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QTL mapping of soybean cyst nematode race 9: a generalized linear modeling approach

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Abstract

The Female Index (FI) is a relative measure of host suitability of a soybean line for a particular nematode population and often shows a non-normal distribution. Moreover, most quantitative trait loci (QTL) mapping methods assume that the phenotype follows a normal distribution such as composite interval mapping (CIM). Therefore, a generalized linear modeling (GLM) approach was employed to map QTL for resistance to race 9 of the soybean cyst nematode (SCN) using a total of 83 simple sequence repeat markers (SSR). Two GLM models were tested: model 1, where the FI was treated as a continuous variable, assuming a Gamma distribution with a logarithmic link function; and model 2, where the FI was treated as a categorical trait in a five-item hierarchy, assuming a multinomial distribution with a cumulative logit link function. The FI data of 108 recombinant inbred lines (RIL) confirmed the non-normal distribution for race 9 of the SCN (Shapiro-Wilk's w=0.86, P<0.0001, skewness=1.52 and kurtosis=2.93). Eight RIL were confirmed to be resistant (FI≤10), and 23 to be highly susceptible (FI≥100). Both GLM models identified one QTL for SCN on the molecular linkage group G, between the markers Satt275 and Satt038 at 48.4 centiMorgans (P=0.017 and 0.033, for models 1 and 2, respectively). Additionally, these results were also compared with the CIM and Bayesian interval mapping (BIM) methods, assuming experimental data with a non-normal response, to determine the robustness and statistical power of these two methods for mapping QTLs. The results make clear that generalized linear modeling approach can be used as an efficient method to map QTLs in a continuous trait with a non-Gaussian distribution. CIM and BIM were robust enough for a reliable mapping of QTLs underlying nonnormally distributed data.

Keywords: Female index; *Generalized linear model*; *Glycine max*; *Heterodera glycines*; microsatellite.
Abbreviations: ANOVA_Analysis of variance; BIM_Bayesian interval mapping; CIM_composite interval mapping; FI_female index; GLM_generalized linear model; IM_interval mapping; LG_linkage group; MAS_marker assisted selection, QTL_quantitative trait loci; RJ-MCMC_reversible jump Markov chain Monte Carlo; RIL_recombinant inbred lines; SCN_soybean cyst nematode; SSR_simple sequence repeats.

Introduction

Heterodera glycines Ichinohe, commonly known as the soybean cyst nematode (SCN) is the most devastating pathogen of soybeans (*Glycine max* L. Merr.) globally. It is present in most soybean producing countries, and results in annual yield losses of approximately $1.5 billion in the United States alone (Wrather and Koenning, 2006). Several SCN races have been reported in different countries including the USA, Argentina, Brazil, China, Japan, and Russia (Ye, 2012). The soybean has become the most important Brazilian agricultural product in recent years. Brazil is the world's second largest producer of soybeans, and production is growing at twice the global rate (Goldsmith and Hirsch, 2006). The SCN was first found in the growing season of 1991/92 (Matsuo et al., 2012). Eleven SCN races (1, 2, 3, 4, 4+, 5, 6, 9, 10, 14 and 14+) have been detected in ten states, with an estimated area of over 2.0 million hectares (EMBRAPA, 2008). The grain yield losses in these states can reach 90% depending on the degree of infestation, cultivar susceptibility, soil fertility and the nematode race (Dhingra et al., 2009). However, cultivars available for cropping in Brazil have shown high resistance only to races 1 and 3 and moderate resistance to the other races (EMBRAPA, 2006). With the large number of SCN races identified in Brazil, there is a great interest in conducting Marker-Assisted Selection (MAS) for SCN resistance in Brazilian breeding programs. Understanding the nature of soybean resistance is needed to develop SCN-resistant varieties. Molecular mapping of resistance to SCN using SSR markers provides a powerful tool for the characterization of the genetic basis of soybean resistance (Guo et al., 2005). MAS can be less time-consuming than phenotypic selection and can select for a greater number of genotypes that carry resistance genes to

several SCN races (Cervigni et al., 2007). The Female Index (FI) is a relative measure of host suitability of a soybean line for a particular nematode population, and this index has also been used to describe the vulnerability of soybeans to damage by the nematode (Young, 1990). However, there is a challenge in data analysis because the FI usually shows a non-normal distribution (Guo et al., 2005; Cervigni et al., 2007; Wu et al., 2009; Ferreira et al., 2011) although the effect of non-normality on quantitative trait loci (QTL) mapping data analysis is expected to be significantly reduced due to the use of cofactor markers in composite interval mapping (CIM) developed by Zeng (1993) and permutation tests for the determination of threshold values (Churchill and Doerge, 1994). Interval mapping methods are the most commonly used method for mapping QTL, and typically apply to quantitative traits that have a continuous, normal distribution. In agricultural crops, the phenotypes of some traits are measured as discrete variables. For example, traits measured as counts are usually modeled by the Poisson distribution. Binary traits are also common in agricultural experiments (Coffman et al., 2005; Mora and Serra, 2014).
The accuracy of QTL mapping must, therefore, be as high as possible (Mora et al., 2010; Arriagada et al., 2012). Although some transformations can be used to improve the normality of traits, not all traits can be transformed (Xu and Hu, 2010). The generalized linear model (GLM) approach was developed in 1972 by Nelder and Wedderburn (1972). It is based on exponential distributions (termed "exponential family distributions"), and uses methods similar to traditional linear modeling for normal data distribution (Myers et al., 2002). The GLM approach is the most appropriate method for analyzing traits with non-normal distributions and has been widely applied to map QTL for special traits (e.g., binary traits (Yi and Xu, 2000; Deng et al., 2006), ordinal traits (Hackett and Weller, 1995; Rao and Xu, 1998) and Poisson traits (Cui et al., 2006; Cui and Yang, 2009). In this study, a generalized linear modeling approach was applied to map QTL underlying the resistance of soybean to Race 9 of the cyst nematode, as the assumption of normality of the female index data was not met. Additionally, we compared the results of the GLM evaluations to the CIM and Bayesian methods, assuming experimental data with a non-normal response.

