GENETIC DISSECTION OF FIELD RESISTANCE TO SUDDEN DEATH SYNDROME (SDS) IN SOYBEAN

By

Ruijuan Tan

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Plant Breeding, Genetics, and Biotechnology – Crop and Soil Sciences – Doctor of Philosophy

2018

ABSTRACT

GENETIC DISSECTION OF FIELD RESISTANCE TO SUDDEN DEATH SYNDROME (SDS) IN SOYBEAN

By

Ruijuan Tan

Sudden death syndrome (SDS), caused by the soil-borne fungus *Fusarium virguliforme*, is a devastating disease of soybean and has been found in most soybean growing regions of the United States. Use of SDS resistant cultivars is the most cost-efficient method to manage this disease. Dissecting the genetic architecture of SDS resistance is essential for soybean breeding. In this study, two recombinant inbred line populations with genotypes obtained from Infinium SoySNP6K BeadChip were employed to 1) identify loci underlying the root and foliar resistance to SDS; 2) investigate the effect of epistatic interaction on SDS resistance; and 3) examine the relationship among *F. virguliforme* colonization in root, foliar damage, and yield.

In the population generated from the cross GD2422 × LD01-5907, four quantitative trait loci (QTLs) were identified and mapped on Chromosomes 4, 8, 12, and 18. The resistant parent, LD01-5907, conferred the resistance alleles for the QTLs on Chromosomes 8 and 18, while the susceptible parent, GD2422, provided the resistance alleles for the QTLs on Chromosomes 4 and 12. The minor QTL mapped on Chromosome 12 is novel. The QTLs identified on Chromosomes 8 and 18 overlapped with two loci underlying soybean cyst nematode (SCN) resistance, Rhg4 and Rhg1, respectively. A significant epistatic interaction between the two QTLs on Chromosomes 8 and 18 was detected by disease incidence across two years. Individual effects of these two QTLs together with their interaction effect explained around 70% of phenotypic variance. The epistatic interaction was confirmed by field performance across multiple years at

the genotypic group, progeny line, and single plant levels. In addition, the resistance alleles at the QTLs on Chromosomes 8 and 18 showed recessive inheritance.

In the population derived from the cross U01-390489 × E07080, a weak positive correlation was observed between the *F. virguliforme* content in root and foliar damage. Compared to *F. virguliforme* content, the foliar damage showed stronger negative correlation with plot yield with the disease index showing the highest correlation coefficient. Twelve loci associated with foliar resistance were identified, and four of them were detected by multiple foliar-damage related parameters across several environments. These loci were mapped on Chromosomes 6, 9, and 18. In contrast, only one QTL was identified for resistance to *F. virguliforme* content and mapped on Chromosome 18. It overlapped with the QTL identified by disease index in the same environment. Given that the stability of identified QTLs across environments and higher correlation with plot yield, foliar symptom related parameters, especially disease index, were more valuable for SDS resistance breeding. The molecular markers associated with the identified QTLs and other information present in this research will aid the marker-assisted selection for resistance to SDS in soybean breeding.

Chapter 2 – QTL mapping and epistatic interaction analysis of field resistance to sudden death syndrome (*Fusarium virguliforme*) in soybean has been published in Theoretical and Applied genetics. doi: 10.1007/s00122-018-3110-x

I would like to dedicate this dissertation to my husband (Zixiang Wen), father (Guixiang Tan), mother (Xiuqin Ba), sister (Ruijie Tan), grandmother (Yufang Deng), grandfather (Enxian Ba), brother (Ruidong Tan), and all other people who influenced my life.

ACKNOWLEDGEMENTS

As approaching to the end of my PhD journey, I would like to express my appreciation to many wonderful people who have influenced my life so much.

Firstly, I would like to express my sincere gratitude to my advisor, Dr. Dechun Wang, for the continuous support and guidance on my study and helping me to grow as a research scientist. Besides my advisor, I would like to thank the rest of my dissertation committee: Dr. Amy Iezzoni, Dr. James Kelly, and Dr. Martin Chilvers for their insightful comments and suggestions, and also for the hard questions during my comprehensive exam which incented me to learn broadly and deeply. My sincere thanks also go to Dr. Honglian Li and Dr. Xiaoping Xing, for allowing me to conduct my M.S. research in USA. My greatest appreciation goes to my mentor in the college, Wenlin Jin, (1956.8-2007.4), who had introduced me to the academic research area and devoted his life to the adzuki bean breeding and genetics.

Secondly, I would like to acknowledge North Central Soybean Research Program and Michigan Soybean Promotion Committee which funded my project. Thanks Plant breeding, genetics, and biotechnology program, Graduate school, Department of plant, soil, and microbial sciences, College of agricultural and natural resources at Michigan State University for the financial support on my assistantship and fellowship. I would also like to thank our field technician, John F. Boyse for helping me to conduct the wonderful field experiments and showing me Michigan agriculture. Without his great help, I will not be able to accomplish my Ph.D research successfully. Paul J. Collins helped me a lot on my research. He also revised my manuscripts

and explained many things I did not fully understand to me with great patience. My appreciation also goes to Jie Wang, who has given me a lot of technical support on the quantification of root pathogen, and Janette Jacobs, who prepared a big amount of sorghum inoculum for my artificially inoculated field experiment. In addition, I feel grateful for having my other wonderful lab-mates, collaborators, and friends, Shichen Zhang, Yingdong Bi, Feng Lin, Randall Laurenz, Cuihua Gu, Adam Byrne, Lihong Li, Wei Xiao, Jiazheng yuan, and Shabir Hussain Wani. They all helped and supported my projects.

Thirdly, I would like to acknowledge my parents, Guixiang Tan and Xiuqin Ba, for raising me up and giving me the freedom to be independent and make my own decision. I want to offer special thanks to my grandmother, Yufang Deng, who is no longer with us and gave me unconditioned and undifferentiated love during my grown-up and taught me to be a strong person. Also, wish my grandfather, Enxian Ba, a peaceful and happy rest in the heaven who left us three months ago. I also want to especially thank my sister for supporting me when I was in the college and having taken good care of my parents for years. My gratitude also goes to my uncles, aunts, parents-in-law, and other relatives, including Guojun Tan, Yiming Ba, Yiqin Ba, Bin Wen, Ronglan Wu, Hong Ni, Tao Tan, Xiao Tan, Zili Wen, and Chunlin Zhou for their support and being proud of me.

Last but not the least, I want to give my deepest love and appreciation to my dear husband, Dr. Zixiang Wen, for giving me a great marriage, accompanying me through the tough period of life, always supporting me, loving me, spoiling me, and never giving up on me. Without him, I would not be able to be the current self.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW

Soybean production

Soybean [*Glycine max* (L.) Merri.], one of the principle food plants for humans, is a species of legume native to east Asia (Hymowitz T. 2008). Soybean was introduced to the U.S. in 1765 (Hartman et al. 1999) and large-scale production started in the 20th century. In the crop year 2016/2017, soybean accounted for about 61% of worldwide oil production. Currently, soybean has become the dominant oilseed crops in the United States and accounts for 90 percent of oilseed production (USDA-ERS, 2017). Due to its high content of protein and oil, the majority of soybean are typically processed to extract edible oil and produce soybean meal. Only a small percentage of soybeans are directly processed for human consumption, such as soy sauce, soy milk, soy protein, soy sprouts, tofu, etc. Soybean contains about 20% oil and is the second largest source of vegetable oil. Soybean is also a concentrated source of isoflavone, which may reduce the risk of breast cancer and prostate cancer (Messina 1999). Processed soybean meal is the main source of protein for livestock feed. The high-quality protein meal produced from soybean is up to 994 kg per acre in the United States, based on the 0.73 kg meal production per kilogram soybean and 1361 kg of soybean per acre. Moreover, soybean can be used to produce biodiesel which is cleaner burning than petroleum-based diesel oil and reduces particulate emissions. One bushel of soybeans can yield 1.5 gallons of biodiesel. Based on the yield of 44 bushels per acre, an acre of soybean can produce 66 gallons of biodiesel (Hill et al. 2006). In 2017, US soybean production reached 4.39 billion bushels, two percent more than 2016 with the average yield at a high record of 49.1 bushels per acre (USDA-NASS, 2017). In Michigan, 2,280,000 acres of soybean were planted in 2017 and the estimated economic value is about US $897.2 million (USDA-NASS, 2017).

Soybean sudden death syndrome

Sudden death syndrome (SDS), caused by the soil-borne fungus, *Fusarium virguliforme*, is an important disease of soybean in the United States (Aoki et al. 2003). SDS was first detected in Arkansas by H. J. Walters in 1970s, and M.C. Hirrel coined the name in 1980s (Roy et al. 1997). The pathogen spread from south to northern regions (Scherm and Yang 1999). Currently, SDS has become widespread in most soybean-producing regions of the United States, Argentina and Brazil (Rupe et al. 2001). SDS was ranked as one of the top 10 diseases that suppressed soybean yield in the USA in 11 of 12 years from1996 to 2007 and it is often ranked fifth to second among general soybean diseases (Wrather and Koenning 2009). Between 2003 and 2005, average yield losses due to SDS in the United States exceeded 600,000 metric tons annually (Wrather and Koenning 2006). In 2015, SDS caused the third greatest estimated yield loss in the northern states with about 40.16 million bushels lost, which is more than 14 percent of the total amount of disease-related losses. Yield losses caused by SDS depend on plant age at disease onset and on disease severity (Hartman et al. 2015). The results from Hartman et al. (1995) at University of Illinois at Urbana-Champaign showed that soybean yield was 20-46% lower in severely SDS-infected fields than in less infected fields. Moreover, based on the difference of soybean market price and disease severity in each year, the economic losses due to SDS ranged from US $15.7 million in 1998 to US $669.2 million in 2012 (Navi 2016).

Pathogen of SDS

To date, five different Fusarium species are known to cause SDS in different geological regions. In North America, SDS was caused by *Fusarium virguliforme* (ex. *F. solani* f. sp. *glycines*), while the pathogenic agents of SDS in South America were *Fusarium brasilience, Fusarium crassistipitatum, Fusarium tucumaniae, Fusarium cuneirostrum,* and *Fusarium virguliforme*

(Aoki et al. 2003; Aoki et al. 2005; Aoki et al. 2012; O' Donnell et al. 2010). Recently, *F. virguliforme* was isolated from the soil in Malaysia (Chehri et al. 2014). *F. virguliforme* is a hemi-biotrophic, asexually propagated fungus with only one mating gene idiomorph, *MAT1-1*. In contrast, *F. tucumaniae* has two idiomorphs, with each possessing either the *MAT1-1* or *MAT1-2* mating idiomorph. Therefore, sexual reproduction is possible in this species and it is the only known sexually reproducing species among the seven closely related Fusarium (Hughes et al. 2014). SDS pathogen can only be isolated from soybean roots, never from the above-ground plant tissues (Hartman et al. 2015).

Symptom of SDS

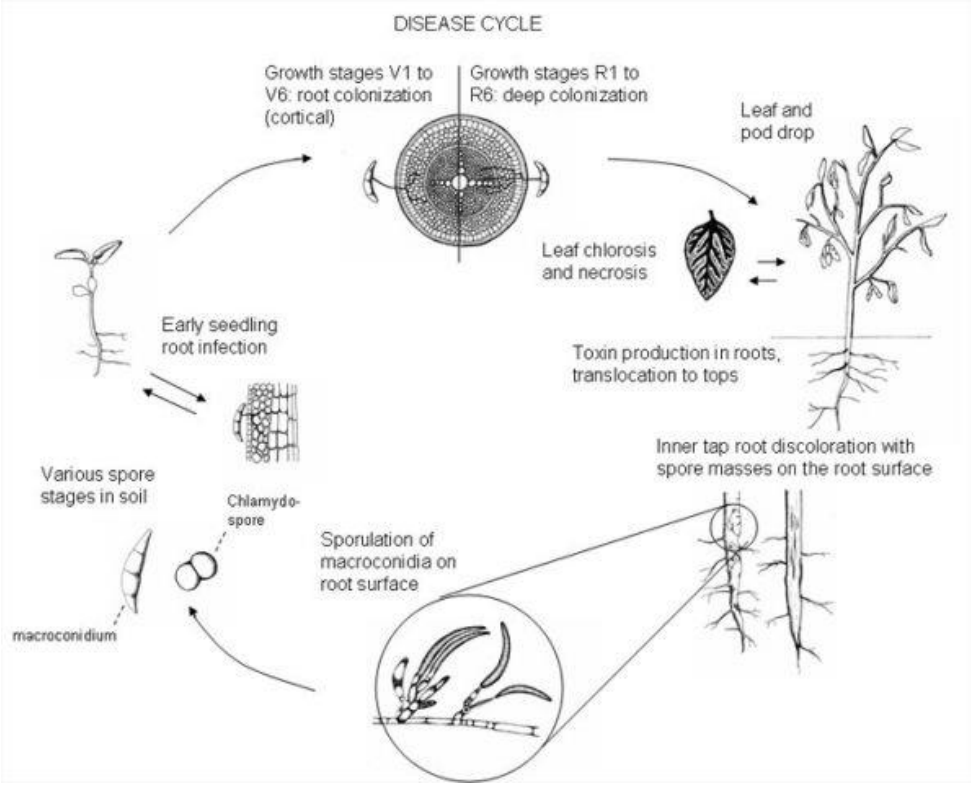
The symptoms caused by SDS damage include root rot and leaf scorch. Root damage caused by SDS may appear similar as those caused by other root-infecting pathogens, such as root discoloration and deteriorated root length. However, the white pith in the stem of SDS-infected plants is a diagnostic feature that can distinguish SDS from brown stem rot. In moist conditions, blue fungal growth may be seen on the root surface. Rupe et al. (1989) found that the epidermis of taproot and lateral roots showed the highest infection. When plants were infected by *F. virguliforme*, different cultivars exhibit significant differences for root length, surface area, and average diameter (Ortiz-Ribbing and Eastburn 2004). Infection of roots may occur early in the season, but leaf symptoms generally do not appear until around the reproductive stage (Vosberg et al. 2017). The typical foliar symptoms of SDS on soybean are caused by the phytotoxins which were produced in the root and translocated to the above-ground part. It often begins as scattered yellow, diffused spots between veins on the leaf. As the disease progresses, chlorosis and necrosis will expand to the whole interval areas while the vein remain green. Under the most severe scenario, the leaves will be detached from petioles and pod abortion occurred. In terms of

the relation between root and foliar symptoms, Navi and Young (2008) demonstrated the difference of colonization area between plant with and without foliar symptoms. The plants with foliar symptoms show discoloration in both xylem and phloem tissues, whereas, the plants without foliar symptoms only show discoloration in xylem tissues.

Disease cycle of SDS

The SDS pathogen can survive in soil, crop residue and inside of soybean cyst nematodes during winter in the form of macroconidia or chlamydospores (McLean and Lawrence 1995; Roy et al. 1997; Navi and Yang 2016). In the spring, with the rising soil temperatures, the chlamydospores or macroconidia near the plant root will be stimulated to germinate and infect the plant roots. During the process of *F. virguliforme* colonizing the soybean root, phytotoxins are produced and translocated to the stem and leaves. Foliar symptoms generally start to express around the reproductive stage, including chlorosis and necrosis. Flowing water and cultivation practices can help spread this disease over long distances.

Figure 1.1 Disease cycle of sudden death syndrome (Westphal et al. 2008)



Phytotoxins related to SDS

SDS disease development starts with *F. virguliforme* colonization in the xylem tissue of roots under suitable conditions, which provides a passage for phytotoxins to move upwards and plays an essential role in the expression of foliar symptoms. To date, four phytotoxins have been identified to be associated with the SDS foliar symptoms. Radicicol was the first phytotoxin to be reported to induce interveinal necrosis and marginal curling on soybean (Baker and Nemec, 1994). Jin et al. (1996) identified a 17-kDa effector which can cause chlorosis and necrosis on soybean cotyledons and leaves detachment. In 2011, FvTox1 was reported to be the major phytotoxin inducing chlorosis and chlorophyll content reduction of leaf disks (Brar et al. 2011) and its function was confirmed in transgenic plants (Brar and Bhattacharyya 2012). In 2016,

Chang et al. (2016) identified multiple phytotoxins produced by *F. virguliforme*, among which FvNIS1 induced the similar foliar symptoms as in the field.

Host response to *F. virguliforme* infection

Recently, a few studies were conducted to explore the molecular mechanisms underlying *F. virguliforme* infection. Metabolomics analysis conducted by Abeysekara et al. (2016) found that the plant immunity inducer pipecolic acid (Pip) and salicylic acid increased in xylem sap and leaves of *F. virguliforme*-infected soybean plants, suggesting Pip plays a major role in inducing host defense responses in above ground parts. Several differentially expressed proteins ,which were involved in the pathway of disease resistance, stress tolerance and metabolism, were identified during the proteomic analysis of *F. virguliforme*-infected soybean roots (Iqbal et al. 2016). Transcriptome analyses of *F. virguliforme* found that several infection-induced genes encoded the enzymes with oxidation-reduction properties and hydrolytic and catalytic activities expressed during late infection play a role in root necrosis (Sahu et al. 2017). *FvSNF1*, the sucrose non-fermenting protein kinase gene of *F. virguliforme*, was reported to regulate cell-wall-degrading enzyme expression, which can affect the virulence of the fungus on soybean (Islam et al. 2017).

SDS management

Fungicides have been used to suppress SDS epidemics, among which fluopyram showed promising results in reducing SDS (Wang 2014). Fluopyram seed treatment has been reported to reduce SDS disease severity, but it reduce the plant population as well (Kandel et al. 2016a). However, grain yield was not influenced by population reduction. A 2-year study involving 12 field experiments in five states found that fluopyram seed treatment and in-furrow application

reduced SDS severity and increased yield (Kandel et al. 2016b). In 2018, A meta-analysis for fluopyram efficacy involved 12 U.S. states and Ontario, Canada found that fluopyram contributed 35% of foliar severity reduction and 7.6% of yield increase (Kandel et al. 2018).

Many studies have been conducted to examine how environmental factors affect the SDS epidemics, including planting time, soil conditions, soil nutrition, crop debris, and microbe community.

