MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 32-2017
2018 FINAL REPORT

Title: Inheritance of a water use efficiency trait in soybean and its integration into improved soybean germplasm with high germinability

INVESTIGATORS: Dr. Jeffery D. Ray
Phone: (662) 686-3036
Email: Jeff.Ray@ars.usda.gov
Dr. James R. Smith
Dr. Larry C. Purcell
Dr. Felix B. Fritschi
Dr. Jason Gillman

TECHNICAL SUMMARY

Only about half (45.6%; 2013 NASS data) of Mississippi soybean acreage is irrigated. Of the acreage that is irrigated, future watering restrictions and rising costs may limit full-season irrigation options. Thus the development of improved water-use-efficient cultivars would likely benefit both irrigated and dryland production systems—i.e., all Mississippi soybean production.

Water-use efficiency (WUE) can be simply defined as yield per unit of water consumed. Previous genome-wide association studies (GWAS) by this team (Ray, Smith, Purcell, Fritschi) identified at least 21 different genetic loci putatively associated with WUE. In order to confirm and refine these QTL associations, a bi-parental F6-derived recombinant-inbred line (RIL) population between soybean lines differing in WUE was developed through funding from a recent United Soybean Board grant.

Under Objective 1, we grew and phenotyped the RIL in 2017 at three field locations: 1) Stoneville, MS (Smith and Ray); 2) Pine Tree Branch Station, AR (Purcell et al.); and 3) Columbia, MO (Fritschi et al.). The phenotypic data collected in 2017 will be used to identify QTLs associated with WUE. Each of the three locations employed their own resources to conduct the field experiments. The funds requested in this proposal covered the WUE assays of the samples from all three locations. Dr. Purcell has a Ph.D. graduate student who will fully analyze the data and conduct the QTL analysis to identify genomic locations associated with WUE. Markers in these locations will useful in the development of cultivars with greater WUE as well as provide specific loci for further physiological and molecular investigations.

In combination with this genetic study, an on-going breeding program combining high WUE and high germinability has been continued (Objective 2). High germinability under high heat conditions results in high-quality seed (reduced disease, wrinkling, green seed, dockage, etc.) as opposed to the typically low quality seed of the common germplasm pool used in most current cultivars.

Agronomically-improved F6:7 breeding lines with high WUE and high germinability were harvested in 2016 and selected F6:8 lines were harvested in Mississippi in 2017. In the 2018 season, we are conducting irrigated yield trials of these lines in Miss., and we will conduct combined irrigated and nonirrigated trials beginning in 2019 in Miss., Ark., Mo., and Ariz. The best, most WUE lines, will be identified for release.

BACKGROUND

Combining WUE with high germinability under high heat has the potential to produce agronomically sound cultivars with increased productivity and higher seed quality, but using less water (irrigated or dryland) than current commercial cultivars. For both the genetic study and the breeding program, much of the foundation work (development and genotyping of the RIL, early-generation breeding, etc.) was funded by a previous USB grant (ended December 2016). The research in this MSPB funded research has allowed the completion of that multi-year project.

It is widely recognized that improved WUE can enhance yield in certain environments; however, directly measuring WUE in large populations is extremely difficult. A surrogate for direct water-use efficiency measurement was developed in the 1980's based on the tissue composition of carbon isotopes 13C and 12C in plant tissue. The molar abundance ratio of 13C/12C in plant tissues is usually less than that in atmospheric CO_2 due to discrimination against the 'heavier' 13C during photosynthesis.

Numerous studies have shown a correlation between carbon isotope composition and WUE. In this report, when discussing WUE, we are specifically referring to WUE as estimated by carbon isotope composition. Previous genome-wide association studies (GWAS) by this team (Ray, Smith, Purcell, Fritschi) identified at least 21 different genetic loci putatively associated with WUE. In order to confirm and refine some of these QTL associations, a bi-parental F6-derived RIL population between soybean lines differing in WUE (PI 416997 [high WUE] x PI 567201D [low WUE]) was developed and genotyped under the auspices of a USB-funded grant that is now ended. Mapping WUE in this RIL population will confirm putative loci and allow the targeting of specific genes along with the identification of associated molecular markers. Confirmation of important loci, identification of specific genes, and identification of molecular markers will be useful in current and future breeding programs for WUE.

