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Unravelling the novel source of resistance and differential reactions on chilli die back and fruit rot through screening of various chilli genotypes

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ABSTRACT

Dieback and fruit rot, caused by Colletotrichum species, are significant pre- and post-harvest diseases affecting chili (Capsicum spp.), with C. capsici being the most prevalent species in India. A study evaluating the resistance of 41 capsicum genotypes to these diseases revealed varying levels of resistance. While some moderately cultivated lines and crosses showed resistance, genotypes such as Bhut Jolokia, PBC-380, and IC-383072 remained symptomless.In vivo, inoculation trials with C. capsici determined that nine days post-inoculation is the optimal time for assessing disease resistance. These screenings identified nine highly resistant and eleven highly susceptible genotypes. Consistently resistant genotypes to dieback included BS-35, BS-20, BS-28, Punjab Lal, Bhut Jolokia, Taiwan-2, IC-383072, and Pant C-1. Additionally, BS-28, CC-0189, and Hisar Sakti demonstrated immunity to fruit rot. Further analysis of genotypes inoculated with C. capsici isolates revealed the presence of two distinct pathotypes. Eleven genotypes, including Bhut Jolokia, BS-27, BS-28, BS-35, BS-37, CC-0189, Hisar Sakti, IC-326272, and IC-383072, exhibited resistance to fruit rot. Challenges of this study were genetic variability among genotypes, environmental factors, selection pressure, lack of clear biomarkers, pest & disease complexes, limited availability of resistant genotypes. These findings contribute in encapturing of resistant genotypes against fruit rot and dieback and succeed. and emphasize the importance of detailed studies on the prevalent pathogen strains in specific regions to enhance resistance breeding efforts. The newly identified resistant genotypes serve as valuable resources for advancing breeding programs targeting dieback and fruit rot resistance.

Keywords: *Die back, Chilli, Genotypes, Fruit rot, Colletotrichum capsica, Screening, Resistant, Susceptible*

Introduction

Capsicum annuum, or chili, is a valuable vegetable and spice crop that is grown all over the world. Andhra Pradesh, Karnataka, Maharashtra, Odisha, Tamil Nadu, Madhya Pradesh, and Rajasthan are among the states in India that produce the most chilies. About 54.41 thousand hectares of chili are produced in Madhya Pradesh, with an average yield of 0.98 tons per hectare and a production of 93.57 thousand metric tons (1). *Colletotrichum* species are important plant pathogens on a global scale, producing illnesses in a variety of crops, including cereals, legumes, vegetables, and fruits, such as die-back, anthracnose, fruit rot, leaf spot, wilt, and damping-off (2). Depending on the chili type, die-back in chili (*Capsicum annuum*) caused by *Colletotrichum capsica* (Syd.) Butler and Bisby can cause yield reductions of 10% to 60%(3). Initial reports of die-back and fruit rot caused by *Colletotrichum brevisporum* emerged from bromeliads (*Neoregalia* sp.) and *Pandanus pygmaeus* in Thailand, its endophytic form in *Lyciumchinense* Miller in Korea, and papaya in Brazil (4). Chili holds a special place in global cuisine, consumed both as a dried spice and as a fresh vegetable. In Punjab, die-back caused by this pathogen has led to yield losses of 66–84%. Chemical control measures for die-back can be expensive and pose environmental hazards, making the development of resistant chili varieties the most practical and sustainable management approach.

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Understanding the nature and mechanisms of resistance to die-back is crucial for designing effective breeding programs. Screening chili genotypes for resistance to die-back is a key step in identifying varieties with natural resistance. This not only reduces reliance on chemical controls but also provides valuable resources for breeding programs aimed at enhancing disease resistance in chili crops. The primary objectives of screening are to:

1. Identify genotypes with natural resistance to die-back disease.

2. Evaluate the response of different genotypes under controlled conditions.

Field tests using artificial inoculation, disease evaluation, cultivar categorization, and biochemical analysis are commonly included in the process. Breeding initiatives can increase chili resilience by discovering resistant genotypes, guaranteeing steady output in die-back-affected areas. Using fungicidal treatments, resistant or tolerant cultivars, and disease-free seeds are some methods of managing die-back.

The purpose of this study was to use artificial inoculation to find resistant cultivars or germplasm against Colletotrichum capsici in field settings. To find those with resistance to die-back disease, 89 genotypes in all were examined.

Material and Methods

In order to screen 89 chili genotypes for resistance to die-back disease caused by Colletotrichum capsici, the experiment was carried out at Jammu College during the 2023–2024 season. During the Kharif season, the study used a Randomized Complete Block Design (RCBD) with three replications and an artificial inoculation approach. Among the genotypes that were evaluated, Ujala was employed as a susceptible check and BS-27 as a resistant control. Assam-10, Bhut Jolokia, BS-27, BS-28, BS-35, BS-37, Byadagi Dabbi-1, Agnirekha, Akola-10, Arka Khyati, Arka Lohit, Arka Meghana, and Arka Mohini were among the 89 chili cultivars that were screened, ByadagiKaddi, California Wonder, CC-0189, CC-0210, EC-341075, EC-510320, EC-378632, EC-405253, EC-566320, EC-566969, EC-599969, EC-622085, Hisar Sakti, Hisar Vijay, IC-537599, IC-545651, IC-545654, IC-545723, IC-208534, IC-208580, IC-208586, IC-208591, IC-214965, IC-226257, IC-255928, IC-255929, IC-255941, IC-255944, IC-297654, IC-326272, IC-344727, IC-383072, IC-387691, IC-395318, IC-545649, IC-545722, IC-545735, IC-570408, Jaganath, Jawahar Mirch, Jawahar Mirch-218, Jwala, Jyanti, Kashi Anmol, Kashi Anmol-2, Kashi Gaurav, Kashi Siduri, Kashi Surkh, Kiran, Lembang-1, Pant C-1, Phule Jyoti, Punjab Lal, Pusa Jawala, Pusa Sadabahar, Rajput Sankeshwar, SH-C-1, SH-C-11, SH-C-14, SH-C-15, SH-C-19, SH-C-4, SH-C-6, SH-C-9, Sikkim Chilli, Sitara, SSB-8, SUM-17, Sunidhi, Taiwan-2, Tanjung-2, Tiwari, and Ujala. Three artificial inoculation treatments were used during the screening process: Inoculation with distilled water (control), Inoculation without injury, and Inoculation with injury. The study aimed to evaluate and identify genotypes with resistance to die-back disease under controlled conditions, contributing to efforts to develop disease-resistant chili varieties (6).

Methodology for Screening of Chili Cultivars

Semi-ripe chili fruits (25 days old) from each of the 25 cultivars/germplasms were selected for the study. A purified, pathogenic isolate of *Colletotrichum capsici* was used based on its proven pathogenicity. Mass multiplication has beenwas carried out and screening was done (7).

