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Symptomatical Study on *Xanthomonas axonopodis* PV. *punicae* Causing Bacterial Blight of *Punica granatum* L. (Pomegranate)

Ramesh Ippikoppa, Kiran Kumar K. C., Premchand U, Mesta R. K., Kulapati Hipparagi, Pallavi H. M., Raghavendra S.

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Abstract Pomegranate (*Punica granatum* L.) is one of the important fruit crops, which is regarded as a fruit of paradise. The crop is native to Iran and being grown in arid as well as semi arid regions world wide. In India, pomegranate is extensively grown in Maharashtra, Karnataka. Total area under pomegranate in India is 1.43 lakh ha out of which Maharashtra stands first (99,140 ha) followed by Karnataka (19,040 ha). In Karnataka major growing districts are Vijayapur, Bellary, Koppal, Bagalkot, Chitradurga and Belgavi. Pomegranate cultivation suffers from large number of pests and diseases, among those bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae*, is major constraint for production and productivity. The bacterial blight initially appeared during in some parts of Karnataka and Maharashtra and later spreased like a wildfire in several pomegranate growing areas in India. Later it lead to drastic reduction in production of pomegranate due to bacterial blight disease. Hence, symptomatological study has been down and understand the expression of symptoms and development of pathogen in leaves, twigs and fruits.

Keywords Bacterial blight, Pomegranate, Symptomatical study, *Xanthomonas axonopodis* pv. *punicae*.

R. Ippikoppa*, K. Kumar K. C.,
Premchand U., Mesta R. K.
Department of Plant Pathology,
College of Horticulture Bagalkot, UHS,
Bagalkot 587104, Karnataka, India

K. Hipparagi
Department of Fruit Science,
College of Horticulture Bagalkot, UHS,
Bagalkot 587104, Karnataka, India

Pallavi H. M., Raghavendra S.
Department of Biotechnology and Crop Improvement,
College of Horticulture Bagalkot,
UHS, Bagalkot 587104, Karnataka, India
e-mail : ramesh.pathology@gmail.com
*Correspondence

Introduction

Pomegranate (*Punica granatum* L.) is one of the important fruit crops, which is regarded as a fruit of paradise. Pomegranate is commonly known as Anar in Hindi and Dalimbe in Kannada belongs to the family Punicaceae. It is a good source of antioxidants, carbohydrates and minerals such as calcium, iron and sulfur and also rich in vitamin-C and citric acid [1]. It has been cultivated since ancient times throughout the Mediterranean regions of Asia. The crop is native to Iran and being grown in arid as well as semi arid

regions world wide. It is cultivated extensively in Spain, Morocco, Egypt, Iran, Afghanistan and other Mediterranean countries. In India, pomegranate is extensively grown in Maharashtra, Karnataka. Total area under pomegranate in India is 1.43 lakh ha out of which Maharashtra stands first (99,140 ha) followed by Karnataka (19,040 ha). In Karnataka major growing districts are Vijayapur, Bellary, Koppal, Bagalkot, Chitradurga and Belgavi [2].

Pomegranate cultivation suffers from large number of pests and diseases which include bacterial blight, wilt, anthracnose, leaf and fruit spots and nematode. Among these diseases, bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae*, is major constraint for production and productivity. The bacterial blight initially appeared during 2001 [3] in some parts of Karnataka and Maharashtra and later spreaded like a wildfire in several pomegranate growing areas in India. In 2002 to 2004 there was drastic reduction in production of pomegranate due to bacterial blight disease. Hence, the present investigations were undertaken to understand the symptomatical and nature of pathogen development i.e. *X. a.*pv. *punicae* (Hingorani and Singh) Vauterin et al., causing bacterial blight of *Punica granatum* L.

Materials and Methods

Collection of samples

Collection of *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh) Vauterin et al. causing bacterial blight of *Punica granatum* L. (Pomegranate) samples were collected from major pomegranate growing area of Karnataka.

