Evaluation of 20 Citrus Varieties Resistance to *Pseudofabraea citricarpa*

Quan Chen[1,2], Wenjing Zhang[1,2], Jiequn Ren[1,2], Pingwei Xiang[1,2] and Jinhui He[3]
[1]*Chongqing Three Gorges Academy of Agricultural Sciences, Wanzhou, 400401, Chongqing, China*
[2]*College of Biology and Food Engineering, Chongqing Three Gorges University, Wanzhou, 404020, Chongqing, China*
[3]*Plant Protection and Fruit Tree Technology Popularization Station in Wanzhou District of Chongqing, Wanzhou, 404155, Chongqing, China*

Keywords: Citrus Target Spot, *Pseudofabraea citricarpa*, Resistance Evaluation.

Abstract: Citrus target spot is one of the most destructive diseases on leaves, shoots and fruits of some citrus in China. In this study, to evaluate the resistance against the disease, the separated leaf inoculation method was used to inoculate *Pseudofabraea citricarpa* pathogen on abatial of citrus leaves under 10℃. Resistance of 20 citrus varieties were evaluated. The results indicated that among the 20 citrus varieties, 18 varieties were classified into highly resistant varieties according to the diameter spots. Orah 091 was moderate resistant and CRIC32-01 was resistant to *Ps. citricarpa*. The study could be used for the further study of citrus resistance genes to *Ps. Citricarpa* and has given suggestions for structure in citrus production areas.

1 INTRODUCTION

Citrus target spot is a newly emerging citrus disease caused by *Pseudofabraea citricarpa* (Yang, Fang, Yu, Bi, Zhou, 2019); (Chen, 2016). And a newly emerging leaf-spotting disease of citrus reported in the year of 2004 which was occurred in late winter and early spring in Chenggu County, Shanxi Province of China (Zhu, Wang, Huang, Zhang, Li, 2012). This fungal pathogen could infect both Satsuma mandarin (*Citrus unshiu*) and kumquat (*Fortunella margarita*) in orchards (Zhu, Wang, Huang, Zhang, Li, 2012). This disease caused considerable economic losses in Wanzhou, Chongqing City, Yichang City, Hubei Province and Jishou City, Hunan Province, China in 2021 (Xiao, Zeng, Wang, Cheng, Li, 2020). These morbidity tendency demonstrated a trend of accelerating propagation from north to south of China (Zhan, 2021). These incidence trend was the same as our previous prediction of suitable area and risk analysis for citrus target spot (Xu, Chen, 2020). Citrus target spot occurs during late winter and early spring and causes severe leaf spotting, defoliation or even fruit-dropping and tree- dead. Once the disease becomes epidemic, existing technology including chemical agents, some agricultural control and physical control are difficult to control effectively (Xiao, Zeng, Wang, Cheng, Li, 2020); (Zhu, 2012). Breeding resistant varieties is the most fundamental means of this disease control.

At present, the research on citrus target spot is still in the initial stage, mainly focusing on the identification of pathogen, identification of pathogenic factors and prediction of suitable areas (Yang, Fang, Yu, Bi, Zhou, 2019). The SCAR molecular detection provides a fast and easy method for early identification of this disease (Yang, Hu, 2018). And the research integrated transcriptomic and secretomic approaches revealed critical pathogenicity factors in *Ps. citricarpa* inciting citrus target spot (Yang, Fang, Yu, Bi, Zhou, 2019). According to the latest research, citrus target spot is at a high risk level in China, and the high and middle suitable areas are mainly concentrated in the citrus dominant area in the upper and middle reaches of Yangtze River (Xu, Chen, 2020). However, there is not a perfect and viable standardized system for the identification of resistance to citrus target spot, which leads to the slow process of screening resistant germplasm and breeding resistant varieties. Therefore, we took the average diameter of the speckle and the incidence as the evaluation standard for resistance (Chen, Liu, 2009); (Hu, Liu, 2015); (Liu, Hu, 2013); (Zhang, Ding, 2013); (Ling, Huang, 2011); (Li, Zheng, 2009) and inoculated different

Chen, Q., Zhang, W., Ren, J., Xiang, P. and He, J.
Evaluation of 20 Citrus Varieties Resistance to Pseudofabraea Citricarpa.
DOI: 10.5220/0011212900003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 397-401
ISBN: 978-989-758-595-1

citrus varieties for identifying resistance to this disease.

