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Short communication

Cross-infectivity of *Ralstonia solanacearum* from marigold grown in Andaman Islands

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ABSTRACT

Bacterial wilt disease, caused by *Ralstonia solanacearum*, is one of the major concerns for marigold cultivation in Andaman Islands. Cross-infectivity potential of the bacterial wilt pathogen, isolated from marigold, was tested in other common vegetable-hosts of the Island. Pathogen identity was confirmed by morphological identification and Biolog based phenotypic fingerprinting. Cross-infectivity tests revealed tomato to be the most susceptible among the three solanaceous hosts tested. Highest wilt incidence was observed in tomato and marigold (100%) plants, followed by 55.6% in brinjal and 22.3% in chilli, upon artificial soil inoculation. Our study enlightens pathogenic potential of the bacterial wilt pathogen in important vegetable crops of Andaman Islands and can help formulate suitable management practices for successful management of the pathogen.

Key words: Marigold, *Ralstonia solanacearum*, bacterial wilt, Andaman Islands

INTRODUCTION

Marigold is one of the most popular traditional crops grown in Andaman and Nicobar Islands. It has gained popularity due to ease in its culture, wide adaptability, profuse flowering, short juvenility, large blooming period, attractive color, shape, size and good keeping quality, attracting the attention of flower growers. Demand for marigold flowers is increasing very rapidly among the local farmers. One of the major constraints faced in cultivation of marigold in the Island is wilt disease which is highly prevalent due to the climatic conditions prevalent on the Island. Yield loss due to the bacterial wilt disease caused by the pathogen, *Ralstonia solanacearum*, is one of the major concerns for farmers in marigold cultivation. It is a gram-negative, soil-borne bacterium belonging to the 5 sub-division of Proteobacteria (Yabuuchi *et al*, 1995) and has been described as a 'species complex' due to its complex phenotypic and genotypic diversity (Palleroni and Doudoroff, 1971). It has a wide host range that includes over 450 plant species belonging to about 50 families (Hayward, 1991) and is spread worldwide in tropical, sub-tropical and temperate regions. *R. solanacearum* species were classified into five races based on host range (Buddenhagen *et al*, 1962), and six biovars based on utilization of disaccharides and hexose alcohols (Hayward, 1964). On the molecular basis, the species is divided into four phylotypes (Fegan and Prior, 2005) corresponding to four, broad genetic groups, each related to a geographic origin (Phylotype I: Asian origin; Phylotype II: American origin; Phylotype III: African origin, and Phylotype IV: Indonesian/Australian origin). Each phylotype can be further subdivided into 'sequevars' (sequence variants) according to the nucleotide polymorphism of the *egl* gene (Fegan and Prior, 2005). Solanaceous vegetables are among the major horticultural crops grown in the Island for livelihood security of farmers. Land area available for cultivation is limited in the Island and, hence, farmers grow flowers and vegetables as mixed cropping. Yield loss is noticed in Solanaceous vegetables due to the wilt disease. Therefore, we attempted a study on the cross-infectivity of the pathogen isolated from marigold in common solanaceous vegetables of the Island, viz., brinjal, tomato and chillies, to ultimately help in

Isolation of bacteria was done by the method of by Kelman (1954). Briefly, stem pieces

of 2-3cm excised from infected plants were washed five times in sterilized, deionized water and blot-dried for 15 min on an autoclaved paper towel. Stem pieces were then placed in test tubes containing 5 ml sterile water (for bacterial exudation) for about 5-10 min. A loopful of ooze was then streaked onto CPG agar (gL^{-1}, Casamino acids 1; Peptone 10; Glucose 10; Agar 15; pH 7.2) amended with 2,3,5 triphenyl tetrazolium chloride (1%), and incubated for 36-48 hrs at 28-30°C. Fluidal, white colonies with a pinkish centre were picked up and maintained in 15% glycerol stock at -80┐ C for long-term storage.

Biolog based phenotypic fingerprinting

All the isolates were subjected to Biolog-based phenotypic fingerprinting, using the Microlog system (Biolog, Inc., Hayward, CA). The bacterial solution (prepared as per the manufacturer's instructions) was pipetted into each of the 96 wells in the Biolog microplate, and the plates were incubated at 28-30┐ C for 16–24h and read using an automated plate reader (Biolog, Inc.).