Preparation of Spore Suspension and Inoculation

A ten-day-old active culture of the fungus was used to extract conidia. Sterilized distilled water was poured to the petri Petri plates, and a camel toothbrush was used to gently scrape the fungus surface in order to collect spores. Through microscopic examination, the resultant spore suspension was moved to a beaker, filtered, and adjusted to a concentration of 10^6 spores/ml. The percentage of infected sites (as determined by lesion development) compared to the total number of inoculation sites was used to calculate the disease incidence on fruits.The score chart will be used to determine the severity of the die-back and fruit rot diseases in chillies. (15).

Grade	Per cent fruit area infection/ branches infected per plant	Reaction
0	0 %	Immune
1	1-10%	Resistant
3	11-25%	Moderately resistant
5	26-50%	Moderately susceptible
7	51-75%	Susceptible
9	>75%	Highly susceptible

The die-back severity will be assessed based on per cent branches infected in each plant and fruit severity will be assed number of rotted fruits per plant using the following formula given by (Wheeler 1969):

$$\text{PDI of the Die-back} = \frac{\text{Sum of numerical disease rating infected}}{\text{Total no.of samples x Maximum of disease rating scale}} \times 100$$

$$\text{PDI of Fruit rot} = \frac{\text{Sum of numerical disease rating infected}}{\text{Total no.of plants x Maximum of disease rating scale}} \times 100$$

This methodology allowed for the identification of resistant, moderately resistant, and susceptible genotypes, aiding in the selection of varieties for further breeding and management strategies (8).

Artificial Inoculation Using Fruit Puncture Method

For the study, mature, dark green chili fruits that were 2-3 cm long and completely developed but not yet red were harvested from robust plants. Twenty-five kinds or germplasms of semi-ripe fruits (25 days old) were chosen. To reduce surface microbial contamination, the fruits were carefully removed, cleaned with sterile distilled water (SDW), and then wiped with cotton soaked in ethanol. An inoculum concentration of 5×10^5 conidia/ml was created in accordance with earlier findings (9). Each fruit was punctured (0.6 mm diameter × 1.2 mm depth) and drops of this conidial suspension were administered to the proximal and distal ends. Three experimental sets of fruits were prepared:

1. On the proximal and distal ends of the fruit surface, two drops (10 µl each) of the conidial suspension were applied.

2. To observe the development of anthracnose lesions, fruits were softly punctured with a sharp blade and then inoculated with two drops (10 µl each) of the conidial suspension at the proximal and distal ends (10).

3. (Control): The fruits were treated with sterile distilled water rather than the conidial suspension.

Following inoculation, the fruits were put in plastic containers measuring 30 x 20 x 7 cm. They were sealed with plastic tape and lined with four layers of moistened paper towels to retain humidity. At 5, 7, and 10 days after vaccination, disease symptoms were noted and assessed by measuring the lesion area. A statistical analysis was conducted on the data (11).

Results and Discussion

Screening of genotypes against chilli dieback (*Colletotrichum capsici*) under epiphytotic situations

The response of *Colletotrichum capsici* was evaluated against 89 chili genotypes under controlled conditions. Three inoculation methods were tested: Application of two drops of sterile distilled water (without injuring the fruit), Application of two drops of conidial suspension (without injuring the fruit), and Application of two drops of conidial suspension (after injuring the fruit). Observations were recorded five, seven, and ten days post-inoculation. Significant differences in disease reactions were observed across all genotypes, as shown in Table 1. Among the inoculation methods, applying conidial suspension after injuring the fruit was the most effective in inducing infection, whereas no infection occurred in the other two treatments (13). Seven days post-inoculation, data from Table 1 revealed that all genotypes exhibited significantly different disease responses to *C. capsici*. The application of conidial suspension after fruit injury consistently resulted in higher infection levels compared to other treatments. In contrast, fruits inoculated without injury showed no signs of infection. Nine genotypes—Bhut Jolokia, BS-27, BS-28, BS-35, BS-37, CC-0189, Hisar Sakti, IC-326272, and IC-383072—displayed resistant reactions. These genotypes showed die-back severity ranging from 0.34% to 9.06% and fruit rot severity ranging from 2.38% to 10.12%, as follows:

Die-back severity (%):Bhut Jolokia (9.06), BS-27 (2.46), BS-28 (0.34), BS-35 (8.50), BS-37 (0.63), CC-0189 (0.76), Hisar Sakti (5.28), IC-326272 (9.34), IC-383072 (1.81).

Fruit rot severity (%):Bhut Jolokia (10.12), BS-27 (2.54), BS-28 (6.78), BS-35 (9.56), BS-37 (6.74), CC-0189 (9.34), Hisar Sakti (5.54), IC-326272 (9.34), IC-383072 (2.38).

The resistance observed in these genotypes may be attributed to genetic, physiological, and biochemical factors. Key resistance mechanisms include: Genetic resistancei.eFaster recognition of pathogens and activation of defense-related proteins (e.g., pathogenesis-related proteins). Antioxidant activity or Higher levels of antioxidants to mitigate oxidative stress caused by the pathogen, maintaining plant health under infection pressure. Structural barriers like Stronger cell walls, thicker waxy coatings, or other physical traits that hinder pathogen entry and spread within the plant (14). In contrast, several genotypes—including Agnirekha, Assam-10, CC-0210, EC-566320, IC-208534, IC-208586, IC-214965, Kashi Anmol, Kashi Anmol-2, Lembang-1, Pusa Jawala, Pusa Sadabahar, SH-C-1, SH-C-6, SH-C-15, Sikkim Chilli, SUM-17, Taiwan-2, and Tanjung-2—showed moderately resistant reactions, with intermediate levels of die-back and fruit rot severity following inoculation with conidial suspension after injury. These findings align with results reported by Rajamanickam and Sethuraman (2014), who evaluated four different inoculation methods under *in vivo*conditions to assess their effectiveness against die-back disease.Twenty two genotypes (Byadagi Dabbi-1, EC-378632, EC-566969, EC-599969, IC-208591, IC-226257, IC-545651, IC-545654, IC-545722, IC-545723, IC-545735, IC-570408, Kashi Gaurav, Kashi Siduri, Pant C-1, Punjab Lal, Rajput, Sankeshwar, SH-C-4, SH-C-9, Sitara, Tiwari) displayed moderately susceptiblereaction against dieback with severity ranging from 2.5-11.3 %. Further eleven genotypes (Akola-10, Arka Lohit, Arka Mohini, EC-510320, IC-255928, IC-344727, Jyanti, Kashi Surkh, Phule Jyoti, SH-C-19, Sunidhi) showcased highly susceptible reaction against dieback with severity of 80.21, 41.24, 52.94, 51.68, 52.50, 41.71, 44.54, 53.50, 34.27, 58.55 and 42.81 % severity respectivly (Table 1). Twenty seven genotypes *viz.*, Arka Khyati, Arka Lohit, Arka Meghana, ByadagiKaddi, California Wonder, EC-341075, EC-405253, EC-622085, Hisar Vijay, IC-208580, IC-255929, IC-255941, IC-255944, IC-297654, IC-387691, IC-395318, IC-537599, IC-545649, IC-545944, Jawahar Mirch, Jawahar Mirch-218, Jwala, Kiran, SSB 8, SH-C-11, SH-C-14, Ujala shown susceptible reaction against dieback and fruit rot with severity ranging from 40-87 % severity respectively. This is due to poor environment adaptation and lower antioxidant capacity (Malathi., 2001).Among the methods tested, spraying spore suspension after pinpricking the fruits resulted in the highest level of infection (Table 1 and 2).

