

## Genetic Analysis of Relative Water Content (RWC) in Two Recombinant Inbred Line Populations of Soybean [*Glycine max* (L.) Merr.]

Salvador Virginia<sup>1</sup>, Megan Pagan<sup>1</sup>, Melissa Cooper<sup>1</sup>, Stella K. Kantartzi<sup>2</sup>, David A. Lightfoot<sup>2</sup>, Khalid Meksem<sup>2</sup>, and My Abdelmajid Kassem<sup>1\*</sup>

<sup>1</sup> Plant Genomics and Biotechnology Lab, Department of Biological Sciences, Fayetteville State University, Fayetteville, NC 28301, USA; <sup>2</sup> Department of Plant, Soil, and Agricultural Systems, Southern Illinois University, Carbondale, IL, 62901, USA.

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### Abstract

**Drought affects soybean [*Glycine max* (L.) Merr.] and other crops productivity in the US and other parts of the world. Relative water content (RWC) is an important indicator for plant water deficit tolerance (WDT). The objective of this study is to map quantitative trait loci (QTL) for RWC and several other leaf traits such as leaf dry weight (LDW), leaf fresh weight (LFW), and leaf turgid weight (LTW) in two soybean recombinant inbred line (RIL) populations, one derived from a cross of 'Essex' and 'Forrest' (ExF, n=94) and the other is derived from a cross of 'PI 438489B' and 'Hamilton' (PlxH, n=50). In the PlxH RIL population, eight QTL were identified and mapped on 6 different linkage groups (LGs) of the soybean genome. No QTL for LFW were identified in this population. In the ExF RIL population, 10 QTL were identified and mapped on 5 different LGs of soybean. Chromosome 18 (LG G) contains clusters of QTL for LFW, LTW, and RWC in the ExF RIL population. This same chromosome contains a QTL for RWC in the PlxH RIL population. The QTL found here are important to be included in breeding programs for soybean water deficit tolerance (WDT).**

**Keywords:** QTL, soybean, drought, water deficit tolerance (WDT), relative water content (RWC).

### Introduction

Soybean [*Glycine max* (L.) Merr.] is an important crop cultivated in the US and worldwide. In 2009, the US exported 1.28 billion bushels of soybeans which accounts for 46% of the world's total production and a profit that exceeded \$21 (Soy Stats, 2012).

Soybean is a great source of proteins and oils (Howell and Carter, 1958; Hartwig, 1973). Soybeans are also used for soy diesel, candles, soy milk, building materials, soy crayons, hand cleaners, and many other products (USB, 2007). Soybean seeds contain numerous nutraceuticals compounds such as isoflavones or phytoestrogens (Eldridge and Kwolek, 1983; Regal et al., 2000). The most studied, in soybean, are daidzein, genistein, and glycitein (Barnes et al., 1990, 1994; Messina and Barnes, 1991; Cassidy et al., 1990, 1994; Huang et al., 1992; Fostis et al., 1993; Jing et al., 1993; Peterson et al., 1993; Anderson et al., 1995; Knight and Eden, 1996; Anthony et al., 1996; Banz et al., 1999; Greer-Baney et al., 1999; Njiti et al., 1999; Regal et al., 2000; Meksem et al., 2001; Munro et al., 2003; Kassem et al., 2004). The last decades witnessed the use of large amounts of soy products including isoflavones; however, several studies showed that they might have positive and negative health effects in humans and animals (Barnes et al., 1990, 1994; Njiti et al., 1999; Regal et al., 2000; Meksem et al., 2001).

\* Corresponding author: mkassem@uncfsu.edu.

Soybeans, like other crops, are subject to numerous diseases and sensitive to drought (Specht et al., 2001). Researchers and plant breeders look to develop soybean cultivars that are drought tolerant or resistant. Drought stress might lower the ability of roots to absorb enough water which leads to wilting. Soybean plants defend themselves against water loss by closing their stomata and avoid dehydration. However, this might reduce the photosynthetic rate as well as the uptake of minerals from the soil. This reduced metabolism, caused by drought, might be reversible when enough water is available. Slow photosynthetic rate has a drastic effect on yield. The stressed plants usually slow or stop growth and flowering, abort grain, drop leaves, and abort pods.

