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Morpho-pathological Variability of Chili Anthracnose (*Colletotrichum capsici* (Syd))Bisby and Butler) in Southern Nations Nationalities Peoples and Oromiya Region, Ethiopia

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Abstract
Chili had immense dietary and economic importance for Ethiopians. Ironically, it suffers profound losses in yield due to anthracnose caused by Colletotrichum capsici. But scientific information on variability of the Colletotrichum spp in the country is insufficient. Thus, the present study was undertaken to determine the morpho-pathological variability to design better management practices of Colletotrichum capsici to avoid losses. Morpho-pathological variations were studied in twenty isolates of C. capsici were collected from main chili growing farms located in southern and oromiya regions. The isolates were cultured and identified. Variations among isolates in terms of Colony size, color, shape, marginal pattern and characteristics had been observed. Nine differential virulence patterns were depicted amongst 20 locally acclaimed chili genotypes. Cultural tests had categorized the isolates into ten different groups. Disease incidence was also. Colonies varied in their cultural attributes ranging from cottony to fluffy, mostly suppressed with regular to irregular margins. Color of colonies ranged between cottony white to dark grey. Growth rate of isolates was between 22.0-69.5mm. Morphological studies of isolates revealed variations in their color, size, shape, acervuli production, setae size and shape, conidia. Average conidial size varied from 18.00-33.3 µm and average setae size varied from 77.2-181.2 µm. In conclusion, the existence of variations among Colletotrichum capsici isolates strengthened the present knowledge on the identification and understanding pathogen which has immense contribution for ultimate anthracnose disease management.

Key Words: *Chili anthracnose, Colletotrichum capsici, Morphological variability, Pathological variability*

Introduction

Chili (*Capsicum frutescence* L.) is an important cash crop grown worldwide (Makari *et al*., 2009; Bosland and Votava, 2000)and in Ethiopia (Seleshi, *et al.*, 2014; EEPA, 2003). It is prone to number of fungal bacterial and viral diseases (Devi and Prakasam, 2014). Which significantly affect its production and quality? However, huge losses to the crop are incurred mostly by fungal diseases. Of these diseases, dieback and fruit rot has assumed the status of major disease in some important chili growing countries(Makari *et al*., 2009; Sharma *et al*.,2005). Anthracnose causes extensive pre and post-harvest damage to chili fruits causing anthracnose lesions (Mehrotra and Aggarwal, 2003). Even small anthracnose lesions on chili fruits reduced their marketable value (Masoodi *et al*., 2013). *Colletotrichium capsici* is most adhesive that adhere to the plant surface and remain latent until such physiological changes occur in the fruit and cause economic losses to the farmers due to low fruit quality and is marketability(Canoon *et al*., 2011; Noireung *et al.*, 2012). Many post-harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until the fruit ripens (Masoodi *et al*., 2013;Agrios, 2005).*Colletotrichum* species have the most adhesive discs that adhere to plant surfaces and remain latent until physiological changes occur in fruits (Mehrotra and Aggarwal, 2003).

Chili anthracnose usually develops under high humid conditions when rain occurs after the fruits have started to ripen with reported losses of up to 84% (Rajapakse *et al.*, 2007; Tameru *et al.*, 2003; Tameru, 2004). Economic losses caused by the disease are mainly attributed to lower quality and marketability. Variability of the progeny exhibits a characteristic that is different from those present in the ancestral individuals or descent individuals, this individual is called a variant (Agrios, 2005).

Sharma *et al.* (2005) studied the pathogenic variability in *C. capsici* studied and found 15 pathotypes of *Colletotrichium capcisi* that existed from 30 isolates studied in India and proposed 15 pathotypes of *C. capsici* existed among 37 isolates from different chili

growing regions in India. In recent years, anthracnose disease has frequently been observed in Ethiopia and is assuming serious proportions causing heavy losses to the crop. It has been revealed that except the evaluation of fungicides against the causal pathogen are incomplete (Seleshi *et al.,* 2014;Tameru*et al.,* 2003; Tameru, 2004). However, no systemic work on the variability of *Colletotrichum capsici* has been conducted in pepper growing regions of Ethiopia where agro-climatic conditions are entirely different from rest of the country (Tameru *et al.,* 2003; Tameru, 2004). The frequent epiphytotics of the disease in valley witnessed during past few years and extent of the damage inflicted by it, has necessitated us to generate basic information on variability of the pathogen. The present studies were therefore initiated to find out the variability of *Colletotrichum capsici* isolates.

Materials and Methods

Sampling Area

Extensive survey was carried out in the South-western, Chili growing parts of Ethiopia during growing season (June-September) 2013 and 2014 for anthracnose disease assessment. Chili fruits from susceptible cultivars exhibiting typical and variable symptoms of *C. capsici* were collected from twelve chili growing locations across four districts of SNNP, two districts of Amhara and two districts of Oromiya viz., Alaba, Maraqo, Shashogo, Humbo-tabala, Adama and Lome districts.

