The establishment of *sclerotinia sclerotiorum* resistant near isogenic lines

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ABSTRACT

Zhong R-888 (*Brassice napus*) had the best resistance to stem rot, Zhong R-783 (*B. napus*) and Ning RS-1 (*B.napus*) had better resistance than Zhongyou821 (*B.napus*). Three BC_5 near isogenic line (NIL) with stem rot resistance were established by using Zhongyou821 as the receptor parent, and Zhong R-888, Zhong R-783, and Ning RS-1 as the donor parents. The resistance to stem rot was significantly different among the three NILs and Zhongyou821 at the $P<0.01$ levels. The fungi-on-seed ratios of the NIL were similar to that of their respective donor parents. Proline content of seeds was significantly different among the NIL at the $P<0.01$ levels. Proline content of seeds of several randomly selected varieties of mustard, *B.campestris*, and the three *sclerotiorum* resistant NIL differed at the $P<0.01$ level. The proline content in seeds of mustard was higher than that of the three sclerotiorum resistant NILs, *B. campestris* and susceptible *B. napus.*

Key word: *Brassica napus* - NIL (near-isogenic line) - Disease resistance - *Sclerotinia sclerotiorum*

INSTRUCTION

The stem rot of *Brassica napus* is a world wild disease, which can badly harm the quality, and the yield of *Brassica napus* and it is especially serious in the middle and lower reaches of the Changjiang River in China where the yield is higher than other area. The cultivated area of *Brassica napus* is over 100 million Chinese unit of area mu (1/15 of a hectare) and the loss of the yield caused by the stem rot is between 10% and 20%. It is an economical and effective way to abate the harm of the stem rot that we shall search and select resistance genes to that disease and by the mean time select and improve resistance cultivars.

It is a common and effective method to search and select the resistance genes using the NIL. We can use the cultivars (lines) of disease resistance as the donor parent, and the excellent cultivars as the receptor parents and backcross parents to create the NIL. Davierwala with his companions used the NIL as the experimental materials and then used molecule marker assistant selecting technique to get a bacterial blight resistance line in rice[1] .Shi with his companions used the wheat stripe rust resistant NIL as the experimental materials and applied the technology of RGAP resistance gene analog polymorphism to identify the resistance gene *Yr9*[2]. Riely with his companions used the technology of map-based-clone to separate and identify the tomato disease resistance gene *Pto* that codes Ser/Thr protein kinase, and got 4 gene *Pto* family numbers [3].

In adversity conditions, the proline always accumulates in the frond. Leul with his companions observed that the activities of superoxide dismutase, catalase, peroxidase and the content of free proline observably increase in *Brassica napus* when they were submerged in water [4]. Saradhi with his companions thought that the paddy, the mustard and mung bean accumulate a plenty of proline to prevent the peroxidition from coming into being when they

were exposed to UV radiation[5]. In addition, jasmone is the signal substance which is produced when the pathogeny infalls in the frond. Zhao with his companions discovered that the chitinase abducted by jasmone in the mustard contains two domains that combine chitin and the structural of chitinase contains a great deal of proline [6]. Therefore the proline accumulated in the frond reflects the resistance to adverse circumstances of plants in some conditions [7].

We used the excellent cultivar Zhongyou821 (*B.napus*) as the female parent, and better disease resistant cultivars Zhong R-888, Zhong R-783 and Ning RS-1 as the donor parents to establish the *sclerotinia sclerotiorum* resistant near isogenic lines. We selected and identified the resistance material according to resistance of the laminas in vitro the frond and the fungi-on-seed ratios combining with the ratios of proline, then primarily analyzed the resistance of the near isogenic line (NIL).

Material and method

1.1 Material

1.1.1 Supplied Material

16 *Brassica napus* cultivars (lins) and near isogenic lines (NILs) from Huazhong agricultural university.

Supplied fungi

The *sclerotinia sclerotiorum* were collected form the diseased *Brassica napus* in the experiment field in Huazhong Agricultural University, which were expanded by the PDA culture medium.

1.2 Method

1.2.1 Establish the NIL

We used the excellent cultivars (lins) Zhongyou821 (*B.napus*) as the female parent, Zhong R-888, Zhong R-783 (*B. napus*) and Ning RS-1 (*B.napus*) that have better resistance than Zhongyou821 (*B.napus*) as the donor parents to establish the *sclerotinia sclerotiorum* resistant near isogenic lines.

1.2.2 Identify the resistance of the laminas in vitro the *Brassica napus*

According to Hu Baocheng's method [8], We selected vigorous, full-developed leaves from the 7-8 real leaves stage plants growing in field to identify the resistance by inoculation. We culled one leave from each individual plant and washed them. Afterward we inoculated them.(The diameter of the inoculate is about 5mm.) After that we closed down them to keep stable humidity and temperature. We cultured them naturally and observed the diameters of the diseased speckles. (Repeated 2~3 times.)

