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# Evaluating the Impact of Climate Change on Wilt Incidence in Chilli (*Capsicum annuum* L.) in Temperate Ecologies: Mitigation Strategies and Adaptation Approaches

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## ABSTRACT

*Chilli holds significant importance among the various vegetables cultivated in temperate regions, particularly in the Kashmir Valley. Climate change has notably increased the frequency and severity of wilt disease in chilli, leading to crop losses ranging from 45 to 60 percent in the Kashmir Valley alone [1],[2] by altering pathogen dynamics. Rising temperatures, changing rainfall patterns, elevated CO2 levels, and extreme weather events all contribute to the evolving landscape of wilt diseases. Tackling these challenges requires a comprehensive approach, including the development of resistant crop varieties, effective disease management strategies, and staying updated on the latest research and technological advancements. By adopting proactive measures, it is possible to reduce the impact of climate change on wilt diseases and ensure the continued productivity and sustainability of agriculture in temperate regions. In this context, the current study focuses on the identification, isolation, and utilization of wilt-resistant lines/hybrids. The experimental material consisted of eighty genotypes including sixty-six crosses, twelve parents and two checks. The experimental material was subjected to screening under both field and in vitro conditions against wilt disease. The results revealed that twelve chilli genotypes showed highly resistant reaction against wilt disease and twenty-six genotypes showed resistant reaction. The rest of the genotypes were moderately resistant, susceptible or highly susceptible. Thus the genotypes showing highly resistant/ resistant reaction to this devastating disease can be further evaluated and released among farmers, so that farmers can use these genotypes and prevent their crop losses posed by climate change.*

**Keywords:** *Chilli, Climate change, Disease resistance, Fusarium wilt, Hybrids, Pathogen Dynamics.*

## Introduction

The word capsicum comes from the Greek word 'kapto,' which means "to bite" or "to swallow". It is a significant vegetable and spice crop that is farmed worldwide in tropical and subtropical climates. It came from the West Indies and Tropical America, and in the seventeenth century, the Portuguese introduced it to India. Ever since then, it has spread throughout the world, including India, as a common vegetable and condiment. Due to its popularity as a spice and vegetable, its production and use have increased significantly over the 20th century, and it is currently a key ingredient in many different types of cuisines all over the world.

It is grown for its two main commercial qualities, which are its red colour and pungency. Some cultivars are prized for their biting pungency, which is linked to the crystalline caustic volatile alkaloid capsaicin ($C_{18}H_{27}NO_3$), while others are noted for their red colour due to the pigment capsanthin ($C_{40}H_{56}O_3$). From a nutritional perspective, the green fruit of the chilli plant is rich in minerals including potassium, magnesium, calcium, and iron in addition to vitamins A, E, C, and P [3].

Globally, agricultural landscapes are changing due to climate change, which also brings with it a number of difficulties that have an impact on crop productivity and health. Chilli (*Capsicum annuum* L.) is one of the several crops that are most susceptible. Since chilli is an essential component of many cuisines around the world and a substantial cash crop in many areas, it is important to investigate how climate change may affect the spread of chilli diseases.

The dynamics of wilt disease development are changing due to climate change in temperate zones, where the climate is defined by moderate temperatures and marked seasonal fluctuations. The growth and activity of pathogens that cause wilt are impacted by rising temperatures. Warmer temperatures are ideal for wilt-causing fungus like Fusarium spp.The pathogens become more aggressive with rising temperatures, which exacerbates the severity of the disease. Elevated temperatures additionally hasten the rates of pathogen reproduction and augment their capacity to infect crops. The development of wilt disease is impacted by modifications in precipitation patterns, such as an increase in the frequency of heavy rains and extended dry spells. Waterlogging from heavy rains also encourage the growth of pathogens and cause root rot and wilt illnesses by generating anaerobic conditions. On the other hand, a

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protracted drought cause stress to plants, increasing their vulnerability to wilt pathogens and decreasing their capacity to recover from infections.

