Characterization of the Genetic Basis and Biological Significance of Heteroblasty in Soybean

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Abstract

Leaf morphology, as a critical measure of plant architecture, has been associated with soybean yield and quality. As yet, conclusive investigations into the genetic basis and biological significance of heteroblasty in soybean remain sparse. To identify and biologically characterize the quantitative trait loci (QTLs) influencing heteroblasty in soybean, 11 traits associated with leaf morphology, seed quality, and grain yield were observed for two recombinant inbred line (RIL) populations grown in two environments. Values of broad-sense heritability (H^2) for leaf traits in two RIL populations varied from 0.62 to 0.99. These high heritabilities, taken together with the Kurtosis and Skewness values indicate that the phenotypic variation in leaf morphology observed for both RIL populations are mainly controlled by quantitative traits. The three observed leaf morphology traits exhibited significant correlations ($P < 0.05$) with more than half of the grain yield and seed quality traits, with correlation coefficients varying from -0.40 to 0.43 , which suggests that leaf morphology can significantly influence soybean yield and quality. A total of 11 QTLs were detected for leaf morphology, with logarithm of odds (LOD) values ranging from 3.40 to 89.6, which accounted for 1.38 ~ 74.53% of genetic variation. Eight of these loci for leaf morphology co-located with those for seed quality and grain yield, which coincided large and extensive leaf morphologies that contributed to seed quality and grain yield formation. Overall, these results provide important information for breeding high yielding crop varieties with improved quality traits produced through optimization of leaf morphology.

Introduction

In generally, leaf morphology, which can be described by leaf length (LL), length width (LW), leaf shape index (LS) and leaf area (LA), is not only able to affect light penetration, light absorption, CO₂ fixation and photosynthetic efficiency, but also may alter the canopy structure of the population which can optimize light distribution, increase light energy utilization efficiency and facilitate ventilation permeability (Sarlikioti et al. 2011; Bhagsari et al. 1986). Therefore, it is not surprising that crops with ideal plant canopy structures will produce higher grain yield. Meanwhile, leaf morphology has also been proven to play roles in regulating fruit quality. For instance, in tomato (*Solanum lycopersicum*), rounder leaflets were associated with enriched fruit sugar content and the potential for improved fruit quality (Eshed et al. 1995; Chitwood et al. 2014).

For a given plant species, one plant may exhibit multiple leaf morphologies, with much of the variation in leaf shape resulting from species-specific patterns of leaf development. As a result, the leaves formed by a single plant can display a remarkable diversity of forms, which is known as heterophylly. Heterophylly may all more efficient use of light energy through affects on canopy structure, gas exchange and light interception of the lower canopy (Hejník et al. 2014; Beyschlag et al. 2017). For example, relatively narrow leaves on top of a plant allow light through to the middle and lower leaves for photosynthetic activity throughout the canopy, while relatively bigger and rounder leaves emerging from middle and lower nodes facilitate absorption of scattered light. In any case, studies to date strongly suggest that breeding crop varieties for high yield and better quality through optimization of leaf morphology is a feasible strategy.

Soybean (*Glycine max* (L.) Merrill), one of the most economically important leguminous crops, is a crucial plant source of proteins and oil for both humans and domesticated animals (Graham et al. 2003). Due to increasing market demands, breeding soybeans for higher yields is an urgent priority. Crop yield is an aggregate of complex traits, which is affected by many morphological, physiological and agronomic traits. Leaves are the major photosynthetic organs in plants, and leaf shape is a major component of plant architecture. It follows, therefore, that suitable leaf shapes may significantly enhance the photosynthetic capacity of soybean plant, and thereby increase yields and potentially improve specific quality traits (Reinhardt et al. 2002).