Germinability under high heat environments, as often experienced in the Early Soybean Production System (ESPS), is related to seed quality traits, including diseases, wrinkling, green seed, dockage, etc. Recent advances in breeding lines with improved germinability developed at Stoneville have germinability levels of greater than 90% even when harvested in July and August, and this is significantly greater than that of commercial cultivars. With the selection of improved F6:7 lines in 2016 at Stoneville, combining WUE and germinability is nearly complete. However, these new breeding lines need thorough testing. Therefore, the second part of this research was to continue with the breeding program to produce and test advanced breeding lines for full yield testing in both irrigated and dryland environments. The best, most WUE lines will be identified for release. These releases will mostly be MG IV lines.
Combining improved germinability (and associated improved seed quality traits) with improved water-use efficiency would result in a soybean more suited to most of the production areas of Mississippi.

OBJECTIVES

Development of soybean cultivars with increased water-use efficiency would benefit all Mississippi soybean producers whether using an irrigated or dryland production system. The future will likely entail increased irrigation costs and perhaps water use restrictions. Thus, cultivars with the ability to more efficiently use water resources would be beneficial. Similarly, such cultivars would potentially result in increased yield in dryland systems for the same amount of input water resources (i.e. rainfall). In addition, combining improved water-use efficiency with high germinability would enhance seed quality under adverse conditions.

Objective 1: Identify Quantitative Trait Loci associated with water-use efficiency in an F6-derived RIL population developed from soybean lines differing in water-use efficiency.

Objective 2: Within an on-going breeding program, select for agronomically sound breeding lines with improved water-use efficiency and high germinability under high heat environments.

APPROACH AND EXPERIMENT CONDUCT

Objective 1.

A bi-parental F6-derived RIL population (196 lines) from parental lines differing in WUE was grown and phenotyped in 2017 in three field locations: 1) Stoneville, MS (Smith and Ray); 2) Pine Tree Branch Station, AR (Purcell et al.); and 3) Columbia, MO (Fritschi et al.). Each location had two replications organized in a randomized complete block design. The RIL parents (PI 416997 [high WUE] x PI 567201D [low WUE]) and five check cultivars (AG4632, Dillon, Maverick, Osage and Pella86) were included at each location. Planting dates were 16 May 2017 for Stoneville, MS, 14 May 2017 for Columbia, MO, and 10 June 2017 for Fayetteville, AR.

Five aboveground plant samples were taken when most of the RILs were at about stage R1 or R2. At each location, the plant samples were dried at approximately 55 °C and then course-ground. The course-ground samples were shipped to Purcell's lab (University of Arkansas) where they were finely ground, weighed, encapsulated, and then shipped to the University of California (Davis) for isotope analysis.

Objective 2.

F6:7 breeding lines were selected in 2016 from advanced generation material differing in WUE and high germinability. In each previous generation (F2, F3, F4, and F5), breeding lines were evaluated and selected for good agronomics, WUE, and germinability. Selections from the 2016 test were grown in Stoneville in 2017 for evaluation and seed increase. Originally, we had planned to conduct an isotope analysis on these lines in 2017, but our field was damaged by dicamba drift that we thought might bias the results. However, we were still able to increase the seed and the lines are currently being evaluated in replicated field tests in 2018 where we will confirm WUE difference by isotope analysis as described above in Objective 1.

The plant samples have been collected and processing is underway. In addition to seed yield, lodging, height, and germinability, etc. will be measured on all lines. As this MSPB grant has ended, the cooperators involved in this study will cover the cost incurred. We have noted variable but moderate (to date) apparent dicamba damage again this year (2018), but do not anticipate it being severe enough to cause problems, and fully expect to harvest enough seed for the multi-location (Ark., Mo., and Miss.) irrigated and nonirrigated yield trials on these lines in 2019. The best, most WUE lines will be identified for release. These releases will mostly be MG IV and V lines.