Increased $CO_2$ can have an impact on pathogen interactions and plant physiology. Elevated $CO_2$ levels can cause a plant to grow faster and change how nutrients are allocated, which can affect the plant's ability to fend off wilt diseases. For example, alterations in the architecture of the roots and the intake of nutrients might affect the plant's vulnerability to diseases like Fusarium spp. Wilt disease issues may get worse because to the rising frequency of extreme weather events like heat waves and strong storms. Heat waves cause stress to plants, lowering their defenses and increasing their vulnerability to diseases.

Wilt diseases, caused by fungi, represent a significant threat to agriculture worldwide. Chilli wilt disease is characterized by brown vascular discoloration followed by withering and chlorosis of leaves and subsequent mortality of the plant [4]. The disease is reported to be induced by a complex of soil borne pathogens [5] which renders most of the management practices ineffective. The use of resistant varieties is the most efficient and cost-effective way to combat the threat of soil-borne diseases, although the chilli cultivars used in Kashmir are more or less sensitive to the disease. Furthermore, there are just a few resistant sources available in the world's chilli germplasm [6].

Developing chilli varieties/ hybrids with resistance to wilt diseases is a crucial strategy to prevent this elite crop from the devastating effects. In this context the current study for isolation, identification and utilization of resistant lines for development of high yielding and wilt resistant crosses was carried out as it is of prime importance and can save the chilli industry in Kashmir.

## Experimental Material (layout, design)

The experimental material for the study consisted of twelve diverse genotypes of chilli (*Capsicum annuum* L.) maintained by Division of Vegetable Science, SKUAST-Kashmir, Shalimar. 66 $F_1$ crosses were generated through 12 x 12 diallel mating design (excluding reciprocals) at Vegetable Experimental Field, Division of Vegetable Science, SKUAST-Kashmir Shalimar. The set of 80 viz., crosses (66) along with their parents (12) and checks (2) were evaluated in augumented block design at Experimental Farm, Division of Vegetable Science, SKUAST-Kashmir Shalimar. The row to row and plant to plant spacing was maintained at 60 x 45 cm. recommended package of practices were adopted to raise a healthy crop except fungicidal sprays to allow for natural epiphytotics and simultaneous screening of parents and crosses for chilli wilt *in vitro* with isolated casual organisms.

Initially, the casual organisms of chilli wilt disease were isolated from wilt affected chilli plants and tried for the isolation of associated fungal pathogen by tissue bit method. The isolated fungal organism was further purified by single spore culture method. Pathogenicity was proved on potted susceptible chilli cultivar (Kashmir- Long-1) by confirming postulates of Koch. Cultural and morphological characteristics of the isolated causal organism(s) were recorded in pure culture and compared with authentic description for its identification.

The experimental material consisting of twelve diverse parents and sixty-six crosses were subjected to screening (*in vivo* and *in vitro*).

The observations on wilt incidence of all the test genotypes were recorded up to harvesting time. The assessment of level of resistance and susceptibility was done according to disease rating scale of Kesavan and Chaudary 1977 as under;

| Disease Rating | Description (%) | Reaction type | Symbol |
|---|---|---|---|
| 0 | 0 | Immune | I |
| 1 | 0.1-10 | Highly Resistant | HR |
| 2 | 10.1-30 | Resistant | R |
| 3 | 30.1-50 | Moderately Resistant | MR |
| 4 | 50.1-80 | Susceptible | S |
| 5 | 80.1-100 | Highly susceptible | HS |

Per cent disease incidence was calculated as per the following formula:

$$\text{Percent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

The genotypes found immune, highly resistant, resistant and moderately resistant under field screening were further screened under artificially inoculated controlled conditions following the inoculation method as adopted by Najar (2001) [7]. The seedling raised under sterilized conditions were transplanted in sterilized soil in pots incorporated seven days before with 10 per cent (w/w) test fungal cultures grown on sand maize meal medium. The seedlings transplanted in un-inoculated sterilized soil were kept as check.

Observation on percent disease incidence was recorded 60 days after sowing to access the level of resistance and susceptibility of each test genotype according to the disease rating scale as given in table. The observations were recorded on five randomly selected plants in each replication. Mean values were worked out for further statistical analysis

## Isolation and purification of causal fungi

The infected samples were collected from various locations across the Kashmir valley viz., Srinagar, Ganderbal, Pulwama, chadoora, causal fungus of the disease was isolated from the stem portion of the affected plants by tissue bit technique (Plate 1). Similar type of fungus was yielded from all the isolations attempted. The cultures so obtained were purified by single spore method and maintained on PDA by repeated sub-culturing at monthly intervals.

