Breeding of a *Brassica napus* cultivar Zhongshuang No. 9 with high-resistance to *Sclerotinia sclerotiorum* and dynamics of its important defense enzyme activity

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ABSTRACT

Zhongshuang No. 9, a new semi-winter *Brassica napus* variety with high resistance to *Sclerotinia sclerotiorum* and lodging, high-yield, double-low quality, was bred by multiple crossing and microspore culture technique. It was registered and released in China in 2002. In Regional Trail of Hubei Province in China, Zhongshuang No. 9 yielded 2482.2 kg/hm^2 averagely in 2000-2002, 15.33% higher than the control variety Zhongyou 821. Erucic acid, glucosinolates and oil contents of Zhongshuang No. 9 were 0.23%, 22.69μmol/g (in meal) and 42%, respectively. In field identification of resistance to *S. sclerotiorum*, disease incidence and disease index of Zhongshuang No. 9 were averagely 13.31% and 6.47, respectively, which decreased those of the control variety Zhongyou 821 by 28% and 36%, respectively. By the inoculation of detached leaves with mycelia, the necrotic lesion area of Zhongshuang No.9 was 4.709cm^2,which reached significant difference compared to the mid-resistant variety Zhongyou821. The possible mechanism of resistance to *S. sclerotiorum* was studied through comparing changes in the activities of phenylalanine ammonia lyase (PAL),exo-chitinase,β-1□3-glucanase, peroxidase (POD) and polyphenoloxidase (PPO) of Zhongshuang No.9 with those of other resistant, mid-resistant and susceptible cultivars.

Key words: Resistance to *Sclerotinia sclerotiorum*; Double-low; Zhongshuang No.9; *Brassica napus*□Defense enzyme; Dynamics

INTRODUCTION

Rapeseed (*Brassica napus*) is the most important oil crop in China. In the breeding of canola varieties, it is still difficult for a variety to possess several desirable traits such as resistance to *S. sclerotiorum* and lodging, high-yield and double-low. *S. sclerotiorum* is a main disease on rapeseed, and has become a limiting factor for rapeseed production. Recently, dynamic changes in the activity of important defense enzymes such as phenylalanine ammonia lyase (PAL),exo-chitinase,β-1□3-glucanase, peroxidase (POD) and polyphenoloxidase (PPO) after inoculation were studied in different plant varieties, and a rapid elevation of an enzyme activity was associated with resistance to disease[1-7]. The paper reported Zhongshuang No. 9, a new semi-winter *Brassica napus* cultivar possessing high resistance to *Sclerotinia sclerotiorum* and lodging, high-yield, double-low quality and extensive adaptability, was bred through multiple crossing and microspore culture technique. The possible mechanism of resistance to *S. sclerotiorum* was studied through comparing to change in activities of PAL, exo-chitinase, β-1, 3-glucanase, POD and PPO of Zhongshuang No.9 with those of other resistant, mid-resistant and susceptible cultivars.

MATERIALS AND METHODS

Plant and fungal materials

Zhongshuang No. 9, a double low variety with high-resistance to *Sclerotinia sclerotiorum;* M083, a resistant strain; Zhongyou 821, a double high and mid-resistance variety; Zhongshuang No. 3, a susceptible variety; Zhongshuang No. 4 and 84004, double low variety (strain). They were plant materials used for the experiments. Mycelia (*S. Sclerotiorum*) were provided by Liu S Y, Key Lab. of Genetics Improvement of oil crops, CAAS.

Procedure of breeding

Zhongshuang No. 9 was bred by multiple crossing and microspore culture technique. A

multiple cross of (Zhongyou 821×84004) × Zhongshuang No. 4 was made in 1994 and 1995. The desirable progenies of it were selected and purified by microspore culture technique from 1996 to 1999. A distinguished strain named 93256 was selected to participate in the Regional Trial of Hubei Province during 2000 to 2002. It was registered and named "Zhongshuang No. 9" in 2002.

Inoculation of leaf surface

Leaves from the same position of plants were excised before flower. Groups of excised leaves were inoculated with mycelia while the other groups remained not (CK). Petri plates with inoculated leaves were placed in a 21-25□ growth chamber and maintained at 100% relative humidity. The excised leaves were cut for 0.5g every time after inoculated time of 0, 12, 24, 36, 48, 60, 72h.

