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Symbiotic N Fixation: Nodulation of Tepary Bean (*Phaseolus acutifolius* A. Gray)[1]

Research article

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Abstract

Tepary Bean (*Phaseolus acutifolius* A. Gray), is known to be a drought-tolerant grain legume and could play a positive role in supporting sustainable role in production of food and feed under changing climatic conditions due to its' ability to Symbiotically fix atmospheric N. However, existence of any rhizobial strain that could nodulate Tepary bean was unknown before the initiation of this study. We evaluated nodulation in 51 Tepary bean accessions after inoculation with a rhizobial strain (Phaseolus Spec #3) from Novozymes BioAg, Inc. to determine existence, if present, of nodulation. Our results indicated that Tepary bean could be successfully nodulated by using Phaseolus Spec #3 strain. We grew 51 Tepary bean accessions in a mixture of sand and vermiculite in two experiments and inoculated seeds with Phaseolus Spec #3 and recorded data on nodule size scores (1= small sized nodules similar in size to mustard seed; 2= medium sized nodules; varying from mustard seed size to soybean seed size and 3= large sized nodules greater in size than soybean seed), nodule number scores (1= less than 5 nodules; 2= more than 10 nodules; and 3= more than 20 nodules). A SPAD (SPAD-502, Minolta Corporation) meter was used to record relative chlorophyll in each plant prior to harvesting. A new variable named "Combined Score" was computed by summing values for nodule size scores, nodule number scores, and SPAD readings. The SPAD unit reading, nodule size score, nodule number score, and combined score means were 27.08, 1.48, 2.12, and 30.68, respectively. SPAD reading exhibited 0.44 and 0.61 correlation coefficient (significant at 1% level), respectively with nodule size score and module number score. Based on results of this study, we conclude that Tepary bean can be nodulated by Phaseolus Spec #3 rhizobial strain.

Keywords: Symbiotic N Fixation; Bradyrhizobium; *Phaseolus acutifolius*; Nodule number; Nodule size; SPAD reading

Introduction

Tepary bean (*Phaseolus acutifolius* A. Gray) is a desert legume native to the south western United States, Texas, New Mexico and Mexico. Tepary bean has served as a staple food for generations of prehistoric Native Americans [1]. Tepary bean's dry seed is consumed due its rich protein and carbohydrate content. "Tepary bean's dry seed is consumed due to its rich protein content varying from 17 to 27 percent and due to its rich carbohydrate content averaging 59 percent" [2]. Biochemical analysis of Tepary bean indicates that Tepary plants produce a high amount of soluble solids in the seed such as glucose and sucrose under sufficient or insufficient water supply [3]. Tepary bean plants flower within 27- 40 days after germination and ripen between 60-80 days [4]. Short duration wild Tepary bean varieties mature within two months in tropical climates. However, its growth period may extend up to 120 days in cooler areas such as in costal Algeria [4].

The United Nations Food and Agriculture Organization estimates that about 795 million people of the 7.3 billion people in the world, or one in nine, were suffering from chronic undernourishment in 2014-2016 (http://www.worldhunger.org/articles/Learn/world%20

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hunger%20facts%202002.htm). In many parts of the world there is increasing scarcity of water for agriculture [5]. Consequently, there is potential for the use of drought-tolerant legumes in agriculture not only for their Symbiotic N Fixation potential but also for reduced water use.

Tepary bean is considered one of the most heat and drought tolerant crops in the world [2]. This legume has the ability to flourish during intense drought with low annual rainwater requirements ranging from 500 mm to 1700 mm [4]. Tepary bean has been introduced to African agriculture due to its ability to mature from a single thunderstorm's down pour or single irrigation [4]. Researchers in Virginia have demonstrated that Tepary bean has the ability to produce well in the southern USA [6].

If proven successful, Tepary bean can be used as a sustainable crop for environmentally-friendly agriculture by lowering grower's dependence on synthetic nitrogen fertilizers. The use of nitrogen fixed by the Tepary bean may eliminate and or reduce applying synthetic inorganic nitrogen fertilizer, thus reducing the possibility of water pollution caused by run-off into streams, lakes, rivers and tributaries.

This study was conducted during 2006 and 2007 as a MS degree thesis project to identify rhizobial strains that can nodulate Tepary bean. At this time, no rhizobial strain was known to nodulate Tepary bean.