Preparation of inoculum and Cultural Characteristics

Cultural variability among the isolates was studied on the basis of the standard suggested by (Tesfaye and Kapoor, 2007). Five millimeter mycehal discs of 7 days old culture of each isolate was transferred to the centre of sterilized Petri plates containing potato dextrose agar medium and incubated at 25±1 ^{0}C. Colony character viz., color and margins were recorded after 10 days of inoculation by taking two perpendicular measurements and their average calculated.

Morphological Variation

The morphological variation among the various isolates of *C. capsici* was studied on artificial culture in the laboratory. Mono-conidial culture of each isolate was first grown on potato dextrose agar medium and then semi-permanent shades prepared from 10 days old culture, stained with cotton blue in lactophenol. The important characters studied were based on Septation, color and length of hyphae; length and breadth of setae; color and size of acervulli and conidia (Masoodi*et al*., 2013).

Study on Pathogenicity tests

Fourteen-day-old cultures of *Colletotrichum* spp. grown on PDA under 12 h of alternate light and dark conditions, maintained at 26–28°C were used for making conidial suspension. The plates were flooded with sterile distilled water and gently scrapped by sterile loop to collect the conidia from the culture. The suspension was filtered between layers of muslin cloth and concentration was adjusted to 10^6 conidia ml^{-1} using a haemocytometer (Masoodi *et al*., 2013).

Locally available fresh chilli fruits of varieties, namely, '*Melkazala, Maraqofana, Melkashote, Weldele, Bako local, Odaharo, Dube medium, Dube short* and*Gojeb local'* were harvested at pre- and post-ripening stages to study the pathogenicity of Colletotrichum isolates by injection method. The fruits were surface-sterilized with 1% sodium hypochlorite solution for 5 min and rinsed with sterile distilled water for two to three times. Ten micro-liters of a conidial suspension were injected at the centre of each fruit using a sterile syringe. The fruits were then kept in moist chambers maintained at 25–26°C and 98% relative humidity. Un-inoculated but wounded fruits served as control. Five fruits of ripe and unripe stage were considered for studying the pathogenicity of each Colletotrichum isolate. Inoculated fruits were evaluated for anthracnose symptoms after 9 days of incubation on the basis of lesion size relative to overall size of fruit. The rate of lesion progression on ripe and unripe fruits was measured every day.

Disease severity was scored on a 0–9 scale (0 = no infection,1 = 1–2%, 3 = 3–5%, 5 = 6–10%, 7 = 11–25% and 9 =>25% infected fruit area) modified from the method used by Siddiqui*et al.*(2008). The experiment was replicated three times to confirm the results. The pathogen was re-isolated after 10 days using direct isolation and was cultured on PDA to morphologically identify and compare with the original isolate in order to fulfill the Koch's postulates(Masoodi*et al*., 2013).

In order to identify physiological races of *C. capsici*, attempts were made to develop a differential set of capsicum varieties. Twenty cultivars of *C. frutescence* originated from nine known genotypic lines were evaluated for resistance to 20 chosen isolates of *C. capsici.* These included Melkazala, Maraqofana, Melkashote, Weldele, Bako local, Odaharo, Dube medium, Dube short and Gojeb local from Melkassa Agricultural Research Center (MARC). The genotypes were diverse (two from each) with respect to their collection sites too.

Detached Fruit Method

In this trial, the fruits collected from chili growing areas were carefully selected and subjected to study based on the method recommend by Tesfaye and Kapoor (2007). Then, the fruits were first washed with distilled water and pin pricked gently with sterilized needle prior to inoculation and then inoculated by placing uniform drop of spore suspension. The inoculated fruits were placed in a humidity chamber and kept at 25±1^{0}C. The growth of the pathogen was recorded up to 7 days after inoculation.

The disease was estimated by visual observation based on the lesion development on the fruit. The disease reaction was scored on the basis of 0-5 point scale modified from the disease reaction was analyzed using Susheela(2012); Where, (+++) no infection, (++) 1-2% fruit area infected, (+) 2.1-5% fruit are infected, (-) 5.1-10% fruit area infected, (- -) 10.1-25% fruit area infected and (- - -) >25% fruit area infected. For pathotype groupings, reaction types of(+++),(++) and (+) or either of these were graded as resistant (+) while those falling in (- - -), (- -) and '-' or either of these were rated as susceptible (-).

Analysis of data

The data of various experiments were subjected to statistical analysis with the help of computer. The data was subjected to appropriate transformations, wherever needed as suggested by Gomez and Gomez (1984) before analysis.

Results

As the experimental findings of this research depicts, on the basis of morphological characters, pathogenicity and comparison with the authentic description Kumar *et al*. (2015) the fungus was identified as *C. capsici.*

Variability Study

Variability amongst the isolates was recorded with respect to cultural, morphological and pathogenic characters.