Results and Discussion

Linkage analysis and genetic map

Twenty-four markers showed distortion of the Mendelian segregation (1:1), and thus, 120 markers were included in the analysis (Supplementary table 1). Eighty-three SSR were grouped into 22 LGs of the genome (Fig 1), which represent the genomic segments of 15 LGs of the soybean consensus linkage map (Song et al., 2004). Similar results were obtained by Ferreira et al. (2011), who found approximately 20% distortion, and obtained eighty SSR that represented genomic segments of 17 LGs of the soybean consensus linkage map. This distortion is frequent in several crops, including soybean. Many markers did not link to any LG, due to their great distances from the other markers, over 40 percent of recombination frequency in the same linkage group, or belonged to an LG that was not represented by any other marker according to the consensus map (Ferreira et al., 2011).

QTL analysis

In the Bayesian results, posterior frequencies for the number of QTLs, performed by 1 million RJ-MCMC iterations, confirmed the presence of one QTL on the LG G. The estimates of posterior modes, calculated using the Kernel density estimation method (and 95% credible intervals) for the posterior distributions of additive variance, additive effect and heritability of the QTL were 0.19 (0.06; 0.81), -20.1 (-30.5; -8.9) and 0.15 (0.03; 0.32), respectively. This result agrees with the CIM analysis, in which the QTL, identified between markers Satt275 and Satt038 on the LG G, explains 20% of the phenotypic variance, and also agrees with previous reports (Cervigni et al., 2007; Ferreira et al., 2011). Fig 2 shows the posterior frequencies of the LG G with the most likely number of QTL. The BIM and CIM agree with GLM-M and GLM-G methods, indicating that the BIM and CIM were fairly robust in erroneously assuming the non-normal data of FI. In recent years, many QTLs associated with resistance to SCN have been identified (Concibido et al., 2004; Guo et al., 2006). The QTL identified in our study has been associated with the *rhg1* gene on chromosome 18 (Kim and Diers, 2013). That locus was detected in many previous reports and was considered to be one of the major genes conferring resistance to SCN (Chang et al., 2011). According to Cervigni et al. (2007), at least two genes participate in the resistance to race 9. In addition, *rhg1* and *Rhg4* are necessary to confer nearly complete resistance to SCN race 3 and 14 (Afzal et al., 2009; Chang et al., 2011). The *rhg1* gene has the greatest impact on the development of SCN from all races in several resistance cultivars including Hartwig (Ferreira et al., 2011; Kandoth et al., 2011). According to previous reports, the LG G has the largest number of QTLs associated with SCN resistance to different races, but in different positions on the LG G. Additionally, *rhg1* have been involved to defense against various stresses. For instance, Kandoth et al. (2011) presented evidence for the potential involvement of a complex stress- and defense-related response, including increased expression of genes involved in the production of ROS, the unfolded protein response, salicylic acid mediated signaling, and plant programmed cell death in *rhg1*-mediated resistance to SCN. Their study demonstrates that a network of molecular events take place during *rhg1*-mediated resistance, leading to a highly complex defense response against a root pathogen, which explains its involvement in resistance to several SCN races. Recently, by sequencing analysis rhg1 was discovered to be a complex locus at which resistance-conferring haplotypes carry up to 10 tandem repeat copies of a 31-kb DNA segment (Cook et al., 2014). Cook et al. (2012) also determined that three very tightly linked genes at rhg1 contribute to SCN resistance, and encode a predicted amino acid transporter (Glyma18g02580), an a-SNAP protein predicted to participate in disassembly of SNARE membrane trafficking complexes (Glyma18g02590), and a protein with aWI12 (wound-inducible protein 12) region but no functionally characterized domains (Glyma18g02610). Furthermore, the DNA encoding these genes is present in multiple copies in SCN-resistant parents, and this causes elevated expression of the genes. Two of the identified genes, Glyma18g02580 and Glyma18g02610 , did not carry amino acid polymorphisms between resistant and susceptible rhg1 haplotypes. However, Glyma18g02590 contain amino acid polymorphisms relative to the reference soybean genome Williams 82, which is SCN-susceptible (Cook et al., 2014).

Table 1. QTL detection details for SCN resistance on linkage group G (three first SSR intervals are shown), which were determined using a generalized linear modeling approach.

SSR interval	Position (cM)	GLM-M		GLM-G	
		Wald χ^2	P > χ^2	Wald χ^2	P > χ^2
Satt163 - Satt275	0.000	0.00	0.990	0.01	0.918
	0.050	0.00	0.983	0.00	0.952
	0.100	0.00	0.945	0.00	0.999
	0.200	0.05	0.828	0.03	0.861
	0.300	0.14	0.706	0.13	0.719
	0.305	0.15	0.702	0.13	0.714
Satt275 - Satt038	0.305	3.69	0.055	3.30	0.069
	0.355	3.96	0.047	3.94	0.047
	0.405	4.23	0.040	4.70	0.030
	0.435	4.37	0.037	5.14	0.023
	0.455	4.45	0.035	5.41	0.020
	0.465	4.48	0.034	5.53	0.019
	0.475	4.51	0.034	5.63	0.018
	0.484	4.53	0.033	5.72	0.017
Satt038 - Sat_163	0.484	0.78	0.378	2.65	0.103
	0.534	0.79	0.374	2.65	0.104
	0.584	0.81	0.370	2.64	0.104
	0.684	0.86	0.354	2.58	0.108
	0.784	0.94	0.332	2.27	0.132
	0.884	0.61	0.435	0.52	0.471
	0.942	0.29	0.589	0.00	0.956

GLM-M: generalized linear model in which the FI was treated as a categorical trait (Multinomial distribution and cumulative logit link function). GLM-G: the FI was treated as a continuous variable (Gamma distribution and logarithmic link function).