Leaf morphologies observed on currently available soybean varieties can be mainly classified into two categories: ovate and lanceolate (Jun et al. 2014; Xia et al. 2018; Baldocchi et al. 1985; Wang et al. 2019). Soybean with lanceolate leaves tend to produce more seeds per pod (SPP) than those with ovate leaves, which are more likely to generate bigger and heavier seeds than soybeans with lanceolate leaves (Domingo et al. 1945; Mandl et al. 1981). Canopies of soybeans with lanceolate leaves lower leaf area indices, but intercept more photo synthetically active radiation than canopies of soybeans with ovate leaves canopies (Bianchi et al. 2020). Nevertheless, each type of leaf morphology has its own distinct advantages, therefore it is not surprising that tests for yield differences among soybeans displaying variation in leaf morphology and canopy architecture have returned mixed results (Sayama et al. 2017; Wells et al. 1993).

In this study, we first explored the molecular mechanisms underlying the formation of soybean leaf morphology through observations of a series of leaf-related traits, including LL, LW, LA, leaf weight, LS, all of which have been widely studied in previous work. According

to published data from soybase.org (<https://www.soybase.org/sitemap.php>), a total of more than 70 quantitative trait loci (QTLs) associated with soybean leaflet shape have been found on 18 of 20 chromosomes, with the highest concentrations found on chromosomes 6, 15 and 19 (20 QTLs). These QTLs align with several annotated genes that have been predicted to regulate leaf morphology and cloned to clarify their functions (Wang et al. 2019; Liu et al. 2019). One single recessive gene *ln* has been found to contribute to lanceolate leaf formation, while, its dominant allele, *Ln*, contributes to ovate leaf formation (Fang et al. 2013). However, all of research outlined above has focused on single types of leaf morphology. None have considered any affects that heteroblasty might have on soybean yield and quality. Therefore, the determination of any significant impacts of heteroblasty on soybean yield and quality, as well as, any common genetic foundations, remains a relevant and potentially critical line of inquiry for realizing gains in soybean productivity sufficient to meet future demands.

In this study, three advanced cultivars contrasting in leaf morphology phenotypes, Jidou17 (JD17, leaf heteroblasty), Jidou12 (JD12, ovate leaves) and Suinong14 (SN14, lanceolate leaves), were used to make crosses of JD17×JD12 and JD17×SN14. The main objectives of this study were (1) to clarify the biological significance of heteroblasty in regards to soybean yield and quality, and (2) to identity genetic markers held in common among significant markers for heteroblasty, yield and quality. This study may facilitate dissecting the elements of ideal soybean plant architecture and, therefore, contribute to breeding high yielding soybean cultivars with containing bean quality improvements realized through optimization of leave morphology.

Materials And Methods

Plant materials

Jidou 17 (JD17) is a high yielding soybean cultivar from the Institute of Cereal and Oil Crops at the Hebei Academy of Agricultural and Forestry Sciences. It is adapted to the Huang-Huai-Hai growing region. It displays a remarkable diversity in leaf shape within single plants at the full seed reproductive growth stage (R6), with the leaf shape from bottom to top on the main stem gradually changing from oval to narrow (Fig. 1-A).

Two RIL populations, with JD17 as a common parent, were constructed to detect QTL for leaf shape-related traits. The other two parents were also cultivars from China. Jidou 12 (JD12) from Hebei Province is characterized by ovate fractures of leaflets during the R6 stage of development. Suinong 14 (SN14) from Heilongjiang Province is characterized by narrow fractures of leaflets during the R6 stage of development. The two RIL populations were designated as JJ (JD17×JD12) and JS (JD17×SN14). These RIL populations were derived by advancing F2 lines of both populations up to six generations through single seed descent (SSD). JJ and JS were composed of 191 and 286 RIL lines respectively.

Field trials and trait evaluation

Two F_{6:10} RIL populations and the three parents were planted in Shijiazhuang of Hebei Province in 2017 and 2018. Experiments included three replicates arranged in a randomized incomplete block design. Each plot contained two 2.0 m long rows spaced 0.5 m apart, with 20 seeds planted per row. Field management followed local practices.

At the R6 growth stage, leaf traits were determined for the three most basal leaflets and the top three fully developed leaflets on the main stem from three plants towards the middle of each plot. The phenotypic values of LL and LW were obtained by measurement. The LS values were defined as the ratio of LL to LW. The leaf shapes of the three top pinnate leaves were labeled LSUP, and the shapes of the three most basal pinnate-ternate leaves were labeled LSDOWN. The leaf shape ratio (LSR = LSUP/LSDOWN) was used to evaluate heteroblasty in the JJ and JS RIL populations.

