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RESEARCH ARTICLE

Biochemical, histological and molecular investigations of coriander genotypes against *Fusarium* wilt

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Abstract

The wilt of coriander is a serious problem and affects the plants in the initial stages causing poor growth and plants become stunted drooping of terminal shoots, followed by withering and drying of leaves. The present investigation was in vertisols under supplemental irrigated conditions. Fifty-four lines of coriander germplasm were screened for wilt under natural. The genotypes were categorized into highly resistant, resistant, moderately resistant, susceptible and highly susceptible groups based on the disease reaction. The native fungal isolate of *Fusarium oxysporum* spp. was isolated, identified at morphological and molecular level, deposited in the NCBI gene bank with ID number OR483968 and used for challenge inoculations. The 2 lines from each disease reaction categories were randomly selected and taken forward to assess the resistance reaction through challenge inoculation. Biochemical studies of genotypes under uninoculated and inoculated conditions revealed that the polyphenol oxidase activity, peroxidase, superoxide dismutase, protein and phenol content were higher in highly resistant category, whereas the catalase and malondialdehyde content was higher in the susceptible category. Histological studies indicated that the stem and root are affected by pathogen significantly. The stem cross section of highly susceptible genotype exhibited discoluration and shredding of pith compared with highly resistant genotype. Study of root cross-section confirmed the pathogen's alleged capacity to enter the plant's root system and colonize it via its vascular system. Whereas, the stomatal characters *viz.,* stomatal count, stomatal length and stomatal width exhibited less variation among various categories of coriander genotypes. The morphological and molecular variation (using ISSR markers) among coriander genotypes was also studied. The resistant genotypes like LCC-169, LCC – 200, LCC-150, LCC- 190 and LCC- 208 were distributed across different clusters indicated that the genetic background of resistance might be different and distinct.

Keywords

disease resistance; *Fusarium* wilt; peroxidase activity; polyphenol oxidase; superoxide dismutase

Introduction

Coriander (*Coriandrum sativum* L.), commonly referred to as Cilantro in English and Dhania in Hindi, is an annual herb from the Apiaceae family. It has a diploid chromosome number of 2n = 2x = 22 (1). In India produces 81100 MT of coriander

annually, from a cultivated area of 640000 ha (2). The productivity of coriander is constrained by multiple factors, one being the production reduction by incidence of diseases. Several diseases affect coriander which includes wilt, powdery mildew, stem gall, root rot and damping off. Among these, the most critical are wilt caused by *Fusarium oxysporum* (3), stem gall caused by *Protomyces macrosporus* (10) and powdery mildew caused by *Erysiphe polygoni*. Coriander is grown in different situations rainfed - vertisols or irrigated -dry inceptisols /alfisols. The wilt disease is prevalent in all these farming situations hampering coriander production. In case of wilt, inadequate knowledge of disease management is the most important production constraint. The disease is managed by Integrated Disease Management (IDM) practices, which is cost effective, but often cumbersome. Traditionally coriander is grown in Rajasthan, Madhya Pradesh, Andhra Pradesh and Gujarat where the crop is cultivated in the same land over years hence the soil borne disease is prevalent. The incidence of the disease was reported from the Indian states of Madhya Pradesh, Gujarat, Rajasthan, Andhra Pradesh which are major coriander growing areas of the country (3). The disease was also reported from Israel (4), USA (5), Egypt (6), Argentina (7), China (8) and Italy (9). Wilt significantly impacts coriander by causing drooping of shoots, yellowing of foliage and withering of leaves, especially in early growth stages. Severe infestation of *Fusarium* leads to complete mortality of plants driving crop failure or results in poor seed quality if affected during flowering (11). Wilt disease can result in up to a 60 percent yield loss in coriander and 15 to 25% was reported in Gujarat (12). In the present investigation, fifty four genotypes were studied for their reaction to wilt in naturally evolved sick plot. Further, the biochemical and histopathological changes after inoculation with Fusarium wilt in resistant and susceptible coriander genotypes were studied to reveal underlying mechanisms of resistance and susceptibility.

The investigation was carried out in 2022 at Horticultural Research Station, Lam, Guntur, India having the coordinates: 16° 18' N latitude, 80° 29' E longitude. The site is located in the Krishna-Godavari Agro-climatic zone of Andhra Pradesh. The soil of the experimental site is vertisols (black cotton soil) with a pH of 8.4, EC of 0.16 mmhos cm^{-1} and good moisture retentive capacity.

Screening for Fusarium wilt resistance

The pathogen of Fusarium wilt was isolated using standard protocol (13) and was identified at morphological and molecular level. The pure culture of pathogen was maintained and multiplied on sand sorghum medium for further use. Screening for *Fusarium* wilt was done under natural incidence conditions and challenge inoculated conditions. Under natural conditions, the screening was done in a naturally evolved sick plot, which was observed for disease expression for the past 5 years. 54 genotypes were screened for *Fusarium* wilt resistance in this sick plot. The experiment was laid out in Augmented Block Design with four checks and five blocks. The genotypes were sown in the month of October, 2022 and harvested during January, 2023. The crop was planted with a spacing of 30 cm x 10 cm and uniform cultural practices were followed. In the sick plot the symptoms were observed from 3 weeks after sowing to 9 weeks after sowing to evaluate the wilting, growth and yield. The percentage of mortality of was recorded in ten days interval. The percent disease incidence was arrived from the final screening data.

The artificial screening of all the 54 genotypes was taken up in the month of February, 2023. Isolation and identification of pathogen was carried out during January 2023. The native isolate, thus derived was used for artificial screening. Under challenge inoculated conditions, the plug trays were sterilized, filled with sterilized cocopeat. The plug trays were artificially inoculated with *F. oxysporum* with a concentration of 1×10^6 conidia/mL. The plug trays were kept in shade net house under ambient conditions. Observations were recorded based on the symptoms and mortality of the plants. The symptoms were recorded from 10 days after planting as some of the genotypes might be susceptible at germination and seedling stage. The screening was done subsequently at 20 days after planting and 30 days after planting. The percent disease incidence was arrived from the final screening data.

