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BACTERIAL OOZE AS A SOURCE OF INOCULUM IN THE SPREAD OF BACTERIAL BLIGHT OF POMEGRANATE CAUSED BY *XANTHOMONAS AXONOPODIS* PV. *PUNICAE*

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ABSTRACT

Bacterial pathogens of plants are known to live in their natural ooze exuded from infected plants, which protects them from various adverse factors. Further dissemination of these plant pathogenic bacteria from one plant to another or to other parts of the same plant is carried out primarily by water, insects, other animals, and humans. As it is known that, wind-blown rain increase the incidence of bacterial blight disease of pomegranate in the field, a study was conducted to find out the role of bacterial ooze in spreading the bacterial blight of pomegranate caused *Xanthomonas axonopodis pv. punicae* (*Xap*) within the plant or from one plant to another plant within the orchard. In the current study, the exudates oozed out from the infected leaves & nodal portion of twigs of the pomegranate plants maintained in the glass house which were artificially inoculated with *Xap* were collected and subjected for re-isolation of the *Xap* on Nutrient Glucose Agar. Upon obtaining pure culture of the bacterium, DNA was extracted from the pathogen and subjected for PCR amplification of *gyr*B gene using a pair of *gyr*B gene specific primers. The PCR amplification resulted in the production of amplicon size of 491 bp or approximately 500 bp, which confirmed the identity of the pathogen as *X. axonopodis* pv. *puniace*. This study has thrown light on the role bacterial ooze exuded form the infected plant portion in the spread of bacterial blight disease of pomegranate. Further, the findings also emphasize the importance of adoption of intensive plant protection measures like selection of healthy planting materials, Phyto sanitation and prophylactic spray of recommended bactericides for the effective management of bacterial blight disease.

Keywords: Bacterial ooze, Bacterial blight, Pomegranate, Spread, *Xanthomonas axonopodis* pv. *punicae*

Introduction

Pomegranate is one of the major commercial fruit crop grown in India. In the past few years the production of quality pomegranate fruits and its export has shown a downward trend mainly due to devastation of pomegranate orchard by bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae*. The bacterial blight of pomegranate caused a huge economic loss (60 – 70%) to the pomegranate growers especially in Maharashtra, Karnataka and Andhra Pradesh (Ravikumar *et al*., 2006; Raghawan, 2007; Benagi *et al*., 2011; Raju *et. al*., 2013 and Yenjerappa, 2014).

The disease was first time reported as leaf spot of pomegranate by Hingorani and Mehta (1952). The disease is characterized by the appearance of small, irregular water soaked, dark coloured spots (Figure-1) on leaves resulting in pre-mature defoliation. Pathogen also infects stem and branches causing girdling and cracking symptoms (Figure-2). Spots on fruits are dark

brown, irregular and slightly raised with oily appearance, which splits opens with 'L/Y' shaped cracks at final stages (Figure-3). Under severe conditions, it destroys the entire orchard and causes huge economic loses up to 70-80 per cent (Benagi *et al*., 2011 and Yenjerappa *et al*., 2014).

It has been well established that, in plant bacterial diseases *viz*., fire blight of apple, citrus canker, black rot of cabbage, bacterial leaf blight of rice the bacterial ooze which is exuded on surface of the infected plant parts like leaves, fruits and branches acts as main source of inoculum in spreading the diseases. In this context, an effort has been made to establish the key role of bacterial ooze of pathogen *X. aoxonopodis* pv. *punicae* present on the infected parts of pomegranate plant as a source of inoculum in the spreading bacterial blight disease.

Material and Methods

The pure and virulent culture of *X. axonopoodis* pv. *punicae* maintained in the Plant Pathology Department, University of Agricultural Sciences-Dharwad was multiplied in Nutrient Glucose Broth (200 ml) in Erlenmeyer flaks by inoculating a loopful of bacterial culture. The inoculated flask was incubated for three days at 28±1°C.

Eight months old bacterial blight susceptible Bhagwa variety pomegranate plants grown in the earthen pots containing sterilized soil amended with necessary nutrients maintained in the glasshouse first sprayed with water and then covered with a polythene sheet for 24 h before inoculation. The pre-incubated pomegranate plants were sprayed with 4×10^7 cfu/ml of bacterial (*Xap*) suspension (200 ml: 800 ml; bacterial suspension: sterile distilled water) with hand sprayer. The sprayed plants were covered with polythene sheets and kept in humid chamber for the next 48 h where in humidity (>95%) and temperature (28±1°C) was maintained. Plants sprayed with sterile distilled water with nutrient broth served as control. Inoculated plants were sprayed twice a day with distilled water to maintain humidity.