RESULTS

Objective 1.

In the 2016 season, we estimated WUE (i.e. using isotope data) on the RIL population at Stoneville, Miss. as part of a previous USB grant. In the 2017 season as part of this MSPB-funded grant, we collected WUE data on the RIL population in three locations (AR, MO, and MS). In total, 1,210 samples from the three locations were collected, processed, and sent to the University of California at Davis's Isotope lab for WUE determination. Although not part of the MSPB-funded portion of the research, we are including the 2016 results in this report. Thus we have four locations/environments: 1) Stoneville, MS in 2016 (MS16), 2) Stoneville, MS in 2017 (MS17), Columbia, MO in 2017 (MO17), and 3) Pine Tree, AR in 2017 (AR17). A preliminary summary of the data is presented in this report as a full analysis will take more time.

Table 1 shows the descriptive statistics of the carbon isotope ratio values for the four locations. The more negative the carbon isotope ratio, the less WUE the soybean line is. Over the four locations, values ranged from a minimum of -30.51 (i.e. the least WUE) in MS16 to a maximum of -26.72 in MO17 (i.e. the most WUE).

The frequency distribution of WUE at each location is shown in Figure 1. At all locations, the RIL population exhibited transgressive segregation (WUE values more extreme than either parent), although more so towards the higher WUE end of the scale (Figure 1). Additionally, the WUE of the RIL population was highly and significantly ($P < 0.0001$) correlated over the four environments, with coefficients ranging of 0.67 to 0.78 (Table 2). We also estimated a moderate average narrow sense heritability across environments of 64%.

Although, the data from the four locations were similarly distributed and well-correlated, the range of WUE values differed among the four environments (Table 1 and Figure 1). Additionally, there was good separation between the parents in 3 of 4 locations. Differences between locations were most likely as a result of local environmental differences such as temperature, and rainfall (amounts, timing, and distribution). These factors and others will be considered in detail in the full analysis.

The distribution of the RIL population along with the correlations among environments and the moderate heritably indicates that we should be able to map genomic regions associated with WUE. Most of the molecular-marker genotyping of the RIL population is complete (some gaps in the map need to be filled in, Figure 2) and a more thorough analysis of the phenotypic data conducted. A Ph.D. graduate student at the University of Arkansas (under Dr. Purcell) will lead the completion of the data analysis and mapping supported by other scientists on the project (ARS in MS and MO, and University of MO).

Objective 2.

In the fall of 2017, single-row plots of multiple F6-derived full-sib breeding lines were bulk- harvested. Harvest conditions were hot, humid, and rainy, and redbanded stink bugs were not adequately controlled. The seed was weighed for yield and assayed for germinability and hard seed.

Previously, each breeding line had been assayed for good agronomic traits, WUE, and germinability in the F2, F3, F4, and F5 generations. In each generation, selections were made for high and low WUE, coupled with high germinability and high yield potential. In each generation, single plants with the best agronomic traits (high pod load, optimum height, low lodging and shattering, etc.) were selected. Hence, each 2017 breeding line was highly selected, in contrast to the totally unselected random lines evaluated in the RIL of Objective 1. Note that all breeding lines from Objective 2 and all RIL lines from Objective 1 were 50% PI 567201B, which had previously been studied in detail by the group for WUE.

Based on 2017 yield and germinability data, 28 breeding lines were planted at Stoneville on 18 April 2018 in replicated 4-row yield plots. Maturities of the 28 selected breeding lines range from MG III to V. Stands for all plots were excellent. Tissue sampling for WUE estimates and DNA extraction were taken on each border row for each line in each replication in June of 2018. All plots are set up to be furrow-irrigated as needed throughout the growing season.

As of this writing, plots have been irrigated one time for a duration of 24 hours. After harvest, seed yield and seed germinability will be estimated for each line. Based on seed yield, germinability, and WUE differentials, two groups of lines will be selected for additional testing at four locations in 2019 (MS, AR, MO, and AZ). Each location will feature irrigation as a factor (replicated irrigated vs. replicated nonirrigated blocks), plus replication within each block for line effects. We will estimate the effect of WUE on seed yield for both irrigated and nonirrigated treatments. The experiment will be repeated in subsequent years.

