Identification of snap bean genotypes with enhanced levels of nitrogen fixation.

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1 and 2 – Summary and introduction

Nitrate is the most common groundwater contaminant in Wisconsin (Kraft, 1994). Nearly 78,000 acres of snap beans (*Phaseolus vulgaris* L.) are produced in Wisconsin annually of which approximately 50% are located in the Central Sands region where the common soil type, Plainfield loamy sand, is susceptible to nitrate leaching. Recommendations for nitrogen fertilizer applications are 40 lbs/acre for a target yield of 1.5 to 6.5 tons (Laboski et. al., 2006). In commercial snap bean production, additional nitrogen fertilizer, either synthetic or organic, is commonly applied to counter abiotic and biotic stress, and nitrogen rates can commonly exceed 100 lbs/acre. The snap bean is a vegetable in the Fabaceae family and does have the capacity to fix atmospheric nitrogen through a symbiotic relationship with *Rhizobium* spp.; nevertheless, the historically easy access to relatively inexpensive nitrogen-based soil amendments has precluded the desire to develop cultivars with improved nitrogen-use efficiency (NUE). Modern snap bean cultivars have been bred without consideration of nitrogen fixation and are adapted to high input nitrate-based soil amendments. As snap bean breeders and organic producers, we have an obligation, an opportunity, and the ability to improve the NUE of the next generation of snap bean cultivars and recapture and enhance the snap bean's inherent ability to fix atmospheric nitrogen.

Our snap bean breeding program at the University of Wisconsin has developed genetic resistance to root rot disease in collaboration with the Midwest Food Processors Association (MWFPA). Additionally, in collaboration with OFRF, Flyte Family Farm, the MWFPA, and Pure Line Seeds, Inc., we have validated the use of OMRI-approved spinosad seed treatments as effective in controlling seed corn maggot (*Delia platura*). The MWFPA and snap bean processors recognize that the demand for organic snap beans far exceeds the supply. The snap bean industry, including seed producers, growers, processors and researchers integrate cooperatively through the MWFPA. The MWFPA and their Executive Director, Nick George, are very interested in responding to the desire of growers and processors to expand organic snap bean production in Wisconsin and are particularly concerned with nitrate leaching from vegetable production fields. The need to reduce the use of nitrogen-based fertilizers in snap bean production fields and enhance the snap bean's inherent ability to fix nitrogen has been presented and discussed at annual meetings of the Raw Products Committee of the MWFPA.

3. Objective statement

Our objective was to identify snap bean genotypes with enhanced nitrogen-use efficiency to reduce the need for application of supplemental nitrogen fertilizers.

5. Project results and materials and methods

Two inbred line populations, TR65 and TR67, were developed by backcrossing lines derived from the original cross between Eagle and Puebla 152. Eagle is a standard Andean snap bean variety developed in 1971 by Seminis Vegetable Seeds, Inc. (formerly Asgrow Seed Co.). Puebla 152, a black-seeded, Mesoamerican dry bean landrace from Mexico, was identified as a genotype with high levels of biological nitrogen fixation based on the aceteylene reduction assay (McFerson, 1983) and later supported by N-isotope analysis (Bliss el al., 1986). Puebla 152 is also the source for root rot resistance (Rosas et al., 1984; Nienhuis and Kmiecik, 1992). The TR67 and TR65 populations represent backcrosses of the original 'Eagle x Puebla' cross to the recurrent parent Eagle (thus providing 75% resemblance) and also to a more modern snap bean cultivar currently gown and processed extensively in Wisconsin, Hystyle. These populations were originally evaluated for nitrogen-use efficiency by Ben Hughey in his 2013 M.S. thesis, 'Snap bean breeding for enhanced nitrogen-use efficiency' – M.S. University of Wisconsin- Madison.

Our objective is to validate and identify snap bean genotypes with improved NUE that would have a large biomass (dry weight) when grown in a low N environment, thus precluding the need for additional application of nitrogen fertilizer.

The 'high' 'low' nitrogen plots were grown on the Hancock Agriculture Experiment station in Hancock, WI and also grown in an organic production field in cooperation with Flyte Family Farms in Plover, WI.