The degree of drought stress can be physiologically estimated by measuring the leaf relative water content (RWC) which can control the plant response to water deficit (Hunt et al., 1987). Mapping QTL for agronomic traits including traits involved in drought tolerance such as water use efficiency (WUE), RWC, and root traits have been identified and mapped in wheat (Dhanda and Sethi, 1998), barley (Teulat et al., 1997, 2003), sunflower (Kiani et al., 2007), corn (Frova et al., 1999), soybean (SoyBase, 2011), and many other crop species. However, the genes controlling these traits and many others are still widely unknown (Keurentjes et al., 2008).

The objective of this study is to genetically map quantitative trait loci (QTL) for leaf fresh weight (LFW), leaf turgid weight (LTW), leaf dry weight (LDW), and leaf relative water content (RWC) in soybean using two recombinant inbred line populations (RIL): One derived from the cross of 'Essex' and 'Forrest' cultivars (ExF,  $n = 94$ ) and the second is derived from the cross of 'PI 438489B' and 'Hamilton' (PlxH,  $n = 54$ ) and to compare results from both RIL populations.

## Materials and Methods

### Plant Materials

The 'Essex' by 'Forrest' RIL population (EXF,  $n = 94$ ) used in this study (Lightfoot et al., 2005) was provided in 2007 (at the F5:16) by Prof. D.A. Lightfoot of SIUC. This ExF RIL population was extensively studied for sudden death syndrome (SDS) re-

sistance, soybean cyst nematode (SCN) resistance, manganese toxicity resistance, seed yield, and several other traits (Kassem et al., 2004a,b, 2006, 2007a,b; Alcivar et al., 2007; Jacobson et al., 2007).

The 'Hartwig' by 'Flyer' RIL population (HxF,  $n=92$ ) used in this study was also provided in 2007 by Prof. D.A. Lightfoot of SIUC. This HxF RIL population was extensively studied for seed yield, sudden death syndrome (SDS) resistance, maturity, and lodging (Kazi, 2005; Kazi et al., 2007, 2008).

### Growth Conditions

Growth conditions were performed as described in Jacobson et al. (2007). Briefly, one seed per pot, with 3 seed of each RIL, were grown in pots of 30 x 30 cm (diameter x depth) containing approximately 1 Kg of PRO-MIX soil. The PRO-MIX is a ready-made, peat based growing mix containing the Canadian sphagnum peat moss (75-85% by volume), limestone (for pH adjustment), perlite, a wetting agent, and vermiculate. The seeds of the two populations (ExF,  $n=20$ ; HxF,  $n=20$ ) were planted in the greenhouse for three months (October – December, 2008). The plants were kept in the greenhouse at  $25 \pm 10^\circ\text{C}$  under natural lighting and harvested after 3 months and the relative water contents (RWC) were measured as described below.

### Relative Water Content Measurement

Relative water content (RWC) was estimated as described previously (Smart and Bingham, 1974). Briefly, the fresh weight of a mixed sample of five young leaves from each plant from each population is determined. The leaves of the same sample were left floating on distilled water, in Petri dishes, for 4 hours and the turgid weight is then recorded. After that, the leaf tissues were dried in an oven at  $65^\circ\text{C}$  for 24 hrs and their dry weight was measured. The RWC is calculated according to the following formula (Smart and Bingham, 1974):

$$\text{RWC} = (\text{Fresh Weight} - \text{Dry Weight}) / (\text{Turgid Weight} - \text{Dry Weight}).$$

**Table 1.** Means of the leaf traits measured in the 'PI438489B' by 'Hamilton' RIL population and parent lines (PI438489B and Hamilton).

