, Cu and Bo.

In 1997, the experimental design was a split-plot with 3 replicates. Main plots were fertiliser N at either 0, 75 or 150 kg/ha, surface applied at sowing as urea. Subplots were 7 *P. vulgaris* genotypes, selected in the previous (1996) experiment for high N_2 fixation (ICA20667, ICA21573) or high yield (RIZ53, UNS171). Check cultivars were Spearfelt, Rainbird and Gallaroy. Plots consisted of 4 rows, 10-m long and 1 m apart. Seed was inoculated with commercial inoculant as above and sown on 21 July. Basal fertiliser was as follows: P (20 kg/ha), K (30 kg/ha), Mg (15 kg/ha) and trace amounts of Zn, Cu and Bo.

Sampling

In 1995, plots were sampled at mid pod-fill (60 days after sowing) for shoot biomass and N_2 fixation. All plants in a 1-m length of row were cut at ground level and immediately separated into leaves and shoot axes (stems plus petioles). The 2 fractions were oven-dried (80°C for 48 h) and weighed to give total shoot biomass. The shoot axes fraction was subsequently extracted for N solute analysis. Grain yield was determined for each plot at maturity from a 2-m length of row.

In 1996 and 1997, two 1-m lengths of row were sampled for shoot biomass and N_2 fixation at 48–50 (flowering) and 63–65 days after sowing (mid pod-fill), as described above. Because of the larger samples, i.e. 2 m of row versus 1 m in 1995, 15 plants were randomly separated from the rest for N solute

Table 1. Relative ureide-nitrogen concentrations and shoot dry matter for the best-performing genotypes for nitrogen fixation and yield and the check cultivars in field experiments at Southedge, far-north Queensland during 1995–97

Total numbers of genotypes sown each year were: 60 in 1995, 13 in 1996, and 7 in 1997. Values in parentheses are the ranking of the genotype

Group or genotype	1995	1996		1997	
	60 DAP	48 DAP	63 DAP	50 DAP	65 DAP
	Relative ureide-nitrogen concentration (%)				
High N_2 fixing					
ICA20667	74 (1)	49 (2)	42 (2)	36 (2)	75 (1)
ICA21573	53 (13)	44 (3)	32 (7)	38 (1)	72 (2)
High yielding					
RIZ53	27 (59)	26 (7)	25 (11)	24 (7)	47 (7)
Check cultivars					
Rainbird	55 (8)	21 (10)	44 (1)	25 (6)	60 (6)
Spearfelt	63 (2)	18 (12)	41 (3)	27 (4)	66 (4)
Gallaroy	38 (40)	n.m.	n.m.	30 (3)	69 (3)
Significance					
Genotype	**	**	*	**	**
s.e.d.	10.7	8.4	6.5	7.4	14.9
	Shoot dry matter (t/ha)				
High N_2 fixing					
ICA20667	2.03 (4)	2.07 (2)	5.13 (2)	1.16 (3)	2.71 (4)
ICA21573	2.11 (3)	2.03 (3)	4.60 (7)	1.27 (2)	3.00 (1)
High yielding					
RIZ53	2.49 (1)	2.35 (1)	5.42 (1)	1.06 (4)	2.57 (5)
Check cultivars					
Rainbird	1.30 (43)	1.85 (6)	4.34 (10)	1.06 (5)	2.73 (3)
Spearfelt	1.10 (59)	1.76 (10)	4.22 (12)	0.92 (6)	2.45 (6)
Gallaroy	1.28 (46)	n.m.	n.m.	1.37 (1)	2.78 (2)
Significance					
Genotype	**	**	*	**	**
s.e.d.	0.27	0.20	0.52	0.09	0.25

* $P<0.05$; ** $P<0.01$; n.m., not measured.

analysis on the shoot axes. All dry weights were recorded and added to determine total shoot dry weight. Grain yield was determined for each plot at maturity from two 2-m lengths of row.

