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Diseases Resistance in Pomegranates: Importance, Sources, Breeding Approaches, and Progress

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In recent years, there has been an increasing interest in commercial production of pomegranates in Florida and other states in the Southeast. One of the most challenging issues in growing pomegranates is the occurrence of several pomegranate diseases that can defoliate trees and reduce fruit quality. Fungal diseases caused by *Cercospora punicae* and *Colletotrichum* sp. appear to be the most problematic in the southeastern United States. However, disease problems also occur in other production areas. In California and other growing areas with a drier climate, *Alternaria* fruit rot (black heart disease) is a major disease. Bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae* has devastated pomegranate production in India. The development of locally adapted pomegranate cultivars with disease resistance would be an important tool for effective management of these diseases. In Florida, several dozen pomegranate cultivars have been screened under natural disease pressure in open orchards or by artificial inoculations of detached leaves and containerized plants to identify sources of resistance to leaf spots caused by *Cercospora* and *Colletotrichum*. Breeding populations have been created through conventional hybridization to improve pomegranate resistance to these diseases.

Pomegranate as an Alternative Fruit Crop in the Southeast United States

Pomegranate (*Punica granatum*) is in the same order as guava (*Psidium* sp.), Myrtales, and in the genus *Punica*, which is the sole genus in Punicaceae (ITIS, 2018). It is an ancient fruit which has been cultivated for thousands of years since 3000 BCE (Stover and Mercure, 2007). There are different ideas as to the origin of the pomegranate. For some the region from Iran to northern India is widely accepted (Morton, 1987), whereas other narrows the area to Persia (Iran) and some surrounding areas (Mars, 2000). Although pomegranate is a vigorous plant that can be grown in many places in the world, it is best adapted to a hot and arid climate (LaRue, 1977). It spreads out and is naturalized in India, China and the Mediterranean region because of their favorable climate. It flourishes particularly well in Spain. Pomegranate was dispersed to the Americas by Spanish missionaries in the 1500s (LaRue, 1977). In 1700s, pomegranate made its way to the United States (U.S.). There is evidence that pomegranate first arrived in Florida and Georgia and then moved to California together with the missionaries (Stover and Mercure, 2007).

In the U.S., California is the largest producer of pomegranates (NASS, 2014). The dominant production area in California is the San Joaquin Valley, where the production area has expanded from 5,600 ha in 2006 to 11,300 ha in 2014 (NASS, 2014). Researches have shown that the antioxidant activity of pomegranate juice is higher than most well-known antioxidant foods, such as orange juice, green tea, grape seed, goji, acai, and red wine (Gil et al., 2000; Henning et al., 2014; Parashar & Badal, 2011). Meanwhile, there is increased interest in the production of pomegranates in southeast U.S. due to a devastating bacterial disease called citrus greening in the citrus industry. As the largest citrus producing state in the U.S., Florida orange acreage and yield have decreased by 26% and 42%, respectively since citrus greening was first reported in Florida (Singerman and Useche, 2016). In addition, pomegranates can be grown using the same tractors, sprayers, cultivation, fertilization and irrigation systems, and fruit transport and storage facilities that have been used in citrus production. Therefore, pomegranate is considered as a potential alternative crop for citrus in Florida, Georgia and Alabama.

Impact of Diseases on Pomegranates

A range of pathogens including fungi, bacteria, viruses and nematodes can attack cultivated pomegranates. These pathogens can cause symptoms such as wilting, stunted growth, dieback of branches, leaf spots, defoliation, and plant death. Pathogens can drastically lower crop yield and affect crop quality by causing fruit spot, fruit rot, and fruit drop. Except for nematodes that typically attack the roots, the other pathogens attacking pomegranate affect the economic part of the plant, the fruit. Pomegranate trees are also grown as ornamental plants (Wolf, 1927). Leaf spots, defoliation and early flower drop caused by some of these pathogens can affect the ornamental value of the plant.

Pomegranate Diseases

Fungal diseases are common on pomegranate plants grown in the southeast U.S. due to the warm and humid weather during the summer, which is favorable to the growth and reproduction of fungal pathogens. Rain splashes also help disperse spores of fungal pathogens. Pathogens such as *Colletotrichum* spp., *Cercospora punicae,* and *Pilidiella granati* have been isolated from diseased tissues of pomegranates grown in Florida. Disease

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Table 1. Summary of pomegranate diseases reported in the United States.

