doi:10.13926/j.cnki.apps.000356

# 地被菊品种对镰孢菌侵染的响应差异及抗性综合评定

张 红[1],牛凯丽[1],聂 梅[1],王 晨[1],牛颜冰[1],吕晋慧[1*],张春来[1],孙文献[2]
([1]山西农业大学,太谷 030801;[2]中国农业大学植保学院,北京 100193)

**摘要**:地被菊枯萎病由镰孢菌引起,是一种土传性病害,严重威胁地被菊生产。本试验用 2 个强致病力镰孢菌菌株,即尖孢镰孢菌(*Fusarium oxysporum*)、茄镰孢菌(*F. solani*)接种 5 个地被菊品种的幼苗,测定了病菌侵染下不同品种的形态、生理和生化响应的差异,以建立抗病品种筛选的生理和生化指标。地被菊品种间抗性水平可根据病情指数划分;受病菌侵染后,叶片叶绿素含量下降,抗病品种'俏粉阁'叶绿素含量相对较高;丙二醛含量增加,抗病品种'乳荷'和'俏粉阁'的丙二醛含量显著低于感病品种'玲珑';在病菌侵染早期,脯氨酸含量、超氧化物歧化酶和过氧化物酶活性在抗病品种受到迅速诱导增加,随后下降。运用隶属函数对其抗病能力进行综合评定,地被菊品种对尖孢镰孢菌(*F. oxysporum*)抗病性从强到弱依次为:'俏粉阁'>'乳荷'>'火焰'>'鲜红'>'玲珑',地被菊品种对茄镰孢菌(*F. solani*)抗病性从强到弱依次为:'俏粉阁'>'乳荷'>'鲜红'>'火焰'>'玲珑',为地被菊生产和开展菊花抗病机理研究提供了依据。

**关键词**:地被菊;枯萎病;抗病性;生理响应;镰孢菌

## Differential responses of *Chrysanthemum morifolium* varieties to *Fusarium* infection and synthetic evaluation of resistance

ZHANG Hong[1], NIU Kai-li[1], NIE Mei[1], Wang Chen[1], NIU Yan-bing[1], LÜ Jin-hui[1*], ZHANG Chun-lai[1], SUN Wen-xian[2] ([1]Shanxi Agricultural University, Taigu 030801, China;[2]College of Plant Protection,China Agricultural University,Beijing100193,China)

**Abstract**: Soilborne disease Fusarium wilt, caused by *Fusarium oxysporum* and *F. solani* threatens chrysanthemum production seriously. However, there was no report on the difference of physiological and biochemical responses between the resistant and susceptible varieties. To establish the physiological and biochemical indexes for the selection of resistant varieties, two *Fusarium* strains with strong pathogenicity were inoculated into the seedlings of five *Chrysanthemum morifolium* varieties, and the morphological, physiological and biochemical responses of different varieties infected with the pathogen were measured in this study.

The results showed that the disease index of *C.morifolium* was negaticely correlated with disease resistance after infection . With the development of the disease, the content of leaf chlorophyll (Chl) content increased slightly and then decreased, and the content of Chl in resistant variety 'Qiaofenge' was higher than the other varieties tested. The content of malondialdehyde (MDA) increased after infection. The MDA content was significantly higher in susceptible variety 'Linglong' than those in resistant varieties, and it was negatively correlated with disease resistance of varieties. The proline content increased rapidly in resistant varieties, showing that the higher the content of peoline, the stronger the disease resistance of varieties, The content of soluble protein (SPC) increased firstly and then decreased during infection and it was higher in resistant varieties than that in susceptible ones. The activities of leaf superoxide dismutase (SOD) and peroxidase (POD) increased first and

**收稿日期**: 2020-01-15; **修回日期**: 2020-05-27; **网络首发时间**: 2020-05-29
**网络首发地址**: http://kns.cnki.net/kcms/detail/11.2184.q.20200528.1328.001.html
**基金项目**: 现代农业产业技术体系建设专项资金资助项目(CARS-21);重要花卉种质资源发掘与创新利用(2013BAD01B07);山西省百人计划合作共建项目(晋组 2017-30)
**通讯作者**: 吕晋慧,教授,主要从事植物遗传育种研究;E-mail:lvjinhui11@126.Com,chunlaiz@hotmail.com
**第一作者**: 张 红,硕士研究生,研究方向为园林植物与观赏园艺;E-mail:820413424@qq.com。

then decreased following infection. SOD and POD activities in resistant varieties increased rapidly and they were higher than susceptible ones.In summary, through evaluation of resistance and measurement of various physiological indicators and disease index after inoculation, the resistance of five Ground cover chrysanthemum varieties to *F. oxysporum* from high to low was 'Qiaofenge' > 'Ruhe'> 'Huoyan '> 'Xianhong' > 'Lingnong', while the disease resistance to *F. solani* from high to low was 'Qiaofenge'> 'Ruhe' > 'Xianhong'> ' Huoyan' > 'Lingnong'. This provides basis for deployment of chrysanthemum cultivars in production and molecular breeding of disease resistant .

**Key words**: *Chrysanthemum morifolium*; Fusarium wilts; resistance to disease; response to infection; *Fuarium*

**中图分类号**: S682.11　　**文献标识码**: A　　**文章编号**: 0412-0914(2021)01-0059-11

枯萎病是一种分布范围广、菌种类型多、防治困难、严重影响作物产量和品质的世界性土传真菌性病害,镰孢菌是引起枯萎病的主要致病菌[1,2],其由土壤传入根系,损坏根系膜系统,致使根系代谢紊乱,导致植物根系养分吸收受阻,最终根系活力下降直至死亡[3],且在植物全生育期内均会发生,给农业生产造成严重的经济损失[4]。近年来,致病性镰孢菌引起的枯萎病受到了国内外学者的广泛关注,报道中被侵染的寄主植物包括水果、蔬菜、粮食等作物[1,5],研究主要集中在对病原菌鉴定、毒素检测及防治等方面[2,6~10]。Yan 等[9]发现尖孢镰孢菌、茄病镰孢菌、木贼镰孢菌是引起白菜枯萎病的主要致病菌,且病菌同源性高达99%~100%。利用非致病性、弱致病性镰孢菌对植株产生的交叉保护作用,诱导植株产生防御措施从而降低发病率[11]。

生产上利用抗病品种是防治枯萎病的有效方法。Zvirin 等[12]发现病原菌对甜瓜感病品种的侵染速度明显快于抗病品种。病害胁迫严重影响植物的生理生化,生理抗性指标在一定程度上反映了植物的抗性[13],而对任何单项指标的研究不能有效、准确地评价植物抗性[14,15]。