A total of five parameters, pod number per plant (PNPP), seed number per plant (SNPP), seed weight (SW), protein content (PC) and oil content (OC) were measured to determine yield and seed quality phenotypes produced by the two RIL populations grown inr two years in Shijiazhuang. SW was measured by weighing 100 random filled seeds. SNPP was calculated by counting the number of seeds per plant after threshing. PNPP values were obtained by counting the number of pods per pod. 50 g of seed from each line were used for PC and OC determination by Fourier-transform near infrared spectrometry (Bruker Vector 22/N, Germany) (Miao et al. 2019).

Statistical analysis

Statistical analysis was performed in SPSS Statistics 17.0. ANOVA was performed using the phenotypic data of the two years, 2017 and 2018. A mixed procedure was used with genotype and location included as fixed effects in order to estimate heritability. A post hoc Turkey's test was used to compare means. The broad-sense heritability of single environments was then calculated by the following formula:

$$H = \sigma^2G / (\sigma^2G + (\sigma^2e/r))$$

where σ^2G is the genotypic variance; σ^2e is the error variance, and r is the number of replications.

GBS library construction and SNP identification

Genomic DNA of parents and their RILs were extracted using the NuClean Plant Genomic DNA Kit (CWBIO, Beijing, China) following the manufacturer's protocol. SNP genotyping was performed using a GBS approach. The GBS library was constructed as previously described (Cheng et al. 2015). Paired-end sequencing was performed on selected tags using an Illumina 2500 platform (Illumina, USA) at the Novogene Bioinformatics Institute, Beijing, China.

The Burrows-Wheeler Aligner (BWA), SAMtools and a custom Perl script were used to identify SNPs in the RIL population (Zhou et al. 2016). The software tool ANNOVAR (Wang et al. 2010) was used to align and annotate SNPs or InDels based on the GFF3 files from the soybean genome annotations provided in the Phytozome database.

Bin map construction and QTL analysis

Chi-square (χ^2) tests were performed for all SNPs to detect segregation distortion. Markers with segregation distortion test p values of less than 0.001, were filtered out along with those containing abnormal bases (Su et al. 2017). All markers were removed if more than 20% of the tested lines returned missing genotypes in TASSEL 5.0. Parental allele assignment and imputation were performed using the Window LD function of FsFHap (Swarts et al. 2014) incorporated into TASSEL 5.0.

After filtering GBS data, redundant markers were binned based on segregation patterns deciphered in the RIL population using the BIN function in IciMapping 4.1 (Meng et al. 2015; Li et al. 2008). Markers segregating with at least one other marker were retained, and one marker was chosen to represent each bin (Zeng et al. 2019). The bins were used as genetic markers for the construction of a linkage map constructed using the MAP foundation for statistical computing by IciMapping Version 4.1 software (<https://www.isbreeding.net/software/>).

Additive QTL were detected using inclusive composite interval mapping (ICIM) in the BIP (bi-parental populations) model of QTL IciMapping software v4.1, with the P values for entering variables (PIN) = 0.01. The threshold of the logarithm of the odds (LOD) score for evaluating the statistical significance of QTL effects was determined using 1000 permutations at the significance level of 0.05 (Pei et al. 2018). As a result, an LOD score of 3.3 was used as the threshold to declare the presence of a significant QTL.

Results

Phenotypic evaluation of parents

To accurately evaluate differences in leaf morphology among the three included parents, a representative leaf from each node was selected to calculate the parameters of leaf length to width ratio (LS). As expected, leaf morphology contrasted between JD12 and SN14, as indicated by respective LS indices of 1.28 ~ 1.58 and 2.90 ~ 3.49. These observations fit the existing categorization of JD12 and SN14 having ovate and lanceolate leaves, respectively. Interestingly, the LS indices of JD17 leaves ranged from 1.40 to 3.80, with the bottom 3 ~ 4 leaves being ovate leaves (LS: 1.48 ~ 1.55), and the top 3 ~ 4 leaves being mostly lanceolate leaves (LS: 2.63 ~ 3.44). These results imply considerable heteroblasty in the canopies of JD17 soybean plants (Fig. 1-B).

