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SHORT COMMUNICATION

Improvement of *Sclerotinia sclerotiorum* resistance in *Brassica napus* by using *B. oleracea*

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Abstract *Sclerotinia* stem rot is one of the most serious diseases in rapeseed (*Brassica napus*) due to the lack of resistance sources. A high level of resistance was reported in *Brassica oleracea* cytodeme, one of parental species of rapeseed. In this study, a panel of 55 resynthesized lines of *B. napus* (RS lines) derived from seven wild and two cultivated types of *B. oleracea* was evaluated for *Sclerotinia* resistance over 2 years. Relative to 'Zhongyou 821', a cultivar of *B. napus* with partial resistance against *S. sclerotiorum*, RS lines exhibited stronger stem resistance. Although the resistant level of RS lines was lower than that of corresponding parental *B. oleracea*, a moderate correlation between resistance of RS line and corresponding parental *B. oleracea* type was found both for leaf ($r = 0.74$, $P = 0.02$) and stem ($r = 0.69$, $P = 0.04$). Our data suggests that the RS lines are important resources to improve *Sclerotinia* resistance of current rapeseed. A breeding strategy is discussed to enhance the *Sclerotinia* resistance of rapeseed by using *B. oleracea*.

Keywords *Brassica napus* · *Brassica oleracea* · Leaf · *Sclerotinia sclerotiorum* · Stem

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Introduction

Sclerotinia stem rot, caused by the fungal pathogen *Sclerotinia sclerotiorum*, is one of the most serious diseases in rapeseed (*Brassica napus*) (Dunker and Tiedemann Dunker and von Tiedemann 2004; Hind et al. 2003; Lamey 2003; Pope et al. 1989). In the field, the stem and pod of rapeseed are infected by this pathogen, resulting in yield loss. To breed resistant rapeseed variety is an economical and ecological sustainable way to control this disease, but complete resistance is unavailable in current rapeseed.

Brassica napus (AACC), originated from interspecific hybridization between *B. rapa* (AA) and *B. oleracea* (CC). It has a narrow genetic basis partly due to its intensive modern breeding and short history of origination and domestication (Becker et al. 1995, Seyis et al. 2003). In contrast, the parental species *B. oleracea*, including various cultivated and wild types, shows a much wider genetic diversity (Mei et al. 2011a; Snogerup et al. 1990) and harbors some elite traits such as high resistance against *Peronospora parasitica* (Mithen et al. 1987; Mithen and Magrath

1992), *Aleyrodes proletella* (Ramsey and Ellis 1994) and *Delia radicum* (Ellis et al. 1999). More recently, we have found high levels of resistance against *Sclerotinia* in *B. oleracea* cytodeme, such as *B. rupestris*, *B. incana*, *B. insularis* and *B. villosa* (Mei et al. 2011b).

Resynthesis of a rapeseed line (RS line) is an effective strategy to improve current rapeseed via widening genetic diversity (Girke et al. 2012a), enhancing heterotic potential (Girke et al. 2012b; Udall et al. 2004), and transferring elite traits from parental species (Abawi et al. 1975; Crouch et al. 1994; Diederichsen and Sacristan 1996; Dreyer et al. 2001; Happstadius et al. 2003; Walsh et al. 1999). In order to verify the hypothesis that the *Sclerotinia* resistance of rapeseed can be improved using resynthesized *B. napus*, a panel of RS lines derived from various types of *B. oleracea* was screened and the resistance against *S. sclerotiorum* was compared with the resistance of the parental *B. oleracea* as identified in our previous research (Mei et al. 2011b). Our data suggest that the *Sclerotinia* stem resistance of rapeseed can be improved by using *B. oleracea*.

Materials and methods

Plant materials

A panel of 55 RS lines, collected from Göttingen University and Southwest University (Supplement Table 1), were sown in two crop seasons, 2010–2011 and 2011–2012, in the experimental field of Southwest University, Chongqing (China). A randomized complete block design with two replications was employed, twenty plants of each plot in two rows, with 30 cm between rows and 25 cm within rows. The donors of C subgenome of the RS lines were seven wild and two cultivated types of *B. oleracea*, which had been identified as resistant against *Sclerotinia* in our previous research (Mei et al. 2011b).

