Resistance of *B.napus* cv of Yanze River Region in China to *Sclerotinia sclerotiorum*

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Abstract: Based on the research of mycella inoculation at the seedlingstage and mycelia borne stick inoculation at the flowering stage, it was found that *B. napus* cv of Yanze river region in China has generally higher resistance to *S. sclerotiorum* than that *B. napus* cv overseas.

B. napus is a major oil crop in the arid and semiarid regions in China. The area of *B. napus* of Yanze river region in China is about 90 % of all over the country. The stem rot on *B .napus* is serious ,but the resistance *B. napus* cultivar is better in this region. In 2000, we evaluated the resistance to *S. sclerotiorum* of *B. napus* cv of Yanze river region in China and *B. napus* cv overseas. So we had carred our some studies. The results obtained were as follows.

1 Materials and Methods

1.1 Materials

The *B. napus* cv of Yanze river region is: Zhongyou821, Canyou5, Zhongshuang2, Xiangyou15, Xiangyou10, Xiangyou14, Huahuang2, Huaza6, Zhongyouza6, Shuyou6, Chuanyou14, Huyou4, Rongxuan2. The *B. napus* cv overseas is:SW038, Midas, Target, Tower, Zephyr, Matador, Wesroona, Wesbrook, Masowick, Alter, H52, Panna. All seeds of *B.napus* cultivar came from The Oilseed Crops Research Institute, Hunan Agriculture University. Zhongyou821, The moderate resistance to *S. sclerotiorum* variety as a control. *S. sclerotiorum* stain was used obtained from The Oilseed Crops Institute, Hunan Agriculture University, this stain was collected in Changsha ,Hunan. purified and preserved in hyphostroma, its number is SS_2.

1.2 Methods

1.2.1 Mycelia inoculation at the *B.napus* seedling stage. Methods were applied according to Luokuan[2], Biwen Zhou[3] ,but with moderate modification.

Put pasteurized soil into 60cm×30cm plastic dish, plant 5 lines, 30 plants in each dish. All dishes were cultured in growth cabinet. When *B. napus* seedlings have 5 leaves, using sterile scalpel cut PDA hypheal mass into pieces of 5mm×5mm, put a piece on the last third leaf of *B. napus* seedlings .cultured these inoculated plants under growth cabinet situation of 22-25℃, humidity above 90%, descend to 80% after 3 days.

Investigating disease percentage, disease index, calculating relative resistance index and resistance degree after culturing 5 days.

$$\text{Diseased plant percentage (P)} = \frac{\text{Number of diseased plants(D)}}{\text{Number of total plants(N)}} \times 100\%$$

$$\text{Disease index (ID)} = \frac{\sum_{i=1}^{k}(Ni \cdot Gi)}{GK \cdot N} \times 100\%$$

Where Gi is the disease degree; Ni is the number of investigated plants; I=0,1,2……k; GK is the highest degree.

Standard of grading: 0 degree is normal, lesion area account for less 10% of total leaf area is 1 degree, lesion area account for 10% -30% of total leaf area is 2 degree, lesion area account for 30% -50% of total leaf area is 3 degree, lesion area account for more 50% of total leaf area is 4 degree.

$$\text{Relative Resistance Index (RRI)} = In\left(\frac{ID_m}{100 - ID_m}\right) \cdot in\left(\frac{ID_{CK}}{100 - ID_{CK}}\right)$$

Where m is the tested material; CK is the control.

Table 1 Resistance type of *B. napus* to *S. sclerotiorum*

	RRI					
	≤−1. 2	≤−0. 7	<0	≥0	>0.9	>2.0
Degree of resistance	HR	MR	LR	LS	MS	HS

1.2.2 Mycelia-borne Stick Inoculation At The Flowering Stage of *B. napus*.

A sterile stick was inserted into a PDA medium and also a small piece of hypheal mass was replanted into the medium. After they were cultured for 3-4days under16-20℃, the stick with hypheal can be used to inoculate. In a field at the flowering stage of *B. napus*, a stick with hypheal was inserted into the main stem of each plant. The location of inoculation with *B. napus* is about 40cm above the ground. In order to keep the location of inoculation wet, a wet cotton ball was attached with it and then wrapped in a plastic film. Every 10 plant was treated for each cultivar or line. The disease percentage and disease index were investigated after one week. The disease was classified according to the following standard: no disease is grade 0; lesion length 1-2mm is grade 1; lesion length 2-3mm is grade 2; lesion length 3-4mm is grade 3 and lesion length more than 4mm is grade 4. The relative resistance index and resistance grade were calculated as shown above.

