

## Research Article

# Screening for chickpea germplasm resistant to *Fusarium* wilt disease under natural conditions of infection

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**Abstract:** Among the best ways to control chickpea wilt disease caused by *Fusarium oxysporum* f.sp. *ciceris* (Padwick) is the use of resistant genotypes. Accordingly, the resistance of forty-one different chickpea genotypes was evaluated, over two growing seasons, under natural field infection conditions. Follow-up experiments revealed that most chickpea genotypes exhibited typical yellowing and wilting symptoms associated with wilt disease. Quantifying disease incidence at different stages revealed considerable variation among chickpea genotypes ranging from 28.13% to 66.15%. Among the genotypes tested, five can be qualified as resistant and sixteen genotypes moderately resistant, while eighteen were susceptible and only two can be considered very susceptible to *Fusarium* wilt. The results show that disease severity increases over time, correlated with disease incidence, and vice versa. Furthermore, grain yield was negatively affected by disease incidence; however, the disease did not affect the hundred-grain weight. The genotypes characterized by resistance to wilt and combined with productive performance can be used as such or integrated into breeding programs to develop *Fusarium* wilt-resistant varieties.

**Keywords:** chickpea, *Cicer arietinum*, *Fusarium oxysporum*, genotype resistance

## Introduction

Chickpea *Cicer arietinum* L. is the second grain legume cultivated in the world, with a total cultivated area of 14.5 million hectares (FAO, 2017) and ranked third among the pulse crops; and accounts for 11.67 million tons annually (Merga *et al.*, 2019). It is mainly used for human consumption and is an essential constituent of the Mediterranean diet.

Chickpea is a good and cheap source of protein, and for this reason, this crop is cultivated on five continents.

One of the major biotic stresses limiting chickpea production is its susceptibility to fungal diseases. However, upon control of this disease, there has only been a marginal increase in chickpea productivity. This is mainly attributed to various biotic (e. g., *Ascochyta* blight, *Fusarium* wilt, and pod borer) and abiotic (e. g., drought, salinity, heat, etc.) stresses. Reducing the losses due to these stresses is important to enhance crop production (Tarafdar *et al.*, 2017; Caballo *et al.*, 2019). Among the biotic stresses, *Fusarium* wilt is a

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devastating fungal disease posing adverse effects on chickpea productivity in most chickpea-growing countries of the world.

*Fusarium* wilt has become a major threat to chickpea production, and the yield losses range from 10-90%, depending upon the severity of the disease and climatic conditions (Kumar *et al.*, 2012; Patil *et al.*, 2015; Sunkad *et al.*, 2019). The disease was first reported in India by Butler in 1918, but its etiology was not correctly determined until 1940 by Padwick. It is widespread in most chickpea-growing areas in Asia, Africa, Southern Europe, and the Americas, but it has not yet been reported in Australia (Cunnington *et al.*, 2007).

*Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is soilborne and root inhabiting in nature which may survive in the soil for a long period (up to six years) (Chen *et al.*, 2011; Muhammad *et al.*, 2020). Since the disease is soilborne, its management through either crop rotation or the application of fungicides is difficult (Sharma *et al.*, 2017), and no single control measure is fully effective (Landa *et al.*, 2004). Incorporating diverse resistance sources is a more effective, economical, and eco-friendly strategy for managing chickpea wilt (Govil and Rana, 1994). Consequently, considerable efforts have been made to identify resistance sources against wilt worldwide (Sharma *et al.* 2012; Saabale *et al.*, 2017), and several are being utilized in breeding programs. Numerous sources of resistance to *Fusarium* wilt in chickpea have been identified previously (Mirzapour *et al.*, 2014; Chobe *et al.*, 2016) and have contributed to the substantial increase of chickpea productivity in semi-arid regions of Africa and Asia (Upasani *et al.*, 2017; Fikre *et al.*, 2018).

Breeding efforts have significantly reduced the *Fusarium* wilt effect on the chickpea crop (Jendoubi *et al.*, 2017). The use of resistant cultivars has been widely recognized as the most effective method for soilborne disease control (Panth *et al.*, 2020). However, the performance of varieties differs from place to place owing to the existence of physiological races among the Foc isolates (Sharma *et al.*, 2014). As chickpea is grown in diverse agroecological zones and

environments, these stable/durable sources can be used in future resistance breeding programs to develop *Fusarium* wilt-resistant cultivars (Sharma *et al.*, 2019).

