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Etiology of Pomegranate Wilt and its Management

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ABSTRACT

Wilt surveys of major pomegranate areas in India carried out during 2005-09 revealed disease prevalence in the states of Maharashtra (49.2%), Karnataka (61.11%) and Andhra Pradesh (8.69%). In general, wilt was prevalent in 47.57% of orchards, of which only 5.82% had severe wilt infections, 10.03% moderate and 31.71% mild wilt infections. Wilt was prevalent on all important cultivars of all ages from 2-20 years. On the basis of microscopic examination of diseased plant parts, cultural studies and pathogenicity tests the causal organism of pomegranate vascular wilt was identified as *Ceratocystis fimbriata* Ellis & Halsted as isolations from about 77.0% of the wilt samples from different locations revealed growth of *C. fimbriata.* Pathogenicity tests revealed that *C. fimbriata* was able to infect plants without any injury on roots and developed symptoms in one to five months incubation period in one and half year old plants. Besides *C. fimbriata*, some other pathogens have also been found associated with wilt infection. Although *C. fimbriata* has been reported on many hosts like *Ipomoea batatas*, *Eucalyptus* spp., *Mangifera indica*, *Coffea* spp., *Citrus* spp., etc. there appears to be host specialization in the pathogen as it had failed to cross infect the other hosts than its own. *In vitro* studies revealed efficacy of many fungicides like carbendazim (0.1%), propiconazole (0.1%), hexaconazole (0.1%), mancozeb (0.2%), captan (0.2%), chemicals like boric acid (0.1%) and bioagent *Trichoderma viride* preparation (0.2%) in providing complete inhibition of the pathogen. Under field conditions soil drenching of affected and adjacent healthy plants with carbendazim or propiconazole (0.2%) + chlorpyriphos (0.2%) has resulted in effective wilt management.

Keywords: *Ceratocystis fimbriata*, control, epidemiology, *Punica granatum*

CONTENTS

INTRODUCTION

Pomegranate (*Punica granatum* L.), an important fruit crop of India, is commercially cultivated in the states of Maharashtra, Karnataka, Andhra Pradesh, Gujarat, Rajasthan and Tamil Nadu. Wilt is one of the important diseases of pomegranate adversely affecting crop cultivation in all major growing regions of the country. Pomegranate wilt was first reported in India from Nashik district in Maharashtra in 1978 and subsequently from Kaladgi and Kanamadi areas of Karnataka in 1988 and Cadapa, Andhra Pradesh in 2002 and disease was attributed to *Ceratocystis fimbriata* (Somasekhara 1999, 2006). Chavan and Dake (2001) observed wilt in Maharashtra, India in 1998 and reported it to be caused by *Fusarium oxysporum*. Studies conducted at National Research Centre on Pomegranate Solapur during 2005-09 revealed pomegranate wilt as one of the important limiting factors in pomegranate cultivation in Maharashtra, Karnataka and Andhra Pradesh and reported *C. fimbriata* as one of the major causes of wilt disease amongst other biotic and abiotic factors (Sharma *et al*. 2008; Sharma 2009). Pomegranate wilt has also been reported from other coun-

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Invited Mini-Review

Table 1 Wilt prevalence and incidence in different sates of India during 2005-2009.

State	No. of orchards surveyed	*Wilt prevalence (%)	**Wilt incidence (%)		
			Severe	Moderate	Mild
Maharashtra	250	123 (49.2)	16 (6.4)	26 (10.4)	81 (32.4)
Karnataka	36	22 (61.11)	2 (5.55)	5 (13.88)	15 (41.66)
Andhra Pradesh	23	02 (8.69)	0 (0.00)	0 (0.00)	2 (8.69)
Total	309	147 (47.57)	18 (5.82)	31 (10.03)	98 (31.71)

*Wilt Prevalence: Per cent orchards revealing wilt out of total surveyed.
**Wilt incidence: Severe (per cent orchards with > 40.0% wilt incidence); Moderate: (per cent orchards with wilt incidence between 11- 40.0%; Mild (per cent orchards revealing wilt incidence ≤ 10.0%)

Fig. 1 (**A**) Partly wilt infected plant. (**B**) Completely wilt-infected plant.

tries like Iran (Banihashemi 1998), China (Huang *et al*. 2003), and Greece (Tziros and Tzavella-Klonari 2008).