Isolation and Purification of *Colletotrichum capsici* Isolates

From a total number of 48 isolates of *C. capsici* obtained, only twenty of them were selected for the experiment. On this basis, 20 different chili growing areas in the 3 surveyed regions (SNNP, Oromiya, and Amhara) in which prevalence was suspected to be high had been considered. Purification had been undertaken in Department of Microbial, Cellular and Molecular Biology laboratory, College of natural and Computational Sciences, Addis Ababa University

Table 1. Variability in cultural characteristics of different isolates of *Colletotrichum capsici* on Potato Dextrose Agar medium

Isolate	Source/Location	Colony Characteristics			Mycelial growth after 7days (mm)
		Type and Color	Shape (Center)	Margin Pattern	
AAUCC1	Alaba	White	Fluffy	Regular, White	54.60
AAUCC2	Alaba	White	Fluffy, Greyish	Regular, V-Shaped	53.52
AAUCC3	Alaba	White	Cottony	Irregular	52.80
AAUCC4	Alaba	Grey	Whitish , Raised	V-Shaped, Irregular	69.5
AAUCC5	Maraqo	White	Suppressed	V-Shaped, regular	45.5
AAUCC6	Maraqo	White	Fluffy, Greyish	irregular	55.0
AAUCC7	Maraqo	White, Fluffy	Whitish Raised	Suppressed	22.0
AAUCC8	Maraqo	White	Cottony	Regular	58.0
AAUCC9	Humbo	Grey, White	White	Suppressed, White	50.2
AAUCC10	Humbo	White	Cottony	Regular	53.5
AAUCC11	Humbo	Dull White, Fluffy	Brown	Irregular	59.0
AAUCC12	Shashogo	Dull Grey	Suppressed	Regular	52.5
AAUCCl3	Shashogo	Dull White,	Greyish	Irregular	55.5
AAUCC14	Shashogo	Dull White	Light Grey, Fluffy	Regular	54.0
AAUCCl5	Shashogo	White	Fluffy	Regular	52.5
AAUCC16	Arsi Neg.	White, Fluffy	Greyish	Regular, White	55.0
AAUCC17	Wonji	Light Brown	Greyish	Irregular	50.0
AAUCC18	Adama	Light Smokey Grey	White Tinge, Raised	Regular	53.5
AAUCCl9	Amhara	brown,	Grey , Fluffy	Irregular	55.5
AAUCC20	Amhara	smoky grey	White, suppressed	Regular, V-Shaped	53.5

**Mean of 20observations, AAUCc:=Addis Ababa University Colletotrichum capsici*

Cultural Variability

Isolates of *Colletotrichum capsici* differed with respect to their cultural characteristics. The characters viz., type and color of colony, growth rate of fungus and pigmentation were recorded.

Type and Color of colony

The twenty isolates of *Colletotrichum capsici* were grown on PDA showed variation in their colony characteristics. Colony color varied from light to dark grey with whitish or brownish tinge. Mostly the colonies had cottony or fluffy mycelial growth with regular to irregular margin (Table 1). The fluffy growth was observed in six isolates viz., AAUCc-1, AAUCc-3, AAUCc-6, AAUCc-14, AAUCc-15 and AAUCc-19 whereas cottony growth in three isolates viz., AAUCc-3, AAUCc-8, and AAUCc-10. Among the studied colonies, suppressed growth was observed in AAUCc-7 and AAUCc-9 and v-shape pattern was depicted on isolate AAUCc-4 and AAUCc-5. From all the colonies studied, raised type of colonies was observed in AAUCc-4, AAUCc-7 and AAUCc-18 isolates.

Variation had been exhibited among isolates in terms of colony colors (Table 1). White colonies were observed in all isolates except three isolates, viz., AAUCc-3, AAUCc-17 and AAUCc-19 which exhibited at least slight greyish color. Isolate AAUCc-4 and AAUCc-17 had shown light brown with white greyish centre.

The colony margins varied from regular to irregular (Table 1). Regular margins were observed in eleven isolates viz., AAUCc-1, AAUCc-2, AAUCc-5, AAUCc-8, AAUCc-10, AAUCc-12, AAUCc-14, AAUCc-15, AAUCc-16, AAUCc-18, AAUCc-20 whereas, eight isolates AAUCc-6, AAUCc-3, AAUCc-4, AAUCc-9, AAUCc-11, AAUCc-13, AAUCc-17 and AAUCc-19 had irregular margins. Margins were whitish in AAUCc-1, AAUCc-9, and AAUCc-16 isolates. Besides, V-shaped margins were observed in isolate AAUCc-2, AAUCc-4 and AAUCc-5 and AAUCc-20. In isolates AAUCc-11, AAUCc-13, and AAUCc-14, the margins were had dull white color.

Growth Rate of Fungus

The result of this research had further revealed (in Table 1) considerable variation in the radial growth (in mm) 7 days after inoculation. Least growth rate of 22.0 mm was recorded in AAUCc-7. Isolate AAUCc-4 with mean radial growth 69.5 mm was the fastest followed by AAUCc-11 (59.0%), AAUCc-8 (58.0%), AAUCc-13 (55.5%) and AAUCc-19 (55.5%).

Morphological Variability:

Variations were observed amongst the isolates with respect to morphological characters like conidia size, shape, acervuli production, setae size and its characters.