Nitrogen fixation

The ureide method was used to assess N_2 fixation activity (Peoples *et al*. 1989*a*, 1989*b*; Herridge and Peoples 1990). Shoot axes were finely ground and extracted in boiling water for analysis for ureides and nitrate-N. The relative abundance of ureide-N was calculated from the molar concentrations of ureides and nitrate, assuming 4 N atoms per ureide molecule. The equation is:

$$\text{Relative ureide-N (\%)} = 100\ (4 \times \text{ureides})/(4 \times \text{ureides} + \text{nitrate})$$

Results

Nitrogen fixation

The relative ureide-N values presented in Table 1 are an index of Pfix. The higher the relative ureide-N values, the higher the proportion of currently-assimilated plant N derived from N_2 fixation. Low values, i.e. 10–40%, indicate suppression of N_2 fixation by soil nitrate and a relatively high utilisation of soil nitrate by the plant.

The outstanding genotype for Pfix was ICA20667, ranking first or second for the 3 years of experiments (Table 1). Genotype ICA21573 also performed strongly. Commercial checks, Rainbird, Spearfelt and Gallaroy, varied from high rankings, e.g. Spearfelt in 1995, Rainbird in the second sampling in 1996, to near to the lowest, e.g. Gallaroy in 1995 and Rainbird in 1997. Overall, relative ureide-N values for the commercial checks were about 25% less than for ICA20667and ICA21573. The high-yielding genotype, RIZ53, had consistently low relative ureide-N values (45% less than the high N_2 fixers).

Biomass yield

In soils low in plant-available N, shoot biomass and N are also indices of N_2 fixation. Accurate determinations of total N fixed, however, can only be made by multiplying crop N by Pfix. Genotype RIZ53 produced the most shoot biomass overall, yielding, on average, about 35% more than the commercial checks (Table 1). The 2 high N_2-fixing genotypes produced about 20% more shoot biomass than the checks.

Genotypic responses to fertiliser nitrogen

Three rates of fertiliser N were used in the 1997 experiment to determine genotypic responses to the suppressive effects of soil nitrate on N_2 fixation (i.e. nitrate tolerance). A strong interaction between fertiliser N rate and genotype for shoot biomass and shoot N would further indicate genotypic differences in

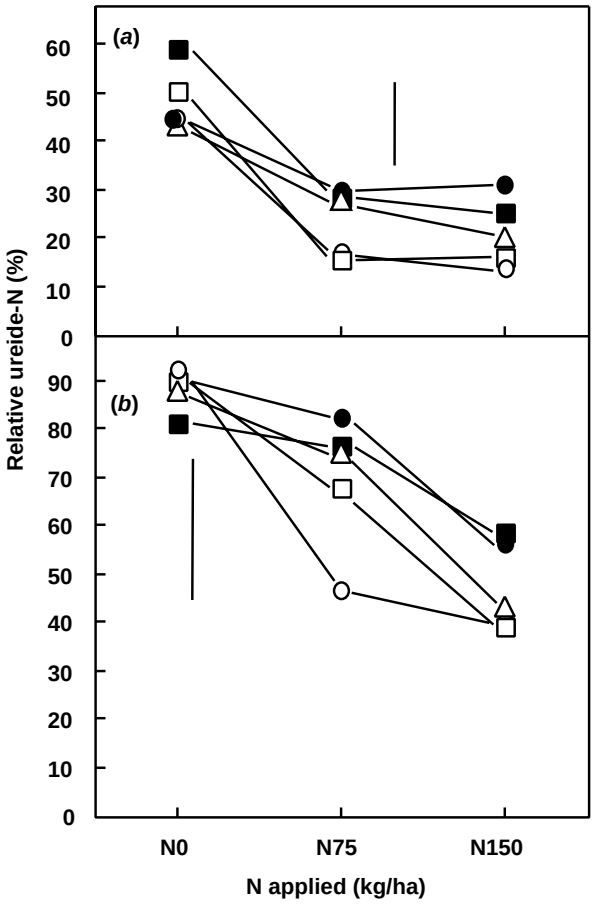

Figure 1. Responses for relative ureide-N at (*a*) 50 and (*b*) 65 days after sowing of high-fixing/high-yielding genotypes, ICA20667 (●) and ICA21573 (■), and commercial checks, cvv. Rainbird (○), Spearfelt (□) and Gallaroy (△), to fertiliser N, at Southedge, far-north Queensland in 1997. Vertical bars represent s.e.d.

