IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO *Sclerotinia sclerotiorum* IN *Brassica napus*

By

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LIST OF ABBREVIATIONS

AFLP: Amplified fragment length polymorphism

DH: Doubled haploid

DNA: Deoxyribonucleic acid

EST: Expressed sequence tags

HEAR: High erucic acid rapeseed

LG: Linkage group

LOD: Logarithmic of odds

LRS: Likelihood ratio statistic

MAS: Marker assisted selection

PCR: Polymerase chain reaction

PDA: Potato dextrose agar

QTL: Quantitative trait loci

RAPD: Random amplified polymorphic DNA

RFPL: Restricted fragment length polymorphism

SHEAR: Super high erucic acid rapeseed

SNP: Single nucleotide polymorphism

SRAP: Sequence related amplified polymorphism

DEDICATION

I dedicate this degree to my beloved father

S. Jagga Singh Behla

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Ravneet Singh Behla

ABSTRACT

Quantitative trait loci (QTL) analysis for *Sclerotinia* stem rot resistance was carried out in five doubled haploid (DH) populations of *Brassica napus*.

Sclerotinia stem rot is caused by the necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. *Sclerotinia* stem rot has worldwide occurrence and causes significant yield losses in many crop species. Several screening methods have been recommended in the literature to evaluate plant resistance to *Sclerotinia* stem rot. Four controlled environment based screening methods: 1) excised leaf assay, 2) cotyledon assay, 3) mycelial stem inoculation technique and 4) petiole inoculation technique compared for their ability to differentiate between plant susceptibility/resistance, their reliability and suitability for large scale screening using eight *B. napus* cultivars/lines of varying reaction to *S. sclerotiorum*. The petiole inoculation technique and the mycelium stem inoculation technique were identified as reliable methods in this study.

Previously developed, five *B. napus* DH populations (H1, H2, H3, DH179 and DH180) segregating for resistance to *Sclerotinia* stem rot were used in this study. The petiole inoculation technique was used to evaluate resistance to *Sclerotinia* stem rot. Data on days to wilting was recorded for a two week period. Twelve plants per line were screened in each evaluation and each population was evaluated three times. Two to three day-old mycelial cultures of *S. sclerotiorum* isolate Canada 77 was used.

QTL analyses were carried out using a LOD threshold value of 2.5 on each individual replicate and on the average of all the replicates. In the H1 population, the

number of QTL detected ranged from four to six in each analysis. In the H2 population, there were three to six QTL in each analysis. There were two to six QTL in each analysis of the H3 population. In the DH179 population, the number of QTL detected ranged from three to five in each analysis. In DH180 population, the number of QTL identified varied from three to six in each analysis. A number of common QTL were found between the replicates of each population. Five common QTL were identified between these populations. The markers linked to these QTL are now available for marker assisted selection.

FOREWORD

This thesis has been prepared in manuscript format as outlined by the Department of Plant Science, University of Manitoba, Winnipeg, Canada. A general introduction and literature review is followed by two manuscripts. The first manuscript follows the style recommended by Canadian Journal of Plant Pathology and the second manuscript follows Theoretical and Applied Genetics journal. Each manuscript contains an abstract, introduction, materials and methods, results and discussion. A general discussion and recommendations for future studies and references follow the manuscripts.

1.0 GENERAL INTRODUCTION

Brassica napus is an important oilseed species in Asia, North America and Europe. The cultivars developed from this species have diverse oil usages, which vary from edible oils, industrial oils and lubrication oils to biodiesel. Oleiferous *Brassicas* are the third most important source of vegetable oils and contribute 14 % of global vegetable oils. Almost all plant parts are valuable to human beings. The nutritious meal remaining after oil extraction is used in animal and poultry feed. The dried plant remains after harvesting are utilized as fuel for cooking in many countries (Dixon, 2006; Gupta and Pratap, 2007).

This crop is under constant biotic and abiotic stresses. Among the biotic stresses, the major diseases vary from one geographic area to another. *Sclerotinia* stem rot or *Sclerotinia* white mold of *Brassicas* is one of the major diseases. It is a threat in all major *Brassica*-growing nations. *Sclerotinia* stem rot is caused by a necrotrophic fungus, *Sclerotinia sclerotiorum* (Lib.) de Bary. *Sclerotinia* stem rot causes significant yield losses. Under favourable disease development conditions, economic losses from *Sclerotinia* stem rot can be as high as 100 % (Purdy, 1979). The percent yield losses range from 0.4 to 0.5 times the percent disease incidence (Del Rio *et al.,* 2007; Morrall *et al.,* 1984). *Sclerotinia sclerotiorum* also causes significant yield losses in other important crops such as soybean, sunflower, chickpea, peanut and dry bean and lentils (Bolton *et al.,* 2006). It is estimated that the United States of America losses $482 million per year due to *Sclerotinia* diseases in various crops. The annual losses for the *B. napus* crop alone are around $ 24 million in the United States of America (United States Department of Agriculture, 2009).

Sclerotinia sclerotiorum is a non-host specific, necrotrophic and ubiquitous fungus. It is a broad spectrum plant pathogen infecting more than 400 plant species (Boland and Hall, 1994). *Sclerotinia sclerotiorum* spends most of its life cycle as vegetative resting structures, the sclerotia, in the soil or plant debris (Adams and Ayers, 1979). Under favourable conditions such as high relative humidity, soil water potentials of 100 kPa and temperature range of 10-25 ^{0}C, sclerotia germinate to produce apothecia which in turn produce ascospores. Ascospores become airborne which act as the primary source of inoculum.

Control measures for *Sclerotinia* stem rot include cultural practices, chemical treatment, biological control and host resistance. The escape mechanisms include upright growth habit of the plants, short open canopies, apetalous inflorescences and lodging resistance (Bardin and Huang, 2001; Jurke and Fernando, 2002). Cultural practices include crop rotation and zero tillage to reduce sclerotia load, and wider plant and row spacing (Bardin and Huang, 2001; Fernando *et al.*, 2004). Seed treatment with fungicides and fungicide spray at flowering can also be helpful in preventing the disease. Some mycoparasitic fungi and bacteria which parasitize sclerotia can also help in reducing the sclerotia load in the soil. None of these control measures, however, are very effective in controlling the disease (Fernando *et al*., 2004). The inheritance of host resistance to *Sclerotinia* stem rot is polygenic in all crop species. There are very limited resistance sources available for this disease. Breeders in the public sector in China have successfully developed some oilseed rape cultivars such as Zhongyou 821 and Huashong3 that are resistant to *Sclerotinia* stem rot in under field conditions.

Several screening methods have been used to evaluate plant resistance to *S. sclerotiorum*. The field evaluations for resistance to *Sclerotinia* stem rot produce inconsistent results. Environmental variation also plays an important role in disease incidence and severity (Jurke and Fernando, 2008; Whipps *et al*., 2002). *Sclerotinia* stem rot screening under controlled environmental conditions may provide more accurate estimations of physiological resistance of the plants. Newman and Barley (1987) reported the use of an ascospore suspension spray to evaluate mature plants for resistance to *S. sclerotiorum* in *B. napus*. Bailey (1987) used a detached leaf assay to evaluate resistance to *Sclerotinia* stem rot. Del Rio *et al*. (2000) developed a petiole inoculation technique for soybean which was later modified by Zhao *et al*. (2004) to evaluate *Sclerotinia* stem rot resistance in *B. napus*. Zhao *et al*. (2004) reported that the petiole inoculation technique was a reliable screening method. Bradley *et al*. (2006) evaluated three controlled environment-based methods and also found that the petiole inoculation technique was reliable screening. Garg *et al*. (2008) reported the use of a cotyledon inoculation method to evaluate resistance to *Sclerotinia* stem rot in *B. napus*. They reported that the cotyledon assay had a positive correlation with field evaluation results. Jurke and Fernando (2008) compared four growthroom techniques using ten *B. napus* cultivars/lines. Most of the techniques showed variable results including field evaluation trails between the replicates. They reported that the mypetal technique was a most reliable screening method as there were no significant trail effects. As outlined above, a number of methods have been suggested in the literature to evaluate *Sclerotinia* stem rot resistance. Different studies

reported different methods as reliable. However, there is no universally accepted *Sclerotinia* stem rot screening method.

The inheritance of host resistance to *Sclerotinia* stem rot is polygenic in nature (Bolton *et al*., 2006). A number of quantitative trait loci (QTL) are thought to provide partial resistance to *Sclerotinia* stem rot in *B. napus* (Yin *et al.* 2010; Zhao and Meng, 2003; Zhao *et al.*, 2006). A considerable amount of progress has been made in QTL mapping using molecular markers. Marker assisted selection has become a routine procedure in many breeding programs. The use of markers linked to QTL associated with selected traits can be effectively utilized to hasten the breeding objectives. The markers linked to *Sclerotinia* stem rot resistance can be used to develop resistant cultivars through marker assisted selections.

The development of a genetic map for the population is important step in QTL mapping. Genetic maps can be developed by using different types of molecular markers. A number of PCR-based molecular marker systems are available but sequence related amplified polymorphism (SRAP) is gaining popularity. It is a simple, high throughput, one step PCR protocol and amplifies more than one allele (Li and Qurois, 2001). The SRAP marker technique has been used to develop an ultra dense genetic map in *B. napus* (Sun *et al*., 2007).

There are very few studies reported on QTL mapping for resistance to *Sclerotinia* stem rot in *B. napus*. The number of QTL reported varies considerably. Zhao and Meng (2003) identified six QTL responsible for *Sclerotinia* resistance in an F_3 population of *B. napus*. Zhao *et al*. (2006) evaluated two DH populations of *B. napus*

for resistance to *Sclerotinia* stem rot and identified 13 QTL in both populations. Yin *et al*. (2010) evaluated one DH population using three screening methods. They reported 21 QTL responsible for resistance to *Sclerotinia*. These studies have reported very little consistency in the results indicating profound environmental effects on resistance evaluation. There were very few common QTL identified between the replicates. None have reported common QTL between the populations. The markers linked to *Sclerotinia* stem rot resistance are not publicly available. Therefore, there is an urgent need to identify QTL responsible for resistance to *Sclerotinia* stem rot in different populations, identify common QTL between the populations and identify marker linked to resistance loci that can be used in marker assisted selection.

The objectives of this study were:

1. To compare four controlled environment-based *Sclerotinia* stem root screening methods to identify a suitable screening method for large-scale screening in *B. napus.*

2. To evaluate five doubled haploid (DH) *B. napus* populations for resistance to *Sclerotinia* stem rot using the screening method identified in objective one.

3. To identify QTL and linked markers for resistance loci to *Sclerotinia* stem rot in each of five DH *B. napus* populations.

4. To identify common QTL among the five DH *B. napus* populations.

2.0 LITERATURE REVIEW

2.1. Brassica Species

The *Brassica* genus belongs to the family Brassicaceae (previously known as Cruciferae). The natural process of interspecific hybridization and development of stable hybrids has lead to new and diverse *Brassica* species. This genus comprises of a large group of highly diverse species and sub-species. For example, *Brassica oleracea* L. offers a number of crops which are morphologically distinct such as Brussels sprouts, cauliflower, broccoli, Chinese kale, kohlrabi, cabbages (white-headed, red-headed and savoy-headed). Similarly, *B. rapa* offers caisin, Chinese cabbage, Pak Choi, turnip rapes, mizuna, tatsoi and oleiferous crops (Dixon, 2006).

The wild diploid species after intercrossing and chromosome doubling resulted in amphidiploids as new species. The Korean botanist U (1935) was the first person to reveal the relationship between the amphidiploid species and their diploid progenitors. The pictorial representation which discloses the relation among these species is famously known as the "triangle of U". According to U (1935) there are three diploid species, *B. campestris* L. (Syn. *B. rapa*), *B. nigra* (L.) Koch. and *B. oleracea* L. Their genomes are designated AA (2n=20), BB (2n=16) and CC (2n=18), respectively. These diploid species intercrossed leading to form the amphidiploids *B. juncea* L. Czern. (AABB, 2n=36), *B. napus* L. (AACC, 2n=38) and *B. carinata* Braun (BBCC, 2n=34).

The Brassica *genus* offers a variety of products for human and livestock consumption. These include leaves for salad and curries, roots or inflorescences as vegetables, whole above-ground plants as fodder or green manure, seeds for edible oils

and condiments (Dixon, 2006). The oil also has its usage in lubricatant industries and can be used for illumination in oil lamps. Dried plant remains are still used as fuel for cooking in many Asian countries. Oleiferous *Brassicas* contribute 14% of global vegetable oils (Gupta and Pratap, 2007).

2.2. History of Rapeseed and Canola in Canada

The terms rapeseed and canola can be applied to both *B. rapa* and *B. napus*. Rapeseed is not a native crop to Canada. Immigrants from Europe and the UK brought rapeseed to Canada. Fred Solvoniuk, a polish immigrant, received a bag of seeds from his home country. He sowed those seeds at his farm at Shellbrook (SK) in 1936. This marked the introduction of rapeseed to Canada. The seeds later were identified to be *B. rapa* (www.oilseedrape.org.uk).

The initial cultivation was restricted to home use with no commercial interest. During the Second World War, however, as the supply of rapeseed oil was cut short, the Government of Canada provided price sureties to encourage growers. After the war, cultivation of rapeseed was again reduced to a minimum. In Canada, breeders in the public sector saw rapeseed as a potential export crop to the Asian market. Their interest resulted in a massive introduction of germplasm from across the world in breeding programs. The initial objectives were to screen germplasm for adaptability and yield. In 1954, Canadian breeders from Agriculture and Agri-Food Canada, Saskatoon released the first *B. napus* cultivar 'Golden'. 'Arlo' was Canada's first *B. rapa* cultivar released in 1958. The cultivars released until 1970 were high in erucic acid (40-45%) and glucosinolates (> 100 µmole/g air-dried oil-free meal) (Stefansson and Downey, 1995).

A number of cultivars were released during 1950's and the main emphasis still was on yield, uniformity, lodging resistance and oil content. But during the 1950's, researchers in human nutrition published many reports regarding the negative effects of high erucic acid and glucosinolate content to human and animals. In 1956, the Canadian Department of National Health and Welfare imposed a ban on the sales of rapeseed oil for edible purposes (www.oilseedrape.org.uk). Breeders all over the world directed their efforts to develop cultivars which were low in erucic acid and in glucosinolates. *Brassica napus* cultivar 'Liho' was identified as a source of low erucic acid content (McVetty *et al.,* 2009). The source of low glucosinolates was identified in a *B. napus* cultivar 'Bronowski' which is still believed to be the only genetic source of low glucosinolate content. Although other sources had been found, the trait could not be stably introgressed to new cultivars from these additional sources (Agnihotri *et al.,* 2007).

In 1974, Dr. Stefansson developed the world's first 'double low' *B. napus* cultivar 'Tower' at the University of Manitoba (Stefansson and Downey, 1995). 'Tower' had less than 1% erucic acid content in oil and less than 30 μmole/g aliphatic glucosinolates in air-dried oil-free meal. The term 'Canola' was coined and trademarked for such double low rapeseed oil. Canola is derived from the description Canadian oil low acid. The term was registered by the Western Canadian Oilseed Crushers' Association. The trademark was later transferred to the Rapeseed Association of Canada in 1980. In 1980, the Rapeseed Association of Canada changed its name to Canola Council of Canada and therefore the trademark was transferred to the council (Gupta and Partap, 2007).

Regardless of the negative health effects when consumed, erucic acid has high demand as industrial oil. The demand for erucic acid has been on a continuous rise.

Breeding programs have arisen to cater to this niche market. The main breeding objectives for such breeding programs are to develop better HEAR (high erucic acid rapeseed) cultivars and SHEAR (super high erucic acid rapeseed) germplasm (McVetty *et al*., 2009). HEAR and SHEAR cultivars are high in erucic acid (>50%) and low in glucosinolates (<20 µmole. g^{-1} seed). A number of HEAR cultivars have been released by the University of Manitoba during last decade (McVetty *et al.,* 2006a; McVetty *et al.,* 2006b; McVetty *et al.,* 2000a ; McVetty *et al.,* 2000b; McVetty *et al.,* 1999).

2.3.1. *Sclerotinia* Stem Rot: Economic Importance

Rapeseed/canola cultivation worldwide suffers from a number of diseases. The importance of disease varies with geographic area of cultivation. In North America, blackleg and *Sclerotinia* stem rot are two major diseases of rapeseed/canola. *Sclerotinia* stem rot causes significant yield losses because it causes loss of plant structural integrity, loss of photosynthetic area, lodging and premature death. The disease is sporadic to regular and varies from location to location and year to year. The losses may be 0 to 100% (Purdy, 1979). In Canada, *S. sclerotiorum* has been reported from almost all the provinces (McDonals and Boland, 2004; McLaren *et al*., 2004). In Manitoba, losses range from 5 to 100% with an average yield loss of 0.4 to 0.5 times the per cent infection (Manitoba Agriculture, 2009; Morrall *et al*. (1984). Del Rio *et al*. (2007) reported that there was 0.5% yield loss for every unit percentage of disease incidence. They further revealed that 17% disease incidence had the same economic loss as the cost of fungicide application. Therefore, 17% disease incidence should be considered as economic threshold.

In 2009, *Sclerotinia* stem rot and blackleg were the most prevalent diseases in Manitoba rapeseed/ canola fields. On average, *Sclerotinia* stem rot was present in 91% of the sampled fields. The average disease incidence ranged from 7 to 37% with a provincial mean of 18% during 2009 (McLaren *et al*., 2010).

In North Dakota and Minnesota the average yield losses were around 13%, however, the losses of up to 50% were reported in some fields during 2008 (Markell *et al.,* 2009). The annual losses due to *Sclerotinia* diseases were estimated to be around $482 million in all host species, and canola crop losses alone were around $24 million in the United States. This large economic impact has led to the development of the National Sclerotinia Initiative to reduce the losses due to *Sclerotinia* diseases (United States Department of Agriculture, 2009). There is an increase in annual yield losses due to *S. sclerotiorum* (Fernando *et al.*, 2004).

2.3.2. *Sclerotinia* Stem Rot: The Disease Symptomatology

Among *Brassica* crops, *S. sclerotiorum* infects cabbage, cauliflower, Brussels sprouts, broccoli and oilseed rape. The disease symptoms vary with hosts (Buchwaldt, 2007; Purdy, 1979). Symptoms under field conditions appear during the flowering stage. The first symptoms appear on the petals as mushy light brown lesions on and around the petals (Markell *et al*., 2009). The symptoms on young stems appear as water-soaked lesions which progress in all directions and cause stem girdling. On leaves, water soaked lesions later turn to blight-like lesions. The leaf lesions are irregular and grayish necrotic spots (Bardin and Huang, 2001; Buchwaldt, 2007). On stems, the symptoms usually start from the axils of leaves as white to grayish lesions. As the disease progresses, these

lesions increase in size and the whole stem shows discolouration due to bleaching of green chlorophyll.

On severely affected plants, the stem weakens and becomes shredded. The structurally weakened plants may die prematurely or lodge with seed weight or heavy winds. At the end of season, black sclerotia can be seen inside or on the surface of stem (Buchwaldt, 2007). Under favourable conditions of high humidity and moderate temperatures, small mushroom like structures, the apothecia may be produced on sclerotia (Markell *et al.,* 2009).

2.4.1. *Sclerotinia sclerotiorum*: The Fungus

Sclerotinia sclerotiorum (Lib.) de Bary is a non-host specific necrotropic fungus. The fungus is known to infect 408 plant species from 278 genera and 75 families of the Plant kingdom (Boland and Hall, 1994). The wide host range indicates its cosmopolitan habitat. There are more than 60 names given to the diseases caused by this fungus (Purdy 1979).

The fungus has been known for almost two centuries. *Sclerotinia sclerotiorum* (Lib.) de Bary was first reported by Libert in 1837 as *Peziza sclerotiorum*. In 1879, Fuckel transferred it to the genus *Sclerotinia* and renamed it as *Sclerotinia libertania* Fuckel. However as per International Code of Botanical Nomenclature, a species must retain its original specific epithet while being transferred from one genus to other. The name *Sclerotinia sclerotiorum* was first given to this fungus by de Bary in 1884 (Purdy, 1979). Thus *Sclerotinia sclerotiorum* (Lib.) de Bary was accepted as a conserved name in 1981 (Bolton *et al.,* 2006; Kohn 1979b).

Sclerotinia sclerotiorum (Lib.) de Bary belongs to the family Sclerotiniaceae of the order Helotiales in the phylum Ascomycota (Bolton *et al.,* 2006). The family Sclerotiniaceae was introduced by Witzel (1945) to include fungal species which produce inoperculate acsi from stipitate apothecia, globose spermatia and stromata. He provided a key depending upon stroma type, presence of functional conidial stage and type of conidia and ascospore color as characters to assign species to the genera. The family has gone through many revisions and changes since its establishment. The family Sclerotiniaceae presently includes thirty three genera including several economically important plant pathogens, such as *Sclerotinia*, *Botrytis* and *Monilia* (Bolton *et al.,* 2006). The general character shared by all member species is the formation of dark sclerotial stroma as perennating structures during unfavourable conditions. The fungi of the family Sclerotiniaceae are inhabitants of temperate to sub-arctic areas of the northern hemisphere (Willetts and Wong, 1980).

The genus *Sclerotinia* was erected by Fuckel in 1870 (Purdy, 1979). Kohn (1979b) delimited the genus to include only species in which the outermost layer of the apothecium is made of globose cells perpendicular to receptacle surface. The fungi of this genus cannot produce asexual spores, the conidia. She provided a detailed key to assign species to the genus *Sclerotinia*. She retained only three species, namely *S. sclerotiorum* (Lib.) de Bary, *S. minor* Jagger and *S. trifoliorum* Eriks out of 250 epithets. Later *Sclerotinia asari* (Wu and Wang, 1983) and *S. nivalis* (Saito, 1997) were also included based on DNA analysis. *Sclerotinia sclerotiorum* (Lib.) de Bary and *S. minor* Jagger are the only two species from this genus reported in Canada (Bardin and Huang, 2001)

Sclerotinia sclerotiorum hyphae are multinucleate, septate, hyaline and branched. The mycelium is generally white in colour and gives a fluffy appearance on plant tissue and artificial culture media (Bolton *et al.,* 2006). The number of nuclei per asci is two and the haploid chromosome number is eight for this fungal pathogen (Kohn, 1979a; Willetts and Wong, 1980). *Sclerotinia sclerotiorum* does not produce asexual spores (Bolton *et al.,* 2006; Kohn, 1979b).

Necrotrophic plant pathogens with a broad host range generally produce a large number of toxic chemicals and hydrolytic enzymes. These molecules have low or no interactions with the host and thus demonstrate low host specificity and are toxic to a broad host range (Hegedus and Rimmer, 2005; Koehler and Ayers, 1972; van Kan, 2006). These hydrolytic enzymes have many isozymic forms which further limit the host defense (Keon *et al*., 1987; Riou *et al*., 1991). *Sclerotinia sclerotiorum*, being a necrotrophic fungus releases a number of hydrolytic enzymes to hydrolyse the host tissue. Oxalic acid produced by *S. sclerotiorum*, also plays an important role as pathogenicity factor (Godoy *et al*., 1990; Rollins and Dickman, 2001).

Sclerotinia sclerotiorum produces a wide range of endo- and exo-polysaccharide degrading enzymes. Riou *et al.* (1991) found cellulosic, hemicellulosic and pectinolytic enzymes secreted by this fungi on 16 carbohydrate substrates. They also found glycoside hydrolase enzymes which help polysaccharidases to further degrade the polysaccharides to monosaccharides. They also found isozymes of various hydrolytic enzymes and suggested that different isozmyes may be regulated by different control mechanisms. Riou *et al*. (1992) purified two pectinolytic enzymes, expolygalacturonase (exoPG) and

expolymethylgalacturonase (exoPMG). The enzymes showed optimal activity at pH 5, temperature 45 ^{0}C and had substrate specificity.

Poussereau *et al.* (2001a) detected acid proteases produced by *S. sclerotiorum* on minimal media containing sunflower extracts. The maximum activity was detected at pH 4.5. On further analysis, they found the two groups of proteases, the acid asypartyl proteases and acid non-aspartyl proteases. In the same study, they cloned and sequenced *acp1*, a gene encoding an acid protease enzyme and reported that its expression was regulated by external pH and plant substrate. Poussereau *et al.* (2001b) isolated and cloned the gene *aspS* that encodes aspartyle protease in *S. sclerotiorum*. RT-PCR analysis further revealed that *aspS* was expressed from the start of infection of sunflower cotyledons. Billon-Grand *et al.* (2002) identified a number of acid proteases and one neutral protease produced by *S. sclerotiorum* on artificial media as well as *in planta*. The enzymatic activities of acidic enzymes were the same both *in vitro* and *in planta* and their levels corresponded to the levels of colonization of the fungus and the amount of host tissue degraded.

Sclerotinia sclerotiorum produces oxalic acid which is an important pathogenicity factor. Noyes and Hancock (1981) identified oxalic acid from sunflower extracts. They further revealed that the level of oxalic acid was 15 times higher in wilted leaves than in the healthy leaves. Marciano *et al.* (1983) reported that the virulence of *S. sclerotiorum* corresponded to the amount of oxalic acid produced. Cotton *et al.* (2003) revealed that the expression of three endoPG encoding genes *pg1-3* was firmly under the control of pH. Godoy *et al.* (1990) demonstrated the role of oxalic acid in the pathogenicity of *S.*

sclerotiorum using a mutant deficient in oxalic acid production. The mutants were nonpathogenic and produced very small lesions on inoculated bean leaves.

Oxalic acid assists pathogenicity of *S. sclerotiorum* in a number of ways. It reduces the pH in the host tissue which is required by many cell wall degrading enzymes. Low pH also inhibits the activity of polygalacturonase-inhibiting proteins produced by the host thus restrains the plant defense. Oxalic acid chelates Ca^{+2} of the middle lamellae therefore disrupting the cell wall integrity. Chelation of Ca^{+2} also inhibits the plant's defense mechanisms. Oxalic acid suppresses the oxidative burst response and activity of polyphenol oxidase which are important host defense mechanisms. Finally, oxalic acid inhibits stomatal closure leading to dehydration (Bolton *et al*. 2006; Hegedus and Rimmer, 2005).

2.4.2. *Sclerotinia sclerotiorum*: The Sclerotia

Under adverse environmental conditions including limited nutrients, mycelia produce sclerotia (Sing: Sclerotium) both *in planta* and on media (Le Tourneau, 1979; Willetts and Wong, 1980). The sclerotia act as vegetative survival structures. Sclerotia can remain viable up to 4 or 5 years under field conditions (Adams and Ayers, 1979). A sclerotium histologically consists of three regions; an outer dark rind, a middle cortex and core which is called the medulla. The outer rind is generally dark in colour due to the deposition of melanin and is usually three cells wide. The cortex is two to four cells in thickness and is made up of pseudoparenchymatous cells. The medulla constitutes prosenchymatous cells (Willetts and Wong, 1980). The melanin deposited in the rind plays an important role in sclerotia survival. Melanin is water insoluble, resistant to concentrated acids, bleach and oxidizing agents (Henson *et al.,* 1999).

Sclerotinia spp. spend almost 90% of their life cycle as sclerotia in the soil (Adams and Ayers, 1979). A number of factors could be responsible for sclerotia initiation. These include temperature, light, mechanical barriers, nutrition limitation and H^+ concentrations (Le Tourneau, 1979; Willetts and Wong, 1980).

The development of sclerotia can be differentiated into three stages namely initiation, growth and maturation (Townsend and Willetts, 1954). Any of the above discussed factors could be responsible for sclerotia initiation, which promotes the formation of sclerotial primordia. There is profuse dichotomous branching of long aerial hyphae followed by their aggregation during the growth phase. The hyphal aggregation increases in size to a maximum limit. During this stage, the sclerotia store nutrients which are essential for their survival. During the maturation stage, sclerotia stop growing in size, deposit pigment in the rind. This is followed by dehydration of sclerotial tissues (Willetts and Wong, 1980).

A limited number of studies have investigated the factors and chemicals involved in the inhibition of sclerotia formation. Continuous sub-culturing on artificial media inhibits sclerotium development. Certain chemicals such as ρ-aminobenoic acid, phenylthourea, Al^{+2} and flurophenylalanine have been identified which inhibit sclerotia formation but these chemicals have inhibitory effects only at particular concentrations. Several of compounds such as metal ions, buffers, cations, anions and low molecular weight carbohydrates also can inhibit sclerotia germination (Le Tourneau, 1979).

