

Crown gall of marigold

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Crown gall disease of marigold (*Tagetes erecta* L.) caused by *Agrobacterium tumefaciens* (Smith and Townsend) was recorded in Nadia, Midnapore, Bankura and 24-Parganas(North) districts of West Bengal. The disease was common in incidence in the rainy season in the early crop. The isolate of the bacterium infected amganda (*Coreopsis picta*), balsam (*Impatiens balsamina*), chrysanthemum (*Chrysanthemum* sp.), sandhyamoni (*Mirabilis jalapa*), zinnia (*Zinnia elegans*), tomato (*Lycopersicon esculentum*), kalanchoe (*Kalanchoe diargremontiana*), nayantara (*Catharanthus rosea*) on artificial inoculation. This bacterium did not hamper rooting in air-layering of guava (*Psidium guajava*), litchi (*Litchi chinensis*) and pomegranate (*Punica granatum*) but prevented rooting in chrysanthemum cutting.

Key words : Crown gall, marigold, *Agrobacterium tumefaciens*

Marigold (*Tagetes erecta* L.) is one of the most important ornamental plants grown throughout the world. It is commercially cultivated in large scale in some parts of Midnapore, Howrah, Nadia and 24-Parganas(North) districts of West Bengal. In surveys conducted during 1990-94 for assessment of the disease situation, a gall type symptom was noted on marigold plants. Such gall type malady was not reported earlier from India. Present study is a detail report on the distribution, symptoms of the disease, identity and biology of the causal pathogen.

MATERIAL AND METHODS

Young, soft, small, whitish galls were used for isolation of the pathogen. Surface sterilisation was done by dipping galls in absolute alcohol for one minute followed by repeated washing in sterile distilled water. A gall was crushed aseptically with the help of a sterilised forcep inside a culture tube containing 5 ml sterile distilled water and the tube was kept for 5 minutes. The bacteria oozed out in water and then the cell suspension was streaked on nutrient agar slants. The slants were incubated at $28^{\circ} \pm 1^{\circ}\text{C}$ for 48 h. Single white dominant colonies were selected and streaked on PDA slants and the bacterial culture was purified following usual method (Shekhawat, 1972).

Young vigorously growing marigold plants were selected for pathogenicity test. Wounds were created on different parts by different ways viz. cutting the top portion of the growing stem or branch, removing one or more leaflets from the leaf. Then 48 h old bacterial culture was smeared on these fresh wounds followed by covering the wounds

with moist cotton. Inoculation was done during the afternoon hours and the inoculated portions were covered with polythene bags for 48 h to create a humid condition. In another set of plants, bacterial culture was applied on unwounded parts.

Host range study was conducted with twenty plant species of different categories. In the rainy season the plants were artificially inoculated by creating a wound with a needle charged with 24 h old culture of the test bacterium at the active growing stage of the plants. Inoculation was done in the afternoon hours. The wound sites were covered with moist cotton.

The whole plant/branch was kept covered for 24 h with polythelene bag. In case of guava, litchi and pomegranate, the bacterium was smeared on the branch after removing the bark at the time of making air-layering.

To study the effect of the pathogen on chrysanthemum cutting ten pots were filled with sterilised sand. In five pots cuttings (5-7 cm long) were planted after treatment with root promoting hormone (Seradix B). The rest five pots were planted with the cuttings after smearing the cut end with Seradix B and 24 h old culture of the test bacterium. In each pot ten cuttings were planted. These were kept under shade for 15 days and observation was made 15 days after planting.

Morphological, biochemical and physiological properties of the causal bacterium were tested following standard methods described by Shekhawat (1972).

RESULTS AND DISCUSSION

Distribution

In West Bengal the disease was first recorded at Kalyani, Nadia in August, 1990. Later surveys were conducted in different places of West Bengal particularly during rainy and winter seasons. The disease was found prevalent in Panskura and Debra blocks of Midnapore district where marigold was grown as a commercial crop. The incidence of the disease was also recorded in Ranaghat of Nadia, Basirhat area of 24-Parganas (North) and Jaypur in Bankura.

Symptoms of the disease

The disease was characterised by the appearance of out growths (galls) on different parts of the host plant. Very often at initial stages, the callus was confused with gall. With the advancement of the disease the galls became prominent, could easily be distinguished from the calli. Usually the symptoms first appeared on the petioles of lower leaves and the basal portion of the stem. In the early stage, the galls were more or less spherical, white or creamy white in colour having smooth surface and were soft.

As the galls became older, a gradual change was noticed in shape, size, colour and surface of the galls. The galls enlarged and became 1-2 mm in diameter with somewhat irregular in shape. Some of the galls developed on the stem became extremely large about 1-2.5 cm in diameter. The surface of the larger galls became convoluted. The colour of the galls gradually turned to brown, and ultimately dark brown. The inner tissues of the galls, however, remained flesh coloured. A number of galls developed in close proximity and often coalesced with each other to form a large galled area.

