Genome-wide association study to identify soybean stem pushing resistance and lodging resistance loci

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Abstract: Lodging resistance is an important objective for soybean [Glycine max (L.) Merr.] breeding, but selection for this trait has been difficult since the resistance is controlled by multiple genes, and these genes interact with the environment. A total of 130 of 139 lines constituting a soybean genome-wide association study panel were phenotyped for stem pushing resistance, which is defined as the push-back strength when the plant stem is inclined, by measuring the force required to push a stem to a 45° angle using a force gauge in a greenhouse, and also for lodging, plant height, seed yield, and maturity at three locations in total in eastern Canada in 2013 or 2017. Two quantitative trait loci (QTLs) for pushing resistance were identified on chromosome 5 and 11, and each QTL accounted for 16.0% of phenotypic variation. In our panel, the alleles for higher pushing resistance were always of lower frequency than the alternate allele. Examining the panel at these QTLs identified that higher pushing resistance was associated with lower lodging on chromosomes 5 and 11, and that the difference for lodging between alleles was significant on chromosome 5. There was no difference in plant height or yield at the QTL on chromosomes 5 or 11, while higher pushing resistance was associated with later maturity at both QTLs. The pushing resistance QTL on chromosome 11 will be useful for decreasing lodging in Canadian short-season soybean.

Key words: GWAS, SNP, QTL, lodging resistance, pushing resistance, soybean.
Introduction
Improvement of lodging resistance is an important breeding objective in various crops such as rice (Setter et al. 1997; Kashiwagi and Ishimaru 2004), wheat (Foulkes et al. 2011), soybean (Cooper 1971), and corn (Fouéré et al. 1995). Improvement of lodging resistance contributes to increased yield performance not only through increased solar radiation interception, which is influenced by canopy architecture, but also through efficient field crop management and mechanical harvesting (Cooper 1971; Setter et al. 1997). In soybean [Glycine max (L.) Merr.], lodging resistance has been steadily improved due to plant breeders’ efforts spanning many years in Canada (Voldeng et al. 1997; Morrison et al. 2000), India (Ramteke et al. 2011), and the USA (Luedders 1977; Wilcox et al. 1979; Wilcox 2001; Rincker et al. 2014; Keep et al. 2016). Since lodging is controlled by multiple genes and these genes interact with the environment (Lee et al. 1996a, 1996b; Liu et al. 2017), selection for lines with excellent lodging resistance has to be repeated in multiple environments.

In general, there is a negative correlation between main stem length and lodging resistance, and a decrease in main stem length contributes to improved lodging resistance (Ramteke et al. 2011; Keep et al. 2016). It is known that enhancement of lodging resistance, however, cannot be explained only by a decrease in the length of the stem, but also by the improvement of stem strength (Shimada et al. 2002; Chen et al. 2011; Yamaguchi et al. 2014; Kitabatake et al. 2019). Since the length of the main stem is an important trait to ensure sufficient aboveground growth, which is closely related to yield, stem strength is a noteworthy trait for lodging resistance (Chen et al. 2011). The pushing resistance method is a method of measuring the push-back strength with a force gauge when the base portion of the stem is inclined at a certain angle (Idris et al. 1975). Cultivar differences in stem pushing resistance measured using this method have been reported in rice (Idris et al. 1975; Terashima et al. 1992), corn (Fouéré et al. 1995), and canola (Wu and Ma 2016). Pushing resistance quantitative trait loci (QTLs) have been reported in Italian ryegrass (Inoue et al. 2004), rice (Kashiwagi and Ishimaru 2004; Yadav et al. 2017), wheat (Hai et al. 2005), and corn (Yang et al. 2016). Varietal differences in soybean stem pushing resistance related to lodging resistance have been identified (Shimada et al. 2002; Chen et al. 2011; Chen et al. 2017) and resulted in the identification of QTLs for stem pushing resistance (Chen et al. 2011; Chen et al. 2017).

Advancements in genetic analysis technology have made it possible to perform genome-wide analyses. A genome-wide association study (GWAS) is a powerful method that can deal with a wide genetic background and a high density of SNP markers can provide extremely high resolution. Sonah et al. (2015) developed an association panel comprised of Canadian soybean varieties and identified 1, 1, 3, 8, and 8 QTLs governing maturity, plant height, seed weight, oil content, and protein content, respectively. The QTLs governing maturity and plant height were considered to be due to E3 (GmPhyA3), a gene previously reported; however, three QTLs controlling oil and protein content were novel. Currently, there are no reports of any QTLs related to stem pushing resistance identified using GWAS in soybean. As previously described, the lodging resistance of modern Canadian soybean varieties tends to be superior to that of older ones (Voldeng et al. 1997; Morrison et al. 2000). The set of Canadian varieties selected by Sonah et al. (2015) for constructing the association panel included both modern and older varieties. Therefore, there is a great opportunity to attempt to identify important genes that have contributed to the improvement of lodging resistance by phenotyping and analyzing this panel through GWAS.