The survival of sclerotia under field conditions depends upon a number of factors. The soil temperature and soil moisture play important roles in the survival of sclerotia in the field. High temperature and high soil moisture have the most damaging effect on

sclerotia survival (Nelson, 1998). Adams and Ayers (1979) reported that soil temperature of 10-30 ^{0}C had no adverse effect on sclerotia survival. Adam (1975) reported that a constant temperature of 35 ^{0}C for three weeks reduced sclerotial survival. Flooding the field for 26 to 31 days killed 100% of the sclerotia (Moore, 1949). Another important factor pertaining to sclerotial survival is soil microbes that parasitize sclerotia. Select bacterial and fungal species can degrade sclerotia for carbon sources. These species include *Coniothyrium minitans*, *Gliocladium* spp. *Fusarium* spp., *Triochoderma* spp. *Penicillum* spp, *Aspergillus* spp., *Stachybotrys* spp. and *Verticillium* spp. (Adams and Ayers, 1979; Fernando *et al.*, 2004 Nelson, 1998).

The studies have little consensus on duration for which sclerotia can remain viable. Brown and Butler (1936) reported that the sclerotia can remain viable for up to ten years under favourable conditions while Adams and Ayers (1979) suggested that the sclerotia can remain viable for four to five years.

2.4.3. *Sclerotinia sclerotiorum*: The Life Cycle

Sclerotinia sclerotiorum under natural conditions spends most of its life cycle as sclerotia in soil or plant debris. Sclerotia can germinate either myceliogenically or carpogenically depending upon the environmental conditions. Myceliogenic germination leads to the development of fungal mycelial hyphae which can infect host plants. The sclerotia can germinate carpogenically to produce apothecia (Adams and Ayers, 1979; Bolton *et al*., 2006). A number of factors including environment play important roles in either type of germination. These include temperature at which sclerotia are formed, their size, dryness and age of sclerotia, cyclic dry and wet conditions, presence of exogenous

nutrients, mechanical injuries to sclerotia, relative humidity (RH) and temperature during germination and preconditioning of sclerotia (Abawi and Grogan, 1979; Adams and Ayers, 1979; Clarkson *et al*., 2004; Huang *et al*., 1998; Kohn, 1979b; Le Tourneau, 1979; Liu and Paul, 2007).

Myceliogenic germination occurs if sclerotia are formed at higher temperature range of 20-25 ^{0}C and are air or desiccant dried (Huang *et al*., 1998). The sclerotia can produce mycelia in the presence of exogenous nutrients, and when the sclerotia are fresh and small in size (Abawi and Grogan, 1979). The subsequent dry and wet cycles, mechanical injuries and sclerotial abnormalities such as lack of pigmentation promote nutrient leakage and myceliogenic germination (Huang *et al*., 1998). Huang *et al*. (1998) reported that both fresh and aged sclerotia grow myceliogenically at 100% RH. They also reported that temperature had no effect during 100% RH whereas interactions were significant at 90% RH. Disease incidence was higher for desiccant dried sclerotia as compared to air dried sclerotia. The myceliogenic germination causes soil-born, below ground infection of very limited extent and therefore it rarely causes epidemics (Abawi and Grogan, 1979).

Carpogenic germination leads to the development of stipe primordia from the medulla of the sclerotia which eventually bear apothecia (Kohn, 1979b). Only the sclerotia present in the top 2-3 cm soil can contribute to apothecial release because the stipes are no longer than 3 cm under field conditions (Abawi and Grogan, 1979).

Le Tourneau (1979) characterized apothecial initials as brown to hyaline interwoven hyphae with dense cytoplasm which divide actively. These initials elongate and emerge from the sclerotial surface and continue to grow. The stipes are positively

phototropic and differentiate to form apothecia only if light of wavelength 390 nm or below is provided. The apothecia are stipitate, cupulate and cinnamon to umber in colour. Each ascus produces 8 ascospores which are hyaline and ellipsoid in shape (Kohn, 1979b). The ascospores are released by a puffing mechanism i.e. forced ejection. Ascospore release is a diurnal process and each apothecium releases up to 7.6 X 10^5 ascospores over 20 days (Clarkson *et al.,* 2003). The temperature is also important factor for the rate of germination and percent germination of sclerotia (Clarkson *et al.,* 2004).

The most important environmental factors for carpogenic germination and spore release are the temperature at which sclerotia were formed, the soil temperature and moisture, RH and light. The sclerotia usually require a pre-conditioning at 10 ^{0}C. The carpogenic germination takes place between 5-25 ^{0}C and there is no to a very small percentage of germination at 26 ^{0}C. The most suitable range is 15-20 ^{0}C. The sclerotia collected from cool climates (10 ^{0}C) germinate faster than the ones collected from hotter climates (25-30^{0}C) (Clarkson *et al*. 2004; Kohn 1979b).

The soil moisture/water potential also plays an important role. Grogan and Abawi (1975) reported that there was carpogenic germination and development of apothecia was at 0 kPa but not at -600 kPa. Clarkson *et al.* (2004) reported that a water potential of approximately – 100 kPa was a threshold for carpogenic germination.

In general, the apothecia are produced during the spring, summer and fall in the case of temperate regions whereas in tropical regions apothecia are produced during seasonal rainfall. These times correspond to suitable moisture and temperature conditions for carpogenic germination and ascospore production (Kohn, 1979b).

Ascospores are a primary source of inoculum. After release, ascospores become airborne and can infect all aboveground plant parts (Abawi and Grogan, 1979; Adams and Ayers, 1979). These ascospores can travel long distances (Adams and Ayers, 1979) and therefore can cause epidemics provided that the environmental conditions are favourable for disease development.

The ascospores from carpogenic germination and mycelium from myceliogenic germination need senescent tissue for their initial growth. The ascospores landing on dead or dying plant parts germinate to produce mycelia in the presence of a water film. Hegedus and Rimmer (2005) called this phase as the opportunistic phase. After colonization, the mycelia invade healthy tissue and decompose the host tissue with the help of hydrolytic enzymes and other toxic molecules. As the host tissue becomes limited or environmental conditions become unfavourable, the mycelia condense together and initiate sclerotial formation. These sclerotia can be formed either in stem pith and/ or fruit cavities and/or on plant parts to complete the life cycle (Hegedus and Rimmer, 2005; Purdy, 1979).

2.5. Disease screening methods

A number of methods have been used to evaluate resistance to *Sclerotinia* stem rot in various crops. In some cases, the same methods were used in different crops. For examples ascospore suspension spray was used for soybean (Cline and Jacobsen, 1983; Hunter *et al.,* 1981) and rapeseed (Newman and Barley, 1987); petiole inoculation technique was used in rapeseed (Zhao *et al*., 2004) and soybean (Del Reo *et al*., 2000; Hoffman *et al*., 2002); stem inoculation was used in soybean (Wegulo *et al*., 1998) and sunflower (Vuong *et al*., 2004) and oxalic acid test in soybean (Wegulo *et al.,* 1998) and

canola (Bradley *et al.,* 2006) to evaluate resistance to *Sclerotinia* stem rot with or without modifications.

The methods used to screen resistance to *S. sclerotiorum* can be broadly divided into two categories (i) field evaluation or (ii) controlled-environment-based evaluation depending upon the site of evaluation. Field evaluation can be performed either by growing the germplasm in naturally infested fields (Bradley *et al*., 2006; Grau *et al*., 1982; Kim *et al*., 2000) or using artificial methods of inoculation to inoculate plants in the field (Bradley *et al*., 2006; Gossen and McDonald, 2007, Wegulo *et al*., 1998; Yin *et al.,* 2010). Disease development in field evaluations entirely depends upon the environmental conditions during the evaluation period. These methods exploit the host-pathogen interaction under natural conditions. However, naturally infested fields are limited to a few locations and artificial inoculation methods under field conditions are laborious, time consuming and frequently result in inconsistent disease pressure. Environmental variations also play an important role in both disease incidence and disease severity. It is therefore difficult to achieve expected and reproducible results in the field (Jurke and Fernando, 2008; Whipps *et al*., 2002).

Controlled-environment-based screening can provide better estimates of physiological resistance. These methods are fairly quick, testing can be done several times per year and most of the methods are inexpensive. A number of controlled environment-based screening methods have been suggested to evaluate *Sclerotinia* stem rot resistance in various crops.

The first controlled-environment-based screening of oilseed rape was reported by Newman and Bailey (1987). They screened eight breeders' selections and eight

commercial cultivars of oilseed rape for resistance to *Sclerotinia* stem rot. They inoculated plants by spraying an ascospore suspension on the plants at mid-flowering stage. The main stem lesion length was measured at 12 -14 days after inoculation. The plants were covered with polythene bags to maintain high humidity. They reported that the results were inconsistent.

Bailey (1987) screened six breeding lines using a detached leaf assay. He compared different inoculum media and reported that mycelium grown on water agar was the most effective method for screening.

Zhao *et al*. (2004) developed a comparatively simple and inexpensive method for screening *B. napus* germplasm for resistance to *Sclerotinia* stem rot using the petiole inoculation technique. The technique was first developed by Del Reo *et al*. (2000) for soybean. In this technique, the fungus was loaded into 1 ml pipette tips by pushing the open end of the tip into advancing edges of three day old mycelial culture. The petioles of the third fully expanded leaves of four week old plants were cut at 2.5 cm away from the main stem. The pipette tips with inoculum were pushed onto the petiole stubs. The data were recorded using two separate scoring systems. In the first, the days to wilt was recorded daily for 7 to 11 days after inoculation. In the second system, lesion phenotypes were recorded on a scale of zero to four starting with zero for no symptoms to four for expanded, sunken, water soaked lesions resulting in wilt of the foliage. They reported that the differences were significant among cultivars in all the experiments. The scores of days to wilt and lesion phenotypes were highly correlated.

Bradley *et al*. (2006) compared the response of canola cultivars to *Sclerotinia* stem rot using the petiole inoculation technique, oxalic acid assay and detached leaf assay

with field testing. They reported that the differences among the cultivars were significant for the petiole inoculation technique and oxalic acid assay but not for the detached leaf assay. They also reported that the interactions for field testing were significant for cultivar-location, year-location, year-cultivar and location-year-cultivar.

Garg *et al*. (2008) developed a cotyledon assay for *Sclerotinia* stem rot resistance evaluation in *B. napus*. They inoculated cotyledons of ten day old plants with mycelial suspension. The cotyledons were wounded and 10 µl of mycelial suspension was applied on each wound. The plants were kept under high humidity conditions for two days and later transferred to normal greenhouse conditions. They reported significant differences among all the cultivars and a positive correlation with field testing.

As mentioned above, a number of methods have been used with varying results and occasional correlation with field screening. Many of these methods also showed varying results across labs and crops. Some methods such as the detached leaf assay, the cotyledon leaf assay and the petiole inoculation technique are suitable for large scale screening while other techniques require more resources. Studies are needed to further develop a universally accepted screening method which is simple, reliable and positive correlated with field screening.

2.6. Molecular markers

Markers which represent the genetic differences among individuals are called genetic markers. Genetic markers may be classified as morphological, biochemical or DNA markers, depending upon the site of predicted variation. Morphological markers indicate the morphological differences between the individuals such as plant color, shape, texture, height etc. Biochemical markers usually represent differences in the properties of

enzymes (e.g. isozymes). Morphological and biochemical markers have a number of limitations e.g. these are available in limited numbers. They are often influenced by environmental factors and may be available only at a particular developmental stage. However, the DNA (molecular) markers indicate differences at the DNA level. These markers overcome the limitations of both morphological and biochemical markers (Collard *et al*., 2005; Winter and Kahl, 1995).

Molecular markers can classified as hybridization-based, polymerase chain reaction (PCR)-based and DNA sequence-based markers (Collard *et al*., 2005). Hybridization-based makers represent differences in the DNA of individuals by hybridizing the target DNA with probes e.g. restriction fragment length polymorphism (RFLP) (Helentjaris *et al*., 1986) and expressed sequence tags (Qin *et al*., 2001). PCR-based markers amplify the target DNA using PCR in thermocyclers e.g. random amplified polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams *et al.,* 1990), microsatellite markers (SSR) (Litt and Luty, 1989), amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995) and sequence related amplified polymorphism (SRAP) (Li and Quiros, 2001). The DNA sequence based markers are usually developed to identify the sequence-based differences between the individuals e.g. single nucleotide polymorphism (SNP) (Vignal *et al.,* 2002).

Each marker system has its own advantages and disadvantages. Hybridization-based techniques are labourious and expensive. There are a number of SNP detection techniques but they require very expensive instruments. These techniques are therefore limited to well funded and equipped labs. PCR-based marker techniques, however, are relatively simple and inexpensive to use. PCR-based maker techniques have their own

advantages and disadvantages. RAPD has low repeatability and shows inconsistent results among the labs. Microsatellite markers (SSR) are more expensive to develop and only one or few loci are amplified per primer pair. Although AFLP amplify multiple loci, this technique requires multiple steps. SRAP technique as compared to others is simple, amplifies multiple loci and is reliable (Li and Quiros, 2001).

Marker development using the SRAP technique requires of two primers, a 15-25 nt forward primer and a 15-25 nt reverse primer. These primers are consisted of 10 or 11 random nt as "filler" sequences at the 5' end, followed by CCGG bases in forward primers and AATT in reverse primers and three selective bases at the 3' end. CCGG sequences are used in SRAP primers to target open reading frame regions as these are rich in GC content (Li and Quiros, 2001).

In the original protocol, the PCR products were separated on acrylamide gels and detected by autoradiography (Li and Quiros, 2001). But later, one of the two primers was labeled with fluorescent dyes and PCR products were detected on an ABI DNA analyzer (Sun *et al*. 2007). This made the SRAP technique high throughput, safe and comparatively less expensive than the original technique. SRAP markers have been used for construction of genetic linkage maps (Chen *et al*. 2010; Sun *et al*. 2007) and gene mapping (Rahman *et al.* 2008; Rahman *et al*., 2007) and QTL analysis (Yuan *et al*., 2008; Zhang *et al*., 2009).

2.7. Quantitative Trait Loci Mapping

Genes are responsible for genetic variation and are the basis for genetic and breeding research. Depending on the type of variation in a segregating population, traits

may be classified as either qualitative or quantitative traits. Qualitative traits are the ones which show discontinuous variation and the progeny can be divided into discrete classes. These traits are governed by one or a few genes thus these are also known as major gene, Mendelian gene or oligogenic traits. On the other hand, the quantitative traits show continuous variation. Nelsson-Ehle (1908) reported that the inheritance of such a quantitative trait is governed by many genes with additive effects. These traits are usually subjected to measurements. The environment has profound effect on quantitative traits. The quantitative traits are also known as minor gene, metric or polygenic traits.

The breeding and selection of qualitative traits is generally easier than for quantitative traits. Most economically important traits, however, such as yield, quality and some types of disease resistance are quantitative in inheritance. The sites or loci on the chromosomes that are associated with a given quantitative trait are called Quantitative trait loci (QTL) (Collard *et al*., 2005). The advent of molecular marker techniques which represent the genetic variation among the individuals has made it possible to relate genetic variation to phenotypic variation. Biometric methods have been developed to co-analyze the segregation of markers and phenotypic values of a population for mapping these QTL (Asins, 2002). Collard *et al*. (2005) defined QTL mapping as "the process of constructing linkage maps and conducting QTL analysis to identify genomic regions associated with traits".

There are several different types of segregating populations which can be used for QTL analysis. The most commonly used are F_2, back cross progenies (BC), recombinant inbred lines (RILs) or double haploid (DH) lines. F_2 and BC populations are easier and require less time to develop. F_2 populations are better than BC because QTL of recessive

alleles from the recurrent parent cannot be detected in BC populations (Asins, 2002). Although DH lines and RILs are more difficult to produce, these provide a number of advantages over the F_2 and BC populations. These populations (DH and RIL) can be replicated and thus provide an opportunity to evaluate any trait as many times as required. The DH and RIL populations can be of small size as compared to BC populations to provide same the quality of results. Carbonell *et al*. (1993) from their simulated populations concluded that DH and RIL populations provide more accurate locations of QTL with less variance. They also reported that power of identification of QTL with low heritability (5%) was 90% for DH and RIL populations as compared to F_2 and BC populations which should have a heritability of 14% to have the same level of predication power.

A number of methods have been proposed to detect QTL. The most commonly used methods are single-marker analysis, simple interval mapping and composite interval mapping. Single-marker analysis uses simple statistical tools such as t-test, analysis of variance and linear regression. It simply detects single marker linked with QTL (Liu 1998). The advantage of single-maker analysis is that QTL analysis can be performed without a complete linkage map. But if the marker is not closely linked to a QTL, the QTL may not be identified (Tanksley, 1993).

Simple interval mapping as the name suggests uses intervals between linked markers along the linkage group and analyzes its association with the phenotypic values as compared to single maker in single-marker analysis. The most commonly used method is composite interval mapping. It integrates both interval mapping and linear regression (Collard *et al*., 2005). Composite interval mapping is more robust at identifying QTL

than other methods. Simple interval mapping and composite interval mapping analyses both provide statistic values such as likelihood ratio statistics (LRS) or logarithmic of odds (LOD) scores. The LRS or LOD values are used to identify the position of a QTL on the map. The two are related to each other as LRS = 4.6 X LOD (Liu, 1998). Any LOD value between 2 to 3 is used as threshold or cutout value to identify QTL. Any QTL with a LOD value higher than this threshold value is deemed significant (Collard *et al*., 2005).

A number of factors influence the QTL analysis and mapping. The major factors are genetic properties, population sizes, environmental factors and experimental errors. As discussed above, the quantitative traits are governed by many genes. But all QTL do not have same phenotypic effect. Thus QTL having small effects may not be detected as their LOD values could be lower than a threshold value. Although there is no rule to calculate optimum size of a population, the larger the population size, the better the mapping studies thus the more accurate QTL analysis. The quantitative traits are highly influenced by environment. Thus environment can result in incorrect phenotypic evaluation and in turn influence the QTL analysis. The experimental errors are the ones that occur at experimenter's level, which may include errors in genotypic and phenotypic data (Collard *et al*., 2005; Liu, 1998; Tanksley, 1993).

The QTL mapping studies help in understanding the genetics of quantitative trait but a major objective of such studies is to find linked markers to major QTL for molecular assisted selection (MAS) (Bernardo, 2008). Although these QTL are difficult to find, once found they can be a real asset for breeders. The heritability of a quantitative trait is usually low thus MAS can help in the selection of genotypes. Concibido *et al*.

(2004) compared the cost of screening soybean cyst nematode resistance with MAS to manual inoculation. They estimated that the disease screening cost ranged between $1.50 to $5.00 per data point for manual inoculation as compared $0.25 to $1.00 per data point for MAS. Another added advantage is that the environmental factors during the genotypic selection do not have any influence on the selection.

Pumphrey *et al*. (2007) used microsatellite markers for *Fhb1* QTL conferring resistant against fusarium head blight in wheat to develop 19 nearly isogenic lines (NILs). They reported that lines with *Fhb1* QTL showed 23% lesser disease severity and 27% lesser infected kernels. Mudge *et al*. (1997) identified two microsatellite markers Satt038 and Satt130 linked with one major QTL conferring against soybean cyst nematode resistance in soybean. They used these two markers for MAS in segregating populations. They reported that these two markers can collectively predict the phenotype with 98% accuracy. Concibido *et al*. (1996) identified RFLP maker linked to QTL conferring soybean cyst nematode resistance in soybean. They found that MAS predication had an accuracy of 83 to 90%.

2.8. QTL identified for resistance to *Sclerotinia* stem rot in *B. napus*

QTL analyses for *Sclerotinia* stem rot disease resistance have been carried out in several crops such as sunflower, soybean, common bean, *B. napus* etc. *B. napus* has been subjected to analysis in only three studies. In these three studies, a total of four populations were used to identify the QTL for resistance to *Sclerotinia* stem rot using different screening methods.

Zhao and Meng (2003) evaluated 128 $F_{2:3}$ families for resistance to *Sclerotinia* stem rot with two different screening methods to identify QTL related to *Sclerotinia* stem rot resistance. Their genetic mapping resulted in 23 linkage groups (LG) from 107 molecular markers, and the QTL analysis was performed using single-maker analysis using one way ANOVA. Three QTL were identified on LG3, 12 and 17 for the excised leaf assay. The phenotypic variance explained by these QTL ranged from 13.6 to 23.2%. Three distinct QTL were identified on LG7, 10 and 15 for the toothpick method. The phenotypic variance for this trait explained by these QTL varied from 17.4 to 39.8%.

Zhao *et al*. (2006) used two DH populations (HUA and MS) to identify QTL for resistance to *Sclerotinia* stem rot using the petiole inoculation technique. The resistance response to *Sclerotinia* stem rot was recorded on two scales, days to wilt and stem lesion length. In the HUA population, seven QTL for stem lesion length were identified on chromosomes N2, 5, 12, 14, 16 and 19 in three evaluations. The phenotypic variance explained by these QTL ranged from 6 to 22% for stem lesion length. Four QTL for days to wilt were identified on chromosomes N3, 12, 16 and 19 in the same population. The phenotypic variance explained by each QTL ranged 6 to 12% for days to wilt. In the MS population, they identified only one QTL each for stem lesion length and days to wilt on N3 explaining 11.1 and 22.7% phenotypic variation respectively.

Yin *et al*. (2010) identified QTL using three different methods in one DH population. All methods were performed on mature plants. The methods were mycelial toothpick inoculation, mycelial plug inoculation in which mycelial plugs were wrapped in contact the stem and infected petal inoculation in which petals were incubated with mycelial suspension and then placed on clasped leaves.

In mycelial toothpick inoculation, the data were recorded on lesion length at 3 days after inoculation (DAI) and 7 DAI and differences between lesion lengths at 7 DAI and 3 DAI (7-3 DAI) were used to identify QTL. Two QTL were identified on chromosomes N10 and N11 for 3 DAI, explaining phenotypic variance of 14.6 and 21.5% respectively. For 7 DAI, a total of three QTL were identified, one on chromosome N7 and two on N10. The phenotypic variance explained by these QTL was 14.6, 12.8 and 36.1% respectively. Four QTL were identified for (7-3 DAI), one each on N4, N7 and two on N12. The same evaluation system was used to identify QTL for resistance to *Sclerotinia* stem rot in the mycelial plug inoculation method. Three QTL were identified for 3 DAI, two QTL on N3 and one QTL on N4. Four QTL were identified for 7 DAI, one on each N3, LG11 and two on N17. For 7-3 DAI, three QTL were identified on N1, 6 and LG11. In the case of the infected petal inoculation method, lesion length was recorded 10 DAI to identify QTL for resistance to *Sclerotinia* stem rot and only one QTL was identified on N12.

The studies reported in the literature show little consistency of identified QTL for *Sclerotinia* stem rot resistance in *B. napus* between replications, methods and populations. This clearly indicates that the environmental factors have marked effects on *Sclerotinia* disease development.

3.0 COMPARISON OF SCREENING METHODS FOR *Sclerotinia sclerotiorum* RESISTANCE IN *Brassica napus* UNDER CONTROLLED ENVIRONMENT CONDITIONS

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3.0 Comparison of screening methods for *Sclerotinia sclerotiorum* resistance in *Brassica napus* under controlled environment conditions

3.1 Abstract

Sclerotinia sclerotiorum (white mold) causes significant losses in many economically important crops. A number of methods have been suggested to evaluate resistance/ susceptibility to *Sclerotinia* diseases. Four controlled environments screening methods: 1) excised leaf assay, 2) cotyledon assay, 3) mycelial stem inoculation technique and 4) petiole inoculation technique were compared for their reliability and suitability for large scale screening in canola / rapeseed using eight *Brassica napus* genotypes varying in *Sclerotinia* resistance / susceptibility. The excised leaf assay showed significant differences among genotypes and replicates while there were no significant differences among genotypes for the cotyledon assay. The mycelial stem inoculation technique and the petiole inoculation technique showed significant differences among genotypes and non-significant differences among the replicates. The mycelial stem inoculation technique and the petiole inoculation technique were identified as reliable screening methods. Spearman rank correlation was used to compare the mycelial stem inoculation technique with the petiole inoculation technique but no correlation was detected between the two at $P = 0.05$, although the known field resistant checks in both methods were rated as resistant. The virulence of four *S. sclerotiorum* isolates from four different countries was also compared in a split plot design using the petiole inoculation technique. The differences for virulence and isolate-genotype interactions were found to be non-significant at $P = 0.05$.

3.2 Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic, non-host specific fungus. It infects more than 100 plant species in Canada (Boland and Hall, 1994). *Sclerotinia sclerotiorum* causes significant yield losses in many economically important crops such as sunflower, soybean, common bean and canola/ rapeseed. The percent yield losses range from 0.4 to 0.5 times the percent infected plants (Morrall *et al*., 1984). Del Rio *et al*. (2007) estimated 0.5 % yield loss per unit percentage of disease incidence in *B. napus*. The annual losses in major crops due to *Sclerotinia* diseases are around 500 million alone in the United States of America (United States Department of Agriculture, 2009).

The conventional disease management strategies include cultural practices, chemical fungicides and host resistance. Cultural practices aim to reduce the inoculum load and relative humidity required for carpogenic germination. The success of chemical controls entirely depends upon disease forecasting for appropriate application timing (Bardin and Huang, 2001; Fernando *et al.*, 2004). There is very limited genetic-based tolerance available in all host species, however, canola/ rapeseed breeders in China have successfully developed some cultivars which have field tolerance to *Sclerotinia*. Field tolerance to *Sclerotinia* is a combination of physiological resistance and escape mechanisms. For simplicity of presentation, field tolerance will be hereafter be termed resistance.

A number of screening methods have been used in various crops to evaluate resistance to *S. sclerotiorum*. Most of these methods share much in common across the

crops with some minor modifications. The available methods can be divided into two categories (i) screening methods in the field and (ii) screening methods under controlled environments. Disease screening under field conditions exploits the host-pathogen interaction under natural conditions and therefore can provide a better estimation of field resistance. However, field screening has some major challenges, for example, naturally infested fields are limited to certain geographical areas and artificial inoculation under field conditions is laborious and time consuming. It is also difficult to obtain reliable and reproducible results in the field due to inconsistent disease pressure (Jurke and Fernando, 2008; Whipps *et al*., 2002).

Sclerotinia sclerotiorum disease resistance screening methods conducted in controlled environments may provide more consistent and reliable results. Controlled environment methods offer a better estimate of physiological resistance than that from field screening since escapes in the field influence final disease scores. Jurke and Fernando (2008) compared four growth-room screening techniques in *B. napus* and found that the mypetal technique was most reliable for physiological resistance evaluation. Zhao *et al*. (2004) developed a petiole inoculation technique for *B. napus*. These authors reported that resistant genotypes identified at an early stage also displayed resistance when evaluated at the flowering stage indicating that petiole inoculation technique can also provide a good estimate of adult plant resistance. Zhao *et al*. (2004) also tested four isolates of *S. sclerotiorum* for virulence and found no significant differences among the isolates. Bradley *et al*. (2006) evaluated three controlled environment-based screening methods to evaluate canola resistance to *Sclerotinia* stem rot and found significant differences among cultivars using the petiole inoculation technique and oxalic acid assay.

They also found that the area under the disease progress curve for petiole inoculation technique had a negative correlation with yield but no correlation with field evaluation results. Garg *et al*. (2008) developed a cotyledon assay for *Sclerotinia* stem rot resistance in which they evaluated 32 *B. napus* genotypes and found significant differences among the genotypes for *Sclerotinia* stem rot resistance.

The inheritance of *S. sclerotiorum* resistance is complex. As outlined above a number of screening methods have been used to screen germplasm for resistance to *S. sclerotiorum*. Different studies have reported a variety of methods as reliable, some with correlation with the field evaluation results. However, there is still no universally accepted screening method available in any crop species. The lack of standardized, reliable indoor resistance screening methods and high variation in field evaluation results along with limited genotypic resistance poses major challenges to developing *Sclerotinia* stem rot resistant cultivars. The objectives of this study were to 1) compare the various controlled environment-based *Sclerotinia* resistance screening methods to identify a reliable and rapid method that could be used for large scale screening of *B. napus* cultivars/ lines for their resistance to *Sclerotinia* stem rot and 2) to evaluate four different isolates of *S. sclerotiorum* obtained from four countries for their virulence using the screening method identified in the first objective.