Resistance assessment

The *S. sclerotiorum* isolate utilized in Mei et al. (2011b) was used in this study. The plugs (6 mm in diameter) punched from the growing margin of 3-day-old culture of *S. sclerotiorum* on PDA (potato dextrose agar, 20 % potato, 2 % dextrose and 1.5 % agar) were used as inoculums in resistance evaluation.

The fourth leaves at nine-to-twelve-leaf stage and stems at flowering stage were detached in the field plots and used to evaluate *Sclerotinia* resistance according to the method of Mei et al. (2011b, 2012). Briefly, an artificial inoculation was performed in closed inoculation chamber in the laboratory, in which the infection temperature and humidity was maintained at 22 °C and 95 %, respectively. Three individuals of each RS line were tested in each evaluation. The lesion size of inoculated leaves and lesion length of inoculated stems 3 days after inoculation (DAI) were collected.

Statistical analysis

A registered rapeseed cultivar in China, 'Zhongyou 821', with partial resistance against *S. sclerotiorum* was used as resistant control. Relative susceptibility (S) to 'Zhongyou 821' was calculated based on the equation $S = V/V_{control}$, 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 general linear model procedure with SAS, version 6.07 (SAS Institute 1992). Pearson's simple correlation coefficients were calculated between variables of interest.

Results

The resistance was screened in stem and leaf among RS lines (Fig. 1). The size of lesion in leaf ranged from 11.53 to 19.60 cm^2 in 2010 and from 5.84 to 11.34 cm^2 in 2011, while the length of lesion in stem ranged from 3.51 to 7.45 cm in 2010 and from 4.12 to 8.23 cm in 2011. In order to compare the resistance of RS lines across 2 years, a relative susceptibility to 'Zhongyou 821' was calculated (supplement Table 1; Fig. 2). The average relative susceptibility of the RS lines was 1.11 and 0.77 in leaf and stem, respectively, ranging from 0.88 to 1.36 in leaf and from 0.42 to 1.02 in stem. It indicates that RS lines have stronger resistance in stem than in leaf relative to 'Zhongyou 821'.

Fig. 1 Symptoms 3 days after inoculation in leaf (**A**) and stem (**B**) of partial resistant check 'Zhongyou 821', parental line of *B. oleracea*, resynthesized line of *B. napus* and parental line of *B. rapa* from left to right

Table 1 shows the result of ANOVA for the relative susceptibility among RS lines. Significant differences for leaf and stem resistance were detected among RS lines, as well as among types of parental *B. oleracea* ($P < 0.01$). Significant differences of resistance for year-by-leaf interaction ($P < 0.01$), but not for year-by-stem interaction ($P = 0.37$) were detected, indicating that the stem resistance is more stable than leaf

Table 1 ANOVA of relative susceptibility to 'Zhongyou 821' in leaf and stem among resynthesized *B. napus*

Source	MS in leaf	MS in stem
Years	0.01	6.91*
Replications within years	0.12	0.01
Parental types of *B. oleracea*	0.29*	0.29*
Genotypes	0.09*	0.03*
Genotypes x Years	0.10*	0.01

* Significance at $P = 0.01$ level

resistance in RS lines. A low and non significant correlation of resistance between leaf and stem ($r = -0.18$, $P = 0.25$) suggested that different genetic mechanisms separately control stem and leaf resistance of RS lines.

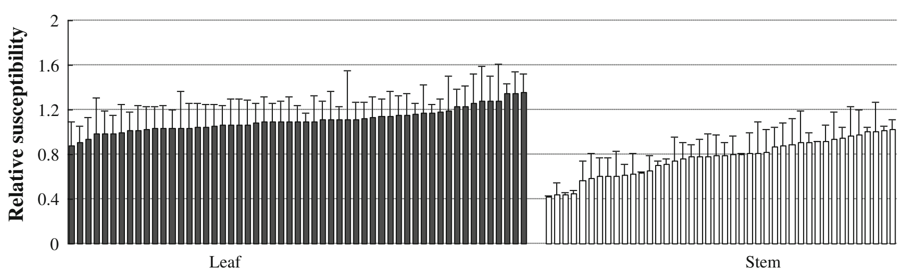
To investigate the resistance transfer from *B. oleracea* into *B. napus*, the mean resistances of all RS lines derived from the same type of *B. oleracea* were compared with those of corresponding type of *B. oleracea* as identified in the previous study (Mei et al. 2011b). Although the resistant level of RS lines was lower than that of corresponding parental *B. oleracea*, a moderate correlation of resistance was found between them in both leaf ($r = 0.74$, $P = 0.02$) and stem ($r = 0.69$, $P = 0.04$) (Fig. 3).

