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## ORIGINAL RESEARCH ARTICLE

## Crop Breeding &amp; Genetics

## Agronomic performance and cooking quality characteristics for slow-darkening pinto beans

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**Abstract**

Slow-darkening (SD) pinto beans (*Phaseolus vulgaris* L.) possess a desirable new trait, conditioned by the recessive *sd* gene, that slows seed coat darkening under delayed harvest and under storage. The effect *sd* may have on performance needs investigation. We examined agronomic performance and cooking quality of SD pinto beans. There were 30 (15 SD and 15 regular darkening [RD]) recombinant inbred lines (RILs) from each of two biparental inbred populations. The 60 RILs were tested across three locations in North Dakota and Washington. In addition, advanced SD and RD pinto breeding lines were tested in trials from 2010 to 2012 and in 2018. Across 2010–2012 trials, the “early generation bred” SD pintos, as a group, had significantly lower emergence, increased lodging, less seed yield, and smaller seed size than the RD group. Conversely, in the 2018 trial, “recently bred” SD pinto breeding lines had competitive agronomic performance to RD lines for seed yield, reduced lodging, and increased emergence. Further research on cooking time is warranted given that SD RILs cooked 20% faster than the RD RILs in one population. Overall, SD pintos exhibited slightly better canning quality than RD pintos. Whether raw or cooked, SD pintos were much lighter in color than RD pintos, emphasizing the need to keep them separated as distinct market classes. Breeders should continue to focus on improving agronomic performance for emergence, lodging, seed yield, seed size, and canning quality of SD pinto beans.

**1 | INTRODUCTION**

Pinto bean (*Phaseolus vulgaris* L.) is the most important edible dry bean market class annually produced (0.55 Tg; USDA-NASS 2015) and consumed in the United States and is one of the top three market classes produced in Mexico (0.25 Tg). Pinto bean is produced or imported for

consumption by many other countries across the globe as well, including Brazil, the Dominican Republic, Colombia, Japan, Kenya, and Lesotho, to name a few. Pinto bean originates from the semiarid highlands of Mexico and is assigned to the Durango race within the Middle American gene pool (Singh, Gepts, & Debouck, 1991). Pinto beans are highly productive, with higher seed yields than smaller (navy, black) and larger (kidney) seed-sized market classes grown in the United States (Singh et al., 2007). In addition to pinto beans with higher yields (Brick & Grafton, 1999; Vandemark, Brick, Osorno, Kelly, & Urrea, 2014), breeders

**Abbreviations:** CDBN, Cooperative Dry Bean Nursery; QTL, quantitative trait loci; RD, regular darkening; RIL, recombinant inbred line; SD, slow darkening; SSR, simple sequence repeat.

have incorporated resistance to numerous diseases (Singh & Schwartz, 2010; Terán, Lema, Webster, & Singh, 2009) and improved harvestability by achieving more upright architecture (Kelly, 2001; Soltani et al., 2016). Seed quality of pinto bean is assessed by visual factors such as size, shape, and color. Medium-sized seeds (35–45 g 100 seeds<sup>-1</sup>) is a common characteristic for beans within the Durango race (Brick & Grafton, 1999). Pinto bean is further characterized by a flattened rhombohedral seed shape with tan to light brown background color overlain by a mottled pattern of darker brown color (Bassett, 2007; Freytag and Debouck, 2002). Postharvest changes in seed color, characterized by a gradual darkening of the light cream background color, can have a detrimental effect on pinto bean quality. The seed coat darkening worsens with time in storage (Junk-Knievel, Vandenberg, & Bett, 2007). Rapid darkening of the seed coat can also result from delayed harvests due to adverse weather conditions (Osorno et al., 2017). The price for pinto bean with darkened seed coats is discounted because consumers presume them to be older and slower cooking (D. Fuglesten, Central Valley Bean Co-Op, personal communication, 2020).

During the last 15–20 yr, a trait that slows seed coat darkening was discovered, characterized, and is being widely deployed into new commercial pinto bean cultivars. The trait is controlled by a single recessive gene *sd* (Elsadr, Wright, Pauls, & Bett, 2011; Junk-Knievel, Vandenberg, & Bett, 2008) located on chromosome Pv07 (Felicetti et al., 2012) near the *P* gene (Alvares et al., 2019; Islam, Marsolais, & Dhaubhadel, 2018), which conditions seed coat color (Bassett, 2007; McClean et al., 2018). The first slow-darkening (SD) “bright pinto” cultivars ‘Saltillo’ (Sanchez-Valdez, Acosta-Gallegos, Ibarra-Perez, Rosales-Serna, & Singh, 2004) from Mexico and ‘CDC White Mountain’ (formerly breeding line 1533-15) from Canada released in 2009 (Canadian Food Inspection Agency [CFIA] Registration no. 6606), have been sought by consumers. Another source for *sd* is the SD University of Idaho pinto bean germplasm release SDIP-1 (Singh, Terán, Lema, Dennis, & Hayes, 2006). Allelism tests and linked marker analyses together have shown that the three genotypes possess the same *sd* gene (Felicetti et al., 2012; Junk-Knievel et al., 2008). Although these cultivars and lines exhibit desirable seed color characteristics, they do not have optimum agronomic performance when grown in U.S. production regions. Saltillo is photoperiod sensitive and thus cannot be produced in the United States because it will not flower under the long-day-length conditions during the growing season. CDC White Mountain has bush determinate Type 1 growth habit and early maturity, good traits for regions of Canada with shorter growing seasons, but those same traits contribute to low seed yield potential in the United States under longer growing seasons, making

it less attractive to growers. Similarly, SDIP-1 exhibits low yield potential, smaller seed size with a more cylindrical shape, and prostrated growth habit, which is undesirable for mechanical harvesting. These shortcomings and others have challenged breeders in developing SD pinto bean cultivars with either competitive or superior agronomic performance. Our objectives were to examine agronomic performance and cooking quality in SD pinto bean breeding lines and populations.