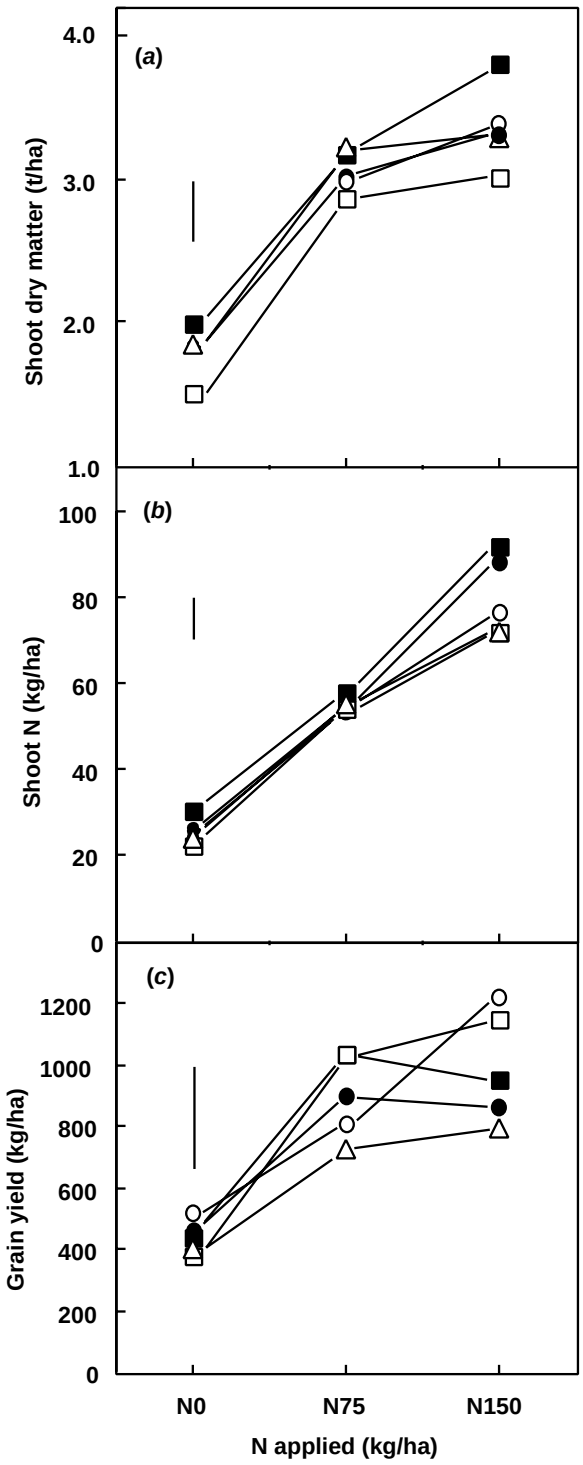

Figure 2. Responses for (*a*) shoot dry matter, (*b*) shoot N and (*c*) grain yield of high-fixing/high-yielding genotypes, ICA20667 (●) and ICA21573 (■), and commercial checks, cvv. Rainbird (○), Spearfelt (□) and Gallaroy (△), to fertiliser N at Southedge, far-north Queensland in 1997. Vertical bars represent s.e.d.

N_2 fixation capacity, with efficient genotypes producing high biomass yields in the absence, as well as in the presence, of fertiliser N.

