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Content will focus on resilience to climate change in agricultural systems, exploring the latest research investigating strategies to adapt to and mitigate climate change. Innovation and imagination backed by good science, as well as diverse voices and perspectives are encouraged. Where are we now and how can we address those challenges? Abstracts must reflect original research, reviews and analyses, datasets, or issues and perspectives related to objectives in the topics below. Authors are expected to review papers in their subject area that are submitted to this virtual issue.

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ARTICLE

Agronomic Application of Genetic Resources

Assessment of soybean test weight among genotypes, environments, agronomic and seed compositional traits

Brant T. McNeece¹ | Jay H. Gillenwater² | Zenglu Li³ | M.A. Rouf Mian^{1,2} ¹ Soybean and Nitrogen Fixation Research Unit, USDA-ARS, 3127 Ligon Street, Raleigh, NC 27607, USA² Dep. of Crop and Soil Sciences, North Carolina State Univ., Raleigh, NC 27695, USA³ Dep. of Crop and Soil Sciences, and Institute of Plant Breeding, Genetics, and Genomics, Univ. of Georgia, Athens, GA 30602, USA

Correspondence

M.A. Rouf Mian, Soybean and Nitrogen Fixation Research Unit, USDA-ARS, 3127 Ligon Street, Raleigh, NC, 27607, USA.
Email: rouf.mian@usda.gov

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Abstract

The value of soybean [*Glycine max* (L.) Merr.] depends on quantity (yield) and quality, such as seed composition and test weight (TW). Test weight is a bulk density measurement for grain quality evaluation, and higher TW grains are preferred for storage, transport, and export. Thus, soybean breeding should focus on improvement of TW. The objectives of this study were to determine genotypic and environmental effects on TW of soybean and explore relationships of TW with yield, maturity, 100 seed weight (SDWT), seed quality (SQ), seed oil concentration (SOC), and seed protein concentration (SPC). Three sets of breeding populations (BPs), two mapping populations (MPs), and five different high vs. low seed protein near-isogenic lines populations (NILPs) were used. The BPs and MPs had an average range of 3.5 kg hectoliter (hL)⁻¹ in TW among genotypes. The average ranges of TW in the NILPs were equal for both low protein (LP) and high protein (HP) lines (2.7 kg hL⁻¹). Broad-sense heritability estimates for TW ranged from .62 to .95. The genotypic and G × E effects on TW were significant in all trials. The SDWT, SQ, SOC, and SPC had strong significant relationships with TW in multiple trials, but were inconsistent overall. Test weight of NILPs differing by presence of ‘Danbaekkong’ high protein allele on chromosome 20 had mostly significant positive and negative relationships with SPC and SOC, respectively.

1 | INTRODUCTION

The United States was second in global soybean [*Glycine max* (L.) Merr.] production with 96.8 Tg accounting for approximately US\$31 billion in 2019 (USDA NASS, 2020). The value of soybean in the United States is not only dependent upon quantity (yield), but also quality, such as seed composi-

tion and test weight (TW). Test weight is a bulk density measurement for grain quality evaluation. In the United States, TW is currently characterized by the Federal Grain Inspection Service (FGIS) as an informational factor for soybean, but was a grade-determining factor prior to 2007 and is still used to evaluate grain at many elevators (GIPSA, 2006). In soybean, TW is reported to the nearest 10th in units of pounds per bushel (lb bu⁻¹) with a conversion factor of 1.287 to express in metric units of kilograms per hectoliter (kg hL⁻¹) (GIPSA, 2006). Unlike wheat (*Triticum aestivum* L.), where TW is a strong factor of cultivar development, soybean samples for TW are not cleaned before acquiring measurements at elevators and foreign material has shown to negatively impact

Abbreviations: BP, breeding population; CCRS, Central Crops Research Station, Clayton, NC; MAS, marker-assisted selection; MD, maturity day; MP, mapping population; NILP, near-isogenic line population; SDWT, seed weight; SOC, seed oil concentration; SPC, seed protein concentration; SQ, seed quality; TRS, Tidewater Research Station, Plymouth, NC; TW, test weight.

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TW (GIPSA, 2007; Naeve & Miller-Garvin, 2019). Soybean is graded according to TW with 72.1, 69.5, 66.9, and 63.1 kg hL⁻¹ representing No. 1, 2, 3, and 4 grades of soybean, respectively (U.S. Soybean Export Council, 2015). Discount factors are applied for TWs <63.1 kg hL⁻¹.

Apart from TW being related to the quality, it is also important for storage, transportation, and export of soybean. The U.S. soybean exports accounted for 49.7 Tg and 33% of the global market share in 2019, second only to Brazil (USDA NASS, 2020). Although the United States is still a major competitor in the global market share of soybean exports, there has been a steady decline since 2016 (USDA FAS, 2020). A key element in this decline involves the transportation efficiency of soybean from farm to endpoint (Salin & Somwaru, 2014). Soybean transportation within the United States consists of three main transportation modes: rail, barge, and truck. The U.S. soybean is mainly exported through ports in two major regions, the Mississippi Gulf and the Pacific Northwest (Denicoff et al., 2014). Transportation to the ports is largely conducted by barge (54%), with truck and rail representing 16 and 30% of tonnage, respectively (Chang, Caffarelli, Gastelle, & Sparger, 2019). Soybean for domestic use in the United States are mainly transported by truck, rail, and barge representing 81, 16, and 3% of tonnage, respectively (Chang, Caffarelli, Gastelle, & Sparger, 2019). Trucks can haul 24,766 kg of soybean based on 77.2 kg hL⁻¹ benchmark according to the Soy Transportation Coalition. Yet, TW of soybean in the United States falls short of this standard, with the average TW of soybean in 2018 being 73.1 kg hL⁻¹ (Naeve & Miller-Garvin, 2019). Low TW soybean occupy the same volume but possess lower mass than higher TW soybean, so they may require more trucks to transport the same mass. Therefore, high TW soybean could improve efficiency by decreasing the transportation cost for the same mass of seed. The U.S. soybean transportation costs have been significantly lower than those of Brazil, but Brazil has committed resources for improvement of their transportation infrastructure to close this gap (Salin & Agapi, 2014). Therefore, it is critical for the United States to continue to invest in avenues to improve and maintain this edge. Thus, TW is a valuable parameter to be taken into consideration in development of new soybean cultivars.