These results indicate that the resistance of resynthesized rapeseed lines is associated closely with that of the parental *B. oleracea* type, and that the stem resistance of rapeseed against *Sclerotinia* can be improved by using *B. oleracea*.

Discussion

In comparison with 'Zhongyou 821', most RS lines exhibited higher resistance level especially in stem, indicating diverse resistance mechanism in RS lines and current rapeseed. More recently, two major

Fig. 2 The relative susceptibility to 'Zhongyou 821' in leaf and stem among resynthesized lines of *B. napus*



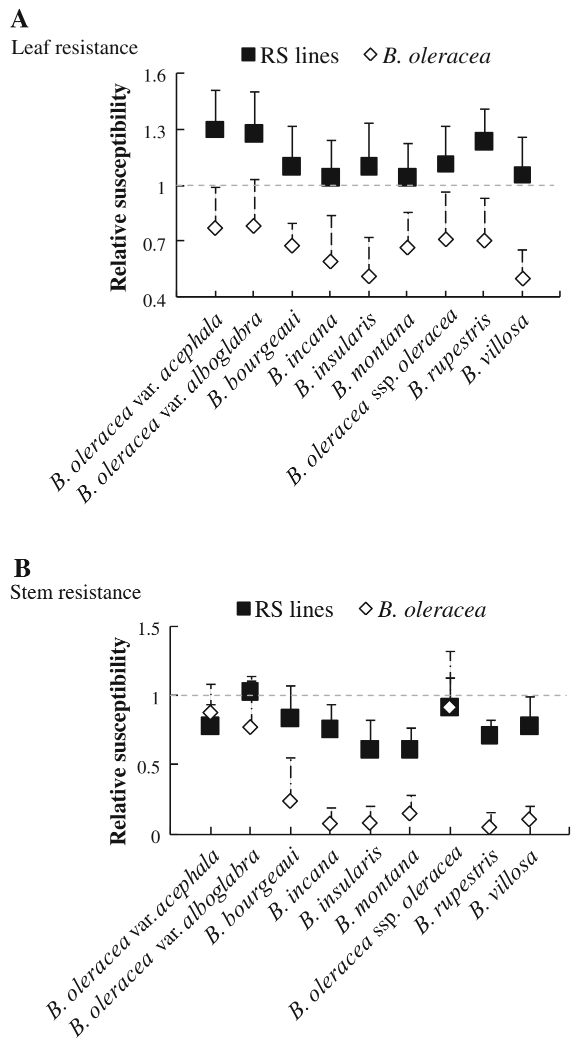


Fig. 3 Relative susceptibility to 'Zhongyou 821' in leaf **A** and stem **B** for resynthesized lines classified by the type of parental *B. oleracea* and corresponding types of *B. oleracea* reported in the previous study (Mei et al. 2011b)

quantitative trait loci (QTL) for *Sclerotinia* resistance from *B. incana*, a wild of *B. oleracea*, were mapped on the chromosome C09 (Mei et al. 2013), whereas several independent studies on mapping for *Sclerotinia* resistance in rapeseed (Yin et al. 2010; Zhao and Meng 2003; Zhao et al. 2006) did not find any major QTL on N19 which corresponds to C09 of *B. oleracea*. Therefore, the RS lines are important resources to improve *Sclerotinia* resistance of current rapeseed.

In the previous study (Mei et al. 2011b), the *Sclerotinia* resistance level of rapeseed was found in the middle of that of its two parental species, *B. oleracea* with high level of resistance and *B. rapa* with high level of susceptibility. It was in accordance with the present study, in which the resistance level of RS line was usually lower than that of its parental types of *B. oleracea*, though the resistance of parental lines of *B. rapa* was not listed. These findings show that additive genetic effects possibly play important roles in the *Sclerotinia* resistance of RS lines. If so, it will be an interesting breeding strategy to improve the *Sclerotinia* resistance of rapeseed by transferring resistance from *B. oleracea* into *B. rapa* due to the high colinearity between their genomes (Cheung et al. 2009; Rana et al. 2004), and then resynthesizing rapeseed to pyramid the resistance from *B. oleracea* and *B. rapa*.

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