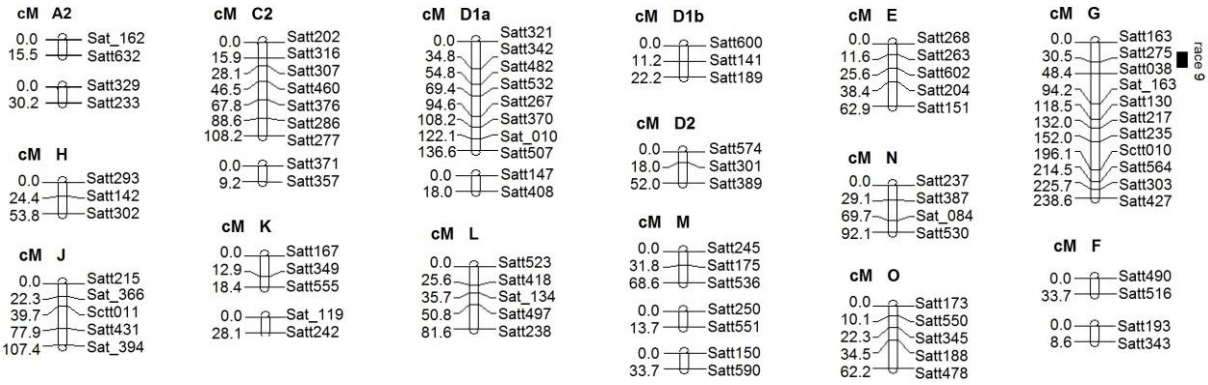

Fig 1. Linkage map constructed using a RIL population ($F_{6:7}$) derived from the cross Hartwig × Y23. QTL underlying the resistance of soybean to race 9 is indicated by a bar on the right of linkage group G. Marker names and genetic distances (in cM) are shown on the right and left, respectively.

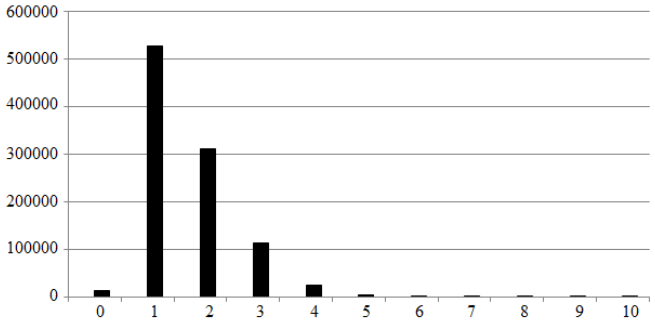

Fig. 2. Posterior frequencies for the number of QTLs carried out by 1 million iterations of the Reversible Jump method.

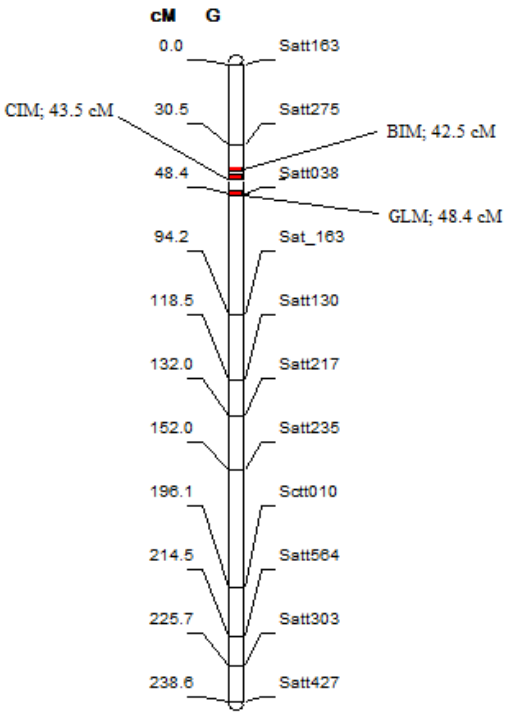

Fig 3. Linkage group G, showing the power of three methods for detection of QTL using non-normal distribution data. Generalized linear model (GLM), Bayesian interval mapping (BIM) and Composite interval mapping (CIM).

