Original Research Article

In-vivo Screening of Cherry Tomato [*Solanum lycopersicum* L. var. *cerasiforme* (Dunnal) A. Gray] Genotypes and Hybrids against *Fusarium* Wilt in Arunachal Pradesh, India

ABSTRACT

Cherry tomato [*Solanum lycopersicum* L. var. *cerasiforme* (Dunnal) A. Gray] is becoming popular among various tribes of Arunachal Pradesh due to its unique taste, flavor and appearance. Owing high rainfall and high humidity, successful cultivation of cherry tomato is becoming restricted in this state due to various biotic factors like infection of wilt complexes as well as pests like root-knot nematodes. The aim of the present investigation was to evaluate eighteen cherry tomato genotypes (nine) and hybrids (nine) against Fungal wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* resistance in the Pasighat condition of Arunachal Pradesh, India. For screening purpose, artificial pathogen inoculation method was applied through pin-prick method in the 35 days old cherry tomato seedlings that were grown in pot culture containing sterile soil and data for the pathogen infection was recorded by following Completely Randomized Design (CRD) in 60 days and 120 days after pathogen inoculation. Among 18 genotypes and hybrids, G4xG6 (DI:0.00 %, PDI:0.00 %), G9 (DI:0.00 %, PDI:0.00 %) and G5 (DI:0.00 %, PDI:0.00 %) showed highest resistance towards *Fusarium* wilt, while genotype G3 (DI:65.56 %, PDI:63.60 %) exhibited susceptible system. The disease incidence was ranged from 0.00%-65.56%. The use of resistant genotypes and hybrids to manage the population of fungus is a very cost effective method and can be exploited commercially in breeding programmes and for vegetable grafting.

Keywords: Cherry tomato, Fusarium oxysporum f. sp. lycopersici, Screening, Resistance

1. INTRODUCTION

Since the inception of the agriculture, individuals have been breeding for desirable traits in an effort to improve the existing crops. However, the current standard conventional crop breeding techniques are insufficient to improve crop growth at the necessary rate to meet the increasing need for the population's demand. The development of long-lasting, broad-spectrum resistance is also crucial due to the increased danger that pests and diseases provide to contemporary agriculture. The development of resilient, broad-spectrum

resistance is also crucial due to the higher risk of pests and diseases to the contemporary agriculture with the increasing of pests and disease infestations for the recent as well as the upcoming days. Hence, crop protection through the development of resistant cultivars is seen as a sustainable and ecologically benign strategy [1]. Crop varieties with resistance features assist in creating a genetic resource of resistant genes that may be exploited in crop improvement programme for newly emerging diseases and pests.

Cherry tomato, *Solanum lycopersicum* L. var. *cerasiformae* [2], is regarded as the ancestor plant (2n=24) of all domesticated tomatoes. Cherry tomatoes are little fruit having variety of shapes and colours. Cherry tomatoes sometimes referred to as the "salad tomatoes," have grown in popularity around the globe as a result of its high vitamin A and C content, protein content, flavorful texture, and ability to maintain its firmness even at high temperatures [3]. The production of cherry tomatoes is subject to a variety of biotic and abiotic challenges, including seasonal environmental factors like temperature and relative humidity as well as diseases and insect pests.

In North-East India, the successful cultivation of cherry tomatoes is hindered by frequent insect invasions and disease infections. The main factors limiting tomato production in all areas of India are mainly diseases like bacterial wilt, causes by *Ralstonia solanacearum* [4], fusarium wilt causes by *Fusarium oxysporum* f.sp. *lycopersici* and pests like phytoparasitic nematodes (*Meloidogyne* spp.) have economic importance.

Fusarium oxysporum f.sp. *lycopersici*, which causes fusarium wilt or fungal wilt in tomatoes, continues to pose a significant threat to tomato production across the world. Additionally, it is a soil-borne vascular wilt disease that turns the lower leaves of the plant to yellow and gradually leads to wilt and dry out of the entire plant [5]. There have been reports of many *Fusarium* races, including races 1, 2, and 3, being able to overcome host resistance. North East Indian states have a higher probability of supporting this soil-borne disease due to excessive rainfall and humidity. *Fusarium* has been blamed for yield losses of up to 50% in the North East region of India. Farmers are still unsure of the most effective fusarium wilt management techniques. Physical, chemical, and biological control techniques can occasionally cause more problems than cultural practices because of the vast host range of the pathogen. By directly using excessive amounts of pesticides in vegetables may cause health issues. Currently, research on vegetable grafting as a combination management technique for lowering biotic and abiotic stresses for long-term sustainability is also being explored.

