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Screening of the chilli cultivars for resistance to fusarium wilt in Northern Karnataka

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Abstract
Twenty seven chilli germplasm lines were screened for resistance against *F. solani* and GPM 21 was resistant for all three weeks using rapid root dip technique. Quality characters especially capasaicin content showed negative significant correlation for disease incidence in chillies. This helps in the area specific requirement of the breeding material to combat the deadly disease of Fusarium wilt.

Keywords: *Fusarium solani,* PCR-ITS, capsaicin, chilli

Introduction
Chilli (*Capsicum annuum* L.) is one of the very important commercial spice crops of India. *Fusarium oxysporum and F. solani* are reported as the most common species of Fusarium found associated with wilt of chilli in India, whereas, *F. moniliforme and F. pallidoroseum* as causal agents are found in some parts of India (Naik *et al*., 2006). The yield loss due to the disease is known to vary from 10-80 per cent worldwide depending upon the variety being grown. (Devikarani *et al* 2008) [4].

India ranks second among world's chilli exporting countries and has showed a steady decline in chilli trade due to increased domestic consumption along with biotic and abiotic stresses. The chilli area, production and productivity are in decreasing trend even though it is a highly profitable commercial spice and vegetable crop due to murda complex (leaf curls and mosaics), wilts and Anthracnose. The most important quality character in chillies is the pungency and the colour. The pungent principle is Capsaicin, maximum is seen in pericarp and seeds have negligible concentration (0.0005%). Colour is because of carotenoids ie., red pigment in chilies is contributed by Capsanthin (35%), Capsolubin(6.4%) and Zeaxanthin (2.3%).

Byadagi Dabbi and Byadagi Kaddi which have attained the status of geographic indicators (GI) are special local cultivars of Karnataka. Byadagi Dabbi is severely suffering from fungal wilt caused by *Fusarium solani*. Presence of *Meloidogyne* is increasing the incidence of the Fusarium wilt and recently has become a serious problem in almost all chilli growing tracts of India, especially in black cotton soils leading up to 20 per cent yield loss (Devika Rani, 2006; Kumar *et al.*,2009; Raghu and Benagi, 2014) [5, 15, 8, 16].

The wilt appears both in seedling and later stage but the highest mortality occurs at flowering and fruiting stage, as a result whole plant wilts leading to a complete loss. Although the disease first appears in patches in a field, it can extend to the entire field if chilli is cultivated repeatedly in the same field. During the same season it gets transferred with irrigation water, when crop is grown during off season i.e.., winter and summer seasons. The disease management strategies using chemicals like fungicides for *Fusarium* were costly, time consuming as well as threat to the environment. Other biological control methods using suppressive soils with beneficial microbes chitinolytic bacteria, antagonistic *Fusarium, Trichoderma and Mycorrhiza* application are still required commercialization. But resistant cultivars for these diseases are the most promising approach. Jabeen *et al.,* (2007) [5] has identified phenols as the important parameter for disease resistance in chilli crosses using Arka lohit as resistant parent for *Fusarium pallasadorium*. Tewksbury *et al.* (2008) [20] reported capsaicin content alters the infection rates of hemipteran insect as well as *Fusarium* in wild chillies. Non pungent chillies in the population were more infected than pungent ones. Thus investigation was carried out to see role of different biochemicals in resistance mechanism in chillies .

Materials and Methods

Plant Materials: Twenty seven chilli genotypes known to be resistant to wilt diseases throughout India were collected. Ujwala, Anugraha (KAU, Kerala), Byadagi Kaddi, Byadagi Dabbi, GPM5, GPM7, GPM8, GPM21, GPM22, GPM206 (HRS, Devihosur), Arka lohit, Pusa Jwala, CO4,, Sitara, (Highly susceptible) KDC1, BSS414, G4, Utkal Roshani, Utkal Ava, Phule Jyothi, Pant C1, COO713, EC341094, EC467636., LCA 334, CM 334, Murda variant. (IIVR Varanasi)