## Morphology based identification of the pathogen

The cultural and morphological characters of the fungus were studied on potato dextrose agar (PDA) medium. The purified culture of the fungus initially produced, submerged, profuse fluffy, fussy white cottony colonies which gradually turned creamy white with purplish pigmentation and developed brownish colour at backside of the petriplate. The fungus attained a growth of 90 mm in 7 days of incubation at 25±1°C. Microscopic observations revealed that mycelium was smooth, branched, septate and cylindrical and measured 1.2-3.6 μm in width (Plate 2). Microconidia and macroconidia were abundantly present. Microconidia were cylindrical to oval shaped, 0-1 septate and measured 4.8-9.6 x 1.8-2.4 μm (Plate 2). Macroconidia were sickle-shaped, slightly curved at apex with foot shaped basal cell, 3-5 septate and 9.6-21.6 x 2.4-4.8 μm in size (Plate 2). Chlamydospores were intercalary, produced singly or in chains, nearly spherical, hyaline and measured 8.4-12 μm in width (Plate 2).

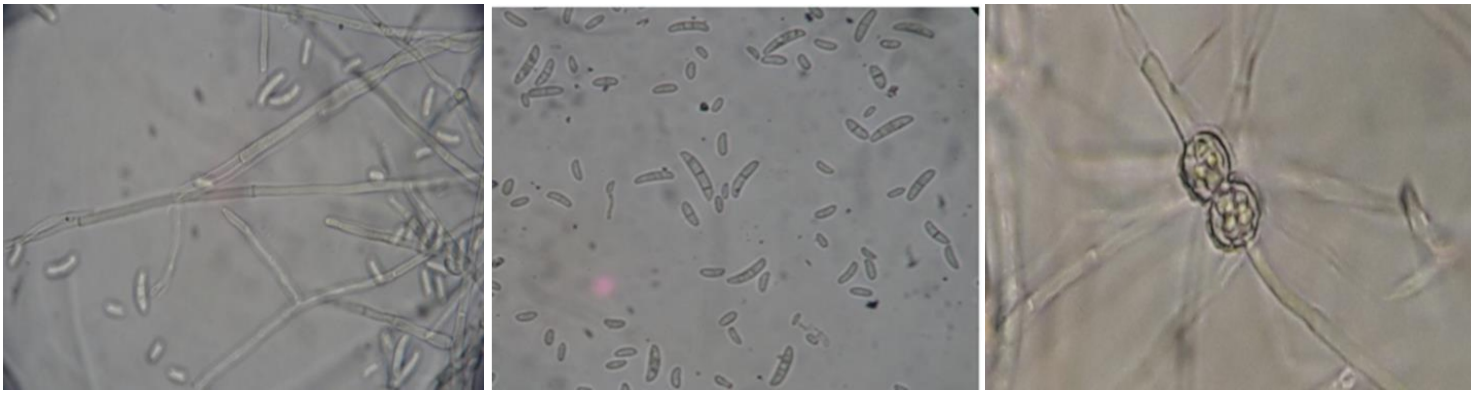
*Table 1: Cultural and morphological characteristics of Fusarium spp.*

| Fungal propagule | Colour | Shape | Size* | Septation |
|---|---|---|---|---|
| Colony | Initially white gradually turning creamish with purplish pigmentation | Floccose, sparse aerial mycelium | 90 mm dia. in 7 days | - |
| Mycelium | Hyaline | Smooth, cylindrical, branched | 1.2-3.6 µm (wide) | Septate |
| Micro conidia | Hyaline | Cylindrical to oval shaped | 4.8-9.6 x 1.8-2.4 µm | 0-1 septate |
| Macro conidia | Hyaline | Sickle-shaped, slightly curved at apex and foot-shaped at basal end | 17.3-23.1 x 2.4-4.8 µm | 3-5 septate |
| Chlamydospore | Hyaline | Spherical, smooth, intercalary, single or in chains | 8.4-12 µm (wide) | - |