Previous researchers have studied about the morphological and biochemical natures of *Fusarium oxysporum* f.sp. *lycopersici* [6,7], about various screening techniques [8,9] and categorized some genotypes, cultivars and hybrids of tomato as resistant, susceptible and tolerant according to their various responses towards the pathogen [10,11,12]. A sensible solution to these issues is to hunt for resistant sources from regionally accessible, novel tomato genotypes with appropriate breeding techniques for
introducing those genes in the commercial cultivars.

2. MATERIAL AND METHODS

2.1 Experimental area

The current study was conducted in the College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh, which is situated in the eastern foothills of the Himalayas, in a naturally ventilated polyhouse. The experimental site is located 155 m above mean sea level with latitude of 28.07° North and a longitude of 95.33° East.

2.2 Experimental materials

Table 1. List of cherry tomato genotypes and their hybrids with respective sources

Sl. No.	Genotype	Sources
1.	Genotype 2 (G2)	Kohima, Nagaland
2	Genotype 3 (G3)	Ziro, Arunachal Pradesh
3	G2xG3	CHF, Pasighat
4	Genotype 7 (G7)	SASARD, Nagaland
5	G2xG7	CHF, Pasighat
6	Genotype 4 (G4)	SASRD, NU
7	Genotype 5 (G5)	Boleng, Arunachal Pradesh
8	G4xG5	CHF, Pasighat
9	G2xG4	CHF, Pasighat
10	Genotype 9 (G9)	Senapati, Manipur
11	G4xG9	CHF, Pasighat
12	G2xG9	CHF, Pasighat
13	G9xG10	CHF, Pasighat
14	G1x G10	CHF, Pasighat
15	G4xG6	CHF, Pasighat
16	Genotype 11 (G11)	Jorhat, Assam
17	Genotype 12 (G12)	Phek, Nagaland

18	Genotype 13 (G13)	Karbi Anglong, Assam

Nine different cherry tomato genotypes, collected from different states of North-East India and nine hybrids developed at the Vegetable Research Farm of College of Horticulture and Forestry in Pasighat, Arunachal Pradesh, through diallel mating, were used for the present study. In Table 1, a list of all nine genotypes, nine hybrids and their sources are provided. The nursery activity was carried out in September 2022 to raise the seedlings of all the F_1 hybrids and cherry tomato genotypes. The parents of the hybrids, namely Genotypes no. 2, 3, 4, 5, 6, and 9 were found to be superior in terms of the general combining ability (GCA) impact and mean performance during the heterosis investigation of the hybrids and parents. Based on specific combining ability SCA effects, heterosis, and mean performances, the hybrids G2xG7, G2XG3, G2XG4, G2XG9, G4XG5, G4XG6, G4XG9, G9xG10, as well as G1xG10, were better. Some wildly growing genotypes named as G11, G12, and G13 were gathered from various locations of North-East India. The seedlings were transplanted after 28 days (October) in the plastic pots that contained sterile media for growing and kept inside a naturally ventilated polyhouse for taking observations.

2.3 Isolation, preparation and artificial inoculation of pathogens

The direct plating technique developed by Okhuoya *et al.* (2012) [13] was used for the isolation of pathogen. Fungi was isolated from the sick-soil of a heavily wilt infected plot. Ten grams (10 g) of each soil sample were serially diluted one to seven times (10^{-1} to 10^{-7}) in 90 ml of sterile, distilled water. First and fourth to seventh fold dilutions were duplicated and plated out in one-tenth of a millilitre ($1/10^{th}$ ml) amounts on Potato Dextrose Agar (PDA) medium that was sterilized in an autoclave at 121°C for 15 minutes. The fungi isolated on the petri plates were incubated at 25-27°C for 72 hrs. With the aid of a sterilized inoculating needle, a small pinch of mycelial mass from isolates sub-cultured in PDA broth for inoculating into the healthy plants was kept at 28±2°C for 48 hours.

According to the instructions given by Ford *et. al.*, (2015) [14], the cherry tomato genotypes and hybrids were allowed to get infected by the pathogen at the root area by creating a shallow groove in the root of the plant's base and placing the adjusted conidial suspension of FOL to $1.3x\ 10^3$ spores/ml of conidial suspension of FOL that was adjusted by a hemacytometer from the one-week old pure culture of *Fusarium oxysporum* f.sp. *lycopersici*. The required amount of fungal spores were injected near the groove of the seedling's root of 35 days old seedlings in the pots and covered with the soil separately in three replications following Completely Randomized Design (CRD). Then infection related signs were observed in the seedlings in the polyhouse condition.

2.4 Observation recorded

With slight modifications, the Winstead and Kelman (1952) [15] 0–5 scale was used to rate the severity of the wilt symptoms in terms of number of wilted plants for each cherry tomato genotypes and hybrids. According to the modified rating scale the cherry tomato genotypes and hybrids were divided into the following categories: highly resistant (HS), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), and highly susceptible (HS).