2 METHODS AND MATERIALS

Fungal Pathogens. *Ps. citricarpa* strains Pc-WZBY1was isolated from Eureka lemon leaves in Wanzhou District, Chongqing City. Direct tissue isolation of causal agents was performed reported by Zhu et al. (Zhu, Wang, Huang, Zhang, Li, 2012) The materials were surface-sterilized with 75% ethanol for 30 s, 1% NaOCl for 1 min, and then rinsed with sterile distilled water for 5 times. Small sections (3 × 5 mm) from the margins of diseased and healthy tissues were placed onto potato dextrose agar (PDA) in petri dishes. Small drops of suspension were placed on a glass slide and examined under a dissecting microscope at ×20 magnification. Drops that contained only a single spore were transferred to fresh PDA plates. The plates were incubated at 20°C until the mycelium covered approximately three-quarters of the plates (about 15 to 20 days). Pure single-spore cultures grew on PDA were then transferred onto fresh PDA and stored at 4°C for further study.

Citrus Varieties. *C.grandis* (Shatianyou, Hongbaoshiyou, Taiguoqingyou, Dianjiangwanyou, DianjiangBaiyou, Liangpingyou, Sanhongyou), *C.sinensis* (Tarocco Blood Orange NO.8, Tarocco Blood Orange NO.9, Newhall Navel Orange), *C.reticulata* (Shiranui, Aiyuan NO.38, Daya mandarin, Tango, Orah 091, Gold Nugget) and *C.tangerine* (Hongjv, CRJC32-01), *C.junos*(Tanaka), *C.aurantium*(Poncirus trifoliata) were collected from citrus germplasm resource nursery of Chongqing Three Gorges Academy of Agricultural Sciences.

Resistance Identification Experiment. The inoculation experiment was conducted in laboratory of Chongqing Three Gorges Academy of Agricultural Sciences in 2020-2021, following a modified method of placing the mycelial plugs on the excised leaves reported by Lin et al. (Lin, Huang, 2011).

15 separated leaves inoculated two sits on each leaf, for 30 spots in total, were inoculated with a typical strain WZBY1 in every variety. Leaves were washed, air-dried, and surface-disinfested with 75% ethanol using cotton swabs, and then put it into a sterile tray covered with sterile gauze soaked with distilled water. Subsequently, pricking ten times (but not pierced) on the lower surface with an insect needle. Two sclertiums (5 mm diameter) were placed on the abaxial of each wounded leaf. For the non-inoculated controls, leaves were put with the same size of PDA only. All treatments were cultured separately under 10 °C in a moisture box and investigating the average diameter of the speckle and incidence rate in in 28 days after inoculation. The resistance was evaluated with the method of average diameter of the speckle (Chen, Liu, 2009); (Hu, Liu, 2015); (Liu, Hu, 2013). The leaves were recorded as infected if inoculated sits displaying disease symptoms (Zhang, Ding, 2013); (Lin, Huang, 2011). Assessment of the resistance grades of citrus varieties to *Ps. Citricarpa* was according to Li et al. (Li, Zheng, 2009). Incidence was obtained using the formula: incidence (%) = (infected leaves/inoculated leaves) × 100%.

All experiments were performed in triplicate. The entire experiment was conducted triplicate.

Statistical Analysis. The database was analyzed by IBM SPSS 16.0 (New York) and and Microsoft Excel. The data are means of 30 replicates in triplicate.

3 TEST RESULTS

3.1 Incidence Rate of Different Citrus Cultivars

There were various degrees of differences on the pathogenicity to test-cultivars, and even got significant difference($P<0.05$) among some varieties. There was 8 varieties of incidence rate were under 20%, 7 varieties among 21-50%, 2 varieties among 51-80%, 3 varieties displayed 100% incidence. Newhall Navel Orange (*C.sinensis*), Orah 091 (*C.reticulata*) and CRIC32-01 (*C.tangerine*) were got 100% incidence. The disease didn't infect Shatianyou, Hongbaoshiyou, Tarocco Blood Orange No.8, Tanaka, Poncirus trifoliata. Incidence rate of this 5 citrus cultivars were all 0%. Sanhongyou, Daya mandarin and Taiguoqingyou displayed 3.33%, 6.67% and 20% for incidence. Incidence rate of Newhall Navel Orange, CRIC32-01 and Orah 091 reached 100%, and Dianjiangwanyou, DianjiangBaiyou and Liangpingyou were showed 40%-50%. The other varieties of *C. grandis* were showed the incidence under 20% (Fig. 1, Fig. 2).