地被菊(*Chrysanthemum morifolium*)为菊科菊属多年生经济、观赏植物[16,17],其花型、花色等具有较高的观赏价值,是主要的观赏性花卉,推广应用前景广泛[18]。目前,非生物胁迫、病害等严重危害菊花生长,造成产量与品质下降。其中枯萎病严重降低了菊花的观赏价值,甚至造成品种丢失。利用 DGGE 分析、定量 PCR 和 ITS 序列分析发现镰孢菌是菊花枯萎病的主要致病菌[19~22],切花菊品种 Yellow Delaware 较 White Iceberg 感病[23]。近年来,对菊花枯萎病的研究多集中在病原菌鉴定及防治方面,但关于不同菊花品种抗性评价及生理响应方面的研究鲜有报道。因此本实验通过两种镰孢病菌,尖孢镰孢菌(*Fusarium oxysporum*)和茄镰孢菌(*F. solani*)侵染 5 个品种地被菊幼苗,并对其生理生化指标及抗性进行系统分析,综合评价各地被菊品种抗枯萎病的强弱,为地被菊栽培、选育和推广提供理论依据。

## 1　材料与方法

### 1.1　实验材料

供试地被菊品种分别为'俏粉阁'、'乳荷'、'火焰'、'鲜红'和'玲珑',选取生长状态一致的插穗,扦插于温室,待其生根后移栽至一次性花盆中(下口径 7 cm,上口径 8 cm,高 13 cm,底部打孔),采用沙土、营养土、蛭石配比为 3∶2∶1 的基质。培养 15 d,生根后即为供试地被菊幼苗。

供试菌种为尖孢镰孢菌(*F. oxysporum*)和茄镰孢菌(*F. solani*),由南京农业大学菊花研究所鉴定为菊花枯萎病的主要致病菌[21]。将病原菌菌饼接种到 PDA 平板上,25℃活化培养 7 d 后,然后切取 3~5 个直径约为 0.7 cm 的菌饼,接种到 100 mL 的 PDB 培养液中,25℃、170 $r\cdot min^{-1}$恒温摇床培养 7 d,血球计数板测定孢子浓度达到 $2.5\times10^7$ 个$\cdot mL^{-1}$。

参考甘蓝枯萎病分级标准[24],建立地被菊枯萎病分级标准。0 级:叶片无任何枯萎症状;1 级:底部 1~2 片叶褪绿发黄;2 级:叶片变黄褐色且萎蔫下垂不超过整株叶片数的 1/2;3 级:1/2~3/4 的叶片枯萎且生长受到抑制,有的植株茎杆呈红褐

色;4 级:全株严重萎蔫以致枯死。

病情指数(DI)= ∑[(各级病株数×各级代表数值)/(调查总株数×最高级代表数值)]×100

地被菊抗性水平分为高抗(HR)、抗(R)、中抗(MR)、感病(S)、高感(HS)。

抗性评价标准按病情指数划分为 HR:0<DI≤15;R:15<DI≤35;MR:35<DI≤52;S:53<DI≤73;HS:74<DI。

### 1.2　实验设计

实验于 2018 年在山西农业大学林业站温室内进行,地被菊培养时相对温度为 25℃±2℃,相对湿度保持在 40%~50%,光照为日光,光照时长 16 h。选取生长健壮且长势一致的地被菊幼苗从花盆中连根拔起,清洗干净,采用伤根浸泡法,轻剪伤根,浸入浓度 $2.5\times10^7$ 个·$mL^{-1}$ 的尖孢镰孢菌(*F. oxysporum*)和茄镰孢菌(*F. solani*)孢子悬浮液中,浸泡 30 min,每个处理 30 株,3 个重复,以无菌水为对照。将植株移栽至花盆中,进行侵染处理,于 4、8、12、16 和 20 d 取样测定不同抗性地被菊幼苗侵染的生理指标,观察发病情况、计算病情指数。

### 1.3　生理生化指标的测定

取植株中部叶片,清洗干净,进行测定,叶片叶绿素含量参考丙酮研磨法[25];可溶性蛋白质含量采用考马斯亮蓝 G-250 染色法、脯氨酸(Pro)含量采用茚三酮显色法[26]。超氧化物歧化酶(SOD)活性采用氮蓝四唑光还原法测定,过氧化物酶(POD)参考愈创木酚法测定,丙二醛(MDA)含量参考硫代巴比妥酸比色法测定[27]。

### 1.4　数据统计与分析

采用 Microsoft Excel 软件处理数据,用 SPSS23.0 软件对数据进行方差分析,差异显著性运用 Duncans 检验法进行多重比较。

## 2　结果

### 2.1　不同地被菊品种对镰孢菌的抗性分析

地被菊枯萎病苗期的病发症状表现为染病初期植株萎蔫下垂,自下部叶片开始褪绿黄化,随着病害程度加重,植株生长延缓,叶片黄化数量和面积不断向上延伸,有的茎杆呈现红褐色,病情严重时可致植株枯萎死亡。

2 种镰孢菌侵染 5 种地被菊后,病情指数随侵染时间的增加而升高,由表 1、表 2 可知,被镰孢菌侵染后,地被菊幼苗病情指数差异显著,侵染‘俏粉阁’‘乳荷’后期(第 16 和 20 d)病情指数显著低于‘鲜红’($P<0.05$)。其中‘俏粉阁’病情指数上升速率较慢,侵染‘俏粉阁’第 20 d 时,病情指数分别为 32.50 和 31.25,根据地被菊苗期抗性评价标准,其病情指数低于 35,表现为 R。镰孢菌侵染第 4~12 d,‘乳荷’病情指上升速率较快,之后发病速度变慢,侵染第 20 d 的病情指数分别为 43.75 和 37.08,病情指数在 35~52 之间,其抗性表现为 MR。镰孢菌侵染‘火焰’和‘玲珑’后随着时间的变化,病情指数快速增加,侵染第 20 d 时,‘火焰’的病情指数分别是 55.42 和 53.75,‘玲珑’的病情指数分别是 62.92 和 57.50,两个品种的病情指数在 53~75 之间,其抗性均表现为 S。镰孢菌侵染‘鲜红’第 20 d 的病情指数分别为 54.17 和 46.67,其抗性表现分别为 S 和 MR。

Table 1　Disease index of five *Chrysanthemum morifolium* varieties after inoculated with *Fusarium oxysporum*

| Varieties | Disease index | | | | |
|---|---|---|---|---|---|
| | 4 d | 8 d | 12 d | 16 d | 20 d |
| Ruhe | 9.58±0.72 bc | 18.75±3.31 cd | 31.67±4.02 b | 39.17±3.15 c | 43.75±3.31 c |
| Huoyan | 12.92±3.15 ab | 22.08±1.91 ab | 33.75±2.50 b | 45.42±3.15 b | 55.42±2.60 b |
| Xianhong | 14.58±1.44 a | 25.42±3.15 ab | 35.42±3.83 ab | 45.00±1.25 b | 54.17±2.60 b |
| Qiaofenge | 7.50±2.50 c | 15.42±2.60 a | 21.25±2.50 c | 27.50±3.31 d | 32.50±3.75 d |
| Linglong | 16.67±1.91 a | 29.17±3.82 a | 40.42±1.91 a | 51.67±1.91 a | 62.92±1.91 a |

Note: Significant differences between varieties ($P<0.05$).