Dieback disease severity

Genotypes such as Arka Khyati, Arka Lohit, Arka Meghana, ByadagiKaddi, California Wonder, EC-341075, EC-405253, EC-622085, Hisar Vijay, IC-208580, IC-255929, IC-255941, IC-255944, IC-297654, and IC-387691 were identified as susceptible, exhibiting die-back disease severity of 80% against *Colletotrichum capsici* under epiphytotic conditions, as illustrated in the heatmap (Fig. 1). In contrast, genotypes like Bhut Jolokia, BS-27, BS-28, BS-35, BS-37, CC-0189, and Hisar Sakti displayed elevated resistance levels, as evident in the heatmap, which highlights their breeding potential and resistance traits against *Colletotrichum*species. These results are consistent with Rajamanickam and Sethuraman's (2014) findings. Significant differences between all kinds that were artificially screened are also seen in Fig. 1. Certain genotypes, including ByadagiKaddi, California Wonder, EC-341075, EC-405253, EC-622085, Hisar Vijay, IC-208580, IC-255929, IC-255941, IC-255944, IC-297654, IC-387691, and Arka Khyati, Arka Lohit, and Arka Meghana, showed a 20% illness severity. The longevity and innate behavioral characteristics of the germplasm lines may be responsible for this variation in disease response.

Because of their vulnerability to extreme weather, these genotypes may be more susceptible, which might affect pathogen entry and distribution and increase the severity of the disease(11).

Categorization of chilli genotypes based on reaction against *Colletotrichum capsica*causing die back

During the screening, disease reactions were classified based on severity levels. Eleven genotypes, including Akola-10, Arka Lohit, Arka Mohini, EC-510320, IC-255928, IC-344727, Jyanti, Kashi Surkh, Phule Jyoti, SH-C-19, and Sunidhi, were identified as highly susceptible to die-back, with a disease severity of 75%, as depicted in Fig. 2. Moderately resistant reactions were observed in genotypes such as IC-214965, Kashi Anmol, Kashi Anmol-2, Lembang-1, Pusa Jawala, Pusa Sadabahar, SH-C-1, SH-C-6, SH-C-15, Sikkim Chilli, SUM-17, Taiwan-2, and Tanjung-2, showing a disease severity ranging from 11% to 25% (16).Moderate susceptibility, with a disease severity of 26% to 50%, was observed in the following genotypes: Byadagi Dabbi-1, EC-378632, EC-566969, EC-599969, IC-208591, IC-226257, IC-545651, IC-545654, IC-545722, IC-545723, IC-545735, IC-570408, Kashi Gaurav, Kashi Siduri, Pant C-1, Punjab Lal, Rajput, Sankeshwar, SH-C-4, SH-C-9, Sitara, and Tiwari.

Classification of genotypes into different clusters for die back disease

Resistant Genotypes includes varieties like Bhut Jolokia and BS-27. These are found to be ideal for cultivation in areas prone to the disease as they exhibit strong resistance. Moderately Resistant Genotypes were observed in genotypes viz., Agnirekha, Assam-10, CC-0210, EC-566320, IC-208534, IC-208586, IC-214965, Kashi Anmol, Kashi Anmol-2, Lembang-1, Pusa Jawala, Pusa Sadabahar, SH-C-1, SH-C-6, SH-C-15, Sikkim Chilli, SUM-17, Taiwan-2, Tanjung-2. These are observed to be perform reasonably well but may still show mild disease symptoms. Moderately Susceptible Genotypes includes Byadagi Dabbi-1, EC-378632, EC-566969, EC-599969, IC-208591, IC-226257, IC-545651, IC-545654, IC-545722, IC-545723, IC-545735, IC-570408, Kashi Gaurav, Kashi Siduri, Pant C-1, Punjab Lal, Rajput, Sankeshwar, SH-C-4, SH-C-9, Sitara, Tiwari. These are noticed to be prone to disease under unfavorable conditions but may be manageable with appropriate care (16). The resistance of genotypes like Bhut Jolokia, BS-27, BS-28, BS-35, BS-37, CC-0189, Hisar Sakti, IC-326272, and IC-383072 to dieback disease can be attributed to genetic resistance, oxidative stress management, production of phytoalexins etc. Susceptible Genotypes include Arka Khyati, Arka Lohit, Arka Meghana, ByadagiKaddi, California Wonder, EC-341075, EC-405253, EC-622085, Hisar Vijay, IC-208580, IC-255929, IC-255941, IC-255944, IC-297654, IC-387691, IC-395318, IC-537599, IC-545649, IC-545944, Jawahar Mirch, Jawahar Mirch-218, Jwala, Kiran, SSB 8, SH-C-11, SH-C-14, Ujala which were presumed to be cause disease resulting in susceptibility. A total of eleven genotypes viz., Akola-10, Arka Lohit, Arka Mohini, EC-510320, IC-255928, IC-344727, Jyanti, Kashi Surkh, Phule Jyoti, SH-C-19, Sunidhi were declared as highly susceptible due to varied climatic conditions and virulence of pathogen as depicted in Table 1. The findings are in align with Malathi., (2001). These genotypes are not ideal for disease-prone areas as they suffer significant damage. These genotypes are the most vulnerable and are highly unsuitable for cultivation in areas with disease pressure.

Heat map and bar graph visualization of various chilli genotypes based on die back disease severity against *Colletotrichum capsici* under epiphytotic situations

Figure 1 illustrates the categorization of chili genotypes based on the Disease Rating Scale and Severity using a heatmap-style visualization. The X-Axis (Genotype)displays various chili genotypes evaluated in the study, with each genotype associated with one or more severity levels. The Y-Axis (Disease Rating Scale) outlines the categories of disease response: Highly Susceptible, Susceptible, Moderately Susceptible, Moderately Resistant, Resistant, and Immune. These categories indicate increasing levels of resistance or decreasing susceptibility to the disease.The heatmap uses a color gradient to represent disease severity, with blue indicating low severity (genotypes with minimal disease symptoms) and red representing high severity (genotypes with severe disease symptoms). Each rectangle corresponds to a specific combination of a genotype and its associated disease rating and severity.The distribution reveals that genotypes are predominantly clustered in the Moderately Resistant and Resistant categories, which are associated with lower disease severity. A small number of genotypes exhibit absolute immunity, indicated by the presence of blue rectangles and the absence of red in the Immune category. Conversely, as disease severity increases, the number of genotypes decreases, with fewer genotypes categorized as Highly Susceptible or Susceptible.A negative correlation is evident in the data: genotypes in higher resistance categories (Immune and Resistant) are associated with lower severity scores (blue zones), while genotypes in susceptible categories correspond to higher severity scores (red zones). (13).

