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A Review on morphological and molecular characterization of *Colletotrichum* Species Associated with Mango Anthracnose in Konkan Region of Maharashtra State

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Abstract

The mango (*Mangifera indica* L.*)* is the premier fruit among the tropical fruits and has been in cultivation in the Indian subcontinent since several centuries. Among the various growth stages of mango, a flowering stage has been most sensitive in successful mango production due to having direct effects on yield. The major problems limiting mango yields are associated with flowering behaviour, *viz*., alternate bearing, irregular bearing, late bearing, Diseases like Anthracnose and Some Insect Pest Infestation. It is prone to many fungal diseases, leading to heavy loss in yield. Anthracnose caused by *Colletotrichum gloeosporioides,* is recognized as one of the most important pre and post-harvest disease limiting mango fruit production worldwide. Morphological characteristics of fungi is not sufficient for the identification of *Colletotrichum* spp. Therefore characterizations of fungi up to the species level requires molecular identification with using the ITS region. Inspite the development of chemical and cultural control measure for this disease significant losses to production during harvesting are still a reality. Identification and Characterization of *Colletotrichum* isolates associated with mango anthracnose in Konkan region, Maharastra, India is unexplored. In this review paper we are trying to revealed the work of many researchers on this aspect with Morphological characters along with molecular phylogeny of *Colletotrichum spp* infecting different mango fruits need to be established.

Keywords: Morphological, Molecular, characterization, colletotrichum sp., Mango, Anthracnose, Konkan Region

Introduction

Mango (*Mangifera indica L.*) is unarguably considered as king of fruits and Cv. Alphonso is called as king of all mango varieties in India, owing to its luscious taste, captivating flavor and high nutritive quality. It belongs to family Anacardiaceae. It is fifth most widely produced fruit crop in the world after Banana, Citrus, Grapes and Apple and third most important fruit crop of tropics after Banana and Citrus. It is the evergreen plant, originated from South East Asia, the Indo Burma Region (Bose, 1985) [13]. It is under cultivation in India for more than 4000 years and hence conspicuous bonds have been formed between the fruits and cultural history of country.

The fruit of mango is very popular with the masses due to its wide range of adaptability, high nutritive values and richness in variety, delicious taste and excellent flavor. It is a rich source of vitamins A and C. The fruit is consumed as raw or ripe. Good mango varieties contain 20 per cent of total soluble solids. Raw fruits of local varieties of mango are used for preparing various traditional products like raw slices in brine, amchur, pickles, murabba, chutneys, panhe (sharabat) etc. (Anonymous, 1980) [6].

Maharashtra state is an emerging as the leading mango growing state, currently occupying 4.7 lakh ha area with production of 5.97 lakh tons and productivity 3.93 tons/ha. The other states in India which produces mangos (lakh tons) are Andhra Pradesh (4.05), Uttar Pradesh (3.58), Karnataka (1.688), Bihar (0.995), Gujarat (0.856), Tamilnadu (0.762), West Bengal (0.578), Orissa (0.577), Kerala (0.373) (Anonymous, 2019-20) [7].

The Konkan region of Maharashtra is emerging as one of the biggest mango growing belt in India. As four districts of Konkan in Maharashtra accounts only one per cent of total geographical area of country and occupies 8 per cent of total area under mango. Konkan region blessed with production of choicest mango variety Alphonso due to its favourable climate and soil type for mango production and productivity. There are more than 3000 named varieties such as Pairi, Alphonso, Dashehari, Langra, Fajali, Chausa, Totapuri, Neelam, Safeda, Rataul, Banganpalli (Baneshan), Mallika, Amrapali, Swarnarekha etc. are being cultivated for commercial purposes. Among them, Alphonso tops the list. It is grown along west cost of India in Gujarat, Maharashtra, Goa and Karnataka which acclaimed as one of the best Indian mango varieties. Alphonso has a great export potential due to its appreciable qualities like sugar acid blend ratio, attractive colour, pleasant aroma, tasty pulp without fiber and long keeping quality. Alphonso has more than 80 per cent share in export of mango products.