Observations were recorded every day for the appearance and development of symptoms. When artificially inoculated plants expressed symptoms on leaves and stem, with the help of sterile needle, the dried, yellow-crystalline ooze present on lower surface of the infected leaves and nodal portion of the infected twigs were collected in the micro-centrifuge tubes (1.5 ml capacity). Such dried, yellow-crystalline ooze collected in the micro-centrifuge tubes were dissolved in 50 µl of sterile water to prepare bacterial suspension. By using sterile loop, a loopful of bacterial suspension was streaked on the Petri-dishes (90 mm diameter) containing Nutrient Glucose Agar (NGA) medium and incubated at 28±1°C for 96 h. Upon re-isolation, the bacterial culture was compared with original culture and further confirmation was done by subjecting the DNA extracted from the pathogen for PCR amplification of *gyr*B gene using a pair of *gyr*B gene specific primer (*gyr*B F- 5' GTTGATGCTGTTCACC AGCG 3'; *gyr*B R- 5' CATTCATTTCGCCCAAG CCC 3') as described by Mondal and Kumar (2011).

Results and Discussion

Inoculation of *X. axonopodis* pv. *punicae* to pomegranate plants

The characteristic symptoms on leaves observed after six days of inoculation. Initially, the symptoms on leaves appeared as small water soaked lesion, later turned to brown to black colour. Later on such spots were developed into angular to irregular shaped spots along the veins and veinlets of leaf lamina leading to marginal necrosis (Basamma, 2013; Madhu *et al*., 2015). The infected young branches were also turned to black colour at nodal region.

At 15 days after inoculation, when the lower surface of the infected portion of the leaves and nodal regions of infected branches were observed very closely, then the yellow-coloured, crystalline, dried bacterial ooze was observed (Figure-4, Figure-5a & 5b). Similar observations were also made by several earlier researchers who studied bacterial blight of rice (Reddy and Shang-Zhi, 1989) and citrus canker diseases (Gottowald *et al*., 1989; Pruvost *et al*., 2002).

Isolation of *X. axonopodis* pv. *punicae* from ooze

Isolation of *X. axonopodis* pv. *punicae* was carried out on NGA medium from bacterial ooze present on the leaves and twigs of inoculated pomegranate plant showing typical symptoms of bacterial blight. The morphological characters of the bacterial colonies appeared was light yellow, raised, convex and glistening after 72 h of incubation at 28±1°C (Yenjerappa, 2009; Basamma, 2013; Giri *et al*., 2015) and resembled the original culture of *X. axonopodis* pv. *punicae* used for inoculation (Figure-6a & 6b). The PCR amplification of genomic DNA extracted from the pathogen resulted in the production of amplicon size of 491 bp or approximately 500 bp (Figure-7), which confirmed the identity of the pathogen as *X. axonopodis* pv. *puniace.* Similar result was also obtained by Basamma (2013).

During the several field visits to the pomegranate orchard, it was observed that, the severity of bacterial blight was more on the leaves and fruits surrounding

the twigs with nodal blight (Figure-8). This may be due to the carrying of bacterial cells in the exudates present on the infected twig by rainwater or mist and deposition of the same on the surrounding leaves and fruits. Similar types of observations were also made in other bacterial diseases by earlier workers. Reddy and Shang-Zhi (1989) reported that, rice leaves infected with *X. campestris* pv. *oryzae* produce exudates in the form of milky or opaque dew drops that can be easily observed in the morning hours. They dry up during the day to form small, yellowish beads that drop into the rice field water, spreading the disease from field to field along with the water. Citrus bacterial canker disease incidence is directly related to the occurrence of abundant rainfall in the spring season when new growth flushes develop and typhoons in autumn season (Goto, 1992; Koizumi, 1985; Stall, 1988). Goto (1992) reported that rain water present on infected citrus foliage may contain 10^5 to 10^8 cfu/ml of *X. campestris* pv. *citri*. Strong winds cause injuries on leaves and twigs and facilitate water soaking on host tissues with *X. campestris* pv. *citri* containing water.

The bacteria present in the exudates may serve as source of inoculum for short distance as well as long range dispersal of the bacterial plant pathogens through rain splashes and wind-blown rains (Goto, 1962; Stall *et al*., 1980; Civerolo, 1984, Timmer *et al*, 2000). Civerolo (1992) reported that *X. campestris pv. citri* (*Xcc*) overwinters in lesions on leaves and shoots infected during the previous autumn. When free moisture is present, bacteria ooze out from lesions. Leaves and young shoots in the first spring flush are infected by *Xcc* from overwintering lesions, through stomatas, lenticels or wounds within six weeks of initiation of growth. Similarly, studies conducted by several other researcher revealed that, the bacterium *X. axonopodis* pv. *citri* multiplies in lesion in leaves, stems and fruits of citrus. When there is free moisture on the lesion surface, bacteria are released from an extracellular polysaccharide matrix and dispersal to new growth by rain splash (Gottowald *et al*., 1989; Pruvost *et al*., 2002) and the force of wind-blown rain droplets (Goto, 1992; Timmer *et al*., 1991).

This study has thrown light on the role bacterial ooze exuded form the infected plant portion in the spread of bacterial blight disease of pomegranate. Further, the findings also emphasizes the importance of adoption of intensive plant protection measures like selection of healthy planting materials, phytosanitation and prophylactic spray of recommended bactericides for the effective management of bacterial blight disease.