Preparation of enzyme and its assay procedure

Preparation of PAL, POD and its assay procedure were undertaken according to Xiao Shuansuo *et al*[5]; Preparation of exo-chitinase, β-1□3-glucanase and its assay procedure were done according to Xiaoshuansuo *et al* and Zhou Lecong [6]; Preparation of PPO and its assay procedure were done according to Li Baoju [7]. One unit of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of □OD/gfw·min (fw:fresh weight). Change curves of enzyme activity were drawn based on the net means (means of enzyme activity of treated groups minus those of CK).

RESULTS

Yield of Zhongshuang No. 9

Zhongshuang No. 9 yielded 2482.2 kg/hm^2 averagely, 15.33% higher than the control cultivar Zhongyou 821 in the Regional Trail of Hubei Province from 2000 to 2002(Table 1).

Table 1 The yield of Zhongshuang No. 9 in Regional Trail of Hubei from 2000 to 2002

Year	Average yield (kg/hm^2)	Compared to Zhongyou 821	
		(kg/hm^2)	(%)
2000-2001	2608.8	2295.3	+13.66*
2001-2002	2355.6	2008.95	+17.25*
Mean	2482.2	2152.13	+15.33

note□The results were from the report of Hubei Regional Trail (2000-2002) in China;

*: Significant of 1% probability level

The characteristics of quality

Erucic acid, glucosinolates of Zhongshuang No. 9 were 0.23% and 22.69μmol/g (in meal), respectively. The oil content of it was 42%.

Resistance to *S. sclerotiorum*

In field identification of resistance to *Sclerotinia sclerotiorum* in the Regional Trail of Hubei in china from 2000 to 2002, means of incidence and disease index of Zhongshuang No. 9 were averagely 13.31% and 6.47 respectively, which respectively decreased those of the control variety Zhongyou 821 by 27.6% and 36.1%. Zhongshuang No. 9 was the most resistant one to *S. sclerotiorum* in all the 18 tested varieties.

The inoculation of detached leaves with mycelia indicated that the necrotic lesion area of Zhongshuang No. 9 was the smallest in 4 tested varieties (Table 2).

Table 2 Difference of the lesion area of tested varieties by detached leaves with mycelia

Variety	Lesion area□cm^2□	5% probability level	1% probability level
Zhongshuang No. 3	6.871±0.453	a	A
Zhongyou 821	5.933±0.571	ab	AB
M083	5.571±0.587	bc	AB
Zhongshuang No. 9	4.709±0.708	c	B

Change of enzyme activity

Change of PAL activity

PAL activity of Zhongshuang No. 9 started to increase after inoculation and was the highest among those of the 4 varieties. It reached the highest point at the time of 36h and was 2-fold of the control (Fig.1)

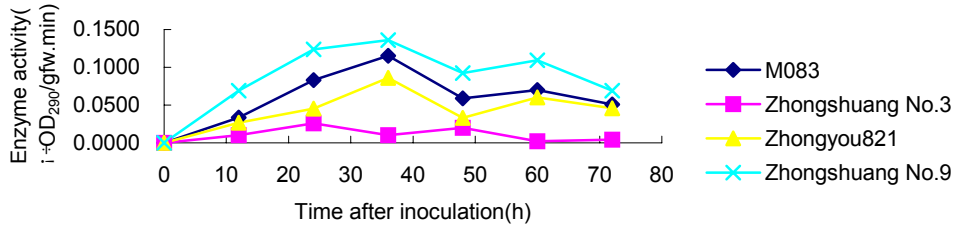


Fig. 1 Changes of PAL activity among different varieties after inoculation

Change of exo-chitinase activity

Exo-chitinase activity of Zhongshuang No. 9 started to increase after inoculation, and the trend kept until the time of 60h. It peaked for 3 times at the time of 12h, 36h and 60h consequently after inoculation. Its increasing rage was the highest among the 4 varieties (Fig.2).

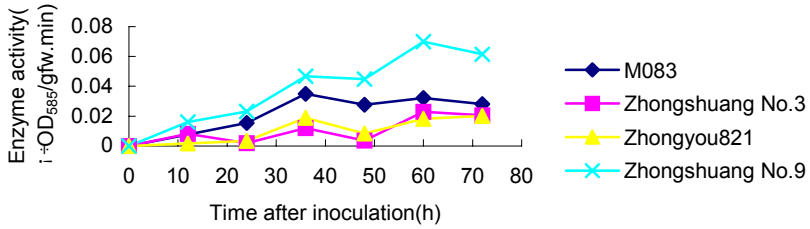


Fig. 2 Changes of exo-chitinase activity among different varieties after inoculation

Change of β-1□3-glucanase activity

β-1□3-glucanase of all the tested varieties started to increase before the time of 36h after inoculation, and then decreased. The activities of resistant varieties (strain) were higher than that of the susceptible variety Zhongshuang No.3, while that of Zhongshuang No. 9 was the highest (Fig.3).