CONCLUSIONS

The MSPB funded the collection and processing of plant samples on the WUE efficiency RIL population in Stoneville, MS in 2017 as well as the carbon isotope measures on the RIL samples collected from Stoneville, MS, Pine Tree, AR and Columbia, MO grown in 2017. AR and MO covered the cost of growing and sampling the WUE RIL at their locations. At all three locations the field experiments were successful; sampling occurred as planned and the carbon isotope measures were completed. Preliminary analysis of the data indicates that the RIL is segregating for WUE. In previous research (funded by the USB) we genotyped the WUE RIL. Dr. Purcell at the University of Arkansas is funding a Ph.D. graduate student to complete the analysis of the WUE data from all three locations and map WUE genomic loci. The yield evaluation of breeding lines developed for WUE and germinability is continuing and we expect multi-location irrigated and nonirrigated experiments in the 2019 season. We will target the best lines for a germplasm release. It should be noted that in the 2017 and 2018 seasons we have observed dicamba damage in these experiments. It is not clear how this may affect our results.

PROJECTED IMPACT OF RESULTS ON MISSISSIPPI SOYBEAN PRODUCTION

In 2015, 2,270,000 acres of soybean were harvested in Mississippi and production was valued at $1,028,537,000. Overall, the 2015 production was estimated at 104,420,000 bushels (National Agricultural Statistics Service). This is about 46 bushels per acre. Every one bushel per acre increase in yield represents an increase of over $22 million in state production value. The greatest potential impact of WUE is on dryland production; however, WUE may also benefit irrigated production in two ways—reduced use of water for irrigation (i.e. reduced cost) and higher yields may be obtained on those fields not optimally irrigated.

EXPECTED END PRODUCT(S)

We expect the release of germplasm with improved WUE and high germinability and the identification of molecular markers useful for marker assisted breeding, as well as multiple peer-reviewed scientific articles from the genetics study and the breeding program. Furthermore, these results will be presented at national scientific meetings and at the soybean breeder's workshop. The data will also be used in a Ph.D. dissertation and as a component of the requirements for the completion of a Ph.D. degree from the University of Arkansas. WUE-germplasm lines will be transferred to commercial and public soybean breeders. These breeders will incorporate the latest herbicide technologies for the development of new cultivars with improved WUE, high yield, and herbicide tolerance.

Table 1. Carbon isotope ratio descriptive statistics of a RIL population grown in four environments. More negative carbon isotope values correspond to less water use efficiency (WUE) WUE.

	Environment†			
Statistic	**MS16**	**MS17**	**AR17**	**MO17**
Number of RILs	197.00	196.00	196.00	196.00
Minimum	-30.51	-30.14	-29.41	-29.08
25% Percentile	-29.90	-29.53	-28.80	-28.61
Median	-29.71	-29.29	-28.57	-28.40
Mean	-29.60	-29.22	-28.40	-28.32
75% Percentile	-29.46	-29.06	-28.15	-28.15
Maximum	-28.13	-27.70	-26.56	-26.72
Range	2.38	2.44	2.85	2.36
C.V.	1.45%	1.51%	2.24%	1.49%

†Location of test environments: MS16 = Stoneville, MS in 2016; MS17 = Stoneville, MS in 2017; AR17 = Pine Tree, AR in 2017; MO17 = Columbia, MO 2017.

Table 2. Pearson correlation coefficients between environments for water use efficiency (WUE) as estimated by the carbon isotope ratio. All correlations are significant ($P \leq 0.0001$).

Environment†	**MS16**	**MS17**	**AR17**	**MO17**
MS16		0.69	0.76	0.74
MS17	0.69		0.72	0.67
AR17	0.76	0.72		0.78
MO17	0.74	0.67	0.78	

†Location of test environments: MS16 = Stoneville, MS in 2016; MS17 = Stoneville, MS in 2017; AR17 = Pine Tree, AR in 2017; MO17 = Columbia, MO 2017.

