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Popping and Yield Characteristics of Nuña Bean Lines Developed for Temperate Climates

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ABSTRACT

Nuña bean is a special market class of common bean (*Phaseolus vulgaris* L.) indigenous to the Andean mountains of Ecuador, Peru, and Bolivia. Nuña bean is often referred to as "popping" or "pop" beans because the seed expands rapidly when heated in oil. South American nuña bean cultivars are not adapted to production in the United States because they are photoperiod sensitive and have an aggressive climbing, indeterminate growth habit. The popping characteristic from nuña bean was successfully introgressed into 10 dry bean lines with determinate growth habit and adaptation to the temperate climates of North America. The objective of this research was to evaluate the nuña breeding lines for yield, popping ability, and seed size at three locations in Colorado. Two lines, CO49956 and CO49957, had the highest popping frequency (70 and 68%, respectively) among the lines tested. Mean seed yield among lines varied from 1321 to 1794 kg ha^{-1} and mean seed size varied from 34 to 44 g 100 seeds^{-1}. All lines had determinate growth habit with Type I architecture. Our findings indicate that four of the lines, CO49956, CO49957, CO50004, and CO49991, were the most productive and had the highest popping frequency. Nuña popping bean has potential for commercial on-farm production in suitable temperate locations and as a commercial product to the American consumer.

Nuña beans are a market class of common bean indigenous to the Andean mountains of Ecuador, Peru, and Bolivia 2000 to 3000 m above sea level (masl). They have been grown as a food crop dating back to Pre-Colombian times (http://naturalscience.com/ns/news/news38.html, verified 18 Apr. 2012). Nuña bean is often referred to as a popping or pop beans because the seed expands rapidly when heated 2 to 4 min in oil or a microwave oven (Fig. 1). Unlike popcorn, starch granules of nuña bean do not gelatinize or melt, and thus, do not contribute to popping ability (Spaeth et al., 1989). Expansion of cotyledon mesophyll tissue in nuña bean occurs in cell walls and occluded intercellular spaces, which results in a softening and rapid expansion of the entire seed. Nuña beans became an important food crop at high altitudes of Andean South America because the traditional methods of cooking beans by boiling in water for 2 to 4 h required extensive cooking fuel, and because water boils at a lower temperature in the high altitude regions, cooking occurs at a much slower rate than at lower elevations. In regions of the developing world where cooking fuel is limited, nuña bean may be advantageous to preserve precious cooking fuel resources.

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Nuña beans have unique potential as a snack food in the United States because they have a nut-like taste and malted-milk ball texture. They also have beneficial nutritional aspects of higher starch, amylase, and Cu than other dry bean market classes. Conversely, nuña bean has slightly lower digestibility than boiled dry beans (Spaeth et al., 1989; National Research Council, 1989). Even though nuña bean has potential as a new market class of dry beans in the temperate regions of the United States, cultivars developed in South America are not suitable for production because they are photoperiod sensitive and only flower during short daylength, and have a climbing growth habit not suitable for mechanical harvest (Zimmerer, 1992).

Nuña beans are classified as race Peru of the Andean Center of Domestication (COD) (Singh et al., 1991) of common bean. The Andean COD is one of two CODs that also includes the Middle American COD. Market classes of dry bean produced in the United States originate from both CODs, including pinto, great northern, and navy beans classified into the Middle American COD, and are characterized by small- to medium-sized seed, indeterminate semi-vine or upright growth habit. Light and dark red kidney, and cranberry market classes grown in the United States are classified into the Andean COD and are characterized by having large seed size, determinate growth habit, and early maturity. The nuña beans from race Peru of the Andean gene pool is characterized as having medium to large seed, indeterminate strong climbing growth habit, very late maturity, and short-daylength photoperiod sensitivity.

Tohme et al. (1995) recognized that nuña beans from the Andean region of South America were unsuitable for commercial production in temperate zones because they were photoperiod sensitive. To develop nuña beans for commercial production in the temperate zones, they suggested that popping ability be combined with bush (determinate) growth habit,

Abbreviations: COD, Center of Domestication.