Trait	RILs (n=54)				Parents	
	Mean	St. Dev.	Skewness	P<W*	PI438489B mean	Hamilton mean
Leaf Fresh Weight (g)	0.1924	0.0219	0.8784	0.0023**	0.2636	0.1614
Leaf Turgid Weight (g)	0.3565	0.0299	0.7045	0.0955ns	0.4888	0.4694
Leaf Dry Weight (g)	0.0614	0.0058	1.0375	0.0126*	0.0968	0.0772
Relative Water Content	0.3791	0.0351	0.6530	0.0024**	0.4255	0.2147

\*H0= Data is from Normal distribution. P < 0.05 rejects H0

**Table 2.** Means of the leaf traits measured in the 'Essex' by 'Forrest' RIL population and parent lines (Essex and Forrest).

Trait	RILs (n=94)				Parents	
	Mean	St Dev.	Skewness	P<W*	Essex mean	Forrest mean
<b>Leaf Fresh Weight (g)</b>	0.4221	0.1497	0.3599	0.0951 <sup>ns</sup>	0.2478	0.3630
<b>Leaf Turgid Weight (g)</b>	0.5600	0.1765	0.4603	0.0147*	0.3734	0.5210
<b>Leaf Dry Weight (g)</b>	0.1666	0.0593	0.6248	0.0281*	0.1124	0.1418
<b>Relative Water Content</b>	0.6425	0.1195	0.7224	0.0282*	0.5188	0.5833

\*H0= Data is from Normal distribution. P <0.05 rejects H0

**Table 3.** Pearson correlation coefficients for the leaf traits measured in the 'PI438489B' by 'Hamilton' RIL population and parent lines (PI438489B' and Hamilton).

Traits	Leaf Turgid Weight (g)	Leaf Dry Weight (g)	Relative Water Content
<b>Leaf Fresh Weight (g)</b>	0.7374***	0.7948***	0.7572***
<b>Leaf Turgid Weight (g)</b>		0.9628***	0.3136*
<b>Leaf Dry Weight (g)</b>			0.3913**

\*Significant at P<0.05 probability level. \*\* Significant at P<0.01 probability level. \*\*\* Significant at P<0.001 probability level.

**Table 4.** Pearson correlation coefficients for the leaf traits measured in the 'Essex' by 'Forrest' RIL population and parent lines (Essex and Forrest).

Traits	Leaf Turgid Weight (g)	Leaf Dry Weight (g)	Relative Water Content
<b>Leaf Fresh Weight (g)</b>	0.9444***	0.9438***	0.5293***
<b>Leaf Turgid Weight (g)</b>		0.9671***	0.2364*
<b>Leaf Dry Weight (g)</b>			0.3110**

\*Significant at P<0.05 probability level. \*\* Significant at P<0.01 probability level. \*\*\* Significant at P<0.001 probability level.

**Table 5.** Comparison of overall means, standard deviations and range of leaf traits measured in the two RIL populations (PI438489B by Hamilton and Essex by Forrest).

Trait	Population	Mean	St Dev	Range
<b>Leaf Fresh Weight (g)</b>	<b>PI438489B x Hamilton</b>	0.1924 <sup>b</sup> *	0.0219	0.1860-0.7106
	<b>Essex x Forrest</b>	0.4221 <sup>a</sup>	0.1497	0.0286-0.6030
<b>Leaf Turgid Weight (g)</b>	<b>PI438489B x Hamilton</b>	0.3565 <sup>b</sup>	0.0299	0.2964-0.8854
	<b>Essex x Forrest</b>	0.5600 <sup>a</sup>	0.1765	0.0660-0.8780
<b>Leaf Dry Weight (g)</b>	<b>PI438489B x Hamilton</b>	0.0614 <sup>b</sup>	0.0058	0.0850-0.3084
	<b>Essex x Forrest</b>	0.1666 <sup>a</sup>	0.0593	0.0092-0.1806
<b>Relative Water Content</b>	<b>PI438489B x Hamilton</b>	0.3791 <sup>b</sup>	0.0351	0.3668-1.0740
	<b>Essex x Forrest</b>	0.6425 <sup>a</sup>	0.1195	0.0877-0.8195

\*Levels not connected by the same letter are significantly different at P<0.05 according to Tukey-Kramer test.