1.2.3 Determine the fungi-on-seed ratios of the *Brassica napus* seeds

We putted 30 seeds into each culture dishes which had been sterilized in high pressure, marinated them with the sterile water, putted them on the sterile worktable and then marinated the seeds with 0.1% Hg liquor for 3 minutes. The seeds were washed with sterile water for 3 times and then dispersed and tidily arranged on the culture dishes. Afterward we putted the culture dishes into the culture chest in the condition of 25 and illumination. Three days later, we investigated the numbers of the seeds that grew the hypha and determine the fungi-on-seed ratios of the seeds. (Repeated 3 times.) [9].

1.2.4 Determine the ratios of proline

The defatted seeds meal was extracted by sulfosalicylic acid and reacted with acid ninhydrin , then determine the ratios of proline. (Repeated 3 times.)

1.2.5 Methods to Stat. and analyze.

The data was analyzed in Microsoft Excel. We used LSR method (Duncan) to do multiple comparisons.

RESULTS

1.1 Determine the *sclerotinia sclerotiorum*.

We used Zhongyou821 (*B.napus*) as the female parent, Zhong R-888,Zhong R-783 (*B. napus*) and Ning RS-1 (*B.napus*) that have better resistance than Zhongyou821 (*B.napus*) as the donor parents to establish the *sclerotinia sclerotiorum* resistant near isogenic lines. We selected and established the NILs according to their disease resistance of the laminas in vitro the frond. The agronomical traits, leaf shape and flowering time were similar to Zhongyou821 (*B.napus*).

Compared with Zhongyou821, we determined the resistance of three BC_5 using the method of laminas in vitro infecting. In table 2,we can see that the sequence of the resistance is zhongyou821×zhong R-888 BC_5 zhongyou 821×ning RS-1 BC_5 zhongyou 821×zhong R-783 BC_5. There were significant differences among the cultivars at the $P<0.01$ level. The diameters of the diseased speckles are consistent with the sequence of the resistance of their own donor parents. We also observed that the resistance of BC_5 is different in different years.

2.2 The analysis of the fungi-on-seed ratios

Table 2 The diameters of the disease spots from different backcross offsprings of *B.napus*

A diameter of the disease spot in the table are the average of 30~120 diameters

Cultivars (lins)	Diameters of the disease spots cm					Average value
	BC1	BC2	BC3	BC4	BC5	
Zhongyou821	3.00 A	3.03 b A	3.11 A	3.07 b B	3.25 A	3.09 A
Zhongyou821×Zhong R-783 BC_5	2.51 B	3.08 a A	2.94 B	2.33 a A	2.87 C	2.95 B
Zhongyou821×Ning RS-1 BC_5	2.35 C	2.88 c B	2.84 C	3.00 c B	3.02 B	2.82 C
Zhongyou821×Zhong R-888 BC_5	2.16 D	2.45 d C	2.48 D	2.80 d C	2.77 D	2.53 D

The sequence of the fungi-on-seed ratios is zhongyou821×zhong R-888 BC_5 zhongyou 821×ning RS-1 BC_5 zhongyou 821×zhong R-783 BC_5 which are consistent with the sequence of the fungi-on-seed ratios in their own donor parents(zhong R-888 ning RS-1 zhong R783. Ai jia zao and You guang ye are susceptible cultivars, and their fungi-on-seed ratios are very high. There were significant difference among the cultivars at the $P<0.01$ level.(Table 3)

According to the results in table 2 and table 3,we can draw to the conclusion that the sequence of resistances to the disease is consistent with the sequence of that in donor parents. Among all the experimental cultivars, Zhong R-888 (*Brassice napus*) had the best resistance to

stem rot, and its BC_5 had much more resistance than other BC_5.

Table 3 The identify results of the ratios of the fungi in different cultivars (lines) *B.napus* seed

Cultivars (lines)	Amount of the seeds bringing fungi per repetition			Ratio of the seeds bringing fungi
	1	2	3	
Zhong R-888	0	0	0	0 A
Zhongyou821	0	1	0	1.1 A
Ning RS-1	0	1	1	2.2 A
Zhong R-78	2	2	1	5.6 A
Zhongyou821×Zhong R-888 BC_5	0	0	0	0 A
Zhongyou821×Ning RS-1 BC_5	1	0	1	2.2 A
Zhongyou821×Zhong R-783 BC_5	2	1	1	4.4 A
Ai jia zao	21	15	20	62.2 B
You guang ye	19	17	16	57.8 B