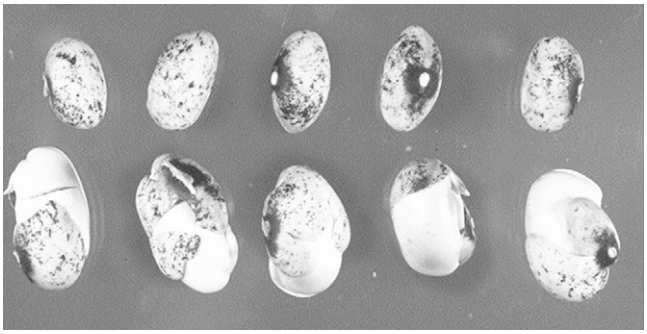

Fig. 1. Nuña bean seeds (top) before and (lower) after seed expansion from heating in a microwave oven for 2 min.

early maturity, and photoperiod insensitivity. In a previous study, we made single crosses between nuña bean germplasm and the commercial light red kidney bean cultivar 'Sacramento' to combine popping ability with photoperiod insensitivity, determinate growth habit, and adaptation to temperate environments (Ogg et al., 1998). The $F_{3:4}$ lines derived from single-crosses in that study had low popping frequency (8%); however, two lines had popping frequency >40%. These results demonstrated that single-crosses between nuña lines and the cultivar Sacramento resulted in limited recovery in popping frequency; however, many lines were adapted to temperate climates. Consequently a backcross system was used to increase popping frequency in lines with determinate growth habit and early (>105 d) plant maturity. The objectives are to report the performance of advanced backcross nuña bean lines that were developed for improved yield, popping ability, and seed size at three locations in Colorado.

MATERIALS AND METHODS

Advanced Line Development

Ten advanced backcross lines were developed by the Dry Bean Breeding Project at Colorado State University, Fort Collins, CO. Initially, two single crosses between nuña bean germplasm and the commercial light red kidney bean cultivar Sacramento (Sacramento/PI 293356 and Sacramento/Piemco) were made to combine popping ability with photoperiod insensitivity, determinate growth habit, and adaptation to temperate environments (Ogg et al., 1998). Two $F_{3:4}$ lines derived from the single-crosses were identified with moderate popping frequency (40–45%) and used as recurrent parents to backcross with nuña bean parents PI 316018 and Piemco. All nuña PIs were obtained from the USDA Western Regional Plant Introduction Station, Pullman WA, and the parental line we termed "Piemco" was obtained from a package of commercial nuña beans purchased in California that was imported from Peru, South America. The identity of the germplasm from which Piemco is derived is unknown. The resultant BC_1F_1 plants were increased in the greenhouse and BC_1F_2 progeny were planted in the field at Fruita, CO. Plants in the BC_1F_2 progeny segregated 3:1 for growth habit (indeterminate: determinate, $P < 0.05$) as expected because the recessive *fin* allele responsible for determinate growth habit segregated in the BC_1F_2 populations (Ogg et al., 2008). Ninety BC_1F_2 plants were selected for determinate (Type I) growth habit and photoperiod insensitivity, then planted to progeny rows the following year. Among the 90 $BC_1F_{2:3}$ progeny rows, 34 of the most adapted progeny rows were identified and 10 to 15 plants selected from each row based on early maturity and suitable agronomic traits. Seed from these plants was harvested and pedigree selection was imposed for popping frequency and agronomic traits for two generations in field nurseries at Fruita, CO. Ten $F_{4:5}$ advanced lines were selected and bulked for field testing at three Colorado locations, Fort Collins, Fruita, and Rocky Ford. Seed production of these advanced bean breeding lines was conducted at Fruita, CO, in 2004.

