Infection of *Sclerotinia sclerotiorum* to rapeseed and sunflower and its virulence differentiation

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ABSTRACT

Isolation, culture and purity of single-sclerotium of *S. sclerotiorum* from rapeseed and afterreap sunflower in Dali county of Shaanxi province, as well as *S. sclerotiorum* of sunflower from Aletai, Xinjiang province. The isolates were divided into three types: A, B and C, according to the growth rate and the yield of mycelium, the characteristic of colony, degree of pathogenicity and oxalic acid accumulation. Type A was from rapeseed and sunflower in Dali county of Shaanxi province, and type B and C from sunflower in Aletai of Xingjiang province. The study showed that the virulence of *S. sclerotiorum* on different hosts was different. Growth of type A and B was normal, and their colonys were even and vigorous. The virulence of type B was stronger on rapeseed, sunflower and soybean, and yield of its oxalic acid was higher, and the virulence of type A was strong only on rapeseed and sunflower, and it was very weak on soybean, but yield of its oxalic acid was highest. The growth of type C was abnormal, its colony was rare and not even, and its virulence was weak on all three crops, and the yield of its oxalic acid was low. In 2001, the field investigation showed that type A isolates resulted in *sclerotinia* stem rot, not only on rapeseed but also on sunflower (root rot type), but it was not found on soybean. Molecular genetics was utilized to further study virulence differentiation.

Keyword: *Sclerotinia sclerotiorum (Lib) de Bary—infection*—virulence—compatibility—RAPD

INTRODUCTION

Sclerotinia sclerotiorum (Lib) de Bary has a wide host range and can cause economic loss to oil crops such as rapeseed, sunflower and soybean. In China, it causes average annual yield loss of rapeseed of about 10-20%, in some severe year it can reach even up to 50%(Chengqing Liu et al) ;In main producing district of sunflower, it causes average annual yield loss of about 30%(Haiyan Lan et al),so it is badly affecting the production of rapeseed and sunflower . Recently, we found that in rotation plough land of rapeseed to soybean, rapeseed to sunflower *S. sclerotiorum* were strong virulent to rapeseed and sunflower, but were not found virulent to soybean in Shaanxi province; However in Aletai, Xingjiang province, this kind of fungus could not only infect sunflower strongly but also infect rapeseed and soybean slightly. Further research was done, its aim was to study the characteristics of strain and its pathogenicity differentiation of *S.sclerotiorum* so as to provide scientific basis for disease resistance breeding and rational cultivation.

MATERIALS AND METHODS

Isolates for trial and resource A total of 40 single-*sclerotium isolates* of *S. sclerotiorum* was used, including 18 from rapeseed in Dali of Shaanxi province and 22 from sunflower in Dali of Shaanxi province and Aletai of Xinjiang province.

Cultural characteristics Active cultures of isolates were obtained by placing small mycelial plugs from stock cultures on PDA(potato dextrose agar),further subculture for comparison of growing speed of mycelia was done by transferring growth active mycelium(agar plugs with diameter of 6mm) to the center of culture dish with rationing PDA(25ml/per plate), then incubated at 22 , colony size was measured every 8 h, colonial morphology and sclerotia production were observed at the same time, four replicates was set; Amount of mycelia growth was done by transferring representative isolates with different growth pattern to rationing potato-sucrose culture fluid, cultures were incubated for 5 days at 23 on an orbital shaker at 100rpm, filtrated mycelia were weighted, amount of oxalic acid accumulation was detected according to the methods of Bateman, four replicates was set.

Compatibility test Mycelial plugs(with diameter of 6mm *)of Sclerotinice sclerotiorum* were transferred to PDA plates by pairs, incubated at 22 .Mycelia compatibility was observed in the same isolate and between different isolates.

virulence tests Rapeseed variety Qinyou No.2, Qinyou No.7, Zhongyou821 and soybean variety Qindou 8 were from Hybrid Rapeseed Research Center of Shaanxi Province, sunflower

varieties G_{101}, F_{51}, and F_{52} were from Seed Manage Station of Shaanxi Province. "Excised leaves" method of inoculation were used to test virulence of the representative isolates to rapeseed, sunflower and soybean. 25 excised leaves were inoculated by each isolate with 3 replicates, after 48h,72h of inoculation, lesion size on leaves were measured, and the virulence was assessed according to the mean of lesion size.

DNA extraction DNA extraction was done as method described by Hao Wang.The representative isolates were cultured in potato-sucrose culture fluid at 22 , mycelia were collected by filtration after mycelia were full of liquid medium, then were washed 2 times with axenic distilled water. The desiccated mycelia by filter paper were ground to powder in a mortar with liquid nitrogen.

RAPD analysis:

Reaction system the RAPD-PCR amplification reactions(20µl) consisted of 2.0µl of 10×reaction buffer 125µM of each dNTP, 0.625µM 10mer random primer 1.0 U of Taq DNA polymerase and 20ng of fungal DNA.

Reaction process denaturation at 95 for 3min; followed by 40 cycles of denaturation at 94 for 50s, 1.5min of annealing at 38 ,and extension for 1min at 72 ;The final extension was 10min at 72 .

Electrophoresis examine 12µl of reaction products was mixed with 3µl loading buffer(including 0.25% bromophenol blue 40 sucrose) amplification products were separated on 1.4% agarose gels(including 0.5µlg/ml Ethidium bromide, EB)at 5Vcm^{-1} for 1-2h and visualized under UV light .