3.3 Materials and methods

Fungal isolates and growth conditions

Four different *S. sclerotiorum* isolates Canada 77, UK-1, USA-43 and China-30 were provided by Dr. D. Fernando, University of Manitoba, Canada. Isolate Canada 77

was used to compare the screening methods and all the four isolates were later used in another experiment to compare their virulence. Sclerotia of each isolate were surface sterilized by immersing them in 2% Sodium Hypochloride for one minute solution and then in 70% ethanol for 30 seconds. The sclerotia were subsequently washed three times in sterilized water. Each surface sterilized sclerotium was cut in half. Each half was slightly embedded in potato dextrose agar (PDA) media (Fisher). The PDA media plates were kept at 20 ^{0}C in an incubator. The fungus was sub-cultured by taking mycelial plugs (8 mm in diameter) from the advancing fronts. One plug was placed in the centre of a petri-dish. The plugs from advancing front of the first sub-cultured plate were used to prepare the actual inoculum. The mycelial suspensions for the cotyledon assay were prepared as described by Garg *et al*. (2008). Seven plugs (8 mm) were sub-cultured in 75 ml potato dextrose broth (Fisher) in a 250 ml flask. The flask was constantly rotated at 120 rpm and 20 ^{0}C for three days. The mycelia were collected through four layers of autoclaved Mira-cloth and then washed with sterilized water. The mycelial mass was then transferred to 75 ml potato dextrose broth and macerated with a blender for three minutes and filtered through four layers of autoclaved Mira-cloth. The final concentration was adjusted to 1 X 10^{3} to 1 X 10^{5} mycelial fragments per ml using a haemocytometer. The suspension solution was constantly shaken during the inoculation process.

Plant materials and growth conditions

Eight *B. napus* genotypes (Westar, MillenniUM 03, Sentry, Topas, Huashuang 3, Zhongyou 821, Ning 89-56 and O7H2) varying in resistance to *Sclerotinia* were used to identify the reliability and suitability of the cotyledon assay, the mycelial stem inoculation technique and the petiole inoculation technique. Seven *B. napus* genotypes

(Westar, MillenniUM 03, Sentry, Topas, Huashuang 3, Zhongyou 821 and Huazhong) were used to evaluate the excised leaf assay. Westar, MillenniUM 03, Sentry, Topas are summer canola or rapeseed cultivars. Zhongyou 821 is a semi-winter rapeseed cultivar. Huashuang 3 and breeding lines Ning 89-56 and O7H2 are winter rapeseed types. Breeding lines Ning 89-56 and O7H2 were kindly provided by Dr. Jiefu Zhang, Jiangsu Academy of Agricultural Sciences, China. The seeds of the remaining cultivars were obtained from University of Manitoba. Huashuang 3 and Zhongyou 821 were identified as *Sclerotinia* resistant checks and Westar was a susceptible check under field conditions. Breeding lines Ning 89-56 and O7H2 are moderately field resistant. All genotypes were grown in flats containing Sunshine® LA3 mix. The seedlings were watered daily and kept in 16 hour light and 8 hour dark photoperiod in University of Manitoba greenhouses.

Controlled environment screening methods

Four *Sclerotinia* disease resistance screening methods, the excised leaf assay (Kim *et al.*, 2000), the cotyledon assay (Garg *et al*., 2008), the mycelial stem inoculation technique (Jurke and Fernando, 2008) and the petiole inoculation technique (Zhao *et al*., 2004) were compared for their reliability and suitability for large scale screening. Each experiment was conducted in randomized complete block (RCB) design with three replicates and 12 plants per replicate.

In the excised leaf assay, the fully expended young leaves from four week-old plants were detached. The detached leaves were kept in a humidity chamber made from transparent polyethylene sheet. High humidity was maintained using two humidifiers (Vicks ultrasonic – V5100N). Mycelial plugs (8 mm in diameter) were cut from the

advancing fronts of two to three day-old mycelial cultures. The mycelial side of each plug was placed on the dorsal surface of the leaf and lesion length was measured 48 hours after inoculation.

In the cotyledon assay, the cotyledons of 10 day-old seedlings were wounded using a pair of forceps. One wound was made on each cotyledon. A 10µl drop of mycelial suspension was applied on each wound using a micropipette. The suspension solution was constantly stirred to maintain the homogeneity of the suspension during inoculation. The inoculated plants were kept in a humidity chamber for two days. After two days, the humidifier chambers were shut off. The lesion length was measured four days after inoculation.

For the mycelial stem inoculation technique, two week-old plants were transferred to a cold chamber for six weeks of vernalization. In the vernalization chamber, the plants were grown in 16 hour of light (1900 lm m^{-2}) per day and the temperature was maintained at 4 ^{0}C. The plants were later transplanted into 15 cm pots (one plant per pot). The potting mixture was prepared by mixing soil, sand and peat in 2:2:1 ratio. The plants were fertilized with 15 ml of 20:20:20 (NPK) fertilizer per 4 L of water every three weeks. At the mid flowering stage, the stem of each plant was inoculated in two spots with mycelial plugs (5 mm in diameter). The mycelial plugs were obtained from advancing fronts of two to three day-old mycelial culture and were wrapped around the stem using Parafilm. The stem lesion length was measured 14 days after inoculation.

A modified version of the petiole inoculation technique (Zhao *et al.*, 2004) was used where seven to 10 day-old plants were transplanted to a soil-less Sunshine LA4 mix.

One plant was transplanted into each 10 cm pot. The petiole of three week-old plant was severed approximately 2.5 cm from the stem. The mycelia from actively growing edges were loaded into 200 µL micropipette tips. Two plugs were loaded by pushing the open end of micropipette tip in the two to three day-old PDA media. Plants were inoculated by pushing the open end of pipette tip containing inoculum on the petiole stub. Days to wilting for a two week period were recorded. A plant was deemed wilted when the stem showed complete girdling or the leaves were flaccid.

Statistical Analysis

All statistical analyses were carried out using Statistical Analysis System 9.2 (SAS Institute Inc. NC). Analysis of variance for the excised leaf assay, the mycelial stem inoculation technique and the petiole inoculation technique was performed using PROC GLM and for cotyledon assay using PROC MIXED procedure. Genotypes were manually ranked one to eight for the mycelial stem inoculation and the petiole inoculation techniques to calculate the Spearman rank correlation coefficient where “one” was the most resistant and “eight” was the most susceptible to *S. sclerotiorum*. The analysis was performed using PROC CORR Spearman procedure. Virulence of four isolates was compared using a split-plot design experiment with two replicates and 12 plants in each replicate. The isolates were randomly assigned to whole plots and genotypes to subplots. The analysis of variance was carried out using PROC MIXED procedure.

3.4 Results and discussion

Excised Leaf Assay

The excised leaf assay is a fairly quick method. The total numbers of days required from seeding to the final screening result was only 30 days. The symptoms appeared as necrotic lesions (Fig. 3.1A). The lesion length varied from 2.01 to 2.77 cm (Table 3.1). The analysis of variance (Table 3.2) showed significant differences among the genotypes and significant differences among the replicates ($P < 0.05$). The significant replication effect suggests that this method is not reliable or repeatable or needs more replicates. Using the excised leaf assay, Bradley *et al*. (2006) did not find any significant differences in *Sclerotinia* resistance among 19 canola cultivars using the excised leaf assay, while Wegulo *et al.* (1998) found significant differences in three out of five experiments among 12 soybean cultivars. The results of this study are in agreement with previous studies (Bradley *et al.,* 2006; Wegulo *et al.,* 1998) that in the excised leaf assay is a less reliable method. In this study, Zhongyou 821 which is supposed to be most field resistant check to *Sclerotinia* stem rot produced the second longest lesions. The excised leaf assay measures the resistance of leaf tissue against *Sclerotinia* mycelium. However, the pathogen mainly affects stems not leaves. The reaction of plant leaves to *Sclerotinia* may not be a good indicator of disease reaction of the stem. In the excised leaf assay, the inoculated plants were kept under high humidity conditions requiring humidifiers and humidity chambers. Since this method requires additional equipment. It may not be suitable for large scale screening.

Cotyledon Assay

The cotyledon assay required 14 days from seeding to final disease resistance evaluation, making this method the most rapid screening method used in this study. The disease assessments were done on 10 day-old seedlings thus this assay required the least

space as compared to other screening methods. Necrotic flecks were seen two to three days after inoculation around the wound on some cotyledons. Similar findings were reported on *B. napus* by Garg *et al*. (2008). The lesion length varied from 5.9 to 7.7 mm (Table 3.1). The analysis of variance showed non-significant differences among the cultivars (Table 3.2). The disease failed to develop on many cotyledons (Fig. 3.1C). This could be due to non-viable mycelia because of vigorous stirring. This method did not show discrete lesion differences between the resistant and susceptible checks (Fig. 3.1B). In contrast, Garg *et al.* (2008) reported this method as reliable. The inoculation conditions, for example, maintenance of humidity, temperature and inoculum preparation need further investigation. Use of ascospores instead of mycelial inoculation may be another option that has not been reported to date. However, ascospores are difficult to produce under laboratory conditions and trans-national shipment of ascospores is difficult because of quarantine regulations.

Mycelial Stem Inoculation Technique

The mycelial stem inoculation required more than 90 days from seeding to final screening results due in part to the need to vernalize the winter canola/ rapeseed lines used in this study. The lesions started to appear two to three days after inoculation as water soaked fronts which progressed in all directions (Fig. 3.1E). In the susceptible genotypes, the whole stem became bleached as the disease progressed and plant died in two to three weeks. Stem lesion length varied from 4.97 to 10.98 cm (Table 3.1). Analysis of variance showed significant differences among the genotypes at $P<0.0001$, the differences among the replicates were non-significant (Table 3.2) thus this method could be considered as repeatable and reliable. The stem lesion length of the resistant

cultivars (Zhongyou 821 and Huashaug 3) was significantly less than the susceptible check Westar (Table 3.1) (Fig. 3.2 D and E). Using the mycelial stem inoculation technique, Wegulo *et al*. (1998) found significant differences for stem lesion length among 12 soybean cultivars. The results this study confirm the results of Wegulo *et al*. (1998), suggesting that the mycelial stem inoculation technique can used to evaluate *Sclerotinia* stem rot resistance. Even though the ranks of cultivars varied between the experiments, the field resistant cultivars produced shorter lesions compared to the susceptible checks used in the study of Wegulo *et al*., 1998. In contrast, Jurke and Fernando (2008) found non-significant differences for lesions length among 10 *B. napus* genotypes. This difference may be due to the fact that, Jurke and Fernando (2008) measured the lesion length at seven days after inoculation while in this study, lesion length was measured at 14 days after inoculation. Longer duration after inoculation could have allowed clearer differences among cultivars/ lines in this study than Jurke and Fernando (2008). Using this screening method, the disease testing is done on mature plant stems of similar age to plants infested by *Sclerotinia* under field conditions. Thus, this method may detect the true physiological resistance to *Sclerotinia* stem rot. Since vernalization is required for winter type of germplasm and screening is done on mature plant stems, this method may require too much time and space to be a suitable for large scale screening.

Petiole inoculation technique

In the petiole inoculation technique, symptoms appeared as water soaked lesions as early as 24 hours after inoculation. The susceptible plants showed complete stem girdling in two to four days (Fig. 3.2 A). Resistance appeared to involve both

development of a corky layer around the point of entry, impeding the movement of the mycelium into the stem and a slowing down of the progress of mycelium in the stem. Days to wilting ranged from 3.8 to 10.9 (Table 3.1). The analysis of variance showed significant differences among the genotypes ($P<0.0001$) and differences among the replicates were non-significant at $P = 0.05$ (Table 3.2), therefore this method could be considered as repeatable. Zhao *et al*. (2004) successfully used this method to differentiate resistant and susceptible checks. They found significant differences among the 47 accession using the petiole inoculation technique. Bradley *et al*. (2006) also found significant differences among 19 canola type cultivars using the petiole inoculation technique. They also reported that this method could successfully differentiate between the susceptible and resistant *B. napus* genotypes. Comparing with the detached leaf assay and the oxalic acid assay, Bradley *et al*. (2006) found the petiole inoculation technique was a more suitable screening method. The results of this study are in agreement with previous studies of Bradley *et al.* (2006) and Zhao *et al.* (2004). Zhao *et al.* (2004) reported that Chinese bred cultivars/ lines were more resistant than Canadian bred cultivars/ lines, we identified similar results. Furthermore, they reported that rapeseed type cultivars/ lines had more resistance to *Sclerotinia* stem rot than canola types. In this study, MillenniUM 03, a high erucic acid rapeseed cultivar showed better resistance than the canola types Westar and Sentry (Table 3.1).

A modified version of the petiole Inoculation technique was used in this study. In this modified petiole inoculation technique, three week-old plants for inoculation instead of four week-old plants and two plugs were loaded to a 200 µl micropipette tip compared to one plug in 1 ml micropipette tip. The summer type *B. napus* cultivars/ lines started

bolting by the 4th week whereas winter type *B. napus* cultivars/ lines remained in the rosette stage. Thus inoculating plants at the four week stage may lead to differences in disease score because of differences in growth habit of cultivars/ lines. Inoculation at the three week-old stage overcomes this potential problem. Two plugs instead of one ensured better contact of the mycelial plugs on the petiole. The 200 µl micropipette tips were lighter than 1 micropipette ml therefore put less pressure on the petiole and ensured better success of infection.

Spearman rank correlation was calculated to identify correlation between the mycelial stem inoculation technique and the petiole inoculation technique. No correlation was observed ($r_s = 0.32$, $P = 0.43$) between the two methods. However, it is important to note that the field tolerant genotypes Zhongyou 821 and Huashaung 3 were identified as resistant and the susceptible check Westar was identified susceptible in both techniques. Non-significant correlations between the screening methods have been reported in previous studies (Jurke and Fernando, 2008; Kim *et al*., 2000; Wegulo *et al*., 1998).

Virulence of *S. sclerotiorum* Isolates

The four isolates used in this experiment did not show significant differences for days to wilting at P = 0.05. Isolate-genotype interactions were also non-significant at P = 0.05 (Table 3.3). The isolates tested were collected from Canada, China, UK and USA. The results indicate that *S. sclerotiorum* isolates collected from different geographical areas have similar virulence. Similar results have been reported in many previous studies (Maltby and Mihail, 1997; Zhao *et al*., 2004). *S. sclerotiorum* is a non-host specific fungus that utilizes several plant tissue degrading enzymes with each enzyme having a

number of isozymes (Bolton *et al*., 2006). Thus the host-pathogen interactions are non-specific resulting in no difference in virulence pattern among the isolates.

The results of this study indicate that both the mycelial stem inoculation technique and the petiole inoculation technique are reliable methods for screening *Sclerotinia* resistance. The petiole inoculation technique requires less time and space compared to the mycelial stem inoculation technique. The petiole inoculation technique is the preferable screening method for large scale screening. Although the cotyledon assay is the most suitable method for large scale screening, it failed to distinguish between the resistant and susceptible cultivars/ lines in this study. This method would require further improvement and verification before it could be recommended. The excised leaf assay was unsatisfactory in this study, in agreement with several previous literature reports.

A modified version of the petiole inoculation technique is the controlled environment *Sclerotinia* screening technique of choice for *B. napus*, especially when winter or semi-winter canola/ rapeseed cultivars are to be evaluated for their *Sclerotinia* stem rot resistance/ susceptibility.

Table 3.1. Mean phenotypic data recorded for four screening methods

Genotypes	Excised leaf assay Leaf lesion length (cm)	Cotyledon assay Cotyledon lesion length (mm)	Mycelial stem inoculation technique Stem lesion length (cm)	Petiole inoculation technique Days to wilting (d)
Westar	2.2	7.7	11.0	3.8
MillenniUM 03	2.8	7.2	6.0	5.3
Sentry	2.3	5.7	8.4	4.5
Topas	2.5	6.7	5.0	6.1
Huashuang 3	2.0	6.2	5.1	8.1
Zhongyou 821	2.7	6.2	5.9	10.9
Ning 89-56	N.A*	6.7	7.2	8.9
O7H2	N.A	5.9	10.2	8.8
Huazhong	2.4	N.A	N.A	N.A
Standard Error	0.23	†	0.35	0.3
CV	9.44	†	4.76	4.1
LSD‡ (0.05)	0.40	†	0.61	0.5

*NA- Genotype was not tested in this method

‡ Least significant difference at P=0.05

† Differences among the genotypes were non-significant (Not calculated)

Table 3.2. The analysis of variance for four controlled environment screening methods

Source	DF	Type III SS	Mean Square	F value	Pr>F
			Excised Leaf Assay		
Genotype	6	1.285	0.214	4.17*	0.0170
Replicate	2	0.941	0.471	9.16*	0.0038
			Cotyledon Assay		
Genotype	7	14.862	2.123	2.91	0.16
Replicate	2	1.894	0.947	0.98	0.42
			Mycelial Stem Inoculation		
Genotype	7	111.860	15.980	130.70**	<.0001
Replicate	2	0.155	0.077	0.63	0.5460
			Petiole Inoculation Technique		
Genotype	7	142.433	20.348	251.91**	<.0001
Replicate	2	0.356	0.178	2.20	0.1473

Table 3.3. Analysis of variance of a split plot design experiment for virulence testing of four *Sclerotinia sclerotiorum* isolates

Source	DF	Type III SS	Mean Square	F value	Pr>F
Isolates	3	0.14	0.04	0.07	0.97
Genotype	7	306.82	43.82	83.94	<0.0001
Replicate	1	0.16	0.16	0.25	0.65
Isolate X Genotype	21	6.14	0.29	0.56	0.91
Isolate X Replicate	3	1.91	0.63	1.22	0.32

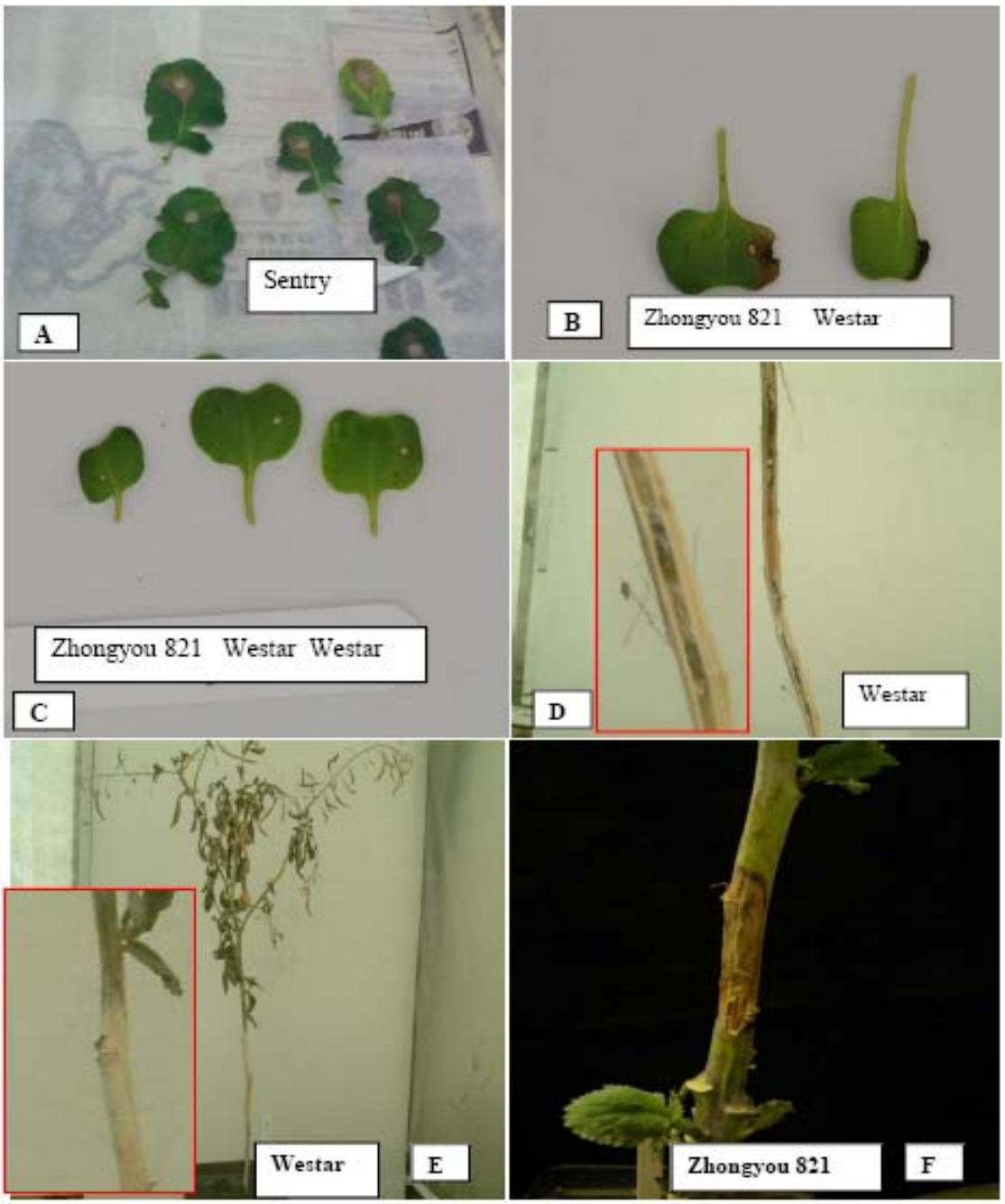

Figure 3.1. (A) Leaf lesion developed two days after inoculation on Sentry in excised leaf assay (B) Lesion development on cotyledons of Zhongyou 821 and Westar in cotyledon assay showing no differential disease reaction on resistant check Zhongyou 821 and susceptible check Westar (C) Zhongyou 821 and Westar cotyledon showing unsuccessful disease development and hypersensitive reaction (D) Sclerotia formation inside the stem of susceptible check Westar in mycelial stem inoculation (E) The disease reaction on susceptible check Westar 14 days after inoculation resulting in stem bleaching and complete wilting of plant in mycelial stem inoculation (F) Disease reaction on resistant check Zhongyou 821 plant showing small stem lesion 14 days after inoculation in mycelial stem inoculation

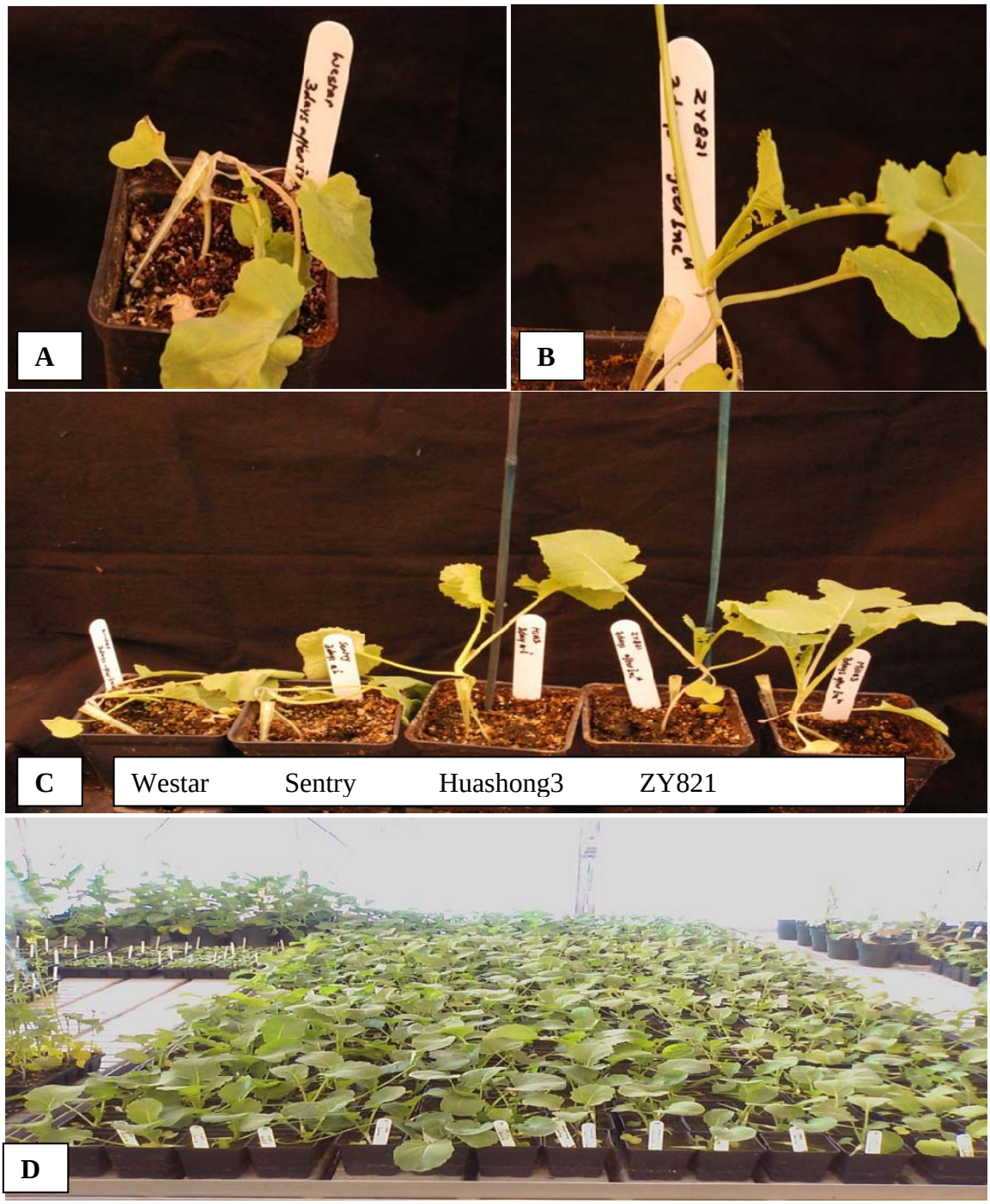

Figure 3.2. (A) Disease development on susceptible check Westar three days after inoculation showing complete stem girdling in the petiole inoculation technique (B) Disease reaction on resistant check Zhongyou 821 showing intact stem three days after inoculation in the petiole inoculation technique (C) Disease reaction on various checks in petiole inoculation technique showing complete stem girding on Westar and Sentry and intact stems of Huashong3 and Zhongyou 821 three days after inoculation (D) Picture showing efficacy of the petiole inoculation technique for large scale screening. A total of 576 plants can be screened on 1.83 X 4.27 m bench

4.0 IDENTIFICATION OF COMMON QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO *Sclerotinia sclerotiorum* IN *Brassica napus* L.

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4.0 Identification of common quantitative trait loci for resistance to *Sclerotinia sclerotiorum* in *Brassica napus* L.

Thesis author, Ravneet Behla, evaluated five DH populations for resistance to *Sclerotinia* stem rot, developed four genetic maps for the H1, H2, DH179 and DH180 populations and identified QTL for resistance to *Sclerotinia* stem rot in all of the five populations.

