

**MARKER ASSISTED BACKCROSSING FOR TRANSFERRING  
FUSARIUM WILT (*Fusarium oxysporum* f. sp. *tracheiphilum*)  
RESISTANCE INTO YARDLONG BEAN (*Vigna unguiculata* subsp.  
*sesquipedalis* (L.) Verdcourt)**

*by*

**SHAHIBA A. M.**

**(2018-21-020)**

**THESIS**

Submitted in partial fulfilment of the requirements for the degree of

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**DEPARTMENT OF PLANT BREEDING AND GENETICS**

**COLLEGE OF AGRICULTURE**

**VELLAYANI, THIRUVANANTHAPURAM-695522**

**KERALA, INDIA**

**2023**

## DECLARATION

I, hereby declare that this thesis entitled “**MARKER ASSISTED BACKCROSSING FOR TRANSFERRING FUSARIUM WILT (*Fusarium oxysporum* f. sp. *tracheiphilum*) RESISTANCE INTO YARDLONG BEAN (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)**” is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani

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**Shahiba A. M.**

(2018-21-020)

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Certified that this thesis entitled “**MARKER ASSISTED BACKCROSSING FOR TRANSFERRING FUSARIUM WILT (*Fusarium oxysporum* f. sp. *tracheiphilum*) RESISTANCE INTO YARDLONG BEAN (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)**” is a record of research work done independently by **Ms. Shahiba A. M.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship, or associateship to her.



**Dr. Jayalekshmy V. G.**

Vellayani

Date: 3/07/2023

(Major Advisor, Advisory Committee)

Professor and Head

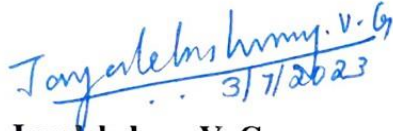
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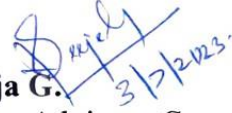
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## CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Shahiba A. M.** a candidate for the degree of **Doctor of Philosophy in Agriculture** with major in Genetics and Plant Breeding, agree that the thesis entitled **“MARKER ASSISTED BACKCROSSING FOR TRANSFERRING FUSARIUM WILT (*Fusarium oxysporum* f. sp. *tracheiphilum*) RESISTANCE INTO YARDLONG BEAN (*Vigna unguiculata* subsp. *sesquipedalis* (L.) *Verdcourt*)”** may be submitted by Ms. Shahiba A.M. in partial fulfillment of the requirement for the degree.

  
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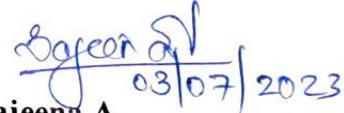
**Dr. Jayalekshmy V. G.**  
(Major Advisor, Advisory Committee)  
Professor and Head  
Department of Seed Science and  
Technology  
College of Agriculture, Vellayani

  
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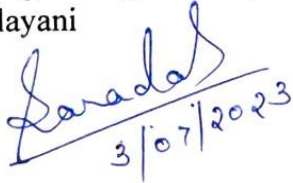
**Dr. Seeja G.**  
(Member, Advisory Committee)  
Professor and Head  
Department of Plant Breeding and Genetics  
College of Agriculture,  
Vellayani

  
03/07/2023

**Dr. Beena Thomas**  
(Member, Advisory Committee)  
Assistant Professor  
Department of Plant Breeding and Genetics  
College of Agriculture,  
Vellayani

  
03/07/2023

**Dr. Sajeena A.**  
(Member, Advisory Committee)  
Assistant Professor  
Department of Plant Pathology  
IFSRS, Karamana

  
3/07/2023

**Dr. Sarada S.**  
(Member, Advisory Committee)  
Assistant Professor  
Department of Vegetable Science  
College of Agriculture,  
Vellayani

  
3 7 23  
**DEAN**

**External Examiner**

Anbil Dharmalingam  
Agrl. College & Res. Instt.  
Tamilnadu Agricultural University  
Tiruchirappalli-620 027.

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## CONTENTS

<b>Sl. No.</b>	<b>CHAPTER</b>	<b>Page No.</b>
1	INTRODUCTION	1-3
2	REVIEW OF LITERATURE	4-58
3	MATERIALS AND METHODS	59-86
4	RESULTS	87-149
5	DISCUSSION	150-171
6	SUMMARY	172-176
7	REFERENCES	177-235
	APPENDICES	236-238
	ABSTRACT	

### LIST OF TABLES

Table No.	Title	Page No.
1.	<i>Fusarium oxysporum</i> races and their effect on various legumes	10
2.	Genetic resources in the legumes that impart <i>Fusarium</i> wilt resistance	22-23
3.	Type of QTL/gene contributing <i>Fusarium</i> wilt resistance in legumes	37-38
4.	Phenotypic and genotypic coefficient of variation of different characters in cowpea	48-52
5.	List of cowpea germplasm used for <i>Fusarium</i> wilt screening	65-67
6.	List of SSR markers linked to <i>Fusarium</i> wilt resistance used for the study	76
7	Morphological characterization isolated of <i>Fusarium</i> spp.	89
8.	The disease score (0-4) in 115 cowpea genotypes	93-95
9.	The number of days taken to wilt, disease intensity and disease reaction of 115 cowpea genotypes to <i>Fusarium</i> wilt	96-98
10.	Biometric and quality parameters in <i>Fusarium</i> wilt resistant and susceptible trailing-type cowpea genotypes	100-101
11.	PCV, GCV, heritability and genetic advance of twelve characters in trailing type cowpea genotypes	102
12.	The genotypic correlation coefficient of twelve characters in trailing-type cowpea genotypes	107
13.	The phenotypic correlation coefficient of twelve characters in trailing-type cowpea genotypes	108

14	The direct and indirect effects of twelve characters in trailing-type cowpea genotypes	111
15	Biometric and quality parameters in Fusarium wilt resistant and susceptible bush-type cowpea genotypes	113-114
16	PCV, GCV, heritability and genetic advance of twelve characters in bush-type cowpea genotypes	118
17	The genotypic correlation coefficient of twelve characters in bush-type cowpea genotypes	120
18	The phenotypic correlation coefficient of twelve characters in bush-type cowpea genotypes	121
19	The direct and indirect effects of twelve characters in bush-type cowpea genotypes	123
20	Percentage of pod set after hybridization between Githika and IRON	127
21	Percentage of germination in $F_1$ s and $BC_1F_1$ generations	127
22	Morphological evaluation of $F_1$ s along with recurrent and donor parents	127
23	Disease scoring of $BC_1F_1$ lines on a 0-4 scale	129-133
24	Number of $BC_1F_1$ lines in each category of disease reaction for chi-square test	135
25	Chi-square test for goodness of fit in $BC_1F_1$ lines	135
26	Performance on biometric parameters of $BC_1F_1$ resistant lines and frequency distribution	137-140
27	Proximity dissimilarity matrix by Euclidean distance with recurrent parent using biometric traits in $BC_1F_1$ resistant lines	142-144
28	Disease scoring of $BC_2F_1$ lines from 25 resistant $BC_1F_1$ lines on a 0-4 scale	147-149

## LIST OF PLATES

<b>Plate No.</b>	<b>Title</b>	<b>Pages Between</b>
1.	Symptoms of Fusarium wilt of yardlong bean	60-61
2.	<i>Fusarium</i> spp. multiplied in PDA Medium	60-61
3.	<i>Fusarium</i> spp. mass multiplied in sand: maize medium (9:1)	60-61
4.	Inoculation of Fusarium culture in 3 week old yardlong bean seedling rhizosphere	64-65
5.	Score (0-4) diagram for assessing the severity of Fusarium wilt in cowpea	68-69
6.	Field evaluation view	68-69
7.	Hybridization steps to produce F <sub>1</sub> s	78-79
8.	Hybridization block for producing F <sub>1</sub> plants	78-79
9.	Evaluation of the F <sub>1</sub> population and the recurrent parent	78-79
10.	Artificial screening of Fusarium wilt BC <sub>1</sub> F <sub>1</sub> lines	80-81
11.	Artificial screening of Fusarium wilt BC <sub>2</sub> F <sub>1</sub> lines	80-81
12.	Characterization of Fot1 isolate	88-89
13.	Characterization of Fot4 isolate	88-89
14.	Characterization of Fot7 isolate	90-91
15.	Characterization of Fot8 isolate	90-91

16.	Characterization of Fot9 isolate	90-91
17.	Standardization of methods for inoculation of Fusarium pathogen	92-93
18.	Virulence testing of Fusarium isolates	92-93
19.	Standardization of Fusarium inoculum percentage for screening	92-93
20.	Fusarium wilt resistant genotypes	98-99
21.	Fusarium wilt moderately resistant genotypes	98-99
22.	Fusarium wilt moderately susceptible genotypes	98-99
23.	Fusarium wilt susceptible genotypes	98-99
24.	Fusarium wilt highly susceptible genotypes	98-99
25.	Reaction of KAU released varieties to Fusarium wilt	98-99
26.	Amplification profile of UASBC59 SSR marker in parental line	126-127
27.	Fusarium screening of the donor parent with KAU-released varieties	126-127
28.	Fusarium wilt resistant BC <sub>1</sub> F <sub>1</sub> lines (score '0')	128-129
29.	Fusarium wilt moderately resistant, moderately susceptible, susceptible BC <sub>1</sub> F <sub>1</sub> lines	128-129
30.	Fusarium wilt highly susceptible BC <sub>1</sub> F <sub>1</sub> lines (Score '4')	128-129
31.	Comparison of Fusarium wilt resistant and susceptible BC <sub>1</sub> F <sub>1</sub> lines in vascular rotting.	134-135

32.	Comparison of Githika, BC <sub>1</sub> F <sub>1</sub> lines and IRON for the reaction to Fusarium wilt	134-135
33.	Variability in pod length (A) Githika, (B) IRON, and (C) BC <sub>1</sub> F <sub>1</sub> resistant lines	134-135
34.	Variability in seed colour and size (A) Githika, (B) IRON, and (C) BC <sub>1</sub> F <sub>1</sub> resistant lines	134-135
35.	Amplification profile of UASBC59 SSR marker in Fusarium wilt resistant BC <sub>1</sub> F <sub>1</sub> lines	146-147
36.	Fusarium wilt resistant (R) BC <sub>2</sub> F <sub>1</sub> lines (score '0')	149-150
37.	Fusarium wilt moderately Resistant (MR) BC <sub>2</sub> F <sub>1</sub> lines (Score '1')	149-150
38.	Fusarium wilt moderately Susceptible BC <sub>2</sub> F <sub>1</sub> lines (Score '2')	149-150
39.	Fusarium wilt susceptible (S) BC <sub>2</sub> F <sub>1</sub> lines (Score '3')	149-150
40.	Fusarium wilt highly Susceptible (HS) BC <sub>2</sub> F <sub>1</sub> lines (Score '4')	149-150
41.	Comparison of BC <sub>2</sub> F <sub>1</sub> lines with Githika and IRON for the reaction to Fusarium wilt	149-150
42.	Transplanted resistant BC <sub>2</sub> F <sub>1</sub> lines under greenhouse condition	149-150
43.	Comparison of Githika and IRON plants after Fusarium inoculation	165-166

## LIST OF FIGURES

<b>Fig. No.</b>	<b>Title</b>	<b>Pages Between</b>
1.	Phylogenetic analysis of isolate with the <i>Fusarium oxysporum</i> database from NCBI	92-93
2.	Frequency of cowpea genotypes and disease intensity (DI)	98-99
3.	Number of cowpea genotypes under different disease grades	98-99
4.	Heatmap with hierarchical clustering of trailing-type cowpea genotypes based on biometric and quality parameters	106-107
5.	Heatmap with hierarchical clustering of bush-type cowpea genotypes based on biometric and quality parameters	116-117
6.	Disease scoring of NBPGR cowpea genotypes	155-156
7.	Disease scoring of AVRDC cowpea genotypes	155-156
8.	Disease scoring of local cowpea genotypes	155-156
9.	Disease scoring of KAU-released cowpea genotypes	155-156
10.	Scatterplot diagram of characters by pod yield per plant in trailing-type cowpea	157-158
11.	PCV and GCV of twelve characters in trailing-type cowpea genotypes	157-158
12.	Heritability and genetic advance of twelve characters in trailing-type cowpea genotypes	157-158

13.	Genotypic correlation matrix of twelve characters in trailing-type cowpea genotypes	159-160
14.	Phenotypic correlation matrix of twelve characters in trailing-type cowpea genotypes	159-160
15.	Path diagram showing direct effect and genotypic correlation of twelve characters in trailing-type cowpea genotypes	159-160
16.	Scatterplot diagram of characters by pod yield per plant in bush-type cowpea genotypes	161-162
17.	PCV and GCV of twelve characters in bush-type cowpea genotypes	161-162
18.	Heritability and genetic advance of twelve characters in bush-type cowpea genotypes	161-162
19.	Genotypic correlation matrix of twelve characters in bush-type cowpea genotypes	161-162
20.	Phenotypic correlation matrix of twelve characters in bush-type cowpea genotypes	161-162
21.	Path diagram showing direct and genotypic correlation of twelve characters in bush-type cowpea genotypes	163-164
22.	Average performance of morphological characters in parents and $F_1$ population	165-166
23.	Frequency of disease score in $BC_1F_1$ lines	167-168
24.	Frequency distribution and probability plot of vine length in $BC_1F_1$ resistant lines	167-168

25.	Frequency distribution and probability plot of primary branches per plant in $BC_1F_1$ resistant lines	167-168
26.	Frequency distribution and probability plot of pod weight in $BC_1F_1$ resistant lines	169-170
27.	Frequency distribution and probability plot of pod length in $BC_1F_1$ resistant lines	169-170
28.	Frequency distribution and probability plot of seeds per pod in $BC_1F_1$ resistant lines	169-170
29.	Frequency distribution and probability plot of crop duration in $BC_1F_1$ resistant lines	169-170
30.	Frequency distribution and probability plot of total phenols in $BC_1F_1$ resistant lines	169-170
31.	Frequency distribution and probability plot of crude fibre content in $BC_1F_1$ resistant lines	169-170
32.	Frequency distribution and probability plot of crude protein content in $BC_1F_1$ resistant lines	169-170
33.	Proximity dissimilarity matrix Euclidean distance of $BC_1F_1$ lines with a recurrent parent using biometric traits	171-172
34.	Constellation plot of $BC_1F_1$ lines using Euclidean distance with a recurrent parent using biometric traits	171-172

## LIST OF APPENDICES

<b>Sl. No.</b>	<b>Title</b>	<b>Appendix No.</b>
1.	ITS-rDNA sequence of Fot1 Fusarium isolate	I
2.	The homologous similarity search of Fot1 sequence in NCBI through BLAST	II
3.	ITS-rDNA sequence of the isolate submitted to the NCBI with accession number MZ706472.1	III

### LIST OF ABBREVIATIONS AND SYMBOLS USED

<sup>o</sup> C	Degree Celsius
%	Per cent
@	At the rate of
&	And
cfu	Colony forming units
CD (0.05)	Critical Difference at 5% level
cm	Centimetre
mg	Milligram
RBD	Randomised Block Design
CRD	Complete Randomized Design
DAS	Days After Sowing
<i>et al.</i>	And others
Sec	Seconds
Min.	Minutes
h	Hours
g	Gram
BLAST	Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
Kg	Kilo gram
DAS	Days after sowing
SSR	Simple Sequence Repeats
KAU	Kerala Agricultural University
AVRDC	Asian Vegetable Research and Development Center
NBPGR	National Bureau of Plant Genetic Resources
bp	Base pairs

PCR	Polymerase Chain Reaction
ITS	Internal Transcribed Spacer
PDA	Potato Dextrose Agar
DAI	Days after inoculation
rDNA	Ribosomal DNA
FW	Fusarium wilt
<i>Fo</i>	<i>Fusarium oxysporum</i>
l	Litres
Temp.	Temperature
<i>Via</i>	Through
No.	Number
rpm	Rotation per minute
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
GCV	Genotypic Coefficient of Variation
PCV	Phenotypic Coefficient of Variation
<i>viz.</i>	Namely
d.f	Degrees of freedom
S. E	Standard Error
mg g <sup>-1</sup>	Milligram per gram

# ***INTRODUCTION***

## 1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.)) is a multipurpose legume as it provides food (grains, tender leaves, and pods) for human consumption, maintains soil-ecology balance through nitrogen fixation by facilitating symbiosis with nodulating bacteria, provides fodder for livestock, and well utilized as a cover crop or an erosion-preventing crop that aids in weed suppression and moisture retention (Omomowo and Babalola, 2021). The crop is grown in many parts of India and elsewhere in the world, mainly in semi-arid regions and is cultivated throughout the year in Kerala.

Cowpea is an annual leguminous crop, a diploid ( $2n=22$ ) member of the family Fabaceae with an estimated genomic size of 620 Mb (Lonardi *et al.*, 2019). Having originated in Africa (OECD, 2016), where the large genetic diversity of wild types occurs (D'Andrea *et al.*, 2007), the crop later spread to all continents, now mainly grown in Asia, Europe, the USA, Central America, and South America (Tetteh *et al.*, 2020). The adaptability of the cowpea in all places is due to its high resilience to harsh conditions, especially hot and dry environments, and poor soils (Boukar *et al.*, 2018). Cowpea is the richest source of nutrients. Its dry grains contain 22-23% protein, high content of thiamine, riboflavin, and niacin and are richer than cereals in iron and calcium content. The leaves are a significant source of  $\beta$ -carotene and ascorbic acid (Carvalho *et al.*, 2017). Cowpea also contributes significantly to economic productivity and environmental sustainability (Cardona-Ayala *et al.*, 2020).

Yardlong bean or asparagus bean (*Vigna unguiculata* ssp. *sesquipedalis*,  $2n = 2x = 22$ ) is one of the subspecies of cowpea (*Vigna unguiculata*), a warm-season and drought-tolerant crop with wide cultivation in East and Southeast Asia (Ehlers and Hall, 1997). It has very long slender pods reaching upto a length of 90cm and is consumed as a fresh vegetable. China, India, Japan, Korea, and Thailand are among the major yardlong bean producing countries (OECD, 2016).

Tender pods are rich in crude protein (3.5-5%), vitamin A (940 IU), vitamin C (13 mg), iron (2.5 mg), and dietary fibre (2g) (Singh *et al.*, 2001). It is an inexpensive source of vegetable protein which is easily digestible, relatively cheaper and has high biological values. It is a well-adapted vegetable crop under Kerala's agro-climatic conditions. However, the production of yardlong bean in Kerala is challenged by a variety of fungal diseases, of which vascular wilt caused by *Fusarium oxysporum* is considered to be the most destructive.

Fusarium wilt caused by the fungal pathogen, *Fusarium oxysporum* f. sp. *tracheiphilum* (Fot), is one of the soil-borne diseases that causes a major threat in many of the cowpea growing areas. The disease incidence substantially reduces the yield by 30 to 100 per cent with high plant mortality, resulting in adverse effects on cowpea production. The occurrence and epidemic spread of the pathogen depends on various soil factors like nutrient status, temperature (25-28°C), moisture, and resistant varieties (Omoigui *et al.*, 2018). The pathogen invades the plant through roots and enters the vascular system, and subsequently, the infected plants exhibit leaf chlorosis, necrosis, wilting, vascular discolouration, and death with severe economic loss. The yield loss due to Fusarium wilt mainly depends on the stage of the attack, where it can reach upto 100 per cent during the pre-pod stage (Okiror, 2002). A wide range of host plants including family members of Fabaceae, Malvaceae, and Solanaceae, and the formation of dormant and thick-walled asexual chlamydospores in the soil crust are the major constraints in controlling the pathogen completely.

It is a soil-borne pathogen and is very difficult to manage using fungicides alone. The continuous use of fumigants for soil fumigation, mainly methyl bromide leads to environmental damage as well as the development of resistant strains of the pathogen. Crop rotation could not be recommended as a sole control measure due to the formation and retention of chlamydospores in the soil crust for several years. Utilization of host plant resistance is a cost-effective and environmentally safe method for the management of Fusarium wilt in cowpea (Ramapura and Babu, 2007).

Screening and identification of Fusarium wilt resistant donors in cowpea is a prerequisite for developing superior varieties with field resistance. The introgression of desired genes using traditional protocols is time-consuming and complicated as well. Hence, molecular markers linked to Fusarium wilt resistance can be used to trace the gene for resistance during early developmental stages, leading to an effective crop improvement in cowpea. The knowledge available on the inheritance of Fusarium wilt resistance and the molecular markers linked with the resistant genes increases the efficiency and speed of resistant breeding programs (Chitwood-Brown *et al.*, 2021). Fusarium wilt resistance in cowpea is mostly controlled by a single dominant gene, the marker-assisted backcross breeding method could be adopted to introgress the gene for resistance to well-adapted varieties (Bharadwaj *et al.*, 2021).

With this background, the present study was undertaken with the objective to transfer the Fusarium wilt resistant gene into yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) through marker-assisted backcrossing.

# ***REVIEW OF LITERATURE***

## 2. REVIEW OF LITERATURE

Yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis*) is considered one of the top ten Asian vegetables that belong to the important subspecies of cowpea. The crop is well adapted to Kerala conditions due to favourable agro-climatic conditions and has come to occupy a prime position among the vegetable crops raised in the state. The crop is highly preferred among the Keralites due to its nutritional factors, soil-improving properties, and easy cultivation.

Among the diseases that occur at different growth stages of the yardlong bean, Fusarium wilt caused by the pathogen *Fusarium oxysporum* f sp. *tracheiphilum* results in major economic yield losses. Symptoms of Fusarium wilt begin with sudden yellowing of foliage to defoliation. Under severe conditions, the lower portion and upper part of the tap root system together form a swollen structure that slowly becomes shredded and disintegrated, leading to the complete death of the plant. Yield loss reaches 100% when it attacks during the pre-pod stage (Okiror, 2002).

So, the present study deals with the identification of the Fusarium wilt resistance donor parent and the introgression of the gene for wilt resistance into yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt). The literature related to symptomatology and characterization of the pathogen, genetic resources and resistant loci, conventional and molecular breeding methods for Fusarium wilt resistance, and biometric and quality characterization in vegetable cowpea is reviewed and presented in this chapter. The studies on other crops are also being reviewed here to supplement the information.

### 2.1. CROP DESCRIPTION

#### 2.1.1. Cowpea (*Vigna unguiculata* L. Walp)

The cowpea is a *Dicotyledonea*, belonging to Fabales, *Fabaceae*, sub-family *Faboideae*, tribe *Phaseoleae*, sub-tribe *Phaseolinae*, genus *Vigna* and section *Catjang*.

It is grown in tropical Africa, Asia, and North and South America mostly as a grain and as a vegetable and fodder crop. It is known by different names in different places, such as ‘*seub*’ and ‘*niao*’ in Senegal, ‘*wake*’ or ‘*bean*’ in Nigeria, ‘*niebe*’ in Africa, ‘*luba hilu*’ in Sudan, ‘*blackeye bean*’ ‘*blackeye peas*’ ‘*crowder peas*’ and ‘*southern peas*’ in the United States, ‘*lobia*’ in the Indian subcontinent, ‘*caupi*’ in Brazil, and ‘*long bean*’ or ‘*asparagus bean*’ in China (OECD, 2016). There are three cultivated (*unguiculata*, *sesquipedalis*, and *cylindrica*) and two wild (*dekindtiana* and *momensis*) subspecies of cowpea (*Vigna unguiculata* (L) Walp). More than 20 binomials were described for the cultivated *Vigna unguiculata* species, but it has two botanical varieties, the cultivated *Vigna unguiculata* var. *unguiculata* and the wild form *Vigna unguiculata* var. *spontanea* (Kebede and Bekeko, 2020).

The probable progenitor of cultivated cowpea is *Vigna unguiculata* var. *spontanea* (Padulosi and Ng, 1997; OECD, 2016). Based on the pod, seed, and ovule characteristics, it has been divided into five subspecies viz., *unguiculata* (grown as a pulse), *metanopthalmus* (black eye pea types), *catiang* (used as forage), *sesquipedalis* (grown as vegetables) and *textilis* (used for fibres) (Pasquet, 1999). But there is no apparent hindrance to recombination or hybridization between members of subspecies or with wild progenitors (OECD, 2016). Cowpea is a predominantly self-pollinated, cleistogamous crop, and flowers are arranged with an axillary racemose or intermediate inflorescence at the distal ends of a 5 to 60 cm long peduncle (Ige *et al.*, 2011). About 50% of the flower buds do not open due to their cleistogamous nature, which advocates self-pollination.

Cowpea is the best companion crop in mixed cropping systems useful for suppressing weed infestation, improving soil fertility, and reducing water evaporation (Meena *et al.*, 2015). It can thrive under low fertility and dry land growing conditions making it one of the most resilient crops suitable for low input and water-limited production (Meena *et al.*, 2015). It performs well in all ecological zones, especially where the annual rainfall ranges between 500 and 1200 mm (Madamba *et al.*, 2006)

along with maximum and minimum temperatures of 30 and 28°C during the growing period (Craufurd *et al.*, 1996). Some cowpea varieties are used to induce suicidal germination of *Striga hermonthica* or to suppress the population of the nematode *Scutellonema cavenessi*, which parasitizes pearl millet, sorghum, and peanut (Badiane *et al.*, 2014).

Cowpea is an important nutritional food in the human diet due to its high protein (rich in lysine and tryptophan) and carbohydrate content with relatively low-fat content and a complementary amino acid pattern to that of cereal grains (Jayathilake *et al.*, 2018). It is a valuable source of protein that is cheaper than fish, meat, or poultry helping to fight malnutrition for low-income farmers (Badiane *et al.*, 2014). Cowpea grain contains protein (250 mg/g), iron (53.2 mg/kg), zinc (38.1 mg/kg), calcium (826 mg/kg), and magnesium (1915 mg/kg) (Boukar *et al.*, 2019). It is also a rich source of phenolic compounds which act as radical scavengers and reducing agents, and have anti-oxidant properties (Weidner *et al.*, 2018; Sombie *et al.*, 2018). The crop gained more attention from consumers and researchers, because of its health benefits including anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory, and anti-hypertensive properties (Jayathilake *et al.*, 2018).

Cowpea leaves are complex, with two asymmetrical side leaflets and one symmetrical central terminal leaflet. Due to the variability of the side leaflets, the central leaflet of a trifoliate is typically used to classify leaf shape. Leaf shape is crucial for taxonomic categorization and for identifying different types of cowpea varieties. Many parts of Africa, enjoy eating young cowpea leaves as a pot herb. The cowpea shoots and leaves are rich sources of phosphorous, calcium, and Vitamin B (Maynard, 2008). The fresh cowpea leaves contain crude protein (28-42 g/ 100g dry weight), crude fibre (10.09-25.51g/100g dry weight) beta-carotene (0.25–36.55 mg/100 g dry weight), Vitamin C (70–203 mg/100 g dry weight) and iron (0.17–75.00 mg/100 g dry weight). Cowpea leaves can be eaten raw, cooked, blanched, dried, or fermented (Owade *et al.*, 2020). But it has antinutritional factors such as phytates, oxalates and nitrates which

can cause a negative impact on nutrient intake. The presence of these antinutritional factors could be reduced by fermentation (38.4% and 8.3% reduction of nitrate and oxalate contents respectively (Muchoki, 2007)) or the method including para-boiling, sun-drying and draining (reduction of 73.9%, 33.3%, 70.7%, and 85.9% in the saponins, tannins, polyphenols, and flavonoid contents, respectively (Chikwendu *et al.*, 2014).

### **2.1.2. Yardlong Bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)**

Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt, 2n= 2X= 22), is a warm season and drought-tolerant crop, widely cultivated in East and Southeast Asia (Fang *et al.*, 2006). Apart from subsp. *unguiculata*, it has a climbing growth habit and produces long pods up to 30-90 cm with narrow kidney-shaped seeds usually 8-12 mm long, which are harvested at the immature ‘snap’ stage (Ehlers and Hall, 1997). Long pods are the characteristic trait of the subsp. *sesquipedalis* which is believed to be the result of intensive local domestication after it was brought to Asia from Africa (Fang *et al.*, 2006). Diverse landraces of yardlong bean were found throughout Asia and have never been observed in Africa (Xu *et al.*, 2011).

It is also known as asparagus bean, string bean, snake bean, sitao and vernacularly known as ‘*Achingapayar*’, ‘*Kurutholapayar*’, ‘*Vallipayar*’ ‘*Vanpayar*’, ‘*Pathinettumaniyan*’ etc., is evenly distributed and preferred in all the districts of Kerala. It is harvested while its pod is still tender thereby providing a very good source of proteins, minerals, vitamins, and dietary fibre (Jayathilake *et al.*, 2018). 100 g of green tender pods contain 4.3 g protein, 2.0 g fibre, 8.0 g carbohydrates, 74 mg phosphorus, 2.5 mg iron, 13.0 mg vitamin-C, and 0.9 mg minerals. Yardlong bean is one of the top crops, that help combat malnutrition and food insecurity in most developing countries due to its high nutritional value and low requirement for cultivation management (Xu *et al.*, 2017). The tender pods of the yardlong bean can be

harvested up to four times in a short season, making it a high-yielding crop that can be grown year-round in greenhouses.

Slowing the rate of seed development to prolong the production of attractive immature pods favourable for vegetable use with more pods per plant is the main objective of the yardlong bean breeding program (Timko *et al.*, 2007). The yardlong bean is believed to have evolved from the short pod cowpeas of the spp *unguiculata* group somewhere in Southeast Asia, perhaps in India (Lush *et al.*, 1980). Even though the genetic bases are very similar, the diversification between spp *unguiculata* and spp *sesquipedalis*, in terms of long pods, seeds colour, and pattern, and long seeds in vegetable cowpea fit in the long pods are not yet understood (Xu *et al.*, 2011). The single nucleotide polymorphism from genotyping by sequencing method in vegetable, fodder, and grain type cowpeas using 130 cowpea accessions by Wu *et al.* (2022) reported the differences in chromosomes Vu03 and Vu08 composition, which might be due to discrete selection and divergence, especially in vegetable type cowpea compared to grain and fodder type cowpeas.

A marker-assisted backcross or introgression breeding approach can be used to accelerate additional genetic variation into yardlong bean (ssp. *sesquipedalis*) breeding programs to ensure genetic gain and transfer of valuable resistant traits from cowpea (ssp. *unguiculata*) (Xu *et al.*, 2010).

### **2.1.3. The comparison of cowpea (ssp. *unguiculata*) and yardlong bean (ssp. *sesquipedalis*) genomes**

The chromosome number in both ssp. *unguiculata* and ssp. *sesquipedalis* is the same,  $2n=2x=22$ , but the genome size varies to some extent. The genome size of ssp. *unguiculata* was reported as 620Mb (Lonardi *et al.*, 2019), while ssp *sesquipedalis* was reported as 632.8Mb (Xia *et al.*, 2019) based on the whole genome shotgun sequencing strategy. The GC content in ssp. *unguiculata* was reported as 32.99% with 49.50% repeat elements, while ssp. *sesquipedalis* had 28.78% and 46.47% GC content and

repeat elements respectively (Xia *et al.*, 2019). The yardlong bean genome assembly was significantly larger than the cowpea genome assembly. The scaffold N50 size of the yardlong bean was greatly longer at 2.7 Mb than the common cowpea genome size analyzed by the next-generation sequencing technology (Xia *et al.*, 2019).

## 2.2. FUSARIUM WILT

### 2.2.1. Fusarium wilt (*Fusarium oxysporum*) in legumes

*Fusarium oxysporum* is an ascomycete fungus, in the class Sordariomycetes, order Hypocreales, family Nectriaceae and genus *Fusarium* (Wollenweber and Reinking, 1935). It is the most economically significant and often encountered species of *Fusarium*. There are both pathogenic (plant, animal, and human) and non-pathogenic *Fusarium* strains which are soil-borne asexual fungi (Leslie and Summerell, 2006). Without creating symptoms, the non-pathogenic forms invade the living tissues in the cortex of plant roots and have a negative interaction with pathogenic soil organisms. In a variety of hosts, the pathogenic forms produce root rot, foliar necrosis, yellowing, and, vascular wilting. Both forms are indistinguishable but exhibit high functional and genetic diversity.

The host range of *Fusarium oxysporum* varies from monocots to dicots, perennials to annuals, and land-based to aquatic plants. Wilt caused by *Fusarium oxysporum* is the most destructive fungal disease, that severely affects global legume production (Haware *et al.*, 1978; Kraft *et al.*, 1998; Kaiser *et al.*, 2000; Fall *et al.*, 2001). Some of the *Fusarium oxysporum* races and their effect on various legumes are listed in Table 1 (Jha *et al.*, 2020).

### 2.2.2. Fusarium wilt (*Fusarium oxysporum* f sp. *tracheiphilum*) in cowpea

#### 2.2.2.1. Incidence of *Fusarium* wilt

Fusarium wilt (*Fusarium oxysporum* f sp. *tracheiphilum*) is a vascular disease of cowpea. The fungus has been detected in cowpea-growing regions throughout the

**Table 1. *Fusarium oxysporum* races and their effect on various legumes**

<b>Crop</b>	<b>Causal organism</b>	<b>Races</b>	<b>Symptoms</b>	<b>% Yield loss</b>	<b>References</b>
Chickpea	<i>Fusarium oxysporum</i> f. sp. <i>ciceri</i> ( <i>Foc</i> )	Eight races (0, 1B/C, 1A, 2, 3, 4, 5, and 6)	Destruction of vascular bundles leads to weakening of plant water relation; drooping of rachis, leaflets, and petioles; yellowing syndrome caused by races 0 and 1B/C.	10-40% and even 100% under favourable conditions	Sharma and Muehlbauer, 2007; Castro <i>et al.</i> , 2010; Jimenez-Diaz <i>et al.</i> , 2015.
Pea	<i>Fusarium oxysporum</i> Schl. f. sp. <i>pisi</i> Snyd. And Hans. ( <i>Fop</i> )	Four races (1, 2, 5, and 6)	Foliage becomes grey-green coloured, thickening of the internodal base, downward curling of leaves, chlorosis, wilting, yellow to orange discolouration in vascular tissue, and death of the entire aerial part. Complete death in the case of races 1 and 5.	Up to 100%	Coyne <i>et al.</i> , 2000; Haglund and Kraft, 2001; Jain <i>et al.</i> , 2015.
Cowpea	<i>Fusarium oxysporum</i> f. sp. <i>tracheiphilum</i> ( <i>Fot</i> )	Four races (1, 2, 3, and 4)	Wilting, leaf chlorosis, and stunting of entire plants with discolouration of vascular tissues. Symptoms are more evident during flowering and early pod development stages with high mortality and severe overall yield loss.	Up to 100%	Davis and Frate, 2007; Pottorff <i>et al.</i> , 2014.
Red gram	<i>Fusarium udum</i> Butler	-	Stunting and overall death of plants.	30-100%	Okiror, 2002.
Common bean	<i>Fusarium oxysporum</i> (Schlecht) f. sp. <i>phaseoli</i> Kendrick & Synder ( <i>FOP</i> )	Two races (2, 4)	Phloem blockage, chlorosis, necrosis and wilting of older and younger leaves, red-brown colored vascular tissue, reduced plant lifecycle and plant death.	Up to 100%	Buruchara and Camacho, 2000; Fall <i>et al.</i> , 2001; Xue <i>et al.</i> , 2015.
Lentil	<i>Fusarium oxysporum</i> f. sp. <i>lentis</i> ( <i>Fol</i> )	Eight races/pat hotypes	Wilting during seedling, flowering, and pod stages. Stunting and vascular discoloration in lower stem.	Up to 100%	Tosi and Cappelli, 2001; Hiremani and Dubey, 2018.
Faba bean	<i>Fusarium oxysporum</i> f. sp. <i>fabae</i> ( <i>FOF</i> )	-	Vascular discolouration and wilting of plants.	-	Rehim, 1962.

world and is an economically significant problem in Asia, the United States, the Central Valley of California (Smith *et al.*, 1999), Australia, Brazil, and Nigeria (Assuncao *et al.*, 2003). It had been reported for the first time in the USA (Orton, 1902) and in India by Singh and Sinha (1955). Reddy *et al.* (1990) reported that *Fusarium* wilt is the major threat in cowpea cultivation and causes substantial yield losses of 30% to 100%. The incidence of *Fusarium* wilt has been noticed in farmer's fields in the Thiruvananthapuram district of Kerala since 1995-96 (Reghunath *et al.*, 1995). The pathogen is predominant in tropics, temperate, desert, alpine, and arctic area, where the harsh climate prevails (Francis and Burgess, 1975) and the distribution covers soils, and organic substrates all over the world (Di Pietro *et al.*, 2003). *Fusarium oxysporum* was the most frequent isolate from diseased plants in all cowpea fields, while *F. solani* and *F. moniliforme* were also present. It was assessed by inoculating *Fusarium* wilt pathogens on two-week-old cowpea seedlings (Senthilkumar, 2003).

Poor drainage and aeration, an unfavourable chemical, and physical condition of the soil, prolonged wet or dry conditions, extremes in temperatures, and storm damage influence the disease development (Brake *et al.*, 1995). Steven *et al.* (2003) reported that the occurrence and spread of soil-borne disease mainly depend on factors like soil nutrient levels, temperature, moisture stress, and the resistance of varieties.

Brake (1990) reported that the growth and survival of pathogens were favoured under dry soil conditions, whereas a four-fold increase in wilted plants under heavy rainfall and waterlogged conditions was argued by Peng *et al.* (1999) and Pattison *et al.* (2014). Temperature is the most critical factor in wilt development (Peng *et al.*, 1999), pathogen growth was inhibited above 33°C and below 17°C and was great at 28°C (Moore, 1995). Wilt disease is more serious in light sandy soils than in heavy clay soils, and suppressive soils with high pH, organic carbon, and clay texture, generally suppress the growth of the *Fusarium* pathogen (Pattison *et al.*, 2014).

#### 2.2.2.2. *Fusarium* pathogen and Disease cycle

*Fusarium* species belong to the class Ascomycetes and family Hypocreaceae, which are filamentous, and the most important phytopathogenic and toxigenic fungi (Okungbowa and Shittu, 2012). The main symptoms of *Fusarium* are wilts, blights, root rots, and cankers (Ma *et al.*, 2013). Thus, the diseases are named *Fusarium* yellows, *Fusarium* blights, and *Fusarium* wilt, and diseases with rotting symptoms are called basal rot, *Fusarium* stem rot, or crown and root rot (Edel-Hermann and Lecomte, 2019).

There are different species complexes in *Fusarium*, but *Fusarium oxysporum* (*Fo*), is the most common species that spreads through the soil by causing vascular wilt and root rot in wide plant species (Agrios, 2005) and it was considered on the list of quarantine in several destinations (Dean *et al.*, 2012). More than 120 *formae speciales* (ff. spp.) have been identified based on the host specificity of *Fusarium oxysporum* (Michielse and Rep, 2009) but, 144 forms of *Fusarium oxysporum* were reported after scrutiny (Lombard *et al.*, 2019). Accordingly, the strain causing *Fusarium* wilt in cowpea is *Fusarium oxysporum* f. sp. *tracheiphilum* (Wollenweber, 1913).

To distinguish rot-producing strains from wilt-producing strains, the term “*radicis*” is used in the naming of the strain (Weimer, 1944) for example *Fusarium oxysporum* f. sp. *radicis-lycopersici* causes root rot in tomato (Williams, 1996). *Fusarium oxysporum* strains are occasionally reported as a part of a consortium of pathogens (Beccari *et al.*, 2010).

Four races of *Fusarium oxysporum* f. sp. *tracheiphilum* (1,2,3, and 4) have been identified based on pathogenic reactions to different cowpea genotypes, and currently, among *Fot* races, race 3 has been reported to occur globally (Hare, 1957; Smith *et al.*, 1999). Race 1 also caused wilt in ‘Yelredo’ soybean (*Glycine max*) and ‘Encore’ chrysanthemum (*Chrysanthemum morifolium*) (Armstrong and Armstrong, 1980). The life cycle of a fungus divides into dormant, parasitic, and saprophytic stages (Agrios,

2005). In the absence of a host, dormant chlamydospores remain in the soil for several years (Roncero *et al.*, 2003). During the presence of the host, the fungal spores germinate and enter the plant system through the roots by secreting cell wall degrading enzymes and secondary metabolites to facilitate penetration (Turra *et al.*, 2015). Root penetration occurs through the natural openings without forming any specialized structures, mainly at intercellular junctions of cortical cells or through wounds (Perez-Nadales and Di Pietro, 2011).

Once entering the root system, the fungal hyphae occupy the root cortex, reach the xylem vessels by penetrating the endodermis, and grow vertically through the xylem (Perez-Nadales *et al.*, 2014). It moves, multiplies in the system, and colonizes until the entire plant wilts. Upon plant death, profuse sporulation, and dispersal of micro- and macro- conidia on the soil for the next cycle of infection occur. At the saprophytic stage, it degrades lignin and carbohydrates associated with soil debris (Agrios, 2005).

### ***2.2.2.3. Symptomatology of Fusarium wilt***

Vascular wilting and root rotting are the two types of symptoms produced by pathogenic *Fusarium oxysporum* strains (Gorden, 2017). In the case of vascular wilting, the pathogen enters the plant system through the root and invades the vascular tissue (Beckman, 1987). *Fusarium* wilts first appear as slight vein clearing on the outer portion of the young leaves, followed by downward drooping (epinasty) of the older leaves (Michielse and Rep, 2009).

Infected plants will wilt and die soon after symptoms appear during the seedling stage, but in older plants, leaf epinasty and vein clearing are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves and finally death of the entire plant (Agrios, 2005). Gokulapalan *et al.* (2006) reported yellowing, wilting, and drooping of leaves, blackening, and drying of veins and the abnormal fluttering of

the stem along the growing tip in Fusarium wilt-affected plants. The flowers become small and sterile resulting in a yield reduction (Di Pietro *et al.*, 2003).

Vascular browning or discolouration of xylem tissues is strong evidence of Fusarium wilt and the plants are more prone to wilt during the period between blossoming and fruit maturation (Smith *et al.*, 1988; Pottoroff *et al.*, 2012). The discolouration can be easily seen when the stem splits vertically or is cross-sectioned (Jimenez-Diaz *et al.*, 2015). The accumulation of fungal mycelium and the gels or gum production as defence responses, blocking or plugging the vessels leading to symptoms resembling water stress (Di Pietro *et al.*, 2003).

Rotting mainly affects plants with storage organs such as bulbs, corms, tubers, and rhizomes (Manici and Cerato, 1994). It progresses in the roots and hypocotyl cortical tissues without reaching the vascular system (Jarvis and Shoemaker, 1978). Brown or black necrotic spots due to discolouration of root tissues end up in the rotting of the plant (Trujillo and Ohno, 1963; Boerema and Hamers, 1989).

## 2.3. CHARACTERIZATION OF FUSARIUM PATHOGEN

### 2.3.1. Morphological and culture characterization of *Fusarium* spp.

Accurate and rapid identification of the pathogen is necessary for appropriate disease management (Singh *et al.*, 2006). The characterization of morphological features is key to the identification and taxonomy of *Fusarium* spp. (Leslie and Summerell, 2006). The macroscopic and microscopic characters, *viz.*, culture characters, length and shape of macroconidia, number, shape, and arrangement of microconidia, and presence and absence of chlamydospores are the key features for *Fusarium* species differentiation (Larone, 1995; De Hoog *et al.*, 2000). The Fusarium pathogen was described as having no sexual morph, but the possible presence of a cryptic sexual cycle was reported (Aoki *et al.*, 2014; Gorden, 2017). The similarity of micro- and macro-conidial morphology and dimensions was considered in the grouping of *Fusarium oxysporum* and numerous varieties and forms (Wollenweber, 1913).

Strains of *Fusarium oxysporum* are known to produce a cocktail of polyketide secondary metabolites with some unknown functions and toxicities (Manici *et al.*, 2017). Some known toxins produced are beauvericin (Lopez-Berges *et al.*, 2013), fusaric acid (Lopez-Diaz *et al.*, 2018) and fumonisins (Rheeder *et al.*, 2002). *Fusarium* spp. has been recognized as a natural source of coloured polyketidic secondary metabolites, among which red naphthoquinone pigments like bikaverin, aurofusarin, fusarubin, bostrycoidin, javanicin, and novarubin, are most abundant followed by hydroxyanthraquinone pigments, but there is no relationship between pathogenicity and the pigments produced (Caro *et al.*, 2017). But, the intensity of colour varied with age and temperature (Honnareddy and Dobey, 2006).

Isolates of *F. solani* causing wilt of patchouli showed moderate growth and maximum mycelial growth within 6-10 days in agar plates. The colony was sparse to dense, greyish white to pinkish in colour. The pathogen produced abundant microconidia, hyaline, cylindrical, with single or two-celled. Macroconidia were 3-4 septate and one-celled hyaline chlamydospores with spherical shapes (Chavan, 2007).

*F. oxysporum* f. sp. *phaseoli* was characterized by Mwangombe *et al.* (2008), which causes Fusarium wilt in common bean. The mycelial texture was either fluffy or fibrous with purple, pink, and white colony colours. The hyphae were highly branched, slender, septate, and produced conidia and chlamydospores. A 0-1 and 3-7 septate microconidia and macroconidia were produced respectively with terminal chlamydospores.

Honnareddy and Dubey (2006) described different isolates of *F. oxysporum* f. sp. *ciceris* causing chickpea Fusarium wilt, the isolates produced normal white to violet, brown, reddish violet, greenish violet, yellowish pink, and dark green coloured pigments in the medium. Mycelium was aerial to fluffy, with tremendous sporulation. Abundant microconidia and macroconidia were produced with terminal or intercalary chlamydospores.

White floccose mycelium with violet pigmentation on PDA, orange sporangia, macroconidia born in sporochia, medium-sized with 3-4 septate, straight, or slightly curved, microconidia produced false heads on short mono phialides, 0-3 septate, oval or reniform shaped, and abundant chlamyospores were observed in *Fusarium oxysporum* f. sp. *vasinfectum* causing Fusarium wilt in cotton (Zhu *et al.*, 2021).

Pothiraj *et al.* (2021) characterized *Fusarium oxysporum* f. sp. *lycopersici* (Fusarium wilt in tomato) as white aerial mycelium with loosely floccose, delicate, purple pigmentation, conidiospores were branched and unbranched mono phialides, microconidia largely single-celled, kidney or oval shaped, produced in false heads, macroconidia were thin-walled and delicate, slightly curved with foot-shaped basal cell, the attenuated apical cell with 4-6 septation, chlamyospores appeared singly or in pairs.

*Fusarium oxysporum* f. sp. *tracheiphilum*, the causal organism of Fusarium wilt in cowpea showed white, pink, violet, or dark coloured pigments on PDA, macroconidia were fusiform, arched with thin extremities and 3-5 septate, microconidia had 0-1 septum, were elliptic, ovoid or reniform in shape, and had intercalary chlamyospores and microconidia were generally more numerous than macroconidia (Metsena *et al.*, 2021).

Characterization of *Fusarium oxysporum* based on distinctive characters of the shapes and sizes of macro- and microconidia, presence, or absence of chlamyospore, colony appearances, pigmentation, and growth rates on agar medium was studied in different species including *F. oxysporum* f. sp. *tracheiphilum* in cowpea (Rodrigues and Menezes, 2005), *Fusarium oxysporum* f. sp. *lycopersici* in tomato (Singha *et al.*, 2016; Adhikari *et al.*, 2020), *Fusarium oxysporum* f. sp. *phaseoli* in common bean (de Carvalho *et al.*, 2022).

### **2.3.2. ITS- Sequence based molecular identification of *Fusarium* spp.**

Species-level detection of the pathogen could be assessed from the characteristic difference in ribosomal DNA (rDNA). The rDNA repeat unit consists of a genic region like a copy of 18S, 5.8S, and 2.8S rDNA and non-genic or spacer regions viz., Internal Transcribed Spacers (ITS) and Inter Genic Spacers (IGS) (O'Donnell, 1992). Highly conserved rDNA genes have been used to analyze major evolutionary events, whereas ITS regions are more variable and used for species-level relationship investigation (Bruns *et al.*, 1991). It has been utilized for fungal species classification due to its systematic and taxonomic usefulness (Chillali *et al.*, 1998).

Molecular methods for pathogen identification are faster but more sensitive than other methods (De Biazio *et al.*, 2008). The use of PCR and DNA sequence analysis of the ITS region has been utilized for the detection, identification, classification, and phylogenetic analysis of many fungi at the species level (Hibbett, 1992; Oliver, 1993; Taylor *et al.*, 2000).

Shahnazi *et al.* (2012) distinguished the isolates of *Fusarium proliferatum* and *Fusarium oxysporum* from ITS sequencing data amplified using ITS1-ITS4 universal primers and concluded that these sequences were more variable and informative for phylogenetic analysis. The pathogens causing Fusarium wilt (*Fusarium* spp.) and anthracnose (*Colletotrichum* spp.) diseases in vegetable cowpea were confirmed through ITS-rDNA sequence analysis. Four *Fusarium* spp. and eight *Colletotrichum* spp. isolated were confirmed to be 90-100% similar to the corresponding pathogen database available in NCBI (Sreeja, 2014).

## **2.4. SCREENING AND SCORING FOR FUSARIUM WILT RESISTANCE**

### **2.4.1. Inoculation methods of *Fusarium* spp.**

The inoculation methods that could produce wilt symptoms in plants were very necessary for disease resistance screening (Junior *et al.*, 2016). Symptoms produced in

the field were not reproducible in inoculation tests, and it takes more time for typical symptoms to appear (Alves *et al.*, 2011). The inoculation method, age of the plant, plant environment, and amount of pathogen has a large impact on disease symptom development (Gabrekiristos *et al.*, 2018).

Jarek *et al.* (2018) inoculated *Fusarium* spp. in peach seedlings through different methods, like, conidial suspension ( $1 \times 10^6$  conidia·mL<sup>-1</sup>) injected into the roots, immersion of roots in the conidial suspension, root wounding, and conidial inoculation, and added ground corn *Fusarium*-colonized medium to the soil at 40g dm<sup>-3</sup>, and concluded that *Fusarium*-colonized ground corn for root inoculation was an effective method and reduced the level of inoculation damage to the plants.

The root tips were trimmed around a one-third portion of the ten-day-old seedlings and immersed in the inoculum suspension for massive penetration of the pathogen directly into the vascular system of the plants allowing faster and stronger symptom development in the root tip method (Eynck *et al.*, 2009). The hydroponic method of inoculation was the more efficient and sensitive approach in determining the virulence of *Fusarium oxysporum* f. sp. *pisi* (*FOP*) in peas than the root tip method, which showed severe *Fusarium* wilt symptoms within 10-13 days after inoculation, and was 8 days shorter than the root tip method (Sakoda *et al.*, 2018).

Wounding and dipping banana roots into the *Foc* conidial suspension ( $1 \times 10^6$  conidia/ml) guaranteed pathogen infection and 100% wilting in the seedlings (Gabrekiristos *et al.*, 2018). The wounding of the seedling roots before inoculation helped in the direct penetration of the pathogen thereby ensuring successful infection up to vascular tissues (Sudarsono, 2004; Sakamoto and Gordon, 2006).

*Fusarium oxysporum* and *Fusarium solani* isolates were inoculated to the pepper seedlings through the root tip method, in which trimmed roots were dipped in the spore suspension ( $1 \times 10^6$  spores ml<sup>-1</sup>) for 30 minutes before transplanting, and the soil infestation method, in which *Fusarium* multiplied in rice-grain was inoculated to

the seedlings by making two holes and it was concluded that both isolates were effective for inducing foliar root rot symptoms when soil infestation was done with the rice-grain method (Guney and Guldur, 2018).

Omoigui *et al.* (2018) inoculated the parents, F<sub>1</sub>, F<sub>2</sub>, and BC<sub>1</sub> progeny of the different crosses in cowpea with *Fusarium oxysporum* f. sp. *tracheiphilum* (*Fot*) using the seed soak method, in which surface sterilized seeds were soaked in spore concentrations of  $2 \times 10^6$  per ml of *Fot* and removed from the inoculum after 6 hours and placed on sterile filter papers.

#### **2.4.2. Mass screening and scoring of genotypes against *Fusarium* spp.**

##### **2.4.2.1. Field screening**

Mass screening in the field or under controlled conditions could be performed with a large amount of genetic material using different parameters, based on whole plant, leaves or xylem observations and root aspects (Sampaio *et al.*, 2020). Naturally and artificially wilt-infected (wilt-sick plots) fields have been used for field screening of the mass population (Bayaa *et al.*, 1998; Pouralibaba *et al.*, 2015).

Madhukumar (2006) inoculated the *Fusarium* pathogen mass multiplied in rice-bran medium at 5g pit<sup>-1</sup> to the furrows in the field for screening of 30-yard-long bean varieties, the inoculum was applied to the field seven days after sowing and the disease intensity was recorded. The disease intensity ranged from 13.83 to 65 per cent. Among the genotypes, two were moderately resistant, 18 were moderately susceptible, and 10 genotypes were susceptible to *Fusarium* wilt. Wilt sick plot (WSP) was used for the screening of thirty-four garden peas with highly susceptible check variety (Arkel) under field conditions and compared the disease reaction (Shubha *et al.*, 2016).

In the field screening techniques, the *Fusarium* wilt symptoms were assessed by calculating the disease incidence or percentage of dead plants (Sharma *et al.*, 2012; Mohammadi *et al.*, 2012; Pouralibaba *et al.*, 2015; Sharma *et al.*, 2019), a scale based

on the percentage of leaves showing symptoms (Navas-Cortes *et al.*, 1998; Landa *et al.*, 2006; Singh *et al.*, 2017) and scale based on the percentage of xylem discolouration (Emberger and Welty, 1983). The severity of Fusarium wilt in cowpea was assessed based on the 0-4 rating scale (Senthilkumar, 2003; Madhukumar, 2006; Sreeja, 2014).

The presence of naturally occurring additional soil pathogens, heterogenization of disease pressure across the field, and involvement of natural factors to a large extent might reduce the efficiency of identifying complete resistance of germplasm against wilt disease under field screening (Bayaa *et al.*, 1998; Infantino *et al.*, 2006; Navas-Cortes *et al.*, 2008). Mixed FW pathotypes, unreliable soil, and crop conditions, a lack of assurance of uniformity in inoculum level, and high experimental error make field screening less accurate and multiple tests need to be conducted to assess the stability of plant response to FW (Sanogo and Zhang, 2016).

#### **2.4.2.2. Controlled screening**

Screening under controlled conditions, including greenhouse and growth chambers, is considered an efficient approach and allows screening out of crop season, clearly all year round (Infantino *et al.*, 2006).

Nair (2006) studied the disease severity in L×T progenies of yardlong bean by seed application of Fusarium multiplied in rice bran medium at 1g/5 seeds, and soil application in pots at 5g/kg soil, and evaluated disease intensity. The plant mortality ranged from 29.49 to 94.48 per cent among L×T progenies, 59.96 to 99.98 per cent among lines, and 60.97 to 75.40 per cent among testers.

Disease incidence or percentage of dead plants (McPhee *et al.*, 2012; Hiremani and Dubey, 2018), percentage of infected leaves (Bani *et al.*, 2012; Risipail *et al.*, 2015), scale based on the percentage of leaves showing symptoms (Bayaa *et al.*, 1995; Fall *et al.*, 2001; Risipail and Rubiales, 2014; Landa *et al.*, 2006), the visual scale at the leaf level (Neumann and Xue, 2003; Arvayo-Ortiz *et al.*, 2012; Merzoug *et al.*, 2014; Pouralibaba *et al.*, 2016; Leitao *et al.*, 2020), the scale for root symptoms (Lebeda *et*

*al.*, 2010), a scale based on percentage xylem discoloration (Fall *et al.*, 2001; Zian *et al.*, 2013; Pottorff *et al.*, 2012) and infrared imaging for plant temperature determination (Rispaill and Rubiales, 2015) are the commonly used methods for assessing wilt disease symptoms under controlled screening.

The extent of *Fusarium* wilt damage in cowpea genotypes was assessed from the phenotypic vascular discoloration measured by uprooting the entire plants and vertical slicing of the stem, and then the damage was evaluated on a 0-5 scale basis (Pottorff *et al.*, 2012; Omoigui *et al.*, 2018).

The detailed screening methods for studying the cellular basis of host *Fusarium oxysporum* interaction were performed by light microscopy (Benhamou and Garand, 2001; Pouralibaba *et al.*, 2017), scanning electron microscopy (Joshi *et al.*, 2012; Pereira *et al.*, 2013), transmission electron microscopy (Bishop and Cooper, 1983), fluorescence microscopy (Ramírez-Suero *et al.*, 2010) and laser confocal microscopy (Nino-Sanchez *et al.*, 2015; Upasani *et al.*, 2016). The detailed 3-dimensional cellular structures were very useful for morpho-functional characterization (Miranda *et al.*, 2015).

The disease severity was greater at the flowering or harvesting stages than at the seedling stage, due to the infection of the vascular tissues and its damage. *Foc* infection in bananas was more severe and showed wilting symptoms at 30 days after inoculation (DAI) than at 10 DAI (Gabrekiristos *et al.*, 2018).

## 2.5. GENETIC RESOURCES, MECHANISM AND INHERITANCE OF GENES CONTRIBUTING TO FUSARIUM WILT RESISTANCE

### 2.5.1. Genetic resources in legumes against *Fusarium* wilt resistance

Genetic resources in legumes that impart *Fusarium* wilt resistance have already been reported and are listed in Table 2.

**Table 2. Genetic resources in the legumes that impart fusarium wilt resistance**

<b>Crop</b>	<b>Genetic resource</b>	<b>Name of the institute</b>	<b>References</b>
Chickpea	ICC 11322 (WR 315), <i>C. bijugum</i> , <i>C. judaicum</i> , <i>C. pinnatifidum</i> , <i>C. reticulatum</i> , <i>C. echinospermum</i> , and <i>C. cuneatum</i> , ICCV2, ICCV3, ICCV4, and ICCV5 (against race I, three lines derived from MABC based C 214 × WR 315 cross, ICCVs 98505, 07105, 07111, 07305, 08113, and 93706 (highly resistant)	ICRISAT, Patancheru	Singh <i>et al.</i> , 1974; Nene and Haware, 1980; Kumar <i>et al.</i> , 1985; Varshney <i>et al.</i> , 2014; Sharma <i>et al.</i> , 2019.
	Digvijay, BG-212, Annigeri	India	Sharma <i>et al.</i> , 2005; Upasani <i>et al.</i> , 2016.
	Super Annigeri and Improved JG74 (resistant against foc4)	ICRISAT, Patancheru ARS, Kalaburagi JNKVV, Jabalpur	Mannur <i>et al.</i> , 2019.
	SCGP-WR 28, H 10-05, GL 10023, IPC 2006-77, and CSJK 72	IARI, New Delhi, India	Dubey <i>et al.</i> , 2017.
	ICC-7520	Iran	Sharma <i>et al.</i> , 2005.
	Surutato-77, Sonora-80, Tubutama, UC-15 and UC-27, Gavilan	Mexico	Morales, 1986; Buddenhagen <i>et al.</i> , 1988; Helms <i>et al.</i> , 1992.
	ICCV 2 and UC 15 FLIP 85-20C, FLIP 85- 29C, and FLIP 85-30C	Hudeiba Research Station, Ed-Damer, Sudan	Ali <i>et al.</i> , 2002.
	FLIP 84-43C (against race 0), ILC-5411, FLIP 85-20C (against race 5), FLIP 85-29C, FLIP 85-30C, ILC-127 (against race 0), ILC-219 (against race 0), ILC-237, ILC-267, and ILC-513 (against race 0)	Santaella, Cordoba, Spain	Jimeinez-Diaz <i>et al.</i> , 1991.

Pigeon pea	ICP 9145, ICPL 20109, ICPL 20096, ICPL 20115, ICPL 20116, ICPL 20102, ICPL 20106, and ICPL 20094	ICRISAT, Patancheru	Reddy <i>et al.</i> , 1995; Sharma <i>et al.</i> , 2016.
	IC0574574	IIPR, Kanpur	Singh <i>et al.</i> , 2011.
Cowpea	CB46, CB3, 7964, 8514, California Blackeye 27, California Blackeye 46, California Blackeye 50 (Fot race 3), CB27, and 524B	University of California, Riverside (UCR)	Roberts <i>et al.</i> , 1995; Ehlers <i>et al.</i> , 2000; Muchero <i>et al.</i> , 2009.
Common bean	HF 465-63-1, RWR 950 and G 685, CAAS260205	Yunnan, China	Pastor-Corrales and Abawi, 1987; Buruchara and Camacho, 2000; Xue <i>et al.</i> , 2015.
Pea	J1412, J11760, P633 ( <i>P. sativum</i> ssp. <i>arvense</i> ), P42( <i>P. sativum</i> ssp. <i>elatius</i> ) against Fop race 2		Bani <i>et al.</i> , 2018.
Lentil	ILL 422, ILL 813, ILL 1220, ILL 1462, ILL 2313, and ILL 2684	ICARDA, Syria	Sarker <i>et al.</i> , 2001.
	ILWL 79 and ILWL 113 of <i>L. culinaris</i> ssp. <i>orientalis</i> ILWL 138 of <i>L. nigricans</i> ssp. <i>Ervoides</i> , 81S15, FLIP2007-42 L and FLIP2009-18 L, BGE016363, BGE025720, BGE032290, and BGE040548	Tel Hadya farm, Northern Syria	Bayaa <i>et al.</i> , 1995; Mohammadi <i>et al.</i> , 2012; Pouralibaba <i>et al.</i> , 2015.
	PL101 and L4076	AICRP, India	Parihar <i>et al.</i> , 2017
Faba bean	Assiut-215, Roomy-3, Marut-2, and Giza-2	Assiut University, Egypt	Mahmoud and Abd El-Fatah, 2020.

### **2.5.2. Cowpea genetic resources for Fusarium wilt resistance**

Considerable genetic variability has been reported in cowpea genotypes in Fusarium wilt response (Roberts *et al.*, 1995). The primary source of Fusarium wilt resistance in cowpea was obtained by growing the susceptible cultivars in high inoculum density field plots or sick plots and identifying the plants surviving the disease infestation. From the field screening, the varieties 'Iron,' 'Victor' and 'Brabham' were selected as possible breeding stocks, and the crosses between these varieties with susceptible varieties yielded resistant progenies (Orton, 1902).

The varieties, Mangolia, Iron PI293520, Iron TVu 990, Iron TVu 1072, and Iron TVu 1611 did not show any wilting or external symptoms of Fusarium wilt from the number of plants tested and were resistant to all races of Fusarium (Armstrong and Armstrong, 1980). The variety Iron (PI293520) was recorded as resistant to all races of pathogen and TVu 347 was resistant to races 1 and 2 (Orton, 1902).

The cross between M455 (a probable derivative of the cultivar 'Iron', which is resistant to all races of Fusarium pathogen) and Brown Sugar Crowder (susceptible to all races), resulted in disease resistance against the pathogen races 1, 2, and 3 (Hare, 1957). Races of the pathogen have been distinguished based on varietal susceptibility, *ie.*, cv 'Iron' was resistant to all races of the pathogen, 'Arlington' was resistant to races 1 and 2 but susceptible to race 3, 'Grace' was resistant to race 2 but killed by race 1, and 'Extra Early Blackeye' susceptible to race 2 but not race 1 (Armstrong and Armstrong, 1980). California Blackeye 5 showed the highest yield reduction due to Fusarium wilt susceptibility (Shihata, and Gad-El-Hak, 1989). Shihata *et al.* (1989) observed that the Fusarium population in the susceptible cultivar (Blackeye) reaches its peak level on the 50<sup>th</sup> day after sowing. There were low disease symptoms and Fusarium populations observed in the resistant cultivar (TVu 1560) and the xylem extracts of this cultivar were more toxic to the pathogen than those obtained from

healthy wilt susceptible (Blackeye) cowpea plants, thus TVu 1560 was the most resistant to all 3 races of *Fusarium* (Shihata, and Gad-El-Hak, 1989).

California Blackeye 46 (CB 46) cowpea and California Blackeye 88 (CB 88) cowpea were developed and released by the University of California in 1987 and 1988 respectively, and are resistant to *Fusarium* wilt. CB 46 was resistant to race 3 and made from the cross between California Blackeye 5 (CB5) X PI166146, while the resistant source PI 166146 was originally collected from India. California Blackeye (CB 88) originated from a mass-selected F<sub>4</sub> family from the cross CB5 X 7977 and was resistant to race 3. The parent 7977 was selected from the breeding line of the cross CB5X PI166146 (Helms *et al.*, 1991).

Other varieties are California Blackeye 27 (CB 27), a single plant selection from line H8-8 with resistance to both races 3 and 4 (Ehlers *et al.*, 2000), California Blackeye 3 (CB 3) (Harris and Ferris, 1991), California Blackeye 50 (CB 50) (Muchero *et al.*, 2009) 7964 and 8514 (Roberts *et al.*, 1995) were also reported as resistance against *Fusarium* wilt.

A high degree of disease susceptibility was observed by Madhukumar (2006) in a field screening of *Fusarium* wilt resistance in thirty-yardlong bean genotypes based on disease intensity percentage. The genotypes Thiruvananthapuram local-1 and Thiruvananthapuram local-3 recorded the highest yield with moderate resistance against *Fusarium* wilt.

*Vigna unguiculata* subsp. *sesquipedalis* varieties, viz., Fei 8, UCR5040, Zhijiang dwarf No. 1, Jiicaidou, Heiziyacao, Fan, Zhuyan long bean and Qiyezi, and *Vigna unguiculata* subsp. *unguiculata* varieties, viz., CB 46 and IT93K\_503\_1, recorded zero disease index based on leaf damage and vascular discolouration, and these were considered immune genotypes in *Vigna* spp. (Wu *et al.*, 2015).

### 2.5.3. Mechanism of host plant resistance against *Fusarium wilt*

Resistance against *Fusarium oxysporum* occurs at different stages of the infection process. As an extracellular defence mechanism, fungal penetration and colonization were prevented or retarded at the root epidermis, cortex, and endodermis regions. In contrast, the vascular resistance mechanism prevents it at the xylem or in the plant stem (Jiménez-Fernández *et al.*, 2013; Bani *et al.*, 2018). The main host plant-resistant mechanism operates among the root tissues, where the *Fo* enters the plant system (Sampaio *et al.*, 2020).

The root exudates are known to cause the germination of *Fusarium* pathogens, so plants in different species or within species tend to produce different metabolite compositions to inhibit or stimulate pathogen germination, as a pre-penetration defence mechanism (Kumar *et al.*, 2016). Phytoalexins were commonly detected as antimicrobial compounds produced by the resistant accessions of legumes (Bani *et al.*, 2018). Other compounds *viz.*, chitinase, glucanases, protease, and lipid transformation proteins were also reported to have inhibitory effects on conidial germination and hyphal growth of *Fo* (Nobrega *et al.*, 2005).

After penetration, almost all the resistant plants efficiently stop the pathogen from reaching the xylem vessels by strengthening the cell wall with lignin deposition, which was observed as a common resistance phenomenon (Stevenson *et al.*, 1997; Upasani *et al.*, 2016; Bani *et al.*, 2018). Increased concentrations of the lignin biosynthetic enzyme, caffeoyl-CoAO-methyltransferase in resistant pea and their upregulation in resistant chickpeas inoculated with *Fo* have been detected through proteomic and transcriptomic analysis (Castillejo *et al.*, 2015; Upasani *et al.*, 2017).

Once the FW pathogen is established in the root system of the recognized host, it leads to the integration of various signal transduction pathways, which are mediated by mitogen-activated protein (MAP) kinase cascades (Widmann *et al.*, 1999). Thus, transducing the signal downstream to the intracellular targets in response to the signal

received from different receptors at the cell surface during host infection (Husaini *et al.*, 2018).

The MAP has an important role in controlling FW pathogenicity and several genes were responsible for host colonization and pathogenicity, including *FWO1* (Inoue *et al.*, 2002), *chitin synthase V*, *DCW1*, *mannose- 6- phosphate isomerase gene*, *FOXG\_11097* (Michielse *et al.*, 2009), secreted in xylem (SIX) protein encoding genes (Thatcher *et al.*, 2016), *SIX1*, *SIX6*, *FTF1* genes (Ninpo-Sainchez *et al.*, 2015) *ClcI* (Canero and Roncero, 2008) and *XlnR* (Calero-Nieto *et al.*, 2007).

The Pathogen Triggered Immunity (PTI) by Pathogen Associated Molecular Patterns (PAMPs) and Effector Triggered Immunity (ETI) by pathogen effectors comprise the two tiers of the plant defence system (Husaini *et al.*, 2018). The first line of plant defence occurs through PTI when PAMPs are recognized by the host *via* receptor proteins, Pathogen Recognition Receptors (PRRs) (Zipfel and Robatzek, 2010; Beck *et al.*, 2012; Lanubile *et al.*, 2015). The plant elicits oxidative burst and ion influx, which transduce the signals to various pathways by reducing the downstream signalling network from MAPK and protein phosphorylation (Boudsocq *et al.*, 2010; Lanubile *et al.*, 2015; Bigeard *et al.*, 2015). Thus, it activates multiple Transcription Factors (TFs) in the host system that induce different defence-responsive genes including Pathogenesis Related (PR) genes, leading to hormone biosynthesis and signalling, protein, and sugar metabolism (Castillejo *et al.*, 2015; Kumar *et al.*, 2016).

If the PTI fails, the second line of defence response handled by ETI, in which the plant defends itself through immune receptors encoded by the Nucleotide Binding Leucine Rich Repeats (NB-LRR) class of R genes, recognizes the effector molecules from the pathogen (Zipfel and Robatzek, 2010; Bigeard *et al.*, 2015; Ma and Ma, 2016). Transcription of R genes imparts plant innate immunity that inhibits pathogen attack (Dodds and Rathjen, 2010). But, the more virulent races of the pathogen could overcome the host-resistant gene and break the resistance. Broad spectrum and durable

host resistance are highly needed (Yin and Qiu, 2019; Li *et al.*, 2020). The Resistant (R) genes *I*, *I-2*, and *I-3* were introgressed from tomato wild species against their corresponding effector genes *Avr 1*(*Six 4*), *Avr 2* (*Six 3*), and *Avr 3* (*Six 1*) under the gene classes LRR-RLP, CC-NB-LRR-RLP and SRLK respectively, and provided complete resistance against *Fol* races (Chitwood-Brown *et al.*, 2021).

The proteins related to plant defence mechanisms, like trypsin and papain inhibitors, glucan hydrolases, and variant vicilins, which could inhibit different phytopathogenic fungi and yeast were identified from cowpea seeds (Gomes *et al.*, 1996; De *et al.*, 2004). The presence of enzymes,  $\beta$ -1,3-glucanases, and chitinases, that could break the cell wall components of the pathogen was identified in several *Fusarium* wilt-resistant genotypes (Houterman *et al.*, 2007) and increased peroxidase activity could act as a barrier to pathogen penetration and proliferation (Vieira *et al.*, 2010). The calmodulin encoding gene, *Csa2G007990* was highly expressed in the FW-inoculated cucumber plants, which bound to the effector peptide protein *Avrblb2*, leading to a hypersensitive response (Dong *et al.*, 2019).

The transgenic banana overexpressed a banana lysine motif-containing receptor-like kinases 1 (MaLYK1) protein, which plays a major role in Tropical Race 4 (TR4) resistance mediated by conserved microbe-associated molecular pattern (MAMP)-activated defence response and had smaller leaf lesions after TR4 treatment than wild type plants (Zhang *et al.*, 2019).

#### **2.5.4. Inheritance of *Fusarium* wilt resistance**

Single major resistant (R) genes were recognized and responded to pathogen avirulence (Avr) genes in the case of *Fusarium* wilt resistance (Lu *et al.*, 2011). Two types of resistance (type A and type B) have been reported in *Brassica oleracea* against *Fusarium* wilt (Blank, 1937). A single dominant gene that is stable at temperatures higher than 24°C contributes to type A resistance, whereas polygenic genes that are

unstable at temperatures above 24°C contribute to type B resistance (Walker, 1953; Sherf and MacNab, 1986).

The action of three independent loci *h1*, *h2*, and *h3* were identified in the resistant reaction against the *Foc1* gene in chickpea (Upadhyaya *et al.*, 1983; Singh *et al.*, 1987) and the involvement of three separate loci for *Foc2* resistance was also reported (Kumar, 1998). Digenic-resistant inheritance for 0 and 2 races of *Foc2* (Tullu *et al.*, 1999; Rubio *et al.*, 2003), monogenic inheritance of *Foc3* and *Foc5* (Tekeoglu *et al.*, 2000; Sharma *et al.*, 2004), monogenic and recessive nature of *Foc4* resistance in WR 315 genotype (Tullu *et al.*, 1999) recessive and digenic action of *Foc4* resistance in Surutato 77 genotype (Tullu *et al.*, 1998) and single gene control against 1A, 2, 3, 4 and 5 races of *Foc* gene (Sharma *et al.*, 2005) have been reported in different studies. Sharma *et al.* (2005) proposed that the complete resistance against four races of *Fo* f. sp. *ciceris* (races 2,3,4, and 5) in chickpeas was governed by single genes that were present in the same linkage group (LG), LG2 (Sharma *et al.*, 2004).

FW (races 1, 2, 5, and 6) resistance in peas was controlled by different genes of a dominant nature (Coyne *et al.*, 2000; Haglund and Kraft, 2001). The *Fop1* resistance was controlled by a single dominant gene (*Fw*) and was subsequently mapped onto Linkage Group III (Grajal-Martin and Muehlbauer, 2002). Single dominant genes against *Fop* (races 1, 5, and 6) resistance have been reported by Bani *et al.* (2012) whereas, race 2 followed a quantitative pattern (McPhee *et al.*, 2012).