Recently, Matsye et al. (2012) suggested that an amino acid polymorphism in the Glyma18g02590 contributes to SCN resistance given that, the SNAP protein is likely involved in vesicle trafficking and may influence exocytosis of products that alter feeding site development or nematode physiology (Frei dit Frey and Robatzek, 2009)

Power and accuracy of QTL detection

The accuracy of the QTL position, however, was dependent on the mapping method used. While the models GLM-M and GLM-G identified the major QTL at 48.4 cM (P=0.017 and 0.033, for models 1 and 2, respectively, Table 1), the BIM and CIM methods identified the QTL at 42.5 cM (posterior mean) and 43.5 cM, respectively (Fig 3). Moreover, this QTL for race 9 has been mapped in the same interval in previous studies using the CIM method at 3.0 cM from marker Satt038 (Cervigni et al., 2007; Ferreira et al., 2011). Additionally, the marker Satt038 has also been mapped close to the *rhg1* gene in several studies (Kazi et al., 2010; Kim and Diers, 2013). The accuracy of QTL mapping must be as high as possible (Mora et al., 2008). The power and precision of QTL mapping depends on the coverage of linkage maps for QTL analysis, given that the distance between the markers affects the position of the QTL. Mayer et al. (2004) for example, found that the reduction of the marker interval size from 10 cM to 5 cM led to a higher power in QTL detection and their effect estimates, as well as a remarkable improvement of the QTL position estimation. An adequate statistical procedure is also important to improve accuracy. Mora et al. (2010) compared the GLM and CIM methods with simulated data with a Gamma distribution, and found that the QTL position differed by 5 cM and was located at different marker intervals. They concluded that the GLM method has superior performance in its ability to map QTL in a trait with a non-Gaussian distribution. Moreover, Yin and Zhang (2006) showed that the GLM approach had certain advantages such as power of detection and QTL position estimation for ordinal traits, given that the QTL position obtained by the GLM approach was closer to the true value with smaller standard errors than that obtained by the linear model approach. Kadarmideen et al. (2000) compared generalized interval mapping for binary traits with linear regression interval mapping, and both methods had a similar power to detect the QTL and similar estimates of QTL location and effects. The results of these studies agree with our results; the QTL position varied between 2.5 and 7.4 cM. According to Yin and Zhang (2006), the accuracy of QTL position estimates increases with the increases in the heritability and QTL effect. Currently, there is little available information on the heritability of this locus that controls resistance to SCN race 9. However, Ferreira et al. (2011) determined that the heritability of the resistance to SCN race 9 in Hartwig is approximately 0.34 and our results indicated a moderate heritability of the QTL identified (h^2 = 0.15). Therefore, in this context, the QTL detected in this study explains a significant percentage of the total heritability of the resistance trait (~ 40%). Moreover, the QTL contributed a large proportion of the additive effect (20%), which is similar to other reports (Concibido et al., 2004; Guo et al., 2006; Cervigni et al., 2007; Wu et al., 2009). Therefore, similar results for the three methods used in this study may be due to the moderate heritability and the additive effect.

Discussion

Methods and data distribution

The problems underlying QTL mapping have been summarized by Banerjee et al. (2008). For example, the predictor variables in the regression are not observed, and the genomic loci on the same chromosome are correlated. Complex traits, involving the participation of multiple genes and the mapping of QTL, requires inference of the genetic architecture (number of genes, their positions, and their effects) underlying these complex traits. According to Li et al. (2007), from a statistical perspective, different methods for QTL mapping are based on three broad classes: regression, maximum-likelihood and Bayesian models. These methods include the analysis of variance (ANOVA), Interval mapping (IM), CIM, multiple interval mapping (MIM), mixed linear models and BIM (Wu et al., 2009; Peixoto et al., 2014). Most markers or QTLs associated with SCN resistance that were identified in prior studies used ANOVA (Silva et al., 2007) and the IM method (Schuster et al., 2001). The CIM method has been commonly used in recent SCN QTL mapping investigations because the IM method can distort the QTL position and effects when there are multiple QTLs in the linkage group (Wu et al., 2009). However, most QTL mapping methods share a common assumption, which is, the phenotype follows a normal distribution; however, many phenotypes of interest do not satisfy this assumption (Yin and Zhang, 2006). According to our results and those from other reports, the GLM approach is an efficient method to map QTL and is particularly suited to address discrete traits or other traits deviating from a normal distribution. Furthermore, according to Rao and Xu (1998), the power and accuracy of QTL parameter estimation can be reduced substantially if a categorical trait is analyzed using linear models (Che and Xu 2012). However, the advantage of the GLM method is related to the number of categories of the

trait. For binary traits, the GLM method was more advantageous than for the four-category trait (Yin and Zhang 2006).

Materials and Methods

Plant material and SCN assay

A population of 180 $F_{6:7}$ recombinant inbred lines (RILs) from a cross of cv. Hartwig (resistant) and line Y23 (susceptible) was used in this study. This population was developed from F_2 plants obtained from five F_1 plants selfed until the F_6 generation using the Single Seed Descent (SSD) method (Brim, 1966). The response to race 9 of the SCN was evaluated on 108 RIL. Inoculum of race 9 was maintained on the roots of a susceptible variety (cv. Peking) growing in the greenhouse at 25°-30°C. The seeds were germinated in sand at 25°C. Each seedling, at two to three days of age, was transplanted into clay pots with a 0.5 L capacity (filled with a 1:2 mixture of soil and sand), then, each plant was inoculated with 4,000 SCN eggs according to the method of Dias et al. (2009). The soybean plants were grown in a greenhouse at 25-30°C under long-day conditions (16 h light). Thirty days after inoculation, plant roots of each RIL were washed with tap water and cysts were collected on 60 mesh sieves. The experiment was carried out in a completely randomized block design with three to six plants per treatment (RIL). Cysts were counted and transformed into the FI, estimated by: FI = 100 (number of cysts and females in a given plant / average number of cysts and females present on Y23). The FI was also calculated to confirm the identity of the inoculated races, according to the method of Riggs and Schmidt (1988).