4.1 Abstract

Sclerotinia stem rot is one of the most devastating diseases of canola/ rapeseed. Quantitative trait loci (QTL) analyses were carried out to identify loci responsible for resistance to *Sclerotinia* stem rot in five previously developed doubled haploid (DH) populations (H1, H2, H3, DH179 and DH180). The DH populations were derived from microspore derived embryos of F_1 plants from crosses Zhongyou 821 X DHBao604 (H1), Zhongyou 821 X DH6576 (H2), Zhongyou 821 X Westar (H3), Huashong3 X MillenniUM 03 (DH179) and Huashong3 X Sentry (DH180). The first parent of these populations Zhongyou 821 or Huashong3 were field resistant cultivars. The second parent was either a known susceptible cultivar/line (DHBao604 and Westar) or was chosen to be genetically distinct (DH6576, MillenniUM 03 and Sentry) from the resistant parent. The petiole inoculation technique was used to phenotype all populations for resistance to *Sclerotinia* stem rot by recording days to wilting after inoculation for up to two weeks. The phenotyping of each population was carried out in three independent replicates with 12 plants per line in each replicate. Genetic maps of the H1, H2, DH179 and DH180 populations were developed using sequence related amplified polymorphism (SRAP) markers. In the H1 population genetic map, 508 markers were assigned to 19 linkage groups with a total genetic distance of 1526.5 cM. The genetic map of the H2 population was consisted of 478 markers, which were allocated to 19 linkage groups with a total genetic distance of 1247.7 cM. A previously published genetic map of the H3

population consisted of 1055 markers assigned to 19 linkage groups with a total genetic distance of 1604.8 cM. In the DH179 population genetic map, 397 markers were assigned to 19 linkage groups with a total genetic distance of 985.2 cM. The genetic map of the DH180 population comprised of 512 markers on 19 linkage groups with a total genetic distance of 1470.7 cM. QTL analysis was carried out for each replicate separately as well as on the average of all the replicates. The putative QTL for resistance to *Sclerotinia* stem rot were identified using a LOD threshold value of 2.5. In the H1 population, the numbers of identified QTL in each analysis varied from four to six. The phenotypic variance explained by each QTL ranged from 5.7 to 28.5%. There were three to six QTL identified in each analysis of the H2 population and the phenotypic variance explained by these QTL ranged from 7.6 to 17%. QTL identified in the H3 population ranged from two to six in each analysis. The phenotypic variance explained by these QTL ranged from 9.9 to 35.2%. In the DH179 population, the numbers of QTL detected in each analysis ranged from three to five. The phenotypic variance explained by individual QTL varied from 7.1 to 23.5%. The numbers of QTL identified in each analysis of the DH180 population ranged from three to six. The phenotypic variance explained by these QTL varied from 7.1 to 15.4%. A number of common QTL were identified between the replicates of each population. Two common QTL were identified on linkage group N07 and N16 between the H1 and H3 populations. There was one common QTL on N09 between the H2 and H3 populations. There were two common QTL between the DH179 and DH180 populations on linkage group LG03 and LG08. There was one common QTL between the H3 and DH179 populations on linkage group N12. Markers linked to these QTL could be useful for marker assisted selection of *Sclerotinia* stem rot resistant genotypes.

4.2 Introduction

Brassica oilseed cultivars belong to *Brassica napus* L., *B. rapa* L., *B. juncea* L. Czern. and *B. carinata* Braun. *Brassica rapa*, *B. juncea* and *B. carinata* cultivars are cultivated mainly on the Indian sub-continent and China. *Brassica napus* is an important *Brassica* species and predominantly grown in North America, Europe and Asia. Oleiferous *Brassicas* are grown for diverse oil properties such as edible oils, industrial oils and biodiesel (Gupta and Pratap, 2007). The crop has high economic value in North America, Europe and Asia. There has been a steady increase in the area of oleiferous *Brassicas* cultivation in Australia and South America over past decade. *Sclerotinia* stem rot is one of the major diseases in almost all *Brassica* growing areas. *Sclerotinia* stem rot is caused by a ubiquitous necrotrophic fungus, *Sclerotinia sclerotiorum* (Lib.) de Bary. It infects almost all plant parts. This fungus causes significant yield losses and affects seed and oil quality. Under favourable disease development conditions, yield losses from *Sclerotinia* stem rot can be as high as 100% (Purdy, 1979).

Conventional and chemical controls for *Sclerotinia* stem rot are not very effective and are often not economically feasible. Breeding for disease resistance is a desirable and the most effective approach to reducing the losses from *S. sclerotiorum*. However, in many crops including *B. napus*, there are very limited sources of genetic-based resistance (Bolton *et al*., 2006). Field resistance is a combination of avoidance mechanisms and physiological resistance. The avoidance mechanisms include upright growth habit, short open canopies, apetalous inflorescences and lodging resistance (Bardin and Huang, 2001; Jurke and Fernando, 2002). The genetic inheritance of resistance to *S. sclerotiorum* is polygenic, which further obstructs the development of *Sclerotinia* stem rot resistant

cultivars. Breeding for disease resistance entirely relies on identification of true genetic-based resistance. Since the evaluation of resistance under the field conditions are influenced by environmental factors, escapes mechanisms and inconsistent disease pressure, identification of physiological resistance is highly desirable.

Several screening methods have been recommended to evaluate physiological resistance of plants to *S. sclerotinia* in *B. napus* (Bradley *et al*., 2006; Jurke and Fernando, 2008; Zhao *et al.*, 2004). The petiole inoculation technique has been successfully used to evaluate germplasm for resistance to *S. sclerotiorum* in *B. napus* (Bradley *et al*., 2006; Zhao *et al*., 2006; Zhao *et al.,* 2004). The phenotyping of segregating populations is an important step in the identification of QTL linked to a particular trait.

The concept of QTL analysis was developed a long time ago (Sax, 1923), but with the advent of DNA/molecular markers, considerable progress has been made in QTL mapping. PCR-based molecular markers are currently the most commonly used markers because these markers are easy to analyse, produce results quickly, are cost effective and comparatively safe to use. Among the PCR-based marker systems, the SRAP marker procedure compared to others is simple, amplifies multiple loci, reliable and can be used comparatively across plant species (Li and Quiros, 2001).

A few studies have been carried out to identify QTL for resistance to *S. sclerotiorum* in *B. napus*. Zhao and Meng (2003) identified six QTL for resistance using two inoculation methods in a F_3 population of *B. napus*. They identified three QTL for leaf resistance to *S. sclerotiorum* using an excised leaf assay. These QTL explained 23.2,

16.6 and 13.6% of phenotypic variance, respectively. Another three QTL were identified for stem resistance using a toothpick screening method. These QTL accounted for 39.8, 17.4 and 30.4% phenotypic variance, respectively. They could not find a common QTL between these screening methods. Zhao *et al*. (2006) evaluated two DH populations using a petiole inoculation technique. They recorded data on days to wilt and stem lesion length. They identified seven QTL responsible for stem lesion length in the first population. The phenotypic variance explained by individual QTL varied from 7.7 to 22.0%. Four QTL were identified for days to wilt. The phenotypic variance explained by each QTL ranged from 5.9 to 14.9%. In the second population, they identified two QTL, one each for stem lesion length and days to wilt. These QTL explained phenotypic variance of 11.1 and 22.7% respectively. In their study, Zhao *et al*. (2006) identified two QTL which were detected more than once between the replicates. However, they could not find a common QTL between the populations. Yin *et al.* (2010) evaluated one DH population using three different screening methods. They identified a total of 21 QTL for resistance to *Sclerotinia* stem rot. The phenotypic variance explained by individual QTL varied from 10.2 to 36.1%. Yin *et al*. (2010) identified only two common QTL with some overlapping regions between two out of three screening methods. There were only three common QTL between their multi-screening method-year evaluations. In these studies, there were very few common QTL between the replicates and screening methods. There is no common QTL reported so far between the populations.

The objectives of this study were to 1) identify QTL responsible for *Sclerotinia* stem rot resistance in five genetically diverse *B. napus* DH populations 2) identify

markers linked to these QTL and 3) identify and compare common QTL between these five populations.

4.3 Materials and methods

Plant materials

Five previously developed *B. napus* DH populations (H1, H2, H3, DH179 and DH180) were used in this study (Table 4.1). The H1, H2 and H3 populations were developed by Dr. Fengqun Yu, and the DH179 and DH180 were developed by Dr. Genyi Li and Dr. Zudong Sun. A total of 106, 84, 58, 92 and 96 DH lines of the H1, H2, H3, DH179 and DH180 populations, respectively, were evaluated for resistance to *Sclerotinia* stem rot in this study. The populations consisted of microspore derived doubled haploids of F_1 plants. The parents of the F_1 plants of the H1, H2, H3, DH179 and DH180 populations were Zhongyou 821 X DHBao604, Zhongyou 821 X DH6576, Zhongyou 821 X Westar, Huashong3 X MillenniUM 03 and Huashong3 X Sentry, respectively. Zhongyou 821 is a Chinese semi-winter type rapeseed cultivar that is a well known *Sclerotinia* stem rot resistant cultivar in China. DHBao604 is a Chinese winter type rapeseed breeding line, highly susceptible to *Sclerotinia* stem rot under field conditions in Wuhan, China (Personal communication with Dr. Fengqun Yu). DH6576 is a spring type Japanese breeding line. The field resistance of DH6576 is not known but it was chosen as one of the parents because the population derived from Zhongyou 821 X DH6576 was expected to be highly polymorphic because the two parents were genetically distinct. Westar is a Canadian spring type canola cultivar susceptible to *Sclerotinia* stem rot under field conditions (Personal communication with Dr. Fengqun Yu). Huashong3 is a

Chinese winter type rapeseed cultivar developed by Prof. Jiangsheng Wu of Huazhong Agricultural University, Wuhan, China. It was identified as resistant to *Sclerotinia* stem rot in field nurseries in Wuhan, China (Personal communication with Dr. Jaifu Zhang). MillenniUM 03 is a Canadian spring type high erucic acid rapeseed cultivar. Sentry is a Canadian spring type canola cultivar. The field resistances of MillenniUM 03 and Sentry to *Sclerotinia* stem rot are not known but each was chosen as one of the parents because these were genetically distinct from second parent Huashong3. In a previous study, Zhongyou 821 and Huashong3 were identified as resistant, and MillenniUM 03, Sentry and Westar as susceptible to *Sclerotinia* stem rot using the petiole inoculation technique. DHBao604 and DH6576 lines were not tested as seeds of these lines were not available.

Inoculum preparation

The isolate of *S. sclerotiorum* used for this study was Canada 77. Sclerotia of this isolate were field-collected from Manitoba, Canada. Sclerotia were surface sterilized by immersing them in 2% Sodium Hypochloride solution for one minute and followed by 70% ethanol for 30 seconds. Sclerotia were subsequently washed three times in sterilized water. Sclerotia were cut into two halves and placed on potato dextrose agar (PDA) media (Fisher). The media plates were kept at 20 ^{0}C in an incubator. Mycelial plugs (8mm) from actively growing edges were sub-cultured on another set of PDA plates. Mycelial plugs taken from the first sub-culture were used to prepare experimental inoculum using the same procedure.

Phenotypic evaluation

The petiole inoculation technique (Zhao *et al*., 2004) with some modifications was used to evaluate all the five DH populations for resistance to *S. sclerotiorum*. In this modified technique, seeds were sown in flats containing soil-less Sunshine LA3 mix. Seven to 10 day-old seedlings were transplanted into 10 cm pots containing soil-less Sunshine LA4 mix. The petiole of the second leaf of three week-old plants were severed approximately 2.5 cm from the main stem. Two mycelial plugs from actively growing edges were loaded into sterilized 200 µL micropipette tips by pushing the open end of micropipette tip into the growing edges of two to three day-old culture plates. The micropipette tip containing the inoculum was pushed on to the petiole stub. The plants were observed for two weeks and days to wilting were recorded every day. A plant was deemed wilted when stem showed complete girdling or the leaves were flaccid. Each population along with resistant and susceptible checks was screened three times and 12 plants per DH line were evaluated for resistance to *Sclerotinia* stem rot in each replicate.

DNA extraction and SRAP markers amplification

DNA was extracted from approximately 1.5 gm fresh leaf tissue in 15 ml tubes using a modified CTAB method (Li and Quiros, 2001). DNA quality was analyzed using ethidium bromide-stained agarose gels. DNA was stored in 384-well plates (VWR®) at 4 ^{0}C. SRAP is a PCR-based technique consisting of two primers (Li and Quiros, 2001). One of the two primers is fluorescently labeled with one of the four fluorescent dyes 6-FAM (blue), VIC (green), NET (yellow) or PET (red). PCR products were amplified using the original SRAP protocol (Li and Quiros, 2001). All PCR reactions for the H1, H2, DH179 and DH180 populations were carried out using a total PCR cocktail volume of 6.5 µl per PCR reaction. The genetic map of the H3 population was developed by Sun

et al. (2007). One hundred and three SRAP primer pairs and two publicly available microsatellite markers (Piquemal *et al*., 2005) were used to develop a genetic map for the H1 population. One hundred primer pairs and two publicly available microsatellite markers were used in genetic map construction of the H2 population. Sun *et al.* (2007) used 1634 primer combinations to develop genetic map for the H3 population. Markers for the DH179 and DH180 population genetic maps were developed using 105 SRAP primer combinations.

Marker detection and data analysis

The PCR products were detected with an ABI 3100 DNA analyzer (ABI, California) using 500-LIZ (orange) as a size standard. The ABI 3100 DNA analyzer can detect five fluorochromes simultaneously. To multiplex, the PCR products of four different dye colours were pooled together with the standard 500-LIZ (ABI) and formamide. A premix of 4 µl 500-LIZ standard per 1 ml formamide was used. The total volume of the standard and formamide was 7.5 µl. The pooled products were denatured at 94 ^{0}C for 4 minutes. The products were then analyzed with the ABI 3100 DNA analyzer using the 36-cm 16-channel arrays.

The data collected from ABI 3100 DNA analyzer were analysed with the GenScan software (ABI). The data from GenScan were converted to images using Genographer 1.6.0 (http://hordeum.oscs.montana.edu/genographer). Each polymorphic locus was scored as a dominant marker using bins, and marker data from Genographer were copied to Microsoft Excel (2003) spread sheets.

Map construction and QTL analyses

Maps were constructed using JoinMap 3.0 at grouping LOD threshold values 2 to 15 to assemble markers into 19 linkage groups. QTL analyses were carried out using WinQTLCart 2.5 software (North Carolina State University, USA). QTL were identified using a composite interval mapping (CIM) procedure with an interval walking speed of 2.0 cM and a threshold LOD value of 2.5. QTL were named using a prefix of name of the population in which they were identified followed by word "R" for replicate analysis or "AV" for average analysis. The word "R" was followed by a digit depending upon the replicate number. It was followed by a digit depending upon the number of QTL identified in each analysis.

Statistical analysis

All statistical analyses were carried out using Statistical Analysis System 9.2 (SAS Institute Inc., NC). Analysis of variance was performed using PROC GLM procedure. Pearson rank correlations were calculated using the PROC CORR Pearson procedure.

4.4 Results

Phenotypic analysis

All DH populations along with checks were evaluated for resistance to *Sclerotinia* stem rot. Disease symptoms appeared as water-soaked lesions on stems as early as 24 hour after inoculation. Susceptible plants showed complete girdling of the stem in two to four days after inoculation. The resistant checks Zhongyou 821 and Huashong3 always had significantly higher number of days to wilting as compared to the susceptible checks Westar, MillenniUM 03 and Sentry (Table 4.2).

The histograms for days to wilting for each replicate along with the average of each DH population are presented in Fig 4.1, 4.2 and 4.3. The average days to wilting varied from 3.9 to 10 in the H1 population, 3.6 to 7.6 in the H2 population and 3.8 to 10.5 in the H3 population, 4 to 11.2 in the DH179 population and 3.8 to 9.5 days in the DH180 population. Analysis of variance showed significant differences for days to wilting among the DH lines in all the five populations. The differences for days to wilting among the replicates were non-significant in the H3 population, however, the differences among the replicates were significant (P=0.05) in the remaining populations (Table 4.3). The replicates of each population showed significant positive rank correlation ($P < 0.001$) with each other (Table 4.4).

Genotypic analysis

The results of genotyping using the SRAP procedure are discussed for each population as follows:

Population H1 (Zhongyou 821 X DHBao604)

A total of 508 polymorphic markers were allocated to 19 linkage groups in the H1 population. These 506 SRAP markers and two microsatellite markers were developed using 103 SRAP primer pairs and two microsatellite primer pairs (Table 4.5). The numbers of polymorphic loci generated by each SRAP primer pair ranged from 1 to 16. Each SRAP primer pair on average produced 4.9 markers. The total genetic distance of 1526.5 cM was covered. On an average there was one marker in every 3 cM of genetic distance. The genetic sizes of individual linkage groups varied from 18.3 to 133.8 cM with an average of 80.3 cM. Thirteen linkage groups were assigned N number depending

upon the common SRAP markers between the H1 and H3 genetic maps and remaining six linkage groups were randomly assigned number ‘1’ to ‘6’.

Population H2 (Zhongyou 821 X DH6576)

In the H2 population, 478 polymorphic loci were assigned to 19 linkage groups. One hundred primer pairs including 98 SRAP primer pairs and two microsatellite primers were used to develop the genetic map of the H2 population (Table 4.6). On an average, each SRAP primer pair produced 4.8 polymorphic loci. The numbers of markers generated by each primer pair ranged from 1 to 16. A total genetic distance of 1247.7 cM was covered. The average marker density was 2.61 cM in the H2 genetic map. The genetic distance covered by individual linkage groups ranged from 31.6 to 174.4 cM with an average of 65.7 cM. Thirteen linkage groups were assigned N number depending upon the common SRAP markers between the H2 and H3 genetic maps and remaining six linkage groups were assigned number ‘1’ to ‘6’ depending upon common SRAP markers between the H1 and H2 genetic maps (Table 4.7).

Some SRAP primer pairs used in the H1 and H2 populations were identical. Some common markers were identified between the H1 and H2 populations. The common markers between these two populations ranged from 1 to 21 for each linkage group (Table 4.7). These common markers allowed aligning the linkage groups of the H1 and H2 populations. Thirteen linkage groups were assigned to the common N linkage groups using 25 SRAP and two microsatellite markers in the H1 population. Twenty SRAP and three microsatellite markers were used to anchor 13 linkage groups to the common N linkage groups in the H2 population.

Population H3 (Zhongyou 821 X Westar)

The genetic map of the H3 population was developed by Sun *et al.* (2007). A total of 1055 markers were allocated to 19 linkage groups using 1634 primer pairs. This genetic map of the H3 population covered a genetic distance of 1604.8 cM. The average marker density was 8.54 markers per cM. The genetic distance of individual linkage groups ranged from 34.5 to 134.4 cM. The linkage groups were assigned to their corresponding N number using 55 microsatellite markers.

Population DH179 (Huashong3 X MillenniUM 03)

In the DH179 population, 397 SRAP markers were assigned to 19 linkage groups using 105 primer pairs (Table 4.8). The total genetic distance of 985.2 cM was covered. On an average, there was one marker for every 2.5 cM. The genetic distance of individual linkage groups varied from 23.1 to 121.6 cM. The numbers of molecular markers generated by each primer pair ranged from 1 to 11 (Table 4.8). Each primer pair on average generated 3.8 SRAP markers. All linkage groups were randomly assigned number '1' to '19'.

Population DH180 (Huashong3 X Sentry)

In the DH180 population, 512 SRAP markers were allocated to 19 linkage groups using 105 primer pairs (Table 4.9). The total genetic distance of 1470.7 cM was covered. The average marker density in this genetic map was 2.9 cM. The genetic distance of individual linkage groups varied from 33.5 to 124.1 cM. The numbers of markers generated by each primer pair varied from 1 to 13 (Table 4.9). Each primer pair on average generated 4.8 markers. Each linkage group was assigned same LG number as

that of the DH179 genetic map when there were one or more common SRAP markers between the DH179 and DH180 linkage groups (Table 4.10).

The populations DH179 and DH180 shared one common parent (Huashong3) and there were many identical primer combinations used to develop these two genetic maps. Some common molecular markers were identified between the DH179 and DH180 populations. The numbers of common molecular markers in each linkage group (LG) ranged from 4 to 24 (Table 4.10). There were 45 common SRAP markers between the DH179 and H3 population genetic maps (Table 4.11). There were 60 common SRAP markers between the DH180 and H3 population genetic maps (Table 4.11). There were only a few identical primer pairs between the H1and H2 population, and the DH179 and DH180 population, no common marker could be identified between these pairs of populations.

QTL analysis

Several QTL for *Sclerotinia* stem rot resistance were detected in each population. In the H1 population, the number of QTL in each analysis varied from four to six (Table 4.12). Five QTL were identified while analyzing the average of all replicates. One QTL was identified on N07, three on N16 and one on N17. The phenotypic variance explained by individual QTL ranged from 5.7 to 28.5% (Table 4.12). In replicate 1, four QTL were identified on linkage groups N07, N16, N19 and LG04 (Table 4.12). The phenotypic variance explained by each QTL varied from 6.9 to 10.6%. In replicate 2, four QTL were identified, one QTL on N07, two on N16 and the remaining one on LG02. The phenotypic variance explained by these QTL ranged from 6.5 to 12.1%. Six QTL were

identified in replicate 3, two QTL on linkage group N07 and N17 and four on N16. The explanation of phenotypic variance varied from 7.0 to 22.7%.

QTL analyses of the H2 population detected a number of QTL responsible for resistance to *Sclerotinia* stem rot (Table 4.13). Three QTL were identified while analyzing the average of all replicates. There was one QTL on N03 and two on N09. The phenotypic variance explained by these QTL ranged from 8.9 to 14% (Table 4.13). Three QTL were identified in replicate 1. Two QTL were identified on N13 which explained the phenotypic variance of 10.2 and 15.4% respectively. One QTL was identified on LG06 which explained 13.9% of the phenotypic variance of the population (Table 4.13). In replicate 2, six QTL were detected, one QTL on N05, two on N09, one on LG05 and two on LG06. The phenotypic variance explained by each QTL varied from 7.6 to 10.8%. Three QTL, one each on N05, N07 and LG06 were identified in replicate 3. The phenotypic variance explained by individual QTL ranged 9 to 17%.

In the H3 population, two QTL were identified while analyzing the average of all replicates. The QTL were located one each on the N12 and the N16 linkage groups. These QTL explained 13.1 and 35.2% of phenotypic variance of the population respectively (Table 4.14). Three QTL were identified in replicate 1, one each on N06, N12 and N16 (Table 4.14). The phenotypic variances explained by these QTL were 11.8, 19.4 and 20.5% respectively. In replicate 2, six QTL were identified one QTL each on N07, N12 and N14 and three on N16. The phenotypic variance explained by these QTL ranged from 9.9 to 22.8%. Four QTL were identified in replicate 3, two QTL were identified on N03 and one each on N09 and N15. Phenotypic variance explained by these QTL varied from 12.9 to 18.2%.

In the DH179 population, identified QTL ranged from three to five in each analysis (Table 4.15). Three QTL were identified while analyzing the average of all the replicates. One QTL was identified on LG04 and two were detected on LG19. The phenotypic variance explained by these QTL ranged 7.1 to 12.1% (Table 4.15). In replicate 1, three QTL were identified, one each on LG02, LG03 and LG05. The phenotypic variance explained by individual QTL varied from 9.1 to 23.5%. Five QTL were identified in replicate 2, two each on LG03 and LG04 and one on LG05. The phenotypic variance explained by individual QTL ranged from 7.8 to 20.5%. In replicate 3, four QTL were detected, one QTL each on LG04, LG07, LG08 and LG10. The phenotypic variance explained by these QTL ranged from 9.9 to 20.7%.

In the DH180 population, the numbers of QTL detected in each analysis ranged from two to six (Table 4.16). Three QTL were identified while analyzing the average of all the three replicates. Two QTL were identified on LG08 explaining phenotypic variance of 15.4 and 8.9% respectively. One QTL was identified on LG12 explaining 7.9% phenotypic variance (Table 4.16). In replicate 1, six QTL were identified in total. One QTL was identified on LG03, two on LG08, two on LG12 and one on LG13. The phenotypic variance explained by individual QTL ranged from 7.6 to 15.4%. Three QTL were detected in replicate 2. One QTL was identified each on LG02, LG10 and LG17. The phenotypic variance explained by these QTL ranged from 7.9 to 15.3%. In replicate 3, four QTL were identified. Two QTL were identified on LG08 and one each on LG13 and LG19. The phenotypic variance explained by individual QTL varied from 7.1 to 19%.

There were QTL regions that were detected in more than one replicate or average in each population. In the H1 population, the genomic region of QTL *H1AV-1* on N07 identified in average analysis (Table 4.12) was also detected in replicates 1, 2 and 3 (Fig.4.5). The QTL *H1AV-3* identified on N16 in the average analysis had overlapping regions with QTL *H1R1-2*, *H1R3-3* and *H1R3-4* of replicates 1 and 3. Similarly, QTL *H1AV-4* was identified at the same location on N16 as that of *H1R2-3, H1R3-5* identified in replicates 2 and 3. Another QTL *H1AV-2* identified on N16 shared the same genomic region as that of *H1R2-2* identified in replicate 2.

In the H2 population, QTL *H2AV-1* identified on N03 in the average analysis (Table 4.13) was localized to the same genomic region as that of *H2R2-1* (Fig. 4.6). Similarly, QTL *H2AV-2* and *H2R2-3* were identified at the same location on N09 during the average and replicate 2 analyses (Fig.4.6). The genomic region of the QTL *H2R3-3* detected on LG06 in replicate 3 was also detected in QTL *H2R1-3* of replicate 1 and *H2R2-5* and *H2R2-6* of replicate 2. The same region was also detected in the average analysis but with only a weak effect (LOD value of 1.8).

In the H3 population, QTL *H3AV-2* identified on N16 during the average analysis (Table 4.14) had overlapping regions with *H3R1-3*, *H3R2-5, H3R2-6* identified in replicate 1 and 2 (Fig. 4.8). Similarly, QTL *H3AV-2* had overlapping regions with *H3R1-2, H3R2-2* identified on N12.

In the DH179 population, QTL *179AV-1* identified on LG04 in the average analysis shared common genetic regions with *179R2-3* and *179R3-1* detected during replicate 2 and 3, suggesting that these QTL could be the same QTL (Fig. 4.8). The QTL

179R1-2 and *179R2-1* identified in replicate 1 and 2 shared the same region on LG03 (Fig. 4.8).

In the DH180 population, all the QTL regions detected in the average analysis were also detected in one or more replicate analyses. The QTL *180AV-1* identified on LG08 during the average analysis shared same genomic region with QTL *180R1-2* and *180R3-1*. The QTL *180AV-2* detected on LG08 shared the same region as that of *180R1-3, 180R3-2* (Fig 4.9). The QTL *180AV-3* was also detected in replicate 1 (*180R1-4*).

4.5 Discussion

Several disease screening methods have been reported in the literature to evaluate resistance to *Sclerotinia* stem rot. *Sclerotinia* stem rot resistance was evaluated in this study using the petiole inoculation technique. Susceptible plants showed complete stem girdling two to four days after inoculation. Huang *et al*. (2008) observed that *S. sclerotiorum* formed infection cushions on the stem of susceptible *B. napus* plants within two days after inoculation. Resistant checks Zhongyou 821 and Huashong3 in the current study only wilted after nine and eight days respectively.

There were significant replicate effects in four populations (H1, H2, DH179 and DH180). The resistance to *Sclerotinia* stem rot is a quantitative trait. Quantitative traits are highly influenced by environmental conditions. Slight changes in the environmental conditions can lead to significant differences in final phenotype of the same genotype. The development of *Sclerotinia* stem rot disease is highly dependent on environmental conditions (Bardin and Huang, 2001; Bolton *et al*., 2006; Yin *et al*., 2010). There is also a possibility that moderately susceptible and moderately resistant DH lines behaved

differently depending upon the environmental conditions; for example, a moderately resistant DH line might have given a susceptible disease reaction when the environment was suitable for disease development and *vice versa*. Significant replicate effects for *Sclerotinia* stem rot resistance have been reported in many previous studies (Mestries et al. 1998; Yue et al. 2008; Zhao et al., 2006). The differences between the replicates were significant, however, there were significant positive rank correlations among the replicates of each population. Given this between replicate variation there were a number of QTL identified in more than one replicate, and for the average of all replicates.

The markers that are common between the populations can be used to anchor a linkage group to its corresponding chromosomal N number using marker information of already anchored linkage groups. The profile generated from a SRAP primer pair produces many markers of varying visual intensity. The SRAP markers of strong visual intensity are very stable and can be used to anchor linkage groups to the corresponding N linkage groups. The efficiency of SRAP markers in anchoring linkage groups of the H1 and H2 genetic maps to chromosome N16 was determined using microsatellite markers. Two publicly available microsatellite markers were used to anchor N16 linkage groups. SRAP profiles of the H1 and H2 populations were revisited to identify primer pairs which produced very strong profiles and from these profiles, markers which were strong and polymorphic between Westar and Zhongyou 821 were selected. These selected primer pairs were used to amplify PCR products from the H3 population. The locations of strongly amplified loci in the three DH populations (H1, H2 and H3) were the same (Fig. 4.4). The molecular data collected from these selected primer pairs in the H3 population were analyzed with the already published data of Sun *et al*. (2007) to find chromosomal

locations. One SRAP marker (SA7PM5-448) was anchored on the N16 linkage group of the H3 population and the same marker was also found in linkage groups N16 of the H1 and H2 populations, which were already anchored by microsatellite markers. This demonstrated that SRAP markers could be used successfully to anchor linkage groups to their corresponding chromosomal N numbers. Thirteen linkage groups were anchored to their corresponding N numbers in the H1 and H2 populations. Anchoring a linkage group to chromosomal N number was important to facilitate the comparison the results of this study with previous studies.