Isolation of fungal pathogens

The infected plants showing typical symptoms of the diseases were used for the isolation of pathogen. The standard tissue isolation procedure was followed to isolate the pathogens. The infected parts were surface sterilized with 1:100 Sodium hypochlorite solution for 60 seconds and washed thrice in sterilized distilled water to remove the traces of the chemical if any and then transferred to sterilized petri-plates containing potato dextrose agar (PDA). The petriplates were incubated at room temperature (27± 1°C) and observed periodically for the growth of pure colonies. The pure colonies which developed from the bits were transferred to PDA slants and incubated at 27±1°C for 10 days. Then such slants were used to study the pathogen characters (Raghu and Benagi 2014) [15, 16]

The identification of *Fusarium* spp. was done based on the spore morphology and colony characters of the fungus by referring to the "Illustrated genera of Imperfect fungi" (Barnett and Hunter, 1972) [1]. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture under aseptic conditions. Such culture tubes were preserved in a refrigerator at 5 ^{o}C and used for further studies.

Mass multiplication of the pathogen

The fungus was mass multiplied on potato dextrose broth (PDB). The mycelial disc cut from the margin of a week old culture grown on Petri dish was inoculated into PDB under aseptic conditions. The flasks were incubated at 28±1^{0} C for 15 days. The mycelium mats were collected after 15 days by filtering with Whatman No 42 filter paper disc of 12.5 cm diameter and washed with sterile water. The spore suspension was prepared using pestle and mortar to disturb the spores in sterile water and filtered through cheese-cloth before use and spore load per ml was computed by using a haemocytometer and adjusted to 1X 10^{6} conidia per ml.

Pathogenicity test by Rapid root dip Transplanting Technique

Rapid root dip transplanting technique method developed by Naik *et al.* (1996) [11]. Chilli seedlings were raised in a plastic trays containing sterilized cocopeat: vermicompost mixture in a nylon net house and protected with two insecticidal sprays of Neem oil (0.3%) and Imidachloprid (0.05%) to prevent the viral disease. Four weeks old seedlings were removed, roots thoroughly washed in running tap water and 3mm tip of roots were cut and immersed in spore suspension of *F. solani* and planted in a plastic pots containing sterilized soil. Then plants were transferred to inoculated pots. The experiment was conducted in randomized block design with three replications and five plants were used per replication. In cases where isolates produced typical wilting symptoms, the fungus was successfully re-isolated and Koch's postulates proved.

Per cent wilt disease incidence (PWI): The incidence of fusarial wilt was recorded at weekly interval after inoculation by using the following formula

Number of wilted plants

$$\text{PWI or Wilt incidence (\%)} = \frac{\text{Number of wilted plants}}{\text{Number of total plants}} \times 100$$

Fusarium wilt incidence was categorized using the following classification ((Devika rani *et al*., 2006) [5].

PWI	Disease Reaction
0	Immune(I)
1-10	Resistant(R)
11-25	Moderately resistant(MR)
26-50	Moderately susceptible(MS)
51-75	Susceptible(S)
76-100	Highly susceptible(HS)

Biochemical parameters

Ascorbic acid (mg/100g) was estimated by volumetric method, phenol (mg/100g) was estimated using FCR method using catechol as a standard and Capsaicin (mg/100g) by colourimetric method (Sadashivam and Manickam 1996) [17].

Results and Discussion

Universally, *F. solani* species complex (FSSC) has an extensive host range and very high levels of diversity in pathogenicity and morphology (Brasileiro *et al.*, 2004) [2]. However, the classification system based only on morphology has not provided an accurate tool for the identification of FSSC, neither has morphological classification system resolved the relationship of isolates within FSCC. So, a molecular approach is promising in establishing the objective (O'Donnell and Gray, 1995; Zhang *et al*., 2006; O'Donnell *et al*., 2008) [21, 14]. Among the methods which researchers have used to analyze the phylogenetics of *F. solani* species are rDNA-IGS, rDNA-ITS regions, large submit RNA gene and translation elongation factor-alpha (tef)

In the present study, the morphology of the *Fusarium* as well as spore shape, size and pathogenesity preliminarily suggests the pathogen as *Fusarium solani* morphotype II *(*Figure 1 A and 1B). The symptoms of highly susceptible (Ujwala) and resistant (GPM 21) are depicted in Figure 1C and 1D. l. Similar results were obtained by Raghu (2014) [15, 16].