Efficacy of breeding programs relies on knowledge of inheritance for desired traits. Studies in common wheat, durum wheat (*T. turgidum* L. ssp. *durum*), oat (*Avena sativa* L.), and maize (*Zea mays* L.) have shown significant effects on TW due to genotype, environment, and genotype × environment interactions (Doehlert et al., 2001; Huang et al., 2006; Li et al., 2013; Vázquez-Carrillo et al., 2016). Heritability estimates among studies in other crops have been consistently high for TW and have resulted in effective selection for TW in breeding programs. Reported estimates of heritability for TW in rice, wheat, oat, and maize are .91, .96, .80, and .60,

Core Ideas

- The gradual decline of soybean test weight in United States needs to be reversed.
- Test weight of soybean exhibited high heritability ($H^2 = .62-.95$).
- Inconsistent weak correlations of maturity and yield with test weight of soybean were found.
- Inconsistent strong relationships of test weight with protein, oil, and seed weight were observed.

respectively (Brandariz & Bernardo, 2019; Cabral et al., 2018; Holland & Munkvold, 2001; Singh et al., 2011). Molecular genetics approaches to TW have also resulted in identification of quantitative trait loci (QTL) for TW in crops such as wheat (Cabral et al., 2018; Sun et al., 2009) and maize (JunQiang et al., 2011). Genetic information regarding TW in other plant systems is readily available, yet soybean research in this area is lacking.

Relationships of TW with disease, management practices, physical properties of seed, and seed composition have also been found. Specifically, susceptibility to fungal pathogens have shown to negatively influence TW. Soybean seeds infected with *Phomopsis sojae* were found to have lower TW than non-symptomatic seed (Hepperly et al., 1978). Similarly, susceptibility to the fungal pathogen, *Parastagonospora nodorum*, decreased TW in wheat (Salgado et al., 2015). Under stress of crown rust (*Puccinia coronata* var. *avenae*) in oat, resistance was positively correlated with TW (Holland & Munkvold, 2001), as was fungicide treatment (Cruppe et al., 2017). Production practices also influence TW. In wheat, a decrease in TW was observed due to delayed harvest (Czarnecki & Evans, 1986) and increased nitrogen levels (>34 kg ha⁻¹) (Roth et al., 1984). Plant density was positively associated with TW in wheat (Roth et al., 1984), oat (Weisberger et al., 2019), and maize (Widdicombe & Thelen, 2002). Row spacing in wheat and maize was not significantly associated with TW (Roth et al., 1984; Widdicombe & Thelen, 2002). In oat, Weisberger et al. (2019) found delayed planting date to negatively impact TW.

Soybean seed composition is the primary factor determining its value. Soybean seed protein accounts for nearly two-thirds of the world's protein meal supply (Hoffman & Falvo, 2004; OECD/FAO, 2016). Also, soybean oil is the second leading source of vegetable oil and the primary feedstock for biodiesel (Irwin, 2017). As with soybean, seed composition of other major crops also determines their value. Studies in other crops have attempted to identify relationships with seed protein concentration (SPC) and TW. In wheat, effects of SPC on TW has been found positive (Gürsoy et al., 2010), negative (Bonfil et al., 2015), and insignificant (Rharrabti, Elhani, Martos-Núñez, & García del Moral, 2001). As with wheat,

a study in maize also found the relationship to be inconclusive (Butts-Wilmsmeyer et al., 2019). A functional analysis of NAC-type transcription factors in maize, showed TW to be effective in differentiating higher SPC from lower SPC transgenic lines (Zhang et al., 2019). Currently, relying on TW for assessments of SPC is debatable.

The objectives of this study were to determine genotypic and environmental effects on TW of soybean and explore relationships of TW with yield, maturity, SDWT, SQ, SOC, and SPC utilized in soybean breeding for increased seed protein. For a comprehensive assessment of the impact of these traits on TW in high and low seed protein breeding lines, multilocation trials were conducted with three breeding populations (BPs) – one consisting of 400 $F_{4:6}$ lines in 2018 (NC18) and two with 60 genotypes each in 2017 (NC17-1 and NC17-2); four mapping populations (MPs) trials derived from two biparental MPs and further divided by relative maturity in 2019 (NC19-1, NC19-2, NC19-3, and NC19-4); and a single location evaluation of five near-isogenic line populations (NILPs) in 2019 (BC₄F₄, HIF-1, HIF-2, HIF-3, and HIF-4).