In this study, a QTL was deemed significant at LOD value ≥ 2.5. A LOD threshold value between 2 to 3 has been suggested in the literature as an appropriate value for QTL analysis (Collard *et al*., 2005). Yin *et al.* (2010) used a LOD threshold value of 2.5 to identify QTL for resistance to *Sclerotinia* stem rot in *B. napus*. In this study, a number of QTL for resistance to *Sclerotinia* stem rot were detected in the five DH populations at a LOD threshold value of 2.5.

Transgressive segregation was also observed in DH populations studied (Fig. 4.2 and 4.3). Some resistance alleles were donated by the susceptible parent in each population resulting in transgressive segregants. These resistant transgressive segregants received the resistance alleles from both parents therefore performed better than the resistant parent.

A QTL which is repeatedly detected in a population or between two or more populations should be considered reliable. The most reliable QTL identified in each population are listed in Table 4.19. There were some common linkage groups detected

during QTL analysis between the populations. Five QTL were identified, one on N07, three on N16 and one on LG04 in the average analysis of the H1 population (Table 4.17). The linkage group N07 was detected in the H2 population in replicate 3 (Table 4.13) and the H3 population in replicate 2 (Table 4.14). Yin *et al*. (2010) also used the same H1 population and repeatedly detected QTL on N07 while analyzing data collected from a mycelial toothpick screening method. Genomic regions on N16 were also identified in the H3 population both in the average as well as in the replicate analyses. Linkage group N16 has also been reported in previous studies for *Sclerotinia* stem rot resistance in *B. napus* (Yin *et al*., 2010, Zhao *et al*., 2006). In the H2 population, three QTL were identified from the average disease reaction values, one on N03 and two on N09 (Table 4.13). Two QTL were detected on N03 of the H3 population in replicate 3 (Table 4.14). Zhao *et al*. (2006) and Yin *et al.* (2010) also detected QTL on N03. One QTL was identified on N09 in the replicate 3 of the H3 population. In the H3 population, two QTL were identified, one on N12 and another one on N16 in the average analysis (Table 4.17). Yin *et al.* (2010) detected one QTL on N12 whereas Zhao *et al*. (2006) identified two QTL on N12. Genomic regions of linkage groups N16 have also been identified for stem lesion length caused by *S. sclerotiorum* in the H3 population (Kutcher, 2011). Three QTL were identified in the average analysis of the DH179 population, one on LG04 and two on LG19 (Table 4.18). One QTL was identified on LG19 in replicate 3 of the DH180 population (Table 4.16). Three QTL were identified in the average analysis of the DH180 population, two QTL on LG08 and one LG12 (Table 4.18). The linkage group LG08 was also detected in replicate 3 of the DH179 population (Table 4.15).

Several common QTL were also identified between the populations. No study so far has reported any common QTL between populations used for QTL detection of *S. sclerotiorum* resistance. SRAP marker SA7BG68-314 linked to QTL *H1R3-2* on N16 of the H1 population was located 1.6 cM away from QTL *H3R2-4* in the H3 population suggesting it could be the same region in both mapping populations (Table 4.20). Similarly, SRAP markers SA7BG53-271 and SA7PM37-185 were located near (0.7 and 1.6 cM respectively) the QTL *H3R2-4* of the H3 population. These markers were located within 2 cM area of QTL *H1R1-2*, *H1R3-2* and *H1R3-3* whereas QTL *H1R3-4* and *H1AV-4* were located about 4 cM from these markers in the H1 population, suggesting that this could be a large chromosome fragment responsible for resistance to *Sclerotinia* stem rot in both populations. The genomic regions on N16 have been reported to be involved in *Sclerotinia* stem rot resistance (Zhao *et al*., 2006; Kutcher 2011). Although there is no direct evidence, *H1R2-3*, *H1R3-5* and *H3AV-2* could be the same genetic region because these loci were about 15-20 cM down stream of common markers in both populations. SRAP marker SA7BG35-381, linked to the QTL *H1R1-1, H2R2-1, H1R3-1* and *H1AV-1* on N07 of the H1 population was found near the location of QTL *H3R2-1* suggesting all these QTL on N07 could be one QTL from same genetic region in the H1 and H3 populations (Table 4.20). Yin *et al.* (2010) detected loci on linkage group N07 for *Sclerotinia* stem resistance. SRAP marker SA7PM17-263 linked to QTL *H2AV-3* of the H2 population was found linked to *H3R3-3* on N09 of the H3 population suggesting that these genomic regions on N09 are common between the H2 and H3 populations (Table 4.20).

One SRAP marker (ODD3PM40-191) linked to the QTL *179R1-2* of the DH 179 population was located just 0.5 cM from QTL *180R1-1*, suggesting that the QTL *179R1-2* and *179R2-1* and *180R1-1* might come from the same genomic locus in both populations (Table 4.20). SRAP marker ME2BG25-390 linked to QTL *180R1-2, 180R3-1* and *180AV-1* of the DH180 population was also found in QTL *179R3-3* of the DH179 population. Another marker (SA7BG1-125) in QTL *179R3-3* of the DH179 was located only 2.7 cM from *180R1-3, 180R3-2* and *180AV-2*, suggesting that all QTL on linkage group LG08 might come from the same chromosomal fragment in both populations. SRAP markers (ODD4PM1-152 and BG23BG14-122) linked to QTL *179R3-4* on LG10 of the DH179 were also found in QTL *H3AV-1, H3R1-2* and *H3R2-2* of the H3 population (Table 4.20). The linkage group LG10 of the DH179 genetic map had 11 common SRAP markers with N12 of the H3 linkage group so probably LG10 is linkage group N12 (Table 4.11).Yin *et al.* (2010) and Zhao et al. (2006) identified linkage group N12 as containing *Sclerotinia* stem rot resistance QTL.

Quantitative traits are controlled by a number of loci with additive effects. In this study, several QTL responsible for resistance to *Sclerotinia* stem rot were identified in each population. Yang *et al*. (2007) detected more than 300 genes that expressed differentially in inoculated *B. napus* plants as compared to control. All these genes might not be involved in host resistance since the plant pathogen also induces the expression of certain host genes for virulence (Agrios, 2005). Yang *et al*. (2007) identified several genes that were involved in the plant resistance response, for example, genes involved in reactive oxygen response, heat stress proteins, plant defensins and jasmonate biosynthesis.

The main objective of QTL mapping is to use the flanking markers that are linked to QTL for marker assisted selection (MAS). It is critical to identify common QTL in QTL mapping because common QTL are most likely the real QTL. Marker linked to these common QTL might be useful in developing cultivars resistant to *Sclerotinia* stem rot.

In this study, five DH populations of *B. napus* were evaluated for *Sclerotinia* stem rot using a petiole inoculation technique. Previously developed genetic map of the H3 population was used for QTL analysis and four genetic maps for remaining populations (H1, H2, DH179 and DH180) were developed using the SRAP technique. A number of common QTL responsible for resistance to *S. sclerotiorum* were identified. Most of the linkage groups identified as responsible for resistance to *Sclerotinia* stem rot in this study have been reported in previous studies on *B. napus*. Marker linked to these common QTL might be useful in developing cultivars resistant to *Sclerotinia* stem rot.

Table 4.1. DH populations used in QTL analysis, their parentage and populations size

Population	Parentage	Number of lines
H1	Zhongyou 821 X DHBao604	106
H2	Zhongyou 821 X DH6576	84
H3	Zhongyou 821 X Westar	58
DH179	Huashong3 X MillenniUM 03	92
DH180	Huashong3 X Sentry	96

Table 4.2. Performance of checks evaluated during *Sclerotinia* stem rot resistance screening for all the five DH populations.

Checks	Replicate 1	Replicate 2	Replicate 3	Average
		Days to wilting		
		Population: H1 (Zhongyou 821 X DHBao604)		
Westar (S)	4.3	4.3	3.5	4.0
MillenniUM 03 (S`)	5.7	4.2	4.3	4.7
Sentry (S`)	5.0	4.9	4.2	4.7
Huashong3 (R)	7.3	8.7	7.9	8.0
Zhongyou 821 (R)	11.0	9.2	9.3	9.8
LSD*		1.7		
		Population: H2 (Zhongyou 821 X DH6576)		
Westar	3.9	3.8	3.5	3.7
MillenniUM 03	4.5	5.5	4.1	4.7
Sentry	5.0	4.7	4.6	4.8
Huashong3	7.5	7.8	9.7	8.3
Zhongyou 821	8.8	9.3	8.3	8.8
LSD*		1.2		
		Population: H3 (Zhongyou 821 X Westar)		
Westar	4.3	3.4	4.8	4.2
MillenniUM 03	4.9	4.1	3.9	4.3
Senrty	5.0	5.3	5.5	5.3
Zhongyou 821	9.3	8.5	9.4	9.1
LSD*		1.4		
		Population: DH179 (Huashong3 X MillenniUM03)		
Westar	4.1	4.3	4.0	4.1
MillenniUM 03	5.4	5.3	4.4	5.0
Huashong3	8.2	8.2	8.0	8.1
Zhongyou 821	10.6	9.9	9.8	10.1
LSD*		1.3		
		Population: DH180 (Huashong3 X Sentry)		
Westar	4.3	4.2	4.4	4.3
MillenniUM 03	5.2	4.7	4.5	4.8
Sentry	4.5	3.7	3.8	4.0
Huashong3	8.2	7.1	6.8	7.4
Zhongyou 821	9.6	9.2	9.5	9.4
LSD*		1.9		

* Least significant difference of mean at P=0.05.

“S” represents that check was identified as susceptible using the field trials.

“S`” represents that checks were identified as susceptible using the petiole inoculation technique.

“R” represents that checks were identified as tolerant using the field trials

Table 4.3. The analysis of variation for days to wilting for all five DH populations

Source	DF	Type III SS	Mean Square	F value	Pr>F
H1 (Zhongyou 821 X DHBao604)					
Genotype	105	633.90	6.03	5.24**	<.0001
Replication	2	18.07	9.04	7.84*	0.005
H2 (Zhongyou 821 X DH6576)					
Genotype	83	292.82	3.52	5.88**	<.0001
Replication	2	4.77	2.38	3.98*	0.021
H3 (Zhongyou 821 X Westar)					
Genotype	57	333.77	5.86	7.40**	<.0001
Replication	2	2.80	1.40	1.77†	0.175
DH179 (Huashong3 X MillenniUM03)					
Genotype	91	544.57	8.84	5.98**	<.0001
Replication	2	29.26	14.63	21.61**	<.0001
DH180 (Huashong3 X Sentry)					
Genotype	94	519.48	5.53	3.68**	<.0001
Replication	2	195.15	97.57	64.95**	<.0001

* Differences were significant at 0.05 levels of significance
** Differences were significant at 0.01 levels of significance
† Differences were non-significant at 0.05 levels of significance

Table 4.4. Pearson rank correlation coefficients for days to wilting between the replicates of the H1, H2, H3, DH179 and DH180 populations

Populations	R1 R2*	R2 R3	R1 R3
H1	0.61†	0.62	0.54
H2	0.65	0.63	0.58
H3	0.68	0.64	0.78
DH179	0.76	0.70	0.74
DH180	0.40	0.55	0.52

*R1, R2 and R3 are replicates 1, 2 and 3
† All Pearson rank correlation coefficients were significant at P<.0001

Table 4.5. Primer pairs used to construct the H1 genetic map and the number of markers developed by each primer pair

Primer pairs	No. of Markers	Primer pairs	No. of Markers	Primer pairs	No. of Markers	Primer pairs	No. of Markers
BG23BG11	2	EM1BG13	5	ODD3PM4	4	SA7BG40	8
BG23BG14	8	FC1BG2	3	PM88BG10	4	SA7BG43	6
BG23BG37	3	FC1BG35	3	PM88BG2	8	SA7BG5	5
BG23BG38	4	FC1BG37	3	PM88BG35	1	SA7BG53	12
BG23BG4	7	FC1BG4	6	PM88BG37	1	SA7BG55	7
BG23BG41	5	FC1BG43	7	PM88BG41	1	SA7BG62	10
BG23BG44	7	FC1BG48	4	PM88BG43	3	SA7BG63	2
BG23BG5	1	FC1BG67	8	PM88BG5	2	SA7BG68	7
BG23BG53	3	FC1BG70	3	PM88BG53	2	SA7BG70	6
BG23BG55	3	FC1BG72	8	PM88BG55	2	SA7PM1	6
BG23BG62	9	FC1PM5	5	PM88BG62	2	SA7PM16	7
BG23BG63	2	FC1PM6	6	PM88BG63	1	SA7PM18	5
BG23BG68	4	ME2BG11	6	PM88BG68	4	SA7PM30	7
BG23BG69	7	ME2BG14	2	PM88BG70	1	SA7PM32	12
BG23PM1	5	ME2BG53	4	PM88BG73	7	SA7PM34	7
BG23PM16	7	ME2BG55	3	PM88PM1	5	SA7PM37	8
BG23PM18	5	ME2BG62	9	PM88PM16	6	SA7PM4	1
BG23PM30	7	ME2BG63	4	PM88PM17	3	SA7PM41	9
BG23PM32	11	OD20PM18	3	PM88PM34	6	SA7PM5	8
BG23PM34	7	ODD3PM1	13	PM88PM37	3	SA7PM6	3
BG23PM37	2	ODD3PM16	5	PM88PM4	1	SA7BG69	1
BG23PM4	5	ODD3PM17	2	PM88PM5	1	SA7PM17	6
BG23PM5	3	ODD3PM18	6	PM88PM6	2	CB501	1
BG23PM6	2	ODD3PM30	4	SA12PM17	3	CB526	1
DC1PM30	3	ODD3PM32	9	SA7BG1	2		
DC1PM32	3	ODD3PM34	16	SA7BG35	3		
EM1BG10	5	ODD3PM37	3	SA7BG39	5		

Table 4.6. Primer pairs used to construct the H2 genetic map and the number of markers developed by each primer pair

Primer pairs	No. of Markers	Primer pairs	No. of Markers	Primer pairs	No. of Markers	Primer pairs	No. of Markers
BG23BG11	2	EM1BG10	3	ODD3PM18	5	SA7BG35	5
BG23BG14	7	EM1BG13	7	ODD3PM30	2	SA7BG39	8
BG23BG38	3	FC1BG2	5	ODD3PM32	10	SA7BG40	2
BG23BG4	5	FC1BG35	4	ODD3PM34	10	SA7BG43	3
BG23BG41	3	FC1BG37	2	ODD3PM37	1	SA7BG5	3
BG23BG44	8	FC1BG4	2	ODD3PM4	7	SA7BG53	5
BG23BG53	4	FC1BG43	2	PM88BG10	2	SA7BG62	8
BG23BG62	5	FC1BG48	1	PM88BG2	5	SA7BG63	8
BG23BG63	2	FC1BG66	5	PM88BG35	3	SA7BG68	9
BG23BG67	2	FC1BG67	9	PM88BG5	1	SA7BG69	4
BG23BG68	6	FC1BG70	9	PM88BG53	2	SA7BG70	4
BG23BG69	4	FC1BG72	5	PM88BG62	1	SA7PM1	7
BG23BG73	2	FC1PM5	5	PM88BG63	3	SA7PM16	7
BG23PM37	3	FC1PM6	5	PM88BG67	7	SA7PM17	8
BG23PM1	6	ME2BG11	4	PM88BG68	7	SA7PM18	6
BG23PM18	4	ME2BG14	3	PM88BG73	5	SA7PM30	5
BG23PM30	8	ME2BG53	3	PM88PM16	4	SA7PM32	11
BG23PM32	4	ME2BG62	10	PM88PM17	1	SA7PM34	5
BG23PM34	3	ME2BG63	5	PM88PM34	5	SA7PM37	9
BG23PM4	3	OD20PM1	2	PM88PM37	3	SA7PM4	12
BG23PM5	7	OD20PM18	3	PM88PM5	2	SA7PM5	16
BG23PM6	2	OD20PM4	4	PM88PM6	6	SA7PM6	4
ME2BG14	2	ODD3PM1	5	SA12PM16	7	SA7BG66	6
DC1PM30	2	ODD3PM16	7	SA12PM17	2	CB211	2
DC1PM32	2	ODD3PM17	5	SA7BG1	5	CB501	1

Table 4.7. The number of common markers identified in each linkage group for the H1 and H2 populations

Population H1	Population H2	No. of Markers
N01	N01	15
N03	N03	5
N05	N05	4
N06	N06	21
N07	N07	9
N09	N09	11
N10	N10	9
N11	N11	5
N12	N12	8
N13	N13	12
N16	N16	8
N17	N17	4
N19	N19	11
LG01	LG01	1
LG02	LG02	2
LG03	LG03	3
LG04	LG04	5
LG05	LG05	3
LG06	LG06	6

Table 4.8. Primer pairs used to construct the DH179 genetic map and the numbers of markers developed by each primer pair

Primer pairs	No. of Markers	Primer pairs	No. of Markers	Primer pairs	No. of Markers	Primer pairs	No. of Markers
BG23BG11	6	BG23PM96	2	ODD3PM1	3	SA7BG35	2
BG23BG14	8	EM1BG10	1	ODD3PM34	6	SA7BG37	3
BG23BG25	2	EM1BG13	3	ODD3PM37	1	SA7BG5	6
BG23BG37	4	FC1BG18	2	ODD3PM40	5	SA7BG53	11
BG23BG4	1	FC1BG53	9	PM88BG10	6	SA7BG62	8
BG23BG5	5	FC1PM58	2	PM88BG11	1	SA7BG68	4
BG23BG53	3	ME2BG11	9	PM88BG35	1	SA7BG70	2
BG23BG62	3	ME2BG14	5	PM88BG5	2	SA7BG72	8
BG23BG70	7	ME2BG25	3	PM88BG53	3	SA7BG94	1
BG23BG72	3	ME2BG33	2	PM88BG62	3	SA7BG95	5
BG23BG86	2	ME2BG35	5	PM88BG68	2	SA7PM101	9
BG23BG94	1	ME2BG37	3	PM88BG70	1	SA7PM1	1
BG23PM101	5	ME2BG62	2	PM88BG72	1	SA7PM16	2
BG23PM110	4	ME2BG68	8	PM88PM101	2	SA7PM30	5
BG23PM113	5	ME2BG70	1	PM88PM110	2	SA7PM34	1
BG23PM1	2	ME2BG72	1	PM88PM113	5	SA7PM37	7
BG23PM32	6	ME2PM101	5	PM88PM32	5	SA7PM4	5
BG23PM33	6	ME2PM110	7	PM88PM37	1	SA7PM41	4
BG23PM34	2	ME2PM113	6	PM88PM77	3	SA7PM77	6
BG23PM37	3	ME2PM18	1	PM88PM78	3	SA7PM78	3
BG23PM41	2	ME2PM47	5	ME2PM77	4	SA7PM89	2
BG23PM4	4	ME2PM78	5	SA7PM113	7	SA7PM90	5
BG23PM52	2	ME2PM89	4	SA7BG1	8	SA7PM96	5
BG23PM77	2	ME2PM90	8	SA7BG16	2	ODD3PM4	1
BG23PM78	1	ME2PM96	1	SA7BG17	3		
BG23PM89	3	ODD20PM1	2	SA7BG31	3		
BG23PM90	6	ODD20PM4	3	SA7BG33	5		

Table 4.9. Different primer pair combinations used to construct the genetic map for the DH 180 population and the number of markers developed by each primer pair

Primer pairs	No. of Markers	Primer pairs	No. of Markers	Primer pairs	No. of Markers	Primer pairs	No. of Markers
BG23BG11	9	BG23PM96	3	ODD3PM1	6	SA7BG37	5
BG23BG14	9	EM1BG10	3	ODD3PM34	4	SA7BG5	8
BG23BG19	1	EM1BG13	7	ODD3PM37	1	SA7BG53	6
BG23BG25	3	FC1BG18	8	ODD3PM40	4	SA7BG55	5
BG23BG35	2	FC1BG2	2	ODD3PM4	2	SA7BG62	13
BG23BG37	4	FC1BG53	4	PM883BG5	2	SA7BG68	8
BG23BG4	2	FC1BG55	3	PM88BG10	6	SA7BG70	7
BG23BG5	5	FC1PM58	11	PM88BG11	1	SA7BG72	9
BG23BG53	2	ME2BG11	10	PM88BG18	1	SA7BG94	2
BG23BG55	2	ME2BG14	10	PM88BG68	2	SA7BG95	5
BG23BG62	3	ME2BG25	3	PM88BG53	5	SA7PM101	5
BG23BG68	2	ME2BG31	1	PM88BG62	4	SA7PM110	7
BG23BG70	9	ME2BG35	4	PM88BG70	2	SA7PM113	9
BG23BG72	3	ME2BG37	3	PM88BG72	1	SA7PM1	2
BG23BG86	4	ME2BG62	2	PM88PM101	2	SA7PM16	6
BG23BG94	2	ME2BG68	6	PM88PM113	9	SA7PM30	7
BG23PM101	4	ME2BG70	3	PM88PM1	2	SA7PM34	3
BG23PM113	7	ME2BG72	3	PM88PM32	8	SA7PM37	5
BG23PM1	6	ME2PM101	6	PM88PM33	3	SA7PM41	8
BG23PM32	5	ME2PM110	4	PM88PM34	1	SA7PM4	5
BG23PM33	6	ME2PM113	7	PM88PM37	1	SA7PM45	8
BG23PM34	4	ME2PM18	4	PM88PM96	7	SA7PM89	7
BG23PM37	3	ME2PM47	8	SA7BG1	7	SA7PM90	5
BG23PM41	8	ME2PM89	8	SA7BG16	7	SA7PM96	12
BG23PM4	6	ME2PM90	3	SA7BG17	7		
BG23PM89	5	ME2PM96	2	SA7BG31	3		
BG23PM90	6	ODD20PM4	3	SA7BG35	4		

Table 4.10. The numbers of common markers identified in each linkage group for the DH179 and DH180 populations

Population DH179	Population DH180	No. of Common markers
LG01	LG01	24
LG02	LG02	8
LG03	LG03	23
LG04	LG04	6
LG05	LG05	9
LG06	LG06	8
LG07	LG07	9
LG08	LG08	16
LG09	LG09	24
LG10	LG10	13
LG11	LG11	11
LG12	LG12	9
LG13	LG13	4
LG14	LG14	4
LG15	LG15	4
LG16	LG16	6
LG17	LG17	5
LG18	LG18	6
LG19	LG19	5

Table 4.11. The numbers of common markers between the DH179, DH180 and the H3 population and their corresponding N numbers

Linkage groups	# of common markers in DH 179	# of common markers in DH 180	N Linkage groups of H3 population
LG01	7	6	N04
LG02	2	3	N13
LG03	6	6	N07
LG04	-	-	-
LG05	-	-	-
LG06	3	3	N02
LG07	1	4	N11
LG08	-	-	-
LG09	7	9	N09
LG10	11	16	N12
LG11	4	3	N05
LG12	2	2	N17
LG13	2	4	N10
LG14	-	-	-
LG15	1	4	N19
LG16	-	-	-
LG17	-	-	-
LG18	-	-	-
LG19	-	-	-

Table 4.12. Putative QTL identified for resistance to *Sclerotinia* stem rot in the H1 population (Zhongyou 821 X DHBao604)

Evaluation	QTL	LOD	LG†	Position (cM)	Flanking Markers	R^{2**}	Additive effect*
R1							
	H1R1-1	4.1	N07	107.7	FC1PM5-273 SA7BG35-381	10.6	0.66^{z}
	H1R1-2	2.8	N16	45.3	BG23PM32-375 BG23PM32-134	7.1	0.55^{z}
	H1R1-3	2.8	N19	28.01	BG23BG44-384 BG23BG62-206	7.0	0.47^{z}
	H1R1-4	2.8	LG04	31.3	PM88BG73-149 SA7PM16-447	6.9	0.45^{z}
R2							
	H1R2-1	4.5	N07	105.8	SA12PM17-320 SA7BG35-381	12.1	0.65^{z}
	H1R2-2	2.7	N16	0.02	ODD3PM32-309	8.8	0.49^{b}
	H1R2-3	2.5	N16	56.4	SA7BG62-357 SA7PM5-144	6.5	0.45^{b}
	H1R2-4	2.5	LG02	27.11	SA7BG53-194 FC1BG4-232	8.2	0.53^{z}
R3							
	H1R3-1	7.8	N07	103.7	ODD3PM32-303 SA7BG35-381	22.1	0.83^{z}
	H1R3-2	2.6	N16	41.7	SA7PM5-116 SA7BG68-314	7.0	0.46^{z}
	H1R3-3	2.7	N16	45.1	BG23PM32-375 BG23PM32-134	7.4	0.47^{z}
	H1R3-4	4.3	N16	48.5	H1ME2BG63-503 ODD3PM4-388	11.3	0.46^{z}
	H1R3-5	3.1	N16	56.4	SA7BG62-357 SA7PM5-144	8.7	0.50^{z}
	H1R3-6	2.6	N17	39.5	BG23BG69-249 SA7BG39-351	22.7	0.79^{z}
Average							
	H1AV-1	11.0	N07	105.7	ODD3PM32-303 SA7BG35-381	28.5	0.85^{z}
	H1AV-2	2.7	N16	0.02	ODD3PM32-309	5.7	0.36^{b}
	H1AV-3	6.6	N16	48.5	BG23BF37-153 BG23PM1-279	16.8	0.63^{z}
	H1AV-4	3.5	N16	55.3	ME2BG11-544 SA7PM5-144	10.0	0.46^{b}
	H1AV-5	3.6	LG04	49.5	PM88BG10-248	7.8	0.40^{z}

* 'b' denotes that the resistance alleles were donated by the susceptible line DHBao604 and 'z' denotes that the resistance alleles were from the resistant cultivar Zhongyou 821.

** R^2 represents the percent phenotypic variation explained by each QTL.

† LG = Linkage Group.

Table 4.13. Putative QTL identified in the H2 population for resistance to *Sclerotinia* stem rot (Zhongyou 821 X DH6576)

Evaluation	QTL	LOD	LG†	Position (cM)	Flanking Markers	R^{2**}	Additive effect
R1	*H2R1-1*	3.0	N13	30.7	BG23BG4-301 FC1BG43-364	10.2	0.41^{z}
	H2R1-2	3.7	N13	46.8	PM88PM34-149 PM88BG10-140	15.4	0.52^{d}
	H2R1-3	3.1	LG06	43.2	PM88BG68-327	13.9	0.48^{d}
R2							
	H2R2-1	2.9	N03	54.3	PM88BG73-478 SA7PM4-377	8.6	0.37^{d}
	H2R2-2	3.7	N09	27.3	BG23PM32-469 SA7BG1-264	10.7	0.41^{d}
	H2R2-3	3.7	N09	36.3	PM88BG68-92 SA7PM4-295	10.8	0.42^{z}
	H2R2-4	2.6	LG05	25.2	SA7PM5-95	7.6	0.36^{d}
	H2R2-5	2.7	LG06	16.7	SA7BG39-348	8.1	0.39^{z}
	H2R2-6	2.7	LG06	28.4	SA12PM16-383 ODD3PM16-383	7.6	0.36^{z}
R3							
	H2R3-1	4.0	N05	31.8	BG23BG69-456	17.0	0.55^{d}
	H2R3-2	3.1	N07	15.2	BG23BG4-235 SA7PM16-295	9.0	0.40^{d}
	H2R3-3	4.4	LG06	29.3	SA7BG39-348 PM88BG68-327	14.9	0.53^{z}
Average							
	H2AV-1	2.8	N03	54.3	PM88BG73-478 SA7PM4-377	8.9	0.34^{d}
	H2AV-2	4.5	N09	36.3	PM88BG68-92 SA7PM4-295	14.0	0.41^{z}
	H2AV-3	4.1	N09	43.7	SA7PM17-263	13.3	0.41^{d}

*'d' denotes that the resistance alleles were donated by DH6576 and 'z' denotes that the resistance alleles were donated by the resistant cultivar Zhongyou 821.