In this study, we aim to phenotype an association panel developed by Sonah et al. (2015) for stem pushing resistance to detect QTLs for this trait, and to evaluate the relationship between these QTLs and both lodging resistance and other agronomic traits measured in field experiments.

Materials and Methods
Field experimentation
The association panel of short-season (MG 000 to MG 0) Canadian soybean varieties (n = 139) used in this study has been previously described in Sonah et al. (2015). Field data (lodging, plant height, maturity, and yield) from plants grown in the summer of 2013 at the University of Guelph in Woodstock, ON (43.141463, −80.801617; hereafter WSK), and Centre de recherché sur les grains Inc. in St-Mathieu-de-Beloeil, QC (45.586648, −73.248716; hereafter SMB), and from plants grown at the Ottawa Research and Development Centre in Ottawa, ON (45.387855, −75.71637; hereafter OTW) in 2017 were used in this study. The experiments in WSK and SMB were arranged in a generalized lattice design with two replications. The experiment in OTW was arranged in a modified augmented design with one replication; however, only 126 lines were used in the analysis since some were lost due to poor establishment. At maturity, lodging was visually scored in each plot as...
1 (erect) to 5 (prostrate). Maturity was defined as the number of days from sowing to maturity (when 95% of the pods have reached their full mature color). Individual field experiments were designed and analyzed with AGROBASE Generation II SQL (Agronomix Software Inc., Winnipeg, MB) using default models (generalized lattice factors included replication, incomplete block, and genotype; modified augmented design as a type 2 design with the most efficient row-column or regression used for adjustment) for each experimental design.

Evaluation of pushing resistance in the greenhouse

Stem pushing resistance of the panel was evaluated in a greenhouse experiment at the Ottawa Research and Development Centre. Six seeds of each line were planted into a 15 cm fiber pot in a potting mixture of three parts Promix BX (Premier Tech Horticulture, Delson, QC) to one part top soil. The plants were thinned to two plants per pot at the unifoliate leaf stage. Due to seed quality, only 130 lines were used in the stem strength analysis. The experiment was replicated in time with two dates of seeding (1 Dec. 2017 and 26 Jan. 2018). Day lengths were extended to 16 h with metal halide lamps supplementing natural day length, and liquid fertilizer (20–20–20 N–P₂O₅–K₂O at 2 g L⁻¹) was supplied in the irrigation water weekly.

Prior to stem pushing resistance measurements at 6 wk following planting, plants were cut off at a height of 20 cm above the soil surface and their branches were removed to eliminate interference of the duplicate plant in a pot when measuring this trait (Fig. 1). The amount of force required to manually push a plant stem to an approximate 45° angle from a vertical position (pushing resistance) was measured in Newtons by placing a digital force gauge ZTA (Imada, Aichi, Japan) on the stem and manually pushing the stem to the desired angle (45°) as marked on a plastic protractor centered on the main stem. Analysis of variance (ANOVA) was performed with SAS version 9.4 (SAS Institute Inc., Cary, NC). Genotypes were considered fixed effects, while all other factors were considered random.

Fig. 1. The procedure for measuring pushing resistance. (a) Prior to stem pushing resistance measurements, plants were cut off at a height of 20 cm above the soil surface and their branches were removed. (b) The force gauge was set on the plastic protractor perpendicularly to the main stem of the tested plants at a height of 10 cm above the soil surface. (c) The plant stem was manually pushed to an approximate 45° angle from a vertical position. The amount of force was measured as pushing resistance.

Genome-wide association study and statistical analysis

The SNP genotyping, genetic diversity, and population structure of the association panel of short-season (MG 000 to MG 0) Canadian soybean varieties (n = 139) used in this study was previously described by Sonah et al. (2015). Additional SNPs developed by Torkamaneh et al. (2018) were included in the analysis. GWAS was performed with a mixed linear model (MLM) with principal component analysis (PCA) + K to account for population structure and kinship, in TASSEL software version 5.2.59 (Bradbury et al. 2007). Site min count (104) was calculated as follows: 130 (number of lines) × 0.8 (1–0.2; this will result in eliminating markers with more than 20% missing genotypic data). Site minimum allele frequency (MAF) was set at 0.05; eliminating minor allele frequency less than 5% (Sonah et al. 2015). Markers with −logP > 4.0 were considered to be QTLs associated with pushing resistance. Pushing resistance QTLs were used to divide the lines into two allelic classes, at each QTL, and mean lodging scores, plant height, yield, and maturity were compared between the two classes using ANOVA.