Considering the cool soil temperature and high soil moisture in early spring, late planting was once recommended to manage SDS. However, inconsistent results were concluded in different studies. Wrather et al. (1995) found that in the non-tilled field, SDS symptom was less for late planting than early planting, but yield decreased as well. On the contrary, a 2-year study conducted in four states found no correlation between planting date and SDS severity, therefore they concluded that delaying planting did not avoid yield losses due to SDS (Kandel et al. 2016a). The effect of planting date on SDS development is usually complicated by the cultivar, seed treatments, and agronomic practice. Marburger et al. (2016) identified significant planting date × cultivar interactions associated with the SDS development and they concluded that early planting coupled with appropriate cultivar selection can maximize yield potential.

Crop rotations and plant residues in the field can affect the SDS infestation. Rupe (1997) and Xing et al. (2009) observed that the corn-soybean rotation system did not provide benefits for reducing SDS severity or lowering the *F. virguliforme* population density. Freed et al. (2017) found that soybean plants planted in corn and sorghum crop residues showed higher foliar disease. Navi et al. (2016) found that a clean corn harvest can lower the SDS risk because corn kernels left in the field could support the survival and colonization of *F. virguliforme*.

Soil related factors exert influence on the SDS epidemics as well. In general, root rot of SDS is favored by wet and cool conditions in the planting time. Meanwhile, high soil moisture and moderate temperatures can accelerate foliar symptoms during reproductive stages (Scherm and Yang 1996). Rupe et al. (1993) found that SDS severity was higher when the available soil P, Mg, and organic matter were higher. Although many soil variables did not show consistent association with SDS in the research conducted by Scherm et al. (1998), Sanogo et al. (2001) observed a 21% increase of SDS disease severity when soil pH was 7.7, compared with when soil PH was 5.5, and potassium chloride amendment reduced SDS severity. In addition, a soil components study including 45 soybean fields across three states (Srour et al. 2017) detected significant differences in bacterial and fungal community structure between SDS- diseased and healthy field. Biochar in the soil has not been found to show either systemic or indirect effects on the SDS root rot severity (Rogovska et al. 2017).

The effect of flooding and anaerobic conditions on SDS epidemics was studied and the result suggested that short-term flooding can increase SDS severity, while long-term flooding decreased SDS severity (Abdelsamad et al. 2017). The soybean defense genes and *F. virguliforme* virulence genes were also down-regulated under anaerobic conditions.

It is often observed that SDS co-occurs with the *Heterodera glycines*, Soybean Cyst Nematode (SCN), in the field. Xing and Westphal (2006) found that SDS severity and root necrosis was higher when SCN present in the field, compared to that without SCN presence. Westphal et al. (2014) identified the association between SDS foliar symptoms and the population density of *H. glycines* in the soil. On the other hand, Gao et al. (2006) found that the root infection by SCN did not affect *F. virguliforme* colonization in root. Marburger et al. (2014) detected a negative

association between the occurrence of SCN and SDS and concluded that SCN and SDS did not depend on each other for colonization in the field. In summary, SCN may increase the severity of SDS, but it is not required for SDS infestation. The co-occurrence may be due to similar favorable environmental factors.

Breeding for plant resistance is an important and most cost-effective approach to manage SDS. Brzostowski et al. (2014) at Kansas State University found that soybean genotypes with partial SDS resistance produced 36% higher yield than the SDS-susceptible genotypes in highly SDS-infested soils. So far, several thousand germplasm accessions have been screened and a limited number of potential resistance sources have been identified (Stephens et al. 1993; Hartman et al. 1997; Huang and Hartman 1998; Mueller et al. 2002). These resistant germplasms have been widely used in the genetic study and integrated into the breeding process. The identified SDS resistance associated molecular markers will aid marker-assisted breeding to enhance the SDS resistance. However, the polygenic and quantitative inheritance of SDS resistance poses a challenge to the breeding.

Since SDS infection is unpredictable and relies on several factors, integration of several management approaches, such as appropriate planting time, crop rotation, clean harvest, resistant cultivar, soil nutrition control, and fungicide treatment, is necessary and will result in promising SDS management.

SDS resistance phenotyping

Considering the root and foliar damage of SDS to soybean plants, different methods have been developed for SDS evaluation. The most commonly used method is foliar-symptoms based evaluation, which consists of three disease parameters: disease severity (DS), disease incidence

(DI), and disease index (DX). Disease severity and disease incidence are rated directly, while disease index is derived from disease severity and disease incidence (DX = DS × DI / 9) (Njiti et al. 1998). In the field, evaluation of foliar symptoms was conducted at R6 stage, when pods at one of the uppermost nodes with a completely expanded leaf contained full-size green seeds (Fehr et al. 1971). Disease severity was rated on a scale ranging from 0 to 9. The estimation criteria used are listed in Table 1.1.

Table 1.1 Estimation criteria of disease severity

Disease severity	Foliar symptom
DS=0	No evidence of sudden death syndrome
DS=1	1–10% of the leaf surface chlorotic or 1–5% necrotic
DS=2	11–20% of the leaf surface chlorotic or 6–10% necrotic
DS=3	21–40% of the leaf surface chlorotic or 11–20% necrotic
DS=4	41–60% of the leaf surface chlorotic or 21–40% necrotic
DS=5	> 60% chlorotic or > 40% necrotic
DS=6	up to 1/3 premature defoliation
DS=7	1/3 to 2/3 premature defoliation
DS=8	> 2/3 premature defoliation
DS=9	plant death before normal defoliation due to senescence

Disease incidence was estimated from 0 to 100% with increments of 5%. In contrast, foliar symptoms evaluation conducted in the greenhouse at two to six weeks after planting and *F. virguliforme* infested sand-cornmeal or sorghum is used to inoculate the soybean plants (Abdelmajid et al. 2012; Zhang et al. 2015; Luckew et al. 2017). Moreover, Swaminathan et al. (2016) developed a stem-cutting assay to screen for SDS resistance to phytotoxins, in which 21-day-old seedlings were cut and put into cell-free *F. virguliforme* culture filtrates. This method directly detected the soybean ability to defend the phytotoxin, without involvement of root resistance.

Compared to foliar symptoms evaluation, root evaluation is more complicated and labor-intensive. Several parameters have been used to estimate SDS root damage. Root rot severity

was rated based on the percentage of root area showing brown or black discoloration (Abdelmajid et al. 2012). Root infection severity was defined as the mean percentage of taproot slices with detectable *F. virguliforme* evidence on restrictive media (Meksem et al. 1999; Prabhu et al. 1999; Kazi et al. 2008a). Root lesion severity measures the severity of root lesion caused by *F. virguliforme*, ranging from 1 (no lesion) to 10 (most severe lesion). Root retention was measured by comparing the dry root weights of inoculated plants and uninoculated plants (Bao et al. 2015). Recently, several quantitative polymerase chain reaction (qPCR) assays have been developed to diagnose the causal pathogen and provided more accurate and less time-consuming evaluations. *F. virguliforme* qPCR assays have been developed in the past few years (Gao et al. 2004; Li et al. 2008; Mbofung et al. 2012; Westphal et al. 2014). In 2015, Kandel et al. (2015) conducted an experiment to compare detection efficacy of six qPCR assays developed by different institutes, and concluded that the assay developed by Wang et al. (2015), which targets the ribosomal DNA intergenic spacer (IGS) region, was most useful due to its high specificity and sensitivity.

Remote sensing has also been used for SDS investigation. Herrman et al. (2018) measured the canopy reflectance to detect the SDS symptom incurred by *F. virguliforme*, and resulted in high accuracy. It showed the potential of using canopy and leaf spectral data for the SDS monitoring, evaluation, and management. It is expected that accurate pathogen detection method and efficient disease symptom evaluation would greatly improve the breeding progress towards to the SDS resistance.

Genetic study of SDS resistance

The most commonly used approach for genetic study related to SDS resistance is bi-parental population-based QTL mapping. It generally starts with germplasm screening, then two germplasm with distinct difference for the target trait are crossed to develop the mapping population. Two parental lines are screened by molecular markers to detect the polymorphisms. Polymorphic markers are then used to genotype the mapping population and construct the genetic linkage map subsequently. Lastly, the genotypic and phenotypic data together with genetic map will be combined to identify the genomic loci underlying the target trait.

In 1990s, over 800 soybean plant introductions (PIs) from China were screened for SDS resistance in greenhouse and growth chamber, and no cultivar with complete resistance was identified (Hartman et al. 1997). However, a few lines that showed less SDS severity or no significant difference than the resistance check were used in the genetic study and breeding, including Forrest, Hartwig, Ripley etc. In 2002, over 6000 soybean PIs were screened for SDS resistance in the greenhouse and less than one percent of germplasm show insignificant difference from the resistant check (Mueller et al. 2002).

Genetic linkage map construction is an important step of bi-parental population-based QTL mapping. In 1990, Keim et al. (1990) constructed the first genetic linkage map of soybean using an F2 segregating population with 150 Restriction Fragment Length Polymorphisms (RFLPs) and resulted in 26 linkage groups. In 1999, Cregan et al. (1999) combined the linkage map from three populations with 606 Simple Sequence Repeat (SSR) markers into the first version of integrated soybean genetic linkage map, which consists of 20 homologous linkage groups, corresponding to the 20 pairs of soybean chromosomes. Later, Song et al. (2004) constructed the

second version of integrated soybean genetic linkage map with five populations which contained over 1800 markers. In 2010, Hyten et al. (2010) added 2500 additional Single Nucleotide Polymorphism (SNP) markers to the soybean integrated linkage map. Whole genome sequence of soybean was publicized in 2010 (Schmutz et al. 2010) and integrated with physical and high-density genetic linkage map. Recently, Song et al. (2016) constructed two high density linkage maps to include more molecular markers and to improve the reference genome assembly. The improvement of molecular markers development and integrated genetic linkage map, as well as accurate genome sequence information will significantly accelerate the discovery of QTLs and gene cloning.

To date, a total of 88 SDS resistance related quantitative trait loci (QTLs) detected in bi-parental populations have been documented on Soybase (www.soybase.org). The population types employed in these studies include RIL and near-isogenic line (NIL) populations, and SDS resistance QTLs were mapped on 19 of 20 chromosomes, except Chromosome 12 (Linkage Group H). The details of reported QTL were summarized in Table 1.2 and shown in Figure 1.2. The resistance sources used in these studies included Forrest, Hartwig, Nior 1, Ripley, Pyramid, PI438489B, MD96-5722, LS94-3207, and LS98-0582. In 2018, Chang et al. (2018) summarized all the QTLs reported until 2017 and proposed a new nomenclature with *Rfv* as prefix for those QTL identified by three or more studies. The evaluation methods involved in these studies included foliar symptom evaluation, culture filtrates screening, root rot estimation, and selective culture of root pathogens.

Figure 1.2 Genetic location of recorded SDS resistance QTLs on the soybean genetic linkage map. Note: chromosome number/genetic linkage group

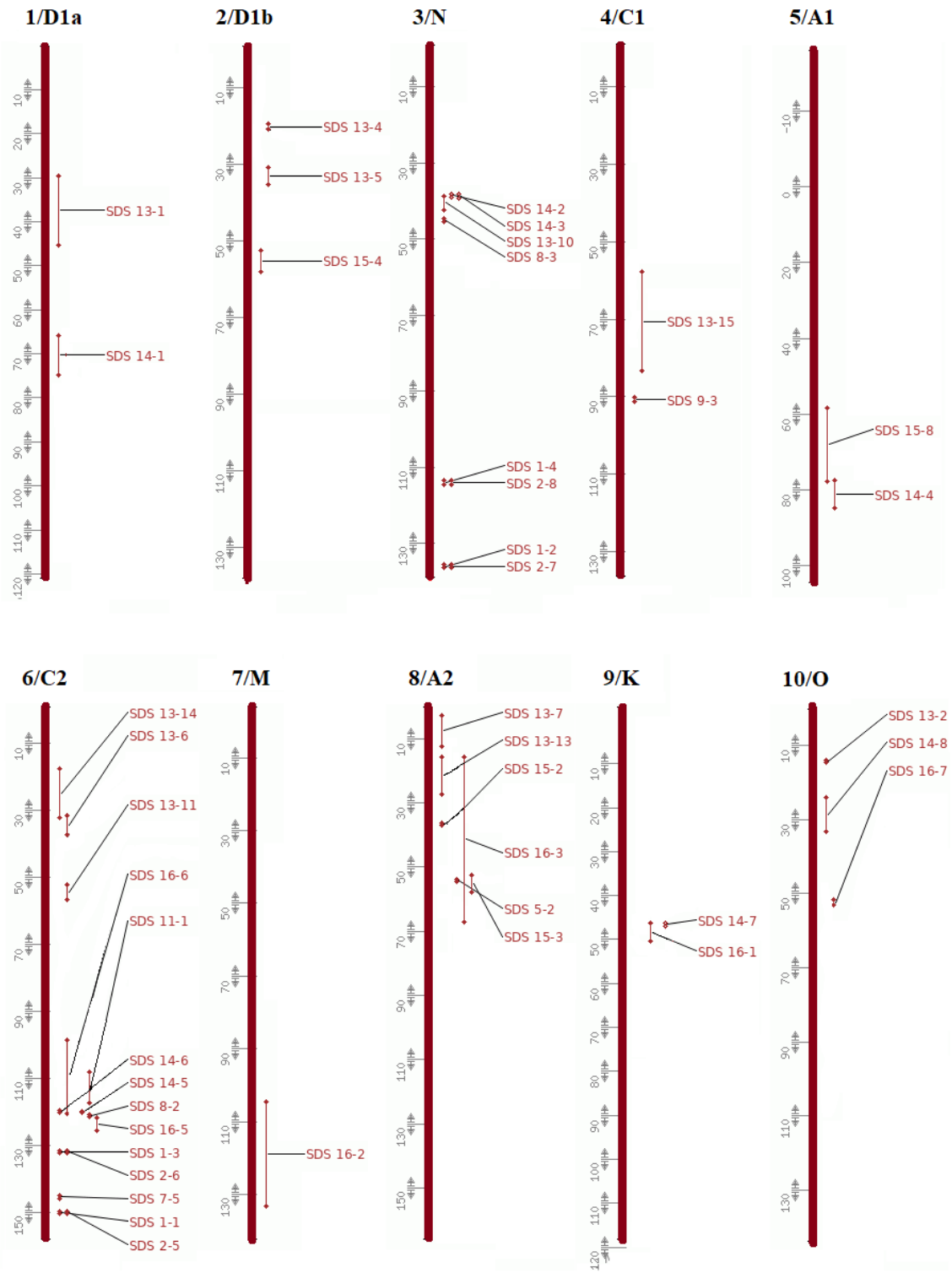


Figure 1.2 (cont'd)

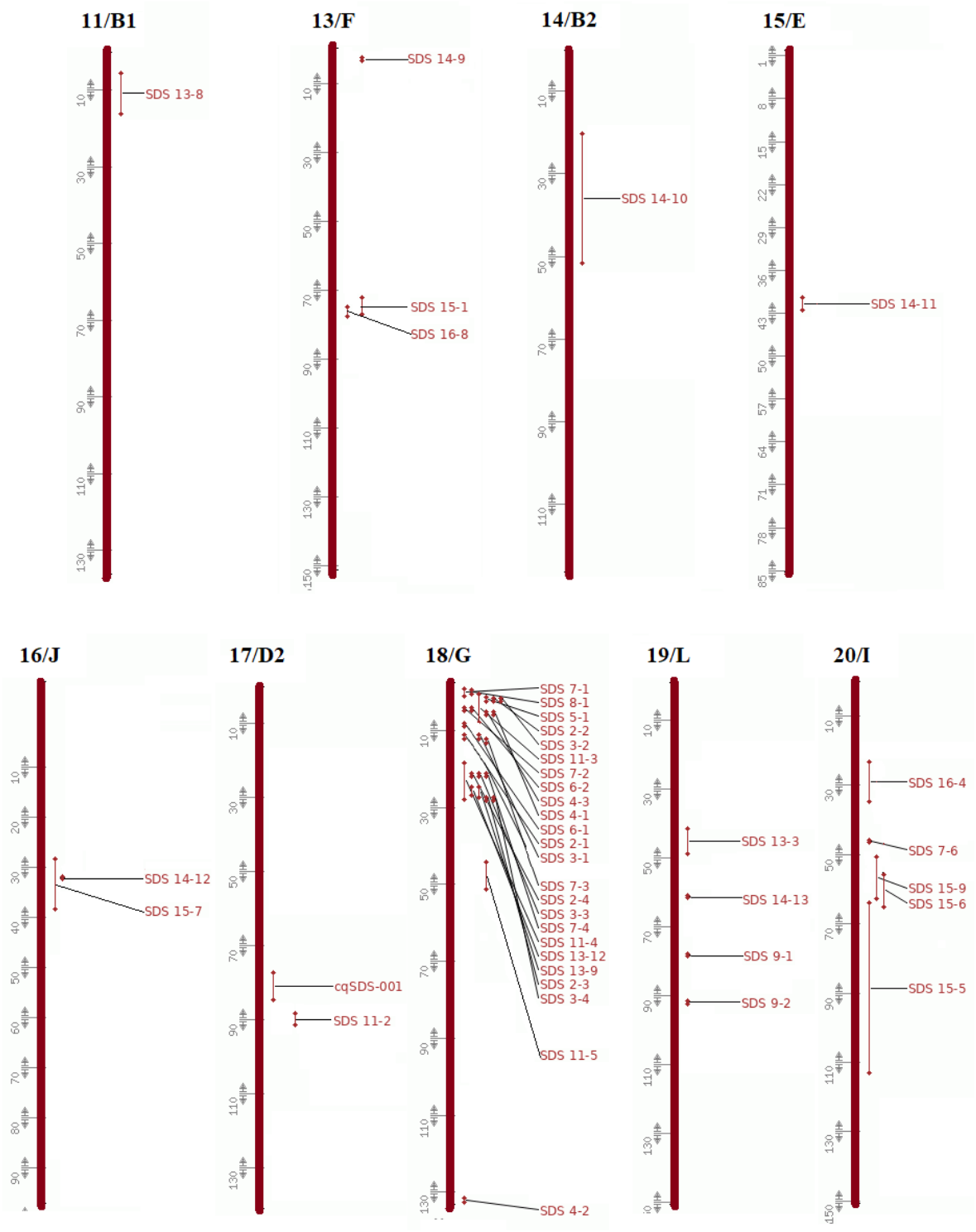


Table 1.2 Recorded SDS resistance QTLs on Soybase

References	Parents		Pop[c].	No. of Markers	QTL[d]	LG[e]	CHR[f]	Position(cM)	
	S[a]	R[b]						Start	End
(Hnetkovsky et al. 1996)	Essex	Forrest	F5:7RIL, F5:11 RIL	**70**	SDS1-1	C2	6	149.00	151.00
					SDS1-2	N	3	135.00	137.00
					SDS1-3	C2	6	131.00	133.00
					SDS1-4	N	3	113.00	115.00
(Chang et al. 1996)	Essex	Forrest	F5:11 RIL	**111**	SDS2-1	G	18	10.60	12.60
					SDS2-2	G	18	1.00	3.00
					SDS2-3	G	18	26.70	28.70
					SDS2-4	G	18	20.60	22.60
					SDS2-5	C2	6	149.00	151.00
					SDS2-6	C2	6	131.00	133.00
					SDS2-7	N	3	135.00	137.00
					SDS2-8	N	3	113.00	115.00
(Chang et al. 1996)	Essex	Forrest	F5:11 RIL	111	SDS3-1	G	18	10.60	12.60
					SDS3-2	G	18	1.00	3.00
					SDS3-3	G	18	20.60	22.60
					SDS3-4	G	18	26.70	28.70
(Njiti et al. 1998)	Essex	Forrest	F5:13 RIL		SDS4-1	G	18	4.60	6.60
					SDS4-2	G	18	131.00	133.00
					SDS4-3	G	18	4.60	6.60
(Prabhu et al. 1999)	Flyer	Hartwig	F5:6 RIL		SDS5-1	G	18	0.84	2.84
					SDS5-2	A2	8	53.20	55.20
(Meksem et al. 1999)	Essex	Forrest	F5:9:13 RIL		SDS6-1	G	18	7.56	9.56
					SDS6-2	G	18	3.53	5.53
(Iqbal et al. 2001)	Essex	Forrest	RIL,	400	SDS7-1	G	18	-1.50	1.50
					SDS7-2	G	18	3.53	5.53
					SDS7-3	G	18	11.74	13.74
					SDS7-4	G	18	20.60	22.60
					SDS7-5	C2	6	144.48	146.48
					SDS7-6	I	20	45.22	47.22

