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Identification of Sources of Resistance to Wilt caused by *Ceratocystis fimbriata* in Pomegranate

G. Abhishek[1] , G. Manjunatha[2*] , R.K. Mesta[3] , R. Gunnaiah[4] and R. Suma[5]

[1]Ph.D. Scholar, Department of Plant Pathology, College of Horticulture, Bagalkot, (Karnataka), India.
[2]Assistant Professor (Plant Pathology), College of Horticulture, Mysore, (Karnataka), India.
[3]Professor and Special Officer, Project Planning and Monitoring Cell (PPMC), University of Horticultural Sciences, Bagalkot, (Karnataka), India.
[4]Assistant Professor (Molecular Biology), Department of Biotechnology and Crop Improvement, College of Horticulture, Bagalkot, (Karnataka), India.
[5]Associate Professor, Department of Soil Science and Agricultural Chemistry, University of Agricultural Sciences, Bangalore, (Karnataka), India.

(Corresponding author: G. Manjunatha)*
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ABSTRACT: A glass house experiment was conducted at the College of Horticulture, Bagalkot, Karnataka, during 2019 to identify potential rootstocks resistant/tolerant to *Ceratocystis fimbriata*. Wilt is one of the important diseases of pomegranate adversely affecting crop cultivation in all major growing regions of the country. The pathogen survives in the soil for many years that results disease mitigation is challenging. Managing wilt in pomegranate through an integrated approach has been suggested, and the use of resistant varieties is one of the economical methods. A total of 182 accessions were collected and screened under glasshouse condition by using sick pot method to identify source of resistance to pomegranate wilt. The results indicated that majority of the pomegranate genotypes exhibited complete susceptibility after pathogen inoculation. Only three cultivars Bedana Suri, Yercaud and Yercaud local displayed delayed wilting symptom upto 360 days after pathogen inoculation. These materials could be of significance to the development of resistant rootstocks. Therefore, the methodology used in this experiment is an efficient method for screening disease resistant genotypes since it can minimize the probability that resistant plants selected under controlled conditions will fail in the field condition.

Keywords: Pomegranate, *Ceratocystis fimbriata*, Pomegranate wilt, Host-plant resistance.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a popular fruit crop with significant profit margins. It has a lot of therapeutic and nutraceutical properties. The increased understanding of the health benefits of this fruit crop has resulted in a great demand for fruit consumption in today's dietary system all over the world. India is the world's highest pomegranate producer, with 2.34 lakh hectares' area, 28.45 lakh MT of production, and an average yield of 12.16 t/ha (Anonymous, 2019). Maharashtra is currently India's leading state, accounting for about 70% of the country's total area and production. Other states that grow pomegranates include Karnataka, Gujarat, Andhra Pradesh, Telangana, Madhya Pradesh, and Rajasthan. Pomegranate in India is largely limited by fungal wilt caused by *Ceratocystis fimbriata* (Somashekar *et al*., 1999). Wilt disease in pomegranate is one of the major biotic constraints in India, as well as many other pomegranate growing regions around the world, such as China, Iran, and Pakistan (Xu *et al*., 2011; Huang *et al*., 2003; Harrington, 2014). *C. fimbriata* is a fungal plant disease that infects a wide variety of tropical and temperate plant species. Significant genetic variation and a diverse host range suggest that *C. fimbriata* has numerous cryptic species. (Barnes *et al*., 2001; Marin *et al*., 2003). Yellowing of foliage, stem cracking with evident dark greyish-brown to purple stains in vascular and neighbouring cortical tissue upon splitting of root and stem bark, particularly in lower branches, are typical symptoms of fungal wilt produced by *C. fimbriata* in pomegranate (Sharma *et al*., 2010) (Fig. 1). Management options include preventive bio formulations and curative synthetic fungicides, which raise production costs and result in residual toxicity. The most promising technique will be the identification of resistant or tolerant cultivars in pomegranate for eco-friendly wilt management. Chemical control does not give complete protection and is expensive, limiting profit margins as well as threats to the environment and human health. As a result, breeding is seen as an appropriate disease management strategy for acquiring resistant cultivars. Disease-resistant cultivars are a useful tool for decreasing disease impact, and cultivar development is thought to be the most cost-effective