## 2 | MATERIALS AND METHODS

### 2.1 | Recombinant inbred populations performance trials

Two F<sub>2</sub> populations (Population I, 23ST; Population II, SF108), used to tag the *sd* gene with simple sequence repeat (SSR) markers (Felicetti et al., 2012), were subsequently developed into recombinant inbred populations of F<sub>5,7</sub> lines by single seed descent method to examine effect of the SD trait on agronomic performance and cooking quality traits. The parents and pedigrees, well described by Felicetti et al. (2012), are briefly presented here. Population I derives from Z0818-23/‘Stampede’. The SD Z0818-23 F<sub>4</sub> breeding line possesses the *sd* gene from a four-way cross OT0643-44/OT0635-14//SDIP-1/OT0643-79 involving three advanced regular-darkening (RD) pinto breeding lines from the USDA-ARS bean breeding program at Prosser, WA, and the *sd* source SDIP-1 (Singh et al., 2006). Stampede pinto (Osorno, Grafton, Rojas-Cifuentes, Gelin, & Vander Wal, 2010) combines high yield potential with upright architecture, resistance to lodging, and multiple disease resistance. Population II derives from ‘Santa Fe’/PS08-108. The SD PS08-108 F<sub>3</sub> breeding line derives from PT7-1/4/Z0720-54/3/PT7-2//1533-15/PT7-2, which includes advanced RD pinto breeding lines from the USDA-ARS program and the *sd* source 1533-15 (i.e., CDC White Mountain). Santa Fe (Kelly, Varner, & Long, 2010) has large seeds and multiple disease resistance. Although Santa Fe is categorized as a commercial pinto, its darkened seed appearance has kept it from becoming a popular, widely grown cultivar.

Population I consisted of 51 recombinant inbred lines (RILs): 29 RD, 18 SD, and four segregating as determined by the accelerated aging ultraviolet light test (Junk-Knievel et al., 2007). Population II consisted of 49 RILs: 22 RD, 22 SD, and five segregating as observed by the ultraviolet test. Homozygous vs. heterozygous presence of *sd* gene was confirmed by DNA assays for linked SSR markers following the protocol of Felicetti et al. (2012). Fifteen homozygous SD and 15 homozygous RD RILs were randomly chosen from each population and then combined

together in a yield trial planted at three locations in 2012: Othello, WA, and Hatton and Prosper, ND. The Washington State University Research Farm in Othello (46°49' N, 119°10' W) has a silt loam (Inceptisol) soil type. Othello represents the southern most commercial dry bean production zone within the Columbia River basin in Washington State. Prosper (47°00' N, 96°47' W) and Hatton (47°37' N, 97°23' W) represent low and middle portions of the Red River Valley, which spans the common border between North Dakota and Minnesota and represents the most prominent dry bean production region in the United States. Soil at Prosper was a Kindred–Bearden silty clay loam (I373A; fine-silty, mixed, superactive, frigid Typic Endoaquolls and fine-silty, mixed, superactive, frigid Aeric Calciaquolls, respectively), with pH 7.6 and 3.0% organic matter. Soil at Hatton was a Glyndon silt loam (Gm; coarse-silty, mixed, superactive, frigid Aeric Calciaquolls) with pH 8.0 and 3.0% organic matter. The trials included both *sd* donor sources (SDIP-1 and CDC White Mountain) and both commercial pinto parents (Stampede and Santa Fe) used in the last cross of each population. The 60 RILs and four parental lines were arranged in an eight-by-eight square lattice design with three replications. The Othello trial was planted on 13 June 2012, using four-row plots with 3-m length and 0.56-m row spacing. Hatton and Prosper were planted on 18 and 25 May 2012, respectively, using two-row plots each 3.6 m long. All trials were managed (herbicide, fertilizer, cultivation, and irrigation [Othello only]) for high yield potential. However, North Dakota trials did not have supplemental irrigation, since most dry bean production in this region is rainfed.

Seed weight ( $\text{g } 100 \text{ seeds}^{-1}$ ) and yield ( $\text{kg ha}^{-1}$ ) adjusted to 14% seed moisture were obtained from the center two harvested rows in Othello, and the entire two-row plots were harvested in Hatton and Prosper. Harvest maturity (d after planting) was recorded in Othello and Prosper. Canopy height (cm) was measured at the R2 to R3 mid-pod-fill growth stage (Schwartz & Langham, 2010) in the North Dakota trials. Emergence was scored at the V2 growth stage on a scale from 1 = complete to 9 = no plants emerged, and lodging was scored at physiological maturity (R7 to R8) on a scale from 1 = no lodging to 9 = completely lodged, on a plot basis in Othello.