The Cv. Alphonso is poorest yielder with average productivity varying from 2.3 to 3 tons/ha which perhaps lowest in the country. Among the several factors ascribed for low yield, susceptibility to pests (Mango hopper), disease (Powdery mildew) and occurrence of alternate bearing (Pandey, 1989). Among these, alternate bearing is most important physiological disorder of mango where, trees carry a heavy load of crop in one year (‘on’ year) and show tendency towards reduced yield in the following year (‘off’ year). Experimental evidences indicated that maturity of terminal shoots and accumulation of carbohydrates in the shoot apex are in some way associated with the synthesis of floral stimulus, the absence can result lack of flowering or biennial bearing in some mango cultivars. When tree produces heavy crop in on year, it gets exhausted nutritionally and is unable to put forth new flush thereby failing to yield in following season also flowering will occurs on mature shoots of one year old only. Most of the commercial south and north varieties of mango are alternate bearer (Chacko, 1992) [15].

.Mango is susceptible to a range of pest and diseases that can significantly reduce the commercial production as well as the fruit quality. Number of diseases infect mangoes at all the stages of its development right from nursery to the fruit in storage or transit. Mango is prone to many fungal diseases like Anthracnose, Rhizopus rot, Stem end rot, Penicillum rot, Black mold rot, Mucor rot, Phyllosticta rot, Pestalotia rot, Macrophoma rot and powdery mildew, leading to heavy loss in yield (Ploetz, 2001) [24]. Among these diseases, anthracnose is the major disease of mango as it occurs at all the mango growing parts. The anthracnose is caused by the fungal pathogen *Colletotrichum gleosporioides*. The disease affects the flowers, leaves and fruit at various stages of growth and is a major cause of fruit rots in the postharvest supply chain. Despite the development of chemical and cultural control measures for this disease, significant losses to production during harvest are still a reality, especially in years where wet weather is experienced. Over recent years several areas of pathogen / host interactions have been studied that may lead to new management options for the disease.

The genus *Colletotrichum* is an important and wide spread group with more than 900 species reported from wide range of host (Jeffries *et al* 1990). But based on conidial morphology, the number of species has been reduced to 40 accepted taxa (Sutton 1992). Although the genus has been fairly well defined, the species concept isstill complicated due to overlapping morphology and a certain degree of confusion still exists (Freeman *et al* 1998), especially between C. *gleosporioides* and *acutatum* groups, which are considered as species complex composed of diverse sub populations (Sutton1992; Freeman *et al* 1998, 2001.

Before the application of molecular-based phylogenetic analysis using multiple markers for the taxonomic and systematic standing of *Colletotrichum spp*., only two species, *Colletotrichumacutatum* and *Colletotrichumgloeosporioides*, were reported to be associated with anthracnose symptoms in many tropical fruit crops. However, recent multigene phylogenetic analysis established that these two species belong to two separate species complexes called “*acutatum* sp” and “*gloeosporioides* sp.”, with several other sub species Latiffah (2021). Studies on the systematics of *Colletotrichum* species recently have placed species of this genus into nine major clades plus small clusters and isolated species, with clades probably representing species complexes (Cannon *et al*. 2012).

Traditional identification and characterization of *Colletotrichum* species was usually based on morphological characteristics (Hyde *et al*. 2009; Photita *et al*. 2005), such as conidial morphological, production of perithecia (Freeman *et al*. 2000), and sensitivity to benomyl (Valero *et al*. 2010). However, it was found that morphological differences are not sufficient to separate *Colletotrichum* species due to differences for the same species when grown in the lab and under different growth conditions. Hence, molecular techniques are needed to separate species of *Colletotrichum* and to examine genetic variation within and between species and populations (Cai *et al*. 2009; Hyde *et al*. 2009; Phoulivong *et al*. 2010).

The internal transcribed spacer region (ITS) of ribosomal DNA has been frequently used to separate *Colletotrichum* species (Freeman *et al*. 2000) but not always satisfactorily (Cannon *et al*. 2012). Other markers, such as actin (ACT), β-tubulin (TUB2), chitin synthase 1 (CHS-1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene have been used to resolve *Colletotrichum* species (Weir *et al*. 2012). Species differentiation is important to formulate disease control strategies.