In order to further clarify this heteroblasty, we calculated the LSR index as the ratio of the LS indices of the top 3 ~ 4 leaves (LSUP) to the bottom 3 ~ 4 leaves (LSDOWN). Values derived from observations of at least ten representative plants revealed LSR indices of ~ 1.96 for JD17 plants, while, LSR indices of JD12 and SN14 canopies were ~ 0.99 and ~ 1.14, respectively (Fig. 1-C). These observation of LSR indices among the three parents confirmed strong heteroblasty in the canopies of JD17 soybeans.

Phenotypic variation among RILs

In order to explore the genetic base of heteroblasty, the phenotypic variation in leaf morphology traits, including LSUP, LSDOWN and LSR, were evaluated within two RIL populations reared under field conditions in 2017 and 2018, with the results being summarized in Table 1. With coefficient of variation (CV) values of 0.07 ~ 0.32 suggesting significant phenotypic variation (CV) for these traits, the mean values observed for each RIL populations all fell between the average parent values, while the maximum and minimum values fell beyond the extremes of the parent values. These results imply that there might be extensive transgressive heritability in both RIL populations, which is indispensable for QTL tests.

Table 1
Phenotypic variation in leaf morphology-observed in two RIL populations.

Population	Traits ^a	Parents		RIL							
		JD17	JD12/SN14	Year	Mean $\pm$ SD (%)	Min	Max	CV/% ^b	Skew	Kurt	H ² % ^c
JD17×JD12 (JJ)	LSR	1.95	1.19	2017	1.40 $\pm$ 0.25	1.01	2.34	18	0.85	0.87	62.4
		2.15	0.98	2018	1.57 $\pm$ 0.37	0.94	2.67	24	0.74	-0.11	
	LSUP	3.01	1.48	2017	1.85 $\pm$ 0.35	1.21	3.42	19	1.08	2.03	69.95
		3.08	1.3	2018	2.08 $\pm$ 0.53	1.23	3.88	25	0.99	0.65	
	LSDOWN	1.54	1.28	2017	1.33 $\pm$ 0.09	1.08	1.64	7	0.57	1.15	74.84
		1.43	1.32	2018	1.32 $\pm$ 0.10	1.02	1.68	8	0.52	1.24	
JD17×SN14 (JS)	LSR	1.95	1.15	2017	1.49 $\pm$ 0.34	0.84	2.88	23	0.78	0.7	92.37
		2.15	1.11	2018	1.62 $\pm$ 0.46	0.73	3.13	28	0.47	-0.54	
	LSUP	3.01	3.34	2017	2.84 $\pm$ 0.61	1.6	4.44	21	0.3	-0.47	90.92
		3.08	3.28	2018	3.06 $\pm$ 0.64	1.83	4.83	21	0.28	-0.31	
	LSDOWN	1.54	2.91	2017	2.01 $\pm$ 0.64	1.24	3.46	32	0.39	-1.46	98.62
		1.43	2.95	2018	2.02 $\pm$ 0.63	1.21	3.84	31	0.47	-1.145	

^aLSR, leaf shape ratio. LSUP, top pinnate leaf shape. LSDOWN, basal ternate pinnate leaf shape. ^bCV, coefficient of variation. ^cH², broad-sense heritability.

Table 2
Description of stable QTL for leaf shape related traits.