2 | MATERIALS AND METHODS

2.1 | Plant materials

Plant materials selected for this assessment have all been developed for their potential in producing desirable agronomic lines with high SPC and represent various populations used in development of such lines in high SPC-breeding programs. The plant row (or progeny row) trial of BPs (NC18) evaluated in this study consisted of 400 $F_{4:6}$ soybean lines. Lines were selected from approximately 1,200 lines of the $F_{4:5}$ generation progeny rows in 2017 to obtain the broadest of SPC. Therefore, 200 lines exhibited high SPC (376–453 g kg⁻¹) and 200 lines exhibited low SPC (317–375 g kg⁻¹) (Supplemental Figure S1). The SPCs are reported on 13% moisture basis. For NC18, two replicates were grown at both Central Crops Research Station in Clayton, NC (CCRS) and Tidewater Research Station (TRS) in Plymouth, NC. Two other yield trials of BPs (NC17-1 and NC17-2) consisted of 60 genotypes ($F_{4:7}$) each, with three replications per trial. The SPCs in NC17-1 and NC17-2 ranged from 334–407 and 342–431 g kg⁻¹, respectively. The MP yield trials, NC19-1 and NC19-2 were selections from the cross of LMN09-119 × N09-09, while NC19-3 and NC19-4 were selections from the cross of LMN09-19 × N13-47. All four parental lines LMN09-119, N09-09, LMN09-19, and N13-47 were developed by USDA-ARS. Test weight distribution of MPs are available in Supplemental Figure S2. Mapping population yield trials derived from the same cross were divided into two separate yield trials based on maturity. Both NC17 and NC19 yield trials were grown as a full-season crop

at TRS and at the Lower Coastal Plain Experiment Station (Caswell Research Farm) near Kinston, NC, in 2017 and 2019, respectively.

The NILPs of high vs. low SPC consisted of one BC₄F₄ population and four heterogeneous inbred family (HIF) populations. In both the BC₄F₄ and HIFs (HIF-1, 2, 3, and 4), NIL were selected based on the presence of the Danbaekong (Dan) high protein (HP) allele on chromosome 20 via marker-assisted selection (MAS). The BC₄F₄ population is derived from the cross of cultivars Wyandot (St Martin et al., 2006) × HighPro1 (PI 678444) (Mian et al., 2017), using Wyandot as the recurrent parent. The BC₄F₄ population consisted of 22 lines homozygous for the LP allele and 29 lines homozygous for the Dan HP allele on chromosome 20 and was grown at CCRS in 2019. The four HIF populations HIF-1, 2, 3, and 4 evaluated in this study were derived from crosses R05-655 × HighPro1, R09-4095 × HighPro1, R02-6268F × HighPro1, and HighPro1 × ‘NC-Roy’ (PI 617045) (Burton et al., 2005), respectively. In all NILPs, cultivar HighPro1 was the source of the Danbaekong allele. The MAS for the high protein allele was performed at the F_5 generation. Thirty-four plant rows, representing four crosses were found to be heterogeneous and were planted in nonreplicated rows in summer of 2018 at CCRS. Of these 34 plant rows, MAS identified 8, 27, 9, and 16 LP plants and 7, 18, 8, and 15 HP plants from four crosses to develop HIF-1, HIF-2, HIF-3, and HIF-4, respectively.

2.2 | Marker-assisted selection

Tissue for initial identification of segregating $F_{4:5}$ plant rows was collected by growing seedlings in rolled wet towels and excising root tips from a minimum of six seedlings per plant row for 199 rows. In 2018, 20 random plants from 34 heterogeneous rows (680 plants total) were tagged in the field for individual leaf tissue collection at V3 stage to verify segregation within the plot and selection of individuals with homozygous alleles at protein locus on chromosome 20. Samples were then freeze-dried using a SP Scientific freeze dryer. The lyophilized samples were homogenized using a MP Biomedicals’ FastPrep-96 homogenizer. Genomic DNA was extracted by a modified CTAB method (Allen et al., 2006). The DNA was diluted to 100 ng µl⁻¹ in H₂O for KASP (Competitive Allele Specific polymerase chain reaction [PCR]) genotyping. Primers for the KASP assay were provided by the University of Georgia Soybean Breeding and Genetics Lab (Supplemental Table S1). Reaction mix components consisted of 2 µl DNA, 2 µl LGC Biosearch Technologies KASP 2x master mix, 0.06 µl assay mix. Reactions were run on a Bio-Rad C1000 thermal cycler at 95 °C for 15 min, 10 cycles with 15 min at 95 °C, and 1 min at 65 °C, then 30 cycles with 15 min at 95 °C and 1 min at 57 °C, then 4 °C termination.

2.3 | Experimental design and phenotyping

Each multi-environmental trial (NC17-1, NC17-2, NC18, NC19-1, NC19-2, NC19-3, and NC19-4) was arranged in a randomized complete block design. NC17-1, NC17-2, NC19-1, NC19-2, NC19-3, and NC19-4 consisted of three replications per genotype, whereas NC18 consisted of two replications per genotype. Conventional check cultivars were included in all multi-environmental trials for comparison of MD, Yield, SPC, and SOC. Plot length was approximately 4.3 m for NC18 in two locations with seed density of approximately 23 seeds m^{-1} . For the NC17 and NC19 trials, plots consisted of three 5.8-m rows with seed density of 26 seeds m^{-1} , and end-trimmed to 4.6 m before harvest. Only the center row was harvested for yield and seed trait analysis. The NILPs were planted in non-replicated rows 6.1 m in length at a seed density of 26 seeds m^{-1} at CCRS in 2019. The row spacing for all trials was 97 cm. All plots were harvested at maturity using plot combines.

