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ORIGINAL ARTICLE

Assessment of *Colletotrichum capsici* and *C. gloeosporioides* Resistance among Chilli Accessions Available in Sri Lanka

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Abstract

Anthracnose is one of the most destructive fungal diseases in chilli (*Capsicum annuum* L.), which affects the seed production and post-harvest quality of the product. There are no reports available on the resistance levels of chilli varieties to anthracnose caused by *Colletotrichum* spp. in Sri Lanka. Therefore, the present investigation was carried out to find sources of resistant for anthracnose in locally available chilli accessions. One isolate from each, *C. capsici* and *C. gloeosporioides* species were selected for the study. Ripened chilli pods of twenty-one chilli accessions were screened against both *Colletotrichum* spp. (droplet of spore suspension-10^5 spores/ml) separately under in-vitro conditions (28 ˚C-temperature, 100%-RH) during Maha 2016/17 with three replicates. The results showed that the disease reaction of *C. capsici* and *C. gloeosporioides* isolates on different chilli accessions were not significantly different from each other ($p>0.23$). However, a significant difference ($p < 0.0001$) in Disease Severity Index (DSI) for anthracnose among chilli accessions was observed for both isolates. The highest DSI value of 79.47 for both *C. capsici* and *C. gloeosporioides* isolates, respectively. DSIs of Jaffna Purple for both *Colletotrichum* spp. were significantly different from the DSIs of the other accessions. The lowest DSI values were observed in MI Hot (19.47 and 19.47), Hen miris (24.73 and 24.73) and *Galkiriyagama* selection (23.21 and 27.03) for *C. capsici* and *C. gloeosporioides*, respectively; which, also grouped together. According to the results, MI Hot, *Galkiriyagama* selection and Hen miris showing lower DSI values can become sources for developing anthracnose resistant/ tolerant chilli lines.

Keywords- *Anthracnose, Chilli accessions, Colletotrichum spp., Resistance*

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1. Introduction

Chilli (*Capsicum annuum* L, Family *Solanaceae*) is one of a top ranked vegetable crop as well as an important spice, and a medicinal plant. Additionally, with high consumption, nutritional aspects and cash value, chilli is important for both farmers and consumers. China is the largest chilli producer in the world with around 17 million tonnes production in year 2017 (FAOSTAT 2017). In Sri Lanka, chilli is an economically important crop and considered to be an essential ingredient in Sri Lankan cuisine. Chilli is cultivated in the dry and intermediate zones of Sri Lanka. *Anuradhapura, Moneragala, Ampara, Puttalam, Vavuniya, Kurunegala* and *Hambanthota* are the major chilli producing areas in Sri Lanka (Agstat 2015). In year 2017, the extent of green chilli cultivation was around 10,937 ha. The annual production of dry chilli was about 51,827 t, and the average yield was around 4.74 tha^{-1}. Therefore, an amount of 51,692 t had to be imported in 2016 (AgStat 2017). The low yield in chilli is mainly due to high pests and diseases incidence, such as leaf curl complex and anthracnose. Anthracnose caused by *Colletotrichum* spp. (Kingdom Fungi; Phylum Ascomycota, Class Sordariomycetes; Order Phyllachorales and Family Phyllachoraceae), is a destructive fungal disease in chilli cultivation, worldwide. Anthracnose disease of chilli was first reported by Halstead from New Jersey, USA in 1890 (Than et al. 2008). Yield losses due to anthracnose is reported to be up to 80% in Thailand (Montri et al. 2009), 66-84% in India (Begum et al. 2015), 15% in Korea (Kim and Park1989), 50% in Malaysia (Sariah 1994) and 21-47% in Sri Lanka (Rajapakse et al. 2002). Several *Colletrotrichum* spp.; *C. capsici* (Sydow) Butl. and Bisby, *C. gloeosporioides* (pent.) Penz. & Sacc., *C. acutatum* Simmonds, *C. coccodes* (Wallr.) Hughes and *C. graminicola* (Ces.) Wils are known to cause anthracnose in chilli in different parts of the world (Hadden and Black 1989). *C. capsici* has been identified as the major pathogen associated with anthracnose in chilli in Asia, Australia, Africa, and North and Central America. *C. gloeosporioides* and *C. capsici* have been identified as the causal organisms of chilli anthracnose in Sri Lanka (Sariah 1994; Rajapakse and Ranasighe 2002). Anthracnose disease is known to occur in three main phases; (i) seedling blight or damping off stage, which is prevalent in nurseries, (ii) leaf spotting and die back stage, which is initiated during different stages of growth, and (iii) fruit rot stage in which the ripen fruits are infected (Begum et al. 2015). Infection of ripen pods causes extensive damage to the fruits, since it reduces the market quality of the product. The pathogen can be spread by air and contaminated seeds and affects seed germination as well as plant vigour (Begum et al. 2015). Presence of high relative humidity and high temperature accelerate the anthracnose epidemic in chilli cultivation (Roberts et al. 2001). Relative humidity plays a critical role in the development of anthracnose. In addition, the grower's negligence plays an important role in spreading the disease at an alarming rate. Pathogen inocula may spread from the previous crop residues to soil or to