Table 1.2 (cont'd)

(Njiti et al. 2002)	Douglas	Pyramid	F6:10 RIL	112	SDS8-1	G	18	-1.00	1.00
					SDS8-2	C2	6	120.27	122.27
					SDS8-3	N	3	44.14	46.14
(Njiti and Lightfoot 2006)	Minsoy	Nior 1	F7:14	247	SDS9-1	L	19	77.23	79.23
					SDS9-2	L	19	91.00	93.00
					SDS9-3	C1	4	89.72	91.72
(De Farias Neto et al. 2007)	Spencer	Ripley	F5:8 NIL	113	cqSDS-001	D2	17	76.69	85.15
(Kazi et al. 2008a)	Flyer	Hartwig	F5 RIL	144	SDS11-1	C2	6	107.58	117.87
					SDS11-2	D2	17	87.66	92.12
					SDS11-3	G	18	0.00	8.42
					SDS11-4	G	18	17.85	28.35
					SDS11-5	G	18	43.77	51.68
(Abdelmajid et al. 2012)	Hamilton	PI438489B	F6:13 RIL	679	SDS13-1	D1a	1	29.15	45.75
					SDS13-2	O	10	13.28	15.06
					SDS13-3	L	19	41.00	49.40
					SDS13-4	D1b	2	18.75	21.44
					SDS13-5	D1b	2	30.23	35.75
					SDS13-6	C2	6	30.79	38.04
					SDS13-7	A2	8	2.00	13.00
					SDS13-8	B1	11	5.07	16.70
					SDS13-9	G	18	24.09	28.03
					SDS13-10	N	3	38.20	42.85
					SDS13-11	C2	6	51.50	57.30
					SDS13-12	G	18	24.09	27.48
					SDS13-13	A2	8	14.99	27.90
					SDS13-14	C2	6	17.10	32.74
					SDS13-15	C1	4	57.30	83.90
(Anderson et al. 2015)	Spencer	MD96-5722	F5:7 RIL	5376	SDS14-1	D1a	1	65.28	75.29
					SDS14-2	N	3	38.5	38.67
					SDS14-3	N	3	38.63	38.67

Table 1.2 (cont'd)

					SDS14-4	A1	5	77.09	85.57
					SDS14-5	C2	6	119.20	120.79
					SDS14-6	C2	6	118.95	120.79
					SDS14-7	K	9	46.38	46.67
					SDS14-8	O	10	23.36	33.69
					SDS14-9	F	13	3.04	3.07
					SDS14-10	B2	14	19.87	52.03
					SDS14-11	E	15	40.11	42.76
					SDS14-12	J	16	31.57	32.77
					SDS14-13	L	19	60.27	62.27
(Swaminathan et al. 2016)	A95-684043	LS94-3207	F7 RIL	1536	SDS15-1	F	13	72.97	78.08
					SDS15-2	A2	8	14.99	51.86
					SDS15-3	A2	8	51.86	58.43
					SDS15-4	D1b	2	51.86	58.43
					SDS15-5	I	20	63.33	113.76
					SDS15-6	I	20	55.09	65.62
					SDS15-7	J	16	27.99	38.70
					SDS15-8	A1	5	57.79	78.44
					SDS15-9	I	20	50.11	63.33
(Swaminathan et al. 2016)	A95-684043	LS98-0582	F7 RIL	1536	SDS16-1	K	9	45.74	50.93
					SDS16-2	M	7	103.98	133.83
					SDS16-3	A2	8	14.99	67.86
					SDS16-4	I	20	22.84	35.34
					SDS16-5	C2	6	121.26	126.23
					SDS16-6	C2	6	97.83	121.26
					SDS16-7	O	10	51.00	53.66
					SDS16-8	F	13	74.12	78.05

[a] S: susceptible parent
[b] R: resistant parent
[c] Pop: population type and generation
[d] QTL: quantitative trait locus

[e] LG: linkage group
[f] CHR: chromosome

Genome-wide association study (GWAS) is another important approach to study SDS resistance in soybean. Different from the bi-parental QTL mapping, GWAS does not require population development. It takes advantage of historical recombination in the germplasm collection. The first GWAS study for SDS resistance based on the foliar symptoms in the field was published in 2014 using about 700 accessions (Wen et al. 2014). Among the total 20 loci identified in this study, seven of them confirmed the previously reported QTLs and 13 were reported as novel. In 2015, Bao et al. (2015) utilized a GWAS approach with the investigation for both root and foliar symptoms to identify loci underlying SDS resistance in early maturing soybean germplasm. Two new loci were detected on Chromosomes 3 and 18 and another two loci confirmed the previously reported QTLs. At the same time, genomic selection was conducted to predict the SDS resistance and the accuracy was 0.64 for the root lesion severity and much lower for the other three traits. In the same year, Zhang et al. (2015) conducted another GWAS to study the genetic architecture of soybean SDS resistance using 214 germplasm accessions. Besides several loci identified, 12 epistatic interactions were detected, and the proportion of phenotypic variance explained by the identified loci was greatly improved when taking epistatic interaction into account.

Aims of dissertation research

Considering that SDS resistant sources are limited, a thorough understanding of underlying genetic control is crucial, and can be achieved by integrating these resistance sources in different genetic background. Moreover, the complicated pathogenesis of *F. virguliforme* infection necessitates the exploration of the relationship between root and foliar damage as well as the genetic control underlying root and foliar resistance. Therefore, in this dissertation, two bi-parental populations were evaluated in SDS-infected field to study the genetic architecture of SDS resistance. In the

population developed from GD2422 × LD01-5907, the objectives were to: 1) detect the loci associated with field resistance to SDS; 2) study the effect of epistatic interaction on SDS resistance; 3) analyze the inheritance pattern of the identified loci. In the population derived from U01-390489 × E07080, the objectives were to: 1) dissect the correlations among root and foliar damage; 2) analyze the correlation of root damage vs yield, as well as foliar damage vs yield; 3) investigate the genetic control underlying root and foliar resistance. The discoveries from this research will aid in marker-assisted breeding and guide breeders to use appropriate selection methodologies in the SDS resistance breeding.

REFERENCES

REFERENCES

Abdelmajid KM, Meksem K, Wood AJ, Lightfoot DA (2007) Loci underlying SDS and SCN resistance mapped in the Essex by Forrest soybean recombinant inbred lines. Rev Biol Biotechnol 6:2–10.

Abdelmajid KM, Ramos L, Leandro L, et al. (2012) The " PI 438489B " by " Hamilton " SNP-Based Genetic Linkage Map of Soybean [*Glycine max* (L .) Merr .] Identified quantitative trait loci that underlie seedling SDS resistance. J Plant Genome Sci 1:18–30. doi: 10.5147/jpgs.2012.0053

Abdelsamad NA, Baumbach J, Bhattacharyya MK, Leandro LF (2017) Soybean sudden death syndrome caused by *Fusarium virguliforme* is impaired by prolonged flooding and anaerobic conditions. Plant Dis 101:712–719. doi: 10.1094/PDIS-04-16-0534-RE

Abeysekara NS, Swaminathan S, Desai N, et al. (2016) The plant immunity inducer pipecolic acid accumulates in the xylem sap and leaves of soybean seedlings following *Fusarium virguliforme* infection. Plant Sci 243:105–114. doi: 10.1016/j.plantsci.2015.11.008

Ahn SJ, Costa J, Emanuel JR (1996) PicoGreen quantitation of DNA: Effective evaluation of samples pre- or post-PCR. Nucleic Acids Res 24:2623–2625. doi: 10.1093/nar/24.13.2623

Anand SC (1992) Registration of "Hartwig" soybean. Crop Sci 32:1069–1070.

Anderson J, Akond M, Kassem MA, et al. (2015) Quantitative trait loci underlying resistance to sudden death syndrome (SDS) in MD96-5722 by "Spencer" recombinant inbred line population of soybean. 3 Biotech 5:203–210. doi: 10.1007/s13205-014-0211-3

Aoki T, O'Donnell K, Scandiani MM (2005) Sudden death syndrome of soybean in South America is caused by four species of *Fusarium*: *Fusarium brasiliense* sp. nov., *F. cuneirostrum* sp. nov., *F. tucumaniae*, and *F. virguliforme*. Mycoscience 46:162–183. doi: 10.1007/s10267-005-0235-y

Aoki T, O 'donnell K, Homma Y, Lattanzi AR (2003) Sudden-death syndrome of sobyean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex - *F.virguliforme* in North America and *F.tucumaniae* in South America. Mycologia 95:660–684. doi: 10.2307/3761942

Aoki T, Scandiani MM, O'Donnell K (2012) Phenotypic, molecular phylogenetic, and pathogenetic characterization of *Fusarium crassistipitatum* sp. nov., a novel soybean sudden death syndrome pathogen from Argentina and Brazil. Mycoscience 53:167–186. doi: 10.1007/s10267-011-0150-3

Baker, R. A., and Nemec, S. 1994. Soybean sudden death syndrome: Isolation and identification of a new phytotoxin from cultures of the causal agent, *Fusarium solani*. Phytopathology

84:1144.

Bao Y, Kurle JE, Anderson G, Young ND (2015) Association mapping and genomic prediction for resistance to sudden death syndrome in early maturing soybean germplasm. Mol Breed 35:1–14. doi: 10.1007/s11032-015-0324-3

Brar HK, Swaminathan S, Bhattacharyya MK (2011) The *Fusarium virguliforme* toxin FvTox1 causes foliar sudden death syndrome-like symptoms in soybean. Mol Plant-Microbe Interact 24:1179–1188. doi: 10.1094/MPMI-12-10-0285

Brar HK, Bhattacharyya (2012) Expression of a single-chain variable-fragment antibody against a *Fusarium virguliforme* toxin peptide enhances tolerance to sudden death syndrome in transgenic soybean plants. Molecular Plant-Microbe Interactions 25: 817–824. Doi: 10.1094/MPMI-12-11-0317

Brucker E, Carlson S, Wright E, et al. (2005) Rhg1 alleles from soybean PI 437654 and PI 88788 respond differentially to isolates of *Heterodera glycines* in the greenhouse. Theor Appl Genet 111:44–49. doi: 10.1007/s00122-005-1970-3

Brzostowski LF, Schapaugh WT, Rzodkiewicz PA, et al. (2014) Effect of host resistance to *Fusarium virguliforme* and *Heterodera glycines* on sudden death syndrome disease severity and soybean yield. Plant Heal Prog 15:1–8. doi: 10.1094/PHP-RS-13-0100

Chang H-X, Domier LL, Radwan O, et al. (2016) Identification of multiple phytotoxins produced by *Fusarium virguliforme* including a phytotoxic effector (FvNIS1) associated with sudden death syndrome foliar symptoms. Mol Plant-Microbe Interact 29:96–108. doi: 10.1094/MPMI-09-15-0219-R

Chang HX, Roth MG, Wang D, et al. (2018) Integration of sudden death syndrome resistance loci in the soybean genome. Theor Appl Genet 131:757–773. doi: 10.1007/s00122-018-3063-0

Chang SJC, Doubler TW, Kilo V, et al. (1996) Two additional loci underlying durable field resistance to soybean sudden death syndrome (SDS). Crop Sci 36:1684–1688. doi: 10.2135/cropsci1996.0011183X003600060044x

Chehri K, Salleh B, Zakaria L (2014) *Fusarium virguliforme*, a soybean sudden death syndrome fungus in Malaysian soil. Australas Plant Dis Notes 9:128. doi: 10.1007/s13314-014-0128-z

Chong SK, Hildebrand KK, Luo Y, et al. (2005) Mapping soybean sudden death syndrome as related to yield and soil/site properties. Soil Tillage Res 84:101–107. doi: 10.1016/j.still.2004.09.001

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971. doi: 10.1534/genetics.107.080101

Cook DE, Lee TG, Guo X, et al. (2012) Copy number variation of multiple genes at Rhg1 mediates nematode resistance in soybean. Science 338:1206–9. doi:

10.1126/science.1228746

Cregan PB, Jarvik T, Brush AL, et al. (1999) An integrated genetic linkage map of the soybean genome. Crop Sci 39:1464–1490.

De Farias Neto AL, Hashmi R, Schmidt M, et al. (2007) Mapping and confirmation of a new sudden death syndrome resistance QTL on linkage group D2 from the soybean genotypes PI 567374 and "Ripley." Mol Breed 20:53–62. doi: 10.1007/s11032-006-9072-8

Fehr WR, Caviness CE, Burmood DT, S. PJ (1971) Stage of development descriptino for soybeans, Glycine max (L.) Merrill. Crop Sci 11:929–931. doi: 10.2135/cropsci1971.0011183X001100060051x

Freed GM, Floyd CM, Malvick DK (2017) Effects of pathogen population levels and crop-derived nutrients on development of soybean sudden death syndrome and growth of *Fusarium virguliforme*. Plant Dis 101:434–441. doi: 10.1094/PDIS-04-16-0524-RE

Gao, X., Jackson, T.A., Lambert, K.N., Li, S., Hartman, G.L.,Niblack TL (2004) Detection and quantification of *Fusarium solani* f.sp. *glycines* in soybean roots with real-time quantitative polymerase chain reaction. Plant Dis 88:1372–1380.

Gao X, Jackson T a, Hartman GL, Niblack TL (2006) Interactions between the soybean cyst nematode and *Fusarium solani* f. sp. *glycines* based on greenhouse factorial experiments. Phytopathology 96:1409–15. doi: 10.1094/PHYTO-96-1409

Giammaria SL, Rupe JC, Chen P, Mozzoni LA (2014) Genetic analysis of resistance to sudden death syndrome caused by *Fusarium virguliforme* in Hartwig soybean. J Crop Improv 28:758–771. doi: 10.1080/15427528.2014.933754

Graham PH, Vance CP (2014) Update on legume utilization legumes : Importance and constraints to greater use. Plant Physiol 131:872–877. doi: 10.1104/pp.017004.872

Grant D, Nelson RT, Cannon SB, Shoemaker RC (2010) SoyBase, the USDA-ARS soybean genetics and genomics database. Nucleic Acids Res 38:843–846. doi: 10.1093/nar/gkp798

Hartman, G.L., G.R. Noel, and L.E. Gray. 1995. Occurrence of soybean sudden death syndrome in east-central Illinois and associated yield losses. Plant Disease 79: 314-318.

Hartman G, Huang Y, Nelson R, Noel G (1997) Germplasm evaluation of *Glycine max* for resistance to *Fusarium solani*, the causal organism of sudden death syndrome. Plant Dis 81:515–518. doi: 10.1094/PDIS.1997.81.5.515

Hartman, G. L., Sinclair, J.B., and Rupe, J.C, ed. 1999 Compedium of soybean disease, 4th ed. American Phytopathological Society, St. Paul, Minnesota.

Hartman GL, Chang HX, Leandro LF (2015) Research advances and management of soybean sudden death syndrome. Crop Prot 73:60–66. doi: 10.1016/j.cropro.2015.01.017

Herrmann I, Vosberg SK, Ravindran P, et al. (2018) Leaf and canopy level detection of *Fusarium virguliforme* (sudden death syndrome) in soybean. Remote Sens 10:1–19. doi: 10.3390/rs10030426

Hill A., Kurki A., Morris M., (2006), Biodiesel: The sustainability dimensions, ATTRA Publication. Butte, MT: National Center for Appropriate Technology. Pages 4-5

Hnetkovsky N, Chang SJC, Doubler TW, et al. (1996) Genetic mapping of loci underlying field resistance to soybean sudden death syndrome (SDS). Crop Sci 36:393–400. doi: 10.2135/cropsci1996.0011183X003600020030x

Holland J (2001) Epistasis and plant breeding. Plant Breed Rev 21: 27-92

Huang YH, Hartman GL (1998) Reaction of selected soybean genotypes to isolates of *Fusarium solani* f. sp. *glycines* and their culture filtrates. Plant Dis 82:999–1002. doi: 10.1094/PDIS.1998.82.9.999

Hughes TJ, O'Donnell K, Sink S, et al. (2014) Genetic architecture and evolution of the mating type locus in fusaria that cause soybean sudden death syndrome and bean root rot. Mycologia 106:686–697. doi: 10.3852/13-318

Hymowitz T. (2008) The History of the Soybean. In: Johnson, L.A., White, P.J. and Galloway, R. (eds) Soybeans: chemistry, production, processing, and utilization. AOCS Press, Urbana, IL, USA, pp. 1–31.