The genotypes and checks were grown in 3' plots with 15 plants/plot and ten bordered plants were harvested from each plot. The genotypes were gown in two nitrogen levels: low, in which no supplemental nitrogen was applied, and high, in which the plots were side-dressed before flowering with the equivalent of 40 units of nitrogen in the form on Urea. The evaluation of NUE is based on the following formula, termed Nitrogen Stability Index (NSI), as a measure of Nitrogen-use efficiency (NUE):

(Dry weight in high nitrogen – dry weight in low nitrogen), this difference divided by the dry weight in low nitrogen, all expressed as a ratio. Thus, the most NUE genotypes would have a NSI of zero, indicating their growth at low levels of nitrogen was close to equivalent to growth at high levels of nitrogen fertilizer.

The check cultivars included the following:

Eagle	A standard Andean snap bean variety developed in 1971 by Seminis Vegetable Seeds, Inc. (formerly Asgrow Seed Co.).
Hystyle	A newer widely grown Andean snap bean cultivar for processing. Developed by Harris-Moran Seed Co in the early 1980's
Huntington	A new Andean snap bean processing cultivar which is non-nodulating or poorly nodulating and thus "responds well to high input environments"
R99	A Mesoamerican dry bean, white seeded, non-nodulating mutant
Pueba 152	A black-seeded, Mesoamerican dry bean landrace from

	Mexico was identified as a genotype with high levels of biological nitrogen fixation
Note: not all checks were grown and evaluated in all locations and years.	

Results

Prior results: in 2011 and 2013 we evaluated 100 genotypes derived from the TR65 and TR67 inbred backcross populations and identified the breeding lines with the best nitrogen-use efficiency (Table 1).

Table 1. Ranking of best genotypes identified in an evaluation of nitrogen-use efficiency in 2011 and 2012 at the Hancock ARS. Genotypes in **bold**, were the genotypes validated as having high NUE in the present study, and thus provide an independent Assessment of NUE.

Hughey, Benjamin. 2012. Snap bean breeding for enhance nitrogen-use efficiency. M.S. thesis, Univ. of Wisconsin - Madison.

Genotype TR65		Genotype TR76
TR65-031-21100000 **TR65-055-111000000**		TR67-018-1110000 **TR67-015-21200000**
TR65-016-25100000		**TR67-029-1210000**
TR65-031-1110000		**TR67-006-1110000**
TR65-009-21200000		TR67-013-1110000
TR65-037-11100000		TR67-026-1110000
TR65-045-211000000		TR67-011-2110000
TR65-024-11100000		TR67-005-21100000
TR65-034-11100000BR		TR67-042-2210000
TR65-042-21100000		TR67-005-2210000

One of our principal objectives was to validate the nitrogen-use efficiency of these genotypes in a broader array of locations, including organic production (Flyte Family Farm). Evaluation of test genotypes and checks for nitrogen-use efficiency at two locations, Hancock ARS (conventional) and Flyte Family Farm (organic), indicated significant differences among genotypes, and no genotype x environment interaction (indicating consistent ranking of the cultivars (Table 2).

These contrasting conventional and organic production environments suggest that genotypes with high levels of NUE in conventional environments will also have high levels of NUE in an organic production system.

The mean NUE values were consistent with expectations, the largest NSI values (a measure of nitrogen-use efficiency) was associated with the non-nodulating check 'R99' – this large value indicates a large difference in growth (dry weight) when grown in a 'high' vs a 'low' nitrogen environment, which is consistent with this genotype being mutant for non-nodulation. The snap bean cultivars, 'Hystyle', 'Eagle' and the non-nodulating or poorly nodulating cultivar 'Huntington' were also tended to have large NSI deviations, indicating low levels of nitrogen fixation. The check cultivar selected for its high level of biological nitrogen fixation, Puebla 152, had a very low NSI deviation, likely due to it's ability to supplement growth in the low nitrogen environment with nitrogen derived by biological nitrogen fixation – this was our objective.

We did identify and validate that TR65 and TR67 inbred-backcross derivative lines had nitrogen-use efficiency (low NSI values) similar to the high biological nitrogen fixation check, Puebla 152 (Table 3). The high levels of NUE (low NSI) values is a validation of the lines previously identified as having superior NUE in the trials conducted in 2011 and 2012 by Benjamin Hughey in his 2013 thesis, and included **TR65-031-21100000, TR65-031-1110000, TR67-015-21200000, TR67-029-1210000 and, TR67-006-1110000** (Tables 1 and 3, respectively).

Table 2. ANOVA of nitrogen stability index (NSI) of snap bean genotypes evaluated as dry weight of seedlings grown in high and low levels of nitrogen at Hancock Ag. Research Station, Hancock, WI and Flyte Farms, Coloma, WI.