3.2 Diameter of Infection Sits of Different Citrus Cultivars

Shatianyou, Hongbaoshiyou, Sanhongyou, Tarocco Blood Orange No.8, Tanaka and Poncirus trifoliata were 0mm in diameter of infection sits, and

displayed immune phenotype. Taiguoqingyou, and Daya mandarin displayed 0.1m for average lesion diameter, respectively. 6 varieties ranged from 0.2mm to 1.0mm for average diameters of lesions, and 5 varieties were 1.1mm-5.0mm (Fig. 3).

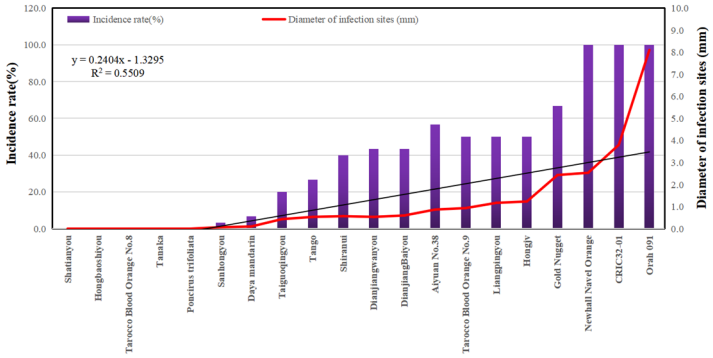
In the 20 varieties, 18 (90 percent of all test varieties) ranged from 0 to 3 mm in spots diameter which can be classified into highly resistant varieties. Orah 091 got 8.1mm for average lesion diameter of which the maximal diameter could reach 14mm which was defined as moderate resistant. CRIC32-01 gotting 3.8mm for average lesion diameter and 100% for incidence which showed resistant to *Ps. Citricarpa.* There was a particular example Newhall Navel Orange, of which the average lesion diameter got 1.8 mm but incidence was 100%, which was identified with highly resistant variety according to the diameter spots.

In *C.grandis*, except Liangpingyou, the other 6 test varieties were all classified to highly resistant. In *C.reticulata*, Orha 091 was the most susceptible variety(100% for the incidence, 8.1mm for the diameter spots). Daya mandarin was classified to highly resistant to *Ps. Citricarpa* according to the diameter spots. In *C.tangerine*, incidence and epidemic degree of CRIC32-01 was more severe, gotting second diameter(3.8mm) in the test varieties and reaching 100% for incidence. While the Hongjv displayed significantly lower incidence (50%) of *Ps. Citricarpa* (Fig. 2, Fig. 3), which was classified into highly resistant group.

The results also suggested that the incidence rate of varieties correlated linearly with lesion diameter(R^2=0.55) (Fig. 2). In all test objects, the high incidence, such as CRIC32-01 and Orah 091, existed a tendency to strong virulent and a high risk to infected by *Ps. Citricarpa* in citrus production areas in our country. In addition, for reducing the risk of disease and economic losses, late-maturing, suitable and strong resistant varieties should be planted, including increasing Tarocco Blood Orange No.8, Tango, Shiranui in upper and middle reaches of Yangtze River. In western Hubei and western Hunan, according to the current structure of citrus industry and climatic conditions, the susceptible citrus, such as Orah 091 should be appropriately reduced. Depending on the natural advantages, Aiyuan No.38, CRIC32-01 and Taiguoqingyou should be with due consideration.

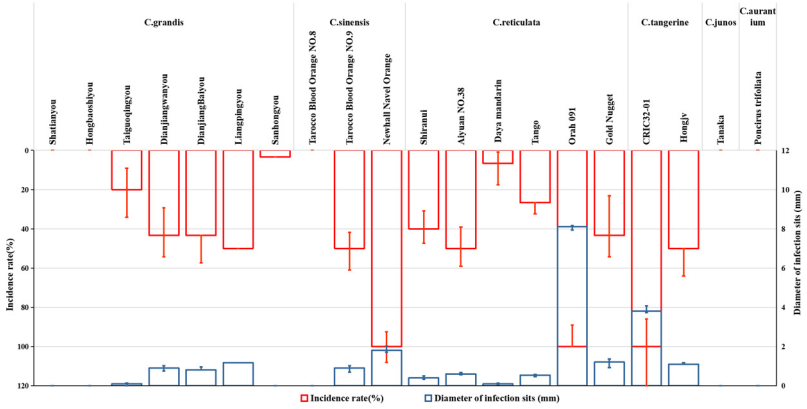
Figure 1: Symptoms of some citrus varieties after inoculation.