technique of disease control in crop production (Pertot *et al.*, 2017). Santos *et al*. (2013) screened cocoa genotypes for identification of *Ceratocystis cacaofunesta* resistant genotypes, and they found TSH 1188 as resistant. There was variability in the resistance reactions against *Ceratocystis colombiana* in the coffee genotypes tested and twelve genotypes were found resistant (Castro Caicedo *et al*., 2013). Late wilt symptoms (16.33 months) was recorded in the rootstock Jallore Seedless, while the earlier wilt symptoms (12 months) were observed in Alandi, Patna-5 and Muscat (Ahire and Suryawanshi, 2021). For the control of diseases and pests, the use of chosen resistant rootstocks is an ancient practise widely utilised in the cultivation of a range of woody trees (Mudge *et al*., 2009). The use of disease-resistant cultivars is universally acknowledged as the safest, most cost-efficient, and most successful way to protect crops against disease (Johnson and Jellis, 1992). Additionally, resistant genotypes from diverse genetic resources are required to develop *C. fimbriata* resistant varieties. The identification of resistant rootstock will pave the new ways for developing durable resistance and to reduce the expenditure of management. Therefore, the goal of this research is to find the source of resistance to *C. fimbriata* wilt. We tested 182 genotypes for resistance to *C. fimbriata* in order to identify genotypes for future resistance breeding initiatives.

Fig. 1. Symptoms of *Ceratocystis fimbriata* affected pomegranate plants.

MATERIAL AND METHODS

Isolation of *Ceratocystis fimbriata* from infected plant parts. Symptomatic *Ceratocystis fimbriata* plant samples were collected from a pomegranate field in the Bagalkot district of Karanataka. India in sterilised polyolefin bags (16.1864° N, 75.7390° E). The samples were taken to a lab to isolate and identify the fungus. Standard mycological procedures were used to isolate the pathogen from symptomatic roots on PDA (Potato Dextrose Agar, Himedia, India) plates. Stem parts with vascular staining were recovered, and the cut piece was surface sterilised with 1:1000 mercuric chloride solutions for 60 seconds then washed in 70% alcohol and twice with sterile water to eliminate traces of mercuric chloride. The pathogen was isolated using the carrot bait approach (Moller and DeVay, 1968), in which stems were put in between the carrot discs and cultured at 25±2 °C under a 12-hour photoperiod in a humid chamber (Moller & DeVay, 1968). After perithecium formation, a portion of these fungi was transferred to freshly prepared PDA to allow the full development of fungi. On PDA media *C. fimbriata* produced dark grayish growth consisting of septate mycelium, endoconidia, aleurioconidia and long-necked perithecia. Pathogenicity was proved for isolated cultures using 12 months old plants (cv. Bhagwa) by artificial inoculation of the pathogen. The inoculum was prepared from culture grown on 2% potato detrose broth maintained at 28 ±2 °C.

Plant materials. An experiment was carried at the College of Horticulture, Bagalkot, Karnataka, during 2019 to identify potential rootstocks resistant/tolerant to *Ceratocystis fimbriata*. Collections of accessions/germplasm/ varieties were made in collaboration with ICAR-Indian Institute of Horticultural Research and ICAR-National Research Centre on Pomegranate. Local types were also collected from different parts of the country in the project mode sponsored by state govt. of Karnataka. A total of 182 accessions were collected. The cuttings were grown in pot with diameter of 33×36 cm containing sterile potting mixture Sand: Soil: FYM (in 2:1:1 ratio). The hardwood cuttings were raised up to 6 months providing necessary supporting nutrients. The plants were recorded as susceptible if characteristics symptom expression of wilt disease was observed.