Hyten DL, Choi IY, Song Q, et al. (2010) A high density integrated genetic linkage map of soybean and the development of a 1536 universal soy linkage panel for quantitative trait locus mapping. Crop Sci 50:960–968. doi: 10.2135/cropsci2009.06.0360

Iqbal MJ, Majeed M, Humayun M, et al. (2016) Proteomic profiling and the predicted interactome of host proteins in compatible and incompatible interactions between soybean and *Fusarium virguliforme*. Appl Biochem Biotechnol 180:1657–1674. doi: 10.1007/s12010-016-2194-5

Iqbal MJ, Meksem K, Njiti VN, et al. (2001) Microsatellite markers identify three additional quantitative trait loci for resistance to soybean sudden-death syndrome (SDS) in Essex x Forrest RILs. Theor Appl Genet 102:187–192. doi: 10.1007/s001220051634

Islam KT, Bond JP, Fakhoury AM (2017) FvSNF1, the sucrose non-fermenting protein kinase gene of *Fusarium virguliforme*, is required for cell-wall-degrading enzymes expression and sudden death syndrome development in soybean. Curr Genet 63:723–738. doi: 10.1007/s00294-017-0676-9

J.C.Rupe (1989) Frequency and pathogenicity of *Fusarium solani* recovered from soybeans with sudden death syndrome.PDF. Plant Dis 73:581–584. doi: 10.1094/PD-73-0581

JIN H, HARTMAN GL, NICKELL CD, WIDHOLM JM (1996) Characterization and purification of a phytotoxin produced by *Fusarium solani*, the causal agent of soybean

sudden death syndrome. Phytopathology 86:277–282.

Kandel YR, Haudenshield JS, Srour AY, et al. (2015) Multilaboratory comparison of quantitative PCR assays for detection and quantification of *Fusarium virguliforme* from soybean roots and soil. Phytopathology 105:1601–1611. doi: 10.1094/PHYTO-04-15-0096-R

Kandel YR, Wise KA, Bradley CA, et al. (2016a) Effect of planting date, seed treatment, and cultivar on plant population, sudden death syndrome, and yield of soybean. Plant Dis 100:1735–1743. doi: 10.1094/PDIS-02-16-0146-RE

Kandel YR, Wise KA, Bradley CA, et al. (2016b) Fungicide and cultivar effects on sudden death syndrome and yield of soybean. Plant Dis 100:1339–1350. doi: 10.1094/PDIS-11-15-1263-RE

Kazi S, Shultz J, Afzal J, et al. (2008a) Separate loci underlie resistance to root infection and leaf scorch during soybean sudden death syndrome. Theor Appl Genet 116:967–977. doi: 10.1007/s00122-008-0728-0

Kazi S, Shultz J, Afzal J, et al. (2008b) Separate loci underlie resistance to root infection and leaf scorch during soybean sudden death syndrome. Theor Appl Genet 116:967–977. doi: 10.1007/s00122-008-0728-0

Keim P, Diers BW, Olson TC, Shoemaker RC (1990) RFLP mapping in soybean: Association between marker loci and variation in quantitative traits. Genetics 126:735–742.

Kim M, Hyten DL, Bent AF, Diers BW (2010) Fine mapping of the SCN resistance locus rhg1-b from PI 88788. Plant Genome J 3:81. doi: 10.3835/plantgenome2010.02.0001

Kisha TJ, Sneller CH, Diers BW (1997) Relationship between genetic distance among parents and genetic variance in populations of soybean. Crop Sci 37:1317–1325. doi: 10.2135/cropsci1997.0011183X003700040048x

Li D, Sun M, Han Y, et al. (2010) Identification of QTL underlying soluble pigment content in soybean stems related to resistance to soybean white mold (*Sclerotinia sclerotiorum*). Euphytica 172:49–57. doi: 10.1007/s10681-009-0036-z

Li S, Hartman GL, Domier LL, Boykin D (2008) Quantification of *Fusarium solani* f. sp. *glycines* isolates in soybean roots by colony-forming unit assays and real-time quantitative PCR. Theor Appl Genet 117:343–352. doi: 10.1007/s00122-008-0779-2

Lightfoot DA (2015) Two decades of molecular marker-assisted breeding for resistance to soybean sudden death syndrome. Crop Sci 55:1460–1484. doi: 10.2135/cropsci2014.10.0721

Liu S, Kandoth PK, Lakhssassi N, et al. (2017) The soybean GmSNAP18 gene underlies two types of resistance to soybean cyst nematode. Nat Commun 8:1–11. doi: 10.1038/ncomms14822

Liu S, Kandoth PK, Warren SD, et al. (2012) A soybean cyst nematode resistance gene points to a new mechanism of plant resistance to pathogens. Nature 492:256–260. doi: 10.1038/nature11651

Luckew AS, Leandro LF, Bhattacharyya MK, et al. (2013) Usefulness of 10 genomic regions in soybean associated with sudden death syndrome resistance. Theor Appl Genet 126:2391–2403. doi: 10.1007/s00122-013-2143-4

Luckew AS, Swaminathan S, Leandro LF, et al. (2017) "MN1606SP" by "Spencer" filial soybean population reveals novel quantitative trait loci and interactions among loci conditioning SDS resistance. Theor Appl Genet 1–11. doi: 10.1007/s00122-017-2947-8

Marburger DA, Smith DL, Conley SP (2016) Revisiting planting date and cultivar effects on soybean sudden death syndrome development and yield loss. doi: 10.1094/PDIS-12-15-1411-RE

Marburger D, Conley S, Esker P, et al. (2014) Relationship between *Fusarium virguliforme* and *Heterodera glycines* in commercial soybean fields in Wisconsin. Plant Heal Prog 15:11–18. doi: 10.1094/PHP-RS-13-0107

Mbofung GYC, Harrington TC, Steimel JT, et al. (2012) Genetic structure and variation in aggressiveness in *Fusarium virguliforme* in the Midwest United States. Can J Plant Pathol 34:83–97. doi: 10.1080/07060661.2012.664564

McLean KS, Lawrence GW (1995) Development of *Heterodera glycines* as affected by *Fusarium solani*, the causal agent of Sudden Death Syndrome of soybean. J Nematol 27:70–7.

Meksem K, Doubler TW, Chancharoenchai K, et al. (1999) Clustering among loci underlying soybean resistance to Fusarium solani, SDS and SCN in near-isogenic lines. Theor Appl Genet 99:1131–1142. doi: 10.1007/s001220051317

Meksem K, Pantazopoulos P, Njiti VN, et al. (2001) "Forrest" resistance to the soybean cyst nematode is bigenic: Saturation mapping of the Rhg1 and Rhg4 loci. Theor Appl Genet 103:710–717. doi: 10.1007/s001220100597

Messina MJ (1999) Legumes and soybeans: Overview of their nutritional profiles and health effects. Am J Clin Nutr. doi: 10.1093/ajcn/70.3.439s

Mueller DS, Hartman GL, Nelson RL, Pedersen WL (2002) Evaluation of *Glycine max* germplasm for resistance to *Fusarium solani* f. sp. *glycines*. Plant Dis 86:741–746. doi: 10.1094/PDIS.2002.86.7.741

Navi, S. S., and Yang, X. B. 2008. Foliar symptom expression in association with early infection and xylem colonization by *Fusarium virguliforme* (formerly *F. solani* f. sp. *glycines*), the causal agent of soybean sudden death syndrome. Online. Plant Health Progress doi:10.1094/PHP-2008-0222-01-RS

Navi S (2016) Sudden death syndrome - a growing threat of losses in soybeans. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour. doi: 10.1079/PAVSNNR201611039

Navi SS, Yang XB (2016) Impact of crop residue and corn-soybean rotation on the survival of *Fusarium virguliforme* a causal agent of sudden death syndrome of soybean. J Plant Pathol Microbiol 7:1–7. doi: 10.4172/2157-7471.1000330

Njiti VN, Doubler TW, Suttner RJ, et al. (1998) Resistance to soybean sudden death syndrome and root colonization by *Fusarium solani* f. sp. *glycines* in near-isogeneic lines. Crop Sci 38:472–477.

Njiti VN, Lightfoot DA (2006) Genetic analysis infers *Dt* loci underlie resistance to *Fusarium solani* f. sp *glycines* in indeterminate soybeans. Can J Plant Sci 86:83–90. doi: 10.4141/P05-046

Njiti VN, Meksem K, Iqbal MJ, et al. (2002) Common loci underlie field resistance to soybean sudden death syndrome in Forrest, Pyramid, Essex, and Douglas. Theor Appl Genet 104:294–300. doi: 10.1007/s001220100682

Nyquist W, Baker R (1991) Estimation of heritability and prediction of selection response in plant populations. Crit Rev Plant Sci 10:235–322.

Ortiz-Ribbing LM, Eastburn DM (2004) Soybean root systems and sudden death syndrome severity: Taproot and sateral root infection. Plant Dis 88:1011–1016. doi: 10.1094/PDIS.2004.88.9.1011

Prabhu, R. R, Njiti, et al. (1999) Selecting soybean cultivars for dual resistance to soybean cyst nematode and sudden death syndrome using two DNA markers. Crop Sci 39:982–987.

Pudake RN, Swaminathan S, Sahu BB, et al. (2013) Investigation of the *Fusarium virguliforme* fvtox1 mutants revealed that the FvTox1 toxin is involved in foliar sudden death syndrome development in soybean. Curr Genet 59:107–117. doi: 10.1007/s00294-013-0392-z

Rogovska N, Laird D, Leandro L, Aller D (2017) Biochar effect on severity of soybean root disease caused by *Fusarium virguliforme*. Plant Soil 413:111–126. doi: 10.1007/s11104-016-3086-8

Roy KW, State M, Rupe JC, et al. (1997) Sudden death syndrome of soybean. Plant Dis 81:1100–1111.

Rupe JC, Correll JC, Guerber JC, et al. (2001) Differentiation of the sudden death syndrome pathogen of soybean, *Fusarium solani* f.sp *glycines*, from other isolates of F-solani based on cultural morphology, pathogenicity, and mitochondrial DNA restriction fragment length polymorphisms. Can J Bot Can Bot 79:829–835. doi: 10.1139/cjb-79-7-829

Rupe JC, Robbins RT, Becton CM, et al. (1999) Vertical and temporal distribution of *Fusarium solani* and *Heterodera glycines* in fields with sudden death syndrome of soybean. Soil biology and biochemistry 31: 245-251

Rupe JC, Robbins RT, Gbur EE (1997) Effect of crop rotation on soil population densities of *Fusarium solani* and *Heterodera glycines* and on the development of sudden death syndrome of soybean. Crop Prot 16:575–580. doi: 10.1016/S0261-2194(97)00031-8

Rupe JC, Sabbe WE, Robbins RT, Gbur EE (1993) Soil and plant factors associated with sudden death syndrome of soybean. Jpa 6:218. doi: 10.2134/jpa1993.0218

Sahu BB, Baumbach JL, Singh P, et al. (2017) Investigation of the *Fusarium virguliforme* transcriptomes induced during infection of soybean roots suggests that enzymes with hydrolytic activities could play a major role in root necrosis. PLoS One 12:1–22. doi: 10.1371/journal.pone.0169963

Sanogo S, Yang XB (2001) Relation of sand content, pH, and potassium and phosphorus nutrition to the development of sudden death syndrome in soybean. Can J Plant Pathol 23:174–180. doi: 10.1080/07060660109506927

Scherm H, Yang XB (1999) Risk assessment for sudden death syndrome of soybean in the north-central United States. Agric Syst 59:301–310. doi: 10.1016/S0308-521X(99)00011-6

Scherm H, Yang XB (1996) Development of sudden death syndrome of soybean in relation to soil temperature and soil water matric potential. Phytopathology 86:642–649.

Scherm H, Yang XB, Lundeen P (1998) Soil variables associated with sudden death syndrome in soybean fields in Iowa. Plant Dis 82:1152–1157. doi: 10.1094/PDIS.1998.82.10.1152

Schmutz J, Cannon SB, Schlueter J, et al. (2010) Genome sequence of the palaeopolyploid soybean. Nature 463:178–183. doi: 10.1038/nature08670

Shi Z, Liu S, Noe J, et al. (2015) SNP identification and marker assay development for high-throughput selection of soybean cyst nematode resistance. BMC Genomics 16:1–12. doi: 10.1186/s12864-015-1531-3

Song Q, Jenkins J, Jia G, et al. (2016) Construction of high resolution genetic linkage maps to improve the soybean genome sequence assembly Glyma1.01. BMC Genomics. doi: 10.1186/s12864-015-2344-0

Song QJ, Marek LF, Shoemaker RC, et al. (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128. doi: 10.1007/s00122-004-1602-3

Srour A, Afzal AJ, Blahut-Beatty L, et al. (2012) The receptor like kinase at *Rhg1*-a/*Rfs2* caused pleiotropic resistance to sudden death syndrome and soybean cyst nematode as a transgene by altering signaling responses. BMC Genomics 13:368. doi: 10.1186/1471-2164-13-368

Srour AY, Gibson DJ, Leandro LFS, et al. (2017) Unraveling microbial and edaphic factors affecting the development of sudden death syndrome in soybean. Phytobiomes 1:91–101. doi: 10.1094/PBIOMES-02-17-0009-R

Stephens PA, Nickell CD, Lim SM (1993) Sudden death syndrome development in soybean

cultivars differing in resistance to Fusarium solani. Crop Sci 33:63–66.

Swaminathan S, Abeysekara NS, Liu M, et al. (2016) Quantitative trait loci underlying host responses of soybean to *Fusarium virguliforme* toxins that cause foliar sudden death syndrome. Theor Appl Genet 129:495–506. doi: 10.1007/s00122-015-2643-5

Tan R, Serven B, Collins PJ, et al. (2018) QTL mapping and epistatic interaction analysis of field resistance to sudden death syndrome (*Fusarium virguliforme*) in soybean. Theor Appl Genet. doi: 10.1007/s00122-018-3110-x

Tischner T, Allphin L, Chase K, et al. (2003) Genetics of seed abortion and reproductive traits in soybean. Crop Sci 43:464–473. doi: 10.2135/cropsci2003.0464

Triwitayakorn K, Njiti VN, Iqbal MJ, et al. (2005) Genomic analysis of a region encompassing *QRfs1* and *QRfs2* : genes that underlie soybean resistance to sudden death syndrome. Genome 48:125–138. doi: 10.1139/g04-103

Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78. doi: 10.1093/jhered/93.1.77

Vosberg SK, Marburger DA, Smith DL, Conley SP (2017) Planting date and fluopyram seed treatment effect on soybean sudden death syndrome and seed yield. Agron J 109:2570. doi: 10.2134/agronj2017.04.0232

Wang, J., Jacobs, J., and Chilvers, M. 2014. Management of soybean sudden death syndrome by seed treatment with fluopyram. Phytopathology 104 (Suppl.):127

Wang J, Jacobs JL, Byrne JM, Chilvers MI (2015) Improved diagnoses and quantification of *Fusarium virguliforme* , causal agent of soybean sudden death syndrome. Phytopathology 105:378–387. doi: 10.1094/PHYTO-06-14-0177-R

Wen Z, Tan R, Yuan J, et al. (2014) Genome-wide association mapping of quantitative resistance to sudden death syndrome in soybean. BMC Genomics 15:809.

Westphal, A., T.S. Abney, L.J. Xing and G.E. Shaner. Sudden death syndrome of soybean. 2008. *The Plant Health Instructor*. DOI:10.1094/PHI-I-2008-0102-01

Westphal A, Li C, Xing L, et al. (2014) Contributions of *Fusarium virguliforme* and *Heterodera glycines* to the disease complex of sudden death syndrome of soybean. PLoS One 9:1–13. doi: 10.1371/journal.pone.0099529

Wrather JA, Kendig SR, Anand SC, et al. (1995) Effects of tillage, cultivar, and planting date on percentage of soybean leaves with symptoms of sudden death syndrome. Plant Dis. 79:560–562.