Classification of genotypes into different clusters for fruitrot

This data categorizes genotypes based on their reactions to aeminent fruit rot disease, organizing them into six levels of susceptibility: Immune, Resistant,Moderately Resistant, Moderately Susceptible, Susceptible, and Highly Susceptible as depicted in table 3. Immune (3 genotypes) includes BS-28, CC-0189, Hisar Sakti. Resistant (11 genotypes) has Bhut Jolokia, BS-27, BS-35, BS-37, IC-326272, IC-383072, Pant C-1, Pusa Sadabahar, SH-C-1, Taiwan-2, Tanjung-2. Moderately Resistant (24 genotypes) contains Agnirekha, Assam-10, CC-0210, EC-378632, EC-566320, IC-545654, IC-208534, IC-208586, IC-214965, IC-226257, IC-545735, IC-570408, Kashi Anmol, Kashi Anmol-2, Kashi Gaurav, Lembang-1, Punjab Lal, Pusa Jawala, SH-C-15, SH-C-4, SH-C-6, Sikkim Chilli, Sitara, SUM-17. Moderately Susceptible (39 genotypes) includes Arka Khyati, Arka Lohit, Arka Meghana, Byadagi Dabbi-1, ByadagiKaddi, California Wonder, EC-341075, EC-405253, EC-566969, EC-599969, Hisar Vijay, IC-537599, IC-545651, IC-545723, IC-208580, IC-208591, IC-255929, IC-255941, IC-255944, IC-297654, IC-344727, IC-387691, IC-395318, IC-545649, IC-545722, Jaganath, Jawahar Mirch, Jwala, Jyanti, Kashi Siduri, Pant C-1, Phule Jyoti, Rajput, Sankeshwar, SH-C-11, SH-C-14, SH-C-9, Sunidhi, Tiwari. Susceptible (11 genotypes): Arka Lohit, Arka Mohini, EC-510320, EC-622085, IC-255928, Jawahar Mirch-218, Kashi Surkh, Kiran, SH-C-19, SSB 8, Ujala. Highly Susceptible (1 genotype) possess genotypes like Akola-10. This detailed classification provides valuable insights for selecting genotypes suitable for breeding programs or cultivation based on disease resistance. Categorizing genotypes by their disease reactions is a crucial aspect of plant breeding, agriculture, and disease management.

Immune Genotypes exhibit no disease symptoms, indicating complete resistance. This immunity may stem from specific resistance (R) genes that recognize and neutralize pathogens early. Immune genotypes, like Hisar Sakti, are ideal for breeding programs focused on developing disease-resistant varieties, especially for regions with high disease pressure. Resistant Genotypes such as Bhut Jolokia and BS-27 show minimal disease symptoms even under high disease pressure. Although not completely immune, these plants possess strong defense mechanisms, including structural barriers or biochemical responses, to limit disease progression. Resistant varieties are particularly valuable in areas with moderate to high disease prevalence, as they reduce reliance on chemical controls. Bhut Jolokia, a well-known chili variety, can thrive in disease-prone regions, minimizing losses while ensuring good yields. Moderately Resistant Genotypes like Kashi Anmol and Punjab Lal display moderate disease symptoms but maintain acceptable productivity. These genotypes delay disease onset, allowing time for early control measures. They are often included in breeding programs to combine moderate resistance with other desirable traits such as high yield or flavor. For instance, Kashi Anmol, a commercial variety, strikes a balance between disease management and productivity.

Moderately Susceptible Genotypes like Jwala and Arka Meghana are affected by fruit rot disease but can still produce reasonable yields under good management. These plants require integrated pest management (IPM) practices, including fungicides, proper spacing, and irrigation control, to mitigate disease impact. Despite their susceptibility, varieties like Jwala remain popular for their marketable traits, such as flavor or appearance. Arka Meghana could be a viable option for farmers willing to invest in crop protection strategies (Table 3). Susceptible Genotypes such as Kashi Surkh are highly affected by the disease, leading to significant yield losses. These genotypes are unsuitable for cultivation in areas where the disease is prevalent unless robust control measures are available. In regions with frequent outbreaks, susceptible varieties are often replaced by resistant ones. However, Kashi Surkh might still be grown in areas with low disease pressure or where its high-value traits justify additional protective efforts. Highly Susceptible Genotypes like Akola-10 are extremely vulnerable to the disease, resulting in uneconomical yields. These varieties are unsuitable for cultivation in disease-prone areas and are often excluded from consideration unless exceptional control measures are implemented (17).

Fruit rot disease severity of different chilli genotypes against *Colletotrichumcapsici*

Figure 3 provides a visual representation of the mean fruit rot disease severity across various chili genotypes. Higher values indicate greater susceptibility, while lower values signify resistance or immunity. Each bar corresponds to the mean disease severity of a specific genotype, with the bar heights reflecting susceptibility levels: High Disease Severity genotypes like Akola-10, Arka Lohit, and SH-C-19 show tall bars, representing high susceptibility (e.g., severity >80%). These genotypes are less suitable for cultivation in disease-prone regions unless effective control measures are available. Low Disease Severity genotypes such as BS-28, CC-0189, and Hisar Sakti exhibit short bars, indicating minimal disease severity (close to 0%). These genotypes demonstrate strong resistance or immunity due to robust genetic defense mechanisms. They are ideal for cultivation in areas with high disease pressure and for use in breeding programs aimed at developing resistant varieties.

Intermediate Disease Severity genotypes like Bhut Jolokia, Kashi Anmol, and Punjab Lal show moderate bar heights (e.g., 20-50% severity). These genotypes display partial resistance and are suitable for regions with moderate disease pressure. However, integrated disease management strategies may be necessary to optimize productivity. This figure highlights the relative susceptibility of chili genotypes to fruit rot disease, with resistant genotypes like BS-28 standing out as valuable for breeding and cultivation, particularly in disease-prone areas. Highly susceptible genotypes like Akola-10 can serve as controls in research on disease progression. Meanwhile, moderately susceptible genotypes, such as Jwala, may still be cultivated with proper disease management practices. The data presented in Figure 3 serves as a critical resource for breeders, farmers, and researchers, enabling informed decisions on genotype selection for enhanced productivity, reduced chemical dependency, and sustainable agricultural practices (18).