RESULTS

Cultural characteristics of isolates 40 isolates of *S. sclerotiorum* were divided into A,B and C altogether 3 types according to growing speed of mycelia , sclerotia production and distribution on PDA plate and amount of mycelia growth in rationing potato-sucrose culture fluid .Mycelia of Type A isolates spread evenly on plate with compact colony and fast growing speed of about 4.5 5.6cm/d and was not easily aged, the amount of mycelia incubated for 5days was 0.24 0.288g dry weight/ flask. Sclerotia production was 20-28 grains/plate with sclerotia forming concentric circles; Colony morphology of type B isolates was the same as that of type A, but with more slow growing speed of about 3.5 4.2cm/d, the amount of mycelia was about 0.1g less than that of type A, but its sclerotia production was about 18 grains/plate much more; Mycelia of Type C isolates was loose and was easily aged to change color, it spread unevenly and fan-shapedly with slow-growth rate of 1.1 2.6cm/d,the amount of mycelia for 5 days was lowest of 0. 095 0.11g/flask, Sclerotia production was 7-15grains/plate with sclerotia scattered every where on colony.

Pathogenic differentiation of isolates Significant difference in virulence and oxalic acid accumulation were found among 3 types of isolates(table1) .Oxalic acid accumulation was A>B>C.Virulence to rapeseed was A>B>C, to sunflower was B>A> C, to soybean was B>C > A.

Table1. Oxalic acid accumulation and virulence of different types of isolates

Isolates type	oxalic acid accumulation (mmol/l)	lesion size(cm)		
		rapeseed	sunflower	soybean
A	3.45	4.12	4.27	0.77
B	2.79	3.62	5.38	3.18
C	2.28	0.74	2.14	1.86

lesion size was the mean value of that of 72 hours post-inoculation of the representative isolates on excised leaves of the same crops.

Mycelial compatibility of different isolates From the test results, it was found that there were 3 types of reaction among hyphae. In the first type, hyphae could fuse each other well; In the second type, light reaction line appeared between the two isolates with hyphae developing in the reaction zone; In the third type, a obvious broad reaction line between the two isolates developed and no hyphae existed in the interaction zone. The first reactive type existed only in the same isolates. The second and third reactive type existed between all the isolates, but isolates belonging to the second type were obtained from same region, most of isolates belonging to the third type were obtained from different region. Further cluster analysis of reactive type of 15 representative isolates showed that they were clustered into different groups according to Euclidean distance, and the reactive type in some extent was related to genetic relationships among variant isolates. The difference caused by host and pathogenicity was

much less than caused by geographical origin, Mycelial compatibility of different isolates had no relationship to their pathogenicity.

RAPD analysis 40 of the 120 random primers could amplify polymorphic DAN fragments in which 20 primers with plenty and stable bands and good replication were selected to amplify DNA of 17 representative isolates.182 DNA fragments were obtained. Among these,161 were polymorphic fragments, amount to 88.5 per cent of the total DNA fragments. This demonstrated that plentiful genetic polymorphism existed among 17 isolates. Cluster analysis showed in different similarity coefficients these isolates could clustered into different population, but population of isolates clustered by polymorphism of amplified DAN fragments had no significant correlation with pathogenic population.

DISCUSSION

S. sclerotiorum has very wide host range and geographic distribution. The virulence of individual strain was affected both by its genetic variation and infection factors and by geographic ecological environment and nutritional environment. Through this study, it was shown that different isolates had specific virulence to different host. Isolates of type A were isolated from rapeseed and from sunflower whose fore-crop was also rapeseed and that was planted firstly in Dali, Shaanxi Province. By inoculation of excised leaves, they were strong virulence to rapeseed and sunflower, but were weak virulence to soybean. Isolates of type B were isolated from sunflower in Aletai, Xinjiang province, they were strong virulence not only to rapeseed and sunflower but also to soybean, and this might be the result of long mutual interaction between isolate and host. In addition, different ecological environment of different region where stem rot occurred, different infectious ways of different isolate to different host, different growth habit and susceptible stage of host all could cause the virulence difference of isolate population. For example, *S. sclerotiorum* can infect sunflower by mycelium and ascospore leading to root rot, stem rot and head rot, but it infect petal and senescent leaves of rapeseed and soybean only mainly by ascospore. These factors resulted in time difference of interaction that isolates were between different region and different host and special virulence on different host.

Oxalic acid as a kind of toxin of *S. sclerotiorum* taking part in pathogenicity had been reported much. In this study, oxalic acid accumulation of 3 types of isolates demonstrated that pathogenicity was not completely correlated with the production of oxalic acid, such as in type A isolates, production of oxalic acid was the highest, but its pathogenicity to sunflower and soybean, except for rapeseed, was much weaker than that of type B isolates.

Mycelial compatibility of different isolates was different in this study, but there had no significant correlation between mycelial compatible groups and pathogenic groups. By RAPD of polymorphism of DAN fragments, it was found that there existed very plentiful genetic diversity in 17 representative *S.sclerotiorum* isolates, but there also had no significant correlation between their genetic relationship and virulence. This might be the main genomic zones analyzed by RAPD that were not in pathogenic functional zone or far from it, so the polymorphic DAN fragments amplified by random primer couldn't be directly used for correlated analysis with virulence. The polymorphic marker closely linked to virulence should be screened firstly, then be applied to reveal virulence and differentiation mechanism in molecular level, and special marker closely correlated with virulence will be screened to analyze and forecast disease.

Different isolates of *Sclerotinia sclerotiorum* had specific pathogenicity to different host, and the viability of *sclerotia* in soil was very long, so the crops, such as wheat and maize resistant to this fungus could be used for crop-rotation with long cycle to susceptible rapeseed, sunflower and soybean. In addition, by regulating sowing-season based on local climate, susceptible stage of susceptible crops to stem rot could be escaped. Disease control could also be done by not planting sunflower after rapeseed and other Cruiferae crops.

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