** R^2 represents the percent phenotypic variation explained by each QTL.

† LG = Linkage Group.

Table 4.14. Putative QTL identified for resistance to *Sclerotinia* stem rot in the H3 population (Zhongyou 821 X Westar)

Evaluation	QTL	LOD	LG†	Position (cM)	Flanking Markers	R^{2**}	Additive effect*
R1	*H3R1-1*	3.2	N06	65.7	*830Fg168 *901Fy239	11.8	0.59[z]
	H3R1-2	4.3	N12	100.5	*1129Db500 *1203Ey229	19.4	0.79[z]
	H3R1-3	5.2	N16	44.0	*0128Dg214	20.5	0.59[z]
R2							
	H3R2-1	4.0	N07	31.3	*815Br300 *815Cy167	13.1	0.72[z]
	H3R2-2	3.2	N12	83.2	*817Bg474 *1231Cg186	9.9	0.57[z]
	H3R2-3	3.4	N14	80.2	*0505Ab171	10.0	0.75[z]
	H3R2-4	3.1	N16	22.9	*812By442 *819Fg134	10.8	0.67[w]
	H3R2-5	4.5	N16	30.6	*1215Fg232 *815Cb361	14.9	0.97[w]
	H3R2-6	6.0	N16	42.1	*718Dg129	22.8	0.63[z]
R3							
	H3R3-1	3.8	N03	19.4	*0210Ay250 *817Cg465	13.5	0.62[z]
	H3R3-2	3.6	N03	79.0	*0129Bg182 *1202Eb214	12.9	0.47[z]
	H3R3-3	4.2	N09	5.2	*0119cr164 *0213Dy305	15.2	0.51[z]
	H3R3-4	4.9	N15	26.1	*718Fg174 *901Br462	18.2	0.55[z]
Average							
	H3AV-1	3.7	N12	90.9	*819Dg375 *1129Db500	13.1	0.55[z]
	H3AV-2	7.7	N16	42.1	*822Ab378 *718Dg129	35.2	0.58[z]

* 'w' denotes that the resistance alleles were donated by the susceptible cultivar Westar and 'z' denotes that the resistance alleles were donated by the resistant cultivar Zhongyou 821.

** R^2 represents the percent phenotypic variation explained by each QTL.

† LG = Linkage Group.

Table 4.15. Putative QTL identified for resistance to *Sclerotinia* stem rot in the DH179 population (Huashong3 X MillenniUM 03)

Evaluation	QTL	LOD	LG†	Position (cM)	Flanking Markers	R^{2**}	Additive effect*
R1	*179R1-1*	3.4	LG02	7.0	FC1BG53-455	15	0.55[m]
	179R1-2	8.5	LG03	77.3	SA7BG1-168 ODD3PM40-191	23.5	0.53[h]
	179R1-3	3.3	LG05	28.2	BG23PM96-627 ME2PM47-310	9.1	0.49[h]
R2	*179R2-1*	4.0	LG03	86.1	BG23PM33-478	12.1	0.62[h]
	179R2-2	2.8	LG03	110.7	SA7BG72-590	20.5	0.77[h]
	179R2-3	5.0	LG04	8.2	BG23PM101-170	13.9	0.67[h]
	179R2-4	2.8	LG04	17.2	ME2PM47-137	7.8	0.49[h]
	179R2-5	3.4	LG05	12.8	SA7PM101-526 ME2BG11-474	8.4	0.50[h]
R3	*179R3-1*	3.2	LG04	8.2	BG23PM101-170 SA7PM16-289	17.1	0.81[h]
	179R3-2	2.7	LG07	10.9	BG23PM37-144	20.7	0.79[h]
	179R3-3	4.3	LG08	3.9	SA7BG33-402 ME2BG25-366	11.9	0.71[h]
	179R3-4	3.7	LG10	1.115	SA72PM113-628 SA7BG53-597	9.9	0.60[h]
Average	*179AV-1*	4.5	LG04	11.4	BG23PM101-170 SA7PM16-289	12.1	0.56[h]
	179AV-2	4.4	LG19	10.0	SA7PM78-269	11.4	0.84[h]
	179AV-3	2.8	LG19	16.75	SA7PM78-257	7.1	0.57[m]

*'m' denotes that the resistance alleles were contributed by the susceptible cultivar MillenniUM 03 and 'h' denotes that the resistance alleles were contributed by resistant cultivar Huashong3.

** R^2 represents the percent phenotypic variation explained by each QTL.

† LG = Linkage Group.

Table 4.16. Putative QTL identified for resistance to *Sclerotinia* stem rot in the DH180 population (Huashong3 X Sentry)

Evaluation	QTL	LOD	LG†	Position (cM)	Flanking Markers	R^{2**}	Additive effect*
R1	*180R1-1*	4.7	LG03	50.3	BG23PM4-275	12.9	1.1[s]
	180R1-2	4.0	LG08	58.6	ME2BG25-390	10.6	0.60[s]
	180R1-3	2.8	LG08	65.4	BG23PM34-291 ME2PM47-160	7.6	0.56[s]
	180R1-4	3.3	LG12	22.1	ME2PM113-327 BG23PM113-328	8.3	0.58[h]
	180R1-5	2.9	LG12	27.2	ME2PM47-519	7.6	0.56[h]
	180R1-6	5.7	LG13	26.1	BG23PM1-217 SA7PM4-194	15.4	0.79[h]
R2	*180R2-1*	3.4	LG02	0.0	SA7BG16-365	9.6	0.45[s]
	180R2-2	3.7	LG10	10.7	FC1PM58-379 SA7PM110-493	15.3	0.58[h]
	180R2-3	2.8	LG17	7.7	BG23PM89-118 PM88PM96-593	7.9	0.41[h]
R3	*180R3-1*	6.5	LG08	58.6	FC1BG18-412 ME2BG25-390	19	0.81[s]
	180R3-2	2.7	LG08	65.4	ME2PM47-160	8.5	0.53[s]
	180R3-3	4.3	LG13	0.0	BG23PM33-131	12.3	0.62[s]
	180R3-4	2.6	LG19	18.8	SA7PM41-356	7.1	0.48[h]
Average							
	180AV-1	5.7	LG08	58.6	ME2BG25-390	15.4	0.57[s]
	180AV-2	3.1	LG08	65.4	BG23PM34-291 ME2PM47-160	8.9	0.42[s]
	180AV-3	3.0	LG12	22.1	ME2PM113-327 SA7PM113-327	7.9	0.41[h]

*'s' denotes that the resistance alleles were contributed by the susceptible cultivar Sentry and 'h' denotes that the resistance alleles were contributed by the resistant cultivar Huashong3.

** R^2 represents the percent phenotypic variation explained by each QTL.

† LG = Linkage Group.

Table 4.17. The comparison of QTL identified on average days to wilting in the H1 (Zhongyou 821 X DHBao604), H2 (Zhongyou 821 X DH6576) and H3 (Zhongyou 821 X Westar) populations

Population	QTL	LOD	LG†	Position (cM)	Flanking Markers	R^{2**}	Additive effect*
H1	*H1AV-1*	11.0	N07	105.7	ODD3PM32-303 SA7BG35-381	28.5	0.85[z]
	H1AV-2	2.7	N16	0.02	ODD3PM32-309	5.7	0.36[b]
	H1AV-3	6.6	N16	48.5	BG23BF37-153 BG23PM1-279	16.8	0.63[z]
	H1AV-4	3.5	N16	55.3	ME2BG11-544 SA7PM5-144	10.0	0.46[b]
	H1AV-5	3.6	LG04	49.5	PM88BG10-248	7.8	0.40[z]
H2	*H2AV-1*	2.8	N03	54.3	PM88BG73-478 SA7PM4-377	8.9	0.34[d]
	H2AV-2	4.5	N09	36.3	PM88BG68-92 SA7PM4-295	14.0	0.41[z]
	H2AV-3	4.1	N09	43.7	SA7PM17-263	13.3	0.41[d]
H3	*H3AV1-1*	3.7	N12	90.9	*819Dg375 *1129Db500	13.1	0.55[z]
	H3AV1-2	7.7	N16	42.1	*822Ab378 *718Dg129	35.2	0.58[z]

* 'z' denotes that the resistance alleles were donated by resistant cultivar Zhongyou 821, 'b' denotes that the resistance alleles were denoted by the susceptible cultivar DHBao604 and 'd' denotes that the resistance alleles were donated by DH6576.

** R^2 represents the percent phenotypic variation explained by each QTL.

† LG = Linkage Group.

Table 4.18. The comparison of QTL identified on average days to wilting in DH179 (Huashong3 X MillenniUM 03) and DH180 (Huashong3 X Sentry) populations

Population	QTL	LOD	LG†	Position (cM)	Flanking Markers	R^{2**}	Additive effect*
DH179	*179AV-1*	4.5	LG04	11.4	BG23PM101-170 SA7PM16-289	12.1	0.56 [h]
	179AV-2	4.4	LG19	10.0	SA7PM78-269	11.4	0.84[h]
	179AV-3	2.8	LG19	16.75	SA7PM78-257	7.1	0.57 [m]
DH180	*180AVG-1*	5.7	LG08	58.6	ME2BG25-390	15.4	0.57[s]
	180AVG-2	3.1	LG08	65.4	BG23PM34-291 ME2PM47-160	8.9	0.42[s]
	180AVG-3	3.0	LG12	22.1	ME2PM113-327 SA7PM113-327	7.9	0.41[h]

* 'h' denotes that the resistance alleles were donated by the resistant cultivar Huashong3, 'm' denotes that the resistance alleles were donated by the susceptible cultivar MillenniUM 03 and 's' denotes that the resistance alleles were donated by the susceptible cultivar Sentry.

** R^2 represents the percent phenotypic variation explained by each QTL

† LG = Linkage Group

Table 4.19. Most reliable QTL identified in all the five DH populations

Population	QTL	LOD	LG†	Position (cM)	Flanking Markers	R^{2**}	Additive effect*
H1	*H1AV-1*	11.0	N07	105.7	ODD3PM32-303 SA7BG35-381	28.5	0.85[z]
	H1R3-2	2.6	N16	41.7	SA7PM5-116 SA7BG68-314	7.0	0.46[z]
	H1AV-3	6.6	N16	48.5	BG23BF37-153 BG23PM1-279	16.8	0.63[z]
H2	*H2AV-1*	2.8	N03	54.3	PM88BG73-478 SA7PM4-377	8.9	0.34[d]
	H2AV-2	4.5	N09	36.3	PM88BG68-92 SA7PM4-295	14.0	0.41[z]
	H2AV-3	4.1	N09	43.7	SA7PM17-263	13.3	0.41[d]
H3	*H3R2-1*	4.0	N07	31.3	*815Br300 *815Cy167	13.1	0.72[z]
	H3R2-2	3.2	N12	83.2	*817Bg474 *1231Cg186	9.9	0.57[z]
	H3AV-2	7.7	N16	42.1	*822Ab378 *718Dg129	35.2	0.58[z]
DH179	*179R1-2*	8.5	LG03	77.3	SA7BG1-168 ODD3PM40-191	23.5	0.53[h]
	179R3-3	4.3	LG08	3.9	SA7BG33-402 ME2BG25-366	11.9	0.71[h]
	179R3-4	3.7	LG10	1.115	SA72PM113-628 SA7BG53-597	9.9	0.60[h]
	179AV-1	4.5	LG04	11.4	BG23PM101-170 SA7PM16-289	12.1	0.56[h]
DH180	*180R3-1*	6.5	LG08	58.6	FC1BG18-412 ME2BG25-390	19	0.81[s]
	180AV-2	3.1	LG08	65.4	BG23PM34-291 ME2PM47-160	8.9	0.42[s]
	180AV-3	3.0	LG12	22.1	ME2PM113-327 SA7PM113-327	7.9	0.41[h]

*'z' denotes that the resistance alleles were donated by the resistant cultivar Zhongyou 821, 'd' denotes that the resistance alleles were donated by DH6576, 'h' denotes that the resistance alleles were donated by the resistant cultivar Huashong3, 'and 's' denotes that the resistance alleles were donated by the susceptible cultivar Sentry.

** R^2 represents the percent phenotypic variation explained by each QTL.

† LG = Linkage Group.

Table 4.20. The common QTL among the different DH populations

QTL 1	QTL 2	Linkage group	Linked marker (s)	Populations
H1R3-2	*H3R2-4*	N16	SA7BG68-134	H1 and H3
H1R1-2	*H3R2-4*	N16	SA7BG53-271 and SA7PM37-185	H1 and H3
H1AV-1	*H3R2-1*	N07	SA7BG35-381	H1 and H3
H2AV-3	*H3R3-3*	N09	SA7PM17-263	H2 and H3
179R1-2	*180R1-1*	LG03	ODD3PM40-191	DH179 and DH180
179R3-3	*180AV-1*	LG08	ME2BG25-390	DH179 and DH180
179R3-3	*180AV-2*	LG08	SA7BG1-125	DH179 and DH180
179R3-4	*H3R2-2*	N12	ODD4PM1-152 and BG23BG14-122	DH179 and H3

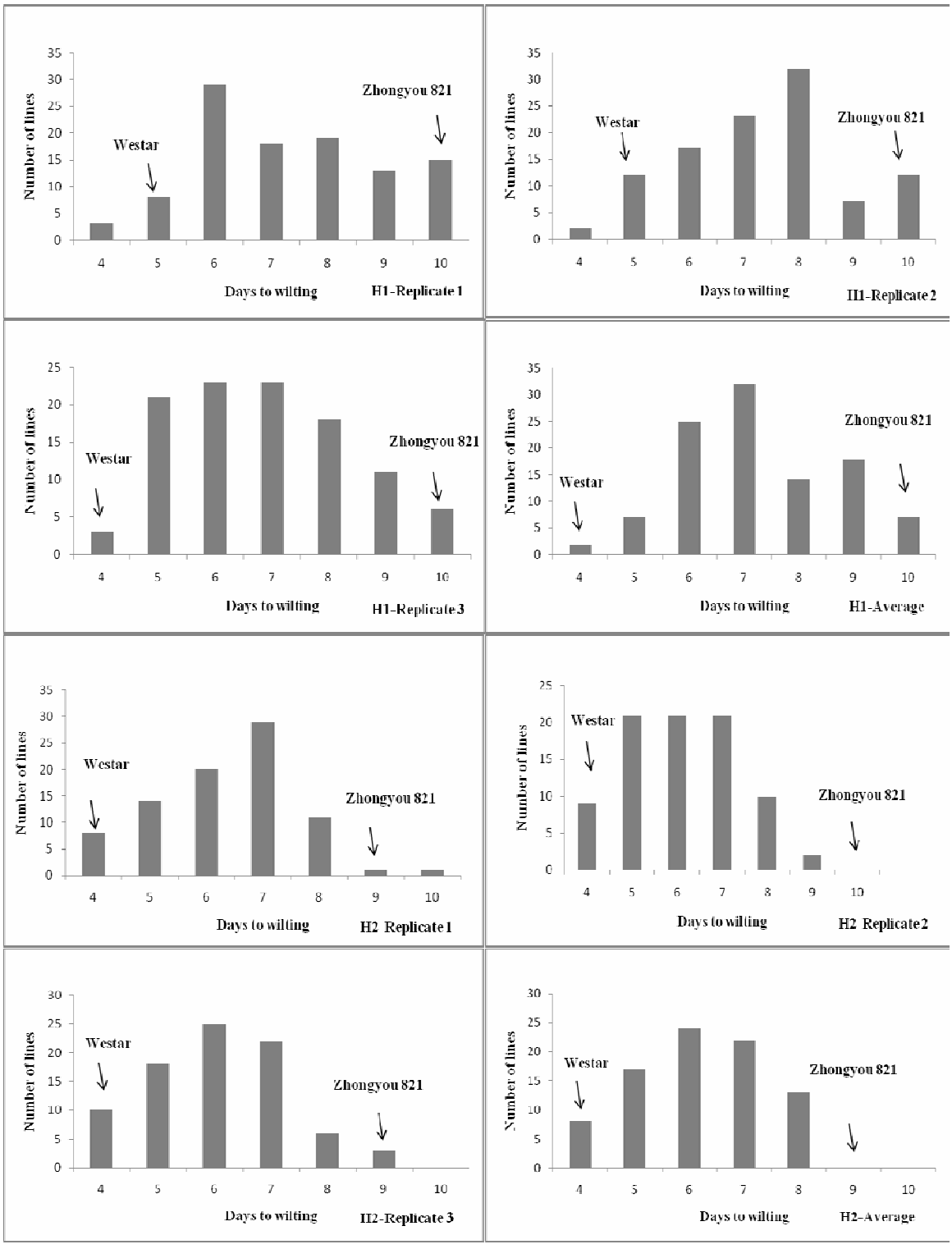

Figure 4.1 Frequency distribution graphs of days to wilting of each replicate as well as average in the H1 and H2 populations. Performance of checks Westar and Zhongyou 821 is indicated on each graph. The graphs are showing continuous variation for days to wilting revealing quantitative inherence of resistance to *Sclerotinia* stem rot. Susceptible and resistant checks differed significantly for days to wilting in each replicate

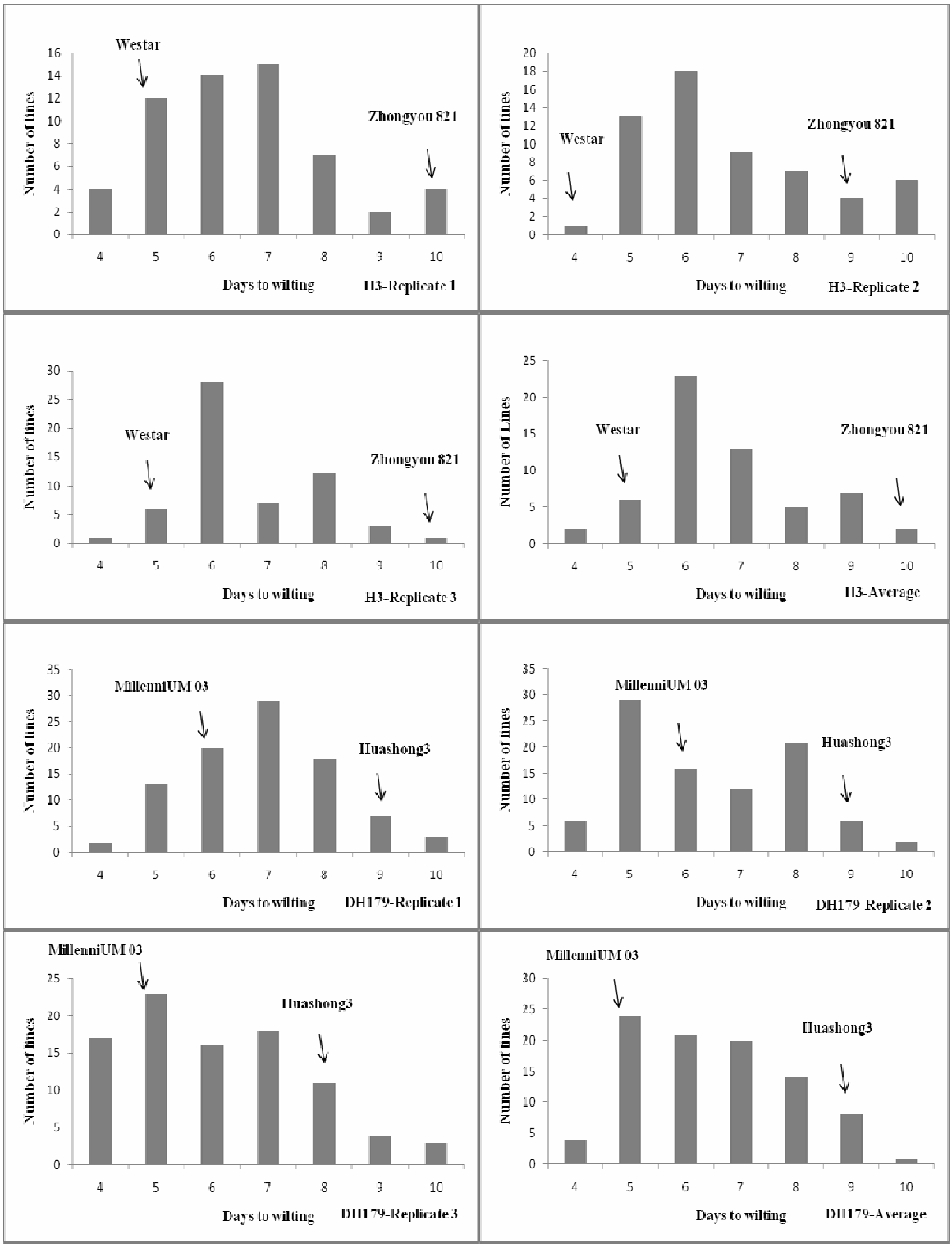

Figure 4.2 Frequency distribution graphs of days to wilting of individual replicates and average in the H3 and DH179 populations. Performance of parental cultivars is indicated on each graph. The graphs are showing continuous variation for days to wilting revealing quantitative inherence of resistance to *Sclerotinia* stem rot. Susceptible and resistant checks/ parents differed significantly for days to wilting in each replicate

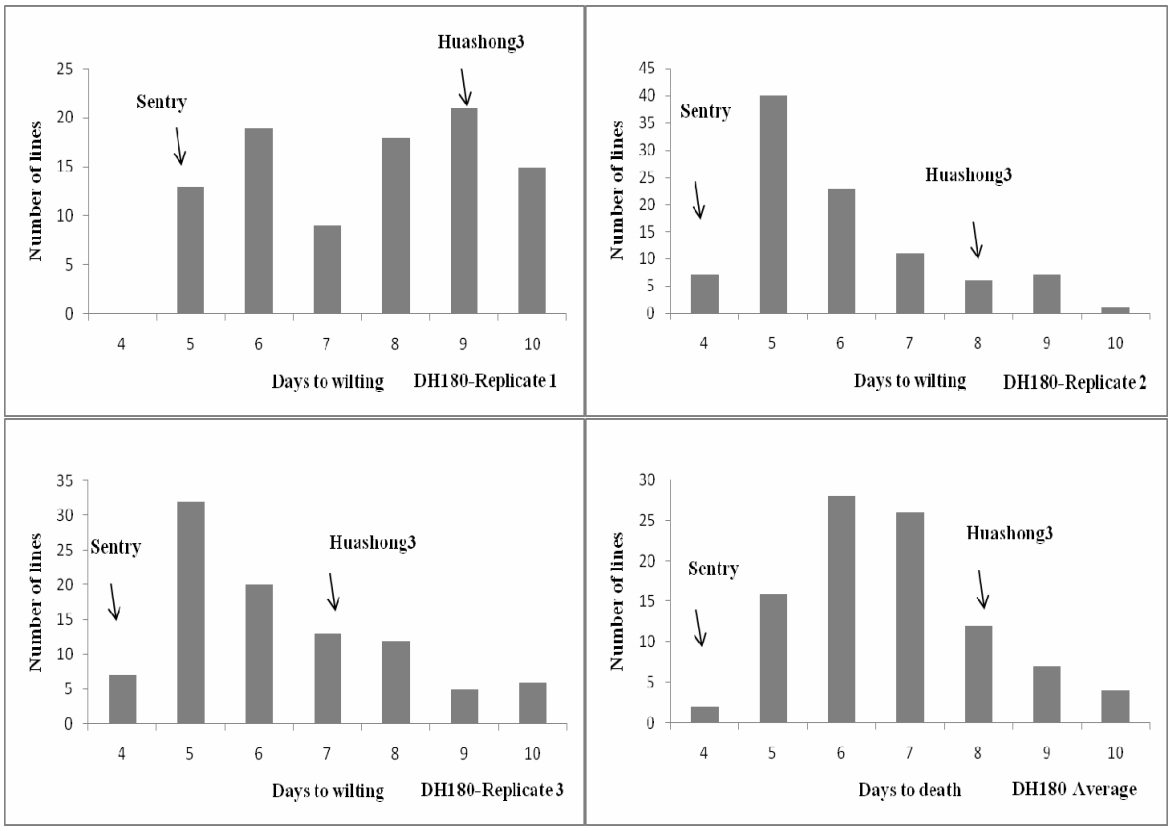

Figure 4.3 Frequency distribution graphs of individual replicate as well as average of DH180 population. Performance of parental lines is indicated on each graph. The graphs are showing continuous variation for days to wilting revealing quantitative inherence of resistance to *Sclerotinia* stem rot. Susceptible and resistant parents differed significantly for days to wilting in each replicate

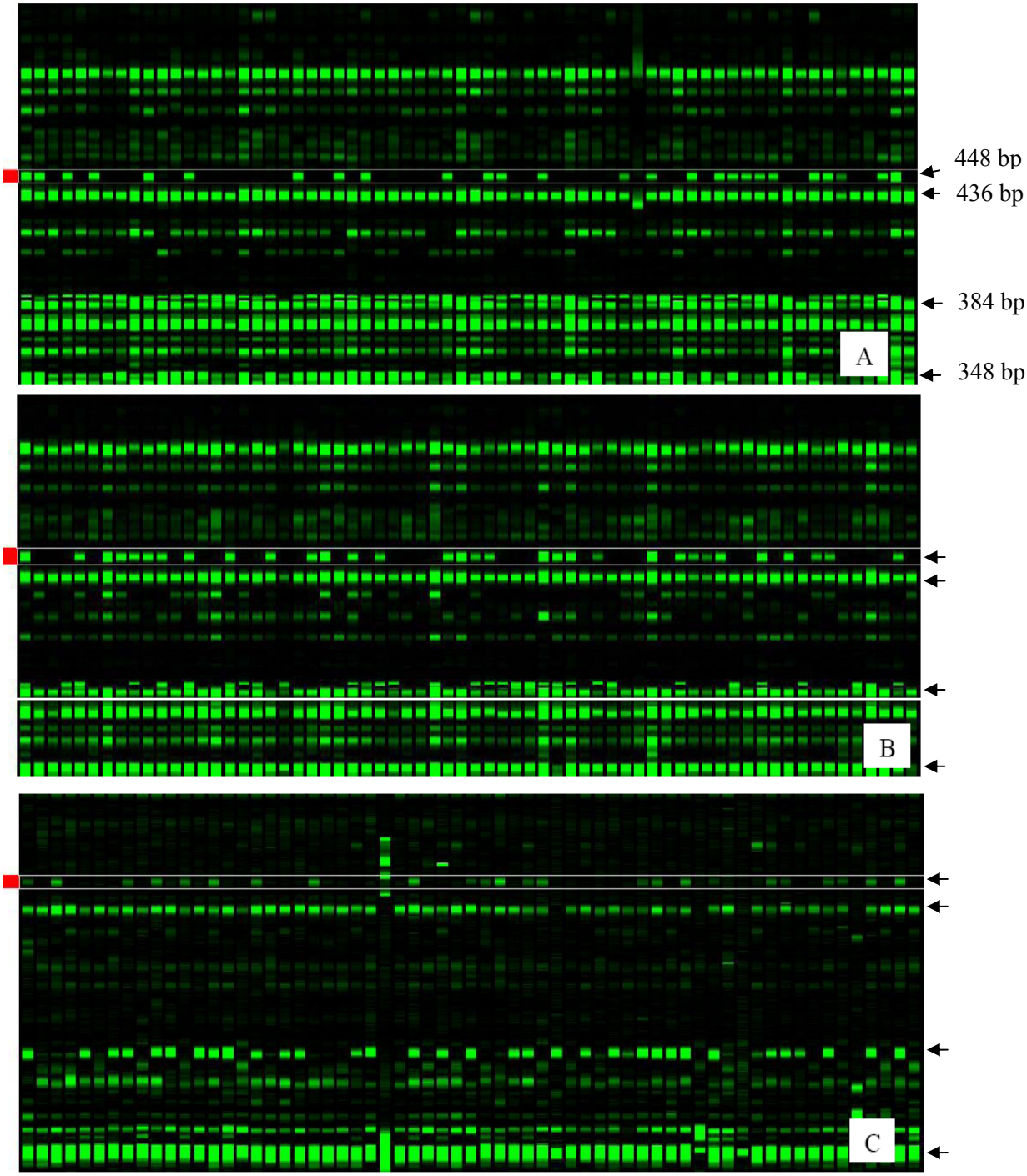

Figure. 4.4 Sequence related amplified polymorphism (SRAP) profiles amplified with SA7 and PM5 primer pairs in three doubled haploid populations: H1 (A), H2 (B) and H3 (C). Marked bins in each profile show the locations of marker SA7PM5-448. SRAP profiles of all three populations were same. For example, alleles of size 436 and 348 base pairs (bp) were amplified in all three populations and were non-polymorphic, whereas, allele of size 384 bp was also amplified in all three population but was non-polymorphic in the H1 and H2 populations but was polymorphic in the H3 population