Source	d.f.	M.S.	P>f
Location	1	3497	<0.001
Rep(loc.)	4	939	-
Genotype	16	6697	0.11
Genotype x Location	16	5018	n.s.

error	60	273	

Table 3. Mean nitrogen stability index (NSI) of snap bean genotypes evaluated as the dry weight differential of platns grown in contrasting high and low levels of nitrogen at Hancock Ag. Research Station (conventional), Hancock, WI and Flyte Farms, Coloma, WI (organic).

Genotype	Mean ranking Student's t[z]				NSI[y] mean
TR65-055-111	A				**29.4**
R99	A	B			**21.8**
Hystyle	A	B	C		**21.1**
TR67-018-111	A	B	C		**17.2**
Eagle	A	B	C	D	**15.8**
TR65-016-251	A	B	C	D	**12.8**
Huntington	A	B	C	D	**12.4**
TR65-069-113		B	C	D	**9.8**
TR67-019-211		B	C	D	**7.8**
TR67-044-221		B	C	D	**7.3**
TR65-038-111		B	C	D	**7.2**
TR67-015-212		B	C	D	**5.9**
TR65-031-211		B	C	D	**3.9**
Puebla 152		B	C	D	**3.2**
TR67-006-111		B	C	D	**2.8**
TR67-029-121			C	D	**-0.0**
TR65-031-111				D	**-3.0**

[z] levels not connected by same letter are significantly different

[y] Nitrogen stability index = [(Dry weight high-dry weight low) / dry weight low]*100

The TR65 and TR67 derivative lines and checks were evaluated in multiple environments over multiple years, including evaluation in an organic production environment in cooperation with Flyte Family Farm, Coloma, WI in 2016. Over all locations and years, the check cultivars 'Eagle', 'Hystyle' and the non-nodulating check all tended to have larger NSI values, indicating, as expected, low nitrogen-use efficiency (Table 4). In contrast, lower NSI values were associated with the high-nitrogen fixing check, 'Puebla', indicating its ability to compensate for reduced supplemental nitrogen though enhanced noduation and nitrogen fixation (Table 4). Among the TR65 and TR67 derivative families with the lowest values of NSI (indicating enhanced nitrogen-use efficiency) were TR65-0310111, TR67-006-111 and Tr67-029-111 (indicated in **bold** in Table 4).

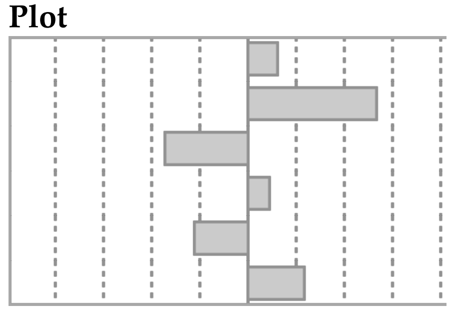
Table 4. Nitrogen Stability Index (NSI) values over locations and years.				
	Flyte Farm Organic 2016	Hancock ARS E5 2014	Hancock 2015	Hancock K18 2016
Eagle	8.4	54.1	31.6	23.3
Hystyle	15.2	5.3	33.4	26.9
Puebla 152	1.8	9.9	-10.4	4.7
R99	6.1	27.3	-	37.6
TR65-016-251	5.9	48.5	-18.0	19.7
TR65-031-111	**-15.4**	**6.06**	**-12.9**	**9.3**
TR65-031-211	-3.0	4.8	25.1	10.9
TR65-038-111	17.5	22.3	31.6	-3.0
TR65-055-111	10.0	-8.83	156.4	48.9
TR65-069-113	9.4	28.2	-15.3	10.1
TR67-006-111	**2.4**	**-2.1**	**-0.3**	**3.2**
TR67-015-212	11.1	40.7	14.9	0.7
TR67-018-111	13.5	-18.5	-7.4	20.9
TR67-019-211	3.0	64.0	-25.9	12.5
TR67-029-121	**-11.27**	**10.0**	**-36.1**	**11.2**
TR67-044-221	-8.2	-18.6	1.7	22.8

In spite of the general consistency of the data over locations and years, the rank correlations over years were generally low (Table 5). The largest positive rank correlation (0.53) was between the ARS and E5 location at the Hancock Ag. Res. Station location. The lack of greater consistency over locations is likely due to larger than expected experimental errors associated with the data. The magnitude of experimental errors may be reduced by using larger plots and including additional replications. Nevertheless, in spite of the large experimental errors, the TR65 and TR67 lines with enhanced nitrogen-use efficiency were generally among the lines with the lowest NSI values.