In a mixed population of *Colletotrichum* species, sensitivity of one species to a certain fungicide as oppesed to the other may cause a shift in population structure. (Freeman *et al* 1998). Considering recent changes in classification of *Colletotrichum* species, and the lack of information on the identity and characteristics of isolates causing mango anthracnose in Konkanregion, Maharashtra, India. Thus, this study is undertaken to determine the identity of *Colletotrichum* species isolated from anthracnose of mango leaves and fruit by using morphological characteristics and sequencing of ITS regions and beta tubulin gene.

Mango (*Mangifera indica* L) is one of the most popular fruit gown throughout the tropics and subtropics worldwide. It is prone to many fungal diseases, leading to heavy loss in yield. Anthracnose caused by *Colletotrichum gloeosporioides*, isrecognized as one of the most important pre and post-harvest disease limiting mango fruit production worldwide.

Morphological characteristics of fungi is not sufficient for the identification of *Colletotrichum* spp. Therefore characterizations of fungi up to the species level requires molecular identification with using the ITS region.

Investigation on the identification and characterization of *Colletotrichum* isolates to provide useful information about *Colletotrichum* spp. associated with mango in Konkan region of Maharastra, India. Through morphology and sequencing of ITS regions and beta tubulin gene for to suggest strategies to prevent and control pathogen.

It is need to better understanding of *Colletotrichum* species involved and the efficacy of different management tools will enable growers to make more successful integrated management decisions. Improving existing methods for species identification, applying resistance management practices, registering new mode of action and breeding tolerant cultivars will further increase the available option for integrated management.

Reviews on Morphological and Molecular characterization of *colletotrichum* Species Associated with Mango Anthracnose:

Pallem Chowdappa *et al*. (2009) found that anthracnose, caused by *Colletotrichum sp*., is a major disease causing severe yield losses. The objective of the present study was to understand diversity and distribution of *Colletotrichum* species prevalent in major grape-growing areas in India. A total of 30 isolates of *Colletotrichum*, collected from major grape growing regions of Andhra Pradesh, Karnataka and Maharashtra between 2003 and 2005, were characterized using morphological criteria, species-specific PCR and ITS sequencing. Three morphological types were distinguished based on colony growth rate and conidial morphology. Morphological type I (*C. gloeosporioides*) produced cylindrical conidia with both ends rounded and colony growth rates ranged from 11.21 to 12.08 mm/day. Morphological type II (*C. acutatum*) produced fusiform conidia with tapered/rounded ends and growth rate was 6.29 mm/day. Morphological type III (*C. acutatum*) had fusiform and elongated conidia with mostly tapered ends and colony growth rate was 4.66 mm/day. The universal primer pair ITS1 and ITS4 amplified, a 560-bp fragment from genomic DNA of isolates belonging to all three morphological types. Morphological type I isolates were identified as C. *gloeosporioides* on the basis of species-specific diagnostic PCR and rDNA-ITS sequences while morphological types II (isolate CG 5) and III (CG33) were identified as *C. acutatum*. All three morpho groups could also be distinguished based on ITS-RFLP profiles as generated by restriction enzyme, MboI. The present study, thus, clearly established *C. gloeosporioides* as the dominant pathogen (93.33%) with very limited occurrence of C. *acutatum* (6.66%). This is the first report of occurrence of *C. acutatum* and existence of diverse molecular groups among *C. gloeosporioides* and *C. acutatum* populations associated with grape anthracnose in India.

Bally *et al*. (2013) initiated a screening program in mango accessions in the Australian National Mango Genebank to look for any significant resistance to *C. gloeosporioides* in fruit as they ripened. Screening was conducted by rating reactions to natural infection of anthracnose and reactions to artificially inoculating fruit with virulent isolates of *C. gloeosporioides*. A range of reactions to the pathogen were identified, with strong resistance found in one accession of the species M. laurina. This accession was used as the pollen parent in a controlled crossing program with a M. indica hybrid from the Australian Mango Breeding Program (AMBP). Sixty successful hybrids between the species have been generated. The hybrid population will be screened for resistance to anthracnose and used for gene discovery investigations to identify markers for anthracnose resistance.