**Table 2 Disease incidence index of five *Chrysanthemum morifolium* varieties after inoculated with *Fusarium solani***

| Varieties | Disease index | | | | |
|---|---|---|---|---|---|
| | 4 d | 8 d | 12 d | 16 d | 20 d |
| Ruhe | 7.50±1.25 c | 16.25±1.25 c | 24.17±1.44 b | 33.33±1.91 c | 37.08±1.91 c |
| Huoyan | 11.67±1.91 bc | 17.50±3.31 bc | 27.92±3.15 b | 42.08±3.15 ab | 53.75±3.31 a |
| Xianhong | 12.92±4.39 ab | 21.25±3.31 ab | 30.00±2.17 ab | 38.33±2.60 b | 46.67±3.15 b |
| Qiaofenge | 8.33±1.91 bc | 13.33±2.60 c | 20.42±1.91 c | 26.25±3.31 d | 31.25±1.25 d |
| Linglong | 13.33±1.44 a | 24.17±1.91 a | 36.25±2.50 a | 46.67±1.44 a | 57.50±1.25 a |

Note：Significant differences between varieties（$P<0.05$）.

### 2.2 镰孢菌侵染对地被菊叶绿素含量的影响

由图 1 可知,2 种镰孢菌侵染地被菊后,叶片叶绿素含量变化明显呈先升后降的趋势,侵染第 8 d 时,叶片叶绿素含量均达到最大值,不同品种间差异不显著($P<0.05$),侵染第 12 d 时,叶片叶绿素含量明显下降,侵染第 16 和 20 d 时,抗病品种‘俏粉阁’叶绿素含量最高,感病品种‘玲珑’叶绿素含量显著低于抗病品种‘俏粉阁’($P<0.05$),第 20 d 时不同地被菊叶片叶绿素降到最低。镰孢菌侵染第 20 d 时叶片叶绿素含量从高到低依次为‘俏粉阁’>‘乳荷’>‘鲜红’>‘火焰’>‘玲珑’。

### 2.3 镰孢菌侵染对地被菊可溶性蛋白含量的影响

2 种镰孢菌侵染地被菊后,叶片可溶性蛋白含量随着感病时间的增加呈先升高后降低的趋势(图 2)。镰孢菌侵染第 8 d 时,‘乳荷’、‘火焰’和‘俏粉阁’的可溶性蛋白含量达到最高值,‘乳荷’可溶性蛋白含量分别比对照增高了 30.41% 和 17.75%,‘火焰’可溶性蛋白含量分别比对照增高了 29.12% 和 23.57%,‘俏粉阁’可溶性蛋白含量分别比对照增加了 119.84% 和 87.75%,镰孢菌侵染‘玲珑’第 12 d 时,可溶性蛋白含量达到最大值,分别比对照提高了 13.73% 和 5.49%。镰孢菌侵染‘鲜红’后可溶性蛋白含量出现高峰值的时间不同,尖孢镰孢菌(*F. oxysporum*)侵染第 12 d 时可溶性蛋白含量最高,比对照增加了 7.9%;茄镰孢菌(*F. solani*)侵染第 8 d 时叶片可溶性蛋白含量达到最大值,比对照增高了 18.43%。

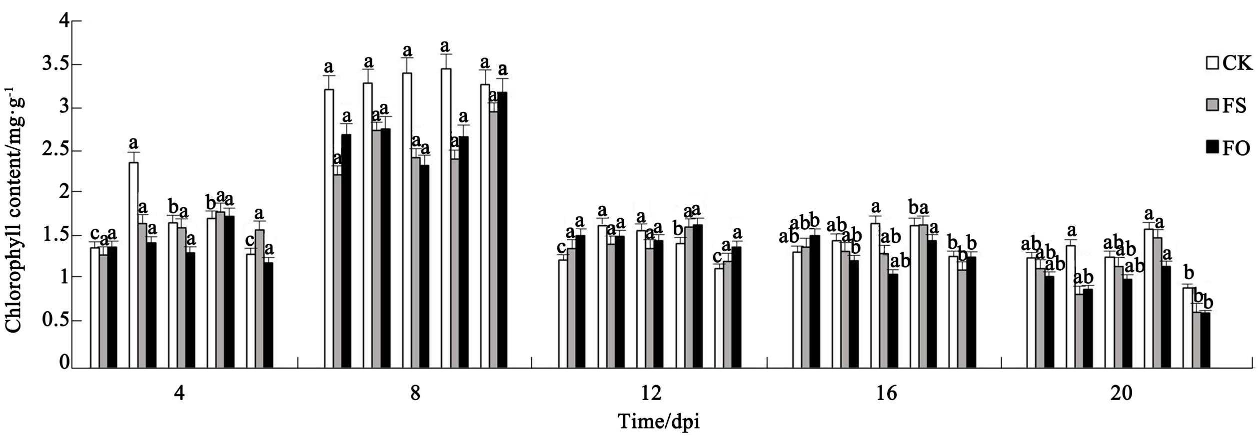


**Fig. 1 Effect of *Fusarium oxysporum*(FO) and *F. solani* (FS) infection on chlorophyll content of five *Chrysanthemum morifolium* varieties**

From left to right, the data of the same group of days in the figure are ‘Ruhe’,‘Huoyan’,‘Xianhong’,‘Qiaofenge’,‘Linglong’. Small letters indicate significant differences among varieties($P<0.5$).

### 2.4　镰孢菌侵染对地被菊脯氨酸含量的影响

2 种镰孢菌侵染后,地被菊叶片脯氨酸含量迅速积累(图 3),随侵染时间的增加脯氨酸含量先升后降。侵染第 16 d 时,叶片脯氨酸含量均达到最高值。尖孢镰孢菌(*F. oxysporum*)侵染后,‘乳荷’、‘火焰’、‘鲜红’、‘俏粉阁’和‘玲珑’分别是对照的 5.58、4.85、2.03、2.07 和 1.94 倍。茄镰孢菌(*F. solani*)侵染分别是对照的 5.73、4.34、2.25、2.05和 1.65 倍。2 种镰孢菌侵染第 20 d 时,叶片脯氨酸含量从大到小依次为:‘俏粉阁’>‘乳荷’>‘鲜红’>‘火焰’>‘玲珑’。

### 2.5　镰孢菌侵染后地被菊叶片超氧化物歧化酶、过氧化物酶活性的影响

由图 4 可知,2 种镰孢菌侵染 5 种地被菊后,叶片超氧化物歧化酶活性呈先升高后降低的趋势,镰孢菌侵染‘乳荷’和‘俏粉阁’后超氧化物歧化酶活性显著增加($P<0.05$),‘玲珑’超氧化物歧化酶活性最低,显著低于其他品种($P<0.05$)。因品种不同超氧化物歧化酶活性出现高峰的时间不同,尖孢镰孢菌(*F. oxysporum*)侵染‘乳荷’和‘俏粉阁’第 8 d 时超氧化物歧化酶活性最强,分别是对照的 1.56 和1.71倍;第16 d时‘火焰’和‘鲜红’的超氧化物