Maturity day (MD) was recorded as the 1st day when 95% of the pods matured. Seed quality (SQ) was rated visually on harvested seed samples using a 1–5 scale established by Green et al. (1965), where 1 indicates very good seed quality and 5 indicates very poor seed quality. Following harvest, the paper bags with seed from each plot were opened and placed on a bench in an environmentally controlled greenhouse to ambiently dry down to a moisture content of ~7%. The Model 6.0-6-SHL-20-G Galvanized Steel Single Spiral Separator (Profile Industries) was used to clean the dry seed. An 80 g subsample of cleaned whole soybean from each plot was used for SPC and SOC analysis.

A Perten DA 7250 Near Infrared Reflectance (NIR) Analyzer (Perten Instruments) was used to obtain SPC and SOC percentages expressed on dry weight basis and converted to a 13% moisture basis. Seed weight (SDWT) represented the mass in grams of 100 seed. Test weight values and moisture content were obtained by the Dickey John GAC UGMA 2500 Grain Analysis Computer (Dickey-john). The volume of the GAC 2500 UGMA test cell was 412 cm^3 . Plot samples insufficient in volume for accurate TW measurement were excluded from analysis. To align values with TWs taken at grain elevators, all TW values were converted to 13% moisture content according to Liu et al. (2019).

2.4 | Statistical analysis

PROC MIXED in SAS Version 9.4 was used to assess the genotypic and environmental effects and obtain least squares means (LS Means), where genotype was treated as a fixed effect and environment, genotype \times environment, and replication(environment) as random effects for ANOVA and com-

bined analyses; for individual location analyses, genotype was treated as fixed and replication was treated as random (SAS Institute, Cary, NC, 2012). Statistical significance at $\alpha = .05$ was assessed using Fisher's LSD for post hoc comparisons. Correlation analysis of TW with MD, yield, SQ, SDWT, SOC, and SPC was performed using the appropriate genotypic LS means with PROC CORR (SAS Institute, Cary, NC, 2012). PROC TTEST was used for TW comparison between locations and backgrounds of NILPs at $\alpha = .05$ (SAS Institute, Cary, NC, 2012). Correlations were analyzed by trial (combined) and locations within each trial. Broad-sense heritability estimates (H^2) were obtained using the "sommer" package in R (Covarrubias-Pazaran, 2016, 2018), with H^2 calculated on an entry-mean basis by the formula:

$$H^2 = \frac{\sigma_g^2}{\frac{\sigma_e^2}{rt} + \frac{\sigma_{ge}^2}{t} + \sigma_g^2}$$

where σ_g^2 was estimated genetic variance, σ_e^2 was residual error variance, σ_{ge}^2 was the genotype \times environment interaction variance, r was replications, and t was location (testing environment).

3 | RESULTS

3.1 | Genotypic and environmental effects on test weight in breeding populations and mapping populations

The genotypic effect on TW was significant in all trials (Table 1). The environmental effect of TW was significant in five of seven trials (Table 1). NC19-3 and NC19-4 did not show a significant effect of environment with TW, and the genotypic effect accounted for most of the variability in these trials (Table 1). Locations within trials often had significantly different TW values (Figure 1, Supplemental Tables S2 and S3). The Plymouth location, representing all yield trials, revealed significantly higher TW values than the paired location in five of the seven replicated trials (Figure 1). The genotype \times environment interaction was found statistically significant in all trials (Table 1). Only the two NC17 trials and NC19-2 exhibited a significant effect of replication within environment on TW (Table 1). The strong genotypic effects shown here across seven separate trials spanning 3 yr support potential for effective selection for TW in breeding programs.

3.2 | Broad-sense heritability

The broad sense heritability for TW in this study ranged from .62 to .95, with six trials exhibiting heritability estimates

TABLE 1 Analysis of variance of soybean test weight observed in replicated trials from 2017–2019 grown in North Carolina

Source of variation	NC17-1		NC17-2		NC18		NC19-1		NC19-2		NC19-3		NC19-4	
	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS
Genotype (G)	49	1.33***	59	2.40***	393	4.77***	44	3.02***	44	3.73***	44	2.74***	43	2.06***
Environment (E)	1	284.47***	1	110.88***	1	101.96***	1	7.00***	1	8.03*	1	0.46	1	0.03
Rep (E)	2	0.80***	2	0.86***	2	0.01	2	0.09	2	0.27*	2	0.18	2	0.04
G × E	49	0.53***	59	0.49***	393	0.47***	44	0.21***	44	0.20***	44	0.15***	43	0.28***
Residuals	167	0.14	199	0.16	764	0.25	86	0.1	88	0.08	86	0.07	86	0.09

Note: MS = Mean Squares.

*Significant at the .05 probability level. **Significant at the .01 probability level.

***Significant at the .001 probability level.

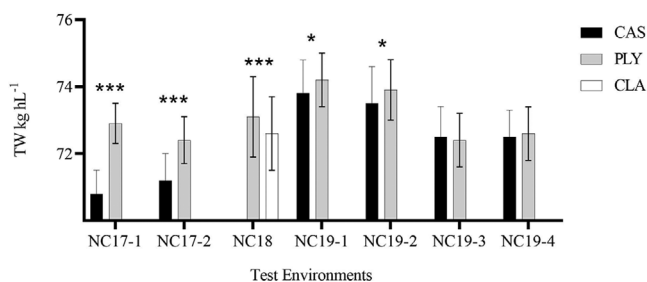


FIGURE 1 Comparison of soybean test weight values by locations within replicated trials grown in North Carolina from 2017–2019. Results of student's paired *t* test showing six out of the seven trials exhibiting numerically higher test weight (TW) values in the Plymouth, NC, location, with five of those trials being significantly different. *Statistical significance at the .05 probability level. **Statistical significance at the .01 probability level. ***Statistical significance at the .001 probability level

≥0.80 (Table 2). Liu et al. (2019) reported heritability of soybean TW to range from .84 to .97. These observations are comparable to those in other crops and their relatively high value indicates soybean TW may be improved by breeding and selection.