Conidial size

The mean conidial size of isolates ranged from 18.19 - 37.30 x 1.00 - 5.31 µm (Table 2). The average maximum conidial length (37.30µm) observed in AAUCc-1 was significantly higher than other isolates, whereas minimum length (18.19 µm) in AAUCc-6 was recorded. The mean maximum conidial breadth of 5.31 µm was observed in AAUCc-12 while minimum conidial breadth of 1.00 µm observed in AAUCc-9. The second least conidial length (18.22 µm) and breadth (1.56 µm) was in AAUCc-13 and AAUCc-2, respectively.

Conidial shape

It has been revealed in this study that all the isolates had fusiform to falcate type of conidia (Table 2). Nine isolates viz., AAUCc-2, AAUCc-3, AAUCc-5, AAUCc-6, AAUCc-7, AAUCc-8, AAUCc-10, AAUCc-11, AAUCc-13, AAUCc-14, AAUCc-15 were having fusiform conidia and the rest 11 isolates, viz., AAUCc-1, AAUCc-4, AAUCc-9, AAUCc-12, AAUCc-16, AAUCc-18, AAUCc-19, AAUCc-20 having falcate conidia.

Variability in acervuli production and setae size:

Table 3 showed that the variation in setae size and acervuli production. Irrespective of the isolates, setae was measured to have 50.0-193.6 x 3.35 - 6.6 µm. The maximum size of the setae have been observed in isolate AAUCc-5 measuring 102.4 - 193.6 x 4.34-6.6 µm followed by isolate AAUCc-9 having an average measure of 131.22 x 4.66 µm acervuli length and breadth, respectively.

An insight into data further reveals that as irrespective of the isolates, Acervuli production ranged from 24 - 57mm mycelia disc. The least production of acervuli, with 5mm mycelia disc, was recorded in isolate AAUCc-16 (24 mm), followed by AAUCc-20 (26mm) and AAUCc-17 (28mm). To add up, isolates varied significantly in their characteristics (Table 3). AAUCc-2, AAUCc-4, AAUCc-6, AAUCc-8, AAUCc-9, AAUCc-12, AAUCc-15, AAUCc-17, AAUCc-18, and AAUCc-20 were submerged and scattered. Isolates AAUCc-1, AAUCc-5, AAUCc-7, AAUCc-10, and AAUCc-11 were raised and scattered whereas, the rest five isolates named AAUCc-3, AAUCc-13, AAUCc-14, AAUCc-16, and AAUCc-19 were appeared raised with concentric ring.

Table 2. Variability in conidial shape and size of *Colletotrichum capsici* isolates

Isolate	Length (µm)* Range	Length (µm)* Mean	Breadth (µm)" Range	Breadth (µm)"	Shape
AAUCC	7.2-29.7	37.30	0.78-3.280	2.03	Falcate
AAUCC	9.8-32.9	30.70	0.25-3.950	1.56	Fusiform
AAUCC	10.2-42.8	27.20	0.35-2.950	2.50	Fusiform
AAUCC	11.3-43.00	29.01	0.65-10.50	3.86	Falcate
AAUCC	10.9-42.00	19.20	0.53-2.100	2.88	Fusiform
AAUCC	11.5-27.4	18.19	0.55-3.300	3.16	Fusiform
AAUCC	12.5-43.4	18.40	0.25-11.40	3.56	Fusiform
AAUCC	10.2-55.8	31.70	0.65-3.980	2.50	Fusiform
AAUCC	11.2-31.8	28.10	0.42-3.280	1.00	Falcate
AAUCC	10.1-56.8	27.60	0.72-2.380	2.23	Fusiform
AAUCC	10.5-35.5	26.40	0.65-3.330	3.30	Fusiform
AAUCC	12.3-36.8	25.10	3.12-3.660	5.31	Falcate
AAUCC	7.9-21.00	18.22	2.65-14.12	3.11	Fusiform
AAUCC	11.2-32.3	33.60	0.52-15.22	3.33	Fusiform
AAUCC	8.2-48.1	24.10	1.32-22.12	5.11	Fusiform
AAUCC	12.2-32.3	25.10	3.31-14.12	5.44	Falcate
AAUCC	10.2-49.1	27.09	0.32-13.12	5.21	Falcate
AAUCC	10.1-37.2	20.10	0.32-11.62	1.00	Falcate
AAUCC	8.9-43.00	31.20	0.28-14.6	2.36	Falcate
AAUCC	9.8-45.00	19.40	2.22-15.7	3.22	Falcate

*Mean of 20 observations, AAUCc:=Addis Ababa University *Colletotrichum capsici*

Table 3. Variability in acervuli production and setae size of different isolates of *Colletotrichum capsici* on potato dextrose agar medium