Bar plot categorization of different chilli genotypes based on fruit rot disease reaction against *Colletotrichum capsici* under epiphytotic conditions

Fig 4 effectively highlights the distribution of chilli genotypes across disease severity categories. It underscores the need for focused breeding programs to develop more resistant varieties and reduce reliance on susceptible genotypes. For growers, this data aids in selecting the most suitable genotypes for their specific conditions, ensuring better yield and profitability. The graph categorizes chilli genotypes based on their fruit rot disease severity rating scale, showing the distribution of genotypes across different levels of disease response. In Fig 4, X-Axis (Fruit Rot Disease Rating Scale) represents different levels of disease response, categorized into: Highly Susceptible, Moderately Resistant, Moderately Susceptible, Resistant, Susceptible. Y-Axis (Number of Chilli Genotypes) indicates the count of genotypes corresponding to each disease severity category. The bars show the number of genotypes in each category, further divided by reaction percentages, 1-10% (Yellow):Strong resistance or near immunity, 11-25% (Green): Resistant, 26-50% (Orange): Moderate resistance, 51-75% (Purple): Moderate susceptibility, 75% (Blue): High susceptibility. Only one genotype falls in highly susceptible category (blue, >75% severity). This genotype (e.g., Akola-10) is highly vulnerable to fruit rot. Such genotypes are not suitable for cultivation without significant disease management efforts.A moderate number of genotypes (~24) fall in the moderately resistant category (yellow and green bars, 1-25% severity).These genotypes exhibit partial resistance and are more robust against fruit rot than susceptible ones.They are viable for cultivation in areas with mild to moderate disease prevalence, requiring minimal external control.The largest category (~39 genotypes) is moderately susceptible (purple and blue bars, 51-75% severity). These genotypes are affected by the disease but can still produce acceptable yields under proper management.A smaller group (~11 genotypes) shows resistance (yellow and green bars, 1-25% severity). Jeyalakshmi and Seetharaman (1998) reported that these genotypes have strong defenses against fruit rot and are ideal for breeding programs and cultivation in high-risk areas.A significant number of genotypes (~11) fall under the susceptible category (blue and purple bars, >50% severity). These genotypes are at high risk and require heavy disease control measures. This is due to high Proportion of Susceptible and Moderately Susceptible Genotypes. The scarcity of genotypes in the resistant categories highlights the importance of preserving and utilizing these genotypes for breeding and it is said that Resistant Genotypes are best suited for endemic regions and long-term sustainability (16).

Heat map representation of different chilli genotypes based on fruit rots disease severity and scale rating against *Colletotrichum capsici*

A detailed summary of chili genotypes arranged according to their fruit rot disease grading scale is shown in Figure 5, where a color gradient denotes the associated disease severity levels. This graph illustrates the significance of both genetic resistance and external management techniques in disease control and offers insight into how various chili genotypes react to fruit rot disease, ranging from immunity to high vulnerability. Very Few genotypes fall into the category of Highly Susceptible Genotypes, where the greatest disease severity levels (deep red) indicate an impact of more than 80%. This category includes the genotype Akola-10, for instance. Moderate to High Disease Severity: A significant proportion of genotypes exhibit red to purple hues, which indicate moderate to high disease severity. Due to their susceptibility to fruit rot, these genotypes necessitate intensive disease control measures. Genotypes That Are Moderately Susceptible: The majority of genotypes fall into this category. The illness severity exhibited by these genotypes varies; some exhibit moderate susceptibility (purple shades, 30-50% severity), while others exhibit high susceptibility (red shades, >50% severity). With the right disease control measures, these genotypes can still be controlled. The term "moderately resistant genotypes" refers to those that exhibit moderate resistance, with blue to purple hues signifying disease severity ranging from 10 to 40%. These genotypes can be grown in regions with mild to moderate disease pressure because they are more disease-resistant. The resistant genotypes include a select few that exhibit the lowest disease severity (dark blue), indicating near immunity or strong genetic resistance. These genotypes are ideal candidates for breeding programs or cultivation in regions with high disease risk. Notably, some genotypes within the same disease rating category show varying degrees of severity, such as different shades of purple and red within the moderately susceptible group. This variation highlights the role of environmental factors and specific resistance mechanisms in disease performance. While a significant number of genotypes fall under the moderately susceptible category, suggesting that most varieties are neither fully resistant nor highly vulnerable, the data indicates that resistant genotypes hold the greatest potential for effective disease management and breeding in high-risk areas(17).

The safest, most economical, and ecologically friendly way to control disease may be through crop plants' natural resistance or tolerance to pathogen infection. It was found that when the chilli germplasm/varieties with resistance to the fruit rot pathogen, i.e., *Colletotrichum capsici*, were detected in the field, Kashi Anmol, Kashi Anmol-2, Lembang-1, Pusa Jawala, and Pusa Sadabahar displayed a moderately resistant reaction in both settings. Using the detached technique, the smallest lesion size and the least amount of spore generation were seen on chilli fruits of DC-4, Anka lohit, LCA 301, LCA-235, and LCA-333. In addition to their resistance to fruit rot disease, these germplasm lines could be employed in hybridization operations to create cultivars with other desired characteristics.

The resistance of different chilli germplasm lines to the fruit rot pathogen has also been evaluated by numerous researchers. Types BGI and Lorai had the smallest lesion diameters (5.75 and 6.00 mm, respectively) and were consequently classed as resistant to fruit rot, according to Thind and Jhooty's (1985) laboratory examination of 19 different varieties of red ripe fruits.

Conclusion

In summary, nine genotypes were shown to be dieback and fruit rot resistant and can be used to breed chili cultivars that are resistant to the disease: Bhut Jolokia, BS-27, BS-28, BS-35, BS-37, CC-0189, Hisar Sakti, IC-326272, and IC-383072. Future studies should concentrate on introducing the resistance genes from these genotypes into susceptible commercial cultivars of *Capsicum annuum*, as there are currently no commercial cultivars of the plant that are resistant to anthracnose. Bhut Jolokia, BS-27, BS-35, BS-37, IC-326272, IC-383072, Pant C-1, Pusa Sadabahar, SH-C-1, Taiwan-2, and Tanjung-2 were among the nine genotypes found to be resistant to fruit rot disease, while eleven genotypes were found to be susceptible, including Akola-10, Arka Lohit, Arka Mohini, EC-510320, IC-255928, IC-344727, Jyanti, Kashi Surkh, Phule Jyoti, SH-C-19, and Sunidhi. Given that environmental conditions and particular resistance mechanisms influence disease performance, the variance in disease severity within the categories emphasizes the significance of both genetic resistance and appropriate management approaches. Since there are currently no commercially available cultivars of *Capsicum annuum* that are resistant to dieback or fruitrot, current research should concentrate on introducing these resistance genes into commercial cultivars of *C. annuum* that are vulnerable.