Average values of the percent disease incidence (PDI) of Fusarium wilt at first week, second week and third week interval are presented in Table 1. First week after inoculation highest PDI was observed in Sitara (88.25%) followed by Pusa Jwala (80%), Lowest PDI was observed in KDC1 (4.5%) followed by GPM21(8.5%) and GPM22(9.5%). These three KDC1, GPM21 and GPM22 were resistant and others are susceptible. Second week after inoculation highest PDI was observed in Sitara (100%) followed Ujwala (82.5%), Anugraha (81.5%), GPM3 (80.5%), GPM8 (80.5%), GPM206 (80.5%), and Pusa Jwala (80%). Details of the germplasm lines categorized into different classes immune to highly susceptible are shown in Table 2 at weekly intervals. Data reveals that GPM 21, KDC1 and GPM22 can be used as resistant sources for further crop improvement programmes. Similar findings were obtained by Singh *et al.* (1998) [18] screened chilli germplasm against Fusarial wilt caused by *Fusarium oxysporum* in Himachal Pradesh during 1993-1994.

Out of 30 genotypes evaluated, nine were moderately resistant and remaining 21 were either susceptible or highly susceptible none of the germplasm lines were found highly resistant to wilt. Naik *et al*., 2008 [10] reported IHR3018 as resistant using rapid root dip and transplanting method against *Fusarium solani.* Raghu (2014) [15, 16] also reported Sitara as highly susceptible to *Fusarium* wilt followed by Byadagi Dabbi and recently Singh *et al* (2017) [19], evaluated chilli cultivars/varieties against *F. oxysporum* causing wilt in chilli under *in vitro* conditions by water culture technique revealed that out of the twenty-two chilli cultivars/varieties, most of the cultivars/varieties were susceptible to the disease but only one cultivar CO-4 showed resistant reaction (6.67% disease incidence), Pant C-1, Punjab Lal and Kashi Sinduri were moderately susceptible (33.33 to 48.99% DI). The cultivars Pusa Sadabahar and Arka Abhir were highly susceptible to wilt disease with 100 per cent disease incidence.

However, it is too much to expect in case of soil borne pathogens such as *Fusarium* to have a stable resistance because of variability of the pathogen. Nevertheless, resistance in soil borne diseases would continue to play an important role for the simple reason that although high resistance may not always be attainable, even moderate resistance may help to make other measures more effective.

Biochemical studies

Observations on quality traits like ascorbic acid content, phenols and capsaicin were also taken, which can help for the optimum choice of the cultivars in future breeding programmes. Average values of all the germplasm lines are listed in table 1. Analysis of the variance(ANOVA) for these along with percent disease incidence shows significance differences for Ascorbic acid content, phenols, Capsaicin content and Percent Disease incidence(PDI) for Fusarium wilt. (Table 3). Mean values of Ascorbic acid was 79.5mg/100g and it ranged from 26.32 to 394.74mg/100g. There was high variation in phenol content which ranged from 1.92mg/100g -13.22mg/100g with average of 6.02 mg/100g. All the biochemical parameters shows high PCV, high GCV (>20%) and high heritability (>60%) (Table 4). Diseases resistance in chilli is governed by different barriers. Ascorbic acid, phenols and capsaicin provides some degree of resistance; also they determine quality characteristics in green and red chillies. Phenols are known as one of component of the resistance (Jabeen *et al*., 2007) [5] for *Fusarium pallidoroseum* (Cooke) Sacc. But in our study there is no significant association for *Fusarium solani*.

Tewksbury *et al.,* (2008) [20] used wild chillies to show that chemical defense of ripe fruit reflects variation in the risk of microbial attack. Capsaicinoids are the chemicals responsible for the well known pungency of chili fruits. *Capsicum chacoense* is naturally polymorphic for the production of capsaicinoids and displays geographic variation in the proportion of individual plants in a population that produce capsaicinoids. This variation is directly linked to variation in the damage caused by a fungal pathogen of chilli seeds. They found that *Fusarium* fungus is the primary cause of predispersal chilli seed mortality, and experimentally demonstrated that capsaicinoids protect chilli seeds from *Fusarium*. As a secondary infection after damage by hemipteran insects also depends on the variation in plants in a population producing capsaicinoids and suggests a strong antifungal role for capsaicinoids.