For the Othello and Hatton trials, harvested seeds from plots for five SD and five RD RILs randomly chosen from each population were sent to the USDA-ARS Food Legume Quality Genetics Laboratory in East Lansing, MI, for cooking time analysis. For both locations, the harvested seed, placed in brown paper bags, was maintained on shelves at ambient temperatures in short-term seed storage facilities for ~280 d before they were cooked. For each line, a sample of 30 seeds were weighed and soaked for 12 h in deionized water. After 12 h, water was drained, and seed weight

was measured. Next, 25 of the soaked seeds were cooked with a Mattson bean cooker in boiling deionized water (Wang & Daun, 2005). The cooking time was recorded for the time needed for 80% of the plungers to pierce the seeds. A colorimeter, Hunter Labscan XE (Hunter Associate Laboratory) was used for extracting color parameters from raw and cooked beans. For analysis, three measurements of color were extracted: CIELAB color space channels  $L^*$  (lightness from black to white),  $a^*$  (from green to red), and  $b^*$  (from blue to yellow).

These agronomic and cooking time data, combined across locations, were analyzed by PROC GLIMMIX, (SAS Institute, 2017). Lines and locations were considered fixed, and replications and blocks were considered as random effects. Least square means were generated and compared within and across populations and parents using contrasts. The Tukey–Kramer pairwise test ( $P < .05$ ) was used to identify significant differences among line means.

## 2.2 | Advanced breeding lines performance trials

Field performance trials, which included both RD and SD advanced pinto bean breeding lines and cultivars, were conducted in 2010 (13 RD and 10 SD lines), 2011 (advanced yield trial = 4 RD and 8 SD; preliminary yield trial = 26 RD and 23 SD lines), 2012 (8 RD and 10 SD lines), and 2018 (26 RD and 19 SD lines) at Othello, and in 2012 (4 RD and 19 SD lines) at Prosper, Hatton, and Johnstown. These materials were bred separately from the RIL populations except for two individual RILs, 23ST-27 (Population I) and SF103-8 (Population II), which were eventually released as new SD pinto bean cultivars ‘Scout’ (unpublished) and ‘ND-Palomino’ (Osorno et al., 2017), respectively. For these performance trials, plots were arranged in randomized complete block design with three to four replications depending on seed availability. An individual plot contained four rows with 0.56-m spacing and 6-m length in Washington and four-row plots of 3.6 m each row and 0.91 m between rows for North Dakota trials. The trials were managed for optimum seed yield as described above. Harvest maturity, seed weight, and yield for both Washington and North Dakota trials, and emergence and lodging scores for Washington and canopy height for North Dakota trials were obtained as described above. Different RD and SD pinto breeding lines and cultivars and combinations were tested across these trials; therefore, these data are analyzed by PROC GLM (SAS Institute, 2017) and summarized separately by year and state. A contrast was used to examine whether the group of RD lines differed ( $P < .05$ ) from the group of SD lines tested within each trial for the different traits measured.

Canning tests were also conducted on seed harvested from some of these advanced performance trials. The seed from two replications for select lines from the 2011 and 2018 Washington performance trials were canned by the USDA-ARS Dry Bean Food Legume Quality Laboratory (East Lansing, MI) about 40–50 d postharvest. Canning quality was measured on a 90-g sample using a small scale canning protocol (Hosfield, Uebersax, & Isleib, 1984) and in 2018 on a 135-g sample. One month after the beans were canned, visual appeal was evaluated by trained panelists on a hedonic scale of 1–5, with 1 being least desirable and 5 most desirable. This scale takes into account whole bean integrity, uniformity of size, and brine color (Mendoza, Kelly, & Cichy, 2017). This scale converts to a canning quality rating of 1 = unacceptable, 2 = poor, 3 = average, 4 = excellent, and 5 = exceptional. In 2011, 12 panelists evaluated the samples, and in 2018, 11 panelists evaluated the samples. Sample ratings were averaged across all panelists. Canned bean color was measured with a Hunter Labscan XE colorimeter (Hunter Associate Laboratory).