Results for the high N_2-fixing/high-yielding genotypes, ICA20667 and ICA21573, and commercial checks are presented in Figs 1 and 2. Relative ureide-N values declined for all genotypes with increasing rates of fertiliser N. However, the average decline for the high N_2-fixing/high-yielding genotypes was slightly less (46 and 33% for the 2 samplings, comparison of N0 and N150) than for the commercial checks (57 and 55%). In other words, genotypes ICA20667 and ICA21573 maintained higher Pfix values in the presence of elevated soil nitrate than the commercial cultivars.

Shoot biomass and N and grain yields of both groups of genotypes responded strongly to fertiliser N (Fig. 2). For the high-fixing/high-yielding genotypes, average increases were 62% (N75) and 86% (N150) for shoot biomass, and 100% (N75) and 227% (N150) for shoot N. For commercial checks, biomass increases were 75% (N75) and 87% (N150), whilst shoot N increases were 138% (N75) and 217% (N150). The greater effect of fertiliser N on shoot N resulted from increased shoot N concentrations. Average concentrations (over all genotypes) were 1.4% (N0), 1.9% (N75) and 2.4% (N150). Average grain yields of the two groups were more than doubled by fertiliser N, even at the lower rate of 75 kg/ha (Fig. 2*c*).

Discussion and conclusion

The final outcome of this 15-year program, involving almost 1500 genotypes of *P. vulgaris,* was to identify 2 genotypes, ICA20667 and ICA31573, that were marginally more efficient at fixing N_2 than current commercial cultivars. The genotypes maintained higher levels of *P*fix in the presence of soil nitrate and produced greater yields of shoot biomass. Both are large-seeded 'Andean' types from the Colombian national breeding program, as distinct from the small-seeded 'meso-American' types. They were not suitable for commercial release, however, because they both lacked resistance to bean rust (*Uromyces appendiculatus*) and produced large, mottled seeds rather than the small white seeds preferred by the Australian market. More importantly, ICA20667 and ICA31573 required fertiliser N when grown in low nitrate soils for high yields, suggesting that they did not have sufficient N_2 fixation capacity to satisfy crop N demand.

One option for improving N_2 fixation of ICA20667 and ICA31573 was to cross the 2 genotypes in an effort to pyramid genes for yield and N_2 fixation, and then to cross the progeny with agronomic types. The other option involved an acceptance of the N_2 fixation limitations of *P. vulgaris* and to seek enhanced N_2 fixation capacity through interspecific hybridisation. Both options have merit without guarantee of success, but do require a long-term breeding commitment. There was not scope in this program to explore these options.

Clearly, the recommendation that navy and culinary beans be fertilised with N at rates of 75–150 kg/ha should remain. In the 1997 experiment that included 3 rates of fertiliser N, grain yields in the nil N plots were 400–500 kg/ha, but were increased to economic levels of 700–1000 kg/ha with just 75 kg/ha fertiliser N and to 1200 kg/ha with 150 kg N/ha.

Our results support the theory that *P. vulgaris* has been essentially bred as a vegetable crop reliant on fertiliser N and the capacity for N_2 fixation may have become superfluous. The plant, particularly the early maturing, bush (erect) types, has become very efficient in using mineral N as a source of N for growth. George and Singleton (1992), in a field study of *P. vulgaris* and soybean, confirmed results of earlier research (e.g. Piha and Munns 1987*a*, 1987*b*) indicating that *P. vulgaris* had a generally low capacity for N_2 fixation, which appeared to be associated with inefficient nodule function rather than with low nodule numbers. The George and Singleton (1992) study also showed that *P. vulgaris* was more efficient at taking up N from the soil (natural N fertility and applied fertiliser N) and that this efficiency may have been due to a larger and more efficient root system. It is likely that this efficiency in using mineral N was developed through breeding and selection of elite lines in high N fertility soils and resulted in the concomitant decline in the efficiency of nodule function.

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