Chr	QTL Loci	Traits ^a	QTL	Population	Years	Marker interval	LOD ^b	PVE ^c (%)	Add ^d
1	<i>qLM1</i>	LSR	<i>qLSR1</i>	JJ	2017/2018	Gm01:48868375 ~ Gm01:49131130	3.42– 4.93	5.49– 8.03	0.07–0.08
		LSUP	<i>qLSUP1</i>	JJ	2017	Gm01:49432006 ~ Gm01:49761132	7.16	11.49	0.12
3	<i>qLM3</i>	LSUP	<i>qLSUP3</i>	JS	2018	Gm03:39064883 ~ Gm03:39119155	4.55	3.02	-0.13
		LSDOWN	<i>qLSDOWN3</i>	JJ	2018	Gm03:38132250 ~ Gm03:38684678	4.84	10.68	0.03
10	<i>qLM10-1</i>	LSDOWN	<i>qLSDOWN10</i>	JS	2018	Gm10:1528492 ~ Gm10:1931561	4.09	1.94	-0.09
	<i>qLM10-2</i>	LSR	<i>qLSR10</i>	JS	2018	Gm10:45298496 ~ Gm10:45654439	3.46	2.41	0.07
		LSUP	<i>qLSUP10</i>	JS	2017/2018	Gm10:45004437 ~ Gm10:45265551	7.24– 8.73	4.93– 5.68	0.15–0.16
12	<i>qLM12</i>	LSR	<i>qLSR12</i>	JJ	2018	Gm12:3549384 ~ Gm12:3738868	4.06	6.57	0.09
14	<i>qLM14</i>	LSUP	<i>qLSUP14</i>	JS	2018	Gm14:277700 ~ Gm14:449709	4.99	3.43	0.13
15	<i>qLM15</i>	LSR	<i>qLSR15</i>	JJ	2017	Gm15:8280651 ~ Gm15:9085621	3.4	5.49	0.06
		LSDOWN	<i>qLSDOWN15</i>	JS	2017	Gm15:11854815 ~ Gm15:11878190	3.74	1.38	-0.08
17	<i>qLM17</i>	LSUP	<i>qLSUP17</i>	JJ	2018	Gm17:10209159 ~ Gm17:12449434	3.75	6.94	0.15
18	<i>qLM18</i>	LSR	<i>qLSR18</i>	JS	2017/2018	Gm18:55804271 ~ Gm18:56171564	13.13– 15.12	10.56– 12.53	0.12–0.16
		LSUP	<i>qLSUP18</i>	JS	2017/2018	Gm18:55570282 ~ Gm18:56171564	13.19– 23.98	9.11– 19.43	0.20–0.32
19	<i>qLM19</i>	LSR	<i>qLSR19</i>	JJ	2017/2018	Gm19:45157936 ~ Gm19:45286223	12.73– 15.01	23.05– 28.12	0.12–0.18
		LSUP	<i>qLSUP19</i>	JJ	2017/2018	Gm19:45157936 ~ Gm19:45286223	11.89– 16.18	19.21– 27.34	0.16–0.29

^aLSR, leaf shape ratio; LSUP, top pinnate leaf shape; LSDOWN, basal ternate pinnate leaf shape; ^bLOD, logarithm of odds. ^cPVE, phenotypic variation estimated from marker regression against phenotype. ^dAdd, additive effect of QTL.

Chr	QTL Loci	Traits ^a	QTL	Population	Years	Marker interval	LOD ^b	PVE ^c (%)	Add ^d
20	<i>qLM20</i>	LSR	<i>qLSR20</i>	JS	2017/2018	Gm20:35767199 ~ Gm20:35910712	41.91– 44.17	43.69– 44.75	0.22–0.32
		LSDOWN	<i>qLSDOWN20</i>	JS	2017/2018	Gm20:35767199 ~ Gm20:35910712	81.20– 89.60	71.56– 74.53	-0.54– -0.57
^a LSR, leaf shape ratio; LSUP, top pinnate leaf shape; LSDOWN, basal ternate pinnate leaf shape; ^b LOD, logarithm of odds. ^c PVE, phenotypic variation estimated from marker regression against phenotype. ^d Add, additive effect of QTL.									

To further clarify the origination of the observed phenotypic variation, broad-sense heritability (H^2) for tested traits was observed over 2 years of field experiments. In these tests, H^2 values varied from 0.62 to 0.75 and from 0.91 to 0.99 for the JJ and JS populations, respectively. This indicated that the phenotypic variation observed among RILs in both populations was mainly derived from genetic variation, especially in the JS population (Table 1). Meanwhile, according to the values of Kurtosis and Skewness calculated over the 2 years of data collection, all tested traits, except for LSDOWN in JS, approximately fit into normal distributions, which indicates that the observed leaf traits are quantitative traits (Table 1).