All the lines were screened based on the disease severity using a 0-5 point disease rating scale (14). On the basis of disease incidence percentage, they were categorized into highly resistant (HR), resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS). Among the 54 genotypes, top 2 lines were selected from different categories of reaction to wilt and subjected to morphological, biochemical, histological and molecular studies. Further, one genotype each from highly resistant and highly susceptible categories was observed for stem and root damage using microscope (Olympus, 40x magnification) for comparison.

Morphological studies

The morphological evaluation was laid out during January, 2023 and harvested during March, 2023. The study was taken up to evaluate the *per se* performance of the genotype in disease free environment. The trial was laid out with 10 genotypes in Randomized Block Design with 4 replications in disease free plot. The evaluation accommodated 2 genotypes each from HR (LCC-150, LCC- 169), R (LCC - 200, LCC- 208), MR (LCC- 170, LCC- 190), S (LCC- 105, LCC-206) and HS (LCC-1, LCC-99) categories. The observations on growth, yield and yield attributes were recorded for all the genotypes.

Histological studies

Histological studies were carried out with one genotype each from HR (LCC- 150) and HS (LCC-1) categories.

Stem cross section and root cross section

The samples were collected from susceptible and resistant plants of coriander genotypes on 20^{th} day after inoculation (DAI), by which time, all the genotypes usually shown full symptoms after inoculation. The time of collection of samples was decided based on the earlier inoculation trials. Plant samples were preserved by immersion in 70% alcohol. These samples were subsequently converted into permanent specimens using glycerin-gelatin, with cross-sections obtained from both stems and roots, as detailed by (15) and observed microscope.

Stomatal characters

The mean stomatal number was determined using the polyvinyl acetate method, where polyvinyl acetate was applied to both surfaces of the leaf to create a thin film. After a 5 min interval, the polyvinyl acetate layer was carefully peeled off and mounted onto a clean slide for observation under a microscope, following

the procedure outlined (16). Stomatal length and width were determined using the slides.

Biochemical analysis

The assay was carried out 20 days after sowing, in healthy and inoculated plants, which were raised in pots separately. The inoculation in the pots in disease induction group was done at the time of sowing. The evaluation accommodated 2 genotypes each from HR (LCC- 150, LCC- 169), R (LCC - 200, LCC- 208), MR (LCC- 170, LCC- 190), S (LCC- 105, LCC-206) and HS (LCC-1, LCC-99) categories. The leaf samples were used to estimate Phenols (17), proteins (18) and enzyme assays viz., catalase (19), peroxidase activity (20), polyphenol oxidase activity (21), superoxide dismutase (22) and malondialdehyde (23) to assess the changes in metabolites under each group during course of infection.

Molecular marker analysis in coriander

Ten coriander genotypes were grown in a plug trays and leaf material from 3-4 weeks old plants was used for DNA extraction *via* the CTAB method (24). The extracted DNA was stored at -20 ° C. DNA quantification and quality assessment were performed using a Nano Drop spectrophotometer. The concentration and purity of the DNA were measured and potential degradation or contamination was checked by running an aliquot on a 0.8% agarose gel stained with ethidium bromide. The gel was visualized using a gel documentation system. For the present study, 8 ISSR primers were selected from the published literature as indicated in Table-1. DNA was amplified *in vitro* using PCR with ISSR primers. The reaction, conducted in an Eppendorf Master Cycler, included 5 µL $MgCl_2$ (50 mM) - 1 µL dNTPs (10 mM) -0.5 µL, Primer - 2 µL, Taq DNA polymerase (5 U/µL) -0.2 L DNA (100 ng/ µL) -2 µL, Molecular biology grade water - 15.8 µL, totaling 25 µL PCR conditions we re: initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, primer annealing at 42-66 °C for 1 min, extension at 72 °C for 2 min, final extension at 72 ° C for 7 min and store at 4 °C. Products were separated on a 1.5% agarose gel, stained with ethidium bromide and visualized under UV light. Amplified fragments were scored for presence or absence and analyzed with NTSYS-pc to construct a dendrogram using UPGMA (25).

Results

Pathogen isolation and identification

After isolation, one week old cultures were purified with a single spore on PDA to purify the pathogen. The pure culture of the isolate was maintained in PDA slants in a refrigerator at 4 ºC for further investigations. After the pathogen's purification, it was observed through macroscopic and microscopic investigations, the pathogen produced 2 different forms of conidia *i.e.,* macroconidia and microconidia. Macroscopic observations revealed that the growth appearance of pathogen was cottony and white colonies surface while the texture and surface characteristics was dense aerial mycelium, white to pink colour on the lower side. The observations indicated that the pathogen in investigation was *Fusarium oxysporum* and it was further identified at molecular level.

Screening of coriander against the Fusarium wilt under natural and challenge inoculated conditions

Under natural conditions, based on the disease incidence percentage, 4 genotypes were found highly resistant (3-7%), seven genotypes were resistant (13-20%), 9 were moderately resistant (23-30%), 13 genotypes were susceptible (40-50%) and the remaining 21 genotypes were highly susceptible (83-100%). In artificial screening, 4 genotypes were found highly resistant (5.2-10%), 7 genotypes were resistant (12.7-18.9%), 9 were moderately resistant (21.1-29%), 10 genotypes were susceptible (39.5-49.8%) and the remaining 24 genotypes were highly susceptible (65.9-99%).