### 3 | RESULTS AND DISCUSSION

#### 3.1 | Agronomic traits

##### 3.1.1 | Recombinant inbred populations

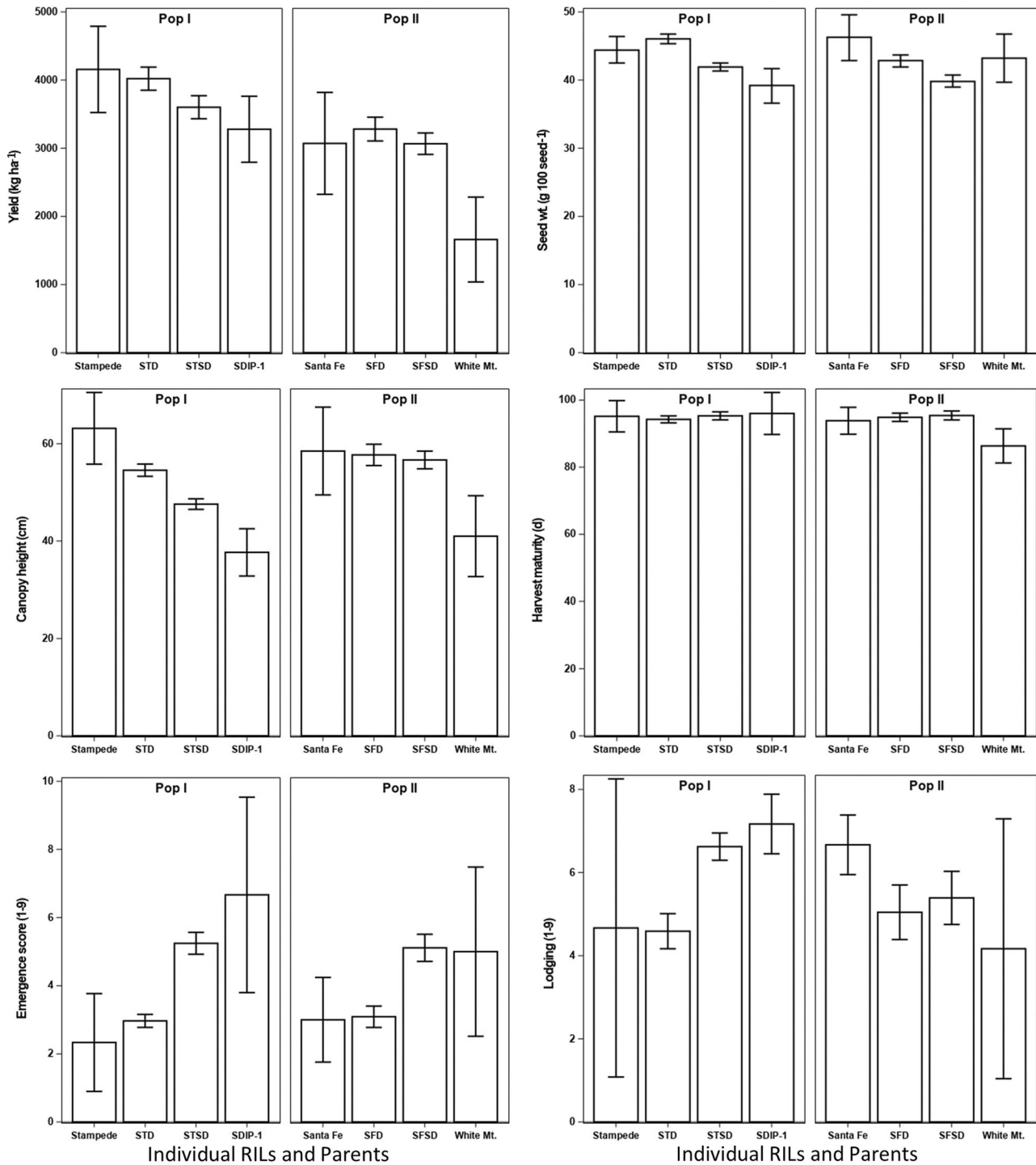
The performance attributes' combined means across the three locations (Othello, Prosper, and Hatton) revealed that the commercial pinto parents Stampede for Population I and Santa Fe for Population II significantly outperformed the respective *sd* sources SDIP-1 and CDC White Mountain for most agronomic traits (Figure 1, Supplemental Table S1). Similarly, for each population, the RD group of 15 RILs outperformed the SD group of 15 RILs for most agronomic traits, and the mean differences for each trait between RD and SD groups were generally greater in Population I than in Population II.

The commercial pinto Stampede had 27% higher seed yield than the *sd* source SDIP-1 across locations (Figure 1). The SD RILs for Population I as a group had 9% lower seed yield than the RD group of RILs, and the RD group was not significantly different from Stampede. The commercial pinto Santa Fe had 102% higher seed yield than the *sd* source CDC White Mountain. The low seed yield for CDC White Mountain can be attributed to its early maturity and determinate bush growth habit. Similarly, for Population II, the SD group of RILs yielded 9% less than the RD group, and the RD group of RILs did not differ significantly from Santa Fe. Interestingly, seed yield for 3 and 11 individual SD RILs in Populations I and II, respectively, were not significantly different from their respective commer-

cial pinto parents Stampede and Santa Fe (Figure 2). Additional field testing of these lines allowed individual RILs 23ST-27 (Population I) and SF103-8 (Population II) to be released as SD pinto bean cultivars 'Scout' (PVP no. 201457) and 'ND-Palomino' (Osorno et al., 2017).

Seed weight ( $\text{g } 100 \text{ seeds}^{-1}$ ) for the *sd* sources SDIP-1 and CDC White Mountain were 11 and 8% less than the respective Stampede and Santa Fe parents (Figure 1). For both populations, the SD group of RILs had 7% lower seed weight than the RD group of RILs. The seed weight for the SD group in Population I was intermediate between the parent (Stampede) and *sd* source (SDIP-1), whereas for Population II, the seed weight for the SD group was lighter than both parent (Santa Fe) and *sd* source (CDC White Mountain). Only 4 of 15 and 1 of 15 individual SD RILs in Populations I and II, respectively, had similar seed weight as the RD parents Stampede and Santa Fe (Figure 2). The *sd* gene located on chromosome Pv07 is near the *P* (pigment) locus at 40.39 Mb (Alvares et al., 2019). The *P* locus is near quantitative trait loci (QTL) affecting seed size (McClean et al., 2018); thus, genetic linkage may explain in part the seed weight differences observed between the SD and RD groups. Although the smaller seed size for many of the individual SD RILs is within the commercially acceptable range (35–45  $\text{g } 100 \text{ seeds}^{-1}$ ), these results continue to support the reported challenges of breeders for obtaining SD pinto beans with desirable seed size.

