JOURNAL OF PURE AND APPLIED MICROBIOLOGY, APRIL 2014. Vol. **8**(2), p. 1523-1534

Resistance Analysis of *Lilium oriental* on Bulb Rot

Songping Hu[1]*, Zhigang Wei[1]*, Sixiang Zheng[2], Yuanyauan Nie[3], Lin Zhang[3], Tingting Pei[1] and Xia Yu[1]**

[1]College of Bioscience and Bioengineering, Jiangxi Agricultural University, Nanchang City of Jiangxi Province - 330045, China.
[2]Agricultural Sciences Institute of Zhuzhou, Zhuzhou City of Hunan Province - 412007, China.
[3]College of Science, Jiangxi Agricultural University, Nanchang City of Jiangxi Province - 330045,China.

(Received: 09 January 2014; accepted: 18 March 2014)

In order to understand the mechanisms of bulb rot pathogenesis and disease resistance of *Lilium oriental* as soon as possible, the study was conducted using *Lilium oriental* materials from the Agricultural Sciences Institute of Zhuzhou in Hunan. The bulb rot pathogen was isolated by the mercuric chloride disinfection method. Then, the effect of seven inoculation methods and completed disease resistance identification of 30 self-cultivation *Lilium oriental* materials were compared. At the same time, the total content and antifungal activity of saponin in materials were also investigated. The results suggested that *Fusarium oxysporum* was the pathogen causing *Lilium oriental* bulb rot. According to the clustering analysis, 30 *Lilium* materials tested were divided into three groups of 3 resistant, 16 medium resistant and 11 susceptible materials. It also showed that resistant materials had significantly higher saponin content than susceptible materials did. Additionally, the saponin content was significantly correlated to resistance levels (r=0.819). With the mixed liquid plate culture method, it was discovered that the saponin extract solution, whose total saponin content was 1.194mg/mL, had a certain inhibition rate of 29.39% on pathogen's mycelial growth.

Key words: *Lilium oriental*; bulb rot; *Fusarium oxysporum*; resistance identification; saponin content.

Lily bulb rot disease (referred as bulb rot herein) which is also called basal decay and wilt disease is one of most serious diseases for production of lilies, the perennial herb. *Lilium oriental* (*Lilium* spp.) is one of the three lily cultivars. It is popular for its gorgeous flowers, strong aroma, beautiful color and other features, and therefore has high ornamental value. However, the domestic and international studies indicate that *Lilium oriental* has poor resistance to bulb rot[1]. Most of commercial bulbs sold on the current market are medium resistant or susceptible varieties[2].

To date, China has put a lot of effort in developing domestic *Lilium oriental* bulbs in order to put an end to the unfavorable situation of heavy dependence of lily bulbs on imports by independent research[3-4]. The control and prevention of bulb rot is one of the key techniques to realize domestic production of *Lilium oriental* bulbs. There is an eager requirement to master the technique. Academically, the selection of disease-resistant varieties is the best way to solve the disease problems. The pathogen study and resistance identification is the basis for resistant breeding. Yan Liu *et al.*,[5] used the germination rate after

* Authors contributing equally.
**To whom all correspondence should be addressed.
E-mail: zhanglin06@163.com

fungus inoculation as a resistance index to identify resistance of 44 *Lilium oriental* materials to Fusarium bulb rot. Xiumei Yang *et al*. [2,6] used the scale inoculating method to identify resistance of tens of lily germplasm resources. Lvchun Peng *et al*.[7] performed the study on aseptic identification of the resistance of lily varieties to toxin filtrate from Fusarium oxysporum f. sp. Lilii for the first time. Lulin Ma *et al*.[8] investigated resistance of 20 lily resources using the root-cutting inoculation method. Lili Zhang *et al*.[9] studied the resistance of 9 lilies using the scale inoculating method and investigated factors inducing the disease. However, the bulb rot pathogenesis mechanisms and resistance of *Lilium oriental* are still under intense study and are not very unclear yet. Moreover, there is no report about the critical operation and fine technique.