Fig. 1 : Leaf infected with bacterial blight showing necrotic spots

Fig. 2 : Nodal blight

Fig. 3 : Fruit cracking

Fig. 4 : Leaf showing initial water soaked lesions with bacterial ooze

Fig. 5a : Yellow-crystalline bacterial ooze from nodal portion of twig

Fig. 5b. Yellow-crystalline bacterial ooze from nodal portion of twig

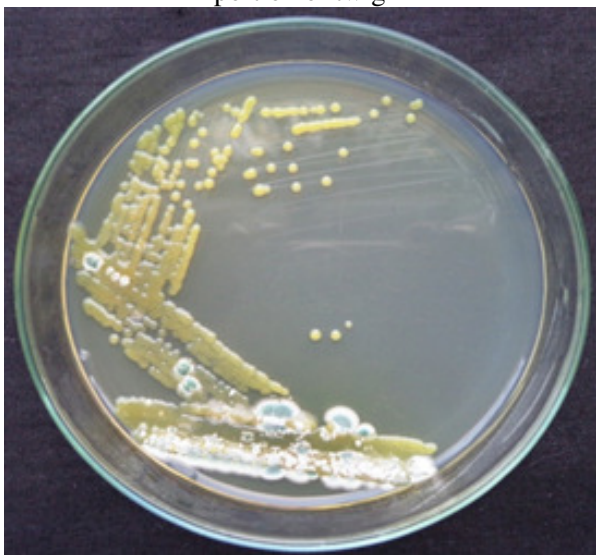


Fig. 6a : Colonies of *X. axonopodis* pv. *punicae* isolated from the bacterial ooze collected from infected leaves.

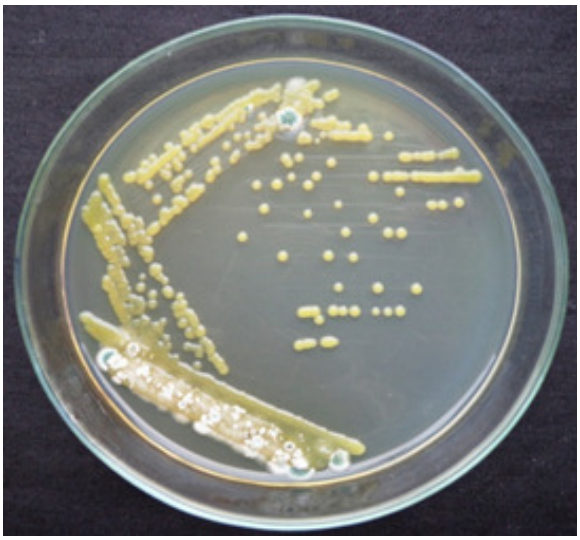
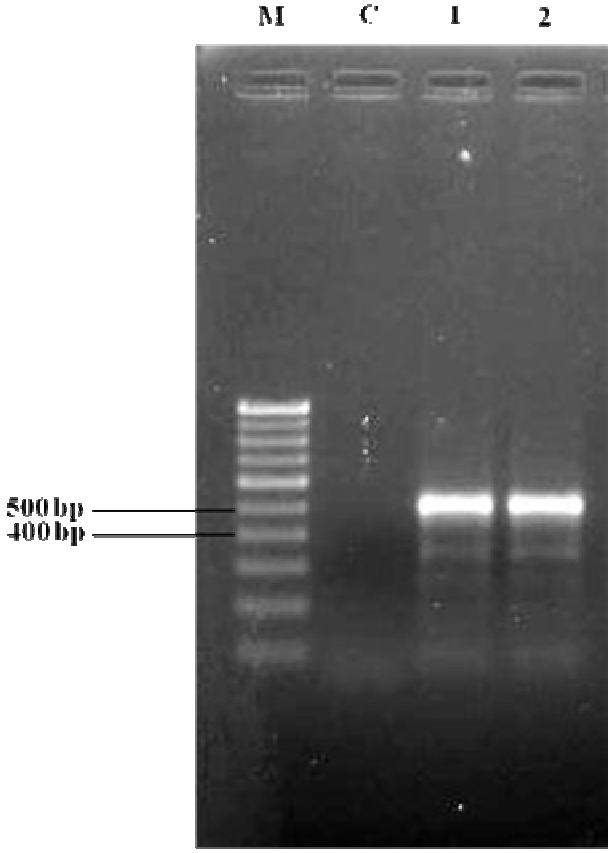


Fig. 6b : Colonies of *X. axonopodis* pv. *punicae* isolated from the bacterial ooze collected from infected twig.



M- Marker; C- Control; 1- *Xap* from leaf ooze; 2- *Xap* from twig ooze

Fig. 7 : PCR amplification of *gyr*B gene in *X. axonopodis pv. punicae*

Fig.8 : Bacterial blight infected pomegranate twig surrounded by severely infected leaves in the close vicinity

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