Field Test Environments

Fort Collins

The elevation at the Agricultural Research Demonstration and Extension Center, Fort Collins, CO, is 1554 masl and has an average of 142 frost-free days. The soil is a Fort Collins loam (superactive, mesic Aridic Haplustalfs). At this location, the experiment was a randomized, complete block design. Experimental units were 1.5 by 6.4 m (two 0.76-m rows). The previous crop in 2006 and 2007 was corn (*Zea mays* L.). Urea (46–0–0) was broadcast at a rate of 49.5 kg N ha^{-1} in 2006 and 56 kg N ha^{-1} in 2007 and incorporated with a roller harrow before planting. In 2006, S-metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide) was applied at a rate of 1.1 kg ha^{-1} a.i. and incorporated with a spike-toothed harrow. In 2007, EPTC (*S*-ethyl dipropylthiocarbamate) was applied 1 June 2007 before emergence at a rate of 1.96 kg ha^{-1} a.i. and incorporated with a spike-tooth harrow. Mid-season weed-control was provided by using a tank-mix of imazamox (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid), bentazon (3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide), dimethenamid ((RS) 2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl)acetamide), and clethodim ((E,E)-(±)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) at rates of 0.035 kg ha^{-1} a.i., 1.12 kg ha^{-1} a.i., 0.63 kg ha^{-1} a.i., and 0.19 kg ha^{-1} a.i., respectively.

Planting occurred 1 June 2006 and 27 May 2007 using a four-row precision planter. The seeding rate in both years was approximately 195,000 seeds ha^{-1}. The field was cultivated 8 July 2006 and 1 July 2007. In 2006, the field was furrow-irrigated and in 2007 the field was irrigated with a linear-move sprinkler system. In both years the field was irrigated as needed to maintain available soil moisture at an optimum for plant growth. Plots were undercut and windrowed with a rod cutter windrower on 15 and 17 Oct. in 2006 and 2007, respectively, and threshed with a Vogel stationary thresher on 25 and 15 Oct. in 2006 and 2007, respectively.

Fruita

The elevation is 1402 masl with average frost-free growing season of 180 d. The soil is a Billings silty clay loam [fine-silty, mixed (calcareous), mesic Typic Torrifluevents]. The experiment was a randomized, complete block design with three replicates. Plot size was 1.5 m wide by 7.6 m long (two 0.76-m rows). In 2006, the field was fallowed the previous year and in 2007 the previous crop was corn. Diammonium

phosphate fertilizer (18–46–0) was broadcast applied at rate of 224 kg ha^{-1} before planting. A tank mix of dimethenamid-P [(*S*)-2-chloro-*N*-[(1-methyl-2-methoxy)ethyl]-*N*-(2,4-dimethyl-thien-3-yl)-acetamide] at 0.63 kg ha^{-1} a.i.and 1.96 kg ha^{-1} a.i. of EPTC (*S*-ethyl dipropylthiocarbamate) was applied in 187 L ha^{-1} at 172 kPa. Immediately following application of the herbicides two incorporation passes were performed with a roller harrow (cultipacker).

Planting occurred on 8 June 2006 and 31 May 2007 with an air planter modified for planting plots. Seeding rate was approximately 221,000 seeds ha^{-1}. The experiment was furrow-irrigated as needed during the growing season and irrigation water was not a limiting factor for crop production in both years. Plots were cut with a Pickett One-Step rod cutter windrower on 1 Nov. 2006 and 12 Oct. 2007 and threshed on 2 Nov. 2006 and 19 Oct. 2007 using a small plot combine equipped to harvest dry beans.

Rocky Ford

The elevation at Rocky Ford is 1274 masl and has an average frost-free growing season of 179 d based on a threshold temperature of –1.9°C. The soil is a Rocky Ford silty clay loam (mixed, mesic Ustic Torriorthents). The experiment was a randomized, complete block design with three replicates. Plot size was 1.5-m wide by 7.6-m long (two 0.76-m rows). In 2006, the previous crop was soybean [*Glycine max* (L.) Merr.] and in 2007 the previous crop was corn. Two hundred twenty-four kilograms of 11–52–0 ha^{-1} (24.6 kg N and 116 kg P ha^{-1}) were broadcast and incorporated before planting in 2006 and 2007. Weeds were controlled in 2006 using tillage and with hand hoeing. Before planting in 2007, a tank mix of glyphosate [*N*-(phosphonomethyl)glycine] at 1.1 kg ai ha^{-1} and [(*S*)-metolachlor] at 1.2 L ha^{-1} a.i. with ammonium sulfate at 1% solution was applied on 21 May 2007 using 187 L ha^{-1} at 172 kPa. As in 2006, hand hoeing was used a needed to control weeds.

