

Comparison of cultural characteristics and pathogenicity between *Sclerotinia sclerotiorum* and a sclerotia-forming fungus causing stem rot of welsh onion

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Abstract

Sclerotinia sclerotiorum was collected from rapeseed in 12 different regions of China and a sclerotia-forming fungus causing stem rot of welsh onion was collected from Dali County of Shaanxi Province. According to the mycelial growth rate and the colony morphological characteristics, sclerotial yield and formation pattern on colony, and the pathogenicity on rapeseed, the strains were divided into three types: A, B and C. Types A and B were from rapeseed, whereas the type C was from welsh onion. The pathogenicity sequence of the three types was A>B>C. The three type strains were incubated at 16°C, 20°C, 24°C, 28°C and 32°C. Under each temperature, mycelial growth of the type A strains was quicker than the type B strains, and the growth of type A and B were quicker than the type C strains. The optimum temperature for growth and sclerotial production of the three types of strains was 24°C. The result of investigation in the field indicated pathogenic fungi of stem rot of welsh onion (Type C strains) did not infect rapeseed, so rapeseed may be grown with welsh onion for crop rotation.

Key words: *Sclerotinia.sclerotiorum*, stem rot of welsh onion, temperature, pathogenicity

Stem rot of rapeseed (*Brassica napus*) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the worldwide spread diseases on rapeseed. It not only results in the loss in rapeseed yield, but also results in rapeseed quality and oil content^[1]. The host range of *S. sclerotiorum* is wide, including 75 families 278 genera and 408 species and 42 subspecies or varieties of plants^[2]. This pathogen has a few related species, including *S. trifoliorum* Eriks. and *S. minor* Jagg.^[3] Li and Wang^[4,5] reported that *S. trifoliorum* and *S. minor* had pathogenic potential on natural hosts of *S. sclerotiorum*. It is just for unsuitable environment factors that make them unable to infect some natural host plants of *S. sclerotiorum*, such as rapeseed, non-heading Chinese cabbage and broad bean. In this study, stem rot of welsh onion is one kind of fungal disease caused by *Ciborinia allii* (Saw.) Kohn. The pathogen has narrow nature host range. At present, we found that it can infect welsh onion, onion, garlic and leek. This pathogen is similar to *S. minor* and *S. sclerotiorum* in yield and morphological characteristics of sclerotia. Therefore, a study was initiated to compared mycelial growth, characteristics of sclerotia and pathogenicity on rapeseed between *Ciborinia allii* (Saw.) Kohn and *S. sclerotiorum*. and evaluated the possibility to rapeseed pathogenic potential.

1. Materials and Methods

1.1 Tested strains

Twelve strains of *S. sclerotiorum* were isolated from the rapeseed of the different rapeseed planting regions in China, respectively, and 10 strains of *C. allii* of welsh onion were isolated from Dali County of Shaanxi Province.

1.2 Comparison of cultural characteristics

All tested strains were inoculated on potato dextrose agar (PDA) and incubated at 20°C for 2 days. Mycelial plugs (7.5 mm diam.) were removed from the colony margin of each fungal strain, and transferred to the center of Petri dishes each containing 25ml of PDA (90mm diam.), one plug per dish. The dishes were incubated at 16, 20, 24, 28 and 32°C in an incubator. The colony size was measured at every 8 h for calculating the radial growth rate. Meanwhile, characteristics of mycelia and colony morphology, as well as the quantity and morphology and distribution of sclerotia were observed. There were five dishes (replicates) for each strain.

1.3 The pathogenicity tests of different types strains to rapeseed

The tested rapeseed varieties Youyan No.9, Wanyou14, Xiwang 98, Qinzayou No.1, Qinyou No.7 and Zhongyou 821 were collected from Hybrid Rapeseed Research Center of Shaanxi Province. The pathogenicity tests of all strains were determined on excised leaves collected from rapeseed seedlings and in the field at the flowering stage using the inoculation method described by Li et al. (2005)^[6] with 3 replicates for each strain. The disease severity of the representative strains to rapeseed was valued through the determination results of the two methods

2. Result analysis

2.1 The cultural characteristics of strains

According to the mycelial extension rate, morphology of colony, sclerotial morphology and formation pattern, and pathogenicity on rapeseed, 22 tested strains were divided into three types A, B and C. Among these strains, 12 strains of *S. sclerotiorum* from rapeseed belonged to types A and B, whereas other 10 strains of *Ciborinia allii* from welsh onion belonged to C. Mycelia of the type A strains was dense, and colony was thick with the radial extension rate of 2.5 cm/d, and sclerotia were formed concentrically. Mycelia of the type B strains were thin in dark color with the radial extension rate of was 2.1 cm/d, and sclerotia were formed in a single circle on PDA. Sclerotial yield of type A and B strains ranged from 28 to 46 sclerotia per dish. Colonies of type C strains was thin in white-greyish color, and produced loosely aerial mycelia, and the average extension rate was 1.4 cm/d, and sclerotia were very small in size, flatly-shaped and scattered in concentric rings. The average sclerotial yield was 776 sclerotia per plate. At 16-32°C, the type A and B strains could both grow on PDA plate and produce sclerotia, but at 32°C, the extension rate of colony was reduced. However, the type C strains could grow just at 16-28°C, and could not grew at 32°C, and the extension rate of the three types of strains was highest at 24°C(Fig.1), and their growth rates were arranged in a sequence A>B>C under each temperature, and at 16-28°C, the yield of sclerotia of the three types of strains increased with increasing temperature, but the size of sclerotia and the weight of single grain reduced gradually. Especially type C, some strip sclerotia were becoming granule gradually with rising temperature.