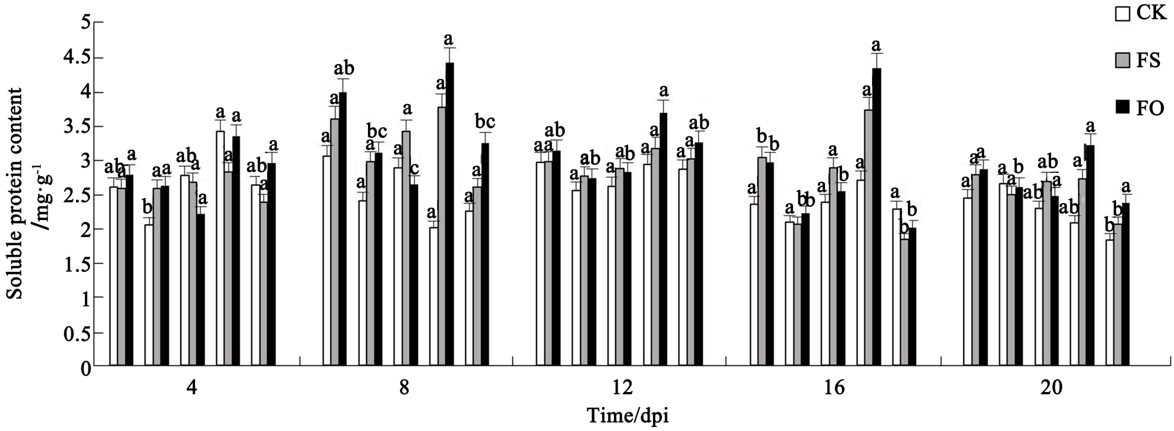


Fig. 2　Effect of *Fusarium oxysporum*(FO) and *F. solani* (FS) infection on soluble protein content of five *Chrysanthemum morifolium*

From left to right, the data of the same group of days in the figure are ‘Ruhe’,‘Huoyan’,‘Xianhong’, ‘Qiaofenge’,‘Linglong’. Small letters indicate significant differences among varieties($P<0.5$).

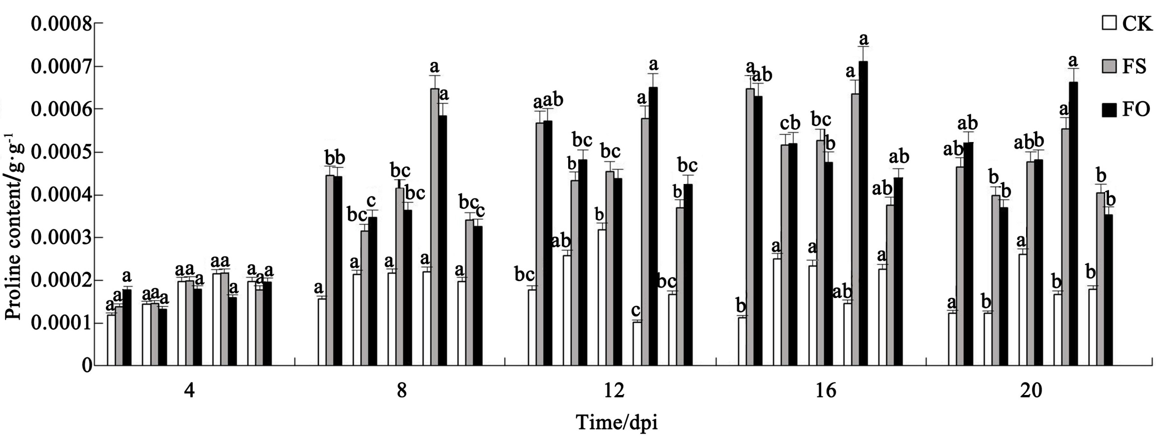


Fig. 3　Effect of *Fusarium oxysporum*(FO) and *F. solani* (FS) infection on proline content of five *Chrysanthemum morifolium*

From left to right, the data of the same group of days in the figure are ‘Ruhe’,‘Huoyan’,‘Xianhong’, ‘Qiaofenge’,‘Linglong’. Small letters indicate significant differences among varieties($P<0.5$).

歧化酶活性达到最高值,分别是对照的 1.52 和 1.56 倍;尖孢镰孢菌(*F. oxysporum*)侵染‘玲珑’第 10 d 时超氧化物歧化酶活性最强,是对照的 1.06 倍。茄镰孢菌(*F. solani*)侵染‘乳荷’、‘鲜红’、‘俏粉阁’和‘玲珑’第 12 d 时,超氧化物歧化酶活性达到高峰,分别是对照的 1.34、1.32、1.38 和 1.22 倍;‘火焰’第 5 d 时活性最强,是对照的 1.6 倍。2 种镰孢菌侵染地被菊第 20 时,超氧化物歧化酶活性从高到低依次为‘乳荷’>‘俏粉阁’>‘鲜红’>‘火焰’>‘玲珑’。

由图 5 可知,2 种镰孢菌侵染地被菊后,叶片过氧化物酶活性随感病时间增加先升后降,侵染第 16 d 时地被菊过氧化物酶活性达到最高峰,其中‘乳荷’和‘俏粉阁’过氧化物酶活性显著高于其他品种($P<0.05$);尖孢镰孢菌(*F. oxysporum*)侵染‘俏粉阁’‘乳荷’‘火焰’‘鲜红’‘玲珑’过氧化物酶活性分别是对照的 1.91、1.52、1.52、1.6 和 1.55 倍,茄镰孢菌(*F. solani*)侵染后分别是对照的 1.71、1.62、1.48、1.59 和 1.3 倍。2 种镰孢菌侵染地被菊第 20 d 时,过氧化物酶活性从高到低依次为‘乳荷’>‘俏粉阁’>‘鲜红’>‘火焰’>‘玲珑’。