Shubha *et al.* (2016) studied the inheritance of *Fop* resistant genes using F<sub>1</sub>, F<sub>2</sub>, B<sub>1</sub> and B<sub>2</sub> progenies of three crosses, Pusa Pragati × GP-55, Pusa Pragati × GP-6, and Arkel × GP-17, and concluded that the resistance was governed by monogenic dominant gene and showed mendelian segregation.

Fusarium wilt (*FOP*) resistance in common bean showed the presence of a single gene (Cross *et al.*, 2000; Fall *et al.*, 2001), oligogene (Salgado *et al.*, 1995;

Batista *et al.*, 2017), and polygenes (Salgado *et al.*, 1995; Cross *et al.*, 2000; Batista *et al.*, 2017). While, QTL for *FOP4* resistance was also reported (Fall *et al.*, 2001).

The inheritance of pigeon pea Fusarium wilt resistance was reported as controlled by two complementary genes (Pathak, 1970), dominant monogenic (Kotresh *et al.*, 2006; Karimi *et al.*, 2010), recessive monogenic (Jain and Reddy, 1995; Odeny *et al.*, 2009) one dominant and one recessive gene with dominant suppressive epistatic (Saxena *et al.*, 2012), and polygenic inheritance (Pal, 1934). Recent studies done with four resistant and four susceptible populations reported that FW resistance was governed by the dominant genes in BDN 2004-1 and BDN 2001-9, two duplicate dominant genes in BWR 133, and two dominant complementary genes in IPA 234 varieties (Singh *et al.*, 2016).

Kamboj *et al.* (1990) reported the involvement of five independently segregating genes for *Fol* resistance in a lentil based on allelic tests of the crosses with three *Fol* resistant lines (L 234, JL 446, and LP 286) and two *Fol* susceptible lines (L 9-12 and JL 641). Dominant monogenic inheritance of *Fol* resistance was proposed by Eujayl *et al.* (1998) in F<sub>2:4</sub> progenies of the cross ILL 5588 (resistant) × L 692-16-1 (susceptible).

The California cowpea cultivars ‘7964’ and ‘CB3’ showed a single dominant resistant gene for both races 2 and 3 and it was noted that the race 3 gene in ‘CB3’ also conferred incompletely, dominant resistance to race 2, while the race 2 gene in ‘7964’ conferred incompletely, dominant resistance to race 3 (Rigert and Foster, 1987). Hare (1957) reported that the resistance genes for each race 1, 2 and 3 were controlled by the dominant gene from the genetic studies conducted using M455 (resistant cultivar) and Brown Sugar Crowder (susceptible cultivar). Whereas, oligogenic resistance was reported for resistance to *Fusarium oxysporum* (Beckman and Roberts, 1995).

Omoigui *et al.* (2018) investigated the inheritance of *Fusarium oxysporum* f.sp. *tracheiphilum* (*Fot*) in cowpea lines by studying the F<sub>1</sub>, F<sub>2</sub>, and backcross populations.

Concluded that the *Fot* rot resistance in cowpea lines was controlled by dominant genes and there is no evidence of a maternal effect in the inheritance of the resistance. The allelism test suggested that the gene in TVu134 was the same in TVu410 and TVu 109-1 for Fusarium wilt resistance.

Inheritance of FW resistance in cucumber is controlled by a single gene (Vakalounakis, 2015), and multiple genes (Liu *et al.*, 2003; Wang, 2005; Dong *et al.*, 2019). A dominant gene (FW1) on chromosome 2B confers resistance to race 1 of the pathogens in strawberries (Pincot *et al.*, 2022).

## 2.6. CROP IMPROVEMENT STRATEGIES FOR FUSARIUM WILT RESISTANCE

It is very hard to eradicate the Fusarium pathogen by employing any diseases management measures due to the ability of the soil-borne pathogen to survive in the soil for several decades in the absence of a host (Rubiales *et al.*, 2015; Panth *et al.*, 2020). In addition, the pathogen can reside at some depth in the soil and can cause disease at low inoculum levels too (Pittaway *et al.*, 1999). The disease incubation period is very long, and there is no practical and effective way of detecting an infected plant until external symptoms are expressed (Hennessy *et al.*, 2005).

Altering the soil pH using lime or nitrogen amendments (Jones and Woltz, 1983), creating long fallow without cultivating any crops for a certain duration of time (Correll and Jones, 2014), crop rotation (Jones *et al.*, 1983; De Corato *et al.*, 2020), soil fumigation using methyl bromide (Cao *et al.*, 2019) and application of chemicals fungicides, are some of the management measures adopted. Although these methods reduced disease incidence, they did not eliminate crop losses from Fusarium (Chitwood-Brown *et al.*, 2021).

It is necessary to develop and exploit a sustainable host plant resistance mechanism, which is the most durable, economical, and eco-friendly strategy to minimize crop loss due to Fusarium wilt and limit the use of hazardous chemical fungicides (Saxena, 2008; Jain *et al.*, 2015). The plant traits that impart Fusarium wilt

resistance need to be considered, and improvement over those traits could generate durable and broad-spectrum resistance in plants (Bohra *et al.*, 2014).

### **2.6.1. Conventional breeding methods**

Host plant resistance is the most effective way to control *Fusarium* wilt (Chitwood-Brown *et al.*, 2021). The basic breeding methods include the identification of resistant sources and the introgression of genomic regions conferring *Fusarium* wilt resistance to elite but susceptible genotypes through a complex crossing selection scheme (Bohn and Tucker, 1939; Gaur *et al.*, 2007). The hybridization involves three-way or multiple crosses with more parents depending on the complexity of breeding goal (Salimath *et al.*, 2007).

Singh *et al.* (2008) back-crossed F<sub>1</sub> (susceptible × resistant genotypes) with the recurrent parent (wilt-susceptible cultivar) up to BC<sub>4</sub> generation and each back-cross F<sub>1</sub> generation was raised under wilt sick plot conditions in safflower against *Fusarium oxysporum* Schlecht f.sp. *carthami* resistance breeding. The bulk breeding method, by which targeted crosses were advanced under disease-free conditions as bulk up to F<sub>4</sub> generation, and single plant progeny selection from F<sub>5</sub> generation onwards under wilt sick-plot conditions were practiced at ICARDA (Rahman *et al.*, 2009). Three breeding lines in chickpea (ICCV 05527, ICCV 05528, and ICCV 96818) with moderate levels of *Fusarium* wilt resistance and stable performance across the environments were identified by Genotype and Genotype × Environment (GGE) biplot analysis (Sharma *et al.*, 2012).

The more information available on the genetic basis of resistance, the more direct approaches could be applied. The back cross method is commonly adapted to introgress the resistant gene into well-adapted but susceptible varieties in cases of resistance controlled by single genes (Gaur *et al.*, 2012; Rubiales *et al.*, 2015). A combination of bulk and pedigree methods was often used for selection among the segregating generation of chickpeas (Gaur *et al.*, 2012).

Recombination breeding, a selection-crossing-selection cycle has been practiced in chickpea, lentil, and red gram by Choudhary *et al.* (2013), for Fusarium wilt resistance by controlled crossing between an agronomically superior genotype with the wilt-resistant donor, followed by pedigree selection. GGE Biplot analysis revealed that IL1(BGM 20211) and IL4(BGM 20212) genotypes in chickpeas performed well in yield and were highly stable across the six environments under Fusarium wilt conditions (Bharadwaj *et al.*, 2022).

Conventional breeding approaches to obtain Fusarium wilt-resistant varieties were time-consuming and not very efficient in the case of complex resistant traits. It demands sufficient genetic variation in the breeding material and greater manpower for hybridization and handling of segregating populations (Yin and Qiu, 2019).

Precision breeding approaches involving molecular innovations have been applied to developing *Fo* resistant varieties to increase the efficiency and speed of breeding programs (Sampaio *et al.*, 2020; Jha *et al.*, 2020).

## **2.6.2. Molecular breeding methods**

### ***2.6.2.1. Resistant loci and molecular markers associated with Fusarium wilt resistance***

Locating or mapping resistant genes against FW races in different crops was facilitated using DNA marker technologies (Tullu *et al.*, 1999; Tekeoglu *et al.*, 2000; Cobos *et al.*, 2005). The *Fw* locus controlling FW in lentil (*Fol*) at 10.8cM was tagged using the RAPD marker, OPK-15<sub>900</sub> (Eujayl *et al.*, 1998). The presence of the *Foc01/foc01* gene on Linkage Group (LG) 5 was confirmed by two flanking markers, OPJ20600 (RAPD) and TR59 (STMS), in the RIL population of chickpea, CA2139 × JG62 (Cobos *et al.*, 2005). Another gene *Foc02/foc02* was reported in the mapping population, CA 2156 × JG 62 (RIL), using two flanking markers, TS47 (STMS) and TA59 (STMS) (Rubio *et al.*, 2003; Halila *et al.*, 2009). The gene encoding chitinase activity which hydrolyzes fungal cell wall components (chitin and β-glucan) has been

used successfully to inhibit phytopathogenic fungi in many crops (Chhikara *et al.*, 2012; Nookaraju and Agrawal, 2012).

Genetic linkage of SSR (Loridon *et al.*, 2005), SCAR (Okubara *et al.*, 2005), TRAP (Kwon *et al.*, 2013), and AFLP (McClendon *et al.*, 2002) markers with Fusarium wilt resistance in pea was identified. SSR and RAPD markers, SSR59-2B and p17m30710 respectively were used for the identification of the *Fw* locus on LG6 in lentil (Hamwieh *et al.*, 2005). The SSR markers TA103 (Varshney *et al.*, 2014), TA110 and H3A12 (Gowda *et al.*, 2009) were identified for confirming the presence of the *Foc1* gene in chickpea.

Anti-apoptosis genes which cause inhibition of cell death and some animal genes associated with apoptosis inhibiting, *B-cell lymphoma-xl (Bcl-xL)*, *Cell death protein-9 (Ced-9)*, and *Bcl-2 3' untranslated region (3' UTR)* exhibited improved resistance to the *Foc* Race 1 in banana (Paul *et al.*, 2011). Tolerance to Tropical Race 4 (TR 4) of the *Foc* pathogen was improved in transgenic banana with a secreted endo-chitinase gene from *Trichoderma harzianum (chit42)* (Hu *et al.*, 2013).

The cucumber *Foc2.1* gene linked SSR marker was reported by Wang (2005) in a genetic interval of 5.98cM and the *Foc4* resistant gene was located between the QTLs SSR17631 and SSR00684 on Chr2 (Zhou *et al.*, 2015). Two TIR-NBS-LRR coding protein genes (*Bra012688* and *Bra012689*) were reported as candidate genes for Fusarium wilt resistance in *B. rapa* (Shimizu *et al.*, 2014).

Pottorff *et al.* (2012) studied the RIL population of cowpea, California Blackeye 27 (resistant) X 24-125B-1 (susceptible), for Fusarium race 3 resistance through SNP marker 1\_1107, which was co-segregating with *Fot3-1*, and concluded that a 3.5cM genomic region on LG1 contributes to *Fot3-1* resistance. Four candidate genes from the genomic region of *Fot3-1* were identified as leucine-rich repeat serine/threonine protein kinases by comparative analysis between cowpea and soybean

(Pottorff *et al.*, 2012). In pea, two minor QTLs on LG3 were reported as resistance against *Fop* race 2 (McPhee *et al.*, 2012).

Three RIL populations IT93K-503-1 X CB46, CB27 X 24-125B-1, and CB27 X IT82E-18/Big Buff, were studied for mapping loci conferring resistance to *Fot* race 4, and two independent loci that confer *Fot* race 4 resistance, *Fot4-1* and *Fot4-2* were identified in cowpea (Pottorff *et al.*, 2014). Comparative analysis between cowpea and soybean reported a role for candidate genes underlying *Fot4-1* and *Fot4-2*, QTLs that code for TIR–NBS–LRR proteins and leucine-rich repeat serine/threonine protein kinases (Pottorff *et al.*, 2014). One major QTL (*GSSR 18-TC14801*) identified by Jingade and Ravikumar (2015) on LG1 for *Foc1* resistance, another major QTL, *FW-Q-APR-2-1* on CaLG02 and two minor QTLs *FW-Q-APR-4-1* and *FW-Q-APR-6-1* on CaLG4 and CaLG6 against *Foc1* and *Foc3* respectively were reported by Garg *et al.* (2018) in chickpea.

A CAPS marker that could be used for screening FW resistance in pea was identified on the LG3, which was 0.9cM distant from the *Fw* locus (Jain *et al.*, 2015). Eleven significant Marker Trait Associations (MTAs) (on LG1, 3, 4, 6, 8, 9,10, and 11) and seven significant MTAs (on LG 3, 6, 10, 11) in GWAS of 96 cowpea genotypes explained 4% phenotypic variation (PV) related to leaf damage traits and 9.7% PV related to vascular discolouration respectively (Wu *et al.*, 2015).

Two SNPs *viz.*, 1\_0691 and 1\_1369 showed proximity to the QTL *Fot3-1* and *Fot4-2* among the significant MTAs identified from cowpea genotypes through GWAS (Wu *et al.*, 2015). Two candidate genes (*LOC101514038* and *LOC101499491*) for the disease resistance in chickpea were identified from 27 annotated genes and fine-mapped the gene *Foc01/foc01* on LG5 within an interval of 2 cM using Near Isogenic Lines (NILs) (Jendoubi *et al.*, 2016). FW resistant candidate genes in red gram were analyzed through SSR (Patil *et al.*, 2017) and SNP (Singh *et al.*, 2016; Saxena *et al.*, 2017) markers.

Omoigui *et al.* (2018) screened 2000 SSR markers in cowpea to identify closely linked markers for *Fot* race 1 and identified that the C13-16 could be used to screen cowpea genotypes for resistance to *Fot*. The candidate gene was responsible for phytoalexin biosynthesis, hypersensitive response, and plant primary metabolism in common bean against the FOP-SP1 race 6 strain of *F. oxysporum* f. sp. *phaseoli* located on Pv04, Pv05, Pv07, and Pv08 chromosomes and was reported using nine significant SNPs among the 162 diverse Portuguese accessions (Leitao *et al.*, 2020).

Two genes encoding antimicrobial peptides, *antimicrobial peptide (Ace-AMPI)* and *ferredoxin-like protein (pflp)* transformed banana plants exhibited increased resistance to *Foc* race 1, with a vascular discolouration index of 10-20%, as compared to non-transformed plants (vascular discolouration index of 96%) (Sunisha *et al.*, 2020). The expression of the gene *HG\_GLEAN\_10011803* was upregulated in FW- infected bottle gourd plants (YD-4), and the presence of the gene was confirmed using the marker BGRSe\_00818 (Li *et al.*, 2021).

Raghu *et al.* (2021) tested 162 genic SSR markers derived from Fusarium wilt resistant genomic loci of chickpea for cross-genera transferability across nine other legumes, in which the highest gene transferability was observed in cowpea (32.14%) followed by rice bean (31.55%). Type of QTL/gene contributing to FW resistance in some of the crops are listed in Table 3.

#### **2.6.2.2. Marker assisted selection for Fusarium wilt resistance**

Marker aided breeding methods impart durable resistance against multiple FW races using efficient QTL pyramiding into new cultivars (Kaiser *et al.*, 1994). Boosting crop improvement to satisfy the future need for nutritious foods, the “5Gs” breeding technique (Genome assembly, Germplasm characterization, Gene function identification, Genomic breeding, and gene editing) has been recently proposed (Varshney *et al.*, 2019). Whereas, rapid strategies for developing disease-resistant cultivars using genome-wide SSR markers for foreground and background selection

**Table 3. Type of QTL/gene contributing Fusarium wilt resistance in legumes**

Crops	Mapping population	QTL/gene	Type of marker and name	References
Chickpea	C-104 × WR-315	Single recessive gene (race 1 and race4)	CS-27700, UBC-170550 (RAPD)	Tullu <i>et al.</i> , 1998.
	<i>C. arietinum</i> × <i>C. reticulatum</i>	races 4 and 5	STMS and a SCAR	Winter <i>et al.</i> , 2000.
	CA2139 × JG62	One gene resistance for Fusarium wilt race 0 ( <i>Foc0</i> )	OPJ20(600) (RAPD) TR59(STMS)	Cobos <i>et al.</i> , 2005.
	C214 × WR315	<i>FW-Q-APR-6-1</i> ( <i>Foc1</i> ) and <i>FW-Q-APR-6-2</i> ( <i>Foc1</i> )	CaM1402 and CaM1101 (STMS)	Sabbavarapu <i>et al.</i> , 2013.
	JG62 × WR315	Wilt 1 (race 1), Wilt 2 (race 1)	TA27-TA59 (STMS)	Patil <i>et al.</i> , 2014.
Cowpea	CB5 × CB3, CB5 × 7964, F1, F2, and BC	One dominant gene (race 2), one dominant gene (race 3)	-	Rigert and Foster, 1987.
	California Blackeye 27 × 24-125B-1	<i>Fot3-1</i>	SNP 1_0860 and 1_1107 1_1484 and 1_09	Pottorff <i>et al.</i> , 2012.
	IT93K-503-1 × CB46	<i>Fot4-1</i> and <i>Fot4-2</i>		Pottorff <i>et al.</i> , 2014.
	A panel of 96 genotypes	17 significant MTAs for Fusarium wilt resistance	SNP 1_0075, 1_1111,1_1147, 1_0251, 1_0895, 1_0691, 1_0897, 1_0298, 1_0410, 1_0857, 1_0981, 1_1369, 1_0691, 1_0330, 1_1062, 1_0629, 1_0318, and 1_1504	Wu <i>et al.</i> , 2015.
	F1, F2, and BC	-	SSR, C13–16	Omoigui <i>et al.</i> , 2018.
Pea	Green arrow × PI179449	<i>Fw</i>	ACG:CAT_222(AFLP)	McClendon <i>et al.</i> , 2002.

	Lifter × Radley and Shawnee × Bohatyr	<i>Fw</i>	THO (CAPS marker)	Jain <i>et al.</i> , 2015.
Lentil	ILL 5588 × L 692-16-1(s)	<i>Fw gene</i>	SSR59-2B, p17m30710	Hamwieh <i>et al.</i> , 2005.
	ILL5588 × L692-16-1(s)	<i>Fw gene</i>	OP-BH800 and OP-DI5500	Eujayl <i>et al.</i> , 1998.
Pigeonpea	GS1xICPL87119	<i>One gene</i>	RAPD (OPM03704 and OPAC11500)	Kotresh <i>et al.</i> , 2006.
	ICPL 20096 × ICPL 332	<i>C.cajan_03203</i> <i>C.cajan_07078</i> <i>C.cajan_07124</i> <i>C. cajan_0296</i>	SNP	Singh <i>et al.</i> , 2016.
	ICPB 2049 × ICPL 99050	qFW1.1,qFW2.1	SNP, S1_2827280-S1_4263752	Saxena <i>et al.</i> , 2017.
	ICPL 20096 × ICPL 332	qFW3.1,qFW4.1	S2_16115010-S2_15580586	
	ICPL 85063 × ICPL 87119	qFW6.1,qFW11.1	S3_18695411-S3_17153283	
qFW11.2,qFW11.3		S4_597553-S4_1108184 S4_597553-S4_1108184 S6_22726005-S6_23553522 S11_37262913- S11_37133265 S11_43777543- S11_37133265 S11_20607023- S11_16809228 S11_4243778-S11_22408748		

for recovery of recurrent parent genome is an environment-independent and precise method (Bharadwaj *et al.*, 2021).

Closely linked markers in the RIL population have been used for the development of Near Isogenic Lines (NILs) in chickpea for Fusarium wilt resistance (Castro *et al.*, 2010). Gametophytic selection for wilt resistance in chickpea was practiced using molecular markers linked to the H1 and H2 loci (Ravikumar *et al.*, 2013).

The FW resistant genomic regions *foc1* and *foc2* were transferred from JG315 to C 214 (Varshney *et al.*, 2014) and from Vijay to Pusa 256 (Pratap *et al.*, 2017) through marker assisted backcrossing. High to moderate resistance against FW (*foc1*) under field conditions was recorded in MABC- bred lines carrying favorable alleles like ICCX-100175-349-2-2, ICCX-100175-382-4-6, and ICCX-100175-389-3-2 in chickpea (Varshney *et al.*, 2014).

The target genomic region conferring FW resistance in chickpea (*foc4*) was transferred from WR 315 to FW-sensitive elite chickpea cultivars, Annigeri 1 and JG 74 through the marker-assisted backcrossing (MABC) scheme. Thus, MABC-derived varieties *viz.*, Super Annigeri 1 and JG 74315-14 of chickpea showed an 8% increase in yield and disease resistance over Annigeri and 35.5 % yield and disease resistance over JG74 respectively (Mannur *et al.*, 2019).

A high yielding Fusarium wilt-resistant chickpea variety, BGM 20211 (Pusa Manav) was developed through gene pyramiding by crossing Pusa 391 (recurrent parent) and WR 315 (donor), using polymorphic foreground markers, TA27, TA96, and GA16 (Bharadwaj *et al.*, 2022). The varieties included early flowering (51 days) and maturing (106 days) lines.

## 2.7. BIOMETRIC AND QUALITY PARAMETERS IN COWPEA

### 2.7.1. Genetic variability

Genetic variability in breeding material is a prerequisite for a successful plant breeding programme. It is the basis for the selection of suitable breeding material for breeders in crop improvement (Sharma *et al.*, 2017). Understanding the level of genetic diversity in the germplasm is essential for effectively conserving and utilising germplasm resources (Huaqiang *et al.*, 2012). Genotypic variability could be efficient for selection depending on heritability, genetic advance, and correlation among the individual traits (Bilgin *et al.*, 2010).

Mofokeng *et al.* (2020) evaluated fifty cowpea accessions for genetic variability in yield and yield related traits and reported that the number of branches per plant, pod number per plant, days to flowering, and number of pods per plant, were significantly different, while the traits, pod weight per plant, hundred seed weight, seed number per pod, seed number per plant and grain yield were non-significant among the accession. Significant GXE interaction was observed in agronomic traits such as the number of branches, number of pods per plant, number of seeds per pod, pod diameter, plant height, pod length, hundred seed weight, grain yield, days to flowering maturity, plant height, grain yield, and number of seeds per pod in cowpea (Martos *et al.*, 2017).

Gerrano *et al.* (2015) studied the genetic variability in twenty-five cowpea (*V. unguiculata* (L.) Walp.) genotypes using sixteen characters and observed that the genotypes used for the study were highly significant for all the traits indicating the high genetic variability among genotypes.

#### 2.7.1.1. Biometric Characters

##### 2.7.1.1.1. Vine length

Significant variability in vine length was observed by Mishra and Dash (2009), in thirty-three varieties of yardlong bean and ranged from 271.67 to 504.40 cm. Plant

height varied from 9.93 cm (IC 198361) to 29.88cm (C-152) in the thirty-five varieties of cowpea studied (Makanur *et al.*, 2013). Variations of morphological characters in cowpea under normal and water stress conditions were assessed by Mafakheri *et al.* (2017) who reported an average height of 45.13 cm and 37.66 cm under normal and water stress situations respectively.

Vine length (cm) was evaluated in twenty-four yardlong bean varieties and recorded in the range of 45.00 to 356.40 cm with an average length of 274.16 cm (Bhagavati *et al.*, 2018). Savithiri *et al.* (2018) studied sixty-two varieties of yardlong bean and reported a vine length of 187.87 cm with a range of 61.27 to 348.43 cm.

Growth attributes were analyzed in seven cowpea genotypes at 15, 25, and 35 DAS, and it was reported that plant height had a highly significant variation (1% level of probability) among the genotypes. The highest plant height recorded in the genotype Long Yard Bean at 15, 25, and 35 DAS was 27.59, 45.20, and 104.69 cm respectively (Kandel *et al.*, 2019).

George (2022) evaluated the mean performance of yardlong bean hybrids along with the parents, and significant variation was observed among the treatments for vine length. The range of vine length in parents varied from 171.34±3.21 cm (VU 53) to 454.00±2.85 cm (Vellayani Jyothika), and the maximum vine length observed in the hybrid Vellayani Jyothika × VU 53 (427.89±2.22 cm) was greater than the minimum vine length in Githika × VU 53 (363.81±3.38 cm). A genetic analysis study by Verma and Deepanshu (2022) with twenty-seven cowpea genotypes showed significant variation in plant height, in which the shortest genotype was BARDWAR 2 (38.53cm) and the tallest was Black Seed Local 6 (64.39 cm).

#### **2.7.1.1.2. Primary branches per plant**

Significant differences in primary branches per plant in yardlong bean genotypes were observed in different studies in which, the range includes 4.63 to 9.55 (Madhavi, 2012), 5.89 to 9.44 (Hossain *et al.*, 2013), 2.91 to 4.74 (mean 3.83) (Rout *et*

*al.*, 2018), 3.93 to 7.23 (mean 5.07) (Bhagavati *et al.*, 2018), and 4 to 7.3 (mean 4) (Savithiri *et al.*, 2018). The number of branches per plant studied in seven genotypes of cowpea at 15, 25 and 35 DAS by Kandel *et al.* (2019) reported significant variation at 15 and 35 DAS, in which more branches were produced by IT04K-227-4 (2 Nos) and IT86F-2062-5 (2 Nos) at 15 DAS and by Gajale bodi (9.53 Nos) at 35 DAS.

Thangam *et al.* (2020) studied variability in vegetable cowpea germplasm from Goa and reported a range of 4.10 to 8.25 numbers of primary branches with a mean of  $5.29 \pm 0.24$  numbers. The genotype VCG4 produced the lowest (4.10) and VCG2 produced the highest (8.25) number of primary branches. Mali *et al.* (2021) evaluated the cowpea genotypes for growth, yield, and yield attributing characters, and recorded maximum primary branches per plant in Arka Garima (7.60) and minimum branches in AKCP- 2 (5.43).

#### **2.7.1.1.3. Number of pods per plant**

Mafakheri *et al.* (2017) assessed genetic diversity in sixty-two cowpea genotypes using morphological characterization and recorded significant variation in pods per plant, which ranged from 37.67 to 55.67 with an average of 45.13 under normal conditions, whereas range from 30.67 to 40.44 (mean 37.66) was recorded under drought stress condition. A range of 73.08 to 179.07 (mean 124.15) pods in a yardlong bean was reported by Bhagavati *et al.* (2018).

Verma *et al.* (2019) studied the genetic variation for green pod yield and its components in the F<sub>1</sub> generation of cowpea genotypes and reported 30 to 60 pods per plant. The pod and seed traits in cowpea crosses produced from five different genotypes in F<sub>1</sub> and F<sub>2</sub> generations were evaluated by Zaki and Radwan (2022) and recorded as the maximum number of pods per plant in Cream 7 (58.90) among the parental genotypes, Dokki 331 × Asian Introduction (71.0) among the F<sub>1</sub> generation and Cream 7 × Asian Introduction (48.2) among the F<sub>2</sub> generation.

#### **2.7.1.1.4. Pod yield per plant**

Mafakheri *et al.* (2017) assessed variation in cowpea genotypes under normal and water-stressed conditions for economic yield. Minimum and maximum economic yield under normal conditions varied from 54.80 g and 193.73 g respectively, whereas under water-stressed conditions the yield varied from 31.50g to 195.24 g.

The study by Rout *et al.* (2018) in thirteen yardlong bean genotypes recorded an average of 242.12 g pod yield per plant. While 1.87 kg pod yield per plant was reported by Bhagavati *et al.* (2018) using twenty-four yardlong bean genotypes in genetic variability studies. Green pod yield per plant ranged from 424.3 to 1512.24 g in different yardlong bean genotypes studied (Savithiri *et al.*, 2018).

Genetic variability in different quantitative traits in cowpea was studied by Verma *et al.* (2019) who recorded green pod yield per plant of 195.418 g, with a range of 128.42 to 256.47 g. Significant variation in yield per plant was observed by Tambitkar *et al.* (2021), from the evaluation of forty-one cowpea genotypes and recorded as 20.89 to 37.54 g yield per plant variation among the genotypes.

George (2022) evaluated yield per plant and yield per plot in yardlong bean hybrids, in which the hybrid Deepika × VU53 showed the highest yield per plant ( $1416.00 \pm 126.09$ g) and yield per plot ( $14.49 \pm 1.14$  kg) and the lowest was by the hybrid Vellayani Jyothika × VU53 ( $1196.67 \pm 39.27$ g and  $11.96 \pm 0.39$ kg).

#### **2.7.1.1.5. Pod weight**

The pod weight varied from 11.4 to 16.53g (mean 14.45) (Rout *et al.*, 2018), and 10.67 to 35.05g (mean 22.56) (Savithiri *et al.*, 2018) in the different yardlong bean varieties studied. The cowpea genotypes evaluated for growth, yield, and yield attributing characters by Mali *et al.* (2021), recorded significant variation in pod weight, which ranged from 9.40 g (AKCP- 8-4) to 3.36 g (Konkan Sadabahar). Zaki and Radwan (2022) reported the highest pod weight of 2.7 g in the F<sub>1</sub> progenies of the

cross between Colossus and Commercial 1, the lowest pod weight of 1.6 g in the F<sub>1</sub> progenies of the cross between Cream 7 and Asian Introduction and the highest pod weight of 3.7 g in Commercial 1 among the parental progenies studied.

#### **2.7.1.1.6. Pod length**

The crop yardlong bean is known for its long pods, which reach more than 50cm on average. Many studies were done for understanding the variability in pod length and the ranges include, 38.94 to 49.22 cm (mean 42.56 cm) (Rout *et al.*, 2018), 23.53 to 64.63 cm (mean 42.15 cm) (Bhagavati *et al.*, 2018), 15.95 to 72.35 cm (average 44.07 cm) (Savithiri *et al.*, 2018) and 20.66 to 54.00 cm (mean 38.18 cm) (George, 2022).

Pod length in grain cowpea varies from vegetable cowpea, many studies were done for evaluating grain cowpea and reported pod length variations, which includes, 13.99 to 19.69 cm (mean 15.71 cm) under normal irrigation, 13.39 to 18.48 cm (mean 15.33 cm) under drought stress conditions (Mafakheri *et al.*, 2017), 14.25 to 32.00 cm (mean 19.94 cm) (Ugale *et al.*, 2020), 6.00 to 27.20 cm (Nkhoma *et al.*, 2020) 10.37 to 18.01 cm (mean 13.23 cm) (Verma and Deepanshu, 2022).

#### **2.7.1.1.7. Number of seeds per pod**

The number of seeds per pod in fourteen yardlong bean genotypes varied from 17.86 to 22.80 with a mean value of 20.23 seeds per pod (Rout *et al.*, 2018). Whereas, Bhagavati *et al.* (2018) reported a range of 13.33 to 17.73 seeds per pod in twenty-four yardlong bean genotypes. The cowpea variety Arka Garima produced a minimum (12.20) number of seeds per pod and VCG13 produced a maximum (17.91) number of seeds per pod from the genetic variability evaluation of twenty-nine cowpea genotypes (Thangam *et al.*, 2020).

Nkhoma *et al.* (2020) assessed the genetic diversity of a hundred cowpea germplasm using nine agronomic traits and observed significant variation among the genotypes in which the number of pods per plant varied from 3.00 to 56.00. Verma and

Deepanshu (2022) recorded variation in the number of seeds per pod in each genotype studied which varied from 7.64 to 14.06 (average 11.25) and significant genetic variability was observed in the trait.

#### **2.7.1.1.8. Crop duration**

Patel *et al.* (2016) evaluated genetic variability in cowpea and recorded that the genotype Pusa Phalguni had a shorter crop duration (78.97 days) and Phule CP 5040 had a longer crop duration (93.28 days). The variability in yardlong bean studied by Asoontha and Abraham (2017) recorded Accession 1 with the maximum growing period (84.33 days) and minimum in Adityapuram local (74.67 days). The crop duration varied from 37.84 to 50.57 days (mean 43.29 days), among the yardlong bean germplasm evaluated (Bhagavati *et al.*, 2018).

#### **2.7.1.1.9. Number of harvests**

Manju (2006) characterized sixty-six accessions of vegetable cowpea and recorded a maximum of eight harvests in VS-52 and VS-59 and a minimum of 2.50 harvests in VS-7 and VS-34.

A study on genetic variability in thirteen yardlong bean genotypes for vegetative growth, flowering, pod, and pod yield parameters by Rout *et al.* (2018), reported a range of 7.33 to 12.66 number of harvests, with an average value of 9.94 number of harvests.

#### **2.7.1.2. Quality Characters**

##### **2.7.1.2.1. Total phenols**

Nair (2006) evaluated L×T progenies of yardlong bean for yield and Fusarium wilt resistance and recorded the range of total phenols as 10.95 mg/100g (Malika) to 11.20 mg/100g (VS-86) among the lines, 10.68 mg/100g (TVM-3) to 11.38 mg/100g

(TVM-1) among the testers and 10.37 mg/100g (P1×TVM-3) to 11.13 mg/100g (Vellayani local×TVM-1, P1×TVM-1 and Malika×TVM-3) among the crosses.

The highest phenol content was recorded in VU 17 (11.36 mg/100g) and the lowest in Vu 15 (8.783 mg/100g) from the evaluation of thirty yardlong bean genotypes (Madhukumar, 2006). Total phenol content ranged from 0.026 to 0.832 g/100 g in one hundred and twenty cowpea genotypes (Padhi *et al.*, 2022).

#### **2.7.1.2.2. Crude fibre content**

The genetic analysis of yield and Fusarium wilt resistance was evaluated in the L×T progenies of the yardlong bean by Nair (2006) and reported maximum fibre content in the line P-1 (2.37%) among lines and TVM-3 (2.03%) among testers, and Varuvila-2 × TVM-3 (2.07%) among crosses. Crude fibre content significantly varied in the yardlong bean genotypes studied by Madhukumar (2006) which ranged from 1.76% (Vu 41) to 2.26% (Vu 29).

Jithesh (2009) evaluated fifty genotypes of yardlong bean for fifteen yield and yield related traits and recorded a range of crude fibre content of pods from 1.97% (G<sub>40</sub>) to 5.26 (G<sub>24</sub>).

#### **2.7.1.2.3. Crude protein content**

Per cent seed protein content varied from 22.64 to 25.92% in sixty diverse genotypes of cowpea germplasm lines (Sharma *et al.*, 2017). Bhagavati *et al.* (2018) reported that protein content in yardlong bean varied from 20.13 to 31.9 mg/100g with a mean value of 27.10 mg/100g.

The crude protein values ranged from 15.42 to 46.9% with an average value of 20.69% in four hundred and fifty RIL populations of cowpea, when evaluated under non-stress conditions, however under water-stressed conditions the values ranged from 12.6 to 28.89% with mean value of 20.34, but the crude protein concentration between both situations was not significantly different (Alidu *et al.*, 2020).

Padhi *et al.* (2022) evaluated one hundred and twenty cowpea germplasm for nutrient mining for nutrient-dense accessions and reported varied amounts of seed protein content in each genotype, which ranged from  $19.35 \pm 0.42$  g/100g (EC 724681) to  $27.89 \pm 1.19$  g/100g (EC 390248).

## **2.7.2. Genetic Parameters**

### ***2.7.2.1. Phenotypic and Genotypic Coefficient of Variation***

Phenotypic and genotypic coefficients of variation are widely used for the assessment of variability (Verma and Deepanshu, 2022). Generally, the phenotypic coefficient of variation was relatively higher than the genotypic coefficient of variation due to the presence of environmental factors controlling the qualitative as well as quantitative characters (Ubi *et al.*, 2001; Nwosu *et al.*, 2013). The phenotypic and genotypic coefficient of variation of different characters in cowpea were studied by many researchers, some of the reports are listed in Table 4.

### ***2.7.2.2. Heritability and Genetic Advance***

The expression of a trait is not only influenced by genetic factors but also by environmental factors, and creates varying relationship patterns, which are highly effective for selection (Magloire, 2005). The selection of elite genotypes from diverse genetic populations could be assessed using measures of heritability (Selvi *et al.*, 2003), and genetic advance measures the amount of progress that could be expected with the selection of characters. High broad sense heritability indicates the predominance of additive gene action in the expression of the traits (Manggoel *et al.*, 2012). So, a single plant selection improves the trait under consideration and these traits could be easily transferred from parents to offspring (Rashwan, 2010). The characters with high heritability coupled with high genetic advance could be improved through direct selection (Tefera *et al.*, 2017). High heritability coupled with high genetic advance was observed by Resmi *et al.* (2004) the characters such as pod yield per plant, pods per kg,

**Table 4. Phenotypic and genotypic coefficient of variation of different characters in cowpea**

Characters	PCV	GCV	References
Vine length	H	H	Eswaran <i>et al.</i> , 2007; Hasan <i>et al.</i> , 2015; Savithiri <i>et al.</i> , 2018; Panchta <i>et al.</i> , 2020; Ugale <i>et al.</i> , 2020; George, 2022.
	H	M	Bhagavati <i>et al.</i> , 2018; Rout <i>et al.</i> , 2018; Thangam <i>et al.</i> , 2020.
	M	M	Rambabu <i>et al.</i> , 2016; Verma <i>et al.</i> , 2019; Haque <i>et al.</i> , 2021.
	M	L	Verma and Deepanshu, 2022.
Number of branches per plant	H	H	Bhagavati <i>et al.</i> , 2018; Khan <i>et al.</i> , 2015; Thangam <i>et al.</i> , 2020; Panchta <i>et al.</i> , 2020; Haque <i>et al.</i> , 2021; Verma and Deepanshu, 2022.
	H	M	Tambitkar <i>et al.</i> , 2021.
	M	M	Eswaran <i>et al.</i> , 2007; Rambabu <i>et al.</i> , 2016; Verma <i>et al.</i> , 2019; Rout <i>et al.</i> , 2018; Savithiri <i>et al.</i> , 2018.
	M	L	George, 2022.
Leaf length	M	M	Eswaran <i>et al.</i> , 2007; Thangam <i>et al.</i> , 2020.
	M	L	Rambabu <i>et al.</i> , 2016.
	L	L	Bhagavati <i>et al.</i> , 2018; Rout <i>et al.</i> , 2018; Savithiri <i>et al.</i> , 2018; George, 2022.
Leaf breadth	M	M	Rambabu <i>et al.</i> , 2016; Thangam <i>et al.</i> , 2020.
	M	L	Bhagavati <i>et al.</i> , 2018; Rout <i>et al.</i> , 2018.

	L	L	George, 2022.
Days to 50% flowering	H	H	Panchta <i>et al.</i> , 2020; Haque <i>et al.</i> , 2021.
	M	M	Rout <i>et al.</i> , 2018; Thangam <i>et al.</i> , 2020; George, 2022.
	L	L	Hasan <i>et al.</i> , 2015; Rambabu <i>et al.</i> , 2016; Bhagavati <i>et al.</i> , 2018; Gupta <i>et al.</i> , 2019; Ugale <i>et al.</i> , 2020; Tambitkar <i>et al.</i> , 2021.
Crop duration	H	H	Panchta <i>et al.</i> , 2020.
	M	M	Nigude <i>et al.</i> , 2004; Savithiri <i>et al.</i> , 2018; Haque <i>et al.</i> , 2021.
	M	L	George, 2022.
	L	L	Hasan <i>et al.</i> , 2015; Rambabu <i>et al.</i> , 2016; Bhagavati <i>et al.</i> , 2018; Rout <i>et al.</i> , 2018; Tambitkar <i>et al.</i> , 2021.
Number of harvests	H	M	Rout <i>et al.</i> , 2018; Thangam <i>et al.</i> , 2020.
	L	L	Ugale <i>et al.</i> , 2020.
Number of pods per cluster	H	H	Gupta <i>et al.</i> , 2019; Verma <i>et al.</i> , 2019.
	M	L	Hasan <i>et al.</i> , 2015.
	L	L	Rambabu <i>et al.</i> , 2016; Bhagavati <i>et al.</i> , 2018; Rout <i>et al.</i> , 2018.
Number of pods per plant	H	H	Vidya, 2000; Selvam <i>et al.</i> , 2000; Suganthi and Murugan, 2008; Jithesh, 2009; Sivakumar, 2012; Kumar <i>et al.</i> , 2013;

			Rout <i>et al.</i> , 2018; Verma <i>et al.</i> , 2019; Gupta <i>et al.</i> , 2019; Thangam <i>et al.</i> , 2020; Haque <i>et al.</i> , 2021; George, 2022.
	M	M	Ugale <i>et al.</i> , 2020; Kusmiyati <i>et al.</i> , 2022.
	L	L	Tambitkar <i>et al.</i> , 2021.
Pod weight	H	H	Jithesh, 2009; Sivakumar, 2012; Kumar <i>et al.</i> , 2013; Savithiri <i>et al.</i> , 2018; Verma <i>et al.</i> , 2019; Gupta <i>et al.</i> , 2019; Thangam <i>et al.</i> , 2020; Haque <i>et al.</i> , 2021; George, 2022.
	M	L	Rout <i>et al.</i> , 2018.
	L	L	Ugale <i>et al.</i> , 2020.
Pod length	H	H	Hazra <i>et al.</i> , 1999; Jithesh, 2009; Sivakumar, 2012; Kumar <i>et al.</i> , 2013; Khan <i>et al.</i> , 2015; Rajput, 2016; Khandait <i>et al.</i> , 2016; Rambabu <i>et al.</i> , 2016; Bhagavati <i>et al.</i> , 2018; Savithiri <i>et al.</i> , 2018; Gupta <i>et al.</i> , 2019; Thangam <i>et al.</i> , 2020; Haque <i>et al.</i> , 2021; Kusmiyati <i>et al.</i> , 2022; George, 2022.
	M	M	Hasan <i>et al.</i> , 2015; Tambitkar <i>et al.</i> , 2021.
	L	L	Rout <i>et al.</i> , 2018.
Pod diameter	H	H	Haque <i>et al.</i> , 2021.
	M	M	Nigude <i>et al.</i> , 2004; Rambabu <i>et al.</i> , 2016; Rout <i>et al.</i> , 2018.
	M	L	Bhagavati <i>et al.</i> , 2018; Ugale <i>et al.</i> , 2020.

	L	L	George, 2022.
Number of seeds per pod	H	H	Srinivas <i>et al.</i> , 2017; Gupta <i>et al.</i> , 2019.
	M	M	Nigude <i>et al.</i> , 2004; Hasan <i>et al.</i> , 2015; Thangam <i>et al.</i> , 2020; Ugale <i>et al.</i> , 2020; Kusmiyati <i>et al.</i> , 2022; George, 2022.
	M	L	Rambabu <i>et al.</i> , 2016; Bhagavati <i>et al.</i> , 2018; Panchta <i>et al.</i> , 2020.
	L	L	Rout <i>et al.</i> , 2018.
Seed length	M	M	Haque <i>et al.</i> , 2021.
Seed breadth	M	M	Haque <i>et al.</i> , 2021.
Weight of 100 seeds	H	H	Jithesh, 2009; Haque <i>et al.</i> , 2021; Bhagavati <i>et al.</i> , 2018; Verma <i>et al.</i> , 2019; George, 2022; Thangam <i>et al.</i> , 2020.
	M	L	Ugale <i>et al.</i> , 2020.
	M	M	Hasan <i>et al.</i> , 2015.
	L	L	Tambitkar <i>et al.</i> , 2021; Kusmiyati <i>et al.</i> , 2022.
Plant germination percentage	L	L	Gupta <i>et al.</i> , 2019.
Pod yield per plant	H	H	Vidya, 2000; Selvam <i>et al.</i> , 2000; Vidya <i>et al.</i> , 2002; Jithesh, 2009; Kumar <i>et al.</i> , 2013; Khanpara <i>et al.</i> , 2015; Rambabu <i>et al.</i> , 2016; Bhagavati <i>et al.</i> , 2018; Rout <i>et al.</i> , 2018; Savithiri <i>et</i>

			<i>al.</i> , 2018; Thangam <i>et al.</i> , 2020; Haque <i>et al.</i> , 2021; George, 2022.
	M	M	Tambitkar <i>et al.</i> , 2021.
	M	L	Ugale <i>et al.</i> , 2020.
Vitamin C	H	H	Rambabu <i>et al.</i> , 2016.
Seed protein content	H	H	Sivakumar, 2012.
	M	M	Savithiri <i>et al.</i> , 2018.
	M	L	Bhagavati <i>et al.</i> , 2018; George, 2022; Tambitkar <i>et al.</i> , 2021.
	L	L	Jithesh, 2009; Rambabu <i>et al.</i> , 2016; Hasan <i>et al.</i> , 2015.
Ascorbic acid	H	H	Bhagavati <i>et al.</i> , 2018.
	M	M	Savithiri <i>et al.</i> , 2018.
Crude fibre	L	L	Manju, 2006; Jithesh, 2009; Savithiri <i>et al.</i> , 2018.
Total Phenols	L	L	Madhukumar, 2006.

PCV- Phenotypic Coefficient of Variation, GCV- Genotypic Coefficient of Variation

H- High; M-Moderate; L-Low

Where, High: >20 per cent, Medium: 10-20 per cent and Low: <10 per cent (Sivasubrahmanian and Menon, 1973)

inflorescence per plant, pod weight, and keeping quality of pods among the twenty-four biometric characters studied in thirty diverse genotypes of yardlong bean.

High heritability was observed for all the traits studied in the cowpea genotypes except the number of branches, which had the lowest value (Gerrano *et al.*, 2015). Nineteen characters were studied in forty yardlong bean varieties by Rambabu *et al.* (2016) reported that pod length, pod girth, plant height, pod yield per plant, terminal leaf breadth, seed protein content, number of clusters per plant and seed number per pod had high heritability coupled with high genetic advance.

The characters, vine length, number of primary branches per plant, terminal leaf breadth, number of clusters per plant, number of pods per plant, pod length, 100 seed weight, pod yield per plant, pod yield per plot, pod yield per hectare and ascorbic acid content were reported as high genetic advance with high heritability in twenty-four yardlong bean genotypes (Bhagavati *et al.*, 2018). Because of the additive gene action of these characters, the simple direct selection improves the yardlong bean performance.

The number of harvests, days to 50% flowering, number of flowers per cluster, and average pod yield per plant, had high heritability and genetic advance from the twenty characters studied in thirteen yardlong bean genotypes (Rout *et al.*, 2018)

Haque *et al.* (2021) evaluated twenty-five yardlong bean varieties for fourteen characters and concluded that all the characters studied were highly heritable, and high heritability with high genetic advance was observed for the traits, the number of pods per plant, plant height, pod length, and seed breadth. These characters could be considered for direct selection for crop improvement. Whereas, pod length, number of pods per plant, number of seeds per pod, and weight of 100 seeds showed high heritability with a low genetic advance in the F<sub>5</sub> population of yardlong bean, which required various breeding methods for crop improvement (Kusmiyati *et al.*, 2022). Verma and Deepanshu (2022) assessed genetic diversity in cowpea genotypes with sixteen characters and recorded high

heritability and genetic advance in the number of primary branches (61.32 and 37.66), 10 pod weight (74.69 and 28.00), and days to germination (84.04 and 57.30), whereas low heritability and genetic advance were observed for the traits plant height (27.35 and 9.20), number of pods per cluster (17.86 and 6.90), percentage of pod setting per cluster (41.26 and 15.17), number of seeds per pod (22.82 and 9.24) and green pod yield per plant (22.19 and 6.66).

Genetic variability studies in an early segregating population of cowpea by Selvi *et al.* (2022) confirmed the high heritability and genetic advance of the characteristics namely, the number of pods per plant and single plant yield and days to first flowering in the cowpea crosses between RC101×Vyjayanthi, RC101×Vellayani Jyothika and ACM 05-02×Ettumanoor local. High heritability and high genetic advance were recorded for protein content in pods by Manju (2006) and Jithesh (2009), whereas high heritability with medium genetic advance was reported by George (2022) in yardlong bean parents and hybrids. Anwar *et al.* (2023) studied heritability and correlation to determine the selection criteria in the F<sub>5</sub> population of yardlong bean and concluded that the number of leaves, number of pods, and weight of 100 seeds had a high broad sense of heritability.

#### **2.7.2.3. Correlation Coefficient Analysis**

The interrelationship between the traits could be identified through correlation analysis (Owusu *et al.*, 2018; Mofokeng *et al.*, 2020). The selection of a trait of interest indirectly improves the associated traits (Gerrano *et al.*, 2015). The green pod yield in yardlong bean under Fusarium wilt condition was directly correlated with days to 50% flowering (0.452), days to first harvest (0.500), pod clusters per plant (0.642), pods per plant (0.770), pod weight (0.482), pod length (0.358), pod breadth (0.379) seeds per pod (0.288) and total phenols (0.355) (Madhukumar, 2006). The soluble protein content ( $r = -0.5995$ ) and total phenol content ( $r = -0.5313$ ) showed a negative correlation with Fusarium wilt incidence (Arzoo *et al.*, 2012). Pod length had a strong positive significant correlation with the number of seeds per pod, 50% flowering, leaf area, leaf area index, and plant height and was negatively correlated with the number of pods per plant, which

might be due to some functional relationship between vegetative and yield traits, and leaf length showed a moderate positive correlation with leaf area and leaf area index and a weak positive correlation with the number of seeds per pod. A highly significant positive correlation was reported between grain yield per plant and leaf width, leaf area, leaf area index, and harvest index in cowpea genotypes (Gerrano *et al.*, 2015).

The total fibre content in cowpea seeds was positively correlated with the number of green pods per cluster (0.2175) and pod diameter (0.2503), whereas it was negatively correlated with days to first flowering (-0.2971), days to 50% flowering (-0.2870), pod length (-0.03111) and number of seeds per pod (-0.4026). While protein content showed a significant positive association with plant height (0.2955), number of nodes on main branches (0.2851), number of clusters per plant (0.2755), number of green pods per plant (0.2881), and pod yield per plant (0.2328) (Jogdhande *et al.*, 2017). So, the direct selection of the characters improves the yield too. A significant negative correlation of days to first flower (-0.513), days to 50% flowering (-0.387) and fibre content (-0.644) with yield per plot were reported by Manisha *et al.* (2018) in the F<sub>5</sub> generation of cowpea.

Walle *et al.* (2018) analyzed cowpea landraces in Ethiopia and revealed a negative and significant correlation of seed yield with days to flowering, days to maturity, and grain filling period, and a positive significant correlation with seed length, seed thickness, seed width, hundred seed weight, number of pods per plant, number of seeds per pod, pod length, peduncle length, number of pods per peduncle and number of racemes per plant. Paghadar *et al.* (2019) concluded a positive and significant correlation between green pod yield per plant and the number of primary branches per plant, plant height, pod length, number of pods per plant, and number of seeds per pod at both genotypic and phenotypic levels. Days to physiological maturity were positively correlated with 100 seed weight due to the long grain filling period and larger seed size (Sharma *et al.*, 2017). A significant positive correlation between pod number per plant and pod weight, seed number per pod, and grain yield was observed by Lazaridi *et al.* (2017) and Mofokeng *et al.* (2020).

A significant negative correlation was observed between days to flowering and the number of branches (Mofokeng *et al.*, 2020).

Plant height at maturity and 100 seed weight correlated positively and it was concluded that improving plant height would directly increase the seed weight due to the large amount of light intercepted during the grain filling period (Owusu *et al.*, 2021). Grain yield had a significant positive correlation with the number of pods per plant, the number of seeds per plant and pod yield, while the number of days to 50% flowering was positively correlated with the number of days to 90% pod maturity and 100 seed weight in 16 advanced breeding lines of cowpea, concluding that early maturity does not always result in small seed size (Owusu *et al.*, 2021). Yield per plant in yardlong bean was directly associated with the number of branches per plant, number of pods per plant, pod length and number of seeds per pod, with a significant negative association between days to flowering and days to fruit set (Haque *et al.*, 2021).

A positive and significant association of yield per plant with harvest index (0.8652) and negative significance with days to maturity (-0.4294) was reported by Snehal *et al.* (2021). The number of primary branches had a significant positive correlation with the number of flowers per cluster, number of clusters per plant, number of green pods per cluster, and number of green pods per plant (Nanda *et al.*, 2022) in the yardlong bean genotypes studied. The substantial relationships between seven nutritional parameters were studied by Padhi *et al.* (2022) in 120 cowpea accessions and reported that a highly significant and negative correlation was observed between starch and protein content ( $r=-0.63$ ), phenol and starch content ( $r=-0.326$ ) and phytic acid and amylose ( $r=-0.226$ ), whereas phytic acid and protein content were positively correlated ( $r= 0.238$ ).

#### **2.7.2.4. Path Coefficient Analysis**

The genotypic correlation between yield and yield components was partitioned into direct and indirect effects and measures the relative importance of the causal factors individually. A positive significant direct effect of days to 50%

flowering (0.075), pods per plant (0.786), pod weight (0.654) and seeds per pod (0.136) and a negative significant direct effect of pod length (-0.117) and total phenols (-0.070) on yardlong bean yield under *Fusarium* wilt conditions with a residual effect of 0.1415 were reported by Madhukumar (2006). Pods per plant recorded a positive direct effect on seed length (0.4368), pod weight (0.2372), vine length (0.1779), pod protein (0.1247), pod girth (0.1091) and the number of harvests (0.1033) and negative direct effect on 100 seed weight (-0.1529) was concluded by Manju (2006) from the characterization of vegetable cowpea with 27 characters. Jithesh (2009) recorded a positive direct effect of pod yield per plant with the number of pods per plant (0.4669), seeds per pod (0.1626), and 100 seed weight (0.1699) in the genetic analysis of yardlong bean.

Manggoel *et al.* (2012) concluded a positive direct effect of the number of peduncles per plant (0.94), flowers per plant (1.40), and 100 seed weight (1.45) on seed yield in cowpea. Meena *et al.* (2015) studied the character association between seed yield and its component traits in cowpea and reported a positive direct effect of days to maturity (1.51), plant height (0.16), pods per plant (0.35), pod length (2.36) and 100 seed weight and negative direct effect of days to 50% flowering (-2.21) and seeds per pod (-1.68) with seed yield. A positive direct effect of the characters, number of branches per plant, number of clusters per plant, number of green pods per plant, pod diameter, 100 seed weight and number of seeds per pod, and, a negative direct effect of the characters, plant height, days for first flowering, number of pods per plant, pod length, average pod weight and fibre content on plant yield were reported by Jogdhande *et al.* (2017) in thirty cowpea genotypes studied.

Manisha *et al.* (2018) revealed that the characters, number of pods per plant (1.525), 100 seed weight (0.319), fibre content (0.295), leaf area (0.285), primary branches per plant (0.193), plant height (0.144), days to first flower (0.131), pod length (0.051), and number of seeds per pod (0.046) had a positive direct effect on yield in the F<sub>5</sub> generation of cowpea evaluated. The characters viz., number of pods per cluster (-0.452), number of clusters per plant (-0.268), average pod weight (-0.226), and days to 50% flowering (-0.189) had a negative direct effect on yield.

A significant direct effect of the number of primary branches per plant (0.308), pod length (0.3827), and number of pods per plant (0.2733) was reported by Paghadar *et al.* (2019) in sixty genotypes of vegetable cowpea.

Days to maturity (0.2508), number of pods per plant (1.6407), number of clusters per plant (0.1915), number of pods per cluster (0.0692), and plant height (0.0919) showed a positive direct effect on seed yield per plant (Snehal *et al.*, 2021). Whereas, the maximum positive and direct effect of plant yield was contributed by pods per plant and seed per pod in yardlong bean (Haque *et al.*, 2021). Nanda *et al.* (2022) investigated correlation and path analysis in 51 yardlong bean genotype using twelve different traits and observed that flowers per cluster (11.844), green pod length (4.3048), days to first picking (2.7420), number of primary branches per plant (1.4122), days to 50 per cent flowering (0.6889) and number of clusters per plant (0.4498) had a direct effect on yield per plant, vine length (-0.0861), and days to first flowering (-2.263) had the highest negative effect on yield.

# ***MATERIALS AND METHODS***

### 3. MATERIALS AND METHODS

The present study entitled “Marker assisted backcrossing for transferring Fusarium wilt (*Fusarium oxysporum* f. sp. *tracheiphilum*) resistance into Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)” was carried out at the Department of Plant Breeding, College of Agriculture (CoA), Vellayani and Integrated Farming System Research Station (IFSRS), Karamana, during the year 2019-2022. The details of materials and methods followed for the study are presented in this chapter.

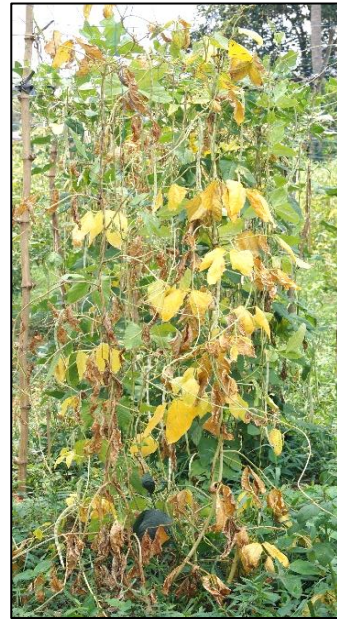
#### 3.1. ISOLATION, IDENTIFICATION, AND MULTIPLICATION OF FUSARIUM WILT CULTURE

##### 3.1.1. Isolation of pathogen associated with Fusarium wilt in yardlong bean

Fusarium wilt disease in yardlong bean plants was identified in the field by its characteristic symptoms (Plate 1). The infected yardlong bean plants (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) were collected from the field, and the stem, as well as roots, were washed in running water to remove the adhered soil particles. The infected collar portion and root bits were cut into small pieces 1-2 mm in size, and surface sterilized with 0.1% mercuric chloride (HgCl<sub>2</sub>) followed by three times washes with sterile water. Five small bits were transferred aseptically into a Petri plate containing solidified Potato Dextrose Agar (PDA) medium amended with antibiotic streptomycin sulfate and tetracycline hydrochloride to minimize any chances of bacterial contamination (Selim and El Zanaty, 2014). The plates were incubated at 28±2°C and observations were made periodically. From the three days old fungal culture plates, a five mm disc of the fungus was cut using a sterilized cork borer and kept at the centre of each sterilized fresh Petri plate containing 15 ml of sterilized and solidified PDA medium. The plates were incubated for 3 days at 28±2°C (Plate 2). The fungus was subcultured using a single hyphal tip method and pure culture was maintained in PDA slants at 4°C (Aneja, 2003).



**A. Foliar yellowing and defoliation**



**B. Yellowing and drying up of plants**



**C. Damage to tap roots and lateral roots**

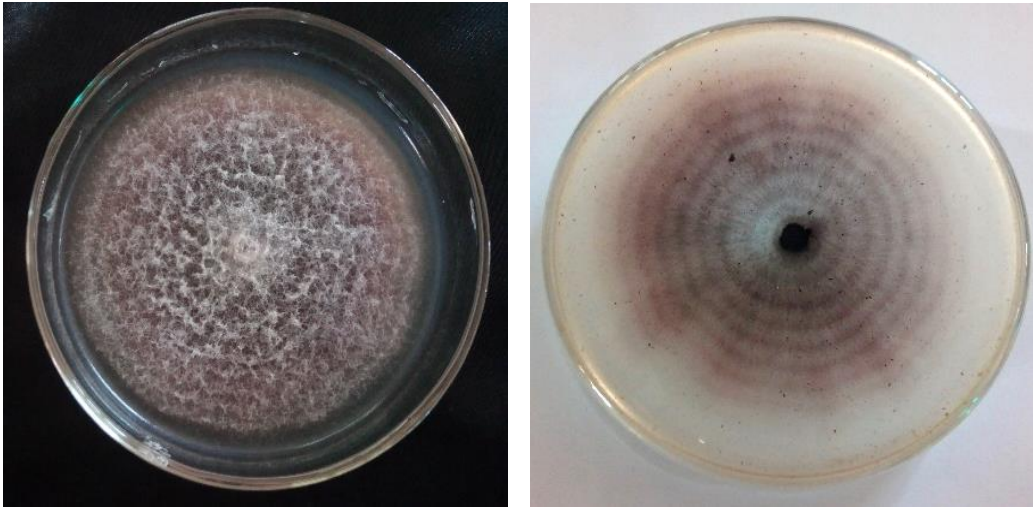


**D. Basal stem swelling and shredding**

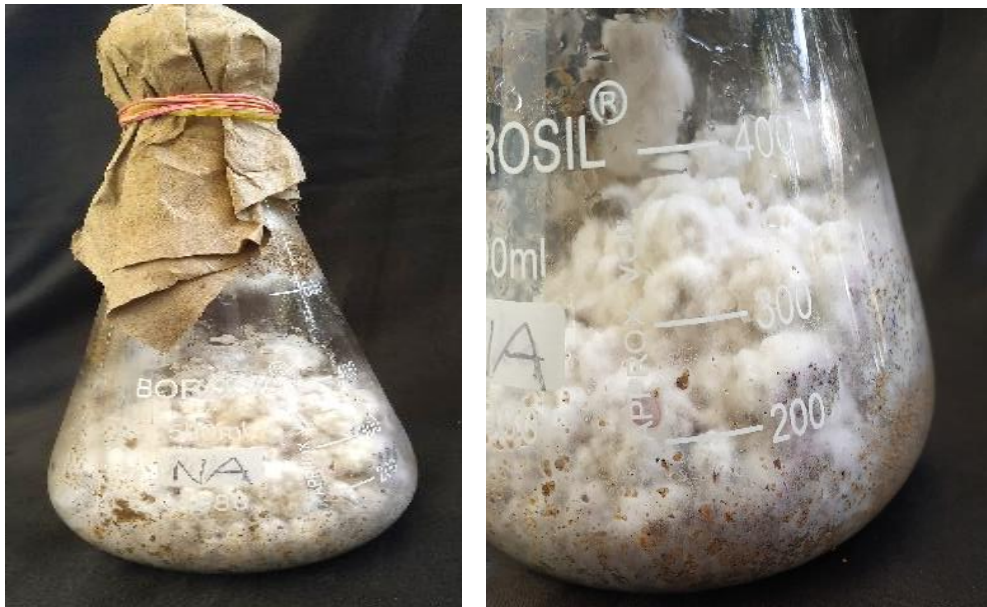


**E. Damage to conducting tissues**

**Plate 1. Symptoms of Fusarium wilt of yardlong bean**



**Plate 2. *Fusarium* spp. multiplied in PDA Medium**



**Plate 3. *Fusarium* spp. mass multiplied in sand: maize medium (9:1)**

### ***Composition of Potato Dextrose Agar (PDA)***

Peeled and sliced potatoes	200g
Dextrose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> )	20g
Agar	20g
Distilled water	1000ml (to make up)

### **3.1.2. Identification and multiplication of the pathogen associated with Fusarium wilt of yardlong bean**

#### ***3.1.2.1. Morphological and culture characterization of Fusarium wilt-associated pathogen***

The mycelial growth, colony characters, and spore specifications were recorded three days after inoculation (DAI) daily. The dimensions of microconidia, macroconidia, and chlamydospores were recorded. The pathogen was characterized under a microscope.

#### ***3.1.2.2. Mass multiplication of Fusarium wilt-associated pathogen***

The isolated Fusarium wilt fungal pathogen was multiplied in a sand-maize medium containing 9 parts of sand and 1 part of maize meal. The medium was prepared by mixing 90g of sterilized riverbed sand and 10g of maize meal and moistened with 20 ml of distilled water in 250 ml of an Erlenmeyer flask. The medium was sterilized at 15 lbs for one hour continuously for 2 days. After cooling, 5 mm culture discs of the fungus were aseptically transferred into the flask and closed airtightly; incubated for 15 days at room temperature (28± 2°C) for mass multiplication (Ashwathi *et al.*, 2017) (Plate 3).

#### ***3.1.2.3. Selection of inoculation method for screening***

Different inoculation methods, including soil inoculation of the fungal culture multiplied in the sand: maize (9:1) media cultured isolates, pin pricking of stem and leaves followed by disc inoculation, and soil inoculation with sand maize (9:1) media cultured isolates along with control containing soil alone were used for

screening. The Inoculation method which could reproduce the disease symptoms was used as the screening method for further studies.

#### ***3.1.2.4. Virulence testing and standardization of pathogen inoculum for screening***

Healthy yardlong bean seedlings (seven days old) were planted in cups filled with artificially inoculated soil (sterile soil containing pathogen multiplied in sand maize medium) of each isolate of the pathogen separately. The isolate which induced wilt symptoms within a shorter period was considered the most virulent isolate and was selected for further studies. The pathogenicity of the virulent isolate was proved by following Koch's postulates.

Standardization of inoculum was done by growing healthy yardlong bean seedlings in different concentrations of pathogen multiplied in sand maize medium (1, 2, 5, 10, 15, 20, and 25%). The lowest percentage of inoculum that showed early symptoms of Fusarium wilt was used for further studies.

#### **3.1.3. Molecular characterization of Fusarium wilt-associated pathogen by partial sequencing of Internal Transcribed Spacer (ITS) region of rDNA**

##### ***3.1.3.1. DNA isolation using NucleoSpin® Plant II Kit (Macherey-Nagel)***

Mycelium (100g) was homogenized in 400 µl of buffer PL1. RNase A solution (10 µl) was added to it and inverted to get mixed up and the homogenate was incubated at 65°C for 10 min. The lysate was centrifuged at 11000 x g for two min. after transferring it to the NucleoSpin filter. The flow-through liquid was collected and the filter was discarded. Buffer PC (450 µl) was added and mixed well, and the solution was then transferred to a NucleoSpin Plant II column, centrifuged for one min. and the flow through liquid was discarded. The buffer PW1 (400 µl) was added to the column, centrifuged at 11000 x g for one min., and flow through liquid was discarded. Then, buffer PW2 (700 µl) was added to the column, centrifuged at 11000 x g for one min., and flow through liquid was discarded. Finally, buffer PW2 (200 µl) was added and centrifuged at 11000 x g for two min. to dry the silica membrane. The column was transferred to a new 1.7 ml tube and

50 µl of the buffer PE was added and incubated at 65°C for 5 min. The column was then centrifuged at 11000 x g for 1 minute to elute the DNA, and DNA was stored at -20°C.

### **3.1.3.2. Agarose Gel Electrophoresis of isolated fungal DNA**

The quality of isolated DNA was checked using agarose gel electrophoresis. One µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer at pH-8.0) was added to 5µl of DNA and loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until the bromophenol dye front migrated to the bottom of the gel. The gels were visualized in a UV trans illuminator (Genei) and the image was captured under UV light using a Gel documentation system (Bio-Rad).

### **3.1.3.3. PCR Analysis with ITS primers**

The internal transcribed spacer (ITS) ribosomal DNA regions were amplified by PCR using universal primer pairs, ITS1-ITS4 (White *et al.*, 1990). The PCR reaction mixture contained 200 ng fungal genomic DNA, 5 µl of 2x Phire Master Mix, 4 µl distilled water, and 0.25 µl of each primer.

Target	Primer name	Direction	Sequence (5'→ 3')
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTTGCGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC

Amplification was done in a Gene Amp-9700 thermal cycler (ABI, USA) as one cycle of denaturation at 98°C for 30 sec, followed by 40 cycles of denaturation at 98° C for 5sec, annealing at 58° C for 10sec, and extension at 72° C for 15sec, and final one cycle of extension at 72° C for 60 sec.

### **3.1.3.4. Agarose Gel Electrophoresis of PCR products**

The PCR products were checked in 2% agarose gel prepared in 0.5 X TBE buffer containing 0.5 µg/ml of ethidium bromide. A 2 µl of 6X gel-loading buffer

was mixed with 8 µl of PCR product and loaded in the electrophoresis unit. The electrophoresis was performed at 75 V power supply with 0.5X TBE as a buffer until bromophenol blue dye has migrated to 3/4<sup>th</sup> of the agarose gel. 2- log DNA ladder (NEB) was used as molecular standards for reference. The gel was visualized in a UV transilluminator (Genei) and the image was captured under UV light using a Gel documentation system (Bio-Rad).

### ***3.1.3.5. Sequencing using BigDye Terminator v3.1***

The sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA). The PCR mix contains,

PCR Product (ExoSAP treated)	10-20 ng
Primer	3.2 pM (either F/R)
Sequencing mix	0.28 µl
5X Reaction buffer	1.86 µl
Sterile distilled water	makeup to 10 µl

The sequencing PCR temperature consisted of 96°C for two min. for 1<sup>st</sup> cycle, followed by 30 cycles of 96°C for 30 sec, 50°C for 40 sec, and 60°C for 4 min. for all the primers used.

### ***3.1.3.6. Sequence analysis and submission of sequences in NCBI***

The sequences were trimmed and assembled, the consensus sequences were obtained using Biological Sequence Alignment Editor and Analysis (BioEdit) software (Hall, 1999). The homology of ITS-rDNA conserved region sequences in the Fusarium wilt-associated pathogen of yardlong bean was identified by performing a similarity search using Basic Local Alignment Search Tool (BLAST) in the National Centre for Biotechnology Information (NCBI) database and the sequences were matched with the existing available database for species confirmation. Based on the sequence similarity, the obtained sequence of the pathogen was deposited in the NCBI database and an accession number was obtained.

### ***3.1.3.7. Phylogenetic analysis of isolated pathogen with the sequences of Fusarium available in the NCBI database***

The nucleotide sequence of the isolated pathogen was compared with the *Fusarium oxysporum* database from the NCBI. Phylogenetic evolutionary relationship analysis was performed using Molecular Evolutionary Genetic Analysis software (MEGA 7) by Neighbour-joining method with 100 bootstraps run.

## **3.2. ARTIFICIAL INOCULATION OF *Fusarium oxysporum* AND SCORING OF DISEASE INCIDENCE IN COWPEA GERMPLASM**

### **3.2.1. Collection of cowpea germplasm**

Cowpea germplasm was collected from the Asian Vegetable Research and Development Centre (AVRDC), Taiwan (30 Nos), National Bureau of Plant Genetic Resources (NBPGR), New Delhi (30 Nos), and Department of Plant Breeding and Genetics, College of Agriculture, Vellayani (45 Nos), along with Kerala Agricultural University (KAU) released varieties (10 Nos). The details of 115 cowpea germplasm collected is given in Table 5.

### **3.2.2. Artificial inoculation and scoring**

The experiment was conducted in a completely randomized design (CRD) with three replications and control without pathogen inoculum. Ten per cent of fungus inoculum multiplied in sand maize medium was mixed with sterilized field soil. 250g of multiplied pathogen inoculum-soil mixture was transferred into the pots, watered, and well maintained for 4 days. Cowpea seedlings at 10 days after sowing (DAS) (4 leaf stage) were transplanted to each pot (Plate 4) and the number of days taken to initiate wilting, disease score (0-4), disease intensity (%), and disease reactions were recorded.

#### ***3.2.2.1. Number of days taken to wilt (days)***

The number of days taken to induce the *Fusarium* wilt disease symptoms in inoculated plants compared to control plants was recorded in days.



a. Fungal culture in the sand: maize medium



b. 50 g of medium



c. Mixed with 500g soil



d. Transplanted three-week-old seedling into the mix

**Plate 4. Inoculation of *Fusarium* culture in 3 week old yardlong bean seedling rhizosphere**

**Table 5. List of cowpea germplasm used for Fusarium wilt screening**

<b>NBPGR Germplasms (30 Nos)</b>	
SI. No.	Name
1.	TCR79
2.	TCR80
3.	TCR81
4.	TCR83
5.	TCR84
6.	TCR85
7.	TCR86
8.	TCR87
9.	TCR88
10.	TCR89
11.	TCR101
12.	TCR104
13.	TCR105
14.	TCR106
15.	TCR107
16.	TCR108
17.	TCR109
18.	TCR110
19.	TCR111
20.	TCR112
21.	TCR113
22.	TCR115
23.	TCR116
24.	TCR117
25.	TCR118
26.	TCR119
27.	TCR122
28.	TCR124
29.	TCR125
30.	TCR126

<b>AVRDC Germplasms (30 Nos)</b>	
SI. No.	Name
31.	VI001920 A-R
32.	VI034386
33.	VI034392
34.	VI036720
35.	VI036721
36.	VI040951
37.	VI041083
38.	VI041680
39.	VI041703-A
40.	VI046192
41.	VI046645-B
42.	VI046648-B
43.	VI046653
44.	VI047617
45.	VI048020
46.	VI048484
47.	VI048488
48.	VI048533
49.	VI050893
50.	VI050896
51.	VI050941
52.	VI055471
53.	VI057906-A
54.	VI057936-B
55.	VI061014
56.	VI061032
57.	VI061064
58.	VI061095
59.	VI062338
60.	VI064556 (IRON)

**Table 5. (Contd.) List of cowpea germplasm used for Fusarium wilt screening**

<b>Local accessions from Dept. of Plant Breeding and Genetics (45 Nos)</b>			
<b>SI. No.</b>	<b>Name</b>	<b>SI. No.</b>	<b>Name</b>
61.	Aryanadu local	83.	Nenmara local V
62.	Ayyanthole local	84.	Nenmara local VI
63.	Cherthala local I	85.	Omallur local I
64.	Cherthala local II	86.	Padavalam payar
65.	Elamadu local II	87.	Palakkad local I
66.	Kadambarakonam local	88.	Palakkad local (meter payar)
67.	Kallicaud local	89.	Peyad local
68.	Kallicaud local II	90.	Puthenpeedikayil local
69.	Kanjikuzhi local	91.	Vellavalli payar
70.	Kochi local	92.	Vlathankara local I
71.	Kollam local I	93.	Vlathankara local II
72.	Kollamcode local	94.	Wayanadu local I
73.	Koovappally local	95.	Wayanadu local II
74.	Kottayam thattathi local	96.	Muttathkonam local
75.	Kumil local	97.	Kottayam local I
76.	Mavelikara local	98.	Kalliyoor local
77.	Nelladu local III	99.	Aranmula local
78.	Nellanadu local	100.	Kilimanur local
79.	Nellanadu local II	101.	Elamadu local I
80.	Nenmara local II	102.	Kulashekharam local I
81.	Nenmara local III	103.	Nilamel local
82.	Nenmara local IV	104.	Kottarakkara local
		105.	Anchal local II

**Table 5. (Contd.) List of cowpea germplasm used for Fusarium wilt screening**

<b>KAU released varieties (10 Nos)</b>			
<b>SI No.</b>	<b>Genotypes</b>	<b>Year</b>	<b>Parentage</b>
1	Malika	1992	Single plant selection from “Trivandrum local”
2	Sharika	1993	Single plant selection from “Valiyavila local”
3	Bhagyalakshmi	2001	Introduction followed by selection from IT-83E-911 (IITA)
4	Lola	2001	Pureline selection from local collection from Kanakkari, Kottayam
5	Vyjyanthi (VS-21-1)	1998	Pureline selection from Perumpadavam local
6	Vellayani Jyothika	2006	Selection from “Sreekaryam local”
7	Githika	2015	Local collection and selection from Vellayani
8	Manjari	2018	Backcross breeding KMV1 X CO6, CO6 donor parent for CABMV resistance
9	Anaswara	2002	Pureline selection from local collection “Elenji”
10	Kanakamony	1977	Pureline selection from “Kunnamkulam local”

#### **3.2.2.2. Disease score (0-4 scale)**

The severity of Fusarium wilt in each genotype was recorded by following the 0-4 rating scale (Plate 5) (Senthilkumar, 2003).