alternative hosts. The poor field sanitation along with these conditions leads to epidemics of the disease. Characteristics and virulence of the pathogen can vary depending upon environmental fluctuations, wide host range and inappropriate chemical applications. As a result, the degree of anthracnose incidence could vary from field to field and season to season. Thus far, the disease is mostly managed by using agro-chemicals including systemic and contact fungicides, which results in the accumulation of harmful pesticide residues in to the eco system. However, they do not provide a satisfactory level of control, especially under wet conditions (Rajapakse and Ranasighe 2002). On the other hand, application of chemicals to control anthracnose is also difficult due to smallholder and homegarden farming systems present in Sri Lanka. Application of agro-chemicals in controlling chilli anthracnose is impossible when the disease occurs during the storage. Management of disease through host resistance is one of best options available in modern agriculture. Therefore, the present investigation was carried out to find out the sources of resistance to anthracnose in different chilli accessions available in Sri Lanka.

2. Materials and methods

2.1 Plant materials

Twenty-one chilli accessions (Table 1) were grown in experimental fields and under greenhouse conditions at the Field Crops Research and Development Institute, Mahailluppallama, Sri Lanka during Maha 2016/17 under the recommended agronomic practices of the Department of Agriculture, Sri Lanka. These twenty-one chilli accessions consisted of local, introduced and imported commercial varieties, which have been recommended by the Department of Agriculture for cultivation in different agro-ecological regions of Sri Lanka.

2.2 Collection and Isolation of Pathogen

Ten pods samples, with characteristic anthracnose symptoms, were randomly collected from different locations of the Field Crops Research and Development Institute, Mahailluppallama, Sri Lanka during 2016/17 *Maha* season. Tissues of 5 mm diameter were collected from the edges of lesions were surface sterilized with 70% ethanol for 30s, followed by 1% NaOCl for 1 min and washed twice with sterilized double distilled water. They were dried on sterile filter papers and placed on potato dextrose agar (PDA) media supplemented with streptomycin (50 mgL^{-1}). Cultured plates were incubated at 28 ˚C for 7 days. Koch's Postulation procedure was followed to confirm the causal organism.

Table 1: List of chilli accessions used to evaluate anthracnose disease resistance

No	Accession Name	Parents/Source
01	Arunalu	MI-2 x Santaka
02	CAH 36	From International chilli nurseries
03	Galkiriyagama Selection	Selected from landraces
04	Hot beauty	From AVRDC
05	Hen miris	Improved landrace
06	ICPN	From international chilli nurseries
07	Kochchi 1	Not known
08	Kochchi 2	Not Known
09	KA-02	MI-2 x PC-1
10	MI – 02	Selection from MI-1
11	MI Hot	(BL 39 x IR) x KA-2
12	MI Green	(MI-2 x IR) (MI-2 x 142A)
13	MICH3	MI-1 x Wander Hot
14	MICH HY1	Galkiriyagama Selection x MI waraniya 1
15	Jaffna Selection	Selected from a landrace
16	Waraniya	Selected from a landrace
17	Waraniya purple	Not known
18	PBC 380	From Malayasia
19	Line 985.3	From AVRDC
20	Jaffna purple	Farmer collection
21	*Capsicum baccatum*	Obtained from Plant Genetic Resources Centre of Sri Lanka

2.3 Morphological and cultural examination

Mycelial discs were obtained from actively growing edges of 5 days old cultures and transferred to PDA media supplemented with 50 $mg^{-1}L$ streptomycin. Plates were incubated at 28 ˚C for 7 days. Pathogen was identified by colony morphology on PDA and spore characters were observed under the microscope and referring to published literature. Two species of the pathogen were identified as the *C. capsici* and *C. gloeosporioides*. The Diameter of colonies was recorded each day along two axes perpendicular to each other across the culture. Isolates of the pathogen were grown on PDA slants were stored at 4 ˚C for further studies.