3.3 | Test weight performance of check cultivars

Check cultivar performance for TW in soybean is not readily available. In our trials, several commonly used conventional check cultivars in Maturity Group (MG) V, VI, and VII for high yield, SPC, and SOC were evaluated. For comparison, TW and MD of these checks along with four to six of the top TW-breeding lines from yield trials in the study are presented (Tables 3 and 4). Maturity Group V check cultivars Osage (PI 648270) (Chen et al., 2007) and NC-Miller (PI 665018) (Burton, Miranda, Carter, & Bowman, 2012) were evaluated in three and two trials, respectively. Test weight of 'Osage' was significantly less than many breeding lines, but TW of 'NC-Miller' was not significantly different than those of the breeding lines (Tables 3 and 4). Maturity group VI checks 'NC-Dunphy' (North Carolina State University) and 'NC-Roy' (PI 617045) (Burton, Carter Jr. & Bowman, 2005) were included in six and two trials, respectively. Test weight of NC-Dunphy was significantly less than many breeding lines, but TW of NC-Roy, except for one breeding line in NC19-2, was not significantly different from the top TW-breeding lines (Tables 3 and 4). Maturity Group VII

TABLE 2 Broad sense heritability estimates of soybean test weight, yield and other traits observed in replicated trials grown in North Carolina from 2017–2019

Trial	NC17-1	NC17-2	NC18	NC19-1	NC19-2	NC19-3	NC19-4
	H^2 ^a	H^2	H^2	H^2	H^2	H^2	H^2
TW ^a	.62	.80	.90	.93	.95	.95	.87
MD	.52	.77	.88	.65	.52	.69	.64
SDWT	.75	.84	.86	.90	.89	.89	.87
SQ	.42	.97	.58	.26	.02	.48	.30
Yield	.71	.82	–	.13	.60	.69	.59
Protein ^b	.87	.94	.92	.95	.94	.95	.95
Oil ^b	.91	.93	.94	.92	.93	.94	.90

Note: H^2 = broad-sense heritability estimate; TW = test weight; MD = maturity day; SDWT = 100-seed weight; SQ = seed quality, 1 = very good, 5 = very poor (Green et al., 1965).

^aTW = Test weight normalized to 13% moisture content by Lui et al. (2019) method.

^bProtein and oil in g kg⁻¹ expressed on a 13% moisture basis.

TABLE 3 Comparison of soybean test weight between check cultivars and breeding lines observed in replicated trials grown in North Carolina in 2017 and 2018

NC17-1			NC17-2			NC18		
Line	TW ^a	MD ^b	Line	TW	MD	Line	TW	MD
	kg hL ⁻¹	d (1 Oct. = 1)		kg hL ⁻¹	d (1 Oct. = 1)		kg hL ⁻¹	d (1 Oct. = 1)
Osage ^c	70.9	11	NC-Dunphy ^c	70.8	16	NC-Miller	73.9	18
NC-Miller ^c	72.5	16	N16-1152	73.1	22	NC-Dunphy	72.5	19
N16-931	72.8	13	N16-600	72.8	14	NC-Wilder ^c	74.1	32
N16-970	72.8	18	N16-869	72.6	24	N17-2398	75.1	16
N16-1216	72.7	17	N16-1172	72.6	16	N17-2484	75	13
N16-1182	72.7	12	N16-1153	72.5	22	N17-2119	75	17
N16-989	72.5	15	N16-1183	72.5	13	N17-2562	74.9	30
LSD .05	0.9	5	LSD .05	0.9	5	LSD .05	1	6

^aTW = Test weight normalized to 13% moisture content by Lui et al. (2019) method.

^bMD = Maturity days after 1 October.

^cCheck cultivar: Osage and NC-Miller = Maturity Group (MG) V, NC-Dunphy and NC-Roy = MG VI, NC-Wilder = MG VII.

TABLE 4 Comparison of soybean test weight of check cultivars to those of breeding lines observed in replicated trials grown in North Carolina in 2019

NC19-1			NC19-2			NC19-3			NC19-4		
Line	TW ^a	MD ^b	Line	TW	MD	Line	TW	MD	Line	TW	MD
	kg hL ⁻¹	d (Oct 1 = 1)		kg hL ⁻¹	d (Oct 1 = 1)		kg hL ⁻¹	d (Oct 1 = 1)		kg hL ⁻¹	d (Oct 1 = 1)
Osage ^c	72.2	19	Osage	72.4	19	NC-Dunphy	72.1	24	NC-Dunphy	72.4	27
NC-Dunphy ^c	72	21	NC-Dunphy	72.4	27	NC-Raleigh ^c	71.5	30	NC-Raleigh	71.8	28
NC-Roy ^c	74.7	22	NC-Roy	75	24	NC-Dilday ^c	72.9	26	NC-Dilday	73.4	30
N18-1704	75.8	18	N18-1561	75.4	22	N18-1944	74.1	19	N18-1971	74.3	23
N18-1626	75.4	18	N18-1675	75.3	20	N18-1861	73.7	21	N18-1849	73.8	29
N18-1659	75.3	20	N18-1614	75.3	22	N18-1946	73.6	27	N18-1851	73.8	23
N18-1737	75	18	N18-1821	75.2	22	N18-1932	73.5	26	N18-1841	73.6	26
LSD .05	0.7	2	LSD _{0.05}	0.6	5	LSD .05	0.6	5	LSD .05	0.8	5

^aTW = Test weight normalized to 13% moisture content by Lui et al. (2019) method.