**Mean of 100 microscopic observations*

*Plate 1: Fusarium cultures isolated from samples of various locations and typical Wilt symptoms in Chilli (Capsicum annuum L.)*

*A) 7 Days Old Cultures*



*B) Mycelium*

*C) Microconidia & Macroconidia*

*D) Chlamydospore*

*Plate 2: Cultural and morphological characteristics of Fusarium spp.*

***Plate 3: Germplasm screening under field and artificially inoculated controlled conditions***

**Sequencing and Bioinformatics analysis**. The PCR product was sequenced, and identified through DNA sequencing. The pathogens identified were various species of *Fusarium oxysporum* and *Fusarium Solani.*

**BLAST (Basic Local Alignment Search Tool)** was used to find regions of similarity between the query sequence and the sequence present in the NCBI (www.ncbi.nlm.in) database.

**Sequence submitted to GenBank**. The sequences designated as Khursheed_hussain4, Khursheed_hussain5, Khursheed_hussain6, *Fusarium oxysporum* isolate S, *Fusarium solani* isolate P, *Fusarium solani* isolate G, with accession No. OM033474.1, OM033475.1, OM033476.1, ON795838.1, ON795837.1 and ON793813.1 and size 511, 483, 488, 569, 582 and 579 base pairs respectively were successfully published in GenBank.

**Pathogenicity**. The pathogenicity of isolated fungi was established by confirming postulates of Koch on potted chilli plants cv. Kashmir Long-1. The initial disease symptoms of *Fusarium spp* inoculated seedlings developed after 7-10 days of inoculation. The re-isolation of *Fusarium spp.* From the inoculated chilli plants was also carried and compared with their original inoculated culture. The re-isolated pathogen completely resembled the original inoculated fungi in their morphological, cultural and pathogenic characteristics, so satisfied Koch's postulates.

## Results and Discussion

The fungi associated with chilli wilt was isolated from diseased plants by tissue bit technique and further purified by single spore technique.

Isolation from the stem portion of chilli plants with typical symptoms of wilt disease revealed the presence of *Fusarium spp* as the pathogen of the disease. On PDA medium, *Fusarium spp* formed white fluffy or cottony colonies which later turned creamy white with purple pigmentation. On the basis of morphological and molecular characteristics and comparison with authentic descriptions the pathogens were identified as various strains of *Fusarium oxysporum and Fusarium solani.* The *F. oxysporum and Fusarium solani* as causal organism of wilt disease has also been reported earlier by various workers Viz., Joshi, Singh, Pandita *and* Farwah [8],[9],[10],[2]. The fungal pathogen isolated from wilted chilli plants, when artificially inoculated through rhizosphere inoculation technique on potted chilli plants produced typical disease symptoms. Re-isolation of pathogen from the artificially inoculated and infected plants confirmed postulates of koch. The initial disease symptoms of *Fusarium spp.* inoculated seedlings as yellowing and wilting in younger leaflets, epinasty, stunning and yellowing of older leaves, clinging to the plant of dried leaves, brown vascular discoloration of collar portion. The present observations on symptomatological development of chilli plants due to *F. oxysporum* infection are more or less in agreement with those reported by Tembhurne and Thoyajakshi [11], [12].

**Germplasm screening under field conditions and artificially inoculated controlled conditions.**

A total of eighty genotypes including sixty-six crosses, twelve parents and two check were screened under field conditions against wilt disease. The results revealed that twelve chilli genotypes showed highly resistant reaction (disease incidence ranging from 0.1-10 percent) against wilt disease and twenty-six genotypes showed resistant reaction with disease incidence ranging from 10.10 to 30 per cent (Figure1-5). The rest of the genotypes were moderately resistant, susceptible or highly susceptible. In order to validate the field reaction of screened chilli genotypes, they were further screened under artificially inoculated controlled conditions against the isolated fusarium cultures. The results confirmed the that twelve chilli genotypes showed highly resistant reaction (disease incidence ranging from 0.1-10 percent) against wilt disease and twenty-six genotypes showed resistant reaction with disease incidence ranging from 10.10 to 30 per cent The rest of the genotypes were moderately resistant, susceptible or highly susceptible. (Figure 1-5).

