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Research Article

Agronomic Survey and Screening of Genotypes for Anthracnose Infection in *Capsicum annuum* L.

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Abstract

Background and Objective: Chilli anthracnose disease cause a huge commercial loss in India. The study was designed to assess the prevalence of anthracnose disease and to understand the reaction of anthracnose disease with respect to *Colletotrichum capsici* in different chilli genotypes. **Materials and Methods:** Chilli plants raised from fourteen different genotypes were evaluated for their disease reaction nature based on disease incidence, percent disease index against anthracnose pathogen and prevalence of anthracnose infection was checked in chilli growing regions of south Karnataka. **Results:** Plants (30-day-old) raised from the seeds of different genotypes sprayed with a standard conidial suspension of the pathogen (5×10^5 conidia mL⁻¹) showed a different grade of disease reaction in which plants of G4 genotype recorded maximum disease incidence (96%) and 74.11 PDI viewing highest susceptible reaction. Plants of Hot Pepper (HPH-22) showed moderate resistance wherein disease incidence of 36% and 24.2 PDI was noticed. Agronomic field survey recorded maximum anthracnose prevalence in Hassan region and no anthracnose prevalence was observed in Bengaluru. **Conclusion:** The current study provides comprehensive information of genotypes showing the varied degree of disease reaction against anthracnose pathogen particularly of regional choice that has much consequence to the scientists, breeders and the farmers as well.

Key words: Chilli, *Colletotrichum capsici*, agronomic survey, genotypes screening, anthracnose

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is the most consumable crop in the world predominantly in India as its aid taste, flavour, pungency and colour to the food dishes. It contains a good number of vitamins (A, C) and other essential nutrients which serve as an important source in medicines^{1,2}. India is the largest producer and exporter of chilli. On a global scale, India roughly produces about 1.4 million t (21.4%) of chilli and has about 42.2% (801,600 ha) area for chilli cultivation^{3,4}. India exports about 38% of dry chilli to other nations such as West Asia, East Asia, USA, Sri Lanka and Bangladesh⁵. Global plant crop production is principally declined due to hasty spread of the plant diseases particularly caused due to biotic stresses⁶. Chilli is affected by a crucial fungal disease named anthracnose caused by *Colletotrichum capsici* causing gigantic economic loss all over the globe including India^{1,2}.

The agronomic survey helps in the documentation of the relative prevalence of the plant disease and pathotype distribution. Pathogen virulence is varied in terms of heterozygosity, somatic hybridization, sexual processes and selection of plants. Also, it is chiefly dependant on host plant genotype and environment⁷. The genotype screening helps in monitoring the origin and spread of new virulent pathotype in commercial genotypes. Also, any shift from virulence to avirulence in commercial genotypes can be detected⁸. There is a wide gap in the availability of adequate research field data on the occurrence of plant diseases all over the world predominantly of national and regional importance⁹. With this gap and raising concern in literature, the present paper was aimed to check for anthracnose disease prevalence in different chilli growing farmers' fields of south Karnataka, India and different genotypes that are grown in farmers' fields were obtained from a reliable source to check for their disease reaction against anthracnose pathogen-*C. capsici*.

MATERIALS AND METHODS

Agronomic survey in major chilli growing farmers' fields of south Karnataka to check for anthracnose disease prevalence: The field survey was conducted to screen for the prevalence of anthracnose disease symptoms in different chilli growing districts of south Karnataka differing in agroclimatic regions for one year i.e., from December, 2014 to December, 2015. Fields affected with anthracnose disease symptoms were noted down and also other details such as crop stage, variety, month and year of the visit were recorded.

Source of chilli seeds samples: Chilli seeds of different genotypes were procured from the reliable source labelled with variety name, place, producer or seed company name to check for disease reaction nature against anthracnose pathogen.

Source of Anthracnose pathogen: The *C. capsici* was isolated from the infected chilli seed samples collected from agricultural farms and identified based on morphological, cultural and conidial characters. The pathogen was molecularly identified using ITS 1 and ITS 4 primers sequence of the pathogen was deposited in GenBank, NCBI with accession number MH703532¹⁰.