Pathogens	Diseases	Symptoms	State(s)	References
Alternaria spp.	Black heart	Fruit decay within the rind without external symptoms	CA	(Zhang and McCarthy, 2012)
Botrytis cinerea	Grey mold rot	Fruit decay in storage with a grey coat of spores	CA	(Tedford, 2005)
Cercospora lythracearum	Leaf blotch	Circular or angular brown lesion areas on leave	FL, TX, AL	(Wolf, 1927)
Cytospora punicae	Wood canker and branch dieback	Yellowing of leaves, branch and limb dieback, wood lesions, and canker formation	CA	(Peduto Hand et al., 2014)
Neofusicoccum parvum	Shoot blight and stem canker	Death of shoots; sunken cankers on stem	FL, CA	(Kc and Vallad, 2016b; Michailides, 2011)
Pilidiella granati	Fruit rot and leaf spots	Dry rot and mummified fruit; leaf spots with concentric margins on leave	FL, CA	(KC and Vallad, 2016a; Michailides, 2011)
Lasiodiplodia gilanensis	Die back	Sunken lesions and cankers on trunks and branches	CA	(Úrbez-Torres et al., 2017)

symptoms including leaf and fruit spots, stem and tip die back, and fruit rot are observed throughout the year. In areas with dry climates such as California, black heart and grey mold are common. Table 1 summarizes diseases that have been reported on pomegranate in the United States.

Black heart

Black heart, also known as heart rot, is caused by *Alternaria* spp. or *Aspergillus* spp. It is a major disease impacting pomegranate production in California (Zhang and McCarthy, 2012). This disease has been also reported in India (Benagi et al., 2009), Turkey (Pala et al., 2006), Spain (Vicent et al., 2016) and Greece (Tziros et al., 2008).

It is generally accepted that the pathogen infects the fruit during the bloom and early fruit set stage, remaining latent until the fruit is ripe. *Alternaria alternata* penetrates the plant through the stigmata of open flowers (Ezra et al., 2015). Infected fruit are often found containing black and dry rot arils inside with no external symptoms on the outer peel and the hard rind except for a slightly abnormal skin color and light weight (Ezra et al., 2015; Heber et al., 2006). Hence, it is challenging to identify black heart-diseased fruit in a processing line or packinghouse. Zhang and McCarthy (2012) have found a potential nondestructive technique called T_2-based MR imaging to detect black heart in pomegranate fruit.

Anthracnose

Anthracnose is one of the most significant and common diseases affecting annual and perennial tropical plants. It has been reported on pomegranate in Brazil (Mendes, 1998), India (Sharma et al., 2015) and Iran (Rahimlou et al., 2014) In India, *C. gloeosporioides*-incited anthracnose is the second most important disease after bacterial blight (Joshi et al., 2014). Anthracnose typically occurs on fruit as brown irregular lesions, progressing inside the fruit and infecting arils and rind, eventually resulting in fruit decay. Anthracnose can also be found on leaves, leading to necrosis, severe defoliation, and reduced tree vigor.

Leaf and fruit blotch

Pseudocercospora punicae was been reported on pomegranate causing leaf spots in Florida in 1984 (Alfieri Jr, 1984). Prior to this, *Cercospora lythracearum* was identified causing blotches on pomegranate leaves and fruit in Florida (Wolf, 1927). Another specie *Pseudocercospora purpurea* is well known as the most important disease on avocado in Florida (Horst, 2013). On pomegranate plants, irregular brown or black spots (less than 12 mm in diameter on fruit and less than 4 mm in diameter on leaves) have been observed (Phengsintham et al., 2011; Wolf, 1927). Spots may coalesce, causing large necrotic areas if they are numerous. *Pseudocercospora* spores need water to germinate and penetrate (Agrios, 1988), the humid and prolonged rainy season in Florida offers a suitable environment for this disease to develop.

Pilidiella granati (*Coniella granati*) fruit rot

Pilidiella granati, also known as *C. granati*, has been reported to cause fruit rot in Florida (KC and Vallad, 2016), Spain (Palou et al., 2010), Israel (Levy et al., 2011), and Mexico (Cintora-Martínez et al., 2017). *Pilidiella granati* may also cause leaf spots (KC and Vallad, 2016) and crown rots (Çeliker et al., 2012). The symptom of *P. granati* fruit rot initially appears as dark brown lesions, increases in size gradually, and eventually results in fruit rot (Cintora-Martínez et al., 2017) . Abundant dark brown to black spherical pycnidia are often found on the rind of diseased fruit (Palou et al., 2010). *Pilidiella granati* is the main causal agent of preharvest fruit rot in Turkey; this fungal species has become the most important factor limiting the production of pomegranate there (Uysal et al., 2018).

