Identification of resistant sources against *Sclerotinia sclerotiorum* in *Brassica species* with emphasis on *B. oleracea*

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Abstract:

Stem rot caused by *Sclerotinia sclerotiorum* is one of the most devastating diseases of rapeseed (*Brassica napus* L) which causes huge loss in rapeseed production. Genetic sources with high level of resistance have not been found in rapeseed. In this study, 68 accessions in six *Brassica* species, including 47 accessions of *B. oleracea*, were evaluated for leaf and stem resistance to *S. sclerotiorum*. Large variation of resistance was found in *Brassica,* with maximum differences of five and 57 folds in leaf and stem resistance respectively. *B. oleracea*, especially its wild types such as *B. rupestris*, *B. incana*, *B. insularis* and *B. villosa* showed high level of resistance. Our data suggest that wild types of *B. oleracea* possess tremendous potential for improving *S. sclerotiorum* resistance of rapeseed.

Key words: *Brassica napus*, *Brassica oleracea*, *Sclerotinia sclerotiorum*, leaf resistance, stem resistance

Introduction

Rapeseed (*Brassica napus*, AACC) is an important oilseed crop in the world. However, *S. sclerotiorum* causes serious yield losses in rapeseed production. As compared with fungicides, breeding of rapeseed varieties with high level of resistance is an economical and ecological sustainable alternative to control *S. sclerotiorum*.

Although great efforts have been made to understand the resistance mechanism in rapeseed via QTL mapping (Zhao and Meng 2003; Zhao et al. 2006) and microarray analysis (Yang et al. 2007; Zhao et al. 2007; Zhao et al. 2009), and to improve resistance through traditional breeding programs (Wang et al. 2004; Yu et al. 2010), practical benefits from these programs were low due to lack of germplasm with high level of resistance in rapeseed gene pool (Zhao et al. 2004).It is possible to explore sources with strong resistance to *S. sclerotiorum* from its relatives, such as *B. oleracea* which has rich genetic diversity (Lannér et al. 1997; Mei et al. 2010). This study was carried out to identify resistant sources against *S. sclerotiorum* in *Brassica species* with emphasis on *B. oleracea*, and to map QTLs for resistance against *S. sclerotiorum*.

Materials and methods

Plant materials and field trials

A panel of 68 accessions of six *Brassica* species was used for this study. They included 47 *B. oleracea* accessions, of which 29 accessions were from eight wild taxa and 18 accessions were from seven cultivated *B. oleracea*, five *B. napus* accessions, including one susceptible line and four partially resistant lines (Zhongyou 821, Ning RS-1, M83 and Zhongshuang 9), four *B. rapa* accessions, four *B. nigra* accessions, four *B. juncea* accessions and four *B. carinata* accessions.

Randomized complete block designs with two replications were employed in two crop seasons, 2007-2008 and 2008-2009. Twenty plants of each accession were planted in two rows, with 30 cm between rows and 25 cm within rows. Since different variants of *Brassica* varied in flowering time, three accessions of *B. napus* (Ning RS-1, Zhongyou 821 and Zhongshuang 9) as checks were sown for two times to synchronize the growth stage of the accessions and the checks for inoculation.

Culture of *S. sclerotiorum* and assessments of resistance to *S. sclerotiorum*

A *S. sclerotiorum* isolate, obtained originally from the field sown rapeseed plants in Southwest University, Chongqing, was maintained and cultured on potato dextrose agar (PDA) medium (20% potato, 2% dextrose and 1.5% agar) under dark at 20 ℃. Five to ten individuals of each plot were tested for resistance. The resistance was assessed by inoculating leaf and stem with inoculums as described by Zhao and Meng (2003) with minor modifications shown below.

Assessment of leaf resistance - The third fully expanded leaf at the nine- to twelve-leaf stage was excised for the leaf resistance assay in the laboratory. Moist towels were placed at the bottom of a big container (1 m by 2 m). Filter papers were placed over the towels, and the detached leaves were placed on the filter papers. Two plugs (6 mm in diameter) punched from the growing margin of 3-day-old culture of *S. sclerotiorum* on PDA medium were placed separately on two sides of the midrib of leaf. The container was sealed with plastic film to keep humidity, and temperature maintained at 21℃ in laboratory. The lesion size (S) was calculated with the formula $S = \pi * a * b/4$, where 'a' and 'b' represent the long and short diameter of like-elliptic lesion which were measured 3 days after inoculation.

Assessment of stem resistance - The inoculation of stems was carried out about ten days after end of flowering in the field. Sterilized toothpicks were placed in a radial arrangement in petri dishes with PDA medium and co-cultured with the fungus under dark at 20 ℃. Stems were pierced at a height of 20 cm above the ground with an electric drill (3-mm in diameter), and mycelium-covered toothpicks were inserted into the drilled holes. Inoculated stems were subsequently wrapped around with parafilm^{TM} to keep moist. Lesion lengths were measured 10 days after inoculation.

Statistic analysis

Zhongyou 821, a registered cultivar with partial resistance against *S. sclerotiorum* (Zhao et al. 2009), was used as control to calculate the relative susceptibility (R) of accessions tested, based on the equation $R = V/V_{control}$, where V is the value of the accession tested for leaf (lesion size) or stem resistance (lesion length), while $V_{control}$ is that of Zhongyou 821.