Isolate	Size (µm)" Length (range)	Breadth (range)	No. of acervuli 5mm dia. Mycelialdisc	Characteristics
AAUCC-1	77.20 (50.0-109.8)	4.35(4.14-6.6)	35	Raised, scattered
AAUCC-2	89.80 (64.4-113.6)	3.84(3.5-5.5)	31	Submerged, scattered
AAUCC-3	96.50 (67.8-119.6)	4.65(4.4-6.6)	34	Raised, concentric rings
AAUCC-4	86.00 (75.6-119.8)	4.06(4.04-6.6)	57	Submerged, scattered
AAUCC-5	174.50 (102.4-193.6)	4.40(4.34-6.6)	43	Raised, scattered
AAUCC-6	118.88 (105.0-139.8)	4.80(4.34-5.5)	48	Submerged, scattered
AAUCC-7	124.45 (111.0-150.6)	4.35(3.11-6.6)	43	Raised, scattered
AAUCC-8	79.59 (75.0-102.7)	4.35(3.14-6.6)	32	Submerged, scattered
AAUCC-9	131.2 (125.6-140.6)	3.48(4.34-5.5)	49	Submerged, scattered
AAUCC-10	111.59 (114.6-122.7)	4.80(4.34-6.6)	45	Raised, scattered
AAUCC-11	133.95 (127.8-139.6)	3.48(3.34-5.5)	44	Raised, scattered
AAUCC-12	121.11 (118.0-124.8)	4.55(4.24-6.6)	42	Submerged, scattered
AAUCC-l3	99.49 (98.0-120.7)	4.65(4.24-6.6)	52	Raised, concentric rings
AAUCC-14	106.12 (105.5-110.6)	4.85(4.14-6.6)	48	Raised, concentric rings
AAUCC-l5	80.11 (76.0-122.7)	4.48(4.14-5.5)	45	Submerged, Scattered
AAUCC-16	108.22 (103.0-108.6)	4.88(4.14-6.6)	24	Raised, concentric rings
AAUCC-17	112.33 (108.8-119.6)	4.88(4.24-6.6)	28	Submerged, Scattered
AAUCC-18	117.11 (108.6-119.8)	4.75(4.34-5.5)	32	Submerged, Scattered
AAUCC-l9	131.22 (108.2-140.2)	4.66(4.24-5.5)	30	Raised, concentric rings
AAUCC-20	93.30 (86.0-100.0)	4.52(4.14-5.5)	26	Submerged, Scattered

*Mean of 20 observations, AAUCc:=Addis Ababa University *Colletotrichum capsici*

Pathogenic Variation

Totally, forty-eight isolates of *Colletotrichum capsici* from chili fruits were collected from four districts of SNNP, 2 districts of Oromiya and three districts of Amhara Regions. From these, only 20 randomly selected isolates were considered and inoculated on to a set of twenty different chili genotypes taken as differential lines and observations on disease reaction types were recorded after 7 days (Table 4). Both the lowest and highest diseases intensity was recorded in AAUCc-12 against OH2 (10.1%) and WD2 (35.5%), respectively. Isolate AAUCc-8 had shown the second highest disease intensity against BL1 (33.3%). The second lowest disease intensity was observed on AAUCc-5, AAUCc-18 and AAUCc-5 against LV1 (10.4%), MS2 (10.4%) and MZ1 (10.4%), respectively.

Table 4. Variation in *Colletotrichum capsici* isolates and their disease intensity on various chili genotypes

	Disease Intensity*(%)																			
Isolate	**O**	**O**	**W**	**W**	**B**	**MF**	**M**	**M**	**M**	**B**	**M**	**M**	**D**	**D**	**L**	**G**	**G**	**D**	**D**	**L**
AAUCC	33.	17.	26.	28	26	22.	22	18	16	11	26	18.	23.	14	17	16	14	14	22	23
AAUCC	18.	29.	22	28	28	29.6	18	15	21	12	16	18.	25.	15	19	14	15	16	18	14
AAUCC	21	28	26.	25	22	27.1	23	14	31	20	22	14.	14.	19	10	13	29	19	19	12
AAUCC4	27	23.	27	24	28	31.1	23	27	21	14	22	17.	28.	26	12	20	23	17	15	18
AAUCC	21.	19.	31.	21	31	24.5	26	27	30	16	31	16.	12.	22	10	22	25	11	16	17
AAUCC	22.	19.	22	30	21	11.1	29	19	25	24	31	14.	31.	25	15	30	17	21	16	17
AAUCC	17.	22.	10.	22	19	13.1	22	15	29	13	30	24.	22	22	19	16	19	15	21	23
AAUCC	10.	31.	17.	25	33	13.1	25	12	24	18	31	211	28.	22	15	11	19	18	21	25
AAUCC	22.	16.	25.	19	17	15.7	24	25	19	23	24	23.	21.	23	19	21	22	17	21	16
AAUCC	24	20.	26.	18	20	21.2	21	24	25	12	22	19.	16.	21	19	17	29	26	21	14
AAUCC	25.	27.	25.	17	17	23.3	26	21	21	11	18	26.	24.	25	14	23	22	31	24	23
AAUCC	10.	10.	16.	35	22	19.6	15	26	25	23	18	17.	31.	21	11	14	26	16	25	31
AAUCCl	27.	27.	21.	23	24	15.5	20	18	15	27	29	23.	30.	24	17	17	29	21	24	32
AAUCC	17.	16.	19.	22	15	16.1	15	19	16	15	24	23.	11.	25	21	21	20	24	21	31
AAUCCl	11.	27.	17.	17	16	11.5	26	27	13	22	22	22.	11	26	11	25	17	21	21	31
AAUCC	15.	26.	31.	15	30	22.2	25	21	16	20	23	217	17.	19	16	24	18	22	21	15
AAUCC	16.	23.	23.	28	31	24.2	13	21	20	14	19	21.	17.	18	12	24	13	26	25	16
AAUCC	15.	29.	22.	16	14	15.2	14	21	10	23	11	32.	15.	17	20	21	11	16	21	31
AAUCCl	16.	28.	20.	14	12	12.1	20	25	10	22	10	22.	22.	22	18	22	14	16	25	21
AAUCC	23.	27.	24.	13	25	30.0	14	22	12	18	22	25.	18.	24	10	21	21	30	22	19