Screening of pomegranate genotypes for *Ceratocystis fimbriata.* *Ceratocystis fimbriata* culture was isolated from pomegranate infected plant was multiplied on sorghum grains and kept for 15 days for multiplication in the laboratory at 25±1°C. After 15 days, the inoculum grown in sorghum was ground in a blender and 100 g of inoculum was mixed with 1 kg of talc powder for soil inoculation (Fig. 2). After mixing this inoculum with the sterilized soil in pots with different accessions and they were kept for observation of symptoms. Disease severity was recorded at 90, 180, 270 and 360 days after pathogen inoculation using 1-5 rating scale with slight modification in El-Bramawy and Wahid, 2007, score 1 represents no symptoms (free from yellowing and wilting), whereas, score 5 marks as susceptible with more than 50% of yellowing and wilting. The phenotypic scores 1, 2, 3 are considered as wilt resistant in the pomegranate and can be used for breeding programs and data were collected accordingly in the screening experiments (Fig. 3).

Fig. 2. Inoculation of *Ceratocystis fimbriata* for screening of pomegranate genotypes.

Fig. 3. Disease rating scale 1–5, where 1- Immune, 2- Resistant, 3-Moderately resistance, 4- Moderately susceptible, 5- susceptible.

RESULTS AND DISCUSSION

Isolation of the pathogen and morphological charterization. Isolation of *C. fimbriata* carried out using carrot baiting technique as it was found to be efficient in producing abundant perithecia within week after incubation. Ascospores produced were typically hat-shaped, large, viscous and opaque. Typical characteristic of the fungus long necked non-septate, globular perithecia, septate mycelium, hyaline cylindrical endoconidia, ellipsoidal aleurioconodia were observed (Fig. 4). Inoculation of the *C. fimbriata* along with the potting mixture recorded wilt symptom such as yellowing of leaf and dried foliage after 45 days of inoculation.

Screening of pomegranate genotypes for *Ceratocystis fimbriata.* The 182 selected genotypes were screened for pomegranate wilt. A large majority of the pomegranate genotypes exhibited complete susceptibility (Fig. 5) after pathogen inoculation. It is found that 137 (75.29 %) genotypes started showing symptoms after 90 days of pathogen inoculation, 33 genotypes (18.13 %) started showing symptoms after 180 days of pathogen inoculation. 9 genotypes (4.94 %) started showing symptoms after 270 days of pathogen inoculation and 3 genotypes (1.64 %) started showing symptoms after 360 days of pathogen inoculation i.e showed susceptible reaction after 360 days of pathogen inoculation.