### Statistical Data Analysis

Means, standard errors, skewness and Shapiro-Wilk test for determining the normality of distribution were calculated for PI438489B x Hamilton RIL population. Parental means and standard errors were also computed. Pearson's correlation coef

ficients between all trait combinations were determined as well. Results were reported in a Pearson correlation matrix. All analyses were performed by JMP 8.0.2 (SAS Institute Inc., Cary, NC, USA).

**Table 6.** The leaf traits QTL identified in the two RIL populations (PI438489B by Hamilton and Essex by Forrest) of soybean. Leaf fresh weight (LFW), leaf turgid weight (LTW), leaf dry weight (LDW), relative water content (RWC). QTL were named according to the Soybean Genetics Committee's recommendations as revised in March 2007. <http://soybase.org/resources/QTL.php>.

Trait	No.	QTL	Chr./LG	Marker/Interval	Position (cM)	LOD	R <sup>2</sup> (%)
<b>PlxH</b>							
LTW	1	qLTW001	6/C2	ss107912725–ss107925078	39.8–43.3	2.9	0.14
	2	qLTW002	17/D2	ss107913096–ss107916109	7.6–12.8	2.7	0.19
	3	qLTW003	17/D2	ss107929768–ss107913565	15–19.8	4.8	0.25
LDW	1	qLDW001	6/C2	ss107912725–ss107913565	39.4–43.3	2.7	0.15
	2	qLDW002	10/O	ss107930848–ss107917019	56–85	3.3	0.19
RWC	1	qRWC001	2/D1b	ss107912689–ss107929181	39.2–49.5	2.6	0.01
	2	qRWC002	3/N	ss107912938–ss107913615	36.8–38.5	2.7	0.02
	3	qRWC003	18/G	ss107923024–ss107921048	14.8–18.3	2.7	0.01
<b>ExF</b>							
LFW	1	qLFW001	18/G	ATGCGA190–ACCCTC220	92.8–99.8	4.1	0.29
	2	qLFW002	10/O	Satt358–Satt132	0–10.1	3.9	0.38
LTW	1	qLTW001	8/A2	Sat_089–Sat_129	3–16.1	2.6	0.24
	2	qLTW002	4/C1	Satt399–Satt195	0–13	2.6	0.13
	3	qLTW003	18/G	Satt324–Satt356	50.3–59.9	4.6	0.40
	4	qLTW004	18/G	Satt570–Satt122	70.8–73.8	5.3	0.48
LDW	1	qLDW001	4/C1	Satt399–Satt195	0–13	2.7	0.14
	2	qLDW002	18/G	Satt324–Satt356	49.5–60.3	4.4	0.37
	3	qLDW003	18/G	Satt570–Satt122	70.8–77.5	3.8	0.36
RWC	1	qRWC001	11/B1	Satt546–Satt604	54.3–73	2.6	0.18

## Results and Discussion

### Distribution of Leaf Traits

The frequency distribution of 'PI438489B' by 'Hamilton' population for 3 out of 4 studied leaf traits (leaf fresh weight, leaf dry weight, and relative water content) departed significantly from normality ( $P < 0.05$ ). Shapiro-Wilk test revealed that only values of leaf turgid weight were normally distributed (Table 1 and Figure 1).