In this study, the pathogen isolation of *Lilium oriental* bulb rot was investigated, several inoculation methods were compared, resistance identification was performed and the total saponin content was measured. The results may provide technical methods and theoretical basis to further understand the disease and resistance mechanisms of *Lilium oriental* bulb rot.

MATERIALS AND METHODS

Materials

The *Lilium oriental* bulbs with bulb rot were used for pathogen isolation. The regular hybrid offspring of *Lilium oriental* were used for resistance identification. These materials were all from the plant tissue culture laboratory in the Agricultural Science Research Institute of Zhuzhou. The control dioscorea saponin (purity over 98%) was purchased from Nanjing Spring & Autumn Biological Engineering Co., Ltd.

Isolation and observation of the pathogen

Isolation and purification: the materials were sterilized following the steps of 75% ethanol 30s)-sterile water (once)-0.1% mercuric chloride (15min)-sterile water(three times). The Fungi were obtained after isolation and inoculation.

Single-spore isolation: the spore suspension with a certain concentration was made using generated strains. The method of dilution plating was applied to generate single-spore cultures, which were inoculated onto a new medium[10].

Feature description

What has been observed include colony shape, surface moisture, color, edge morphology, the presence of exudate, etc and the colony growth rate, the change of fungus suspension and the change of medium color were recorded. Mycelia were taken to make slides and the mycelium morphology, spore shape, sporogenous cells and other special features were observed through an optical microscope.

Determine pathogenicity of the pathogen

The strains were washed and cultured using sterile water for ten days and then were used to make spore suspension (1.5×10^6 cells/mL). Several two-year old lily bulbs and seedlings were divided into control and experimental groups. Two groups were inoculated with spores and sterile water, respectively, and were planted in fine sand. Thin film was used to maintain moisture for four days followed by conventional planting.

The incidence of disease/%= the number of infected seedlings/the total number of seedlings×100.

The infected materials in the pathogenicity test were sterilized according to the above steps for pathogen isolation. The pathogen was separated again and compared to the pathogen obtained from the first isolation. According to "Laboratory guide to the identification of Fungi" and "The genus Fusarium"[11,12], the fungus was identified based on the index and feature description.

Comparison of different inoculation methods

The authors compared the practical application effect of seven inoculation methods and found the best approach for resistance identification.

Fungus-carrying cultivation inoculation (M1): mycelium blocks were inoculated into cultivation materials.

Direct inoculation with spore-soaked cotton(M2)

The cotton soaked in the spore suspension was directly used to cover seedling bulbs for inoculation.

Stab-injection inoculation (M3) the seedling base was stabbed and injected with a little spore suspension for inoculation.

Stab-soaking inoculation (M4): the seeding base was stabbed and soaked in the spore suspension for half an hour.

Detached scale stab-soaking inoculation (M5): the detached scale was stabbed and soaked in the spore suspension for twenty minutes, put in a plate with moistened filter paper and cultured at 25℃.

Stab-injection inoculation (small bulb seedlings, M6): the procedure was the same as "stab-injection inoculation (M3)" except that small bulb seedlings were used as inoculation materials. Fungus-carrying cultivation + stab-injection inoculation (M7): the combination of "Fungus-carrying cultivation inoculation(M1)" and "stab-injection inoculation(M3)"

Among them, M6 used small bulb seedlings which had grown in a greenhouse for one year as inoculation materials. The same type of tissue cultured seedlings was used for inoculation in the other methods. The relative control group was used for each method. After the treatment, the incidence rate and disease grade were analyzed. The disease grade was defined based on the decay situation.

Resistance identification

The test was performed in a glass greenhouse of the Agricultural Science Research Institution of Zhuzhou from Oct. 2012 to May 2013. There were 30 sets of tested materials, and 30 individual plants were used for the test from each set.

Lily seedlings with two or three leaves were inoculated with the pathogen and plated in fine sand medium under a maintained temperature and moisture condition. One month later, the tested seedlings were observed. The detailed disease symptoms were recorded and used to determine the disease grade (Table 1). The disease condition and resistance index of each material were then calculated.