Preparation of *C. capsici* inoculum: Fifteen-day-old active pure culture of *C. capsici* cultured on Potato Dextrose Agar (PDA) media was taken and 10 mL of Sterile Distilled Water (SDW) was added. The plates were scraped using L-shaped glass rod to ooze out the conidial spores from acervuli and final conidial concentration was adjusted to 5×10^5 conidia mL^{-1} with the help of Haemocytometer^{10,11}.

Growth of chilli plants: Chilli seeds of different genotypes were individually surface sterilized with 0.2% sodium hypochlorite for 1-2 min, rinsed with SDW, blot dried and were sown in pots containing sterilized sand, farmyard manure and red soil [ratio-1:1:2]. The set up was maintained under greenhouse conditions [$25 \pm 2^\circ\text{C}$ at 80% Relative Humidity (RH)] and watered daily for growth and development up to 30 days.

Evaluation of chilli plants of different genotypes for their disease reaction nature against *C. capsici* under greenhouse conditions: Thirty day-old chilli plants of each genotype were sprayed with *C. capsici* inoculum at a standard concentration of 5×10^5 conidia mL^{-1} and a separate set of control plants i.e., not inoculated with pathogen was also maintained. Daily observations of characteristic anthracnose symptoms were made and disease was rated using a standard disease rating scale of 0-9 wherein 0 = no symptoms, 1 = small lesions covering less than 1% of leaf area, 3 = small lesions covering less than 1-10% of leaf area, 5 = Big lesions but not coalescing covering 11-25% leaf area, 7 = lesions on leaves covering 26-50% leaf area, 9 = lesions on leaves covering 51% or more area¹². The whole experimental setup was maintained for 30 days at $25 \pm 2^\circ\text{C}$ at 80% RH and percent disease incidence, Percent Disease Index (PDI) were calculated using the standard formulas described by Wheeler¹³:

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants evaluated}} \times 100$$

$$\text{PDI} = \frac{\sum (\text{Rating number} \times \text{No. of plants with the rating})}{\text{Total No. of plants} \times \text{Highest rating}} \times 100$$

Disease reaction/response of chilli genotypes into Susceptible (S), Highly Susceptible (HS), Moderately Resistant (MR) and Resistant (R) were done based on PDI scale described by Bansal and Grover¹⁴ wherein 0 represents-Immune (I), 1-5% PDI represents-R, 6 to 25% PDI represents-MR, 26-50% PDI represents-S and 51-100% PDI represents-HS.

Statistical analysis: The studies of anthracnose disease reaction on different chilli genotypes were performed in randomized complete block design and consisted of 25 plants per genotype with four replicates. The experimental data

were subjected to Arcsine Transformation and Analysis of Variance (ANOVA) using SPSS v. 18.0 (SPSS Inc., Chicago, IL, USA). Means were separated using Tukey's Honest Significant Difference (HSD) test and the significance was detected with F-value ($p \leq 0.05$). Anthracnose prevalence in respective villages of different districts of south Karnataka was represented in terms of percentage (%) and a Heat map was constructed to depict the same using NCSS statistical software ver. 2020.

RESULTS

Agronomic survey in major chilli growing farmers' fields of south Karnataka to check for anthracnose disease prevalence:

A total of 99 fields (three from each village) were surveyed from December, 2014-2015 for one year irrespective of the crop stage in sixteen different taluks and six different districts of south Karnataka (Table 1). During the survey, it was

Table 1: Agronomic survey for the prevalence of anthracnose infection in chilli growing farmers' fields of south Karnataka for the period 2014-2015