*Means of Replications, = Resistance; + = Susceptible; OH=OdaHaro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=MaraqoFana; GL=Gojeb local; MZ= MelkaZala; DM=Dube medium; DM=Dube short Types

As depicted in table 5, the data of the current study revealed that the isolates exhibited at different virulent pattern when inoculated on the 20 chili differential genotypes. In all, 20 virulent isolates were identified based on similarity or dissimilarity in reaction types exhibited by these differential lines. The isolates comprised AAUCc-1, AAUCc-15 showing resistant reaction on 9 differential lines viz., OH1, OH2, DS1, WD2 , WD1, BL1, GL1, DS2, LV2 and susceptible reaction on the other 11 differential lines viz., MF1, MF2, MS1, MS2, BL2, MZ1, MZ2, DM1, DM2, LV1, GL2.

Three isolates, namely, AAUCc-2, AAUCc-6, AAUCc-16 showing resistant reactions on OH1, WD1, WD2 , MZ1, DM2, GL1, DS1 and susceptible reaction on rest of genotypes. The isolates comprised AAUCc-3 and AAUCc-10 (Group 3) showing resistant response on differential host genotypes OH1, GL1, DS1, DM2 whereas; susceptible reaction was exhibited on rest of cultivars.

Isolates AAUCc-9, AAUCc-12, AAUCc-13 and AAUCc-20 has shown resistant reaction on three differential lines viz., OH1, MF2, MZ2 and susceptible response on rest of the differential host genotypes. The isolates comprised AAUCc-4 showing resistant response on differential host genotypes viz., MF2 and DS1, whereas susceptible reaction exhibited on rest of the cultivars. The isolates comprised of the *Colletotrichum capsici* isolates AAUCc-17, AAUCc-19 showing resistant reaction on WD2 ,DM2, MF2, GL1 chili genotypes and susceptible reaction on rest of genotypes.

Table5. Virulence pattern of *Colletotrichum capsici* isolates on various chili genotypes

Isolate	OH1	OH2		WD2	BL1	MF1	MF2	MS1	MS2	BL2	MZ	MZ	DM	DM2	LV1	GL1	GL2	DS1	DS2	LV2
AAUCC1	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
AAUCC2	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC3	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
AAUCC4	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
AAUCC5	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+
AAUCC6	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC7	+	-	-	-	+	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-
AAUCC8	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-
AAUCC9	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
AAUCC1	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
ÂAUCC1	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+
AAUCC1	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
AAUCCl	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
AAUCC1	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
AAUCCl	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
ĀAUCC1	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC1	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
AAUCC1	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	-
AAUCCl	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
ÂAUCC20	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-

=Resistance; + = Susceptible; = Resistance; + = Susceptible; OH=OdaHaro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=MaraqoFana; GL=Gojeb local; MZ= MelkaZala; DM=Dube medium; DM=Dube short Types

The isolates comprised AAUCc-5 and AAUCc-11 (Group 4) showing resistant response on differential host genotypes viz., OH1, OH2, WD2 , MF1, MZ1, DM1, DM2, LV1, GL1, DS1, LV2 chilies whereas the susceptible reaction was exhibited on the rest of the cultivars. Similarly, the isolate AAUCc-7 was clubbed under (Group 8) showing resistant reaction on differential host like OH1, BL1, MF2, MS1, DM1, LV1, and susceptible response on rest of the differential host genotypes. The isolate comprised AAUCc-8 showing the resistant reaction on five differential lines viz., OH1, MF2, DM1, GL1, DS1, and susceptible reaction on rest of the genotypes. Isolate comprised AAUCc-14 had shown resistant response on OH1, DM2 and susceptible reactions on rest of the genotypes. The isolate AAUCc-18 shown resistant reaction on six differential lines viz., OH1, MS2, BL2, DM1, GL1, DS1, and susceptible reaction on rest of the genotypes

Further, as indicated in table 6, eleven pathotype groups were identified based on similarity or dissimilarity in reaction types exhibited by these differential lines. The group-1 comprised of isolates AAUCc-1, AAUCc-15 showing resistant reaction on 9 differential lines viz., OH1, OH2, DS1, WD2 , WD1, BL1, GL1, DS2, LV2 and susceptible reaction on the other 11 differential lines viz., MF1, MF2, MS1, MS2, BL2, MZ1, MZ2, DM1, DM2, LV1, GL2. The group-2 comprised of the AAUCc-2, AAUCc-6, AAUCc-16 showing resistant reaction onOH1, WD1, WD2 , MZ1, DM2, GL1, DS1and susceptible reaction on rest of genotypes. The group 3 comprised of isolates AAUCc-3, AAUCc-10 showing resistant response on differential host genotypes OH1, GL1, DS1, DM2whereas, susceptible reaction was exhibited on rest of cultivars.