Table 2. List of gradation use for Reaction and Wilting

Grade	Reaction	Wilting
0	Highly resistant (HR)	Plants did not show any wilt symptom (0% wilted)
1	Resistant (R)	1-20% plants wilted
2	Moderately resistant (MR)	21-40% plants wilted
3	Moderately susceptible(MS)	41-60% plants wilted
4	Susceptible (S)	61-80% plants wilted
5	Highly susceptible (HS)	More than 80% plants wilted

Number of plants that succumbed due to *Fusarium* wilt was counted at initial disease assessment date (60 days after inoculation) and final disease assessment date (120 days after inoculation) after inoculation was calculated as below:

Disease incidence (DI%)

$$= \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \text{ X } 100$$

Applying the formula [16] as shown below, percent disease intensity (PDI) was estimated based on the numerical rating observed.

Percent disease index (PDI%)

$$= \frac{\text{Sum of individual ratings x } 100}{\text{Total number of observation x Maximum rating grade}}$$

3. RESULTS AND DISCUSSION

3.1 Screening of cherry tomato genotypes and hybrids against *Fusarium oxysporum* f.sp. *lycopersici* resistance in the pot culture

Eighteen cherry tomato genotypes and hybrids collected and maintained at the Department of Vegetable Science, College of Horticulture and Forestry, Pasighat, Arunachal Pradesh were screened through *in- vivo* inoculation method in pot culture during October, 2022- April, 2023. The first visible symptom for fusarium wilt was observed 12 days after inoculation in hybrid G4xG5, followed by 14 days after inoculation in G4xG9 and 17 days after inoculation in Genotype 3, while it took 36 days in hybrid G1xG10 after inoculation. The results for disease incidence, percent disease index and the host status were presented in the Table 3.

Maximum *fusarium* wilt as a whole disease incidence (DI) was recorded in Genotype 3 (65.56%) at 120 days after inoculation followed by G4xG9 (63.66%) and G4xG5 (63.30%), and lowest disease incidence was recorded in Genotype 13 (17.11%), followed by G2xG7 (18.36%) and G2xG9 (35.17%). No incidence was observed in hybrid G4xG6, Genotype 9 and Genotype 5 at the final disease assessment date.

With the lowest percent disease index (PDI) of fusarium wilt Genotype 13 (16.00%) showed resistance towards *Fusarium* infection followed by G2xG7 (18.00%), while G2xG9 (11.00%)

moderately resistant for the fungal pathogen. High susceptibility was recorded in Genotype 3 (63.60%), followed by G4xG9 (61.80%), and G4xG5 (61.00%). Genotype 9, Genotype 5 and hybrid G4xG6 recorded zero PDI. Genetic regulation stands out as the most cost-effective and efficient approach for mitigating *Fusarium* wilt in tomatoes [17]. The process of domestication and selective breeding has facilitated the adaptability of cherry tomato to various geographic regions, resulting in the high yields. Nevertheless, it is worth noting that numerous indegenious cherry tomato genotypes from various states of Nort-East India lack genes that confer resistance to a broad spectrum of that pathogen. Hence, for instance, only few cherry tomato genotypes from North-East India exhibit resistance to *Fusarium* wilt.

Table 3. DI%, PDI % and screening status of cherry tomato genotypes and hybrids for fusarium wilt

Genotypes/ Hybrids	Percent disease index % (PDI)	Disease incidence (%)		Status
		IDAD (Initial Disease Assessment Date)	FDAD (Final Disease Assessment Date)	
G2	50.00 (45.00)	16.65 (24.08)	52.05 (46.20)	**Moderately susceptible(MS)**
G3	63.60 (52.90)	37.67 (37.86)	65.56 (54.67)	**Susceptible(MS)**
G2xG3	56.00 (48.45)	20.00 (26.56)	57.44 (49.81)	**Moderately susceptible(MS)**
G7	52.00 (46.15)	20.00 (26.57)	54.44 (48.13)	**Moderately susceptible(MS)**
G2xG7	18.00 (25.10)	0.00 (2.50)	18.36 (25.79)	**Resistant (R)**
G4	58.00 (49.60)	17.44 (24.68)	59.33 (50.06)	**Moderately susceptible(MS)**
G5	00.00 (2.50)	0.00 (2.50)	0.00 (2.50)	**Highly resistant (HR)**
G4xG5	61.00 (51.35)	33.33 (35.26)	63.30 (51.95)	**Moderately susceptible(MS)**
G2xG4	57.00 (49.03)	25.55 (30.36)	58.11 (49.51)	**Moderately susceptible(MS)**
G9	00.00 (2.50)	0.00 (2.50)	0.00 (2.50)	**Highly resistant (HR)**
G4xG9	61.80 (51.83)	33.33 (35.26)	63.66 (53.53)	**Susceptible(S)**
G2xG9	34.00 (35.67)	15.11 (22.87)	35.17 (36.08)	**Moderately resistant (MR)**