Place of collection						
Districts surveyed	Taluk	Village	Crop stage	Genotype	Month and year of visit	Prevalence
Mysuru	Mysuru	Daripura	V	Pusa Jwala	September, 2015	-
		Chikkanahalli	F	Arka Lohit	December, 2015	+
		Hosahundi	V	USM- Ashwini	September, 2015	-
		Maratikyathanahalli	F+FL	S-701	September, 2015	-
		Hebbadi	FH	AS-02	September, 2015	+
	Nanjangud	Kadakola	F	MHCP317	September, 2015	-
		Mullur	F	Sonal	October, 2015	+
		Saragooru	F	NS-1701	October, 2015	-
		Hedathale	F+FL	USM- Ashwini	October, 2015	+
		Parasayana Hundi	FH	Pusa Jwala	March, 2015	+
	Heggadadevana (H.D) Kote	Chikkanandi	V	Sonal	March, 2015	+
		Hosahalli	FH	AS-02	March, 2015	+
		Devarahalli	F+FL	Arka Lohit	August, 2015	+
		Naganahalli	V	HPH-22	August, 2015	-
		Bannikuppe	FH	AS-02	August, 2015	+
Mandya	Malavalli	Chottanahalli	V	HPH-22	August, 2015	-
		Dodda Byadarahalli	FH	NS-1701	February, 2015	+
	Pandavapura	Baleathiguppe	FH	MHCP317	March, 2015	-
		Kodagahalli	V	HPH-22	January, 2015	-
		Shivapura	V	SHP-4884	February, 2015	-
Chamarajanagara	Maddur	Yaraganahalli	F+FL	USM- Ashwini	April, 2015	-
		Arepara	F	SHP-4884	December, 2015	-
	Gundlupet	Devarahalli	F+FL	MHCP317	December, 2015	-
		Nallur	F	NS-1701	December, 2015	+
		Chamarajanagara	Nallur	F	NS-1701	December, 2015
Hassan	Arsikere	Channapura	FH	Guntur (G4)	December, 2014	+
	Hassan	Haralahalli	F+FL	NCH-12	December, 2014	-
	Channarayapatna	Dasarahalli	V	Pusa sadabahar	October, 2014	+
		Byadarahalli	F	Pant C-1	December, 2014	+
		Belakere	V	Pusa Jwala	February, 2015	-
Ramanagara	Channapatna	Belakere	V	Pusa Jwala	February, 2015	-
	Magadi	Chikkenahalli	F+FL	Arka lohit	January, 2015	+
		Mathikere	FH	Guntur (G4)	January, 2015	+
Bengaluru	Anekal	Koppa	V	SHP-4884	November, 2015	-
	Bengaluru	Alur	F	HPH-22	November, 2015	-

+: Prevalence/appearance of anthracnose infection in the chilli, -: No prevalence/appearance of anthracnose infection in the chilli, V: Vegetative, FL: Flowering, F: Fruiting, FH: Fruiting and Harvesting stage



Fig. 1(a-i): Different parts of chilli with anthracnose infection spotted during a field visit, (a) Infected field, (b) Infected plant, (c) Infected stem, (d-e) Infected leaves and (f-i) Infected fruits

found that the anthracnose disease affected all parts of the chilli plant i.e., stem, leaf, fruit and prevalence was confirmed based on the characteristic symptoms i.e., irregular circular/ angular sunken lesions with concentric rings of acervuli that coalesce to form black spots on leaves and fruits (Fig. 1a-i). The prevalence of the anthracnose disease was found to be more in Hassan district followed by Ramanagara and Mysuru district. No anthracnose prevalence was observed in Bengaluru district during the visit (Table 1, Fig. 2).

Evaluation of chilli plants of different genotypes for their disease reaction nature against *C. capsici* under greenhouse conditions: All the chilli plants raised from the seeds of different genotypes responded differently and significantly to *C. capsici* infection. Amongst the fourteen genotypes screened, plants of seven genotypes showed high susceptibility reaction wherein G4 plants recorded highest anthracnose incidence (96%) and PDI of 74.11 followed by Pusa sadabahar which documented 81% disease incidence and 69.2 PDI respectively. None of the plants of the genotype

Table 2: Evaluation of plants raised from seeds of different chilli genotypes for their disease reaction against *C. capsici* (MH703532)