Other common diseases

Bacterial blight is a devastating disease that has led to severe losses of pomegranate in India (Kumar et al., 2006). This disease is caused by *Xanthomonas spp*. Sixty to 80% of pomegranate fruit can be lost due to bacterial blight (Kumar et al., 2006; Ramesh and Ram, 1991). This bacterial pathogen infects all parts of the plant and causes water-soaked lesions on leaves and fruit (Jadhav and Sharma, 2009). Luckily, this disease has not been reported in the U.S. Grey mold caused by *Botrytis cinerea* is the most significant postharvest disease, causing 30% postharvest fruit loss in California in 1999 and 2000 (Tedford, 2005).

Disease Management

Effective disease management is crucial for successful production of pomegranates. Chemical applications and cultural practices are widely used in the major pomegranate production countries. In the United States, Scholar (Syngenta Crop Protection LLC, Greensboro, NC) (active ingredient fludioxil) is registered for control of grey mold in California (Puckett et al., 2013), but to date no chemicals have been registered for use on pomegranates in Florida. Excessive use of chemicals may result in environmental and/or health concern issues.

Sources of disease resistance

Using disease-resistant cultivars would be a more economic and sustainable strategy to manage diseases in pomegranates. The first step toward developing new disease-resistant cultivars is to screen existing germplasm and identify sources of resistance to major diseases in the region. Studies have been conducted in India to identify resistance to *C. gloeosporioides*. Nineteen pomegranate cultivars were inoculated with *C. gloeosporioides* using detached leaves, but none of these cultivars showed resistance; 'Arakta', 'Ganesh', and 'Kesar' were more susceptible than other cultivars (Jayalakshmi et al., 2015). Joshi et al. (2014) showed that 'Arakta' and 'Bhagwa' were both highly susceptible to *C. gloeosporioides,* while two other cultivars 'Yarcud Local' and 'Bedana' exhibited uniform resistance to six isolates of *C. gloeosporioides*. Resistance to black heart disease has been sought by Kahramanoglu et al. (2014). The authors reported differences among three cultivars in disease incidence: 20.3%, 14.9%, and 9.8% for cultivars 'Acco', 'Herskovitz', and 'Wonderful', respectively. In addition, another cultivar grown at the Wolfskill Experimental Orchard in California, 'Kara Gul' showed a higher infection rate than 'Wonderful' (Puckett et al., 2013). These results indicate the existence of variation among pomegranate cultivars in susceptibility to black heart. Kumar et al. (2017) found that 'Bhagwa' and 'Mridula' were more susceptible to *A. alternata* black spot disease while 'Ruby' was comparatively less susceptible among the five cultivars tested. Cultivars 'Daru' and 'Nana' have been reported to exhibit resistance against bacterial nodal blight in India (Jalikop et al., 2005). As a wild cultivar grown abundantly in the Himalayas region, 'Daru' is considered to be disease-resistant (Holland et al., 2009; Sharma and Sharma, 1990).

Recently, a molecular marker (PGCT001) has been associated with bacterial blight resistance (and fruit weight) in pomegranate (Singh et al., 2015). The 153-bp allele at this marker locus is associated with less bacterial blight severity and smaller fruit size (Singh et al., 2015). Efforts are being made to develop high throughout markers and to break the linkage between the two traits that are currently tightly linked (Singh et al., 2015).

Thirteen pomegranate cultivars ('Angel Red', 'Arakta', 'Bhagwa', 'Cedar Key Sunset', 'Don Summer North', 'Eve', 'Girkanets', 'Grenada', 'Larkin', 'Mridula', 'Parfyanka', 'Rosazaya', and 'Sakerdze') are being evaluated in Florida for susceptibility to *C. gloeosporioides*. Preliminary results seem to indicate that 'Arakta' and 'Bhagwa' are highly susceptible to leaf spots incited by *C. gloeosporioides*. One local Florida variety 'Cedar Key Sunset' seems to have a good level of resistance against *C. gloeosporioides*.

Breeding Approaches

Conventional breeding

Conventional breeding methods like seedling selection, hybridization and induction of mutations have been used in pomegranate breeding to improve fruit quality, seed mellowness, juice content and fruit yield (Jalikop, 2010). So far, no cultivars have been released with disease resistance. However, according to Kumar (2016), the Indian Institute of Horticultural Research has developed a hybrid cultivar from wild cultivars in the Himalayan region and commercial cultivars which is resistant to bacterial blight. Conventional breeding is being conducted at the University of Florida/IFAS's Gulf Coast Research and Education Center. Forty-four varieties are being evaluated for disease resistance to leaf spots under natural conditions in central Florida. Seedlings from 16 crosses among 11 selected parents were planted in 2015 and are being screened for leaf spot resistance (Deng, 2018).