Latiffah Zakaria *et al*. (2015) investigated *Colletotrichum* species are well-known causal agent of anthracnose. A study was conducted to determine the identity of *Colletotrichum* associated with anthracnose of mango (*Mangifera indica*) fruits. Thirty five *Colletotrichum* isolates were isolated from anthracnose lesion of two mango cultivars, Chokanan and Harum Manis. Based on the conidial morphology, two morphotypes (I and II) of *C. gleosporioides* were identified. Based on BLAST search of ITS regions and β-tubulin sequences, majority of the isolates showed 99-100% similarity with *Colletotrichum sp*. from mango and other hosts and three isolates, 100% similarity with *C.asianum*. From phylogenetic analysis using maximum likelihood method of combined datasets, the isolates from mango formed three clades, which corresponded to *C. gloeosporioides* sensulato and *C. asianum*. Therefore, the present study showed that the isolates associated with anthracnose of mango belong to *C. gleosporioides* sensulato and *C. asianum*.

Amsula Abera *et al*. (2016) identify and characterize *Colletotrichum* species isolates responsible for anthracnose of mango in Southwest of Ethiopia. Samples of infected mango leaves, panicles and immature fruits were collected from home gardens of nine districts in Southwestern part of Ethiopia. Among them eight isolates of *Colletotrichum* species with distinct morphology on PDA were observed in each group. Colony color, shape and diameter of every culture were recorded, conidial size and shapes were computed from 20 conidia per isolate. The results showed that *Colletotrichum* species isolates were grouped into three distinct morphological types*: Colletotrichum gloeosporioide* morph type I (37%) with hyaline cylindrical conidia rounded both ends, Colletotrichum acutatum morphotype II (38%) conidia mass in the center and fusiform tapered to a point in both ends, and *Colletotrichum asianum* morph type III (25%) cylindrical conidia with obtuse to slightly rounded ends. The length of conidia ranged from 10.5-17.8 μm, width (3.22 to 6.9 μm). Among six media tested, highest mean colony diameter of 51.9 mm was recorded on Potato dextrose agar and the lowest mean mycelial growth of 18.4 mm was recorded on Tap water agar. All isolates had good sporulating capacity on general media. Based on the results of this study, it could be concluded that, *C. gloeosporioides C. acutatum and C asianum* were found to be the major causal agents of mango anthracnose. Additional study on the epidemiology of anthracnose of mango is needed for further disease management strategies.

AshafulAlam *et al*. (2017) investigated that *Mangifera indica* L. (mango) is affected by various diseases at different developmental stages. One of the most common diseases of mango is anthracnose caused by *Colletotrichum gloeosporioides* in the world. Proper identification of causal organism is difficult because of the morphological variation. The objective of this study was to characterize the morphological variation of *C. gloeosporioides*. One hundred and forty isolates of *C. gloeosporioides* were taken from anthracnose lesions on fruits, leaves, flower clusters and on twigs of mango from geographic nine regions of Bangladesh. Cultural methods (mycelial growth rate, color, texture, acervuli, conidial size and setae) and microscopic measurements (ocular micrometer and stage micrometer) were used to characterize the isolates. The experiment was conducted by following Completely Randomized Design (CRD) with five replicates. One way analysis of variance was done to check the significant ($p<0.05$) differences. All of the isolates varied significantly ($p<0.05$) among different plant parts and also among the origins. They followed the order of F<FLC<T<L and S2<S4<S9<S1<S8<S3<S6<S5<S7,

respectively. Mycelial growth, size of conidia, acervuli (No. cm–2) ranged from 9.5-10.6 mm day–1, 17.82-30.26 and 1.00-5.40 µm, respectively. Mycelialcolor, texture and setae were present. Isolates were clustered into four distinct groups. Conclusion: It is concluded that morphological variations of *C. gloeosporioides* among different plant parts and their origins were found in respect of their mycelial growth rate, conidial size, acervuli production, mycelial color, texture and setae.

Pavitra Kumari *et al.* (2017) found that mango (*Mangifera indica* L.) is one of the world's most important and esteemed fruits of the tropical and subtropical countries. By the virtue of its wide range, delicious taste, superb flavor, very high nutritive and medicinal value as well as great religion-historical significance, it is called the "King of the fruits". Mango is affected by a number of diseases at all stages of the development right from nursery to post harvest including storage and transit. Its production is drastically affected by *Colletotrichum gloeosporioides*, is one of the most damaging pathogen cause mango anthracnose. This paper reviews the research and development of Anthracnose of mango during the precedent in relation to pathogen taxonomy, distribution, biology, disease cycle and management.