The DPS7.05 software was applied for clustering analysis to obtain the optimal clustering results. According to the best clustering distance, 30 *Lilium oriental* materials tested were divided into different resistance groups[13].

Table 1. Disease grading standards

Disease grade	Morbidity symptom	Disease value
Grade 0	Not infected, without any disease symptom	0
Grade 1	Leaf tip blight or leaf chlorosis, no obvious basal decay	1
Grade 2	Leaf blight or chlorosis, minor bulb rot	2
Grade 3	Leaf blight, obvious bulb rot	3
Grade 4	Withered leaves, serious bulb rot	4
Grade 5	Withered leaves, complete bulb rot, death of the whole plant	5

Formula for disease index: disease index/%=["(the number of seedlings at each disease grade × disease value)/(the number of inoculated seedlings× the biggest disease value)]×100. Formula for resistance index: resistance index/%=100-disease index/%.

Determination of total saponin content

5.0mg of dioscorea saponin was accurately weighed and dissolved in methanol up to 50mL to generate the standard control solution (100mg/L).

Small lily scales were taken, cleaned and then dried at 80°C. The dried scales were ground into powder. 1.0g of the powder was weighed and ultrasonic extraction was performed in 20mL of methanol for 1h. Precipitation was removed by filtering. The filtered solution was put at 80°C to remove methanol. 10mL of distilled water was added and the same volume of petroleum was used for extraction. The ether layer was discarded when it was colorless. The same volume of water saturated n-butanol was used to extract the water layer. The water layer was discarded when the water saturated n-butanol was colorless. The water saturated n-butanol layer was dried at 80! using a rotary evaporator. The obtained sample was dissolved in 20mL of methanol to generate the extract of total saponin[14,15].

0.4, 0.6, 0.8, 1.0, 1.2, 1.6 and 2.0mL of the standard solution was put into the test tubes, respectively. The tubes were dried at 80! to remove methanol and allowed to cool. Next, 2mL of 5% vanillin-glacial acetic acid solution and 0.8mL of perchloric acid were added. The solution was mixed,

sealed and put in a 60°C water bath for 20min. The tubes were allowed to cool in a refrigerator for 5min. Exact 5mL of glacial acetic acid was added into each tube. The solution was mixed well and incubated for 10min. The bland solution was used as the control. The absorbance of each standard solution was determined at 540nm[15,16]. The saponin quality and the absorbance were used as X-coordinate and Y-coordinate, respectively, to make the standard curve.

Certain amount of sample extract was used to detect its absorbance according to the above procedure. The saponin content was calculated based on the standard curve. The SPSS 19.0 software was applied to analyze the relevance between the resistance index and saponin content of samples.

The inhibition effect of the saponin extract on the pathogen

A certain amount of above mentioned saponin extract whose total saponin content was 1.194mg/mL was taken and dried at 80! to remove methanol. A certain amount of 0.1%DMSO was added to dissolve. The obtained solution was mixed into the prepared potato sucrose medium (PSA) followed by high temperature sterilization. The solution was poured into plates to form saponin-containing plates. The pathogen blocks were inoculated in the middle of the plates. PSA plates with the same amount of 0.1%DMSO solution were used as the control. The plates were incubated at 28! for three days. The crossing method was applied to measure colony diameter.

RESULTS

Bulb rot symptoms

Bulb rot caused by pathogenic microorganisms may occur at any growth stage of lilies. The pathogens go into plants through a wound or no wound invasion from lily root or bulb base. Then, they gradually infect the upper region along the plant vascular bundle and eventually result in rot in the plant root and bulbs. The inside vascular bundle is full of pathogens and turns brown. The leaf tip gradually becomes yellow, withered and purple. Serious disease even causes completely withered leaves. Plant stem dries off from the base. The roots are detached from bulbs due to decay. The outside scales in a bulb are badly decomposed and the inside scales also fall into slight decay(Fig.1). When the air humidity is relatively high, obvious white to pink basal mycelia can also be observed around the infected bulbs.