## 2.6　镰孢菌对地被菊丙二醛含量的影响

由图 6 可知,地被菊丙二醛含量随 2 种镰孢菌侵染时间的增加先升高后降低再升高。尖孢镰

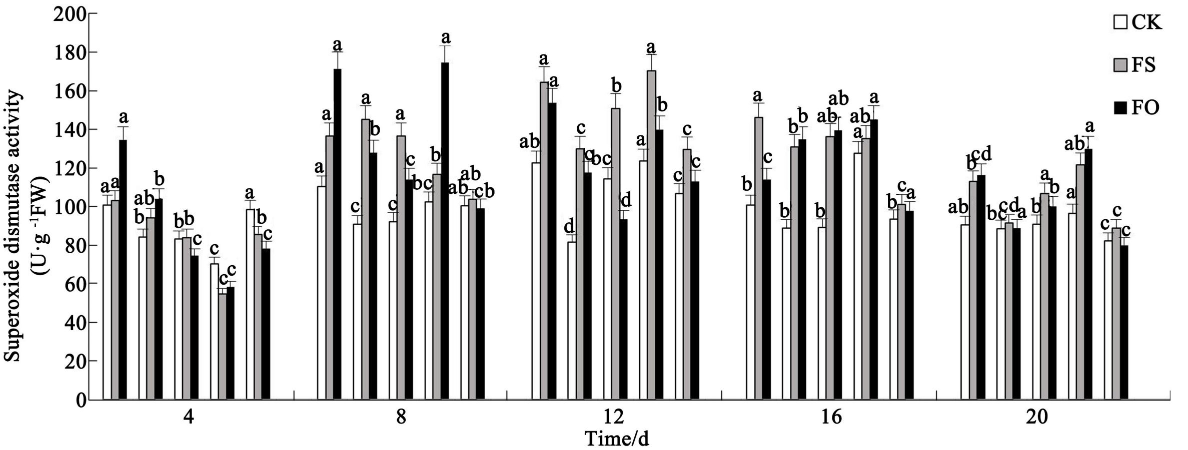


Fig. 4　Effect of *Fusarium oxysporum*(FO) and *F. solani*(FS) infection on superoxide gasifying enzyme activity of five *Chrysanthemum morifolium* varieties

From left to right, the data of the same group of days in the figure are ‘Ruhe’, ‘Huoyan’, ‘Xianhong’, ‘Qiaofenge’, ‘Linglong’. Small letters indicate significant differences among varieties($P<0.5$).

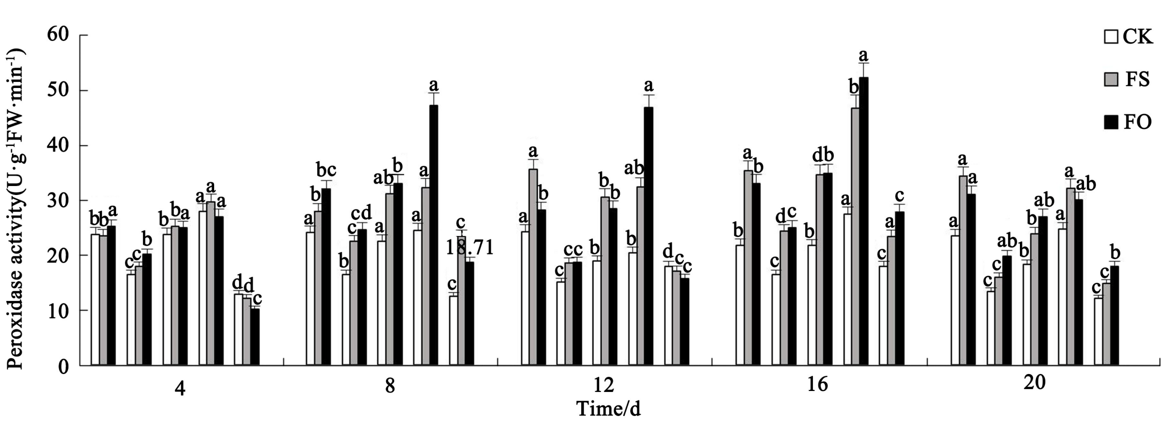


Fig. 5　Effect of *Fusarium oxysporum*(FO) and *F. solani*(FS) infection on peroxidase activity of five *Chrysanthemum morifolium* varieties

From left to right, the data of the same group of days in the figure are ‘Ruhe’, ‘Huoyan’, ‘Xianhong’, ‘Qiaofenge’, ‘Linglong’. Small letters indicate significant differences among varieties($P<0.5$).

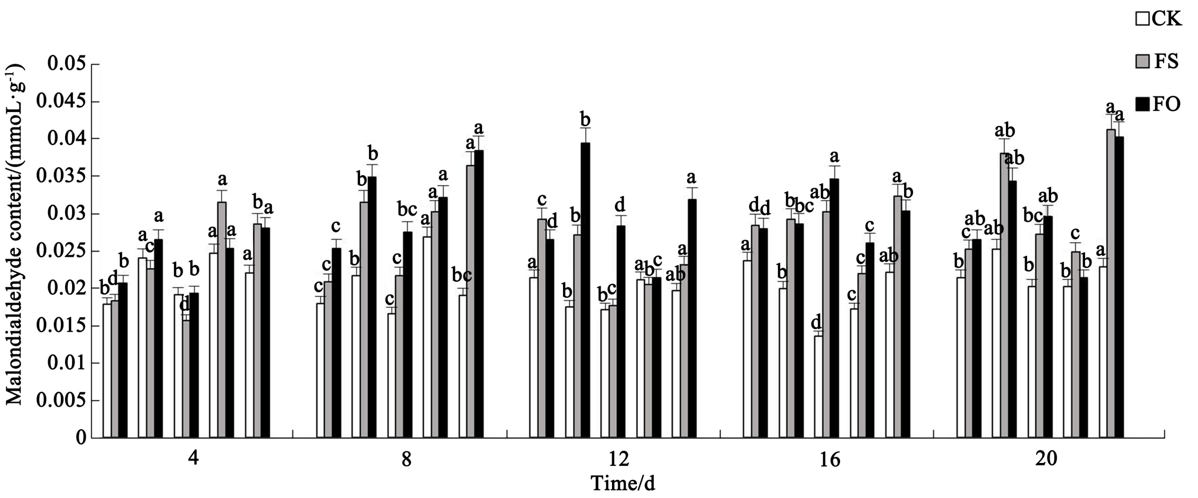


Fig. 6 Effect of *Fusarium oxysporum*(FO) and *F. solani*(FS) infection on malondialdehyde content of five *Chrysanthemum morifolium* varieties

From left to right,the data of the same group of days in the figure are'Ruhe','Huoyan','Xianhong','Qiaofenge','Linglong'. Small letters indicate significant differences among varieties($P<0.5$).