The present review article describes the etiology and epidemiology of pomegranate wilt and different practices which have been adopted for the management of the disease.

WILT DISTRIBUTION AND SEVERITY

Surveys carried out during 2005-09 in the states of Maharashtra, Karnataka and Andhra Pradesh of India revealed wilt prevalence in 47.57% orchards of which 5.82% orchards had wilt in severe form (> 40.0% incidence), 10.03% orchards had moderate incidence (11-40.0%) and 31.71% orchards had mild (up to 10.0%) wilt incidence (**Table 1**). In Maharashtra, wilt prevalence was quite high in the districts of Satara (91.66%), Pune (90.0%), Nashik (66.66%), Solapur (47.05%) and Ahmednagar (50.0%). In Karnataka (Koppal, Bagalkot and Bijapur districts) wilt prevalence was 61.11% of which 5.55% orchards revealed mild wilt incidence. In Ananthpur district of Andhra Pradesh wilt was observed in mild proportion in only 8.69% of the orchards (Anonymous 2007, 2008, 2009). Somasekhara *et al*. (2009), during surveys from 1996-2000, reported 5.96% wilt incience which amounted to monetary losses of Rs 34.3 million.

Banihashemi (1998) observed pomegranate decline in Iran where as Huang *et al*. (2003) reported pomegranate wilt incidence up to 10.61% from Yunnan in China. Wilt symptoms were observed on two year old pomegranate trees cv. 'Wonderful' in Northern Greece (Tzires and Tzavella-Klonari 2008).

SYMPTOMS

Initial wilt symptoms manifested as yellowing of foliage of one or a few branches of a tree followed by yellowing and drooping of foliage of the entire tree (**Fig. 1A, 1B**). At times only one or two stems of the tree showed wilting and it took a few weeks to some months for the entire tree to completely wilt. Although yellowing of leaves normally proceeded acropetally, occasionally some plants revealed wilt symptoms all of a sudden by senescing the entire plant's foliage at once. Wilt-infected plants often revealed dried foliage and fruits attached to the branches for many months.

Fig. 2 Severely wilt affected orchard in Nashik district of Maharashtra.

Vertical stem cracking was also observed as one of the peculiar characteristic symptoms in some wilt infections. In many orchards diseased trees were observed to die due to wilt in patches, thereby indicating the spread of the disease from an infected to an adjacent healthy tree (**Fig. 2**). However, in some orchards wilt infections were spotted unevenly at different locations.

Splitting of root and stem bark and particularly lower branches, or cross and vertical sections of diseased plant parts generally revealed dark grayish-brown streaks in vascular and adjoining cortex tissues (**Fig. 3**).

ETIOLOGY

C. fimbriata was first reported from India by Somasekhara (1999) whereas Huang *et al.* (2003) described pomegranate wilt caused by *C. fimbriata* from Yunnan, China. Based on disease symptoms, microscopic examination of incubated diseased plant parts (cross sections of roots and stems) in moist conditions cultural studies and pathogenicity tests the

Fig. 3 Vertical section of *C. fimbriata* infected stem revealing grayish streaks in vascular and cortex tissues.

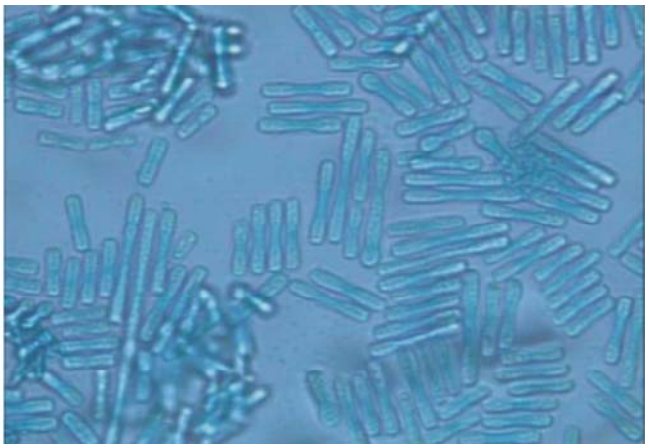

Fig. 4 Endoconidia of *C. fimbriata* in culture (X400).

Fig. 5 Aleurioconidia fo *C. fimbriata* in culture (X400).

causal organism of pomegranate has been identified as *C. fimbriata* Ellis and Halsted (Anonymous 2007).

