

## MANGO LEAF ASSAY,



an Effective Technique for Plant Pathological Research

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Though leaf anthracnose is very common in mango canopies, anthracnose lesion is unusual to be induced artificially on detached mango leaf as uniform as an index for *in situ* assessment of the infection caused by the fungi belonging to *Collectorichum gloeosporioides* group. Mango Leaf Assay was a procedure where the positive infection by *C. gloeosporioides* was induced uniformly on freshly detached mango leaves. The assay was developed during 2007-2008 in order to solve main problems dealing with harvesting season as well as area order to solve main problems dealing with harvesting season as well as area requirement in laboratory in association with *in situ* experiments conducting on mango fruit. Soon after healthy light green colour Nam-dok-mai mango leaves of lately young stage were detached from the canopies, they were dust removed gently with running tap water, surfaced sterilized with 1% NaOCI, and blotted. *C. gloeosporioides* spores were suspended for  $1\times10^\circ$  spores/ml in broth medium formulated with 10g glucose, 0.5g yeast extract, 2g peptone, 0.5g KH<sub>2</sub>PO<sub>4</sub>, and 0.5g Mg<sub>2</sub>SO<sub>4</sub>-7H<sub>2</sub>O in 1L of water, then, 2O µl-drops of the suspension were placed onto the pre-determined sites on mango leaves. The inoculated leaves were included the development in build chamber amonget 25, 28°C, 12 were inclubated for disease development in *humid* chamber amongst 25-28°C, 12 hr-light a day. Basing on consequent lesion diameter, the assay provided positive result of artificial inoculation affected by neither distal nor basal side of the leaf. Through the assay, antagonistic spectrum of *Bacillus megaterium* isolate 3103 upon the infection caused by 14 *C. gloeosporioides* isolates was assessed successfully. Intra-specific variation in pathogenicity was also strongly revealed among diversity of *C. gloeosporioides* group.

Keywords: anthracnose, Colletotrichum gloeosporioides, mango, Mango-leaf assay

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tested CG isolate	Average lesion diameter after inocultion (mm.)			
	3 days		6 days	
	Distal side	Basal side	Distal side	Basal side
405	1.38	1.29	5.00	5.92
426	3.63	3.38	13.95	15.67
788	3.17	3.67	13.38	10.00
Average	2.7 <b>3</b> ª	2.78ª	10.7 <b>8</b> <sup>b</sup>	10.53 <sup>b</sup>

Lesions at 3 days 🦲, and 6 days 📕 after inoculation.

18 16 (mm) 14 12 Lesion diameter 10 8 6 4 2 ٥ distal-end basal-end distal-end basal-end distal-end basal-end distal-end basal-end 405 426 788 Average among the pathogen isolates Tested C. gloeosporioides isolates GPYB BM-3103 GPYB BM-3103 100 100 fruit surface (%) 80 80 (%) 60 60



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Mango Leaf Assay was a very effective technique for plant pathological research. The antagonistic spectrum of Bacillus megaterium isolate 3103 showed promising hostility towards field infection established by C. gloeosporioides. Anthracnose disease on mangoes harvested from BM-3103 sprayed canopies were significantly less severe than those harvested from water or GYP broth medium.

Farungsang, N., U. Farungsang and S. Sangchote. 1998. Factors affecting the consistency of leaf disk assay, pp 131-136. In Disease Control and Storage Life Extension, ACIAR Proceedings No. 81.

Farungsang, U., N. Farungsang, L. Phavaphutanon, C. Rattankreetakul and C. Kunprom. 2008. Benefit of mango-leaf assay on the assessment of antagonistic spectrum of the *Bacillus megaterium* isolate 3103 upon diversity of postharvest pathogen, *Colletotrichum gloeosporioides*. Agricultural Sci. J. 39(3)(Suppl.):43-46



The procedure for Mango Leaf Assay was much easier, more effective, and significantly less time consume than the one for the Leaf disk assay (Farungsang et al., 1998). The naturally imitative infection process of the pathogen was allowed to occurred on un-wounded fresh leaves via Mango Leaf Assay, but on 30 sec-boiled leaf disks through the leaf disk assay where saprophytic colonization should rather occurred. Infection severity was indicated by lesion diameter on fresh leaves, instead of the timeconsuming assessment of fruiting body formation and sporulation on semikilled leaf disks.

Using Mango Leaf Assay, we coped with not only once a year of mango fruit harvesting season, but also budget consuming for mango commodity. Furthermore, consumption of incubation area limit, much greater fungal diversity could be examined once a time.



Broadly antagonistic spectrum of Bacillus megaterium isolate 3103 upon the infection process of 14 C. gloeosporioides isolates was obviously indicated by significant reduction in lesion diameter on freshly detached mango leaves through the Mango Leaf Assay (Farungsang et al., 2008). The figure also presented intra-specific variation in pathogenicity among C. gloeosporioides group diversing in the nature.

Farungsang, U., S. Sinlapasunthorn, C. Rattanakreetakul, L. Phavaphutanon and N. Farungsang. 2011 Impact of preharvest application of *Bacillus megaterium* isolate 3103 on postharvest incidence of mango fruit anthracnose. Agricultural Sci. J. 42(1)(Suppl.):209-212.

Farungsang. 2013. Bacillus megaterium isolate 3103: antagonistic spectrum on Colletotrichum gloeosporioides diversity and impact of field application on postharvest incidence of mango fruit anthracnose. Actahorticulturae 973:81-88