DNA extraction and genetic map

DNA samples were extracted from soybean leaves using the CTAB method (Keim et al., 1988), quantified in a spectrophotometer, and stored at -20°C until use. A total of 144 microsatellite markers (http://www.soybase.org) were initially used. DNA amplification was performed in reactions containing 30 ng DNA, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 2.4 mM $MgCl_2$, 0.1 mM of each dNTP, 0.6 µL of forward and reverse primers and 1 unit of Taq DNA polymerase. Amplifications were performed in 30 cycles, each consisting of one denaturation step at 94ºC for 1 min, one annealing step at 50ºC for 1 min, and one extension step at 72ºC for 2 min. The final cycle was followed by a 7 min extension step at 72ºC. The SSR products were resolved in a 3% agarose gel immersed in TBE (90 mM Tris-borate buffer; 1 mM EDTA, pH 8.0), or in a vertical, non-denaturing, 10% polyacrylamide gel using TAE buffer (40 mM Tris- Acetate buffer, 1 mM EDTA). Gels were stained with ethidium bromide (10 mg/ml) and photodigitalized using the Eagle Eye II system (Stratagene, La Jolla, CA) according to the method of Cervigni et al. (2007). Markers were tested for segregation distortion using the chi-square test. Markers with segregation ratios significantly different from 1:1 ($P < 0.05$) were initially set aside (Supplementary table 1). The genetic map was constructed with Mapmaker/exp version 3.0 (Lincoln and Lander, 1993). Linkage groups were established with a threshold LOD score of 3.0 and a maximum recombination frequency of 0.4. The Kosambi mapping function was employed for map length estimations. The genetic map was drawn using the WinQTLCart 2.5 program (Wang et al., 2011).

QTL mapping

The statistical procedure, the generalized linear model (GLM), was used to map a QTL controlling SCN Race 9, as the assumption of normality of the female index data was not met (Shapiro-Wilk's w=0.86, P-value<0.0001; skewness= 1.52, kurtosis=2.93). Two GLM models were tested: model 1, where FI was treated as a continuous variable, assuming a Gamma distribution with a logarithmic link function; and model 2, in which FI was treated as a categorical trait to quantify the effect of heterogeneity on disease incidence relationships in a five-item hierarchy. The hierarchy was as follows: 1, resistant (IF≤10); 2, moderately resistant (IF=11-30); 3, moderately susceptible (IF=31-60); 4, susceptible (IF=61-99) and 5, highly susceptible (IF≥100). This categorical trait assumes multinomial distribution with a cumulative logit link function.

The density and probability function for the observed response (y) can be expressed as:

$$f(y_i;\theta_i;\phi) = \exp\left\{\frac{y_i\theta_i - b(\theta_i)}{a_i(\phi)} + c(y_i,\phi)\right\},$$

where $a_i(\phi)$, $b(\theta_i)$ and $c(y_i,\phi)$ are specific functions. The parameter θ is related to the mean of the distribution, and ϕ, the dispersion parameter, is known and is usually related to the variance of the distribution (Myers et al., 2002). Assuming a GLM model for QTL analysis with an exponential family distribution, the following general model was constructed around the linear predictor to test for a QTL located in the interval between markers i and $i+1$:

$$\eta_j = b_0 + b^* x_j^* + \sum_{k\neq i,\,i+1}^{c} b_k x_{jk},$$

where c is the number of markers selected, b_0 is the intercept, b* is the effect of the putative QTL in the interval between markers i and $i+1$, x_j^* is an indicator variable, b_k is the partial regression coefficient of the phenotype on the kth marker, x_{jk} is a known coefficient for the kth marker in the jth individual. The model is found through the use of a link function: $\eta_i = g(\mu_i)$, where μ_i is the expectation of the response variable (Myers et al., 2002). GLM models were run in the GENMOD procedure of SAS. Co-factors were previously determined using the same procedure, but without the additive effects of the putative QTL for each genetic linkage group independently. The chi-square of the Wald test was used to test for statistical significance of the QTL (or the additive effect of the putative QTL) according to the method of Myers et al. (2002).

The GLM method was compared with the Composite Interval Mapping (CIM) and the Bayesian interval mapping (BIM) methods, assuming experimental data with a non-normal response, in the program WinQTLCart 2.5. The CIM analysis was conducted using Model 6 with forward and backward stepwise regression, a window size of 10 centiMorgans (cM), five control markers and scanned at 1 cM (Wang et al., 2011). The LOD thresholds to declare significant QTLs were set at 2.4 based on 1,000 permutation tests and a type I error of 5% (Churchill and Doerge, 1994). For the BIM, the posterior marginal parameter distributions were computed using the Reversible Jump Markov Chain

Monte Carlo (RJ-MCMC) algorithm. A Poisson distribution was assumed for the number of QTL, according to the method of Silva and Leandro (2009).

Conclusion

In summary, given that the female index is often non-normally distributed, the generalized linear modeling approach can be used as an efficient method to map QTLs in a trait with a non-Gaussian distribution. The five-item hierarchy proposed for the female index (treated as a categorical trait) was useful for mapping purposes, and the results agreed with the GLM method assuming data with a Gamma distribution. We also want to highlight that the BIM and CIM were fairly robust in erroneously assuming the non-normal data of FI.