Table 1. List of ISSR primers used in the present study

Primer	Sequence (5'→3')	Tm(ºC)
UBC 807	AGAGAGAGAGAGAGAGT	50.36
UBC 808	AGAGAGAGAGAGAGAGC	52.77
UBC 810	GAGAGAGAGAGAGAGAT	50.36
UBC 812	GAGAGAGAGAGAGAGAA	50.36
UBC 818	CACACACACACACACAG	52.77
UBC 815	CTCTCTCTCTCTCTCTG	52.14
UBC 825	ACACACACACACACACT	50.36
UBC 826	ACACACACACACACACC	50.19
UBC 807	AGAGAGAGAGAGAGAGT	50.36

Table 2. Germplasm under different categories of disease response under natural and artificial conditions

D.I %	Category	List of genotypes		No. of genotypes	
		Under natural conditions	Under artificial conditions	under natural conditions	under artificial conditions
1-10	HR	LCC-69, LCC-79, LCC-150, LCC-169	LCC-69, LCC-79, LCC-150, LCC-169	4	4
11-20	R	LCC-63, LCC-170, LCC-188, LCC-200, LCC-208, LCC-318, LCC-345	LCC-63, LCC-170, LCC-200, LCC-208, LCC-306, LCC-318, LCC-345	7	7
21-30	MR	LCC-138, LCC-147, LCC-190, LCC-196, LCC-197, LCC-201, LCC-227, LCC-306, LCC-344	LCC-138, LCC-147, LCC-188, LCC-190, LCC-196, LCC-197, LCC-201, LCC-227, LCC-344	9	9
31-50	S	LCC-22, LCC-28, LCC-29, LCC-40, LCC-48, LCC-59, LCC-60, LCC-90, LCC-105, LCC-202, LCC-206, LCC-235, LCC-309.	LCC-22, LCC-29, LCC-48, LCC-105, LCC -120, LCC-130, LCC-202, LCC-206, LCC-235, LCC-309.	13	10
>50	HS	LCC-1, LCC-7, LCC-12, LCC-14, LCC-15, LCC-19, LCC-23, LCC-26, LCC-27, LCC-30, LCC-34, LCC-49, LCC-99, LCC-100, LCC-119, LCC-120, LCC-130, Suguna, Sudha, Susthira, AD-1	LCC-1, LCC-7, LCC-12, LCC-14, LCC-15, LCC-19, LCC-23, LCC-26, LCC-27, LCC-28, LCC-30, LCC-34, LCC-40, LCC-49, LCC-59, LCC-99, LCC-100, LCC-119, LCC-120, LCC-130, Suguna, Sudha, Susthira, AD-1	21	24
	LSD (p=0.05)	10.941		**C.D.**	**4.876**
	SE(m)	**3.551**		**SE(m)**	**1.715**

HR- highly resistant, R-resistant, MR- moderately resistant, S-susceptible, HS-highly susceptible

Morphological studies

The morphological study showed that there was significant variation among the genotypes under the investigation. Among the genotypes, the genotype LCC-1 recorded the maximum plant height (54.1 cm), while the minimum was observed in LCC-200 (40.2 cm). Maximum number of primary branches per plant was recorded in LCC-99 (4.51), while LCC-200 had minimum (3.2). Secondary branches ranged from 6.18 (LCC-206) to 11.3 (LCC-169). Earliest flowering was seen in LCC-105 (34.4 days). Days taken for maturity varied from 82.2 days (LCC-206) to 89.4 days (LCC-169). The number of umbels per plant ranged from 19.9 (LCC-170) to 28.5 (LCC-208) and umbellets per umbel ranged from 4.1 (LCC-200) to 5.69 (LCC-190). Seeds per umbel varied from 18.12 (LCC-200) to 31.8 (LCC-169) and seed yield per plant ranged from 2.96 g (LCC-200) to 5.85 g (LCC-208). Seed length ranged from 3.76 mm to 5.98 mm (LCC-105). Seed width varied from 2.6 mm to 3.22 mm and test weight ranged from 12.34 g (LCC-105) to 13.64 g (LCC-208) (Table 3).

Histological studies

Cross sectional view of shoot and root damage was observed in healthy and inoculated seedlings at 20th day of sowing and compared (Fig. 2a & b). The stomatal characters were observed under microscope in healthy plants of selected genotypes of each group on 20 days after sowing.

Root cross section

Cross-section of infected coriander roots revealed that the organized arrangement of root tissues especially xylem and phloem vessels was observed in HR genotype (Fig. 2a). Whereas, disorganized and shrinkage of tissues was seen in HS genotype. The presence of fungal mycelium in the infected HS root tissue showed that the fungus was able to infiltrate the root tissue, but it was unable to survive in the HR genotype as it was unable to create any symptoms of the wilt disease or any browning of internal tissues. This might be due to more proliferation of pathogen in the HS genotype, while the growth of fungus was