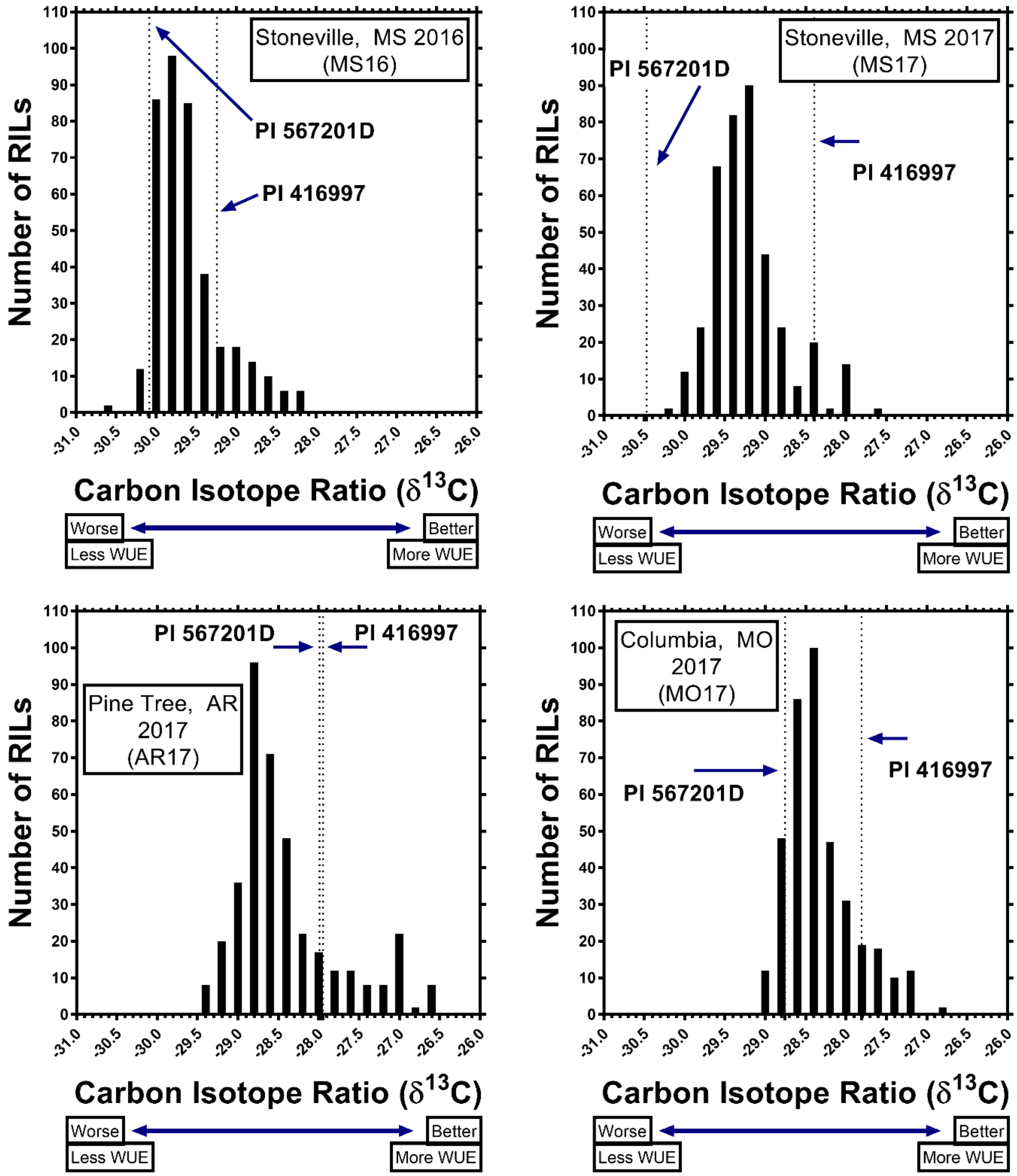

Figure 1. Distribution of water use efficiency (WUE) as estimated by the carbon isotope ratio on a segregating RIL population in four environments.