Planting occurred on 25 May 2006 and 29 May 2007 using a 900 International plot planter. Seeding rate was approximately 222,395 seeds ha^{-1}. Lambda-cyhalothrin insecticide, [1α(S*), 3α(Z)]-(±)-cyano-(3-phenoxyphenyl)methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate, was applied on 27 July 2006 at a rate of 26.4 mg a.i. ha^{-1} and esfenvalerate insecticide, (S)-cyano(3-phenoxyphenyl) methyl (S)-4-chloro-α-(1-methylethyl) benzeneacetate, on 27 July 2007 at 34.8 mg a.i. ha^{-1} to control a Mexican bean beetle (*Epilachna varivestis*) infestation. The experiment was furrow-irrigated as needed during the growing season and irrigation water was not a limiting factor for crop production in both years. Field plots were hand harvested on 24 Oct. 2006 and 9 Oct. 2007 and threshed with a Vogel stationary thresher on 2 Nov. 2006 and 19 Oct. 2007.

Popping Evaluations

Seeds from all advanced breeding lines were evaluated for popping frequency within 3 mo of harvest according to procedures described by Ogg et al. (1998). That procedure used seeds harvested from yield trials, placed in a ceramic crucible and microwave cooked at 50% power for 2 min. Ten sets of 10-seed samples from each replicate were evaluated and the sum of popped beans was used to calculate popping frequency as percent popped seeds.

Statistical Analyses

Analyses of variance were performed for seed yield, popping frequency, and seed weight using the SAS Mixed procedure (SAS Institute, 2008). The mixed model assumed that years and replicates nested in location and years were random model factors, with entry and location assumed as fixed factors Least square pairwise means for all variables were tested using the pdiff option in the LSMEANS command. Pair-wise differences were judged significant when *P* values were <0.05. Least square means for Fort Collins and Fruita were based on the average over years and replicates.

RESULTS AND DISCUSSION

The 10 advanced nuña breeding lines were evaluated in Colorado at three locations in 2006 and 2007. Probability values for the main effects of nuña line and location, and nuña line × location interactions for nuña popping, seed yield, and seed size are presented in Table 1. The main effects for nuña lines and location were significant for seed yield, popping frequency, and seed size. The line × location interaction for popping frequency and seed weight were also significant.

Popping Frequency

Popping frequency among lines varied from 81 to 17% across locations (Table 2). Mean popping frequency across locations and lines was 53%. The highest mean popping frequency (69%) occurred at Rocky Ford followed by Fort Collins (59%) and Fruita (31%). The heritability of popping frequency of nuña bean has been shown to be high (0.87±0.07) based on the heritability of temperate-adapted nuña bean populations developed in Wisconsin (Vorwald and Nienhuis, 2009b).

We considered location effects as a fixed model factor in this study because we wanted to test the specific environments for effects on yield, popping frequency, and seed weight. The data suggested that Rocky Ford is a better environment for high popping frequency in nuña beans; however, only 1 yr of data was collected at that site compared to 2 yr for Fort Collins and Fruita. Lines CO49956 and CO49957 had the highest popping frequency among the 10 lines (70 and 68%, respectively). While there was an overall line × location interaction for popping frequency, CO49956 and CO49957 had the highest frequency at each of the three locations. The highest observed popping frequency was 81%, by line CO49957 at Rocky Ford.

Table 1. Probability values for Type I error for popping frequency, seed yield, and seed weight for line, location, and line × location interaction from the mixed model analysis of variance.