Materials and Methods

Before initiation of this study, existence of any rhizobial strain that could nodulate Tepary bean was unknown. After several attempts, EMD Crop BioScience, Inc. (Now Novozymes BioAg, Inc., Milwaukee, Wisconsin USA 53209) identified one rhizobial strain (Phaseolus Spec #3) as a potential inoculent for tepary bean and provided this strain for our studies. This strain was a mixture of Nitragin strain numbers 127D3, 127E12, and 127N2. Nodulation in 51 accessions of Tepary bean was studied in two experiments during 2006 and 2007 using Phaseolus Spec #3 rhizobial strain. The first experiment was planted on February 1, 2006 and harvested on March 11, 2006. The second experiment was planted on November 26, 2007 and harvested on January 22, 2008.

These experiments were conducted in the greenhouse at the Randolph Agricultural Research Station, Petersburg, Virginia. The

Table 1: Symbiotic N fixation related traits of tepary bean accessions upon inoculation with Phaseolus Spec #3 rhizobial strain.

No.	Accession	SPAD	Nodule Size Score	Nodule Number Score	Combined Score
1	W6-15687	19.32[a]	1.40[b]	2.20[c]	22.92[d]
2	PI-196932	30.00	1.50	2.50	34.00
3	PI-200749	21.38	1.40	2.20	24.98
4	PI-209480	24.60	1.60	2.60	28.80
5	PI-309881	26.22	1.67	2.00	29.88
6	PI-313205	23.80	1.80	1.80	27.40
7	PI-313488	20.60	1.25	2.00	23.85
8	PI-319443	24.03	2.00	2.00	28.03
9	PI-319447	29.05	1.17	2.00	32.22
10	PI-319551	28.94	1.20	1.40	31.54
11	PI-321637	27.74	1.20	1.80	30.74
12	PI-321638	18.14	1.00	1.80	20.94
13	PI-406633	25.28	1.20	2.00	28.48
14	PI-440785	25.42	1.50	2.00	28.92
15	PI-440788	31.28	1.80	2.40	35.48
16	PI-440789	38.64	1.40	2.40	42.44
17	PI-440792	26.60	1.00	2.20	29.80
18	PI-440793	25.78	1.50	2.00	29.28
19	PI-440795	26.98	1.25	2.00	30.23
20	PI-440799	24.27	1.50	2.00	27.77
21	PI-440800	24.06	1.60	2.20	27.86
22	PI-440801	20.65	1.00	2.00	23.65
23	PI-440802	32.42	1.60	2.20	36.22
24	PI-440803	31.24	2.00	2.20	35.44
25	PI-440804	33.57	1.83	2.00	37.40
26	PI-440806	33.10	1.40	1.80	36.30
27	PI-477032	17.62	1.33	2.17	21.12

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28	PI-477033	27.48	1.00	2.25	30.73
29	PI-477035	31.18	1.00	2.00	34.18
30	PI-477036	22.54	1.80	2.20	26.54
31	PI-477037	27.02	1.80	2.20	31.02
32	PI-477038	21.46	1.20	2.80	25.46
33	PI-502216	29.35	29.35	1.67	32.35
34	PI-502217	24.60	2.00	2.00	28.60
35	PI-527335	20.02	1.40	1.80	23.22
36	PI-533453	31.30	1.80	2.20	35.30
37	PI-533454	34.40	1.75	2.00	38.15
38	PI-533455	30.22	1.60	2.20	34.02
39	PI-535206	16.97	1.17	2.17	20.30
40	PI-535208	22.58	1.75	2.00	26.33
41	PI-535214	19.43	1.50	2.50	23.43
42	PI-535226	47.32	1.50	2.17	50.98
43	PI-535227	36.83	1.50	2.33	40.67
44	PI-535229	30.98	1.60	2.60	35.18
45	PI-549447	29.38	1.00	2.20	32.58
46	PI-549787	29.45	1.50	2.00	32.95
47	PI-638923	27.40	1.75	2.25	31.40
48	PI-638924	28.00	1.60	2.20	31.80
49	PI-638925	26.00	1.60	2.20	29.80
50	PI-638926	25.00	1.40	2.60	29.00
51	PI-640957	23.85	2.00	2.00	27.85
Mean		**27.08**	**1.48**	**2.12**	**30.68**
LSD(5%)		**9.39**	**ns**	**0.49**	**9.53**
R^2		**72.78**	**70.08**	**90.66**	**75.81**
CV(%)		**27.30**	**34.22**	**18.30**	**24.47**

