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Screening of different mango cultivars against bacterial canker disease caused by*Xanthomonas campestris* pv. *mangiferae-indicae* under Gangetic alluvial region of West Bengal

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Thirty nine mango cultivars were screenedby artificial inoculation under field and laboratory conditions to find out resistant/tolerant sources of mango cultivar(s) against *Xanthomonas campestris* pv. *mangiferae-indicae* (*Xcmi*) inciting mango bacterial canker disease (MBCD). Field trial was carried out at University Mango orchard under All India Coordinated Research Project on Subtropical Fruits, Central Research Farm, Gayespur, BCKV, West Bengal, Indiaduring 2013-15.Among 39 cultivars Zardalu, Fazli, Sunder langra, Manjeera, Suvarnarekha, IARI-Hy-165were found resistant both in field and laboratorytrials while Dasehari, Bombai, Himsagar were found susceptible.

Key words: Mango, Bacterial canker, *Xanthomonas campestris* pv. *mangiferae indicae*, screening , cultivars, resistant, susceptible.

INTRODUCTION

Diseases caused by plant pathogenic bacteria constitute an emerging threat to global food security. *Xanthomonas* is a large genus of gram-negative bacteria that cause disease in several host plants leading to considerable losses in productivity and quality of harvests. Mango bacterial canker disease (MBCD) which is also known as mango canker, bacterial spot, leaf spot, black spot, mango blight, bacterial black spot caused by *Xanthomonas campestris* pv. *Mangiferae indicae* (*Xcmi*) (Gupta and Sharma, 2000)and is a major mango pathogen in tropical and sub-tropical areas (Gagnevin and Pruvost, 2001).

Bacterial canker is widespread in the Eastern Hemisphere (Gagnevin and Pruvost 2001), moved to West Africa (Pruvost *et al.* 2011), and was also reported in Hawaii (Yasuhara-Bell *et al.* 2013)and subsequently other neighbouring countries and America (Sanahuja *et al*. 2016).The pathogen affects all the above ground plant parts like leaf lamina, petiole, twigs and fruits. *Xanthomonas campestris* pv. *mangiferae indicae* (*Xcmi*) can infect a wide range of mango cultivars and induces raised, angular, black leaf lesions, sometimes with a chlorotic halo. Fruit symptoms appear as small, water-soaked spots on the lenticels that become star-shaped, erumpent, and exude an infectious gum (Pruvost *et al*. 2011).Typical disease symptoms arise on all plant parts including leaves, petiole, twigs, shoot and fruit. Initial symptoms appeared on leaves as tiny small to large angular water. The pathogen affects all the above ground plant parts like leaf lamina, petiole, twigs and fruits.Typical disease symptoms arise on all plant parts including leaves, petiole, twigs, shoot and fruit. Initial symptoms appeared on leaves as tiny small to large angular water soaked lesions surrounded with yellow halo. On advance stage, lesions merged with each other, infected tissue dries up and gives canker like appearance. More often, water soaked lesion progressed along with mid rib starting from petiole. Under severe infections, leaves became yellow with gummy droplets of ooze and shed off. Freshly raised lesions were seen as water soaked but later on became brown to dark brown. Short distance dissemination of the pathogen occurred by splashing rainfalls associated with high speed of wind and long distance by infected plant materials.Canker lesions on surface reduce aes

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thetic value of marketable fruits. Mango canker disease has assumed importance in recent years, destroying choicest varieties. During early sixties, the disease was considered as a minor, but now it is posing a great threat to the commercial cultivars (Dashehari, Mallika and Amrapali) as well as seedling cultivars grown in the country. MBCD is considered a major threat for quality mango production in West Bengal condition as the average severity of the disease on fruits in West Bengal have been estimated to be 39.10 per cent (Anonymous, 2008). Among all possible management strategy host resistance in an important solution of disease management and can be employed easily to avoid chance of residual toxicity of chemicals and evolution of virulent strain of pathogens. Keeping this view in mind, this study was aimed to screen resistant sources of mango germplasm against *Xanthomonascampestrispv.mangiferaeindicae.*

MATERIALS AND METHODS

Survey and isolation of the pathogen Xanthomonas campestris pv. mangiferae indicae and selection of virulent isolate

Bacterial canker infected leaf as well as fruit samples were collected from different mango orchards and carried to the laboratory in polythene bags. The diseased sample (leaf/fruit) was washed properly under running tap water and air dried. Surface sterilization of leaves was made with 70% alcohol. The canker infected portions were then cut into small pieces. The leaf spots /fruit spots were cut along with a portion of healthy green tissue into pieces of 0.5mm diameter using a sterile razor and pieces were chopped in a drop of sterile distilled water. The resulting suspension was streaked on plates containing Sucrose Peptone Agar medium (SPA)[Sucrose-20g/ L; Peptone-5g/L; K_2 HPO4 - 0.5g/L; MgSO4 .7H2 O-0.25g/L; Agar20g/L; Deionised distilled water-1L] (Bochand Bonas,2010) followed by incubation at 28^{0}C for 72 hours.