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Supplementary data

QTL mapping of soybean cyst nematode race 9: a generalized linear modeling approach

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Tab supplementary 1. Microsatellite markers (SSR) with segregation ratio 1:1 ($P < 0.05$ according to the Chi-square test) employed to map QTL for resistance to race 9 of the soybean cyst nematode

SSR	Linkage Group	GenBank Accession	Forward (5' → 3')	Reverse (5 → 3')
Satt454	A1	BH126628	GCTTTTCTTAGAACACAAATTACAAG	CAACCATGATAAATGTGAGTGAG
Satt545	A1	BH126713	CAATGCCATTCCATATTTGTT	CAATTGCCCTAGTTTTGATAG
Satt449	A1	CC453967	GCGTGCTTCTTATATTAGGTGTTAGT	GCGCATTGGAGTTTTTGCTTTT
Satt329	A2	BH126520	GCGGGACGCAAAATTGGATTTAGT	GCGCCGAATAAAACGTGAGAACTG
Satt233	A2	BH126434	AAGCATACTCGTCGTAAC	GCGGTGCAAAGATATTAGAAA
Sat_162	A2	BH126790	GCGTGGTTTTTCGCTGGATATA	GCGCATTTCGTAACATATTTTTCAC
Satt632	A2	BH126793	GGGCTATGAAGGGAATGGAAAGGA	CCCATATTGAAGATTTGAAGTAAT
Satt207	A2	BH126413	GCGTTTTTCTCATTTTGATTCCTAAAC	GCGATTGTGATTGTAGTCCCTAAA
Satt341	A2	BH126532	GCGGAGCTTACCAACATAAAAAAACT	GCGGTCCAACATTGAGGCAAGAATAC
Satt332	B1	BH126523	GCGCATCCAGGGCTTGCAACAAAG	GCGGTCCTTATATATGGAAGATCA
Satt444	B1	BH146217	TGCAAAAATACGGGTTCATAAT	AGAGGAAGCGAGACTAATAGAAG
Satt484	B1	BH126656	GCGTTTAATAAAACTAATTTAATTGTACT	GCGTTCCCTTTCTCTCCTTTCTTTCTT
Satt416	B2	BH126595	TATAGCCCAGCAAAAAAAAACAGAGAT	ATCAAAACCGACCAATGAACAAAAAAA
Sat_009	B2	CC453672	CACACGTATTGTCTTACCAC	CTCCGAGAAGCACGTA
Satt534	B2	BH126703	CTCCTCCTGCGCAACAACAATA	GGGGGATCTAGGCCATGAC
Satt556	B2	BH126723	GCGATAAAACCCGATAAATAA	GCGTTGTGCACCTTGTTTTCT
Satt476	C1	BH126648	TTTGCTGATTAAAAAAACAAAAACTG	TTGTTAGAATGGGGACTACTTCACTA
Satt180	C1	BH126388	TCGCGTTTGTCAGC	TTGATTGAAACCCAACTA
Satt190	C1	BH126397	GGGAGTGTGAACTTACATTGTCT	GGGCCTTGAATTTTGTGCTAT

Satt277	C2	BH126473	GGTGGTGGCGGGTTACTATTACT	CCACGCTTCAGTTGATTCTTACA
Satt307	C2	BH126498	GCGCTGGCCTTTAGAAC	GCGTTGTAGGAAATTTGAGTAGTAAG
Satt202	C2	BH126409	GGAATGCATGAGTATTAACCTCTTAT	GGGCTAACGAACATGTAACTTATCAAC
Satt371	C2	BH126558	TGCAAACTAACTGGATTCACTCA	GAGATCCCGAAATTTTAGTGTAACA
Satt460	C2	BH126633	GCGCGATGGGCTGTTGGTTTTTAT	GCGCATACGATTTGGCATTTTTCTATTG
Satt357	C2	BH126546	CCTGAGCAATTCATACTCC	TAACCGATCCGATCCTTGACA
Satt316	C2	BH126507	GTGAGAAACTAGCCAAGAATAGA	CAATTGTTTCCAAATGACACT
Satt079	C2	BH126324	AGTCGAAGATACACAATTAGAT	CTTTTAGACACAAATTTATCACT
Satt557	C2	BH126724	GCGGGATCCACCATGTAATATGTG	GCGCACTAACCCTTTATTGAA
Satt286	C2	BH126480	GCGGCGTTAATTTATGCCGGAAA	GCGTTTGGTCTAGAATAGTTCTCA
Satt376	C2	BH126563	GCTACGCATTTGGTTTGTTA	ACATGCAATACTTTTTTTTCAT
Satt147	D1a	BH126359	CCATCCCTTCCTCCAAATAGAT	CTTCCACACCCTAGTTTAGTGACAA
Satt267	D1a	BH126463	CCGGTCTGACCTATTCTCAT	CACGGCGTATTTTTATTTTG
Satt321	D1a	BH126512	CACCGTCGTAAAAACTGTGTCGT	GCGTGTCAAAGAGTTTTAGACATC
Satt342	D1a	BH126533	GGTGCAAGGGAAAATGGAAATAA	GATACAACGTCGTGCTACTATCCAAATA
Satt370	D1a	BH126557	GCGGTTAAGGGAATTTGTAACTTGAA	GCGATCATGCATTTATTTGAGATA
Satt408	D1a	BH126588	GCGGTCCGTGCTGTTAATTCTATA	GCGTGATTTATTCATGATATATTTTTG
Satt482	D1a	BH126654	GCGCGTTAGTTTAACGTAAAAGGAAAT	GCGTCACCTCAATGGATATTTATTTTA
Satt507	D1a	BH126678	GCGCTCAGCCTTGTTAAATCACTT	GCGCTACTCTCGTGTCGTTAGTTA
Satt531	D1a	BH126700	GCATGCAACTGAGGGAGCAGAT	GCCACAAATTATGCAGAATATA
Satt532	D1a	BH126701	GCGCCAATATTATCATGCTTTATGT	GCGTGTAAAAATCTTTGAATCTTGA
Satt189	D1b	BH126396	CCATACGCAGCATTAGAG	GCTATTTGCATGTTGAGAA
Satt600	D1b	BH126765	GCGCAGGAAAAAAAAACGCTTTTATT	GCGCAATCCACTAGGTGTTAAT
Satt141	D1b	BH126353	CGGTGGTGGTGTGCATAATAA	CCGTCATAAAAAGTCCCTCAGAAT
Satt301	D2	BH126492	GCGAAACACTCCTAGTTGATTACAAA	GCGATATAATGCACAAAGAAATTAAAGA
Satt574	D2	BH126740	GCGCCTCTCATATGGTAT	GCGGGGGGGAAATGTAGA
Satt389	D2	BH126574	GCGGCTGGTGTATGGTGAAATCA	GCGCCAAAACCAAAAGTTATATC
Satt151	E	BH126363	ATTGCCTAATTTCTGTTTGTTGTAA	CCAAAATTCAAGGCAGTGAC
Satt204	E	BH126411	GCCATCTTGTTACAATGCAGGTA	CCTTACTCACTCCATTGGCATAATA
Satt212	E	BH126418	CCAATCCAAACAAATCCACT	CAGCAATGATGATAATGAATGA
Satt263	E	BH126460	CACCCAATCATGATAGCATTTTAT	CTCATGGAATTGTCTTTCAGTTTC
Satt268	E	BH126464	TCAGGGGTGGACCTATATAAAATA	CAGTGGTGGCAGATGTAGAA
Satt452	E	BH126626	GCGGTCGCTGCGTTCAATAT	GCGCCCAATTATCATGGTAGA
Satt602	E	BH126767	GCGGCGTTAGTGGAATAGAACTA	GCGGGTATACCAAATGAGATAAT