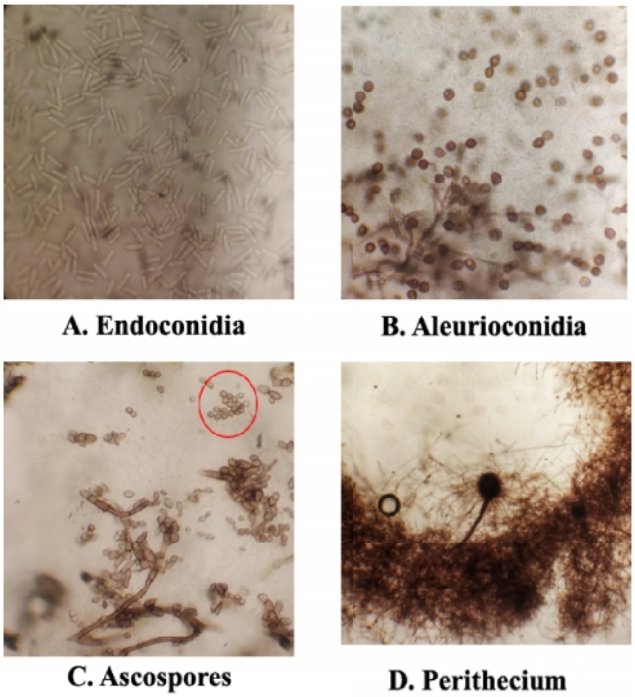

Fig. 4. Morphological characteristics of *Ceratocystis fimbriata.*

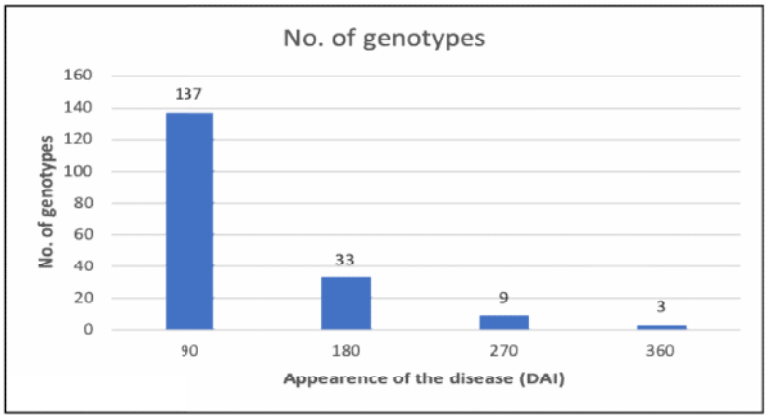

Fig. 5. Expression of pomegranate wilt symptoms in genotypes after pathogen inoculation (DAI).

From our observation, it is clear that no commercial varieties have resistance against the pathogen. Most of the collected genotypes recorded wilting symptoms within 90 days of pathogen inoculation showing susceptibility towards the wilt disease. However, few of the accessions recorded delayed disease expressions up to 180 days. Only three cultivars Bedana Suri, Yercaud and Yercaud local displayed delayed wilting symptom up to 360 days after pathogen inoculation (Table 1). These lines may be useful for further crop improvement program. While identifying the tolerant sources, it is observed that collected lines of pomegranate shows that most of them are susceptible to the pathogen exhibiting characteristic symptoms of wilt. Among screened lines Yeracaud Local, Yeracaud and Bedana Suri did not show any wilting symptom up to 360 days of pathogen inoculation. Ahire and Suryawanshi, 2021, undertook a similar investigation to determine the source of resistance and found that the rootstock Jallore Seedless had late wilt symptoms (16.33 months), while Alandi, Patna-5, and Muscat had earlier wilt symptoms (12 months). Therefore, Effective management of *Ceratocystis* wilt on pomegranate orchards can be accomplished by integrating the use of resistant rootstocks with cultural practices to ensure that grafts are free from pathogens. A similar strategy has been suggested for the management of mango trees by *Ceratocystis* (Oliveira *et al*., 2016). Similar study was conducted by Oliveira *et al*., 2021 in their experiment they studied on cultivars of varied in levels of susceptibility, with disease severity ranging from 40 to 100%. Considering the length of stem lesions, cv. Chieftain showed the lowest level of severity at 40%, while no wilt symptoms were observed at 45 days after inoculation. In addition to the seven cultivars, a half-sibling progeny with 618 plants of the rootstock cv. Bruno was also assessed, but only seven individuals were resistant. Similarly, among the 175 soybean genotypes screened by sick pot method for soil borne fungal diseases, only eight genotypes namely G00005, G00389, G0056, G00168, G00013, G00021, G00149 and G00322 were not affected with seedling mortality diseases (Rahman *et al*., 2018). However, resistance for other wilt pathogens and root knot nematode infestations need further experimentation. Ahire, 2021 carried out an experiment to detect root knot nematode resistant rootstock and discovered that the average range of nematode galls was 25.33-36.00.

Table 1: Expression of *Ceratocystis fimbriata* symptoms after pathogen inoculation.