Pathogen

C. fimbriata has been isolated on potato dextrose agar (PDA) medium from wilt infected plant parts (Anonymous 2007, 2008, 2009). Isolation of the pathogen from soil was made by using carrot slices bait method (Laia *et al.* 2000). The pathogen was successfully baited from infected plant material by placing a small piece of infected plant material between two slices of fresh carrot in moist conditions at 26.0°C (Moller and De Vay 1968). A one-week old culture of *C. fimbriata* on PDA medium revealed dark grayish green growth consisting of septate mycelium, endoconidia, aleurioconidia and long-necked perithecia. Endoconidia were hyaline, cylindrical and formed endogenously in hyphae and their size varied between 10.24-42.11 × 2.35-4.57 µm, with average size of 19.83 × 3.30 µm (**Fig. 4**). Aleurioconidia were thick-walled ellipsoidal, pyriform or obpyriform, truncate at the base, golden-brown (**Fig. 5**) and borne singly or in chains and were intercalary, lateral or terminal on hyphae, size varied from 7.55-35.61 × 6.08-6.33 µm with an average size of 16.48 µm. Perithecia were blackish to brown-blackish in colour, globose to subglobose and measured 137.14-286.98 × 130.36-263.4 µm with an

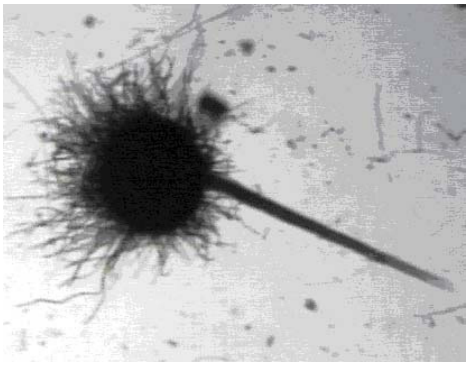

Fig. 6 Perithecium of *C. fimbriata* in culture (X40).

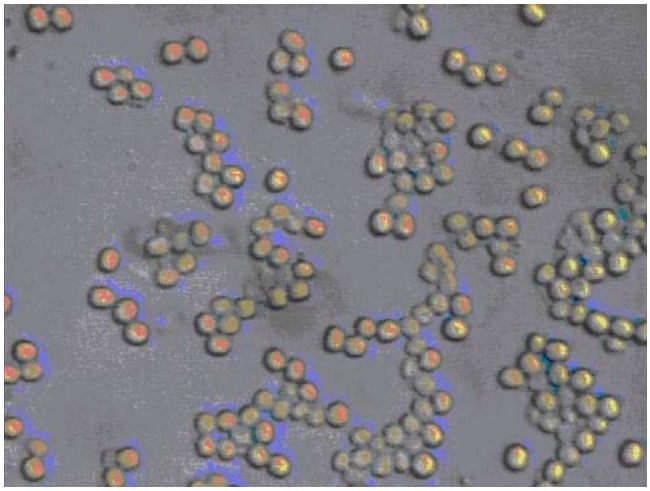

Fig. 7 Ascospores of *C. fimbriata* in culture (X400).

average size of 197.90 × 181.40 µm with a characteristically long neck measuring 109.72-713.14 µm with an average size of 470.09 µm (**Fig. 6**). Perithecia produced ascospores which were hyaline, ovate to galeate and measured 3.03-5.58 × 2.59-3.64 µm with an average size of 4.18 × 3.25 µm (**Fig. 7**). Ascospores were liberated by early ascus deliquescence and were discharged passively through the ostioler hyphae towards the end of perithecial neck. *Chalara* sp. has been observed to be an anamorph of *C. fimbriata* (Anonymous 2008). However, according to Pauline *et al*. (2002) *Chalara* spp. are anamorphs of discomycetes and the genus *Thielaviopsis* is now an anamorph of *Ceratocystis* spp.

Other causes of pomegranate wilt

As mentioned earlier, though *C. fimbriata* was observed to be the main cause of pomegranate vascular wilt, as the pathogen was isolated from 77.0% of diseased samples collected from different locations of Maharashtra and Karnataka during the surveys, other below mentioned pathogens were found associated with wilt infections occurring in different orchards.