Results

Evaluation of pushing resistance in the greenhouse and the identification of QTLs by GWAS

Pushing resistance of the 130 tested lines showed a continuous distribution that ranged from 1.80 to 6.52 N (average of the experiments with two different planting times) (Fig. 2). Analysis of variance for pushing resistance revealed that the genotype effect was significant as well as those of replicate, duplicate within a pot, and genotype × replicate interaction at the P < 0.001 level (Table 1).

GWAS for pushing resistance (~156K SNPs before filtering and ~20K after filtering) revealed two QTLs that mapped to chromosomes 5 (qLR05-1 (QTL for lodging resistance)) and 11 (qLR11-1) with the MLM approach (Fig. 3), and each QTL accounted for 16.0% of phenotypic variation. To determine the extent of the differences between alleles at each QTL, the lines were divided into two allelic classes at the two QTLs detected with MLM, and the pushing resistance scores between the two allelic classes were compared for each QTL. One-way ANOVA revealed
that significant differences between alleles were observed among the two QTLs. Within our panel, the alleles at qLR05-1 and qLR11-1 that were associated with lower pushing resistance had higher frequencies compared with the high pushing resistance alleles (Table 2).

The effect of the identified pushing resistance QTLs on the lodging evaluated in the field

The lodging score (1–5) of tested lines showed continuous distribution from 0.9 to 2.1 at SMB in 2013, 0.3 to 3.3 at WSK in 2013, and from 1.0 to 3.0 at OTW in 2017 (Fig. 2). Genotypes were significantly different in each environment for lodging (13SMB \( P = 0.0003 \), 13WSK \( P = 0.0158 \), 17OTW control plot means, and SE of 1.45 ± 0.26).

To investigate the effect of the QTLs for pushing resistance on lodging in the field, the lines were divided into the two allelic classes for each of the pushing resistance QTL determined in greenhouse pushing tests, and the mean lodging scores from field trials were compared. This comparison revealed that at qLR11-1, field lodging differences between the classes were significant. Lines
The effect of QTLs on other agronomic traits

Since lodging can be a function of aboveground plant growth (plant biomass and the distribution of that mass), and stem strength, pushing resistance QTLs are not likely the only path to improving lodging resistance, and changes in aboveground growth morphology might also be important. To address this relationship, the effects of the pushing-resistance QTLs identified in this study were examined for traits (mean across locations) including length of the main stem, seed yield, and maturity. Main stem length and yield differences between alternative pushing resistance classes were not significant at all pushing resistance QTLs (Table 3). The effects of qLR05-1 and qLR11-1 on maturity were significant, and the maturity of lines with alleles contributing to higher

Table 2. Soybean stem pushing resistance QTLs identified by GWAS, the SNP position, mean stem pushing resistance for allelic classes evaluated in a greenhouse, and number of lines in each allelic class.

| Chr | QTL name | SNP position | P value | R² | Pushing resistance associated with SNP allele [mean ± standard error (N)] |
|-----|----------|--------------|---------|----|---------------------------------------------------------------|
| 5   | qLR05-1  | 32,834,159   | 7.04E−5 | 0.16 | 3.43 ± 0.09 (N = 98) — | 4.15 ± 0.21 (N = 27) — |
| 11  | qLR11-1  | 34,203,032   | 7.31E−5 | 0.16 | 3.45 ± 0.08 (N = 105) 4.21 ± 0.22 (N = 22) — |

Note: ***, P ≤ 0.001. Code letters A, T, G, and C stand for the DNA base chemicals adenine, thymine, guanine, and cytosine, respectively.

Table 3. Mean values of agronomic traits with respect to the allelic classes at QTLs for pushing resistance from 126 soybean lines grown at Saint-Mathieu de Beloeil in 2013, Woodstock in 2013, and Ottawa (lodging and maturity only) in 2017.

| QTL   | SNP | Lodging (1–5) | Plant height (cm) | Yield (kg·ha⁻¹) | Maturity (d) |
|-------|-----|---------------|------------------|-----------------|--------------|
| qLR05-1 | A   | 1.30 ± 0.05   | NS               | 68.1 ± 1.6      | NS           | 2714 ± 79 | NS | 113.8 ± 0.9 | *** |
|       | G   | 1.41 ± 0.03   | NS               | 66.0 ± 0.9      | NS           | 2552 ± 42 | NS | 111.0 ± 0.5 |  **   |
| qLR11-1 | C   | 1.23 ± 0.05   | **               | 68.2 ± 1.9      | NS           | 2732 ± 76 | NS | 114.2 ± 1.0 | *** |
|       | G   | 1.41 ± 0.03   | NS               | 66.2 ± 0.9      | NS           | 2559 ± 42 | NS | 110.9 ± 0.4 |  **   |

Note: Values are shown as mean ± standard error. Bold letters indicate the allelic variations (SNP) with higher pushing resistance for the identified QTLs. **, P ≤ 0.01; ***, P ≤ 0.001; NS, not significant.

with the C allele at qLR11-1 showed higher pushing resistance (Table 2) and reduced field lodging (Table 3).
pushing resistance were on average 2.8–3.3 d later maturing than those with the alternative alleles (Table 3).