^bMD = Maturity days after 1 October.

^cCheck cultivar: Osage = Maturity Group (MG) V, NC-Dunphy and NC-Roy = MG VI, NC-Raleigh and NC-Dilday = MG VII.

checks ‘NC-Dilday’ (North Carolina State University), ‘NC-Raleigh’ (PI 641156) (Burton et al., 2006), and ‘NC-Wilder’ (North Carolina State University) were evaluated in two, two, and one trial(s), respectively. Test weight of NC-Raleigh was significantly less than many breeding lines. Cultivar NC-Dilday was not significantly different from three of the top four TW breeding lines in ‘NC19-4’, but significantly less than three of the top four TW breeding lines in ‘NC19-3’ (Tables 3 and 4). Test weight of NC-Wilder was not significantly different from the top TW breeding lines of ‘NC18’ (Tables 3 and 4). This data shows that some of the check cultivars used in southern soybean breeding are no. 2 grade in terms of TW. These results indicate that improvement of TW should be a consideration in U.S. soybean cultivar breeding.

3.4 | Correlations of test weight with other traits

3.4.1 | Test weight and seed composition

The SPC was significantly ($p < .01$) positively correlated with TW in ‘NC18’. The correlation coefficients (r) for TW with SPCs were .14, .21 and .20 in Clayton (Cla), Plymouth (Ply), and across environments, respectively (Table 5). Seed oil concentration (SOC) was significantly negatively correlated in the combined analysis of ‘NC18’ and ‘NC19-2’ ($r = -.26$ and $-.42$, respectively). Individual locations of these trials also exhibited significant negative correlations of TW with SOC (Table 5). In the NILPs, SOC of HP backgrounds within populations was significantly

TABLE 5 Correlations of agronomic, physical, and compositional qualities of soybean with test weight in replicated trials grown in North Carolina from 2017–2019

Trial	Location ^a	MD ^b	SDWT ^c	SQ ^d	Yield	Protein ^a	Oil ^a
NC17-1	Cas	.06	–.18	–.24	.12	.02	.23
	Ply	.14	–.21	–.29*	–.11	.12	–.05
	Combined	.10	–.23	–.31*	–.04	.07	.09
NC17-2	Cas	.32*	–.23	–.20	.06	–.07	.02
	Ply	.12	–.45***	–.12	–.15	–.02	.01
	Combined	.26*	–.42***	–.16	–.11	–.06	.01
NC18	Cla	.21***	–.02	–.13**	–	.14**	–.20***
	Ply	.32***	–.08	–.21***	–	.21***	–.29***
	Combined	.28***	–.03	–.17***	–	.20***	–.26***
NC19-1	Cas	–.12	–.20	.12	–.01	.05	–.10
	Ply	–.19	–.23	.14	.03	.08	–.15
	Combined	–.16	–.22	.15	.03	.07	–.13
NC19-2	Cas	–.21	–.26	–.04	–.14	.26	–.40**
	Ply	–.10	–.20	.18	–.13	.20	–.41**
	Combined	–.16	–.24	.12	–.15	.24	–.42***
NC19-3	Cas	–.13	–.07	–.10	–.12	.14	–.10
	Ply	–.17	–.35*	–.20	.04	.17	–.10
	Combined	–.17	–.21	–.13	–.06	.15	–.11
NC19-4	Cas	–.06	.16	.21	.24	–.06	–.08
	Ply	.07	–.20	–.09	.14	–.01	–.07
	Combined	–.05	–.06	.09	.22	–.06	–.04

Note: Cas = Caswell, NC; Ply = Plymouth, NC; Cla = Clayton, NC; MD = maturity day; SDWT = 100-seed weight; SQ = seed quality, 1 = very good, 5 = very poor (Green et al., 1965).

^aProtein and oil in g kg^{–1} expressed on a 13% moisture basis.

TABLE 6 Correlations of seed protein and oil concentrations with test weight within soybean near isogenic line (NIL) populations differing in the presence of the Danbaekong allele on chromosome 20 grown in North Carolina in 2019

Trait	BC4F4			HIF-1			HIF-2			HIF-3			HIF-4		
	LP ^a	HP ^b	Combined	LP	HP	Combined	LP	HP	Combined	LP	HP	Combined	LP	HP	Combined
Protein ^c	.27	.38*	.49***	–.01	.97***	.57*	.65***	.49*	.57***	–.11	–.04	.31	–.09	.47	.20
Oil ^c	–.53*	–.45*	–.55***	.19	–.86*	–.43	–.67***	–.74**	–.61***	–.68*	–.78*	–.71***	–.13	–.73**	–.40*

^aLP = Low seed protein background absent (–/–) for the Danbaekong allele on chromosome 20.

^bHP = High seed protein background homozygous (+/+) for the Danbaekong allele on chromosome 20.