a : Average of readings from ten randomly selected leaves using SPAD-502 chlorophyll meter (Minolta Camera Company)
b : Nodule number score: 1 = Less than 5 nodules; 2 = 5 to 20 nodules; and 3 = more than 20 nodules.
c : Nodule size score: 1 = small size nodules similar to mustard seed size; 2 = medium size nodules varying in size from mustard to soybean seed; and 3 = large size nodules larger than soybean seed.
d : A combined index developed by summation of SPAD reading. Nodule number score, and nodule size score.

experimental design used was a completely random design with three replications. Each combination of tepary line and *bradyrhizobial* strain Phaseolus Spec #3 was planted in three different pots (three replications). Thirty cm tall plastic pots were filled with moistened planting material (1:1 sterilized vermiculite and filtered sand). Two seeds were placed in each pot in a hole about 3 cm deep. A pipette was used to draw the liquid inoculant mixture out of a sterile container and then released from the pipette on the seed. This was done repeatedly for every set of three of the 51 lines. After saturation, the seeds were immediately covered with planting material and then watered liberally.

Data recording

At harvest, plants were harvested, their roots gently washed, and nodule size and nodule number scores were recorded each using the following scale: Nodule size scores (1= small sized nodules similar in size to mustard seed; 2= medium sized nodules; varying in size from mustard to soybean seed and 3= large sized nodules greater in size than soybean seed), nodule number scores (1= less than 5 nodules; 2= more than 10 nodules; and 3= more than 20 nodules). A SPAD (SPAD-502, Minolta Corporation) meter was used to record relative chlorophyll in each plant prior to harvesting. A new variable named "Combined Score" was computed by summing values for nodule size scores, nodule number scores, and SPAD readings.

All data were analyzed using SAS [7].

Results and Discussion

Results indicated that significant variation existed among 51 tepary bean accessions, after inoculation with rhizobial strain Phaseolus Spec #3, for all traits under consideration (Table 1) except for nodule size score, which was only significant at 10 % level of significance. The SPAD unit reading, nodule size score, nodule number score, and combined score means were 27.08, 1.48, 2.12, and 30.68, respectively. Values of Coefficients of Determination varied from 70 to 91 percent indicating that variation among 51 Tepary bean accessions accounted

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Table 2: Symbiotic N fixation traits of 51 tepary bean accessions upon inoculation with Phaseolus Spec #3 rhizobial strain.

Trait	SPAD[a]	Nodule Size Score[b]	Nodule Number Score[c]	Combined Score[d]
Erect Stem	27.29	1.47	2.12	30.89
Viny Stem	21.33	1.67	2.11	28.11
Mean	27.08	1.48	2.12	30.68
LSD(5%)	ns	ns	ns	ns
R^2	26.7	43.17	82.09	35.03
CV(%)	34.75	36.58	19.66	31.10
Narrow leaf	28.70	1.57	2.13	32.40
Broad leaf	26.23	1.43	2.12	29.78
Mean	27.08	1.48	2.12	30.68
LSD(5%)	2.41	0.14	ns	2.44
R^2	29.34	44.76	82.06	37.58
CV(%)	34.12	36.06	19.67	30.48
Seed Coat Color				
Black	32.41	1.58	2.13	36.12
Brown	23.46	1.44	2.06	26.96
Grey	19.32	1.40	2.20	22.92
Tan	27.62	1.51	2.11	31.25
White	26.33	1.44	2.14	29.91
Mean	27.08	1.48	2.12	30.68
LSD(5%)	6.47	ns	ns	6.55
R^2	32.79	43.80	82.19	40.51
CV(%)	33.69	36.83	19.85	30.13
Country of Origin				
Puerto Rico	36.81	1.53	2.35	42.69
Mexico	26.94	1.47	2.08	30.49
Costa Rica	26.22	1.67	2.00	29.88
USA	25.96	1.47	2.10	29.53
Nicaragua	24.60	1.60	2.60	28.80
El Salvador	21.38	1.40	2.20	24.98
Mean	27.08	1.48	2.12	30.68
LSD(5%)	7.95	ns	0.37	8.06
R^2	39.22	43.51	83.38	48.12
CV(%)	32.17	37.08	19.45	28.79