Wrather JA, Koenning SR (2009) Effects of diseases on soybean yields in the United States 1996 to 2007. Plant Heal Prog Online. doi: 10.1094/PHP-2009-0401-01-RS

Wrather JA, Koenning SR (2006) Estimates of disease effects on soybean yields in the United States 2003 to 2005. J Nematol 38:173–80. doi: 10.1094/PHP-2009-0401-01-RS

Xing L, Westphal A (2009) Effects of crop rotation of soybean with corn on severity of sudden death syndrome and population densities of *Heterodera glycines* in naturally infested soil. F Crop Res 112:107–117. doi: 10.1016/j.fcr.2009.02.008

Xing LJ, Westphal A (2006) Interaction of *Fusarium solani* f. sp. *glycines* and *Heterodera glycines* in Sudden Death Syndrome of Soybean. Phytopathology 96:763–770. doi: 10.1094/PHYTO-96-0763

Kandel YR, McCarville MT, Adee EA, et al. (2018) Benefits and profitability of fluopyram-amended seed treatments for suppressing sudden death syndrome and protecting soybean yield: A meta-analysis. Plant Disease 102: 1093-1100. https://doi.org/10.1094/PDIS-10-17-1540-RE

Zhang J, Singh A, Mueller DS, Singh AK (2015) Genome-wide association and epistasis studies unravel the genetic architecture of sudden death syndrome resistance in soybean. Plant J 84:1124–1136. doi: 10.1111/tpj.13069

Zhang S, Zhang Z, Bales C, et al. (2017) Mapping novel aphid resistance QTL from wild soybean, Glycine soja 85-32. Theor Appl Genet 130:1941–1952. doi: 10.1007/s00122-017-2935-z

CHAPTER 2

QTL MAPPING AND EPISTATIC INTERACTION ANALYSIS OF FIELD RESISTANCE TO SUDDEN DEATH SYNDROME (*FUSARIUM VIRGULIFORME*) IN SOYBEAN

The work presented in this chapter has been published:

Ruijuan Tan, Bradley Serven, Paul J. Collins, Zhongnan Zhang, Zixiang Wen, John F. Boyse, Cuihua Gu, Martin I. Chilvers, Brian W. Diers, Dechun Wang (2018) QTL mapping and epistatic interaction analysis of field resistance to sudden death syndrome (*Fusarium virguliforme*) in soybean. Theor Appl Genet. doi: 10.1007/s00122-018-3110-x

Abstract

Sudden death syndrome (SDS), caused by *Fusarium virguliforme*, is a major disease of soybean [*Glycine max* (L.) Merr.] in the United States. Breeding for soybean resistance to SDS is the most cost-effective method to manage the disease. The objective of this study was to identify and characterize quantitative trait loci (QTLs) underlying field resistance to SDS in a recombinant inbred line population from the cross GD2422 × LD01-5907. This population was genotyped with 1786 polymorphic single nucleotide polymorphisms (SNPs) using SoySNP6 K iSelect BeadChip and evaluated for SDS resistance in a naturally infested field. Four SDS resistance QTLs were mapped on Chromosomes 4, 8, 12 and 18. The resistant parent, LD01-5907, contributed the resistance alleles for the QTLs on Chromosomes 8 and 18 (qSDS-8 and qSDS-18), while the other parent, GD2422, provided the resistance alleles for the QTLs on Chromosomes 4 and 12 (qSDS-4 and qSDS-12). The minor QTL on Chromosome 12 (qSDS-12) is novel. The QTL on Chromosomes 8 and 18 (qSDS-8 and qSDS-18) overlapped with two soybean cyst nematode resistance-related loci, Rhg4 and Rhg1, respectively. A significant interaction between qSDS-8 and qSDS-18 was detected by disease incidence. Individual effects together with the interaction effect explained around 70% of the phenotypic variance. The epistatic interaction of qSDS-8 and qSDS-18 was confirmed by the field performance across multiple years. Furthermore, the resistance alleles at qSDS-8 and qSDS-18 were demonstrated to be recessive. The SNP markers linked to these QTLs will be useful for marker-assisted breeding to enhance the SDS resistance.

For a full text of this work, please go to https:// doi: 10.1007/s00122-018-3110-x

CHAPTER 3

GENETIC CONTROL UNDERLYING ROOT AND FOLIAR RESISTANCE TO SUDDEN DEATH SYNDROME (*FUSARIUM VIRGULIFORME*) IN SOYBEAN

Abstract

Use of resistant cultivars is the most cost-efficient approach to manage sudden death syndrome (SDS) of soybean, which is caused by *Fusarium virguliforme*. The objectives of the present study were to 1) map the loci associated with root and foliar resistance to *F. virguliforme* infection, and 2) decipher the relationships between root infection, foliar damage, and plot yield. A mapping population consisting of 153 F_4 derived recombinant inbred lines (RILs) from the cross U01-390489 × E07080 was genotyped by SoySNP6K BeadChip assay. Both foliar damage and *F. virguliforme* content in roots were investigated in the field and weak positive correlation were identified in between. Foliar damage showed stronger negative correlation with plot yield than the *F. virguliforme* content. Twelve loci associated with foliar damage were identified and four of them were detected by multiple parameters across environments. In contrast, only one loci associated with root resistance to *F.virguliforme* content and was mapped on Chromosome 18. It colocalized with the loci associated with foliar damage in same environment. The locus on Chromosomes 6, qSDS6-2, associated with resistance to SDS phytotoxins and the locus on Chromosome 18, qSDS18-1, related to resistance to *F. virguliforme* content in root. Both loci exhibited effects on plot yield. The stability of identified loci and higher correlation with plot yield make the foliar damage-related parameters, especially disease index, more valuable as selection criteria for SDS resistance breeding. The information provided by this study will aid marker-assisted selection to improve SDS resistance in soybean.

Introduction

Sudden death syndrome (SDS), caused by the soil-borne fungus, *Fusarium virguliforme* (formerly *F. solani* f.sp. *glycines*), is one of the most important diseases of soybean [*Glycine max* (L.) Merr.] (Aoki et al. 2003). Since its first description in Arkansas in 1971 (Roy et al. 1997), this disease has spread to all soybean-growing states of North America (Hartman et al. 2015). Soybean SDS begins with the root infection by *F. virguliforme*, then phytotoxins are produced in the root and translocated through the vascular tissue to the above-ground parts of soybean plants. The pathogen, *F. virguliforme*, is rarely found in the above-ground parts (Hartman et al. 2015). The root damage caused by *F. virguliforme* is very similar to other root-infecting pathogens, such as necrosis and dieback. The distinguishable characteristics with *F. virguliforme* infection is the brownish vascular tissue with white pith at the lower stem of diseased plants (Hartman et al. 2015). Blue spots of fungus sporulation on the root surface may be observed under certain conditions. The typical foliar symptoms, interveinal chlorosis and necrosis, are invoked by phytotoxins. To date, four phytotoxins associated with SDS foliar symptoms have been identified, including Radicicol, a 17-kDa effector, FvTox1, and FvNIS1 (Chang et al. 2016). Among these phytotoxins, FvTox1 has been reported to be the major toxin causing foliar symptoms of SDS (Brar et al. 2011; Pudake et al. 2013), and its function has been confirmed in transgenic plants (Brar and Bhattacharyya 2012).

A United Soybean Board-supported project has reported that the average of estimated yield loss due to SDS during the period of 1996 to 2009 was about 0.82 million metric tons per year, with the highest yield loss of 2.07 million metric tons in 2000 (Koenning and Wrather 2010). The management options for SDS are limited and use of SDS resistant cultivars has been considered the most effective approach to manage this disease. Chong et al. (2005) conducted a two-year

study using a moderately-resistant cultivar and concluded that when disease index increased 10%, the corresponding yield decreased 5 to 7%. Another study using four genotypes with different SDS resistance levels found that a SDS-resistant cultivar can yield 36% more than the SDS-susceptible cultivar (Brzostowski et al. 2014). These studies have highlighted the importance of SDS resistance breeding. However, since SDS pathogenesis is complicated, whether root or foliar damage caused by SDS has a stronger negative correlation with yield is still unclear. The trait with strongest correlation coefficient with yield would be the best parameter to be used in the SDS-resistant cultivar breeding.

Several studies have been conducted to identify the loci underlying resistance to SDS by investigating root symptoms (Prabhu et al. 1999), foliar symptoms (Chang et al. 1996; Iqbal et al. 2001; Njiti et al. 2002; Njiti and Lightfoot 2006; Abdelmajid et al. 2007; De Farias Neto et al. 2007; Abdelmajid et al. 2012; Wen et al. 2014; Anderson et al. 2015; Zhang et al. 2015; Swaminathan et al. 2016), or both (Njiti et al. 1998; Meksem et al. 1999; Triwitayakorn et al. 2005; Kazi et al. 2008a; Luckew et al. 2013; Bao et al. 2015; Luckew et al. 2017). To date, only one study particularly focused on phytotoxins resistance using *F. virguliforme* culture filtrates (Swaminathan et al. 2016). Root resistance evaluations in these studies were accomplished by either observing the morphology of soybean diseased root or culturing *F. virguliforme* on a selective medium, which may not be able to accurately reflect the *F. virguliforme*'s infection on soybean due to the presence of similar pathogens and the plasticity of *F. virguliforme*'s morphology (Aoki et al. 2003). In recent years, quantitative Polymerase Chain Reaction (qPCR) assays have been developed for causal pathogen diagnosis which provides a more accurate determination approach. Based on the comparisons of six qPCR assays for *F. virguliforme* detection from different institutes, the assay developed by Wang et al. (2015), which targets the

ribosomal DNA intergenic spacer (IGS), was shown to be potentially most useful due to its high specificity and sensitivity (Kandel et al. 2015). Comparably, foliar investigation is much easier to conduct. Two of the three foliar damage related parameters, disease severity (DS) and disease incidence (DI), were directedly estimated from the foliar symptoms and the third parameter, disease index, was calculated as DS×DI/9. Most genetic studies on foliar resistance to SDS were conducted in the field and greenhouse, based on a natural infection or artificial inoculation of *F. virguliforme*. Since foliar symptoms result from the successful infection of *F. virguliforme* in root and translocation of the phytotoxins to above-ground parts, we expect that soybean plant have two mechanisms to defend this disease, one reacting against the *F. virguliforme* colonization and the other one detoxifying the phytotoxins or impeding the translocation of phytotoxins. However, the questions about how *F. virguliforme* colonization in the root correlated with the followed foliar symptoms, and whether foliar evaluation has the capability to identify the QTL underlying root resistance to *F. virguliforme* colonization are still unclear.

The relationship between soybean cyst nematode (SCN) and SDS epidemics is complicated and inconsistent results have been shown by different studies based on field performance (Gao et al. 2006; Xing and Westphal 2006; Marburger et al. 2014; Westphal et al. 2014). Njiti et al. (2002) identified a SDS resistance quantitative trait locus (QTL) which colocalized with the SCN resistance locus – Rhg1, and SDS resistance alleles at this locus were provided by Pyramid, a PI 88788-type soybean. Likewise, in our previous study using a RIL population generated from the cross between a Peking-type soybean and a Chinese germplasm, two SDS resistance loci were identified and they colocalized with the two SCN resistance loci, Rhg1 and Rhg4. Both loci were required for SDS resistance, which is same as Peking-type SCN resistance (Tan et al. 2018). Since SDS and SCN resistance QTLs shared the same genomic location and resistant sources.

The question arising will be whether the beneficial alleles at these resistance loci are sufficient for SDS resistance.

A recombinant inbred line (RIL) population derived from two PI88788-type parental lines (Figure 3.3a) was tested in *F. virguliforme*-infected field and evaluated for foliar damage as well as the root damage using *F. virguliforme*-specific qPCR assay. The objectives were to: 1) Map the loci underlying foliar resistance to SDS as well as root resistance to *F. virgulifome* colonization; 2) Explore the relationship between *F. virguliforme* colonization and foliar damage; 3) Dissect the effects of *F. virguliforme* colonization and foliar damage on the plot yield.

Material and methods

Plant materials

The population generated from the cross U01-390489 by E07080 consisted of 153 F_4-derived RILs. E07080 is an elite line from Michigan State University which is partially resistant to SDS in multiple-years of field evaluation. U01-390489 is an elite line form University of Nebraska and susceptible to SDS.

Plant DNA isolation and genotype screening

Genomic DNA was extracted with a hexadecyltrimethylammonium bromide (CTAB) method (Kisha et al. 1997) for each F4-derived progeny line. One non-expanded trifoliate leaf from each of 10 plants of each line was collected and bulked for DNA isolation. DNA concentration was measured by PicoGreen assays (Ahn et al. 1996). Two parental lines and all $F_{4:5}$ progeny lines were screened using the Illumina SoySNP6K iSelect Beadchip (Illumina, San Diego, Calif. USA) which consists of 5361 Single Nucleotide Polymorphisms (SNPs) at Michigan State

University. Genotypes were called by the Genome Studio program (Illumina, San Diego, Calif. USA).

Phenotype evaluation

In 2013, 2014, and 2015, the mapping population was planted in a randomized complete block design (RCBD) with four replications at Decatur, Michigan, USA, which is a naturally infected field. Each plot was a single row, 3m long with row-spacing of 0.76m. The average number of plants in each plot was 90. In 2013 and 2014, the population was planted in RCBD with four replications at East Lansing, Michigan, USA, which was an artificially inoculated field. *F. virguliforme*-infested sorghum was used as inoculum and the inoculation rate was approximately 6.56 cm^{-3} per meter. Each plot was a single row, 1 meter long with row spacing of 0.76m. The average number of plants in each plot was 26.

Phenotypic evaluations were conducted at both locations (Table 3.7a). Three foliar damage-related parameters, disease severity (DS), disease incidence (DI), and disease index (DX), were estimated at R6 growth stage (Fehr et al. 1971) on a per plot basis. DS was scored using 0-9 scale, with 0 as no symptoms and 9 as premature death (Tan et al., 2018). DI was estimated as the percentage of plants showing SDS symptoms in a plot, ranging from 0 to 100 percent with increments of 5%. Disease index (DX) was calculated as DS×DI/9 (Njiti et al. 1998). Yield under SDS-infection was recorded for each plot in 2013 and 2014 at Decatur field. Root damage-related parameters, including root dry weight and *F. virguliforme* content in soybean root, were determined with three replications in 2014 and 2015 at Decatur, MI. Roots of five plants from each progeny line within each replication were collected at R5 stage, then bulked, washed, oven-dried and ground into powder, and the root dry weight was recorded individually. Total root

DNA was isolated from 70 milligram root powder for each sample using CTAB method. Total root DNA concentration was determined by PicoGreen assay and the *F. virguliforme* DNA was quantified using the real-time PCR protocol with absolute quantification procedure. Real-time PCR experiments were carried out with three technical repeats using a Roche LightCycler 480 system. A standard reference curve was established using pure *F. virguliforme* DNA with one to ten serial dilution from 10 ng/µl to 1pg/µl. The Second Derivative Maximum method was used to estimate the *F. virguliforme* amount in each sample. The qPCR reaction was performed as described in Wang et al. (2015). The *F. virguliforme* content used for analysis was obtained from the amount of *F. virguliforme* DNA being divided by the total root DNA amount within each sample and then multiplied by 10^5.

Genetic map construction

A high-density genetic linkage map was constructed for the mapping population using the Maximum Likelihood mapping option described in the manual of Joinmap 4.0 software (Van Ooijen 2006). Groupings were created using independence LOD, and a LOD score of 3.0 was used to calculate the distance among markers. The linkage groups were assigned to specific chromosomes according to the soybean consensus map (Song et al. 2004). The genetic linkage map was drawn using MapChart 2.2 software (Voorrips 2002).

Statistical and QTL analysis

PROC GLM function of SAS 9.4 (SAS Institute Inc., Cary, NC) was used to obtain the basic statistics and estimation of the broad-sense heritability. The broad-sense heritability was calculated according to Nyquist and Baker (1991). Pearson correlation coefficients were obtained

based on the mean of each parameter using the PROC CORR function in SAS 9.4 (SAS Institute Inc., Cary, NC).

For QTL analysis, the means of root and foliar damage related parameters were used to map the associated genomic loci. Composite interval mapping (CIM) was performed for all parameters to identify QTL with QTL Cartographer V2.5 using the standard model Zmapqtl 6 (Wang et al. 2012). The forward and backward option was used to select markers as cofactors. The walking speed chosen for CIM was 0.5 centimorgan (cM). The empirical LOD threshold for each trait at 5% probability level was determined by a 1,000-permutation test (Churchill and Doerge 1994). Each QTL was defined within the range of +/- 1 LOD from the peak LOD, which corresponds to a 95% confidence interval.

Results

Phenotypic variation and heritability estimation

The mapping population derived from the cross U01-390489 × E07080 was evaluated for SDS resistance at two locations, one at Decatur from 2013 to 2015, and the other at East Lansing in 2013 and 2014. U01-390489 consistently showed more severe foliar damage than E07080 at both locations across years. Phenotypic distributions of the population were continuous for all traits and some RILs showed either higher resistance than the resistant parent or more susceptibility than the susceptible parent (Table 3.1, Figure 3.1). Significant genotype by location, genotype by year, and location by year interactions were detected for all three foliar damage related parameters (Table 3.8a). In brief, SDS infection was less severe at East Lansing, compared to Decatur. The broad-sense heritability of foliar resistance at Decatur ranged from 0.64 to 0.73, higher than East Lansing, which ranged from 0.44 to 0.52. Plot yield was recorded in 2013 and 2014 at Decatur as well. Higher plot yields were consistently observed in E07080,

compared to U01-390489. Also, the plot yield of RILs in 2014 is lower than that in 2013, with the mean of 643.3 grams in 2013 and 549.2 grams in 2014. The distribution pattern of plot yield in the two years is similar and the broad-sense heritability was 0.76. The *F. virguliforme* content was used to estimate root resistance to *F. virguliforme* colonization. The susceptible parental line, U01-390489, consistently showed higher *F. virguliforme* content than E07080. The overall *F. virguliforme* colonization in 2014 was heavier than 2015. Broad-sense heritability of *F. virguliforme* content was 0.52. Inconsistency was observed when comparing root dry weight between two parental lines. The root of U01-390489 weighed more than E07080 in 2014, and less than E07080 in 2015. The broad-sense heritability of root dry weight was 0.55. Overall, foliar damage related parameters showed higher broad-sense heritability than the root related parameters, which implies that foliar symptoms are more stable across environments.