^cProtein and oil in g kg^{–1} expressed on a 13% moisture basis.

negatively related to TW (Table 6), and SPC of HP lines in HIF-1 population exhibited the strongest positive correlation with TW ($r = .97$). When NILPs were analyzed as a whole, all SPC and SOC correlations with TW were positive and negative, respectively (Table 6). However, the relationships with TW and seed composition varied between opposing backgrounds within NILPs (Table 6). Although the relationship with specific compositional properties varied, on average, TW of HP lines was higher when compared to LP lines of the same population (Figure 2). This provides preliminary evidence that incorporation of the HP Danbaekong allele on

chromosome 20 may improve TW alongside the increase in SPC.

Although statistically significant correlations were found between seed composition and TW in several replicated trials of this study, the values were inconsistent among trials. The significant correlations found with these variables and TW were predominantly in the ‘NC18’ trial. These results are most likely due to the broader range of genotypic TW, SPC, and SOC in this trial (Supplemental Table S2 and S4). However, ‘NC19-2’ possessed a narrower range in TW and SOC compared to ‘NC18’ but displayed the strongest correlation of

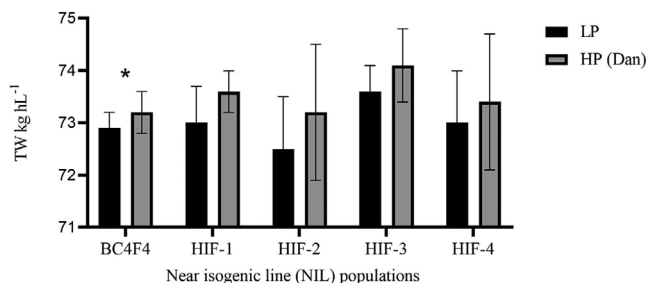


FIGURE 2 Comparison of test weight values between high seed protein (HP) and low seed protein (LP) lines within soybean near isogenic lines (NIL) populations developed by marker-assisted selection (MAS) grown in North Carolina in 2019. High protein NILs homozygous for the Danbaekkong allele on chromosome 20 (+/+) had numerically higher test weight (TW) values compared to opposing NILs homozygous for the absence of the Dan allele (–/–) within the same population, for all NIL populations. Results of student's *t* test of TW values between HP (Dan) and LP backgrounds within NIL populations. *Significant at the .05 probability level

TW with SOC of all trials in this study ($r = -.42$) (Table 5, Supplemental Tables S3 and S5). Similar to the finding of TW and SPC in maize transgenic lines, NILs of this study with higher SPC genetic backgrounds (HP lines) had higher TW than the low SPC (LP lines) within each population (Figure 2, Supplemental Table S6).

3.4.2 | Yield, maturity, and test weight

Seed yield is the most important trait in developing and marketing a soybean cultivar. Genetically increasing TW while maintaining yield would add value by increasing the storage and transport efficiency. No significant correlations were found between yield and TW in this study (Table 5). These results suggest that TW is not directly related to yield in soybean. Therefore, genetic improvement of TW in soybean could potentially be obtained without direct impact on yield. The correlation values between yield and TW in soybean have also been reported as inconsistent among trials and environments (Lui et al., 2019). Maturity exhibited a significant positive relationship with TW only in 'NC17-2' and 'NC18' (Table 5).

3.4.3 | Relationship of physical properties of seed with test weight

When discussing bulk density measurements of seed, physical properties of the seed such as length, width, and seed weight are often mentioned. The 100-seed weight (SDWT) measurement was one of the two physical properties of seed examined in our study. In 'NC17-2', SDWT was significantly negatively

correlated with TW in the combined analysis (Table 5). The SDWT was also significantly negatively correlated in the Ply location of 'NC19-3' ($r = -.35$). Correlations of TW with SDWT were consistently negative in all trials and all locations, except one individual location within a trial (Table 5). Differences observed in combined analyses vs. single location (environment) analyses within the same trial are most likely attributed to the environmental effect on TW observed in this study. Our study shows soybean SDWT to be inversely related to TW, meaning that smaller seed size may result in higher TW in soybean. A possible explanation for our result differing from those of other grain crops is the roundness and uniform shape of soybean seeds, so the varieties with smaller seed size (lower SDWT) allow a larger amount of seed into a specified unit of volume (i.e., better packing efficiency). Liu et al. (2019) also reported SDWT as negatively correlated with TW in soybean.

Seed quality in this study is an estimated visual rating of the seed, where 1 = very good and 5 = very poor quality (Green et al., 1965). Seed quality (SQ) scores in 'NC17-1', and 'NC18' were significantly negatively correlated with TW ($r = -.31$ and $-.17$, respectively). In other words, good quality seed resulted in higher TW.

4 | DISCUSSION

In this study, we examined the genotypic and environmental effects on TW in soybean, as well as the relationship of TW with seed composition, agronomic traits, and physical properties commonly used in soybean-breeding programs. Relatively high heritability for TW has been observed in other grain crops. Maize reports on heritability are consistently around .60 (Brandariz & Bernardo, 2019; Qiang et al., 2011), while heritability in wheat, oat, and rice are $>.80$ (Cabral et al., 2018; Holland & Munkvold, 2001; Pixley & Frey, 1991; Singh et al., 2011; Sun et al., 2009). The significant genotypic effect and relatively high broad-sense heritability of TW in soybean provide evidence to effectively improve this trait by breeding and selections, as has been demonstrated in oat (Klein et al., 2010). However, the significant environment and $G \times E$ interactions also indicate that improvement of TW through breeding may be environment specific in some cases. Liu et al. (2019) also observed high heritability of TW with significant environmental effects in soybean populations.