***Fusarium* spp.:** A dry root rot disease of pomegranate reported from Maharashtra, India was found to be caused by *Fusarium solani* (Kore and Mitkar 1993). Chavan and Dake (2001) reported pomegranate wilt due to *Fusarium oxysporum* from the Ahmednagar and Solapur districts of Maharashtra, India. Clump rot caused by *Fusarium* sp. severely affected pomegranate plants up to the age of 28 months in Karnataka, India (Ravikumar *et al*. 2001). Isolations from the root and collar stem portion of the wilt-infected plants occasionally revealed association of *Fusarium* spp. (*F. solani* and *F. oxysporum*) and the pathogen was found to cause root rot and wilt, particularly in young plants (Anony-

mous 2008).

***Macrophomina phaseolina*:** The pathogen has been isolated from the root and collar portion of wilt-infected plants causing rotting of roots and thereby resulting in wilting of plants (Anonymous 2008).

***Meloidogyne incognita* (root-knot nematode):** Verma (1985) reported the susceptibility of some pomegranate varieties to *M. incognita*. Studies of Darekar *et al.* (1989) revealed 33% yield losses in pomegranate due to root-knot nematode infestations. In some orchards and nursery plants, critical examination of roots of wilt-infected plants revealed development of knots on main and secondary roots which in turn revealed association of eggs and adults of *M. incognita* (Anonymous 2008, 2009; Sharma *et al*. 2008).

***Phytophthora* spp.:** Isolations from the roots of wilt-infected plants seldom revealed the growth of *Phytophthora* spp. Alavi and Zackii (1985) reported crown and root rot of pomegranate caused by *Phytophthora cactorum* from Iran. *Phytophthora nicotianae* var. *nicotianae* has been observed to cause seedling blight and damping-off, particularly in young pomegranate plants (Anonymous 2009).

***Rhizoctonia bataticola*:** Isolations from some wilt-infected plants in nurseries and orchards revealed the presence of *R. bataticola*, which normally causes girdling of root and collar stem portion and thus results in wilting of plants (Raghuvanshi 2007; Anonymous 2008).

***Rosellinia necatrix*:** Sztejnberg and Madar (1979) reported white root rot in pomegranate caused by *R. necatrix* resulting in the death of plants.

***Verticillium dahliae*:** *V. dahliae* as an agent of pomegranate wilt has been reported in Greece (Tziros and Tzavella-Klonari 2008).

***Xyleborus fornicatus* (Shot hole borer):** Shot hole borer infestations conspicuous by pin hole symptoms in the root and collar portion of infected plants in some orchards of Solapur district resulted in drying and wilting of plants (Kulkarni and Gupta 2007; Anonymous 2008). Somasekhara (2006) also reported the presence of pin holes with discoloration due to shot hole borer in the root and collar regions in 368 of 1852 fields surveyed and the incidence of Scolytid beetles which were found to contain *C. fimbriata* was 0.86%.

EPIDEMIOLOGY

Source of primary inoculum and survivability

Wilt pathogen *C. fimbriata* probably survives adverse conditions as mycelia within the plant host or as thick-walled aleurioconidia in the soil or in plant host or debris. Aleurioconidia, because of the thick wall, are probably the most common fungal survival structures in soil and most initial infections arise from such inoculum (Accordi 1989). The fungus survives in infected pomegranate plant parts up to 190 days (Somasekhara *et al*. 2009) and in the soil for at least four months (Anonymous 2009).

Infection of above-ground plant organs by such wilt pathogens usually takes place through wounds, however, infections have been observed in soft tissues under artificial conditions (Zalasky 1965). In contrast, wounds are not necessary for root infections by *Chalara elegans* in some plant species (Christou 1962). Studies carried out at NRCP Solapur revealed that *C. fimbriata* could infect the roots of plants directly without the presence of any wound (Anonymous 2009).

Secondary spread

Secondary spread of the pathogen generally takes place through infected seedlings, irrigation and rain water, root contact, insects, implements, pruning and budding tools. Somasekhara and Wali (2000) indicated the role of scolytid beetle (*Xyleborus fornicatus*) in the spread of the wilt pathogen as they were able to isolate the pathogen from the beetles. In a few wilt-infected orchards wilted plants revealed both shot hole borer (*Xyleborus fornicatus*) infestation and *C. fimbriata* infection, thereby, suggesting the possibility of the beetle in spread of the pathogen (Anonymous 2008).