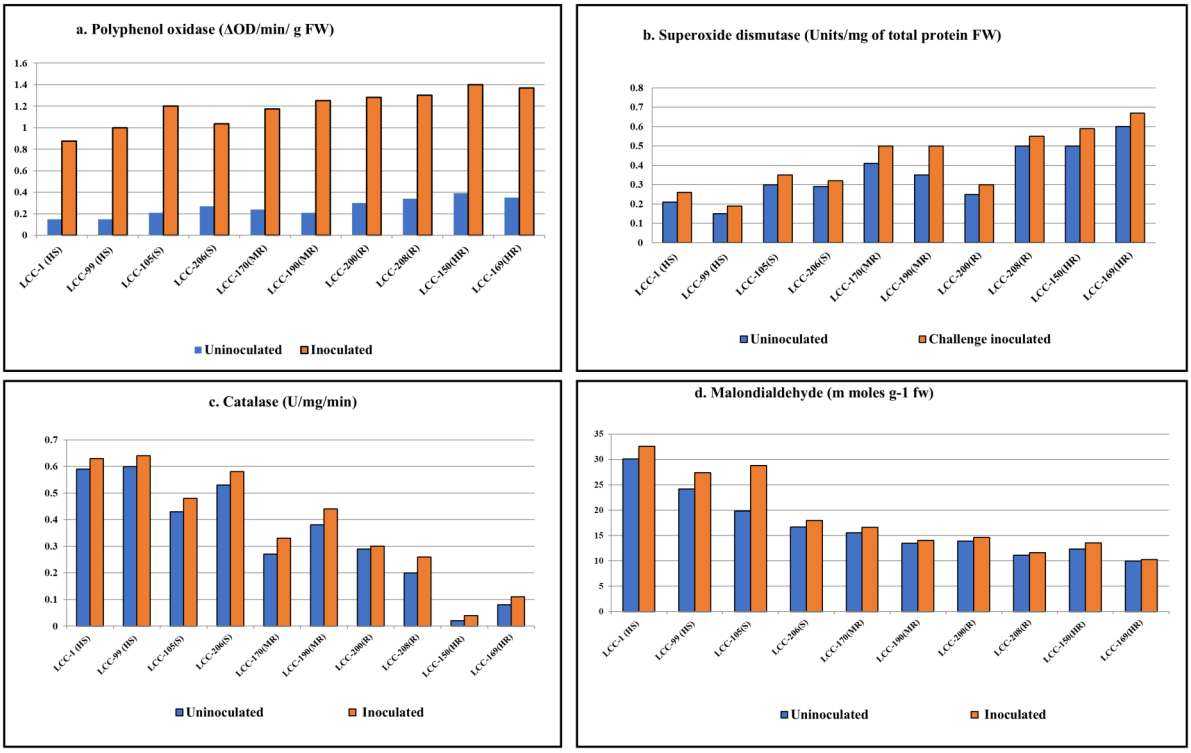

Fig. 1. Antioxidant variation in selected coriander genotypes exhibiting various disease reactions. a. Polyphenol oxidase b. Superoxide dismutase c. Catalase d. Malondialdehyde.

Table 3. Morphological data for ten selected coriander genotypes

Geno type	PH	NPB	NSB	DFF	DFIF	DM	NUP	NUU	NSU	SYP	SL	SW	TW
LCC-1	54.1	3.44	7.28	37.5	45.6	85.1	22.8	5.0	26.7	4.1	3.9	3.2	12.84
LCC-99	47.7	4.51	8.01	39.7	51.1	86.1	21.7	4.4	23.9	3.2	4.4	3.1	13.64
LCC-105	50.3	3.81	7.81	34.4	41.8	82.1	26.8	5.2	27.8	5.4	5.9	3.3	12.34
LCC-150	41.5	3.39	10.21	35.4	47.8	87.8	20.4	4.6	25.1	3.1	4.8	3.0	13.44
LCC-169	46.9	3.69	11.31	39.7	45.1	89.4	21.8	5.2	31.7	4.9	4.8	2.6	12.44
LCC-170	52.7	3.99	9.21	36.1	50.1	87.8	19.9	5.4	27.7	3.5	4.3	2.2	12.84
LCC- 190	53.7	3.29	7.51	37.9	46.6	85.8	22.4	5.6	24.9	3.5	4.0	2.6	12.64
LCC-200	40.1	3.19	8.61	37.6	45.4	84.8	27.3	4.0	18.1	2.9	4.3	2.6	12.84
LCC-206	40.6	3.74	6.1	37.0	41.5	82.9	23.1	5.4	25.4	3.9	3.7	2.8	13.16
LCC-208	41.6	3.84	8.7	40.3	45.8	84.9	28.4	5.0	27.5	5.8	4.8	3.1	12.66
C.D @ 5%	2.602	0.193	0.347	1.973	2.127	1.562	0.688	0.043	0.844	0.076	0.05	0.056	0.029
SE(m)	0.869	0.065	0.116	0.659	0.71	0.522	0.23	0.014	0.282	0.025	0.017	0.019	0.01

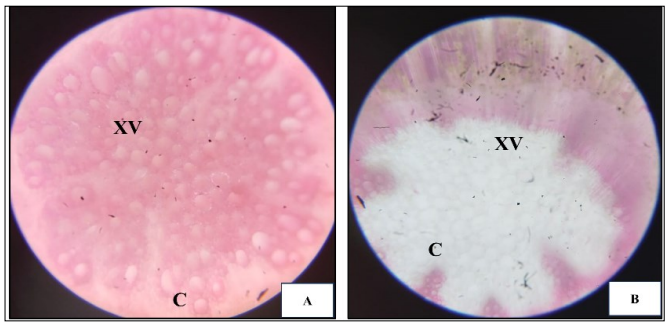

Fig. 2(a). Root cross section of Healthy and infected plant tissues. A. Resistant healthy Root B. Susceptible infected Root C-Cambium, XV-Xylem vessels.

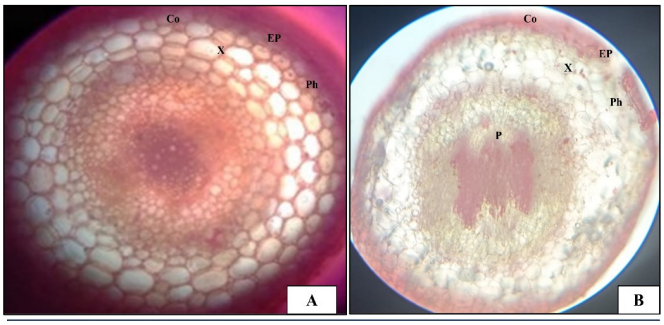

Fig. 2(b). Stem cross section of Healthy and infected plant tissues. A. Resistant inoculated Stem and B. Susceptible inoculated stem. EP-Epidermis, Co-Cortex, Ph -Phloem, P-Pith, X-Xylem.

restricted in HR genotype. *Fusarium oxysporum* colonization was also observed in roots of analysed coriander plants.

Stem cross section

The observations on stem histology presented in Fig. 2b. It was discovered that all the tissue cells in the resistant plant were intact, whereas those in the susceptible diseased tissue were observed to disintegrate. In pathogen inoculated plants, highly susceptible genotype showed a discoloration of pith and vascular bundles. The findings obtained in the current investigation are corroborated with the findings of (26-28).

It was also observed that the discoloration of nearby tissues and the shredding of the pith are significant in the infected HS genotype than HR one. The pith actually serves as the major connecting link between the shoot and root. Shrinkage of pith as witnessed in HS genotype might cause hurdles in anchorage, transportation and distribution of nutrients and minerals. The fungal infection was found to be similar across the genotypes of various groups; however, marked differences were observed in different groups.