In the same way, the AAUCc-9, AAUCc-12 AAUCc-18, and AAUCc-20, isolate were clubbed under group-5 showing resistant reaction on differential lines viz., OH1, MF2, MZ2 and susceptible response on rest of the differential host genotypes. The group-7 comprised of isolateAAUCc-4 showing resistant response on differential host genotypes viz., DS1, MF2 whereas susceptible reaction exhibited on rest of the cultivars. The group-6 comprised of the *Colletotrichum capsici* isolates AAUCc-17 andAAUCc-19 showing resistant reaction on WD2 , DM2, MS2, and GL1chili genotypes and susceptible reaction on rest of genotypes. The group-4 comprised of isolates AAUCc-5, AAUCc-11 showing resistant response on differential host genotypes viz.,

OH1, OH2, WD2 , MF1, MZ1, DM1, DM2, LV1, GL1, DS1, LV2lines whereas the susceptible reaction was exhibited on the rest of the cultivars.

On the contrary, isolate AAUCc-7, group-8, showing resistant reaction on differential hosts, such as OH1, BL1, MF2, MS1, DM1, LV1, and susceptible response on rest of the differential host. The group 9 comprised of isolate AAUCc-8 showing the resistant reaction on five differential lines viz., OH1, MF2, DM1, GL1, DS1, and susceptible reaction on rest of the genotypes. Similarly group 10 comprised of isolate AAUCc-14 that showed resistant response on OH1, DM2 and susceptible reaction on rest of the genotypes. Group 11 comprised isolate AAU Cc-18 showing resistance reaction on six differential lines, viz., OH1, MS2, BL2, DM1, GL1, and DS1.

Table 6. Pathotype groups of *Colletotrichum capsici* isolates on various chili genotypes

Patho. Gr.	Isolates	OH1	OH2	WD1	WD2	BL1	MF1	MF2	MS1	MS2	BL2	MZ1	MZ2	DM1	DM2	LV1	GL1	GL2	DS1	DS2	LV2
= R	AAUCC1,15	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
G2	AAUCC2, 6,	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
G3	AAUCC3,10	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
G4	AAUCC5, 11	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+
G5	AAUCC9, 12,13, 20	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
G6	AAUCC17,1	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
G7	AAUCC4	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
G8	AAUCC7	+	-	-	-	+	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-
G9	AAUCC8	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-
G1	AAUCC14	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
G1	AAUCC18	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	-

= Resistance; + = Susceptible; = Resistance; + = Susceptible; OH=OdaHaro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=MaraqoFana; GL=Gojeb local; MZ= MelkaZala; DM=Dube medium; DM=Dube short Types

The intensity of disease reaction has been indicated in table 7. In view of that, isolates AAUCc-1 has shown a highly resistance reaction on OH2 and BL1 genotypes. Isolate AAUCc-15 has shown a highly resistant reaction on OH1, OH2, WD1, WD2, BL1, DS1, DS2 and LV2 genotypes. Isolates AAUCc-2, AAUCc-6, AAUCc-17 and AAUCc-14 had shown a highly resistant reaction on MZ1, OH1 and WD2; OH1and OH1 genotypes, respectively. On the other hand, isolates AAUCc-1, AAUCc-2, and AAUCc-9 had shown a highly susceptibility reaction on single genotypes each, viz., MS1, MF2 and BL2, in order. Isolates AAUCc-4, AAUCc-8, and AAUCc-20 had shown a highly susceptibility reaction on two genotypes each, viz., MS2 and GL2, WD2 and MF1; and BL1 and GL2, respectively.

Discussion

The present work unraveled that there was variations among isolates of *C. capsici* from different locations of the country. Twenty representative isolates collected from 20 locations of the rift valley varied in their cultural, morphological, pathogenic characteristics. Isolates of *C. capsici* varied in their cultural characteristics viz., colony type, color, margin, and segmentation and growth rate. Colonies were cottony or fluffy and mostly suppressed with color ranging from white to grey. Similarly, several workers have also reported cultural, morphological, pathogenic variability among isolates *Colletotrichum* spp.(Sharma *et al.,* 2005; Masoodi *et al.*, 2013).

It has been indicated that conidia and appressions presence or absence of setae, sclerotia, acervuli and teleomorph state and such as colony, growth rate and texture (Photita, *et al.,* 2005; Than, 2008a,b).Isolates of *Colletotrichum capsici* were studied for morphological variation in their setae, conidia and acervuli production(Devi and Prakasam, 2014; Thaung, 2008. [5,23]. In this study the acervuli production among the isolates of *C. capsici* ranged from 32-55 µm but acervuli number and dimensions could not be taken with definiteness for determining the relatives virulence of the isolates.