To test for potential relations between leaf morphology and productivity, three soybean grain yield traits (PNPP, SNPP and SW) and two quality traits (PC and OC) were also observed in the two RIL populations. In these observations, the H^2 values for all tested traits varied from 0.56 ~ 0.94, while the absolute values of Kurtosis Skewness for all of 5 observed traits were all lower than 3 (Table S1 and S2). These results strongly suggest that grain yield and quality traits in the observed RILs were also mainly controlled by quantitative traits.

Correlation among leaf shape, grain yield and quality traits

To further test for effects of heteroblasty in improving soybean yield and quality, correlation analysis was performed for the tested traits. In this analysis, correlations between leaf shape and yield or quality traits were not consistent. For example, the three observed leaf shape traits were significantly correlated with most of the seed yield and quality traits in the JS population ($0.14 < r < 0.43$), while few relationships were established in the JJ population, none of which involved bottom leaf shape (Fig. 2). This implies that JD17 and JD12 share a common genetic base underlying the LSDOWN trait. Meanwhile, LSR and LSUP in the JJ population were only significantly correlated with SW, PC and OC ($0.16 < r < 0.37$) (Fig. 2). Taken together, these results strongly suggest that both grain yield and seed quality traits may be significantly influenced by heteroblasty, as well as, specific LS traits, depending on the parents. Incorporating heteroblasty measurements into soybean selection efforts may be valuable for programs seeking to breed high yielding or better-quality soybean cultivars.

Genetic linkage map with bin markers

In order to outline the genetic basis for heteroblasty in soybean, two high-density genetic maps were constructed based on the JJ and JS populations using GBS methods. After filtering and combining markers, 1970 bins and 2170 bins remained for mapping in the JJ and JS populations, respectively. The genetic linkage maps constructed from JJ and JS markers covered a genetic distance of 2535.40 and 2559.97 cM, respectively, with an average length of 126.77 and 127.99 cM per respective linkage group. For genetic maps from the JJ population, chromosome 18, at 191.43 cM, was the longest, and chromosome 01 was the shortest at 80.86 cM. The average number of bin makers on each linkage group was 98.50, with chromosome 18 having the most at 136 bin makers, while chromosome 11 harbored the fewest at 62 bin makers. The average distance between adjacent bin markers varied between 0.94 ~ 2.05 cM. Meanwhile, for JS genetic maps, chromosomes 13 and 14 were the longest and shortest, with respective genetic distances of 170.77 and 95.06 cM. The average number of bin makers on each linkage group was 108.50, with the average distance between adjacent bin markers varying between 0.88 ~ 2.07 cM (Table S3). These results verified that the two high-resolution linkage maps constructed in this study were accurate and useful in further studies.

Identification of QTLs for leaf morphology, seed quality, and grain yield traits

In order to further determine the common genetic basis for leaf morphology traits in the two RIL populations, QTL analysis was performed for leaf morphology, quality, and yield traits. This analysis returned a total of 11 significant loci containing 18 QTLs for leaf morphology traits, with 7, 7 and 4 of these QTLs being associated with LSR, LSUP and LSDOWN, respectively. For these QTLs, the LOD values ranged from 3.40 to 89.60 and could explain 1.38 to 74.53% phenotypic variation (PVE). Among these loci, *qLM18*, *qLM19* and *qLM20* were considered as stable and major loci for regulating leaf morphology, because they were returned as significant in each of the two years of experiments with high LOD values (11.89 ~ 89.60) that explain 9.11%~74.53% of phenotypic variation. Within the two populations, 8 and 10 QTLs contributed to leaf shape in the JJ and JS RIL populations, respectively. Interestingly, there were no common QTLs shared between the two populations for a given trait, suggesting that each population has its own unique genetic basis underlying leaf morphology traits.

In QTL mapping for grain yield traits, a total of 14 significant loci containing 22 QTLs were identified among the two soybean RIL populations observed in two years of field experiments, with the LOD values of these QTLs ranging from 3.31 to 29.38, and these loci accounting for 2.88%~31.54% of the observed phenotypic variation. Among these loci, *qGY10-2*, *qGY19* and *qGY20-1*, with LOD values of 3.31 ~ 29.38, and explaining 3.16 ~ 31.54% phenotypic variation, were considered as three major loci for controlling grain yield traits (Table S4). In addition, a total of 15 significant loci containing 20 QTLs were identified for variation in seed quality traits, with the LOD values of these QTLs ranging from 2.50 to 10.77, and these loci accounting for 3.89%~13.74% of the observed phenotypic variation (Table S5).