Stomatal characters (count /m², length µm) and width(µm)

Stomatal count was significantly highest in the LCC- 206 (25) and LCC- 110 (24) followed by the lowest stomatal count observed in LCC-1(10). Stomatal length (µm) was significantly highest in the LCC- 1 (35.03) followed by LCC- 110 (33.40) and the lowest stomata length was observed in LCC-150 (24.50). Stomatal width (µm) was significantly highest in the LCC- 1(32.70) followed by LCC- 99 (29.40) and the lowest stomata length was observed in LCC-150 (22.50) as presented in Fig. 3 and Table 4.

Biochemical Analysis

Table 4. Stomatal characters in selected genotypes exhibiting various disease reactions

Genotype	Stomatal length (µm)	Stomatal width (µm)	Count/m²
LCC-1 (HS)	35.03	32.70	10
LCC-99 (HS)	32.10	29.40	16
LCC-105 (S)	33.40	26.19	24
LCC-206(S)	30.10	23.80	25
LCC-170(MR)	29.40	26.50	12
LCC-190(MR)	30.30	27.40	13
LCC-200(R)	28.90	26.30	18
LCC-208(R)	27.10	28.31	14
LCC-150(HR)	24.50	22.50	19
LCC-169(HR)	28.60	25.39	15
C.D @ 5%	2.022	2.222	1.791
SE(m)	0.681	0.748	0.603

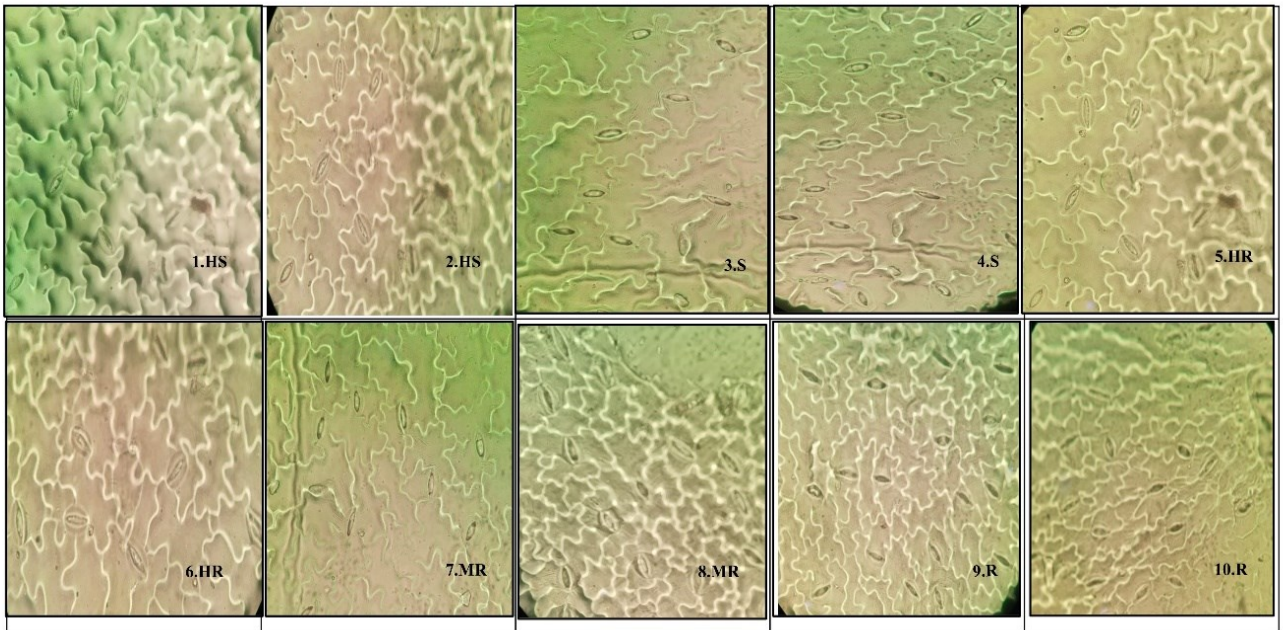

Fig. 3. Comparison of stomatal distribution and stomatal size in 1.LCC-1, 2. LCC-99, 3.LCC-105, 4.LCC-206, 5. LCC-150, 6.LCC-169, 7. LCC-170 8.LCC-190, 9.LCC -200 and 10.LCC-208.HS -Highly susceptible, S-Susceptible, HR- Highly resistant, MR- Moderately resistant, R-Resistant.

The changes in metabolites of each group were observed in healthy and inoculated seedlings at 20th day of sowing and compared (Fig. 2).

Polyphenol oxidase (ΔOD/min/g FW)

In uninoculated coriander genotypes, polyphenol oxidase (PPO) activity ranged from 0.15 to 0.39 ΔOD/min/g FW, with the highest in LCC-150 (HR) and the lowest in LCC-99 (HS) and LCC-1 (HS) (Fig. 1). The inoculated plants exhibited increased PPO activity (0.87 to 1.4 ΔOD/min/g FW); the maximum activity was in LCC-150 (HR). Several previous reports indicated the spike PPO activity upon disease expression (29, 30).

Peroxidase (ΔOD/min/ g FW)

In the group of uninoculated genotypes, LCC-150 (HR) and LCC-169 (HR) showed the highest peroxidase activity, with values of 3.95 and 3.84 ΔOD/min/g FW respectively, while LCC-99 (HS) had the lowest at 1.46 ΔOD/min/g FW (Fig. 1). Among challenge-inoculated genotypes, LCC-150 (HR) had the highest activity at 4.21 ΔOD/min/g FW, followed by LCC-169 (HR) at 4.09 ΔOD/min/g FW.

Superoxide dismutase (Units/mg of total protein FW)

SOD activity in coriander genotypes ranged from 0.15 to 0.50 Units/mg of total protein FW in uninoculated genotypes and from 0.19 to 0.67 Units/mg of total protein FW in inoculated ones (Fig. 1). In uninoculated genotypes, LCC-169 (HR) and LCC-150 (HR) showed the highest SOD activity at 0.50 Units/mg FW, while LCC-99 (HS) had the lowest at 0.15 Units/mg FW. In inoculated genotypes, LCC-169 (HR) had the highest activity at 0.67 Units/mg FW, followed by LCC-150 (HR) at 0.59 Units/mg FW, with LCC-99 (HS) the lowest at 0.19 Units/mg FW. The role of superoxide dismutase in resistance to fungal infections was reported earlier (31).