Sample code	Genotype	Name of the seed company	Disease incidence (DI) [%]	Percent disease index (PDI)	Disease reaction
N-01	Sonal	HyVeg., Tamil Nadu, India	75.00 ± 1.00 ^{bc}	64.00 ± 0.73 ^{bcd}	HS
N-02	Arka Lohit	IIHR, Bengaluru, Karnataka, India	57.00 ± 1.91 ^{efg}	46.80 ± 1.15 ^{gh}	S
N-03	Pusa Sadabahar	National seed corporation (NSC), Bengaluru, Karnataka, India	81.00 ± 1.91 ^b	69.20 ± 0.83 ^{ab}	HS
N-04	AS-02	Ashoka seeds, Bengaluru, Karnataka, India	69.00 ± 1.91 ^{cd}	58.40 ± 1.26 ^{de}	HS
N-05	Pant C-1	National seed corporation (NSC), Bengaluru, Karnataka, India	78.00 ± 1.15 ^b	67.20 ± 0.56 ^{bc}	HS
N-06	SHP-4884	Seminis vegetable seeds Inc., Mumbai, India	42.00 ± 1.15 ^{ij}	31.00 ± 1.14 ^k	S
N-07	Guntur (G4)	National seed corporation (NSC), Bengaluru, Karnataka, India	96.00 ± 1.63 ^a	74.11 ± 0.68 ^a	HS
N-08	USM-Ashwini	Unisem Agritech. Pvt. Ltd., Bengaluru, Karnataka	48.00 ± 1.63 ^{hi}	35.60 ± 1.05 ^{jk}	S
N-09	NCH-12	Nirmal seeds, Maharashtra, India	73.00 ± 1.91 ^{bc}	62.40 ± 0.86 ^{cd}	HS
N-10	S-701	Solar seeds, Bengaluru, India	60.00 ± 1.63 ^{ef}	49.20 ± 0.69 ^{fg}	S
N-11	Sierra-MHCP317	Mahyco®, Mumbai, India	51.00 ± 1.91 ^{gh}	40.60 ± 1.96 ^{ij}	S
N-12	Hot pepper (HPH-22)	Syngenta India Ltd., New Delhi, India	36.00 ± 1.63 ^j	24.20 ± 0.88 ^l	MR
N-13	NS-1701	Namdhari seeds Pvt. Ltd., Bengaluru, Karnataka, India	55.00 ± 1.91 ^{gh}	43.20 ± 1.66 ^{hi}	S
N-14	Pusa Jwala	National seed corporation (NSC), Bengaluru, Karnataka, India	64.00 ± 1.63 ^{de}	53.00 ± 1.32 ^{ef}	HS

Values are means of four independent replicates (n = 4) and ± indicate standard errors. Mean values followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD. Genotypes disease reaction to *C. capsici* was assessed based on PDI scale of Bansal and Grover, 1969 wherein 0 represents- Immune (I), 1-5% PDI- Resistant (R), 6-25% PDI-Moderately Resistant (MR), 26-50% PDI-Susceptible (S) and 51-100% PDI-Highly Susceptible (HS)

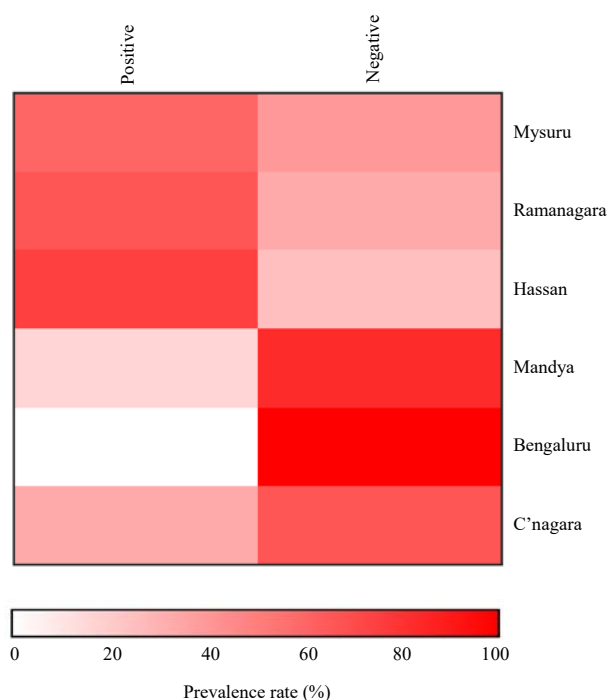


Fig. 2: Heat map depicting the prevalence rate Positive (+) and Negative (-), in terms of percentage (%) against chilli anthracnose disease in respective districts of south Karnataka

was found to be resistant against the test pathogen. However, plants of Hot Pepper (HPH-22) showed moderate resistance to *C. capsici* (Table 2).