- 0- Healthy plants
- 1- Slight yellowing of leaves
- 2- Yellowing and necrosis, initiation of basal swelling
- 3- Basal swelling, yellowing, and necrosis of leaves
- 4- Basal swelling distortion, yellowing, and necrosis of leaves. Total wilting

#### **3.2.2.3. Disease intensity (%)**

The intensity of disease in each genotype was assessed by using the formula developed by Chattopadhyay and Sen (1996).

$$\text{Disease intensity} = \frac{\text{Sum total of scores}}{\text{Total number of plants assessed}} \times \frac{100}{\text{maximum grade}}$$

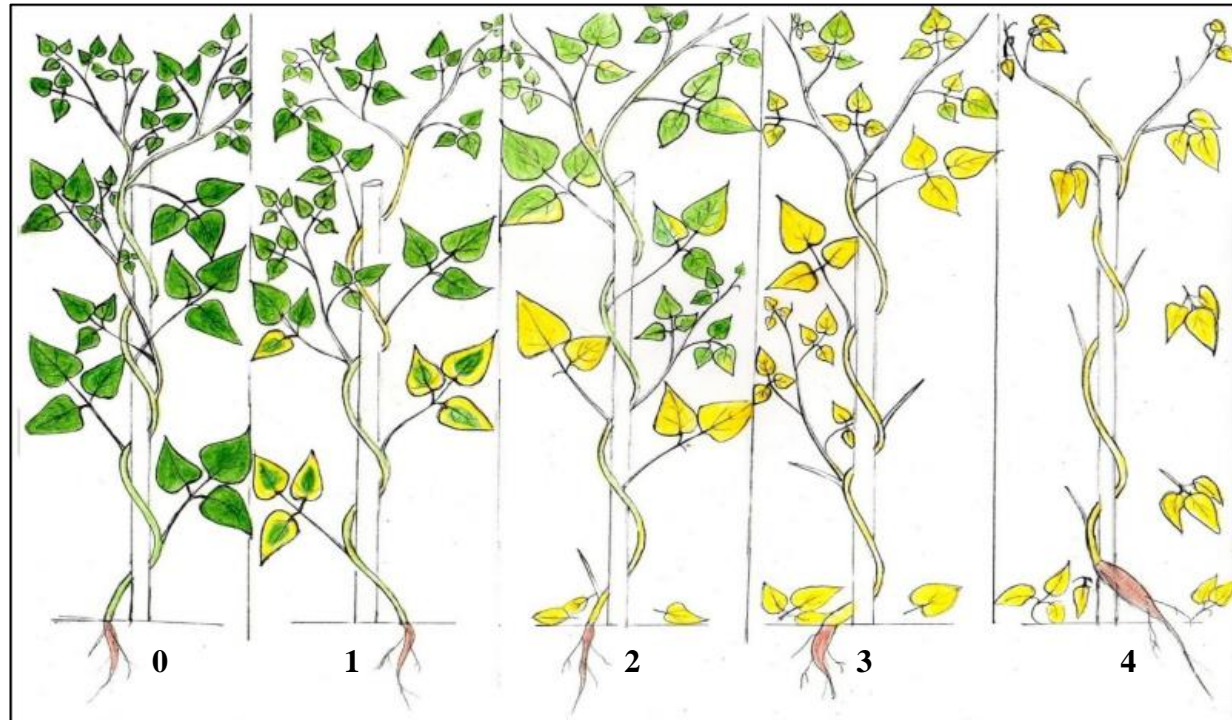
#### **3.2.2.4. Disease reaction**

According to the disease scoring scale, the disease grade was fixed (Popoola *et al.*, 2015) and recorded in each genotype as follows:

- 0- Resistant (0%)-R
- 1- Moderately Resistant (0-25%)-MR
- 2- Moderately Susceptible (26-50%)-MS
- 3- Susceptible (51-75%) -S
- 4- Highly Susceptible (>75%)-HS

### **3.3. EVALUATION OF RESISTANT GENOTYPES FOR YIELD AND QUALITY LINKED WITH FUSARIUM WILT RESISTANCE**

The resistant genotypes obtained from the artificial screening against Fusarium wilt were evaluated in the field for yield and quality parameters (Plate 6).



**Plate 5. Score (0-4) diagram for assessing the severity of Fusarium wilt in cowpea**



**Field preparation**



**Seedlings planted on furrows**



**Field view at 20 days after transplanting**



**Field view at 45 days after transplanting**

**Plate 6. Field evaluation view**

Eighteen genotypes (12 trailing and 6 bush-type cowpeas) along with four susceptible KAU released varieties (3 trailing and 1 bush-type cowpea) were evaluated in the field in Randomized Block Design (RBD) with three replications during the year 2019-2020. Bush and trailing type genotypes were sown in different spacing at 60×45 cm and 1.5×0.45 m respectively and evaluated separately. Each replication was consisting of twenty plants, in which random five plants were considered observation plants, and the following biometric and quality parameters were recorded and tabulated.

### **3.3.1. Biometric Parameters**

#### ***3.3.1.1. Vine length (cm)***

The length of the vine in centimetres was recorded at the time of final harvest from the ground level to the topmost leaf of the plants.

#### ***3.3.1.2. Primary branches per plant***

The number of branches arising from the main stem from all the observation plants at the peak harvest was recorded and the average was calculated.

#### ***3.3.1.3. Pods per plant***

The total number of pods produced per plant till the last harvest was counted from each observation plant and the average was worked out.

#### ***3.3.1.4. Pod yield per plant (g)***

The average weight of all pods harvested from the observation plants was recorded and expressed in grams (g).

#### ***3.3.1.5. Pod weight (g)***

Five pods were selected at random from each observation plant at the peak harvest period. The weight of the pods was taken down, the average was calculated and expressed in grams (g).

#### **3.3.1.6. Pod length (cm)**

Pod length was measured using twine and scale as the distance from the point of pedicel attachment to the apex of the pod and the average worked out and expressed in centimetres (cm).

#### **3.3.1.7. Seeds per pod**

Seeds extracted from the dried pod from the peak harvest were counted and the average was recorded.

#### **3.3.1.8. Crop duration (days)**

The number of days from the date of sowing to the drying of vines of the observation plants was counted and the average was calculated.

#### **3.3.1.9. Number of harvests**

The number of pod harvests in observation plants was recorded and the average was calculated.

### **3.3.2. Quality parameters**

#### **3.3.2.1. Total phenols (mg/100g)**

The total phenol content in the sample was estimated with the Folin-Ciocalteu reagent (Sadasivam and Manickam, 1992).

Reagents:

- i. 80% ethanol
- ii. Folin-Ciocalteu reagent
- iii. 20% Sodium carbonate ( $\text{Na}_2\text{CO}_3$ )
- iv. Standard (100 mg Catechol in 100 ml water), diluted 10 times for working standards

Procedure:

One gram of sample was ground with pestle and mortar in ten times the volume of 80% ethanol and centrifuged the homogenate at 10,000 rpm for 20

minutes for collecting supernatant. Reextracted the residue with five times the volume of 80% ethanol, and centrifuged for supernatant. Evaporated the supernatant to dryness and dissolved the residue in a 5 ml volume of distilled water, pipetted out different aliquots (0.2 to 2 ml) into test tubes, and made the volume in each tube 30 ml with water. 0.5 ml of Folin-Ciocalteu reagent was added and waited for 3 minutes to add 2 ml of 20% Na<sub>2</sub>CO<sub>3</sub> solution to each tube. Then, mixed the tube thoroughly and placed it in boiling water for exactly one minute. Cooled and measured the absorbance at 650nm against a reagent blank. A standard curve was prepared using different concentrations of catechol and the concentration of phenols in the test sample was found, which was expressed in mg/100g.

### **3.3.2.2. Crude fibre content (%)**

The crude fibre content in seeds was estimated by acid and alkali digestion methods (Sadasivam and Manickam, 1992).

Reagent:

- i. 0.128M H<sub>2</sub>SO<sub>4</sub>
- ii. 0.313 N NaOH

Procedure:

Two grams of dried and grounded sample (W<sub>s</sub>) were boiled with 200ml of 0.128M H<sub>2</sub>SO<sub>4</sub> for 30 minutes with pumping chips. It was filtered through muslin cloth and washed with boiling water until washings were no longer acidic. Then, the filtrate was boiled with 200ml of 0.313 N NaOH solution for 30 minutes and again filtered through muslin cloth, washed with boiling water to remove the basic residues. The filtrate was removed and collected on a clean crucible. It was dried in a hot air oven for 2hrs at 130±2°C, cooled in a desiccator and weighed (W<sub>1</sub>). The residue was ignited in a muffle furnace for 2 hours at 600±15°C, cooled in a desiccator and weighed (W<sub>2</sub>). The crude fibre content was calculated by using the following formula and expressed in per cent (%).

$$\text{Crude fibre content (\%)} = \frac{W_1 - W_2}{W_s} \times 100$$

Where,  $W_1$  = Weight of the crucible with fibre,  $W_2$  = Weight of the crucible with ash,  $W_s$  = Weight of the sample

### 3.3.2.3. Crude protein content (%)

Crude protein content was estimated as per the procedure described by AOAC (2000).

Reagents:

- i. 2% boric acid
- ii. 40% NaOH solution
- iii. 0.1 M HCl

Procedure:

The Kjeldahl method described in AOAC (2000) was used to determine the nitrogen content, which involves two major procedures *i.e.*, distillation and titration. For each accession, five millilitres (5ml) of the digested samples were measured and placed into 50ml conical flasks. The purple colour was produced after five millilitres (5 ml) of a 2% boric acid solution were added. The digestion tube was then fixed to the distilling end in the presence of 5 ml of 40% NaOH solution. The colour of the sample was thus changed from purple to light green. After that, the distillate was titrated against 0.1 M HCl till a colour change was observed. For each accession, the titre values were noted. The entire process was repeated using pure water free of ammonia as the blank.

Calculation of percentage nitrogen was done using the formula,

$$\%N = \frac{\text{titre value} \times 0.01 \times 14 \times V \times 100}{1000 \times W \times \text{aliquot pipetted}}$$

where N is nitrogen, V is the extraction volume (100ml) and W is the weight of the powdered sample (0.1g).

Per cent crude protein was calculated by using the formula,

$$\% \text{ Crude Protein} = \%N \times 6.25$$

### 3.4. GENOTYPING OF THE RESISTANT LINES USING SSR MARKER LINKED TO FUSARIUM WILT RESISTANCE

#### 3.4.1. Genomic DNA isolation in cowpea

##### *CTAB Buffer (100 ml)*

CTAB (Hexadecyl trimethyl-ammonium bromide)	2 g
1 M Tris pH 8	10 ml
0.5 M EDTA pH 8 (Ethylenediaminetetra Acetic acid Di-sodium salt)	4 ml
5 M NaCl	28 ml
H <sub>2</sub> O	40 ml
PVP 40 (polyvinyl pyrrolidone (vinylpyrrolidone homopolymer)	1 g
β- Mercaptoethanol	2 ml

(Adjust all to pH 5.0 with HCl and make up to 100 ml with H<sub>2</sub>O)

Genomic DNA was isolated using the CTAB method (Doyle and Doyle, 1990). 200mg of cowpea plant tissue was ground with autoclaved pestle and mortar to fine paste in approximately 500µl of pre-warmed CTAB buffer. Transferred the mixture to a microcentrifuge tube and incubated for 1 hour at 60°C in a water bath with continuous shaking every 10 minutes. After incubation, centrifuged the mix at 12000 rpm for 10 minutes to spin down the cell debris and the supernatant was transferred into clean microcentrifuge tubes. An equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added to each tube, mixed with the solution, and centrifuged at 12000 rpm for 10 minutes. After that, the supernatant was collected to clean microcentrifuge tubes and an equal volume of Chloroform: Isoamyl alcohol (24:1) was added, mixed with the solution, and centrifuged at 12000 rpm for 10 minutes. Again, the supernatant was collected to a new microcentrifuge tube, an equal volume of ice-cold isopropanol was added, inverted the tube was slowly several times to precipitate the DNA. The tube was placed at -20°C for overnight for the alcohol precipitation of DNA. After that, centrifuged the sample at 12000rpm for 10 minutes, decanted the supernatant without disturbing the pellet, and subsequently washed with 200 µl of ice-cold 70% ethanol twice.

Decanted the ethanol, and dried the pellet long enough to remove excess alcohol, but without completely drying the DNA. Dissolved the DNA pellet in 80  $\mu$ l of TE buffer (10mM Tris, PH 8, 1mM EDTA) and added 5  $\mu$ l of RNase A solution and incubated at 37°C for 1 hour. Stored at -20°C after dissolving the DNA into TE buffer.

### **3.4.2. Agarose Gel Electrophoresis**

#### ***Stock solution***

Tris base	240 g
Acetic acid	57.1 ml
0.5 M EDTA (pH=8.0)	186.12 g
The final volume (Distilled H <sub>2</sub> O)	1000 ml

#### ***6X loading dye***

Sucrose	4.0 g
Bromophenol blue	0.025 g
Volume (Distilled H <sub>2</sub> O)	10 ml

(Loading dye solution was stored at 4°C)

Agarose gel electrophoresis was carried out to resolve the genomic DNA in a horizontal gel electrophoresis Unit of BIO-SYS. Agarose (0.8g) was weighed and melted in 0.5X TBE buffer. After cooling the solution at 42-45°C, ethidium bromide was added at the rate of 3  $\mu$ l for 100 ml. The solution was then poured onto a preset, gel casting tray with a comb fixed in position, to a height of 3-5 mm. The gel was allowed to solidify for 15-20 min. After removing the comb, the casting tray was submerged in an electrophoresis tank filled with 0.5X TBE buffer ensuring that the buffer covered the gel to the height of 1mm. The required amount of DNA sample and loading dye (glycerol 30% + bromophenol blue) were mixed in the ratio 5:1 and loaded the slots of gel using a micropipette near the negative terminal. The negative terminal (cathode) and positive terminal (anode) of the electrophoresis unit were attached to the power supply and set a voltage of 60V to run the gel. The

power was turned off when the loading dye reaches about 3/4th of the gel. The gel was visualized under UV light using the SYNGENE gel documentation system in order to check the intactness and shearing of DNA and RNA contamination.

### **3.4.3. Quantitative and qualitative analysis of genomic DNA**

DNA quantification was done using spectrophotometric (Systronics) measurement of UV absorption at wavelengths of 260 and 280 nm. The TE buffer in which the DNA was already dissolved was taken in a cuvette to calibrate the spectrophotometer at 260 and 280 nm wavelengths. The optical density of the DNA samples dissolved in TE buffer was recorded both at 260 and 280 nm wavelengths. The quality of DNA could be judged from the ratio of the O. D. values recorded at 260 and 280 nm. A ratio between 1.8 and 2 indicates good quality. The quantity of DNA in the sample was estimated by using the following formula:

$$\text{The concentration of DNA (ng/}\mu\text{l)} = A_{260} \times 50 \times \text{dilution factor}$$

### **3.4.4. Amplification of genomic DNA by using SSR markers**

The SSR markers, specific to Fusarium wilt resistance genes were selected for the study (Table 6). The reaction was executed in a 25  $\mu\text{l}$  reaction mixture consisting of 20ng template DNA, 2  $\mu\text{l}$  of dNTP mix, 2.5  $\mu\text{l}$  of PCR buffer, 2.5  $\mu\text{l}$  of  $\text{MgCl}_2$  and 0.3  $\mu\text{l}$  Taq DNA polymerase and 1  $\mu\text{l}$  of both forward and reverse primers. Amplification compassed in an Eppendorf master cycler nexus PCR.

Following PCR conditions were used: An initial denaturation at 95° C for 5 minutes followed by 35 cycles of denaturation at 94 ° C for 30 seconds, the annealing temperature of 55 ° C for 30 seconds, primer extension at 72 ° for 1 minute, and final extension 5 at 72°C for 8 minutes.

### **3.4.5. Electrophoresis and visualization of amplified products**

The amplified products were separated on 3% agarose gels, visualized by staining with ethidium bromide and viewed under UV light.

**Table 6. List of SSR markers linked to Fusarium wilt resistance used for the study**

SI No	Marker name	Forward/reverse primers (5'-3')	SSR motifs	Expected product size (bp)	References
1	C13-16	GTCAAAGCAATGGACTAA/ TGAATTTGATACACACACTACT	-	180	Omoigui <i>et al.</i> , 2018 (Cowpea)
2	UASBC34	AGGGAACAACACCCAGATAGTC/ TTAAGAAGGGTTGGGCTCAC	(AT)21	398	Raghu <i>et al.</i> , 2021 (Chickpea)
3	UASBC36	GCTCCAAAAGATTCACAAAAGG/ AGCTGCACTTAATGGAAATGGT	(AAT)12	387	
4	UASBC43	GCTTGTGGAGTGATGGTTTTAT/ AGAGTTGAATGAGAGAGAACGG	(TA)6,(A)10, (AT)7	335	
5	UASBC51	CAAGCTCTGGTAAGCATAAGCA/ CTTTACTTCTACACTCAACGGATCA	(TA)10	369	
6	UASBC52	ACGCTCTTGTCTTTTGTCACTT/ AGTAGTCATCCTCCGGTAATCA	(T)11	389	
7	UASBC59	TCAGCCACACCATTCTAATCAC/ TAAAGTCACCAACCAAAACCCT	(TC)1	190	
8	UASBC60	TTACTTTTACGAGGCGACTTCC/ ACAATTCGAGCACACAAATCAC	(GA)16	384	
9	UASBC79	TTTTCTGAGGCAACATGCTCTT/ CCGAATGACAAGATTCCACACT	(TA)19	305	
10	UASBC70	CCAAACTCTCCTCCCTACCTCT/ AGACATTCCATCTCCCAAGAAA	(TCA)10	386	
11	UASBC83	ATGTGTGTTGTATCCATTGCGT/ GATTGATGCCTAAAATGCAGGT	ATCT)22	355	
12	UASBC90	GTGTCATTGGCTTTGAGATTGT/ AACAAACAGCAGGTTTCGTCCTAT	(TC)7	264	
13	UASBC114	ATTCGGATAGGTTGGTTTGAGT/ CTTTGTTTCATTTAGTCCTCGG	(TA)17	234	
14	UASBC131	CTCTTTTGCATGAATTGGAGC/ GCCACACACAGAACTCAACTTT	(ATA)8	297	
15	UASBC149	AGAGGCAATAATGGGTGTGTTT/ TGGCCTGACCTAGAAGCATATT	(AT)11	250	
16	UASBC170	ACCGTCCAATAAAGAATGTGCT/ GAAGTGAAAGCCGAATCAAAG	(AT)16	397	

**Protocol:**

1. The gel tray was set by taping the open ends and placed on a level surface.
2. Agarose (3%) gel was prepared in 0.5X TBE buffer and cooled and added ethidium bromide at 0.3 µg/ml. The agarose solution was poured into the gel tray with the comb in place, avoiding air bubbles, and allowed to set for 20 minutes.
3. After removing the comb, the gel was placed in the electrophoresis tank containing 0.5X TBE buffer till the gel fully emerged.
4. 10 µl of PCR sample mixed with 2 µl of loading dye (glycerol 30% + bromophenol blue) were transferred into the wells and a 100bp DNA ladder was used to assess the size of the PCR product. The negative terminal (cathode) and positive terminal (anode) of the electrophoresis unit were attached to the power supply and set a voltage of 60V to run the gel.
5. The run was stopped when the bromophenol blue dye reached almost 2/3<sup>rd</sup> of the length of the gel.
6. The gel was visualized under UV light using the SYNGENE gel documentation system and photographed.

### 3.5. HYBRIDISATION OF RESISTANT GENOTYPE WITH SUPERIOR BUT SUSCEPTIBLE VARIETY RELEASED FROM KAU FOR DEVELOPING F<sub>1</sub>.

#### 3.5.1. Selfing and crossing techniques

The anthesis will occur from 6 to 8 a.m. in cowpea. The well-developed cowpea flower buds which are expected to open the next day morning (20 hours before flower opening) were selected and emasculated on the previous evening. The appearance of the corolla just above the calyx is the perfect stage for emasculation and is also recognized by the large size and yellowish colour of the back of the standard petal. Emasculation was done by removing the stamens of the selected bud with a pair of forceps by gently pushing the keel petals apart and bagging using butter paper covers. Cut the bud in the middle, about two-thirds of the way down from its straight edge during evening hours using tiny, highly pointed forceps,

dissecting scissors, scalpels, or even long thumbs and a portion of the style, stigma, and stamens become free and visible. On the next day morning (between 6 and 8 a.m.) emasculated flower buds were pollinated by the male parent. The ripe anthers were collected and pressed against the stigma of the emasculated flower. Only the disc-shaped stigma at the tip of the style is receptive, not the hairy portion beneath. The pollinated buds were again bagged with butter cover and labelled. Fertilized flowers remain in the stalk after 24 hrs, indicating pollination's success. Then the butter paper cover was removed and the crossing was labelled (Myers, 1996). The crossed pods were harvested separately on maturity and seeds were collected (Plate 7). Selfing by bagging the flower buds of each generation was also done, properly tagged and seeds were collected on maturity (Plate 8).

The percentage of pod set was calculated by the total number of flowers pollinated and the number of pods set for the cross and expressed in percentage.

$$\text{Percentage pod set} = \frac{\text{Number of pod set}}{\text{Number of flowers pollinated}} \times 100$$

### 3.6. RAISING AND MORPHOLOGICAL EVALUATION OF F<sub>1</sub> AND THE PARENTS

The F<sub>1</sub> plants and parents were evaluated morphologically and the following biometric parameters were recorded (Plate 9).

#### 3.6.1. Vine length (cm)

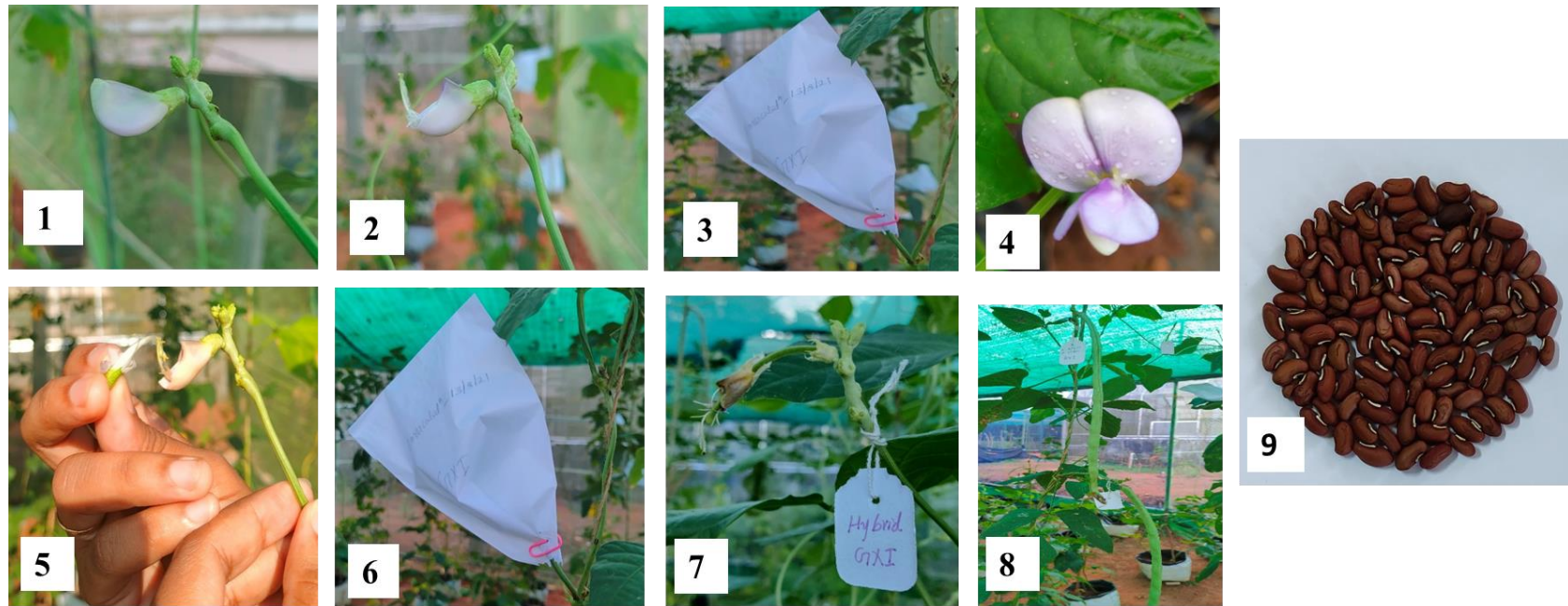
The vine length was observed as per 3.3.1.1. and the mean value was calculated.

#### 3.6.2. Primary branches per plant

Primary branches per plant were recorded as per 3.3.1.2. and the average was calculated.

#### 3.6.3. Pod weight (g)

Pod weight in grams was recorded as per 3.3.1.5. and the mean value was recorded.



**Plate 7. Hybridization steps to produce F<sub>1</sub>s, (1) Unopened flower of Githika (Recipient Parent), (2) Emasculation in the evening, before flower opening, (3) Bagging of emasculated flower to avoid pollen contamination, (4) Selection of opened flower of Iron (Donor parent) the next morning, (5) Pollen dusting from donor flower to emasculated recipient flower, (6) Bagging after pollination, (7) Labelling of hybrid, (8) Hybrid pods, and (9) Seed extraction.**



**Plate 8. Hybridization block for producing F<sub>1</sub> plants**



**Plate 9. Evaluation of the F<sub>1</sub> population and the recurrent parent**

#### **3.6.4. Pod length (cm)**

The pod length was calculated as per 3.3.1.6. and the average was recorded.

#### **3.6.5. Seeds per pod**

The number of seeds per pod was recorded as per 3.3.1.7. and the mean value calculated

#### **3.6.6. Crop duration (days)**

Crop duration was tabulated as per 3.3.1.8. and the average value was calculated.

### **3.7. BACKCROSSING OF F<sub>1</sub> FOR THE PRODUCTION OF BC<sub>1</sub>F<sub>1</sub> LINES.**

The crossing was done as per 3.5.1. and the seeds were harvested. Here, the F<sub>1</sub> plants were considered as the female parent and the pollen from the recurrent parent. The seeds from the female parent were extracted and used for further studies.

### **3.8. EVALUATION OF BC<sub>1</sub>F<sub>1</sub> LINES AT THE SEEDLING STAGE THROUGH ARTIFICIAL SCREENING AND SCORING**

The BC<sub>1</sub>F<sub>1</sub> lines were evaluated at the seedling stage for Fusarium wilt resistance through artificial screening using pathogen multiplied in sand maize media (9:1) (10%) and scored 0-4 disease rating scale basis (Senthilkumar, 2003). The screening and disease scoring was done as per 3.2.2. The resistant BC<sub>1</sub>F<sub>1</sub> lines were advanced to the next generation of backcrossing (Plate 10).

### **3.9. MORPHOLOGICAL AND QUALITY EVALUATION OF RESISTANT BC<sub>1</sub>F<sub>1</sub> LINES AND THE RECURRENT PARENT**

The resistant BC<sub>1</sub>F<sub>1</sub> lines were evaluated for morphological and quality parameters. The readings from each plant were recorded and analysed. The six morphological characters were recorded as per 3.6 and three quality parameters were recorded as per 3.3.2. The frequency distribution, kurtosis and skewness of the characters were studied.



**Plate 10. Artificial screening of Fusarium wilt in BC<sub>1</sub>F<sub>1</sub> lines**



**Plate 11. Artificial screening of Fusarium wilt in BC<sub>2</sub>F<sub>1</sub> lines**

### 3.10. CONFIRMATION OF GENE FOR RESISTANCE IN BC<sub>1</sub>F<sub>1</sub> LINES USING SSR MARKER LINKED TO FUSARIUM WILT

The gene for resistance in BC<sub>1</sub>F<sub>1</sub> resistant lines was confirmed using an SSR marker linked to Fusarium wilt. The same procedure as per 3.4 was followed.

### 3.11. EVALUATION OF BC<sub>2</sub>F<sub>1</sub> LINES AT THE SEEDLING STAGE THROUGH ARTIFICIAL SCREENING AND SCORING

The resistant BC<sub>1</sub>F<sub>1</sub> lines were backcrossed with the recurrent parent to produce BC<sub>2</sub>F<sub>1</sub> lines. Artificial screening and scoring to Fusarium wilt resistance in BC<sub>2</sub>F<sub>1</sub> lines were done as per 3.2.2. (Plate 11).

### 3.12. STATISTICAL ANALYSIS

#### 3.12.1. Analysis of Variance (ANOVA) for CRD

The disease intensity (%) observation was transformed using arc sine transformation method and ANOVA for CRD was calculated.

Sources of variation	d.f	Sum of squares	Mean squares	F ratio
Replications	t-1	TrSS	$TrMS = \frac{TrSS}{t-1}$	$\frac{TrMS}{EMS}$
Error	n-t	ESS	$EMS = \frac{ESS}{n-t}$	
Total	n-1	TSS		

Where, n= The total number of observations, t= number of treatments, TrSS= sum of squares for treatments, ESS= sum of squares for error,

$$\text{Critical Difference, } CD = t_{\alpha} \sqrt{EMS \left( \frac{1}{r_i} + \frac{1}{r_j} \right)}$$

Where,  $t_{\alpha}$  students' t table value distribution at error d.f with the level of significance  $\alpha$  (5% or 1%).

### 3.12.2. Kruskal Wallis test, one-way ANOVA on ranks

The observation of disease score was subjected to nonparametric equivalent one-way ANOVA, Kruskal-Wallis test. The significant difference between values identified according to Duncan's multiple range test at  $\alpha=0.05$ .

### 3.12.3. Analysis of Variance (ANOVA) for RBD

Per replication mean value of biometric traits in each genotypes is used to work out the Analysis of Variance (Panse and Sukhatme, 1967).

Sources of variation	d.f	Sum of squares	Mean squares	F ratio
Replications	t-1	SSR	$MSR = \frac{SSR}{t-1}$	$\frac{MSR}{MSE}$
Treatment	r-1	SST	$MST = \frac{SST}{r-1}$	$\frac{MST}{MSE}$
Error	(t-1)(r-1)	SSE	$MSE = \frac{SSE}{(t-1)(r-1)}$	
Total	rt-1	TSS		

Where, r= number of replications, t= number of treatments, SSR= sum of squares for replication, SST= sum of squares for treatments, SSE= sum of squares for error,

$$\text{Critical Difference, } CD = t_{\alpha} \sqrt{\frac{2MSE}{r}}$$

Where,  $t_{\alpha}$  students't table value distribution at error d.f with level of significance  $\alpha$  (5% or 1%).

### 3.12.4. Estimation of Genetic Parameters

#### 3.12.4.1. Genetic Components of Variance

Phenotypic and genotypic components of variance were estimated for each character by equating expected value of mean squares (MS) to the respective variance components (Jain, 1982).

- i. Genotypic Variance ( $V_G$ ),  $V_G = \frac{MST - MSE}{r}$
- ii. Environmental Variance ( $V_E$ ),  $V_E = MSE$
- iii. Phenotypic Variance ( $V_P$ ),  $V_P = V_G + V_E$

#### 3.12.4.2. Coefficient of Variation

Genotypic, Phenotypic and Environmental Coefficient of Variation were estimated from  $V_P$ ,  $V_G$  and  $V_E$ , expressed in percentage for each trait.

- i. Genotypic coefficient of variation,  $GCV = \frac{\sqrt{V_G}}{X} \times 100$
- ii. Phenotypic coefficient of variation,  $PCV = \frac{\sqrt{V_P}}{X} \times 100$
- iii. Environmental coefficient of variation,  $GCV = \frac{\sqrt{V_E}}{X} \times 100$

Where, X= Grand mean

Sivasubrahmanian and Menon (1973) reported following categories for the range of variation,

- i. High: >20 percent
- ii. Medium: 10-20 percent
- iii. Low: <10 percent

#### 3.12.4.3. Broad Sense Heritability

Ratio of genotypic variance to the total observed variance in the population and calculation expressed in percentage.

$$H^2 = \frac{V_G}{V_P} \times 100$$

Range of Heritability estimation (Johnson *et al.*, 1955) is,

- i. High: >60 percent
- ii. Medium: 30-60 percent
- iii. Low: <30 percent

#### **3.12.4.4. Genetic Advance**

The expected genetic gain or improvement in the next generation by selecting superior genotype under certain amount of selection pressure. Genetic advance estimated by using Burton (1952) formula.

$$GA = KH^2\sqrt{V_P}$$

Where K= selection differential, At 5% selection intensity K=2.06,

H<sup>2</sup>= Heritability, V<sub>p</sub>= Phenotypic variance

#### **3.12.4.5. Genetic Advance as Percent of Mean**

$$GAM = GA/X \times 100$$

Where, GA= Genetic Advance, X= Grand Mean

Ranges of genetic advance by Johnson *et al.* (1955) is,

- i. High= >20 percent
- ii. Medium= 10-20 percent
- iii. Low= 10 percent

#### **3.12.4.6. Estimation of Correlation**

Degree and direction of association between two variables refers the correlation. Genotypic and phenotypic correlations were calculated by using Falconer (1964) formula.

- i. Genotypic coefficient of correlation ( $r_g$ ) =  $r(x_i, x_j)_g = \frac{Cov((x_i, x_j)_g)}{\sqrt{v(x_i)_g \cdot v(x_j)_g}}$

- ii. Phenotypic coefficient of correlation ( $r_p$ ) =  $r(x_i, x_j)_p = \frac{Cov((x_i, x_j)_p)}{\sqrt{v(x_i)_p} \sqrt{v(x_j)_p}}$
- iii. Error coefficient of correlation ( $r_e$ ) =  $r(x_i, x_j)_e = \frac{Cov((x_i, x_j)_e)}{\sqrt{v(x_i)_e} \sqrt{v(x_j)_e}}$

**3.12.4.7. Path Coefficient Analysis**

It is a standardized partial regression coefficient which separates the correlation coefficients into direct and indirect effects (Dewey and Lu, 1959).

$$r_{1y} = P_{1y} r_{11} + P_{2y} r_{12} + P_{3y} r_{13} \dots \dots \dots + P_{ny} r_{1n}$$

$$r_{2y} = P_{2y} r_{21} + P_{2y} r_{22} + P_{3y} r_{23} \dots \dots \dots + P_{ny} r_{2n}$$

$$r_{ny} = P_{1y} r_{n1} + P_{2y} r_{n2} + P_{3y} r_{n3} \dots \dots \dots + P_{ny} r_{nn}$$

Where,

1,2.....n =independent variables

y = dependent variable

$r_{1y}, r_{2y} \dots \dots \dots r_{ny}$  =coefficient of correlation between independent variables

1 to n on dependent variable y.

$P_{1y}, P_{2y} \dots \dots \dots P_{ny}$  =direct effect of character 1 to n on character y.

The above equation can be written in matrix form

$$\begin{bmatrix} r_{1y} \\ r_{2y} \\ \cdot \\ \cdot \\ \cdot \\ r_{ny} \end{bmatrix} \begin{bmatrix} 1 & r_{12} & r_{13} & \cdot & \cdot & r_{1n} \\ r_{21} & 1 & r_{23} & \cdot & \cdot & r_{2n} \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ r_{n1} & r_{n2} & r_{n3} & \cdot & \cdot & 1 \end{bmatrix} \begin{bmatrix} P_{1y} \\ P_{2y} \\ \cdot \\ \cdot \\ \cdot \\ P_{ny} \end{bmatrix}$$

Then  $B=C^{-1}A$  where  $C^{-1} = \begin{bmatrix} C_{11} & C_{12} & C_{13} & \cdot & \cdot & C_{1n} \\ C_{21} & C_{22} & C_{23} & \cdot & \cdot & C_{2n} \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ C_{n1} & C_{n2} & C_{n3} & \cdot & \cdot & C_{nn} \end{bmatrix}$

Direct effects:

$$P_{1y} = \sum_{i=1}^k c_{1i} r_{iy}$$

$$P_{2y} = \sum_{i=1}^k c_{2i} r_{iy}$$

$$P_{ny} = \sum_{i=1}^k c_{ni} r_{iy}$$

$$\text{Residual effect } PR_y = \sqrt{1 - r^2}$$

$$\text{Where, } r^2 = (P_{1y}r_{1y} + P_{2y}r_{2y} + P_{3y}r_{3y} \dots \dots \dots + P_{ny}r_{ny})$$

$P_{iy}$  = direct effect of  $X_i$  on  $y$

$r_{iy}$  = correlation coefficient of  $X_i$  on  $y$

$i = 1, 2, 3, \dots$

### 3.12.5. Chi-square test

Goodness of fit for resistant loci to an expected 1:1 segregation ratio was tested by means of chi-square analysis in  $BC_1F_1$  population controlling by single resistant gene.

Following expression was used to calculate the chi-square values,

$$\chi^2 = \sum(O_i - E_i)^2/E_i \text{ at d.f.} = n-1$$

Where,

$n$  = number of phenotypic classes

d.f = degrees of freedom

$O$  = Observed number of plants in each phenotypic class

E= Expected number of plants in each phenotypic class

### 3.12.6. Euclidean Distance

Proximity dissimilarity matrix was analysed using Euclidean distance method for six morphological characters of BC<sub>1</sub>F<sub>1</sub> using the formula.

$$\text{Euclidean Distance} = \sum_{k=1}^j \left( \frac{X_{ik} - X_{jk}}{S_k} \right)^2$$

Where,

X<sub>ik</sub>= performance of the i<sup>th</sup> parent for k<sup>th</sup> character

X<sub>jk</sub>= performance of the j<sup>th</sup> parent for k<sup>th</sup> character

S<sub>k</sub>= Standard deviation of the kth character

Genetic distance among the BC<sub>1</sub>F<sub>1</sub> with the recurrent parent was measured by Euclidean distance method (Cruz and Regazzi, 1994) using Statistical Package for the Social Sciences (SPSS version 29.0).

## ***RESULTS***

## 4. RESULTS

The results obtained from the study on “Marker assisted backcrossing for transferring Fusarium wilt (*Fusarium oxysporum* f. sp. *tracheiphilum*) resistance into Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)” are presented in the following headings.

- 4.1. Isolation, identification, and multiplication of Fusarium wilt culture
- 4.2. Artificial inoculation of *Fusarium oxysporum* and scoring of disease incidence in cowpea germplasm
- 4.3. Evaluation of resistant genotypes for yield and quality linked with Fusarium wilt resistance
- 4.4. Genotyping of the resistant lines using SSR marker linked to Fusarium wilt resistance
- 4.5. Hybridisation of resistant genotype with superior but susceptible variety released from KAU for developing F<sub>1</sub>.
- 4.6. Raising and morphological evaluation of F<sub>1</sub> and the parents
- 4.7. Backcrossing of F<sub>1</sub> for the production of BC<sub>1</sub>F<sub>1</sub> lines
- 4.8. Evaluation of BC<sub>1</sub>F<sub>1</sub> lines at the seedling stage through artificial screening and scoring
- 4.9. Morphological and quality evaluation of resistant BC<sub>1</sub>F<sub>1</sub> lines and the recurrent parent
- 4.10. Confirmation of resistant gene in BC<sub>1</sub>F<sub>1</sub> lines using SSR marker linked to Fusarium wilt
- 4.11. Evaluation of BC<sub>2</sub>F<sub>1</sub> lines at the seedling stage through artificial screening and scoring

#### 4.1. ISOLATION, IDENTIFICATION, AND MULTIPLICATION OF FUSARIUM WILT CULTURE

##### **4.1.1. Isolation of pathogen associated with Fusarium wilt in cowpea**

The pathogen-causing Fusarium wilt of yardlong bean was isolated from the infected root and stem of the crop showing typical Fusarium wilt symptoms collected from fields of Vellayani. A total of 10 isolates of the pathogen were obtained (Fot1 to Fot10), from which only five isolates (Fot1, Fot4, Fot7, Fot8, and Fot9) showed a characteristic appearance of *Fusarium* spp. (Plates 12-16).

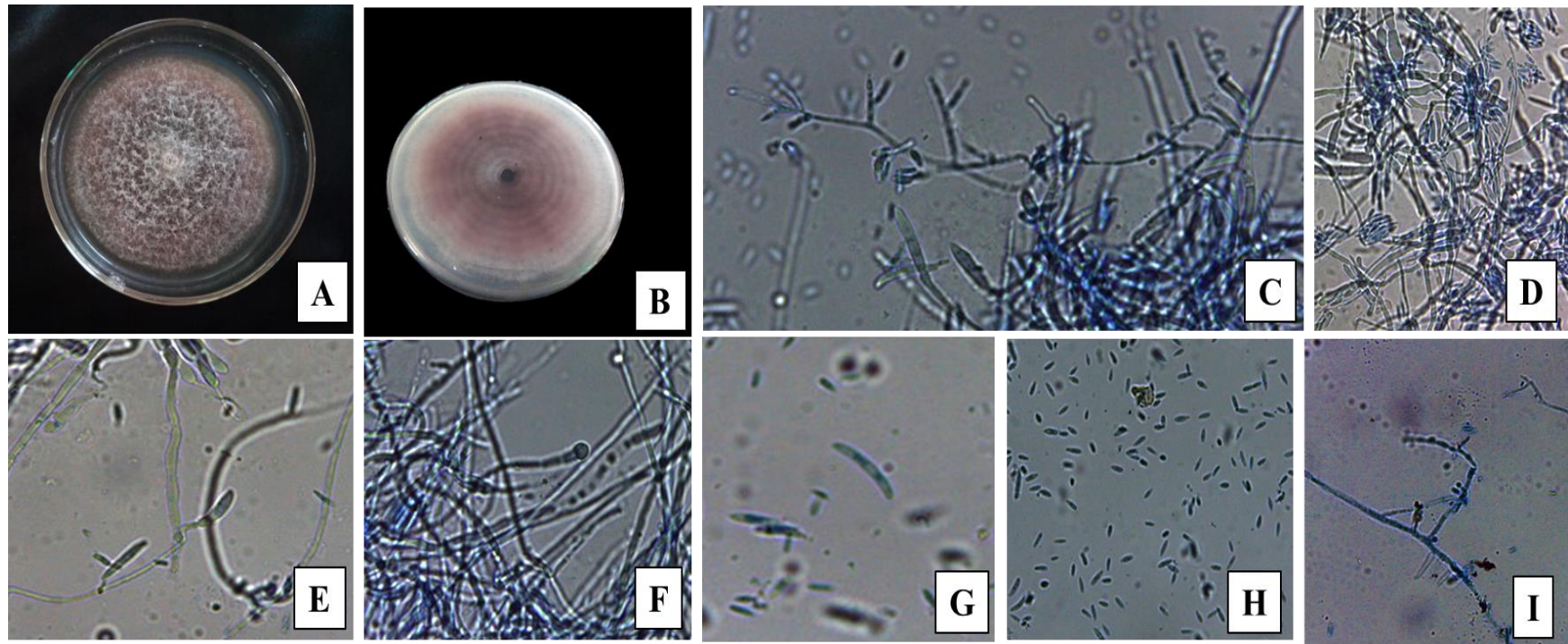
##### **4.1.2. Identification and multiplication of pathogen associated with Fusarium wilt in cowpea**

###### ***4.1.2.1. Morphological and culture characterization of Fusarium wilt-associated pathogen***

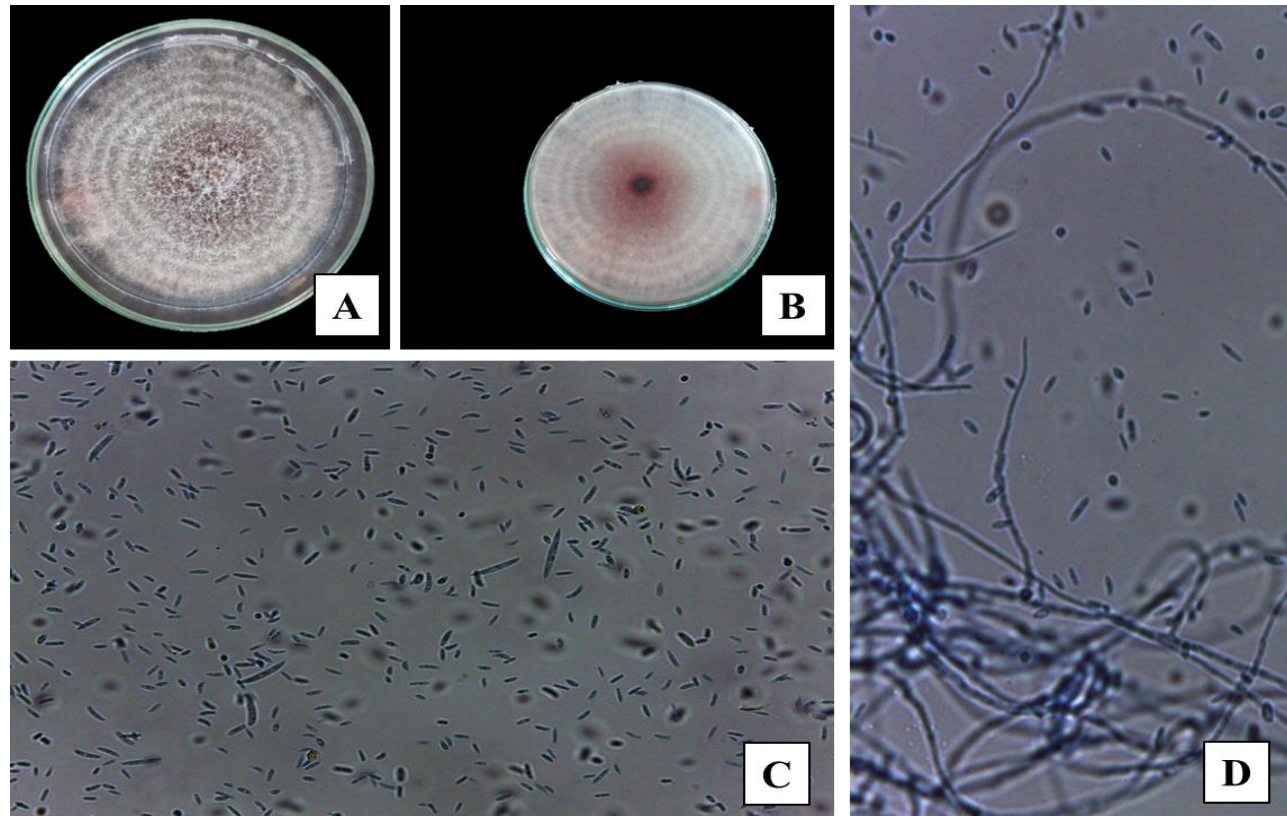
The five isolates of *Fusarium* spp. obtained were cultured on PDA medium for their morphological and cultural characterization and the results are presented in Table 7.

The isolate Fot1 completed its growth in Petri plates within 11 days at 28°C. The colony was floccose in texture, white on the upper surface and reddish brown on the lower surface of the Petri plate. The phialides and conidiophores arose from aerial mycelium. The microconidia were abundant, aseptate, 12.879 x 3.570 µm in size, spherical in shape and the macroconidia were 3-4 septate, thin-walled, and little sickle-shaped. Intercalary and terminal chlamyospores were present (Plate 12).

The second isolate was Fot4, which completed growth within 8 days at 28°C in Petri plates. The colony was floccose in texture, white on the upper surface and pink on the lower surface of the Petri plates. The microconidia were abundant without septation and 11.830 x 3.914 µm in size. The macroconidia were 30.528 x 4.762 µm with two septations. The chlamyospores were present (Plate 13).



**Plate 12. Characterization of Fot1 isolate: (A) Surface of the colony on PDA, (B) Reverse of the colony on PDA, (C) Phialides on aerial mycelia, (D) False head appearance of microconidia, (E) Conidiospores on aerial mycelia, (F) Chlamydo-spore, (G) Macroconidia, (H) Microconidia, and (I) Monophialides.**



**Plate 13. Characterization of Fot4 isolate: (A) Surface of the colony on PDA, (B) Reverse of the colony on PDA, (C) Macroconidia and Microconidia, (D) Monophialides and Chlamydoconidia**

**Table 7. Morphological characterization isolated of *Fusarium* spp.**

<b>Isolates</b>	<b>Features</b>
Fot1	Completed growth in Petri plates within 11 days at 28°C. White floccose mycelia with reddish brown culture appearance; aseptate microconidia (12.879 x 3.570 µm); 3-4 septate macroconidia (32.409 x 5.297 µm), little sickle-shaped; intercalary and terminal chlamydo spores present; false head appearance; phialides and conidiospores on aerial mycelium; pigments produced.
Fot4	Completed growth in Petri plates within 8 days at 28°C. White floccose mycelia with little pink coloured culture; aseptate microconidia (11.830 x 3.914 µm ); 2 septate macroconidia (30.528 x 4.762 µm); axial chlamydo spores present; pigments produced.
Fot7	Completed growth in Petri plates within 6 days at 28°C. White raised mycelia with some pink to magenta coloured culture; aseptate microconidia (8.339 x 2.159 µm); 3 septate macroconidia (28.659 x 2.751 µm); intercalary and axial chlamydo spores present; pigments produced.
Fot8	Completed growth in Petri plates within 7 days at 28°C. White raised mycelia with some yellow-coloured culture; aseptate microconidia (9.802 x 1.974 µm); 2 septate macroconidia (26.692 x 2.662 µm); chlamydo spores were present; pigments produced.
Fot9	Completed growth in Petri plates within 7 days at 28°C. White raised mycelia with red to dark brown coloured culture; aseptate microconidia (10.548 x 2.843 µm); 2 septate macroconidia (27.764 x 3.541 µm); intercalary chlamydo spores were present; pigments produced.

The third isolate Fot7 completed its growth in Petri plates within 6 days at 28°C. The mycelia were white on the upper side and pink to magenta on the lower side of the Petri Plates. The aseptate microconidia (8.339 x 2.159 µm) and three septate macroconidia (28.659 x 2.751 µm) were present with intercalary and terminal chlamydospores (Plate 14).

The fourth isolate was Fot8, which completed its growth in Petri plates within 6 days at 28°C. The mycelia were raised with yellow-coloured culture with aseptate microconidia (9.802 x 1.974 µm) and two septate macroconidia (26.692 x 2.662 µm). Chlamydospores were also present (Plate 15).

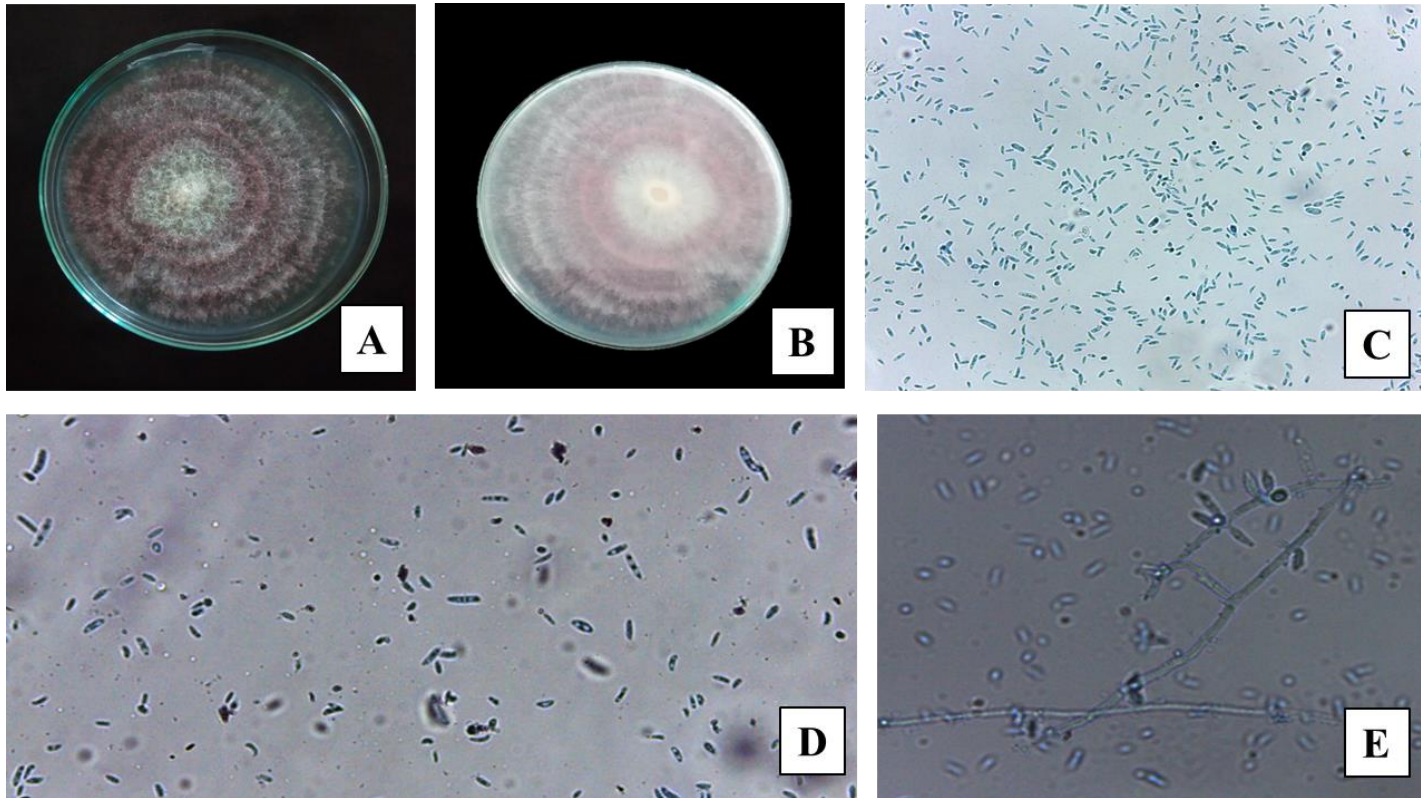
The fifth isolate Fot9 had completed its growth in Petri plates within 7 days at 28°C. The colony was floccose in texture, white on the upper surface and red to dark brown on the lower side of the Petri Plate. The microconidia were abundant without septation (10.548 x 2.843 µm) and macroconidia with two septations (27.764 x 3.541 µm). Intercalary chlamydospores were present (Plate 16).

#### ***4.1.2.2. Mass multiplication of Fusarium wilt-associated pathogen***

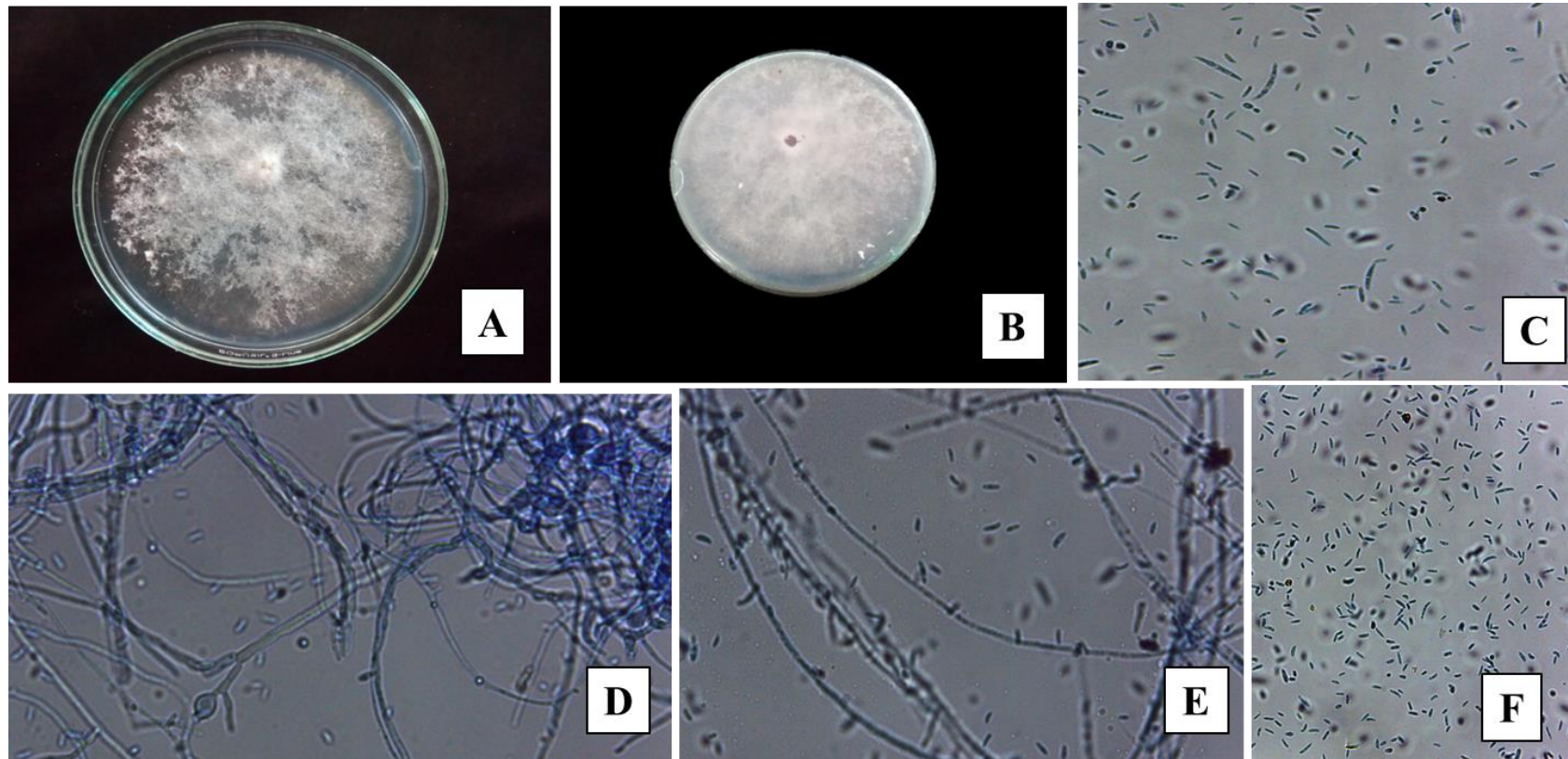
All five isolates obtained were mass multiplied in the sand: maize (9:1) medium at 28°C. The fungus covered the medium completely with white mycelium within two weeks.

#### ***4.1.2.3. Selection of method of inoculation for screening***

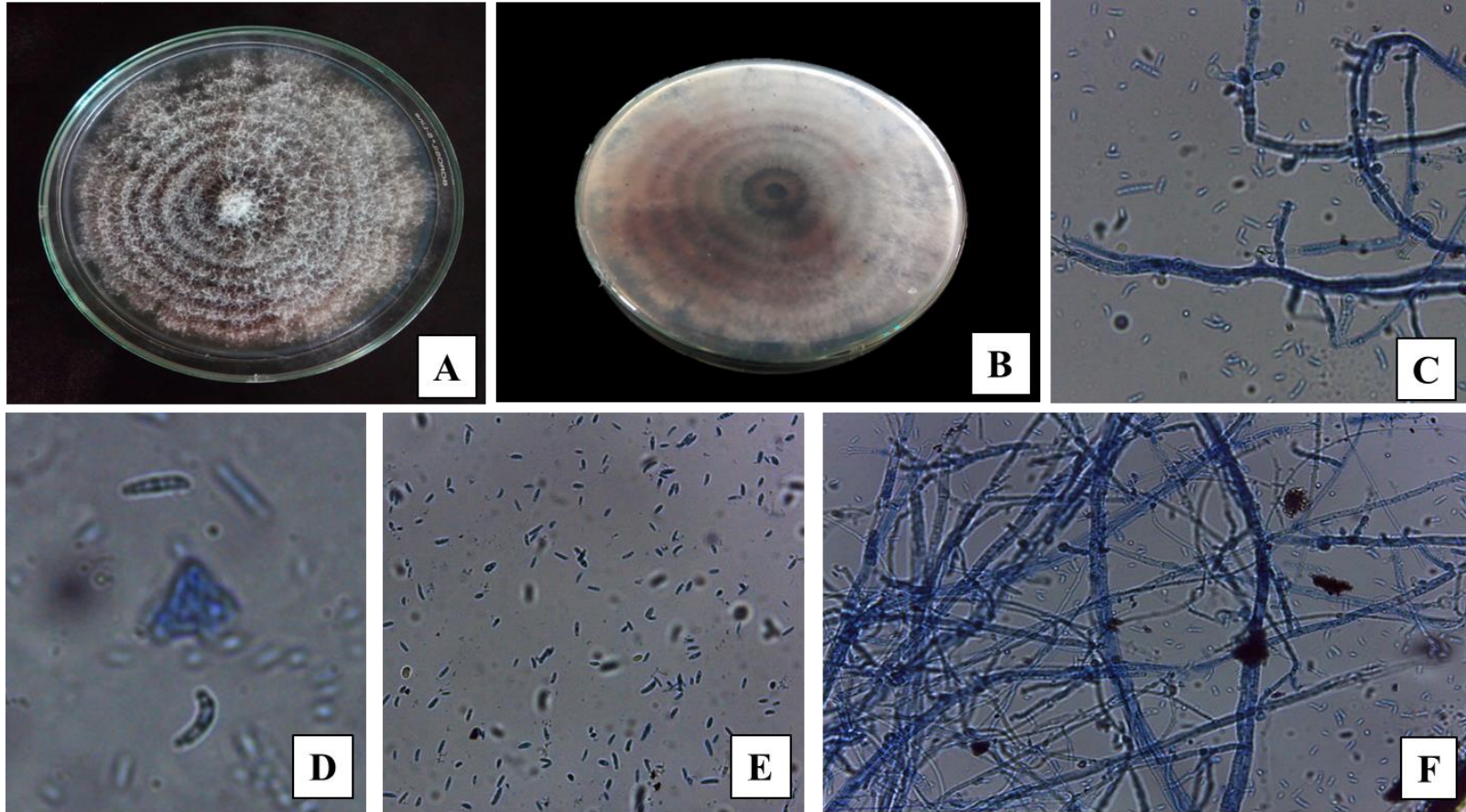
The three methods of inoculation showed differential reactions for Fusarium wilt. Soil inoculation with the pathogen multiplied in the sand: maize medium (9:1) produced wilt symptoms including vascular discolouration, yellowing and necrosis of leaves with complete wilting of plants within five days of inoculation (DAI). The pin pricking and disc inoculation methods did not show any Fusarium wilt symptoms but induced some rotting at the inoculation point. Soil inoculation of the fungus multiplied in the sand: maize (9:1) medium along with pin pricking and disc inoculation methods resulted in basal rotting, yellowing and necrosis of leaves with complete wilting of plants after five DAI. The control plants without inoculation were healthy without Fusarium symptoms (Plate 17).



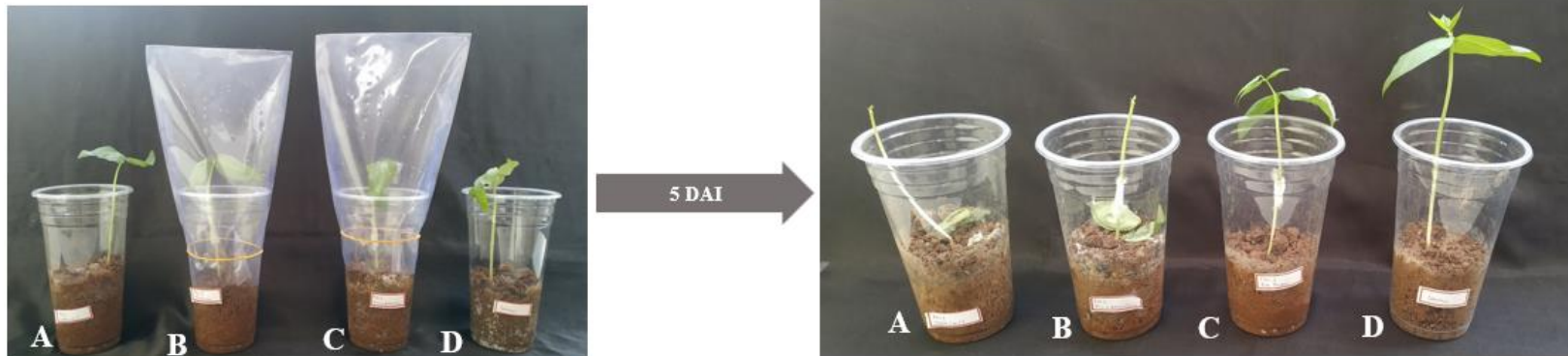
**Plate 14. Characterization of Fot7 isolate: (A) Surface of the colony on PDA, (B) Reverse of the colony on PDA, (C) Microconidia, (D) Macroconidia, and (E) Monophialides and Chlamydo spores**



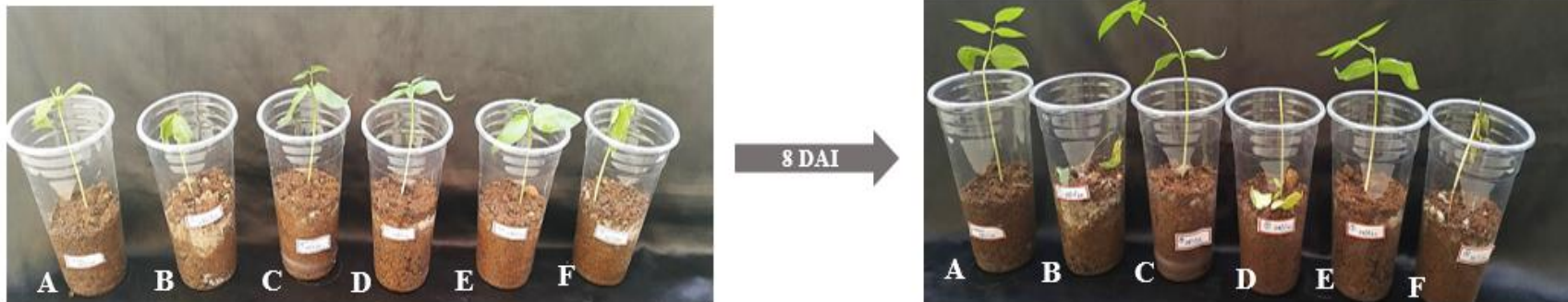
**Plate 15. Characterization of Fot8 isolate: (A) Surface of the colony on PDA, (B) Reverse of the colony on PDA, (C) Macroconidia, (D) Chlamydospore, (E) Monophialides, and (F) Microconidia**



**Plate 16. Characterization of Fot9 isolate: (A) Surface of the colony on PDA, (B) Reverse of the colony on PDA, (C) Chlamydozoospores, (D) Macroconidia, (E) Microconidia, and (F) Monophialides**



**Plate 17. Standardization of methods for inoculation of Fusarium pathogen. (A) Soil inoculation with pathogen multiplied Sand maize media alone, (B) Pin pricking with disc inoculation (stem and leaves) (C) Pin pricking with disc inoculation + Soil inoculation with pathogen multiplied Sand maize media (D) Control, DAI- Days After Inoculation**



**Plate 18. Virulence testing of Fusarium isolates, (A) Control, (B) Fot1, (C) Fot4, (D) Fot7, (E) Fot8 (F) Fot9, DAI- Days After Inoculation**

#### ***4.1.2.4. Virulence testing and standardization of inoculum for screening***

The five isolates obtained were tested for virulence to induce early symptoms of *Fusarium* wilt. Out of the five isolates tested, three induced yellowing, vascular discolouration, and total wilting of cowpea at eight days after inoculation (DAI). The isolate Fot1 took seven days to develop the symptoms and was found to be the highly virulent isolate followed by Fot7 and Fot9. The isolates Fot4 and Fot8 did not show *Fusarium* wilt symptoms implicating their lower virulence (Plate 18).

Among seven different percentages of sand: maize (9:1) medium in which the pathogen multiplied were mixed with sterile soil for inoculation on healthy cowpea plants, 10, 15, 20 and 25% of the inoculum produced the typical *Fusarium* symptoms at 8 DAI (Plate 19).

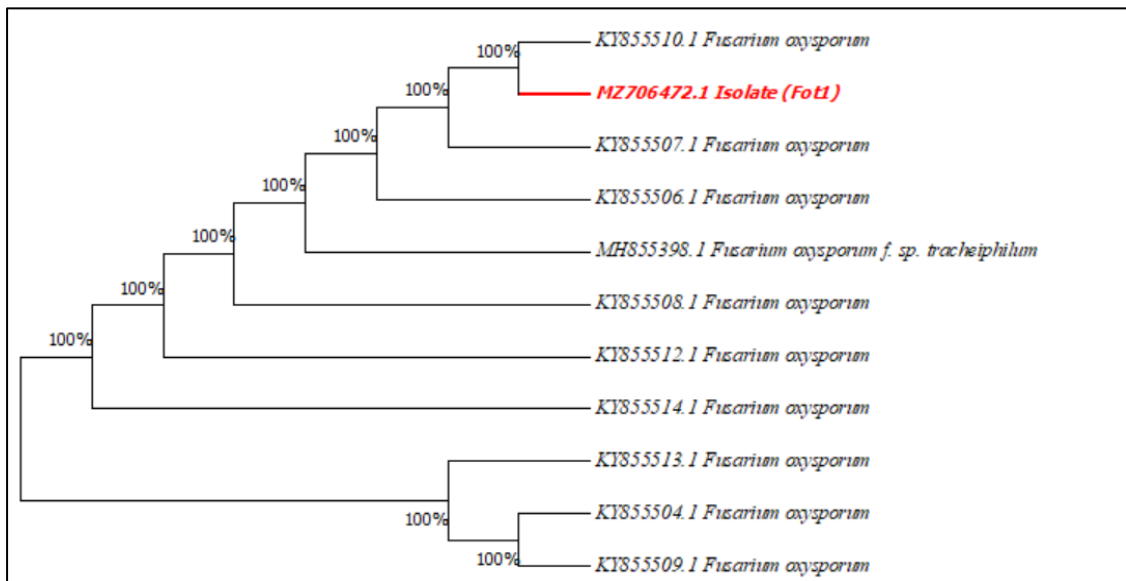
#### **4.1.3. Molecular characterization of *Fusarium* wilt-associated pathogen by partial sequencing of Internal Transcribed Spacer (ITS) region of rDNA**

The ITS-rDNA amplification of obtained *Fusarium* spp. isolate with the universal primers ITS1 and ITS4 produced an amplicon with a product size of 500bp. The amplified DNA was trimmed and assembled. The consensus sequences were obtained using Biological Sequence Alignment Editor and Analysis. The consensus sequence obtained is given in Appendix 1. The homologous similarity search in NCBI through BLAST analysis recorded 99-100% similarity with the *Fusarium oxysporum* database available in the NCBI (Appendix 2).

Ten nucleotide sequences of *Fusarium oxysporum* from the NCBI database were used for phylogenetic analysis. The phylogenetic analysis of the nucleotide sequence of the isolate with the *Fusarium oxysporum* database from the NCBI showed similarity with 100% bootstrap support (Figure 1). The ITS-rDNA sequence of the isolate was submitted to the NCBI with accession number MZ706472.1 (Appendix 3).



**Plate 19. Standardization of *Fusarium* inoculum percentage for screening (A) Control, (B) 1%, (C) 2%, (D) 5%, (E) 10%, (F) 15%, (G) 20%, and (H) 25%**



**Figure 1. Phylogenetic analysis of isolate with the *Fusarium oxysporum* database from NCBI**

## 4.2. ARTIFICIAL INOCULATION OF *Fusarium oxysporum* AND SCORING OF DISEASE INCIDENCE IN COWPEA GERMPLASM

The 115 cowpea genotypes collected from different sources along with 10 KAU-released varieties were artificially screened for Fusarium wilt reaction. The disease reaction was scored on a 0-4 scale, in which the lowest score indicated the highest resistance.

### 4.2.1. Disease score (0-4 scale)

The disease scoring was done based on a 0-4 scale and the mean value was calculated. The mean disease score of each cowpea genotype is given in Table 8. The lowest score '0' was recorded in ten genotypes, '0.3' was scored by eight genotypes, '0.7' by three genotypes, and '1' by twenty-three genotypes. The '1.3' and '1.7' were scored by fifteen and seven genotypes respectively. The score '2' was obtained in eight genotypes, and '2.3' in one genotype. The score of '2.7' in fourteen genotypes, and of '3' in eleven genotypes. The scores '3.3', '3.7' and '4' were scored by three, three and nine genotypes respectively.