Jianyou Mo *et al.* (2018) [18] investigated sixty-five isolates were obtained from mango leaves with anthracnose symptoms, and these were further characterized based on morphology and DNA sequencing. Twenty-nine isolates from different areas were selected for sequencing and analyses of the internal transcribed spacer region, glyceraldehyde-3-phosphate dehydrogenase, partial actin, b-tubulin, and chitin synthase genomic regions. The most common fungal isolates were these three species. *Colletotrichum asianum, C. fructicola, and C. siamense. C. asianum* was the most common and widely distributed in Guangxi (51.7%), followed by C. fructicola (37.9%) and C. siamense (10.2%), both found in Tiandong, Tianyang, and Wuming counties. There was no evidence of geographical specialization of the different species. Pathogenicity assays showed that all iso-lates, were pathogenic to mango leaves and fruit (cultivar Tainong). No relationship was found between origin of isolates and their virulence. This is the first description of *C. asianum, C. fructicola, and C. siamense*as causal agents of mango leaf anthracnose from Guangxi province, China.

Sohail A *et al* (2018) [25] fouused to isolate and identify filamentous fungi from different environmental sources in northern eastern Jordan Deseret. The fungal species were isolated from soil and plant part (Fruits and leaves) samples collected from different geographical location in northern eastern Jordan Deseret. Isolation of fungi from leaves and fruits was implemented by inoculating (1ml) from serial dilutions (10-3- 10-6) on Potato Dextrose Agar (PDA) plates, the plates were incubated at 28˚C for one week then the fungal colonies were observed and pure cultures were maintained. The identification of fungi at the genus level was carried out by using macroscopic and microscopic examinations depending on colony color, shape, hyphae, conidia, conidiophores and arrangement of spores. For molecular identification of the isolated fungi at species level the extracted fungal DNA was amplified by PCR using specific internal transcribed spacer primer (ITS1/ITS4), the PCR products were sequenced and compared with the other related sequences in Gen Bank (NCBI). Eight fungal species were identified as *Aspergillus niger, Aspergillus tubingensis, Alternaria tenuissima, Alternaria alternate, Alternari agaisen, Rhizopus stolonifer, Penicillium citrinum, and Fusarium oxysporum*, results showed that the *Aspergillus niger* was the most abundant fungus obtained from all locations and resources while the *Alternaria tenuissima* was the less one. We also noticed that two of *Alternaria* species were colonized the leaves of plants at different locations. *Rhizopus stolonifer, Aspergillus tubingensis, and Fusarium oxysporum* were isolated and identified from all resources and locations.

Latiffah Zakaria *et al* (2021) found that in tropical fruit crops, anthracnose is mainly caused by species belonging to the fungal genus, *Colletotrichum*. These phytopathogens can infect several parts of the fruit crops; however, infection during postharvest or ripening stages is responsible for major economic losses. Due to the formation of black to dark brown sunken lesions on the fruit surface, anthracnose reduces fruit quality and marketability. Among the most common tropical fruit crops susceptible to anthracnose are mango, papaya, banana, avocado, guava, and dragon fruit; these are economically relevant products in many developing countries. It is important to document that the newly recorded *Colletotrichum* spp. associated with fruit anthracnose can infect multiple hosts, but some species may be host-specific. By using multiple markers, many phylogenetic species of *Colletotrichum* have been reported as anthracnose-causing pathogens. Taking into account that disease management strategies strongly rely on adequate knowledge of the causative agents, updated information on *Colletotrichum* species and the hazard posed by the most recently identified species in tropical fruit plantations and harvested fruits becomes vital. Besides, the newly recorded species may be important for biosecurity and should be listed as quarantine pathogens, considering that tropical fruits are traded worldwide.

Conclusion

From the above reviews expressed by different reasearchers from worldwide on this aspect it is concluded that, various morphological and molecular examination methods needs to be used to different fungal isolates of mango anthracnose and it should be screened for their morphological and molecular characterization. There is need to work on morphology and DNA sequence comparisons for this same issue in Mango. In upcoming researches from globe it is part of attention to do identification of Pathogenecity and virulence of isolates and molecular phylogenetic analysis will help to identification and characterization of *Colletotrichum* species.

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