### Correlation Coefficients of Leaf Traits

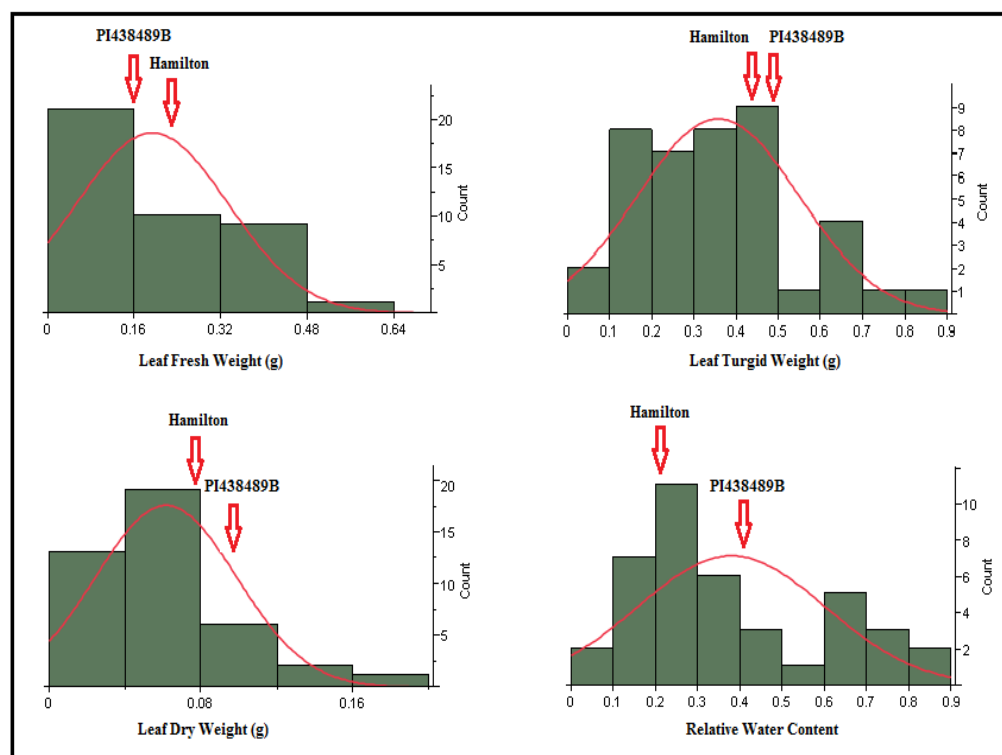
Correlation coefficients were estimated for each pair-wise leaf trait combination for PI438489Bx Hamilton (Table 2). All traits were found significantly ( $P < 0.05$ ) correlated. The highest correlation was estimated for leaf turgid weight and leaf dry weight ( $r = 0.9628^{***}$ ) and the lowest but statistically significant between leaf turgid weight and relative water content ( $r = 0.3136^*$ ).

### QTL Analysis of Leaf Traits

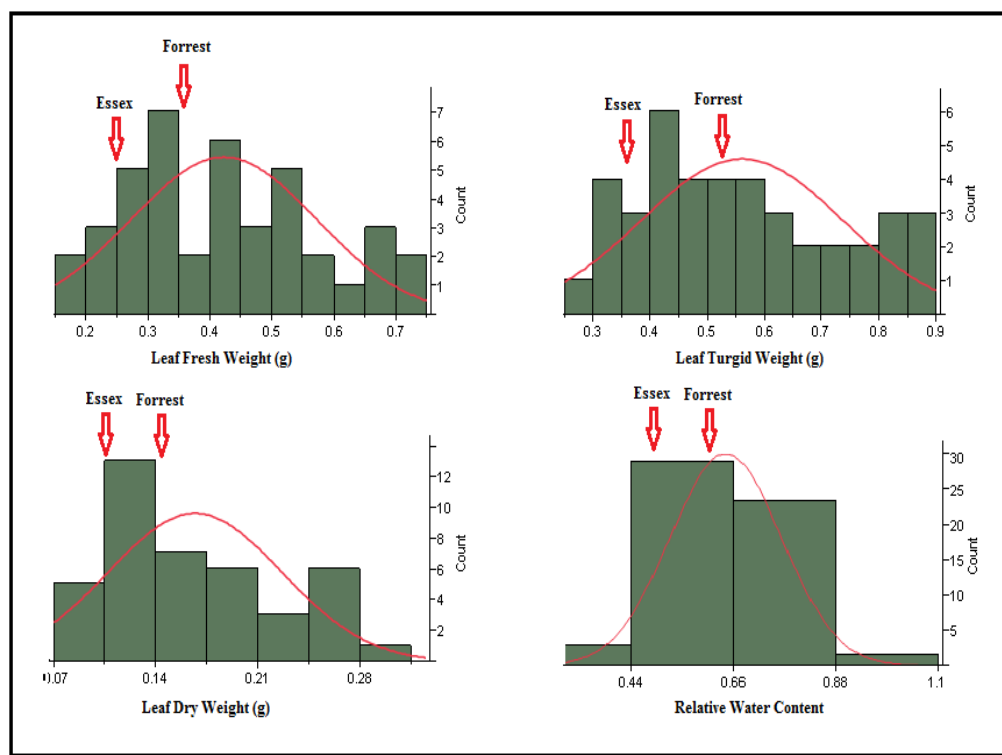
Relative water content (RWC) is an important indicator on