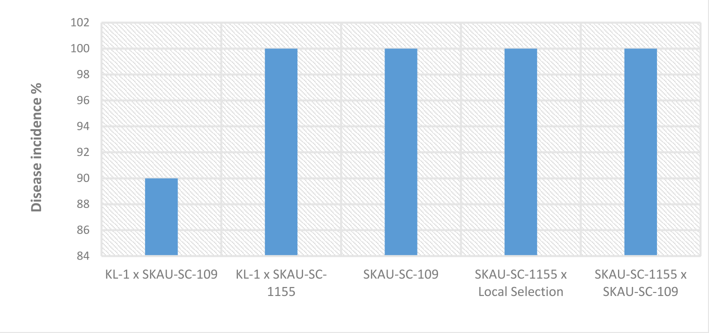


***Figure 1: Genotypes showing highly susceptible disease reaction under both field and in-vitro conditions***

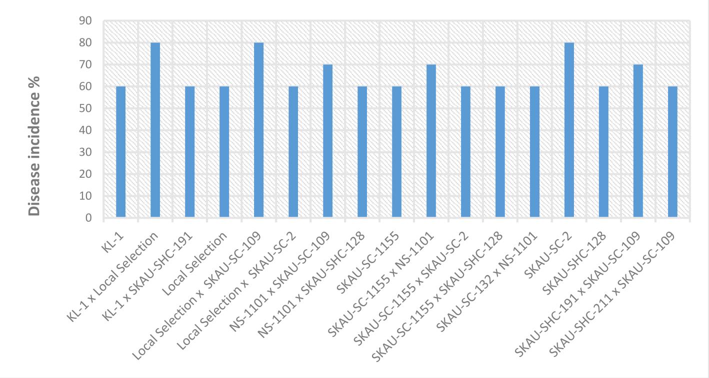


***Figure 2: Genotypes showing susceptible disease reaction under both field and in-vitro conditions***

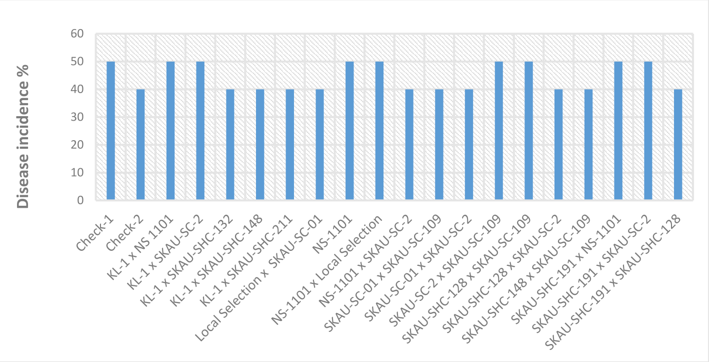


***Figure 3: Genotypes showing moderately resistant disease reaction under both field and in-vitro conditions***

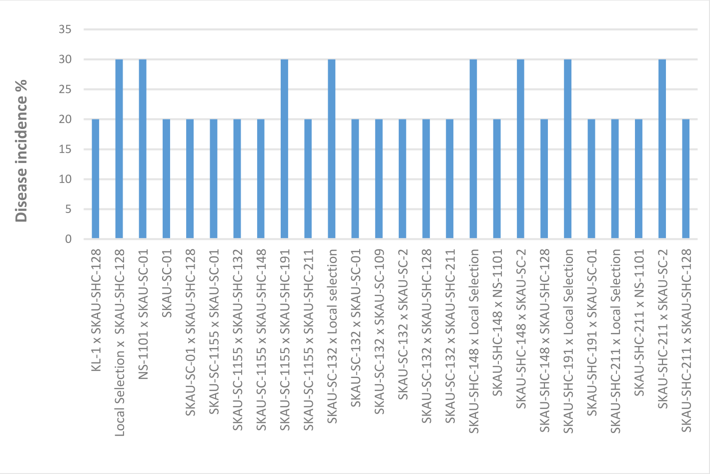


***Figure 4: Genotypes showing resistant disease reaction under both field and in-vitro conditions***

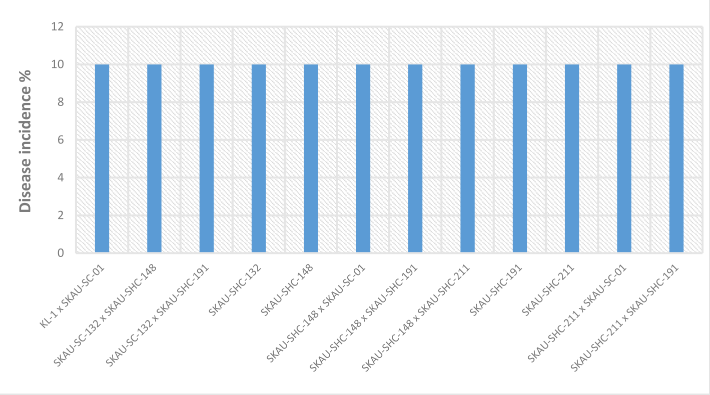


***Figure 5: Genotypes showing highly resistant disease reaction under both field and in-vitro conditions***

## Conclusion

Wilt is one of the important disease of chilli crop, occurring in both temperate and warmer regions of the world. The disease has been reported to cause 10-50 per cent losses around the world and 10-80 per cent in India [12]. Host plant resistance has been a choice in all crop improvement programmes and is the best method available to tackle the soil borne diseases in particular. *Fusarium* wilt is a typical soil borne disease which can be mitigated appropriately by the use of disease resistant cultivars. Further the use of resistant variety will go a long way not only in reducing loss due to disease but also in avoiding fungicidal toxicity likely to occur due to their application [11].

A total of eighty chili genotypes, including sixty-six crosses, twelve parent varieties, and two checks, were screened under both field and in-vitro conditions for their resistance to wilt disease. The results indicated that twelve genotypes—four parent varieties (SKAU-SHC-132, SKAU-SHC-148, SKAU-SHC-211, SKAU-SHC-191) and eight crosses (KL-1 x SKAU-SC-01, SKAU-SHC-132 x SKAU-SHC-148, SKAU-SHC-132 x SKAU-SHC-191, SKAU-SHC-148 x SKAU-SHC-211, SKAU-SHC-148 x SKAU-SHC-191, SKAU-SHC-148 x SKAU-SC-01, SKAU-SHC-211 x SKAU-