A: Dwarfing and turn purple B: Leaf tip yellowing C: Basal mycelium

Fig. 1. Symptoms of *Lilium* Oriental bulb rot

Isolation of the pathogen

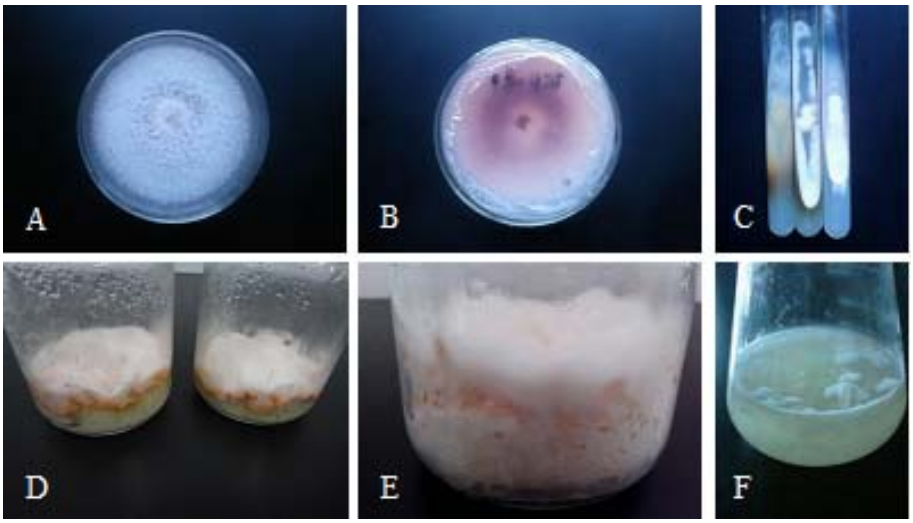
The infected lily bulb scales were washed with water, sterilized, inoculated into 11 bottles and cultured for 6 days. Microorganisms around the scales were observed in 7 bottles. Among them, most of microorganisms were pure white filamentous Fungi. The mycelia were inoculated into plate culture media for purification. After the single spore isolation, a purified strain of filamentous Fungi was achieved.

Pathogen characteristics

Culture characteristics

The colonies in PSA were round with smooth edges. They were white, rich and villous. Aerial mycelia were well developed and the color was white with a little bit pink. On the back, it was pink to purple red. Few concentric rings were observed. The mycelia in mashed potato media were white at the early stage of culture. At the late stage, the edge of mycelia became pink. The mycelia

in rice media were pure white in the beginning and gradually turned into pink. In the end, the mycelia were full of the medium and the color was white touched with pink. In PS liquid media, the fungus liquid was turbid white at first and became pink later. In the end, the media were bright pink and turbid. A lot of flocculation appeared in the bottom of the bottle(Fig.2).



A: Plate culture of PSA (Front) B: Plate culture of PSA (Back) C: Slant culture of PSA
D: Culture of mashed potatoes E: Culture of rice F: Liquid culture of PS

Fig. 2. The culture characteristics of pathogens

The crossing method was used to measure colony diameter. The results showed that the colony diameter in PSA plates was 2.90cm~3.30cm after light culture at 25°C for 3d, and the average diameter was 3.13cm. At Day 9, the diameter reached 8.40cm^ÿ9.05cm and the average was 8.62cm. The colony growth was continuously observed for 7 days, and a curve was made based on the results. It indicated that the average growth rate of mycelia was 9.1cm/d.

Morphological characteristics

Septate hyphae were observed and they were smooth with many branches. The micro-conidium was oval or ovoid. The number was huge and most of them were nonseptate hyphae. Only a few had one separator and the size was 5.08~9.86 um × 1.57~4.24 um. The macro-conidium looked like scimitars. Most of them had sharp ends. The number was less. Generally, they had 3~5 separators and the size was 10.04~48.77 um × 2.32~4.28 um. The sporogenous structure was single phialide and acrospore. The shape of chlamydospore was round. They were acrospore or aleuriospore. Two to three parallel spores were observed accidentally(Fig.3). The edge was smooth. There was no sexual stage.