Screening of germplasms

In vivo experiment was conducted at mango orchard of AICRP, Subtropical Fruits, Central Research Farm, Gayespur, B.C.K.V. during the month of July. Bacterial suspension of most virulent isolate was prepared from 48-72 hrsold culture suspended in sterile water (1.0x10^{7}cfu/ml).Disease free young mango leaves of different mango germplasm were inoculated by Leaf injection-infiltration techniquewith bacterial suspension containing 1.0x10^{7}cfu/ml determined by dilution plate technique. The leaves of check plants were also subjected to similar treatment with sterile water. Disease observations were made to estimate the disease severity based on artificial inoculation of *Xanthomonascampestris*pv*. mangiferae-indicae*isolate for screening of mango germplasms under natural disease pressure in field conditions. Four branches of each plant at different directions (N-E-W-S) were selected and three replications per branch / treatment were maintained including controls. Appearance and subsequent disease development of MBCD were recorded at 24 hrs interval up to 8th day.

In vitro screening of germplasms was carried out in laboratory condition. Wounding of leaves and smearing of bacteria was done by rotating the inoculating needle with culture in a circular fashion. Three replications were taken for each treatment (germplasm) and arranged as complete randomized block design(CRD). Same experiment was repeated three times to make three replication of severity.Observations on disease severity were recorded on leaves and fruits by using a scale of 0-5 (Table 1) as follows : 0= No symptoms on the leaf, 1= 0-5 per cent leaf, 2= 6-20 per cent leaf / fruit area infected, 3=21-40 per cent leaf/ fruit area infected, 4= 41-70 per cent leaf/ fruit area infected, 5= >71 per cent leaf / fruit area infected as developed by Mayee and Datar(1986) and per cent disease severity was worked out by using formula given by Wheeler (1969).

Table 1: Disease-rating scale used to determine the level of resistance / susceptibility of mango bacterial canker

Score	Per cent leaf area infected	Reaction
0	0%	Resistant
1	0-5%	Resistant
2	6-20%	Moderately susceptible
3	21-40%	Moderately susceptible
4	41-70%	Susceptible
5	>71 %	Susceptible

The Disease severity % was calculated by using the following formula.

All the Cultivars were categorized into resistant, moderately susceptible and susceptible taking 0

$$\text{Disease Severity \%} = \frac{\text{Sum of individual ratings}}{\text{No. of leaves examined x Maximum disease scale}} \times 100$$

Table 2: Response of 39 mango cultivars to artificial inoculum of *Xanthomonas campestris pv. mangiferae indicae* under *in situ*