Table 7.Intensity of reactions of *Colletotrichum capsici* isolates on 20 chili genotypes

	Reaction Types on:-																			
Isolate	**O**	**O**	**W**	**W**	**B**	**M**	**M**	**M**	**M**	**B**	**M**	**M**	**D**	**D**	**L**	**G**	**G**	**D**	**D**	**L**
AAUCC1	+	++	+	+	+	-	-	- -	-	-	-	-	-	-	-	+	-	+	+	+
AAUCC2	+	-	+	+	-	- -	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC3	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
AAUCC4	-	-	-	-	-	-	+	-	- -	-	-	-	-	-	-	-	- -	+	-	-
AAUCC5	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+
AAUCC6	++	-	+	++	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC7	+	-	-	-	+	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-
AAUCC8	+	-	-	- -	-	- -	+	-	-	-	-	-	+	-	-	+	-	+	-	-
AAUCC9	+	-	-	-	-	-	+	-	-	- -	-	+	-	-	-	-	-	-	-	-
AAUCC10	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
AAUCC11	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+
AAUCC12	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
AAUCCl3	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
AAUCC14	++	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
AAUCCl5	++	++	++	++	++	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
AAUCC16	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC17	- -	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
AAUCC18	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	-
AAUCCl9	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
AAUCC20	+	-	-	-	- -	-	+	-	-	-	-	+	-	-	-	-	- -	-	-	-

+++ = Immune, ++= Highly Resistant,; + = Resistant; - = Susceptible; - - Highly Susceptible; OH=OdaHaro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=MaraqoFana; GL=Gojeb local; MZ= MelkaZala; DM=Dube medium; DM=Dube short Types

However, this may need more investigation in order to establish such relationship among the isolates. The results achieved are in conformity to the works of Masoodi *et al.* (2013); Kumar *et al.*(2015); Sharma *et al.*(2005); and Sangde *et al.*(2011).Isolates of *C. capsici* varied little in their color of colony were nearly whitish, grey, light brown. Twelve isolates whitish, five isolates grey and two isolates were found to be brown. Shape of conidia was another criterion studied for variation among the isolates. Most of the isolates had Fusiform to Falcate conidia with slight differences in their shape. Ten isolates had fusiform whereas other ten isolates falcate conidia. Similar observations were also observed by Masoodi *et al.* (2013); Sharma *et al*. (2005) and Akhtar and Singh (2007).

The present study on pathological variability among the isolates were made by recording the disease response on a set of 20 chili genotypes, originated from 10 known cultivars, selected arbitrarily from the chili genotype lines, on the basis of their consistent reaction to a few *C. capsici* isolates.These isolates were takenas differential lines for *C. capsic.* Such a selection of differential lines had been adhered to an account of the fact that the differential for pathogenic and variability in isolates of *C. capsici.* In line with this, Sharma *et al.*,(2005)had used the comparable sets of differentials. On the basis of disease reactions expressed by the differential lines, ten groups (races) of *C. capsici* were identified. The group 1 comprised of isolates AAUCc-1, AAUCc-15 whereas group 2 included the isolate AAUCc-2, AAUCc-6, and AAUCc-16. The AAUCc-3 and AAUCc-10 were included in group-3 whereas group-4 included the isolate AAUCc-5 and AAUCc-11. The group 5 comprised of isolates AAUCc-9, AAUCc-12, AAUCc-13, and AAUCc-20. The group 6 comprised of AAUCc-17 and AAUCc-19. The groups 7, 8, 9, 10 and 11 comprised of isolates AAUCc-4, AAUCc-7, AAUCc-8, AAUCc-14 and AAUCc-18, respectively. This corresponds with the findings of Masoodi *et al.*(2013) who found ten patho-groups. The presence of these eleven different groups or races can account for varied pathogenic response to the different genotypes. The resistant behavior of a chili genotype to *C. capsici* in different parts of the country could also be understood by existence of such a variability occurring among these isolates. The existence of different virulent types of the isolates of *C. capsici* in the area so that the evolved variety shows resistance to all the virulence groups/types of the pathogen as to Masoodi *et al.* (2013)..

Ten isolates of *C. acutatum* against seven cultivars reportedly susceptible species of capsicum (Lin *et al.*, 2004) and resistant species such as capsicum Chinese 'PBC 932' (Lin *et al.*, 2004). In contrast to *C.baccatum*, susceptibility of the *C. frutescence* cultivars have been reported in several studies (Lin *et al.*, 2004; Park, 2007) while evolving 79 varieties of capsicum for

resistance to *C. capsici* in field trials. Kumar *et al.*(2015) have also evaluated12 chili varieties against anthracnose and found resistance only in one cultivars, whereas, remaining cultivars were either moderately susceptible or highly susceptible.

Conclusion

The current study indicated that virulence is more severe in tender fruits than ripened ones. The morho-cultural study showed that there is variability among the selected isolates. The pathogen was identified as per the morphological, cultural and pathogenic behavior as *C. capsici*. These studies indicated prevalence of pathogenic variability among the isolates of *C. capsici*. In all 20 isolates, pathogenicity was observed on their pathogenic characteristics on 20 chili host genotypes and accessions depending upon their pathogenic behavior.

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