Satt343	F	BH126534	CATGGCGGAAAGCGAAACA	TCCCAATTCACCTCTTCA
Satt516	F	BH126686	GCGTTAGCACTATTTTTTTACAAGA	GCGCCGTTCCTCTTTACTTTAT
Satt193	F	BH126400	GCGTTTCGATAAAAATGTTACACCTC	TGTTCGCATTATTGATCAAAAAT
Satt490	F	BH126661	GCGGCACGAGTCAACTTTCTGTTTCCT	GCGGAAGAAGATTTTCGTTTTTAT
Sat_163	G	BH126791	GCGGTATATATGTTTGCAAGACATATT	GCGGAATCTCGCCCAGGAGGAACTT
Satt038	G	CC453951	GGGAATCTTTTTTTCTTTCTATTAAGTT	GGGCATTGAAATGGTTTTAGTCA
Satt130	G	BH126344	TAAACGAAATTTAGTTTTAAGACT	TGAATGGCTAAAAACGTGATT
Satt163	G	BH126374	AATAGCACGAGAAAAGGAGAGA	GTGTATGTGAAGGGGAAAAACTA
Satt217	G	BH126421	AATGATTTTGCGTATGTAAGATGA	GCGGATGACATTAATAGTTTTTAGA
Satt235	G	BH126436	GCGGGCTTTGCCAAGAAGTTT	GCGGTGAGGCTGGCTATAAG
Satt275	G	BH126471	GCGGGATAATTGGTTTTACGAAAATGC	GCGCCTAATCACCTAAAAAAACGTTTA
Satt303	G	BH126494	AAAAGCGACGACCTATG	TGAACGTTCTATCAACACA
Satt309	G	BH126500	GCGCCTTCAAATTGGCGTCTT	GCGCCTTAAATAAAACCCGAAACT
Satt356	G	BH126545	CATGCCTGGTCCATTTTG	TCAAGCCACGATAACAGTA
Satt427	G	BH126606	GCGAGTATCCACCCTTTTATAATAAT	TCTCCACGCCACCTTATTTCCTCTCC
Satt564	G	BH126730	GCGCTTCCACCACAATAACA	GCGGCAGAGGACTGACAGCTA
Sctt010	G	BH126786	CGCATGTGCAAGTAAC	TAGTTGGGGAGAAACAG
Satt293	H	BH126486	GCGCAGAAGGTTTGCATAAAAAGAAT	GCGGGCTAAAAAGTTGATGTAATGTG
Satt142	H	BH126354	GGACAACAACAGCGTTTTTAC	TTTGCCACAAAGTTAATTAATGTC
Satt302	H	BH126493	GCGAACTGTAGTTTACTAAAAATAAGTG	GCGGACTGAATTAATATTGGTGTTGAATT
Satt317	H	BH126508	GCGAACAAACTTTCTATACATGATAACA	GCGGGTATATTTTTGTACATAAGTTGGAA
Satt353	H	BH126542	CATACACGCATTGCCTTTCCTGAA	GCGAATGGGAATGCCTTCTTATTCTA
Satt354	I	BH126543	GCGAAAATGGACACCAAAAGTAGTTA	GCGATGCACATCAATTAGAATATACAA
Satt431	J	CC453966	GCGTGGCACCCTTGATAAATAA	GCGCACGAAAGTTTTTCTGTAACA
Sctt011	J	CC454081	CTCCGTTGCTGAT	TAAGCTGAATTAGTAAAA
Satt456	J	CC453968	GGGCCTTCGTTTGAGTTCATAG	GGGATCATTGGTTAATTGTTGTAAGA
Satt215	J	CC453956	GCGCCTTCTTCTGCTAAATCA	CCCATTCAATTGAGATCCAAAATTAC
Sat_366	J	CC453892	GCGGCACAAGAACAGAGGAAACTATT	GCGGACATGGTACATCTATATTACGAGTATT
Sat_394	J	CC453919	GCGGACAGTGTGCTCCTCATATAATAG	GCGTGACTCGGACTTGAAGATAATAATG
Sat_119	K	BH126289	TAGGCTTTCAATTTGCAGAACT	GTTAGGTGTCCCAAGCAACTTA
Satt167	K	BH126378	GATTTACGGGTACTTGGATTCAATA	AGCTACCCAATATGATACTCTACACAGT
Satt178	K	BH126386	GGGAAAATTCTTTTCATATAGATG	GGGGTTGAGATATTTTGTTCATAC
Satt196	K	BH126403	TTGGGAAATAGTGATTGAGGTAAAA	AAATCCCCATTGAATGAGAATAAG
Satt242	K	BH126443	GCGTTGATCAGGTCGATTTTTATTTGT	GCGAGTGCCAACTAACTACTTTTATGA