### 4.2.2. Number of days taken to wilt (days)

The number of days taken to wilt, disease intensity and disease reaction of each genotype to Fusarium wilt are detailed in Table 9. Fusarium wilt symptoms initiated in the genotypes at 15 to 53 days after inoculation. Out of 115 cowpea genotypes, nine genotypes took 53 days, six genotypes 44 days, thirty-one genotypes 48 days, three genotypes 40 days, three genotypes 37 days, ten genotypes 34 days, ten genotypes 32 days, eight genotypes 29 days, eleven genotypes 26 days, twenty-three genotypes 21 days and one genotype 15 days to induce Fusarium wilt symptoms.

### 4.2.3. Disease intensity (DI) (%)

The intensity of the disease varied according to the cowpea genotype and ranged from 0 per cent to 80 per cent. Eighty per cent of disease intensity was

**Table 8. The disease score (0-4) in 115 cowpea genotypes**

Sl. No.	Name	Disease score (0-4) *
1	TCR79	4.0 <sup>i</sup>
2	TCR80	1.0 <sup>a-d</sup>
3	TCR81	3.3 <sup>g-i</sup>
4	TCR83	1.3 <sup>a-e</sup>
5	TCR84	1.0 <sup>a-d</sup>
6	TCR85	1.3 <sup>a-e</sup>
7	TCR86	1.0 <sup>a-d</sup>
8	TCR87	0.0 <sup>a</sup>
9	TCR88	1.3 <sup>a-e</sup>
10	TCR89	2.7 <sup>e-i</sup>
11	TCR101	2.0 <sup>c-g</sup>
12	TCR104	2.7 <sup>e-i</sup>
13	TCR105	1.3 <sup>a-e</sup>
14	TCR106	3.0 <sup>f-i</sup>
15	TCR107	1.3 <sup>a-e</sup>
16	TCR108	1.7 <sup>b-f</sup>
17	TCR109	1.7 <sup>b-f</sup>
18	TCR110	2.0 <sup>c-g</sup>
19	TCR111	3.0 <sup>f-i</sup>
20	TCR112	2.0 <sup>c-g</sup>
21	TCR113	2.7 <sup>e-i</sup>
22	TCR115	1.0 <sup>a-d</sup>
23	TCR116	1.0 <sup>a-d</sup>
24	TCR117	3.0 <sup>f-i</sup>
25	TCR118	2.3 <sup>d-h</sup>
26	TCR119	2.7 <sup>e-i</sup>
27	TCR122	3.0 <sup>f-i</sup>
28	TCR124	1.7 <sup>b-f</sup>
29	TCR125	1.0 <sup>a-d</sup>
30	TCR126	1.3 <sup>a-e</sup>
31	VI001920 A-R	4.0 <sup>i</sup>
32	VI034386	0.0 <sup>a</sup>
33	VI034392	1.3 <sup>a-e</sup>
34	VI036720	3.3 <sup>g-i</sup>
35	VI036721	4.0 <sup>i</sup>
36	VI040951	1.0 <sup>a-d</sup>
37	VI041083	1.3 <sup>a-e</sup>
38	VI041680	1.0 <sup>a-d</sup>
39	VI041703-A	3.0 <sup>f-i</sup>
40	VI046192	3.0 <sup>f-i</sup>
41	VI046645-B	3.7 <sup>hi</sup>

(Cont.)

42	VI046648-B	4.0 <sup>i</sup>
43	VI046653	0.3 <sup>ab</sup>
44	VI047617	1.3 <sup>a-e</sup>
45	VI048020	1.0 <sup>a-d</sup>
46	VI048484	2.7 <sup>e-i</sup>
47	VI048488	4.0 <sup>i</sup>
48	VI048533	0.0 <sup>a</sup>
49	VI050893	3.3 <sup>g-i</sup>
50	VI050896	1.7 <sup>b-f</sup>
51	VI055471	1.0 <sup>a-d</sup>
52	VI057906-A	1.0 <sup>a-d</sup>
53	VI057936-B	1.0 <sup>a-d</sup>
54	VI061032	1.7 <sup>b-f</sup>
55	VI061064	1.3 <sup>a-e</sup>
56	VI061095	2.7 <sup>e-i</sup>
57	VI062338	2.0 <sup>c-g</sup>
58	VI064556 (IRON)	0.0 <sup>a</sup>
59	VI050941	1.0 <sup>a-d</sup>
60	VI061014	2.0 <sup>c-g</sup>
61	Aryanadu local	3.0 <sup>f-i</sup>
62	Ayyanthole local	4.0 <sup>i</sup>
63	Cherthala local I	2.7 <sup>e-i</sup>
64	Cherthala local II	3.0 <sup>f-i</sup>
65	Elamadu local II	0.3 <sup>ab</sup>
66	Kadambarakonam local	4.0 <sup>i</sup>
67	Kallicaud local	0.3 <sup>ab</sup>
68	Kallicaud local II	2.7 <sup>e-i</sup>
69	Kanjikuzhi local	0.0 <sup>a</sup>
70	Kochi local	0.0 <sup>a</sup>
71	Kollam local I	1.3 <sup>a-e</sup>
72	Kollamcode local	1.3 <sup>a-e</sup>
73	Koovappally local	0.3 <sup>ab</sup>
74	Kottayam thattathi local	0.0 <sup>a</sup>
75	Kumil local	3.0 <sup>f-i</sup>
76	Mavelikara local	0.0 <sup>a</sup>
77	Nelladu local III	3.7 <sup>hi</sup>
78	Nellanadu local	0.3 <sup>ab</sup>
79	Nellanadu local II	2.7 <sup>e-i</sup>
80	Nenmara local II	0.7 <sup>a-c</sup>
81	Nenmara local III	4.0 <sup>i</sup>
82	Nenmara local IV	2.0 <sup>c-g</sup>
83	Nenmara local V	2.7 <sup>e-i</sup>
84	Nenmara local VI	3.0 <sup>f-i</sup>
85	Omaller local I	2.7 <sup>e-i</sup>

(Cont.)

86	Padavalam payar	2.7 <sup>e-i</sup>
87	Palakkad local I	2.0 <sup>c-g</sup>
88	Palakkad local (meter payar)	3.7 <sup>hi</sup>
89	Peyad local	1.0 <sup>a-d</sup>
90	Puthenpeedikayil local	3.0 <sup>f-i</sup>
91	Vellavalli payar	1.0 <sup>a-d</sup>
92	Vlathankara local I	0.7 <sup>a-c</sup>
93	Vlathankara local II	1.0 <sup>a-d</sup>
94	Wayanadu local I	0.3 <sup>ab</sup>
95	Wayanadu local II	0.3 <sup>ab</sup>
96	Muttathkonam local	0.0 <sup>a</sup>
97	Kottayam local I	1.0 <sup>a-d</sup>
98	Kalliyoor local	1.3 <sup>a-e</sup>
99	Aranmula local	1.0 <sup>a-d</sup>
100	Kilimanur local	1.7 <sup>b-f</sup>
101	Elamadu local I	2.7 <sup>e-i</sup>
102	Kulashekharam local I	0.3 <sup>ab</sup>
103	Nilamel local	1.0 <sup>a-d</sup>
104	Kottarakkara local	0.0 <sup>a</sup>
105	Anchal local II	1.0 <sup>a-d</sup>
106	Malika	1.0 <sup>a-d</sup>
107	Sharika	1.0 <sup>a-d</sup>
108	Bhagyalakshmi	2.7 <sup>e-i</sup>
109	Lola	1.3 <sup>a-e</sup>
110	Vyjayanthi (VS-21-1)	1.0 <sup>a-d</sup>
111	Vellayani Jyothika	1.7 <sup>b-f</sup>
112	Githika	2.0 <sup>c-g</sup>
113	Manjari	0.7 <sup>a-c</sup>
114	Anaswara	1.3 <sup>a-e</sup>
115	Kanakamony	4.0 <sup>i</sup>
	<b>Maximum</b>	<b>4.000</b>
	<b>Minimum</b>	<b>0.000</b>
	<b>Mean</b>	<b>1.786</b>
	<b>Std Deviation</b>	<b>1.178</b>
	<b>p-value</b>	<b>0.482</b>
	<b>KW value</b>	<b>271.312</b>
	<b>Chi-square</b>	<b>113.145</b>

\*Data are means of three replicates; different alphabetic letters indicate a significant difference between values according to Duncan's multiple range test at  $\alpha=0.05$ .

**Table 9. The number of days taken to wilt, disease intensity and disease reaction of 115 cowpea genotypes to Fusarium wilt**

Sl. No.	Name	Number of days taken to wilt	Disease intensity (%)	Disease reaction
1	TCR79	40	80.0 (1.591) <sup>a</sup>	HS
2	TCR80	48	20.0 (0.524) <sup>j</sup>	MR
3	TCR81	44	66.6 (1.149) <sup>c</sup>	S
4	TCR83	44	26.6 (0.615) <sup>i</sup>	MS
5	TCR84	44	20.0 (0.524) <sup>j</sup>	MR
6	TCR85	48	26.6 (0.615) <sup>i</sup>	MS
7	TCR86	53	20.0 (0.524) <sup>j</sup>	MR
8	TCR87	53	0.00 (0.000) <sup>l</sup>	R
9	TCR88	53	26.6 (0.611) <sup>i</sup>	MS
10	TCR89	48	53.3 (0.955) <sup>e</sup>	S
11	TCR101	53	40.0 (0.785) <sup>g</sup>	MS
12	TCR104	48	53.3 (0.955) <sup>e</sup>	S
13	TCR105	48	26.7 (0.616) <sup>i</sup>	MS
14	TCR106	48	60.0 (1.047) <sup>d</sup>	S
15	TCR107	53	26.7 (0.616) <sup>i</sup>	MS
16	TCR108	53	33.3 (0.701) <sup>h</sup>	MS
17	TCR109	53	33.3 (0.701) <sup>h</sup>	MS
18	TCR110	53	40.0 (0.785) <sup>g</sup>	MS
19	TCR111	53	60.0 (1.047) <sup>d</sup>	S
20	TCR112	26	40.0 (0.785) <sup>g</sup>	MS
21	TCR113	26	53.3 (0.956) <sup>e</sup>	S
22	TCR115	26	20.0 (0.524) <sup>j</sup>	MR
23	TCR116	26	20.0 (0.524) <sup>j</sup>	MR
24	TCR117	26	60.0 (1.047) <sup>d</sup>	S
25	TCR118	26	46.7 (0.870) <sup>f</sup>	MS
26	TCR119	26	53.3 (0.955) <sup>e</sup>	S
27	TCR122	26	60.0 (1.047) <sup>d</sup>	S
28	TCR124	26	33.3 (0.701) <sup>h</sup>	MS
29	TCR125	26	20.0 (0.524) <sup>j</sup>	MR
30	TCR126	26	26.7 (0.616) <sup>i</sup>	MS
31	VI001920 A-R	44	80.0 (1.571) <sup>a</sup>	HS
32	VI034386	44	0.00 (0.000) <sup>l</sup>	R
33	VI034392	44	26.7 (0.616) <sup>i</sup>	MS
34	VI036720	44	66.6 (1.149) <sup>c</sup>	S
35	VI036721	44	80.0 (1.571) <sup>a</sup>	HS
36	VI040951	44	20.0 (0.537) <sup>j</sup>	MR
37	VI041083	44	26.6 (0.615) <sup>i</sup>	MS
38	VI041680	44	20.0 (0.524) <sup>j</sup>	MR
39	VI041703-A	44	60.0 (1.047) <sup>d</sup>	S

(Cont.)

40	VI046192	44	60.0 (1.047) <sup>d</sup>	S
41	VI046645-B	44	73.3 (1.277) <sup>b</sup>	S
42	VI046648-B	44	80.0 (1.571) <sup>a</sup>	HS
43	VI046653	44	0.00 (0.000) <sup>l</sup>	R
44	VI047617	44	26.6 (0.615) <sup>i</sup>	MS
45	VI048020	44	20.0 (0.524) <sup>j</sup>	MR
46	VI048484	37	53.3 (0.955) <sup>e</sup>	S
47	VI048488	37	80.0 (1.571) <sup>a</sup>	HS
48	VI048533	44	0.00 (0.000) <sup>l</sup>	R
49	VI050893	44	66.6 (1.149) <sup>c</sup>	S
50	VI050896	44	33.3 (0.701) <sup>h</sup>	MS
51	VI055471	44	20.0 (0.524) <sup>j</sup>	MR
52	VI057906-A	44	20.0 (0.524) <sup>j</sup>	MR
53	VI057936-B	44	20.0 (0.524) <sup>j</sup>	MR
54	VI061032	44	33.3 (0.701) <sup>h</sup>	MS
55	VI061064	44	26.67 (0.615) <sup>i</sup>	MS
56	VI061095	44	53.33 (0.955) <sup>e</sup>	S
57	VI062338	44	40.00 (0.785) <sup>g</sup>	MS
58	VI064556 (IRON)	44	0.00 (0.000) <sup>l</sup>	R
59	VI050941	44	20.0 (0.524) <sup>j</sup>	MR
60	VI061014	44	40.0 (0.794) <sup>g</sup>	MS
61	Aryanadu local	32	60.0 (1.047) <sup>d</sup>	S
62	Ayyanthole local	21	80.0 (1.571) <sup>a</sup>	HS
63	Cherthala local I	21	53.3 (0.956) <sup>e</sup>	S
64	Cherthala local II	40	60.0 (1.047) <sup>d</sup>	S
65	Elamadu local II	21	0.00 (0.000) <sup>l</sup>	R
66	Kadambarakonam local	15	80.0 (1.571) <sup>a</sup>	HS
67	Kallicaud local	21	0.00 (0.000) <sup>l</sup>	R
68	Kallicaud local II	40	53.3 (0.955) <sup>e</sup>	S
69	Kanjikuzhi local	21	0.00 (0.000) <sup>l</sup>	R
70	Kochi local	21	0.00 (0.000) <sup>l</sup>	R
71	Kollam local I	21	26.7 (0.616) <sup>i</sup>	MS
72	Kollamcode local	21	26.7 (0.616) <sup>i</sup>	MS
73	Koovappally local	21	0.00 (0.000) <sup>l</sup>	R
74	Kottayam thattathi local	21	0.00 (0.000) <sup>l</sup>	R
75	Kumil local	21	60.0 (1.029) <sup>d</sup>	S
76	Mavelikara local	21	0.00 (0.000) <sup>l</sup>	R
77	Nelladu local III	21	73.3 (1.277) <sup>b</sup>	S
78	Nellanadu local	21	0.00 (0.000) <sup>l</sup>	R
79	Nellanadu local II	37	53.3 (0.955) <sup>e</sup>	S
80	Nenmara local II	21	13.3 (0.420) <sup>k</sup>	MR
81	Nenmara local III	21	80.0 (1.571) <sup>a</sup>	HS
82	Nenmara local IV	21	40.0 (0.785) <sup>g</sup>	MS
83	Nenmara local V	21	53.3 (0.955) <sup>e</sup>	S

(Cont.)

84	Nenmara local VI	21	60.0 (1.047) <sup>d</sup>	S
85	Omallur local I	21	53.3 (0.955) <sup>e</sup>	S
86	Padavalam payar	32	53.3 (0.955) <sup>e</sup>	S
87	Palakkad local I	21	40.0 (0.789) <sup>g</sup>	MS
88	Palakkad local (meter payar)	32	73.3 (1.277) <sup>b</sup>	S
89	Peyad local	21	20.0 (0.524) <sup>j</sup>	MR
90	Puthenpeedikayil local	21	60.0 (1.047) <sup>d</sup>	S
91	Vellavalli payar	29	20.0 (0.524) <sup>j</sup>	MR
92	Vlathankara local I	29	13.3 (0.420) <sup>k</sup>	MR
93	Vlathankara local II	29	20.0 (0.524) <sup>j</sup>	MR
94	Wayanadu local I	29	0.00 (0.000) <sup>l</sup>	R
95	Wayanadu local II	29	0.00 (0.000) <sup>l</sup>	R
96	Muttathkonam local	29	0.00 (0.000) <sup>l</sup>	R
97	Kottayam local I	29	20.0 (0.524) <sup>j</sup>	MR
98	Kalliyoor local	29	26.6 (0.615) <sup>i</sup>	MS
99	Aranmula local	32	20.0 (0.518) <sup>j</sup>	MR
100	Kilimanur local	32	33.3 (0.701) <sup>h</sup>	MS
101	Elamadu local I	32	53.3 (0.955) <sup>e</sup>	S
102	Kulashekham local I	32	0.00 (0.000) <sup>l</sup>	R
103	Nilamel local	32	20.0 (0.524) <sup>j</sup>	MR
104	Kottarakkara local	32	0.00 (0.000) <sup>l</sup>	R
105	Anchal local II	32	20.0 (0.524) <sup>j</sup>	MR
106	Malika	34	20.00 (0.524) <sup>j</sup>	MR
107	Sharika	34	20.00 (0.524) <sup>j</sup>	MR
108	Bhagyalakshmi	34	53.33 (0.955) <sup>e</sup>	S
109	Lola	34	26.67 (0.616) <sup>i</sup>	MS
110	Vyjayanthi (VS-21-1)	34	20.00 (0.524) <sup>j</sup>	MR
111	Vellayani Jyothika	34	33.33 (0.702) <sup>h</sup>	MS
112	Githika	34	40.00 (0.785) <sup>g</sup>	MS
113	Manjari	34	13.33 (0.420) <sup>k</sup>	MR
114	Anaswara	34	26.67 (0.616) <sup>i</sup>	MS
115	Kanakamony	34	80.00 (1.571) <sup>a</sup>	HS
	<b>Maximum</b>	<b>15</b>	<b>1.591</b>	
	<b>Minimum</b>	<b>53</b>	<b>0.000</b>	
	<b>Mean</b>	<b>35.069</b>	<b>35.17 (0.703)</b>	
	<b>SE(d)</b>	<b>NA</b>	<b>0.017</b>	
	<b>SE(m)</b>	<b>NA</b>	<b>0.012</b>	
	<b>CV (%)</b>	<b>NA</b>	<b>3.018</b>	

Arc sine transformed values are in parentheses. Different alphabetic letters indicate a significant difference between values. R-Resistant, MR- Moderately Resistant, MS- Moderately Susceptible, S- Susceptible, and HS- Highly Susceptible.

observed in nine genotypes, and 0% disease intensity was observed in eighteen cowpea genotypes (Figure 2).

#### **4.2.4. Disease reaction**

The disease reaction was categorized into five groups according to disease intensity: resistant (0% DI), moderately resistant (1-25% DI), moderately susceptible (26-50% DI), susceptible (51-75% DI) and highly susceptible (>75% DI). Accordingly, the 115 cowpea genotypes were categorized into eighteen resistant, twenty-six moderately resistant, thirty-one moderately susceptible, thirty-one susceptible and nine highly susceptible genotypes.

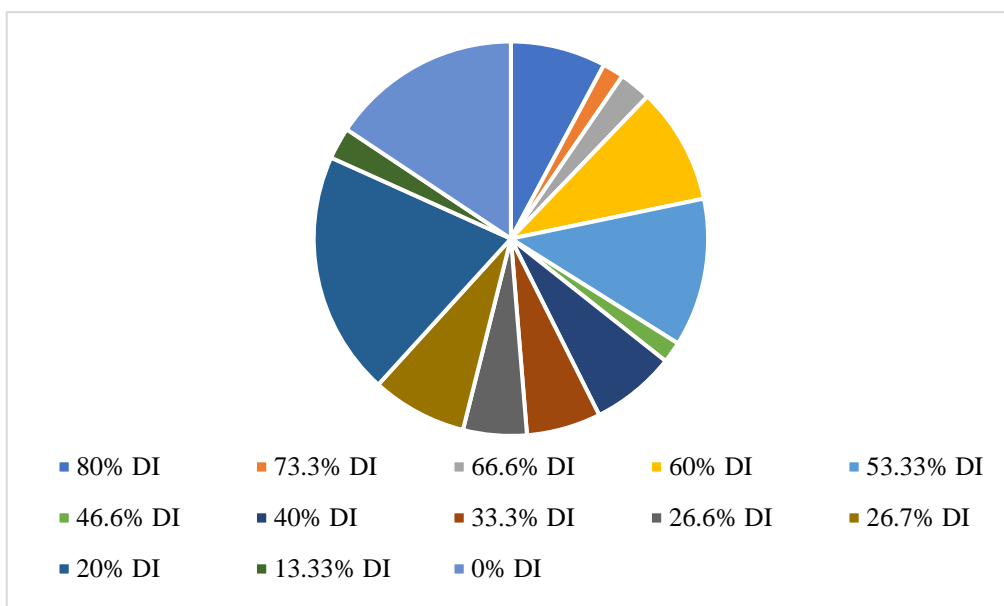
The number of varieties in each category is detailed in Figure 3. Some of the resistant (Plate 20a and b), moderately resistant (Plate 21), moderately susceptible (Plate 22), susceptible (Plate 23) and highly susceptible (Plate 24) varieties along with KAU-released varieties (Plate 25a and b) are furnished.

### **4.3. EVALUATION OF RESISTANT GENOTYPES FOR YIELD AND QUALITY LINKED WITH FUSARIUM WILT RESISTANCE**

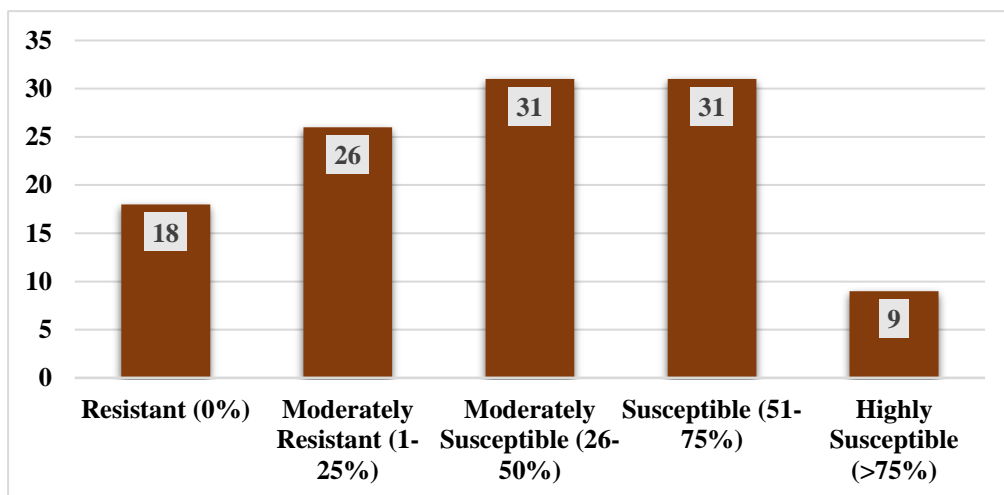
The fifteen trailing-type cowpea genotypes (12 resistant and 3 susceptible) and seven bush-type cowpea genotypes (6 resistant and 1 susceptible) were evaluated in two separate experiments in the field for biometric and quality parameters.

#### **4.3.1. Evaluation of resistant along with susceptible checks in trailing-type cowpea genotypes under field conditions**

The observations on biometric and quality parameters of resistant and susceptible trailing-type cowpea genotypes are given in Table 10. The results pertaining to phenotypic variance ( $V_p$ ), genotypic variance ( $V_g$ ), environmental variance ( $V_e$ ), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad sense heritability ( $h^2$ ) and genetic advance as per cent of mean (GAM) for all the twelve characters of trailing-type cowpea genotypes are furnished in Table 11.



**Figure 2. Frequency of cowpea genotypes and disease intensity (DI)**



**Figure 3. Number of cowpea genotypes under different disease grades**

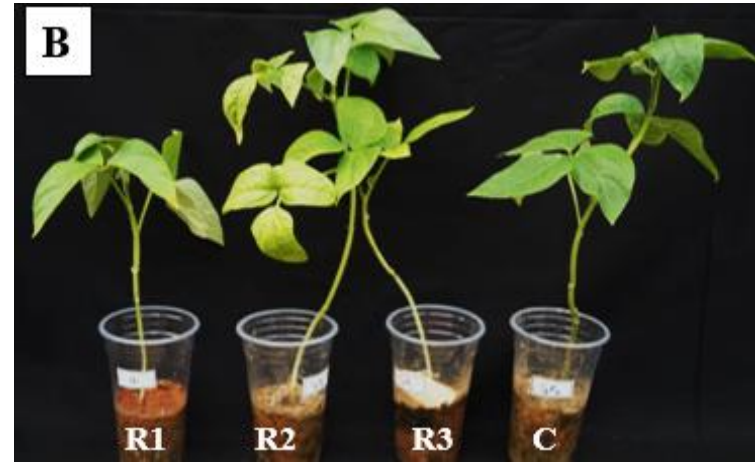
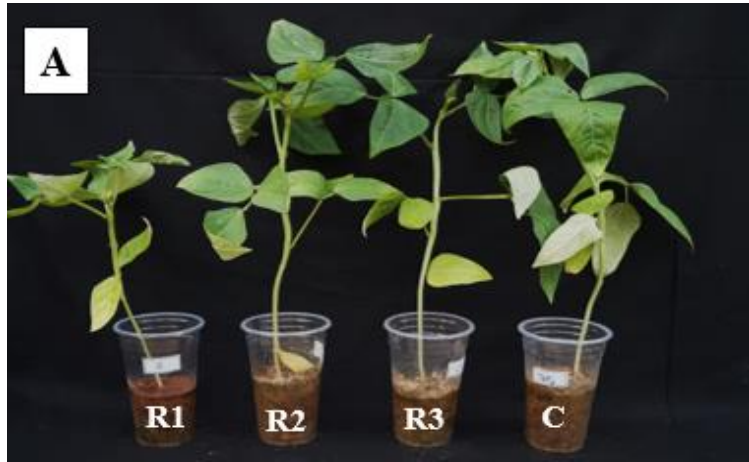


Plate 20. Fusarium wilt resistant genotypes (A) TCR 87, (B) VI034386, (C) Kallicaud Local, (D) Elamadu Local II

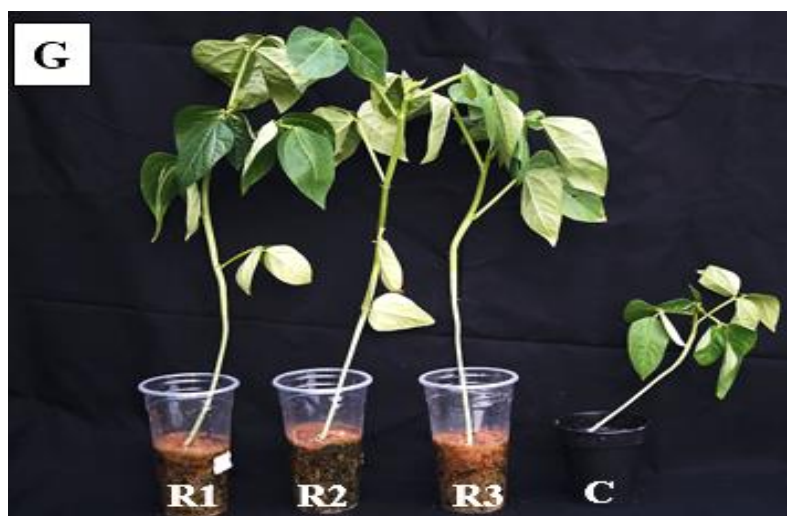
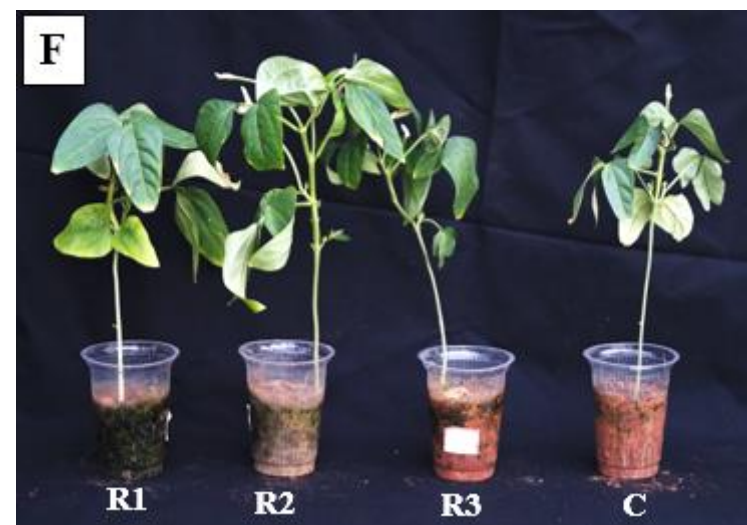
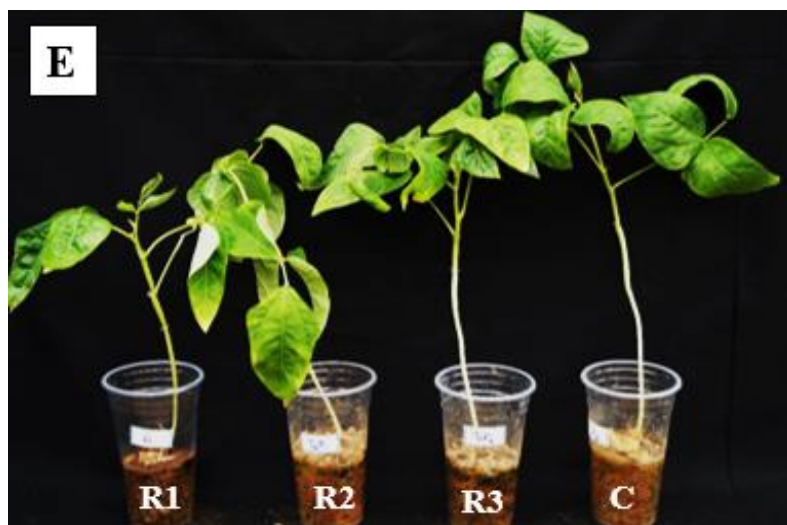


Plate 20 (Cont.). Fusarium wilt resistant genotypes (E) VI046653 and (F) VI048533, (G) VI064556 (IRON), and (H) Kanjikuzhi Local

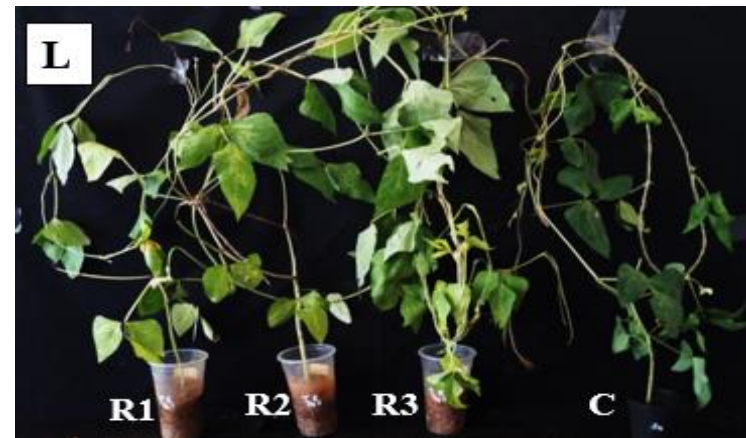
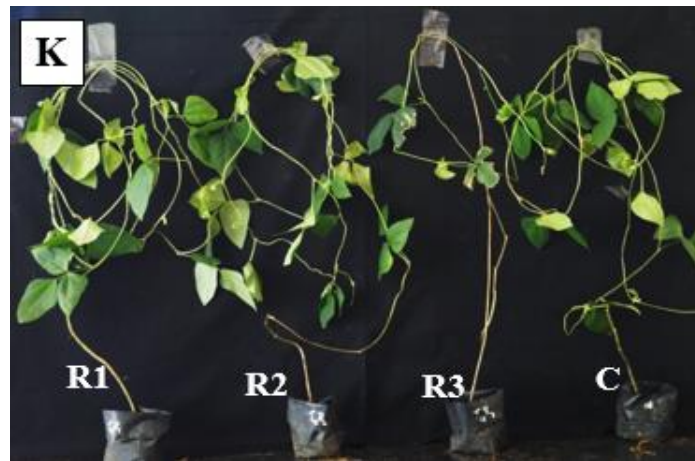


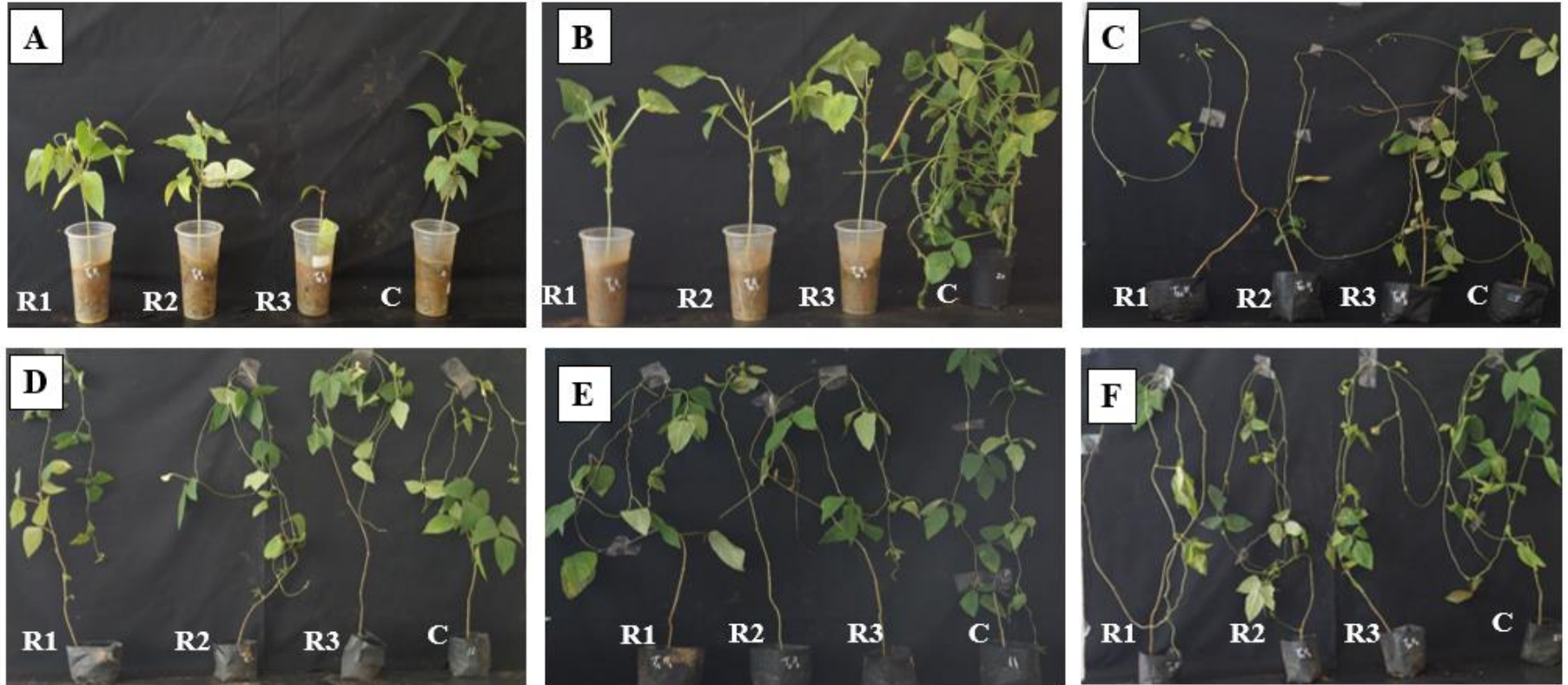
Plate 20 (Cont.). *Fusarium* wilt resistant genotypes (I) Kulashekaram Local, (J) Koovappally Local, (K) Nellanadu Local, (L) Wayanad Local I

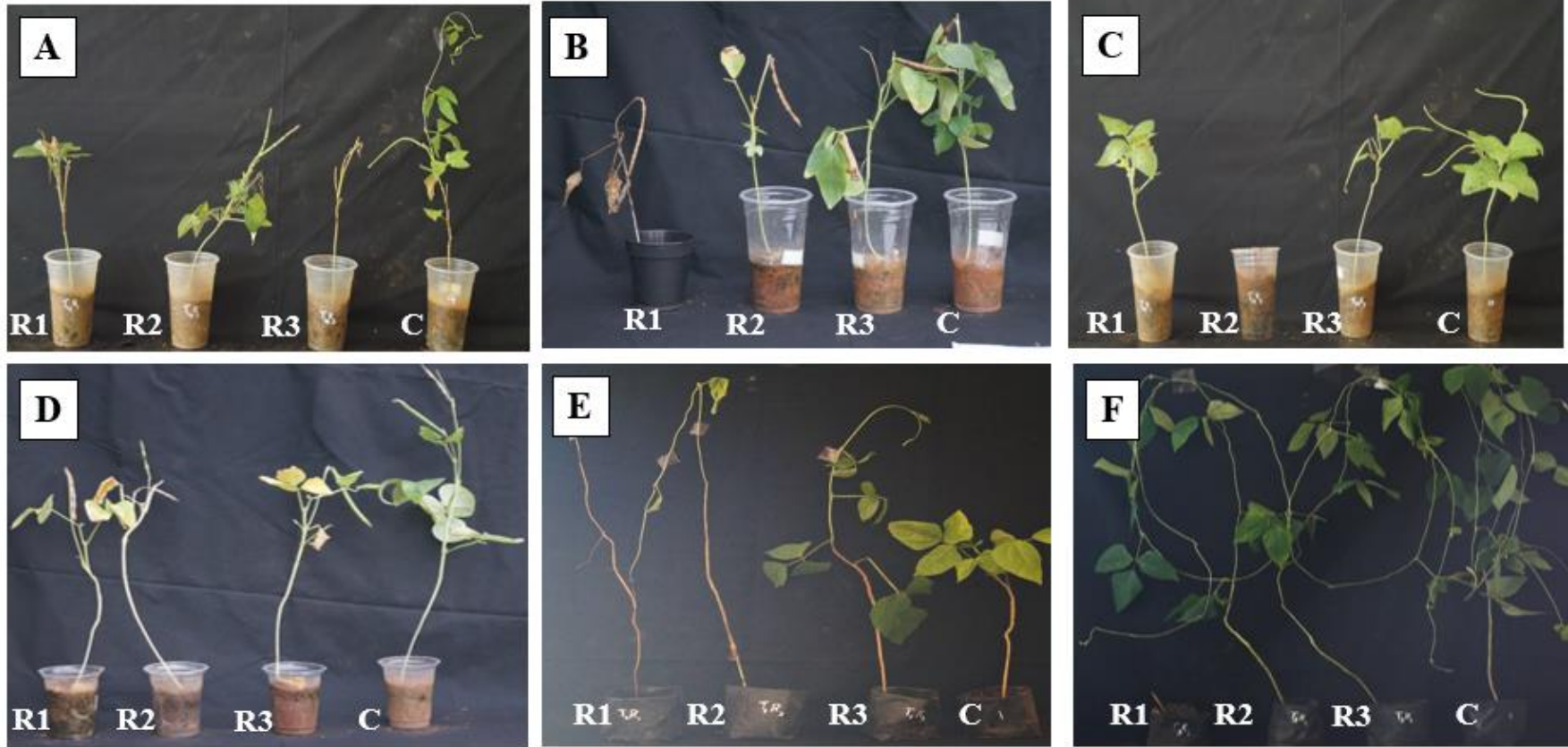


Plate 20 (Cont.). *Fusarium* wilt resistant genotypes (M) Kottarakkara Local, (N) Mavelikkara Local, (O) Wayanad Local II, (P) Muttathkonam Local, (Q) Kochi Local, and (R) Kottayamthattathi Local

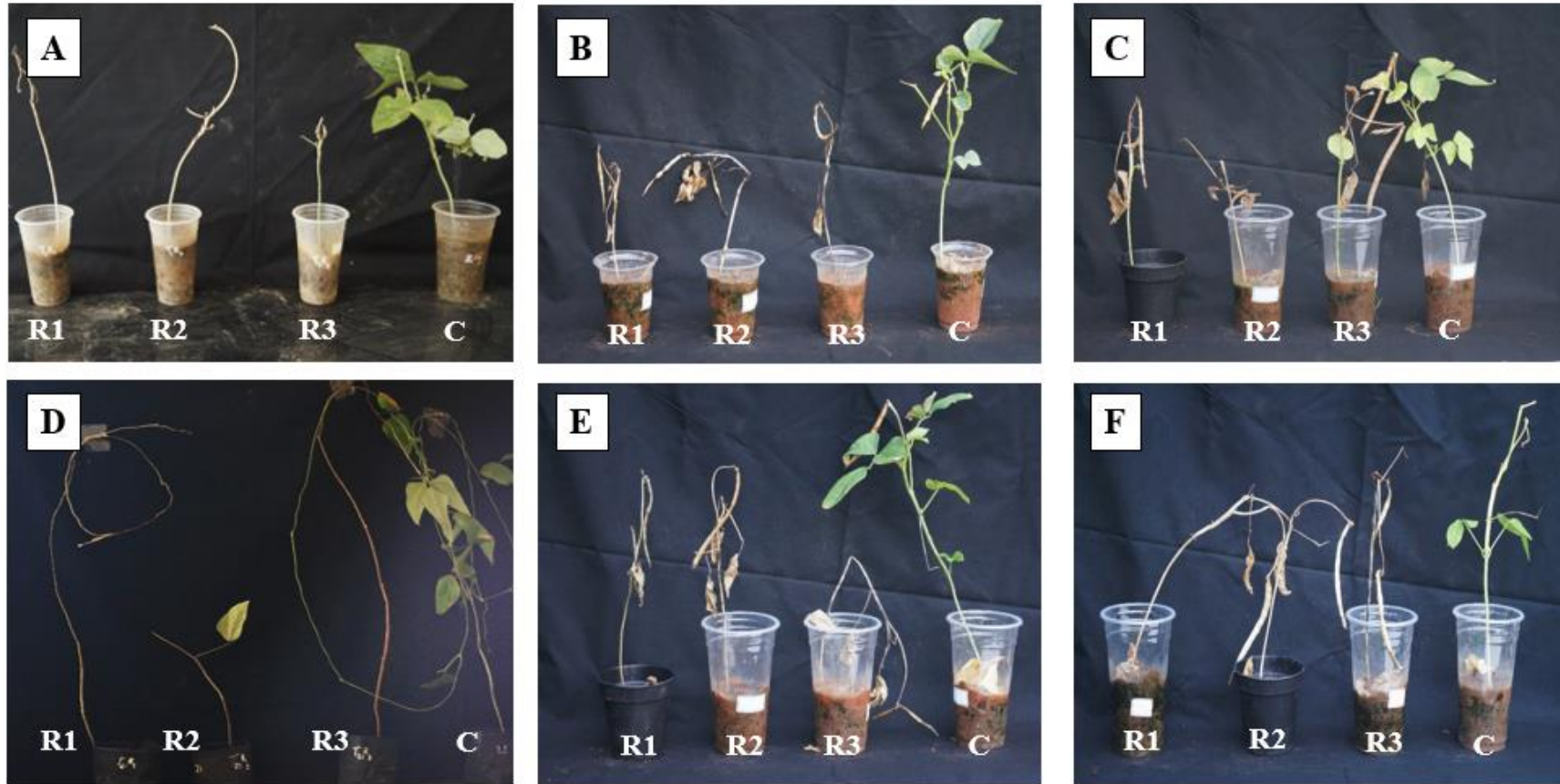


**Plate 21. Fusarium wilt moderately resistant genotypes, (A) TCR 80, (B) Nenmara Local II, (C) VI040951, (D) TCR 81, (E) Peyad Local, and (F) VI041680.**

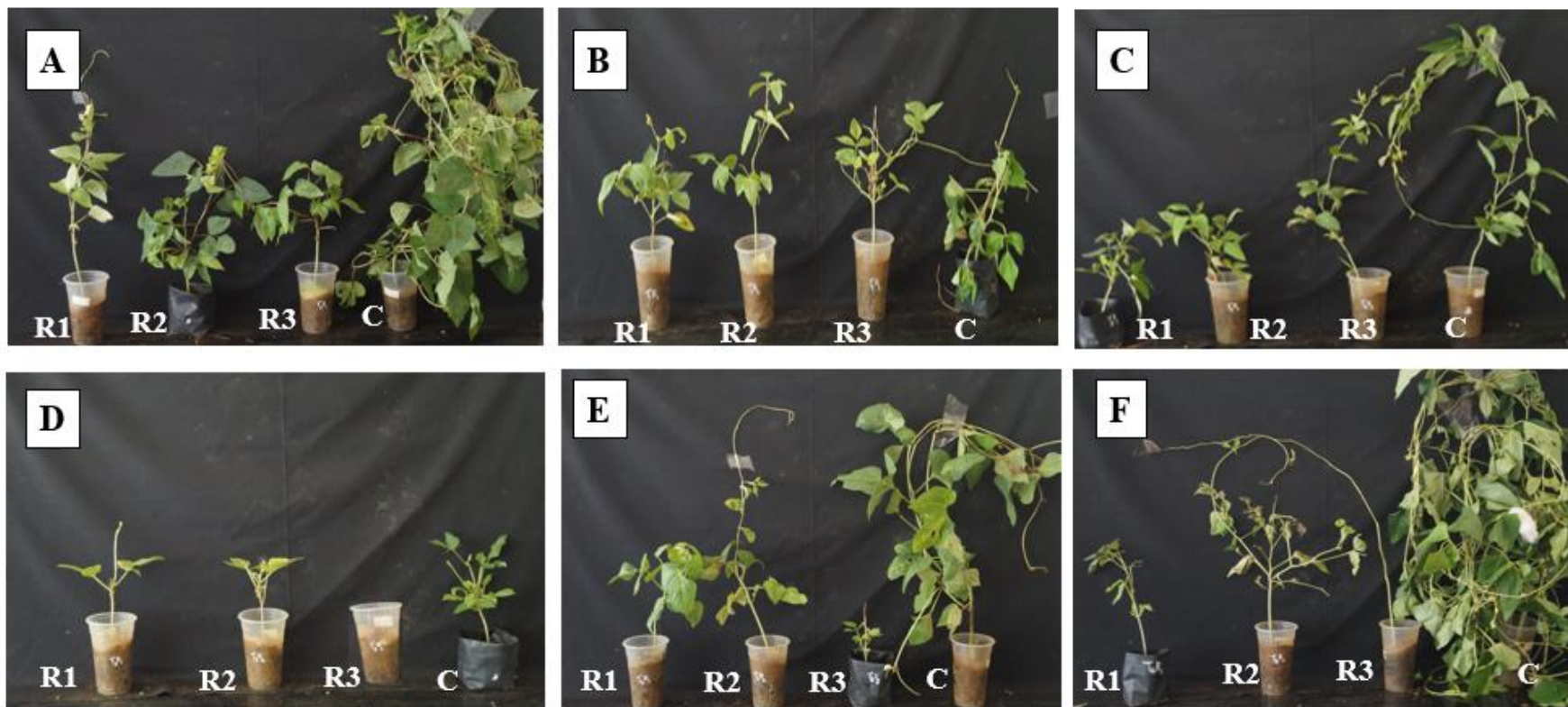




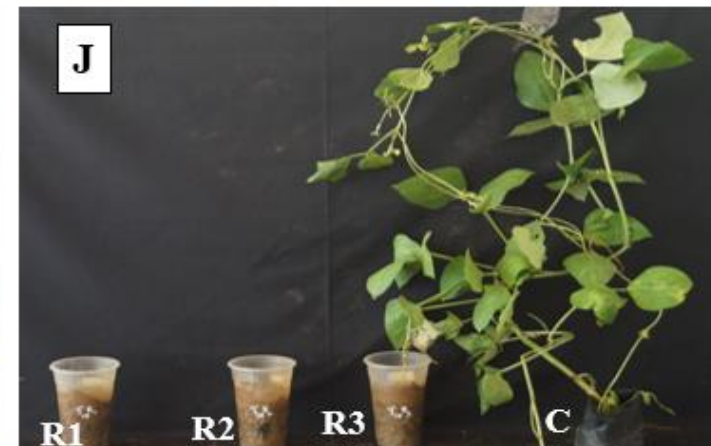
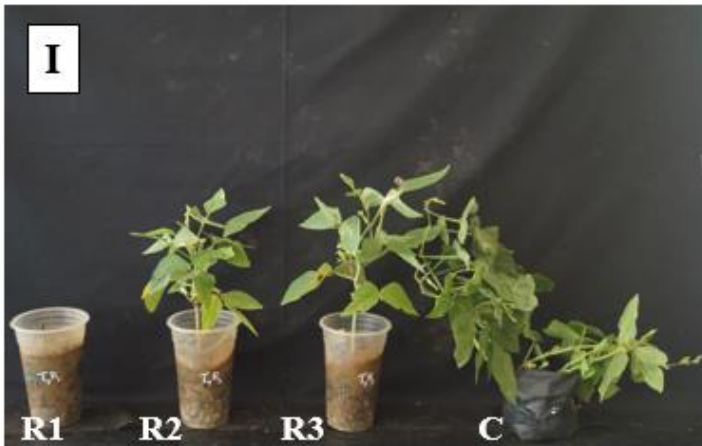
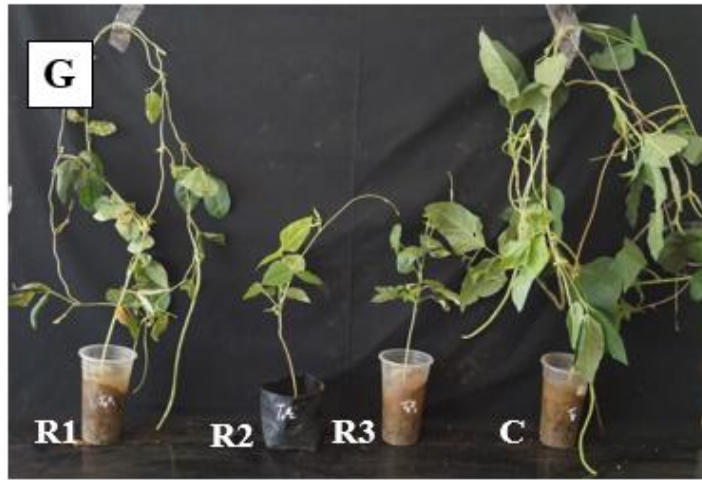
**Plate 23. Fusarium wilt susceptible genotypes (A) TCR 81, (B) TCR 89, (C) VI036720, (D) VI041703-A, (E) Aryanadu Local, (F)Cherthala local.**



**Plate 24. Fusarium wilt highly susceptible genotypes (A) TCR 79, (B) VI001920A, (C) VI036721, (D) Nenmara Local III, (E) VI046648B, (F) VI048488**



**Plate 25. Reaction of KAU released varieties to Fusarium wilt. (A)Malika (MR), (B) Sharika (MR), (C) Vyjayanthi (VS-21-1) (MR), (D) Bhagyalekshmy (S), (E) Lola (MS), and (F) Vellayani Jyothika (MS)**



**Plate 25 (Cont.). Reaction of KAU released varieties to Fusarium wilt (G) Githika (MS), (H) Manjari (MR), (I) Anaswara (MS), and (J) Kanakamony (HS)**

**Table 10. Biometric and quality parameters in Fusarium wilt resistant and susceptible trailing-type cowpea genotypes**

SI No:	Genotypes	Vine length (cm)	Primary branches per plant	Pods per plant	Pod yield per plant (g)	Pod weight (g)	Pod length (cm)	Seeds per pod	Crop duration (days)	Number of harvests	Total phenols (mg/100g)	Crude fibre content (%)	Crude protein content (%)
1	TCR-87 (R)	307.333 <sup>k</sup>	8.500 <sup>b</sup>	22.333 <sup>fg</sup>	267.395 <sup>de</sup>	11.965 <sup>ef</sup>	29.267 <sup>de</sup>	16.717 <sup>defg</sup>	114.778 <sup>ab</sup>	2.667 <sup>abc</sup>	59.000 <sup>c</sup>	18.609 <sup>a</sup>	30.073 <sup>fg</sup>
2	Elamadu Local II (R)	541.833 <sup>c</sup>	6.333 <sup>d</sup>	35.833 <sup>bc</sup>	607.750 <sup>ab</sup>	18.390 <sup>cd</sup>	46.404 <sup>bc</sup>	19.352 <sup>abc</sup>	99.833 <sup>d</sup>	2.667 <sup>abc</sup>	36.120 <sup>j</sup>	13.937 <sup>d</sup>	27.193 <sup>h</sup>
3	Kallicaud Local (R)	647.500 <sup>b</sup>	6.500 <sup>d</sup>	25.220 <sup>ef</sup>	714.058 <sup>ab</sup>	28.568 <sup>a</sup>	66.906 <sup>a</sup>	18.028 <sup>cdef</sup>	105.944 <sup>bcd</sup>	3.000 <sup>ab</sup>	74.753 <sup>a</sup>	9.807 <sup>ij</sup>	28.903 <sup>g</sup>
4	Kanjikuzhi Local (R)	369.000 <sup>ij</sup>	10.333 <sup>a</sup>	26.557 <sup>def</sup>	404.534 <sup>cd</sup>	20.146 <sup>bc</sup>	46.911 <sup>bc</sup>	15.772 <sup>fg</sup>	106.833 <sup>abcd</sup>	2.333 <sup>bcd</sup>	13.493 <sup>l</sup>	10.813 <sup>gh</sup>	30.600 <sup>ef</sup>
5	Koovappally Local (R)	436.500 <sup>fg</sup>	7.833 <sup>bc</sup>	26.167 <sup>def</sup>	633.466 <sup>ab</sup>	22.506 <sup>bc</sup>	55.886 <sup>b</sup>	18.644 <sup>abcd</sup>	108.222 <sup>abcd</sup>	2.333 <sup>bcd</sup>	14.870 <sup>k</sup>	17.197 <sup>b</sup>	34.420 <sup>b</sup>
6	Mavelikkara Local (R)	385.000 <sup>hi</sup>	6.833 <sup>cd</sup>	29.500 <sup>cde</sup>	187.138 <sup>e</sup>	7.753 <sup>f</sup>	23.802 <sup>e</sup>	17.667 <sup>cdef</sup>	117.222 <sup>a</sup>	3.333 <sup>a</sup>	55.620 <sup>d</sup>	8.863 <sup>k</sup>	29.180 <sup>g</sup>
7	Nellanadu Local (R)	312.333 <sup>k</sup>	4.000 <sup>f</sup>	29.833 <sup>cde</sup>	529.347 <sup>bc</sup>	18.854 <sup>cd</sup>	42.358 <sup>c</sup>	16.772 <sup>defg</sup>	97.500 <sup>d</sup>	2.667 <sup>abc</sup>	36.150 <sup>j</sup>	14.713 <sup>c</sup>	31.803 <sup>de</sup>
8	Wayanadu Local I (R)	341.000 <sup>jk</sup>	7.000 <sup>cd</sup>	21.667 <sup>fg</sup>	244.087 <sup>de</sup>	12.001 <sup>ef</sup>	41.389 <sup>c</sup>	14.733 <sup>g</sup>	101.833 <sup>d</sup>	2.000 <sup>cd</sup>	68.793 <sup>b</sup>	18.553 <sup>a</sup>	37.660 <sup>a</sup>
9	Wayanadu Local II (R)	525.500 <sup>cd</sup>	6.833 <sup>cd</sup>	16.500 <sup>g</sup>	283.138 <sup>de</sup>	18.595 <sup>cd</sup>	41.438 <sup>c</sup>	19.306 <sup>abc</sup>	102.722 <sup>d</sup>	1.667 <sup>d</sup>	54.283 <sup>e</sup>	11.240 <sup>fg</sup>	36.647 <sup>a</sup>
10	Muttathkonam Local (R)	414.333 <sup>gh</sup>	5.167 <sup>e</sup>	20.583 <sup>fg</sup>	209.802 <sup>de</sup>	11.848 <sup>ef</sup>	41.319 <sup>c</sup>	20.806 <sup>ab</sup>	113.667 <sup>abc</sup>	2.667 <sup>abc</sup>	51.207 <sup>f</sup>	10.480 <sup>hi</sup>	31.240 <sup>def</sup>
11	Kulashekham Local (R)	327.833 <sup>k</sup>	4.833 <sup>ef</sup>	32.833 <sup>bcd</sup>	204.525 <sup>e</sup>	8.058 <sup>f</sup>	26.792 <sup>e</sup>	16.185 <sup>efg</sup>	103.778 <sup>cd</sup>	3.333 <sup>a</sup>	48.840 <sup>g</sup>	11.403 <sup>fg</sup>	34.183 <sup>b</sup>
12	Kottarakkara Local (R)	475.167 <sup>ef</sup>	4.667 <sup>ef</sup>	16.167 <sup>g</sup>	279.243 <sup>de</sup>	18.721 <sup>cd</sup>	43.451 <sup>c</sup>	20.900 <sup>a</sup>	99.278 <sup>d</sup>	1.667 <sup>d</sup>	51.003 <sup>f</sup>	12.297 <sup>e</sup>	33.160 <sup>bc</sup>
13	Githika (S)	499.083 <sup>de</sup>	9.750 <sup>a</sup>	48.000 <sup>a</sup>	769.013 <sup>a</sup>	24.151 <sup>ab</sup>	57.301 <sup>a</sup>	17.115 <sup>cdef</sup>	101.500 <sup>d</sup>	3.333 <sup>a</sup>	50.247 <sup>f</sup>	8.133 <sup>l</sup>	32.127 <sup>cd</sup>

(Cont.)

14	Vellayani Jyothika (S)	647.667 <sup>b</sup>	8.333 <sup>b</sup>	35.833 <sup>bc</sup>	706.127 <sup>ab</sup>	13.834 <sup>de</sup>	42.138 <sup>c</sup>	17.900 <sup>cdef</sup>	102.500 <sup>d</sup>	3.000 <sup>ab</sup>	43.377 <sup>h</sup>	9.240 <sup>ik</sup>	33.213 <sup>bc</sup>
15	Lola (S)	908.000 <sup>a</sup>	8.500 <sup>b</sup>	37.000 <sup>b</sup>	554.124 <sup>bc</sup>	20.286 <sup>bc</sup>	46.611 <sup>bc</sup>	18.511 <sup>bcde</sup>	100.278 <sup>d</sup>	2.333 <sup>bcd</sup>	40.723 <sup>i</sup>	11.680 <sup>ef</sup>	31.483 <sup>de</sup>
Maximum		908.000	10.333	48.000	769.013	28.568	66.906	20.900	117.222	3.333	74.753	18.609	37.660
Minimum		307.333	4.000	16.167	187.138	7.753	23.802	14.733	97.500	1.666	13.493	8.133	27.193
Mean		475.8722	7.03	28.269	439.583	17.045	42.131	17.894	85.059	2.600	46.565	12.460	32.126
MSE		577.329	0.38	16.078	14255.93	9.223	33.198	2.008	41.888	0.281	0.594	0.166	0.653
SE(d)		19.619	0.503	3.274	97.488	2.48	4.704	1.157	5.284	0.433	0.629	0.333	0.66
SE(m)		13.872	0.356	2.315	68.935	1.753	3.327	0.818	3.737	0.306	0.445	0.236	0.467
CD		40.187	1.031	6.706	199.696	5.079	9.637	2.37	10.825	0.887	1.289	0.682	1.352
CV		5.049	8.771	14.184	27.162	17.817	13.676	7.918	7.609	20.387	1.655	3.273	2.516

(R) Fusarium wilt resistant cowpea genotypes, (S) Fusarium wilt susceptible cowpea genotypes

**Table 11. PCV, GCV, heritability and genetic advance of twelve characters in trailing-type cowpea genotypes**

<b>Characters</b>	<b>Genotypic Variance (Vg)</b>	<b>Phenotypic Variance (Vp)</b>	<b>Environmental Variance (Ve)</b>	<b>PCV (%)</b>	<b>GCV (%)</b>	<b>Heritability (%)</b>	<b>Genetic advance as per cent of mean (%) (i=5%)</b>
Vine length	26473.977	27051.306	577.329	34.562	34.192	97.90	69.679
Primary branches per plant	3.365	3.745	0.380	27.536	26.102	89.90	50.969
Pods per plant	69.233	85.311	16.078	32.674	29.434	81.20	54.623
Pod yield per plant	40463.298	54719.233	14255.935	53.214	45.760	73.90	81.062
Pod weight	32.584	41.807	9.223	37.934	33.489	77.90	60.904
Pod length	105.197	138.395	33.198	27.922	24.344	76.00	43.722
Seeds per pod	2.424	4.432	2.008	11.766	8.701	54.70	13.257
Crop duration	22.314	64.202	41.888	9.420	5.554	34.80	6.745
Number of harvests	0.211	0.492	0.281	26.978	17.667	42.90	23.834
Total phenols	285.875	286.469	0.594	36.348	36.310	99.80	74.721
Crude fibre content	11.628	11.794	0.166	27.553	27.358	98.60	55.960
Crude protein content	7.929	8.582	0.653	9.119	8.765	92.40	17.355

#### ***4.3.1.1. Vine length (cm)***

The vine length varied from 301.33 cm to 908 cm in trailing-type cowpea. The maximum vine length was observed in the genotype Lola ( 908 cm) and the minimum was observed in TCR-87 (301.33 cm). The vine length showed high PCV (34.562%), GCV (34.192%), heritability (97.9%) and genetic advance as per cent of mean (69.679%).

#### ***4.3.1.2. Primary branches per plant***

The maximum number of primary branches was recorded in Kanjikuzhi Local (10.333) which was on par with Githika (9.750). The genotype Nellanadu local recorded a minimum number of primary branches (4.00) and was on par with Kulashekaram Local (4.833) and Kottakarakkal Local (4.667). The trailing type cowpea genotypes recorded high PCV (27.536%) and GCV (26.102%). High heritability (89.9%) with high GA (50.969%) was also recorded for the primary branches per plant.

#### ***4.3.1.3. Pods per plant***

The maximum number of pods per plant was recorded in Githika (48.00) and the minimum in Kottarakkara Local (16.167). The genotypes TCR-87 (22.333), Wayanadu Local I (21.667), Wayanadu Local II (16.500) and Muttathkonam Local (20.583) were on par with Kottarakkara Local for the number of pods per plant. The pods per plant showed high PCV (32.674%), GCV (29.434%), heritability (81.2%) and genetic advance as per cent of mean (54.623) in trailing type cowpea genotypes.

#### ***4.3.1.4. Pod yield per plant (g)***

The highest pod yield per plant was observed in Githika with 769.013g and the lowest in Mavelikkara local with 187.138g. The genotypes Elamadu Local (607.750g), Kallicaud Local (714.058g), Koovappally Local (633.466g) and Vellayani Jyothika (706.127g) were on par with Githika. Whereas, Wayanadu Local I (244.087g), Wayanadu Local II (283.138g), Muttathkonam Local (209.802g), Kulashekaram Local (204.525g) and Kottarakkara Local (279.243g)

were on par with Mevelikkara Local. High PCV (53.214%), GCV (45.760%), heritability (73.9%) and GA as per cent of mean (81.062%) were recorded for pod yield per plant.

#### **4.3.1.5. Pod weight (g)**

The pod weight ranged from 7.753 to 28.568g with a mean value of 17.045g. Mavelikkara Local (7.753g) recorded the minimum pod weight which was on par with TCR-87 (11.965g), Wayanadu Local I (12.001g), Muttathkonam Local (11.848g) and Kulashekham Local (8.058g). The maximum pod weight was observed in Kallicaud Local (28.568g) which was on par with Githika (24.151g). Pod weight recorded high PCV (31.934%), GCV (33.489%) heritability (77.9%) and GA as per cent of mean (60.904%).

#### **4.3.1.6. Pod length (cm)**

The pod length varied from 23.802 to 66.906 cm with a mean value of 42.131 cm. The highest pod length was observed in Kallicaud Local (66.906 cm) which was on par with Githika (57.301 cm). The lowest pod length was recorded in Mavelikkara Local (23.802 cm) which was on par with Kulashekham Local (26.792 cm). Pod length recorded high PCV (27.922%), GCV (24.344%), heritability (76.0%) and GA as per cent mean (43.722%).

#### **4.3.1.7. Seeds per pod**

The seeds per pod in trailing-type cowpeas ranged from 14.733 to 20.900 with a mean of 17.894. The genotype Kottarakkara Local (20.900) had the maximum number of seeds per pod which was significantly on par with Muttathkonam Local (20.806). The genotype Wayanadu Local I (14.733) had the minimum number of seeds per pod which was on par with TCR-87 (16.717), Kanjikuzhi Local (15.77), Nellanadu Local (17.667), and Kulashekham Local (16.187). The seeds per pod showed medium PCV (11.766%), low GCV (8.701%), medium heritability (54.7%) and low GA as per cent of mean (13.257%) in trailing type cowpea genotypes.

#### **4.3.1.8. Crop duration (days)**

The genotype Mavelikkara Local recorded the maximum crop duration (117.222 days) on par with genotypes TCR 87 (114.778 days), Kanjikuzhi Local (106.833 days), Koovappally Local (108.222 days), and Muttathkonam Local (113.667 days). The genotypes Nellanadu Local (97.500 days) recorded the minimum crop duration. Low PCV (9.420%), GCV (5.54%), medium heritability (34.8%) and low GA as per cent of mean (6.745%) were recorded for crop duration.

#### **4.3.1.9. Number of harvests**

The number of harvests varied from 3.333 (Mavelikara Local, Kulashekharan Local, and Githika) to 1.667 (Wayanadu Local II and Kottarakkara Local) with a mean value of 2.600. The number of harvests recorded high PCV (26.978%), GCV (17.667%), medium heritability (42.9%) and high GA as per cent of mean (23.834%).

#### **4.3.1.10. Total phenols (mg/100g)**

The total phenols ranged from 13.497 to 74.753 mg/100g with a mean value of 46.565. The genotype Kallicaud Local (74.753 mg/100g) recorded the maximum phenol content whereas Kanjikuzhi Local (13.497 mg/100g) recorded the minimum total phenols. The total phenols in trailing type cowpea genotypes showed high PCV (36.348%), high GCV (36.310%) high heritability (99.8%), and high GA as per cent of mean (74.721%).

#### **4.3.1.11. Crude fibre content (%)**

The genotype TCR-87 (18.609%) recorded the highest crude fibre content among the trailing type cowpea genotypes, which was on par with Wayanadu Local I (18.553%). The genotype Githika recorded the minimum crude fibre content (8.133%). The crude fibre content in trailing type cowpea genotypes showed high PCV (27.553%), high GCV (27.358%), high heritability (98.6%), and high GA as per cent of the mean (55.960%).

#### ***4.3.1.12. Crude protein content (%)***

The crude protein content ranged from 27.193 to 37.660% with a mean value of 32.126 %. The maximum protein content recorded in the genotype Wayanadu Local I (37.660%) was on par with Wayanadu Local II (36.647 %). The minimum protein content was recorded in Elamadu Local II (27.193%). The crude protein content registered low PCV and GCV (9.119% and 8.765% respectively) and high heritability (92.4%) with medium genetic advance as per cent of mean (17.355%).

#### **4.3.2. Heatmap with hierarchical clustering of trailing-type cowpea genotypes based on biometric and quality parameters**

The hierarchical clustering of the trailing type cowpea genotypes with biometric and quality parameters produced seven clusters (Figure 4). The genotypes TCR-87 and Wayanadu Local I are included in cluster I and Mavelikkara Local, Muttathkonam Local, and Kulashekharam Local in cluster II. Cluster III consisted of two genotypes (Wayanadu Local II and Kottarakkara Local) and cluster IV had two genotypes (Elamadu Local II and Nellanadu Local). Kanjikuzhi Local and Koovappally Local were grouped into cluster V and Kallicaud Local into cluster VI. All the KAU-released varieties Githika, Vellayani Jyothika, and Lola were grouped into cluster VII.

#### **4.3.3. Genotypic and phenotypic correlation among twelve characters in trailing type cowpea genotypes**

The genotypic and phenotypic correlations among the twelve characters in trailing type cowpea genotypes are presented in Tables 12 and 13 respectively, and only significant correlations are discussed below.

##### ***4.3.3.1. Genotypic correlation among twelve characters in trailing type cowpea genotypes***

The pod yield per plant showed a significant and positive correlation with vine length (0.573), primary branches per plant (0.401), pod per plant (0.651), pod

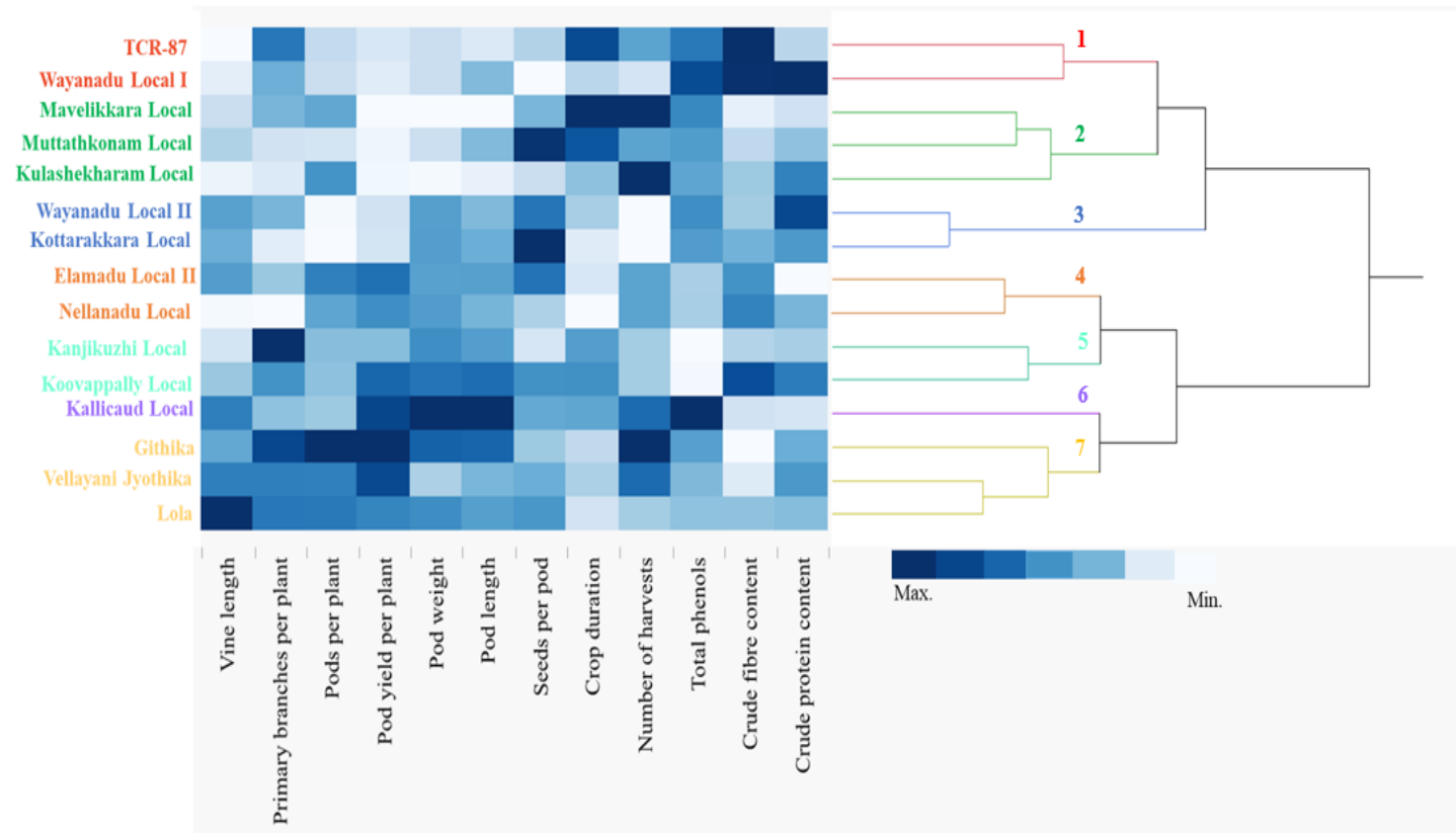


Figure 4. Heatmap with hierarchical clustering of trailing-type cowpea genotypes based on biometric and quality parameters

**Table 12. The genotypic correlation coefficient of twelve characters in trailing-type cowpea genotypes**

Characters	Vine length	Primary branches per plant	Pods per plant	Pod yield per plant	Pod weight	Pod length	Seeds per pod	Crop duration	Number of harvests	Total phenols	Crude fibre content	Crude protein content
Vine length	1											
Primary branches per plant	0.264	1										
Pods per plant	0.341*	0.399*	1									
Pod yield per plant	0.573**	0.401*	0.651**	1								
Pod weight	0.494**	0.279*	0.17	0.769**	1							
Pod length	0.492**	0.078	-0.143	0.626**	0.855**	1						
Seeds per pod	0.43*	-0.408*	-0.291*	0.069	0.199	0.275*	1					
Crop duration	-0.419*	0.235	-0.34*	-0.649**	-0.659**	-0.514**	-0.054	1				
Number of harvests	-0.072	0.122	0.773**	0.272*	-0.274*	-0.362*	-0.402*	0.371*	1			
Total phenols	0.039	-0.284*	-0.223	-0.262	-0.185	-0.149	-0.041	0.157	0.134	1		
Crude fibre content	-0.393*	-0.103	-0.386*	-0.217	-0.132	0.037	-0.297*	-0.001	-0.521**	-0.065	1	
Crude protein content	-0.156	-0.081	-0.329*	-0.283*	-0.162	-0.093	-0.219	-0.409*	-0.605**	0.079	0.274*	1

\*\* Significant @ 1% \* Significant @ 5%

**Table 13. The phenotypic correlation coefficient of twelve characters in trailing-type cowpea genotypes**

Characters	Vine length	Primary branches per plant	Pods per plant	Pod yield per plant	Pod weight	Pod length	Seeds per pod	Crop duration	Number of harvests	Total phenols	Crude fibre content	Crude protein content
Vine length	1											
Primary branches per plant	0.247	1										
Pods per plant	0.312*	0.362*	1									
Pod yield per plant	0.486**	0.329*	0.600**	1								
Pod weight	0.415*	0.247	0.151	0.725**	1							
Pod length	0.424*	0.063	-0.07	0.591**	0.781**	1						
Seeds per pod	0.321*	-0.266	-0.214	-0.034	0.164	0.203	1					
Crop duration	-0.262	0.115	-0.192	-0.218	-0.195	-0.138	0.072	1				
Number of harvests	-0.062	0.037	0.582**	0.257	-0.084	-0.242	-0.166	0.268*	1			
Total phenols	0.038	-0.268*	-0.201	-0.229	-0.166	-0.136	-0.017	0.091	0.093	1		
Crude fibre content	-0.382*	-0.096	-0.358*	-0.189	-0.132	0.024	-0.235	-0.023	-0.336*	-0.066	1	
Crude protein content	-0.149	-0.057	-0.263	-0.209	-0.148	-0.068	-0.165	-0.178	-0.343*	0.077	0.255	1

\*\* Significant @ 1% \* Significant @ 5%

weight (0.769), pod length (0.626), number of harvests (0.272) and crop duration (-0.649) and crude protein content (-0.283) which recorded a significant negative correlation with pod yield per plant.