Catalase (U/mg/min)

In healthy coriander genotypes, catalase content ranged from 0.02 to 0.60 U/mg/min, while in infected genotypes, it varied from 0.04 to 0.64 U/mg/min (Fig. 1). Uninoculated LCC-99 showed the highest catalase activity at 0.60 U/mg/min, comparable to LCC-1 at 0.59 U/mg/min and the lowest was in LCC-150 at 0.002 U/mg/min. Among inoculated genotypes, LCC-99 had the highest activity at 0.64 U/mg/min, similar to LCC-1 at 0.63 U/mg/min, with the lowest in LCC-150 at 0.04 U/mg/min.

Malondialdehyde (MDA) (m moles g-1 fw)

In uninoculated coriander genotypes, MDA activity ranged from 9.96 to 30.13 m moles g^{-1} FW and in challenge-inoculated conditions, it ranged from 10.30 to 32.62 m moles g^{-1} FW (Fig. 1). LCC-1 had the highest MDA content in uninoculated genotypes at 30.13 m moles g^{-1} FW, followed by LCC-99 at 24.17 m moles g^{-1} FW, while LCC-169 had the lowest at 9.96 m moles g^{-1} FW. Among inoculated genotypes, LCC-1 showed the highest MDA content at 32.62 m moles g^{-1} FW, followed by LCC-105 at 28.84 m moles g^{-1} FW, with the lowest in LCC-169 at 10.30 m moles g^{-1} FW.

Total phenol (mg/g)

In uninoculated coriander genotypes, total phenol activity ranged from 0.147 to 0.267 mg/g, while in challenge-inoculated conditions, it varied from 0.164 to 0.285 mg/g (Fig. 1). In uninoculated conditions, LCC-150 (HR) had the highest phenol content at 0.267 mg/g, followed by LCC-169 (HR) at 0.254 mg/g. Under inoculated conditions, LCC-150 (HR) showed the highest phenol activity at 0.285 mg/g, with LCC-169 (HR) at 0.270 mg/g. The higher phenol content in resistant genotypes was observed in banana and cumin (32, 33).

Protein (mg / g)

In uninoculated coriander genotypes, protein activity ranged from 0.16 to 0.29 mg/g, while under challenge-inoculated conditions, it varied from 0.17 to 0.318 mg/g (Fig. 1). The highest protein content in uninoculated conditions was in LCC-150 (HR) at 0.291 mg/g, followed by LCC-169 (HR) at 0.284 mg/g, with the lowest in LCC-1 (HS) at 0.165 mg/g. In challenge-inoculated genotypes, LCC-150 (HR) had the highest protein content at 0.315 mg/g, followed by LCC-169 (HR) at 0.318 mg/g and the lowest in LCC-1 (HS) at 0.17 mg/g. The variation in protein content in relation disease aggression was observed in coriander and cumin (34, 35).

Molecular Characterization

The PIC analysis was carried out to determine the efficiency of each ISSR marker in expressing polymorphic loci in coriander. A PIC value greater than 0.5 indicates loci of high polymorphism, values between 0.25 and 0.5 indicate loci of intermediate polymorphism and less than 0.25 indicate loci of low polymorphism. The PIC values were in between a range of 0.39 to 0.43. The highest polymorphic information content was found for UBC 826 (0.43) followed by UBC 818 (0.42) and UBC 810(0.42), UBC 815(0.40) and UBC 808 (0.39), UBC 807 (0.36). Similar results have been reported (36).

Ten coriander genotypes were used for diversity analysis. The ISSR markers effectively captured the genetic difference among the coriander genotypes demonstrating high polymorphism. Dendrogram (Fig. 4 and Fig. 5) grouped the ten genotypes into 2 major clusters (Cluster I and II). The cluster I comprised of 2 genotypes *viz.,* LCC-169 and LCC-200, which were found to exhibit resistant reactions and recorded a greater number of seeds per umbel. The cluster II was subdivided into four sub clusters *viz.,* A, B, C and D. The sub-cluster A, B and C consisted of one genotype *viz.,* LCC-206, LCC-1 and LCC-170, respectively. While sub-cluster D further sub divided into 2 sub-cluster D1 and D2. The sub-cluster D1 comprised of 2 genotypes *viz.,* LCC-99 and LCC-105 and both the genotypes exhibited susceptible reactions. The sub-cluster D2 comprised of 3 genotypes *viz.,* LCC-150, LCC-190 and LCC-208, which exhibited resistant reactions. LCC-206 and LCC-1 were found to exhibit susceptible reaction for *Fusarium* wilt in coriander while, LCC-170 was found to be moderately resistant. LCC-206 and LCC-170 had similar growth and yield attributes as witnessed form morphological and cluster mean data. Both the genotypes recorded moderate seed yield/plot while LCC-1 recorded higher yields. LCC-208 registered higher yields while LCC-150 and LCC-190 recorded moderate yields. The genotypes LCC-190, LCC-150, LCC-208, LCC-170, LCC-206 were found to be diverse at molecular level. Similar clustering was observed in certain studies. (36, 37).