Table 5. Rank correlation over locations and years.

Variable	**by Variable**	**Spearman ϱ**	**Prob>|ϱ|**
Flyte	E5	0.1214	0.6664
ARS	E5	0.5321	0.0412*
ARS	Flyte	-0.3500	0.2009
K18	E5	0.0929	0.7420
K18	Flyte	-0.2286	0.4126
K18	ARS	0.2321	0.4051

Plot



Genotypes with enhanced nitrogen-use efficiency (measured as NSI) would be expected to have a nitrogen content in foliar tissue. The check cultivar 'Puebla 152) had a higher nitrogen concentration compared to standard snap bean cultivars 'Eagle', 'Huntington', and 'Hystyle'. This result is consistent with expectations of 'Puebla 152's enhanced ability to fix nitrogen. The pattern of nitrogen concentration for the TR65 and TR67 derivative lines was not consistently associated with lines which had low NSI values.

Table 6. Nitrogen concentration in plant tissue (ug/g) of checks and experimental lines.							
Genotype	Mean ranking Student's t[z]						Nitrogen content (ug/g)
TR67-018-111	A						1946
Puebla 152	A	B					1894
TR65-055-111	A	B					1877
TR67-006-111	A	B	C				1631
TR65-038-111	A	B	C	D			1537
TR65-016-251	A	B	C	D			1496
TR65-031-111		B	C	D			1436
TR67-029-121		B	C	D			1394
Eagle			C	D	E		1317
Huntington			C	D	E	F	1266
TR65-069-113			C	D	E	F	1208
TR65-031-211			C	D	E	F	1187
TR67-015-212			C	D	E	F	1128
TR67-019-211				D	E	F	1059
Hystyle					E	F	870
TR67-044-221						F	790
[z] levels not connected by same letter are significantly different							

6. Conclusions and discussion.

1) Field data of nitrogen stability index (NSI) suggests that nitrogen-use efficiency can be evaluated in both conventional and organic production systems.

2) Data on nitrogen content of summer 2016 trials is not yet completed. The samples are currently being dried and ground and should be completed in the spring of 2017.

3) The TR65 and TR67 derivative lines with the best nitrogen use efficiency include the following:

TR67-015-212
TR65-031-211
TR67-006-111
TR67-029-121
TR65-031-111

4) The derivative TR65 and TR67 lines which best combine plant, pod and nitrogen-use efficiency should be increased and trial seed distributed to snap bean seed companies, including Ball Seed Co, West Chicago, IL, Crites Seed Co, Quincy, WA and Pure Line Seed Co, Moscow, ID.

5) The five best performing lines, which best combine plant, pod and nitrogen-use efficiency will be planted in replicated trials at the Hancock Agriculture Research Station in the summer to 2017 and displayed at the Annual Meeting of the Midwest Food Processors Association meetings. This meeting is attended by all or nearly all snap bean seed companies; thus, this presentation will enhance opportunities for licensing of these lines with specific adaptation to low-input and organic snap bean production systems.

6) It may be necessary to backcross an additional generation followed by selection to continue to improve pod and plant characteristics of these nitrogen-use efficient lines.

7. Outreach.

We have sufficient remnant seed of the lines, which demonstrated enhanced nitrogen-use efficiency. We will plant these lines in a replicated trial in the summer of 2017 at the Hancock Agriculture Experiment Station in Hancock, WI and include this evaluation as part of the field day of the Midwest Food Processors meeting. The field day is usually attended by 150+ individuals and professionals representing the snap bean production, processing and seed industry. This will allow us to directly connect with seed producers, such as Crites Seed Co. and Pure Line Seed Co, both of whom are particularly interesting in producing snap bean cultivars specifically adapted to organic production.

We will also provide seed to local organic growers for evaluation by their customers, including:

1) Steve Pincus and Beth Kasmir, TIPI Produce, Evansville, WI

2) Dave Perkins, Vermont Valley Community Farm, Blue Mounds, WI

8. Financial Accounting

This is provided by the University of Wisconsin.

9. Leveraged resources

We have not obtained specific directed grants to continue the research on nitrogen fixation; nevertheless, we have included this research component as part of our formula funds associated with the Regional Hatch Project W-3150, which focuses on development of bean cultivars (dry and snap) adapted to biotic and abiotic stress.

10. REFERENCES

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11. Photos

Although slightly "rough" in terms of seed development and shape, which might preclude these lines for processing, the shape, size and color is acceptable for the fresh market, especially organic market and CSA's.


UW-16


UW-10