how plants withstand drought and become tolerant to WD. In this study, we genetically mapped QTL for leaf fresh weight (LFW), leaf turgid weight (LTW), leaf dry weight (LDW), and leaf relative water content (RWC) using the soybean 'Essex' by 'Forrest' RIL population (ExF,  $n = 94$ ). The phenotypic data for LFW, LDW, LTW, and RWC was compared against the updated ExF genetic linkage map (Kassem et al., 2006) to map QTL for these traits using the CIM of WinQTL Cartographer (Wang et al., 2004).

A total of 8 QTL for LTW, LDW, and RWC were identified and mapped in the 'PI 438489B' by 'Hamilton' RIL population. Three QTL were identified for LTW. The first QTL (qLTW001) was located on chromosome 6 (LG C2), and the second (qLTW002) and third (qLTW003) were located on chromosome 17 (LG D2) (Figure 3, Table 6). These QTL spanned 3.5 cM, 5.2 cM, and 4.8 cM, and have peak LOD scores of 2.9, 2.7, and 4.8, respectively (Figure 3, Table 6). Two QTL were identified for LDW. The first QTL (qLDW001) was located on chromosome 6 (LG C2), and the second (qLDW002) was located on chromosome 10 (LG O). These two QTL spanned 3.9 cM and 29 cM, and have peak LOD scores of 2.7 and 3.3, respectively (Figure 3, Table 6). Three QTL were identified for RWC. The first QTL (qRWC001) was located on chromosome 2 (LG D1b), the second QTL (qRWC002) was located on chromosome 3 (LG N), and the third QTL (qRWC003) was located on chromosome 18 (LG G). these QTL spanned 10.3 cM, 1.7 cM, and 3.5 cM and have peak LOD scores of 2.6, 2.7,

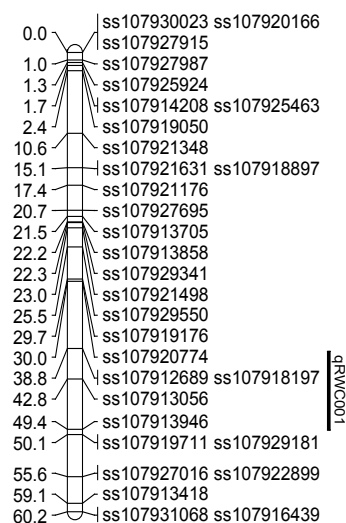


**Figure 1.** Frequency distribution of the leaf traits (leaf fresh weight, leaf turgid, leaf dry weight, and relative water content) in the 'PI438489B' by 'Hamilton' RIL population compared to their parental lines.

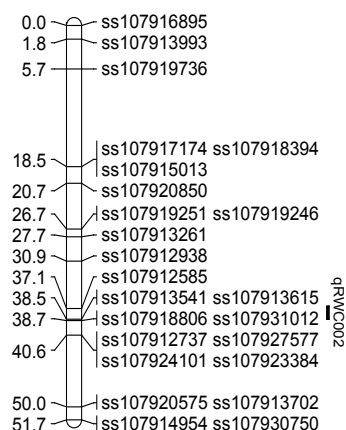


**Figure 2.** Frequency distribution of the leaf traits (leaf fresh weight, leaf turgid, leaf dry weight, and relative water content) in the 'Essex' by 'Forrest' RIL population compared to their parental lines.