Marker assisted breeding

Molecular markers have been used in assessment of genetic diversity and relationships among pomegranate varieties. Type of molecular markers used includes RAPD (Zamani et al., 2007), RFLP (Melgarejo et al., 2009), AFLP (Yuan et al., 2007), and SSR (Hasnaoui et al., 2010; Jian et al., 2012; Singh et al., 2015). Some researchers have concluded that pomegranates are highly polymorphic, whereas others found that the degree of polymorphism was low (Holland et al., 2009). Molecular markers can be extremely useful for tagging and mapping disease resistance genes and selecting new pomegranate varieties with improved disease resistance.

Polyploids

Polyploidy plays an important role in plant breeding. Polyploid plants can be more resistant to diseases and more tolerant to environment stresses than diploids. Tetraploid plants have been induced in 'Nana' by treating in vitro propagated shoots with colchicine (Shao et al., 2003). Tetraploid pomegranate plants have darker green leaves and larger flowers than diploids; their anthers are larger and contain more pollen grains but with lower viability (Shao et al., 2003). So far, data are not available for their resistance to diseases.

Genomics

The genome of a diploid Chinese cultivar 'Dabenzi' has been sequenced and assembled. The assembled genome was 328.83 Mb, with a N50 contig length of 66.97 Kb and a N50 Scaffold length of 1.89 Mb (Qin et al., 2017). Meantime, another widely grown cultivar 'Taishanhong', which produces bright red fruit, was used for genome sequencing (Yuan et al., 2017). The draft pomegranate genome was 274 Mb, covering approximately 81.5% of the estimated 226 Mb genome (Yuan et al., 2017). Among 29,229 clustered, annotated gene models, 6230 orphan genes failed to be clustered with any genes from *P. granatum*, *Arabidopsis thaliana*, *Carica papaya*, *Eucalyptus grandis*, apple, rice, poplar, cocoa, grape, jujube, or peach (Qin et al., 2017). These genes were found enriched in pathways for biosynthesis of secondary metabolites and phenylpropanoids. Compared to eucalyptus, grape, tomato, and *Arabidopsis* sequences, pomegranate has 8854 gene families in common but 1028, 953, and 1435 are unique to grape, *Arabidopsis,* and tomato, respectively (Qin et al., 2017). Pomegranate shares more gene family clusters with *E. grandis,* which is more closely related to pomegranate (Myburg et al., 2014) than to apple, *Arabidopsis,* and grape (Yuan et al., 2017).

Among the annotated pomegranate gene models, 710 R genes were identified (Qin et al., 2017). The availability of these R gene sequences may help us understand the gene-for-gene resistance mechanism between pomegranate plants and pathogens. The identified putative R genes are mapped to nine pseudochromosomes and are non-randomly distributed (Qin et al., 2017). Enrichment of R genes in certain genomic regions indicate the possibility of evolvement from tandem duplication followed by subsequent divergence of linked gene families (Qin et al., 2017).

Transgenic approach

Verma et al. (2014) has shown that pomegranate can be genetically transformed using the *Agrobacterium*-mediated gene transfer technique. The authors obtained transgenic lines in the cultivar 'Kandhari Kabuli' using the *A. tumefaciens* strain EHA 105 and an expression vector carrying the *Cry1A(b)* gene and the *npt-II* gene that can confer kanamycin resistance. Transgenic 'Kandhari Kabuli' plants are expected to express insect resistance from the introduced *Cry1A(b)* gene. This *Agrobacterium*-mediated gene transformation technique can be useful for transferring disease resistance genes to pomegranate cultivars that have good crop yield and fruit quality (Chauhan and Kanwar, 2012).

Future Perspective

Cultivated pomegranates are susceptible to a number of bacterial and fungal diseases. The hot and humid climate in the southeastern United States is highly conducive to the development of these diseases. Screening pomegranate germplasm for disease resistance and developing new disease-resistant varieties should be a priority for any pomegranate breeding effort in Florida and other areas where diseases are a major problem in growing and producing pomegranates. More germplasm needs to be collected and screened for disease resistance. More effective and/or more efficient disease screening techniques need to be developed. As has been shown in other crops, the use of molecular markers can facilitate the identification of disease-resistant germplasm and breeding lines. In this regard, the recently assembled pomegranate whole genome sequences and R gene sequences can be of considerable value to the development of molecular markers for disease resistance in pomegranate. We believe that traditional breeding approaches can play a very important role in improving the resistance of cultivated pomegranates to major bacterial and fungal diseases considering the ease of producing large breeding populations and relatively short breeding cycles. If resources are available, advanced biotechnological tools, such as genotyping-by-sequencing, genomics-based selection, and CRISPER-based gene editing, can be easily applied to pomegranate to improve its resistance to major disease.

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