2.4 Preparation of inoculum

One isolate from each species *C. capsici* and *C. gloeosporioides* was selected and spore suspensions of both isolates were prepared. Ten-day old culture plates were flooded with sterilized distil water and the conidia were gently scraped from the culture plates using a sterilized loop (Montri et al, 2009). The spore concentration was adjusted to 10^5 conidia mL^{-1} (approximately) using a haemocytometer.

2.5 Pathogenicity study

Nine healthy ripened pods plucked from a few plants of each chilli accession (40-50 days after flowering) were surface sterilized with 70% ethanol followed by 2-3 washing with sterilized distilled water in a laminar flow cabinet. The pods were wounded softly with a flame

sterilized needle and inoculated with a 50 µl droplet of *C. capsici* and *C. gloeosporioides* spore suspensions separately. The inoculated pods were placed on aluminium nets, kept in plastic boxes at 28 ˚C. In the boxes the water level was adjusted in a height of 1.5 cm, which was just below the base of aluminium net maintaining 100% relative humidity. The lesion development was measured ten days post-inoculation. Anthracnose symptoms were evaluated based on the developed lesion size compared to the overall size of the pod (Montri et al. 2009). The treatments were arranged in a complete randomized design as a two-factor factorial experiment (two fungal isolates x twenty-one accessions). Data were analysed using SAS software version 9.0 to test the main effect and their interactions. Mean separation was preformed according to Duncan's multiple range test (p<0.05). According to the method described by Montri et al. (2009), disease severity was assessed on a 0 - 9 scale; where, 0 was no infection and 9 was infection greater than 25% (Table 2.). Disease Severity Index (DSI) was calculated for each accession based on equation DSI = (Total sum of numerical rating/ Number of observations X Maximum disease rating scale) X 100 according to Montri et al. (2009).

Table 2. Anthracnose severity scores on chilli pods, chilli resistance level and symptom description

Score	Resistance level	Symptom details
0	HR, highly resistance	No infection
1	R, resistance	1–2% of the fruit area shows necrotic lesion or a larger water-soaked lesion surrounding the infection site
3	MR, moderately resistance	>2–5% of the fruit area shows necrotic lesion, acervuli may be present, or water-soaked lesion up to 5% of the fruit surface
5	MS, moderately susceptible	>5–15% of the fruit area shows necrotic lesion, acervuli present, or water-soaked lesion up to 25% of the fruit surface
7	S, susceptible	>15–25% of the fruit area shows necrotic lesion with acervuli
9	HS, highly susceptible	>25% of the fruit area shows necrosis, lesion often encircling the fruit; abundant acervuli

Montri et al. (2009)

3. Results and Discussion

In the study, isolated chilli anthracnose causal organisms were identified as *C. capsici* and *C. gloeosporioides* according to their colony characteristics (Table 3). According to colony morphology there were two types of conidia (Fig 1; d) sickle shaped conidia (falcate) from *C. capsici* isolate and (Fig 1; h) cylindrical conidia from *C. gloeosporioides* isolate.

Table 3: Morphological characters of *C. capsici* and *C. gloeosporioides* isolates

	C. capsici	***C. gloeosporioides***
Colony colour on PDA	Initial white colonies turned to brown and grey colour with dark centre.	Initial white colour colonies, turned to light orange with time and then turned to grey colour
Reverse of the colony	Grey in colour with dark concentric rings	Dark colour, zonate, colonized, with abundant orange colour conidial masses
Colony shape	Oval shape colonies, fluffy mycelium	Oval shape colonies, fluffy mycelium
Conidia	Hyaline with both ends curved, pointed and gradually tapering towards the both ends.	Cylindrical with rounded ends. Presence of vacuoles
Appressoria on pod surface	Black colour abundant, oval shape	Black colour abundant, oval shape