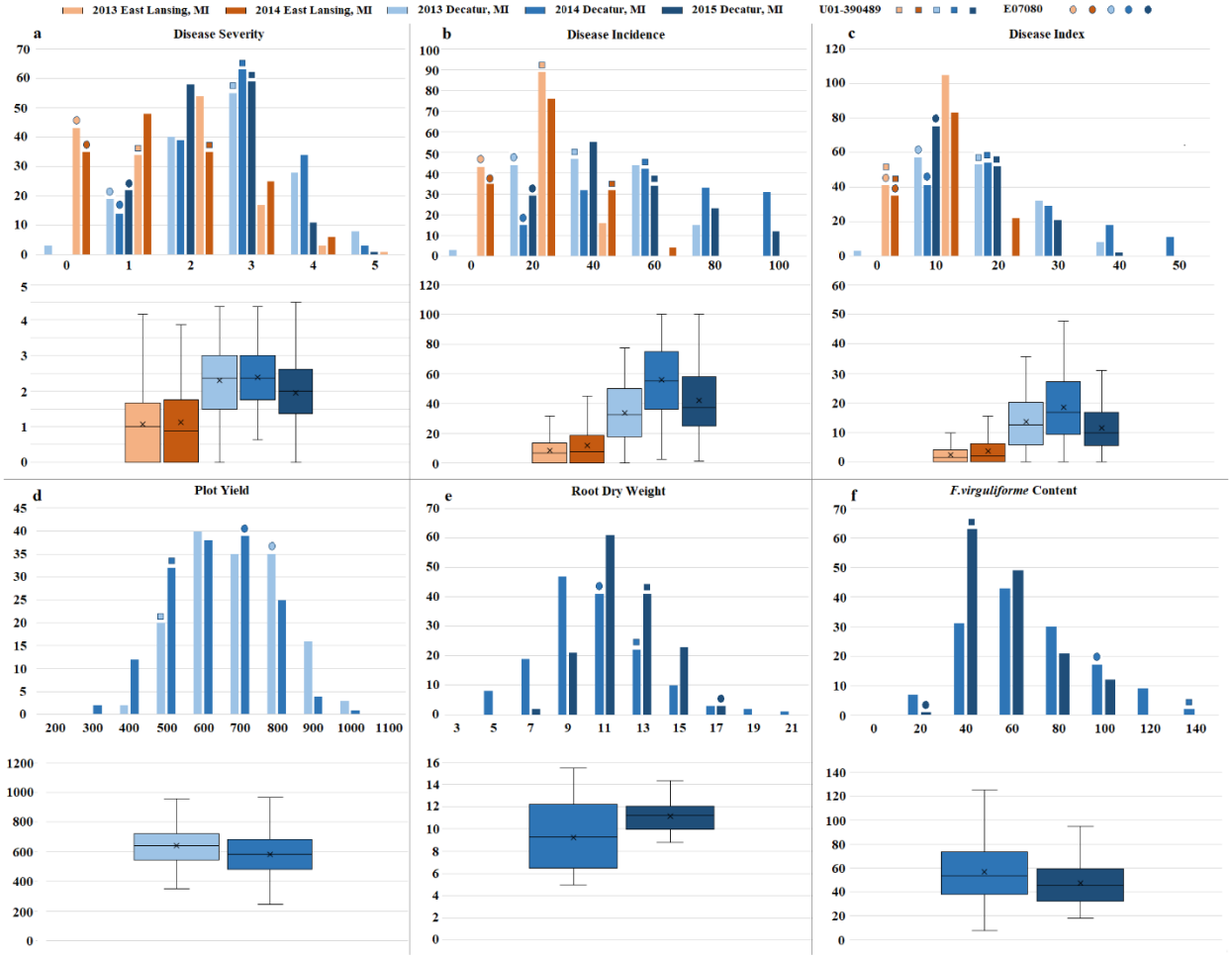
Table 3.1 Estimation of means, ranges of variation, and broad-sense heritability of disease severity (DS), disease incidence (DI), disease index (DX), plot yield (PY), root dry weight (RDW), and *F. virguliforme* content (FvC) for two parental lines and 153 RILs derived from the cross between 'U01-390489' and 'E07080' evaluated in two *F. virguliforme*-infected fields, Decatur and East Lansing, MI, from 2013 to 2015

		2013				2014				2015				H^{2d}
				RILs[c]				RILs				RILs		
Loc	Trt	P1[b]	P2	Mean	Range	P1	P2	Mean	Range	P1	P2	Mean	Range	
DEC[a]	DS	3.13	1.5	2.45	0-4.25	2.5	1.25	2.08	0-3.83	2.88	1.38	2.03	0.25-6.25	0.64
	DI	46.25	15	29.71	0-77.50	51.25	18.75	46.82	0-100	56.25	12.5	42.16	1.25-100	0.73
	DX	18.26	3.54	11.52	0-36.80	16.18	3.47	14.13	0-41.30	22.29	3.82	12.59	0.42-61.11	0.70
	PY	527.9	772.2	643.3	277.3-1028.7	463.6	619.8	549.2	224.50-936.7					0.76
	RDW					12.95	10.80	9.37	3.27-19.34	12.5	16.27	11.03	6.9-16.57	0.55
	FvC					131.57	94.08	56.73	7.71-125.11	35.80	25.42	47.40	18.35-95.02	0.52
EL	DS	1.43	0.6	1.07	0-4.17	1.86	0.25	1.15	0-3.88					0.52
	DI	14.29	3.67	8.68	0-31.67	40	3.13	12.13	0-45.00					0.51
	DX	3.81	0.89	2.55	0-9.91	13.77	0.69	4.12	0-15.49					0.44

[a]DEC: Decatur, EL: East Lansing;
[b]P1: U01-390489, P2: E07080;
[c]RILs: Recombinant inbred lines;
[d]H^2: Broad-sense heritability

Figure 3.1 Frequency distribution of disease severity, disease incidence, disease index, plot yield, root dry weight, and *F. virguliforme* content in the mapping population derived from the cross U01-390489 × E07080 in the *F. virguliforme*-infected fields at Decatur and East Lansing, MI from 2013 to 2015.

Each parameter was shown in both histogram (above) and boxplot (below). Parental lines U01-390489 and E07080 were represented by square and circle with different environments marked by different colors. a: disease severity; b: disease incidence; c: disease index; d: plot yield; e: root dry weight; f: *F. virguliforme* content.



F. virguliforme colonization in the root showed small effect on the foliar damage and root dry weight

To see how *F. virguliforme* colonization in root affect the subsequent foliar symptoms and root dry weight, Pearson correlations among all the disease parameters collected at Decatur in 2014 and 2015 were calculated and summarized in Table 3.2. A weak positive correlation was detected between foliar damage and *F. virguliforme* content in the root at different significance

levels, with the correlation coefficient (*r*) ranging from 0.18 (P<0.05) to 0.23 (P<0.001) in 2014 and 0.28 (P<0.001) to 0.34 (P<0.001) in 2015. Root dry weight was negatively correlated with both foliar damage and *F. virguliforme* content. A stronger negative correlation was found between foliar damage and root dry weight, with *r* ranging from -0.46 to -0.49 (P<0.001) in 2014, and -0.20 (P<0.05) to -0.37 (P<0.001) in 2015. Negative correlations between root dry weight and *F. virguliforme* content in root were -0.20 (P<0.05) and -0.26 (P<0.01) in 2014 and 2015 respectively at different significance levels. In addition, DS showed a significant correlation with DI, with *r*=0.78 in 2014 and *r*=0.64 in 2015 (P<0.001). Correlation coefficient of the same parameters between two years was calculated as well, and a significant correlation was detected for all three foliar damage related parameters and root dry weight, except the *F. virguliforme* content in root between two years was not significantly correlated (Table 3.2).

Table 3.2 Correlation coefficient (*r*) between foliar and root damage related parameters in the RIL population derived from U01-390489×E07080 at Decatur in 2014 and 2015

	DS[a]	DI	DX	FvC	RDW
DS	**0.34***[b]	0.78***	0.84***	0.21**	-0.46***
DI	0.64***	**0.59*****	0.94***	0.18*	-0.47***
DX	0.82***	0.87***	**0.56*****	0.23***	-0.49***
FvC	0.3***	0.28***	0.34***	**0.12NS**	-0.2*
RDW	-0.2*	-0.37***	-0.3***	-0.26**	**0.40*****

Note: Correlation coefficients of disease parameters collected in 2014 at Decatur were summarized in the top right triangle; correlation coefficients of disease parameters collected in 2015 at Decatur were summarized in the bottom left triangle; correlation coefficient of same parameter between 2014 and 2015 was summarized in the diagonal line in bold.
[a] DS: disease severity; DI: disease incidence; DX: disease index; FvC: *F. virguliforome* content in root; RDW: root dry weight.
[b] *: P<0.05; **: P<0.01; ***: P<0.001; NS: not significant

Yield response to root and foliar damage

In 2013 and 2014, plot yield in SDS-infected field was recorded and its correlation with foliar and root damage related parameters were studied. Significant correlations between yield and foliar damage were observed in both years (Table 3.3), except that between plot yield and DI was

barely significant in 2013 (P=0.0595). The negative correlation in 2014 was stronger than 2013, with *r* of -0.37 for DS and r of -0.47 for DX in 2014 (P<0.001). Correlation between plot yield and root related parameters was also calculated in 2014. As shown in Table 3.3, the correlation coefficient between plot yield and the *F. virguliforme* content in root was -0.29 (p<0.001), which was weaker than that between plot yield and foliar damage in 2014. In addition, plot yield showed positive correlation with root dry weight, with the *r* of 0.45 (P<0.001). Correlations of the same parameter from 2013 and 2014 were all significant at P<0.001, with the largest *r* of 0.58 for plot yield.

Table 3.3 Correlation coefficients (*r*) between yield and foliar as well as root damage related parameters in the RIL population derived from U01-390489 × E07080 at Decatur in 2013 and 2014

	DS[a]	DI	DX	FvC	RDW	PY
DS	**0.48*****[b]	0.77***	0.78***			-0.25**
DI	0.78***	**0.51*****	0.98***			-0.15[c]
DX	0.84***	0.94***	**0.51*****			-0.21**
FvC	0.21**	0.18*	0.23**			
RDW	-0.46***	-0.47***	-0.49***	-0.2*		
PY	-0.37***	-0.4***	-0.47***	-0.29***	0.45***	**0.58*****

Note: Correlation coefficients of parameters collected in 2013 at Decatur were summarized in the top right triangle; correlation coefficients of parameters collected in 2014 at Decatur were summarized in the bottom left triangle; correlation coefficient of same parameter between 2013 and 2014 was summarized in the diagonal line in bold.
[a] DS: disease severity; DI: disease incidence; DX: disease index; FvC: *F. virguliforome* content in root; RDW: root dry weight; PY: plot yield.
[b] *: P<0.05; **: P<0.01; ***: P<0.001.
[c] P=0.0595

High-density genetic linkage map

A total of 1222 SNPs showed polymorphism between two parental lines from 5361 SNPs on the SoySNP6K iSelect BeadChip and 1216 were anchored on 20 linkage groups (LGs), corresponding to 20 chromosomes in the consensus map (Song et al. 2004a). The whole genetic linkage map spanned 2170 cM with the largest map length of 214.6 cM for Chromosome 6 (LG

C2). The number of polymorphic SNPs on each chromosome ranged from 8 on Chromosome 4 (LG C1) to 153 on Chromosome 3 (LG N). The average distance between adjacent markers on each LG ranged from 0.6 to 6.2 cM (Table 3.4 & Figure 3.4a).

Table 3.4 Distribution of polymorphic SNPs on each chromosome/linkage group for the population of 153 $F_{4:5}$ RILs from the U01-390489 × E07080

Chr/LG[a]	No. of SNPs	Map Length (cM)	Avg Interval (cM)	Chr/LG	No. of SNPs	Map Length (cM)	Avg Interval (cM)
1/D1a	28	118.6	4.2	**11/B1**	41	90.8	2.2
2/D1b	21	130.4	6.2	**12/H**	23	56.4	2.5
3/N	153	103.1	0.7	**13/F**	118	180.1	1.5
4/C1	8	31.1	3.9	**14/B2**	161	97.8	0.6
5/A1	28	117.2	4.2	**15/E**	111	156.7	1.4
6/C2	116	214.6	1.9	**16/J**	44	93.185	2.1
7/M	54	148.7	2.8	**17/D2**	26	113.4	4.4
8/A2	43	134.6	3.1	**18G**	103	150.5	1.5
9/K	55	99.1	1.8	**19/L**	11	11.0	1.0
10/O	37	55.1	1.5	**20/I**	35	67.3	1.9

[a] Chromosome and linkage group are according to Soybase (Grant et al. 2010)

Loci underlying resistance to foliar damage

Of the 21 traits evaluated, 15 traits were related to foliar damage, including DS, DI and DX at two locations across three years. The average value of each trait was used in the QTL analysis to dissect the loci controlling the resistance to foliar damage. In total, 12 loci were detected and mapped on six chromosomes (Table 3.5), among which four were identified by multiple parameters. The QTL on Chromosome 6 (LG C2), designated as qSDS6-2, was detected by 12 foliar damage related parameters, with resistance alleles consistently originating from the resistant parent E07080. The phenotypic variance explained by this QTL ranged from 6.08 to 17.43% for different parameters across environments. The 95% confidence interval of this QTL was less than one Megabase (Mb), which spanned from 14.05 to 15.03 Mb. Another QTL on Chromosome 6, designated as qSDS6-3, was identified by four parameters, with E07080

providing the resistance alleles as well. The phenotypic variance explained by qSDS6-3 ranged from 7.00 to 9.45%. The QTL mapped on Chromosome 9, designated as qSDS9-1, was associated with four parameters at Decatur. The resistance alleles of the qSDS9-1 was provided by the susceptible parent, U01-390489. This QTL explained 9.0 to 11.7% of the phenotypic variance for different parameters. The QTL on the Chromosome 18 was located on the arm opposite to the Rhg1 locus, and designated as qSDS18-1, and it was associated with five foliar damage-related parameters at East Lansing and one parameter at Decatur. Interestingly, the susceptible parent U01-390489 provided resistance alleles for this locus for all parameters at East Lansing, while the resistance alleles was conferred by E07080 at Decatur. Furthermore, seven more QTL were detected by the foliar damage-related parameters from Decatur and one QTL by disease severity from East Lansing. However, each QTL was associated with only one parameter.

Table 3.5 Summary of quantitative trait loci (QTLs) underlying foliar resistance of SDS detected in the mapping population of 153 $F_{4:5}$ RILs derived from the cross between susceptible parent 'U01-390489' and resistant parent 'E07080' using composite interval mapping method with the field data from Decatur and East Lansing, MI, from 2013 to 2015

QTL	CHR/LG[a]	Traits[b]	Peak Pos[c] (cM)	LOD[d]	R^{2e} (%)	A[f]	95% confidence interval	
							SNP[g]	Mb[h]
qSDS6-1	6/C2	DS2015DEC	54.1	3.08	6.95	0.24	ss245826901- ss245842048	6.33-8.98
qSDS6-2	6/C2	DS2013DEC	124.3	7.55	17.43	0.47	ss245879277- ss245888974;	14.05-15.03
		DI2013DEC	124.3	4.57	8.12	6.53		
		DX2013DEC	124.3	3.53	6.08	2.68		
		DS2014DEC	124.3	3.24	7.10	0.25		
		DX2015DEC	124.3	4.20	8.89	2.98		
		DI2014DEC	128.4	6.84	13.70	9.99		
		DX2014DEC	128.0	7.16	14.29	4.49		
		DS2013EL	127.4	6.03	13.45	0.36		
		DI2013 EL	129.3	5.89	12.18	3.88		
		DX2013 EL	129.3	6.31	13.72	1.28		
		DS2014 EL	129.3	6.19	12.30	0.42		
		DI2014 EL	127.4	4.44	8.69	4.90		
qSDS6-3	6/C2	DS2013DEC	139.1	3.74	9.45	0.34	ss245888974- ss245909007	15.03-16.81
		DI2015DEC	140.1	3.47	7.73	7.07		
		DX2015DEC	140.1	3.19	7.00	2.77		
		DI2014 EL	139.6	3.78	7.87	4.83		
qSDS6-4	6/C2	DX2014DEC	154.4	4.40	9.82	3.70	ss245925990- ss246010254	18.63-39.82
qSDS6-5	6/C2	DX2013DEC	169.0	5.59	11.13	3.11	ss246041195- ss246068439	43.16-44.82
qSDS6-6	6/C2	DX2014DEC	177.4	4.19	8.75	3.43	ss246084690- ss246086447	46.11-46.25
qSDS6-7	6/C2	DX2013DEC	181.0	5.43	10.83	3.04	ss246098726- ss246102570	47.27-57.59

Table 3.5 (cont'd)

qSDS7-1	7/M	DX2013DEC	30.6	4.80	9.93	2.92	ss246280747- ss246282071	14.89-14.99
qSDS9-1	9/K	DI2014DEC	59.6	4.76	9.36	-7.98	ss246827311- ss246949164	7.00-34.27
		DS2014DEC	64.8	4.82	11.67	-0.31		
		DX2014DEC	66.3	3.91	10.96	-3.86		
		DI2015DEC	69.8	3.48	8.99	-7.25		
qSDS17-1	17/D2	DI2013DEC	27.9	3.50	6.50	5.19	ss249273026- ss249290628	4.65-6.82
qSDS18-1	18/G	DI2013 EL	133.9	4.81	9.62	-3.03	ss249931277- ss249984976	58.29-61.89
		DX2013 EL	131.0	4.54	9.45	-1.00		
		DS2014 EL	134.9	5.14	9.90	-0.35		
		DI2014 EL	134.9	6.18	12.96	-5.78		
		DX2014 EL	134.9	7.38	15.81	-2.97		
		DX2015DEC	127.9	3.71	7.89	2.80		
qSDS20-1	20/I	DS2013EL	22.8	3.78	9.83	0.31	ss250304625- ss250327854	1.06-3.76

[a] Chromosome/Linkage group: The linkage group number and chromosome number are according to the SoyBase (Grant et al. 2010)

[b] Traits were named by the order of disease parameter, year, and location. DS: Disease Severity; DI: Disease Incidence; DX: Disease Index; DEC: Decatur; EL: East Lansing. For instance, "DS2015DEC" refers to the disease severity in 2015 at Decatur locations

[c] Peak position was expressed in centimorgan (cM)

[d] The LOD thresholds were estimated by 1000-permutation at 5% level and are summarized in Table 3.9a