DISCUSSION

Capsicum annum L. is widely grown as spice or vegetable that has high nutritional and medicinal properties¹⁵.

It suffers from a major fungal disease named anthracnose that ruins the quality and quantity of chilli fruits (both green and red) causing big profitable losses across the world including India². Agronomic field survey provides a detailed account on the prevalence, severity and relative importance of various plant diseases¹⁶. Keeping this in view, the study was planned to conduct agronomic field survey in different regions of south Karnataka. The results surprised with the prevalence of anthracnose in all the regions of south Karnataka depicting disease severity except Bengaluru and an urgent need for the development of effective disease management strategy. The statement is in line with previous reports who have specified chilli anthracnose as a chronic disease and its prevalence have been reported in different parts of India including North Karnataka causing profitable loss to the poor farmers^{1-2,17-18}.

During the visit, most of the farmers' have planted hybrid chilli seeds on the recommendation of the seed dealers for getting a good yield. Surprisingly, chilli plants raised from seeds of hybrid genotypes showed typical anthracnose symptoms and farmers were managing the disease with the help of chemical fungicides. This suggests the susceptibility of the genotypes advocating a need to continuously screen and checks the genotypes for their disease reaction nature. Also, farmers' dependency on fungicides for the control of anthracnose advocate an urge to come up with effective biological management strategy as these fungicides affect soil texture, soil microflora causing both environmental and human health hazards¹⁹. Despite of the fact of fungicidal toxicity, most of the research scientist recommend the use of chemical fungicides as the managing tool in modern agriculture to bring down the infection level caused by anthracnose pathogen in chilli^{20,21}.

With this obtained source of information, the current study was next aimed to screen different chilli genotypes growing in the farmers' fields for their disease reaction nature against anthracnose pathogen. Out of the fourteen genotypes screened, G4 suggested being the most susceptible genotype to *C. capsici* showing highest disease incidence (96%) and disease index (74.11%) followed by Pusa sadabahar and Pant C-1. However, Hot Pepper (HPH-22) was moderately resistant to pathogen. The statement corresponds with the studies of Sawant *et al.*²² who have reported Pusa jwala genotype showed moderate resistance with PDI range from 24.67 and in our study, it recorded high susceptible reaction to *C. capsici* with disease incidence of 81% and PDI of 69.20.

In the studies conducted by Arunakumara and Satyanarayana²⁰, Pusa Jwala, Pant C-1, Arka lohit reported to show susceptible, moderately susceptible and moderately resistant reaction with mean percent disease index of 67.2, 31.36 and 24.58, respectively. However, in the current study, these genotypes showed high susceptible and susceptible reaction against the test pathogen. Gupta *et al.*²³ have screened for *C. capsici* reaction on fruits of various chilli genotypes wherein Arka Harita, Classica-152 and Madhurima-148 genotypes showed resistant reaction and Pusa jwala, Pant C-1 showed moderate resistance against *C. capsici*. There are various other research reports wherein different chilli genotypes have been screened for the anthracnose disease reaction against *C. capsici*^{24,25}. The variation in disease reaction is suggested to be dependent on various factors i.e., soil, climatic and on specific pathogen strain type that favours the occurrence and spread of the disease²⁶.

CONCLUSION

Based on the results obtained, the study concludes that anthracnose prevalence was predominant in Hassan region followed by Ramanagara and Mysuru of south Karnataka. Among the fourteen genotypes screened, G4 genotype recorded highest susceptible reaction with 96% disease incidence and disease index of 74.11% against *C. capsici* followed by Pusa sadabahar and Pant C-1. However, Hot pepper (HPH-22) recorded a marginal moderate resistance reaction against the test pathogen.

SIGNIFICANCE STATEMENT

The study aimed to check for the anthracnose prevalence in chilli growing farmers' fields to understand the nature of

disease reaction in different genotypes against anthracnose pathogen. The study exploits the most susceptible genotype in chilli with respect to *C. capsici* (MH703532). The study also documented for the first time the prevalence of anthracnose disease in different regions of south Karnataka. The study can be exploited to check for the occurrence of a specific disease and pathotype differential reaction or distribution in commercial plant genotypes that is of much raising concern in today's time to design effective disease control strategy.

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