The galls were mostly found on roots, stems, petioles and leaf veins, but not on petals or flower stalk. Galls were also formed at a height of 30-60 cm and even more above the soil line. Under moist condition numerous root primordia developed on these galls. In some instances, roots developed on the galls became longer, assumed a length of about 2-4 cm and also penetrated the soil where the galls produced on leaves touched loose moist soil. Very often, secondary infections were noticed and the galls were subjected to rotting due to such infections.

The disease was most prominent on the first and second crops which were grown under extreme humid and warm conditions i.e. during the rainy season. Intensity of the disease was low in winter season.

Identity of the pathogen

The causal bacterium was rod shaped ($1.5 - 3.0 \times 0.6-0.8 \mu\text{m}$), gram negative, non-spore forming, motile by 1-6 peritrichous flagella; aerobic; colonies convex, circular, smooth and non-pigmented; catalase and oxidase positive; tyrosinase and lipase negative; ammonium production and hydrogen sulphide production negative; gelatin liquefaction, methyl red test negative; nitrate reduction negative; could not utilise ferric ammonium citrate; could not grow on sodium citrate agar; could not produce 3-ketolactose from lactose and β -indole acetic acid form a medium containing tryptophan; could not hydrolyse starch and caesin. Growth of the bacterium was not inhibited by chloramphenicol, penicillin, ampicillin, erythromycin but was sensitive to terramycin and streptocycline at 100 ppm. On the basis of such characteristics the bacterial pathogen was identified as *Agrobacterium tumefaciens*. From the pathogenicity test it was confirmed that wound was essential for infection.

Host range of the pathogen

In India the disease was reported on plum from Simla Hills (Durgapal and Rai, 1973), on *Pelarigonium graveolens* from Nilgiri Hills (Ramadoss *et al.*, 1985), on *Eucalyptus tereticornis* from Himachal Pradesh (Jindal and Bharadwaj, 1986) and on almond from Solan (Krishan and Sharma, 1988). Crown gall on marigold is recorded first time in India.

Marigold isolate of *A. tumefaciens* exhibited no inhibitory effect on the normal rooting in air-layering of common horticultural crops like litchi, guava, pomegranate but the bacterium inhibited the initiation of roots of chrysanthemum cuttings and no galls developed at the cut ends contaminated with this isolate of *A. tumefaciens* (Table 1). It did not hamper the rooting in air-layering. In addition to marigold, the bacterium infected (Table 1) amganda (*Coreopsis picta*), balsam (*Impatiens balsamina*), chrysanthemum (*Chrysanthemum* sp.) sandhyamoni (*Mirabilis jalapa*), zinnia (*Zinnia elegans*), kalanchoe (*Kalanchoe diagamontiana*), tomato (*Lycopersicon esculentum*) and nayantara (*Catharanthus rosea*) but not china rose (*Hibiscus rosa-sinensis*), dahlia (*Dahlia* sp.), jasmine (*Jasminum* sp.), rose (*Rosa* sp.), tagar (*Tabaranae-montana coronaria*), cestrum (*Cestrum diurnum*), potato (*Solanum tuberosum*), guava (*Psidium guajava*), litchi (*Litchi chinensis*), mango (*Mangifera indica*), pomegranate (*Punica granatum*) and datura (*Datura metel*). Development of root like primordia was noticed on the galls of *Kalanchoe*. De Cleene and De Ley (1976) described litchi, guava, mango, *Kalanchoe* and many others as hosts of *A. tumefaciens*. Such differential behaviour of the pathogen towards different hosts was recorded by Anderson and Moore (1979).

Table 1. Host range of the causal bacterium of crown gall of marigold

Name of the plant	Observation	Name of the plant	Observation
A. Ornamental plants		B. Vegetables	
Amganda (<i>Coreopsis picta</i>)	+	Potato (<i>Solanum tuberosum</i>)	-
Balsam (<i>Impatiens balsamina</i>)	+	Tomato (<i>Lycopersicon esculentum</i>)	+
China rose (<i>Hibiscus rosa-sinensis</i>)	-		
		C. Fruit plants	
Chrysanthemum (<i>Chrysanthemum</i> sp.)	+	Guava (<i>Psidium guajava</i>)	-
Dahlia (<i>Dahlia</i> sp.)	-	Litchi (<i>Litchi chinensis</i>)	-
Jasmine (<i>Jasminum</i> sp.)	-	Mango (<i>Mangifera indica</i>)	-
Rose (<i>Rosa</i> sp.)	-	Pomegranate (<i>Punica granatum</i>)	-
Sandhyamoni (<i>Mirabilis jalapa</i>)	+	D. Medicinal plants	
Tagar (<i>Tabernaemontana coronaria</i>)	-	Datura (<i>Datura metel</i>)	-
		Nayantara (<i>Catharanthus rosea</i>)	+
Zinnia (<i>Zinnia elegans</i>)	+		
Cestrum (<i>Cestrum diurnum</i>)	-		
Kalanchoe (<i>Kalanchoe diargremontiana</i>)	+		

'+' indicates development of gall, '-' indicates no gall development

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