**Discussion**

Improvement of lodging resistance has been an important breeding objective in economically important crops including soybean. Since lodging often varies depending on the growing environment; however, the selection of lines with acceptable lodging resistance is challenging. Multi-year observations in the field are an important and critical requirement in the selection process. In this study, the stem pushing resistance of Canadian varieties in a high-density SNP panel was determined in a greenhouse. Genome-wide association analysis resulted in the identification of two QTLs, qLR05-1 and qLR11-1, for pushing resistance. Each QTL accounted for 16.0% of phenotypic variation, and the alleles for higher pushing resistance were always of lower frequency than the alternate allele in our panel (Table 2).

The stem pushing resistance observations were taken from greenhouse-grown plants in potting mix and could differ across potting mixes or from field observations. However, field lodging scores, which were obtained by averaging the lodging scores evaluated in three different field environments, were lower for lines in the higher pushing-resistance class, and the difference between alleles in qLR11-1 was statistically significant (Table 3). Although the size of population used for the GWAS (N = 130) was not very large, these results validated the effect of qLR11-1 on lodging in fields. We are aware that the observations for lodging at three sites and for stem pushing resistance (n = 4) are limited, and that further data collection would be needed to identify some undetected QTLs for pushing resistance. However, lodging and stem pushing resistance are coincident; therefore, we conclude that this QTL is effective for the improvement of lodging resistance.

To our knowledge, this study is the first report of GWAS being used to identify pushing-resistance QTLs in soybean. Previously, QTL analyses for stem pushing resistance using RILs (Chen et al. 2011; Chen et al. 2017) identified QTLs on chromosomes 4 (qSS-4-1), 6 (qSS-6-1, qSS-6-2, and qSS-6-3), 8 (qSS-8-1 and qSS-8-2), 13 (qSS-13-1), and 14 (qSS-14-1) by composite interval mapping using RILs from the cross between Zhongdou No. 29 (lodging-resistant) and Zhongdou No. 32 (lodging-susceptible). A pushing-resistance QTL was also reported on chromosome 19 (qLS19-1) (Kitabatake et al. 2019). The QTLs identified in this study are located on chromosomes 5 and 11 and are, therefore, novel compared with the previously reported QTLs. Liu et al. (2017) performed modified GWAS with a panel constructed of “Takachi nagaha” and 137 varieties derived from it and identified 31 QTLs on 16 chromosomes. Three out of 31 QTLs were located on chromosome 11 (ss247348244, ss247400714, and ss247434650), whereas no QTL was identified on chromosome 5. The QTLs detected in this study and those of Liu et al. (2017) on chromosome 11 were more than 19 Mbp apart. Therefore, the QTLs identified in this study are considered to be novel QTLs related to stem pushing resistance and (or) lodging resistance.

The effect of the pushing resistance QTLs on main-stem length and seed yield were not significant. This result confirmed that in this panel, increased pushing resistance QTLs were not associated with a change in main-stem length or seed yield. The effects of qLR05-1 and qLR11-1 on maturity were, however, significant. So far, 13 loci (E1-E11, J, and Dt1) have been identified governing maturity in soybean (Liu et al. 2010; Tian et al. 2010; Watanabe et al. 2012; Tsubokura et al. 2013; Lu et al. 2017; Samanfar et al. 2017; Wang et al. 2019). The pushing resistance QTLs identified in this study are different from previously reported loci for time of flowering and maturity. It is possible that the identified QTLs for pushing resistance in this study have pleiotropic effects on maturity, or that some genes that regulate maturity are located in the vicinity of these QTLs. Since maturity is a highly important trait as well as lodging resistance, fine mapping of these QTLs followed by the identification of responsible genes, and comparison of maturity with near-isogenic lines harboring the different alleles will be required in the next step.

In conclusion, we identified two QTLs for soybean stem pushing resistance. The higher pushing resistance alleles were of lower frequency in our panel. There is, therefore, an opportunity to increase the frequency of such desirable alleles, through selection, to improve lodging resistance. In one case, qLR11-1, higher pushing resistance was associated with significantly reduced field lodging. Higher pushing resistance was not associated with plant height or yield for either of the QTLs, but was associated with later maturity on chromosomes 5 and 11.

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**Authors Contributions**

S. Kato, E. Cober, L. O’Donoghue, M.J. Morrison, and I. Rajcan phenotyped the materials. S. Kato, W.A. Bekele, E. Cober, and B. Samanfar performed GWAS and complementary data analysis. D. Torkamanesh and F. Belzile conducted the SNP genotyping. S. Kato, B. Samanfar, and E. Cober wrote the manuscript.

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