The future scope of the study

Unraveling novel sources of resistance and differential reactions to chilli dieback and fruit rot through screening various chilli genotypes offers several potential avenues for research and application:

1. Development of Resistant Cultivars: By identifying and understanding the genetic basis of resistance in specific chilli genotypes, the study could contribute to breeding programs focused on developing chilli varieties with enhanced resistance to dieback and fruit rot. This could help reduce the reliance on chemical pesticides, promoting more sustainable farming practices.

2. Molecular Markers for Resistance: The identification of molecular markers linked to resistance traits could facilitate the development of marker-assisted selection (MAS) tools. This would allow for quicker and more efficient breeding of chilli plants with improved resistance to diseases, significantly shortening the breeding cycle.

3. Mechanisms of Resistance: Further research into the biochemical and molecular mechanisms underlying resistance to dieback and fruit rot could lead to a deeper understanding of plant-pathogen interactions. This could pave the way for novel strategies to enhance resistance, such as the manipulation of specific genes or pathways involved in defense responses.

4. Integrated Disease Management: The insights gained from screening various genotypes could inform integrated disease management (IDM) strategies, combining resistant genotypes with cultural practices, biological control agents, and reduced chemical inputs. This could improve the overall health of chilli crops and reduce the environmental impact of agriculture.

5. Cross-Species Resistance: The study could explore the potential for resistance mechanisms identified in chillies to be applicable to other Capsicum species or related Solanaceae crops, thereby contributing to the broader field of crop protection.

6. Climate Change Adaptation: As climate change continues to affect agricultural productivity, studying the differential reactions of various genotypes to disease could help identify genotypes that are more resilient to changing environmental conditions, such as increased temperatures and humidity, which could exacerbate disease prevalence.

7. Commercial Application and Global Impact: The development of chilli cultivars with enhanced disease resistance could have significant commercial implications, improving yields for farmers and ensuring the supply of high-quality chilli products for global markets. This could also reduce the economic losses associated with disease outbreaks in chilli production.

8. Further Screening of Genetic Diversity: Expanding the study to include a broader range of chilli genotypes, including wild relatives, could uncover additional sources of resistance. This could lead to the discovery of new genes or traits that can be utilized in breeding programs.

By addressing these future directions, the study could contribute significantly to the advancement of both basic and applied agricultural research, improving the sustainability and productivity of chilli farming worldwide.

Acknowledgement

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Conflict of Interest

The authors declare that there is no conflict of interest

Table 1: Classification of genotypes into different clusters for die back disease reaction against Colletotrichum capsiciunder epiphytotic conditions

Disease Reaction	No. of Genotypes	Genotype
Resistant	9	Bhut Jolokia, BS-27, BS-28, BS-35, BS-37, CC-0189, Hisar Sakti, IC-326272, IC-383072
Moderately Resistant	20	Agnirekha, Assam-10, CC-0210, EC-566320, IC-208534, IC-208586, IC-214965, Kashi Anmol, Kashi Anmol-2, Lembang-1, Pusa Jawala, Pusa Sadabahar, SH-C-1, SH-C-6, SH-C-15, Sikkim Chilli, SUM-17, Taiwan-2, Tanjung-2
Moderately Susceptible	22	Byadagi Dabbi-1, EC-378632, EC-566969, EC-599969, IC-208591, IC-226257, IC-545651, IC-545654, IC-545722, IC-545723, IC-545735, IC-570408, Kashi Gaurav, Kashi Siduri, Pant C-1, Punjab Lal, Rajput, Sankeshwar, SH-C-4, SH-C-9, Sitara, Tiwari
Susceptible	27	Arka Khyati, Arka Lohit, Arka Meghana, ByadagiKaddi, California Wonder, EC-341075, EC-405253, EC-622085, Hisar Vijay, IC-208580, IC-255929, IC-255941, IC-255944, IC-297654, IC-387691, IC-395318, IC-537599, IC-545649, IC-545944, Jawahar Mirch, Jawahar Mirch-218, Jwala, Kiran, SSB 8, SH-C-11, SH-C-14, Ujala
Highly Susceptible	11	Akola-10, Arka Lohit, Arka Mohini, EC-510320, IC-255928, IC-344727, Jyanti, Kashi Surkh, Phule Jyoti, SH-C-19, Sunidhi

Table 2- Screening of genotypes against chilli dieback (Colletotrichum capsici) under epiphytotic conditions

S.No	Genotype	Die Back Severity (%)	Reaction	Fruit rot severity (%)	Reaction
1	**Agnirekha**	19.31	MR	22.2	MR
2	**Akola-10**	80.21	HS	81.02	HS
3	**Arka Khyati**	43.56	MS	55.85	S
4	**Arka lohit**	56.13	S	82.54	HS
5	**Arka Lohit**	41.24	MS	52.87	S
6	**Arka meghana**	37.90	MS	55.9	S
7	**Arka mohini**	52.94	S	76.78	HS
8	**Assam-10**	11.65	MR	15.4	MR
9	**Bhut Jolokia**	9.06	R	10.12	R
10	**BS-27**	2.46	R	2.54	R
11	**BS-28**	0.34	I	6.78	R
12	**BS-35**	8.40	R	9.56	R
13	**BS-37**	3.82	R	6.74	R
14	**Byadagi dabbi-1**	37.66	MS	49.81	MS
15	**Byadagikaddi**	48.43	MS	62.09	S
16	**California wonder**	33.46	MS	59.32	S
17	**CC-0189**	0.63	I	9.34	R
18	**CC-0210**	13.43	MR	19.87	MR
19	**EC-341075**	43.14	MS	56.76	S
20	**EC-510320**	51.68	S	76.23	HS
21	**EC-378632**	20.37	MR	43.34	MS
22	**EC-405253**	45.90	MS	67.9	S
23	**EC-566320**	20.22	MR	23.32	MR
24	**EC-566969**	27.50	MS	28.44	MS
25	**EC-599969**	37.21	MS	47.34	MS
26	**EC-622085**	62.72	S	64.86	S
27	**Hisar sakti**	0.76	I	4.54	R
28	**Hisar vijay**	40.08	MS	59.05	S
29	**IC-537599**	38.48	MS	56.75	S
30	**IC-545651**	39.88	MS	46	MS
31	**IC-545654**	19.51	MR	28.77	MS
32	**IC-545723**	28.94	MS	33.42	MS
33	**IC-208534**	12.08	MR	17.59	MR
34	**IC-208580**	44.41	MS	65.44	S
35	**IC-208586**	15.03	MR	17.28	MR
36	**IC-208591**	32.39	MS	36.44	MS
37	**IC-214965**	13.93	MR	16.05	MR
38	**IC-226257**	21.63	MR	45.45	MS
39	**IC-255928**	52.50	S	80.77	HS
40	**IC-255929**	32.88	MS	57.09	S
41	**IC-255941**	40.92	MS	73.47	S
42	**IC-255944**	32.08	MS	56.67	S
43	**IC-297654**	38.33	MS	66.66	S
44	**IC-326272**	5.28	R	9.34	R
45	**IC-344727**	41.71	MS	76.67	HS
46	**IC-383072**	1.81	R	2.38	R
47	**IC-387691**	34.36	MS	61.69	S
48	**IC-395318**	50.34	MS	65.63	S
49	**IC-545649**	42.00	MS	56	S
50	**IC-545722**	32.16	MS	49.03	MS
51	**IC-545735**	20.95	MR	27.38	MS
52	**IC-570408**	21.15	MR	27.65	MS
53	**Jaganath**	44.99	MS	52.52	S
54	**Jawahar Mirch**	33.31	MS	58.43	S
55	**Jawahar Mirch-218**	63.38	S	65.54	S
56	**Jwala**	36.88	MS	56.08	S
57	**Jyanti**	44.54	MS	78.49	HS
58	**Kashi anmol**	17.70	MR	23.03	MR
59	**Kashi anmol-2**	12.51	MR	14.42	MR
60	**Kashi gaurav**	23.71	MR	27.57	MS
61	**Kashi siduri**	27.35	MS	40.34	MS
62	**Kashi Surkh**	53.40	S	78.76	HS