Cross-infectivity assay

A virulent isolate of *Ralstonia solanacearum* from the marigold plant was used for cross-inoculation assay with other important solanaceous hosts, viz., tomato (*Solanum lycopersicum*), brinjal (*Solanum melongena*) and chillies (*Capsicum annuum*). Soil inoculation technique suggested by Kumar (2006) was followed, as described earlier, and the treated plants were incubated at 28-32°C under glasshouse conditions. Three replications of each crop were maintained in each pot, and, for each crop three pots were used. Wilt incidence was recorded at regular intervals upon inoculation with bacterial suspension, and wilt percentage was calculated using the following formula:

$$\text{Wilting percentage} = \frac{\text{Number of plants wilted}}{\text{Total number of plants inoculated}} \times 100$$

Bacterial colonies isolated from infected marigold plants were confirmed by both morphological and biological (biochemical phenotypic) approaches. Morphologically, the isolates were identified as *R. solanacearum* by their typical fluidal colony morphology (Kelman, 1954) showing a spiral, pink centre on CPG agar. Further, the identification was confirmed using 71 carbon sources and 23 chemical sensitivity assays using BIOLOG based phenotypic fingerprinting, which confirmed the identity of the virulent isolate as *R. solanacearum* (>0.80 similarity coefficient). The isolate was named MgA_Rs1 and stored as a water slant, and glycerol stock at 20°C, for short-term and long-term storage, respectively.

Cross-infectivity assay performed with marigold *Ralsonia solanacearum* isolate (MgA_Rs1) on three important solanaceous vegetables, viz., tomato, brinjal and chilli revealed that MgA_Rs1 could infect all three hosts, with minor variation in incubation time. Kumar *et al* (2006) also reported that *Ralstonia solanacearum* isolates from ginger could induce wilt in other commercially-important zingiberaceae plants like small cardamom, large cardamom and turmeric, when inoculated in the greenhouse. Our result also revealed that the virulent strain isolated from marigold could induce wilt symptoms in tomato and marigold plants relatively earlier (7 days post-inoculation) compared to that in brinjal (12 dpi) or chilli (20 dpi) upon artificial inoculation (Table 1). In marigold and tomato, average wilting percentage observed was 100%, as, it could cause wilt in all the nine plants inoculated within two weeks of incubation, whereas, in the case of brinjal and chilli, the wilt percentage was 55.6% and 22.3%, respectively (Fig. 1). MgA_Rs1 could induce wilt in 5 plants out of nine brinjal plants and in two plants in chilli at five week intervals. Earlier, Mondal *et al* (2011) reported that *R. solanacearum* isolated from marigold (host) could infect solanaceous vegetables like tomato and brinjal within 12-15 days

Table 1. Cross-pathogenicity of *Ralstonia solanacearum* isolated from marigold on solanaceous Vegetables

Crop	Days to first wilt	Percent wilt incidence
Marigold	7	100
Tomato	7	100
Brinjal	12	55.5
Chilli	20	22.3

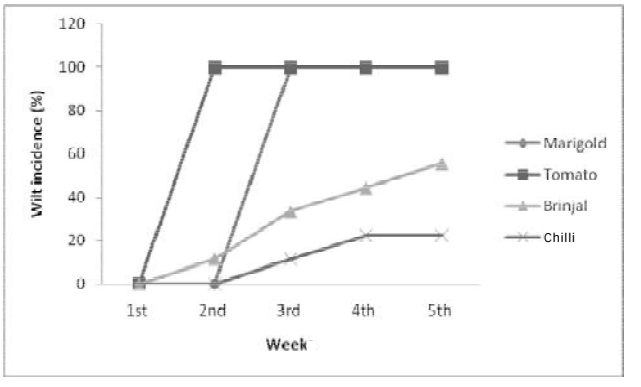

Fig 1. Weekly progress of wilt of marigold caused by a *Ralstonia solanacearum* isolate on various solanaceous crops

period of incubation. Also, our results are in concurrence with findings of Ramesh *et al* (2014) who reported 93% of *Ralstonia solanacearum* isolates collected from various hosts all over India to be pathogenic to eggplant and tomato. The succulent habit of tomato plants (compared to brinjal and chilli) could be the reason for early infection in tomato. Hundred percent wilt incidence was noticed in marigold, as, it is the host plant for this particular pathogen. Percentage of wilt incidence was comparatively low in chilli and brinjal, which could be due to the hardy nature of these crop plants.

Solanaceous vegetables and marigold are important crops grown in the island and yield loss in these crops may be of serious concern to farmers. Our present finding showed that the wilt pathogen of marigold has an equal potential of infecting important solanaceous vegetables like tomato, chillies and brinjal. Our finding is a preliminary study which can help develop effective management practices for controlling the bacterial wilt pathogen that causes serious economic loss in solanaceous vegetables and marigold.

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