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Short communication

Variability in pomegranate fruit spot pathogen

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Pomegranate (*Punica granatum* L) is the most important fruit crop of the tropical and subtropical regions. It is also grown in arid and semi-arid regions of India in a total area of 79,157 ha with a production of 5,09,480 million tonnes. Fruit and leaf spots are important diseases affecting the yield and quality of fruits. Fruit spot caused by *A. alternata* (Fr.) Keissler occurs in pre-harvest and post harvest stages of pomegranate in some areas particularly in western parts of the country. Our surveys in western Rajasthan also revealed that it was major constraints resulting in uprooting of the pomegranate plants of local variety i.e. Jalore seedless in the Jalore District of Rajasthan. Survey revealed that mostly the plants of more than 10-15 years old have been adversely affected and the fruits are not fetching remunerative price even in domestic markets. The test pathogen has also been also reported to cause leaf spots and blights on susceptible cultivars. It is well known that *A. alternata* has different isolates or strains as reported from different crop plants. In pomegranate also, diversified symptoms on different cultivars could be seen. Successful management of this disease depends on the identification of virulent isolates or strains and for that characterization of basic features of pathogen is essentially required. Among different parameters, proteins and amino acid compositions are important biochemical components, which are useful for the chemotaxonomic characterizations of pathogenic isolates. Perhaps, due to genetic factors of the hosts or the diversity among pathogenic populations itself, different characteristic symptoms may be expressed in host plants and therefore, it is imperative to study the basic aspects of the pathogens for the better management of the disease. Therefore, presently, morphological characters and some of the biochemical parameters have been investigated and diversity of different isolates of *A. alternata* of pomegranate is presented.

Pathogenic isolates of *A. alternata* causing fruit spots in pomegranate from Anantapur (Andhra Pradesh), Bikaner (Rajasthan) and Rahuri (Maharashtra) locations were collected and pure culture was made on potato dextrose agar medium. Pathogenic isolates were tested for pathogenicity and maintained for different studies. Cultures were grown over thin layer of medium on sterile microscopic slides and 48 hr. old colonies were stained with cotton blue-lactophenol (HiMedia, Mumbai) and examined under 400x magnifications of light microscope (Olympus, Japan). The mycological attributes such as colour, size and shape of mycelia and conidia of pathogenic isolates were measured using the Software (Dewinter) and digital photographs were also taken using DP-12 Camera of Olympus microscope. Biochemical constituents like total and soluble proteins and amino acids were estimated as per standard procedures (Sadasivam and Manickam, 1992). Isolates were grown in liquid medium of PDA and 15 days old cultured were taken for the proteins estimation. Mycelia mat was collected and ground with phosphate butter (pH 7.0). Constitutive amino acids constituents were estimated from culture filtrates. The results on proteins were expressed in terms of micro gram per 100 mg. of mycelia and total amino acids content was expressed as percentage as per standard procedures.

The results given in Table 1 reveal the existence of morphological variation of three *A. alternata* isolates. Mycelial growth and conidiogenesis were fast in Anantapur and Bikaner isolates as compared to isolate from Rahuri (Maharashtra). Initially olive green and later brown mycelia colonies were noticed in Bikaner isolate while brown colonies from rest of the isolates chains of conidia were observed irrespective of isolates. However, size and shape were dissimilar. Chain of conidia measuring 24.12-33.43 x 7.6-11.53 µm with more number was seen in Anantapur isolate. Roberts *etal.* (2000) reported that accurate identification of small spore *Alternaria* spp. is challenging because of morphological plasticity under non-standard conditions and the common misapplication of the name *A. alternata* to a variety of morphologically distinct taxa. 11.53. Cellular proteins and total amino acids of mycelia of pathogen were also estimated in different isolates. Isolate from Anantapur showed high (1.386 µg/100 mg) total protein followed by isolate of Rahuri (1.295 µg 100 mg) whereas, soluble protein was more (0.824 µg100 mg) in Bikaner isolate followed by 0.64/100 mg in Rahuri isolate. Isolate from Anantapur recorded maximum of 0.16% total amino acids followed by 0.11% in Rahuri and 0.096% in Bikaner isolate. Similarly, variation on percentage of synthesized amino

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Table 1. Diversity on morphologenesis of *A. alternata* isolates of pomegranate.