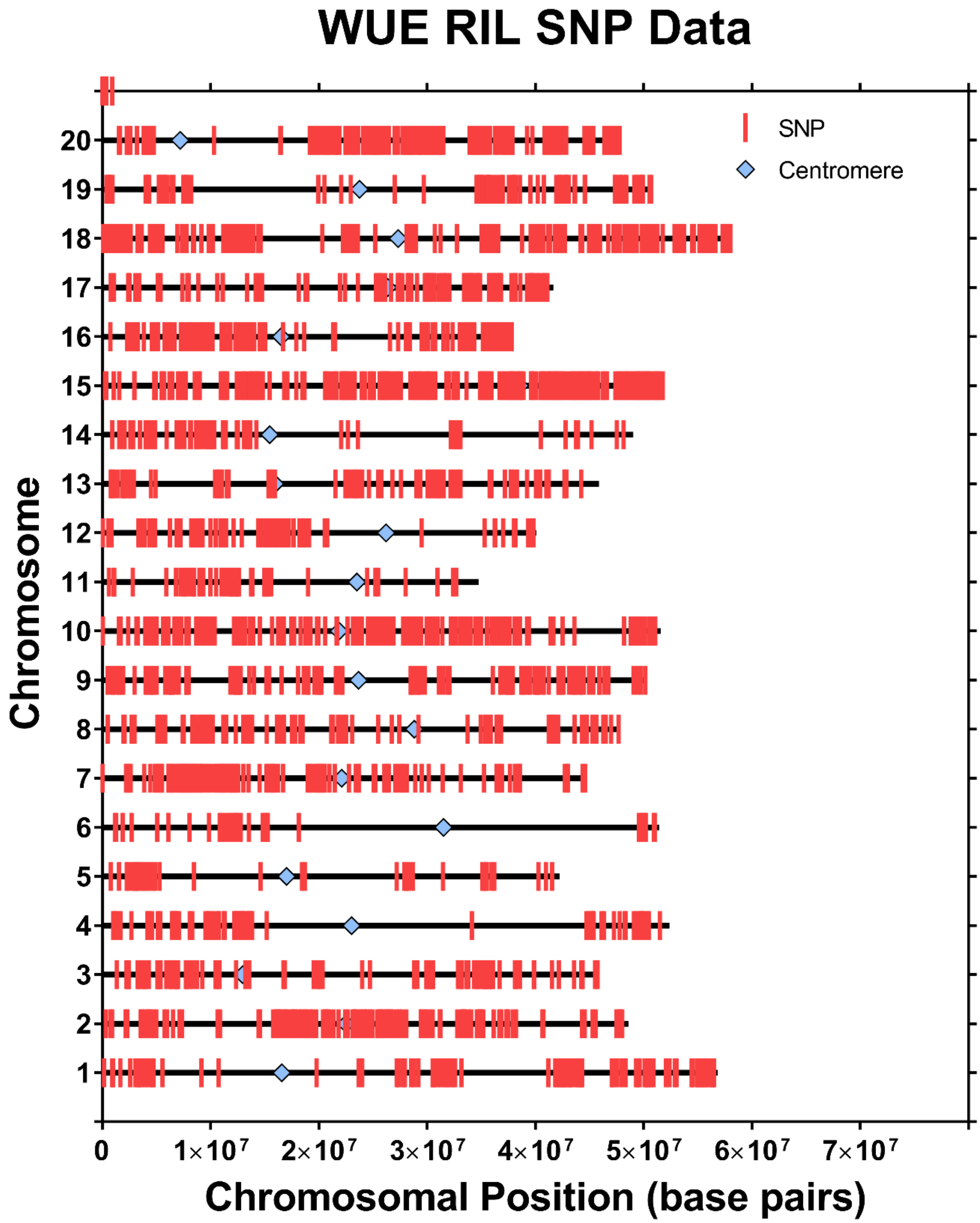

Figure 2. Distribution of SNP molecular markers that will be used for mapping WUE in the RIL population developed for this study.