Cultivar	Disease severity (%)	Gradin g	Reaction	Symptom initiation	Plant parts affected
Dashehari	40.5(39.5)^{s}	3	S	4 th day	Leaf, petiole, fruit
Bombai	42.0(40.4)st	4	S	4 th day	Fruit, leaf
Himsagar	44.5(41.8)^{t}	4	S	5 th day	Leaf, twig, petiole, fruit
Mallika	36.0(36.9)^{q}	3	MS	5 th day	Fruit, leaf, petiole
Neelum	26.5(31.0)^{o}	3	MS	4 th day	Fruit, leaf
Fernandin	30.5(33.5)^{p}	3	MS	4 th day	Leaf, twig, fruit
ArkaPuneet	39.0(38.6)^{qr}	3	MS	4 th day	Fruit, leaf
Vanraj	21.0(27.3)^{lm}	3	MS	4 th day	Leaf, petiole, fruit
Swarna Jahangir	24.0(29.3)^{mn}	3	MS	7 th day	Fruit, leaf
Banganpalli	16.5(24.0)^{k}	2	MS	5 th day	Fruit, leaf
Mulgoa	15.5(23.2)^{k}	2	MS	5 th day	Leaf, twig, petiole, fruit
Neelgoa	23.0(28.7)^{lmn}	3	MS	6 th day	Fruit, leaf
Alphanso	16.0(23.6)^{k}	2	MS	4 th day	Fruit, leaf
Neeluddin	20.0(26.6)^{l}	2	MS	6 th day	Leaf, twig, petiole, fruit
Bangalora	11.0(19.4)^{fghi}	2	MS	5 th day	Fruit, leaf
Neeleshan	10.0(18.4)^{efg}	2	MS	4 th day	Leaf, twig, petiole fruit
Pravashankar	12.5(20.7)^{fghij}	2	MS	6 th day	Fruit, leaf
Rani pasand	13.5(21.6)^{fghij}	2	MS	6 th day	Fruit, leaf
Chatterjee	13.5(21.6)^{fghij}	2	MS	6 th day	Leaf
Al Fazli	12.5(20.7)^{fghij}	2	MS	5 th day	Leaf, twig, fruit
ArkaAnmol	15.0(22.8)^{ij}	2	MS	6 th d ay	Leaf, twig, fruit
Ratna	11.0(19.4)^{fghi}	2	MS	5 th day	Fruit, leaf
A.U. Rumani	10.5(18.9)^{fgh}	2	MS	7 th day	Leaf
Chousa	10.5(18.9)^{fgh}	2	MS	6 th day	Leaf, twig, fruit
Kesar	12.5(20.7)^{fghij}	2	MS	7 th day	Leaf
Amarpali	14.0(22.0)^{ghij}	2	MS	6 th day	Fruit, leaf
Langra	14.5(22.4)^{hij}	2	MS	4 th day	Fruit, leaf
Kishanbhog	6.0(14.2)^{bcd}	2	MS	6 th day	Leaf, petiole, fruit
Sindhu	6.5(14.8)^{cde}	2	MS	7 th day	Leaf, twig, fruit
Gulabkhas	10.0(18.4)^{efg}	2	MS	6 th day	Fruit, leaf
Bombay Green	9.5(18.0)^{def}	2	MS	7 th day	Fruit, leaf
Sorikhas	6.5(14.8)^{cde}	2	MS	7 th day	Leaf, Fruit
Mankurad	10.5(18.9)^{fgh}	2	MS	6 th day	Leaf, Fruit
Zardalu	2.5(9.1)^{ab}	1	R	6 th day	Leaf
Fazli	3.0(10.0)^{Abc}	1	R	7 th day	Leaf, Fruit
Sunder langra	2.0(8.1)^{a}	1	R	6 th day	Leaf
Manjeera	2.0(8.1)^{a}	1	R	6 th day	Leaf
Suvarnrekha	1.0(5.7)^{a}	1	R	6 th day	Leaf
IARI - Hy - 165	4.5(12.2)^{abc}	1	R	5 th day	Fruit, leaf

and 1 score for resistant, 2 and 3 for moderately susceptible and 4 and 5 score for susceptible response respectively (Prakash *et al*. 1994).

RESULTS AND DISCUSSION

Results of field trial revealedthat among 39 mango cultivars three varieties were susceptible;thirtyvarieties weremoderately susceptible;six varieties were resistant to mango bacterial canker disease.Majority of the cultivars screened were moderatelysusceptible towards MBCD.Highest disease severity was observed in cultivar Himsagar (44.5%) followed by Bombai (42.0%) andDashehari (40.5%).Zardalu, Fazli, Sunder langra, Manjeera, Suvarnrekha, IARI-Hy-165were resistant with 2.5%, 3%, 2%, 2%, 1% and 4.5% disease severitytowards

Table 3: *In vitro* screening of 39 mango cultivars against *Xanthomonas campestris pv. mangiferae-indicae*

Cultivar	L esion length (in cm)	Disease severity (%)	Grad ing	Reaction
Dashehari	6.6	71.1(57.5)^{o}	5	S
Langra	4.766667	57.8(49.5)^{jklmn}	4	S
Chousa	3.955556	48.9(44.4)^{fghi}	4	S
Mallika	5.688889	66.7(54.7)^{no}	4	S
Banganpalli	4.3	53.3(46.9)^{ijkl}	4	S
Mulgoa	4.355556	55.6(48.2)^{ijklm}	4	S
Neelum	5.133333	62.2(52.1)^{lmno}	4	S
Alphanso	4.077778	53.3(46.9)^{ijkl}	4	S
Kesar	3.788889	46.7(43.1)^{efgh}	4	S
Fernandin	5.855556	66.7(54.7)^{no}	4	S
Vanraj	5.422222	64.4(53.4)^{mno}	4	S
Bombai	4.044444	48.9(44.4)^{fghi}	4	S
Himsagar	4.277778	55.6(48.2)^{ijklm}	4	S
Amrapali	6.033333	71.1(57.5)^{o}	5	S
Rratna	3.933333	48.9(44.4)^{fghi}	4	S
A.U. Rumani	3.7	46.7(43.1)^{efgh}	4	S
Swarna Jahangir	4.522222	57.8(49.5)^{jklmn}	4	S
Alfazli	3.688889	42.2(40.5)^{efgh}	4	S
ArkaAnmol	4.1	55.6(48.2)^{ijklm}	4	S
ArkaPuneet	5.622222	64.4(53.4)^{mno}	4	S
Neelgoa	4.911111	60.0 (50.8)^{klmn}	4	S
Golapkhas	4.077778	51.1(45.6)^{ghij}	4	S
Fazli	0.266667	11.1(19.5)^{b}	2	MS
Bangalora	2.155556	33.3(35.3)^{cde}	3	MS
Mankurad	2.755556	37.8(37.9)^{def}	3	MS
Bombay Green	3.077778	40.0(39.2)^{defg}	3	MS
Kishanbhog	2.588889	35.6(36.6)^{de}	3	MS
Pravashankar	2.855556	37.8(37.9)^{def}	3	MS
Neeleshan	2.8	40.0(39.2)^{defg}	3	MS
Rani pasand	2.277778	31.1(33.9)^{cd}	3	MS
Chatterjee	1.6	24.4(29.6)^{c}	3	MS
Neeluddin	1.777778	24.4(29.6)^{c}	3	MS
Sorikhas	2.622222	37.8(37.9)^{def}	3	MS
Zardalu	0	0.0(0.0)^{a}	0	R
Suvarnrekha	0	0 .0(0.0)^{a}	0	R
Sunder langra	0	0.0(0.0)^{a}	0	R
Manjeera	0	0.0(0.0)^{a}	0	R
IARI - Hy - 165	0	0.0(0.0)^{a}	0	R
Sindhu	0	0.0(0.0)^{a}	0	R