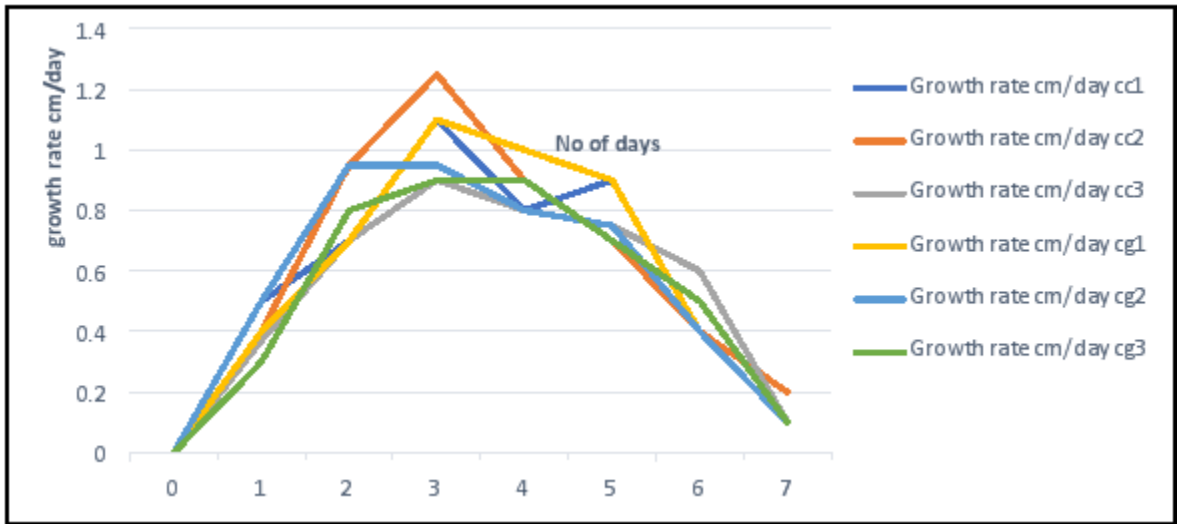

Figure 1. Growth rates of *Colletotrichum capsici* and *C. gloeosporioides* on PDA (tested *C. capsici* isolates named as cc1,cc2 cc3 and *C. gloeosporiodes* isolates cg1,cg2,cg3)

The accessions of *Capsicum* spp. used in the pathogenicity study exhibited a range of host reactions to two pathogen isolates. The results showed that the disease reaction of *C. capsici* (mean = 40.68) and *C. gloeosporioides* (mean = 39.47) isolates on different chilli accessions were not significantly different *(p=0.23*) from each other (Table 4). All tested chilli accessions showed similar reactions in pathogenicity test with respect to *C. capsici* and *C. gloeosporioides*. Therefore, it can be considered that the virulence of *C.capsici* and *C. gloeosporioides* are

almost similar. Colony growth with time on PDA was also found to be similar between the *C. capsici* and *C. gloeosporioides* isolates (Fig.1) and it suggests that both the pathogens have similar adaptations. However, there was a significant difference *($p < 0.0001$)* in DSI of anthracnose among chilli accessions with both the isolates. The highest DSI values (79.47 and 79.47) were observed with Jaffna Local for both isolates and they were significantly different from the DSI of the other accessions. The lowest DSI values were observed with MI Hot (19.47 and 19.47), Hen miris (24.73 and 24.73) and *Galkiriyagama* selection (23.21 and 27.023) for *C. capsici* and *C. gloeosporioides,* respectively (Table 4).

Identification of anthracnose disease resistance in *Capsicum* spp. is important, since the application of agrochemicals to control the disease is environmentally hazardous and practically impossible in certain situations like storage. The 21 tested accessions of this study have different genetic backgrounds (Table 1). Therefore, possibility for differential resistant reactions to anthracnose was expected from the tested accessions. Most of the accessions used in the study showed similar and higher susceptibility reactions with both isolates. However, MI Hot, *Galkiriyagama* selection and Hen miris showed the lowest DSI values, showing their potential to use in developing of anthracnose resistance cultivars (Table 4).

The accession, MI Hot has been developed from (BL 39 x IR) x KA-02 cross (https://www.doa.gov.lk). It showed moderate tolerance to anthracnose disease in this study and field tolerance to other fungal diseases, according to the existing data available at the Field Crops Research and Development Institute (data are not shown here). One of the parents used in the study, KA-02 has shown the high level of DSI values for both isolates: 36.72 and 38.14 for *C. gloeosporioides* and *C. capsici*, respectively.