In the present study negative significant association between Capsaicin and PWI for all three weeks after inoculations was recorded. Increase in capsaicin content (more pungent) reduces the percent wilt incidence and non pungent has more disease. (Table 5). Capsaicin content also differs with different growing conditions. Gangadhar *et al*., (2012) [6] investigated the effect of light emitting diodes (LEDs) on fruit color and primary and secondary metabolites (capsaicinoids) in *Capsicum annuum* L. cv. Cheonyang. High-performance liquid chromatography analysis of acetonitrile extract of chili fruits revealed enhanced capsaicinoid contents in blue LEDs (180 ± 6.32 mg/100 g) when compared with fluorescent light (54 ± 3.12 mg/100 g). Moirangthem *et al* (2014) [9] compared different dates of sowing with different spacing and demonstrated that the productivity shown for 15 September sowing is much higher than the ones produced traditionally in Bhoot Jholokia. Scoville organoleptic test and HPLC method showed highest amount of capsaicinoid content in the fruits produced from a spacing of 105 cm× 105 cm and the crops sown in September 15. Some deviations observed in the present study may also be attributed to different climatic conditions than the origin of the varieties.

Table 1: Average values of chilli varieties for Fusarium wilt resistance

Germplasm lines	PDI_I wk (FW)	PDI_II wk (FW)	PDI_III wk (FW)	Disease reaction	Ascorbic acid mg/100g	Phenols mg/100g	Capsaicin content mg/g
C1 Ujwala	69.50	82.50	100.00	HS	65.79	7.70	0.87
C2 Anugraha	55.00	81.50	81.22	HS	52.63	12.01	1.33
C3 Byadagi Kaddi	36.50	63.50	81.81	HS	65.79	8.03	0.36
C4 Byadagi Dabbi	38.50	76.50	81.91	HS	52.63	5.04	0.39
C5 GPM3	63.50	81.50	90.45	HS	65.79	8.86	0.39
C6 GPM7	35.50	45.50	53.50	S	78.95	3.02	0.57
C7 GPM8	70.00	80.50	80.50	HS	342.11	4.66	0.43
C8 GPM 21	8.50	8.50	9.05	R	78.95	3.66	1.28
C9GPM 22	9.50	10.00	47.50	MS	144.74	6.89	1.00
C10 GPM 206	54.50	80.50	80.50	HS	52.63	5.98	0.77
Arka lohit	33.00	50.00	50.50	MS	52.63	3.90	1.00
Pusa Jwala	80.00	80.00	97.50	HS	65.79	5.10	0.70
CO4	11.50	28.50	50.00	MS	39.47	7.97	1.13
KDC1	4.50	4.50	27.50	MR	65.79	3.78	1.14
Sitara	88.25	100.00	100.00	HS	39.47	2.66	0.57
G4	28.50	32.50	83.50	HS	65.79	6.14	0.91
BSS414	18.50	25.50	33.67	MS	65.79	5.58	1.44
Utkal Roshani	14.50	42.50	42.50	MS	92.11	6.79	0.89
Phule Jyoti	54.50	59.00	87.50	HS	92.11	4.04	0.44

Pant C1	30.50	54.50	77.50	HS	52.63	4.05	0.77
COO713	22.75	45.50	87.50	HS	65.79	4.90	0.24
LCA 334	20.00	30.00	40.50	MS	39.47	2.10	1.04
EC341094	24.50	37.50	60.00	S	39.47	5.46	0.81
EC497636	30.00	42.50	50.00	MS	26.32	6.40	1.34
Utkal Ava	19.00	51.00	80.00	HS	65.79	8.49	1.01
Murda variant	60.00	71.50	97.50	HS	131.58	4.77	0.55
CM334	60.00	72.50	77.50	HS	39.47	6.65	0.92
Grand mean	38.56	52.15	68.51		17.10	18.17	19.28
SE.m+	0.42	0.69	1.25		19.39	1.56	0.23
CV %	1.58	1.90	2.62		9.43	0.76	0.11
CD 5%	0.87	1.41	2.56				
CD 1%	1.69	2.75	4.99				