The vine length registered a significant and positive correlation with pods per plant (0.341), pod weight (0.494), pod length (0.492), and seeds per pod (0.430), and a negative significant correlation with crop duration (-0.419) and crude fibre content (-0.393). The primary branches per plant recorded a positive significant correlation with pods per plant (0.399), and pod weight (0.279), and a negative significant correlation with seeds per pod (-0.408), and total phenols (-0.284).

The positive and significant correlation between pods per plant and the number of harvests (0.773) and the significant negative correlation between pods per plant and seeds per pod (-0.291), crop duration (-0.340), crude fibre content (-0.386) and crude protein content (-0.329) were recorded. The pod weight showed a positive and significant correlation with pod length (0.855) and a significant negative correlation with crop duration (-0.659).

A negative and significant correlation was observed between seeds per pod, the number of harvests (-0.402) and crude fibre content (-0.279). The crop duration showed a positive correlation with the number of harvests (0.371) and a negative correlation with crude protein content (-0.409). The number of harvests was negatively correlated with crude fibre content (-0.521) and crude protein content (-0.605). A positive correlation was observed between crude fibre content and crude protein content (0.274).

#### ***4.3.3.2. Phenotypic correlation among twelve characters in trailing type cowpea genotypes***

The pod yield per plant was significantly and positively correlated with vine length (0.486), primary branches per plant (0.329), pods per plant (0.600), pod weight (0.725), and pod length (0.591). The vine length showed a positive correlation with pods per plant (0.312), pod weight (0.415), pod length (0.424), and seeds per pod (0.321) and a negative correlation with crude fibre content (-0.382).

The primary branches per plant showed a positive and significant correlation with pods per plant (0.362) and a negative correlation with total phenols (-0.268). The pods per plant showed a positive correlation with the number of harvests (0.582) and a negative correlation with crude fibre content (-0.358). A positive and significant correlation was observed between pod weight and pod length (0.781) and crop duration and the number of harvests (0.268). The number of harvests recorded a significant negative correlation with crude fibre content (-0.336) and (-0.343).

#### **4.3.4. Path coefficient analysis using twelve characters in trailing type cowpea genotypes**

Path coefficient analysis splits the genotypic correlation coefficient into direct and indirect effects. The direct and indirect effects of twelve characters of trailing type cowpea genotypes on pod yield per plant are presented in Table 14.

The vine length showed a negative direct effect on pod yield per plant (-0.676). A negative indirect effect through primary branches per plant (-0.178), pods per plant (-0.23), pod weight (-0.334), pod length (-0.333), seeds per pod (-0.291) and a positive indirect effect through crop duration (0.283), crude fibre content (0.265) and crude protein content (0.106) was observed.

The primary branches per plant recorded a positive direct effect on pod yield per plant (0.305), a positive indirect effect through pods per plant (0.122) and a negative indirect effect through seeds per pod (-0.124). A positive direct effect of pods per plant (1.591) was observed on pod yield per plant. The indirect and positive effects of pods per plant through vine length (0.542), primary branches per plant (0.635), pod weight (0.271) and the number of harvests (1.229) were also observed. The indirect and negative effects of pods per plant through pod length (-0.227), seeds per pod (-0.463), crop duration (-0.541), total phenols (-0.335), crude fibre content (-0.614), and crude protein content (-0.523) were recorded.

The pod weight (0.115) and pod length (1.224) showed a positive direct effect on pod yield per plant. The positive indirect effect of pod length through vine

**Table 14. The direct and indirect effects of twelve characters in trailing-type cowpea genotypes**

Characters	Vine length	Primary branches per plant	Pods per plant	Pod weight	Pod length	Seeds per pod	Crop duration	Number of harvests	Total phenols	Crude fibre content	Crude protein content	Genotypic Correlation with the main variable
Vine length	<b>-0.676</b>	0.08	0.542	0.057	0.602	0.463	-0.182	-0.026	0.012	-0.177	-0.121	0.573**
Primary branches per plant	-0.178	<b>0.305</b>	0.635	0.032	0.095	-0.439	0.102	0.045	-0.087	-0.047	-0.063	0.401*
Pods per plant	-0.23	0.122	<b>1.591</b>	0.02	-0.175	-0.313	-0.147	0.282	-0.068	-0.174	-0.255	0.651**
Pod weight	-0.334	0.085	0.271	<b>0.115</b>	1.046	0.214	-0.286	-0.100	-0.056	-0.06	-0.126	0.769**
Pod length	-0.333	0.024	-0.227	0.098	<b>1.224</b>	0.296	-0.223	-0.132	-0.045	0.017	-0.072	0.626**
Seeds per pod	-0.291	-0.124	-0.463	0.023	0.337	<b>1.075</b>	-0.023	-0.147	-0.013	-0.134	-0.17	0.069
Crop duration	0.283	0.072	-0.541	-0.076	-0.629	-0.058	<b>0.434</b>	0.136	0.048	-0.001	-0.317	-0.649**
Number of harvests	0.049	0.037	1.229	-0.031	-0.443	-0.432	0.161	<b>0.365</b>	0.041	-0.235	-0.47	0.272*
Total phenols	-0.026	-0.087	-0.355	-0.021	-0.182	-0.044	0.068	0.049	<b>0.305</b>	-0.029	0.061	-0.262
Crude fibre content	0.265	-0.031	-0.614	-0.015	0.045	-0.32	-0.001	-0.190	-0.02	<b>0.451</b>	0.213	-0.217
Crude protein content	0.106	-0.025	-0.523	-0.019	-0.113	-0.235	-0.177	-0.221	0.024	0.124	<b>0.777</b>	-0.283*

**R= 0.1189**

length (0.602) and seeds per pod (0.337) and a negative indirect effect through crop duration (-0.624), and number of harvests (-0.443) were observed. A positive direct effect of seeds per pod on pod yield per plant was recorded (1.075). Indirect positive effects of seeds per pod through vine length (0.463), and pod weight (0.269) and indirect negative effects through primary branches per plant (-0.439), pods per plant (-0.313), number of harvests (-0.432), crude fibre content (-0.320), and crude protein content (-0.235) were also observed.

Crop duration showed a positive direct effect on pod yield per plant (0.434). The negative indirect effect of crop duration through vine length (-0.182), pod weight (-0.286) pod length (-0.223), and seeds per pod (-0.142) was observed. The direct positive effect of the number of harvests on pod yield per plant (0.365) and an indirect positive effect through pods per plant (0.282) were also recorded. The direct positive effect of total phenols (0.305), crude fibre content (0.451), and crude protein content (0.777) on pod yield per plant was recorded. The overall residual effect of the path coefficient analysis was 0.1189.

#### **4.3.5. Evaluation of resistant and susceptible bush-type cowpea genotypes under field condition**

The observations on biometric and quality parameters of resistant and susceptible bush-type cowpea genotypes are given in Table 15.

##### **4.3.5.1. Vine length (cm)**

The vine length varied from 32.167 cm to 190 cm with a mean value of 118.119 cm. The genotype VI064556 (IRON) recorded the maximum vine length and VI046653 recorded the minimum vine length, which was on par with VI034386 (36.667 cm).

##### **4.3.5.2. Primary branches per plant**

The genotype VI046653 reported the maximum (13.500) number of primary branches per plant and Kottayamthattathi Local recorded the minimum (5.167) number of primary branches per plant. The genotypes Kochi Local (6.000), and

**Table 15. Biometric and quality parameters in Fusarium wilt resistant and susceptible bush-type cowpea genotypes**

SI No.	Genotypes	Vine length (cm)	Primary branches per plant	Pods per plant	Pod yield per plant (g)	Pod weight (g)	Pod length (cm)	Seeds per pod	Crop duration (days)	Number of harvests	Total phenols (mg/100g)	Crude fibre content (%)	Crude protein content (%)
1	VI034386 (R)	36.667 <sup>c</sup>	7.500 <sup>c</sup>	22.111 <sup>e</sup>	198.741 <sup>d</sup>	6.143 <sup>ab</sup>	16.787 <sup>b</sup>	13.583 <sup>c</sup>	83.556 <sup>c</sup>	3.333	64.007 <sup>b</sup>	15.190 <sup>b</sup>	29.163 <sup>a</sup>
2	VI046653 (R)	32.167 <sup>e</sup>	13.500 <sup>a</sup>	31.333 <sup>cd</sup>	306.727 <sup>b</sup>	6.368 <sup>ab</sup>	18.862 <sup>a</sup>	16.559 <sup>b</sup>	85.222 <sup>bc</sup>	2.667	28.887 <sup>e</sup>	15.413 <sup>b</sup>	26.823 <sup>b</sup>
3	VI048533 (R)	107.500 <sup>d</sup>	6.500 <sup>d</sup>	45.000 <sup>a</sup>	242.727 <sup>c</sup>	4.648 <sup>cd</sup>	16.953 <sup>b</sup>	14.288 <sup>c</sup>	87.000 <sup>bc</sup>	2.333	45.627 <sup>d</sup>	16.907 <sup>a</sup>	28.303 <sup>a</sup>
4	VI064556 (IRON) (R)	190.000 <sup>a</sup>	10.500 <sup>b</sup>	42.750 <sup>a</sup>	361.411 <sup>a</sup>	6.770 <sup>a</sup>	17.057 <sup>b</sup>	17.636 <sup>a</sup>	87.667 <sup>b</sup>	3.667	63.373 <sup>b</sup>	17.337 <sup>a</sup>	29.377 <sup>a</sup>
5	Kochi Local (R)	155.833 <sup>b</sup>	6.000 <sup>de</sup>	39.611 <sup>ab</sup>	263.138 <sup>c</sup>	5.499 <sup>bc</sup>	17.261 <sup>b</sup>	17.708 <sup>a</sup>	83.333 <sup>c</sup>	3.000	28.373 <sup>e</sup>	13.560 <sup>c</sup>	29.183 <sup>a</sup>
6	Kottayamthattathi Local (R)	120.000 <sup>c</sup>	5.167 <sup>e</sup>	27.667 <sup>de</sup>	181.686 <sup>d</sup>	4.111 <sup>d</sup>	16.229 <sup>b</sup>	14.297 <sup>c</sup>	83.922 <sup>bc</sup>	2.667	67.623 <sup>a</sup>	12.283 <sup>d</sup>	28.323 <sup>a</sup>
7	Kanakamony (S)	184.667 <sup>a</sup>	5.667 <sup>de</sup>	35.333 <sup>bc</sup>	247.902 <sup>c</sup>	4.950 <sup>cd</sup>	17.188 <sup>b</sup>	16.819 <sup>b</sup>	95.667 <sup>a</sup>	3.333	47.493 <sup>c</sup>	12.007 <sup>d</sup>	26.840 <sup>b</sup>
	Maximum	190.000	13.500	45.000	361.411	6.770	18.862	17.708	95.667	3.667	67.623	17.337	29.377

(Cont.)

Minimum	32.167	5.167	22.111	181.686	4.111	16.229	13.583	83.333	2.667	28.373	12.007	26.823
Mean	118.119	7.833	34.829	257.476	5.498	17.191	15.841	86.623	3.000	49.340	15.099	27.858
MSE	35.25	0.264	12.103	298.057	0.346	0.528	0.186	5.092	0.381	0.693	0.152	0.525
SE(d)	4.848	0.419	2.841	14.096	0.48	0.594	0.353	1.842	0.504	0.68	0.319	0.592
SE(m)	3.428	0.297	2.009	9.968	0.34	0.42	0.249	1.303	0.356	0.481	0.225	0.418
CD	10.562	0.914	6.189	2.179	1.046	1.293	0.768	4.014	NA	1.481	0.694	1.289
CV	5.026	6.558	9.988	6.705	10.698	4.229	2.725	2.605	20.574	1.687	2.66	2.562

(R) Fusarium wilt resistant cowpea genotypes, (S) Fusarium wilt susceptible cowpea genotypes

Kanakamony (5.667) were observed to be on par with Kottayamthattathil Local for the number of primary branches per plant.

#### ***4.3.5.3. Pods per plant***

The pods per plant ranged from 22.111 to 45.00 with a mean value of 34.829. The genotype VI048533 recorded the maximum (45.00) pods per plant which was on par with VI064556 (IRON) (42.750), and Kochi Local (39.611). The minimum pods per plant were recorded in the genotype VI034386 (22.111).

#### ***4.3.5.4. Pod yield per plant (g)***

The genotype VI064556 (IRON) recorded the maximum pod yield per plant (361.411g) and the minimum pod yield per plant in the genotype Kottayamthattathi Local (181.686g).

#### ***4.3.5.5. Pod weight (g)***

The maximum pod weight was recorded in genotype VI046653 (18.862g) which was on par with VI034386 (6.143g), and VI046653 (6.368g). The minimum pod weight was reported in the genotype Kottayamthattathi Local (4.11g) which was on par with VI048533 (4.648g).

#### ***4.3.5.6. Pod length***

The pod length ranged from 16.229 cm (Kottayamthattathi Local) to 18.862 cm (VI046653) with a mean value of 17.191 cm.

#### ***4.3.5.7. Seeds per pod***

The maximum number of seeds per pod was observed in Kochi Local (17.708) and the minimum number of seeds per pod in VI034386 (13.583). The genotypes VI048533 (14.288), and Kottayamthattathi Local (14.297) were on par with VI034386 in the number of seeds per pod.

#### **4.3.5.8. Crop duration (days)**

The crop duration was maximum in Kanakamony (95.667) among the bush-type cowpea genotypes. The minimum crop duration was observed in Kochi Local (83.333) which was on par with VI046653 (85.222), VI048533 (87.00), VI064556 (IRON) (87.667) and Kottayamthattathi Local (83.922).

#### **4.3.5.9. Number of harvests**

The maximum number of harvests was observed in VI064556 (IRON) (3.667) and the minimum number of harvests in VI046653 (2.667) and Kottayamthattathi Local (2.667). There was no significant difference among the bush-type cowpea genotypes for the number of harvests.

#### **4.3.5.10. Quality parameters**

The genotype Kottayamthattathi Local (67.623) registered the highest phenol content and Kochi Local (28.373) had the lowest phenol content. The crude fibre content ranged from 12.007 to 17.337% with a mean value of 15.099%. The maximum crude fibre content was reported in VI064556 (IRON) (17.337%) and the minimum was recorded in Kanakamony (12.007%). The crude protein content ranged from 26.823 to 29.377% with a mean value of 27.8585. The genotype VI046653 recorded the minimum crude protein content (26.823%) and VI064556 (IRON) (29.377%) reported the maximum crude protein content among the bush-type cowpea genotypes.

#### **4.3.6. Heatmap with hierarchical clustering of bush-type cowpea genotypes based on biometric and quality parameters**

The hierarchical clustering of bush-type cowpea genotypes using biometric and quality parameters grouped them into five clusters. Cluster I consisted of the genotypes VI034386 and Kottayamthattathi Local, and cluster II included the genotype VI046653. The genotypes VI048533 and Kochi Local are grouped into cluster III and the KAU released variety Kanakamony is in cluster IV. Cluster V consisted of the genotype VI064556 (IRON) (Figure 5).

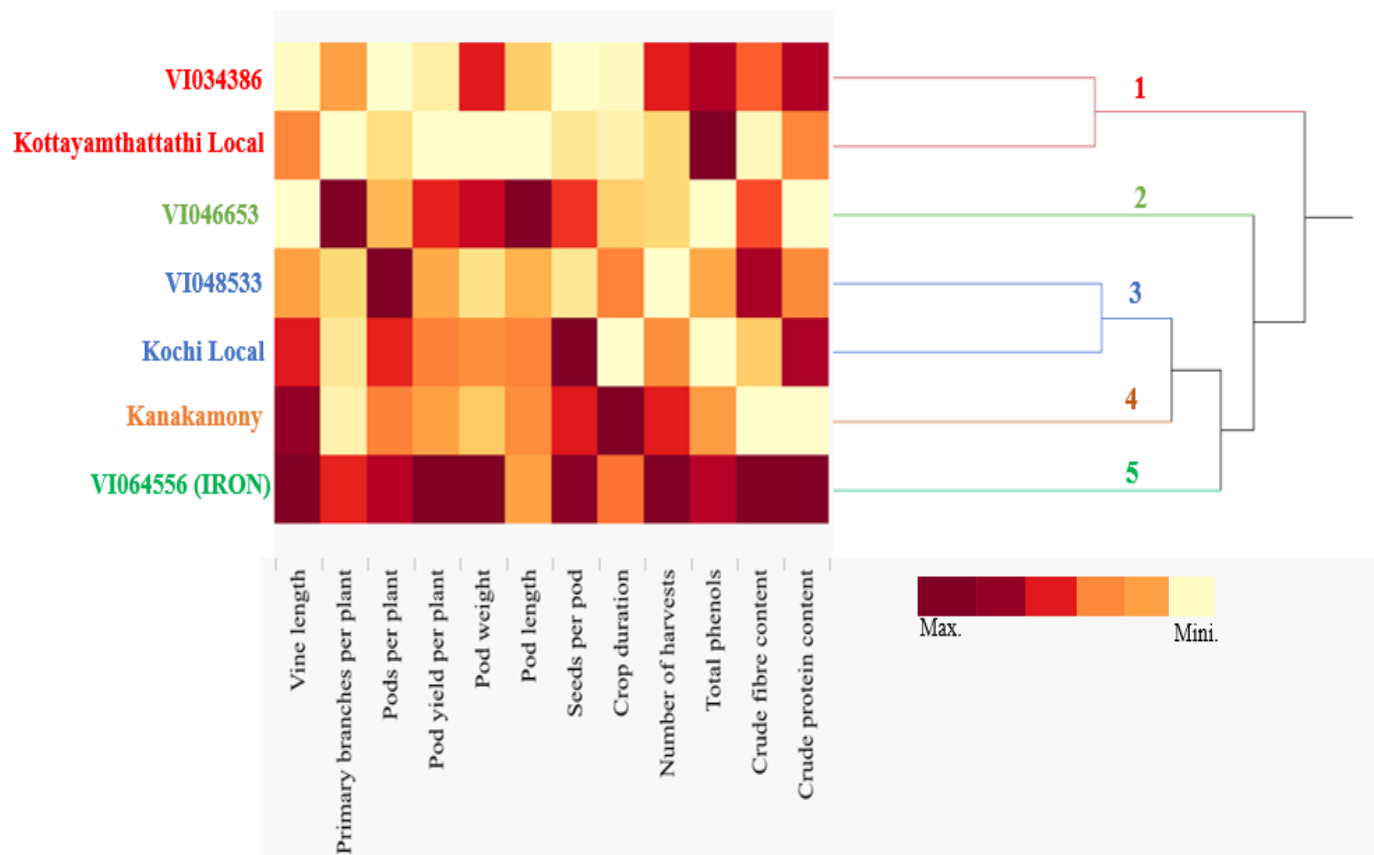


Figure 5. Heatmap with hierarchical clustering of bush-type cowpea genotypes based on biometric and quality parameters

#### **4.3.7. Genetic parameters of the twelve characters in bush-type cowpea genotypes**

The results of phenotypic variance ( $V_p$ ), genotypic variance ( $V_g$ ), environmental variance ( $V_e$ ), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad sense heritability ( $h^2$ ) and genetic advance as per cent of mean (GA) for all the twelve characters of bush-type cowpea genotypes are given in Table 16.

The phenotypic coefficient of variation (PCV) ranged from 4.353% to 54.972% using twelve characters of bush-type cowpea genotypes. High PCV was observed for the characters such as vine length (54.972%), primary branches per plant (39.462), pods per plant (25.221%), pod yield per plant (24.541%), number of harvests (23.006%), and total phenols (33.346%). The medium PCV was shown by the characters' pod weight (19.812%), seeds per pod (11.179%), and crude fibre content (14.610%). Characters like pod length (5.852%), crop duration (5.433%) and crude protein content (4.353%) showed a low phenotypic coefficient of variation.

The genotypic coefficient of variation (GCV) recorded a range of 3.519% to 54.727%. High GCV was observed for the characters' vine length (54.742%), primary branches per plant (38.913%), pods per plant (23.159%), pod yield per plant (23.607%), and total phenols (33.302%), medium GCV was recorded for the characters pod weight (16.676%), seeds per pod (10.842%), number of harvests (10.292%), and crude fibre content (14.366%), and the characters such as pod length (4.047%), crop duration (4.767%) and crude protein content (3.519%) showed a low genotypic coefficient of variation.

The high broad sense heritability and high genetic advance as per cent of mean were observed for the characters such as vine length (99.2% and 112.296%), primary branches per plant (97.2% and 79.047%), seeds per pod (84.3% and 112.296%), pod yield per plant (92.5% and 46.780%), pod weight (70.8% and 28.913%), seeds per pod (94.1% and 21.662%), total phenols (99.7% and

**Table 16. PCV, GCV, heritability and genetic advance of twelve characters in bush-type cowpea genotypes**

<b>Characters</b>	<b>Genotypic Variance (Vg)</b>	<b>Phenotypic Variance (Vp)</b>	<b>Environmental Variance (Ve)</b>	<b>PCV (%)</b>	<b>GCV (%)</b>	<b>Heritability (%)</b>	<b>Genetic advance as per cent of mean (%) (i=5%)</b>
Vine length	4180.988	4216.23	35.250	54.972	54.742	99.2	112.296
Primary branches per plant	9.292	9.556	0.264	39.462	38.913	97.2	79.047
Pods per plant	65.062	77.165	12.103	25.221	23.159	84.3	43.807
Pod yield per plant	3694.515	3992.57	298.057	24.541	23.607	92.5	46.780
Pod weight	0.841	1.187	0.346	19.812	16.676	70.8	28.913
Pod length	0.484	1.012	0.528	5.852	4.047	47.8	5.765
Seeds per pod	2.950	3.136	0.186	11.179	10.842	94.1	21.662
Crop duration	17.054	22.146	5.092	5.433	4.767	77.0	8.618
Number of harvests	0.095	0.476	0.381	23.006	10.292	20.0	9.485
Total phenols	270.013	270.70	0.693	33.346	33.303	99.7	68.517
Crude fibre content	4.442	4.594	0.152	14.610	14.366	96.7	29.101
Crude protein content	0.991	1.516	0.525	4.353	3.519	65.4	5.861

68.517%), and crude fibre content (96.7% and 29.101%). High heritability and low genetic advance as per cent of mean were reported for the characters like crop duration (77.0% and 8.618%), pod length (47.8% and 5.765%) and crude protein content (65.6% and 5.861%) whereas, low heritability and low genetic advance as per cent of mean were observed for the number of harvests (20.0% and 9.485%) respectively.

#### **4.3.8. Genotypic and phenotypic correlation among twelve characters in bush-type cowpea genotypes**

The genotypic and phenotypic correlations among the twelve characters in bush-type cowpea genotypes are presented in Tables 17 and 18 respectively, and only significant correlations are discussed below.

##### ***4.3.8.1. Genotypic correlation among twelve characters in bush-type cowpea genotypes***

A positive and significant correlation was observed between pod yield per plant with primary branches per plant (0.728), pods per plant (0.605), pod weight (0.785), pod length (0.635), seeds per pod (0.795), number of harvests (0.633) and crude fibre content (0.587). Vine length in bush-type cowpea was positively and significantly correlated with pods per plant (0.643), seeds per pod (0.600), crop duration (0.576), and the number of harvests (0.644) and negatively correlated with pod length (-0.438).

Primary branches per plant had a positive correlation with pod weight (0.833), pod length (0.932), and crude fibre content (0.574). The pods per plant showed a positive and significant correlation with seeds per pod (0.542) and crude fibre content (0.451), whereas pod weight showed a significantly positive correlation with pod length (0.737), seeds per pod (0.463), number of harvests (0.809) and crude fibre content (0.627).

The pod length was positively and significantly correlated with seeds per pod (0.511) and negatively correlated with total phenols (-0.886) and crude protein content (-0.694). The seeds per pod had a positive correlation with the number of

**Table 17. The genotypic correlation coefficient of twelve characters in bush-type cowpea genotypes**

Characters	Vine length	Primary branches per plant	Pods per plant	Pod yield per plant	Pod weight	Pod length	Seeds per pod	Crop duration	Number of harvests	Total phenols	Crude fibre content	Crude protein content
Vine length	1											
Primary branches per plant	-0.402	1										
Pods per plant	0.643*	0.016	1									
Pod yield per plant	0.325	0.728**	0.605*	1								
Pod weight	-0.185	0.833**	-0.020	0.785**	1							
Pod length	-0.438*	0.932**	0.071	0.635*	0.737**	1						
Seeds per pod	0.600*	0.322	0.542*	0.795**	0.463*	0.511*	1					
Crop duration	0.576*	-0.133	0.319	0.209	-0.177	0.049	0.356	1				
Number of harvests	0.644*	0.154	-0.216	0.633*	0.809**	-0.229	0.644*	0.502*	1			
Total phenols	0.115	-0.282	-0.347	-0.308	-0.128	-0.886**	-0.51*	-0.001	0.565*	1		
Crude fibre content	-0.200	0.574*	0.451*	0.587*	0.627*	0.259	-0.014	-0.249	0.072	0.029	1	
Crude protein content	0.183	-0.230	0.105	0.007	0.275	-0.694**	-0.082	-0.609*	0.309	0.435*	0.420*	1

\*\* Significant @ 1% \* Significant @ 5%

**Table 18. The phenotypic correlation coefficient of twelve characters in bush-type cowpea genotypes**

Characters	Vine length	Primary branches per plant	Pods per plant	Pod yield per plant	Pod weight	Pod length	Seeds per pod	Crop duration	Number of harvests	Total phenols	Crude fibre content	Crude protein content
Vine length	1											
Primary branches per plant	-0.388	1										
Pods per plant	0.577*	-0.005	1									
Pod yield per plant	0.304	0.686**	0.564*	1								
Pod weight	-0.155	0.714**	0.023	0.618*	1							
Pod length	-0.294	0.660*	0.082	0.484*	0.311	1						
Seeds per pod	0.581*	0.320	0.485*	0.731**	0.379	0.397	1					
Crop duration	0.489*	-0.147	0.263	0.174	-0.07	0.047	0.240	1				
Number of harvests	0.297	0.060	0.011	0.203	0.467*	-0.076	0.292	0.192	1			
Total phenols	0.113	-0.278	-0.31	-0.294	-0.111	-0.594*	-0.489*	0.341	0.237	1		
Crude fibre content	-0.191	0.572*	0.384	0.552*	0.514*	0.197	-0.016	-0.214	-0.023	0.03	1	
Crude protein content	0.144	-0.200	0.125	0.025	0.150	-0.402	-0.078	-0.484*	0.366	0.348	0.295	1

\*\* Significant @ 1% \* Significant @ 5%

harvests (0.644) and a negative correlation with total phenols (-0.510). A significant positive correlation was observed between crop duration and the number of harvests (0.502) and a negative correlation was observed between crop duration and crude protein content (-0.609). The number of harvests had a positive correlation with total phenols (0.565), and total phenols (0.435) and crude fibre content (0.420) had a positive correlation with crude protein content.

#### ***4.3.8.2. Phenotypic correlation among twelve characters in bush-type cowpea genotypes***

The pod yield per plant was phenotypically correlated with primary branches per plant (0.686), pods per plant (0.564), pod weight (0.618), pod length (0.484), seeds per pod (0.731), and crude fibre content (0.552). The vine length was positively and significantly correlated with pods per plant (0.577), seeds per pod (0.581), and crop duration (0.489). The primary branches per plant were positively correlated with pod weight (0.714), and crude fibre content (0.572). A positive and significant correlation was observed between pods per plant and seeds per pod (0.485), and pod weight with the number of harvests (0.467) and crude fibre content (0.514).

A significant negative correlation was observed between pod length and total phenols (-0.594), seeds per pod and total phenols (-0.489), and crop duration and crude protein content (-0.484).

#### **4.3.9. Path coefficient analysis using twelve characters in bush-type cowpea genotypes**

The direct and indirect effect of twelve characters of bush-type cowpea genotypes on pod yield per plant are presented in Table 19.

The vine length showed a positive direct effect on pod yield per plant (0.173). A positive indirect effect through pods per plant (0.111), seeds per pod (0.104) and the number of harvests (0.111).

**Table 19. The direct and indirect effects of twelve characters in bush-type cowpea genotypes**

Characters	Vine length	Primary branches per plant	Pods per plant	Pod weight	Pod length	Seeds per pod	Crop duration	Number of harvests	Total phenols	Crude fibre content	Crude protein content	Genotypic Correlation with the main variable
Vine length	<b>0.173</b>	-0.26	0.117	-0.019	-0.023	0.208	0.136	-0.054	0.01	-0.01	0.046	0.325
Primary branches per plant	-0.069	<b>0.647</b>	0.003	0.085	0.048	0.112	-0.031	-0.013	-0.026	0.03	-0.057	0.728**
Pods per plant	0.111	0.01	<b>0.183</b>	-0.002	0.004	0.188	0.075	0.018	-0.032	0.024	0.026	0.605*
Pod weight	-0.032	0.539	-0.004	<b>0.101</b>	0.038	0.161	-0.042	-0.067	-0.012	0.033	0.069	0.785**
Pod length	-0.076	0.603	0.013	0.075	<b>0.052</b>	0.177	0.012	0.019	-0.08	0.014	-0.173	0.635*
Seeds per pod	0.104	0.209	0.099	0.047	0.027	<b>0.347</b>	0.084	-0.054	-0.046	-0.001	-0.02	0.795**
Crop duration	0.099	-0.086	0.058	-0.018	0.003	0.123	<b>0.236</b>	-0.042	0.118	-0.013	-0.152	0.209
Number of harvests	0.111	0.100	-0.04	0.082	-0.012	0.224	0.119	<b>-0.083</b>	0.051	0.004	0.077	0.633*
Total phenols	0.02	-0.182	-0.064	-0.013	-0.046	-0.177	0.319	-0.047	<b>0.091</b>	0.002	0.109	-0.308
Crude fibre content	-0.034	0.372	0.082	0.064	0.013	-0.005	-0.059	-0.006	0.003	<b>0.052</b>	0.105	0.587*
Crude protein content	0.032	-0.149	0.019	0.028	-0.036	-0.028	-0.144	-0.026	0.039	0.022	<b>0.250</b>	0.007

**R=0.0286**

Primary branches per plant recorded a positive direct effect (0.647) on pod yield per plant in bush-type cowpeas. Positive indirect effects through pod weight (0.539), pod length (0.603), seeds per pod (0.209) and crude fibre content (0.372). Primary branches per plant recorded negative indirect effects through vine length (-0.260), total phenols (-0.182) and crude protein content (-0.149) on pod yield per plant.

The pods per plant observed a positive direct effect (0.183) on pod yield per plant. Vine length had a positive indirect (0.117) effect on pod yield per plant through pods per plant. Pod weight (0.101) and pod length (0.052) positively affected pod yield per plant.

The seeds per pod recorded a positive direct effect (0.347) on pod yield per plant. A positive indirect effect through vine length (0.208), primary branches per plant (0.112), pods per plant (0.188), pod weight (0.161), pod length (0.177), crop duration (0.123) and number of harvests (0.224), and a negative indirect effect through total phenols (-0.177).

The crop duration had a positive direct effect (0.236) on pod yield per plant, positive indirect effects through the number of harvests (0.119) and total phenols (0.319) and a negative indirect effect through crude protein content (-0.144).

The total number of harvests recorded a negative direct effect (-0.083) on pod yield per plant. A positive direct effect (0.091) of total phenols on pod yield per plant and a positive indirect effect (0.118) through crop duration was observed.

The crude fibre content (0.052) and crude protein content (0.250) had a positive direct effect on pod yield per plant. Crude protein content had a positive indirect effect through total phenols (0.109) and crude fibre content (0.105) and a negative indirect effect through pod length (-0.173) and crop duration (-0.152) on the pod yield per plant. The overall residual effect of path coefficient analysis using characters of bush-type cowpea genotypes was 0.0286.

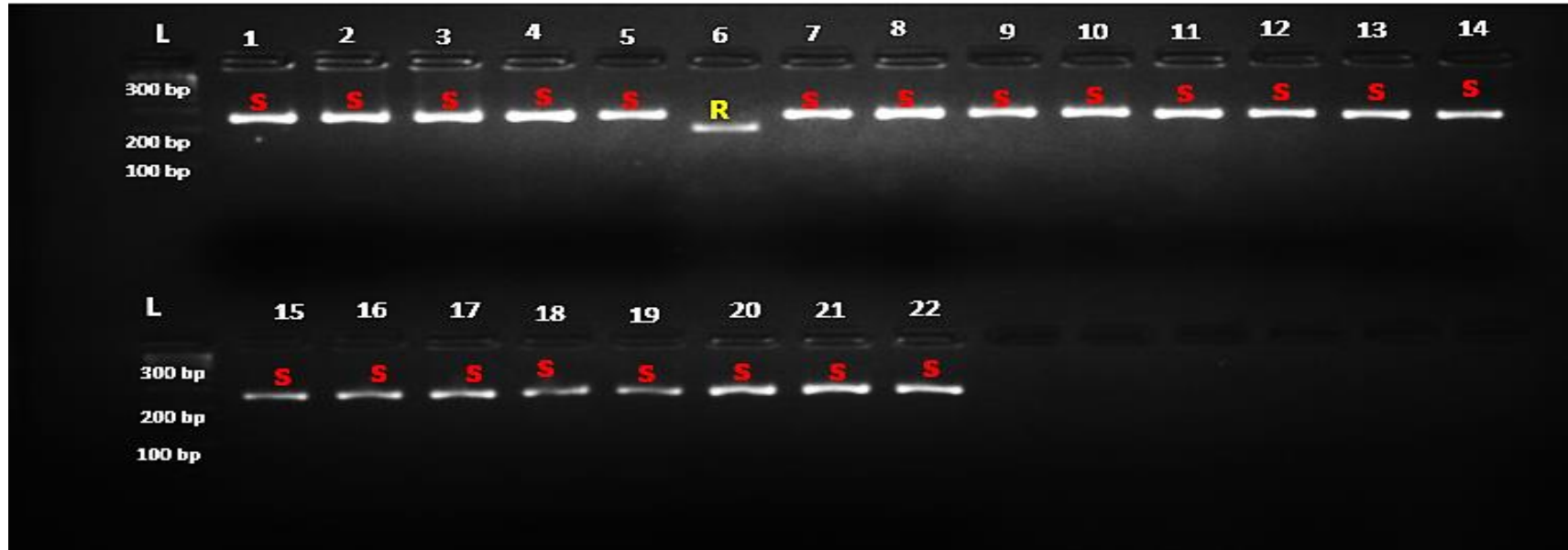
#### 4.4. GENOTYPING OF THE RESISTANT LINES USING SSR MARKER LINKED TO FUSARIUM WILT RESISTANCE

The genomic DNA was isolated from eighteen resistant cowpea genotypes (12 trailing types and 6 bush types) and four susceptible KAU-released varieties (3 trailing and 1 bush-type cowpea) using the CTAB method. The genomic DNA was quantified using a spectrophotometer and the quality was assured. It was amplified using sixteen Fusarium wilt resistance linked SSR markers, in which one marker (C13-16) was from the cowpea genome and others were from the chickpea genome which could be used as transferable markers to the cowpea genome.

The SSR marker C13-16 could not produce an amplicon in resistant lines. A polymorphic band was observed in the genotype VI064556 (IRON) at 190 bp size and the remaining genotypes produced an amplicon size of 250 bp using the SSR marker UASBC59 (Plate 26). The remaining fourteen SSR markers could not produce a polymorphic band between resistant and susceptible lines.

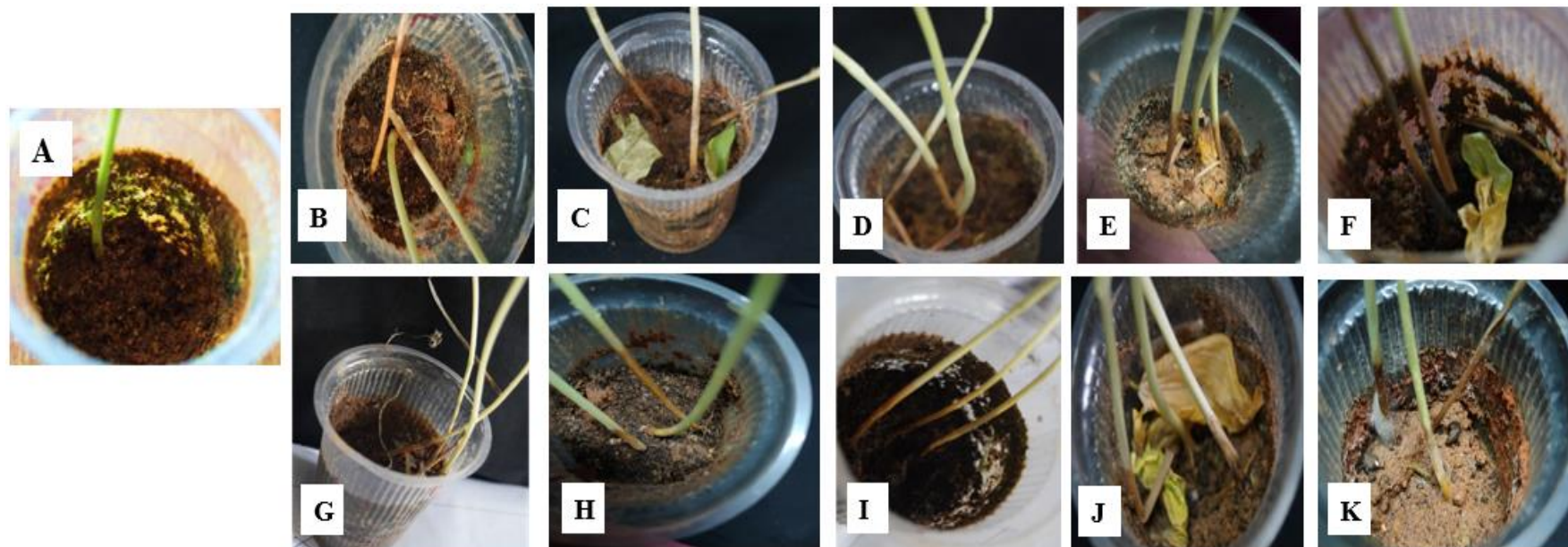
#### 4.5. HYBRIDISATION OF RESISTANT GENOTYPE WITH SUPERIOR BUT SUSCEPTIBLE VARIETY RELEASED FROM KAU FOR DEVELOPING F<sub>1</sub>.

The Fusarium wilt donor parent and KAU-released varieties were artificially screened and all the KAU-released varieties showed basal stem rotting and vascular discolouration of Fusarium wilt (Plate 27). So, the high-yielding and popular yardlong bean variety Githika was selected as the recipient parent in the backcrossing programme. The Fusarium wilt-resistant donor genotype VI064556 (IRON) was crossed with the popular but Fusarium wilt susceptible KAU released variety Githika for the production of F<sub>1</sub> generation. The per cent pod set was calculated by dividing the number of pod sets by the number of flowers crossed into a hundred. Out of 384 flowers crossed, about 46 set into pods so, the pod set per cent in the cross between Githika and IRON was observed to be 12 per cent (Table 20).



**Plate 26. Amplification profile of UASBC59 SSR marker in parental line, S- Susceptible, R- Resistant, L- 100bp ladder**

(1) TCR87, (2) VI034386, (3) VI046653, (4) VI048533, (5) Elamdu Local II, (6) VI064556 (IRON), (7) Kallicaud Local, (8) Kanjikuzhi Local, (9) Kochi Local, (10) Koovappally Local, (11) Kottayamthattathi Local, (12) Mavelikkara Local, (13) Nellanadu Local, (14) Wayanadu Local I, (15) Wayanadu Local II, (16) Muttathkonam Local, (17) Kulashekham Local, (18) Kottarakkara Local, (19) Githika, (20) Vellayani Jyothika, (21) Lola, and (22) Kanakamony



**Plate 27. Fusarium screening of the donor parent with KAU-released varieties**

**(A) IRON, (B), Malika, (C) Anaswara, (D) Manjari, (E) Vellayani Jyothika, (F) Kanakamony, (G) Vyjayanthi, (H) Lola, (I) Bhagyalekshmy, (J) Sharika, and (K) Githika**

#### 4.6. RAISING AND MORPHOLOGICAL EVALUATION OF F<sub>1</sub> AND THE PARENTS

The F<sub>1</sub> plants produced by crossing Githika and IRON were morphologically evaluated along with their parents. A total of 210 F<sub>1</sub> seeds were produced, of which 148 germinated with 70.4 per cent germination (Table 21). The mean performance of F<sub>1</sub>s, Githika and IRON for six morphological characters is given in Table 22.

##### **4.6.1. Vine length (cm)**

The vine length of F<sub>1</sub> plants was above the average of their parents. The F<sub>1</sub> plants, Githika and IRON were 335.600 cm, 445.587cm and 150.667cm in vine length respectively.

##### **4.6.2. Primary branches per plant**

The F<sub>1</sub> plants produced 8.40 primary branches per plant, Githika recorded 8.233 primary branches per plant and IRON produced 8.667 primary branches per plant.

##### **4.6.3. Pod weight (g)**

The F<sub>1</sub> plants, Githika, and IRON recorded pod weights of 18.567g, 24.950g and 7.197g respectively.

##### **4.6.4. Pod length (cm)**

The length of pods in F<sub>1</sub> plants was recorded at 27.950 cm, and their parents Githika and IRON have observed pod lengths of 51.0 cm and 16.543 cm respectively.

##### **4.6.5. Seeds per pod**

The seeds per pod were the maximum in Githika (18.807), and the minimum in IRON (17.527). The F<sub>1</sub> plants produced 17.527 seeds per pod.

**Table 20. Percentage of pod set after hybridization between Githika and IRON**

Number of flowers crossed	Number of pod set	Percentage of pod set
384	46	12%

**Table 21. Percentage of germination in F<sub>1</sub>s and BC<sub>1</sub>F<sub>1</sub> generations**

Generation	Number of plants	Germination percentage
F <sub>1</sub> produced	210	70.4%
F <sub>1</sub> seeds germinated	148	
BC <sub>1</sub> F <sub>1</sub> plants developed	270	73.0%
BC <sub>1</sub> F <sub>1</sub> plants germinated	198	

**Table 22. Morphological evaluation of F<sub>1</sub>s along with recurrent and donor parents**

Population	Vine length (cm)	Primary branches per plant	Pod weight (g)	Pod length (cm)	Seeds per pod	Crop duration (days)
P1 (Githika)	445.587	8.233	24.950	51.000	18.807	114.183
P2 (IRON)	150.667	8.667	7.197	16.543	17.527	93.667
P1 x P2	335.600	8.400	18.567	27.950	17.890	107.333
Mean	310.618	8.433	16.904	31.831	18.074	105.061
SE (m)	87.367	0.741	5.209	10.360	0.695	7.001
CV (%)	48.717	15.211	53.375	56.374	6.664	11.542

#### 4.6.6. Crop duration

The crop duration was the maximum in Githika (114.183 days) and the minimum in IRON (93.667 days), and the F<sub>1</sub> plants completed the crop in 107.333 days.

#### 4.7. BACKCROSSING OF F<sub>1</sub> FOR THE PRODUCTION OF BC<sub>1</sub>F<sub>1</sub> LINES

The F<sub>1</sub> plants were backcrossed with the recurrent parent Githika to produce BC<sub>1</sub>F<sub>1</sub> lines. A total of 270 BC<sub>1</sub>F<sub>1</sub> lines were produced from 148 F<sub>1</sub> plants.

#### 4.8. EVALUATION OF BC<sub>1</sub>F<sub>1</sub> LINES AT THE SEEDLING STAGE THROUGH ARTIFICIAL SCREENING AND SCORING

The 270 BC<sub>1</sub>F<sub>1</sub> lines of seeds were planted for seedlings, of which 198 germinated giving 73% germination (Table 21). The 198 BC<sub>1</sub>F<sub>1</sub> lines were artificially evaluated for Fusarium wilt at the seedling stage (10 days old). The scoring was done using a 0-4 scale in which the lowest score indicated the highest resistance (Table 23). Out of 198 BC<sub>1</sub>F<sub>1</sub> lines, 59 scored '0', 27 scored '1', 14 scored '2', 25 scored '3' and 73 scored '4' representing resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible categories respectively (Plates 28, 29, and 30). Vascular rotting was more easily visible in the susceptible BC<sub>1</sub>F<sub>1</sub> lines than in resistant ones (Plate 31). The comparison of different disease reactions in BC<sub>1</sub>F<sub>1</sub> lines with their parents is shown in Plate 32.

The chi-square test for goodness of fit in BC<sub>1</sub>F<sub>1</sub> lines was calculated for identifying the inheritance pattern of the resistant gene. For the genetic hypothesis, a disease score of 0 or 1 was considered resistant (R) and a disease score of 2, 3 or 4 was considered susceptible (S). The 86 BC<sub>1</sub>F<sub>1</sub> lines were considered resistant and 112 BC<sub>1</sub>F<sub>1</sub> lines were considered susceptible plants (Table 24). The expected ratio for resistant: susceptible was 1:1 in the BC<sub>1</sub>F<sub>1</sub> generation. The expected frequency of resistant and susceptible from the 198 BC<sub>1</sub>F<sub>1</sub> lines was 99 each and the observed frequency was 86 and 112 respectively. The calculated chi-square value was 3.414 and the table value at 1 degree of freedom (at  $\alpha=0.05$ ) was 3.814. So, the null



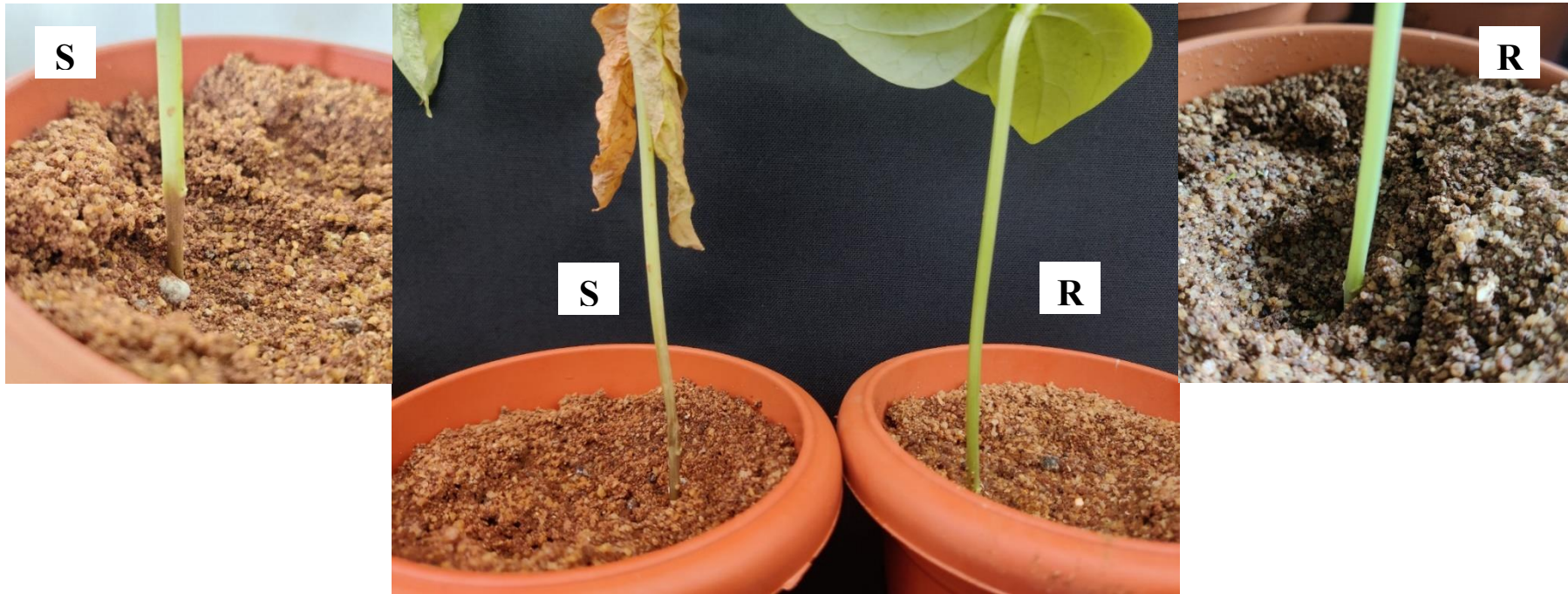
**Plate 28. Fusarium wilt resistant BC<sub>1</sub>F<sub>1</sub> lines (score '0')**



**Plate 29. Fusarium wilt moderately resistant, moderately susceptible, susceptible BC<sub>1</sub>F<sub>1</sub> lines**



**Plate 30. Fusarium wilt highly susceptible BC<sub>1</sub>F<sub>1</sub> lines (Score '4')**



**Plate 31. Comparison of Fusarium wilt resistant and susceptible BC<sub>1</sub>F<sub>1</sub> lines in vascular rotting. S- susceptible and R- resistant**

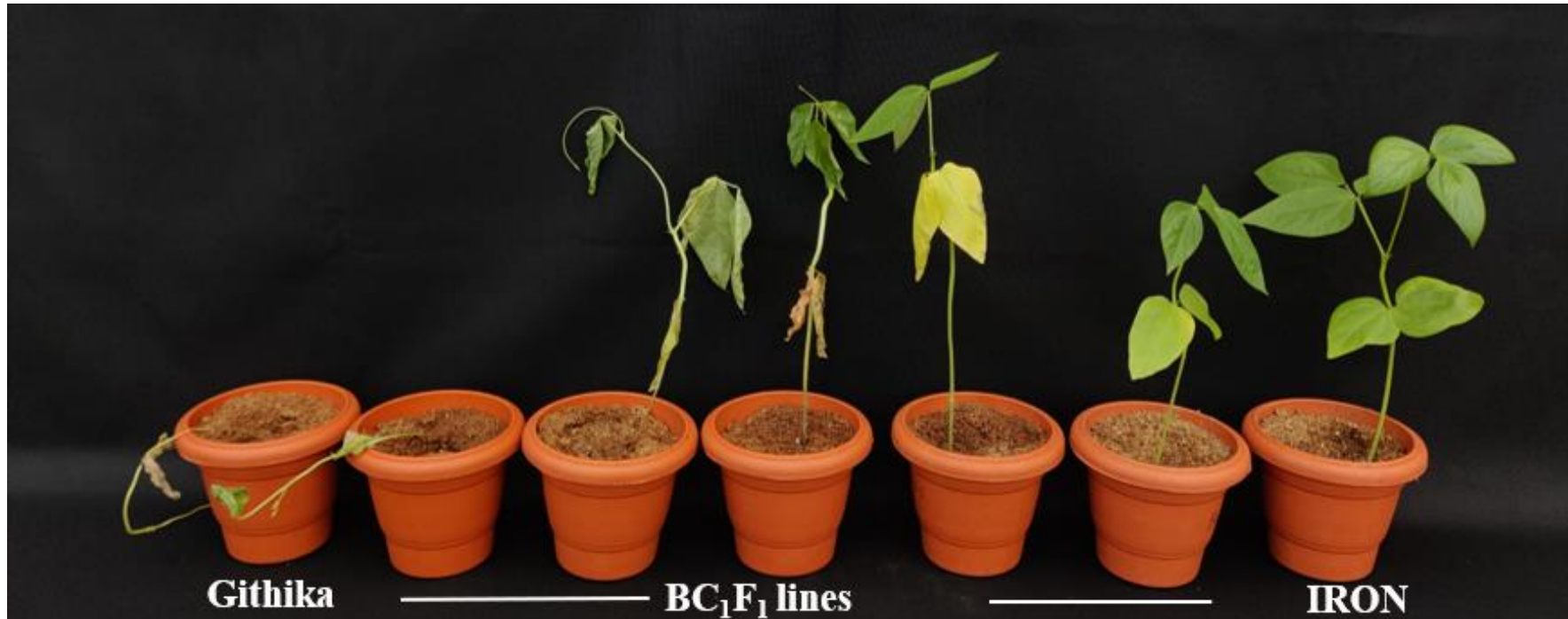


Plate 32. Comparison of Githika, BC<sub>1</sub>F<sub>1</sub> lines, and IRON for the reaction to Fusarium wilt

**Table 23. Disease scoring of BC<sub>1</sub>F<sub>1</sub> lines on a 0-4 scale**

SI No.	Genotypes	Disease Scoring	Disease Reaction
1	BG1	0	Resistant (R)
2	BG 2	4	Highly Susceptible (HS)
3	BG 3	0	Resistant (R)
4	BG 4	0	Resistant (R)
5	BG 5	2	Moderately Susceptible (MS)
6	BG 6	0	Resistant (R)
7	BG 7	0	Resistant (R)
8	BG 8	4	Highly Susceptible (HS)
9	BG 9	0	Resistant (R)
10	BG 10	0	Resistant (R)
11	BG 11	4	Highly Susceptible (HS)
12	BG 12	4	Highly Susceptible (HS)
13	BG 13	0	Resistant (R)
14	BG 14	0	Resistant (R)
15	BG 15	1	Moderately Resistant (MR)
16	BG 16	0	Resistant (R)
17	BG 17	4	Highly Susceptible (HS)
18	BG 18	4	Highly Susceptible (HS)
19	BG 19	0	Resistant (R)
20	BG 20	4	Highly Susceptible (HS)
21	BG 21	4	Highly Susceptible (HS)
22	BG 22	4	Highly Susceptible (HS)
23	BG 23	0	Resistant (R)
24	BG 24	0	Resistant (R)
25	BG 25	0	Resistant (R)
26	BG 26	4	Highly Susceptible (HS)
27	BG 27	4	Highly Susceptible (HS)
28	BG 28	4	Highly Susceptible (HS)
29	BG 29	0	Resistant (R)
30	BG 30	0	Resistant (R)
31	BG 31	1	Moderately Resistant (MR)
32	BG 32	3	Susceptible (S)
33	BG 33	0	Resistant (R)
34	BG 34	0	Resistant (R)
35	BG 35	4	Highly Susceptible (HS)
36	BG 36	4	Highly Susceptible (HS)
37	BG 37	0	Resistant (R)
38	BG 38	0	Resistant (R)
39	BG 39	4	Highly Susceptible (HS)
40	BG 40	4	Highly Susceptible (HS)
41	BG 41	0	Resistant (R)

(Cont.)

42	BG 42	0	Resistant (R)
43	BG 43	0	Resistant (R)
44	BG 44	0	Resistant (R)
45	BG 45	4	Highly Susceptible (HS)
46	BG 46	4	Highly Susceptible (HS)
47	BG 47	1	Moderately Resistant (MR)
48	BG 48	1	Moderately Resistant (MR)
49	BG 49	4	Highly Susceptible (HS)
50	BG 50	0	Resistant (R)
51	BG 51	0	Resistant (R)
52	BG 52	0	Resistant (R)
53	BG 53	0	Resistant (R)
54	BG 54	0	Resistant (R)
55	BG 55	3	Susceptible (S)
56	BG 56	1	Moderately Resistant (MR)
57	BG 57	4	Highly Susceptible (HS)
58	BG 58	1	Moderately Resistant (MR)
59	BG 59	4	Highly Susceptible (HS)
60	BG 60	4	Highly Susceptible (HS)
61	BG 61	2	Moderately Susceptible (MS)
62	BG 62	0	Resistant (R)
63	BG 63	0	Resistant (R)
64	BG 64	1	Moderately Resistant (MR)
65	BG 65	4	Highly Susceptible (HS)
66	BG 66	0	Resistant (R)
67	BG 67	0	Resistant (R)
68	BG 68	4	Highly Susceptible (HS)
69	BG 69	3	Susceptible (S)
70	BG 70	3	Susceptible (S)
71	BG 71	4	Highly Susceptible (HS)
72	BG 72	3	Susceptible (S)
73	BG 73	3	Susceptible (S)
74	BG 74	1	Moderately Resistant (MR)
75	BG 75	4	Highly Susceptible (HS)
76	BG 76	4	Highly Susceptible (HS)
77	BG 77	0	Resistant (R)
78	BG 78	4	Highly Susceptible (HS)
79	BG 79	0	Resistant (R)
80	BG 80	1	Moderately Resistant (MR)
81	BG 81	4	Highly Susceptible (HS)
82	BG 82	1	Moderately Resistant (MR)
83	BG 83	1	Moderately Resistant (MR)
84	BG 84	0	Resistant (R)
85	BG 85	0	Resistant (R)

(Cont.)

86	BG 86	0	Resistant (R)
87	BG 87	0	Resistant (R)
88	BG 88	0	Resistant (R)
89	BG 89	2	Moderately Susceptible (MS)
90	BG 90	1	Moderately Resistant (MR)
91	BG 91	1	Moderately Resistant (MR)
92	BG 92	3	Susceptible (S)
93	BG 93	2	Moderately Susceptible (MS)
94	BG 94	2	Moderately Susceptible (MS)
95	BG 95	0	Resistant (R)
96	BG 96	4	Highly Susceptible (HS)
97	BG 97	4	Highly Susceptible (HS)
98	BG 98	0	Resistant (R)
99	BG 99	4	Highly Susceptible (HS)
100	BG 100	0	Resistant (R)
101	BG 101	0	Resistant (R)
102	BG 102	4	Highly Susceptible (HS)
103	BG 103	4	Highly Susceptible (HS)
104	BG 104	4	Highly Susceptible (HS)
105	BG 105	4	Highly Susceptible (HS)
106	BG 106	2	Moderately Susceptible (MS)
107	BG 107	0	Resistant (R)
108	BG 108	3	Susceptible (S)
109	BG 109	4	Highly Susceptible (HS)
110	BG 110	3	Susceptible (S)
111	BG 111	3	Susceptible (S)
112	BG 112	4	Highly Susceptible (HS)
113	BG 113	4	Highly Susceptible (HS)
114	BG 114	4	Highly Susceptible (HS)
115	BG 115	3	Susceptible (S)
116	BG 116	2	Moderately Susceptible (MS)
117	BG 117	0	Resistant (R)
118	BG 118	1	Moderately Resistant (MR)
119	BG 119	0	Resistant (R)
120	BG 120	1	Moderately Resistant (MR)
121	BG 121	2	Moderately Susceptible (MS)
122	BG 122	4	Highly Susceptible (HS)
123	BG 123	0	Resistant (R)
124	BG 124	0	Resistant (R)
125	BG 125	0	Resistant (R)
126	BG 126	4	Highly Susceptible (HS)
127	BG 127	0	Resistant (R)
128	BG 128	4	Highly Susceptible (HS)
129	BG 129	4	Highly Susceptible (HS)

(Cont.)

130	BG 130	0	Resistant (R)
131	BG 131	4	Highly Susceptible (HS)
132	BG 132	4	Highly Susceptible (HS)
133	BG 133	4	Highly Susceptible (HS)
134	BG 134	4	Highly Susceptible (HS)
135	BG 135	0	Resistant (R)
136	BG 136	2	Moderately Susceptible (MS)
137	BG 137	4	Highly Susceptible (HS)
138	BG 138	0	Resistant (R)
139	BG 139	4	Highly Susceptible (HS)
140	BG 140	4	Highly Susceptible (HS)
141	BG 141	0	Resistant (R)
142	BG 142	0	Resistant (R)
143	BG 143	4	Highly Susceptible (HS)
144	BG 144	4	Highly Susceptible (HS)
145	BG 145	4	Highly Susceptible (HS)
146	BG 146	4	Highly Susceptible (HS)
147	BG 147	4	Highly Susceptible (HS)
148	BG 148	3	Susceptible (S)
149	BG 149	0	Resistant (R)
150	BG 150	4	Highly Susceptible (HS)
152	BG 152	3	Susceptible (S)
153	BG 153	4	Highly Susceptible (HS)
154	BG 154	0	Resistant (R)
155	BG 155	1	Moderately Resistant (MR)
156	BG 156	4	Highly Susceptible (HS)
157	BG 157	3	Susceptible (S)
158	BG 158	0	Resistant (R)
159	BG 159	4	Highly Susceptible (HS)
160	BG 160	4	Highly Susceptible (HS)
161	BG 161	4	Highly Susceptible (HS)
162	BG 162	1	Moderately Resistant (MR)
163	BG 163	4	Highly Susceptible (HS)
164	BG 164	3	Susceptible (S)
165	BG 165	2	Moderately Susceptible (MS)
166	BG 166	1	Moderately Resistant (MR)
167	BG 167	3	Susceptible (S)
168	BG 168	2	Moderately Susceptible (MS)
169	BG 169	1	Moderately Resistant (MR)
170	BG 170	2	Moderately Susceptible (MS)
171	BG 171	4	Highly Susceptible (HS)
172	BG 172	4	Highly Susceptible (HS)
173	BG 173	4	Highly Susceptible (HS)
174	BG 174	3	Susceptible (S)

(Cont.)

175	BG 175	1	Moderately Resistant (MR)
176	BG 176	4	Highly Susceptible (HS)
177	BG 177	3	Susceptible (S)
178	BG 178	2	Moderately Susceptible (MS)
179	BG 179	4	Highly Susceptible (HS)
180	BG 180	3	Susceptible (S)
181	BG 181	1	Moderately Resistant (MR)
182	BG 182	1	Moderately Resistant (MR)
183	BG 183	4	Highly Susceptible (HS)
184	BG 184	3	Susceptible (S)
185	BG 185	3	Susceptible (S)
186	BG 186	3	Susceptible (S)
187	BG 187	2	Moderately Susceptible (MS)
188	BG 188	1	Moderately Resistant (MR)
189	BG 189	4	Highly Susceptible (HS)
190	BG 190	3	Susceptible (S)
191	BG 191	1	Moderately Resistant (MR)
192	BG 192	4	Highly Susceptible (HS)
193	BG 193	3	Susceptible (S)
194	BG 194	1	Moderately Resistant (MR)
195	BG 195	1	Moderately Resistant (MR)
196	BG 196	3	Susceptible (S)
197	BG 197	4	Highly Susceptible (HS)
198	BG 198	3	Susceptible (S)
	<b>Average</b>	<b>2.212</b>	
	<b>p-value</b>	<b>0.487</b>	
	<b>S.D</b>	<b>1.71</b>	
	<b>KW Value</b>	<b>197.0</b>	
	<b>Chi-square value</b>	<b>&gt;113.145</b>	

hypothesis which says there is no significant difference between observed and expected values was accepted (Table 25).

#### 4.9. MORPHOLOGICAL AND QUALITY EVALUATION OF RESISTANT BC<sub>1</sub>F<sub>1</sub> LINES AND THE RECURRENT PARENT

The resistant BC<sub>1</sub>F<sub>1</sub> lines were evaluated for biometric and quality parameters along with their parents.

##### 4.9.1. Biometric characters

The performance on biometric parameters of each resistant segregant and frequency distribution in terms of skewness and kurtosis along with their parents are given in Table 26. The variability in pod length, seed colour and size in resistant BC<sub>1</sub>F<sub>1</sub> lines along with their parents is shown in Plates 33 and 34 respectively.

##### 4.9.1.1. *Vine length*

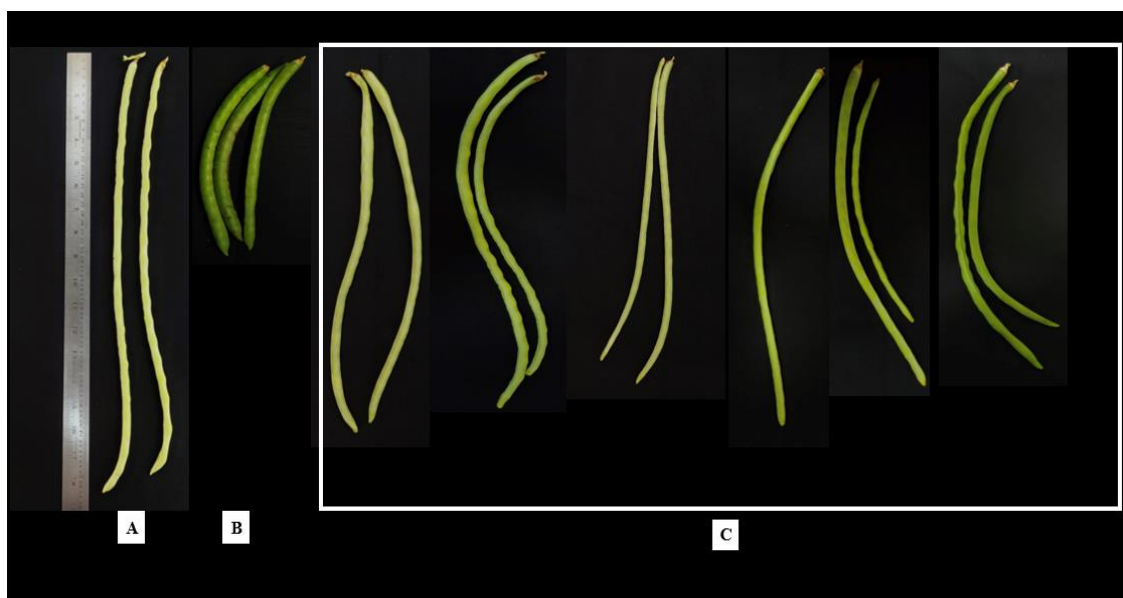
The vine length ranged from 186 cm to 416 cm in resistant BC<sub>1</sub>F<sub>1</sub> lines, 416 cm in Githika and 182 cm in IRON with a mean value of 280.74 cm. The frequency distribution of vine length with skewness of 0.4305 and kurtosis of -0.0998 was recorded.

##### 4.9.1.2. *Primary branches per plant*

The number of primary branches per plant varied from 4.00 to 8.00 in resistant BC<sub>1</sub>F<sub>1</sub> lines, 8 numbers in Githika and 7 numbers in IRON with a mean value of 5.065. The primary branches per plant were distributed with positive skewness (0.9041) and positive kurtosis (0.5539).

##### 4.9.1.3. *Pod weight*

The maximum pod weight was observed in BG124 (25.46 g) and the minimum was observed in BG67 (10.01 g), 25.58 g in Githika and 7.498 g in IRON with an average of 16.973g. The frequency distribution of pod weight in resistant BC<sub>1</sub>F<sub>1</sub> lines was negatively skewed (-0.0687) with positive kurtosis (0.0438).



**Plate 33. Variability in pod length (A) Githika, (B) IRON, and (C) BC<sub>1</sub>F<sub>1</sub> resistant lines**



**Plate 34. Variability in seed colour and size (A) Githika, (B) IRON, and (C) BC<sub>1</sub>F<sub>1</sub> resistant lines**

**Table 24. Number of BC<sub>1</sub>F<sub>1</sub> lines in each category of disease reaction for chi-square test**

Category	Number of BC <sub>1</sub> F <sub>1</sub> lines	
Resistant (0)	59	86 Resistant
Moderately Resistant (1)	27	
Moderately Susceptible (2)	14	112 Susceptible
Susceptible (3)	25	
Highly Susceptible (4)	73	
Total	198	

**Table 25. Chi-square test for goodness of fit in BC<sub>1</sub>F<sub>1</sub> lines**

Number of BC <sub>1</sub> F <sub>1</sub> plants	Expected Ratio	Disease reaction	Expected (E)	Observed (O)	$\chi^2 = \sum(O_i - E_i)^2/E_i$	Table $\chi^2$ (df=1, $\alpha=0.05$ )
198	1:1 (R:S)	R	99	86	3.414	3.814
		S	99	112		

#### ***4.9.1.4. Pod length***

The pod length ranged from 16.50 cm to 43.00 cm in resistant BC<sub>1</sub>F<sub>1</sub> lines, 42.83 cm in Githika and 13.60 cm in IRON. The average value of pod length was observed to be 28.729 cm with positive skewness (0.2629) and kurtosis (0.2859) in the frequency distribution.

#### ***4.9.1.5. Seeds per pod***

The seeds per pod ranged from 10.23 to 20.67 in resistant BC<sub>1</sub>F<sub>1</sub> lines, 20.75 in Githika, and 17.20 in IRON with an average of 14.8929. The frequency distribution of seeds per pod observed positive skewness (0.1809) with negative kurtosis (-0.4867).

#### ***4.9.1.6. Crop duration***

The minimum crop duration was observed in BG88 (101 days) and the maximum in BG7 (120 days) among resistant BC<sub>1</sub>F<sub>1</sub> lines, 121.58 days in Gitika and 100.66 days in IRON with a mean value of 108.827 days. The frequency was distributed for crop duration with positive skewness (0.5539) and negative kurtosis (-0.4071).

### **4.9.2. Quality parameters**

The performance on quality parameters of each resistant segregant and frequency distribution in terms of skewness and kurtosis along with their parents are given in Table 20.

#### ***4.9.2.1. Total phenols***

The total phenols ranged from 46.12 to 59.82 in resistant BC<sub>1</sub>F<sub>1</sub> lines after artificial screening, 22.68 in Githika and 34.39 in IRON. The frequency distribution of total phenols recorded the negative skewness (-2.8225) and the positive kurtosis (12.1270).

**Table 26. Performance on biometric parameters of BC<sub>1</sub>F<sub>1</sub> resistant lines and frequency distribution**

Sl No.	Genotypes	Vine length (cm)	Primary branches per plant	Pod weight (g)	Pod length (cm)	Seeds per pod	Crop duration (days)	Total phenols (mg/100g)	Crude fibre content (%)	Crude protein content (%)
1	BG 1	235.00	4.00	17.030	27.40	14.00	116.00	55.76	10.72	27.42
2	BG 3	253.00	4.00	14.627	27.75	17.00	110.00	48.52	12.56	28.76
3	BG 4	340.00	6.00	12.883	26.99	14.50	112.00	57.46	11.41	27.44
4	BG 6	416.00	8.00	14.447	27.55	16.75	120.00	46.28	12.37	29.84
5	BG 7	284.00	4.00	19.973	30.67	11.50	120.00	50.74	13.35	26.69
6	BG 9	258.00	4.00	17.423	24.25	16.00	118.00	46.44	9.19	20.21
7	BG 10	341.00	5.00	15.895	21.43	16.33	110.00	53.13	10.74	23.57
8	BG 13	325.00	5.00	23.485	38.64	13.20	103.00	55.18	9.20	25.55
9	BG 14	298.00	4.00	10.060	27.88	17.43	108.00	59.52	10.16	20.30
10	BG 16	254.00	4.00	17.213	20.65	12.90	103.00	53.67	8.03	24.78
11	BG 19	229.00	5.00	12.770	21.44	15.32	110.00	48.42	10.98	30.47
12	BG 23	218.00	5.00	21.067	34.17	19.33	115.00	48.22	10.32	25.12
13	BG 24	249.00	5.00	19.043	27.45	18.28	102.00	53.39	9.61	27.58
14	BG 25	236.00	4.00	12.440	26.67	14.00	104.00	52.73	9.37	29.32
15	BG 29	267.00	5.00	12.553	26.11	14.52	107.00	55.92	8.25	31.85
16	BG 30	342.00	6.00	17.743	30.00	12.00	110.00	49.93	9.33	31.89

(Cont.)

17	BG 33	231.00	4.00	12.140	20.53	12.84	104.00	56.16	7.56	30.35
18	BG 34	223.00	6.00	14.635	24.52	12.98	108.00	59.48	9.22	26.69
19	BG 37	329.00	6.00	13.920	21.27	13.33	104.00	50.62	12.36	28.37
20	BG 38	248.00	4.00	11.635	33.00	16.33	114.00	48.76	14.58	27.88
21	BG 41	315.00	5.00	14.177	29.00	14.00	107.00	58.10	10.33	29.27
22	BG 42	373.00	6.00	13.127	27.82	14.41	110.00	53.96	8.58	20.59
23	BG 43	328.00	5.00	16.172	36.71	15.88	109.00	55.45	9.67	26.66
24	BG 44	285.00	4.00	16.180	35.14	15.40	105.00	54.23	11.66	20.38
25	BG 50	237.00	6.00	13.510	29.87	13.45	118.00	58.79	13.26	30.47
26	BG 51	261.00	5.00	19.09	27.63	15.82	110.00	48.06	10.18	33.88
27	BG 52	243.00	5.00	16.72	23.50	15.96	104.00	46.12	8.73	32.56
28	BG 53	186.00	5.00	18.45	27.50	11.43	106.00	59.28	9.67	28.82
29	BG 54	264.00	4.00	21.43	33.25	14.50	110.00	52.63	12.06	20.58
30	BG 62	332.00	4.00	20.42	32.00	15.33	108.00	54.33	13.7	19.57
31	BG 63	264.00	5.00	20.39	28.30	15.21	105.00	49.16	10.36	25.55
32	BG 66	285.00	4.00	17.35	25.11	16.00	108.00	55.59	8.61	28.58
33	BG 67	341.00	6.00	10.01	33.50	15.50	102.00	58.09	9.29	25.37
34	BG 77	289.00	6.00	16.16	28.00	10.38	113.00	59.82	9.93	28.90
35	BG 79	310.00	5.00	13.38	26.00	12.44	115.00	52.26	8.65	29.57
36	BG 84	287.00	5.00	17.53	29.00	10.23	117.00	51.20	7.52	31.26
37	BG 85	250.00	4.00	16.49	35.00	15.67	115.00	53.19	6.78	32.47

(Cont.)