Satt349	K	BH126539	GCGGGAACGAACGGGAAGAAGAAC	GCCATCCAATGTTTAGAAGAAC
Satt555	K	BH126722	GCGGTTGGCTTTGATGATGT	TTACCGCATGTTCTTGGACTA
Satt418	L	BH126597	GCGAAAGCACATATGGGTTTGAAT	GCGAGGGCATATATATGATGAGGTA
Sat_134	L	BH126304	GCGATGAGGAAAGGTGATAGTGAACTTG	GCGCTCAGCTTGCATATATAAAATAATA
Satt497	L	BH126668	GCGGTTTTGGATTGACTTTGTTGA	GGCTCAATTAGAGCATGCAACATC
Satt446	L	BH126621	CCGCATAAAAAACACAACAAATTA	GCGGGCAAATTTGACCTAACTCACAAC
Satt523	L	BH126693	GCGATTTCTTCCTTGAAGAATTTTCTG	GCGCTTTTTCGGCTGTTATTTTTAACT
Satt527	L	BH126697	GCGGTTACATCTTGCAAACTAAATTAAC	GCGGAATTTTGCACATAAATTAATAACT
Sct_010	L	BH126771	TCCCAAAAGCATTGAG	TATGCACGGAAGAGGA
Satt238	L	BH126439	GCGCCATTTTAATGATTTATTTA	GCGGAAAGAAGAGGAAGAAAG
Satt150	M	BH126362	AAGCTTGAGGTTATTCGAAAATGAC	TGCCATCAGGTTGTGTAAGTGT
Satt175	M	BH126384	GACCTCGCTCTCTGTTTCTCAT	GGTGACCACCCCTATTCCTTAT
Satt245	M	BH126445	AACGGGAGTAGGACATTTTATT	GCGCCTCCTGAATTTCAAAGAATGAAGA
Satt250	M	BH126448	CGCCAGCTAGCTAGTCTCAT	AATTTGCTCCAGTGTTTTAAGTTT
Satt463	M	BH126636	TTGGATCTCATATTCAAACTTTCAAG	CTGCAAATTTGATGCACATGTGTCTA
Satt536	M	BH126704	GCGCCACAGAAATTCCTTTTTCTA	GCGCCATAAGGTGGTTACCAAAAGA
Satt551	M	BH126718	GAATATCACGCGAGAATTTTAC	TATATGCGAACCCTCTTACAAT
Satt590	M	BH126756	GCGCGCATTTTTTAAGTTAATGTTCT	GCGCGAGTTAGCGAATTATTTGTC
Sat_084	N	CC453683	AAAAAAGTATCCATGAAACAA	TTGGGACCTTAGAAGCTA
Satt237	N	BH126438	GCGTGATTTCAATCCTTTTTC	GCGGTTGTCCTGTTAGAACCT
Satt387	N	BH126572	GCGTTACGTTTCACTATTTATTTAACAT	GCGGCAGGCTAGCTACATCAAGAG
Satt485	N	BH146218	GCGAATACGCATAAAAAAATCAACAAGA	GCGAAAAGAAAATTTAAAAAAAAATATAT
Satt530	N	BH126699	CATGCATATTGACTTCATTATT	CCAAGCGGGTGAAGAGGTTTTT
Satt549	N	BH126716	GCGGCAAAACTTTGGAGTATTGCAA	GCGCGCAACAATCACTAGTACG
Satt345	O	BH126535	CCCCTATTTCAAGAGAATAAGGAA	CCATGCTCTACATCTTCATCATC
Satt478	O	BH126650	CAGCCAAGCAAAAGATAAATAATA	TCCCCCACAAGAGAACAAGAAGGT
Sat_108	O	CC453689	AAAAATCTATTCACTTTGAGTCTA	TTGAAAGAGTCACGTCTATTCTAT
Satt550	O	BH126717	CGTCAATTAAGCAAAAATGTGA	GCGCGGATGAGCGTGCGTTTTTA
Satt173	O	BH126382	TGCGCCATTTATTCTTCA	AAGCGAAATCACCTCCTCT
Satt358	O	BH126547	GCGGCGCTTTATGTAACAATACGATTT	GCGAGTAAAAGCAGAGTGCGGAGTA
Satt479	O	BH126651	GCGCTTTCAAAAAGTAACAATTAATGAAA	GCGGGAATTGGTTAATCTCATCGTGAC
Sat_274	O	CC453808	GCGCCGATCTTTAGTGAGGTTACAAGT	GCGTTCAGCGAGTCCAGAAATAG
Satt188	O	BH126395	GCGTTTTAATTTTAATTTATTATTTTC	GCGCTGTCTTAATTGGAGATAC