Identifying common genetic regions underlying leaf morphology, seed quality, and grain yield traits

One of the main objectives of this study is to clarify the practicality of increasing soybean seed quality and grain yield through optimization of leaf morphology. To this end, the 95% confidence intervals for QTLs were projected on a common genetic map. The results revealed a total of eight loci on seven chromosomes in which QTLs for leaf morphology that co-locate in overlapping or closely linked confidence regions with seed quality or/and grain yield traits. Five of these overlapping regions influence leaf morphology, seed quality, and grain yield traits (Fig. 3). Among significant loci for leaf morphology, *qLM20*, which contributed the highest LOD and PVE values to effects on leaf morphology, also accounted for the highest variation in 100 seed weight (18.25%), along with 10.73 ~ 11.21% of the variation in pod number per plant, as well as 3.89 ~ 6.73% of the variation in protein content in the JS population. Another confidence locus, *qLM19*, which contributed the highest LOD and PVE values to effects of leaf morphology in the JJ population, also co-located with both *qSW19* and *qOC19*, which accounted for 9.23 ~ 9.53% and 4.99 ~ 5.25% of the variation in 100 seed weight and oil content, respectively. In summary, all the co-localization observed in the included RIL populations coincided with the result that leaf morphology largely and extensively contributed to seed quality and grain yield traits.

Discussion

Plant shoot architecture is a critical, complex and comprehensive trait that could significantly influence crop yield. Leaves are the main organs for photosynthesis and synthesizing chemical compounds needed throughout crop development. As a major component of plant architecture, it is not surprising that leaf morphology and the arrangement of leaves in the canopy are able to affect light capture efficiency and photosynthetic efficiency (Chitwood et al. 2016), and, as such, have attracted increasing attention from crop breeders. In addition, suitable leaf morphology can also enhance sugar content in some fruits, such as tomato, through effects on photosynthetic efficiency, which leads to the question of whether leaf morphology can also affect soybean quality traits (Rowland et al. 2020). For their part, soybean breeders have realized the importance of leaf morphology for grain yield, though the physiological and molecular mechanisms underlying these relationships have not been well documented. Therefore, to discover the genetic mechanisms behind these affects, we studied leaf shape, grain yield and grain quality in two RIL populations.

The JD17 parent, a high yielding soybean cultivar with large values of oil content (> 23%), is one of the most widely planted soybean varieties in Huang-Huai-Hai region. In generally, breeders suppose that JD17 can reach high yields largely due to heteroblasty in its canopy producing an ideal shoot architecture. However, this hypothesis is mainly based on breeder observations without any solid experimental evidence. Therefore, in this study, three leaf morphology traits in two populations, including the LSR index for heteroblasty, were measured and tested for correlation with yield and quality traits. As expected, heteroblasty appeared to exert a great

influence on soybean grain yield and seed quality, as indicated by the high values of correlation between LSR and other traits (Fig. 2). Interestingly, significant positive correlations were found between heteroblasty and three grain yield traits in the JS population, while, only SW was negatively correlated with heteroblasty in JJ population (Fig. 2). These results strongly imply the presence of distinct genetic elements underlying heteroblasty traits in the JS and JJ populations. To test this, QTL analysis was performed for the two populations. In this study, no common QTLs for any leaf morphology trait were found between the two populations. This is strong evidence that the two RIL populations studied herein contain distinct genetic elements affecting leaf morphology.