38	BG 86	246.00	4.00	19.12	24.00	18.52	110.00	58.12	10.96	30.69
39	BG 87	297.00	6.00	19.49	30.65	14.55	102.00	57.94	9.92	27.43
40	BG 88	235.00	4.00	16.59	27.09	15.52	101.00	55.01	12.33	29.69
41	BG 95	351.00	7.00	18.06	27.10	17.00	108.00	55.57	12.31	30.88
42	BG 98	332.00	6.00	21.28	40.67	20.67	104.00	52.23	14.15	21.44
43	BG 100	307.00	5.00	19.06	29.77	18.00	110.00	53.34	12.99	27.47
44	BG 101	195.00	5.00	12.64	24.75	13.50	105.00	55.55	9.87	26.26
45	BG 107	269.00	4.00	11.80	43.00	11.50	107.00	49.83	10.45	31.48
46	BG 117	240.00	4.00	21.46	32.75	19.00	103.00	53.67	11.64	30.22
47	BG 119	243.00	5.00	16.90	37.00	20.00	110.00	58.35	9.47	31.58
48	BG 123	275.00	6.00	23.41	39.33	15.00	116.00	52.93	10.25	27.55
49	BG 124	337.00	7.00	25.46	31.75	11.00	110.00	58.88	8.46	29.28
50	BG 125	279.00	5.00	18.93	32.00	13.50	104.00	52.29	12.04	31.66
51	BG 127	263.00	4.00	18.04	24.88	15.75	108.00	53.18	13.29	30.42
52	BG 130	323.00	6.00	19.63	36.67	15.33	104.00	56.02	10.45	33.44
53	BG 135	220.00	4.00	17.87	22.05	11.00	110.00	56.95	9.78	32.79
54	BG 138	198.00	5.00	17.36	16.50	10.33	105.00	53.23	8.56	28.44
55	BG 141	365.00	5.00	20.21	29.26	12.00	114.00	54.61	9.88	27.63
56	BG 142	290.00	4.00	17.56	23.10	11.00	110.00	51.39	8.52	25.55
57	BG 149	253.00	5.00	18.79	31.00	18.00	102.00	53.74	10.27	26.82
58	BG 154	325.00	6.00	18.33	24.42	17.50	103.00	54.80	12.43	23.57
59	BG 158	264.00	5.00	18.78	22.68	12.00	110.00	57.99	10.08	29.22

(Cont.)

60	Githika	416.00	8.00	25.58	42.83	20.75	121.58	22.68	9.21	26.74
61	IRON	182.00	7.00	7.498	13.60	17.20	100.66	34.39	15.41	24.91
	<b>Minimum</b>	<b>186</b>	<b>4</b>	<b>10.01</b>	<b>16.50</b>	<b>10.23</b>	<b>101.00</b>	<b>46.12</b>	<b>6.7</b>	<b>19.57</b>
	<b>Maximum</b>	<b>416</b>	<b>8</b>	<b>25.46</b>	<b>43.00</b>	<b>20.67</b>	<b>120.00</b>	<b>59.82</b>	<b>14.58</b>	<b>33.88</b>
	<b>Mean</b>	<b>280.74</b>	<b>5.065</b>	<b>16.973</b>	<b>28.729</b>	<b>14.8979</b>	<b>108.824</b>	<b>53.004</b>	<b>10.508</b>	<b>27.765</b>
	<b>Std. Dev</b>	<b>52.095</b>	<b>1.0306</b>	<b>3.729</b>	<b>5.9992</b>	<b>2.6410</b>	<b>5.258</b>	<b>5.876</b>	<b>1.867</b>	<b>3.589</b>
	<b>Variance</b>	<b>2713.96</b>	<b>1.0622</b>	<b>13.906</b>	<b>35.991</b>	<b>6.9749</b>	<b>27.648</b>	<b>34.5328</b>	<b>3.4867</b>	<b>12.882</b>
	<b>Skewness</b>	<b>0.4305</b>	<b>0.9041</b>	<b>-0.0687</b>	<b>0.2629</b>	<b>0.1809</b>	<b>0.5539</b>	<b>-2.8225</b>	<b>0.5273</b>	<b>-0.6641</b>
	<b>Kurtosis</b>	<b>-0.0998</b>	<b>0.5539</b>	<b>0.0438</b>	<b>0.2859</b>	<b>-0.4867</b>	<b>-0.4071</b>	<b>12.1270</b>	<b>-0.1653</b>	<b>-0.0907</b>
	<b>C.V</b>	<b>18.557</b>	<b>20.3466</b>	<b>21.970</b>	<b>20.881</b>	<b>17.727</b>	<b>4.8318</b>	<b>11.0867</b>	<b>17.769</b>	<b>12.926</b>

#### ***4.9.2.2. Crude fibre content***

The crude fibre content was maximum in BG58 (14.58%) and the minimum in BG85(6.78%) among the resistant BC<sub>1</sub>F<sub>1</sub> lines, 9.21% in Githika and 15.41% in IRON. The positive skewness (0.5273) and negative kurtosis (-0.1653) were observed for crude fibre content in the frequency distribution.

#### ***4.9.2.3. Crude protein content***

The crude protein content varied from 19.57 (BG62) to 33.88 (BG51) in resistant BC<sub>1</sub>F<sub>1</sub> lines with an average of 27.765%. The Githika recorded crude protein content of 26.74% and IRON recorded 24.9%. The crude protein content frequency distribution observed negative skewness (-0.6641) and negative kurtosis (-0.0907).

### **4.9.3. Proximity dissimilarity matrix by Euclidian distance with a recurrent parent using biometric characters**

The six biometric characters were used for calculating the proximity dissimilarity matrix by Euclidian distance of resistant BC<sub>1</sub>F<sub>1</sub> lines with the recurrent parent Githika (Table 27).

#### ***4.9.3.1. Vine length***

The minimum coefficient of dissimilarity for vine length in resistant BC<sub>1</sub>F<sub>1</sub> lines was recorded in BG6 (0.060), followed by BG42 (0.370) and BG141 (0.450) compared with the recurrent parent Githika (0.000). The maximum dissimilarity coefficient was observed in BG53 (2.240). The donor parent IRON showed a 2.280 dissimilarity coefficient with the recurrent parent Githika (0.000).

#### ***4.9.3.2. Primary branches per plant***

The coefficient of dissimilarity for primary branches per plant between the resistant BC<sub>1</sub>F<sub>1</sub> lines and the recurrent parent Githika ranged from 0.000 to 4.000. The minimum coefficient (0.000) was observed in BG6, and the maximum.

**Table 27. Proximity dissimilarity matrix by Euclidean distance with recurrent parent using biometric traits in BC<sub>1</sub>F<sub>1</sub> resistant lines**

SI No.	Genotypes	Vine length	Primary branches per plant	Pod weight	Pod length	Seeds per pod	Crop duration	Overall
1	BG 1	1.750	4.000	8.5500	15.428	6.750	5.5800	20.171
2	BG 3	1.570	4.000	10.953	15.078	3.750	11.580	22.670
3	BG 4	0.700	2.000	12.697	15.843	6.250	9.5800	23.400
4	BG 6	0.060	0.000	11.133	15.278	4.000	1.5800	19.387
5	BG 7	1.260	4.000	5.6070	12.161	9.250	1.5800	16.881
6	BG 9	1.520	4.000	8.1570	18.578	4.750	3.5800	21.572
7	BG 10	0.690	3.000	9.6850	21.398	4.417	11.580	26.735
8	BG 13	0.850	3.000	2.0950	4.1880	7.550	18.580	20.830
9	BG 14	1.120	4.000	15.520	14.948	3.320	13.580	26.019
10	BG 16	1.560	4.000	8.3670	22.178	7.850	18.580	31.419
11	BG 19	1.810	3.000	12.810	21.388	5.430	11.580	28.238
12	BG 23	1.920	3.000	4.5130	8.6610	1.420	6.5800	12.385
13	BG 24	1.610	3.000	6.5370	15.378	2.470	19.580	26.082
14	BG 25	1.740	4.000	13.140	16.161	6.750	17.580	28.416
15	BG 29	1.430	3.000	13.027	16.718	6.230	14.580	26.676
16	BG 30	0.680	2.000	7.8370	12.828	8.750	11.580	21.002
17	BG 33	1.790	4.000	13.440	22.298	7.910	17.580	32.690
18	BG 34	1.870	2.000	10.945	18.308	7.770	13.580	26.594
19	BG 37	0.810	2.000	11.660	21.561	7.417	17.580	31.138
20	BG 38	1.620	4.000	13.945	9.8280	4.417	7.5800	19.663

(Cont.)

21	BG 41	0.950	3.000	11.403	13.828	6.750	14.580	24.275
22	BG 42	0.370	2.000	12.453	15.008	6.340	11.580	23.638
23	BG 43	0.820	3.000	9.4080	6.1150	4.875	12.580	17.821
24	BG 44	1.250	4.000	9.4000	7.6880	5.350	16.580	21.646
25	BG 50	1.730	2.000	12.070	12.955	7.300	3.5800	19.663
26	BG 51	1.490	3.000	6.4900	15.198	4.930	11.580	21.041
27	BG 52	1.670	3.000	8.8630	19.328	4.790	17.580	28.212
28	BG 53	2.240	3.000	7.1300	15.328	9.320	15.580	25.088
29	BG 54	1.460	4.000	4.1500	9.5780	6.250	11.580	17.328
30	BG 62	0.780	4.000	5.1600	10.828	5.420	13.580	19.346
31	BG 63	1.460	3.000	5.1950	14.528	5.540	16.580	23.554
32	BG 66	1.250	4.000	8.2330	17.721	4.750	13.580	24.624
33	BG 67	0.690	2.000	15.575	9.3280	5.250	19.580	27.295
34	BG 77	1.210	2.000	9.4230	14.828	10.37	8.5800	22.255
35	BG 79	1.000	3.000	12.200	16.828	8.310	6.5800	23.545
36	BG 84	1.230	3.000	8.0550	13.828	10.52	4.5800	19.956
37	BG 85	1.600	4.000	9.0850	7.8280	5.083	6.5800	15.215
38	BG 86	1.640	4.000	6.4570	18.828	2.230	11.580	23.536
39	BG 87	1.130	2.000	6.0930	12.181	6.200	19.580	24.751
40	BG 88	1.750	4.000	8.9830	15.735	5.230	20.580	28.253
41	BG 95	0.590	1.000	7.5230	15.725	3.750	13.580	22.443
42	BG 98	0.780	2.000	4.3000	2.1610	0.083	17.580	18.353
43	BG 100	1.030	3.000	6.5170	13.055	2.750	11.580	19.095

(Cont.)

44	BG 101	2.150	3.000	12.945	18.078	7.250	16.580	28.904
45	BG 107	1.410	4.000	13.780	0.1720	9.250	14.580	22.495
46	BG 117	1.700	4.000	4.1200	10.078	1.750	18.580	22.039
47	BG 119	1.670	3.000	8.6800	5.8280	0.750	11.580	15.992
48	BG 123	1.350	2.000	2.1700	3.4950	5.750	5.5800	9.3240
49	BG 124	0.730	1.000	0.1250	11.078	9.750	11.580	18.800
50	BG 125	1.310	3.000	6.6500	10.828	7.250	17.580	23.104
51	BG 127	1.470	4.000	7.5400	17.953	5.000	13.580	24.632
52	BG 130	0.870	2.000	5.9550	6.1610	5.417	17.580	20.410
53	BG 135	1.900	4.000	7.7150	20.778	9.750	11.580	27.203
54	BG 138	2.120	3.000	8.2170	26.328	10.42	16.580	34.023
55	BG 141	0.450	3.000	5.3700	13.568	8.750	7.5800	18.872
56	BG 142	1.200	4.000	8.0200	19.728	9.750	11.580	26.460
57	BG 149	1.570	3.000	6.7870	11.828	2.750	19.580	24.256
58	BG 154	0.850	2.000	7.2530	18.411	3.250	18.580	27.424
59	BG 158	1.460	3.000	6.7970	20.153	8.750	11.580	25.964
<b>60</b>	<b>Githika</b>	<b>0.000</b>	<b>0.000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.000</b>	<b>0.0000</b>	<b>0.0000</b>
<b>61</b>	<b>IRON</b>	<b>2.280</b>	<b>4.000</b>	<b>18.082</b>	<b>29.228</b>	<b>3.550</b>	<b>20.920</b>	<b>40.653</b>

coefficient (4.000) was observed in the twenty resistant BC<sub>1</sub>F<sub>1</sub> lines. The IRON also had a maximum dissimilarity coefficient (4.000) with the Githika.

#### ***4.9.3.3. Pod weight***

The minimum dissimilarity coefficient was observed in BG124 (0.1250) and the maximum in BG67 (15.575) with the recurrent parent Githika (0.000). The dissimilarity coefficient in IRON with Githika was found to be 18.082 for pod weight.

#### ***4.9.3.4. Pod length***

The dissimilarity coefficient for pod length between the resistant BC<sub>1</sub>F<sub>1</sub> lines and the recurrent parent Githika varied from 0.1270 to 22.298. The minimum dissimilarity coefficient was observed in BG107 (0.1270) and the maximum in BG33 (22.298) with the recurrent parent Githika (0.000). The donor parent IRON had a 29.228 dissimilarity coefficient with Githika.

#### ***4.9.3.5. Seeds per pod***

The resistant BC<sub>1</sub>F<sub>1</sub> segregant BG98 (0.083) was found to be more similar to recurrent parent Githika (0.000) for the number of seeds per pod. The maximum dissimilarity coefficient was observed in BG84 (10.520) for seeds per pod. The donor IRON was 3.550 dissimilar to the recipient parent Githika.

#### ***4.9.3.6. Crop duration***

The dissimilarity coefficient varied from 1.5800 to 20.580 for crop duration in the resistant BC<sub>1</sub>F<sub>1</sub> lines. The maximum coefficient (20.850) was observed in BG88 and the minimum (1.580) in BG6 and BG7. The donor parent IRON was 20.920 dissimilar to the recurrent parent Githika (0.000).

#### ***4.9.3.7. Overall characters***

All six morphological characters were considered for calculating the overall dissimilarity coefficient among the resistant BC<sub>1</sub>F<sub>1</sub> lines with recurrent parent Githika. The overall dissimilarity coefficient ranged from 9.3240 to 34.023

compared with Githika. Of the 59 resistant BC<sub>1</sub>F<sub>1</sub> lines twenty-five were close to the recurrent parent with the least dissimilarity coefficient. These 25 resistant BC<sub>1</sub>F<sub>1</sub> lines were used to produce BC<sub>2</sub>F<sub>1</sub> lines.

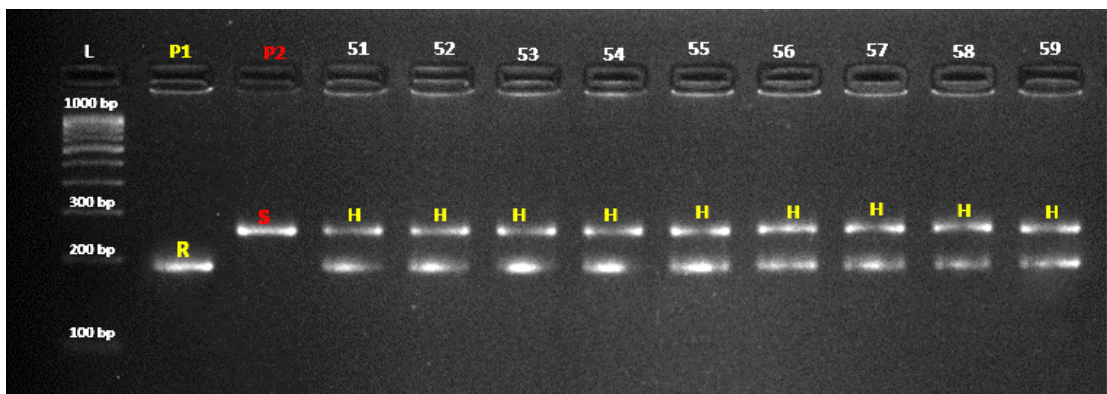
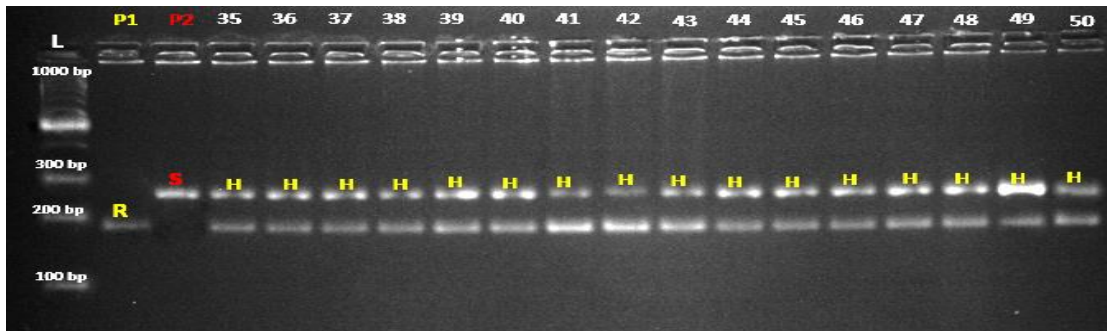
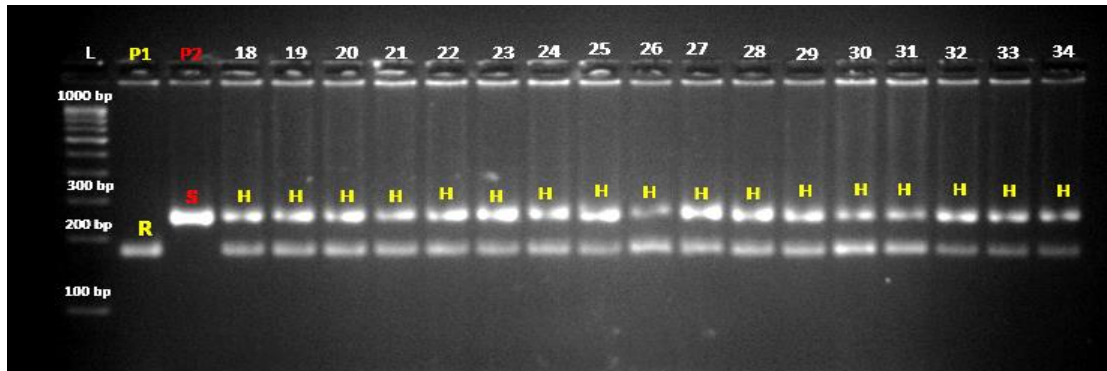
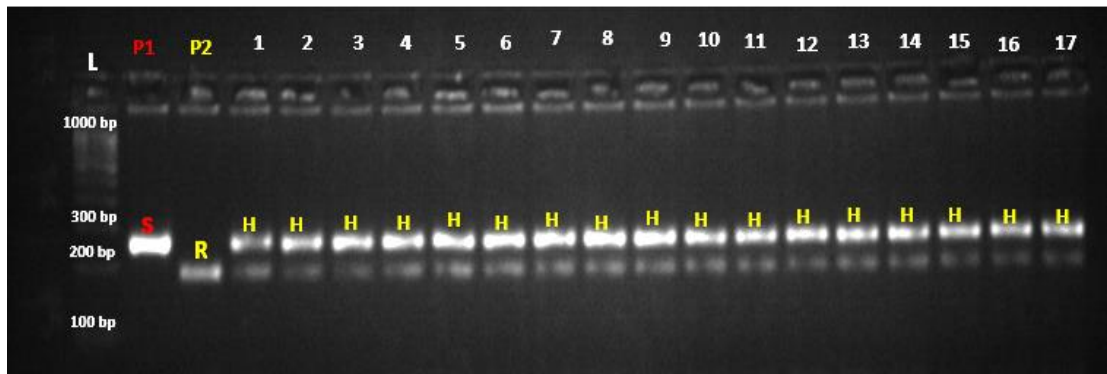
#### 4.10. CONFIRMATION OF RESISTANT GENE IN BC<sub>1</sub>F<sub>1</sub> LINES USING SSR MARKER LINKED TO FUSARIUM WILT

The genomic DNA from all the resistant BC<sub>1</sub>F<sub>1</sub> lines was isolated using the CTAB method. The quantity and quality of genomic DNA were assured using a spectrophotometer and agarose electrophoresis. The genomic DNA of fifty-nine resistant BC<sub>1</sub>F<sub>1</sub> lines was amplified using Fusarium wilt-linked SSR marker UASBC59. The resistant donor IRON produced an amplicon at 190bp (R) and the recipient parent Githika produced an amplicon size of 250bp (r). All the resistant BC<sub>1</sub>F<sub>1</sub> lines produced heterozygous (Rr) bands with two amplicon products at 190 bp and 250 bp size (Plate 35).

#### 4.11. EVALUATION OF BC<sub>2</sub>F<sub>1</sub> LINES AT THE SEEDLING STAGE THROUGH ARTIFICIAL SCREENING AND SCORING

The twenty- five resistant BC<sub>1</sub>F<sub>1</sub> lines which were closely similar to the recurrent parent Githika were backcrossed to produce BC<sub>2</sub>F<sub>1</sub> lines. A total of 128 BC<sub>2</sub>F<sub>1</sub> lines were artificially screened for Fusarium wilt reaction and scored based on a 0-4 scale after two weeks of inoculation (Table 28). Out of 128 BC<sub>2</sub>F<sub>1</sub> lines, thirty-five scored '0' and were Fusarium wilt-resistant lines (Plate 36). Nineteen BC<sub>2</sub>F<sub>1</sub> lines scored '1' representing moderately resistant lines (Plate 37) and twenty-one scored '2' indicating moderately susceptible lines (Plate 38). There were twelve susceptible lines (Plate 39) and forty-one highly susceptible lines (Plate 40) with scores of '3' and '4' respectively. A comparison of BC<sub>2</sub>F<sub>1</sub> lines with their parents is shown in Plate 41.

The thirty-five resistant BC<sub>2</sub>F<sub>1</sub> lines were transplanted to the greenhouse for further evaluation or a backcrossing programme (Plate 42).



**Plate 35. Amplification profile of UASBC59 SSR marker in Fusarium resistant BC<sub>1</sub>F<sub>1</sub> lines, S-Susceptible (250bp), R-Resistant (190bp), L-100bp ladder, 1-59 in resistant BC<sub>1</sub>F<sub>1</sub> lines**

**Table 28. Disease scoring of BC<sub>2</sub>F<sub>1</sub> lines from 25 resistant BC<sub>1</sub>F<sub>1</sub> lines on a 0-4 scale**

Sl No.	Genotypes	Disease Scoring	Disease Reaction
1	BG 1-1	0	Resistant (R)
2	BG 1-2	4	Highly Susceptible (HS)
3	BG 1-3	4	Highly Susceptible (HS)
4	BG 1-4	0	Resistant (R)
5	BG 3-1	3	Susceptible (S)
6	BG 3-2	1	Moderately Resistant (MR)
7	BG 3-3	4	Highly Susceptible (HS)
8	BG 3-4	0	Resistant (R)
9	BG 3-5	3	Susceptible (S)
10	BG 6-1	0	Resistant (R)
11	BG 6-2	0	Resistant (R)
12	BG 6-3	0	Resistant (R)
13	BG 6-4	1	Moderately Resistant (MR)
14	BG 6-5	3	Susceptible (S)
15	BG 7-1	4	Highly Susceptible (HS)
16	BG 7-2	4	Highly Susceptible (HS)
17	BG 7-3	2	Moderately Susceptible (MS)
18	BG 7-4	4	Highly Susceptible (HS)
19	BG 7-5	2	Moderately Susceptible (MS)
20	BG 7-6	0	Resistant (R)
21	BG 7-7	1	Moderately Resistant (MR)
22	BG 9-1	4	Highly Susceptible (HS)
23	BG 9-2	4	Highly Susceptible (HS)
24	BG 9-3	2	Moderately Susceptible (MS)
25	BG 9-4	4	Highly Susceptible (HS)
26	BG 9-5	4	Highly Susceptible (HS)
27	BG 9-6	0	Resistant (R)
28	BG 13-1	1	Moderately Resistant (MR)
29	BG 13-2	4	Highly Susceptible (HS)
30	BG 13-3	4	Highly Susceptible (HS)
31	BG 13-4	4	Highly Susceptible (HS)
32	BG 13-5	2	Moderately Susceptible (MS)
33	BG 13-6	0	Resistant (R)
34	BG 13-7	2	Moderately Susceptible (MS)
35	BG 23-1	0	Resistant (R)
36	BG 23-2	4	Highly Susceptible (HS)
37	BG 23-3	2	Moderately Susceptible (MS)
38	BG 23-4	0	Resistant (R)
39	BG 23-5	2	Moderately Susceptible (MS)
40	BG 30-1	0	Resistant (R)
41	BG 30-2	4	Highly Susceptible (HS)

(Cont.)

42	BG 30-3	2	Moderately Susceptible (MS)
43	BG 30-4	0	Resistant (R)
44	BG 30-5	0	Resistant (R)
45	BG 30-6	1	Moderately Resistant (MR)
46	BG 38-1	4	Highly Susceptible (HS)
47	BG 38-2	2	Moderately Susceptible (MS)
48	BG 38-3	4	Highly Susceptible (HS)
49	BG 38-4	1	Moderately Resistant (MR)
50	BG 38-5	3	Susceptible (S)
51	BG 38-6	4	Highly Susceptible (HS)
52	BG 38-7	4	Highly Susceptible (HS)
53	BG 38-8	4	Highly Susceptible (HS)
54	BG 38-9	2	Moderately Susceptible (MS)
55	BG 38-10	0	Resistant (R)
56	BG 43-1	0	Resistant (R)
57	BG 43-2	1	Moderately Resistant (MR)
58	BG 44-1	2	Moderately Susceptible (MS)
59	BG 44-2	1	Moderately Resistant (MR)
60	BG 44-3	0	Resistant (R)
61	BG 44-4	0	Resistant (R)
62	BG 44-5	1	Moderately Resistant (MR)
63	BG 44-6	4	Highly Susceptible (HS)
64	BG 44-7	2	Moderately Susceptible (MS)
65	BG 44-8	4	Highly Susceptible (HS)
66	BG 44-9	1	Moderately Resistant (MR)
67	BG 44-10	4	Highly Susceptible (HS)
68	BG 44-11	4	Highly Susceptible (HS)
69	BG 44-12	1	Moderately Resistant (MR)
70	BG 44-13	3	Susceptible (S)
71	BG 44-14	0	Resistant (R)
72	BG 44-15	1	Moderately Resistant (MR)
73	BG 50-1	4	Highly Susceptible (HS)
74	BG 50-2	0	Resistant (R)
75	BG 50-3	2	Moderately Susceptible (MS)
76	BG 50-4	0	Resistant (R)
77	BG 50-5	4	Highly Susceptible (HS)
78	BG 50-6	2	Moderately Susceptible (MS)
79	BG 51-1	0	Resistant (R)
80	BG 51-2	0	Resistant (R)
81	BG 51-3	4	Highly Susceptible (HS)
82	BG 51-4	1	Moderately Resistant (MR)
83	BG 51-5	4	Highly Susceptible (HS)
84	BG 54-1	0	Resistant (R)
85	BG 54-2	4	Highly Susceptible (HS)

(Cont.)

86	BG 54-3	3	Susceptible (S)
87	BG 62-1	1	Moderately Resistant (MR)
88	BG 62-2	0	Resistant (R)
89	BG 62-3	0	Resistant (R)
90	BG 62-4	4	Highly Susceptible (HS)
91	BG 62-5	3	Susceptible (S)
92	BG 62-6	4	Highly Susceptible (HS)
93	BG 84-1	1	Moderately Resistant (MR)
94	BG 84-2	4	Highly Susceptible (HS)
95	BG 84-3	2	Moderately Susceptible (MS)
96	BG 84-4	0	Resistant (R)
97	BG 84-5	1	Moderately Resistant (MR)
98	BG 85-1	4	Highly Susceptible (HS)
99	BG 85-2	0	Resistant (R)
100	BG 85-3	3	Susceptible (S)
101	BG 85-4	2	Moderately Susceptible (MS)
102	BG 85-5	4	Highly Susceptible (HS)
103	BG 85-6	4	Highly Susceptible (HS)
104	BG 98-1	3	Susceptible (S)
105	BG 98-2	2	Moderately Susceptible (MS)
106	BG 98-3	0	Resistant (R)
107	BG 100-1	1	Moderately Resistant (MR)
108	BG 100-2	4	Highly Susceptible (HS)
109	BG 100-3	4	Highly Susceptible (HS)
110	BG 100-4	2	Moderately Susceptible (MS)
111	BG 100-5	0	Resistant (R)
112	BG 100-6	2	Moderately Susceptible (MS)
113	BG 100-7	0	Resistant (R)
114	BG 117-1	2	Moderately Susceptible (MS)
115	BG 117-2	4	Highly Susceptible (HS)
116	BG 117-3	4	Highly Susceptible (HS)
117	BG 117-4	1	Moderately Resistant (MR)
118	BG 119-1	3	Susceptible (S)
119	BG 119-2	2	Moderately Susceptible (MS)
120	BG 123-1	0	Resistant (R)
121	BG 123-2	4	Highly Susceptible (HS)
122	BG 123-3	4	Highly Susceptible (HS)
123	BG 123-4	3	Susceptible (S)
124	BG 123-5	0	Resistant (R)
125	BG 130-1	3	Susceptible (S)
126	BG 130-2	0	Resistant (R)
127	BG 141-1	0	Resistant (R)
128	BG 141-2	1	Moderately Resistant (MR)



**Plate 36. Fusarium wilt resistant (R) BC<sub>2</sub>F<sub>1</sub> lines (score '0')**



**Plate 37. Fusarium wilt moderately Resistant (MR) BC<sub>2</sub>F<sub>1</sub> lines (Score '1')**



**Plate 38. Fusarium wilt moderately Susceptible BC<sub>2</sub>F<sub>1</sub> lines (Score '2')**



**Plate 39. Fusarium wilt susceptible (S) BC<sub>2</sub>F<sub>1</sub> lines (Score '3')**



**Plate 40. Fusarium wilt highly Susceptible (HS) BC<sub>2</sub>F<sub>1</sub> lines (Score '4')**



**Plate 41. Comparison of BC<sub>2</sub>F<sub>1</sub> lines with Githika and IRON for the reaction to Fusarium wilt**



**Plate 42. Transplanted resistant BC<sub>2</sub>F<sub>1</sub> lines under greenhouse condition**

## ***DISCUSSION***

## 5. DISCUSSION

Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) is the most popular legume vegetable in Kerala. It is mainly grown for its young leaves, immature pods, and mature dry seeds (Hall *et al.*, 2003; Hazra *et al.*, 2007) and is favoured for nutrition and heritage culinary (Sultana *et al.*, 2020). The crop is a major component of sustainable agriculture due to its short duration (Das *et al.*, 2018). It tolerates heat and slightly dry conditions better than pole bean and is day-neutral. Yardlong beans are harvested at the immature pod stage (45-75 cm) and play an important role in diet due to the high percentage of digestive protein, minerals, vitamins, and micronutrients (Toppo and Sahu, 2020).

Due to difficulties in adapting to their environment and their vulnerability to pests and diseases, the yield of most legumes is still low and inconsistent (Rubiales *et al.*, 2015). The primary factors limiting the output of yardlong beans are pests and diseases. Among the diseases, Fusarium wilt causes high economic losses (Okiror, 2002). Vascular browning, leaf epinasty, stunting, gradual wilting, defoliation, and finally plant death are the characteristic symptoms of the disease (Agrios, 2005). Besides cultural, chemical, and biological control techniques, the use of resistant cultivars is largely recognized as the safest, most practical, and most successful crop protection strategy to manage soil-borne diseases (Sampaio *et al.*, 2020).

Marker-assisted selection can accelerate traditional breeding using DNA markers (Gujaria *et al.*, 2011). With molecular markers, backcrossing can be accomplished using Marker-assisted backcrossing. This strategy is frequently applied to introgress desired traits, such as disease and pest resistance, nutritional factors, etc. by transferring the gene of choice or quantitative trait loci (QTLs) from the donor parent to high-yielding cultivated varieties (Ribaut *et al.*, 2004). Many researchers worked for transferring Fusarium wilt resistance through marker-assisted backcrossing methods in various crops, including Sousaraei *et al.* (2018) in melon, Varshney *et al.* (2014), Pratap *et al.* (2017) and Mannur *et al.* (2019) in

chickpea, Simsek *et al.* (2018) in tomato, Liu *et al.* (2017) in cabbage, Mehraj *et al.* (2020) in *Brassica* spp. and Kekec *et al.* (2022) in eggplant.

With this background, the present study entitled Marker-assisted backcrossing for transferring Fusarium wilt (*Fusarium oxysporum* f. sp. *tracheiphilum*) resistance into yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) was carried out with an objective to introgress Fusarium wilt resistant gene into yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) through marker-assisted backcrossing. The results obtained in the study are discussed below.

#### 5.1. ISOLATION, IDENTIFICATION, AND MULTIPLICATION OF FUSARIUM WILT CULTURE

The pathogen isolated from Fusarium-infected yardlong bean plants from the Vellayani region was aseptically cultured on potato dextrose agar (PDA) medium. The PDA medium was one of the best-supporting media for maximum mycelial growth and abundant sporulation for *Fusarium oxysporum* (Choudhary *et al.*, 2022). The incidence of Fusarium wilt has been widely distributed all through the cowpea fields in Thiruvananthapuram district since 1995-96 (Reghunath *et al.*, 1995). The cowpea Fusarium wilt was also noticed in the farmers' fields in different places in the Thiruvananthapuram district (Senthilkumar, 2003). Of the ten isolates obtained from the infected yardlong bean fields, only five were close to the Fusarium pathogen in culture appearance. It was noted that the Fusarium pathogens occasionally occur as a group of pathogens (Beccari *et al.*, 2010), hence the isolation of these pathogens may also include other pathogens.

The isolated *Fusarium* spp. completed its growth in Petri plates within 7-11 days at 28°C. About 8-9 days with 5 mm per day radial growth of Fusarium pathogen isolated from infected cowpea were reported (Chauhan, 2000). The colonies were floccose in texture, white raised mycelia, and yellow to reddish brown in the culture appearance. *Fusarium* spp. was known to produce secondary metabolites and toxins in the culture plates that may lead to coloured media (Manici *et al.*, 2017). The microconidia were abundant, and aseptate with 8.339-12.879 X

1.974-3.914  $\mu\text{m}$  in dimensions. The macroconidia were 2-3 septate with 32.409-26.692 X 5.927-2.662  $\mu\text{m}$ , thin-walled and sickle-shaped. In all the cultures chlamydospores were present. The specification of *Fusarium* was in accordance with the epi-typification of *Fusarium oxysporum* given by Lombard *et al.* (2019). The primary characteristics used to distinguish between various *Fusarium* species include macroscopic and microscopic characteristics such as cultural characteristics, macro-conidial length and form, micro-conidial number, shape, and arrangement, and presence or absence of chlamydospores (Larone, 1995; De Hoog *et al.*, 2000). The cultural and morphological differences in the *Fusarium oxysporum* causing Fusarium wilt were recorded by Honnareddy and Dubey (2006) in chickpeas, Mwangombe *et al.* (2008) in common peas, Zhu *et al.* (2021) in cotton, Pothiraj *et al.* (2021) in tomatoes and Metsena *et al.* (2021) in cowpea.

Inoculation is the process of introducing alive microorganisms into a host using a medium that will allow their growth, multiplication, and colonisation, which will eventually result in disease (Imathiu *et al.*, 2014). Soil inoculation with 10 per cent sand: maize media fungi multiplied was observed to be the best inoculation method for the *Fusarium* pathogen, which obtained 100% *Fusarium* wilt symptoms in transplanted healthy cowpea seedlings including vascular discolouration, yellowing and necrotic leaves with total wilting of plants. Colonised maize grain is the most effective at significantly causing *Fusarium* head blight in wheat (Imathiu *et al.*, 2014). About 68 to 72 per cent of *Fusarium* wilt incidence on onion was reported by Yadav *et al.* (2017) in transplanted seedlings in the inoculated soil. As a soil-borne pathogen, the *Fusarium* spp. was easily multiplied in the soil after inoculation and an amount of inoculum was available to enter the cowpea vascular system to cause symptoms. The potting mix infested with *Fusarium oxysporum* closer to that of field-level densities produced consistent disease symptoms that were closely related to the cotton cultivar reaction observed in the field (Potter, 2005).

From the virulence rating of the isolated pathogens, out of five isolates, three isolates induced *Fusarium* wilt symptoms and isolate Fot1 developed early

symptoms in healthy cowpea seedlings and was found to be a highly virulent isolate. It was mass multiplied in the sand maize medium and 10 per cent of inoculum produced the typical *Fusarium* symptoms. *Fusarium* pathogen multiplied in rice bran media was inoculated at 5g pit<sup>-1</sup> to the furrows in the field for screening yardlong bean varieties (Madhukumar, 2006). Ashwathi *et al.* (2017) used 5 per cent (w/w) sand: maize media fungi multiplied for the pathogenicity test of *Fusarium* spp. associated with vascular wilt of coriander. About 4 per cent soil inoculation for *Fusarium oxysporum* f.sp. *ciceri* screening in chickpeas (Kala *et al.*, 2016), and 10 per cent inoculation for *Fusarium oxysporum* f.sp. *lycopersici* in tomatoes (Manikandan *et al.*, 2018) confirmed the consistent disease symptoms. Hence, the per cent of *Fusarium* inoculum for screening changes according to the virulent capacity of the pathogen for inducing the symptoms. Screening by a virulent strain of the pathogen may always be reliable and accurate due to enough of a pathogen inoculum to cause disease (Fred *et al.*, 2016).

The detection and confirmation of pathogens are prerequisites for screening purposes. The ITS region contains frequent tandem repeats of ribosomal RNA in the haploid genome, which were particularly helpful in identifying species, hence it has been widely used as a standard marker in DNA bar-coding of fungal species (Janevska and Tudzynski, 2018). The confirmation of the isolated Fot1 pathogen was assessed from the characteristic ITS-ribosomal DNA (rDNA). The internal transcribed spacer (ITS) region was more variable and used for species-level relationship investigation (Bruns *et al.*, 1991). So, the ITS region of Fot1 was amplified using universal primers ITS1 and ITS4, the amplicon (500bp) sequence was trimmed, assembled and a consensus sequence was obtained. The ITS region was utilized for the detection, identification, classification, and phylogenetic analysis of many fungi at the species level (Hibbett, 1992; Oliver, 1993; Taylor *et al.*, 2000; Shahnazi *et al.*, 2012). The amplification of the ITS region of 12 different *Fusarium* sp. isolates using ITS1-ITS4 primers yielded an amplicon size of 500 to 530 bp (Lee *et al.*, 2000; Sreeja, 2014).

The similarity search of the consensus sequence in NCBI through BLAST analysis recorded 99-100 per cent similarity with the *Fusarium oxysporum* database available in the NCBI. Nucleotide sequencing of ITS fragments was required for determining interspecific and intraspecific differences due to the consistency of ITS fragment size across many fungi groups (Batista *et al.*, 2008). The BLAST software evaluates the statistical significance of matches between nucleotide or protein sequences. It is possible to determine the members of gene families and infer functional and evolutionary links between sequences (Boratyn *et al.*, 2013). The phylogenetic analysis of the sequence with the *Fusarium oxysporum* database from the NCBI showed similarity with 100% bootstrap support. The phylogenetic analysis to differentiate *Fusarium* spp. using ITS nucleotide sequences was attempted by researchers as well (Lee *et al.*, 2000; Sreeja, 2014). Hence, the isolated Fot1 pathogen was confirmed as *Fusarium oxysporum* f. sp. *tracheiphilum* and the ITS-rDNA sequence of the isolate was submitted to the NCBI with accession number MZ706472.1.

## 5.2. ARTIFICIAL INOCULATION OF *Fusarium oxysporum* AND SCORING OF DISEASE INCIDENCE IN COWPEA GERMPLASM

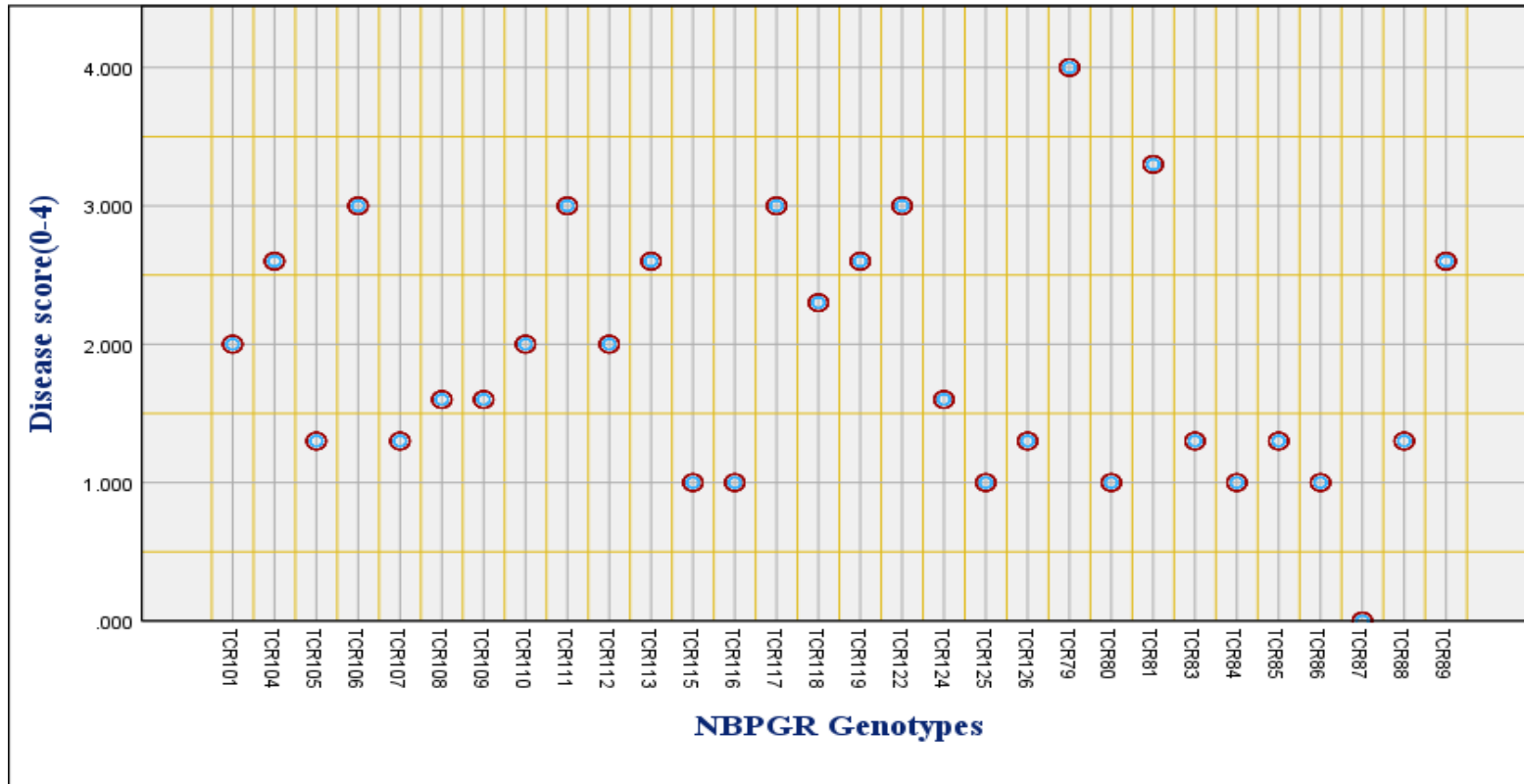
The artificial inoculation of plants with pathogens provides insights into plant-pathogen interactions and aids in the identification of disease-resistant crops (Koch *et al.*, 2020). In the study, artificial screening of 115 cowpea genotypes by soil inoculation with pathogen multiplied in the sand: maize medium (10%) was scored based on a 0-4 scale given by Senthilkumar (2003). Out of 115 cowpea genotypes, eighteen scored 0 to 0.3 with 0% disease intensity which were considered as the *Fusarium* wilt-resistant cowpea genotypes. The scoring of genotypes using a disease rating scale to *Fusarium* wilt was carried out in different crops including cowpea (Madhukumar, 2006; Nair, 2006; Omoigui *et al.*, 2018), eggplant (Partha *et al.*, 2016), chickpea (Venkataramanamma *et al.*, 2018; Yadav and Kumar, 2019; Kumar *et al.*, 2019), tomato (Sanap *et al.*, 2020), lentil (Roy *et al.*, 2021), and peas (Deng *et al.*, 2022). Results also revealed that the genotypes from various sources have variable levels of disease reaction. A genotype collected

from NBPGR (Figure 6), 4 genotypes from AVRDC (Figure 7) and thirteen genotypes from local collections (Figure 8) were found to be resistant to *Fusarium* wilt. The local cowpea genotypes from Kerala, the Thiruvananthapuram local-1 and Thiruvananthapuram local-3 recorded the highest yield with moderate resistance against *Fusarium* wilt (Madhukumar, 2006). In general, landraces have a high degree of genetic variability and disease resistance (Rubiales *et al.*, 2021). The lowest score of '0.7' was recorded in the KAU-released variety Manjari (Figure 9).

The cowpea genotypes took 15 to 53 days to show *Fusarium* wilt symptoms after inoculation. The chickpea genotypes were screened using pot culture compared to the sick plot approach, in which the late wilt percentage was higher than the early wilt percentage (Yadav and Kumar, 2019). The wilting intensity was maximum during the flowering or pod stage than the seedling stage in cowpea genotypes. It might be due to the prolonged exposure of plants to pathogens and the age of seedlings used for screening. Zemouli-Benfreha *et al.* (2014) and Asfaw (2018) reported that the *Fusarium oxysporum* causes *Fusarium* wilt in chickpeas (*Foc*) infects at every stage of crop growth but it was detrimental at blooming and pod-setting growth stages.

In the study, the *Fusarium* disease intensity in the 115 cowpea genotypes varied from 0 to 80 per cent and was categorized into different reaction groups. The 0 per cent disease intensity was observed in the resistant genotypes and 80% in highly susceptible genotypes. The *Fusarium* wilt intensity in thirteen advanced chickpea genotypes was reported as 5.5 to 18.4 per cent under field screening and 0 to 20% in artificial screening with the susceptible check of 95 per cent and 100 per cent under field and artificial screening respectively (Venkataramanamma *et al.*, 2018).

The early wilt incidence varied from 0 to 75 per cent in chickpea lines, whereas late wilt incidence was 0 to 100 per cent (Yadav and Kumar, 2019). Seven cultivars of tomato showed 7.14 to 62.69 per cent of wilt incidence under poly bag culture screening to *F. oxysporum* f. sp. *lycopersici* (Sanap *et al.*, 2020). Deng *et al.* (2022) reported a 10-100 per cent disease index, 0-100 per cent disease rate, and



**Figure 6. Disease scoring of NBPGR cowpea genotypes**

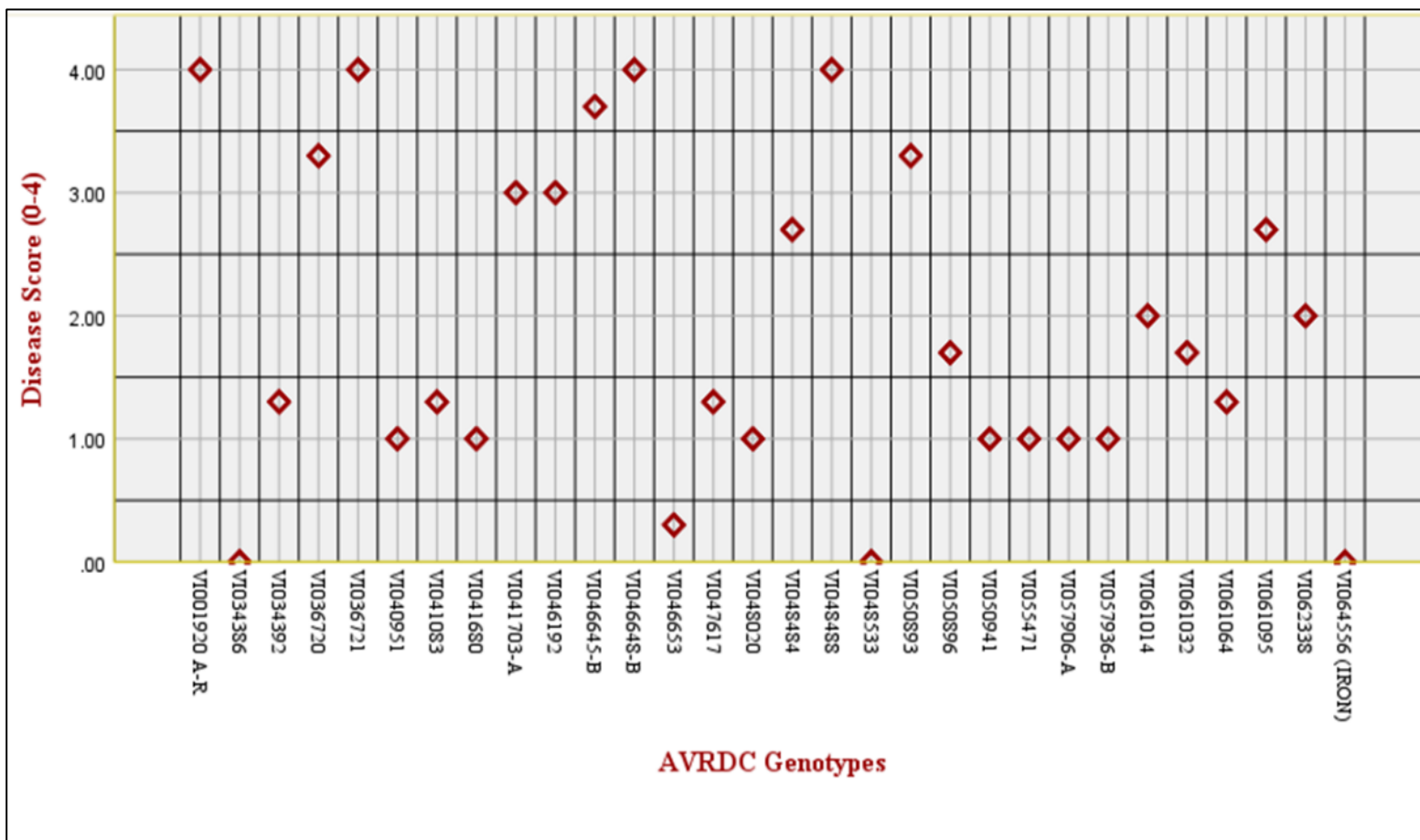


Figure 7. Disease scoring of AVRDC cowpea genotypes

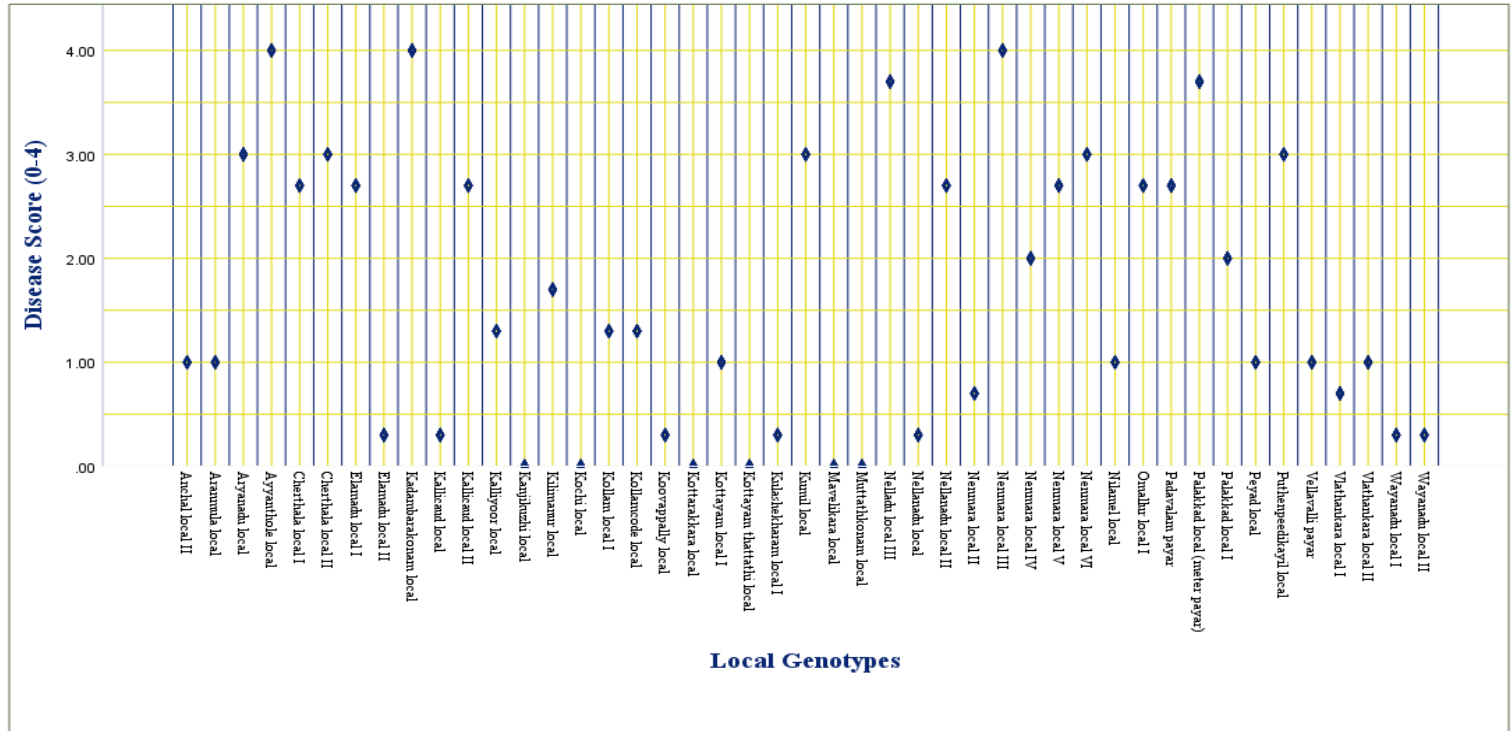
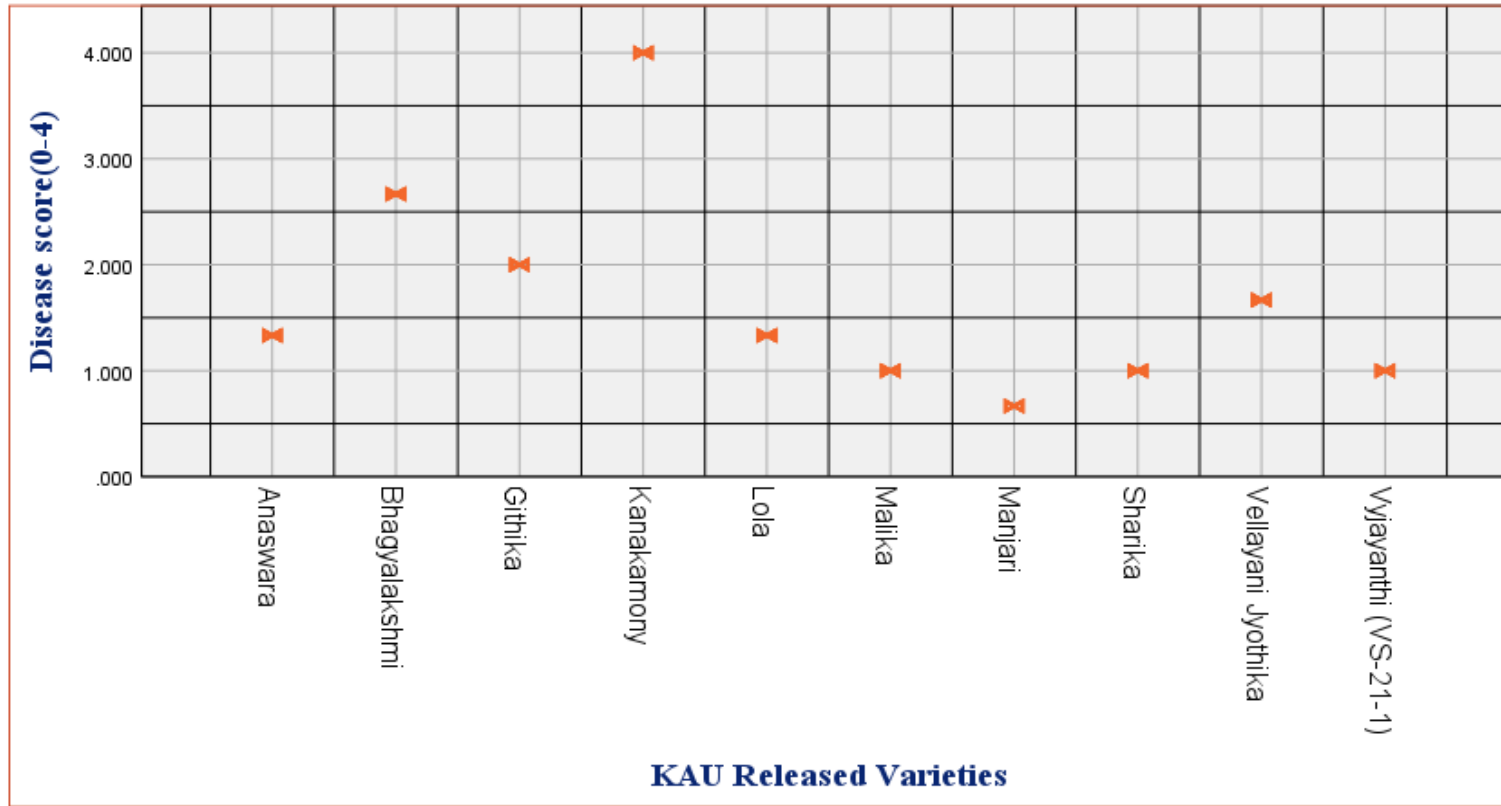


Figure 8. Disease scoring of local cowpea genotypes



**Figure 9. Disease scoring of KAU-released cowpea genotypes**

1.67-100 per cent of leaves showing symptoms in seven different cultivars of peas to *Fusarium oxysporum* f. sp. *pisi* at 28 days post-inoculation (dpi). Hence, the disease reaction intensity depends on the culture of *Fusarium* spp. used, the genotypes under screening and the prevailing environment in terms of soil moisture, temperature, pH, and other factors.

### 5.3. EVALUATION OF RESISTANT GENOTYPES FOR YIELD AND QUALITY LINKED WITH FUSARIUM WILT RESISTANCE

The eighteen resistant cowpea genotypes consisted of twelve trailing types and six bush types. These resistant genotypes along with susceptible KAU-released cowpea varieties, three from trailing type and one from bush type were evaluated under field conditions in two different experiments. The yield attributing characters and quality characters were evaluated in these cowpea genotypes and values were compared.

#### 5.3.1. Evaluation of resistant trailing-type cowpea genotypes along with susceptible checks under field conditions

The resistant and susceptible trailing-type cowpea genotypes were evaluated for nine biometric and three quality parameters. The mean performance of each genotype for the characters showed significant variations with ample scope for improvement. Of the characters, pod weight, pods per plant, pod length, and vine length largely determine the crop yield per plant, thus being considered as the yield attributes (Figure 10a-d). The variability distribution was broad in the case of primary branches per plant, seeds per pod, crop duration, number of harvests, total phenols, crude protein content, and crude fibre content (Figure 10e-k). The distribution of variability was narrow for the characters' pod weight, pods per plant and pod length in trailing-type cowpea genotypes. The tremendous variability in yardlong beans was reported by Bhagavati *et al.* (2018), Savithiri *et al.* (2018), Ugale *et al.* (2020) and George (2022).

Heatmap clustering is a false-coloured image clustering, in which the data values are transformed to colour scale. Similar observations are clustered together

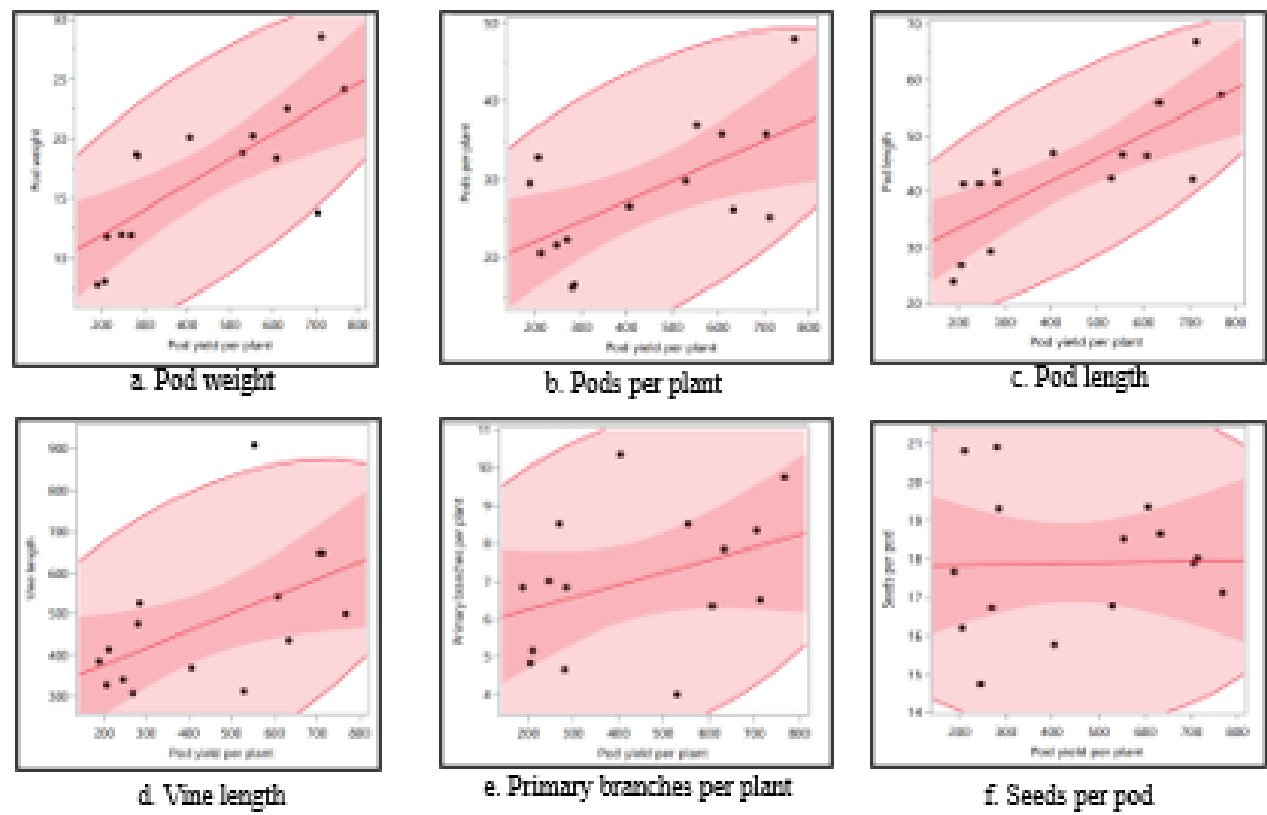
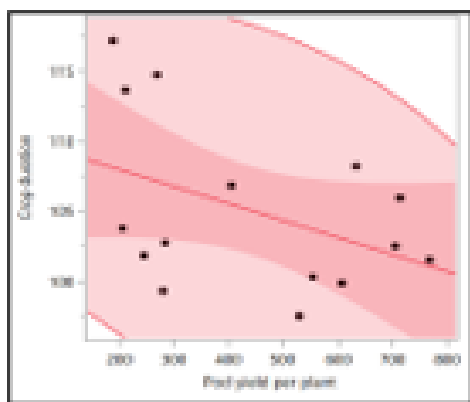
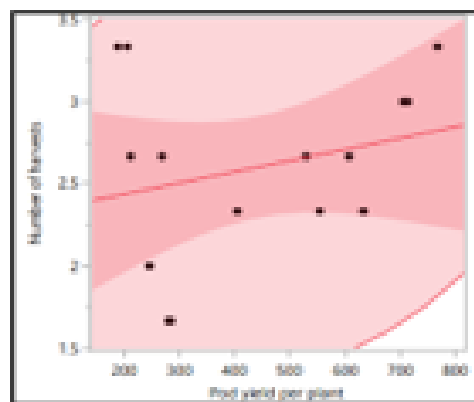


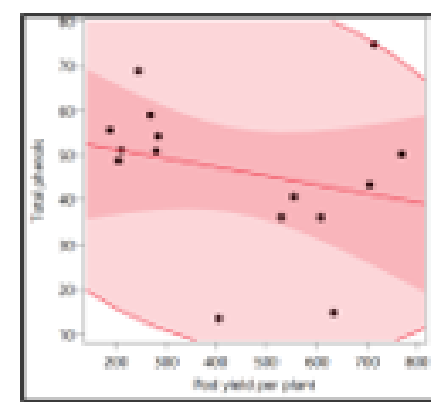
Figure 10. Scatterplot diagram of characters by pod yield per plant in trailing-type cowpea



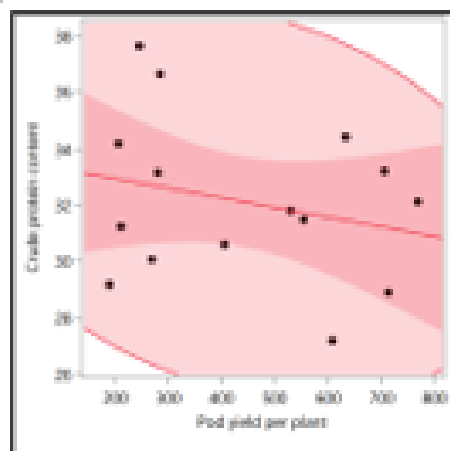
**g. Crop duration**



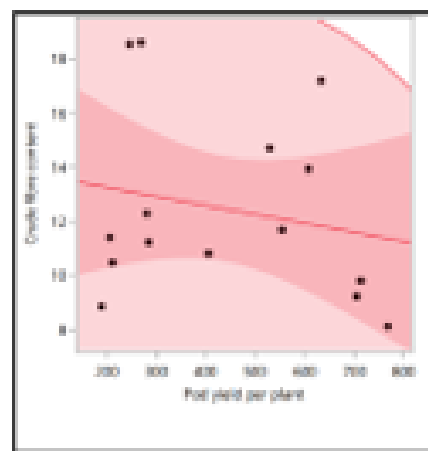
**h. Number of harvests**



**i. Total phenols**



**j. Crude protein content**



**k. Crude fibre content**

**Figure 10 (cont.). Scatterplot diagram of characters by pod yield per plant in trailing-type cowpea**

heat map hierarchical clustering analysis of trailing type genotypes using biometric and quality parameters could produce seven clusters, indicating that the genotypes were genetically divergent. The KAU released susceptible cowpea varieties (Githika, Vellayani Jyothika and Lola) that were grouped into a single cluster. The Lola and Vellayani Jyothika were grouped into the same clusters using seed quality parameters by Aswathi *et al.* (2016). Asoontha and Abraham (2017) grouped the local accessions and Githika in different clusters using ten biometric traits observation values.

The estimates of the coefficient of variation designated the amount of variability present in the population (Kusmiyati *et al.*, 2022). The value of the genotypic coefficient of variation (GCV) was smaller than the phenotypic coefficient of variation (PCV), but a small difference was recorded between PCV and GCV indicating that the environment had less influence towards the trait expression (Figure 11). The presence of a narrow gap between PCV and GCV was also reported by Vidya *et al.* (2002), Savithri *et al.* (2018), Kusmiyati *et al.* (2022), and George (2022) in the yardlong bean genotypes.

Heritability is the heritable component of a character's phenotypic variance and shows the extent to which a character is passed down from one generation to the next. A high heritability estimate reflects a character's low reliance on the environment, making such a character a good candidate for direct selection. Greater genotypic variance on the heritable traits is measured by a character's higher broad sense heritability value rather than phenotypic effect. Heritability estimates combined with predicted genetic advances are more useful than heritability alone for estimating yield under phenotypic selection (Johnson *et al.* 1955). In the present study, the trailing-type cowpea genotypes recorded the high heritability coupled with high genetic advance for the character's vine length, primary branches per plant, pods per plant, pod yield per plant, pod weight, pod length, total phenols, crude fibre content that these traits are predominant in additive gene action (Figure 12). The same findings were reported by Resmi *et al.* (2004) for pod yield per plant and pod weight, Rambabu *et al.* (2016) for pod length, plant height and pod yield

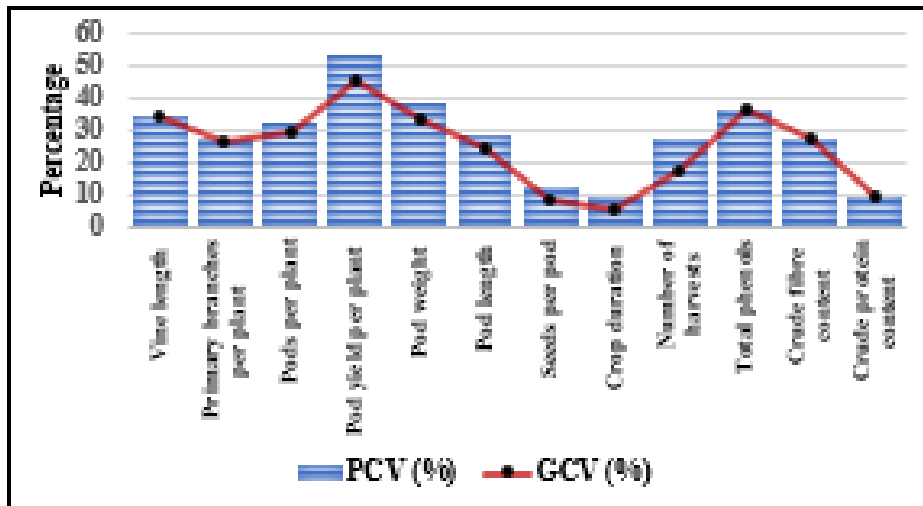


Figure 11. PCV and GCV of twelve characters in trailing-type cowpea genotypes

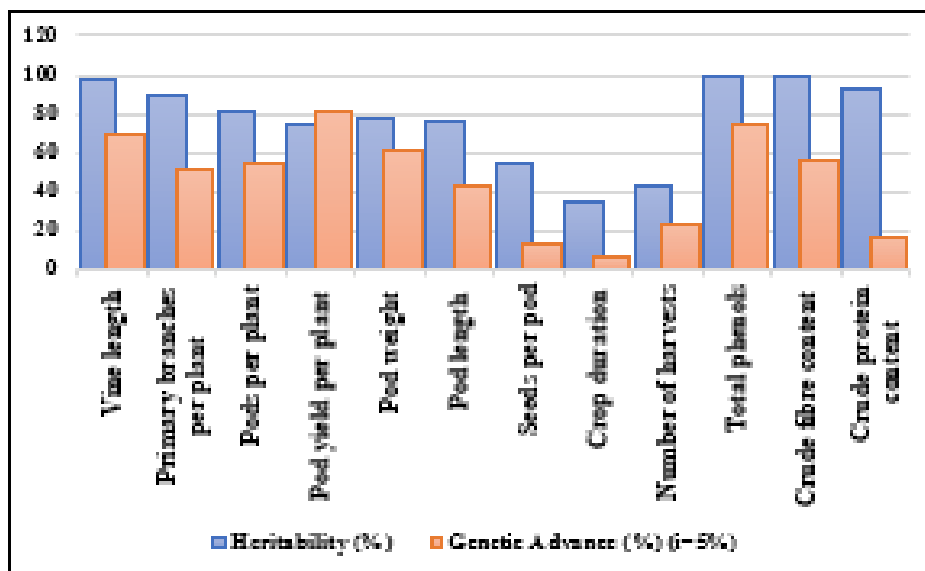


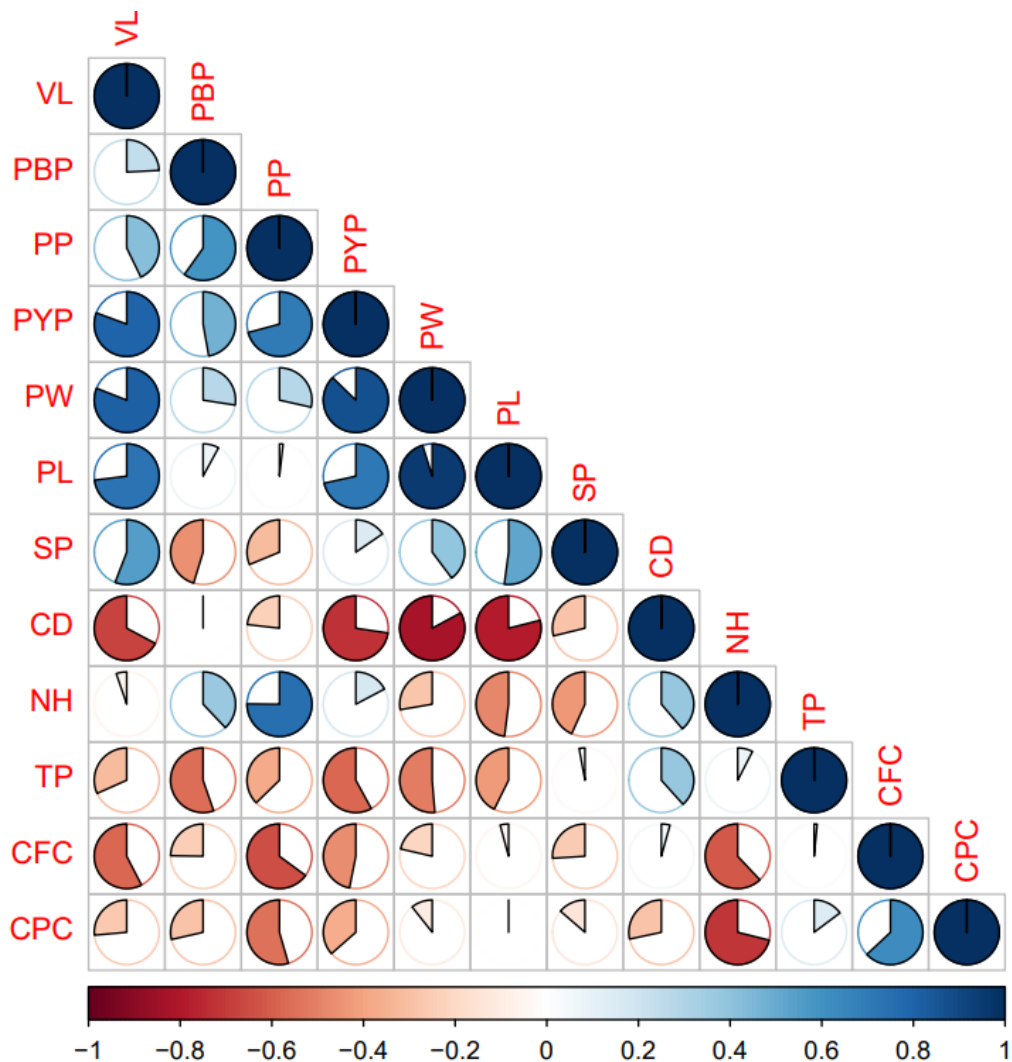
Figure 12. Heritability and genetic advance of twelve characters in trailing-type cowpea genotypes

per plant, and Bhagavati *et al.* (2018) for vine length, the number of primary branches per plant, number of pods per plant and pod yield per plant in yardlong bean varieties.