infection of *Xcmi* respectively.Cultivar Mallika, Neelum, Fernandin, Arka Puneet, Vanraj, Swarna Jahangir, Banganpalli, Mulgoa, Neelgoa, Alphanso, Neeluddin, Bangalora, Neeleshan, Pravashankar, Ranipasand, Chatterjee, Al Fazli, Arka Anmol, Ratna, A.U. Rumani, Chousa, Kesar, Amarpali, Langra, Kishanbhog, Sindhu, Gulabkhas, Bombay Green, Sorikhas and Mankurad was found moderately susceptible with disease severity range of 6% to 39%. Typical canker lesion was seen on all plant parts of susceptible and moderately susceptible cultivars. But in Chatterjee, A. U. Rumani, Kesar, Zardalu, Sunder langra,Manjeera and Suvarnarekha, lesions were confined within leaves and rest plant parts were found free from infection (Table2). Time of symptom initiation varied among the cultivars starting from 4th day after inoculation to 7th day (Table2).

In laboratory condition Dasehari, Langra, Chousa, Mallika, Banganpalli, Mulgoa, Neelum, Alphanso, Kesar, Fernandin, Vanraj, Bombai, Himsagar, Amrapali, Ratna, A.U. Rumani, Swarna Jahangir, Alfazli, ArkaAnmol, Arka Puneet, Neelgoa and Golapkhas were susceptible with a range of 42.2-71.1% disease severity**(**Table 3**).** Zardalu, Sunder langra, Manjeera, Sindhu, Suvarnrekha and IARI-Hy-165 were resistant with 0.0% disease severity. Cultivar Fazli, Bangalora, Mankurad, Bombay Green, Kishanbhog, Pravashankar, Neeleshan, Rani pasand, Chatterjee, Neeluddinwere found as moderately susceptible with a range of 11.1-40.0%

disease severity. It has been observed during the screening of cultivars that few of the cultivars which were earlier found to be resistant against the *Xcmi* infection were found to be moderately susceptible became to susceptible afterwards.

Long back it was found out that Langra, Dashehari, Chousa, Bombai, Gulabkhas, Kesar were moderately resistant cultivars. Bombay green, Himsagar were susceptible cultivars but Mallika, Amrapali, Vanraj were highly susceptible as they screened. Likewise, some North Indian varieties-Dashehari, Chausa, Kishanbhog, Bombay green and Lucknow safeda were found to be resistant in field but susceptible under artificial inoculations. This may be due to the increasing aggressiveness of the pathogen with the course of time or might be due to the environmental factors contributing to the disease development. The variations of resistance / susceptibility in the present studies might be due to differences of geographical location, meteorological conditions, age of the plant observed and method of evaluations etc. It was also shown that North Indian commercial cultivars *viz*., Bappakai, Jahangir, East Indian cultivars *viz*., Bombay Green, Fazari, Kishanbhog, Scipiaand Zardalu were free from canker in field evaluation. Khatua*et al*. (2012) found out that Himsagar and Bombay green were susceptible. However, Neelum, Totapuri, Mallika, Mohanbhog, Bouganpalli, Fazli, Meghlanthan, SafedaMaliahabadi, Rataul, Daseri and Amprapalli were resistant. The cultivars Zardalu, Alphanso and BiswanathChatterjee recorded moderately resistant, while Langra, Bombay yellow, Kishanbhog, Sorikhas and Jehanara were moderately susceptible. These findings support the present studies conducted for screening of mango cultivars.

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