Another issue for breeders can be the difficulty in recovering upright lines resistant to lodging from crosses involving parents that lack upright architecture such as CDC White Mountain and SDIP-1. Both Stampede and Santa Fe have upright architecture as indicated by tall plant canopy heights, but Stampede is more resistant to lodging than Santa Fe (Figure 1). For Population I, the SD group of RILs was 13% shorter and 32% more lodged than the RD group. The *sd* gene located on chromosome Pv07 near 40.39 Mb is close to a candidate gene (46 Mb) with major influence on the lodging trait and associated canopy height trait (Moghaddam et al., 2016). Thus, moderate linkage (estimated to be 24 cM based on  $0.233 \text{ Mb cM}^{-1}$  in euchromatic regions; Schmutz et al., 2014) between *sd* and a gene affecting lodging may be partly responsible for the increased lodging and reduced canopy height for the SD RILs in Population I (Figure 2). For Population II, the SD and RD groups exhibited similar heights as Santa Fe, and both groups were more resistant to lodging than Santa Fe. Perhaps CDC White Mountain contributed favorable traits for canopy height and reduced lodging that were masked by the *fin* gene (Repinski, Kwak, & Gepts, 2012), which conditions bush determinate growth habit. Moreover, the *sd* source from CDC Mountain was transferred into a commercial pinto bean background via four subsequent crosses, which provided

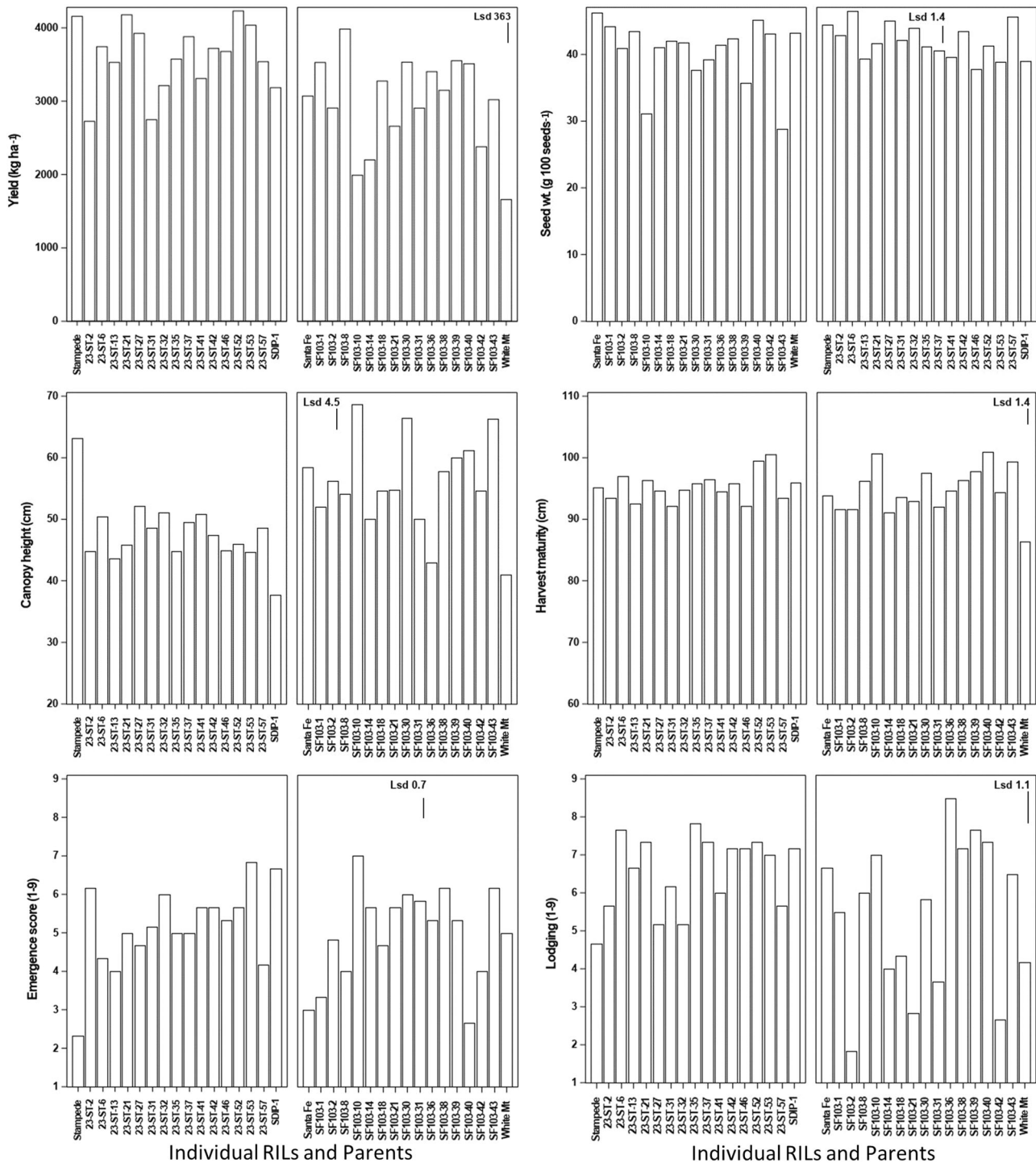


**FIGURE 1** Agronomic performance trait means for two recombinant inbred line (RIL) populations ST (Z0818-23/Stampede) and SF (Santa Fe/PS08-108) grouped by regular-darkening (STD and SFD) and slow-darkening (STSD and SFSD) RILs (15 RILs in each group), regular-darkening parents (Stampede and Santa Fe), and slow-darkening donor parents (SDIP-1 and White Mt.). Means were generated across locations (Othello, WA; Hatton and Prosser, ND) for those traits measured in multiple locations. Bars represent standard error (Supplemental Table S1)

ample opportunity to overcome any negative linkage drag effects.