1.2.3 Extraction and Analysis of Phenol

Measurements were made according to a method introduced by Zhu Guanlian[4] with moderate modification.

Extraction of Phenol: firstly a sample of 5g fresh leaves was weighed. It was mixed with 20ml ethanol and 2ml of 10% tri-chloroacetic acid, rubbing in a mortar. Then the liquid was shifted into 50ml volumetric flask and the mortar was washed with

trichloroacetic acid. Then the volume was fixed to 50ml. After it was kept still for 0.5h, the solution was filtered in a 50ml volumetric flask through a glass filter with filter paper and collected in a test tube.

Making of standard curve: 12 dry test tubes were taken and divided into two groups. Each group was added respectively in-order with 0.1ml, 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml gallic acid solutions. Then distilled water was added to keep 1.0ml liquid in each tube. In addition, another test tube with 1.0ml distilled water was used for control. 5.0ml Folin-Phenol solution I 1ml were added into each test tube. After they were mixed and kept under 25℃ for 10 minutes, 0.5ml Folin-Phnolic solution II was added, mixed immediately and kept under 25℃ for 30 minutes. Absorbance was measured using spectrophoto meter at light wavelength 500nm. Then a standard curve can be made.

Sample Analysis: four test tubes were divided into two groups. 1ml sample solution was added into each tube in two groups respectively. Two different kinds of sample solutions were used for different groups. An additional test tube with 1.0ml distilled water was used for control. According to the method of making standard curve, the absorbance of each sample was measured. Basing on the standard curve, the phenol content in the sample was decided. Then the phenol weight in every gram of fresh sample (μg/g fresh leaf) could be calculated.

2 Result and Analysis

2.1 The resistance to *S. sclerotiorum* on *B. napus* cv of Yanze river region in China and *B. napus* cv overseas at seeding stage

From table 2, we can find that all types of *B. napus* cv of Yanze river region in China has generally higer resistance to *S. sclerotiorum* than that of *B. napus* cv overseas. There are 8 resistant cv (account for 66.7%) and 4 susceptibe ones (31.2%) among the 12 *B. napus* cv of Yanze river region in China evaluated while ther are 1 risistant cv (account for 8.3%) and 11 susceptible ones (91.7%) among the 12 *B. napus* cv overseas.

Table 2 The resistance of *B. napus* cv to *S. sclerotiorum* by mycelia inoculation at seedling stage (Mar,2000)

B. napus cv of Yanze river region in China					
CV	No. of plants inoculated	Rate of disease(%)	Index disease	Relative resistance index	Reaiatance type
Canyou5	150	82.3	76.8	1.18	MS
Zhongshuang2	150	51.2	42.8	-0.32	LR
Xiangyou10	33	75.7	65.4	0.62	LS
Xiangyou14	28	57.1	48.8	-0.07	LR
Xiangyou15	150	29.0	22.3	-1.27	HR
Xiangyou11	150	30.3	35.8	-0.98	MR
Huaza6	150	31.2	34.7	-1.11	MR
Zhongyouza6	150	29.8	33.3	-1.00	MR
Shayou6	30	80.0	67.8	0.72	LS
Chuanyou14	33	72.7	61.0	0.43	LS
Huyou4	35	53.3	45.4	-1.00	MR