The present investigation was carried out in the Department of Plant Pathology, College of Horticulture, Bagalkot, Karnataka.

Symptomatology of the bacterial blight of pomegranate

Various parts of the plants such as leaves, twigs/ branches and fruits showing bacterial blight symptoms were collected from the disease affected pomegranate fields for isolation and identification of the pathogen. The symptoms were studied on leaves, twings and fruits recorded.

Collection of blight affected pomegranate plant parts and confirming the bacterial nature of the disease

Different parts of plant affected by the disease viz., infected leaves, twing and fruits showing typical blight symptoms were collected from farmers field at Sokanadagi village of Bagalkot district and used in the isolation of *X. a.*pv. *punicae*. Bacterial nature of the disease was confirmed by the ooze test. Small bits of infected tissues were cut from affected plant parts and placed in a drop of water taken on glass slide and observed under high power objective lens (100x) of the compound microscope (make LABOMADE) for bacterial ooze from the cut ends of the tissues. Jet of bacterial cells started oozing out within minutes after suspending these cut pieces in water, thus confirming the bacterial nature of the disease.

Isolation, purification and preservation of bacterial blight pathogen

Isolation of the pathogen

Infected plant parts such as leaf, bark of the stem and fruit pericarp showing typical symptoms of bacterial blight were used to isolate the causal agent of bacterial blight. The diseased samples were washed thoroughly with tap water and air dried. The infected portion along with a bit of sarounding healthy part was cut into small pieces and were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for one minute and washed three times serially in sterile distilled water to remove the traces of mercuric chloride. The diseased bits were then suspended in a test tube containing 3 ml of sterile distilled water and squeezed gently with sterilized scalpel. When the water became slightly turbid due to oozing of bacterial cells, the suspension was serially diluted upto 10–3 dilutions in 9 ml sterile water blanks. Pour 1 ml of diluted bacterial cell suspension into the sterilized petridish and over laged with 20 ml sterilized molten luke warm NA medium. The plates were rotated gently in clockwise

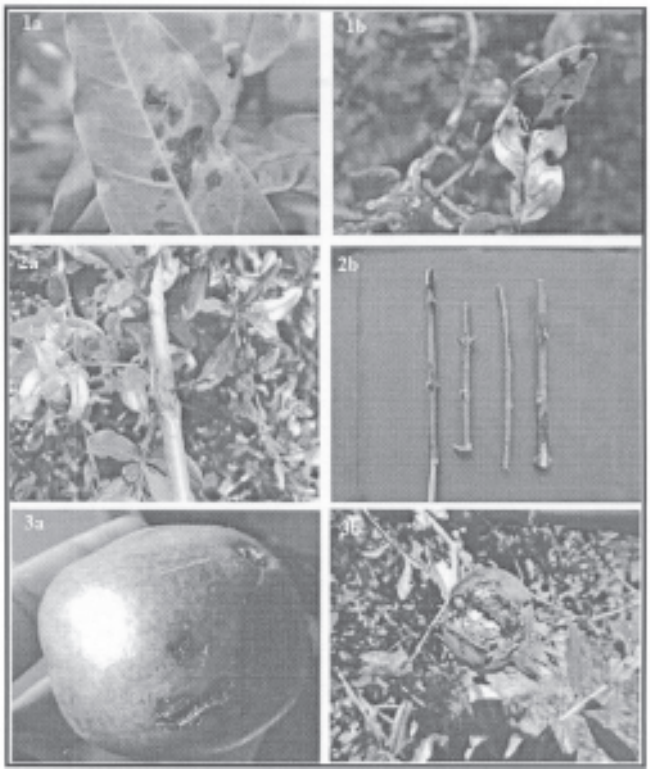

Fig. 1, 2 and 3. Symptomatic expression of pathogen on leaves, twigs/branches and fruits.

and anti-clockwise direction to allow the uniform distribution of bacterial cell suspension in the medium. The inoculated plates were incubated at 30°C for 72 h. After the incubation period, observations were made for the development of well separated, typical, light yellow colored bacterial colonies resembling *Xanthomonas* sp.