Therefore, the present study was designed to evaluate forty-one chickpea genotypes (*Cicer arietinum* L.) against *Fusarium* wilt disease during two cropping seasons under natural infection conditions at the Technical Institute for Field Crops (ITGC) Setif, Algeria, for further utilization.

## Materials and Methods

### Experimental materials and site

The plant material consisted of forty-one genotypes of chickpeas selected by the International Center for Agricultural Research in the Dry Areas (ICARDA), including ILC482 variety as susceptible check, which was evaluated for *Fusarium* wilt of chickpea in the field under natural conditions of infection (Table 1).

The trial was conducted at the Experimental Station of the Technical Institute for Field Crops (ITGC) on a plot historically known for infection by *Fusarium oxysporum* FOC (Debbi, 2010; Abed *et al.*, 2016).

### Experimental design and treatments

The field experiment was arranged in a randomized complete block design (RCBD) with four replications. Each elementary plot was made up of a line 2 meters long on which 10 chickpea seeds were sown. The inter-row, intra-row, and block spacings were 0.4m, 0.2m, and 1m, respectively. The experiments were carried out on durum wheat precedent during the 2017/2018 crop year and following a pea-triticale forage association during the 2018/2019 crop year. The field was plowed deeply with a share-plow, followed by crossing with a cover crop and passing with a rotary harrow. Phosphorous fertilizer (M. A. P: 0.052) was incorporated during the preparation of the seedbed at the rate of 52 kg of Phosphoric anhydride (P<sub>2</sub>O<sub>5</sub>) per hectare. Manual weeding was done three times at the seedling, flowering, and podding stages.

**Table 1** Chickpea genotypes evaluated for their varietal behaviour towards the *Fusarium* wilt disease.

N	Genotypes	Pedigree
1	FLIP10-350 C	X04TH103/X03TH-171XS01132
2	FLIP10-354 C	X04TH122/FLIP97-165XPusa 1053
3	FLIP10-357 C	X04TH131/FLIP95-68XFLIP97-83
4	FLIP10-358 C	X04TH133/FLIP97-91XFLIP98-15
5	FLIP10-368 C	X04TH161/S01250XFLIP98-233
6	FLIP10-376 C	X04TH178/FLIP97-91XFLIP98-137
7	FLIP10-380 C	X04TH184/FLIP97-91XFLIP95-51
8	FLIP10-382 C	X04TH188/ICC 12004XFLIP99-48
9	FLIP11-23 C	X04TH60/X03TH-60XFLIP96-154
10	FLIP11-24 C	X04TH60/X03TH-60XFLIP96-154
11	FLIP11-35 C	X04TH65/X03TH-133XFLIP96-154
12	FLIP11-37 C	X04TH65/X03TH-133XFLIP96-154
13	FLIP11-48 C	X04TH69/X03TH-137XFLIP96-154
14	FLIP11-49 C	X04TH71/X03TH-139XFLIP99-34
15	FLIP11-52 C	X04TH73/X03TH-141XFLIP96-154
16	FLIP11-68 C	X04TH76/X03TH-144XFLIP97-116
17	FLIP11-69 C	X04TH76/X03TH-144XFLIP97-116
18	FLIP11-77 C	X04TH79/X03TH-147XFLIP96-154
19	FLIP11-82 C	X04TH79/X03TH-147XFLIP96-154
20	FLIP11-83 C	X04TH80/X03TH-148XS01076
21	FLIP11-90 C	X04TH96/X03TH-164XS01105
22	FLIP11-115 C	X04TH129/FLIP98-233XFLIP99-48
23	FLIP11-116 C	X04TH129/FLIP98-233XFLIP99-48
24	FLIP11-121 C	X04TH132/FLIP97-90XFLIP97-126
25	FLIP11-122 C	X04TH133/FLIP97-91XFLIP98-15
26	FLIP11-123 C	X04TH133/FLIP97-91XFLIP98-15
27	FLIP11-124 C	X04TH133/FLIP97-91XFLIP98-15
28	FLIP11-142 C	X04TH137/FLIP98-137XFLIP98-15
29	FLIP11-143 C	X04TH137/FLIP98-137XFLIP98-15
30	FLIP11-144 C	X04TH142/FLIP00-4XFLIP97-165
31	FLIP11-149 C	X04TH147/FLIP00-17XFLIP98-230
32	FLIP11-150 C	X04TH148/S00541XFLIP98-232
33	FLIP11-152 C	X04TH151/S01020XFLIP95-68
34	FLIP11-159 C	X04TH182/FLIP98-137XFLIP97-229
35	FLIP11-172 C	X05TH106/FLIP97-131XFLIP00-14
36	FLIP11-176 C	X05TH132/FLIP97-185XFLIP00-14
37	FLIP11-186 C	X06TH113/FLIP03-138XFLIP03-80
38	FLIP11-204 C	X05TH106/FLIP97-131XFLIP00-14
39	FLIP11-223 C	X04TH136/FLIP97-229XFLIP97-126
40	FLIP11-227 C	X05TH141/FLIP97-85XSel03TH10089
41	ILC482	Susceptible control genotype