Seed compositional traits did not result in a conclusive relationship with TW in this study. Although the mass densities of protein and oil differ substantially (1.22 – 1.43 g cm $^{-3}$ and 0.917 g cm $^{-3}$, respectively), the theoretical seed density changes due to these compositional differences did not result in a definitive relationship with TW (bulk density) (Fischer et al., 2004). This is most likely due to the small contribution that true seed density has on the bulk density or TW

measurement (Ghaderi et al., 1971). However, in the ‘NC18 trials’, in which the genotypic range of differences in protein as well as oil concentrations were much larger compared to those in the ‘NC17’ trials (Supplemental Table S4), there were significant positive correlation between TW and SPC, and negative correlation between TW and SOC. Negative correlation of TW with SOC was also found in ‘NC19-2’ (Table 5). It appears that within the normal ranges of SPC and SOC (340–390 g kg⁻¹ and 170–200 g kg⁻¹, on 13% moisture basis, respectively) of U.S. soybean varieties and advanced breeding lines the effects of seed protein or oil on TW is generally small and may not show significant correlations with TW. However, if the range of genotypic differences in a study is larger (for example, ‘NC18’ with SPC and SOC range of 317–453 g kg⁻¹ and 161–229 g kg⁻¹, respectively), such correlations may be larger in magnitude and statistically significant. Liu et al. (2019) also reported a positive correlation of SPC and negative correlation of SOC with soybean TW. Although TW has not shown concordant relationships with *total* SPC in soybean, dissection and analysis of the specific seed proteins and amino acid compositions of the total SPC may lead to a more definitive relationships such as that which Gerde et al. (2016) found when analyzing specific zein proteins in maize.

Use of large numbers of NILs to analyze the tentative impact of seed compositional traits on TW may be an effective way of revealing the true relationship of each trait with TW. The results from the NILPs in this study, particularly for the HP lines, reveal much stronger correlation values of SPC and SOC with TW compared to the other populations. This observation is most likely due to the near-isogenic background of these lines within the NILPs compared to the RILs in the other populations. Since they differ mainly by the Dan locus on chromosome 20, the other traits affecting TW values are very similar among NILs. This allows clearer observation of the effect that seed composition has on TW, as demonstrated by the stronger observed relationships (Table 6). Similarly, Zhang et al. (2019) has demonstrated effectiveness of TW for distinguishing high SPC from low SPC in transgenic lines of maize. Further studies, particularly using NILs, will be necessary to determine the true nature of relationships of soybean TW with seed protein and seed oil concentrations.

Maturity date’s relationship to TW varied in strength and significance among trials. This discrepancy is most likely due to differences in genotypes, planting dates, and location and plot management practices. Yield was found to have no definite relationship with TW of soybean in this study, as direction and strength of the relationship varied among and within trials (Table 5).

Test weight has long been defined as the product of kernel density and packing efficiency, where packing efficiency is the percentage of volume of a certain container occupied

by the grain. Packing efficiency has been highly correlated ($r = .961$) with TW and weakly influenced by true density (Ghaderi et al., 1971). Packing efficiency is affected by seed shape, seed uniformity, harvest location, and surface characteristics (Lloyd et al., 1999). Physical properties of seed factor into packing efficiency and therefore TW. Our results of SDWT and SQ, both physical properties of soybean seed, exhibiting significant correlations to TW support the previous reports. Seed quality score is a factor in our program for advancement from plant rows to yield trials; therefore, the nonsignificant values for SQ in ‘NC17-2’ and ‘NC19’s are most likely due to a diminished number of entries with poor seed quality and susceptibility to fungal pathogens. Our results show SDWT having mostly consistent negative relationship with TW, particularly the relationships of TW with SDWT in ‘N17-2’ trials (Plymouth and combined) were very strong. The check cultivars Osage and NC-Raleigh were influential points in correlations of SDWT with TW, by exhibiting both very low SDWT and TW. Although this went against the consensus trend in the analysis, their values were not omitted in this study because they remained consistent across trials. Liu et al. (2019) also reported significant negative correlation of SDWT with TW in soybean.

In summary, TW in soybean has not been extensively studied when compared to other grain crops. Further investigation will be needed for harnessing the value of this trait in soybean, as projections for the demand of this crop rise. Higher TW soybean seemingly depends on physical and chemical properties of the seed. Physical properties of seed are important criteria for transportation, processing, and storage. A better understanding of these properties of TW has potential to positively impact the whole supply chain for soybean commerce. Further studies of physical properties of soybean are needed to elucidate the relationship of packing efficiency and TW in soybean. Furthermore, the effects of SPC and SOC on TW deserve further investigations, specifically with relation to the components of SPC (amino acids) and SOC (fatty acids), and differences within NIL populations or functional analyses of independent transgenic lines. Our study and a previous study indicate potential positive and negative effects of SPC and SOC, on TW, respectively. Such studies are more important than ever before with the focus on developing high SPC and high SOC soybean for the value-added market systems. The results of this study should be helpful in guiding future TW-related studies and in breeding for improved TW of soybean.

AUTHOR CONTRIBUTIONS

Brant T. McNeece: Formal analysis; Investigation; Methodology; Writing-original draft. Jay H. Gillenwater: Formal analysis; Investigation; Methodology; Writing-original draft. Zenglu Li: Conceptualization; Methodology; Writing-review & editing. M.A. Rouf Mian: Conceptualization; Project

administration; Resources; Supervision; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

M.A. Rouf Mian  <https://orcid.org/0000-0001-6917-0305>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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