A, Shatianyou; B, Hongbaoshiyou; C, Tarocco Blood Orange No.8; D, Tanaka; E, Poncirus trifoliata; F, Sanhongyou; G, Daya mandarin; H, Taiguoqingyou; I, Tango; J,Shiranui; K,Dianjiangwanyou; L, DianjiangBaiyou; M, Aiyuan No.38; N, Tarocco Blood Orange No.9; O, Liangpingyou; P, Hongjv; Q, Gold Nugget; R, Newhall Navel Orange; S,CRIC32-01; T, Orah 091



Note: The data are means of 30 replicates. The experiment was conducted triplicate.

Figure 2: Correlational analyses of the incidence and diameter of infection sits different citrus varieties.



Note: Columns represented means of 30 replicates, and bars represent standard deviation. The experiment was conducted triplicate.

Figure 3: The incidence of different citrus varieties after inoculation.

4 DISCUSSIONS

Citrus target spot has become an important disease infecting such as Satsuma mandarin, kumquat as well as Eureka lemon et al. in some citrus-producing regions reported in Shanxi Province (Zhu, Wang, Huang, Zhang, Li, 2012) Chongqing City (Zhan, 2021), Hubei Province and Hunan Province (Xiao, Zeng, Wang, Cheng, Li, 2020), China, which is leading to substantial economic losses to citrus production. Unlike the most diseases, the disease infected by *Ps. Citricarpa,* prevails only in late winter and early spring (Zhu, Wang, Huang, Zhang, Li, 2012). It causes defoliation, twig dieback, fruit-dropping, and dramatically market value reduction of infected fruits, especially on susceptible citrus varieties. Furthermore, a high proportion of trees become diseased or dead, and some orchards have been destroyed.

Gene is the primary cause of resistance. According to the characteristic of conserved domain of resistance genes, sequence amplification of conserved domain is a common method for identification and discovery of resistance genes (Fenillet, 1997), which has been applied to field pepper (Zhang, Chen, 2008), soybean (Garzon, 2013), wheat (Xi, Wang, 2021) and other plants. The key to study the resistance differences among citrus germplasm, the resistance mechanism and even the interaction between citrus and *Ps. citricarpa* is to study the resistance genes of citrus. Study the resistance genes was the basis of understanding the resistance mechanism which needed to further research.

5 CONCLUSIONS

Based on the results and discussions presented above, the conclusions are obtained as below:

(1) Through the resistance evaluation of dominant citrus varieties with the established method, 18 varieties such as Shatianyou, Hongbaoshiyou, Tarocco Blood Orange No.8, Tanaka, Poncirus trifoliata, Sanhongyou, Daya mandarin, Taiguoqingyou, Tango, Shiranui, Dianjiangwanyou, DianjiangBaiyou, Aiyuan No.38, Tarocco Blood Orange No.9, Liangpingyou, Hongjv, Gold Nugget and Newhall Navel Orange were classified into highly resistant varieties according to the diameter spots. Only Orah 091 was defined as moderate resistant. And CRIC32-01 was resistant cultivars.

(2) According the results in this study, we suggest increasing Tarocco Blood Orange No.8, Tango in upper and middle reaches of Yangtze River. In western Hubei and western Hunan, Orah 091 should be appropriately reduced. Aiyuan No.38, CRIC32-01 and Taiguoqingyou should be with due consideration.

ACKNOWLEDGMENTS

This research was funded by Science and Technology Research Program of Chongqing Municipal Education Commission (Grant No.KJ202101254125241) and Chongqing Wanzhou Science and Technology Program(wzstc-20210211).

REFERENCES

Chen Chen, Gerard J, M Verkley, Sun Guangyu, Johannes Z. Groenewald, Pedro W. Crous. (2016) Redefining common endophytes and plant pathogens in *Neofabraea, Pezicula*, and related genera. Fungal Biology, 120(11):1291-1322.

Chen Zhiyi, Liu Yongfeng, Liu Fengquan, Luo Chuping, Nie Yafeng. (2009) Evaluation of rice varieties resistant to bacterial leaf streak in Jiangsu.Journal of Plant Protection, 36(04):315-318.

Fenillet C, Schachermayr G, Keller B. (1997) Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. The Plant Journal, 11(1):45-52.

