Shoot and Root Control of Ureide Accumulation and Partitioning in *Phaseolus vulgaris* L.

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http://arnoldzwicky.files.wordpress.com/2012/02/seedbeans.jpg

Summary

Ureides are the primary form of organic N transported to the shoots of common bean (*Phaseolus vulgaris* L.). Understanding how production, transport, and accumulation of ureides are regulated in common bean is fundamental to improving biological nitrogen fixation (BNF) in this crop. We used grafting to determine shoot and/or root control of ureide accumulation and partitioning among four genotypes varying in phenotypic traits related to nitrogen fixation (R99, Eagle, Puebla, and R32). Plant ureide content and total N were greater in R32, Puebla and Eagle (nodulating) than in R99 (non-nodulating). R32 (super-nodulator) as a scion or rootstock generally promoted plant N, plant ureide content, and %N derived from BNF. Eagle produced a low number of highly effective nodules; this trait was transferred when Eagle was used as a scion. This study revealed the extent of nodulation, %N derived from N_2 fixation, and nodule effectiveness (mg plant N/nodule) are transferable phenotypic traits.

Conclusions

- Genotypic rankings for total ureide content, total N, biomass, and nodule number varied and were not consistently related.
- Leaf N content was the most consistent trait associated with variation in BNF. But a combination of phenotypic traits (plant N, % N from BNF, and nodule effectiveness) might be more effective in identifying lines with greater capacity for BNF
- Extent of nodulation, %N derived from N_2 fixation, and nodule effectiveness (mg plant N/nodule) are transferable traits that can be manipulated to identify mechanism limiting N accumulation in common beans.

Results

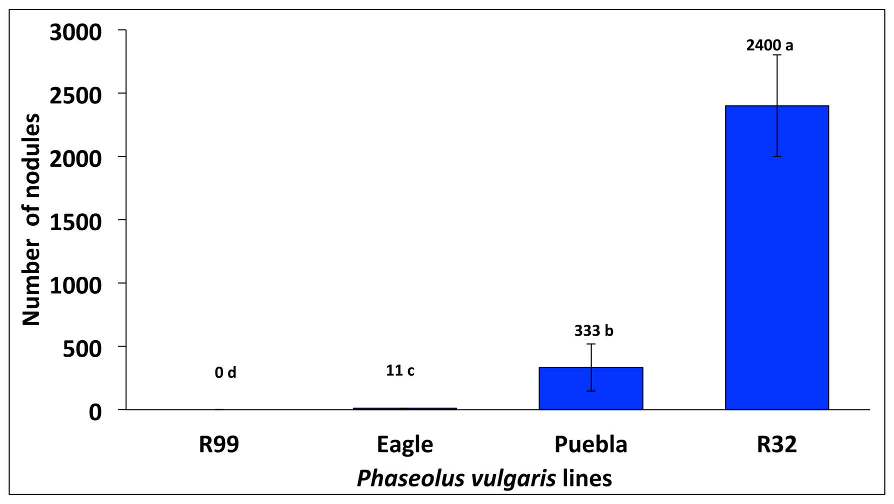
Nodulation, BNF, Plant N and ureide content

Lines selected for study (TABLE 1) varied dramatically in their capacity for nodule formation (FIGURE 1). The non-nodulating line R99 failed to produce nodules; the supernodulating line R32 produced >>2000 nodules per plant. Eagle produced a few large nodules; Puebla typically produced ~300 nodules per plant.

TABLE 1. Characteristics of common bean lines selected for this study.

Common bean lines	Origin	Characteristics
Puebla	Mesoamerican	High capacity for BNF
Eagle	Andean	Low capacity for BNF
R32	Mesoamerican	Super-nodulator
R99	Mesoamerican	Non-nodulator

FIGURE 1: Average nodule number per plant. Plants were grown in the greenhouse and sampled at flowering/early pod set. Nodules were not examined for potential BNF activity.