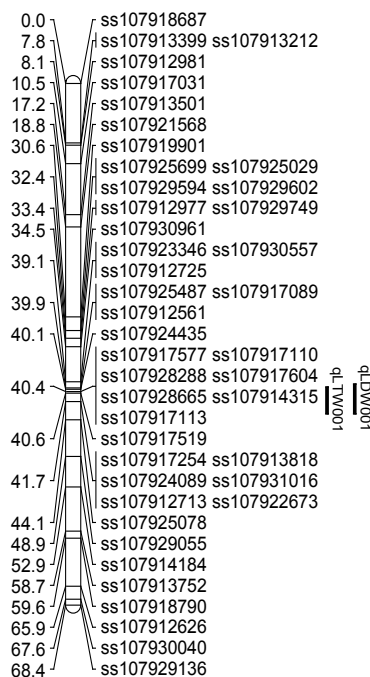
Chr\_2/LG\_D1b



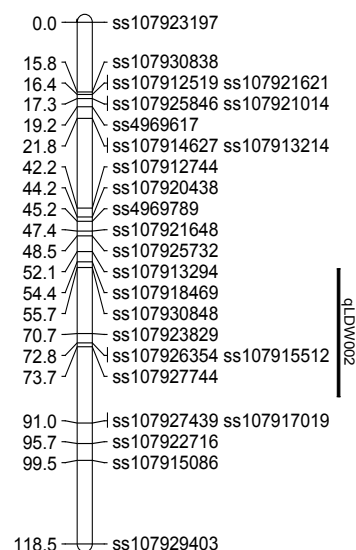
Chr\_3/LG\_N



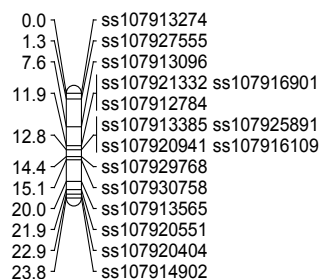
Chr\_6/LG\_C2



Chr\_10/LG\_O



Chr\_17/LG\_D2



Chr\_18/LG\_G

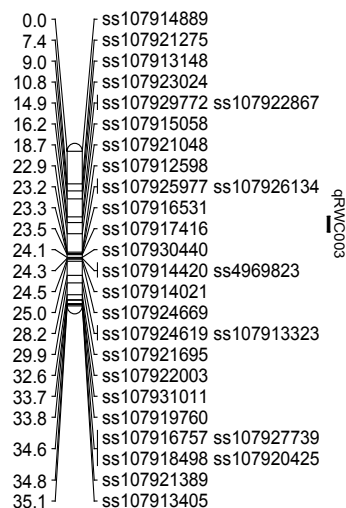
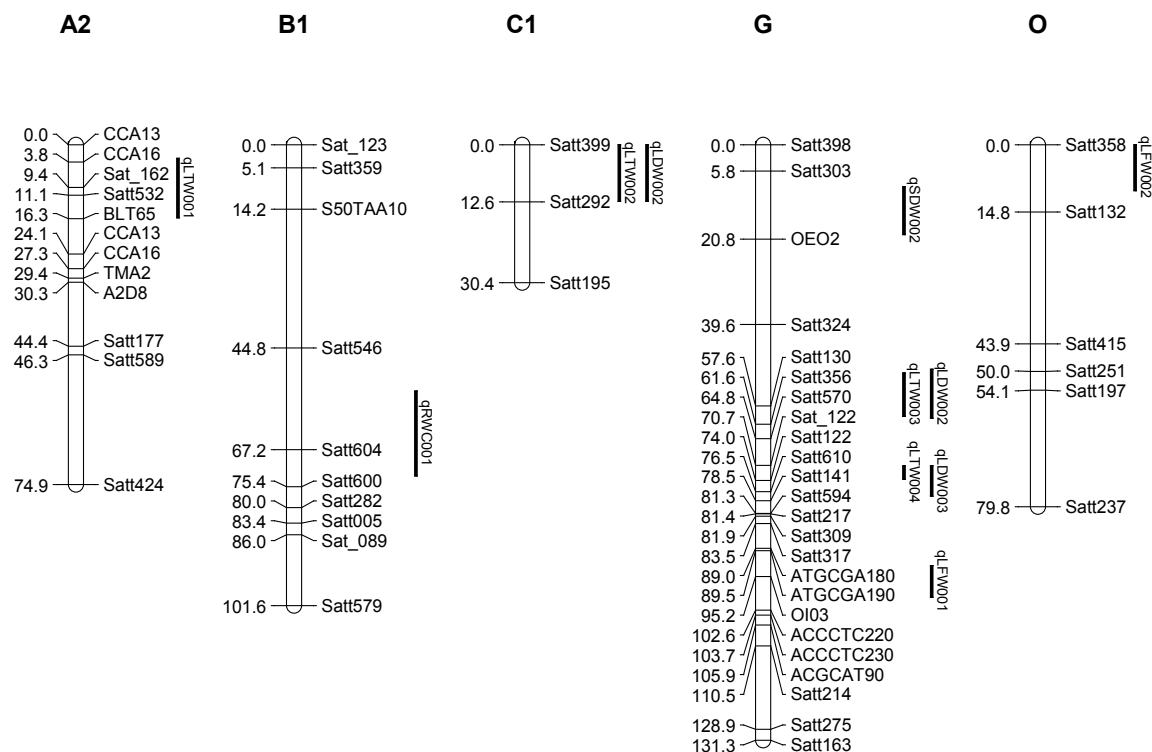