High heritability with medium genetic advance was observed for the characters' number of seeds per pod and crude protein content, medium heritability and low genetic advance for crop duration and medium heritability with high genetic advance for the number of harvests in trailing-type cowpea genotypes. The high heritability might be due to the favourable influence of environment rather than genotype and the simple selection of these characters may not be effective. These characteristics could be improved through transgressive breeding to exploit heterosis or other breeding methods. A similar trend in results was reported by Patel *et al.* (2016) for crop duration, Vidya *et al.* (2002) for the number of seeds per pod, and George (2022) for protein content.

The interrelationship between the characters could be estimated using correlation coefficients (Mofokeng *et al.*, 2020). The correlation of characters with the yield was contributed by genotypic and phenotypic effects. The genotypic coefficient of correlation was more than the phenotypic coefficient of correlation due to the involvement of the environment in the expression of a trait. The characters' vine length, primary branches per plant, pods per plant, pod weight, pod length and the number of harvests had a positive genotypic correlation with the pod yield per plant (Figure 13). The selection of these characters indirectly improves the pod yield per plant in trailing-type cowpea. Similar findings were reported by Madhukumar (2006) for pod weight, Paghadar *et al.* (2019), and Haque *et al.* (2021) for the number of primary branches per plant, plant height, pod length, and number of pods per plant.

A negative genotypic correlation of crop duration and crude protein content was recorded with pod yield per plant. The negative association of crop duration and pod yield per plant might be due to the increased vegetative growth and number of days to first flowering in the plants. The same observation was reported by



**Figure 13. Genotypic correlation matrix of twelve characters in trailing-type cowpea genotypes**

VL-Vine length, PBP- Primary branches per Plant, PP- Pods per plant, PYP-Pod yield per plant, PW-Pod weight, PL-Pod length, SP-Seeds per pod, CD-Crop duration, NH-Number of harvests, TP-Total phenols, CFC-Crude fibre content, and CPC-Crude protein content

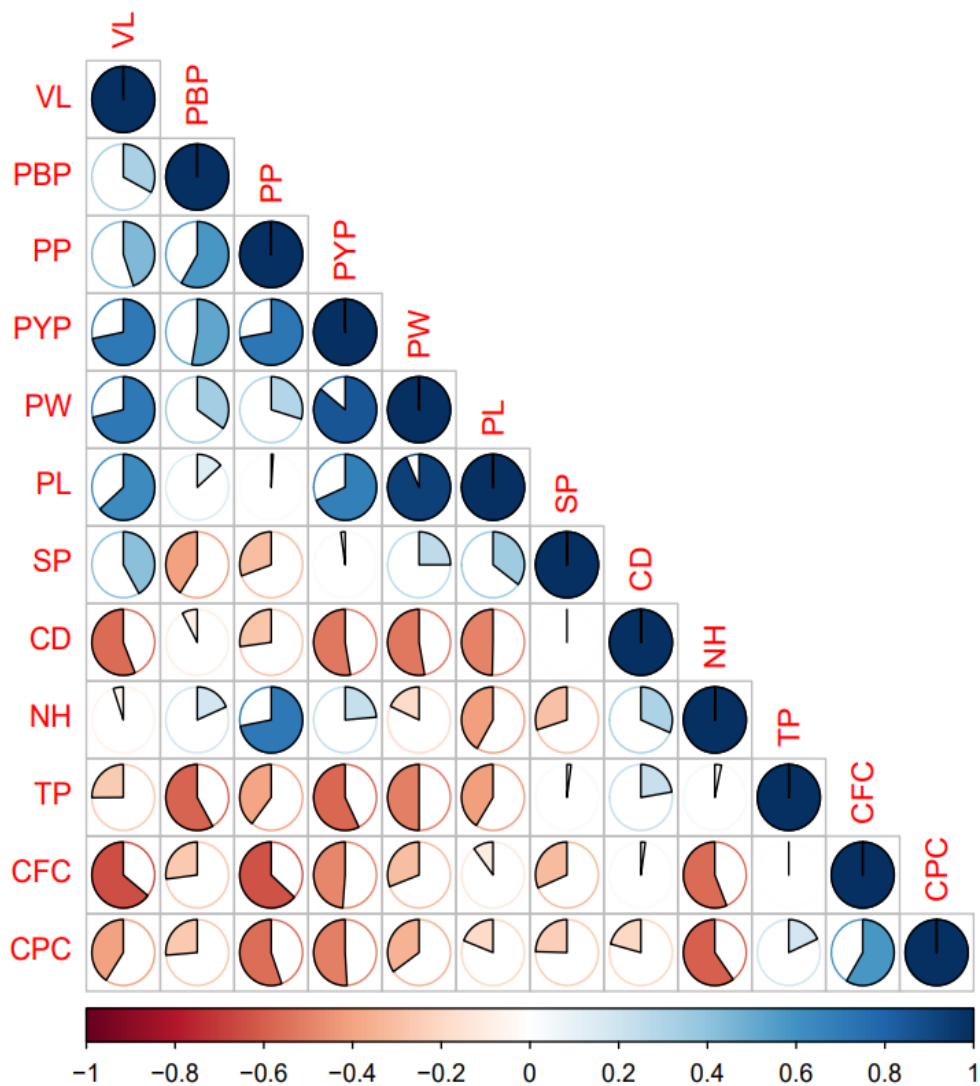
Sreekumar *et al.* (1996), Resmi, (1998) and Vidya and Oommen, (2002) and Ullah *et al.* (2011) in the yardlong bean genotypes.

The phenotypic correlation estimates the expression level association between attributes. The traits showed significant correlation at the genotypic level but not at the phenotypic level due to the contribution of the environment on trait control. So, only vine length, primary branches per plant, pods per plant, pod weight and pod length had a significant positive correlation with pod yield per plant at the phenotypic level (Figure 14). The significant positive phenotypic correlation of pods per plant, pod length, and pod weight (Vidya, 2000; Ullah *et al.*, 2011; Paghadar *et al.*, 2019; Nanda *et al.*, 2022), number of primary branches per plant (Paghadar *et al.*, 2019), and plant height (Paghadar *et al.*, 2019; Thangam *et al.*, 2020) was reported in yardlong beans.

Genotypic correlation between yield and yield components was partitioned into direct and indirect effects in path coefficient analysis and individually measures the relative importance of the causal factor. In the current study, characters such as primary branches per plant, pods per plant, pod weight, pod length, seeds per pod, crop duration, number of harvests, total phenols, crude fibre content and crude protein content recorded positive direct effects on pod yield per plant (Figure 15). The direct selection of these characters directly improves the crop yield. Among the characters pods per plant, pod length and seeds per pod showed a high and positive direct effect on pod yield per plant in trailing-type cowpea genotypes, which could be considered yield contributing traits and direct selection for these traits rewards yield improvement. The positive direct effect of the number of seeds per pod in yardlong beans was reported by Rambabu *et al.* (2016). Paghadar *et al.* (2019) reported the highest positive direct effect of pod length and moderate positive direct effect of the number of pods per plant on vegetable cowpea.

### **5.3.2. Evaluation of resistant bush-type cowpea genotypes along with susceptible check under field conditions**

The bush-type cowpea genotypes identified as resistant and susceptible to Fusarium wilt were evaluated under field conditions for biometric and quality



**Figure 14. Phenotypic correlation matrix of twelve characters in trailing-type cowpea genotypes**

VL-Vine length, PBP- Primary branches per Plant, PP- Pods per plant, PYP-Pod yield per plant, PW-Pod weight, PL-Pod length, SP-Seeds per pod, CD-Crop duration, NH-Number of harvests, TP-Total phenols, CFC-Crude fibre content, and CPC-Crude protein content

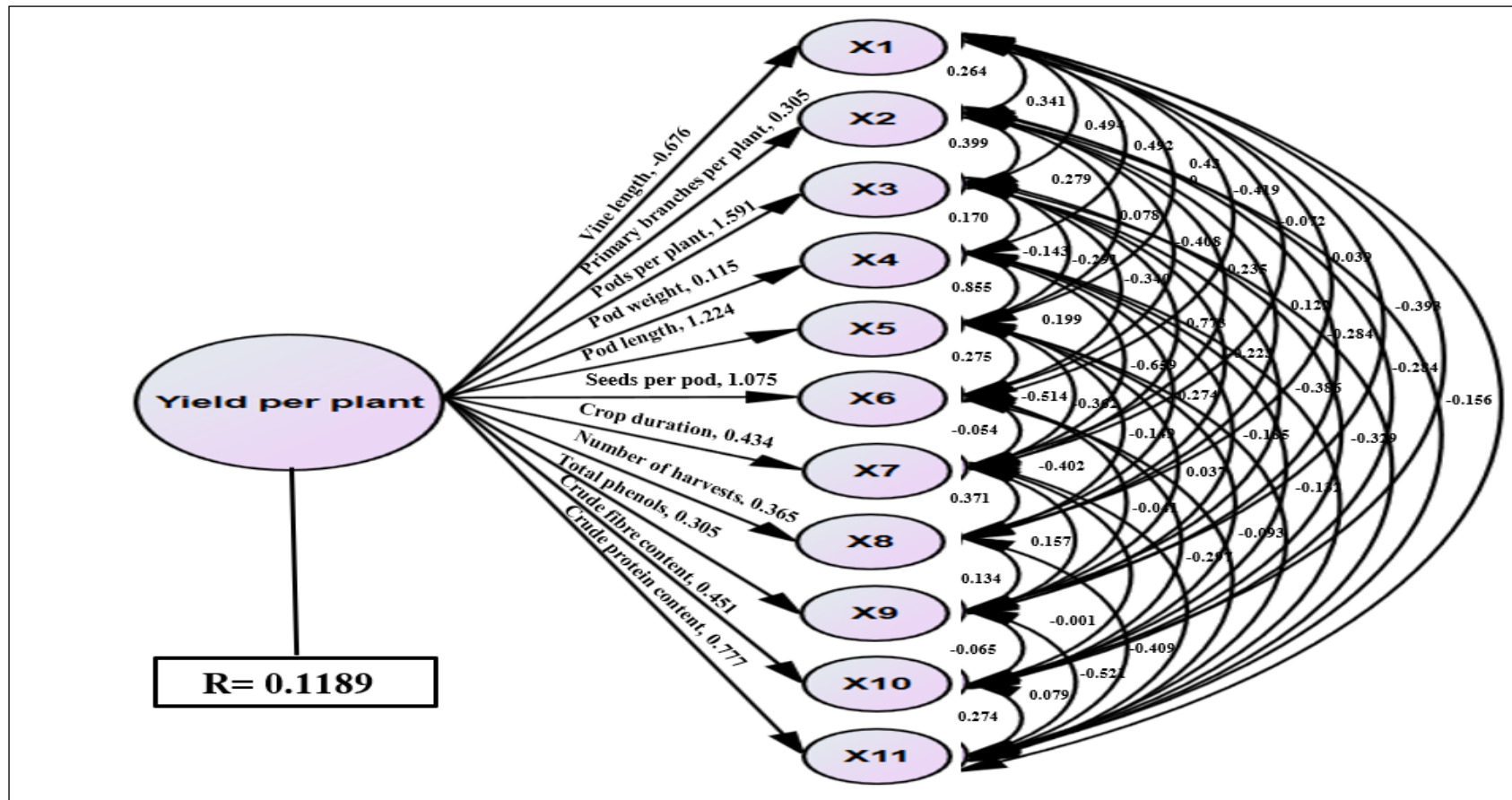
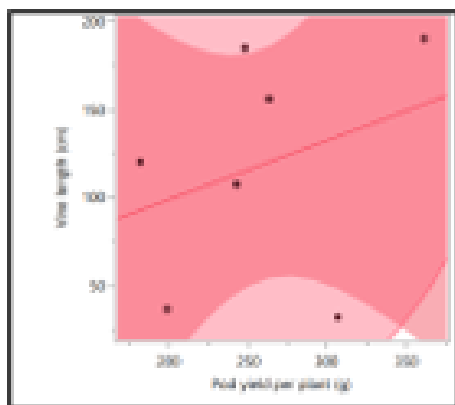


Figure 15. Path diagram showing direct effect and genotypic correlation of twelve characters in trailing-type cowpea genotypes

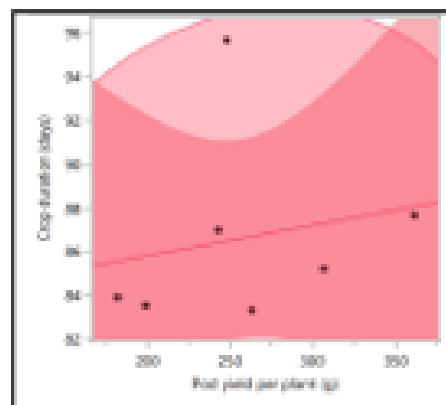
aspects. The genotypes showed a high range of variations in mean value representing more scope for improvement. The highest variability distribution was observed in vine length, crop duration, number of harvests, total phenols, crude protein content, and crude fibre content (Figure 16a-f). The characters such as primary branches per plant, pod weight, pods per plant, pod length and seeds per pod had less variability distribution and are largely dependent on pod yield per plant, which could be considered as yield contributing characters in bush-type cowpea genotypes (Figure 16g-k). The variability in cowpea genotypes was reported by Gerrano *et al.* (2015), Martos *et al.* (2017), Mofokeng *et al.* (2020), and Verma and Deepanshu (2022).

Cluster heatmaps could compress a lot of information into a small area since they have a high data density (Wilkinson and Friendly, 2009). These data-dense visualization techniques are more effective at identifying clusters than unordered heatmaps alone (De *et al.*, 2021). The heatmap hierarchical clustering of bush-type cowpea using biometric and quality parameters produced five clusters, indicating the divergence in the genotypes under study.

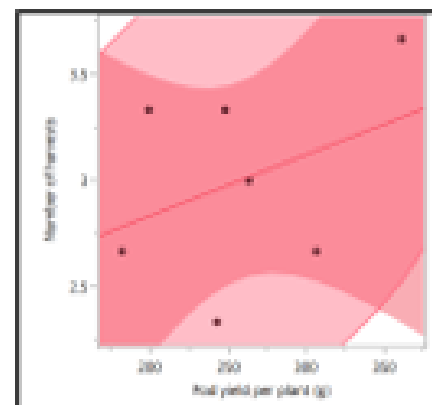
The high PCV and GCV observed in vine length, primary branches per plant, pods per plant, pod yield per plant, and total phenols, moderate PCV and GCV by pod weight, seeds per pod and crude fibre content, and low PCV and GCV by the characters pod length, crop duration and crude protein content in bush-type cowpea genotypes (Figure 17). The differences showed in the variability in PCV and GCV as a result of additive and non-additive gene effects emphasizing the possibility of developing novel varieties or hybrids (Soniya *et al.*, 2008, Verma *et al.*, 2019, Tambitkar *et al.*, 2021, Verma and Deepanshu, 2022). Devi and Jayamani (2018) discovered similar results in cowpea for the number of primary branches per plant, number of pods per plant, and single plant yield. High PCV and GCV contribute to the rich genetic variability of traits and were recorded in the number of pods per plant (Krishnan *et al.*, 2019; Verma *et al.*, 2019), plant height, primary branches per plant, pods per plant, and pod yield per plant (Gupta *et al.*, 2019).



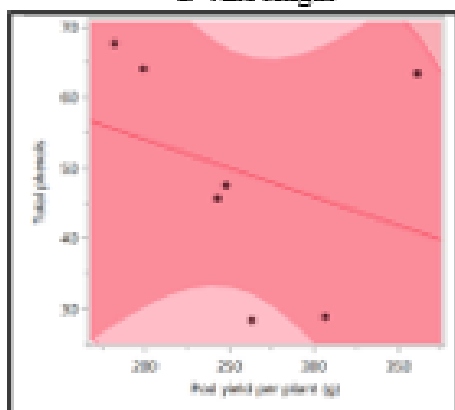
a. Vine length



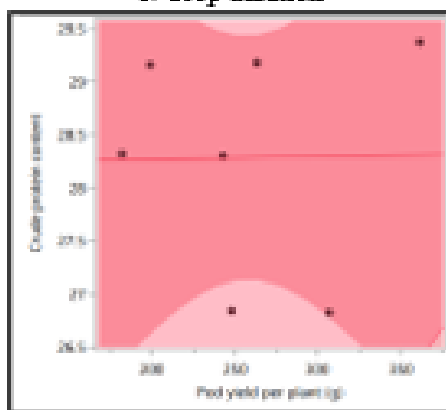
b. Crop duration



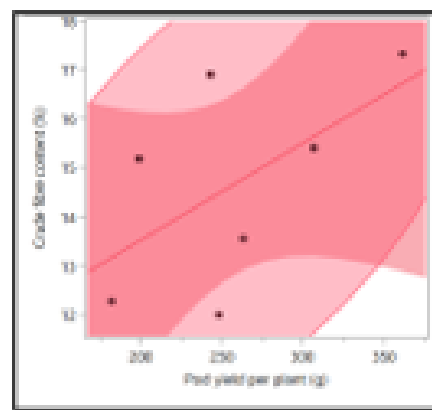
c. Number of harvests



d. Total phenols

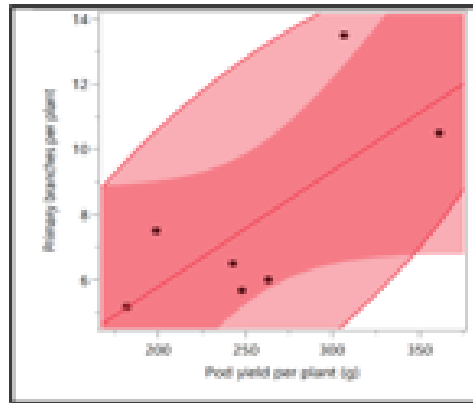


e. Crude protein content

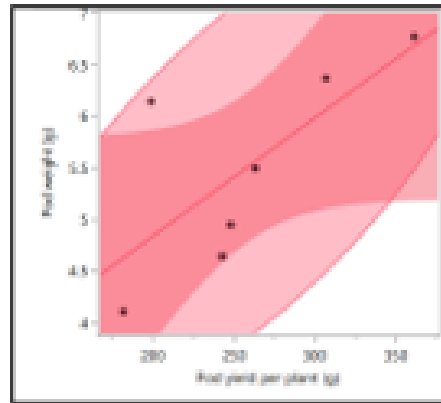


f. Crude fibre content

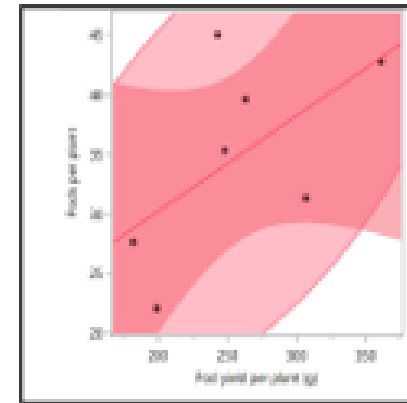
Figure 16. Scatterplot diagram of characters by pod yield per plant in bush-type cowpea genotypes



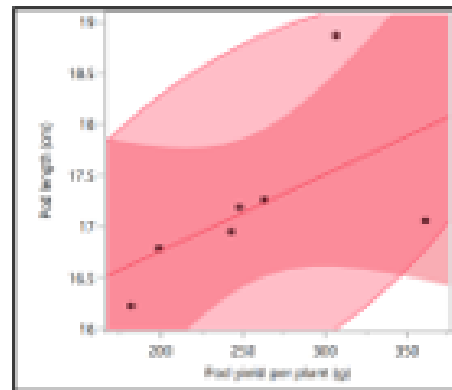
g. Primary branches per plant



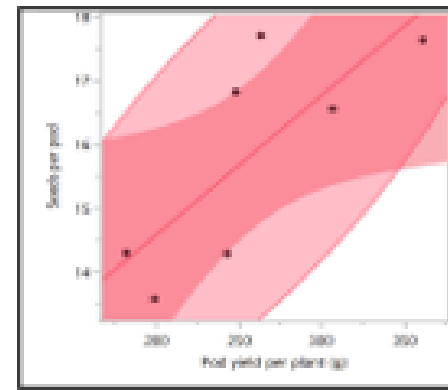
h. Pod weight



i. Pods per plant



j. Pod length



k. Seeds per pod

Figure 16 (Cont.). Scatterplot diagram of characters by pod yield per plant in bush-type cowpea genotypes

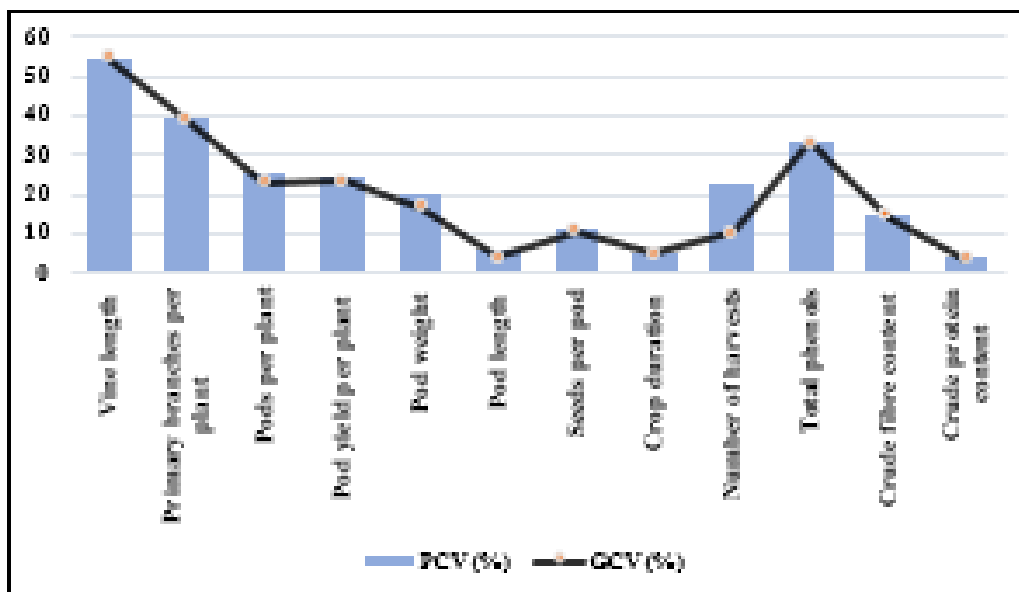


Figure 17. PCV and GCV of twelve characters in bush-type cowpea genotypes

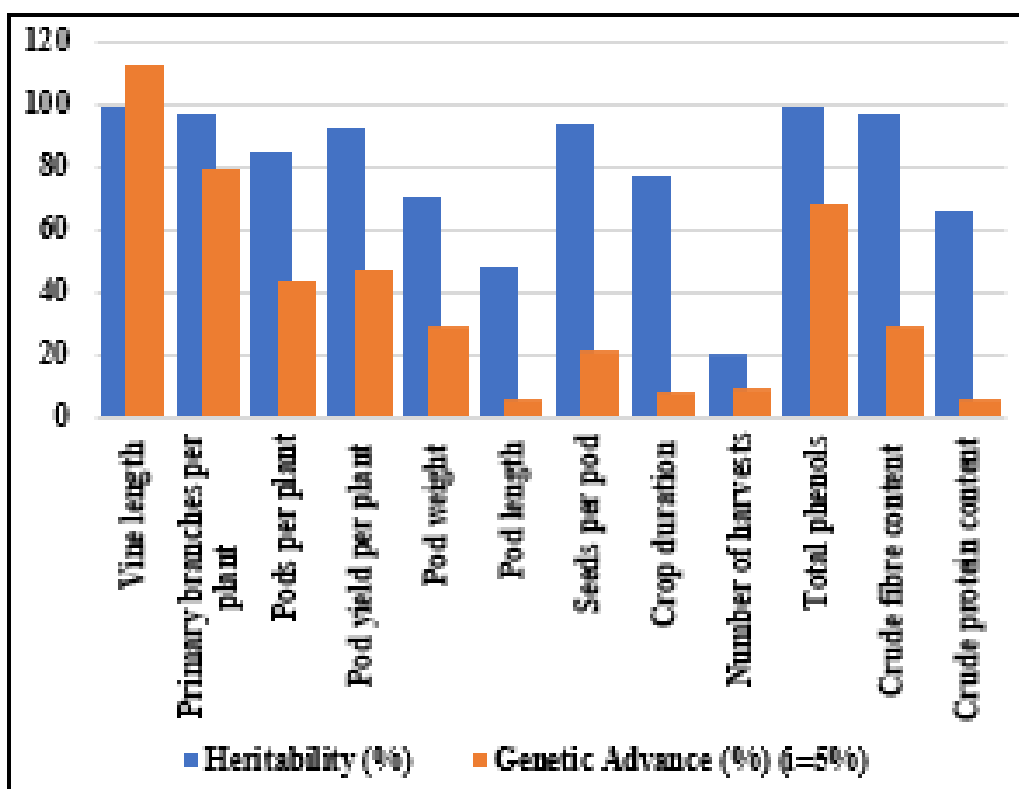
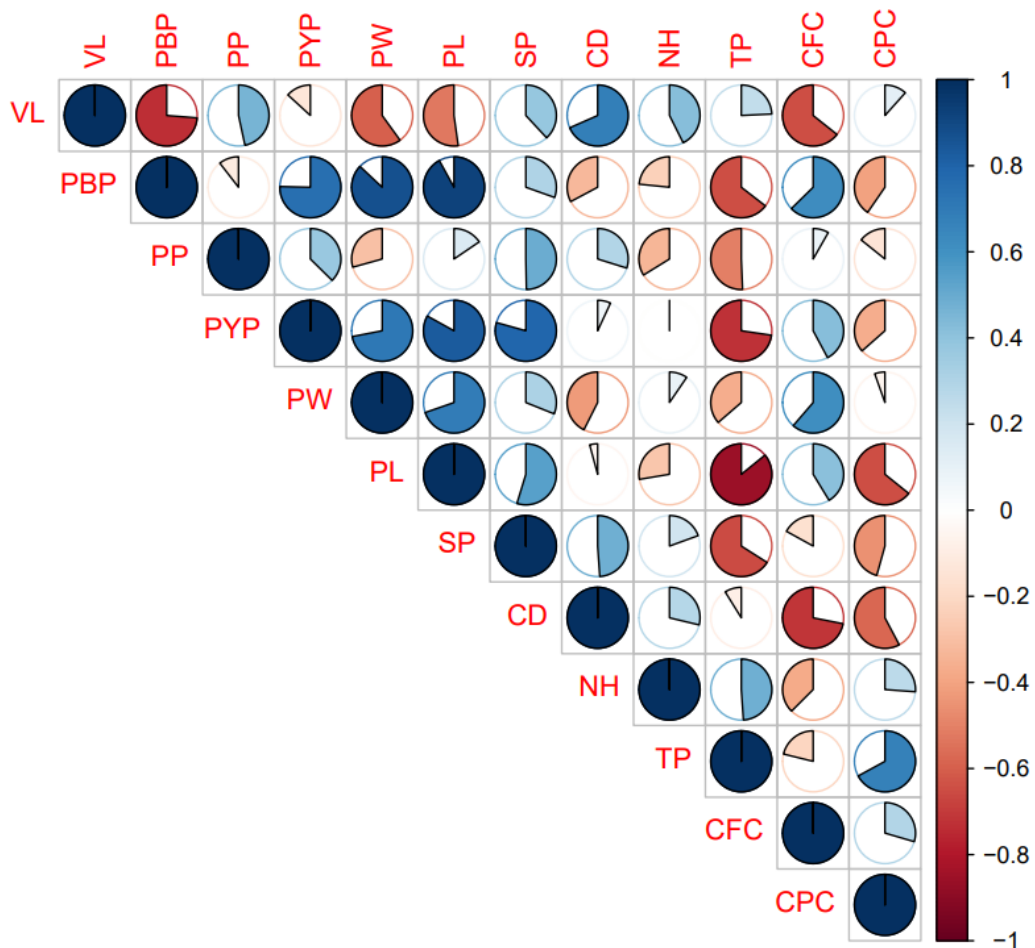


Figure 18. Heritability and Genetic Advance of twelve characters in bush-type cowpea genotypes

Moderate PCV and GCV contribute a greater scope for selection and the traits were moderately contributed by the genetic constitution of genotypes. Moderate PCV and GCV were reported for the number of seeds per pod (Verma *et al.*, 2019; Gupta *et al.*, 2019; Ugale *et al.*, 2020), pod weight (Verma and Deepanshu, 2022), and crude fibre content which supports the results of the present study. Similar results of low PCV and GCV in cowpea genotypes were reported in pod length (Rout *et al.*, 2018), crop duration (Patel *et al.*, 2016; Sarath and Jiji, 2017; Verma and Deepanshu, 2022) and crude protein content (Hasan *et al.*, 2015; Sharma *et al.*, 2017), in which direct selection of these traits will not be effective for crop improvement.

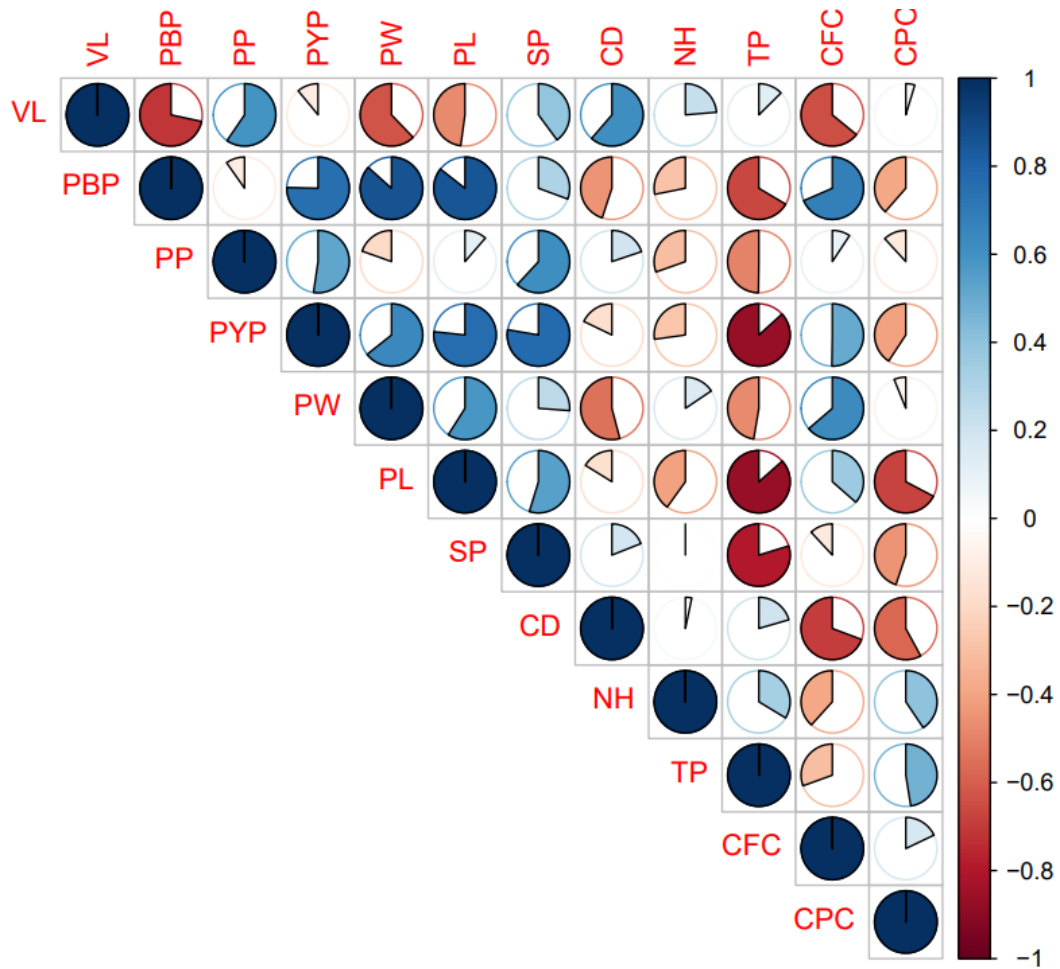
In the present study, high heritability coupled with high genetic advance was observed for the characters' vine length, primary branches per plant, pods per plant, pod yield per plant, pod weight, seeds per pod, total phenols, and crude fibre content in the bush-type cowpea genotypes (Figure 18). The results concurred with previous studies as reported by Belay and Fiseha (2020) for plant height, Ugale *et al.* (2020) for plant height, number of primary branches per plant, and number of pods per plant. High heritability and low genetic advance for pod length, crop duration, and crude protein content indicate that the environment played a role in the control of these traits. Results corroborate those of Devi and Jayamani (2018) for pod length and crop duration.

The correlation between characters was separated into genotypic and phenotypic levels. The genotypic correlation coefficients were larger than their phenotypic correlation coefficients, showing the genetic basis of the relationship. The characteristics of bush-type cowpea genotypes including primary branches per plant, pods per plant, pod weight, pod length, seeds per pod and crude fibre content had a positive and significant correlation with pod yield per plant at both phenotypic and genotypic levels (Figure 19 and 20). The results imply that selection for these characters would lead to simultaneous improvement of pod yield per plant in bush-type cowpeas. The same results were reported by Jogdhande *et al.* (2017) for pod length, Walle *et al.* (2018) and Owusu *et al.* (2021) for the number of pods per plant,



**Figure 19. Genotypic correlation matrix of twelve characters in bush-type cowpea genotypes**

VL-Vine length, PBP- Primary branches per Plant, PP- Pods per plant, PYP-Pod yield per plant, PW-Pod weight, PL-Pod length, SP-Seeds per pod, CD-Crop duration, NH-Number of harvests, TP-Total phenols, CFC-Crude fibre content, and CPC-Crude protein content



**Figure 20. Phenotypic correlation matrix of twelve characters in bush-type cowpea genotypes**

VL-Vine length, PBP- Primary branches per Plant, PP- Pods per plant, PYP-Pod yield per plant, PW-Pod weight, PL-Pod length, SP-Seeds per pod, CD-Crop duration, NH-Number of harvests, TP-Total phenols, CFC-Crude fibre content, and CPC-Crude protein content

number of seeds per pod, and pod length, Tambitkar *et al.* (2021) for the number of primary branches per plant and pod weight.

Cause and effect analysis involves distinguishing direct and indirect effects using Pearson correlation coefficients. The maximum direct effect on pod yield per plant was shown by primary branches per plant followed by seeds per pod, crop duration, crude protein content, pods per plant, and vine length (Figure 21). The low residual effect (0.0286) was noticed in the path analysis indicating the characters taken for analysis were appropriate in determining the yield contributing traits in the cowpea genotypes.

The positive direct effect of pod length, pods per plant and plant height (Patel *et al.*, 2016), number of branches and protein content (Jogdhande *et al.*, 2017) the number of pods per plant (Tambitkar *et al.*, 2021) with pod yield per plant was reported.

Comparing the correlation of characters in both trailing and bush-type cowpea genotypes concluded that the characters such as primary branches per plant, pods per plant, pod weight, pod length, and the number of harvests had positive and significant correlation with pod yield per plant in both cowpea types. These characteristics could be considered yield-attributing traits and the direct selection improves the cowpea yield effectively.

### **5.3.3. Comparison of biometric and quality characters in resistant and susceptible cowpea genotypes**

On comparison of resistant and susceptible trailing-type cowpea genotypes the phenol content and crude fibre content were more in resistant genotypes than in susceptible genotypes. In bush-type cowpeas, the resistant and susceptible genotypes showed variations in crop duration, total phenols, crude fibre content and crude protein content. The crop duration in the resistant genotypes recorded lesser than the susceptible genotype might be to escape from the disease. The total phenols, crude fibre content and crude protein content were higher in resistant genotypes than in susceptible genotypes. The increased protein content in seeds

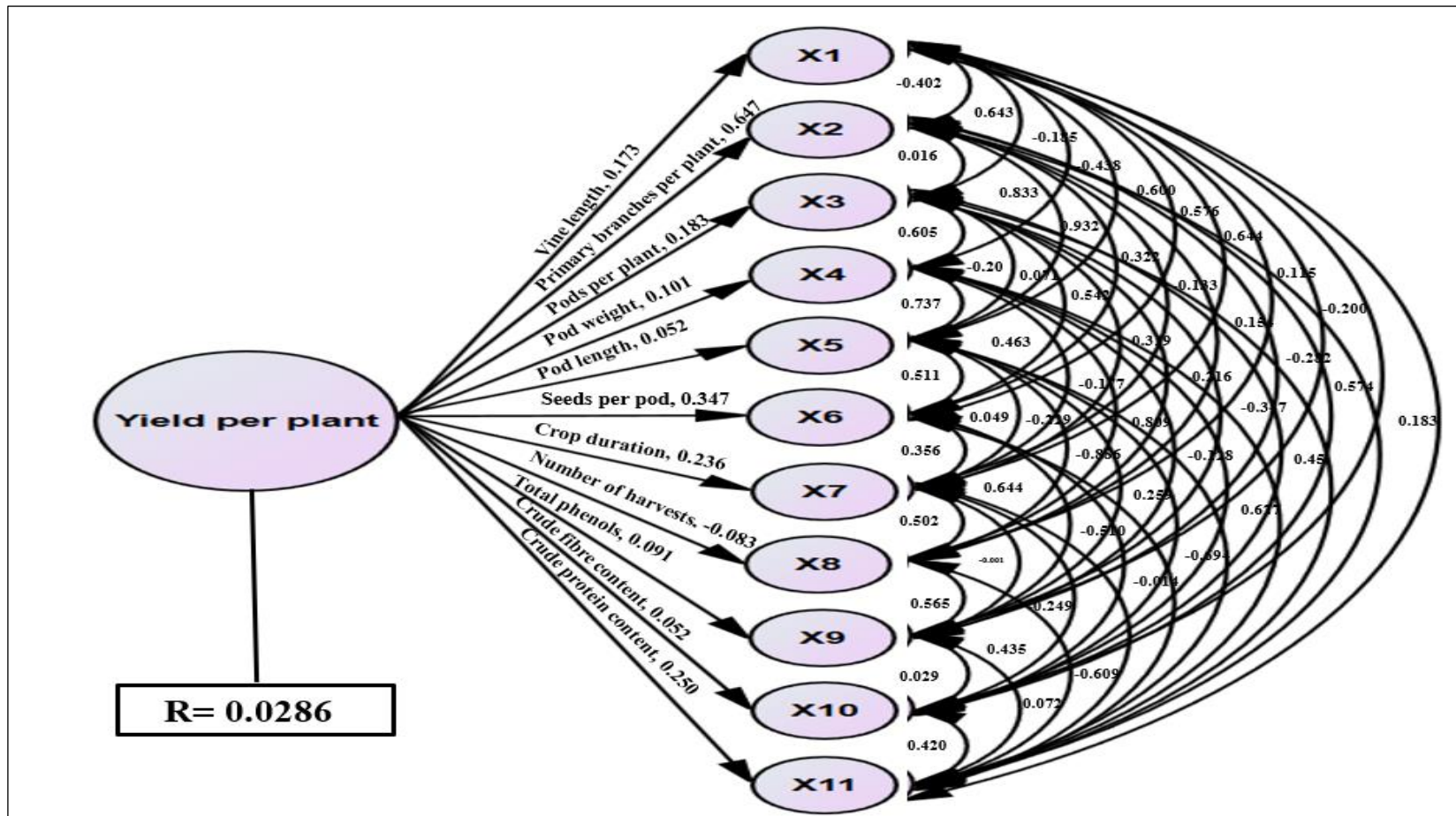


Figure 21. Path diagram showing direct and genotypic correlation of twelve characters in bush-type cowpea genotypes

reduced the conidial germination and hyphal growth of *Fusarium oxysporum* (Nobrega *et al.*, 2005; Castillejo *et al.*, 2015; Kumar *et al.*, 2016). The production of phenol content could be considered an important defence mechanism against *Fusarium oxysporum* attacks (Patel *et al.*, 2015; Bernardi *et al.*, 2018; Pastuszak *et al.*, 2021). The highest fibre content in resistant genotypes against Fusarium wilt was reported in sugarcane (Mahlanza *et al.*, 2014 and in flax (Rozhmina *et al.*, 2019).

#### 5.4. GENOTYPING OF THE RESISTANT LINES USING AN SSR MARKER LINKED TO FUSARIUM WILT RESISTANCE

The marker-assisted selection facilitates sources of differentiation and speeds up the selection of complex features, which is a laborious procedure when assessed phenotypically (Ahmad *et al.*, 2014). The use of molecular markers is a crucial technique for categorizing, describing, and screening for diseases and infections (Yadav *et al.*, 2023).

Simple sequence repeat (SSR) markers have gained importance recently because of their high reproducibility, characterization of co-dominant loci, multi-allelic, high abundance, even distribution throughout the genome, high polymorphism, and ease of use with high throughput methods. These characteristics have made SSR markers an important tool for genotyping, genetic mapping, and marker-assisted selection (MAS) in plant breeding programmes (Miah *et al.*, 2013).

The genomic DNA of resistant and susceptible cowpea genotypes were isolated and the amplification was carried out using Fusarium wilt-linked SSR markers. The SSR marker C13-16 was reported to be linked with Fusarium wilt resistance in cowpea (Omoigui *et al.*, 2018) which, could not produce any amplification product in the genotypes used in the study. These results might be due to the lack of a primer binding site to C13-16 in the genotypes used.

Genetic molecular markers are significant for molecular breeding programmes such as genetic diversity, genetic mapping, MAS, and cross-transferability among related species and genera (Varshney *et al.*, 2005). The

reports by Raghu *et al.* (2021) identified 32.14% of cross-genera transferability of chickpea Fusarium wilt-linked SSR markers to cowpea genome, from that 15 transferable SSR markers were used for the identification of Fusarium wilt resistant genotype in cowpea in the study. Out of 15 SSR markers, only one marker UASBC59 produced polymorphism in the genotypes. The marker UASBC59 showed amplification in all the nine legumes tested including cowpea indicating 100% transferability (Raghu *et al.*, 2021). The most probable reason could be that they originated from the same locus and allelic area of the conserved primer binding sites. The variety IRON showed a polymorphic resistant band at 190 bp and others showed a susceptible band at 250bp.

#### 5.5. HYBRIDISATION OF RESISTANT GENOTYPE WITH SUPERIOR BUT SUSCEPTIBLE VARIETY RELEASED FROM KAU FOR DEVELOPING F<sub>1</sub>.

The bush-cowpea genotype VI064556 (IRON) showed Fusarium wilt resistance at seedling screening and field conditions. The genotype also had high seed protein and fibre content, impeding Fusarium wilt resistance. The soluble protein and total phenol content negatively correlated with Fusarium wilt incidence (Arzoo *et al.*, 2012). The Fusarium wilt-linked SSR marker UASBC59 could produce polymorphic amplicon in IRON also confirming the presence of a Fusarium wilt-resistant gene in the IRON. The presence of Fusarium wilt resistance in the genotype IRON was already reported by Orton (1902), Armstrong and Armstrong (1980), and Hare (1957) and the same variety has been used in breeding programmes for transferring the Fusarium wilt resistance (Shihata, and Gad-El-Hak, 1989; Shihata *et al.*, 1989; Helms *et al.*, 1991; Roberts *et al.*, 1995) and derived varieties showed the same level of resistance to that of IRON. Hence, the genotype IRON was used as the donor parent for Fusarium wilt resistance in the backcross breeding programme.

The vascular discolouration is strong evidence of Fusarium wilt (Ndeve and Roberts, 2019), which was observed in all the KAU-released varieties used for screening indicating the susceptibility to Fusarium wilt. So, the high-yielding long podded KAU-released yardlong bean variety Githika was used as the recurrent

parent in the backcross breeding program. The comparison of Fusarium wilt reaction in donor and recipient parents indicated the differential reaction of these genotypes to the fungal pathogen (Plate 43).

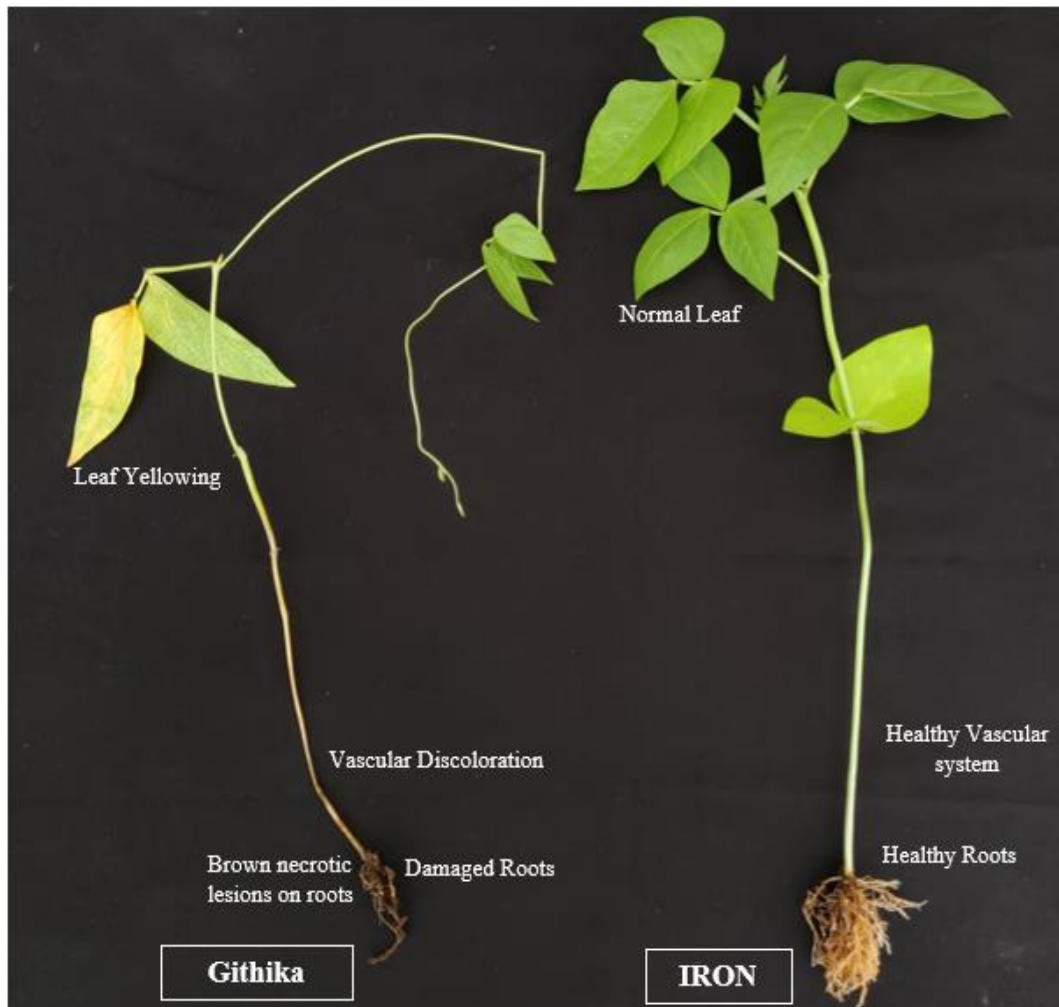
The crossing between IRON and Githika obtained only 12% pod set due to differences in the type of cowpea used. Late flowering and a lesser number of flowers produced in the donor parent led to the non-adequate availability of pollen to pollinate each flower from the recipient parent. The cross between the two erect-type cowpea accessions was reported to be 25% pod set, while its reciprocal cross produced only 20% pod set due to fewer flowers produced in one of the accessions, and concluded that the maternal effects play a significant role in controlling the traits such as the number of pod set, percentage of pod set, pod length and the number of days to pod maturity (Ajayi *et al.*, 2020).

#### 5.6. RAISING AND MORPHOLOGICAL EVALUATION OF F<sub>1</sub> AND THE PARENTS

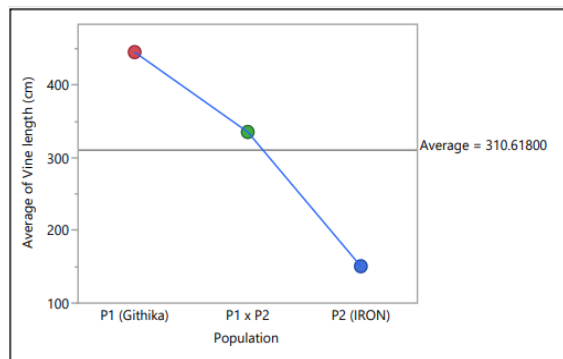
The 210 F<sub>1</sub> seeds were produced by crossing Githika and IRON, among them, 148 germinated with 70.4 per cent germination. The morphological evaluation of F<sub>1</sub>s recorded above-average performance in vine length, pod weight, and crop duration, whereas primary branches per plant, pod length, and seeds per pod were below average between the two parents (Figure 22).

The evaluation of F<sub>1</sub> plants recorded that all the plants were tall with trailing type with an average length of 335.600 cm. The reports by Inasi (1980) from F<sub>1</sub> and F<sub>2</sub> analysis of cowpea concluded that tallness and vining habits were dominant over dwarfness and non-vining habits.

The difference between observations on primary branches per plant in both parents was negligible, so the F<sub>1</sub> plants were observed to be intermediate between the parents. The pod weight, pod length and crop duration in F<sub>1</sub> plants were intermediate to that of their both parents. The intermediate response of pod length in the F<sub>1</sub> generation of cowpea was reported (Lachyan and Dalvi, 2015). The seeds per pod in F<sub>1</sub> were close to that of both parents.

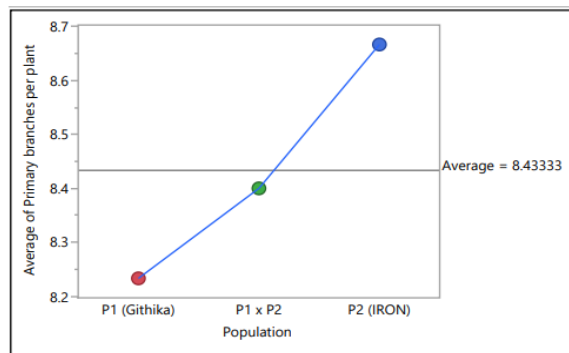


**Plate 43. Comparison of Githika and IRON plants after Fusarium inoculation**



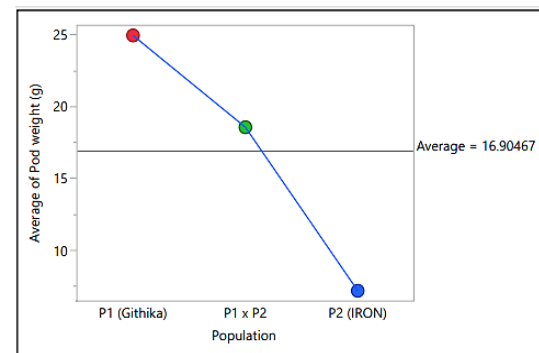
Population ● P1 (Githika) ● P1 x P2 ● P2 (IRON)

a. Vine length



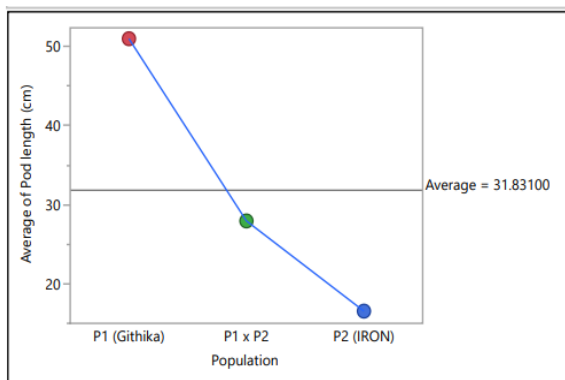
Population ● P1 (Githika) ● P1 x P2 ● P2 (IRON)

b. Primary branches per plant



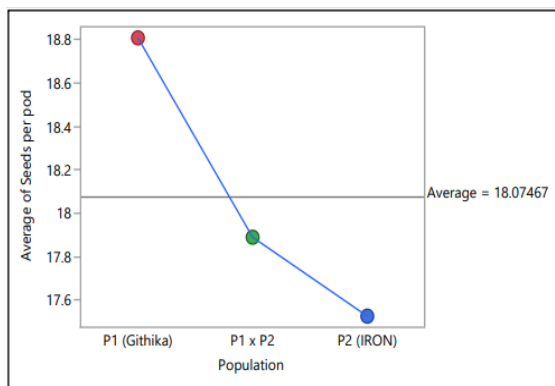
Population ● P1 (Githika) ● P1 x P2 ● P2 (IRON)

c. Pod weight



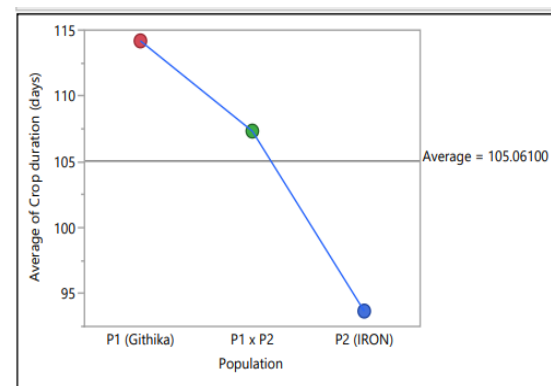
Population ● P1 (Githika) ● P1 x P2 ● P2 (IRON)

d. Pod length



Population ● P1 (Githika) ● P1 x P2 ● P2 (IRON)

e. Seeds per pod



Population ● P1 (Githika) ● P1 x P2 ● P2 (IRON)

f. Crop duration

**Figure 22. Average performance of morphological characters in parents and F<sub>1</sub> population**

## 5.7. EVALUATION OF BC<sub>1</sub>F<sub>1</sub> LINES AT THE SEEDLING STAGE THROUGH ARTIFICIAL SCREENING AND SCORING

All the F<sub>1</sub> plants were crossed with the recurrent parent Githika to produce BC<sub>1</sub>F<sub>1</sub> lines. Ten days old seedlings of 198 BC<sub>1</sub>F<sub>1</sub> were artificially screened for Fusarium wilt reaction and scored on a 0-4 scale, in which 59 scored the lowest score indicating the resistant lines. 27 BC<sub>1</sub>F<sub>1</sub> lines were found to be moderately resistant, 14 were moderately susceptible, 25 were susceptible and 73 were highly susceptible (Figure 23). The Fusarium symptoms were shown by BC<sub>1</sub>F<sub>1</sub> lines after two weeks of inoculation. Early symptoms might be due to the age of the seedlings used for screening. The early seedling screening was very effective for the evaluation of Fusarium wilt disease-tolerant plants, in which two-month-old plantlets (10-15 cm) were effective in the case of bananas (Mak *et al.*, 2004). Fourteen days old seedlings were used for screening Fusarium wilt in pumpkins (Xu *et al.*, 2022), and 2 true leaf stages in cotton (Zhang *et al.*, 2022).

Chi-square analysis for the goodness of fit is a powerful method that allows researchers to assess the degree to which observed data resembles expected theoretical distributions. There have been two hypotheses as the null hypothesis and the alternate hypothesis. The null hypothesis assumes that observed data conforms to the expected distributions, while the alternative hypothesis suggests a deviation from expected distributions. The monogenic dominant inheritance of Fusarium wilt resistance in cowpea was reported by Rigert and Foster (1987), Hare (1957), Beckman and Roberts (1995) and Omoigui *et al.* (2018) and in other legumes (Rubio *et al.*, 2003 and Sharma *et al.*, 2007 in chickpeas, Grajal-Martin and Muehlbauer, 2002 and Bani *et al.*, 2012 in peas, Cross *et al.*, 2000 and Fall *et al.*, 2001 in common beans). So, the expected resistant and susceptible ratio in BC<sub>1</sub>F<sub>1</sub> lines was 1:1, and the same ratio was obtained from the present study. The chi-square analysis concluded that there was no significant difference between observed and expected values by accepting the null hypothesis. Hence, the Fusarium wilt in cowpea was controlled by the monogenic dominant gene.

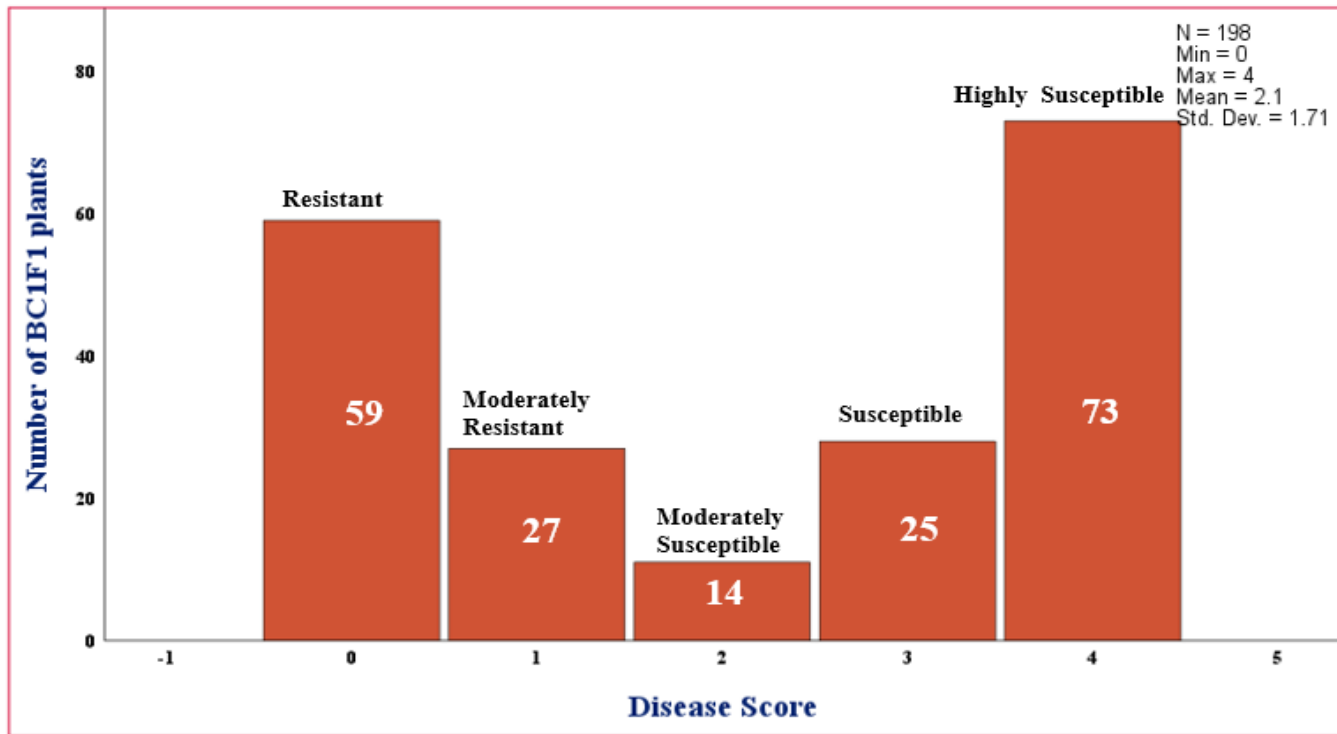


Figure 23. Frequency of disease score in BC<sub>1</sub>F<sub>1</sub> lines

## 5.8. MORPHOLOGICAL AND QUALITY EVALUATION OF RESISTANT BC<sub>1</sub>F<sub>1</sub> LINES AND THE RECURRENT PARENT

Critical analysis of traits in each backcross generation can hasten the recovery of the recurrent parent genome (Joseph *et al.*, 2004; Basavaraj *et al.*, 2010; Baliyan *et al.*, 2018). The frequency distribution for biometric characters on 59 BC<sub>1</sub>F<sub>1</sub> lines revealed different patterns and none of the traits followed the exact normal distribution. Normal distribution follows symmetry with zero skewness and kurtosis (Sahu *et al.*, 2017).

The higher-order statistics of skewness and kurtosis are used to examine the type of gene action (Fisher *et al.*, 1932) and the number of genes influencing attributes (Robson, 1956). The skewed distribution of the characters suggests that environmental factors influence and control non-additive type gene effects (Govintharaj *et al.*, 2017). The skewness describes the positive or negative extent of the individuals in the population and provides an idea of the cluster of deviations that are above or below the measure of central tendency (Pukalenty *et al.*, 2018). Positive skewness is associated with complementary gene action, while negative skewness is associated with duplicate gene action (Sarankumar *et al.*, 2019).

Kurtosis gives an idea of where the population is at its peak; positive kurtosis indicates the presence of gene interaction and is controlled by fewer genes (leptokurtic), whereas negative kurtosis indicates the absence of gene interaction and is ruled by a greater number of genes (platykurtic) (Govintharaj *et al.*, 2017).

The vine length difference of about 234cm was observed between Githika and IRON. So, the tremendous variation in BC<sub>1</sub>F<sub>1</sub> lines for vine length was recorded within a range of 186-416 cm. The distribution of vine length in BC<sub>1</sub>F<sub>1</sub> lines was positively skewed with platykurtic (negative kurtosis) indicating the complementary additive gene action and the trait is controlled by many genes (Figure 24). The positive skewness with the platykurtic distribution of plant height was reported by Prabhu *et al.* (2016) in the BC<sub>1</sub>F<sub>1</sub> generation of groundnut, Govintharaj *et al.* (2017) in the intercrossed backcross population of rice,

Pukalenty *et al.* (2018) and Sarankumar *et al.* (2019) in the BC<sub>1</sub>F<sub>1</sub> population of maize.

The primary branches per plant in Githika and IRON were eight and seven respectively, and it ranged from four to eight in BC<sub>1</sub>F<sub>1</sub> lines. The data on primary branches per plant in BC<sub>1</sub>F<sub>1</sub> lines were distributed with positive skewness and positive kurtosis indicating complementary epistasis gene action in which the trait was controlled by fewer genes (Figure 25). The same pattern of distribution in the number of primary branches per plant in groundnut was reported by Prabhu *et al.* (2016).

The recurrent parent Githika is trailing-type cowpea with long pods whereas, the donor parent IRON is bush-type with short pods so, the pod weight and pod length in recurrent and donor parents were largely varied. These two traits in BC<sub>1</sub>F<sub>1</sub> lines also showed variation greatly.

The pod weight (range of 10.01 to 25.46g) was distributed with negative skewness and positive kurtosis, representing that the trait is controlled by fewer genes with duplicate and epistasis gene action (Figure 26). But the pod length (range of 16.50 to 43.00cm) had positive skewness and leptokurtic showed complementary epistasis gene action controlled by fewer genes (Figure 27). A similar data distribution of pod length in the cross between cowpea and yardlong bean (F<sub>2:3</sub> population) was reported by Garcia-Oliveira *et al.* (2020).

The number of seeds per pod in recurrent and donor parents was almost similar with 20.75 and 17.20 respectively. The BC<sub>1</sub>F<sub>1</sub> lines showed the variation in the number of seeds per pod (10.23 to 20.67) with positive skewness and negative kurtosis. The number of seeds per pod was controlled by many genes with complementary additive gene action (Figure 28).