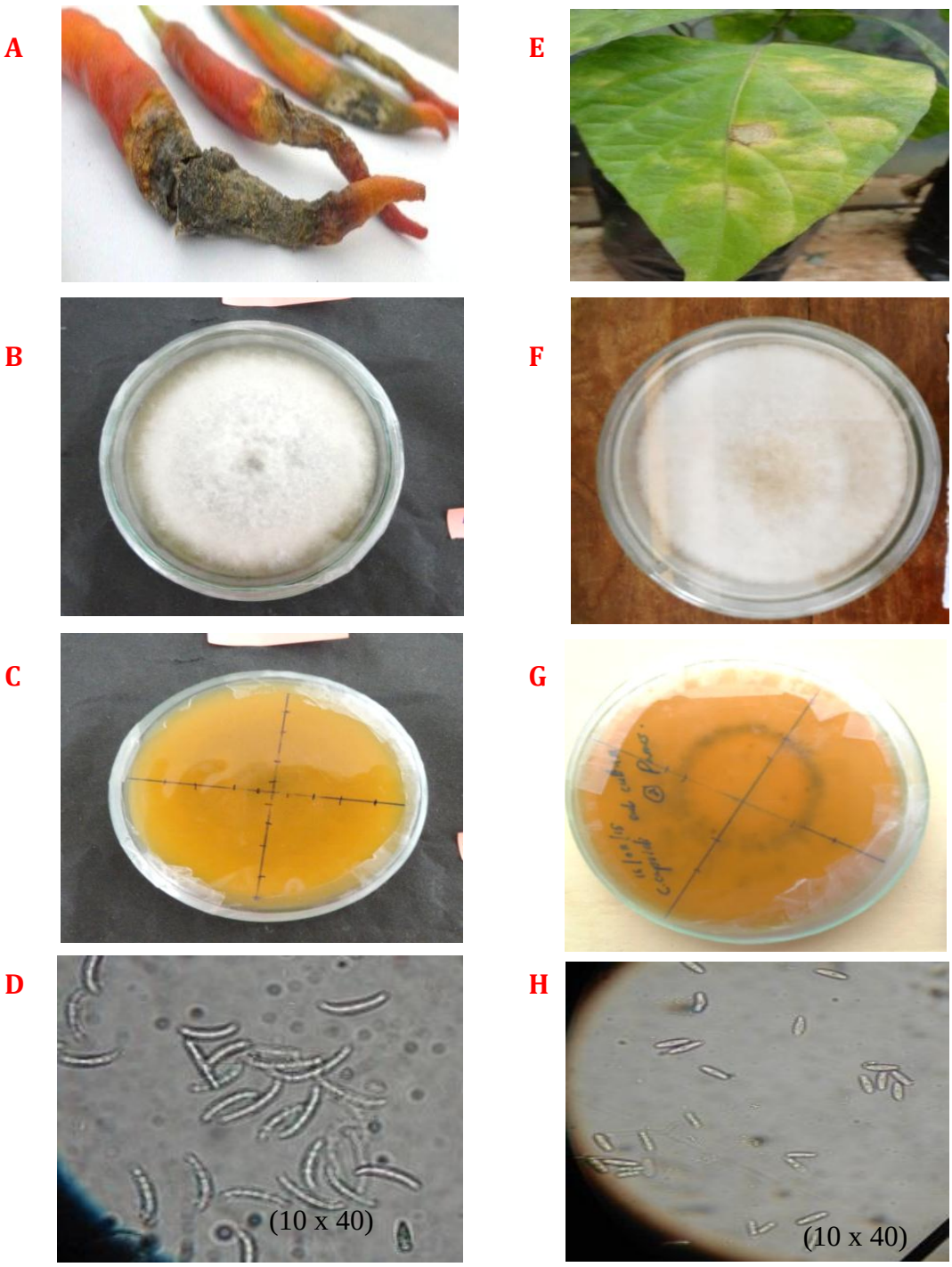

Plate 1. **a.** Anthracnose infected MI-02 pod from FCRDI/MI, b,c and d- 7 day old colony, reverse of colony, and spores of *C. capsici*, respectively e. Anthracnose in infected Kochchi Leaves from FCRDI/MI f, g, and h 7 day old colony, reverse of colony and spores of *C. gloeosporioides,* respectively

Table 4: *In-vitro* differential reaction of twenty-one chilli accessions artificially inoculated by the *Colletotrichum capsici* and *C. gloeosporioides*