Purification and preservation
of bacterial blight pathogen

The isolated colonies of bacterium grown on NA medium were purified by streaking them individually on NA medium. Typical yellow, slimy and glistening bacterial colonies typical to *X. a.* pv. *punicae* were picked up with the help of sterilized inoculation loop and streaked onto the surface of NA medium placed in sterilized petri dishes. The plates were incubated at 32°C for 48 h. Subsequently, the bacterial colonies were streaked on the slants of NA medium and incubated at room temperature and then the slants were stored at 40°C in refrigerator for further use [4].

Proving pathogenicity
(Kochs postulates)

Detached leaf inoculation technique was followed to prove the pathogenicity. Three middle aged leaves were selected and detached from the plants. They were washed well with tap water, wiped with 70% ethanol and air dried. Then injuries were made at several points by pricking with sterilized needle charged with 109 cfu/ml inoculum of *X. a.*pv. *punicae* and also smeared on both sides with culture soaked sterilized cotton swab. The leaves were kept in plates which were lined with sterilized moist filter paper to maintain

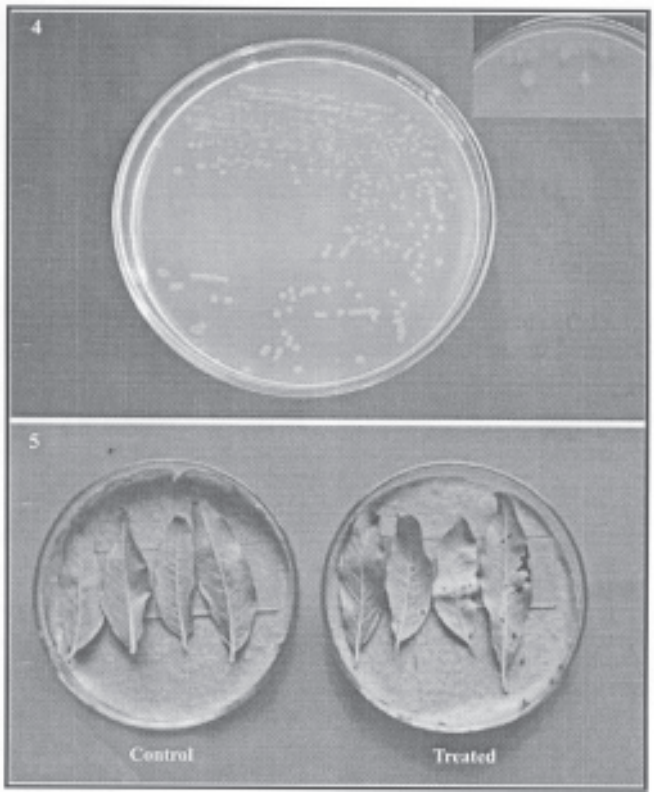

Fig. 4 and 5. Isolation of the pathogen and proving pathogenicity.

humidity ad incubated at 300 C 3 days. The pathogen was reisolated and was compared with the original culture.

Results and Discussion

Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh) Vauterin et al., is known to occur in different pomegranate growing areas of the country viz., Maharashtra, Karnataka and Andhra Pradesh. Pomegranate, the boon commercial fruit crop to the farmer, turned as a big bane after the severe incidence of bacterial blight. The disease continued to damage the crop for the subsequent years till date. Since the bacterial blight of pomegranate was recorded in India by Sharma et al. [5], further in Gamangatti and Patil [6] thoroughly investigated the problem and reported its occurrence has been recorded in different parts of the country. Later reported the disease from Haryana, Madras and Solan region of Himachal Pradesh [7—9].