The crop duration varied between recurrent and donor parents with 21 days difference. The data on crop duration in the BC<sub>1</sub>F<sub>1</sub> lines (101.00 to 120.00 days) showed variation with positive skewness and negative kurtosis representing the trait

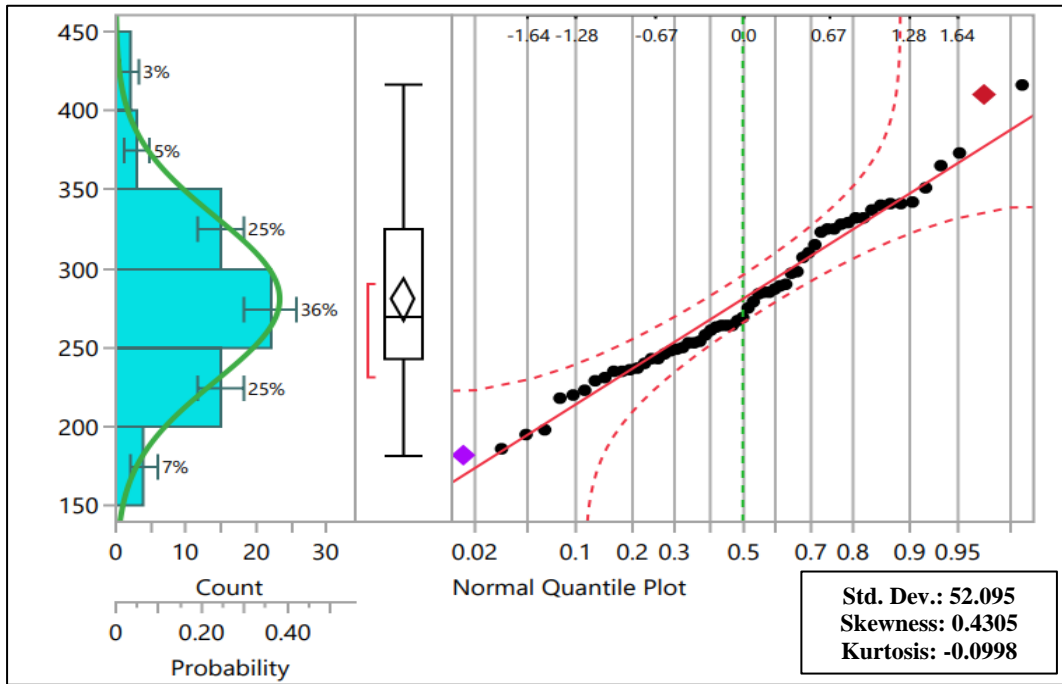


Figure 24. Frequency distribution and probability plot of vine length in  $BC_1F_1$  resistant lines

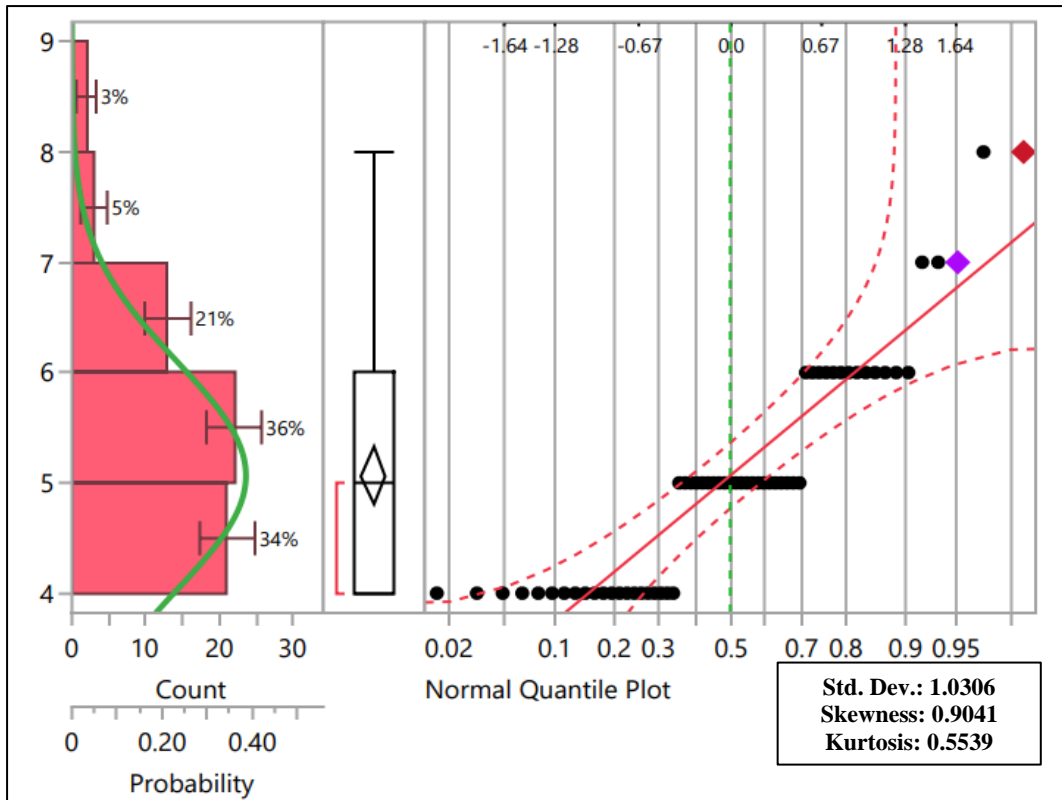
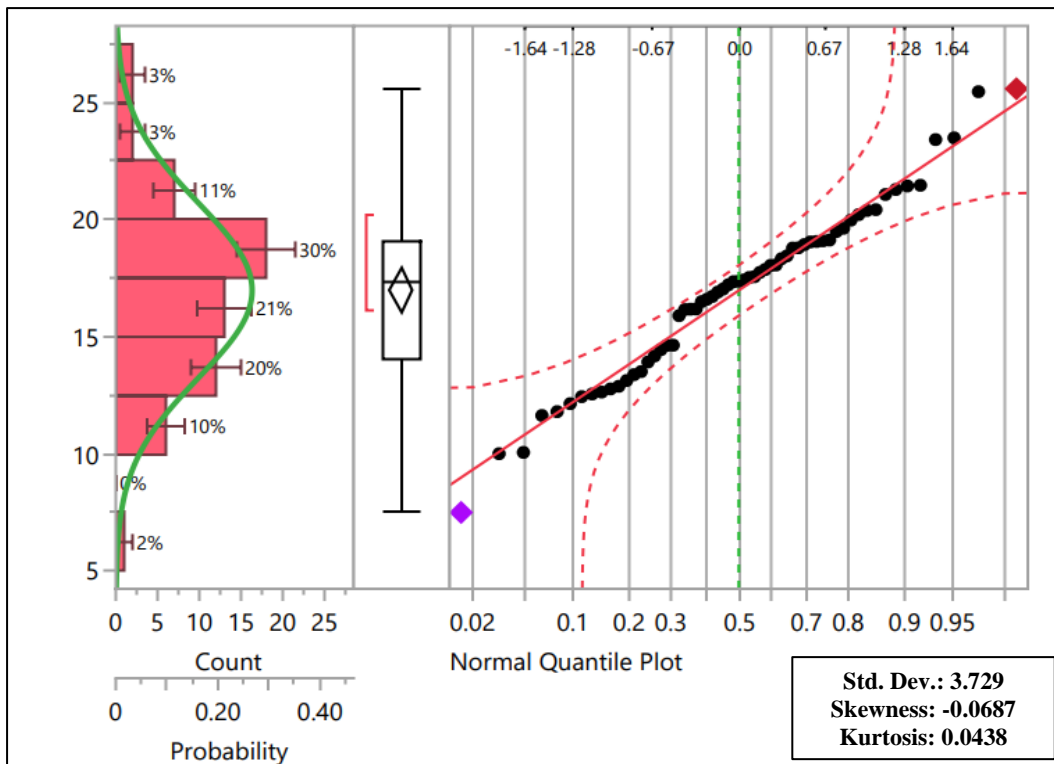
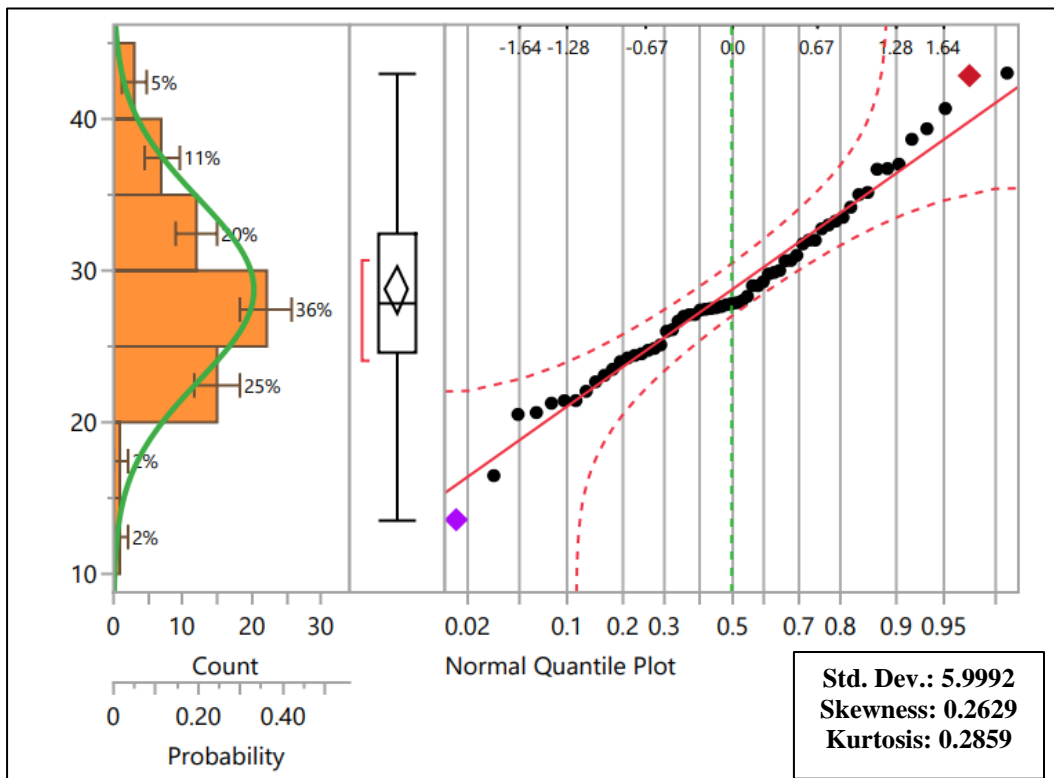


Figure 25. Frequency distribution and probability plot of primary branches per plant in  $BC_1F_1$  resistant lines

◆ Githika    ◆ IRON

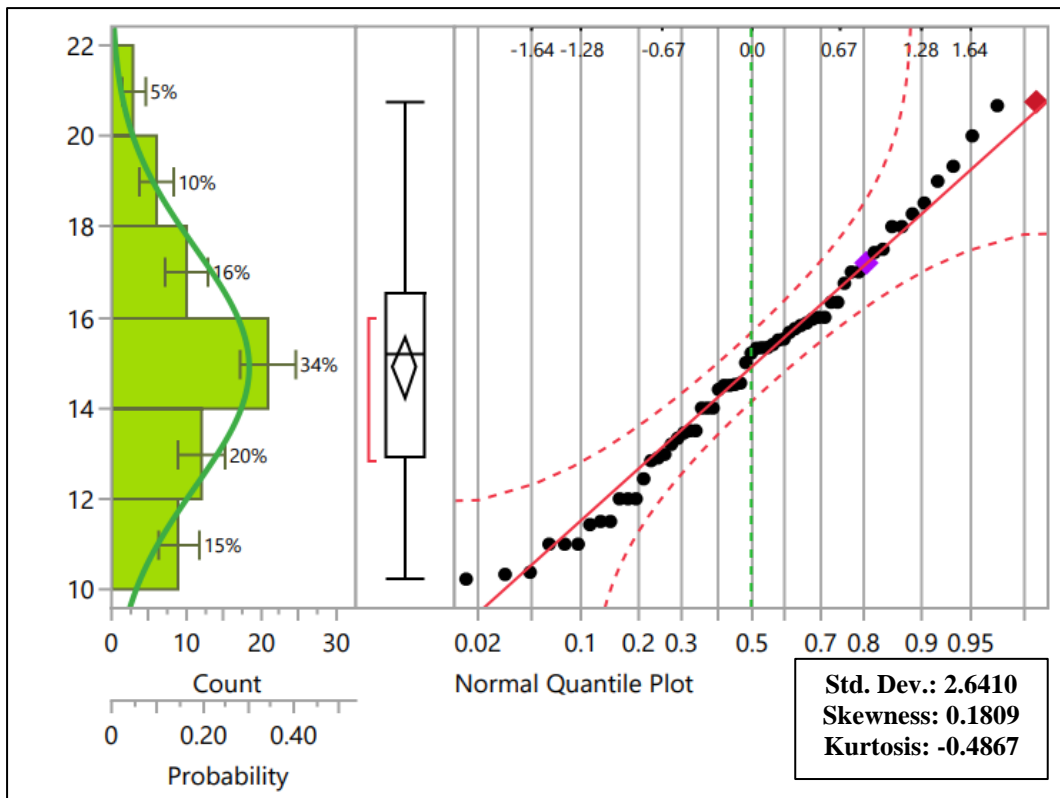


**Figure 26. Frequency distribution and probability plot of pod weight in  $BC_1F_1$  resistant lines**

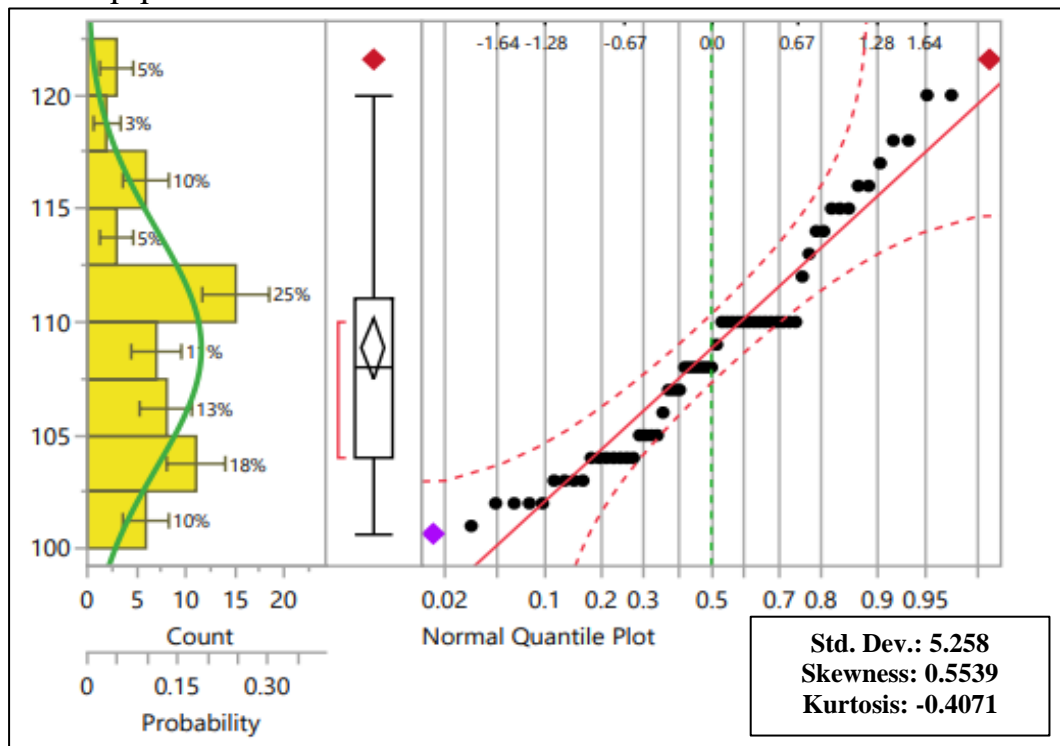


**Figure 27. Frequency distribution and probability plot of pod length in  $BC_1F_1$  resistant lines**

◆ Githika ◆ IRON



**Figure 28. Frequency distribution and probability plot of seeds per pod in BC<sub>1</sub>F<sub>1</sub> resistant lines**



**Figure 29. Frequency distribution and probability plot of crop duration in BC<sub>1</sub>F<sub>1</sub> resistant lines**

◆ Githika ◆ IRON

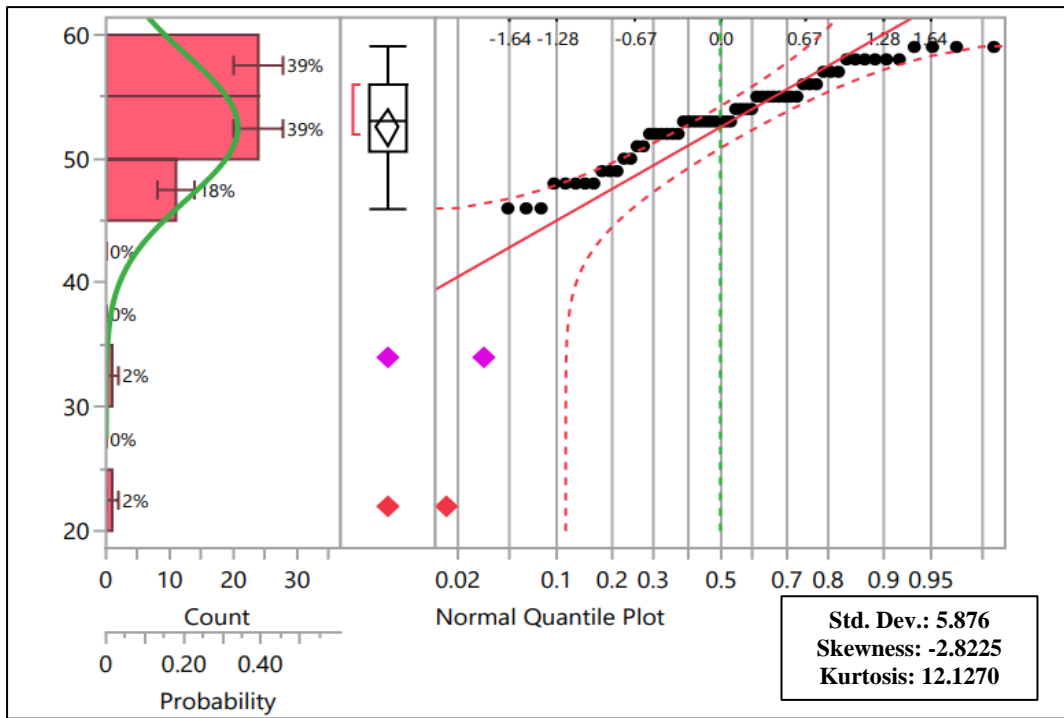


Figure 30. Frequency distribution and probability plot of total phenols in  $BC_1F_1$  resistant lines

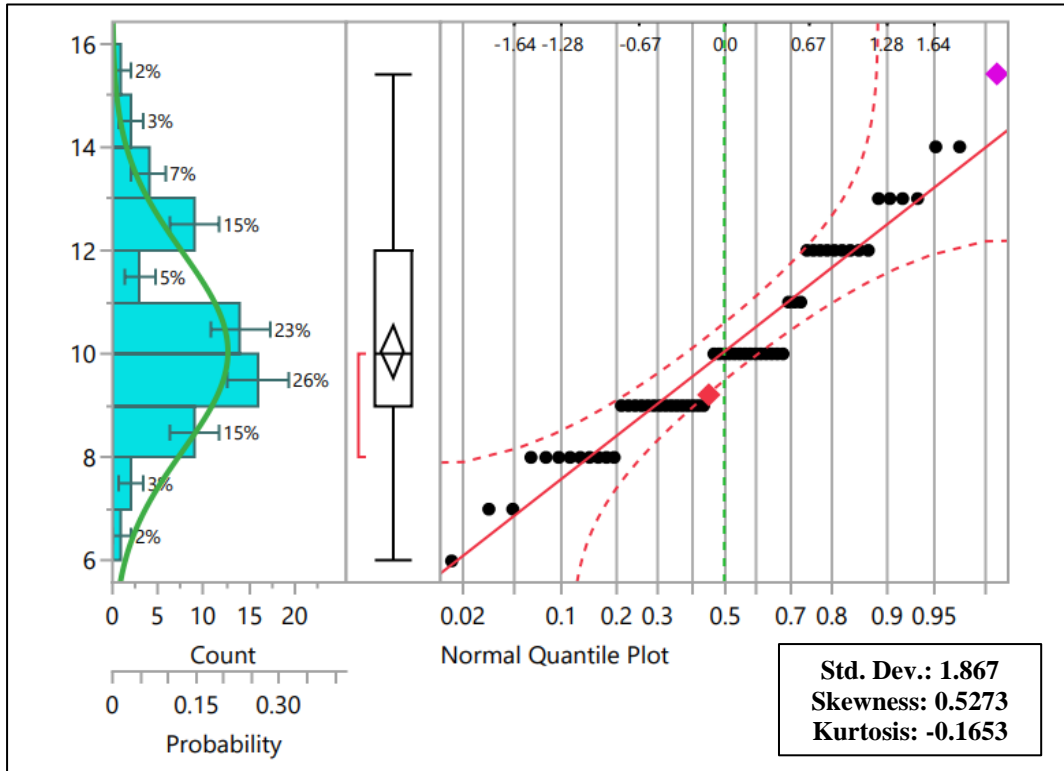
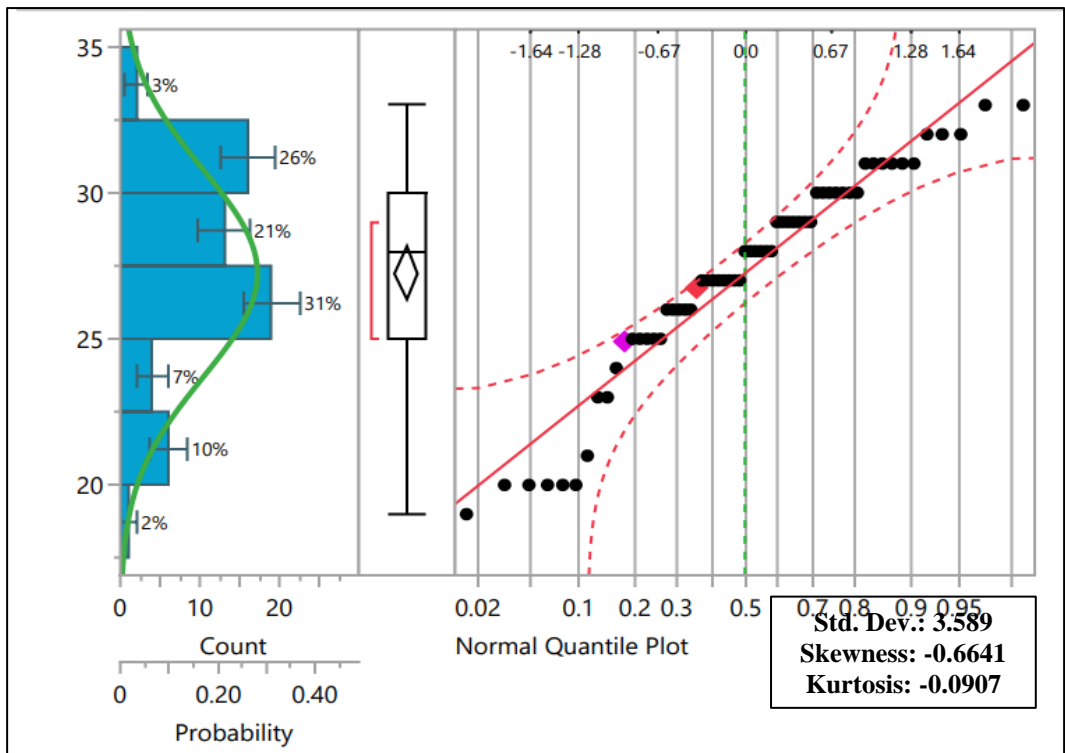


Figure 31. Frequency distribution and probability plot of crude fibre content in  $BC_1F_1$  resistant lines

◆ Githika    ◆ IRON



**Figure 32. Frequency distribution and probability plot of crude protein content in BC<sub>1</sub>F<sub>1</sub> resistant lines**

◆ Githika    ◆ IRON

showing complementary additive gene action under the control of many genes (Figure 29).

Positively skewed distributions show that the population's individuals were clustered around lower mean values, whereas negatively skewed distributions show that they were concentrated around higher mean values (Prabhu *et al.*, 2016). Among the biometric traits used in the study, the vine length, primary branches per plant, pod length, number of seeds per pod and crop duration had positive skewness indicating the data pattern towards the lowest mean value which needs more backcrossing to achieve recurrent parent characters. Only the trait pod weight had negative skewness with clustering towards the highest mean value *i.e.*, towards the recurrent parent.

The frequency distribution of quality parameters in the 59 BC<sub>1</sub>F<sub>1</sub> lines showed varied patterns without normal distribution. The total phenols and crude protein content were negatively skewed and the crude fibre content was positively skewed (Figures 30, 31 and 32). The crude fibre content (19.57 to 33.88%) and crude protein content (6.78 to 14.58%) were platykurtic and total phenols (46.12 to 59.82) were leptokurtic in nature. So, fewer genes with duplicate epistasis gene action controlled the trait total phenols in BC<sub>1</sub>F<sub>1</sub> lines. The crude fibre content showed complementary additive gene action and crude protein content was duplicate additive gene action and both were controlled by many genes.

#### **5.8.1. Proximity dissimilarity matrix by Euclidean distance with a recurrent parent using biometric characters**

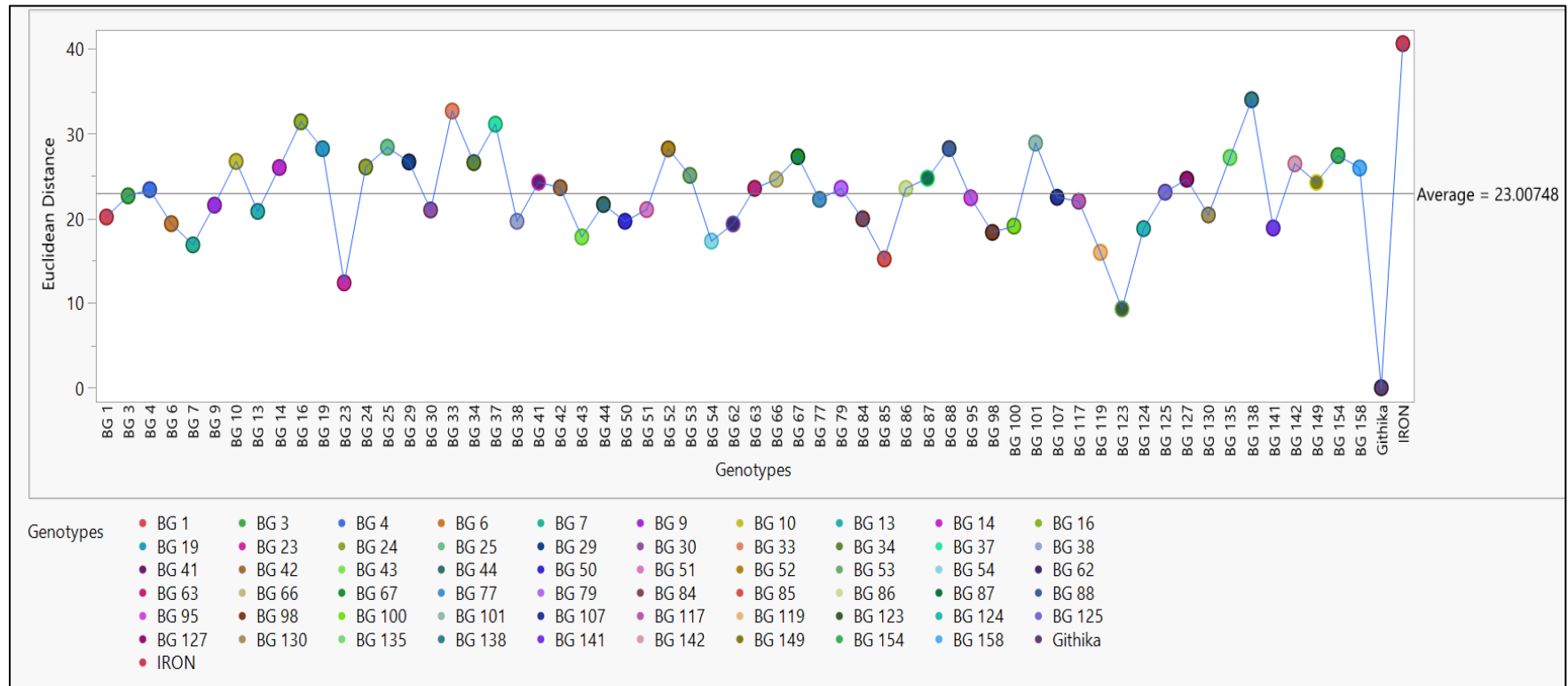
59 resistant BC<sub>1</sub>F<sub>1</sub> lines were analysed for Euclidean distance to identify the lines that were close to recurrent parent Githika. The biometric parameters were used to calculate Euclidean distance using the proximity dissimilarity matrix. The proximity matrix and genetic distance in the population help to ascertain their phylogenetic linkage and evolutionary trends, and sometimes to find out the true nature of the relationship between parents and population (Hoque and Rahman, 2007). The overall Euclidean distance using biometric parameters in the present study varied from 9.3240 to 34.023 with the recurrent parent. The minimum

distance obtained in the BC<sub>1</sub>F<sub>1</sub> line BG123 indicates, a larger similarity with the recurrent parent Githika. The genetic distance between BC<sub>1</sub>F<sub>1</sub> lines and parents and its recurrent gene recovery through the backcrossing method could be assessed by Euclidean distance using proximity dissimilarity analysis. Increasing the number of backcrosses decreases the dissimilarity distance to the recurrent parent. The genetic diversity pattern in wild and local cultivars was estimated using Euclidean cluster analysis (Ogbonnaya *et al.*, 2001). Different qualitative and quantitative traits were used to study the Euclidean distance in different crops (Hanhua, *et al.*, 1995; Gupta *et al.*, 1999). About 25 resistant BC<sub>1</sub>F<sub>1</sub> lines were identified as proximity with the recurrent parent, which was selected and advanced to the next backcross (Figure 33 and Figure 34).

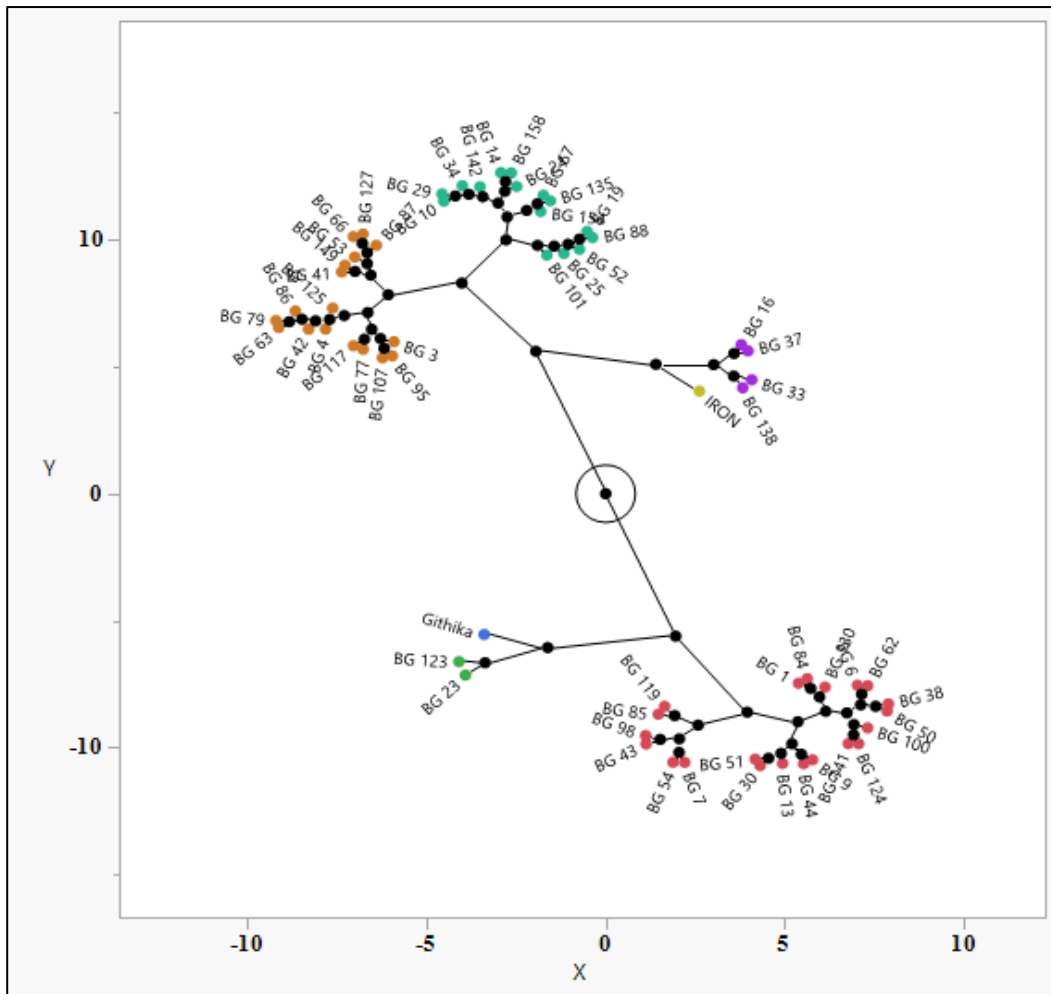
#### 5.9. CONFIRMATION OF RESISTANT GENE IN BC<sub>1</sub>F<sub>1</sub> LINES USING SSR MARKER LINKED TO FUSARIUM WILT

Genomic research is accelerating breeding methods due to applications of molecular markers in crop improvement (Sahu *et al.*, 2020). Markers have been shown to improve the effectiveness of selection and produce unique varieties (Hasan *et al.*, 2021). Genotyping of 59 resistant BC<sub>1</sub>F<sub>1</sub> lines using the SSR marker UASBC59 amplified two bands at 190 bp (Resistant band) and 250 bp (Susceptible band) in all the lines, concluding that the Fusarium wilt resistance in cowpea is controlled by the single dominant gene. The heterozygous BC<sub>1</sub>F<sub>1</sub> lines showed Fusarium wilt resistance phenotypically. The Fusarium wilt phenotyping in cowpea through artificial screening using virulent strains was very effective to identify the resistant genotypes, which were confirmed through genotyping.

The monogenic inheritance of Fusarium wilt resistance in cowpea was reported previously (Brick *et al.*, 2004; Rubio *et al.*, 2003; Augustine *et al.*, 2010). 50% of BC<sub>1</sub>F<sub>1</sub> progenies showed Fusarium wilt resistance in cowpeas (Omoigui *et al.*, 2018). The monogenic inheritance of the gene for Fusarium wilt resistance in chickpeas was confirmed through phenotyping and SSR genotyping (Yadav *et al.*, 2023).



**Figure 33. Proximity dissimilarity matrix Euclidean distance of BC<sub>1</sub>F<sub>1</sub> lines with a recurrent parent using biometric traits**



**Figure 34. Constellation plot of BC<sub>1</sub>F<sub>1</sub> lines using Euclidean distance with a recurrent parent using biometric traits**

## 5.10. EVALUATION OF BC<sub>2</sub>F<sub>1</sub> LINES AT THE SEEDLING STAGE THROUGH ARTIFICIAL SCREENING AND SCORING

The 25 resistant BC<sub>1</sub>F<sub>1</sub> lines which were more similar to the recurrent parent were backcrossed to produce BC<sub>2</sub>F<sub>1</sub> lines. The Fusarium wilt reaction of 128 BC<sub>2</sub>F<sub>1</sub> lines was assessed by artificial screening and scoring based on a 0-4 scale. The virulent strain Fot1 which was isolated from the Vellayani region was used for the artificial screening. Virulent races of diseases require continuous characterization for the screening of germplasm (Ahmad *et al.*, 2014). From the screening, 35 BC<sub>2</sub>F<sub>1</sub> lines were resistant, 19 were moderately resistant, 21 were moderately susceptible, 12 were susceptible and 41 were highly susceptible.

The different reaction category for Fusarium wilt was used for distinguishing the reaction of the plants to the disease. The resistant BC<sub>2</sub>F<sub>1</sub> lines could be used for advanced backcrossing generation by genotyping using molecular markers. The same categorization for Fusarium wilt reaction was done by Chaudhary *et al.* (2007), Dubey and Singh, (2008), Ratan and Biswas, (2010), and Yadav and Kumar (2019). The resistant BC<sub>2</sub>F<sub>1</sub> lines developed in the present study were forwarded for the further backcrossing programme.

The investigation resulted in the identification of the *Fusarium oxysporum* responsible for the Fusarium wilt in cowpeas in the Vellayani region, its morphological and molecular characterisation, and the ITS sequence was deposited into the NCBI database with accession number MZ706472.1. The study also revealed that the sand: maize was the optimal medium for pathogen mass multiplication, and 10% inoculum was standardised as the ideal amount for inoculation for artificially screening cowpea resistance to Fusarium wilt. Fusarium wilt resistance was found in the cowpea variety IRON, and a transferable SSR marker from chickpea, UASBC59, was found to be useful as a Fusarium wilt-resistant linked SSR marker in cowpea. The 35 BC<sub>2</sub>F<sub>1</sub> lines that were discovered to be Fusarium wilt resistant as part of the study could be employed as breeding lines for creating promising varieties with Fusarium wilt resistance.

# ***SUMMARY***

## 6. SUMMARY

The present study on “Marker assisted backcrossing for transferring Fusarium wilt (*Fusarium oxysporum* f. sp. *tracheiphilum*) resistance into Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)” was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani and Integrated Farming System Research Station (IFSRS), Karamana, during the year 2019-2022, with an objective to introgress Fusarium wilt resistant gene into yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) through marker-assisted backcrossing.

The pathogen was isolated from the infected yardlong bean plants cultivated at the College of Agriculture, Vellayani. The isolated pathogens were identified based on morphological and molecular characteristics. A total of 10 isolates of the pathogen were isolated from the field, of which only five appeared as Fusarium pathogens. The isolate Fot1 was found to be more virulent and had the capacity to induce early Fusarium wilt symptoms in healthy yardlong bean plants. The Fot1 pathogen completed its growth in Petri plates containing potato dextrose agar medium within 11 days at 28±2°C and mycelia appeared white and floccose with reddish brown pigmentation. The phialides and conidiophores were present and arose on aerial mycelium. The microconidia were abundant, aseptate, 12.879 x 3.570 µm in size, and spherical in shape. The macroconidia were 3-4 septate, thin-walled, and little sickle-shaped. Intercalary and terminal chlamydospores were also present. Sand: maize medium (9:1) was standardized as the optimum medium for the multiplication of the pathogen and a 10% inoculum level was found to cause disease symptoms on artificial inoculation.

The internal transcribed spacer (ITS) region of the Fot1 pathogen was amplified using the universal primers ITS1 and ITS4, and a consensus sequence was obtained. The similarity search of the sequence was done in NCBI through BLAST analysis, which revealed that the sequence was 99-100% homology with the *Fusarium oxysporum* database available in the NCBI. The phylogenetic analysis of the sequence with the *Fusarium oxysporum* database from the NCBI showed

similarity with 100% bootstrap support. Hence, the isolated Fot1 pathogen was confirmed as *Fusarium oxysporum* f. sp. *tracheiphilum* and the ITS-rDNA sequence of the isolate was submitted to the NCBI with accession number MZ706472.1.

The cowpea genotypes were collected from the Asian Vegetable Research and Development Centre (AVRDC), Taiwan (30 Nos), National Bureau of Plant Genetic Resources (NBPGR), New Delhi (30 Nos), and Department of Plant Breeding and Genetics, College of Agriculture, Vellayani (45 Nos), along with the varieties released from Kerala Agricultural University (KAU) (10 Nos) were used for artificial screening at the seedling stage using *Fusarium* wilt culture. A total of 115 cowpea genotypes were screened in a completely randomized design (CRD) in three replications and scored based on a 0-4 scale given by Senthilkumar (2003). Out of these, eighteen were resistant, twenty-six were moderately resistant, thirty-one were moderately susceptible, thirty-one were susceptible, and nine were highly susceptible. The disease intensity ranged from 0% to 80% and the genotypes were taken 15 to 53 days to induce *Fusarium* wilt symptoms.

The resistant cowpea genotypes were evaluated in the field along with four popular, but susceptible varieties released by KAU in two separate experiments. The twelve resistant and three susceptible trailing-type cowpea genotypes were evaluated in the field at 1.5×0.45m spacing and six resistant and one susceptible bush-type cowpea genotypes were evaluated at 60×45 cm spacing in a randomized block design (RBD) with three replications. Twelve biometric characters including nine morphological and three quality parameters were observed from each genotype and statistically evaluated. Analysis of variance revealed a significant variation among the genotypes for twelve biometric characters. The mean performance of each genotype for the characters showed significant variations with ample scope for improvement. The quality traits including total phenols, crude fibre content and crude protein content were higher in the resistant genotypes than in susceptible genotypes, which might be considered an important defence mechanism against *Fusarium oxysporum*.

The phenotypic and genotypic coefficients of variation, heritability, and genetic advance were high for vine length, primary branches per plant, pods per plant, pod weight, pod yield per plant, total phenols, and crude fibre content in both trailing and bush-type cowpea genotypes indicated the high variability in the characters studied and offering ample scope for selection. A significant positive genotypic correlation was observed for the characters including primary branches per plant, pods per plant, pod weight, pod length, and number of harvests with pod yield per plant in both trailing and bush-type cowpea genotypes. Path analysis of biometric characters revealed the direct positive effect of primary branches per plant, pods per plant, seeds per pod, and crude protein content on pod yield per plant in both trailing and bush-type cowpea genotypes.

The genomic DNA of resistant and susceptible cowpea genotypes were isolated and the amplification was carried out using Fusarium wilt-linked SSR markers. Sixteen SSR markers including one from cowpea and the other fifteen from chickpea which are transferable to the cowpea genome were used. Out of these SSR markers, UASBC59 could produce a polymorphic band in the genotypes at 250 bp (susceptible) and IRON at 190 bp (resistant) product size. The variety IRON showed total wilt resistance during seedling screening and under field conditions. Its high content of seed protein and fibre also indicated its resistance to Fusarium wilt. So, it was selected as a Fusarium wilt-resistant donor parent in the backcross breeding program. The IRON was crossed with Githika, the superior, but susceptible variety released from KAU and obtained about 12% pod set. The 210 F<sub>1</sub> seeds were produced by crossing Githika and IRON, among them, 148 germinated with 70.4 per cent germination. The F<sub>1</sub>s produced were morphologically evaluated with parents for six characters, which concluded that all the characters of F<sub>1</sub>s were in the mid-parental range. The morphological evaluation of F<sub>1</sub>s recorded above-average performance in vine length, pod weight, and crop duration, whereas primary branches per plant, pod length, and seeds per pod were below average between the two parents.

All F<sub>1</sub>s were backcrossed with recurrent parent Githika to produce BC<sub>1</sub>F<sub>1</sub> lines. About 270 BC<sub>1</sub>F<sub>1</sub> lines were produced, of which only 198 lines germinated with 73 per cent germination. The artificial screening of 198 BC<sub>1</sub>F<sub>1</sub> lines using 10% *Fusarium* inoculum, identified 59 as resistant, 27 were moderately resistant, 14 were moderately susceptible, 25 were susceptible and 73 were highly susceptible. The chi-square analysis for the resistant and susceptible genotypes confirmed the 1:1 ratio, indicating that the *Fusarium* wilt in cowpea was controlled by a single dominant gene.

The morphological evaluation of 59 resistant BC<sub>1</sub>F<sub>1</sub> lines using biometric and quality parameters showed that the trait values of lines were distributed within the range of that of parents. The characters including vine length, primary branches per plant, pod length, seeds per pod, crop duration and crude fibre content showed positive skewness indicating complementary gene action whereas pod weight, total phenols, and crude protein content showed negative skewness indicating inhibitory gene action. Positive kurtosis was observed in primary branches per plant, pod weight, pod length and total phenols revealing that these characters were controlled by few genes and negative kurtosis in vine length, seeds per pod, crop duration, crude fibre content and crude protein content, indicating these characters were controlled by many genes. The Euclidean distance using biometric traits derived from proximity dissimilarity matrix analysis in 59 resistant BC<sub>1</sub>F<sub>1</sub> lines found that 25 lines were close to the recurrent parent. The genotyping of 59 resistant BC<sub>1</sub>F<sub>1</sub> lines using the SSR marker UASBC59, showed the heterozygous condition, which also revealed the dominant monogenic inheritance of the *Fusarium* wilt-resistant gene in cowpea.

The 25 resistant BC<sub>1</sub>F<sub>1</sub> lines which were in close proximity to the recurrent parent were backcrossed to produce BC<sub>2</sub>F<sub>1</sub> lines. About 128 BC<sub>2</sub>F<sub>1</sub> lines were artificially screened for *Fusarium* wilt reaction and scored on a 0-4 scale. Out of this, 35 were resistant, 19 were moderately resistant, 21 were moderately susceptible, 12 were susceptible and 41 were highly susceptible. The 35 BC<sub>2</sub>F<sub>1</sub> resistant lines obtained can be carried forward for further evaluation.

From the study, the *Fusarium oxysporum* causing Fusarium wilt in cowpea was isolated from the Vellayani region, identified through morphological and molecular characterization, and the ITS sequence of the isolate was deposited in the NCBI database. The study also identified the sand: maize as the best medium for mass multiplication of the pathogen and 10% inoculum was standardized as the optimum level for inoculation for artificial screening for Fusarium wilt resistance in cowpea. The cowpea variety IRON was identified as a probable donor for Fusarium wilt resistance, and a transferable SSR marker from the chickpea, UASBC59 was identified for use as Fusarium wilt-resistant linked SSR marker in cowpea. The 35 BC<sub>2</sub>F<sub>1</sub> lines found resistant to Fusarium wilt as part of the study could be used as breeding lines for developing promising varieties with commercial traits of Githika and Fusarium wilt resistance of IRON.

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# ***APPENDICES***

## APPENDIX- I

### ITS-rDNA SEQUENCE OF FOT1 FUSARIUM ISOLATE

>MZ706472.1 *Fusarium oxysporum* f. sp. *tracheiphilum*  
isolate fot1 internal transcribed spacer 1, partial  
sequence; 5.8S ribosomal RNA gene and internal  
transcribed spacer 2, complete sequence; and large  
subunit ribosomal RNA gene, partial sequence  
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CGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGT  
AAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGTTCTGGCATCGA  
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TAGTAGTAAAACCCCTCGCAACTGGTACGCGGCGGGCCAAGCCGTTAAACCCCCA  
ACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATAT  
CAATAAGCGA

## APPENDIX- II

### THE HOMOLOGOUS SIMILARITY SEARCH OF FOT1 SEQUENCE IN NCBI THROUGH BLAST

An official website of the United States government [Here's how you know](#)

**NIH** National Library of Medicine  
National Center for Biotechnology Information Log in

BLAST® » blastn suite » results for RID-7VTTPURM016 Home Recent Results Saved Strategies Help

**Take the BLAST survey today** Start survey

[< Edit Search](#) [Save Search](#) [Search Summary](#) [How to read this report?](#) [BLAST Help Videos](#) [Back to Traditional Results Page](#)

**i** Your results are filtered to match records that include: fusarium oxysporum

**Job Title** gb|MZ706472.1  
**RID** 7VTTPURM016 Search expires on 06-06 21:46 pm [Download All](#)  
**Program** BLASTN [Citation](#)  
**Database** nt [See details](#)  
**Query ID** MZ706472.1  
**Description** Fusarium oxysporum f. sp. tracheiphilum isolate fot1 inter...  
**Molecule type** nucleic acid  
**Query Length** 505  
**Other reports** [Distance tree of results](#) [MSA viewer](#)

**Filter Results**

**Organism** only top 20 will appear  exclude  
fusarium oxysporum  
[+ Add organism](#)

**Percent Identity**  to  **E value**  to  **Query Coverage**  to   
**Filter** **Reset**

**Descriptions** [Graphic Summary](#) [Alignments](#) [Taxonomy](#)

**Sequences producing significant alignments** Download Select columns Show 100

select all 6 sequences selected [GenBank](#) [Graphics](#) [Distance tree of results](#) [MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	Fusarium oxysporum f. sp. tracheiphilum isolate fot1 internal transcribed spacer_1, partial sequence: 5.8S ribosoma...	Fusarium oxyspo...	933	933	100%	0.0	100.00%	505	MZ706472.1
<input checked="" type="checkbox"/>	Fusarium oxysporum f. sp. capsici isolate 1 internal transcribed spacer_1, partial sequence: 5.8S ribosomal RNA ge...	Fusarium oxyspo...	931	931	99%	0.0	100.00%	529	QP881816.1
<input checked="" type="checkbox"/>	Fusarium oxysporum isolate RDL2 internal transcribed spacer_1, partial sequence: 5.8S ribosomal RNA gene and J...	Fusarium oxyspo...	931	931	99%	0.0	100.00%	510	QP684354.1
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## APPENDIX- III

### ITS-RDNA SEQUENCE OF THE ISOLATE SUBMITTED TO THE NCBI WITH ACCESSION NUMBER MZ706472.1

An official website of the United States government [Here's how you know](#)

**NIH** National Library of Medicine  
National Center for Biotechnology Information Log in

Nucleotide   Advanced Help

GenBank

#### Fusarium oxysporum f. sp. tracheiphilum isolate fot1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MZ706472.1  
[FASTA](#) [Graphics](#)

[Go to:](#)

**LOCUS** MZ706472 595 bp DNA linear PLN 10-AUG-2021  
**DEFINITION** Fusarium oxysporum f. sp. tracheiphilum isolate fot1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.  
**ACCESSION** MZ706472  
**VERSION** MZ706472.1  
**KEYWORDS** .

**SOURCE** Fusarium oxysporum f. sp. tracheiphilum  
**ORGANISM** Fusarium oxysporum f. sp. tracheiphilum  
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae; Fusarium; Fusarium oxysporum species complex.

**REFERENCE** 1 (bases 1 to 505)  
**AUTHORS** Shahiba,A.M., Jayalekshmy,V.G. and Sajeena,A.  
**TITLE** Fusarium oxysporum  
**JOURNAL** Unpublished

**REFERENCE** 2 (bases 1 to 505)  
**AUTHORS** Shahiba,A.M., Jayalekshmy,V.G. and Sajeena,A.  
**TITLE** Direct Submission  
**JOURNAL** Submitted (04-AUG-2021) Plant Breeding and Genetics, College of Agriculture, Vellayani, Vellayani, Trivandrum, Kerala 695522, India

**COMMENT** ##Assembly-Data-START##  
Sequencing Technology :: Sanger dideoxy sequencing  
##Assembly-Data-END##

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/note="contains internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA"

**ORIGIN**  
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61 cggcccgcca gaggaccctt aaactctgtt tctatatgta actctgagt aaaccataa  
121 ataaatcaaa actttcaaca cggatctct tggttctggc atcgtgag aacgcagcaa  
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**Change region shown**  
 Whole sequence  
 Selected region  
from: begin  to: end

**Customize view**

**Analyze this sequence**

**Related information**

**LinkOut to external resources**  
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**MARKER ASSISTED BACKCROSSING FOR  
TRANSFERRING FUSARIUM WILT (*Fusarium oxysporum* f.  
sp. *tracheiphilum*) RESISTANCE INTO YARDLONG BEAN  
(*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)**

*by*

**SHAHIBA A. M.**

**(2018-21-020)**

**Abstract of the thesis**

**Submitted in partial fulfilment of the requirements for the degree of**

**DOCTOR OF PHILOSOPHY IN AGRICULTURE**

**Faculty of Agriculture**

**Kerala Agricultural University**



**DEPARTMENT OF PLANT BREEDING AND GENETICS**

**COLLEGE OF AGRICULTURE**

**VELLAYANI, THIRUVANANTHAPURAM-695522**

**KERALA, INDIA**

**2023**

## ABSTRACT

The present study entitled “Marker assisted backcrossing for transferring Fusarium wilt (*Fusarium oxysporum* f. sp. *tracheiphilum*) resistance into Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)” was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani and Integrated Farming System Research Station (IFSRS), Karamana, during the year 2019-2022, with an objective to introgress genes for Fusarium wilt resistance into yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) through marker-assisted backcrossing.

The pathogen-causing Fusarium wilt was isolated from the infected yardlong bean plants cultivated at the College of Agriculture, Vellayani, and identified based on morphological and molecular characteristics. The pathogen completed growth in Petri plates containing potato dextrose agar medium within 11 days at 28±2°C and mycelia appeared white and floccose with reddish brown pigmentation. The presence of microconidia, macroconidia, and chlamydospores confirms the pathogen as *Fusarium oxysporum*. Molecular characterization of the identified pathogen was done using Internal Transcribed Spacer (ITS) region universal primers. The BLAST analysis of the amplified nucleotide sequence revealed 99-100 per cent similarity with the sequences of *Fusarium oxysporum* in the NCBI database. The identified sequence of *Fusarium oxysporum* f sp. *tracheiphilum* was deposited in the NCBI GenBank database with the accession number viz., MZ706472.1. Sand: maize medium (9:1) was standardized as the optimum medium for the multiplication of the pathogen and a 10% inoculum level was found to provide disease symptoms on artificial inoculation.

The cowpea genotypes collected from the Asian Vegetable Research and Development Centre (AVRDC), Taiwan (30 Nos), National Bureau of Plant Genetic Resources (NBPGR), New Delhi (30 Nos), and Department of Plant Breeding and Genetics, College of Agriculture, Vellayani (45 Nos), along with the varieties released from Kerala Agricultural University (KAU) (10 Nos) were used for artificial screening at the seedling stage using Fusarium wilt culture. This

experiment was done in a completely randomized design (CRD) with three replications. The scoring for disease incidence was done using the 0-4 scale reported by Senthilkumar (2003). Out of the total 115 cowpea genotypes screened eighteen were resistant, twenty-six were moderately resistant, thirty-one were moderately susceptible, thirty-one were susceptible, and nine were highly susceptible.

Eighteen genotypes found resistant during the screening process at the seedling stage were evaluated in the field along with four popular, but susceptible varieties released by KAU. These cowpea genotypes consisted of fifteen trailing types (12 resistant and 3 susceptible) and seven bush types (6 resistant and 1 susceptible). The trailing and bush-type cowpea genotypes were evaluated separately in a randomized block design (RBD) in three replications. Analysis of variance revealed a significant variation among the genotypes for twelve biometric traits.

The phenotypic and genotypic coefficients of variation, heritability, and genetic advance were high for vine length, primary branches per plant, pods per plant, pod weight, pod yield per plant, total phenols, and crude fibre content in both trailing and bush type cowpea genotypes indicated the high variability in the characters studied and offering ample scope for selection. A significant positive genotypic correlation was observed for the characters including primary branches per plant, pods per plant, pod weight, pod length, and number of harvests with pod yield per plant in both trailing and bush-type cowpea genotypes. Path analysis of biometric characters revealed the direct positive effect of primary branches per plant, pods per plant, seeds per pod, and crude protein content on pod yield per plant in both trailing and bush-type cowpea genotypes.

The variety IRON showed total wilt resistance during seedling screening and under field conditions. Its high content of seed protein and fibre also indicated its resistance to Fusarium wilt. The reported SSR marker C13-16, could not show polymorphism between resistant and susceptible genotypes and hence, 15 transferable SSR markers linked to Fusarium wilt in chickpea were used for

screening, and from among them the SSR marker UASBC59 could produce polymorphic amplicon in IRON. Hence, IRON was chosen as the donor parent for marker-assisted breeding using UASBC59.

The identified *Fusarium* wilt resistant donor variety IRON was crossed with Githika, the superior, but susceptible variety released from KAU. The  $F_1$ s were morphologically evaluated with parents for six characters which revealed that all the characters of  $F_1$ s were in the mid-parental range. The  $F_1$ s were used to produce  $BC_1F_1$  lines and a total of 198  $BC_1F_1$  lines were artificially screened at the seedling stage for *Fusarium* wilt reaction and scored using a 0-4 scale. Among the  $BC_1F_1$  lines, fifty-nine were found to be resistant to *Fusarium* wilt, 27 were moderately resistant, 14 were moderately susceptible, 25 were susceptible, and 73 were highly susceptible. The chi-square goodness of fit test ratio of 1:1 obtained in the  $BC_1F_1$  lines for resistance: susceptible confirmed the monogenic dominant inheritance pattern of the gene for resistance to *Fusarium* wilt in cowpea. The molecular analysis of the resistant  $BC_1F_1$  lines using SSR marker UASBC59 revealed a heterozygous condition and this could confirm the dominant inheritance of the gene for resistance. Evaluation of nine biometric and three quality parameters in the 59  $BC_1F_1$  resistant lines showed significant variations. Euclidean distance using biometric traits derived from proximity dissimilarity matrix analysis found that out of the 59  $BC_1F_1$  resistant lines, 25 had close proximity with the recurrent parent. These lines were used to produce  $BC_2F_1$  breeding lines. The 128  $BC_2F_1$  lines raised from the 25 resistant  $BC_1F_1$  lines were subjected to screening for *Fusarium* wilt resistance at the seedling stage and scored using a 0-4 scale. Out of this, 35 were resistant, 19 were moderately resistant, 21 were moderately susceptible, 12 were susceptible and 41 were highly susceptible. The 35  $BC_2F_1$  resistant lines obtained can be carried forward for further evaluation.

The *Fusarium oxysporum* causing *Fusarium* wilt in cowpea was isolated, identified and deposited in the NCBI database. The study also identified sand: maize as the best media for mass multiplication of the pathogen and standardized 10% inoculum as the optimum level for inoculation for screening *Fusarium* wilt

resistance in cowpea. UASBC59, the transferable SSR marker from the chickpea was identified for use as a Fusarium wilt resistant linked SSR marker in cowpea. The 35 BC<sub>2</sub>F<sub>1</sub> lines found resistant to Fusarium wilt as part of the study could be used as breeding lines for developing promising varieties with commercial traits of Githika and Fusarium wilt resistance of IRON.

**സംഗ്രഹം**

"മാർക്കർ അസ്സിസ്റ്റഡ് ബ്രീഡിങ് ഫോർ ട്രാൻസ്ഫെറിങ് ഫ്യൂസേറിയം വിൽറ്റ് (*Fusarium oxysporum f sp. tracheiphilum*) റെസിസ്റ്റൻസ് ഇന്റു യാർഡ് ലോങ്ങ് ബീൻ (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt)" എന്ന ഗവേഷണ പദ്ധതി 2019-2022 കാലയളവിൽ വെള്ളായണി കാർഷിക കോളേജിന്റെ സസ്യപ്രജനന ജനിതകശാസ്ത്ര വിഭാഗത്തിലും കരമന സമഗ്ര കാർഷിക സമ്പ്രദായ ഗവേഷണകേന്ദ്രത്തിലുമായി നടത്തുകയുണ്ടായി. ഫ്യൂസേറിയം വാട്ടരോഗത്തിന് എതിരെ പ്രതിരോധ ശേഷി നൽകുന്ന ജീനുകൾ മാർക്കർ അധിഷ്ഠിത ബാക്ക് ക്രോസ്സിംഗ് മുഖേന വളളിപ്പയറിലേക്ക് സംക്ഷേപിപ്പിക്കുക എന്നതായിരുന്നു പഠനപദ്ധതിയുടെ ഉദ്ദേശം.

പയറിൽ ഫ്യൂസേറിയം ഉണ്ടാക്കുന്ന വാട്ടത്തിന്റ രോഗകാരിയായ സൂക്ഷ്മാണുവിനെ വെള്ളായണി കാർഷിക കോളേജ് പരിധിയിൽ ഉള്ള ഒരു രോഗബാധിത കൃഷി സ്ഥലത്ത് നിന്നും ഏകീകരിച്ചെടുത്ത ശേഷം രൂപശാസ്ത്രപരമായും തന്മാത്രാ തലങ്ങളിലുമായി പഠനവിധേയമാക്കുകയും സ്വഭാവ സവിശേഷതകൾ രേഖപ്പെടുത്തുകയുമുണ്ടായി. ഏകീകരിച്ചെടുത്ത രോഗകാരികളെ 28±2°C ഡിഗ്രി സെൽഷ്യസ് താപനിലയിൽ പൊട്ടറ്റോ-ഡെസ്റ്ററോസ് അഗർ മാധ്യമത്തിൽ വളർത്തിയപ്പോൾ, 11 ദിവസം കൊണ്ട് അവ വളർച്ച പൂർത്തിയാക്കി. ചുവപ്പ് കലർന്ന തവിട്ട് നിറത്തോട് കൂടി കാണപ്പെട്ട കൾച്ചറിന്, വെളുത്ത നിറത്തിലായി മൈസീലിയകൾ ഉള്ളതായി കണ്ടു. രോഗാണുവിന്റെ മൈക്രോകോണീടിയ, മാക്രോകോണീടിയ, ക്ലാമിഡോസ്പോർസ്, എന്നിവയുടെ രൂപഭാവങ്ങൾ നിരീക്ഷിച്ചതിൽ നിന്നും "ഫ്യൂസേറിയം ഓക്സിസ്പോറം " എന്ന സൂക്ഷ്മാണുവാണു് രോഗകാരി എന്ന് സ്ഥിരീകരിച്ചു. തിരിച്ചറിഞ്ഞ രോഗകാരിയുടെ തന്മാത്രാ തലത്തിലുള്ള സ്വഭാവ സവിശേഷതകൾ ഇന്റർണൽ ട്രാൻസ്ക്രൈബ്ബ് സ്പേസർ (ഐ ടി എസ്) റീജിയൻ യൂണിവേഴ്സ് പ്രൈമറുകൾ ഉപയോഗിച്ചുകൊണ്ട് രേഖപ്പെടുത്തി. വർദ്ധിപ്പിച്ചെടുത്ത ന്യൂക്ലിയോടൈഡ് സീക്വൻസുകൾ BLAST സംവിധാനം ഉപയോഗിച്ച് വിശകലനം ചെയ്തതിൽ നിന്നും, NCBI ഡാറ്റാ ബേസിൽ ഉള്ള 'ഫ്യൂസേറിയം ഓക്സിസ്പോറം' ത്തിന്റെ സീക്വൻസുകളുമായി അവ 99-100% സാമ്യം പുലർത്തുന്നതായി കാണിച്ചു. തിരിച്ചറിഞ്ഞ 'ഫ്യൂസേറിയം ഓക്സിസ്പോറത്തിന്റെ (*Fusarium oxysporum f sp. tracheiphilum*)' സീക്വൻസുകൾ NCBI ജീൻ ബാങ്ക് ഡാറ്റാ ബേസിൽ നിക്ഷേപിക്കുകയും M2706472 എന്ന ആക്സൻ നമ്പറോട് കൂടി രേഖപ്പെടുത്തുകയും ചെയ്തു. തിരിച്ചറിഞ്ഞ രോഗകാരികളെ മണലും ചോളവും 9:1 എന്ന അനുപാതത്തിൽ

കലർത്തിയുണ്ടാക്കിയ മാധ്യമത്തിൽ വംശവർദ്ധനവ് നടത്തുന്നത് ഏറ്റവും അനുയോജ്യമായ രീതിയായി അംഗീകരിക്കപ്പെട്ടു. അതുപോലെ രോഗലക്ഷണങ്ങൾ ഉണ്ടാക്കാനായി കൃത്യമായി രോഗാംശനിവേശനം നടത്തുവാൻ ഏറ്റവും ഉപയുക്തമായ ഇനോക്യൂലത്തിന്റെ അളവ് പത്തു ശതമാനം ആയി കണ്ടെത്തി.

ഗവേഷണത്തിനായി വിവിധ ഇനത്തിൽപ്പെടുന്ന വള്ളിപ്പയറുകൾ ശേഖരിച്ചു. ഏഷ്യൻ വെജിറ്റബിൾ റിസർച്ച് ആൻഡ് ഡെവലപ്മെന്റ് സെന്റർ (AVRDC) തായ്‌വാൻ (30 എണ്ണം), നാഷണൽ ബ്യൂറോ ഓഫ് പ്ലാന്റ് ജനറ്റിക് റിസോഴ്സ് (NBPGR), ന്യൂ ഡൽഹി (30 എണ്ണം), വെള്ളായണി കാർഷിക കോളേജിലെ സസ്യ പ്രജനന വിഭാഗത്തിൽ നിന്നും (45 എണ്ണം) അതിനോടൊപ്പം കേരള കാർഷിക സർവകലാശാല പുറത്തിറക്കിയ ഇനങ്ങൾ (10 എണ്ണം) എന്നിങ്ങനെ വിവിധ പയറിനങ്ങളെ ഫ്യൂസേരിയം കൾച്ചർ ഉപയോഗിച്ച് തൈപ്രായത്തിൽ സ്ക്രീനിംഗ് പ്രക്രിയയ്ക്ക് വിധേയമാക്കി. കംപ്ലീറ്റെലി റാൻറമയിസ്റ്റ് ഡിസൈനിൽ 3 റപ്ലിക്കേഷനുകളിലായാണ് ഈ പരീക്ഷണം നടത്തിയത്. 2003-ൽ സെന്റിൽ കുമാർ നിർദ്ദേശിച്ച 0-4 സ്കെയിൽ ആണ് സ്ക്രീനിംഗ് പ്രക്രിയയ്ക്ക് ഉപയോഗിച്ചത്. സ്ക്രീനിങ്ങിന് വിധേയമായ 115 പയറിനങ്ങളിൽ 18 എണ്ണത്തിനു പൂർണ്ണമായും, 26 എണ്ണം ഭാഗികമായും രോഗ പ്രതിരോധശേഷി ഉള്ളതായി രേഖപ്പെടുത്തി. ബാക്കിയുള്ളവയിൽ 31 എണ്ണം ഭാഗികമായി രോഗത്തിന് കീഴ്പ്പെടുന്നവയും, 31 എണ്ണം രോഗത്തിന് വിധേയമായതും, 9 എണ്ണം വളരെ പെട്ടെന്ന് തന്നെ രോഗം ബാധിക്കുന്നവയാണെന്നും തെളിയിക്കപ്പെട്ടു.

തൈ പ്രായത്തിൽ നടത്തിയ സ്ക്രീനിംഗ് പ്രക്രിയയിൽ രോഗപ്രതിരോധ ശേഷി ഉള്ളതായി തെളിയിച്ച 18 പയറിനങ്ങളെ കേരള കാർഷിക സർവകലാശാല പുറത്തിറക്കിയ ജനകീയമായതും രോഗപ്രതിരോധശേഷി ഇല്ലാത്തതുമായ നാലു പയറിനങ്ങൾക്കൊപ്പം കൃഷിയിടത്തിൽ വളർത്തുകയും വിലയിരുത്തുകയും ചെയ്തു. പഠനത്തിന് വിധേയമാക്കിയവയിൽ പടർന്നു വളരുന്ന 15 ഇനങ്ങളും (12 രോഗപ്രതിരോധശേഷിയുള്ളവ, മൂന്ന് രോഗപ്രതിരോധശേഷി ഇല്ലാത്തവ) ഏഴു കുറ്റിപ്പയറിനങ്ങളും (ആറ് രോഗപ്രതിരോധശേഷി ഉള്ളത്, ഒരു രോഗപ്രതിരോധ ശേഷി ഇല്ലാത്തത്) ഉൾപ്പെടുന്നു. പടരുന്ന ഇനങ്ങളും കുറ്റിപ്പയറിനങ്ങളും വെച്ചേറെ, റാണ്ടമയിസ്റ്റ് ബ്ലോക്ക് ഡിസൈനിൽ മൂന്നു റപ്ലിക്കേഷനുകളിലായി വിലയിരുത്തുകയുണ്ടായി. വിശകലനം ചെയ്ത രൂപശാസ്ത്രപരമായ പന്ത്രണ്ട് സൂചകങ്ങളിലും പയറിനങ്ങൾ വ്യത്യസ്ത പുലർത്തി.

വളളിയുടെ നീളം, ശാഖകളുടെ എണ്ണം, കായകളുടെ എണ്ണം, കായ്കളുടെ ഭാരം, കായ്ഫലം, ഫീനോളിന്റെ ആകെ അളവ്, ക്രൂഡ് ഫൈബറിന്റെ അളവ് എന്നീ സൂചകങ്ങൾ പടരുന്ന ഇനങ്ങളിലും കുറ്റിപ്പയറിനങ്ങളിലും പരിശോധിച്ചപ്പോൾ വ്യതിയാനത്തിന്റെ സ്ഥൂലരൂപത്തിലുണ്ടാകുന്ന ഗുണനഘടകം, ജനിതകമായ ഗുണനഘടകം, പൈതൃകക്ഷമത, ജനിതക മുന്നേറ്റം എന്നിവ ഉയർന്ന നിരക്കിലാണെന്ന് കണ്ടു. പഠനവിധേയമാക്കിയ പ്രതീകങ്ങളിൽ കാണിച്ച ഉയർന്ന വേരിയബിലിറ്റി മികച്ച ഇനങ്ങളുടെ തിരഞ്ഞെടുപ്പിനായി ഉയർന്ന സാധ്യതയാണ് മുന്നോട്ട് വയ്ക്കുന്നത്. പടരുന്നതും അല്ലാത്തതുമായ ഇരു പയറിനങ്ങളിലും ശാഖകളുടെ എണ്ണം, കായ്കളുടെ എണ്ണം, കായ്കളുടെ ഭാരം, കായ്കളുടെ നീളം, വിളവെടുപ്പിന്റെ എണ്ണം എന്നീ പ്രതീകങ്ങളിൽ അനുകൂലവും ഗണ്യവുമായി ജനിതകപരമായ ഒരു പരസ്പരബന്ധം ഉള്ളതായി കണ്ടു. പാത്ത് അനാലിസിസ് രീതിയിൽ നടത്തിയ വിശകലനത്തിൽ നിന്നും പടരുന്ന ഇനങ്ങളിലും കുറ്റിപ്പയറിനങ്ങളിലും പ്രഥമ ശാഖകളുടെ എണ്ണം, കായ്കളുടെ എണ്ണം, കായിലെ വിത്തുകളുടെ എണ്ണം, മാംസ്യത്തിന്റെ അളവ് എന്നിവ ചെടിയുടെ കായ്കലത്തിൽ നേരിട്ടും അനുകൂലവുമായ പ്രഭാവം ചെലുത്തുന്നതായി വെളിവാക്കപ്പെട്ടു.

ക്വഷിയിടത്തിൽ തൈ ചെടികളിൽ നടത്തിയ സ്ക്രീനിംഗിൽ IRON എന്ന ഇനം പൂർണ്ണമായും രോഗപ്രതിരോധ ശേഷി കാണിച്ചു. IRON പയറിനത്തിന്റെ വിത്തുകളിൽ അടങ്ങിയിരിക്കുന്ന മാംസ്യത്തിന്റെയും ക്രൂഡ് ഫൈബറിന്റെയും ഉയർന്ന ശതമാനം ഫ്യൂസേരിയം വാട്ടത്തിനെതിരെയുള്ള പ്രതിരോധശേഷിയെ കുറിക്കുന്നതാണ്. C13-16 എസ് എസ് ആർ മാർക്കറുകൾക്ക് രോഗപ്രതിരോധശേഷി ഉള്ളവയും രോഗപ്രതിരോധ ശേഷി ഇല്ലാത്തവയും തമ്മിലുള്ള ബഹുരൂപത വ്യക്തമാക്കാൻ കഴിയാതിരുന്നതിനാൽ കടലയിലെ ഫ്യൂസേരിയം വാട്ടരോഗത്തിനെ കാണിക്കുന്ന കൈമാറ്റം ചെയ്യാൻ കഴിയുന്ന 15 എസ് എസ് ആർ മാർക്കറുകളാണ് സ്ക്രീനിംഗിനായി ഉപയോഗിച്ചത്. ഈ മാർക്കറുകളിൽ UASBC59 എന്ന എസ് എസ് ആർ മാർക്കറിനു IRON-ൽ, ബഹുരൂപത ഉള്ള ആംപ്ലികോൺ ഉണ്ടാക്കുവാൻ കഴിഞ്ഞു. ഈ കാരണത്താൽ മാർക്കർ അധിഷ്ഠിത സസ്യപ്രജനനത്തിനായുള്ള ഡോണർ പേരേന്റ് ആയി IRON ഇനത്തിനെ തിരഞ്ഞെടുത്തു, UASBC59 എന്ന എസ് എസ് ആർ മാർക്കർ ആണ് ഉപയോഗിച്ചത്.

രോഗപ്രതിരോധശേഷിക്കുള്ള ദാതാവായി തിരിച്ചറിഞ്ഞ IRON നെ കാർഷിക സർവകലാശാലയിൽ നിന്നും പുറത്തിറക്കിയ രോഗപ്രതിരോധശേഷി കുറഞ്ഞ പയറിനമായ ഗീതികയുമായി സങ്കരണം നടത്തുകയുണ്ടായി. കിട്ടിയ F<sub>1</sub> തലമുറയിലെ

സസ്യങ്ങളുടെ ആറ് സ്വഭാവ സവിശേഷതകൾ താരതമ്യം ചെയ്ത് വിലയിരുത്തിയപ്പോൾ, കണക്കിലെടുത്ത പ്രതീകങ്ങളെല്ലാം തന്നെ രക്ഷകർത്താക്കളുടെ സ്വഭാവത്തിന്റെ ശരാശരി ആണെന്ന് കണ്ടെത്തി.  $F_1$  തലമുറയിലെ സസ്യങ്ങളെ  $BC_1F_1$  ലൈൻസുകൾ ഉണ്ടാക്കാൻ ഉപയോഗിച്ചു. ഫ്യൂസേരിയം വാട്ടരോഗത്തിനോട് ഉള്ള പ്രതികരണം മനസിലാക്കുവാനായി 198  $BC_1F_1$  ലൈൻസുകളെ തെരച്ചെടികളായിരിക്കുന്ന ഘട്ടത്തിൽ തന്നെ കൃത്യമായി സ്ക്രീനിംഗ് നടത്തുകയും 0-4 സ്കെയിൽ മാനദണ്ഡത്തിൽ സ്കോറിങ് പ്രക്രിയക്ക് വിധേയമാക്കുകയും ചെയ്തു.

$BC_1F_1$  ലൈൻസുകളിൽ 59 എണ്ണം ഫ്യൂസേരിയം വാട്ടരോഗത്തിനെതിരെ പ്രതിരോധശേഷി ഉള്ളവയും 27 എണ്ണം മിതമായ പ്രതിരോധശേഷി ഉള്ളവയുമായി കണ്ടെത്തി, കൂടാതെ പൂർണ്ണമായും രോഗം വരാൻ സാധ്യതയുള്ള രോഗപ്രതിരോധ ശേഷി ഇല്ലാത്ത 73 എണ്ണവും മിതമായ രോഗസാധ്യത ഉള്ള 14 എണ്ണവും അവയിലുൾപ്പെട്ടിരുന്നു. രോഗപ്രതിരോധശേഷി ഉള്ളതും ഇല്ലാത്തതുമായ  $BC_1F_1$  ലൈൻസുകളെ കൈ സ്ക്വയർ ടെസ്റ്റിന് വിധേയമാക്കിയപ്പോൾ 1:1 അനുപാതം ലഭിച്ചു. ഈ പരീക്ഷണഫലം ഫ്യൂസേരിയം വാട്ടരോഗത്തിനെതിരെയുള്ള പ്രതിരോധശേഷി നിർണയിക്കുന്ന ജീനുകളുടെ മോണോജീനിക് പാരമ്പര്യ കൈമാറ്റ മാതൃക തെളിയിക്കുന്നതാണ്. UASBC59 എന്ന എസ് എസ് ആർ മാർക്കർ ഉപയോഗിച്ച് തന്മാത്രാ തലത്തിൽ നടത്തിയ വിശകലനത്തിൽ നിന്നും  $BC_1F_1$  ലൈൻസുകൾ ഹെറ്റെറോസയ്ഗസ് അവസ്ഥയിലാണ് നിലകൊള്ളുന്നതെന്ന് കാണാം, ഇതുവഴി പ്രതിരോധശേഷി നൽകുന്ന ജീനിന്റെ പാരമ്പര്യ കൈമാറ്റത്തിലെ ആധിപത്യം ഉറപ്പിക്കാൻ കഴിഞ്ഞു.

രോഗപ്രതിരോധശേഷിയുള്ള 59  $BC_1F_1$  ലൈൻസുകളുടെ 9 രൂപശാസ്ത്രപരമായ പ്രതീകങ്ങളും ഗുണമേന്മ നിർണയിക്കുന്ന പ്രതീകങ്ങളും ഗണ്യമായി വ്യതിയാനം കാണിക്കുന്നതായി വിലയിരുത്തി. ബയോമെട്രിക് പ്രതീകങ്ങളുടെ പ്രോക്സിമിറ്റി ഡിസിമിലാരിറ്റി മാട്രിക്സ് അടിസ്ഥാനപ്പെടുത്തിയുള്ള യൂക്ളിഡിയൻ അകലം പരിശോധിച്ചതിൽ നിന്നും, പ്രതിരോധശേഷിയുള്ള 59  $BC_1F_1$  ലൈൻസുകളിൽ 25 എണ്ണം റെക്കറന്റ് പാരന്റിനോട് വളരെ അടുത്ത് നിൽക്കുന്നതായി കണ്ടെത്തി. അവയെ  $BC_2F_1$  തലമുറയെ വികസിപ്പിക്കുന്നതിനായി ഉപയോഗിച്ചു.

രോഗപ്രതിരോധശേഷിയുള്ള 25  $BC_1F_1$  ലൈൻസുകളിൽ നിന്നും ഉൽപ്പാദിപ്പിച്ച 128  $BC_2F_1$  തലമുറയെ തെരച്ചെടികളായി ഫ്യൂസേരിയം വാട്ടരോഗത്തിന്റെ 0-4 സ്കെയിൽ ഉപയോഗിച്ചുള്ള സ്കോറിങ് പ്രക്രിയക്ക് വിധേയമാക്കി. ഇതിൽ നിന്നും 35

രോഗപ്രതിരോധശേഷിയുള്ളത്,19 മിതമായ  
 രോഗപ്രതിരോധശേഷിയുള്ളത്, 21 മിതമായ  
 രോഗബാധിതമാകുന്നത്,12 രോഗപ്രതിരോധശേഷിയില്ലാത്തത്,41  
 ഒട്ടും രോഗപ്രതിരോധശേഷിയില്ലാത്തത് എന്നിങ്ങനെ  
 രേഖപ്പെടുത്തി. രോഗപ്രതിരോധശേഷി കുടിയവയായി ലഭിച്ച 35  
 $BC_2F_1$  സസ്യങ്ങളെ തുടർമൂല്യനിർണ്ണയത്തിനും  
 കണ്ടുപിടുത്തങ്ങൾക്കുമായി ഉപയോഗപ്പെടുത്താവുന്നതാണ്.

പയറിലുണ്ടാകുന്ന വാട്ടാരോഗത്തിന്റെ രോഗകാരിയായ  
 ഫ്യൂസേരിയം ഓക്ലിസ്പോറം എന്ന സൂക്ഷ്മാണുവിനെ ഏകീകരിച്ച്  
 തിരിച്ചറിഞ്ഞ ശേഷം NCBI ഡാറ്റാ ബേസിൽ നിക്ഷേപിക്കാൻ  
 സാധിച്ചു. ഈ പഠന പദ്ധതി വഴി വാട്ടാരോഗത്തിന്റെ  
 രോഗകാരിയായ ഫ്യൂസേരിയത്തിന്റെ വംശവർദ്ധനവിന് മണലും  
 ചോളവും കലർത്തിയ മാധ്യമമാണ് ഏറ്റവും അനുയോജ്യമെന്ന്  
 തിരിച്ചറിഞ്ഞു. അതുപോലെ പയറിന്റെ ഫ്യൂസേരിയം  
 പ്രതിരോധശേഷി നിർണ്ണയിക്കുന്ന സ്ക്രീനിംഗിനായുള്ള  
 രോഗാംശനിവേശനം നടത്താനാവശ്യമായ രോഗാംശത്തിന്റെ  
 ഒപ്പിമൽ അളവ് 10 ശതമാനമായി മാനദണ്ഡപ്പെടുത്തുവാൻ കഴിഞ്ഞു.  
 കടലയിൽ സംവഹനശേഷിയുള്ള UASBC59 എസ് എസ് ആർ  
 മാർക്കറുകൾ പയറിലെ ഫ്യൂസേരിയം വാട്ടാരോഗത്തിന്റെ  
 പ്രതിരോധ ശേഷിയുമായി ബന്ധപ്പെട്ട എസ് എസ് ആർ  
 മാർക്കറായും ഉപയോഗപ്പെടുത്തുവാൻ കഴിയുമെന്ന് ഗവേഷണ  
 പദ്ധതിയിലൂടെ തെളിഞ്ഞു. പഠനത്തിന് ഭാഗമായി ഫ്യൂസേരിയം  
 വാട്ടത്തിനെതിരെ പ്രതിരോധശേഷിയുള്ളതായി കണ്ടെത്തിയ 35  
 $BC_2F_1$  തലമുറയെ ഗീതികയുടെ വാണിജ്യ ഗുണങ്ങളും IRON ന്റെ  
 രോഗപ്രതിരോധശേഷിയും അടങ്ങിയ മികവുറ്റ ഇനങ്ങൾ  
 വികസിപ്പിച്ചെടുക്കാൻ ഉപയോഗിക്കാമെന്നും കണ്ടെത്തി.