Appearance of wilt (DAI)	Accessions/Cultivars
90 DAI	Bhagwa, Achik Dana, Agah, Alandi, Bassein Seedless, Phule Bhagwa, Coimbatore White, D-26, Dorsata Malas, Jallore Seedless, Kabul P1, Kabul yellow, Kabul-29, Kabul IIHR-90, Kaladagi Local, Nana, P-13, P-23, Ruby, Shirin Anar, Sindoori, Soft grafted, Spendanader, Phule super Bhagwa, Surrat Anar, Tabesta, IC318762,IC318706, 1-207, 1-110, 1-147,1-58, 5-36, 2-138, 2-20, 2-40, 3-41, 1-176, 4-63, 1-177, 3-129, 1-191, 5-37, 1-6, 3-284, 1-153, 7-128, 1-1, 8-37, 1-76, 1-127, 2-78, 8-82, 3-5, 3-70, 3-101, 3-90, 5-140, 1-60, 3-157, 4-134, 3-112, 3-117, 1-157, 3-276, 1-2, 3-159, 1-201, 3-13, 1-59, 1-12, 2-66, 1-70, 1-174, 3-182, 1-219, 2-62, 4-62, 1-15, 1-52, 4-25, 3-38, 3-105, 2-65, 5-46, 1-201, 2-56, 3-121, 6-10, 5-52, 1-221, 2-36, 2-31, 3-8, 2-174, 3-87, 3-92, 3-64, 3-283, 3-71, 2-147, 7-93, 2-49, 2-155, 4-32, 2-41, 1-109, 3-259, 1-206, 1-197, 7-122, 4-98, 7-9, 1-74, 3-18, 3-220, 1-89, 7-23, 1-141, 7-86, 7-30, 2-35, 8-12, 1-86, 1-120, 7-190, 3-130, 8-80, 1-183, 5-38, 3-160, 2-76, 3-274, 3-238, 2-48, 7-144, 8-49, 1-103, 4-11, 3-54, 1-314
180 DAI	A.K.Anar, Alah, Phule Arakta, Bedana Thinskin, Boskalinsi, Cranado-de-Elcho, Damini, G-137, Gul-e-Shah Red, Ganesh, Gul-e- shah, Gul-e- shah Rose Pink, Jodhpur Red, Jyoti, Kabul canoor, Kalisirin, Kayaki Anar, Lupinia, Mridula, P-26, P-16, Patna-5, Sarkar, Shiah Sirin, Spinsakhaharil, Sursakkar, Yercaud HRS, Dharu, IC318735, IC318707, ACC8, IC318724, IC318734
270 DAI	Bedana Sedana, K.R.S, Khandhari , Masta, Dholka, Yercaud HRS, RCR CB Plot, Bosekaliniski, P-13
360 DAI	Bedana Suri, Yercaud local, Yercaud

Daru had the lowest number of nematode galls (25.33). In pomegranate wilt is a complex disease with many times association of nematodes and stem borer, which needs to be studied further. Prolonged survivability of the pathogen in soil makes pathogen management difficult, even though when clean planting material is used. With a steady increase of global demand for production of pomegranate a greater care is needed to manage the pathogen and its spread. A clear understanding of different host range, resistant rootstock evaluation and detailed understating of tolerant lines in near future may be helpful by successful breeding program for resistant cultivar development. In southern Brazil, the fungus *C. fimbriata* is developed in the soil and the pathogen can spread to adjacent plants through grafted roots as well as contaminated tools (Piveta *et al*., 2016). As demonstrated in this study, genotypes Bedana Suri, Yercaud and Yercaud local sustain relatively longer period and showed delayed symptoms. The assessment of *Ceratocystis* wilt resistance under different environmental conditions would therefore provide a deeper understanding of the resistance mechanisms of these sources and their potential interest in providing broad and stable resistance required for the development of cultivars with long-lasting resistance. Therefore, the methodology used in this experiment is an efficient method for screening disease resistant genotypes since it can minimize the probability that resistant plants selected under controlled conditions will fail in the field condition. The identification of pomegranate genotypes that are resistant to *Ceratocystis* wilt along with cultural practices that help to identify disease free grafts which is relevant to reduce the impact of the disease.

CONCLUSION

Ceratocystis fimbriata is the major cause of vascular pomegranate wilt. In addition, some pathogens have been reported to destroy root and collar stems of plants, likely to result in isolated wilt infections. Although appropriate wilt management strategies involving cultural practices, sanitation measures and chemical treatment have been developed and identified and provide satisfying wilt control, but there is a need to develop wilt-resistant rootstock to implement appropriate wilt-resistant varieties to ensure a more economical and efficient wilt management strategies. Only three cultivars Bedana Suri, Yercaud and Yercaud local displayed delayed wilting symptom up to 360 days after pathogen inoculation. A clear understanding of different host range, resistant rootstock evaluation and detailed understating of tolerant lines in near future may be helpful by successful breeding program for resistant cultivar development. Our results have made it possible to identify new sources of resistance to *Ceratocystis* wilt in pomegranate genotypes of very diverse sources. These materials could be of significance to the development of resistant rootstocks that can be used for the management of *Ceratocystis* wilt in pomegranate. This is the first comprehensive report on screening of germplasm for tolerance against *C. fimbriata* in pomegranate.

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Conflict of Interest. Nil.

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