A: Micro-conidium B: Macro-conidium C: Chlamydospore

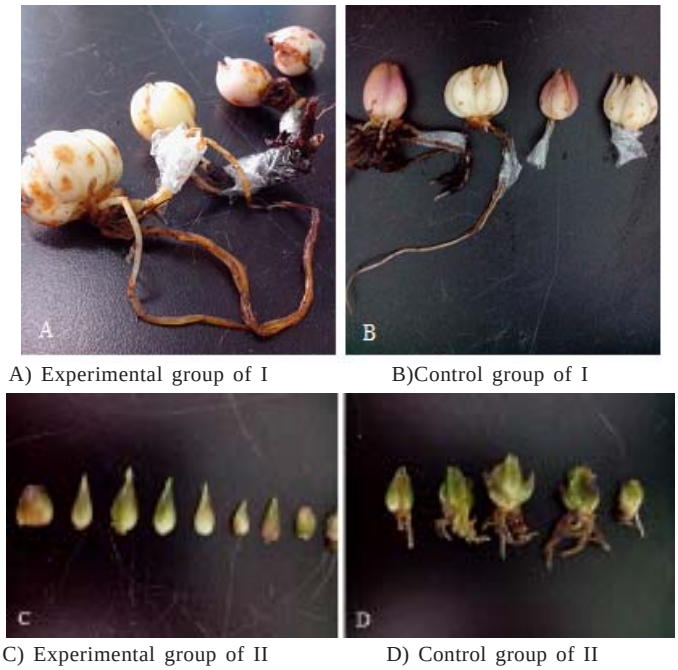
Fig. 3. The morphological characteristics of pathogens

Results of the pathogenicity test

The stab-injection inoculation method was applied to both lily bulbs and seedlings. The bulb and seedling groups were observed at Day 15 and 7, respectively. The results showed that although both control groups had no obvious disease symptoms, the incidence for both experimental groups was 100% , (Tab. 2 and Fig.4). The data indicated that the isolated pathogens did induce lily bulb rot. Moreover, seedling inoculation led to more serious and earlier infection than bulb inoculation, which is consistent with the results reported by Lili Zhang *et al.*,[9].

Table 2. The test of pathogenicity

Number	Inoculation material	Inoculation method	Inoculation result		Incidence	
			Experiment	Control	Experiment	Control
I	Bulb	Stab-injection inoculation	Brown rot occurred in most of roots. At the stab sites, decay was obvious and had rot patches	All bulbs were good. Only few roots had decay. There was no obvious rot at the stab sites.	10 0	0
II	Seedl ing	Stab-injection inoculation	Basel rot was obvious. The base was detached and significant decay regions were observed in each scale.	All seedlings were good. Partial wilt showed up in the root, but new white root appeared.	10 0	0



A) Experimental group of I B)Control group of I

C) Experimental group of II D) Control group of II

Fig. 4. The pathogenicity test results

The materials of experimental group I and II used in the pathogenicity test was selected to perform the pathogen isolation again. The mycelia were generated in some explants after three days' culture. At Day 5, fungi were seen in all the materials. After isolation and purification, the obtained strain had similar colony characteristics, mycelium morphology, spore shape and culture color to the strain generated by the first isolation. Morphological identification implicated that these two strains belonged to the same species.

Based on the first isolation situation, culture characteristics, micro morphology features, pathogenicity and the second isolation situation and with referrence to "Laboratory guide to the identification of Fungi (Jingchao Wei) " and "The genus Fusarium (Booth)" [11,12], conclusion was drawn that the strain isolated from *Lilium oriental* rot scale was *Fusarium oxysporum*. Meanwhile, according to the four-step process of the Koch Law, it was decided that this strain is the pathogen of *Lilium oriental* bulb rot.