1.2 The analysis of the proline content of seeds

From Table 4, there were significant differences in the proline content of seeds among the cultivars at the P<0.01 level. The mustard contains observably more proline than other cultivars at the P<0.01 level. Besides it, the proline content of seeds from *Brassice napus* cultivars (zhongyou821 zhong R-888)BC_5, (zhongyou 821×ning RS-1) BC_5, zhongyou 821× zhong R-783 BC_5 are more than other cultivars. The seeds of *B.campestris* (Ma cheng gui san you cai, Qi xingjian, Sichuan 501) contain less proline than that of *Brassice napus* cultivars. Ai jia zao and You guang ye, which are much easier to be taken ill, contain the least proline in their seeds (significant at the P<0.01 level). The proline content of seeds among the cultivars are consistent with the sequence of resistances to the disease.

DISCUSSION

In this experiment, we can get a result that (zhongyou821×zhong R-888)BC_5,(zhongyou 821×ning RS-1)BC_5, (zhongyou 821×zhong R-783) BC_5 and other NIL have much better resistances to the disease than their backcross parents.

The result of variance analysis indicates that the resistance to stem rot has significant difference among the different cultivars. So we can draw to the conclusion that different cultivars have different resistant genetic basis. Gan and her colleagues analyzed genetic diversity of B*rassica* genus using primer of disease resistance genes in *Arabidopsis thaliana* and found that there are genetic distances among *Brassica napus* (zhongyou821, zhong R-888, ning RS-1, zhong R-783) and that reflects that they have genetic differences. [10] The dissociative proline contents in seeds of different cultivars are analogous to the resistance descendiblity. The sequence of the dissociative proline contents in seeds of the NIL is consistent with the sequence of the resistance in their donor parents.

The dissociative proline contents in seeds of mustard are more than that of any other cultivars. The three NIL of *Brassica napu* contain less proline in their seeds. *B.campestris* cultivars and some *Brassica napu* (Ai jia zao and You guang ye), which are more susceptible to stem rot, contain even less proline in their seeds than the NIL. This sequence is consistent with the sequence of the resistance of different cultivars in the three types of rape, which shows that the dissociative proline contents in seeds can reflect the resistance of different cultivars to a certain extent.

After intruded by the pathogens, the fungi-on-seed ratios are a factor to reflect the resistance to disease. However, it is not absolute. Ning RS-1(*Brassica napus*,)and zhong R-783(*Brassica napus*) have better resistance to disease than Zhongyou821, while their fungi-on-seed ratios are higher than zhongyou821.Ningyou7 is more susceptible to stem rot in the field naturally, but its fungi-on-seed ratios is nil. The reason needs to be farther researched.

REFERENCE

Davierwala AP, Reddy AP, Lagu MD, et al. Gupta VS.Marker assisted selection of bacterial blight resistance genes in rice. **Biochem Genet.** 2001 Aug;39(7-8):261-78

Shi ZX, Chen XM, Line RF, et al. Development of resistance gene analog polymorphism markers for the Yr9 gene resistance to wheat stripe rust. **Genome.** 2001 Aug;44(4):509-16

Riely BK, Martin GB.Ancient origin of pathogen recognition specificity conferred by the tomato disease resistance gene Pto. **Proc Natl Acad Sci.** 2001 Feb 13;98(4):2059-64

Leul M, Zhou WJ. Alleviation of Waterlogging Damage in Winter Rape by Uniconazole Application: Effects on Enzyme Activity, Lipid Peroxidation, and Membrane Integrity. **journal of plant growth regulation**. 1999 Aug;18(1):9-14

Saradhi PP, Alia, Arora S. et al. Proline accumulates in plants exposed to UV radiation and protects them against UV induced peroxidation. **Biochem Biophys Res Commun**.1995 Apr 6;209(1):1-5

Zhao KJ, Chye ML. Methyl jasmonate induces expression of a novel Brassica juncea chitinase with two chitin-binding domains. **Plant Mol Biol.** 1999 Aug;40(6):1009-18

Gan li, Lu jindian, et al. Effcts of *Verticillium albatrum* on proline content photosynthesis and transpiration of cotton leaf. **Acta Agriculturae Boreali- occidentalis Sinica.** 1992 1(1):8-11

Hu Baocheng, Rimm.,SR. Preliminary study of ertificial (tolerance) to *Sclerotinia sclerotiorum* in rapeseed using detached leaves.**Joural of Anhui Agricultural Sciences.** 1989(3) 56-58

Wang Xiaoming, Ji Xiaohua, et al. Discuss of diffuse approach to Sclerophthora macrospora in corn. **Plant Protection.** 2001(5) 18-20

Gan Li, Meng Jinling. Genetic diversity analysis of B*rassica* genus for the loci to resistant disease genes **Acta Agronomica Sinica.** 2000 26 6 450-458