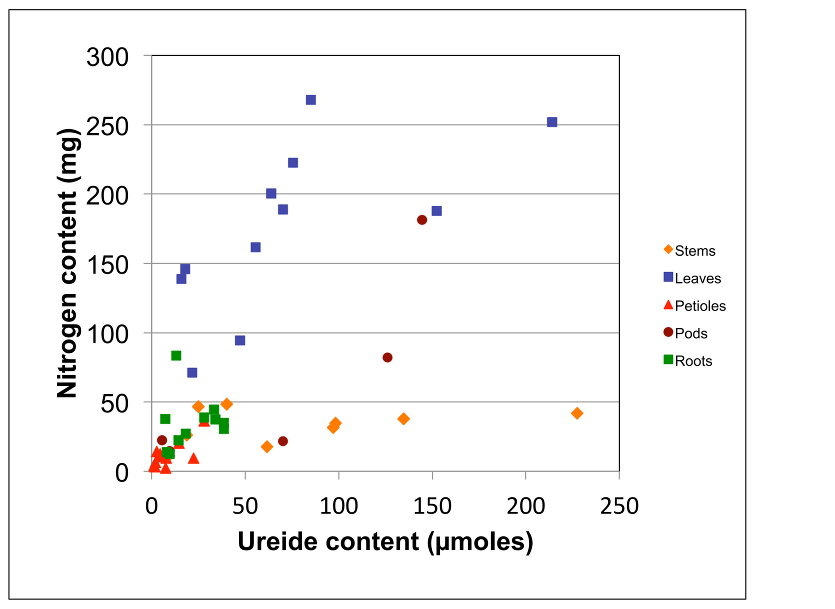


Variation in nodule number was not closely related to the accumulation of dry matter, total plant N, or total plant ureide content (TABLE 2). Ureides detected in R99 evidently were derived from nucleotide metabolism or remobilization of N from older tissues. Most of the N accumulated in leaves; the leaves, stems, and pods were major sites of ureide accumulation (FIGURE 2). There was a modest correlation between total plant N and total plant ureide content (R^2 = 0.73).

TABLE 2: Nodule number, plant biomass, total plant N, and total plant ureide content for four common beans lines. Plant N and ureide values are normalized to the average dry weight for all lines. R32 is a supernodulator, R99 is a non-nodulating line.

Phaseolus vulgaris lines	Number of nodules	Whole plant biomass	Normalized whole plant Nitrogen	Normalized whole plant Ureide content
		(g)	(mg)	(µmol/gDW)
R32	2400 a	8.4 b	356.9 a	340.0 a
Puebla	333 b	13.2 a	292.8 b	121.6 b
Eagle	11 c	9.1 ab	355.2 a	372.8 a
R99	0 d	6.9 b	206.6 c	75.5 b

FIGURE 2. Comparisons of tissue N and tissue ureide contents in stems, leaves, petioles, pods, and roots of Puebla, Eagle, R32, and R99 common bean plants. Each point is from an individual plant.



Grafting to alter %N from BNF and nodule effectiveness

The scion had a major impact on nodule formation (TABLE 3). No nodules formed when R99 was used as a rootstock. Compared to the controls, R32 scion and rootstock promoted nodulation, Eagle scion suppressed it, and R99 scion was as capable as Puebla scion at supporting nodulation. There was little correspondence between total plant N or ureide content (R^2 = 0.49).

TABLE 3: Impact of reciprocal grafting on plant nodulation, biomass, total plant N and total plant ureide content. Data are Mean ± SE of 3 or 4 plants. R32: supernodulating line. R99: non-nodulating line. To enable direct comparison of total plant N and plant ureide content, these data are normalized to the same (average) DW for all plants within a rootstock group. Means in columns within root stock groups followed by the same letter are not significantly different at P=0.1 * Structures counted as nodules on R99 roots are likely pseudo-nodules.

Rootstock/Scion	Number of nodules	Whole plant biomass	Normalized whole plant Nitrogen	Normalized whole plant Ureide content
		(g)	(mg)	(µmol/gDW)
R32 Control	2400 a	8.4 b	356.9 a	340.0 a
R32/R32	2833 a	11.3 b	403.9 a	360.6 a
R32/R99	840 b	17.9 a	325.0 a	326.2 a
R32/Puebla	910 b	10.0 b	342.0 a	294.4 a
R99 Control	0 b	6.9 a	206.6 b	75.5 c
R99/R99	*9 a	6.2 a	426.3 a	147.7 a
R99/Eagle	0 b	9.2 a	152.8 b	92.0 b
R99/Puebla	*3 ab	8.6 a	145.0 b	81.9 c
R99/R32	0 b	9.7 a	162.0 b	90.6 bc
Puebla Control	333 bc	13.2 a	292.8 b	121.6 b
Puebla/Puebla	339 bc	13.5 a	329.5 b	181.4 b
Puebla/Eagle	185 c	12.6 a	290.4 b	187.3 b
Puebla/R99	480 b	11.2 b	299.8 b	161.8 b
Puebla/R32	2400 a	9.3 b	452.4 a	362.5 a
Eagle Control	11 c	9.1 b	355.2 a	372.8 ab
Eagle/Eagle	9 c	6.5 b	178.2 c	88.3 c
Eagle/R99	716 b	13.0 a	295.7 b	349.1 b
Eagle/Puebla	640 b	14.9 a	292.2 b	401.8 ab
Eagle/R32	1194 a	15.7 a	384.1 ab	483.4 a

Control non-grafted plants derived from 29 - 42% of their total N from N_2 fixation as estimated relative to N accumulation by R99 (TABLE 4). Scions of Puebla and R32 apparently increased % N from BNF. R99 scions supported a large number of nodules and a normal level of N derived from BNF.

FIGURE 3: Grafted plants in the greenhouse prior to harvest.