Our results support that harvest maturity is not an issue in breeding SD pintos (Figures 1 and 2). The mean days to

harvest maturity for the commercial parents, *sd* sources, and SD and RD groups of RILs for both populations were similar, except for CDC White Mountain, which was 7–10 d earlier maturing. Pinto bean cultivars with determinate



**FIGURE 2** Agronomic performance trait means for the individual slow-darkening (SD) recombinant inbred lines (RILs) from two RIL populations, ST-23 (Population I [Pop I]: Z0818-23/Stampede) and SF103 (Population II [Pop II]: Santa Fe/PS08-108); regular-darkening parents (Stampede and Santa Fe); and slow-darkening donor parents (SDIP-1 and White Mt.). Means were generated across locations (Othello, WA; Hatton and Prosper, ND) for those traits measured in multiple locations. Mean separation (LSD 0.05) values generated by Tukey–Kramer pairwise comparison tests are indicated for each individual trait

bush growth habits are inherently earlier maturing than beans with indeterminate vining growth habits. None of the RILs in Population II exhibited determinate growth habit because this trait had been selected against during

the development of the SD parental line PS08-108 that was crossed with Santa Fe.

For the Washington breeding program, we have observed in the field that seedling emergence (1–9 scale)

of SD pintos can be inferior to RD pintos. Note that this problem may be addressed with a seed treatment containing fungicides, but to enable selection of lines with inherent differences for improved seedling emergence, we do not use any seed treatment in our pinto bean performance trials. Stampede (2.5) and Santa Fe (2.9) exhibited the best emergence scores, and the *sd* sources SDIP-1 (6.6) and CDC White Mountain (4.8) exhibited the worst (Figures 1 and 2). The RD RILs as a group for each respective population had significantly better emergence scores (3.0 and 3.1) than the SD groups of RILs (5.2 and 5.0). These results support that a concerted effort should be made to select against poor seedling emergence when breeding SD pinto beans. Beans with colored seed coats (dominant *P* gene) have flavonoid compounds that exhibit fungistatic properties (Panche, Diwan, & Chandra, 2016; Prasad & Weigle, 1976), and white beans (recessive *p*) lack these compounds. Perhaps the reduced flavonoid concentration observed for SD pintos in comparison with RD pintos (Beninger et al., 2005; Duwadi et al., 2018) has less fungistatic activity, contributing in part to the poorer seedling emergence observed for SD lines in the Washington trials. Some growers have complained that SD pinto seeds are more prone to splitting and cracking during threshing. If SD pintos are sensitive to damage during harvest operations, then differences in seed germination rates between SD and RD pintos could influence seedling emergence as well. We did not measure germination rates in this study.

### 3.1.2 | Advanced breeding lines

The pattern of difference in agronomic performance for the SD vs. RD groups of advanced pinto breeding lines and cultivars (Table 1) reflected the differences in agronomic performance observed for the SD and RD groups of RILs in Populations I and II (Figure 1). Seed yield ( $\text{kg ha}^{-1}$ ) for the SD breeding lines averaged 8% less than the RD lines across the five Washington trials. Conversely, seed yield was similar for both groups of breeding lines in North Dakota. This difference in yield response between SD and RD groups may result from the higher seed yield potential realized for beans grown in Washington than in North Dakota, in part from the use of irrigation. Seed weight ( $\text{g } 100 \text{ seeds}^{-1}$ ) was significantly reduced for the SD group in all trials averaging 8% less weight than the RD group of advanced breeding lines and cultivars. Similarly, the SD lines as a group had consistently poorer seedling emergence (all five Washington trials) and increased lodging (in three of four Washington trials) compared with the RD group of lines. Canopy height, although significant, was only slightly different between the SD and RD

groups of lines across the three North Dakota locations in 2012.

The poorer agronomic performance (lower seed yield, smaller seed size, poorer seedling emergence, and increased lodging) for the early-generated SD breeding materials compared with RD advanced breeding lines and cultivars in the 2010–2012 trials was less pronounced in the 2018 performance trial, which possesses the most recent “next” generation SD breeding materials (Table 1). When mean data for individual lines and cultivars are examined across these trials (Supplemental Tables S3–S8), there is clear evidence for some progress being made in improving agronomic performance of SD pintos. Currently, there are a few SD lines and cultivars in the 2018 Washington trial that exhibit some combinations of high yield, upright architecture, with acceptable seed size, and good canning quality (Supplemental Table S8). Slow-darkening lines with combinations of such traits were mostly absent in the 2010–2012 trials. Inspection of the agronomic performance traits for the 2016–2018 Cooperative Dry Bean Nursery (CDBN) trials (Urrea, 2016, 2017, 2018) similarly shows that a few recently bred SD pinto breeding lines and cultivars exhibit some combinations of high seed yield, large seed size, upright architecture, and good canning quality. The CDBN from 2016 to 2018 tested SD (four to five lines) and RD (four to seven lines) pintos among other dry bean market classes for agronomic performance across six to eight locations in the United States and one location in Canada.