Figure 3. The locations of the leaf traits QTL identified in the PlxH RIL population.





**Figure 4.** The locations of the leaf traits QTL identified in the ExF RIL population.

and 2.7, respectively (Figure 3, Table 6).

In the 'Essex' by 'Forrest' RIL population, a total of 10 QTL for LFW, LTW, LDW, and RWC were identified and mapped on 5 different LGs. Two QTL were identified for LFW: qLFW001 on chromosome 18 (LG G) and qLFW002 on chromosome 10 (LG O) (Figure 3, Table 6). These QTL spanned 7 cM and 10.1 cM and have peak LOD scores of 4.1 and 3.9, respectively (Figure 3, Table 6). Four QTL were identified for LTW: qLTW001 on chromosome 8 (LG A2), qLTW002 on chromosome 4 (LG C1), and both qLTW003 and qLTW004 on chromosome 18 (LG G). These QTL spanned 13.1 cM, 13 cM, 9.6 cM, 3 cM and had peak LOD scores of 2.6, 4.6, and 5.3, respectively (Figure 3, Table 6). Three QTL were identified for LDW: qLDW001 on chromosome 4 (LG C1) and both qLDW002 and qLDW003 on chromosome 18 (LG G). These QTL spanned 13 cM, 10.8 cM, 6.7 cM and had peak LOD scores of 2.7, 4.4, and 3.8, respectively (Figure 3, Table 6). In this ExF RIL population, one QTL (qRWC001) was identified for RWC on chromosome 11 (LG B1). The QTL spanned 18.7 cM and had a peak LOD score of 2.6 (Figure 3, Table 6).

Clusters of leaf QTL were observed in both populations. In the PlxH RIL population, a cluster of LTW QTL was observed on chromosome 17 (LG D2) in the 7.6 to 19.8 cM interval. Similarly, a cluster of LFW, LTW, and LDW QTL was observed on chromosome 18 (LG G) in an interval spanning approximately 50.3 cM (49.5–99.8 cM) which is in agreement with previous findings of QTL clustering on the soybean genome (Meksem et al., 1999; Ross-Ibarra, 2005; Zhao-Ming et al., 2011). Interestingly, a QTL

for leaf area was identified and mapped within this same region using the PI416937 by Young RIL population (Mian et al., 1998). Similarly, 3 other QTL for leaf area were mapped on chromosome 8 (LG A2) using the PI416937 by Young RIL population. The first QTL fits within the same region of qLTW001 reported here, the second and third QTL were mapped 4 cM and 15 cM, respectively from qLTW001 (Mian et al., 1998; SoyBase, 2011). On chromosome 6 (LG C2), several QTL for leaf area were identified using the PI416937 by Young (Mian et al., 1998; SoyBase, 2011) and Archer by Minsoy (Mansur et al., 1993) RIL populations. These QTL were mapped 53.3 cM to 108 cM from qLTW001 and qLDW001 reported in this study (Mansur et al., 1993; Mian et al., 1998; SoyBase, 2011). The RWC QTL found here maybe important for breeding programs that aim to develop soybean for water deficit tolerance (WDT).

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