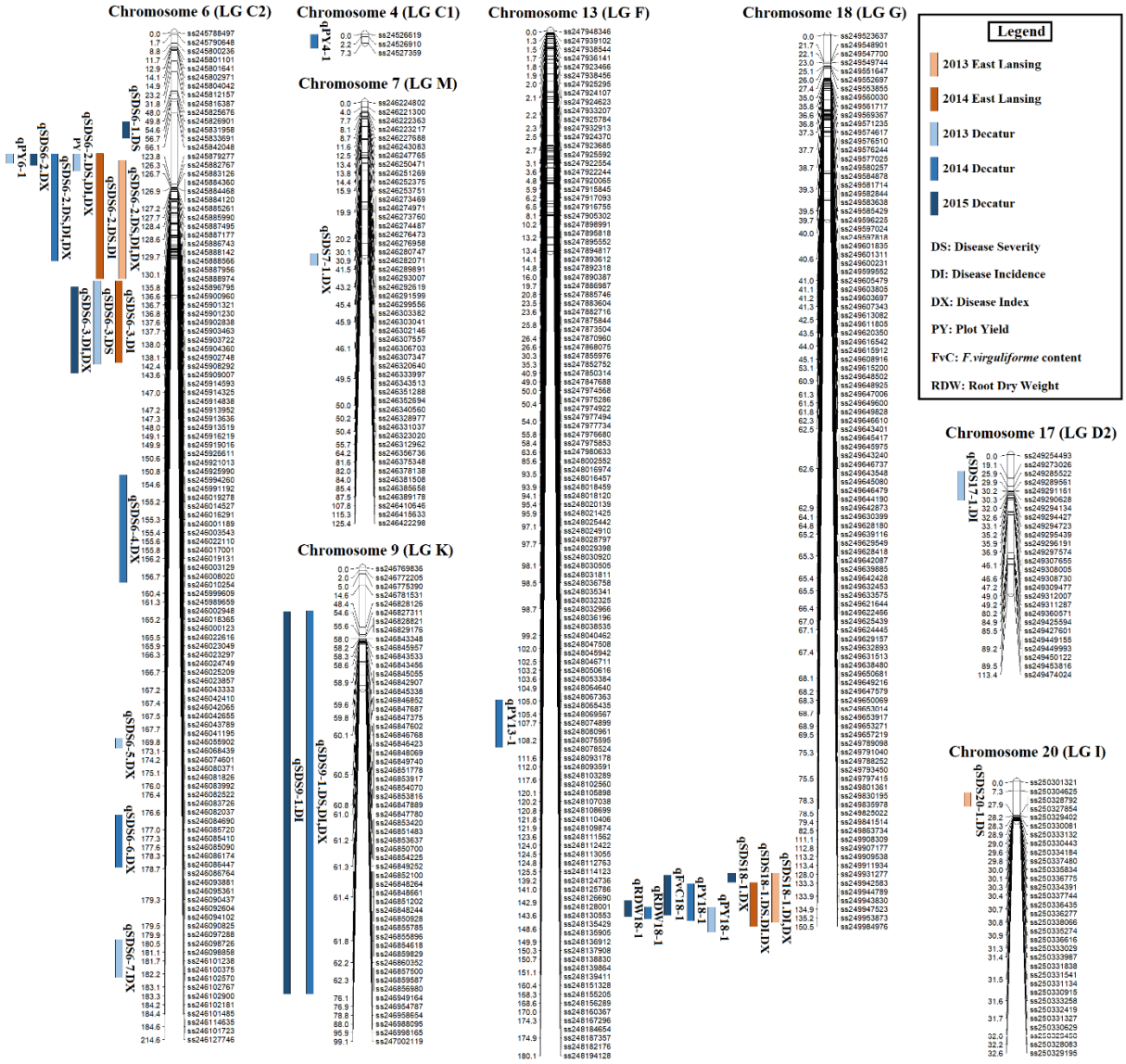
[e] R^2: percentage of phenotypic variance explained by a QTL

[f] Additive effect: the negative value implies that U01-390489 decreases the phenotypic value. The positive value implies that E07080 decreases the phenotypic value

[g] Flanking markers of the 95% confidence interval

[h] Physical map interval in Megabase (Mb)

Figure 3.2 Genomic locations of QTL detected by 21 phenotypic traits using a composite interval mapping method in the RIL population developed from the cross U01-390489 × E07080.



Genomic loci underlying resistance to root damage and plot yield

In total, six QTLs were identified for the root related parameters and plot yield (Table 3.6). for the root resistance to *F. virguliforme* colonization, only one QTL, qFvC18-1, was identified in 2015 at Decatur and mapped on Chromosome 18. It overlapped with the QTL identified by disease index in the same environment. The resistance alleles of qFvC18-1 were conferred by

E07080 and it explained 8.4% of phenotypic variance. One root dry weight associated QTL, designated as qRDW18-1, was detected at Decatur in both 2014 and 2015, and its confidence interval overlapped with qFvC18-1. The alleles from susceptible parent U01-390489 decreased the root dry weight at this locus. Given the positive correlation between root dry weight and plot yield, the beneficial alleles of this locus were provided by E07080. This QTL explained 17.9% of phenotypic variance in 2014 and 20.9% in 2015.

In addition, four plot yield-associated QTL were detected at Decatur across two years. The QTL located on Chromosome 6, designated as qPY6-1, overlapped with qSDS6-2, which associated with multiple foliar damage related parameters. Another QTL associated with plot-yield on Chromosome 18 detected in 2013 and 2014 was designated as qPY18-1, and it overlapped with the qSDS18-1, qFvC18-1 and qRDW18-1. Two other plot-yield related QTLs were mapped on Chromosomes 4 and 13, designated as qPY4-1 and qPY13-1. Except for the beneficial alleles of qPY13-1 originating from U01-390489, E07080 provided the beneficial alleles for the other three plot-yield associated QTLs. The highest phenotypic variance was explained by qPY18-1, with 26.7% in 2013 and 9.1% in 2014.

Table 3.6 Summary of QTL for *F. virguliforme* content in root (FvC), root dry weight (RDW), and plot yield (PY) detected in the mapping population U01-390489 × E07080 using composite interval mapping method at the SDS-infected field, Decatur, MI from 2014 and 2015

QTL	CHR/LG[a]	Trait[b]	Peak Pos[c] (cM)	LOD[d]	R^{2e} (%)	A[f]	95% Confidence Interval	
							SNP[g]	Mb[h]
qPY4-1	4/C1	PY2014DEC	1.0	3.32	6.36	-33.55	ss245560843- ss245567348	47.29-48.08
qPY6-1	6/C2	PY2013DEC	124.3	3.53	4.76	-32.50	ss245879277- ss245882767	14.05-14.42
qPY13-1	13/F	PY2014DEC	107.8	3.29	6.27	33.33	ss248065435- ss248078524	29.38-30.10
qPY18-1	18/G	PY2013DEC	134.9	15.34	26.71	-72.52	ss249931277- ss249984976	58.29-61.98
		PY2014DEC	134.4	4.72	9.12	-41.40		
qFvC18-1	18/G	FvC2015DEC	133.9	3.55	8.42	5.76	ss249931277-ss249953873	58.29-59.83
qRDW18-1	18/G	RDW2014DEC	134.9	8.17	17.88	-1.23	ss249942583-ss249953873	59.07-59.82
		RDW2015DEC	133.9	9.55	20.85	-0.92		

[a] Chromosome/Linkage group: The linkage group number and chromosome number are according to the SoyBase (Grant et al. 2010)

[b] Traits were named by the order of disease parameter, year, and location. PY: Plot yield; FvC: *F. virguliforme* Content in root; RDW: Root dry weight. For instance, "PY2014DEC" refers to the plot yield in 2014 at Decatur

[c] Peak position was expressed in centimorgan (cM)

[d] The LOD thresholds were estimated by 1000-permutation at 5% level and are summarized in Table 3.10a

[e] R^2: percentage of phenotypic variance explained by a QTL

[f] Additive effect: the negative value implies that U01-390489 decreases the phenotypic value. The positive value implies that E07080 decreases the phenotypic value

[g] Flanking markers of the 95% confidence interval

[h] Physical map interval in million base (Mb)

Discussion

The RIL population derived from U01-390489 by E07080 was evaluated at two locations from 2013 to 2015. Since both parental lines carry the same alleles as PI88788 at Rhg1 locus on Chromosome 18, no polymorphism was detected in this region. However, we did observe various degrees of SDS damage in this population, which implied that resistance alleles at the Rhg1 region for PI88788-type soybean were insufficient to confer complete SDS resistance. Overall, SDS infection at Decatur, a naturally infected field, was more severe and the foliar damage related parameters also showed higher broad sense heritability than East Lansing, which was artificially inoculated. It suggests that although the isolates used for inoculation at East Lansing were from Decatur field, different soil variables, including moisture, microbial community, pH, temperature, and other environmental factors affected the level of SDS infection, which have been demonstrated in several studies (Scherm et al. 1998; Sanogo and Yang 2001; Rogovska et al. 2017; Srour et al. 2017).

Root and foliar damage caused by *F. virguliforme* were investigated at Decatur in 2014 and 2015 with heavier infection in 2014. The positive correlation between foliar damage and *F. virguliforme* content in the root was significant in both years and we observed that the correlation coefficient was higher when disease infection was lower. In previous genetic studies, some root resistance QTL overlapped with foliar resistance, but others did not (Njiti et al. 1998; Meksem et al. 1999; Triwitayakorn et al. 2005; Kazi et al. 2008a; Luckew et al. 2013; Bao et al. 2015; Luckew et al. 2017). We speculate that it might be partly due to the difference on disease pressure or pathogen concentrations used in these studies. There might be a critical point for the existence of correlation between root and foliar damage. For instance, when the *F. virguliforme* colonization in the root surpass the critical point, its impact on foliar damage would become

insignificant. Root dry weight showed stronger negative correlation with majority of foliar symptoms than the *F. virguliforme* content in the root for both years, with exception of DS in 2015.

In terms of the correlation with plot yield, foliar damage related parameters showed stronger negative correlation in 2014 than 2013. In 2014, foliar symptoms showed stronger correlation with plot yield than the *F. virguliforme* content in root, which may be due to the comprehensive characteristics of foliar symptoms that combining the defense mechanisms to both *F. virguliforme* and phytotoxins. Furthermore, DX showed the highest correlation coefficient with plot yield compared to DS and DI, which suggests that DX is the best parameter to estimate the effect of SDS damage on the yield. Not surprisingly, root dry weight showed positive correlation with plot yield.

A high density genetic linkage map is crucial for QTL identification, candidate gene discovery, and precision breeding using marker-assisted selection. In the present study, with more than 1200 polymorphic SNPs distributed on 20 chromosomes, twelve QTLs were mapped for the foliar resistance to SDS on six chromosomes, and another six QTLs were identified for plot yield, root dry weight, and *F. virguliforme* content in root. Among QTL underlying foliar resistance to SDS, qSDS6-2 was the most stable locus across different environments, since it was identified by multiple parameters at two locations over three years. This QTL overlapped with the QTL identified by Swaminathan (2016), which was associated with resistance to phytotoxins and designated as SDS16-6 on Soybase. Moreover, with our high-density genetic linkage map, we delimited this QTL from a 15-Mb region to an interval less than 1-Mb. Since this QTL was not detected by the *F. virguliforme* content in our mapping population, we conclude that the

responsible gene within this locus was involved in the procedure of detoxifying the phytotoxins or blocking its translocation. Moreover, qPY6-1 associated with plot yield colocalized with this locus, so the alleles at this locus affected plot yield. An adjacent QTL, qSDS6-3, was mapped to an interval less than 2-Mb based on four foliar damage-related parameters at two locations. The QTL on Chromosome 9, qSDS9-1, was detected by the foliar parameters from Decatur over two years, and it overlapped with SDS16-1 (Swaminathan et al. 2016) and qDX009 (Anderson et al. 2015). The QTL on Chromosome 18, qSDS18-1, was detected by multiple foliar damage related parameters at East Lansing and Decatur. Intriguingly, the resistance alleles for this locus were provided by different parental lines, with U01-390489 at East Lansing and E07080 at Decatur. We suspect that there might be allele by environment effect, since the disease pressure at two locations were different. In addition, qSDS18-1 colocalized with qFvC18-1 in 2015 Decatur trial, which suggested that the foliar damage related parameter has the capability to detect root resistance QTL when disease pressure is low. This locus was also detected for plot yield and root dry weight, with beneficial alleles from E07080, which suggests that the alleles at this locus were able to show their effects the plot and root dry weight. In addition, two more plot yield QTL were identified in this study. One QTL on Chromosome 4, qPY4-1, overlapped with the QTL reported by Li et al (2010), which associated with seed number per plant and seed weight. The other QTL associated with plot yield located on Chromosome 13, qPY13-1, overlapped with the previously reported QTL by Tischner et al (2003), which associated with seed set and later designated as seed set 1-3 on Soybase.

In summary, 1) A positive correlation between foliar damage and *F. virguliforme* colonization was identified, with a higher correlation coefficient in the year of less disease infection; 2) Foliar damage related parameters can detect the loci underlying resistance to both pathogen

colonization and phytotoxins; 3) Disease index showed highest correlation with plot-yield and could be a valuable parameter to estimate SDS damage on yield; 4) The resistance alleles at qSDS6-2 and qSDS18-1 can be integrated into breeding to avoid the yield loss due to SDS damage. The molecular markers identified in this study will be useful in marker-assisted SDS resistance breeding in soybean.

APPENDIX

APPENDIX

Table 3.7a Summary of phenotype evaluation at Decatur and East Lansing, MI from 2013 to 2015 in the F4 derived RIL population generated from the cross U01-390489 × E07080

Location	Year	DS[a]	DI	DX	Yield	RDW	FvC
East Lansing	2013	√	√	√			
	2014	√	√	√			
Decatur	2013	√	√	√	√		
	2014	√	√	√	√	√	√
	2015	√	√	√		√	√

[a] DS: Disease Severity, DI: Disease Incidence; DX: Disease Index; Plot Yield: grain weight per 3m plot; RDW: root dry weight per five plants, FvC: *F.virguliforme* content.

Table 3.8a Genotype by environment interaction detected by ANOVA in the population of U01-390489 × E07080

	Pr>F			
	Genotype × Year	Genotype × Location	Location × Year	Genotype × Location × Year
DS [a]	0.0006	<0.0001	0.0432	0.0636
DI	0.0006	<0.0001	<0.0001	0.7182
DX	<0.0001	<0.0001	<0.0001	0.0415

[a] DS: Disease Severity, DI: Disease Incidence; DX: Disease Index

Table 3.9a The LOD thresholds used in QTL analysis for 15 foliar damage-related parameters

Trait	LOD threshold	Trait	LOD threshold	Trait	LOD threshold
DS2013EL	3.27	DI2013EL	3.22	DX2013EL	3.16
DS2014EL	3.03	DI2014EL	3.07	DX2014EL	3.13
DS2013DEC	3.17	DI2013DEC	3.32	DX2013DEC	3.21
DS2014DEC	3.25	DI2014DEC	3.24	DX2014DEC	3.28
DS2015DEC	3.07	DI2015DEC	3.24	DX2015DEC	3.08

Table 3.10a The LOD thresholds used in QTL analysis for *F. virguliforme* content in root, root dry weight, and plot yield

Trait	LOD threshold	Trait	LOD threshold
PY2013DEC	3.26	PY2014DEC	3.25
FvC2014DEC	3.25	FvC2015DEC	3.37
RDW2014DEC	3.29	RDW2015DEC	3.23

Figure 3.3a Haplotype analysis of the Rhg1 locus of two parental lines, U01-390489 and E07080 compared to Peking and PI88788 using Illumina SoySNP50K iSelect BeadChip data

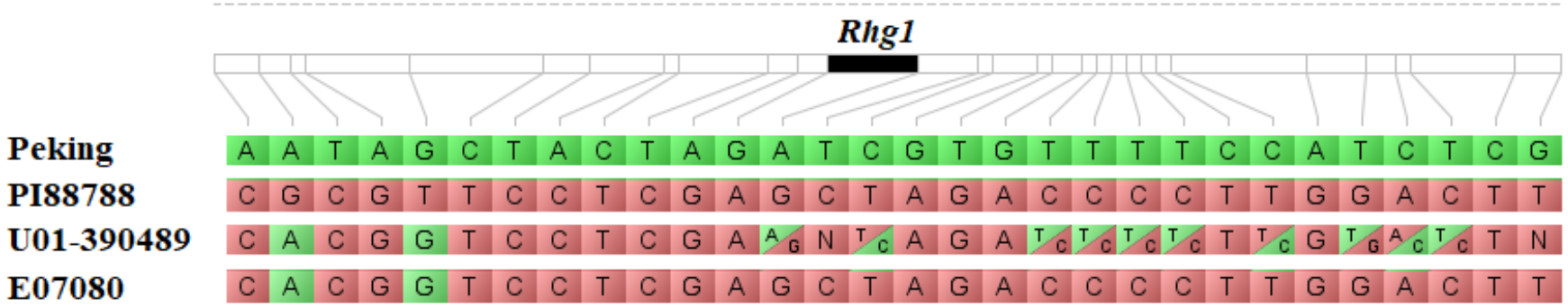


Figure 3.4a Genetic linkage map of the mapping population generated from the cross U01-390489 × E07080