G9xG10	52.00 (46.15)	23.33 (28.88)	54.44 (48.13)	**Moderately susceptible(MS)**
G1x G10	53.00 (46.72)	17.44 (24.68)	54.09 (47.28)	**Moderately susceptible(MS)**
G4xG6	0.00 (2.50)	0.00 (2.50)	0.00 (2.50)	**Highly resistant (HR)**
G11	56.00 (48.45)	11.11 (19.47)	57.44 (49.81)	**Moderately susceptible(MS)**
G12	60.00 (50.77)	33.33 (35.26)	62.22 (51.42)	**Moderately susceptible(MS)**
G13	16.00 (23.58)	0.00 (2.50)	17.11 (24.87)	**Resistant (R)**
SEm ±	**0.38**	**0.19**	**0.36**	
CV (%)	**1.76**	**1.55**	**1.68**	
CD at 5%	**1.11**	**0.55**	**1.03**	
CD at 1%	**1.48**	**0.74**	**1.37**	

(Figures in the parenthesis are angularly transformed values)

3.2 Categorization of the genotypes and hybrids against *Fusarium* wilt resistance

For fusarium wilt resistance, Genotype 9, Genotype 5 and hybrid G4xG6 were grouped in one category that showed highly resistant (HR) during the observation after 120 days of inoculation. Genotype 13 and hybrids G2xG7 were grouped in resistant (R), hybrid G2xG9 was found moderately resistant (MR), five genotypes, Genotype 11, Genotype 12, Genotype 7, Genotype 2 and Genotype 4 with five hybrids G2xG3, G4xG5, G2xG4, G9xG10 and G1xG10 were grouped under moderately susceptible (MS) while Genotype 3 and hybrid G4xG9 were grouped under susceptible (S) category. Since, Genotype 9, Genotype 5 and hybrid G4xG6 were found highly resistant in field condition; these can be used in breeding programme for development of resistant lines against fusarium wilt.

When it comes to disease incidence (DI), environmental conditions like fluctuation of temperature from low to high, relative humidity causes a noticeable breakdown in the level of resistance for the genotypes and hybrids towards the pathogen inside the polyhouse. It was reported by Alexandrov (2005) [18]. Variations of the degree of resistance for *Fusarium oxysporum* by different varieties, cultivars and lines were reported by Fernandes *et al.*, (2023), Sanap *et al.*, (2020), Serife *et al.*, (2018); Onyekachukwu *et al.*, (2017) and others in terms of % of plant infected by the pathogen with different ranges and the responses of the plants with respect to combination of genetic environmental factors.

Percent disease index showed nil in Genotype 9, Genotype 5 and hybrid G4xG6 under this study. Pothiraj *et al.* (2021) reported fusarium wilt PDI range from 0-100% in tomato under various treatment of biocontrol agents. It was supported by Attia *et al.* (2022) and Mohandas *et al.* (2013).The potential resistance of the hybrids to the fungus is attributed to Gene *I-3*. This gene encodes a protein akin to an S-receptor-like kinase, which has the capability to identify the effectors generated by *Fusarium oxysporum* f.sp. *lycopersici*. Upon recognition, it triggers the biochemical defense mechanisms of the plant [21]. Increasing content of enzymatic [22] and non-enzymatic antioxidants [23] also responsible for inducing resistance towards Fusarium within the stressed condition. For confirming their resistance for the pathogen, help of molecular markers could be taken to detect the presence of resistance genes within all genotypes and their inheritance pattern could also be studied in their respective hybrids.

4. CONCLUSION

The farmers of Arunachal Pradesh are showing interest towards the cultivation of cherry tomato as it has importance in their various local cuisines. But, the cultivation is greatly infected by wilt complexes including both pathogens and pests (nematodes). Hence, present investigation was carried out to address the problem of the farmers and to obtain a durable solution for them. Eighteen cherry tomato genotypes (nine) and hybrids (nine) were evaluated for their resistance towards *Fusarium oxysporum* f.sp. *lycopersici.* All genotypes and hybrids responded differently towards the resistance for the pathogens. Out of all, Genotype 9, 5 and hybrid G4xG6 showed high resistance with no disease incidence, while wild Genotype 13 and hybrid G2xG7 were found resistant. A useful alternative to treatments like pesticides that might be an environmentally benign approach has been made possible by the adoption of resistant tomato genotypes or hybrids acquired via breeding programmes. But then also, for confirmation for the presence of resistant gene, molecular approaches need to be carried out. The creation of resistant plant types and the encouragement of resistance in plants would benefit greatly from a thorough understanding of these functions and the use of combinations of various genetic engineering approaches.

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