Discussion

Screening of *Fusarium* wilt resistant germplasm may provide a

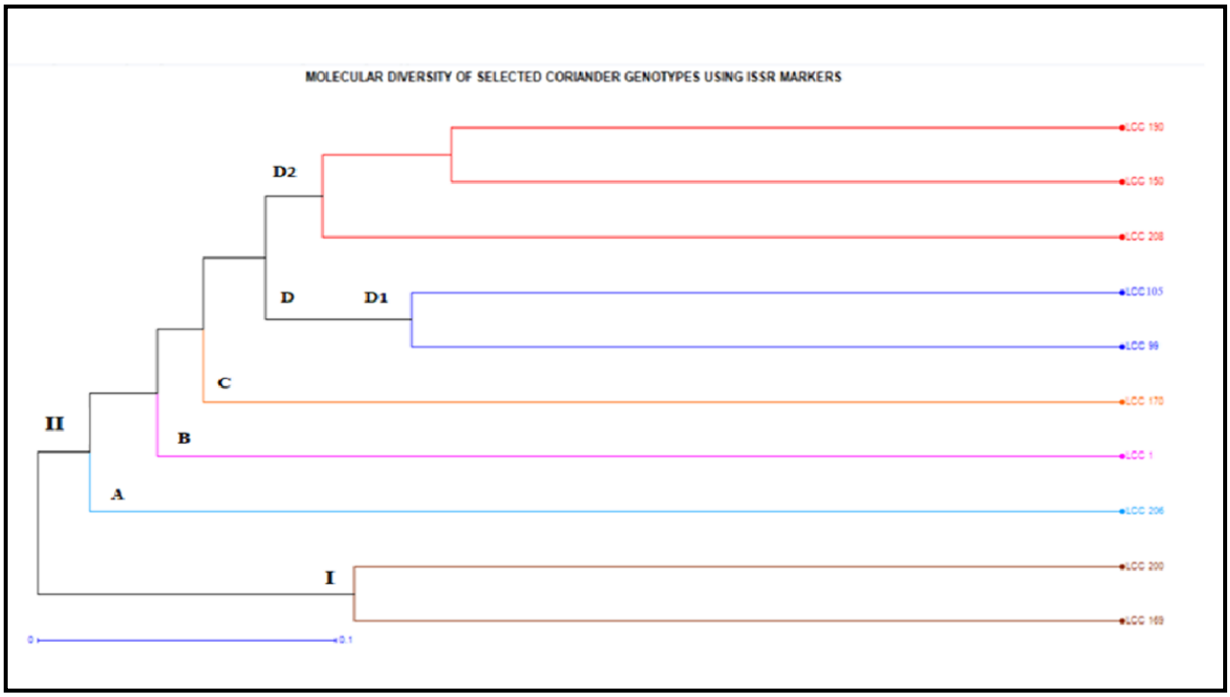

Fig. 4. Molecular diversity of selected coriander genotypes.

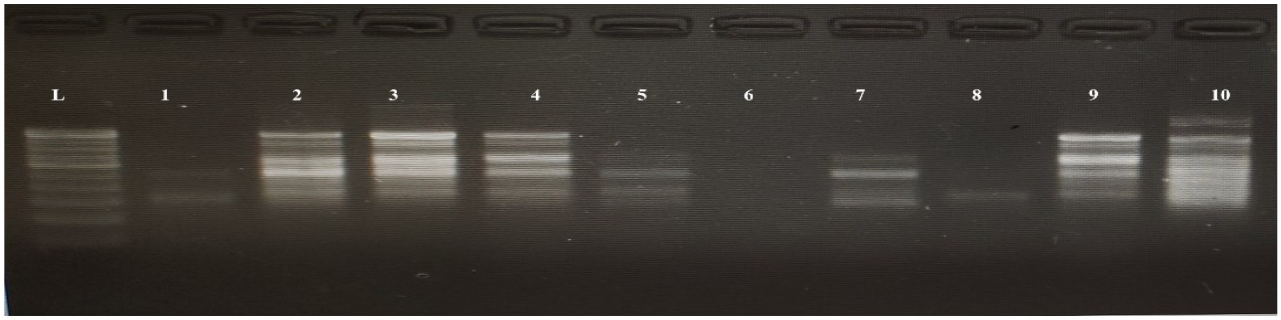

Fig. 5. ISSR profile generated for coriander genotypes by primer UBC 826.

1. LCC-1, 2. LCC-99, 3. LCC-105, 4. 4. LCC-150, 5. LCC-169, 6. LCC-170, 7. LCC-190, 8. LCC-200, 9. LCC-206, 10. LCC-208 -Ladder

strong potential for an efficient application of resistant plant material or genes to improve the resistance in genotypes (38). However, in resistant genotypes, the number of diseased plants was always low or even absent and the growth reduction was much less than in susceptible genotypes. The occurrence of diseased plants in resistant genotypes was likely due to environmental variation (39). The results inferred that all the 54-coriander genotypes performed significantly differently and suggested that there was a chance to uncover valuable genotypes in the screened group. Some genotypes did not consistently exhibit the same level of resistance in field and plug tray conditions, indicating that resistance to *Fusarium* wilt in coriander may be impacted by factors other than genetics. Interaction of soil-borne pathogens with the environmental elements *viz.,* carbon dioxide, temperature, soil texture, soil pH and moisture might have affected disease occurrence. It is also implicated that higher carbon-dioxide or lower pH may favour the multiplication of fungi, weaken defence mechanism of plants and cause disease.

This discovery supports the hypothesis that the pathogen can enter the plant's root system and colonize it via its vascular system. *F. oxysporum* colonization was seen in the examined coriander organs (root and stem), demonstrating the process of colonization through the vascular system. This result confirms the pathogen's alleged capacity to enter the plant's root system and colonize it via its vascular system. Similar process of colonization of *F. oxysporum* was documented (40, 41).

Colonization of pathogen, profuse growth and proliferation of fungus could be seen in the HS genotype, causing blockage of vascular bundles *viz.,* xylem and phloem. Damage in xylem and phloem vessels causes disruption in water conducting mechanism and food translocation from source to sink respectively, leading to wilting of plant. Further vascular bundles spoilage causes epinasty, flaccidity, chlorosis, vascular browning and necrosis of the above ground part, which are the main signs of the disease. Further, plants may wilt partially or entirely, get stunted and eventually die as evinced with HS genotype. Similar results and predictions were presented by (26 -28). While, the up regulation of defense mechanism in the HR genotype conferred resistance and caused negligible damage to plant. Defense mechanism regulates cell wall integrity (CWI) in response to fungi infections, which includes cell wall reinforcement as an active component. In order to protect cell walls from further invasion by fungus, stress lignin might have typically deposited in HR genotypes. Cell wall strengthening would have rendered cells resistant to mechanical penetration, provides resistance to wall disintegration by fungal enzymes through synthesis of phenolic precursors of lignin and free radicals available for the inactivation of fungal membranes, toxins, enzymes and elicitors (42). Thus, tissue deformation was less in HR genotypes and it could be ascertained by the MDA

levels, which is an indicator of cell damage.