63	**Kiran**	51.19	S	52.95	S
64	**Lembang-1**	20.91	MR	24.12	MR
65	**Pant C-1**	10.33	R	11.9	MR
66	**Pant C-1**	27.28	MS	40.2	MS
67	**Phule jyoti**	34.27	MS	77.02	HS
68	**Punjab lal**	15.69	MR	34.53	MS
69	**Pusa Jawala**	19.16	MR	25.34	MR
70	**Pusa sadabahar**	9.45	R	12.43	MR
71	**Rajput**	32.71	MS	48.34	MS
72	**Sankeshwar**	26.92	MS	35.56	MS
73	**SH-C-1**	8.80	R	15.3	MR
74	**SH-C-11**	40.47	MS	53.53	S
75	**SH-C-14**	49.76	MS	66.35	S
76	**SH-C-15**	16.74	MR	22.12	MR
77	**SH-C-19**	58.55	S	77.45	HS
78	**SH-C-4**	16.64	MR	29.35	MS
79	**SH-C-6**	14.03	MR	18.29	MR
80	**SH-C-9**	42.91	MS	49.43	MS
81	**Sikkim Chilli**	17.38	MR	20.05	MR
82	**Sitara**	20.53	MR	26.09	MS
83	**SSB 8**	61.63	S	69.4	S
84	**SUM-17**	15.43	MR	15.45	MR
85	**Sunidhi**	42.81	MS	77	HS
86	**Taiwan-2**	9.39	R	13.89	MR
87	**Tanjung-2**	6.79	R	11.78	MR
88	**Tiwari**	31.52	MS	41.09	MS
89	**Ujala**	64.96	S	74.09	S

Table 3: Classification of genotypes into different clusters for fruit rot disease reaction against Colletotrichum capsici under epiphytotic conditions

Disease Reaction	No. of Genotypes	Genotype
Immune	3	BS-28, CC-0189, Hisar sakti
Resistant	11	Bhut Jolokia, BS-27, BS-35, BS-37, IC-326272, IC-383072, Pant C-1, Pusa sadabahar, SH-C-1, Taiwan-2, Tanjung-2
Moderately Resistant	24	Agnirekha, Assam-10, CC-0210, EC-378632, EC-566320, IC-545654, IC-208534, IC-208586, IC-214965, IC-226257, IC-545735, IC-570408, Kashi anmol, Kashi anmol-2, Kashi gaurav, Lembang-1, Punjab lal, Pusa Jawala, SH-C-15, SH-C-4, SH-C-6, Sikkim Chilli, Sitara, SUM-17
Moderately Susceptible	39	Arka Khyati, Arka Lohit, Arka meghana, Byadagi dabbi-1, Byadagikaddi, California wonder, EC-341075, EC-405253, EC-566969, EC-599969, Hisar vijay, IC-537599, IC-545651, IC-545723, IC-208580, IC-208591, IC-255929, IC-255941, IC-255944, IC-297654, IC-344727, IC-387691, IC-395318, IC-545649, IC-545722, Jaganath, Jawahar Mirch, Jwala, Jyanti, Kashi siduri, Pant C-1, Phule jyoti, Rajput, Sankeshwar, SH-C-11, SH-C-14, SH-C-9, Sunidhi, Tiwari
Susceptible	11	Arka lohit, Arka mohini, EC-510320, EC-622085, IC-255928, Jawahar Mirch-218, Kashi Surkh, Kiran, SH-C-19, SSB 8, Ujala
Highly Susceptible	1	Akola-10

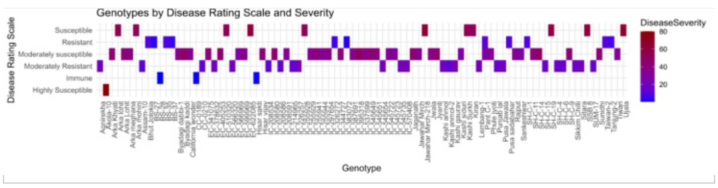


Fig 1: Heat map representation of different chilli genotypes based on die back disease severity and scale rating against Colletotrichum capsici under epiphytotic conditions

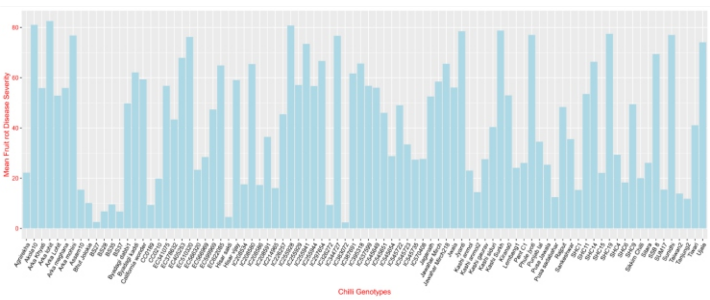


Fig 3: Fruit rot disease severity of different chilli genotypes against Colletotrichumcapsici under epiphytotic conditions