Table 2: Categorization of germplasm lines for Fusarium wilt incidence in sick pots 1 week, 2 week, 3 weeks post inoculation

PWI	Disease Reaction	Germplasm lines I week	Germplasm lines II week	Germplasm lines III week
0	Immune(I)	Nil	Nil	Nil
1-10	Resistant(R)	GPM21, GPM22, KDC1	GPM21, GPM22, KDC1	GPM21
11-25	Moderately resistant(MR)	CO4, BSS414, Utkal Roshani, COO713, LCA334, EC341094, Utkal Ava	Nil	Nil
26-50	Moderately susceptible(MS)	Byadagi Kaddi, Byadagi Dabbi, GPM7, Arka lohit, G4, Pant C1, EC497636,	GPM7, Arka lohit, CO4, G4, BSS414, Utkal Roshani,COO713, LCA334, EC341094, EC497636,	GPM22, Arka lohit, CO4, BSS414, Utkal Roshani, LCA334, EC497636, KDC1
51-75	Susceptible(S)	Ujwala, Anugraha, GPM3, GPM8, GPM206, Phule Jyoti, Murda variant, CM334	Byadagi Kaddi, Phule Jyothi, Pant C1, Utkal Ava, Murda variant, CM334	GPM7, EC341094
76-100	Highly susceptible(HS)	Pusa Jwala, Sitara,	Ujwala, Anugraha, Byadagi Dabbi, GPM3, GPM8, GPM206, Pusa Jwala, Sitara,	Ujwala, Anugraha, Byadagi Dabbi, Byadagi Kaddi, GPM3, GPM8, GPM206, Pusa Jwala, Sitara, G4, Phule Jyoti, Pant C1, COO713, Utkal Ava, Murda variant, CM334

Table 3: ANOVA for biochemical and disease traits in chilli

Source of variation	d.f.	AA	PHE	CC	PDI-FW	PDI-SW	PDI-TW
Genotypes MSS	26	9428.56	10.46	0.23	1117.87	1300.95	1217.83
Replication MSS	1	902.66	0.00	0.07	3.63	12.52	20.31
Error MSS	26	184.81	1.20	0.03	0.37	0.98	3.22
F value		51.02**	8.73**	8.94**	3021.15**	1327.43**	377.68**
CV %		17.10	18.17	19.28	1.58	1.90	2.62
CD 5%		19.39	1.56	0.23	0.87	1.41	2.56
SE.m+		9.43	0.76	0.11	0.42	0.69	1.25

AA: Ascorbic acid (mg/100g); PHE: Phenols (mg/100g); CC: Capsaicin content (mg/g); PDI-FW: Percent disease incidence_First week (FW); PDI-SW: Percent disease incidence_Second week (FW); PDI-TW: Percent disease incidence_Third week (FW)

Table 4: Mean, range and genetic variability components biochemical and disease traits in chilli

Traits	Mean	Min	Max	PCV	GCV	h^2_{bs}	GA	GAM
AA	79.50	26.32	394.74	87.21	85.52	96.20	137.33	172.74
PHE	6.02	1.92	13.22	40.08	35.73	79.50	3.95	65.61
CC	0.83	0.20	1.54	43.01	38.44	79.90	0.58	70.78
PDI-FW	38.56	4.00	89.00	61.33	61.31	99.90	48.68	126.25
PDI-SW	52.15	4.00	100.00	48.93	48.89	99.80	52.48	100.64
PDI-TW	68.50	9.01	100.00	36.07	35.97	99.50	50.63	73.91

PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; h^2_{bs}: Heritability in broad sense; GA: Genetic advance; GAM: Genetic advance as percent of mean; AA: Ascorbic acid (mg/100g); PHE: Phenols (mg/100g); CC: Capsaicin content (mg/g); PDI-FW: Percent disease incidence_First week (FW); PDI-SW: Percent disease incidence_Second week (FW); PDI-TW:Percent disease incidence_Third week (FW)