## 3.2 | Cooking quality traits

### 3.2.1 | Cooking time

Seed color and cooking time were conducted on a subsample of 10 RILs (5 SD and 5 RD) from each population (Supplemental Table S2). Dry seed color was different for the RD and SD lines. For uncooked beans, the  $L^*$  values were higher and the  $a^*$  and  $b^*$  values were lower for the SD groups of RILs, indicating that they exhibited a lighter and brighter seed coat color (Figure 3, Supplemental Tables S1 and S2). Junk-Knievel et al. (2007) observed that the difference for  $L^*$  and  $a^*$  values between uncooked SD and RD pintos increased as the beans aged. Bean cooking times determined for individual RILs, averaged across locations, ranged from 22 min (fastest cooking) to 38 min (slowest cooking). Overall, the RILs grown in Washington took 4 min less time to cook than the same RILs grown in North Dakota. There was no RIL  $\times$  location interaction for cooking time. Among groups of RILs, the RD and SD groups in Population I each cooked in 29 min. Conversely, for Population II, the RD group (35 min) took 7 min longer

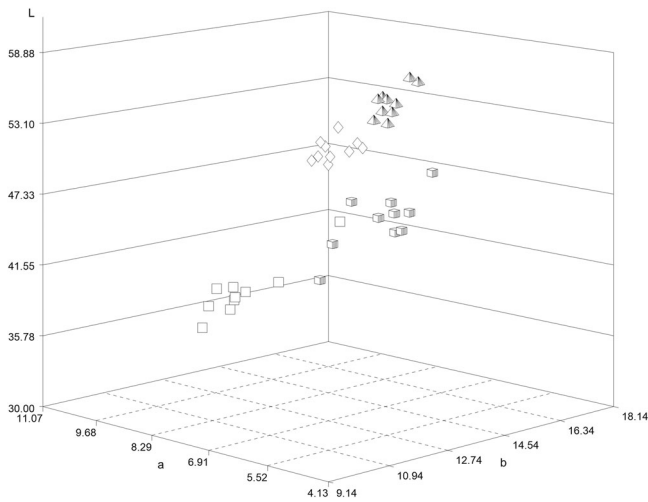


**TABLE 1** Agronomic performance and cooking quality traits means for pinto bean breeding lines and cultivars, grouped by regular-darkening (RD lines) vs. slow-darkening (SD lines) seed coat trait, from performance trials (with three to four replications) conducted in North Dakota and Washington from 2010 to 2012, and 2018. The means for North Dakota 2012 trials represent the combined data across three locations

Trial and trait	RD lines	SD lines	Contrast <i>P</i> value
Othello, WA, 2010	<i>n</i> = 13	<i>n</i> = 10	
Emergence, 1–9 <sup>a</sup>	2.3	4.2	<.0001
Lodging, 1–9 <sup>a</sup>	4.9	6.0	<.0001
Harvest maturity, d	108	109	.1146
Seed weight, g 100 seeds <sup>-1</sup>	40.9	37.3	<.0001
Yield, kg ha <sup>-1</sup>	4136	3,828	<.0001
Othello, WA, 2011, advanced trial	<i>n</i> = 8	<i>n</i> = 10	
Emergence, 1–9	2.0	3.8	<.0001
Lodging, 1–9	4.3	4.8	.1067
Harvest maturity, d	99	99	.3938
Seed weight, g 100 seeds <sup>-1</sup>	40.9	35.5	<.0001
Yield, kg ha <sup>-1</sup>	3,281	2,663	<.0001
Othello, WA, 2011, preliminary trial	<i>n</i> = 26	<i>n</i> = 23	
Emergence, 1–9	2.1	3.5	<.0001
Lodging, 1–9	4.7	5.9	<.0001
Harvest maturity, d	98	99	<.0001
Seed weight, g 100 seeds <sup>-1</sup>	40.0	38.5	<.0001
Yield, kg ha <sup>-1</sup>	4,842	4,596	<.0002
Canned lines	<i>n</i> = 4	<i>n</i> = 9	
Canning quality, 1–5 <sup>a</sup>	2.6	2.7	.4433
<i>L</i> <sup>*b</sup>	39.7	45.3	<.0001
<i>a</i> <sup>*b</sup>	14.9	12.8	<.0001
<i>b</i> <sup>*b</sup>	22.6	23.8	.0028
Othello, WA, 2012	<i>n</i> = 34	<i>n</i> = 23	
Emergence, 1–9	4.1	4.9	<.0001
Harvest maturity, d	103	105	.0196
Seed weight, g 100 seeds <sup>-1</sup>	39.9	37.7	<.0001
Yield, kg ha <sup>-1</sup>	2,834	2,669	.0093
Hatton, Johnstown, Prosper, ND, 2012	<i>n</i> = 4	<i>n</i> = 20	
Canopy height, cm	52.6	50.0	<.0001
Harvest maturity, d	96	97	.0002
Seed weight, g 100 seeds <sup>-1</sup>	42.0	38.5	<.0001
Yield, kg ha <sup>-1</sup>	3,027	2,962	.2818
Othello, WA, 2018	<i>n</i> = 27	<i>n</i> = 21	
Emergence, 1–9	2.2	3.2	<.0001
Lodging, 1–9	3.4	4.2	<.0001
Harvest maturity, d	94	94	.2389
Seed weight, g 100 seeds <sup>-1</sup>	47.0	44.1	<.0001
Yield, kg ha <sup>-1</sup>	4,003	3,864	.0245
Canning quality, 1–5	2.6 ( <i>n</i> = 14)	3.2 ( <i>n</i> = 7)	<.0001

<sup>a</sup>Emergence from 1 = complete to 9 = no plants emerged; lodging from 1 = no lodging to 9 = completely lodged; canning quality from 1 = unacceptable to 5 = exceptional.