孢菌(*F. oxysporum*)侵染'火焰'、'鲜红'和'玲珑'第20 d时,叶片丙二醛含量显著高于'俏粉阁'($P<0.05$),分别比对照提高了36.04%、46.65%和76.27%;尖孢镰孢菌(*F. oxysporum*)侵染'乳荷'和'俏粉阁'第20 d时,分别比对照增高了23.57%和5.77%;茄镰孢菌(*F. solani*)侵染第20 d时,'火焰'和'玲珑'的含量分别比对照升高了50.83%和80.65%,显著高于'乳荷'和'俏粉阁'($P<0.05$),'乳荷'、'鲜红'和'俏粉阁'丙二醛含量较低,分别比对照增加了17.65%、34.64%和22.86%。2种镰孢菌侵染第20 d后丙二醛含量从高到低依次为'玲珑'>'火焰'>'鲜红'>'乳荷'>'俏粉阁'。

## 3 讨论

多项研究表明,采用尖孢镰孢菌(*F. oxysporum*)侵染或其产生的毒素处理植物,首先会诱导植物活性氧迸发,诱导超氧化物歧化酶、过氧化物酶、PAL、蛋白酶、DNAase、崩溃酶caspase-3等活性变化,导致细胞死亡,形成过敏反应[6,28]。从形态、生理和生化指标,研究枯萎病对地被菊的致病性,对培育抗病品种有重大意义。

### 3.1 镰孢菌对地被菊丙二醛含量的影响

植物在衰老或逆境条件下膜脂发生过氧化作用,最终产物为丙二醛,其含量的高低反映了膜脂过氧化和质膜受伤害的程度[29]。当植物受到病原菌侵染时,活性氧迸发导致膜透性增大,细胞内物质外渗,电导率增加,最终导致植物死亡[30],本研究中镰孢菌侵染地被菊第20 d时,感病品种'玲珑''火焰'和'鲜红'叶片丙二醛含量较高,'玲珑'丙二醛含量最高,抗病和中抗品种'俏粉阁'和'乳荷'叶片丙二醛含量显著低于感病品种,表明丙二醛的含量越高,地被菊对镰孢菌抗性越差。很多学者对感染枯萎病的黄瓜[31]、棉花[32]、香蕉[33]等作物进行了研究,发现感病品种丙二醛含量较高,与品种的抗病性呈负相关。

### 3.2 镰孢菌对地被菊叶片酶活性和脯氨酸的影响

逆境胁迫下植物细胞内会产生活性氧(ROS),而ROS过多不能及时清除会引起植物死亡[34]。次生代谢活性增强,合成类黄酮、木质素、肼胝质等防御物质,POD、PAL等是代谢关键酶[6]。抗氧化保护酶(如SOD、POD等)组成了高效专一的ROS清除系统,植物机体内保护系统的重要组成部分,植物正常生长时,在保护酶作用下活性氧处于低水平动态平衡中,利于植物生长[35],抗氧化酶活性变化是植物适应和抵御逆境胁迫的有效途径之一[36]。本研究中侵染地被菊后,超氧化物歧化酶(SOD)、过氧化物酶(POD)活性随着侵染时间的增加先升后降。根据Ma等的时间差

理论[37],植物存在一个耐受胁迫极限,镰孢菌侵染初期地被菊 SOD、POD 酶活性升高,但随着病情加重达到了胁迫极限后,酶活性开始下降。病害胁迫第 20 d 时,抗病品种'俏粉阁'和'乳荷'酶活性显著高于感病品种'火焰'和'玲珑',研究发现,外源 BABA[38]、嘧啶衍生物 BDO-1[39]的诱导能增加 SOD、POD 等酶活性,提高植物对枯萎病的抗性,说明病菌侵染后,地被菊通过增强酶活性来缓解病害胁迫,酶活性越高,品种抗性越强。Duan 等[33]、Zhao 等[40]、Zhang 等[41]、Yang 等[42]认为植物感染枯萎病后抗氧化酶(POD、SOD)活性增强,与其抗病性呈正相关,与本试验结果一致。

脯氨酸(Pro)是植物在逆境胁迫中维持体内水分平衡的渗透物质,通过渗透调节缓解细胞组织受到伤害[43]。Liang 等[44]认为枯萎病抗性强的辣椒品种 Pro 含量较高,本研究中病菌侵染地被菊后抗病和中抗品种'俏粉阁''乳荷'Pro 含量迅速累积,感病品种'火焰'和'鲜红'Pro 含量相对较低且上升速率平缓。表明抗性较强的地被菊品种通过提高 Pro 含量对细胞渗透势进行调节,以提高其对枯萎病的抗性。

### 3.3 镰孢菌对地被菊叶绿素、可溶性蛋白含量的影响

镰孢菌侵染地被菊后,植株叶片褪色变黄且萎蔫下垂,叶绿素含量总体呈先升高后降低趋势,这是由于镰孢菌侵入地被菊根部,破坏其疏水基和维管系统,从而激起地被菊体内的防御机制,产生相应的生理生化反应[45],其抗病品种'俏粉阁'叶片叶绿素含量显著高于感病品种'玲珑',且'俏粉阁'叶绿素含量下降速度相对较慢,与 Bao 等[46]研究西瓜枯萎病根系叶绿素含量变化结果一致。本研究中侵染初期地被菊叶片叶绿素含量升高,植株初期遭受病害胁迫,通过加速叶绿素合成来抵御病害的应激反应,但随着病情的加重,病菌扩散至整株,叶绿素合成受到抑制,含量降低。侵染第 20 d 时,抗病品种'俏粉阁'叶片叶绿素含量显著高于感病品种'玲珑',表明地被菊通过提高叶绿素含量抵抗病害胁迫,可以作为菊花枯萎病的抗性评价生理指标之一。

可溶性蛋白质是植物生命活动的重要物质,参与植物体内各种酶的代谢[47,48],Han[49]、Liu[50]认为对枯萎病抗性较强的植物品种可溶性蛋白质含量高于感病品种。本研究中,镰孢菌侵染地被菊后可溶性蛋白质含量先升后降,研究表明,植物受到病原菌侵染初期通过提高蛋白质含量启动自身的防卫机制抵御病菌侵染[50,51],随着侵染时间的增加,地被菊可溶性蛋白质含量降低,这是由于病菌自身合成的可溶性蛋白质增加,使寄主体内可溶性蛋白质降解,因此寄主可溶性蛋白质含量降低[52];而侵染 20 d 后抗病较强的品种'俏粉阁'和'乳荷'可溶性蛋白质含量显著高于感病品种'玲珑',说明地被菊可溶性蛋白质含量与其抗性呈正相关。

### 3.4 地被菊品种镰孢菌抗病性评价

综上所述,病原菌侵染 5 个品种地被菊后产生的生理生化反应不同,本研究结合隶属函数对病菌侵染地被菊后不同生理指标进行了综合评价,5 种地被菊对尖孢镰孢菌(*F. oxysporum*)的抗性从强到弱依次为'俏粉阁'>'乳荷'>'火焰'>'鲜红'>'玲珑',对茄病镰孢菌(*F. solani*)的抗病性从强到弱依次为'俏粉阁'>'乳荷'>'鲜红'>'火焰'>'玲珑'。因此,'俏粉阁'和'乳荷'对枯萎病致病性镰孢菌,即尖孢镰孢菌(*F. oxysporum*)和茄镰孢菌(*F. solani*)的抗性较强,是抗病品种,而玲珑'是感病品种。枯萎病在菊花连作条件下加重[21],生产上选用抗病品种是预防连作障碍的首选措施,可以利用高抗枯萎病的'俏粉阁'和'乳荷'做进一步的田间试验。生产上也需要不断培育更多的抗病品种,通过抗感品种的比较组学研究,开发抗病基因及其分子标记,可用分子育种快速改良地被菊。