As the disease is damaging the crop both quantitatively and qualitatively, causing severe economic losses with the incidence ranged from 20–90%. Hence, the present investigations were undertaken to understand the symptomatical and biological nature of *X. a.* pv. *punicae* (Hingorani and Singh) Vauterin et al., causing bacterial blight of *Punica granatum* L.

Symptomatology

Various parts of the showing pomegranate plant parts showing bacterial blight symptoms such as leaves, twigs/branches and fruits were used in this study.

The symptoms on leaves, initially appeared as minute water soaked lesions (Fig. 1) which later turned brown to dark brown surrounded by diffused water soaked margin or yellow halo. Collision of several such spots was seen on single leaf, which increased in size, to form irregular lesions (Fig. 1). Severely infected leaves became yellow, distorted and defoliated. On main stems, twigs or branches, long narrow and elongated bluish-black colored lesions were seen (Fig. 2). Later, these lesions became rough, cankerous and appeared dark brown (Fig. 2). With the advancement of disease, bark cracked, dried and died. The symptoms on fruits first appeared as small-diffused oily lesions (Fig. 3), later turned to brown surrounded by diffused water soaked zones. The lesions which were round in the beginning increased in size became irrigular, brown to dark brown. The rind of affected fruits cracks in the middle of the lesion in the form of L, Y or star shape. Later, affected fruits split opened longitudinally through the lesion exposing arils (Fig. 3). Similar symptoms have been reported by the earlier workers from New Delhi [6], Haryana [7], Tamil Nadu [8], Himachal Pradesh [9] and in Karnataka [10]. In Karnataka [4, 11—15] reported similar symptoms on leaves, twigs and fruits.

Isolation of the pathogen and maintenance of pure culture

The causal organism *X. a.*pv. *punicae* (Hingorani and Singh) [16] was isolated from infected leaf, bark of the stem and pericarp of the fruit showing typical symptoms of bacterial blight. Isolation was done by employing the serial dilution planting technique using NA medium. Repeated isolation from the infected plant parts yielded well separated, typical, yellow, mucoid, colonies of bacterium on NA medium after 72 h of incubation at 30°C (Figs. 4 and 5). Colonies were purified by streaking the isolated colony on yeast-dextrose-calcium carbonate agar medium and pure colonies obtained were further streaked on to the NA slants and inoculated to water blanks (vials containing sterile disillded water) for storage. Later kept for incubation at 30°C for 72 h. Cultures obtained were stored in the refrigerator at 5°C for further studies.

The observations made pertaining to the isolation in the present investigation were in conformity with the work of Manjula [11], who obtained pure culture of the seven isolates of the pathogen from infected plant parts on nutrient agar medium by dilution planting technique.

Kiran Kumar [4] isolated the causal organism of bacterial blight of pomegranate by infected parts viz., leaves, twigs and fruits on nutrient agar medium. The bacterial colonies appeared as light yellow, raised, convex and glistering. The colonies were purified and preserved in distilled water taken in sterile polypropylene tubes at 4°C.

Proving pathogenicity (Kochs postulates)

Kochs postulates were followed to prove pathogenic nature of *X. a.* pv. *punicae* isolates. For proving pathogenicity detached leaf inoculation method was followed Sharma and Sharma [17]. The characteristic symptoms were observed on pomegranate leaves after four days of inoculation as small water soaked lesions. After six days of inoculation it turned brown to black colored lesions, which later developed into angular to irregular shaped spots along the margins, veins and veinlets of the leaf lamina. Re-isolated the pathogen from these lesions and compared with original culture to confirm the identity of the pathogen. The re-isolated culture resembled the original mother culture and thus pathogenicity was confirmed (Fig. 5). Tuite, [17] followed the same method for proving pathogenicity. Similarly, Raju [18] also reported the symptoms were observed on pomegranate leaves after four days of inoculation as small water soaked lesions. Later symptoms developed into angular to irregular shaped spots along the margins, veins and veinlets of the leaf lamina. The pathogenicity was proved by re-isolating the pathogen.

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