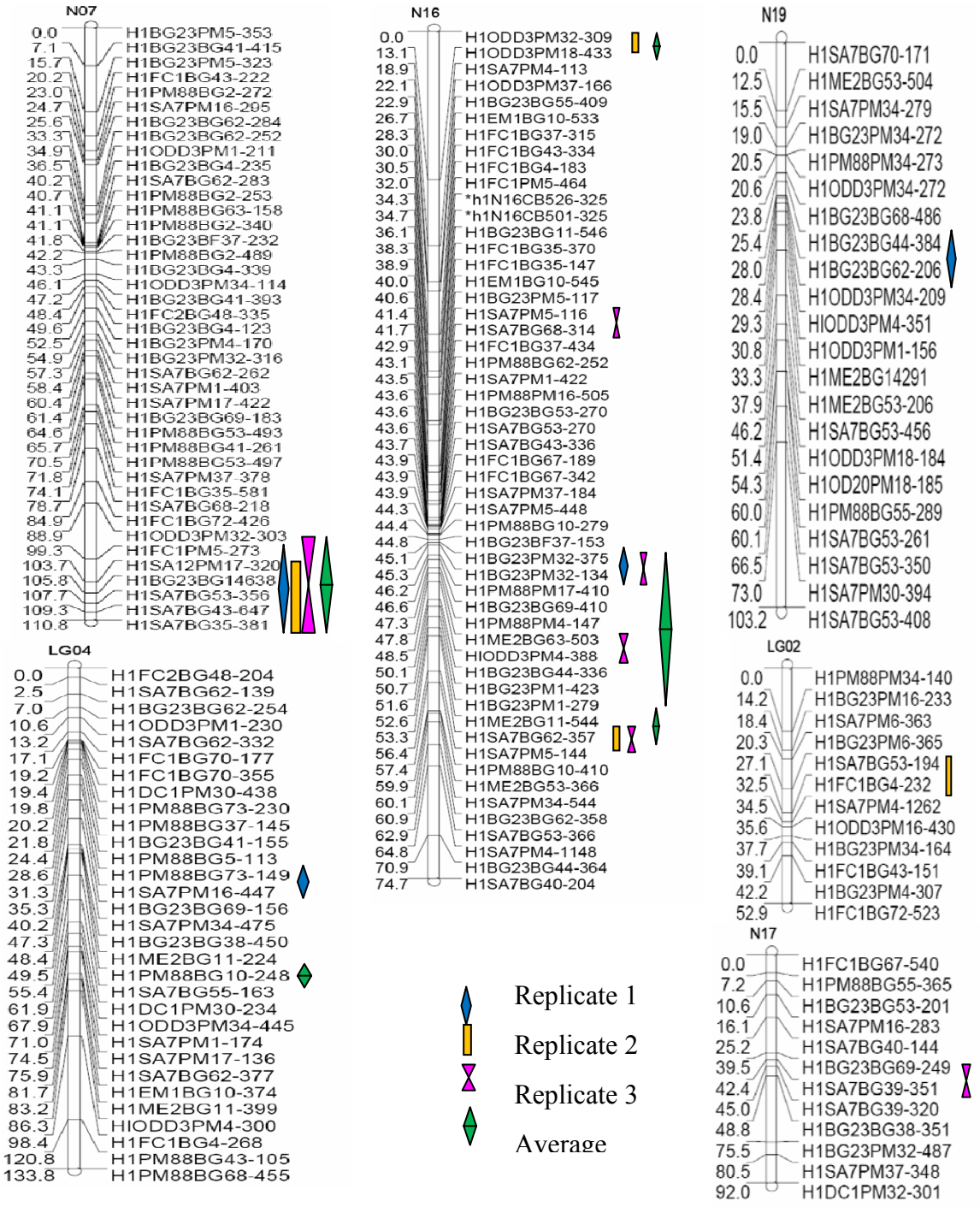

Figure 4.5 The locations of putative QTL for resistance to *Sclerotinia* stem rot in the H1 population (Zhongyou 821 X DHBao604). QTL analyses were performed on days to wilting on the average all replicates as well as on individual replicates. Each legend shows the location of each significant QTL responsible for *Sclerotinia* stem rot resistance. Some common QTL regions were identified between the replicates and the average analysis for example on N07 and N16

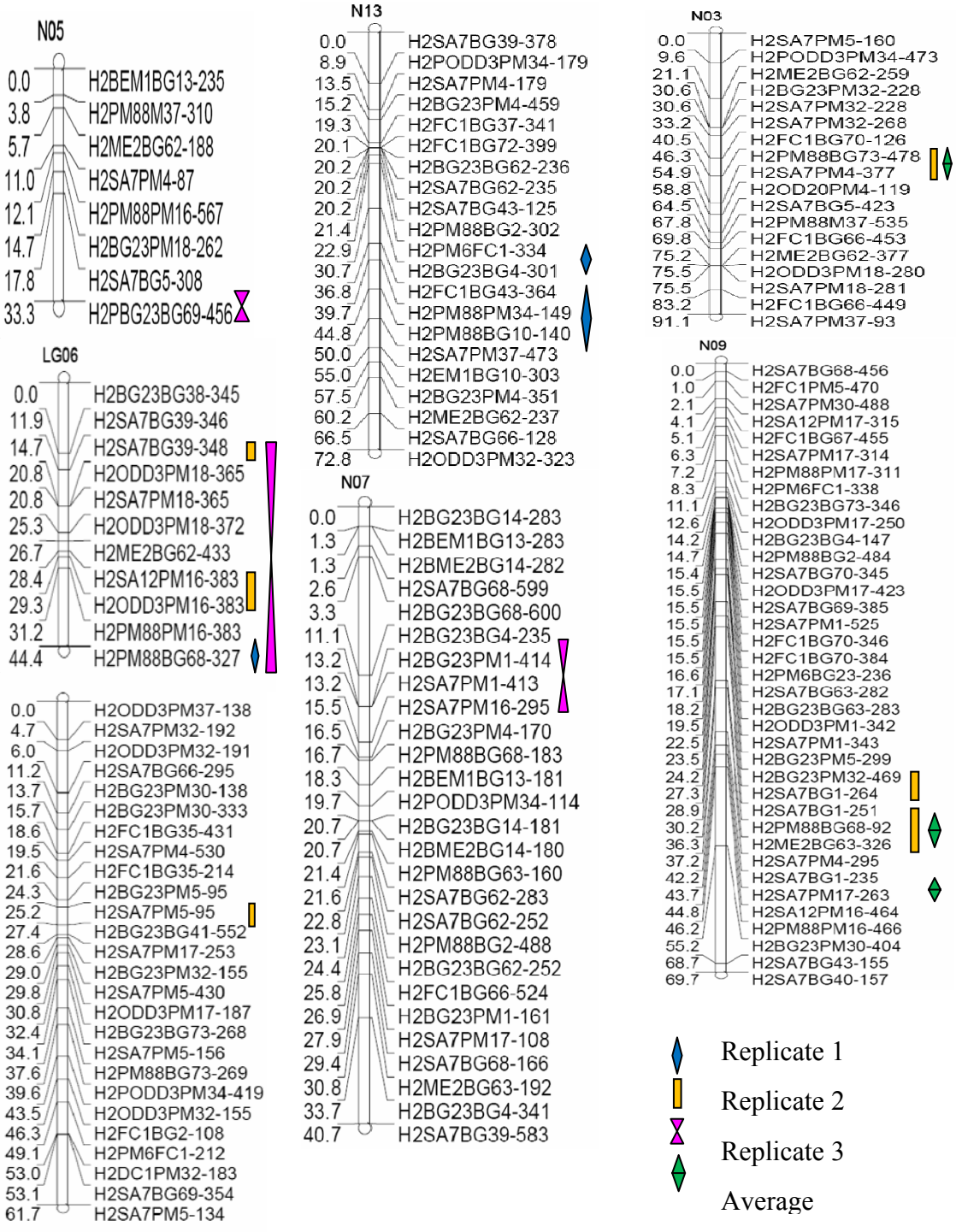

Figure 4.6 The locations of putative QTL for resistance to *Sclerotinia* stem rot in the H2 population (Zhongyou 821 X DH6576). Each legend shows the location of each significant QTL responsible for *Sclerotinia* stem rot resistance. Some common QTL regions were identified between the replicates and the average analysis for example on N03, N09 and LG06

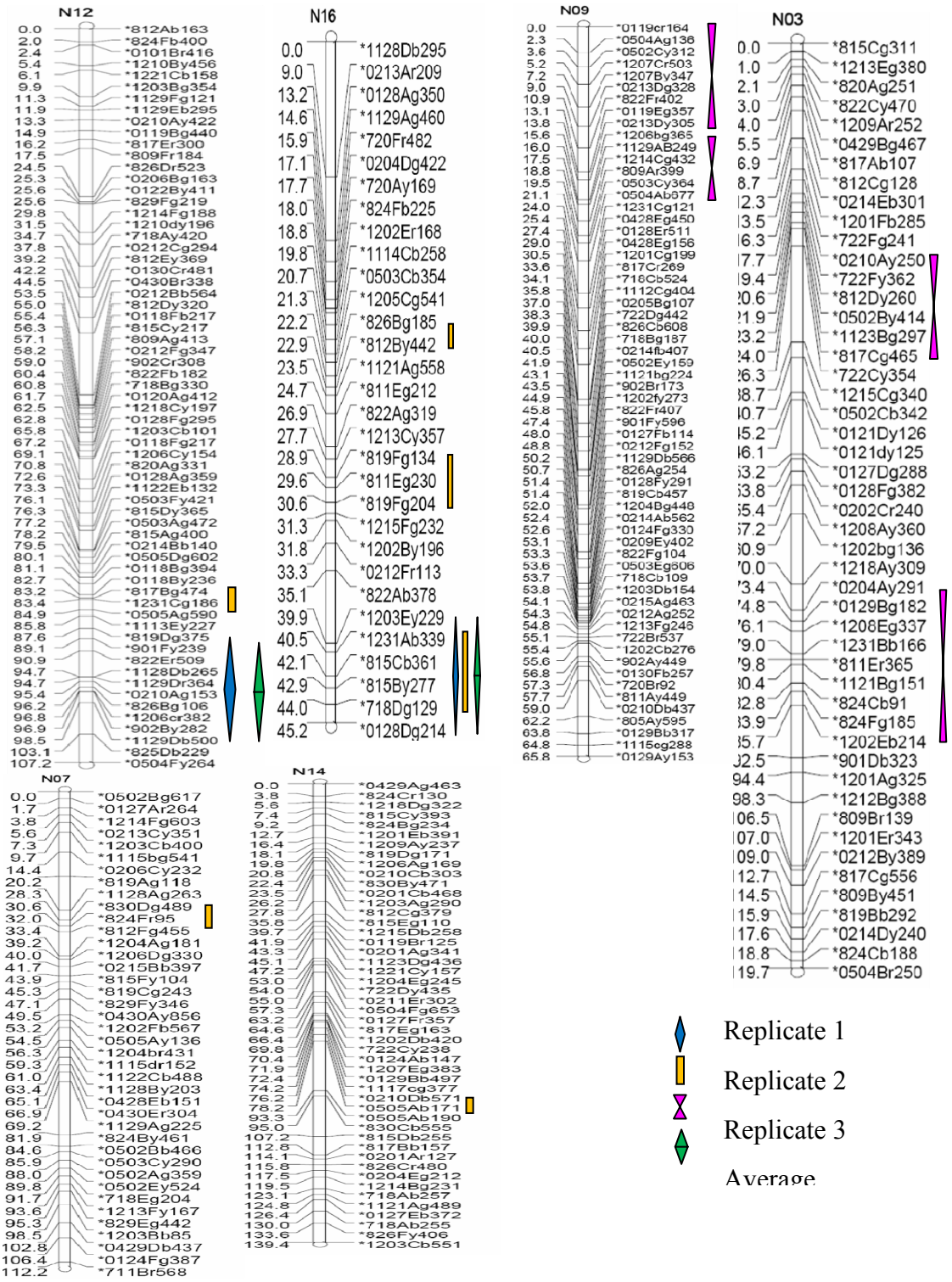

Figure 4.7 The locations of putative QTL for resistance to *Sclerotinia* stem rot in the H3 population (Zhongyou 821 X Westar). Each legend shows the location of each significant QTL responsible for *Sclerotinia* stem rot resistance. Some common QTL regions were identified between the replicates and the average analysis for example on N12 and N16

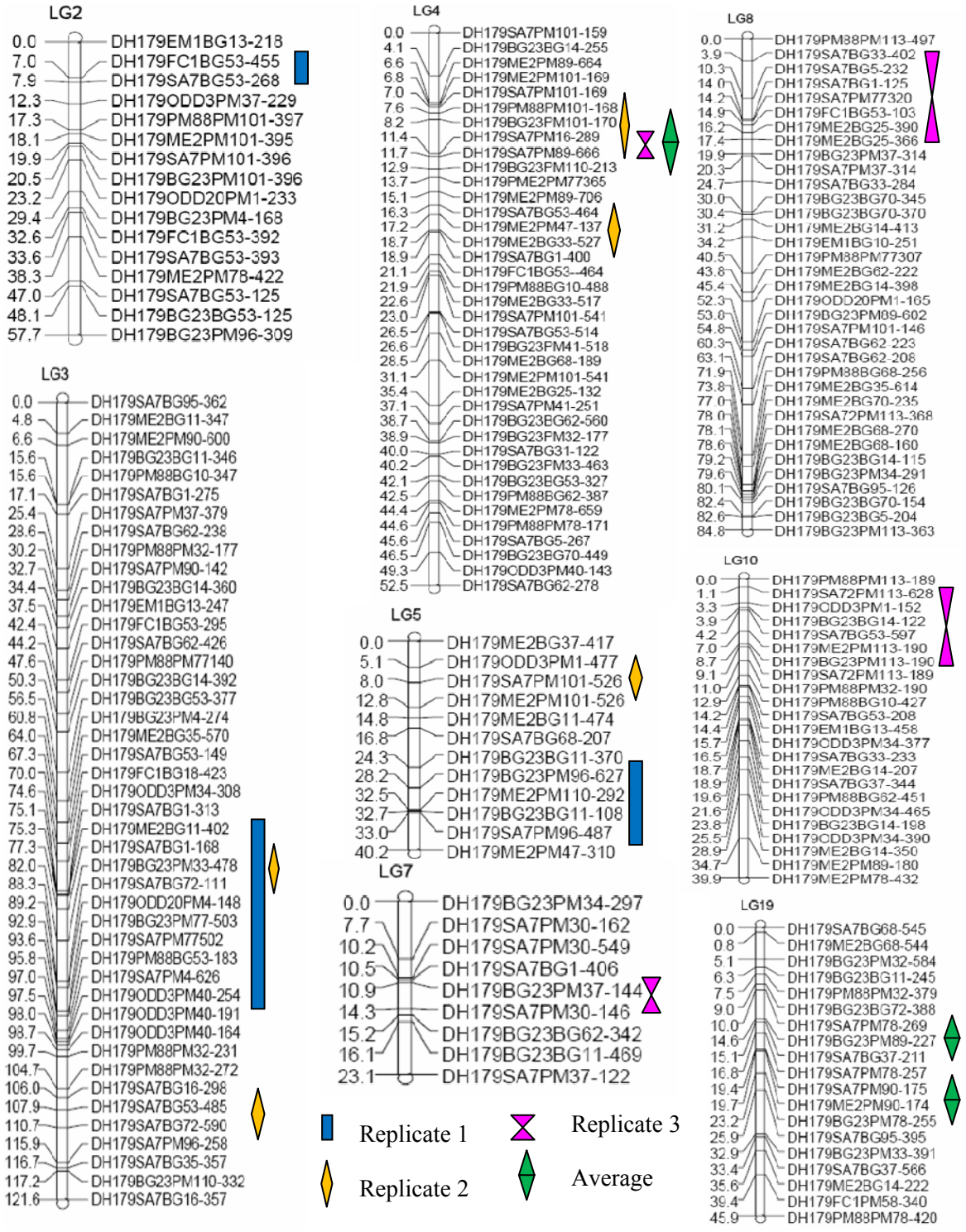

Figure 4.8 The locations of QTL for resistance to *Sclerotinia* stem rot detected in the DH179 population (Huashong3 X MillenniUM03). Each legend shows the location of each significant QTL responsible for *Sclerotinia* stem rot resistance. Some common QTL regions were identified between the replicates and the average analysis for example on LG03 and LG04

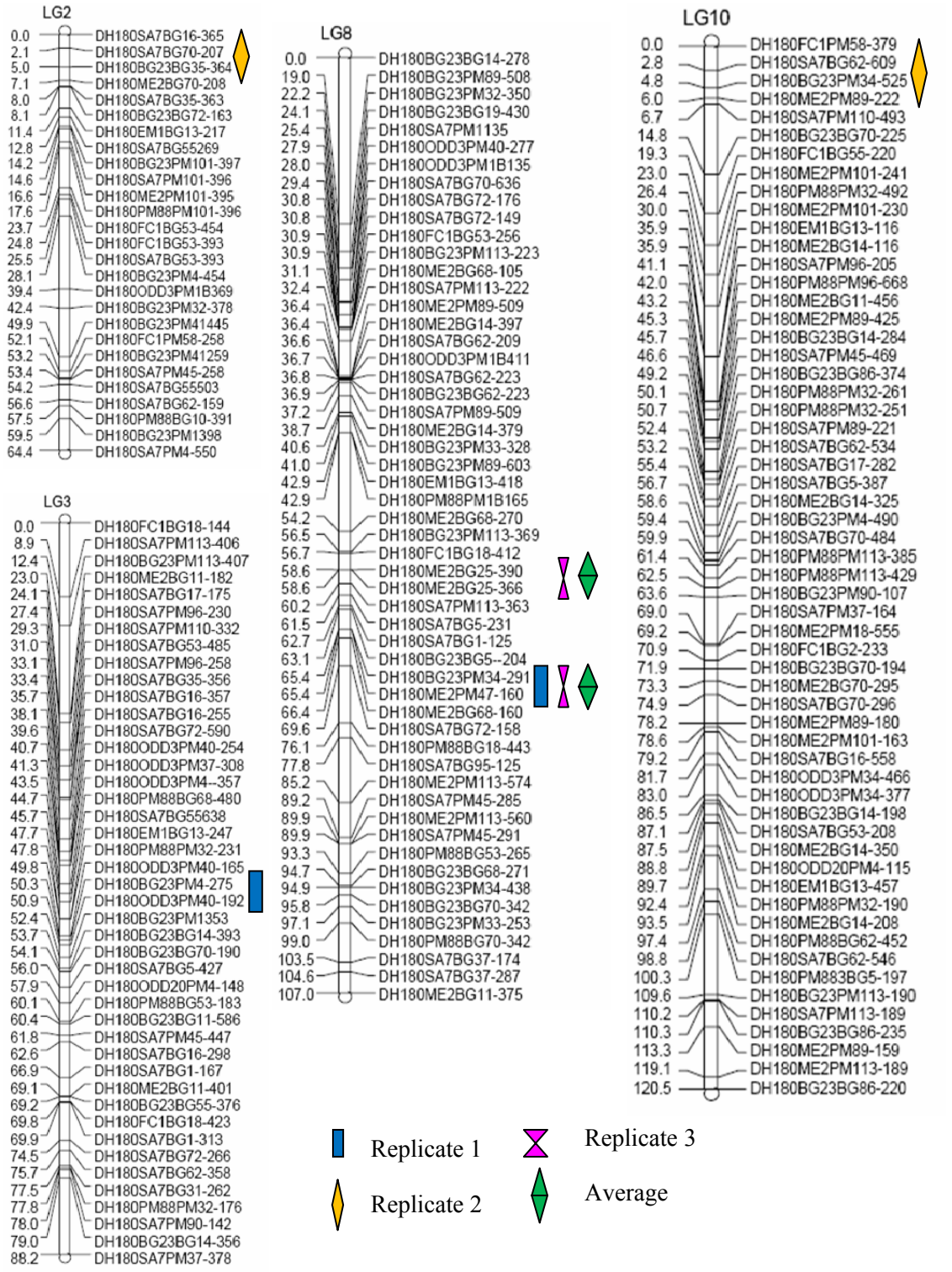

Figure 4.9 The locations of QTL for resistance to *Sclerotinia* stem rot detected in the DH population DH180 (Huashong3 X Sentry) in four analyses. Each legend shows the location of each significant QTL responsible for *Sclerotinia* stem rot resistance. Some common QTL regions were identified between the replicates and the average analysis for example on LG08

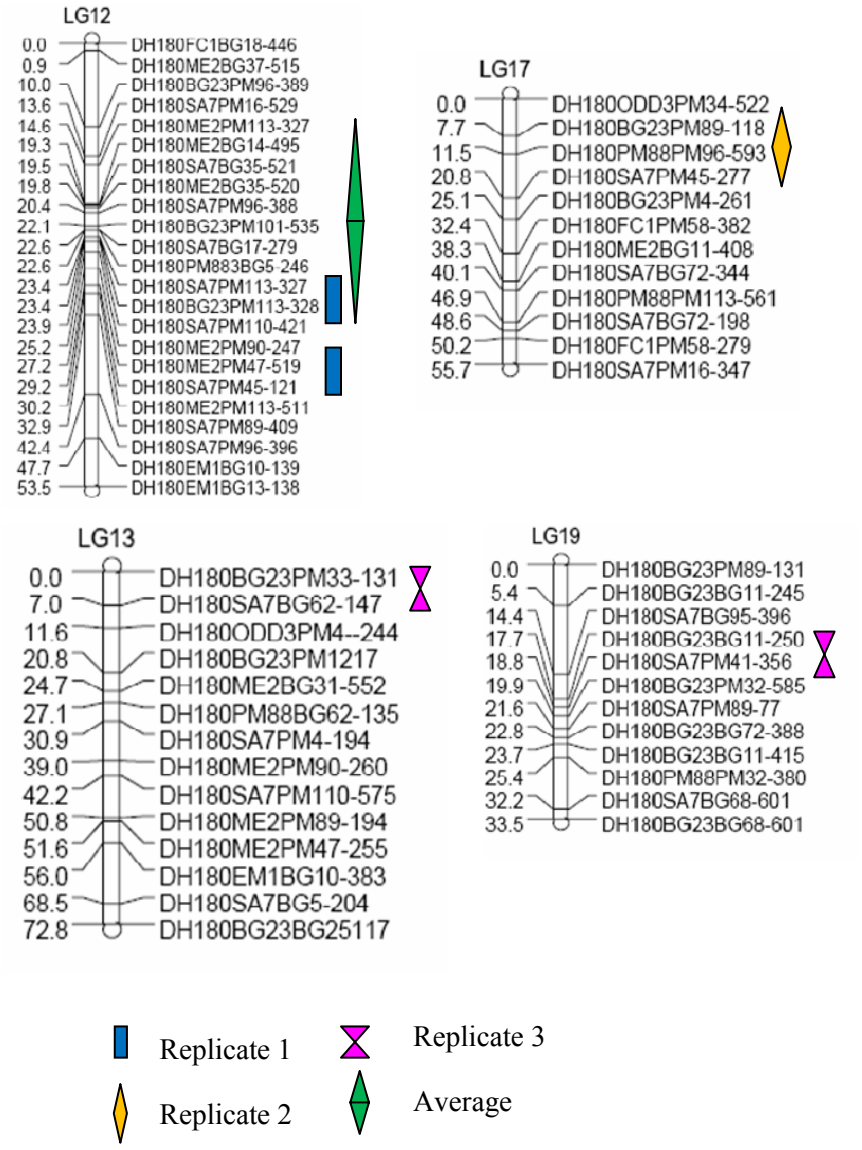

Figure 4.10 The locations of QTL for resistance to *Sclerotinia* stem rot detected in the DH population DH180 (Huashong3 X Sentry) in four analyses. Each legend shows the location of each significant QTL responsible for *Sclerotinia* stem rot resistance. Some common QTL regions were identified between the replicates and the average analysis for example on N12

5.0 GENERAL DISCUSSION AND CONCLUSIONS

In this research, a reliable controlled-environment based screening method was identified to evaluate resistance to *S. sclerotiorum* after comparing four screening methods. The petiole inoculation technique method was selected to evaluate five DH populations of *B. napus*. Four genetic maps were constructed using the sequence related polymorphism (SRAP) marker technique. These four genetic maps, together with a previously published genetic map were used to map the QTL responsible for resistance to *S. sclerotiorum* in these five DH populations.

In the first study, the excised leaf assay, the cotyledon assay, the mycelial stem inoculation technique and the petiole inoculation technique were compared. Each screening method was evaluated for its ability and reliability to differentiate between resistance and susceptibility to *Sclerotinia* stem rot in *B. napus*. The mycelial stem inoculation technique and petiole inoculation technique were identified as reliable methods. In mycelial stem inoculation, mature plant stems were inoculated with fungal mycelia. Under field conditions, disease starts when ascospores germinate on dead or dying floral parts. Mycelium then invades the main stem of infested plant. The mycelial stem inoculation technique could be a better estimate of field tolerance as mature stem resistance to fungal progress is a major factor in disease severity. The petiole inoculation technique was preferred because of space, time and human resource considerations. Research is required to determine the correlation between field evaluation results, and between the mycelial stem inoculation technique and the petiole inoculation technique results.

The rank correlations between the mycelial stem inoculation technique and the petiole inoculation technique were not significant at P = 0.05. However, the field resistant checks Zhongyou 821 and Huashong3, and the susceptible check Westar were correctly identified by both screening methods. It is possible that either the mycelial stem inoculation technique or the petiole inoculation technique, or both, may not be able to reliably evaluate the disease reaction of moderately resistant and moderately susceptible genotypes. This would lead to lack of significant correlation between the two methods.

Breeding programs generally handle large amounts of germplasm at different stages of development so efficiency is important. It is beneficial to have a disease screening method that requires a minimum of time, space and correlates well with field results. The cotyledon assay requires the least space and time compared to the other methods but this assay requires further standardization. The petiole inoculation technique on the other hand is moderately easy and requires less time and space than the mycelial stem inoculation technique. The petiole inoculation technique appears to be the suitable one for large scale screening.

The resistance to *S. sclerotiorum* is a quantitative trait. Quantitative traits are controlled by a number of genes and such traits are highly influenced by environmental factors. There were significant differences among the replicates in four out of five populations. Previous studies have also reported significant replicate effects. Temperature and relative humidity play an important role in *Sclerotinia* stem rot disease development. Environmental sensitivity of *S. sclerotiorum* and its implications on disease development have been mentioned in many previous studies. Although the experiments were

conducted in the same environmentally controlled greenhouse, it was very difficult to maintain a constant and uniform temperature over such a large area and time. Minor changes in the environmental conditions could have resulted in significant differences among the replicates. Although there were significant differences between the replicates, significant positive phenotypic correlations were seen among the replicates of each population. Repeated evaluation of resistance to *S. sclerotiorum* is necessary to find the true genetic potential of each line to resist *S. sclerotiorum*.

The SRAP technique was used to construct four genetic maps in this study. This technique has been used to develop genetic maps in *B. rapa* and *B. napus* in a number of previous studies. Using SSR markers, this study has successfully demonstrated that strong SRAP markers can also be used to assign a linkage group to particular chromosome. Anchoring a linkage group to its chromosome is important to compare the results of one study with other studies, especially when different marker systems are used to develop genetic maps.

QTL analyses were carried out on individual replicates as well as on the average of all replicates for each population. In the previous studies there was little consistency among the identified QTL in different replicates or using different screening methods. A number of common QTL responsible for resistance to *Sclerotinia* stem rot were identified. In this study, some QTL were identified only in one analysis but not in others. These loci must be active only in particular environmental conditions. *Sclerotinia sclerotiorum* produces a large number of hydrolytic enzymes with each enzyme having different isozymes. Optimal enzymatic activity condition requirements of each enzyme

and isozyme differ significantly. This gives evolutionary advantage to the fungus to infect plants in wide range of environmental conditions. Changes in environmental conditions probably trigger a particular set of hydrolytic enzymes which have optimal enzymatic activity in that particular environment, leading to detection of distinct loci.