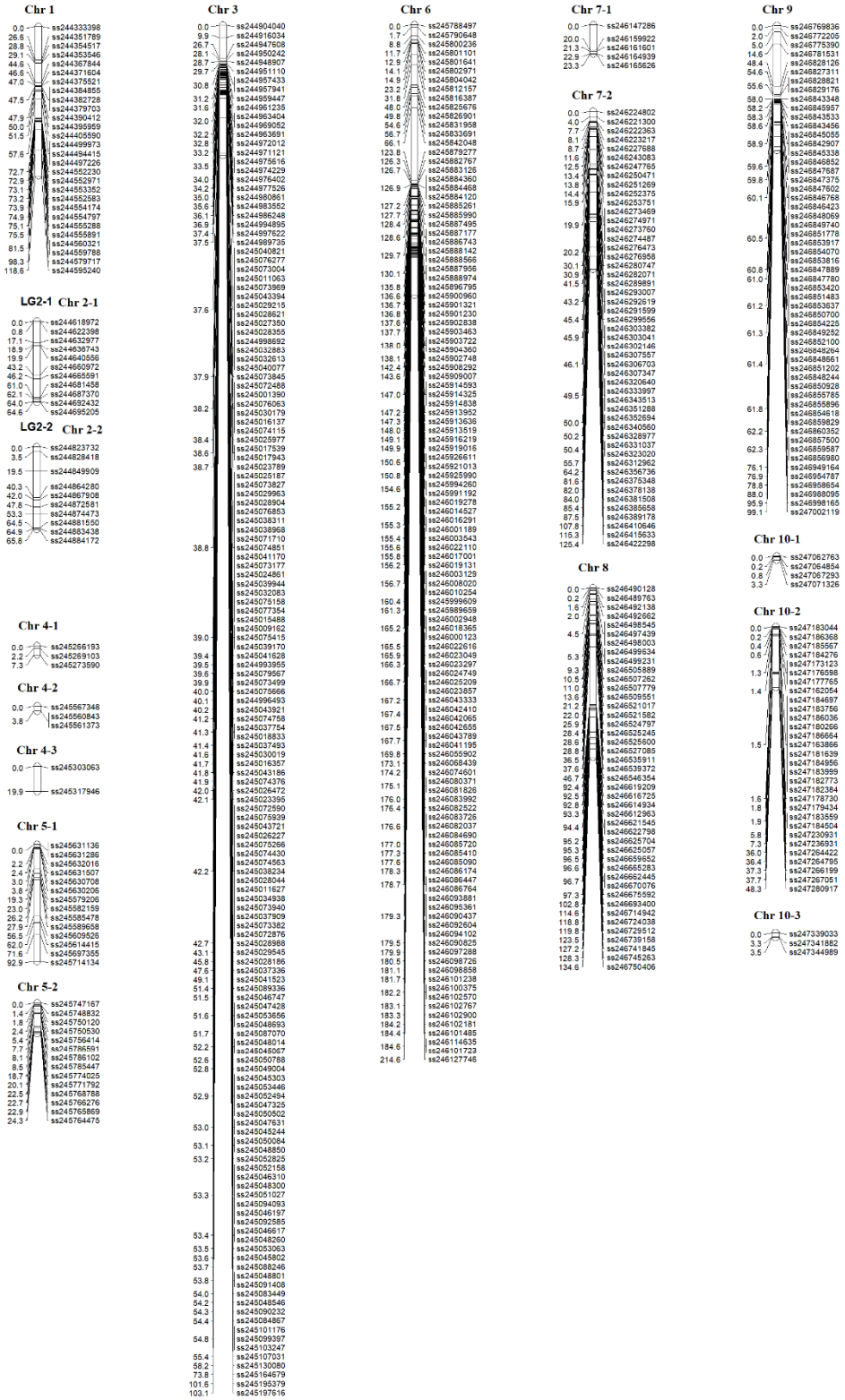
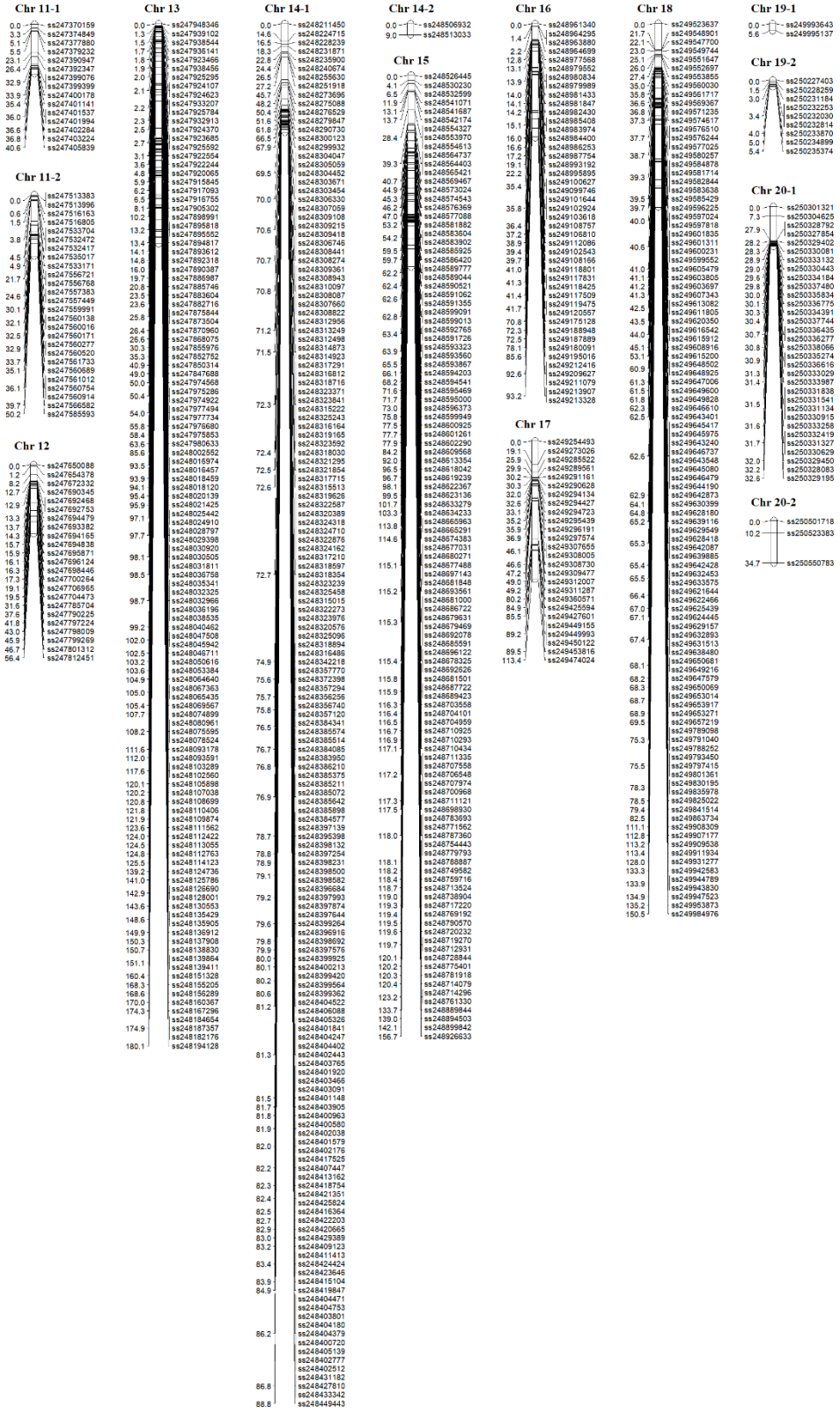


Figure 3.4a (cont'd)



REFERENCES

REFERENCES

Abdelmajid KM, Meksem K, Wood AJ, Lightfoot DA (2007) Loci underlying SDS and SCN resistance mapped in the “Essex” by “Forrest” soybean recombinant inbred lines. Rev Biol Biotechnol 6:2–10.

Abdelmajid KM, Ramos L, Leandro L, et al. (2012) The “ PI 438489B ” by “ Hamilton ” SNP-based genetic linkage map of soybean [*Glycine max* (L .) Merr .] identified quantitative trait loci that underlie seedling SDS resistance. J Plant Genome Sci 1:18–30. doi: 10.5147/jpgs.2012.0053

Ahn SJ, Costa J, Emanuel JR (1996) PicoGreen quantitation of DNA: Effective evaluation of samples pre- or post-PCR. Nucleic Acids Res 24:2623–2625. doi: 10.1093/nar/24.13.2623

Anderson J, Akond M, Kassem MA, et al. (2015) Quantitative trait loci underlying resistance to sudden death syndrome (SDS) in MD96-5722 by “Spencer” recombinant inbred line population of soybean. 3 Biotech 5:203–210. doi: 10.1007/s13205-014-0211-3

Aoki T, O ’donnell K, Homma Y, Lattanzi AR (2003) Sudden-death syndrome of sobyean is caused by two morphologically and phylogenetically distinct species within the Fusarium solani species complex - *F.virguliforme* in North America and *F.tucumaniae* in South America. Mycologia 95:660–684. doi: 10.2307/3761942

Bao Y, Kurle JE, Anderson G, Young ND (2015) Association mapping and genomic prediction for resistance to sudden death syndrome in early maturing soybean germplasm. Mol Breed 35:1–14. doi: 10.1007/s11032-015-0324-3

Brar HK, Swaminathan S, Bhattacharyya MK (2011) The *Fusarium virguliforme* toxin FvTox1 causes foliar sudden death syndrome-like symptoms in soybean. Mol Plant-Microbe Interact 24:1179–1188. doi: 10.1094/MPMI-12-10-0285

Brar HK, Bhattacharyya (2012) Expression of a single-chain variable-fragment antibody against a *Fusarium virguliforme* toxin peptide enhances tolerance to sudden death syndrome in transgenic soybean plants. Molecular Plant-Microbe Interactions 25: 817–824. Doi: 10.1094/MPMI-12-11-0317

Brzostowski LF, Schapaugh WT, Rzodkiewicz PA, et al. (2014) Effect of host resistance to *Fusarium virguliforme* and *Heterodera glycines* on sudden death syndrome disease Severity and soybean yield. Plant Heal Prog 15:1–8. doi: 10.1094/PHP-RS-13-0100

Chang H-X, Domier LL, Radwan O, et al. (2016) Identification of multiple phytotoxins produced by *Fusarium virguliforme* including a phytotoxic effector (FvNIS1) associated with sudden death syndrome foliar symptoms. Mol Plant-Microbe Interact 29:96–108. doi: 10.1094/MPMI-09-15-0219-R

Chang SJC, Doubler TW, Kilo V, et al. (1996) Two additional loci underlying durable field resistance to soybean sudden death syndrome (SDS). Crop Sci 36:1684–1688. doi: 10.2135/cropsci1996.0011183X003600060044x

Chong SK, Hildebrand KK, Luo Y, et al. (2005) Mapping soybean sudden death syndrome as related to yield and soil/site properties. Soil Tillage Res 84:101–107. doi: 10.1016/j.still.2004.09.001

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971. doi: 10.1534/genetics.107.080101

De Farias Neto AL, Hashmi R, Schmidt M, et al. (2007) Mapping and confirmation of a new sudden death syndrome resistance QTL on linkage group D2 from the soybean genotypes PI 567374 and "Ripley." Mol Breed 20:53–62. doi: 10.1007/s11032-006-9072-8

Fehr WR, Caviness CE, Burmood DT, S. PJ (1971) Stage of development descriptino for soybeans, Glycine max (L.) Merrill. Crop Sci 11:929–931. doi: 10.2135/cropsci1971.0011183X001100060051x

Gao X, Jackson T a, Hartman GL, Niblack TL (2006) Interactions between the soybean cyst nematode and *Fusarium solani* f. sp. *glycines* based on greenhouse factorial experiments. Phytopathology 96:1409–15. doi: 10.1094/PHYTO-96-1409

Grant D, Nelson RT, Cannon SB, Shoemaker RC (2010) SoyBase, the USDA-ARS soybean genetics and genomics database. Nucleic Acids Res 38:843–846. doi: 10.1093/nar/gkp798

Hartman GL, Chang HX, Leandro LF (2015) Research advances and management of soybean sudden death syndrome. Crop Prot 73:60–66. doi: 10.1016/j.cropro.2015.01.017

Iqbal MJ, Meksem K, Njiti VN, et al (2001) Microsatellite markers identify three additional quantitative trait loci for resistance to soybean sudden-death syndrome (SDS) in Essex x Forrest RILs. Theor Appl Genet 102:187–192. doi: 10.1007/s001220051634

Kandel YR, Haudenshield JS, Srour AY, et al. (2015) Multilaboratory comparison of quantitative PCR assays for detection and quantification of *Fusarium virguliforme* from soybean roots and soil. Phytopathology 105:1601–1611. doi: 10.1094/PHYTO-04-15-0096-R

Kazi S, Shultz J, Afzal J, et al. (2008) Separate loci underlie resistance to root infection and leaf scorch during soybean sudden death syndrome. Theor Appl Genet 116:967–977. doi: 10.1007/s00122-008-0728-0

Kisha TJ, Sneller CH, Diers BW (1997) Relationship between genetic distance among parents and genetic variance in populations of soybean. Crop Sci 37:1317–1325. doi: 10.2135/cropsci1997.0011183X003700040048x

Koenning, S. R., and Wrather, J. A. 2010. Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. Online. Plant Health

Progress doi:10.1094/PHP-2010-122-01-RS.

Li D, Sun M, Han Y, et al. (2010) Identification of QTL underlying soluble pigment content in soybean stems related to resistance to soybean white mold (*Sclerotinia sclerotiorum*). Euphytica 172:49–57. doi: 10.1007/s10681-009-0036-z

Liu S, Kandoth PK, Lakhssassi N, et al. (2017) The soybean GmSNAP18 gene underlies two types of resistance to soybean cyst nematode. Nat Commun 8:1–11. doi: 10.1038/ncomms14822

Luckew AS, Leandro LF, Bhattacharyya MK, et al. (2013) Usefulness of 10 genomic regions in soybean associated with sudden death syndrome resistance. Theor Appl Genet 126:2391–2403. doi: 10.1007/s00122-013-2143-4

Luckew AS, Swaminathan S, Leandro LF, et al. (2017) "MN1606SP" by "Spencer" filial soybean population reveals novel quantitative trait loci and interactions among loci conditioning SDS resistance. Theor Appl Genet 1–11. doi: 10.1007/s00122-017-2947-8

Marburger D, Conley S, Esker P, et al. (2014) Relationship between *Fusarium virguliforme* and *Heterodera glycines* in commercial soybean fields in Wisconsin. Plant Heal Prog 15:11–18. doi: 10.1094/PHP-RS-13-0107

Meksem K, Doubler TW, Chancharoenchai K, et al. (1999) Clustering among loci underlying soybean resistance to *Fusarium solani*, SDS and SCN in near-isogenic lines. Theor Appl Genet 99:1131–1142. doi: 10.1007/s001220051317

Njiti VN, Doubler TW, Suttner RJ, et al. (1998) Resistance to soybean sudden death syndrome and root colonization by *Fusarium solani* f. sp. *glycines* in near-isogeneic lines. Crop Sci 38:472–477.

Njiti VN, Lightfoot DA (2006) Genetic analysis infers Dt loci underlie resistance to *Fusarium solani* f. sp *glycines* in indeterminate soybeans. Can J Plant Sci 86:83–90. doi: 10.4141/P05-046

Njiti VN, Meksem K, Iqbal MJ, et al. (2002) Common loci underlie field resistance to soybean sudden death syndrome in Forrest, Pyramid, Essex, and Douglas. Theor Appl Genet 104:294–300. doi: 10.1007/s001220100682

Nyquist W, Baker R (1991) Estimation of heritability and prediction of selection response in plant populations. Crit Rev Plant Sci 10:235–322.

Prabhu, R. R, Njiti, et al. (1999) Selecting soybean cultivars for dual resistance to soybean cyst nematode and sudden death syndrome using two DNA markers. Crop Sci 39:982–987.

Pudake RN, Swaminathan S, Sahu BB, et al. (2013) Investigation of the *Fusarium virguliforme* fvtox1 mutants revealed that the FvTox1 toxin is involved in foliar sudden death syndrome development in soybean. Curr Genet 59:107–117. doi: 10.1007/s00294-013-0392-z

Rogovska N, Laird D, Leandro L, Aller D (2017) Biochar effect on severity of soybean root disease caused by *Fusarium virguliforme*. Plant Soil 413:111–126. doi: 10.1007/s11104-016-3086-8

Roy KW, State M, Rupe JC, et al. (1997) Sudden death syndrome of soybean. Plant Dis 81:1100–1111.

Sanogo S, Yang XB (2001) Relation of sand content, pH, and potassium and phosphorus nutrition to the development of sudden death syndrome in soybean. Can J Plant Pathol 23:174–180. doi: 10.1080/07060660109506927

Scherm H, Yang XB, Lundeen P (1998) Soil variables associated with sudden death syndrome in soybean fields in Iowa. Plant Dis 82:1152–1157. doi: 10.1094/PDIS.1998.82.10.1152

Song QJ, Marek LF, Shoemaker RC, et al. (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128. doi: 10.1007/s00122-004-1602-3

Srour AY, Gibson DJ, Leandro LFS, et al. (2017) Unraveling microbial and edaphic factors affecting the development of sudden death syndrome in soybean. Phytobiomes 1:91–101. doi: 10.1094/PBIOMES-02-17-0009-R

Swaminathan S, Abeysekara NS, Liu M, et al. (2016) Quantitative trait loci underlying host responses of soybean to *Fusarium virguliforme* toxins that cause foliar sudden death syndrome. Theor Appl Genet 129:495–506. doi: 10.1007/s00122-015-2643-5

Tan R, Serven B, Collins PJ, et al. (2018) QTL mapping and epistatic interaction analysis of field resistance to sudden death syndrome (*Fusarium virguliforme*) in soybean. Theor Appl Genet. doi: 10.1007/s00122-018-3110-x

Tischner T, Allphin L, Chase K, et al. (2003) Genetics of seed abortion and reproductive traits in soybean. Crop Sci 43:464–473. doi: 10.2135/cropsci2003.0464

Triwitayakorn K, Njiti VN, Iqbal MJ, et al. (2005) Genomic analysis of a region encompassing *QRfs1* and *QRfs2* : genes that underlie soybean resistance to sudden death syndrome. Genome 48:125–138. doi: 10.1139/g04-103

Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78. doi: 10.1093/jhered/93.1.77

Wang J, Jacobs JL, Byrne JM, Chilvers MI (2015) Improved diagnoses and quantification of *Fusarium virguliforme* , causal agent of soybean sudden death syndrome. Phytopathology 105:378–387. doi: 10.1094/PHYTO-06-14-0177-R

Wen Z, Tan R, Yuan J, et al. (2014) Genome-wide association mapping of quantitative resistance to sudden death syndrome in soybean. BMC Genomics 15:809.

Westphal A, Li C, Xing L, et al (2014) Contributions of *Fusarium virguliforme* and *Heterodera glycines* to the disease complex of sudden death syndrome of soybean. PLoS One 9:1–13.

doi: 10.1371/journal.pone.0099529

Xing LJ, Westphal A (2006) Interaction of *Fusarium solani* f. sp. *glycines* and *Heterodera glycines* in Sudden Death Syndrome of Soybean. Phytopathology 96:763–770. doi: 10.1094/PHYTO-96-0763

Zhang J, Singh A, Mueller DS, Singh AK (2015) Genome-wide association and epistasis studies unravel the genetic architecture of sudden death syndrome resistance in soybean. Plant J 84:1124–1136. doi: 10.1111/tpj.13069