Source	Degrees of freedom	*P* values		
		Popping frequency	Seed yield	Seed weight
		%	kg ha^{-1}	g 100 seed^{-1}
Nuña breeding line	9	<0.0001	0.001	<0.0001
Location	2	<0.0001	0.013	<0.0001
Nuña line × Location	18	<0.0001	0.4346	0.001

Table 2. Least square means for popping frequency among nuña lines grown at Fort Collins, Fruita, and Rocky Ford, CO, in 2006 and 2007.

	Popping frequency			
	Location			
Nuña line	Fort Collins	Fruita	Rocky Ford†	Mean*
	%			
CO49956	78a	55a	77a	70A
CO49957	79a	44b	81a	68A
CO49984	66b	27d	74ab	56B
CO49990	62b	35c	67b	55B
CO50004	67b	32cd	65b	55B
CO49979	50c	32cd	66b	49C
CO49982	56c	26d	66b	49C
CO49978	50c	25d	69b	48C
CO49961	40c	18e	65b	41D
CO49991	44cd	17e	59b	40D
Mean	59B	31C	69A	53

* Simple effects least square means separated by different lower-case letters and main effects least square means separated by different upper-case letters are significantly different at $P \leq 0.05$ according to pdiff command in Proc Mixed analysis in SAS.

† Popping frequency at Rocky Ford 2007 not included in analysis due to stand loss.

Table 3. Least square means for seed yield among nuña lines grown at Fort Collins, Fruita, and Rocky Ford, CO, in 2006 and 2007.

	Seed yield			
	Location			
Nuña line	Fort Collins	Fruita	Rocky Ford†	Mean*
	kg ha^{-1}			
CO50004	1861a	1539a	1982ab	1794A
CO49991	1796ab	1402ab	2151a	1783A
CO49979	1359b	1412ab	1928ab	1566AB
CO49978	1332b	1396ab	1912ab	1547AB
CO49984	1583ab	1027b	1728ab	1446B
CO49957	1495ab	1030b	1778ab	1434B
CO49956	1253b	1204ab	1811ab	1423B
CO49990	1404ab	901b	1816ab	1374B
CO49982	1419ab	1112b	1550b	1360B
CO49961	1358b	859b	1745ab	1321B
Mean	1387B	1185B	1840A	1471

* Simple effects least square means separated by different lower-case letters and main effects least square means separated by different upper-case letters are significantly different at $P \leq 0.05$ according to pdiff command in Proc Mixed analysis in SAS.

† Seed yield at Rocky Ford 2007 not included in analysis due to stand loss.

Seed Yield

Seed yield varied from 859 to 2151 kg ha^{-1} among lines at the three locations (Table 3). Mean seed yield across locations and lines was 1471 kg ha^{-1}. The highest mean seed yield, 1840 kg ha^{-1}, occurred at Rocky Ford while seed yields at Fort Collins and Fruita did not differ (1387 and 1185 kg ha^{-1}, respectively). Across locations, lines C050004 and CO49991 had higher seed yield than the lowest six lines, however they did not differ in yield from CO 49979 and CO49978. Because the line × location interaction was not significant for seed yield, the response of the 10 advanced nuña lines was similar across the three diverse Colorado locations.

The parent Sacramento was included in some location years and the average yield of Sacramento across locations and years was 777 kg ha^{-1}. By comparison, the nuña yields were greater than Sacramento. Nevertheless, these nuña bean seed yields are lower than most yields of other dry bean classes that are grown in Colorado.