Table 5: Correlation for biochemical and disease traits in chilli varieties

	AA	PHE	CC	PDI-SW	PDI-TW	PDI-FW
AA	1					
PHE	-0.057	1				
CC	-0.230	0.254	1			
PDI-SW	0.068	0.163	-0.453**	1		
PDI-TW	0.050	0.152	-0.620**	0.831**	1	
PDI-FW	0.148	-0.005	-0.437**	0.929**	0.788**	1

*,** significant at 5% and 1% level of probability, If correlation r => 0.268 and 0.347

AA: Ascorbic acid (mg/100g); PHE: Phenols (mg/100g); CC: Capsaicin content (mg/g); PDI-FW: Percent disease incidence_First week (FW); PDI-SW: Percent disease incidence_Second week (FW); PDI-TW: Percent disease incidence_Third week (FW)

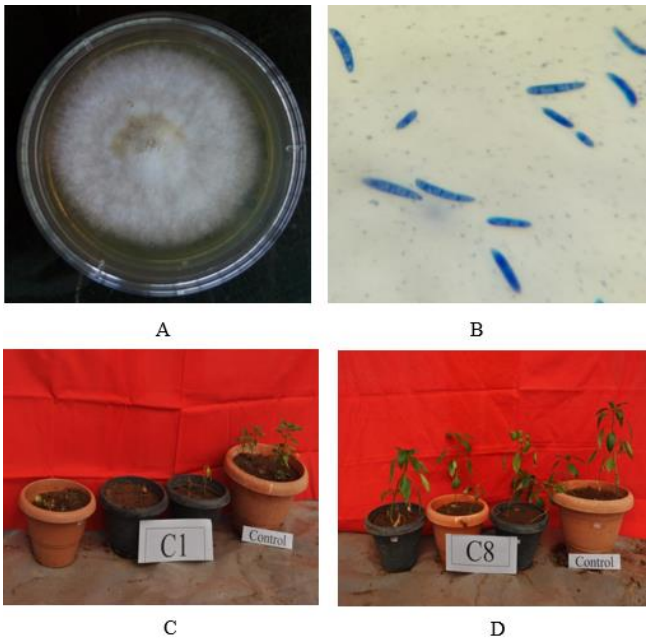

Fig 1: *Fusarium solani* and Highly resistant and highly susceptible chilli germplasm lines three weeks after artificial inoculation. A. *Fusarium solani* after one week of inoculation on PDA B. Micro and macroconidia present in two week old PDB(40X) C. Ujwala(C1) D. GPM21(C8) two weeks after inoculation.

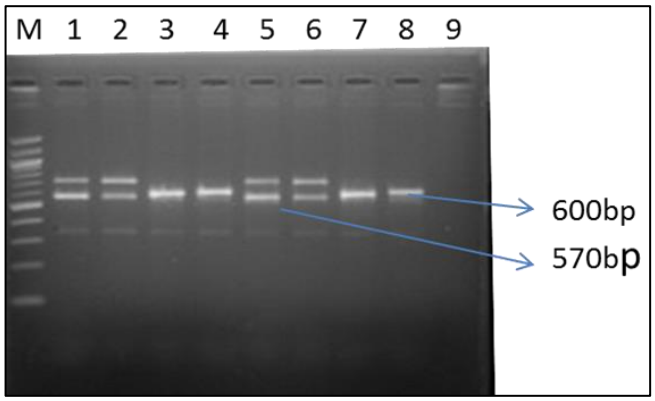

Fig 2: PCR products with ITS 1 and ITS4 primers separated on 2% Agarose. 1, 2 master isolate of *Fusarium solani* and 5, 6 isolated from symptomatic plant, 3,4,7,8 *Trichoderma spp* M-100 bp DNA ladder 9- Negative control

Conclusion

Screening of the genotypes for the resistance in different locality was necessary for targeting the region specific pathotype. GPM 21 identified as resistant for all three weeks of observation will be used in future breeding programmes for introgression of the gene into susceptible but well adopted cultivars. Capsaicin content gene may be closely associated with *Fusarium* wilt resistant gene. If the markers are identified for this we can incorporate only resistant to Fusarium wilt in non-pungent cultivars. Fusarium wilt resistant line GPM21 and other lines with high capsaicin have negative correlation with PWI. It indicates increased capsaicin provides resistance to Fusarium wilt in chilli.

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