FIGURE 4. Examples of common bean roots sampled from a normally-nodulating line (left), a supernodulating line (center), and a scion of a normally-nodulating line grafted onto the non-nodulating line R99 (right)

TABLE 4. Effect of grafting on nodule number, N derived from BNF, and nodule effectiveness. Data are the means of 3 or 4 plants.

--------- Controls Not Grafted --------				
Line not grafted	Average nodule number	Total N from BNF (mg)	Nodule effectiveness (mg N per nodule)	N derived from N_2 fixation (%)
R32	2400	150	0.05	42.1
Puebla	333	86	0.30	29.4
Eagle	11	149	14.5	41.8
R99	0	0	--	0

--------- Grafted Plants ---------				
Line used as a scion	Average nodule number	Total N from BNF (mg)	Nodule effectiveness (mg N per nodule)	N derived from N_2 fixation (%)
R32	2207	237	0.11	59.4
Puebla	630	176	0.27	54.9
Eagle	68	121	1.78	44.4
R99	679	100	0.15	32.7

--------- Grafted Plants ---------				
Line used as a rootstock	Average nodule number	Total N from BNF (mg)	Nodule effectiveness (mg N per nodule)	N derived from N_2 fixation (%)
R32	1746	150	0.09	42.1
Puebla	747	126	0.17	38.0
Eagle	514	95	0.18	31.0
R99	0	0	--	0

This project was supported by a grant from

PULSE CRSP Dry Grain Pulses Collaborative Research Support Program MICHIGAN STATE UNIVERSITY

Methods

Nodulation, Plant N and ureide content, N from BNF

Four common bean lines (R99, Eagle, Puebla 152, and R32) were provided by Dr. Karen Cichy, USDA-ARS, East Lansing Michigan. R99 is a non-nodulating mutant and R32 is a nitrate-tolerant super-nodulating mutant derived from OAC Rico. Eagle is an Andean cultivar developed by Seminis Vegetable Seeds. Puebla 152 is a landrace from Mexico known for its superiority in symbiotic nitrogen fixation. Seeds were germinated on moistened paper towels at 20°C. Germinated seeds were transferred to peat pellets and incubated for five days in a controlled environment chamber at 24°C, light photon flux of 150 to 200 $\mu mol^{-2}s^{-1}$ PAR and 16h photoperiod. Vigorous seedlings were transplanted to commercial potting mix in 4-L free draining pots in a greenhouse at 25°C and 16h photoperiod. One week after transplanting, soil was inoculated with a peat-based BioStacked® inoculant. One week after inoculation, the soil was fertilized with 25 mL of solution containing 100 mg/L N (1.1% ammoniacal N, 11.8% nitrate N and 2.1% urea N (Peter's Excel Water Soluble Fertilizer). This amount of N promoted growth of non-nodulating R99 without inhibiting nodulation of Eagle, Puebla and R32. Plants were arranged in a completely randomized design and watered regularly. Plants were harvested at flowering/early pod set (40 days after planting). Roots were removed and soaked in water to facilitate removal of soil and minimize loss of nodules. Nodules were counted manually and were not examined for internal color to determine whether they were competent for N_2 fixation. Tissue N concentration was measured in ~125 mg of dried, ground tissue using Dumas complete combustion technique. Ureide concentration was determined according to Young and Conway (1942) with modifications (de Silva et al., 1996). Ureides were extracted from 25 mg of dried, ground tissue. Samples were homogenized in 1 mL 0.2N sodium hydroxide (NaOH), boiled at 100°C for 30 min, cooled and centrifuged at 10,000g for 5 min. The supernatant was stored at 10°C until ureide determination. Tissue extract was boiled in 0.5N NaOH for 8 min, cooled, boiled in 0.74N hydrochloric acid (HCl) and 3.3g/kg phenylhydrazine-HCl for 2 min, and cooled. Color was developed with the addition of concentrated HCl and 16.7g/kg KFeCN. Ureide concentration was determined spectrophotometrically against an allantoin standard at 520 nm within 15 min of addition of KFeCN Plant ureide and N derived from N_2 fixation in Eagle, Puebla, and R32 were estimated by difference from ureide and N contents in the non-nodulating line R99.

Grafting to alter %N from BNF and nodule effectiveness

A series of self- and reciprocal-grafts were made among the four common bean lines using each as a rootstock and scion. Vigorous seedlings with healthy unfolded primary leaves were selected for reciprocal grafts using the wedge graft technique. Hypocotyls of each rootstock were detached at mid-point with a razor. The base of the scion [upper hypocotyls with cotyledons] was cut to form a V-shape to fit in a 1-cm deep vertical split on the rootstock [lower half of the hypocotyl and roots]. The graft unions were secured using grafting tape. Grafted and non-grafted seedlings were returned to the growth chambers for three days and covered with transparent plastic covers to minimize transpiration. The successfully grafted plants were transplanted to pots, transferred to the greenhouse and maintained as indicated above.