Comparison of different inoculation methods

It was found that different inoculation methods had different inoculation effect (Table 3). Among them, the effect of the stab-injection inoculation method was the best. The incidence of disease was 100% and the disease severity was 90%. The second method was the combined approach of the fungus-carrying inoculation and stab-injection inoculation. The incidence was 100% and the disease severity was 80%. The third one is the stab-soaking inoculation method. Its incidence was 100% and the disease severity was 70%. The effects of the fungus-carrying cultivation inoculation and direct inoculation with spore-soaked cotton were bad and could not meet the requirements for resistance identification.

Table 3. Comparison of different inoculation methods

Number	Inoculation method	Inoculation days	Incidence	Disease severity	
				Experiment	Control
M1	Fungus-carrying cultivation inoculation	10	0	-	-
M2	Direct inoculation with spore-soaked cotton	10	70	15	-
M3	Stab-injection inoculation	10	100	90	-
M4	Stab-soaking inoculation	10	100	70	-
M5	Stab-injection inoculation (small bulb seedlings)	30	100	50	-
M6	Detached scale stab-soaking inoculation	30	100	-	-
M7	Fungus-carrying cultivation+stab-injection inoculation	10	100	80	-

Note: "-" means no incidence or unrecognized

No infected plant was observed using the fungus-carrying cultivation inoculation method. It may be due to poor survival of mycelia in the medium which has no pathogenicity. The disease severity caused by the direct inoculation method with spore-soaked cotton was very low. Since the seedlings used in this method had no wounds, the low severity may be because of the ineffective invasion of pathogens. In the detached scale stab-soaking inoculation method, pathogenic mycelia appeared in the experimental group, but the detailed disease situation could not be recognized. Hence, the method is not suitable for resistance identification. The stab-injection inoculation (small bulb seedlings) had moderate disease severity and the disease cycle was long. Thus, this method is not suitable for resistance identification too. In contrast, using seedlings as inoculation materials are more feasible[8].

With the inoculation effects, advantages and disadvantages of each method, it is concluded that the stab-injection inoculation method has the

best effect, but it is complicated and time-consuming for large-scale resistance identification. In contrast, the stab-soaking method is relatively simple, fast and feasible, so it is suitable for resistance identification tests which need large-scale inoculation. Ultimately, decisions were made to use the stab-soaking inoculation method to identify resistance of 30 materials.

The resistance identification results of materials

The resistance identification results showed that various materials had different resistant ability to *Fusarium oxysorum* bulb rot. The highest resistance index was 76.00% and the lowest index was 28.67% (Table 4).

Table 4. The resistance identification results of materials

Material number	Test number	The number of infected seedlings at various disease grades						Resistance index %
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	
ZN-2	30	3	1	1	11	0	14	29.33
ZN-3	30	3	8	8	3	3	5	53.33
ZN-4	30	3	3	12	0	1	11	42.67
ZN-5	30	8	4	6	0	0	12	49.33
ZN-6	30	1	2	10	4	1	12	34.67
ZN-7	30	1	2	13	3	0	11	38.67
ZN-8	30	1	3	7	2	1	16	28.67
ZN-9	30	8	1	3	4	2	12	42.00
ZN-10	30	3	6	3	3	3	12	38.00
ZN-11	30	6	6	4	2	0	12	46.67
ZN-12	30	7	11	6	1	0	5	66.00
ZN-13	30	2	9	7	1	1	10	46.67
ZN-14	30	2	5	10	0	2	11	41.33
ZN-15	30	2	7	6	0	1	14	38.00
ZN-16	30	0	2	11	6	0	11	35.33
ZN-17	30	6	6	3	0	0	15	42.00
ZN-18	30	10	4	6	2	1	7	59.33
ZN-19	30	0	6	10	0	1	13	36.67
ZN-20	30	1	8	6	0	0	15	36.67
ZN-21	30	2	8	3	3	4	10	40.67
ZN-22	30	1	14	5	4	0	6	56.00
ZN-23	30	0	7	6	1	2	14	33.33
ZN-24	30	1	5	12	3	0	9	44.67
ZN-25	30	4	7	9	1	0	9	51.33
ZN-26	30	1	10	6	1	0	12	43.33
ZN-27	30	1	15	11	0	0	3	65.33
ZN-28	30	3	3	10	3	1	10	42.67
ZN-29	30	0	2	12	4	2	10	36.00
K45	30	1	9	6	5	1	8	46.67
y	30	13	12	0	0	1	4	76.00