Seed Weight

Seed weight varied from 29 to 51 g 100 seeds^{-1}among lines (Table 4). Mean seed weight of lines across locations was 37 g 100 seeds^{-1}. The highest mean seed weight among lines was produced at Fort Collins and Rocky Ford at 38 and 41 g 100 seeds^{-1}, respectively, while the smallest seed weight was produced at Fruita at 32 g 100 seeds^{-1}. When averaged across locations, the largest seeds were produced by CO49956 and CO49957, lines that also had the highest mean popping frequency. A significant line × location interaction occurred for seed weight; however, both CO49956 and CO49957 produced the heaviest seed at all three locations. Seed size is a valuable criterion for marketing dry beans. All market classes of dry bean have a specific range of seed size that determines commercial acceptance. The market class for nuña beans in temperate zones has not been established and our data on seed size aids in establishing a baseline for nuña beans as a market class in the United States as seed size is an important descriptor of seed quality for any dry bean market class, including nuña. Vorwald and Nienhuis (2009a) noted that the popping percentage and the degree of nuña bean seed expansion was not correlated with seed weight, but they noted that direct selection should be effective in increasing seed weight of nuña bean.

The popping characteristic was introgressed into dry bean lines that possessed determinate growth habit and photoperiod insensitivity which provided adaptation to the temperate climates in Colorado. Even though the popping percentage of these breeding lines was <100%, they represent advancement for popping characteristics and additional testing for optimum environments may provide popping frequencies that are commercially acceptable. The lines may need additional breeding to improve stability across environments for agronomic characteristics suited to North America.

During the development of these lines, photoperiod insensitive material was recovered after the first backcross;

Table 4. Least square means for seed weight among advanced nuña lines grown at Fort Collins, Fruita, and Rocky Ford, CO, in 2006 and 2007.

	Seed weight			
	Location			
Nuña line	Fort Collins	Fruita	Rocky Ford†	Mean*
	g 100 seed^{-1}			
CO49956	44a	38a	49a	44A
CO49957	44a	35b	51a	43A
CO49978	36b	29c	39c	35B
CO49990	36b	29c	39c	35B
CO49984	33c	31c	41bc	35B
CO49961	33c	29c	44b	35B
CO50004	38b	29c	36c	34B
CO49991	34c	30c	38c	34B
CO49979	37b	29c	37c	34B
CO49982	36b	29c	37c	34B
Mean	38A	32B	41A	37

* Simple effects least square means separated by different lower-case letters and main effects least square means separated by different upper-case letters are significantly different at $P \leq 0.05$ according to pdiff command in Proc Mixed analysis in SAS.

† Seed weight at Rocky Ford 2007 not included in analysis due to stand loss.

however, none of the inbred lines had 100% popping frequency. After selection for adaptation, maturity, and determinate growth habit, most lines were reasonably adapted to production in Colorado. CO49956 and CO 49957 had the highest popping frequency at all the three locations; consequently, they represent lines that should be considered for release or for breeding material to enhance popping frequency, agronomic performance, and disease resistance. Lines CO50004, and CO49991 also had high yield and moderate popping frequency; consequently, they may also contribute to development of commercially useful material.

The nuña germplasm reported herein is susceptible to most pathogens that attack dry beans including, bean common mosaic virus and bean common mosaic necrosis virus, foliar rust [caused by *Uromyces appendiculatus* (Pers.) Unger], bacterial common blight [caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye, Syn. with *X. campestris*], and halo blight [caused by *Pseudomonas syringae* pv. *phaseolica* (Burkh.) Dows] (Schwartz et al., 2004). Additional plant breeding will be required to reduce or eliminate the susceptibility of these lines to these pathogens.

The production environment affected the amount of popping of the 10 advanced lines and the location selected for future commercial production of popping beans needs to be carefully considered to obtain high popping frequency, high seed yield, and large seed size.

Based on personal taste by the authors, nuña lines in this study had a nut-like taste and malted-milk ball texture. Maintaining these characteristics in the development of nuña bean will remain important. The unique characteristics of nuña popping bean give it potential as a snack food in the United States. The protein level of nuña beans found in previous studies is similar to the range observed in other market classes of *Phaseolus vulgaris* (van Beem et al., 1992). Thus, as a snack food, nuña bean would provide protein that is not found in many snack foods. Furthermore, a snack food with a nut-like taste and the texture of malted-milk ball is likely to have consumer appeal. We conclude that nuña popping bean has potential for commercial on farm production in suitable temperate locations and as a commercial product to the American consumer and others.

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