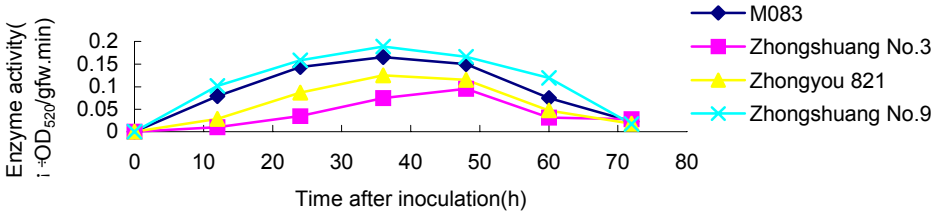


Fig.3 Changes of β-1□3-glucanase activity among different varieties after inoculation

Change of POD activity

The POD activity of Zhongshuang No.9 reached two peaks at the time of 12h and 36h after inoculation with *S. Sclerotiorum*, and was two times higher than that of the control (Fig.4).

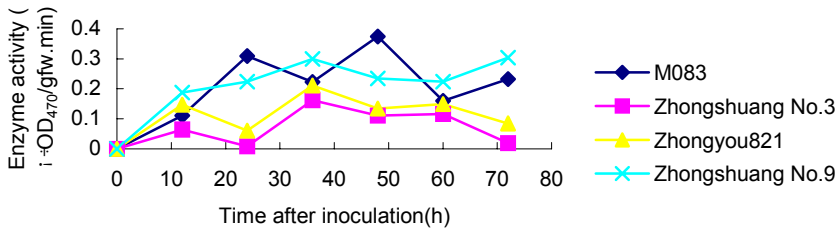
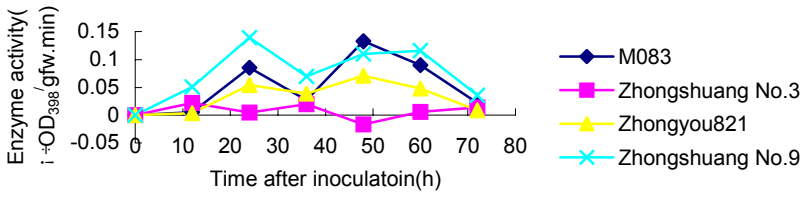


Fig. 4 Changes of POD activity among different varieties after inoculation

Change of PPO activity

PPO activity of three resistant varieties (strains) was significantly higher than that of the



susceptible variety. That of Zhongshuang No.9 increased most rapidly and reached two peaks at the time of 12h and 48h(Fig.5).

Fig. 5 Changes of PPO activity among different varieties after inoculation

DISCUSSION

Both field identification and the inoculation of detached leaves with mycelia showed that Zhongshuang No. 9 was highly resistant to *S. Sclerotiorum.* Based on the results of dynamic changes of different defense enzyme activities, the resistance could be attributed to a rapid increasing of PAL activity stimulating the production of downstream compounds of the phenylpropanoid pathway that inhibits fungal colonization. It could be attributed to a rapid elevation of exo-chitinase and β-1□3-glucanase activity degrading the fungus cell wall to kill them, releasing soluble chitins and β-1□3-glucosans from fungus cell walls, which acted as elicitor to activate defense genes involved in this variety defense reaction, such as the biosynthesis of lignin, phytoalexins. It could also be attributed to a rapid elevation of POD activity that led to the biosynthesis and accumulation of lignin and phenolic compound, which inhibit mycelium growth. Then a rapid elevation of PPO activity could make phenolic compound oxidized to quinones, which poisoned fungus. The stem of Zhongshuang No. 9 was solid and highly lignified, which could inhibit fungal colonisation.

Many useful genes from several parents were recombined and gotten together by multiple crossing, and were purified by microspore culture technique [8]. Zhongshuang No. 9 possesses characteristics of high resistance to *S. sclerotiorum* and lodging, high-yield, high oil content and double-low quality. It is not only a promising variety in production, but also a desirable genetic resource for the breeding of resistance to *S. sclerotiorum* and lodging in *B. napus*.

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