The resistance indexes of various materials were normalized. The Euclidean distance was used as the clustering distance. The deviation square method was used as the clustering method. The obtained clustering effect was consistent with the conventional resistance classification. Based on the clustering analysis results (Fig.5), the 5.00 of Euclidean distance was used as clustering segmentation points. The 30 materials tested were divided into three groups. The first group included three materials, y, ZN-12 and ZN-27, which were resistant materials. The second group included 16 materials, ZN-18, , ZN-22, , ZN-3, , ZN-25, , ZN-5, , ZN-11, , ZN-13, , ZN-30, , ZN-24, , ZN-26, , ZN-4, , ZN-28, , ZN-9, , ZN-17, , ZN-14 and ZN-21, , which were identified as medium resistant materials. The third group had 11 materials, ZN-7, ZN-10, ZN-15, ZN-19, ZN-20, ZN-29, ZN-16, ZN-6, ZN-23, ZN-2

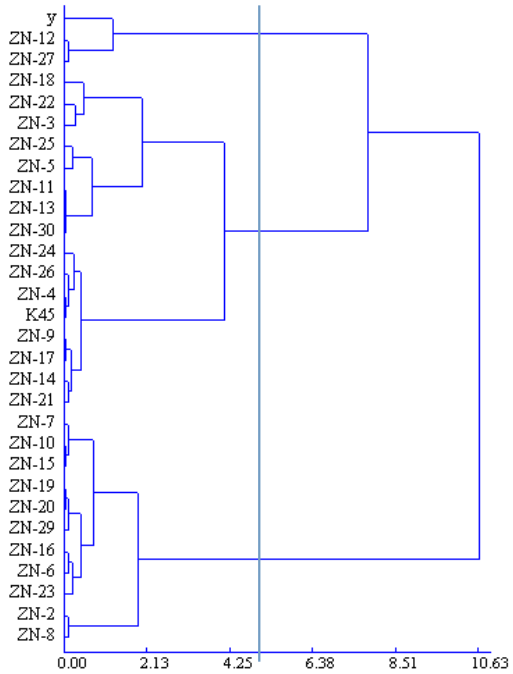


Fig 5. Resistance clustering analysis of 30 *Lilium* Oriental materials to bulb rot.

and ZN-8, which were defined as susceptible materials.

Therefore, most of the tested materials are medium resistant and susceptible materials. It is consistent with previous report that the resistance of *Lilium oriental* materials is not high[2].

Determination of total saponin content

The saponin quality was used as X-coordinate and the absorbance was used as Y-coordinate. The achieved standard curve was "$y=0.0018x + 0.0516, R^2=0.9962$".

Based on the results of resistance identification, several materials with different resistance levels were selected to determine total saponin content in the normal seedling bulbs. The results suggested that the highly resistant material ZN-12 also showed high saponin content, about 4.6907mg/g (dry weight ratio). In contrast, ZN-8, which had weak resistance ability, had low saponin content, only about 2.1925mg/g(Tab. 5). The authors further studied the relevance between the resistance index and saponin content, and the results indicated that the correlation coefficient was up to 0.819 which was significant ($p<0.01$)(Table 6).

Table 5. Determination of total saponin content in different resistant materials

Material number	Resistance index %	Saponin content/(mg/g)
ZN-3	53.33	4.2826
ZN-7	38.67	3.4195
ZN-8	28.67	2.1925
ZN-12	66.00	4.6907
ZN-13	46.67	4.2304
ZM-14	41.33	3.3585
ZN-16	35.33	3.6199
ZN-21	40.67	2.8574
ZN-22	56.00	3.6313
ZN-24	44.67	3.7456
ZN-26	43.33	3.4840