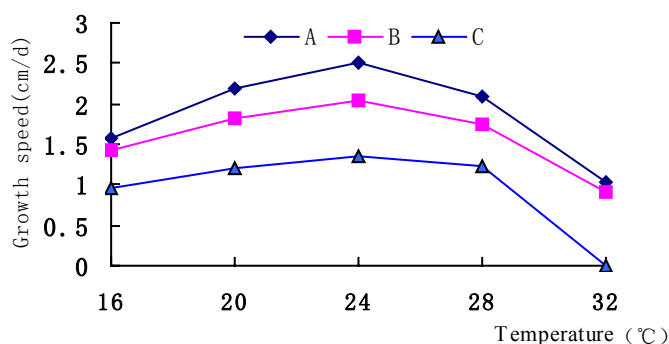


Fig. 1 The radial extension rate of the different types of strains under different temperature

2.2 Pathogenicity of strains to rapeseed

The study showed that the pathogenicity degree of the three types of strains to 6 rapeseed varieties was A>B>C (Table 1). Among them, pathogenicity of the type A and B strains on rapeseed had no qualitative difference, just a little bit of quantitative difference, and the pathogenicity of the type A strains was stronger than that for the type B strains, and the average lesion extension rate was 0.99 cm/d on excised leaves of rapeseed, the disease incidence of the field inoculation was 99.5% and the disease index was 45.9%. Pathogenicity of type B strains was weaker, and average extending speed of the lesion was 0.70 cm/d, the disease incidence of the field inoculation was 90.8%, and the disease index was 41.9%. However, pathogenicity of the type C strains could not infect rapeseed. In indoor inoculation on detached leaves for 3d, the biggest size of lesion was 0.34 cm without further extension. There was no infection in the field inoculation test.

3. Discussion

Stem rot of welsh onion was a kind of fungi disease that was discovered first in Taiwan by Sawada in 1919, He identified the pathogeny as *Sclerotinia* and named it *Sclerotinia allii* Sawada. In the early 1990s, Zhuang Wenying studied further the fungi of *Sclerotiniaceae* Whetzel ex Whetzel, and revised the identity, and classified again it as *Ciborinia* and named *Ciborinia allii* (Saw.) Kohn^[7]. *Ciborinia allii* (Saw.) Kohn and *S. sclerotiorum* both belong to *Sclerotiniaceae* Whetzel ex Whetzel as ascomycetes, and they both are fungi that may produce sclerotia. In cultural characteristics and infection characters to hosts, they have not only significant differences, but also similarities. This study showed that the formed course of sclerotia of *Ciborinia allii* (Saw.) Kohn and *S. sclerotiorum* shape and size and quantities of sclerotia were different, sclerotia of *Ciborinia allii* (Saw.) Kohn. was smaller with more quantities. At 24°C, the sclerotia quantities per plate (90mm diam) was over 600 grains normally, which were over 20 times than sclerotia of *S. sclerotiorum*, but dry weight of sclerotia was lower than *S. sclerotiorum*. Besides, their infection ways were alike, namely infecting their hosts by ascospores and mycelia. Studying pathogenicity of *S. sclerotiorum* and its related species to hosts, Li and Wang considered that *S. trifoliorum* Eriks and *S. minor* Jagg. both may infect natural hosts of *S. sclerotiorum*, rapeseed and non-heading chinese cabbage ect., under the compulsive inoculation of excised leaves, so they had pathogenicity potential to natural hosts of *S. sclerotiorum*. In this study, though there were many similarities between *Ciborinia allii* (Saw.) Kohn and *S. sclerotiorum*. The natural host range of *Ciborinia allii* (Saw.) Kohn was very narrow. From pathogenicity test, *Ciborinia allii* (Saw.) Kohn could not infect rapeseed, which is the natural host of *S. sclerotiorum*, under natural condition, but under the compulsive inoculation of excised leaves, though *Ciborinia allii* (Saw.) Kohn could infect excised leaves of rapeseed to appear lesion, these lesions were small with slow spread, and formed necrotic lesions soon, and could not go on spreading. So, pathogenicity potential for *Ciborinia*

allii(Saw.) Kohn to rapeseed was small. Therefore, welsh onion and rapeseed can be cultivated in rotation.

Table 1 Comparison of pathogenicity of different type strains to different rapeseed

Varieties	Strain type	Field inoculation		Indoor inoculation	
		Disease incidence (%)	Disease index (%)	Lesion extension rate (cm/d)	Lesion size(cm)
Youyan No.9	A	100	54.3	1.28	5.29
	B	93	49.5	0.88	3.93
	C	0	0	0.06	0.23
Wanyou 14	A	100	46.2	0.90	4.79
	B	89	40.3	0.65	3.81
	C	0	0	0.06	0.28
Xiwang 98	A	100	49.3	0.98	4.73
	B	91	45.1	0.65	3.8
	C	0	0	0.09	0.34
Qinzayou No.1	A	99	43.2	0.93	4.4
	B	87.6	40.4	0.72	3.15
	C	0	0	0.07	0.30
Qinyou No.7	A	98	39.8	0.96	4.68
	B	90.5	36.7	0.68	3.85
	C	0	0	0.05	0.20
Zhongyou 821	A	100	42.4	0.87	4.45
	B	93.4	39.5	0.60	3.46
	C	0	0	0.07	0.31
Average	A	99.5	45.9	0.99	4.72
	B	90.8	41.9	0.70	3.67
	C	0	0	0.07	0.28

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