总之,镰孢菌侵染或其产生的毒素还会诱导过敏反应,影响 PAL、蛋白酶、DNAase、崩溃酶 3(caspase-3)等活性,导致细胞死亡[6,28]。研究植物抗病性机制表明,茉莉酸(JA)和乙烯途径在抗镰孢菌等腐生性病菌上起到重要作用[53]。本研究检测到活性氧代谢产物丙二醛和关键酶超氧化物歧化酶活性以及次生代谢酶过氧化物酶的活性在抗、感枯萎病地被菊品种间存在较大的差异,可以推断相应的基因表达水平发生改变。期望用基因组学、转录组学、蛋白质组学等技术,系统研究抗枯萎病地被菊品种'俏粉阁'和'乳荷'对尖孢镰孢菌(*F. oxysporum*)的抗性机制,有利于快速、高效和准确地选育抗枯萎病品种。

**致谢**:供试尖孢镰孢菌(*F. oxysporum*)和茄病镰孢菌(*F. solani*)由南京农业大学菊花研究所陈发棣教授和宋爱萍副教授提供,特此感谢。

## 参考文献


[1] Dugan F M , Hellier B C , Lupien S L. First report of *Fusarium proliferatum* causing rot of garlic bulbs in North America[J]. Plant Pathology, 2003, 52(3): 426-426.

[2] Cao Y F, Shen Z Z, Liu S S, *et al*. Evaluation of effect of *Trichoderma* controlling Fusarium wilt disease and its influencing factors with meta-analysis in China (in Chinese)[J].Acta Pedologica Sinica(土壤学报), 2019, 56(3):716-727.

[3] Brian C K,Katrina D W,Nicholas V N,*et al*. Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi[J]. Biotechnology for Biofuels, 2011, 4(1): 4-28.

[4] Liu X Y, Li F, Chen H R, *et al*. Advances in biocontrol of pathogenic *Fusarium oxysporum* (in Chinese) [J]. Journal of Yunnan University (Natural Sciences Edition) [云南大学学报(自然科学版)], 2008, (S1):89-93.

[5] Ma L J, Geiser D M, Proctor R H, *et al*. *Fusarium* pathogenomics[J]. Annual Review of Microbiology, 2013, 67:399-416.

[6] Hano C, Addi M, Fliniaux O, *et al*. Molecular characterization of cell death induced by a compatible interaction between *Fusarium oxysporum* f. sp. *linii* and flax (*Linum usitatissimum*) cells[J]. Plant Physiology and Biochemistry, 2008, 46(5-6):590-600.

[7] Cox K L, Babilonia K, Wheeler T, *et al*. Return of old foes-recurrence of bacterial blight and *Fusarium* wilt of cotton[J]. Current Opinion in Plant Biology, 2019, 50(1): 95-103.

[8] Mohammed B L, Hussein R A, Toama F N. Biological control of Fusarium wilt in tomato by endophytic rhizobactria[J]. Energy Procedia,2019,157:171-179.

[9] Yan W X, Shi Y X, Li P L, *et al*. Preliminary study on Fusarium species causing Chinese cabbage wilt (in Chinese) [J]. Acta Phytopathologica Sinica (植物病理学报), 2018, 48(5):587-593.

[10] Shi Y R, Xu W H, Lu Z H, *et al*. Inhibiting effects of *Bacillus amyloliquefaciens* LZN01 on *Fusarium oxysporum* f. sp. Niveum (in Chinese) [J].Jiangsu Agricultural Sciences (江苏农业科学), 2019, 47(12): 141-145.

[11] Xu Y G, He Z, Li R, *et al*. Research progress of cucumber Fusarium wilt (in Chinese) [J]. China Cucurbits and Vegetables (中国瓜菜) ,2018,31(6):1-6.

[12] Zvirin T, Herman R, Brotman Y, *et al*. Differential colonization and defence responses of resistant and susceptible melon lines infected by *Fusarium oxysporum* race 1·2 [J]. Plant Pathology, 2010,59(3):576-585.

[13] Sun J, Zeng J, Wang Y J, *et al*. Evaluation and screening of drought stress tolerance in 20 cut *Chrysanthemum* varieties (in Chinese) [J].Journal of Nanjing Agricultural University (南京农业大学学报), 2013, 36(1):24-28.

[14] Zhang Z M, Wan S B, Dai L X, *et al*. Estimating and screening of drought resistance indexes of peanut (in Chinese) [J].Chinese Journal of Plant Ecology (植物生态学报), 2011,35(1):100-109.

[15] Zhang L, Zhang Q X, Gao Y K, *et al*. Study on physiological traits of drought resistance for transgenic *Chrysanthemum* varieties Wanfen (in Chinese) [J]. Acta Agriculturae Boreali-Sinica (华北农学报), 2011,26(5):181-185.

[16] Chen Y L. Economic value of *Chrysanthemum*. Borderland economy and culture (in Chinese) [J]. The Border Economy and Culture (边疆经济与文化), 2007(7):32-33.

[17] Lei Y. Study on shade tolerance of ground-cover *Chrysanthemum* (in Chinese) [D]. Beijing: Beijing Forestry University (北京:北京林业大学),2015.

[18] Xu Z R, Ma J, Hou J, *et al* . Comparison on the regeneration system and genetic transformation condition in 4 ground -cover *Chrysanthemum* cultivars (in Chinese) [J].Chinese Agricultural Science Bulletin (中国农学通报) ,2014,30(7):130-137.

[19] Minuto A, Gullino LM, Garibaldi A. Susceptibility of *Gerbera* and *Chrysanthemum* varieties (*Gerbera jamesoni* and *Chrysanthemum morifolium*) to *Fusarium oxysporum* f.sp. *chrysanthemi*[J]. Communications in

Agricultural and Applied Biological Sciences, 2007,72(4):715-721.

[20] Chen H J, Zhao S, Zhang K K, *et al.* Isolation and identification of *Fusarium oxysporum* from *Chrysanthemum* and the effects of crude toxin on growth of cut *Chrysanthemum* 'Jimba' seedlings (in Chinese) [J]. Journal of Nanjing Agricultural University(南京农业大学学报), 2018,41(4):662-669.

[21] Aiping S , Shuang Z , Sisi C , *et al*. The abundance and diversity of Soil fungi in continuously mono-cropped *Chrysanthemum*[J]. Scientific World Journal, 2013:1-8.

[22] Cao J Y, Shen Tu X P, *et al.* Identification of the pathogens causing Fusarium wilt disease in *Chrysanthemum morifolium* ramat and control effect of *Streptomyces diastatochramogenes* (in Chinese) [J]. Chinese Journal of Biological Control(中国生物防治学报), 2019, 35(2):265-271.

[23] Johnson A W, Littrell R H. Effect of *Meloidogyne incognita*, *M. hapla*, and *M. javanica* on the Severity of Fusarium wilt of *Chrysanthemum*[J]. Journal of nematology, 1969, 1(2):122.