<sup>b</sup>CIELAB color space values *L*\* (lightness from black to white), *a*\* (from green to red), and *b*\* (from blue to yellow).



**FIGURE 3** Mean CIELAB color space values  $L^*$  (lightness from black to white),  $a^*$  (from green to red), and  $b^*$  (from blue to yellow) across locations (Othello, WA, and Prosper, ND) for slow-darkening (SD) recombinant inbred lines (RILs) uncooked (pyramid) and cooked (cube) and regular-darkening (RD) RILs uncooked (diamond) and cooked (square). Five SD and five RD RILs from each Population I (Z0818-23/Stampede) and II (Santa Fe/PS08-108) were tested

on average to cook than the SD group (28 min). The difference in cooking time for SD and RD groups of RILs for Population II suggests that *sd*, or some trait linked to it, may condition reduced cooking time in some genetic backgrounds. Pinto bean canners and processors have mentioned that SD pintos cook faster than RD pintos and that adjustments in protocols may be necessary to accommodate this difference. The color of the cooked seeds was also measured, and the SD groups of RILs retained a lighter brown color than the RD groups, as confirmed by significant differences ( $P < .05$ ) for  $L^*$ ,  $a^*$ , and  $b^*$  values (Figure 3). Cooking time was not correlated with SD or RD seed coat trait segregating in carioca populations stored for 90 or 180 d (Alvares, Pereira, Melo, Miklas, & Melo, 2020).

### 3.2.2 | Canning quality

The canning quality was similarly rated just below average for the groups of SD and RD breeding lines and cultivars for the 2011 Washington trial, but the SD group had a significantly better above average canning score than the RD group in the 2018 Washington trial (Table 1). If you look at the individual line means from the 2011 trial (Supplemental Table S9), only one SD pinto had an excellent rating (4), similar to the high canning quality standard RD check ‘Othello’, but the three RD pintos in the trial also had inferior canning quality compared with the standard check. The canning quality scores for six of seven SD pintos were not significantly different from Othello in the 2018

trial, and two SD pintos scored better than Othello (Supplemental Table S10). None of the 13 RD pintos in the 2018 trial canned as well as Othello pinto.

Michigan is the only location in the CDBN that is canned. The canning tests are performed by the USDA-ARS Dry Bean Quality Laboratory (East Lansing, MI) using the same protocol described above, except that only a single replication is canned. The canning data (Michigan location) for the CDBN from 2016 to 2018 (Supplemental Table S11) support the canning data obtained for the 2011 and 2018 Washington trials (this study), in that canning quality for the same line, whether SD or RD, varies from location to location and year to year. The importance of a genotype  $\times$  environment effect on canning quality is described in the literature (Balasubramanian, Slinkard, Tyler, & Vandenberg, 1999; Osorno et al., 2012). As groups, SD and RD pintos exhibited similar canning quality, with both groups composed of lines representing a wide range in canning quality. Thus, it appears that canning quality, as measured in this study, is uncorrelated with the SD seed coat trait.

The color for the canned samples from Washington 2011 trials mirrored the samples in the Mattson cooker, where  $L^*$  was higher,  $a^*$  was lower, and  $b^*$  was slightly higher for the SD vs. RD group of lines. These consistent color differences for uncooked, cooked, and canned products contribute to the needed separation of SD and RD pintos as two distinct market classes. Comingling of SD and RD pintos in either dry pack or canned bean products will result in an inferior product with mixed shades of brown color.

## 4 | SUMMARY

Slow-darkening pinto beans are gaining popularity in the United States because they maintain a brighter seed coat appearance under delayed harvest situations and retain a brighter, more appealing seed coat appearance after storage. In fact, during the past few years, there have been more pinto bean cultivars released with the SD seed coat trait than those without. We examined the effects of the SD seed coat trait, conditioned by the recessive *sd* gene, on agronomic performance and cooking quality traits in recombinant inbred populations, advanced breeding lines, and cultivars grown in Washington and North Dakota from 2010 to 2018. Across these performance trials, the SD pintos as a group had poorer seedling emergence, shorter plants that lodged more, lower seed yield, and smaller seed size compared with the RD group of materials tested. Trait differences between SD and RD groups were more pronounced in Washington than in North Dakota. Linkage of *sd* with QTL for smaller seed size and with a candidate gene for lodging likely contributed to the poor

performance of the SD pintos for those traits. The *sd* gene may have pleiotropic effect on stand establishment as a result of lower flavonoid production in SD pintos, which has been reported in the literature. However, agronomic performance of SD pintos is improving, as evidenced by reduced differences between SD and RD pintos for seed yield and size, and plant architecture in the most recent 2018 Washington trial and in the CDBN trials from 2016 to 2018. Nonetheless, pinto bean breeders need to remain cognizant of potential agronomic performance issues due to linkage drag and pleiotropy associated with introgressing the SD seed coat trait and be extra cautious when transferring the trait from original sources and “first-generation: breeding lines and cultivars.

Further research on the effect of the SD trait on cooking time is warranted, given that in one population, the SD RILs cooked 20% faster than the RD RILs. Canning quality exhibited a similar range of good and poor canners for both the SD and RD groups of pinto lines tested in this study. Overall, however, SD pintos exhibited slightly better canning quality than RD pintos. Whether raw, cooked, or canned, SD pintos had a much lighter color than RD pintos, emphasizing the need to keep SD and RD pintos separated at all levels of production, storage, and processing.


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## CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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