Garzon L N. , Oliveros OA., Rosen B, Ligarreto G A., Cook D R., Blair MW. (2013) Isolation and characterization of nucleotide-binding site resistance gene homologues in common bean (*Phaseolus vulgaris*). Phytopathology, 103(02):156- 168.

Hu Junhua, Liu Rongping, Wang Xuelian, Zhou Na, Hong Qibin, Yao Tingshan, Li Taisheng, JIANG Dong, Cao Li, Li Hongjun. (2015) Evaluation of Citrus germplasm resistance to Alternaria alternata. Journal of Fruit Science, 32(4): 672-680

Liu Rong-ping, Hu Jun-hua, Yao Tin-shan, Wang Xue-lian, Zuo Pei-pei, Wang Yanjie, Li Hong-jun. (2013) A rapid laboratory evaluation method of citrus brown spot caused by Alternaria alternate. Journal of Fruit Science, 30(5): 889-892

Lin Yueli, Huang Lili, Suolang Lamu, Gao Xiaoning, Chen Yinchao, Kang Zhensheng. (2011) A rapid laboratory evaluation system for apple ring rot. Journal of Plant Protection, 38(01):37-41.

Li Wenyang, Zheng Chunyao, Li Chaoping, Cai Zhiying, Lin Chunhua, Huang Guixiu. (2009) Resistance identification of main rubber cultivars and some rubber germplasm in china to *colletotri-chum acutatum* in laboratory. Tropical Agricultural Engineering, 33 (5): 31-36.

Xiao Xiaoe, Zeng Yating, Wang Wen, Cheng Lan, Li Hongye. (2020) First Report and New Hosts of *Pseudofabraea citricarpa* Causing Citrus Target Spot in China, Plant Health Progress, doi. org/10. 1094/PHP-07-20-0056-RS.

Xu Yonghong, Chen Li, Tang Song, Ding Dekuan, Yang Yuheng. (2020) Prediction of Suitable Area and Risk Analysis for Citrus Target Spot, Scientia Agricultura Sinica, 53(21):4430-4439.

Xi Ling, Wang Yuqi, Yang Xiu, Zhu Wei, Chen Guoyue, Wang Yi, Qin Peng, Zhou Yonghong, Kang Houyang. (2021) Evaluation of Resistance to Stripe Rust and Molecular Detection of Resistance Gene(s) in 243 Common Wheat Landraces from the Yunnan Province. Scientia Agricultura Sinica, 54(4): 684-695.

Yang Yuheng, Fang Anfei, Yu Yang, Bi Chaowei, Zhou Changyong. (2019) Integrated transcriptomic and secretomic approaches reveal critical pathogenicity factors in *Pseudofabraea citricarpa* inciting citrus target spot. Microbial Biotechnology, 12(6):1260–1273.

Yang Yuheng, Hu Junhua, Chen Fajing, Ding Dekuan, Zhou Changyong. (2018) Development of a SCAR Marker-Based Diagnostic Method for the Detection of the Citrus Target Spot Pathogen *Pseudofabraea citricarpa*. BioMed Research International, 7128903.

Zhu L, Wang Xinghong, Huang Feng, Zhang Jinze, Li Hongye. (2012) A Destructive New Disease of Citrus in China Caused by *Cryptosporiopsis citricarpa* sp. nov. Plant Disease. 96:804-812.

Zhan Shuang, Wu Wang, Hu Junhua, Wu Yuzhu ,Qiao Xinhua, Chen Li, Cheng Lan, Zhou Yan. (2021) Pathogen identification and screening of control agent of suspected citrus target spot in Wanzhou, Chongqing. Fruit trees in southern China, 50(1): 1-7.

Zhu Li. (2012) Identification of Five Pathogens Causing Citrus Disease in china. Hangzhou: Zhejiang University.

Zhang Changwei, Ding Guoxiang, Ni Xianlin, Liu Tianpeng, Chen Guoming, Zhao Ganlin. (2013) Resistance identification of the liquor-feedstock sorghum varieties, hybrids and parents to sorghum head smut. Journal of Plant Protection, 40(03): 219-224.

Zhang Liying, Chen Rugang, Zhang Junhong, Ouyang Bo, Xiao Jinghua, Li Hanxia, Ye Zhibiao. (2008) Cloning and Analysis of Resistance Gene Analogs from Pepper (*Capsicum annuum* L.). Scientia Agricultura Sinica, 41(1):169-175.