C. fimbriata is reported to emit volatile substances (mixture of C1-5 aliphatic alcohols and their acetate esters) which have been assumed to be an adaptation for dispersal by insects which are attracted to diseased plants and can become contaminated with sticky spores (Kile 1993).

After entering the host, mycelium and spores move through the xylem in water conducting cells and into ray-parenchyma cells. The fungus causes dark reddish-brown to purple deep-brown or black staining in the xylem. When the bark of affected branches or trunks is removed or such branches are cut in cross sections the staining along the rays gives a distinctive wedge-shaped or starburst-like pattern (Sinclair *et al*. 1987). Dark grayish streaks are observed both in vascular and cortex tissues of the roots and stems (Somasekhara and Wali 2000; Anonymous 2007; Sharma 2009).

Pathogenicity tests

Somasekhara and Wali (2000) observed wilt symptoms in artificially inoculated pomegranate seedlings after 12 months of inoculation. Pathogenicity of *C. fimbriata* studied on one-and-a-half year old plants of cv. 'Ganesh' in pots revealed wilt symptom initiation in inoculated plants after 30 days of pathogen application and symptoms continued to develop till another 5 months when all the treated plants succumbed to infections by the pathogen. Wilt infection was observed in both treatments having plants with wounded and unwounded roots, indicating the entry of the pathogen even without wounds (Anonymous 2009).

Host range and specificity

Although *C. fimbriata* has been reported on numerous host plants like *Theobromae cacao*, *Mangifera indica*, *Ipomoea batatas*, *Platanus orientalis*, *Coffea* spp., *Eucalyptus* spp., *Citrus* spp., *Prunus* spp., *Crotolaria juncea*, *Hevea brasillense*, *Cassia remigera*, *Acacia* spp., *Annona* spp., *Manihot esculenta*, *Herrania* spp., etc., there are reports of apparently host specialized strains called 'races' or 'forms' which may prove to be distinct species (Harrington 2000; Baker *et al*. 2003). Also, cross inoculation studies of some workers have established the host-specificity of some of these *C. fimbriata* types as the isolates failed to cross infect one another (Vogelzang and Scott 1990; Baker *et al*. 2003). Harrington (2000), on the basis of rDNA and allozyme analysis, proposed that cryptic species within the *C. fimbriata* complex fall into three broad geographic clades, the North American, the Latin American and the Asian clades. Somasekhara and Gaddanakeri (2009), studying host specificity, revealed that *C. fimbriata* from *P. granatum* was unable to infect plants of other hosts including *Acharas sapota*, *Carica papaya*, *Citrus aurantifolia*, *Curcuma longa*, *Ficus elastica*, *Mangifera indica*, *Piper betle*, *Murraya koenigi*, *Psidium guajava*, *Saccahrum officinarum*, *Tectona grandis*, *Vitis vinifera*, and *Zizyphus mauritiana*.

Predisposing factors

Pomegranate wilt may become severe during temperatures ranging between 18.0-30.0°C and frequent rains (Huang *et al*. 2003). Some abiotic factors which favor *C. fimbriata* infections in pomegranate include plantation at close spa-

cing, orchards with deep heavy soils and application of excessive irrigation and fertilizers (Raghuvanshi 2007; Anonymous 2008; Jamadar *et al*. 2009). Boron deficiency in soil enhances *C. fimbriata* infections on *Ipomoea batatas* (Hu *et al*. 1999).

WILT MANAGEMENT

Disease can be managed effectively by adopting integrated management practices including sanitation, cultural methods, chemical control and use of resistant cultivars.

Sanitation measures

Sanitation practices help in minimizing and destroying the pathogen inoculum. Removing and destroying dead and partly wilt-infected plants in orchards has been advocated for vascular stain and canker diseases caused by *C. fimbriata* (Szkolinik 1951; Schieber and Sosa 1960; Panconesi 1981). Disinfection of pruning and grafting tools may help control *C. fimbriata* diseases in *Platanus* (Walter *et al*. 1952). Sanitation procedures resulted in a significant decline in the incidence of black rot of sweet potato in the USA (Clark and Moyer 1988). Eradication of wilt-infected plants has been advocated for the management of pomegranate wilt due to *C. fimbriata* (Jamadar *et al*. 2009; Somasekhara *et al*. 2009).