SHC-191, SKAU-SHC-211 x SKAU-SC-01) exhibited a highly resistant reaction to wilt disease, with disease incidences ranging from 0.1% to 10%. Additionally, twenty-six other genotypes displayed resistant reactions, with disease incidence ranging from 10.10% to 27.70%. The remaining genotypes were categorized as moderately resistant, susceptible, or highly susceptible to the disease. This study's findings underscore the significance of identifying chili genotypes with varying levels of resistance, particularly in the face of climate change. As global temperatures rise and weather patterns become increasingly unpredictable, climate change exacerbates the spread of plant diseases, including wilt. Increased temperatures, altered precipitation patterns, and more frequent extreme weather events create favorable conditions for pathogens, increasing the vulnerability of crops like chili to diseases.

Given this context, the identification of highly resistant chili genotypes is crucial for mitigating the impacts of climate change. These resistant genotypes, identified in the current study, represent a valuable resource for breeding programs aiming to enhance the resilience of chili crops. By integrating these resistant varieties into cultivation practices, farmers can better withstand the biotic stressors exacerbated by climate change, such as increased pathogen prevalence. Furthermore, the adaptation of agriculture to changing climatic conditions requires not only the development of disease-resistant varieties but also strategies to manage both biotic and abiotic stresses. Previous studies have also explored the identification of chili germplasm with different levels of resistance to wilt disease (Jabeena; Tembhurne; Singh; Shoba) [13], [11], [9], [14], confirming the importance of such breeding efforts. The genotypes identified as highly resistant or resistant in the current study can be further evaluated for their agronomic traits and then potentially released for commercial cultivation. This approach would contribute significantly to the sustainability of chili production, helping farmers cope with the dual pressures of biotic stress (like wilt) and abiotic stress driven by climate change. In summary, the ongoing evaluation and deployment of disease-resistant chili genotypes are essential in ensuring the continued productivity of chili crops in regions like Kashmir Valley. By addressing both the genetic and environmental challenges posed by climate change, we can safeguard agricultural systems from the increasing risks of plant diseases and maintain food security.

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