Table 6. Correlation analysis between the resistance index and saponin content

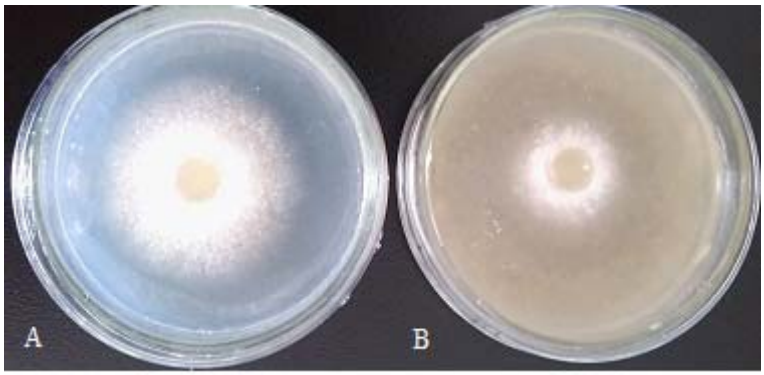
		Saponin content	Resistance index
Saponin content	Pearson correlation	1	0.819 **
	Significance (2-tailed)		0.002
	N	11	11

Note: Correlation is significant at the 0.01 level (2-tailed).

Based on research findings and the literature, is is concluded that the total saponin content can indeed represent the resistance level of materials in a certain level. It may provide a quick way for large-scale screening of high resistant materials[17,18].

The inhibition effect of saponin extract on the pathogen

After incubation for three days, the treatment group had a 29.39% inhibition rate for pathogen's mycelial growth. The average colony diameter of the treatment and control groups was 30.89mm and 40.25, respectively. The diameter of the original fungus colony was 8.40mm(Fig.6). It implicates that the saponin extract inhibits the growth of the pathogen. It may be also possible that the resistance of a material to a pathogen reflects the inhibition effect of its saponin on the pathogen and the saponin content directly influences its resistance ability.



A) Control group B) Treatment group

Fig. 6. The inhibition effect of saponin extract on pathogen's mycelial growth

DISCUSSION

Lily bulb rot is a common disease for lily cultivation. It is very harmful and usually causes huge economic losses on the production of cultivation[19].

Fusarium oxysporum is the pathogen resulting in *Lilium oriental* bulb rot, which is accordant with current reports. However, based on the growth features of lilies and microbial disease characteristics, it is believed that it is very likely that Fusarium oxysporum is not the only pathogen for the disease. The occurrence of bulb rot may be caused by microbial pathogens together with nematodes and mites[12, 20]. In this case, further study on pathogens and disease mechanisms is necessary to understand the detailed pathologic process of bulb rot.

Various species or materials have different disease resistance[21]. It is impacted by the growth period, climate, environment and other factors. Hence, resistance identification cannot be done once and for all. On the contrary, it needs to be performed continuously along with the growth of materials[22]. Using the relative resistance indexes[23, 24] as indicators may be more suitable for analysis and comparison of different materials at different stages. Thus, only those species that show strong resistance to pathogens during the whole growth cycle and even the storage and transport periods are the true resistant varieties. It is particularly important in the production and cultivation practice.

The infection and resistance of plants is a complex process. The internal mechanisms are a subtle system. The internal defense enzyme system, antibacterial metabolites, special morphology and the relationship with pathogens may be all related to disease resistance. The differentiated types, pathogenicity and toxins of pathogens and the collaboration with other soil organisms may be associated with pathogen virulence. Additionally, the environment factors such as temperature, humidity and illumination and the cultivation conditions such as soil, water and fertilizer and management are critical elements for the pathogenesis and disease resistance of plants[9, 12].

Therefore, the study on plant pathogenesis and resistance mechanisms needs to be done from various aspects. The present research on saponin is just one of them. Further investigation should be performed in the future.

ACKNOWLEDGMENTS

This study was jointly supported by grants from the National Natural Science Foundation of China (No. 31260314 and 31160176), Natural Science Foundation Project of Jiangxi Province(No. 2010GZN0038). It was also supported by the Key Project of Jiangxi Education Department (No. GJJ11018), the Personnel Foundation Project of Jiangxi Agricultural University(No. 3136).

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