There was no variation in stomatal characters of the genotypes exhibiting various resistant reactions (Table 4). Different resistance responses displayed by different genotypes were probably not related to studied stomatal characters, might be due to mechanisms in roots and stem and this might be linked to a gene-to-gene interaction (43, 44).

Plants defend themselves against a variety of oxidative stresses through antioxidant enzymes. By enhancing the activity of different antioxidant enzymes like CAT, POD and SOD, it has been shown that H_2O_2 improves the antioxidant capacity of cells. The involvement of polyphenol oxidase in the oxidation of phenolic compounds to quinines, which increases antimicrobial action, is crucial in the defense mechanism against pathogens. It might play a direct role in stopping pathogen development by speeding up the death of cells near the infection site, preventing the spread of the infection, by producing a toxic environment that inhibits the growth of the pathogen inside the cells (45). In the present study, POD activity was observed to have elevated in the highly resistant genotypes. POD scavenges the H_2O_2 and also catalyzes the synthesis of the cell wall, modifying the mechanical properties of the cell wall and membrane integrity during the infestation (46). The increased peroxidase and polyphenol oxidase activity and changes in the phenolic constituents immediately after infection are normal responses of a host plant in putting up an initial defense. This mechanism breaks down as found in susceptible genotypes, probably due to the lower synthesis of phenolic enzymes and substrates. However, it persists in the resistant ones. Plants utilize the SOD enzyme as their first line of defense because it converts superoxide radicals produced by oxidative metabolism into H_2O_2 and O_2 (47). Plants produce antioxidant enzymes in response to pathogen infestation regimes that inhibit damage to cellular integrity and regulate their antioxidant capacity. During plant-pathogen interactions, catalase expression and activity are altered. Host catalase gene expression or enzyme activity during disease progression may rise or fall depending on the interacting partners and spatial and temporal parameters. In the present investigation, the catalase activity is higher in the highly susceptible genotypes than the highly resistant types. Brief increase in catalase activity was reported in *B. cinerea*-infected tomato, but gradually decreased as the disease progressed (48). Similar dynamics in catalase activity was reported in Potato infected by nematodes and bacterial infection (49). Decreased catalase activity in the HR genotype might allow accumulation of H_2O_2 content, contributing to the strengthening of the cell wall, there by triggering a hypersensitive reaction through activation of defense genes and subsequently curtailing the pathogen infection. During an incompatible interaction, pathogen infection is assumed to be halted by reducing plant catalase activity and gene expression. The increase in malondialdehyde reported in the estimation of MDA is crucial since it was a marker for evaluating lipid peroxidation. The biotic and abiotic stress caused by intercellular oxidative stress, led to severe disturbances in the plant cell and increase in the content of MDA.

Due to their potent antioxidant properties, phenols aid in the defense against reactive oxygen species (ROS), which are invariably created when environmental factors hinder photosynthetic metabolism (50). Proteins both directly and indirectly contribute to defensive processes by triggering plant defense mechanisms. Most plants quickly produce protein when a pathogen attacks them. Plants can build resistance to infections through an efficient defense system (51).

Significant variation was observed among the coriander genotypes at the molecular level. The molecular and morphological data were in sync with one another as indicated by the dendrogram. In the diversity analysis the sub cluster A branched of early suggesting that it is genetically distant compared to other genotypes in cluster 2. The genotype might represent an important source of unique traits for breeding program such as elevated level of protected enzymes (polyphenol oxidase, peroxidise) and structural integrity in stem and root tissues upon challenge inoculation. The sub clusters D1 and D2 had high degree of similarity, which might be due to common ancestry or similar selection pressure or derived from common growing areas. The distribution of resistant and tolerant genotypes in different clusters and sub clusters showed the genetic back ground of resistance might be different and distinct among the tolerant/resistant genotypes. Similar classification in terms of disease response of *Fusarium* wilt was noticed in the molecular data as well as in morphological data. Since the resistant genotypes were identified, design and development of a genetic marker associated with resistance will augment further crop improvement programs, facilitating marker-assisted selection of parents and segregating populations (52).

Conclusion

The present study on the resistance of coriander to *Fusarium* wilt has revealed significant insights into the genetic and biochemical mechanisms underlying disease resistance. The screening of 54 genotypes under both natural and artificial inoculation conditions showed that varying degrees of resistance in coriander. The genotypes LCC-150 and LCC-169 were found resistant under both conditions of evaluation. The genotypes showed significant variation in morphological traits. In resistant genotypes, tissue structures were intact compared to the disorganized tissues observed in susceptible ones. Biochemical assessments highlighted elevated levels of key defensive enzymes, including polyphenol oxidase and peroxidase, in resistant genotypes upon pathogen exposure. These enzymes play critical role in plant defense mechanisms, indicating that tweaking these biochemical pathways could be an effective strategy for improving disease resistance in coriander. This is the first study that identified the variation in resistance levels in coriander for *Fusarium* wilt disease under artificial inoculation conditions. Hence, effective disease management through breeding for resistance can mitigate the adverse effects of *Fusarium* wilt on coriander production. The findings advocate for the implementation of Integrated Disease Management (IDM) practices those leverage resistant genotypes to enhance productivity and sustainability in coriander cultivation. Future research should focus on further elucidating the molecular pathways involved in resistance to facilitate targeted breeding programs.

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Authors' contributions

MS conducted the study and wrote the manuscript. KG participated in the design of the study and performed the statistical analysis. BT carried out the molecular diversity studies and approved the final manuscript. TV and MK conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used [NAME TOOL / SERVICE] in order to [REASON]. After using this tool/ service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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