Ln, which encodes a homolog of the *Arabidopsis JAGGED (JAG)* gene that regulates lateral organ development and exerts pleiotropic effects on fruit patterning, was reported as a key regulator of leaflet shape and seed number per pod in soybean (Fang et al. 2013; Jeong et al. 2012). In this study, a major QTL, *qLM20*, with the highest observed LOD and PVE% values of 89.6 and 74.53%, was detected on chromosome 20 in the JS population. Interestingly, the *Ln* gene is located in the confidence region of *qLM20*, which only covered a physical interval of 150 kb. These results strongly imply that effects of *qLM20* are impacted by the *Ln* gene, and this might partly explain the common genetic basis for leaf morphology and grain yield. Meanwhile, according to previously published work, shoot architecture may be impacted by the growth habit of soybean plants, with two well-known genetic loci, *Dt1* and *Dt2*, being cloned and documented as relevant underlying genes. Here, *qLM18* and *qLM19* were identified as two stable major QTLs for leaf morphology with relatively high LOD values in the JJ and JS populations, respectively. Surprisingly, *qLM18* and *qLM19* shared overlapping confidence intervals with *Dt2* and *Dt1*, respectively. The *Dt1* gene (*GmTFL1*) is a homolog of the *Arabidopsis TERMINAL FLOWER1 (TFL1)* gene that plays diverse roles related to signaling pathways controlling growth and differentiation (Thompson et al. 1995; Liu et al. 2010). In *Arabidopsis*, *TFL1* participates in the transition from vegetative to reproductive phases marked by an increase in the length/width ratio of the leaf blade. For its part, *Dt2*, a dominant MADS domain factor gene, promotes the early transformation of SAMs into reproductive development through the inhibition of *DT1* expression in SAMs (Ping et al. 2014). Therefore, we speculate that heteroblasty of soybean is related to the vegetative-to-reproductive phase transition.

Photosynthesis is an essential process for crop growth, and with leaves being the main organs for photosynthesis in plants, leaf morphology and distribution can be expected to significantly affect crop productivity. In related leaf removal experiments, yield and quality components change according to the number of leaves removed, time of leaf removal, and the position of removed leaves (Sackston 1959). However, leaf morphology and shoot architecture effects on productivity have not been well documented in soybean. For example, soybean leaf morphology effects on seed quality have been poorly documented until now. In this study, we elucidated important relations between leaf growth patterns and elements of crop productivity, including yield, protein content and oil content.

In this study, LSR and LSUP were negatively correlated (-0.37) with PC in the JJ population. Interestingly, this strong correlation was not found in the JS population. This might be due to the diversity of genetic backgrounds present among the three crossed parents. However, although we have found seven co-located loci regulating leaf morphology and seed quality traits, the physiological mechanisms employed to realize the observed phenotypes remain largely unknown. Hence, further investigation to finely delimit genetic markers with the goal of cloning candidate genes may help us better understand leaf morphology impacts on soybean yield and seed quality.

In summary, this report provides preliminary information on potential roles for genetic elements associated with leaf morphology in breeding programs aimed at improving soybean yield and seed quality. This work lays the foundation for further efforts to identify valuable genetic resources for MAS breeding programs.

Declarations

Acknowledgments Not applicable.

Authors' contribution QC, CY and MZ designed the experiments and analyzed the data. QC, LA, BL, YW, YF carried out the experiments. LY, QC, XS and BL constructed and genotyped the RILs. QC, SC, HZ and YY wrote the paper.

Ethics approval and consent to participate All data and materials are not related to human and animals. This research is not related to any plant specimens to be deposited as vouchers or any other association for this section. Written informed consent was obtained from individual.

Conflict of interests The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Figures

Figure 1

Phenotypic variation in leaf shape for parents at the R6 stage of soybean development. (A) The leaves arranged from left to right were the 1st , 2nd..... and Nth trifoliolate leaves. The distribution of leaf shape index (LS) (B) and leaf shape ratio (C) on whole plants.

Figure 2

Correlation analysis of leaf shape, grain yield and seed quality related traits. Displayed values are correlation coefficients (r) . The areas and colors of ellipses show the absolute value of corresponding r values. Asterisks indicate significant correlations at the P = 0.05 (*), 0.01 (**) and 0.001 (***) levels. .

Figure 3

Genetic maps of loci associated with leaf shape, seed quality and soybean yield. Colored and bold fonts represent loci for identified genes. Black, green and red blocks represent yield, quality and leaf shape related loci, respectively.

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