[24] LÜ H H, Fang Z Y, Yang L M, *et al*. Research on screening of resistant resources to Fusarium Wilt and inheritance of the resistant gene in cabbage (in Chinese) [J]. Acta Horticulturae Sinica (园艺学报), 2011, 38(5):875-885.

[25] Sarijeva G, Knapp M, Lichtenthaler H K. Differences in photosynthetic activity, chlorophyll and carotenoid levels and in chlorophyll fluorescence parameters in green sun and shade leaves of *Ginkgo* and *Fagus*[J]. Journal of Plant Physiology,2007,164(7):950-955.

[26] Li H S. Principle and technology of plant physiological and biochemical experiments(in Chinese). [M].Beijing: Higher Education Press (北京:高等教育出版社), 2000.

[27] Lu W J. Plant physiology experiment course (in Chinese)[M].Beijing:China Forestry Press(北京:中国林业出版社), 2012.

[28] Singh V K, Singh H B, Upadhyay R S. Role of fusaric acid in the development of 'Fusarium Wilt' symptoms in tomato: Physiological, biochemical and proteomic perspectives[J]. Plant Physiology and Biochemistry, 2017,118:320-332.

[29] Ren L, Zhao X L, Xu J L, *et al*. Varied morphological and physiological responses to drought stress among four tea *Chrysanthemum* cultivars (in Chinese) [J]. Acta Ecologica Sinica(生态学报), 2015,35(15):5131-5139.

[30] Trunova T I, Zvereva G H. Effect of protein synthesis inhibitors of winter wheat[J]. Plant Physiology, 1997, 4:39-40.

[31] Guan X C. Changes of MDA content with Fusarium *Wilt* infection in seedling stage of different cucumber varieties (in Chinese) [J]. Horticulture & Seed(园艺与种苗), 2017(5):8-9.

[32] Song F M, Ge X C, Zheng Z. The roles of active oxygen species and lipid peroxidation in the resistance of cotton seedlings to Fusarium Wilt (in Chinese) [J]. Acta Phytopathologica Sinica(植物病理学报), 2001, 31(2):110-116.

[33] Duan Y J, Zhang X Y, Huang M J. The effect of bean Pulp fermentation broth on control banana *Wilt* pathogenicity(in Chinese) [J].Chinese Agricultural Science Bulletin (中国农学通报), 2011,27(25):215-218.

[34] Lang H Y. Study on the response of chl *APX-overexpressed* populus tomenentosa to stress (in Chinese) [D]. Jinan:Shandong Normal University (济南:山东师范大学), 2014.

[35] Zhao J. Study on the physiolgial response mechanism of pear callus to pathogenic fungi infection (in Chinese) [D].Yangling:Northwest University of Agriculture and Forestry Science and Technology (杨凌:西北农林科技大学), 2012.

[36] Wen X. Study on resistance of vegetation on municipal solid (in Chinese) [D]. Beijing: Beijing Forestry University (北京:北京林业大学), 2011.

[37] Ma Z R, Liu R T. Basic principles of drought resistance physiology of herbage (in Chinese) [M]. Lanzhou: Lanzhou University Press(兰州:兰州大学出版社), 1993.

[38] Xu Q,Li H X,Xian X L, *et al*. Effects of BABA on photosynthetic characteristics and antioxidative system in rhododendron under $NaHCO_3$ stress (in Chinese)

[J].Forest Research(林业科学研究),2018,31(2):133-140.

[39] Shi Y X, Zhang X H, Xu Y F, *et al*. A pyrimidine derivative confers cucumber induced resistance to Fusarium Wilt(in Chinese)[J].Acta Horticulturae Sinica(园艺学报),2019,46(5):877-890.

[40] Zhao X J, Tang X, Cheng Ji W, *et al.* The relationship between malonaldehyde content, enzymatic changes and the resistance of bitter gourd to Fusarium Wilt (in Chinese)[J]. Journal of South China Agricultural University(华南农业大学学报), 2013,4(3):372-377.

[41] Zhang Y N, Wang X X, Li X G, *et al.* Effects of continuous cropping on physiological and biochemical resistance of cotton to Fusarium wilt(in Chinese)[J]. Acta Ecologica Sinica(生态学报), 2016,36(14):4456-4464.

[42] Yang Z A, Zhou L Y, Fu Z, *et al.* Response of three cultivated varieties of *Cucurbita ficifotia* to Fusarium Wilt(in Chinese)[J]. Chinese Journal of Tropical Crops(热带作物学报), 2017,38(1):144-149.

[43] Zhu H,Zu Y G, Wang W J, *et al.* Effect of proline on plant growth under different stress conditions (in Chinese)[J].Journal of Northeast Forestry University(东北林业大学学报),2009,37(4):86-89.

[44] Liang J G, Wang J M. Studies on the pathogen causing *Capsicum Wilt* in Shanxi (in Chinese)[J]. Journal of Shanxi Agricultural University(Natural Science Edition)[山西农业大学学报(自然科学版)], 2002, 22(1):29-31,45.

[45] Chen C Q, Bélanger R R, Benhamou N, *et al*. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (*PGPR*) and *Pythium aphanidermatum*[J]. Physiological and Molecular Plant Pathology, 2000,56(1): 13-23.

[46] Bao W X, Zhong C, Zhao W Z, *et al.* Comprehensive resistance evaluation of grafting watermelon seedlings on different bottle gourd stocks to watermel on Fusarium wilt (in Chinese)[J]. Journal of Southern Agriculture(南方农业学报), 2017,48(3):441-447.

[47] Ding D, Lu C P, Liu F, *et al.* Research progress on Lily Fusarium Wilt (in Chinese)[J].Jiangsu Agricultural Sciences(江苏农业科学), 2009,(3):128-130.

[48] Lv J H, Li Y F, Wang X, *et al.* Impact of shading on growth, development and physiological characteristics of *Trollius chinensis* Bunge (in Chinese)[J]. Scientia Agricultura Sinica(中国农业科学), 2013,46(9):1772-1780.

[49] Han S Q. Mechanism of phenolics on resisting Fusarium Wilt of banana (in Chinese) [D]. Wuhan: Huazhong Agricultural University(武汉:华中农业大学),2014.

[50] Liu F M. analysis of protein and amino acid contents in the seedlings of cotton varieties as related to their resistance to Fusarium wilt (in Chinese)[J].Journal of Southwest Agricultural (西南农业大学学报), 1997(2):161-164.

[51] Xu Z G. General plant pathology (in Chinese) [M]. Beijing:China Agricultural Press(北京:中国农业出版社), 1997.

[52] Zhang Y S. Pathological physiology of plant diseases (in Chinese)[M]. Nanjing:Jiangsu Science and Technology Publishing House(南京:江苏科学技术出版社), 1995.

[53] Zhang C L, Xu D C, Jiang X L, *et al.* Molecular breeding to enhance disease resistance in sugarbeet (in Chinese)[J].China Beet & Sugar(中国甜菜糖业), 2008(2):23-26.

责任编辑:曾晓葳