a : Average of readings from ten randomly selected leaves using SPAD-502 chlorophyll meter (Minolta Camera Company).
b : Nodule number score: 1 = Less than 5 nodules; 2 = 5 to 20 nodules; and 3 = more than 20 nodules.
c : Nodule size score: 1 = small size nodules similar to mustard seed; 2 = medium size nodules varying in size from mustard to soybean seed; and 3 = large size nodules larger than soybean seed.
d : A combined index developed by summation of SPAD unit reading, nodule number score, and nodule size score.

for major variation in traits under consideration.

We compared morphological traits of Tepary bean accessions (Table 2) and observed that plant growth type (erect or viny) did not affect nodulation traits in Tapery bean whereas existence of narrow leaves imparted significant advantage for nodulation traits over broad leaves: narrow leaved-accessions had significantly higher means for SPAD unit readings than accessions for broad leaves (28.70 vs. 26.23, respectively), nodule size score (1.57 vs. 1.43, respectively), and combined score (32.40 vs. 29.78, respectively). Accessions with black seeds had significantly higher SPAD unit readings and combined scores.

Country of origin of Tepary bean accessions (Table 3), affected

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nodulastion traits: accessions originating from Puerto Rico had 38.8 SPAD unit reading and 42.69 combined score both being significantly higher than for accessions originating from Mexico, Costa Rica, USA, Nicaragua, or El Salvador. Tepary bean accessions originating from these countries had SPAD unit readings and combined scores of 26.94 and30.49, 26,22 and 29.88, 25.96 and 29.53, 24.60 and 28.80, and 21.38 and 24.98, respectively.

The combined score also showed that of the 51 tepary bean accessions in the study, PI-535226 was the best cultivar for traits related to nodulation and nitrogen fixation. Using combined score, developed by combining various traits, the best tepary bean lines were PI-535526, PI-440789, PI-535227, PI-533454 and PI-440804. Based on the combined score, PI-535226 was most efficient for nodulation characteristics with a combined score of 50.98, followed by PI-440789 with a combined score of 42.44.

The Minolta Chlorophyll Meter (SPAD-502) has been extensively used to instantly measure the amount of chlorophyll content; an indirect measure of nitrogen content (http://www.specmeters.com/Chlorophyll_Meters/Minolta_SPAD_502_Meter.html). In these studies, mean SPAD unit readings varied from 17.0 to 47.3 with a mean of 27.08 (Table 1). The ten lines with highest SPAD unit readings were PI-535226, PI-440789, PI-535227, PI-533454, PI-440804, PI-440806, PI-440802, PI-533453, PI-440788, and PI-440803. Given that SPAD unit readings are related to nitrogen content of the leaves, we can conclude that these five lines benefited most from inoculation with the Phaseolus Spec #3 inoculum.

The means presented in Table 1 indicate existence of significant variation among 51 tepary bean accessions. This observation along with the observation that three accessions were observed to have SPAD unit readings beyond the positive side of mean ± 1 standard deviation demonstrate that some tepary bean accessions had functioning roots, otherwise these accessions could not have leaves greener than other accessions given that the only source of nitrogen was that from nodules.

Significant correlation existed between SPAD readings, nodule number score, and nodule size scores. SPAD readings exhibited 0.44 and 0.61 Pearson correlation coefficients (significant at 1% level), respectively with nodule size and nodule number score. Apparently, accessions with higher root nodules size or root nodule number implied functionality of these nodules by increased SPAD unit readings.

Based on results of this study, we conclude that Tepary can be nodulated by Phaseolus Spec #3 rhizobial strain. We spoeculate that it is possible that other rhizobial strain might be able to nodulate Tepary bean and this research should be expanded. It is also possible that identification of additional rhizobial strains for nodulation in Tepary bean could lead to existence of specificity between rhizobial strains and Tepary bean lines similar to what we observed in white lupin (*Lupinus albus* L.) during our previous research period [8]. Our results are contrary to those of Duggar [9].

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