The genomic regions on linkage group N03 were repeatedly identified in the H2 and H3 populations. The genomic regions on linkage group N07 were detected for *Sclerotinia* stem rot resistance in all five DH populations. QTL on linkage group N12 were detected in the H3, DH179 and DH180 populations. Similarly genomic regions of linkage group N16 were detected repeatedly in the H1 and H3 populations. Some distinct QTL were identified on linkage groups N05, N06, N14, N17 and N19. QTL for resistance to *Sclerotinia* stem rot were detected on linkage groups N03, N05, N06, N07, N14, N16, N17 and N19 in the previous two studies.

A number of common QTL between the populations were identified. The common genomic regions on N07 and N16 were identified between the H1 and H3 populations. The genomic regions involved in *Sclerotinia* stem rot resistance on LG03 and LG08 were common between the DH 179 and DH180 populations. The resistant parent of the H3 (Zhongyou 821) and DH179 population (Huashong3) were different, however, one common genomic region on N12 was identified between these populations. These results suggest that there were some common defense mechanism between these two resistant parents. No other study has reported any common QTL between populations to date.

Sclerotinia stem rot is a very difficult disease on which to conduct research. Limited reports in the literature on the topic reflect the difficulty of studying the host-pathogen interaction. There is only one cultivar registered in Canada that is tolerant to *Sclerotinia* stem rot. Researchers have been making efforts to identify *Sclerotinia* resistance loci for years. The polygenic inheritance, limited resistance sources, partial resistance, ineffective field screening, unavailability of a single standard indoor screening method and profound environmental effects on disease development are the main reasons for slow progress in the development of *Sclerotinia* resistant cultivars. A considerable amount of progress has been made in this study over previous studies. The modified petiole inoculation method reported in this study overcomes differential growth habits of spring and winter type of genotypes. It also requires less space and time than previously reported methods. The identified QTL showed more consistency than seen in previously reported studies in *B. napus*. Common QTL between the different populations were also identified and reported in the first time.

6.0 RECOMMENDATIONS FOR FUTURE STUDIES

The genetic maps developed in this study would be very useful for future research. Four new genetic maps for four DH populations were developed. Five genetic maps for five *B. napus* DH populations segregating for *Sclerotinia* stem rot resistance are now available. Microsatellite markers should be integrated on these genetic maps to compare the QTL locations with previous studies. Expressed Sequence Tags (EST) markers related to *Sclerotinia* stem rot resistance can also be integrated on the existing genetic maps to identify the genes responsible for resistance to *Sclerotinia* stem rot. These DH populations can be used for repeated evaluation of *Sclerotinia* stem rot resistance. Therefore, these populations can be used to confirm reliable QTL for resistance to *Sclerotinia* stem rot. In this study, some common QTL for resistance to *Sclerotinia* stem rot were found. There are a variety of indoor screening methods available to evaluate *Sclerotinia* stem rot resistance in *B. napus*. These populations should be evaluated for *Sclerotinia* stem rot resistance with other controlled-environment screening methods as well as in the field. The field evaluation of resistance to *Sclerotinia* stem rot should be carried out in locations where disease occurs frequently for instance in China. The QTL detected with different indoor screening methods and field evaluations should be compared to identify common QTL between different screening methods and populations. These common QTL should be considered reliable for use in marker assisted plant selection. The verification of reliable QTL should be carried out by developing nearly isogenic lines for these common QTL using a backcross breeding procedure and marker assisted selection.

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APPENDICES

Appendix i

Days to wilting of each DH line in each replicate and average days to wilting for the H1 population

DH line	Replicate 1	Replicate 2	Replicate 3	Average
H1-120	5.4	7.2	8.5	7.0
H1-124	9.1	8.0	8.0	8.4
H1-126	5.9	7.4	5.3	6.2
H1-135	7.5	5.8	8.0	7.1
H1-136	5.8	6.1	5.6	5.8
H1-140	4.9	7.0	4.7	5.5
H1-148	9.4	7.7	8.1	8.4
H1-150	6.6	6.8	3.7	5.7
H1-152	5.6	6.4	5.0	5.6
H1-155	6.5	6.5	4.1	5.7
H1-158	3.9	3.9	3.8	3.9
H1-162	7.1	7.1	5.6	6.6
H1-164	9.1	8.0	7.5	8.2
H1-166	4.1	5.4	4.7	4.7
H1-177	7.7	7.5	6.2	7.1
H1-179	6.9	7.3	5.6	6.6
H1-181	8.6	7.7	7.9	8.1
H1-183	4.8	5.0	4.6	4.8
H1-184	9.7	7.1	9.6	8.8
H1-185	5.7	7.3	7.2	6.7
H1-186	7.1	7.9	5.3	6.8
H1-188	5.4	4.8	5.5	5.2
H1-191	5.7	5.4	4.6	5.2
H1-195	5.9	7.4	6.7	6.7
H1-201	5.3	7.0	8.5	6.9
H1-203	8.2	7.3	6.8	7.4
H1-204	6.5	5.6	6.7	6.3
H1-205	4.3	4.9	5.2	4.8
H1-206	7.8	8.5	7.5	7.9
H1-210	5.9	5.8	4.6	5.4
H1-214	6.3	5.0	6.2	5.8
H1-216	8.7	8.2	5.8	7.6
H1-227	5.1	6.4	8.3	6.6
H1-228	4.2	5.1	3.5	4.3
H1-318	5.6	4.8	5.4	5.2
H1-319	9.5	9.7	6.2	8.4
H1-324	6.5	6.0	7.0	6.5
H1-330	5.3	5.3	5.0	5.2
H1-332	5.5	7.5	6.0	6.3
H1-388	6.5	9.8	9.6	8.6
H1-391	8.7	7.7	7.4	7.9
H1-392	4.4	6.5	7.1	6.0
H1-400	9.7	9.4	11.0	10.0
H1-401	5.8	4.9	4.1	4.9
H1-402	7.5	7.4	5.0	6.6
H1-403	5.6	5.4	6.1	5.7
H1-406	9.8	11.1	7.4	9.4

H1-407	4.5	6.7	5.8	5.6
H1-408	7.3	4.9	4.5	5.5
H1-409	8.5	6.6	7.1	7.4
H1-411	5.9	4.5	5.6	5.3
H1-413	9.6	7.2	8.0	8.3
H1-414	4.4	5.4	4.7	4.8
H1-510	5.6	4.5	5.3	5.1
H1-512	6.0	5.5	4.3	5.3
H1-515	7.7	7.3	4.6	6.5
H1-520	8.7	9.1	6.3	8.0
H1-525	7.9	5.7	5.7	6.4
H1-528	7.2	5.1	4.9	5.7
H1-534	5.7	7.5	6.6	6.6
H1-537	7.8	7.4	6.9	7.4
H1-539	5.1	7.7	6.8	6.5
H1-544	6.7	9.5	7.0	7.7
H1-548	5.5	6.8	8.0	6.8
H1-550	7.0	7.2	5.2	6.5
H1-553	4.0	3.4	4.3	3.9
H1-561	5.1	6.3	5.3	5.6
H1-562	5.3	5.3	6.5	5.7
H1-563(1)	6.3	6.5	7.5	6.8
H1-564	6.8	6.9	5.9	6.5
H1-566	10.3	10.4	8.8	9.8
H1-570	5.9	7.3	4.8	6.0
H1-571	7.6	6.7	6.1	6.8
H1-573	9.6	6.8	7.8	8.1
H1-574	6.2	7.9	4.6	6.2
H1-575	7.7	7.8	5.1	6.9
H1-600	11.3	9.4	8.0	9.6
H1-601	9.0	9.3	5.8	8.0
H1-602	8.4	9.3	7.8	8.5
H1-604	5.5	7.1	6.4	6.3
H1-605	8.2	7.0	9.0	8.1
H1-633(1)	8.1	9.0	11.5	9.5
H1-633(2)	7.1	7.8	9.2	8.0
H1-657	6.2	8.5	8.7	7.8
H1-660	7.9	6.1	5.8	6.6
H1-664	5.2	5.5	5.4	5.4
H1-665	9.9	8.1	8.7	8.9
H1-667	6.4	7.5	6.6	6.8
H1-668	9.3	7.4	7.5	8.0
H1-673	7.7	6.7	6.3	6.9
H1-769	10.0	6.3	7.3	7.8
H1-773	7.0	4.5	6.1	5.9
H1-774	6.9	5.0	4.2	5.4
H1-780	5.7	5.4	5.4	5.5
H1-781	7.1	6.3	4.8	6.1
H1-782	8.1	8.8	8.5	8.5
H1-786	6.2	6.8	5.5	6.2
H1-787	8.0	12.2	8.5	9.6
H1-788	8.8	10.4	9.4	9.5
H1-791	3.8	4.1	4.4	4.1
H1-792	9.4	9.6	8.6	9.2
H1-793	10.7	6.0	6.3	7.7
H1-797	6.0	6.7	6.3	6.3

H1-799	8.6	8.5	9.5	8.9
H1-802	7.0	7.3	6.2	6.8
H1-825	7.8	7.2	6.5	7.2
Westar	4.3	4.3	3.5	4.0
MillenniUM 03	5.7	4.2	4.3	4.7
Sentry	5.0	4.9	4.2	4.7
Huashong3	7.3	8.7	7.9	8.0
Zhongyou 821	11.0	9.2	9.3	9.8

Appendix ii

Days to wilting of each DH line in each replicate and average days to wilting for the H2 population

DH line	Replicate 1	Replicate 2	Replicate 3	Average
H2-020	5.3	4.3	3.8	4.4
H2-023	7.5	5.1	5.6	6.1
H2-024	5.5	5.0	7.0	5.8
H2-025	5.5	6.4	7.8	6.6
H2-026	7.0	5.5	4.9	5.8
H2-030	6.7	6.0	6.0	6.2
H2-031	4.5	3.8	4.1	4.1
H2-034	7.0	6.2	8.6	7.3
H2-035	4.6	3.4	3.5	3.8
H2-036	5.5	4.5	4.8	4.9
H2-037	6.4	4.8	4.4	5.2
H2-039	6.6	4.8	4.9	5.4
H2-040	6.8	6.8	5.4	6.4
H2-044	5.3	6.4	5.1	5.6
H2-048	4.7	4.6	5.3	4.9
H2-049	6.5	5.4	7.2	6.4
H2-050	7.0	6.3	6.7	6.6
H2-052	6.3	4.6	5.6	5.5
H2-053	7.0	7.0	6.5	6.8
H2-055	7.0	7.0	6.3	6.8
H2-056	4.8	4.5	4.7	4.7
H2-057	6.0	5.8	6.0	5.9
H2-058	4.1	3.3	4.4	3.9
H2-059	7.6	4.3	4.7	5.5
H2-060	3.8	4.4	3.6	3.9
H2-061	7.1	6.7	6.7	6.8
H2-062	6.6	5.0	5.5	5.7
H2-066	3.6	3.7	3.8	3.7
H2-068	5.6	5.0	5.6	5.4
H2-072	3.3	4.4	3.3	3.6
H2-078	5.4	5.6	4.0	5.0
H2-080	7.1	5.6	6.3	6.4
H2-081	5.9	6.7	5.7	6.1
H2-085	7.3	7.5	7.5	7.4
H2-088	7.0	7.1	8.7	7.6
H2-090	8.5	7.5	6.9	7.6
H2-091	9.4	6.8	6.7	7.6
H2-208	6.3	5.8	5.7	5.9
H2-300	5.6	7.3	5.7	6.2
H2-304	7.0	9.0	6.3	7.4
H2-305	7.2	5.7	4.2	5.7
H2-307	5.8	5.4	6.2	5.8
H2-308	5.5	4.6	5.1	5.1
H2-310	5.4	5.3	5.1	5.3
H2-311	6.3	6.3	7.0	6.5
H2-312	6.8	5.6	4.4	5.6
H2-314	4.6	4.9	5.3	4.9

H2-334	6.3	4.6	3.0	4.6
H2-335	5.5	4.9	4.4	4.9
H2-336	5.2	4.3	4.5	4.7
H2-340	7.0	7.3	7.3	7.2
H2-366	6.5	8.3	5.3	6.7
H2-367	4.9	5.5	5.2	5.2
H2-416	4.9	5.7	6.3	5.6
H2-580	6.6	6.2	6.1	6.3
H2-584	6.8	5.4	5.2	5.8
H2-585	5.7	6.1	7.0	6.3
H2-586	6.0	6.0	6.8	6.3
H2-591	6.4	6.3	6.1	6.3
H2-593	3.8	6.4	4.6	4.9
H2-639	4.2	5.5	5.3	5.0
H2-647	5.7	6.8	4.6	5.7
H2-654	7.7	7.7	6.5	7.3
H2-704	4.0	3.9	4.3	4.1
H2-705	3.8	6.3	3.3	4.5
H2-706	6.3	6.1	7.1	6.5
H2-713	6.7	6.5	8.3	7.2
H2-722	5.9	6.0	5.9	5.9
H2-727	7.1	7.0	5.8	6.6
H2-731	7.0	7.2	7.0	7.1
H2-734	4.1	4.0	5.2	4.4
H2-738	6.6	6.6	6.0	6.4
H2-742	3.3	3.5	3.9	3.6
H2-743	7.5	7.2	8.0	7.6
H2-744	6.7	6.0	6.2	6.3
H2-745	7.3	7.3	6.8	7.1
H2-746	7.6	7.9	6.2	7.2
H2-748	5.1	5.3	6.3	5.6
H2-749	4.8	5.1	6.0	5.3
H2-827	4.6	4.9	4.9	4.8
H2-828	4.5	4.2	5.5	4.7
H2-837	4.3	4.0	4.4	4.3
H2-842	4.0	3.9	4.0	4.0
H2-849	6.4	4.4	4.3	5.1
Westar	3.9	3.8	3.5	3.7
MillenniUM 03	4.5	5.5	4.1	4.7
Sentry	5.0	4.7	4.6	4.8
Huashong3	7.5	7.8	9.7	8.3
Zhongyou 821	8.8	9.3	8.3	8.8

Appendix iii

Days to wilting of each DH line in each replicate and average days to wilting for the H3 population

DH line	Replicate 1	Replicate 2	Replicate 3	Average
H3-232	7.0	5.3	5.4	5.9
H3-233	6.5	7.1	7.9	7.2
H3-235	6.4	5.2	5.8	5.8
H3-236	5.2	6.4	5.6	5.7
H3-238	5.7	5.3	5.4	5.5
H3-240	7.5	7.5	5.3	6.8
H3-241	5.5	5.0	5.9	5.5
H3-242	6.9	7.7	6.5	7.0
H3-243	4.9	5.5	5.2	5.2
H3-245(2)	5.1	5.8	7.2	6.0
H3-248	9.8	8.3	7.4	8.5
H3-342	5.3	5.6	5.9	5.6
H3-344	4.5	4.8	5.5	4.9
H3-346(2)	7.0	5.0	7.4	6.5
H3-350	4.0	4.9	5.0	4.6
H3-351	4.7	6.4	6.6	5.9
H3-376	8.5	8.1	8.0	8.2
H3-378	7.4	6.8	7.2	7.1
H3-385	5.4	5.5	5.4	5.4
H3-386	7.3	6.2	7.4	6.9
H3-387	6.1	11.0	7.7	8.3
H3-419	8.0	10.9	6.7	8.5
H3-421	7.2	10.9	8.1	8.7
H3-423	4.7	6.4	5.4	5.5
H3-610	6.5	8.0	5.7	6.7
H3-611	5.5	5.3	5.3	5.4
H3-614	5.0	4.5	5.7	5.1
H3-615	6.0	5.7	6.8	6.1
H3-619	5.3	6.1	5.1	5.5
H3-623	6.8	5.8	8.1	6.9
H3-624	5.0	5.0	5.1	5.0
H3-628	11.3	9.3	11.0	10.5
H3-629	4.8	5.8	5.6	5.4
H3-653	5.8	5.3	5.2	5.4
H3-757	5.3	4.7	6.1	5.4
H3-758	6.1	6.2	5.7	6.0
H3-759	6.4	7.2	5.8	6.5
H3-760	6.5	6.8	5.5	6.3
H3-761	10.4	9.7	8.7	9.6
H3-762	5.4	6.0	5.4	5.6
H3-852	4.7	4.9	4.8	4.8
H3-853	6.5	4.9	5.4	5.6
H3-854	6.6	5.3	7.5	6.5
H3-859	5.2	5.0	5.4	5.2
H3-861	6.4	5.8	6.6	6.3
H3-862	3.8	4.5	3.3	3.8

H3-863	7.5	8.0	7.6	7.7
H3-867	4.3	5.6	4.3	4.7
H3-869	4.5	5.2	5.6	5.1
H3-871	8.7	9.9	7.3	8.6
H3-872	3.6	7.0	5.9	5.5
H3-875	6.9	5.8	6.0	6.3
H3-879	5.6	7.1	5.7	6.1
H3-881	4.8	4.8	5.0	4.8
H3-882	4.8	5.0	4.2	4.6
H3-885	9.6	8.3	7.3	8.4
H3-889	7.1	8.8	6.8	7.6
H3-895	3.1	3.9	4.7	3.9
Westar	4.3	3.4	4.8	4.2
MillenniUM 03	4.9	4.1	3.9	4.3
Sentry	5.0	5.3	5.5	5.3
Zhongyou 821	9.3	8.5	9.4	9.1

Appendix iv

Days to wilting of each DH line in each replicate and average days to wilting for the DH179 population

DH line	Replicate 1	Replicate 2	Replicate 3	Average
DH179-01	7.4	7.3	6.2	7.0
DH179-02	11.3	11.0	11.4	11.2
DH179-03	6.9	6.4	4.3	5.9
DH179-04	6.7	5.9	6.6	6.4
DH179-05	9.1	7.2	9.0	8.4
DH179-06	7.0	7.0	5.0	6.3
DH179-07	7.0	7.6	6.2	6.9
DH179-08	4.9	3.8	4.7	4.4
DH179-09	6.0	5.1	5.2	5.4
DH179-10	9.0	8.5	7.4	8.3
DH179-11	6.2	4.9	5.5	5.5
DH179-12	4.6	4.3	5.0	4.6
DH179-13	4.3	4.6	4.9	4.6
DH179-14	7.4	7.1	6.7	7.1
DH179-15	6.9	4.3	5.7	5.6
DH179-16	7.8	6.4	6.2	6.8
DH179-17	6.1	6.6	6.3	6.4
DH179-18	5.1	5.9	5.0	5.3
DH179-19	5.3	4.3	3.4	4.3
DH179-20	5.2	4.3	5.0	4.8
DH179-21	7.8	8.1	7.9	7.9
DH179-22	6.6	7.1	7.9	7.2
DH179-23	7.3	9.2	7.2	7.9
DH179-24	9.0	8.5	7.5	8.3
DH179-25	9.0	8.0	8.6	8.5
DH179-26	4.6	4.5	3.2	4.1
DH179-27	5.8	4.8	3.7	4.8
DH179-28	4.8	4.1	3.2	4.0
DH179-29	6.1	5.5	3.9	5.2
DH179-30	6.4	5.2	4.3	5.3
DH179-31	5.3	4.8	4.6	4.9
DH179-32	5.3	4.2	3.9	4.4
DH179-33	4.3	4.0	4.5	4.3
DH179-34	5.9	4.6	4.1	4.9
DH179-35	7.6	7.9	7.5	7.6
DH179-36	7.6	7.5	6.2	7.1
DH179-37	6.9	7.2	5.4	6.5
DH179-38	4.5	5.0	3.5	4.3
DH179-39	8.3	6.8	9.0	8.1
DH179-40	5.9	4.6	4.0	4.8
DH179-41	8.0	7.8	9.7	8.5
DH179-42	6.9	5.3	5.5	5.9
DH179-43	7.6	8.0	4.2	6.6
DH179-44	6.9	4.2	5.6	5.6
DH179-46	6.5	5.3	6.5	6.1
DH179-47	5.3	5.7	3.5	4.9

DH179-48	7.9	5.8	4.3	6.0
DH179-49	7.3	6.3	5.0	6.2
DH179-50	6.8	6.8	6.1	6.6
DH179-51	7.9	7.1	6.6	7.2
DH179-52	6.0	6.5	5.6	6.0
DH179-53	8.6	7.1	7.4	7.7
DH179-54	6.0	4.4	5.0	5.1
DH179-55	6.4	5.0	7.0	6.1
DH179-56	6.4	6.4	4.0	5.6
DH179-57	9.7	7.5	7.8	8.3
DH179-58	6.0	4.4	4.6	5.0
DH179-59	6.3	5.0	6.0	5.8
DH179-61	6.5	6.8	4.8	6.0
DH179-62	5.2	4.8	4.8	4.9
DH179-63	6.5	5.3	5.6	5.8
DH179-64	5.3	4.1	5.4	4.9
DH179-65	5.0	6.0	7.7	6.2
DH179-66	7.2	7.9	5.3	6.8
DH179-67	7.7	8.9	5.5	7.4
DH179-68	6.8	5.0	3.9	5.2
DH179-69	6.1	4.8	5.0	5.3
DH179-70	8.3	8.4	10.3	9.0
DH179-71	4.5	4.1	4.4	4.3
DH179-72	7.1	5.1	5.5	5.9
DH179-73	5.5	4.0	4.5	4.7
DH179-75	7.3	5.6	5.9	6.2
DH179-76	6.2	4.2	6.8	5.7
DH179-77	4.5	3.9	3.4	4.0
DH179-78	5.2	3.7	5.1	4.7
DH179-79	5.8	7.3	6.6	6.6
DH179-82	7.9	8.0	7.4	7.8
DH179-84	8.3	8.0	7.0	7.8
DH179-86	6.8	7.3	7.3	7.1
DH179-87	3.8	6.9	3.9	4.8
DH179-88	6.5	4.9	4.8	5.4
DH179-89	6.7	7.3	5.2	6.4
DH179-90	6.0	5.5	6.9	6.1
DH179-91	6.3	8.7	8.4	7.8
DH179-92	4.9	4.8	4.2	4.6
DH179-93	5.3	5.2	3.7	4.7
DH179-94	7.4	5.6	6.2	6.4
DH179-95	6.3	6.3	6.7	6.4
DH179-96	4.1	4.2	3.3	3.9
DH179-97	4.0	3.6	3.8	3.8
DH179-98	7.0	7.3	7.0	7.1
DH179-100	4.8	4.3	3.5	4.2
Westar	4.1	4.3	4.0	4.1
MillenniUM 03	5.4	5.3	4.4	5.0
Huashong3	8.2	8.2	8.0	8.1
Zhongyou 821	10.6	9.9	9.8	10.1

Appendix v

Days to wilting of each DH line in each replicate and average days to wilting for the DH180 population

DH line	Replicate 1	Replicate 2	Replicate 3	Average
DH180-1	5.0	4.3	3.4	4.3
DH180-2	5.7	4.9	4.8	5.1
DH180-3	4.7	3.4	4.1	4.1
DH180-4	9.2	4.1	6.7	6.7
DH180-5	7.2	3.6	4.8	5.2
DH180-6	5.3	4.8	4.8	4.9
DH180-7	5.0	4.1	8.8	6.0
DH180-8	7.5	5.3	4.2	5.7
DH180-9	5.5	5.3	4.4	5.1
DH180-10	4.5	4.3	5.4	4.7
DH180-11	4.4	8.2	6.5	6.4
DH180-12	5.3	3.7	3.2	4.1
DH180-13	7.7	5.1	4.6	5.8
DH180-14	4.8	3.7	3.0	3.8
DH180-15	10.2	6.4	7.4	8.0
DH180-16	7.0	4.5	4.2	5.2
DH180-18	8.3	5.0	6.3	6.5
DH180-19	8.9	7.8	9.1	8.6
DH180-20	9.4	7.3	9.8	8.8
DH180-21	5.1	4.6	5.8	5.2
DH180-22	5.0	5.3	4.4	4.9
DH180-23	5.9	4.8	6.1	5.6
DH180-24	5.9	4.4	5.9	5.4
DH180-25	10.0	4.8	5.6	6.8
DH180-26	6.6	3.9	4.9	5.1
DH180-27	8.0	4.6	5.3	6.0
DH180-28	7.3	4.6	4.4	5.4
DH180-29	5.7	4.7	3.4	4.6
DH180-30	6.5	4.9	6.2	5.9
DH180-31	7.0	6.0	4.9	6.0
DH180-32	7.4	6.3	9.3	7.7
DH180-33	6.8	5.6	4.3	5.5
DH180-34	5.1	5.6	4.9	5.2
DH180-35	6.3	4.6	6.4	5.8
DH180-36	8.9	5.2	4.6	6.2
DH180-37	7.8	6.2	6.1	6.7
DH180-38	9.1	4.2	4.3	5.8
DH180-39	8.8	5.4	5.8	6.7
DH180-40	7.8	5.8	7.3	7.0
DH180-41	7.8	4.3	6.4	6.1

DH180-42	8.8	5.5	4.8	6.4
DH180-43	5.0	5.9	5.1	5.3
DH180-44	9.9	8.5	9.3	9.2
DH180-45	8.5	7.9	8.3	8.2
DH180-46	5.5	5.5	3.8	4.9
DH180-47	5.7	5.0	5.2	5.3
DH180-48	5.7	6.7	6.2	6.2
DH180-49	7.9	6.4	4.4	6.2
DH180-51	5.4	5.8	4.7	5.3
DH180-53	8.4	7.4	6.4	7.4
DH180-54	5.8	4.4	5.0	5.0
DH180-55	8.8	6.2	4.5	6.5
DH180-56	5.2	5.0	4.5	4.9
DH180-57	8.8	4.5	5.4	6.2
DH180-59	5.2	4.3	5.4	5.0
DH180-60	6.9	5.0	5.1	5.7
DH180-62	7.4	5.3	5.9	6.2
DH180-63	9.5	6.5	5.0	7.0
DH180-65	8.8	5.5	4.8	6.4
DH180-66	9.0	8.8	9.1	9.0
DH180-74	8.7	6.0	7.1	7.3
DH180-77	6.4	7.7	8.0	7.4
DH180-78	9.8	6.2	7.7	7.9
DH180-79	7.1	5.1	7.2	6.5
DH180-80	8.5	8.6	8.0	8.4
DH180-81	11.0	8.0	9.0	9.3
DH180-82	12.3	8.8	7.3	9.5
DH180-83	7.6	8.6	6.0	7.4
DH180-87	4.7	4.5	4.5	4.6
DH180-89	7.7	3.7	5.5	5.6
DH180-90	5.3	4.9	6.3	5.5
DH180-91	7.9	4.8	6.3	6.3
DH180-92	5.5	5.3	4.3	5.0
DH180-93	4.3	4.9	4.2	4.5
DH180-95	12.4	6.0	8.3	8.9
DH180-96	9.0	6.9	5.5	7.1
DH180-98	7.5	9.5	4.8	7.3
DH180-99	6.3	5.5	7.5	6.4
DH180-103	8.7	4.3	3.8	5.6
DH180-104	4.8	3.1	4.1	4.0
DH180-105	4.9	4.7	3.0	4.2
DH180-107	8.3	6.7	5.1	6.7
DH180-108	7.9	6.4	7.4	7.2
DH180-109	5.9	5.0	5.1	5.3
DH180-110	13.0	4.8	8.7	8.8
DH180-111	8.3	4.8	5.9	6.3
DH180-112	11.0	4.9	5.3	7.1
DH180-113	10.2	5.6	7.9	7.9

DH180-114	8.2	4.5	7.7	6.8
DH180-116	8.4	4.5	5.5	6.1
DH180-117	7.9	5.2	6.3	6.4
DH180-121	8.3	4.5	4.8	5.8
DH180-122	8.9	5.0	4.4	6.1
DH180-123	4.5	4.4	4.3	4.4
DH180-126	10.3	8.2	9.3	9.2
Westar	4.3	4.2	4.4	4.3
MillenniUM 03	5.2	4.7	4.5	4.8
Sentry	4.5	3.7	3.8	4.0
Huashong3	8.2	7.1	6.8	7.4
Zhongyou 821	9.6	9.2	9.5	9.4