Isolates	Mycelia (µm)	Conidia (µm)	
		length	width
Rahuri	2.43-2.72	19.20-23.45	10.0-11.77
Bikaner	2.35-2.61	12.25-20.43	4.2-6.19
Anantapur	2.75-2.96	24.12-33.43	7.6-11.53

acids in liquid medium showed that maximum of 0.032% from Anantapur isolate followed by 0.024% in Rahuri and 0.02% in Bikaner isolate. Present results are in agreement with those of Lal *et al.* (1976) that variation in amino acids in the growing culture of *Alternaria alternata*. Earlier study on the composition of proteins from mycelial mass of *Alternaria alternata, Aspergillus carbonarius, Penicillium verruculosum, Tyromyces lacteus* and *Coriolus hirsutus* grown in submerged or solid-state conditions showed that albumins and globulins were predominant out of 70% of total proteins (Babitskaya *et al.*, 1989). Portnoy *et al.* (1993) identified representative strains of *A. alternara*. Each strains was grown on two types of solid media and characterized with descriptions of pigmentation and morphology. The biochemical composition of three isolates of *A. brassicae* designated as isolated A, C and D, which produces three distinct spots on leaves of *Brassica carinata* cv. ppcs-1, was investigated. On the basis of host response, isolate A was rated highly virulent, isolate C as moderately virulent and D as avirulent. Isolate A had the highest composition of carbohydrates and the least virulent D isolate had the lowest total carbohydrate content. It was also suggested that higher levels of carbohydrate may be correlated with isolate virulence. Isolate C had significantly higher levels of lipids, proteins, and DNA in comparison with isolates A and D (Vishwanath and Klte, 1997). Very few qualitative differences were found between the amino acids, organic acids and sugars in the mycelium of *A. triticina* and *A. tenuis* (*A. alternata*) providing biochemical support for the suggestion that *A. triticina* may be an ecotype of *A. alternata*. On the basis of amino acid composition it could not be confirmed that *A., solani* which is closely related to either *A. triticina* or *A. alternata* (Vijaya kumar and Rao, 1976 and 1977).

It is concluded that the fruit rot pathogens of pomegranate is variable particularly with respect to amino acid and protein concentrations and further investigations to correlate with their pathogenic virulence and biochemical factors is required.

References

Babitskaya, V. R., Kostsina, A. M. and Shcherba, V. V. 1989. Study of the composition of protein fractions of fungal origin. *Vestsi-Akademii-Belaruskai-SSR,-Biyalagichnykh-Navuk*, No. 3:36-39.

Lal, B. B., Ram, R. P., Shahdeo, S. P. and Prasad, M. 1976. Variation in amino acids in the growing culture of *Alternaria alternata* (Fr.) Keissler. *Current Science.* 45(4): 150-152.

Portnoy, J., Pacheco, F., Barnes, C., Upadrashta, B. Crenshaw, R and Esch, R. 1993. Selection of representative Alternaria strain groups on the4 basis of morphology, enzyme profile and allergen content. *Journal of Allergens Clinical Immunology.* 91(3): 773-782.

Prasad, P and Reddy, M. N. 1987. Estimation of amino acids, phenols, sugars and nitrogen from the mycelim of *Alternaria alternata. Indian Phytopathology.* 40(3): 416-418.

Roberts, R. G, Reymond, S. T. and Andersen, B. 2000. RAPD fragment pattern analysis and morphological segregation of small spored *Alternaria* species and species groups. *Mycological Research.* 104(2): 151-160.

Vijaya Kumar, C. S. K. and Rao, A. S. 1976). Amino acids, organic acids and sugars present in mycelium of *Alternaria triticina* and *A. tenuis Transactions of British Mycological Society.* 67(3): 498-499.

Vijaya Kumar, C. S. K. and Rao, A. S. 1977. Amino acids in the mycelium and culture filtrate of *Alternaria solani. Transactions of British Mycological Society,* 69 (1) : 153-154.

Vishwanath and Kolte, S. J. 1997. Biochemical variation among three isolates of *Alternaria brassicae. Indian Phytopathology.* 50 (3): 437-438.