	Accession Name	DSI (*C. capsici*)	DSI (*C. gloeosporioides*)
01	Arunalu	29.94 ^{e}	27.03^{f}
02	CAH 36	43.93^{d}	43.93^{d}
03	Galkiriyagama Selection	23.21^{f}	27.03^{f}
04	Hot beauty	42.45^{d}	39.56^{d}
05	Hen miris	24.73^{f}	24.73^{f}
06	ICPN	60.23^{b}	61.85^{b}
07	Kochchi 1	49.62^{c}	48.18^{c}
08	Kochchi 2	43.92^{d}	48.76^{c}
09	KA-02	38.14^{d}	36.72^{d}
10	MI – 02	26.74^{f}	28.74^{e}
11	MI Hot	19.47^{f}	19.47^{f}
12	MI Green	38.19^{d}	38.19^{d}
13	MICH3	26.74^{f}	28.74^{e}
14	MICH HY1	40.93^{d}	45.35^{c}
15	Jaffna Selection	39.63^{d}	39.61^{d}
16	Waraniya	61.85^{b}	61.85^{b}
17	Waraniya purple	29.99^{e}	33.73^{e}
18	PBC 380	52.65^{c}	54.07^{c}
19	Line 987.3	35.25^{e}	35.25^{d}
20	Jaffna local	79.47^{a}	79.47^{a}
21	*Capsicum bacatum*	28.55^{f}	26.95^{f}
	C. capsici (mean = 40.68^{a}) and *C. gloeosporioides* (mean = 39.47^{a})		

(Values showing different letters in reactions are significantly different at 0.05 probability level. (CV% = 14.0)

Therefore, the lower susceptibility character observed in the MI Hot accession must have received from the parents BL 39 and or IR. The accession, *Galkiriyagama* Selection has been developed through evaluation and selection from local landraces from the North Central province of Sri Lanka. *Galkiriyagama* Selection has characters like small leaves and dry chilli storability around 5 months without a colour change. Hen miris also an improved variety from selection and purified landrace found in Sri Lanka. According to Mahasuk et al. (2009), *Capsicum baccatum* species have resistance against anthracnose pathogens. In the present experiment *C. baccatum* accession also showed low DSI value for both isolates (26.95 and 28.55 for *C. gloeosporioides* and *C.capsici,* respectively) (Table 4). Yoon et al. (2005) mentioned that genetic resources, which are resistant to anthracnose, are found only in *C. baccatum* in Korea. Further they mentioned that interspecific hybridization is the only way

to transfer the resistance from *C. baccatum* to *C. annuum* varieties.

The mode of inheritance in anthracnose resistance it has been described as single gene or polygenic genes (Ahmed et al. 1991; Lin et al. 2002; Voorips et al. 2004; Lee et al. 2010). According to Pakdeevaraporn et al. (2005) and Kim et al. (2008), anthracnose resistance is governed by dominant or recessive gene actions. Park et al. (1990), Yoon et al. (2005), Lee et al. (2010) reported that anthracnose resistance has additive or non-additive effects. Sun et al. (2015) reported that the resistance of pepper anthracnose depends on a major QTL on chromosome P5. In contrast, some researchers reported that anthracnose resistance is inherited recessively with epistatic effects (Cheema 1984). The results of the present experiment did not show the dominant or recessive gene action for anthracnose resistance and this was evident by the presence of reaction values in a range for resistance. However, identification of the mode of inheritance in chilli anthracnose is complicated. Taylor (2007) and Begum et al. (2015) reported that there are no tolerant varieties against *Colletotrichum* spp. in chilli. The difference in the susceptibility/ resistance of the tested accessions to anthracnose suggests the presence of QTL or additive gene effect. Results revealed that a single or multiple cross using MI hot, Galkiriyagama Selection, Hen miris and *C. baccatum* may generate better resistant varieties for chilli anthracnose caused by *C. gloeosporioides* and *C. capsici*.

4. Conclusions

According to our knowledge this is the first study that evaluated the disease reaction for anthracnose in recommended chilli varieties, landraces and other accessions available in Sri Lanka. This study revealed that local and exotic chilli accessions are carrying different levels of susceptibility reactions, varying from low susceptibility to high susceptibility. The study also suggested the presence of QTL or additive gene effect against *C. gloeosporioides* and *C. capsici*. Accessions, which showed low DSI values; MI Hot, Galkiriyagama selection and Hen miris can be used for developing resistant chilli varieties through breeding programme. In addition, there is a possibility of finding resistance sources for anthracnose in the gene pool, where Galkiriyagama Selection and Hen miris accessions have been derived.

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