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RESEARCH NOTE

Study on etiology of bacterial blight of pomegranate

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Pomegranate (*Punica granatum* L.) is a favourite table fruit in tropical and sub tropical regions of the world which belongs to family Punicaceae. In India, pomegranate is commercially cultivated in Maharastra and small scale plantations are seen in Gujarat, Rajasthan, Karnataka, Tamil Nadu, Andhra Pradesh, Uttar Pradesh, Punjab and Haryana (Chadha, 2001). Pomegranate is grown all over India covering 1.25 lakh hectares. However, maximum area (87,552 ha) under pomegranate is in Maharashtra followed by Karnataka (11,200 ha), Andhra Pradesh (6,000 ha) and Gujarat (3,700 ha). In Maharashtra, Solapur is having maximum area (30,000 ha) followed by Nasik (25,000 ha), Sangli (9,000 ha), Ahmednagar (6,118 ha) and rest of the districts have less than 5,000 ha. In Karnataka it is mainly grown in Bijapur district and in AP in Anantpur. India accounts for 10 per cent of the total world production of fruits and stands second next to China. India is the second largest producer of pomegranate with a production of 7.92 lakh tons (Anonymous, 2007). A plant with wider adaptability and benefits may also fall sick, which may be due to a pest or pathogen attack. Such sick plants grow and produce poorly. Pomegranate as such is affected by many fungal diseases like Colletotrichum rot, Aspergillus rot, Coniella rot, Pestalotiopsis rot, Pseudocercospora leaf spot etc (Snowden, 1998). However, bacterial blight which is assuming serious proportion in view of the fact that the pathogen is present in a plant and translocates easily wherein the wilting of branches are seen one after another, ultimately the whole plant dries and dies. The disease causes spots on leaves leading to defoliation and fruit spots, and cankerous lesions on stem and in severe cases leading to death of plants.

Different parts of plant affected by the disease *viz.*, infected leaves, twigs and fruits were collected from the farmer's field from Koppal, Raichur and Bellary districts which are the predominant pomegranate growing areas of the state. The affected plant parts were surface sterilized in 0.1 per cent mercuric chloride solution followed by three changes in sterile water.The surface sterilized pieces were transferred into two ml of sterilized water in screw cap tubes. After the water became slightly turbid due to oozing of bacterial cells, the suspension was poured on cooled nutrient agar contained in sterilized Petri plates. The bacterial suspension was dispersed with sterilized surface spreader so as to distribute the bacterial cells uniformly on the surface of the nutrient agar medium. The inoculated plates were incubated at the 28^0C for 3 days. Observations were made for development of bacterial colonies on the plates.

The suspected bacterial colonies were picked up with the help of sterilized inoculation loop and streaked on to the surface of yeast extract dextrose calcium carbonate agar (YDCA, Schaad, 1992) contained in sterilized Petriplates. The plates were incubated at 28^0C for 48-72 hours and the observations were made for the development of well separated typical light yellow coloured bacterial colonies. Such pure colonies were further streaked onto the agar slants containing the nutrient agar medium and incubated at 30^0C for 72 hours, then cultures were stored in the refrigerator at 5^0 C, which served as a stock culture for further studies. The culture was equally maintained by sub-culturing it at 15-20 days interval.

Table 1: Morphological and staining characteristics of the three isolates of *Xanthomonas axonopodis* pv. *punicae*

Characteristics	Isolates		
	Xap1 (Raichur isolate)	Xap2 (Bellary isolate)	Xap3 (Koppal isolate)
Morphology			
Shape	Small rods	Rods	Small rods
Occurrence	Single	Single/pairs	Single/pairs
Staining			
Gram's staining	Negative	Negative	Negative
Capsule staining	Positive	Positive	Positive
Spore staining	Negative	Negative	Negative
Flagellation	Monotrichous	Monotrichous	Monotrichous

The identification of the pathogen involved in causation of bacterial blight in pomegranate was made after examining the morphology and staining characteristics of the three isolates of *Xanthomonas axonopodis* pv. *punicae*strains (Xap1-Raichur, Xap2-Bellary, Xap3-Koppal). The morphological characteristics such as shape, Gram reaction, flagellar staining and capsule staining characters were studied as described by Schaad (1992).

Colony morphology of the bacterium was studied from 48 hr old growth which was circular, convex, and yellow to straw yellow with smooth surface and opaque against transmitted light. Based on the colony morphology, Gram's staining, capsule, flagella staining character and growth on the semi selective media. The bacterium was identified as *Xanthomonas axonopodis* pv. *punicae* (Table 1). All the three isolates were negative in Gram's reaction, rod shaped, occurred either singly or occasionally in chains or in pairs and all the isolates were found positive for capsule staining with monotrichous flagellum, negative for spore staining.

The description of bacterium is in accordance with Hingorani and Mehta (1952) upon isolaion of bacterial pathogen from infected pomegranate leaves and proof of pathogenicity. Infection was readily seen by them on tender leaves artificially inoculated plants in seven to ten days after incubation.Studies on morphological characteristics of the bacterium indicated that the bacterium is rod shaped with rounded ends occurred singly or rarely in pairs, gram negative, capsulated, non-spore forming with single polar flagellum. The cells measured 0.4 to 0.25x1.25- 3.0µm in size and were readily stained with crystal violet, and carbol fushcin. The results obtained in the present study on morphological characters were in agreement with the reports of earlier workersKanwar (1976) and Manjula (2002).

In the present study, *Xanthomonas axonopodis* pv. *punicae* was consistently isolated from the diseased plant parts of pomegranate confirming the above report.

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