Analysis of variance (ANOVA) was conducted using the program of general linear model procedure with SAS, version 6.07 (SAS Institute 1992). Pearson's simple correlation coefficients were calculated among variables of interest.

Results

The leaf resistance was assessed in two crop seasons, with two replications each year. High correlation for disease symptom score was detected between two years ($r = 0.890$, $P < 0.01$), and no significant differences of resistance were detected between years ($P = 0.9792$), whereas significant differences of resistance were found among accessions ($P < 0.01$). The lesion size of detached leaf 3 days after inoculation averaged 15.1 cm^2, ranging from 5.6 to 30.0 cm^2 among 68 accessions.

However, owing to serious plant injury by frost in February 2008, we failed to collect the data of stem resistance in the first crop season. In the second crop season, the accessions were divided into two groups to assess stem resistance based on the difference of stem development. Twenty-eight accessions were inoculated on March 30th, while 22 accessions were inoculated on April 28th in 2009. Three accessions of *B. napus* (Ning RS-1, Zhongyou 821 and Zhongshuang 9) separately sown were used in the two assessments. For these three common accessions, the expansion of lesions on stems were faster in the second assessment due to high temperature, but a high correlation ($r = 0.973$, $P < 0.01$) for the lesion length was found between the two assessments.

To compare the resistance between the two evaluation methods and between the two inoculation dates of stem resistance evaluation, the relative susceptibility to Zhongyou 821 was calculated. The relative susceptibility averaged 0.773 for leaf and 0.780 for stem. The extent of variation between the most susceptible and resistant accessions was 5 fold for leaf resistance and 57 fold for stem resistance. The relative susceptibility of stem of *B. oleracea* accessions was highly correlated with that of leaf ($r = 0.652$, $P < 0.01$), indicating that the resistance of stem was closely associated with that of leaf in *B. oleracea*. All the accessions in *B. oleracea*, except two accessions in the stem assay, exhibited high level of resistance as compared with Zhongyou 821.

Among 68 accessions in *Brassica*, the top resistant lines exited in *B. oleracea* (Fig. 1), especially its wild types such as *B. rupestris*, *B. incana*, *B. insularis* and *B. villosa*. It indicates these wild types of *B. oleracea* possess tremendous potential for improving *S. sclerotiorum* resistance of rapeseed.

Discussion

In this study, the stem resistance was highly and positively correlated with the leaf resistance among accessions in *B. oleracea*, and no significant difference of resistance was found between stem and leaf. Our findings differ from previous studies in rapeseed (Yu et al. 2010; Zhao et al. 2004), in which a slight correlation of resistance between stem and leaf was reported. A possible reason is that the accessions employed in these studies were partially resistant to *S. sclerotiorum*, resulting that the phenotypes of lesions observed among their progenies or themselves were liable to environmental

influence (Zhao et al. 2006). Moreover, *B. oleracea* possibly behaves different way against *S. sclerotiorum*, resulting in different response from rapeseed.

In this study, the resistance against *S. sclerotiorum* was investigated in *Brassica* with emphasis on *B. oleracea*. Although few accessions of other five *Brassica* species were used and only one year's data of stem resistance was available for partial accessions, our data suggested that there is tremendous genetic potential to improve rapeseed resistance against *S. sclerotiorum* with wild types of *B. oleracea,* such as *B. rupestris, B. incana, B. insularis* and *B. villosa* which were found to possess high level of resistance. It is interesting to mention that these four wild types of *B. oleracea* which mainly distributed in Sicily, Italia (Snogerup et al. 1990), possess similar genetic background with each other, and exhibit genetic diversity from the other types of *B. oleracea* (Lannér et al. 1997; Mei et al. 2010). Whether the Sicilian region represents one of the resistance source centres against *S. sclerotiorum* needs to be further studied.

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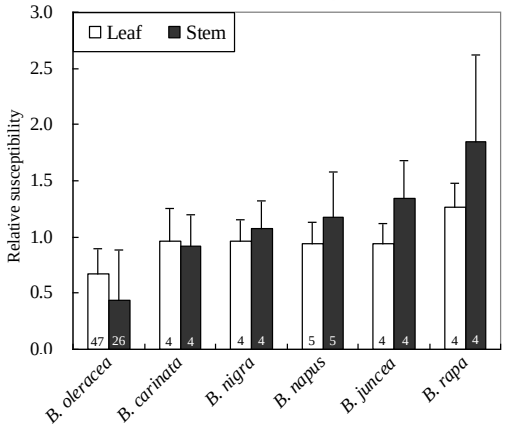
Fig. 1 Relative susceptibility to Zhongyou 821 in leaf and stem among the six *Brassica* species. The number of entries of each species tested is shown on the bottom of column. The relative susceptibility of leaf and stem are presented in white and black respectively. Column and bar represent mean and standard deviation.


Leaf
Stem
Relative susceptibility
3.0
2.5
2.0
1.5
1.0
0.5
0.0
47
26
4
4
4
4
5
5
4
4
4
4
B. oleracea
B. carinata
B. nigra
B. napus
B. juncea
B. rapa