Rongxuan2	32	50.0	40.5	-0.40	LR
Zhongyou821(CK)	150	57.6	50.4	0	
B. napus cv of overseas					
CV	No. of plants inoculated	Rate of disease(%)	Index disease	Relative resistance index	Reaiatance type
SW038	33	96.1	89.2	2.11	MS
Midas	30	76.7	70.2	0.84	LS
Target	36	75.0	64.1	0.56	LS
Tower	29	74.2	68.2	0.74	LS
Zephyr	28	57.1	50.3	0.01	LR
Wesroona	33	66.7	50.7	0.01	LS
Matador	30	83.3	74.6	1.06	MS
Wesbrook	32	62.5	58.0	0.30	LS
Masowick	35	82.8	72.6	0.95	LS
Alter	31	58.0	51.4	0.04	LS
H52	28	89.2	79.6	1.34	MS
Panna	33	60.0	55.6	0.20	LS
Zhongyou821(CK)	150	57.6	50.4	0	

From the comparation above, we can conclude that the resistance of *B.napus* cv of Yanze river region in China to *S. sclerotiorum* is higher than that of *B. napus cv overseas.*

2.2 The inoculation evaluation results of *B. napus* cv of Yanze river region in China and *B. napus* cv overseas at flowering stage

In this study, we evaluate 2 *B. napus* cv Yanze river region in China, 2 *B. napus* cv overseas ,and the control is Zhongyou 821.The results are in table3. From the table 3 above, we can conclude that *B. napus* cv of Yanze river region in China are both high resistant, which is higher to *S. sclerotiorum* than *B. napus* cv overseas in the index of disease, rate of disease and relative resistance index.

Table 3 The resistance of *B. napus* cv to *S. sclerotiorum* by mycelia borne stick inoculation at the flowering stage (Apr,2002)

CV	No. of plants inoculated	Rate of disease(%)	Index disease	Relative resistance index	Reaiatance type
Xiangyou15	10	30	18.5	-1.13	HR
Xiangzayou6	10	30	19.3	-1.00	HR
Tower	10	90	80.2	0.99	MS
Matador	10	100	85.4	2.14	LS
Zhongyou821(CK)	10	60	41.2		

2.3 The change of phenolics content in plant of *B. napus c*v with different resistance to *S. sclerotiorum* after inoculation with *S. sclerotiorum*

This study materials are only 2 cv, they are resistant Xiangyou 15 and susceptible Matador. The results are in the table 4. We can conclude from the table 4, After inoculation with *S. sclerotiorum*, the phenolics content in Xiangyou15 quickly increase ,

and higher than Matador in every stage.36 hours after inoculation with *S. sclerotiorum* the phenolics content come to a higher lever in Xiangyou15, then a little higher. But the phenolics content in Matador come to a climax 48 hours after inoculation with *S. sclerotiorum*, then stablized.

Table 4 The change of phenolics content in leaves of *B. napus* cv with different resistance to *S. sclerotiorum* after in.culation with *S. sclerotiorum*

CV	Content of phenolics ($\mu g \cdot g^{-1} \cdot FW$)					
	CK	16 h after inoculation	24 h after inoculation	36 h after inoculation	48 h after inoculation	72 h after inoculation
Xianyou15	31.8	44.2	66.7	87.5	94.6	98.3
Matador	30.1	33.6	45.2	65.6	68.6	68.3

3 Discussion

There have been some reports relation to the comparison studies about the resistance of *B. napus* to *S. sclerotiorum*. From 1986 to 1993 The Oilseed Crops Research Institute of China agricultural Academy, Hunan Agricultural University, Hunan Academy of Agricultural Science et al evaluate the resistance of 2317 *B. napus* materials to *S. sclerotiorum* by field evaluation and house evaluation, but we find no immunotype material, high resistant materials take up 6%, and we think *B. juncea* in the three types of *B. napus* has a better resistance to *S. sclerotiorum*. Liu Sheng-yi(1999) evaluate by inoculation with *S. sclerotiorum* to 735 of *B. napus, B. capestris and B. jencer* and conclude that there is a extensive variation in above three species, also there are high resistant varieties in every speceis. Brun H(1987) evaluate by field and house inoculation with *S. sclerotiorum*, think the resistance of Asia *B. napus* varieties to *S. sclerotiorum* is much high than European *B. napus* varieties. In *B. napus* varieties we studied, the resistance of which is bred in Yanze river region of China is averagely higher than which is bred oversea, but if all of the varieties are so we must have a further study.

References

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