Cultural measures

Avoidance of already wilt-infested sites and crop rotation with non-host crops may be helpful in the management of pomegranate wilt. Pomegranate plantation in deep heavy soils with poor drainage and at close spacing (to avoid root contact among adjacent plants) should be avoided. Improved drainage and better orchard aeration through efficient weed control have been suggested for the management of *C. fimbriata* in *Coffee* plantations (Szkolinik 1951). Pruning of infected shoots and branches effectively eliminates *C. fimbriata* infections in coffee, almond and stone fruits (De Vay *et al*. 1968).

Chemical measures

Many fungicides and chemicals have been found effective against *C. fimbriata* under laboratory and field conditions. Somasekhara and Wali (2000) and Somasekhara (2009) reported the efficacy of benlate, carbendazim, mancozeb, triadimefon, thiophanate-methyl, ziram and propiconazole against the wilt pathogen under laboratory conditions. In an another study, some of the fungicides and chemicals found highly effective against *C. fimbriata* under *in vitro* conditions include: carbendazim (0.1%), propiconazole (0.1%), hexaconazole (0.1%), tricyclazole (0.1%), myclobutanil (0.1%), mancozeb (0.2%), zineb (0.2%), captan (0.2%), cycloheximide (100 ppm) (antibiotic) and boric acid (0.1%) (Sharma 2009). Somasekhara (2006) reported effective control of pomegranate wilt through soil drenching around the infected and surrounding healthy plants or of the entire orchard with propiconazole (0.1%) + boric acid (0.5%) + phosphoric acid (0.5%).

The insecticide, chlorpyriphos (0.2%) can be used to control shot hole borer and other insect infestations which have seldom been found associated with wilt infections (Kulkarni and Gupta 2007). Wilt infections in soils having shot hole borer and *C. fimbriata* infestations can be managed by soil application of chlorpyriphos (0.2%) alogwith carbedazim (0.2%) or propiconazole (0.2%) (Sharma 2009). Soil sterilization with formalin (0.2%) prior to replanting also controls wilt disease (Somasekhara 2006). Root-knot nematode infestations resulting in wilting can be managed by soil application of carbofuran (3G) or phorate (10G) at 4.0 to 6.0 kg a.i./ha (Mhase 2007).

Biological control

Soil application of a bacterial culture *Bacillus subtilis* was effective in field conditions in reducing pomegranate wilt incidence due to *C. fimbriata* (Somasekhara 2002). Soil application of *Trichoderma* sp. + *Paecilomyces* sp. at 25 g with 2 kg well-decomposed Farm Yard Manure around the trunk of pomegranate trees helps to prevent wilt infections (Raghuvanshi 2007). Application of *Paecilomyces lilacinus* and *Pseudomonas fluorescens* at 10-20 g/m^2 resulted in effective reduction of the root knot nematode population (Mhase 2007). *Trichoderma viride* bioformulation resulted in 86.6% growth inhibition of *C. fimbriata* under *in vitro* conditions (Sharma 2009).

Organic amendments

Soil treatment with neem (*Azadirachta indica*) cake at 2.5 t/ha followed by kranj (*Pongamia pinnata*), mahua (*Bassia latifolia*) and castor (*Ricinus communis*) cakes have also been found effective in the management of root knot nematode (Darekar *et al*. 1989).

Disease resistance

At present, most of the popular pomegranate varieties grown in India, namely, 'Ganesh', 'Bhagawa', 'Arakta' and 'Mridula' are susceptible to *C. fimbriata* and work on selection of suitable wilt resistant rootstock is in progress. Wilt resistance against *C. fimbriata* has been employed in coffee, cacao, and sweet potato (Ikehashi 1986; Iton 1959; Lu *et al*. 1988). Some poplar hybrids and rubber clones have also revealed resistance to *C. fimbriata* (Anonymous 1972; Przybyl 1984).

CONCLUSIONS

Wilt has been observed to be one of the important limiting factors in pomegranate production in all major crop growing states of the country. *Ceratocystis fimbriata* is the main cause of pomegranate vascular wilt. Besides, some other pathogens have also been found to damage root and collar stem of plants and thereby, resulting in isolated wilt infections. Although, suitable wilt management schedules involving cultural practices, sanitation measures and chemical methods have been developed and are providing satisfactory wilt control, there is still a need to develop wilt-resistant rootstock to evolve suitable wilt resistant variety to ensure more economical, feasible and effective wilt management schedule.

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