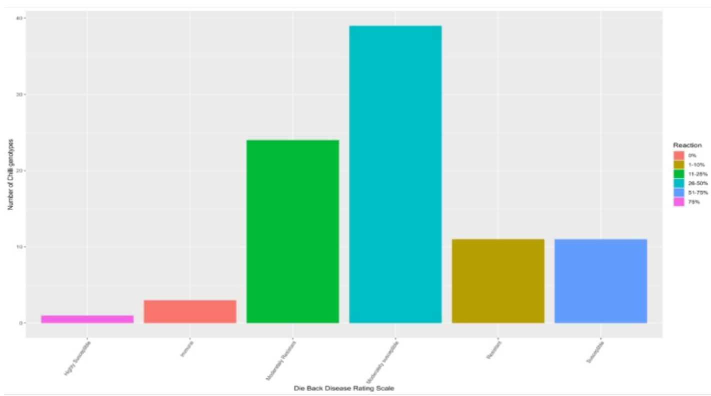


Fig 2: Bar plot categorization of different chilli genotypes based on die back disease reaction against Colletotrichum capsici under epiphytotic conditions

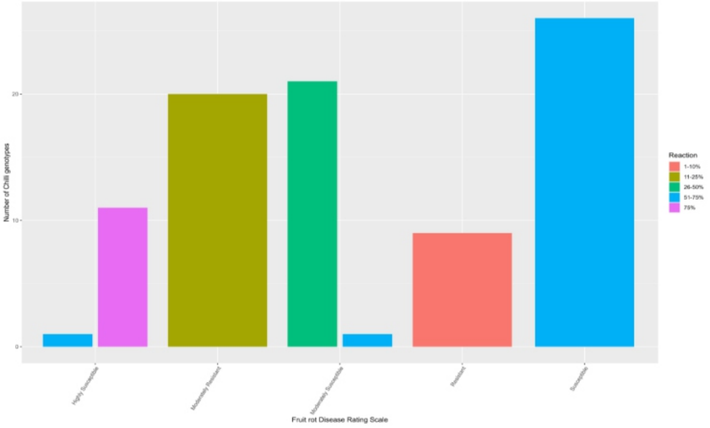


Fig 4: Bar plot categorization of different chilli genotypes based on fruit rot disease reaction against Colletotrichum capsici under epiphytotic conditions

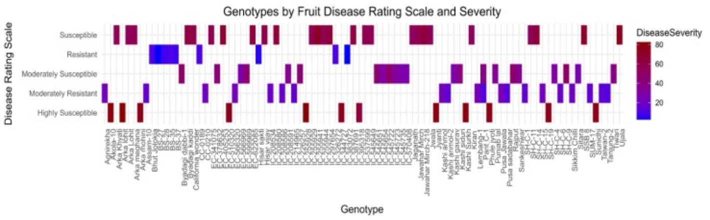
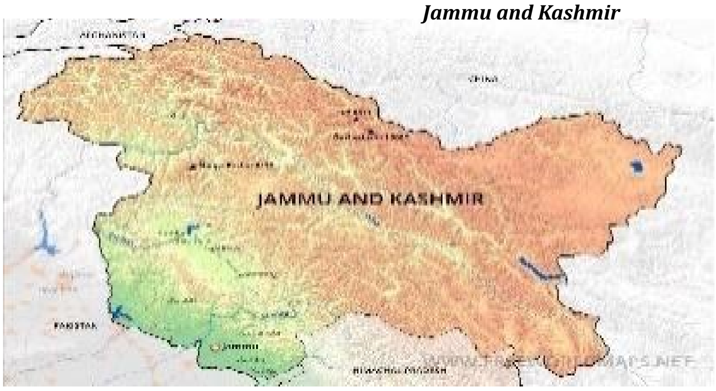


Fig 5: Heat map representation of different chilli genotypes based on fruit rots disease severity and scale rating against Colletotrichum capsici under epiphytotic conditions Schematic representation of the screening of chilli genotypes aginst dieback and fruit rot disease

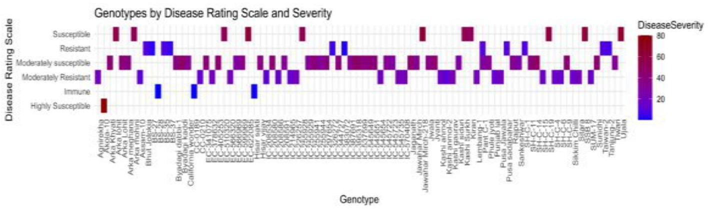
Jammu and Kashmir



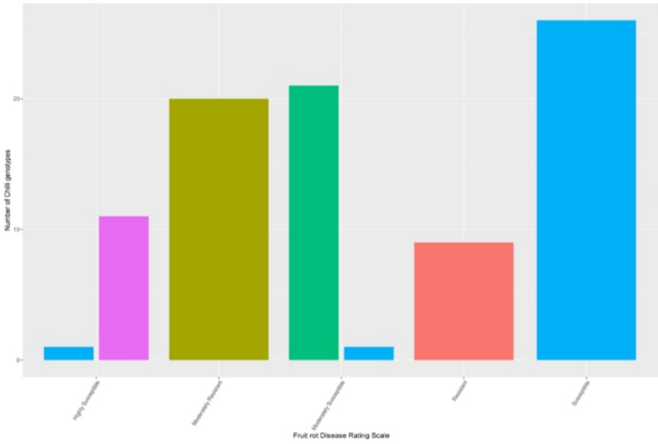
Field sampling of infected fruit rot and dieback samples

Screening of 89 genotypes

Akola-10, Arka Lohit, Arka Mohini, EC-510320, IC-255928, IC-344727, Jyanti, Kashi Surkh, Phule Jyoti, SH-C-19, Sunidhi, Arka Khyati, Arka Lohit, Arka Meghana, Byadagi Kaddi, California Wonder, EC-341075, EC-405253, EC-622085, Hisar Vijay, IC-208580, IC-255929, IC-255941, IC-255944, IC-297654, IC-387691, IC-395318, IC-537599, IC-545649, IC-545944, Jawahar Mirch, Jawahar Mirch-218, Jwala, Kiran, SSB 8, SH-C-11, SH-C-14, Ujala, Byadagi Dabbi-1, EC-378632, EC-566969, EC-599969, IC-208591, IC-226257, IC-545651, IC-545654, IC-545722, IC-545723, IC-545735, IC-570408, Kashi Gaurav, Kashi Siduri, Pant C-1, Punjab Lal, Rajput, Sankeshwar, SH-C-4, SH-C-9, Sitara, Tiwari, Agnirekha, Assam-10, CC-0210, EC-566320, IC-208534, IC-208586, IC-214965, Kashi Anmol, Kashi Anmol-2, Lembang-1, Pusa Jawala, Pusa Sadabahar, SH-C-1, SH-C-6, SH-C-15, Sikkim Chilli, SUM-17, Taiwan-2, Tanjung-2



Heat map representation of different chilli genotypes based on die back disease severity and scale rating against Colletotrichum capsici under epiphytotic conditions



Bar plot categorization of different chilli genotypes based on fruit rot disease reaction against Colletotrichum capsici under epiphytotic conditions

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