**Disease data scoring****Disease incidence of *Fusarium* (DI)**

The DI is expressed as the proportion of plants showing wilt symptoms out of the total plants per plot (Trapero-Casas, 1983). Plants in each row were examined, and the number of plants showing symptoms of yellowing or wilting was noted.

$$\text{Disease Incidence (\%)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

Genotypes were grouped in classes regarding the scale for disease incidence: 0-10%: Highly resistant; 11-20%: Resistant; 21-30%: Moderately resistant; 31-50%: Susceptible; 51-100%: Highly susceptible (Iqbal *et al.*, 1993).

**Disease severity of *Fusarium* (S)**

The ratings of the severity of the attacks were noted 20, 40, and 60 days after sowing based on ten randomly selected plants in a field experiment. The severity of the disease was assessed using a 5-degree rating scale (0 to 4) (0: 0% of yellowed or withered leaves; 1: 1-33% of yellowed or withered leaves; 2: 34-66% of yellowed or withered leaves; 3: 67-100% yellowed or withered leaves; 4: 100% Dead plants). Each degree corresponds to a percentage of leaves showing symptoms of yellowing or wilting (Trapero-Casas and Jiménez-Díaz, 1985; Navas-Cortés *et al.*, 2000). To estimate the severity of the disease, the average index of severity (ISM) was calculated for each plot.

$$\text{Index of Severity Mean (ISM)} = \frac{\sum nj \times ij}{\sum nj}$$

Where n = number of plants characterized by the index i of the severity of disease attributed to plants. ISM was grouped into classes: 0 < ISM < 1: mild disease; 1 < ISM < 2: moderately severe; 2 < ISM < 3: serious disease; 3 < ISM < 4: very serious disease (El-Aoufir, 2001).

**Disease intensity index (DII)**

Disease Intensity Index (DII) is very important to give the relationship between DI and ISM. It is calculated as the percentage of disease incidence X severity index/index maximum severity scale (Luo *et al.*, 2000).

$$\text{Disease Intensity Index (DII)} = \frac{DI \times ISM}{4}$$

Where: **DI**: percentage of disease incidence and **ISM**: Index of severity mean

### Agronomic parameters and analysis

The data for agronomic traits were taken following the standard practice for the field chickpea trial used. Each elementary plot was subjected to several measurements of agronomic parameters, including the emergence rate (number of plants raised out of the total number of seeds sown), the number of ramifications (carried out randomly on five plants in the middle of each line), the flowering period (Number of days to reach 50% of flowers per line), the number of pods per plant (The number of pods harvested divided by the total number of plants), the weight of 100 grains (weight of 100 grains harvested from each line) and the grain yield (the seeds harvested from each elementary plot were weighed and the yield was given in kg/ha for statistical analysis).

### Isolation and identification of the pathogen

Infected chickpea roots showing disease symptoms were sampled from the experimental field for further analysis in the laboratory. The roots were cut into small sections (0.5 cm), washed thoroughly with tap water, and sterilized in 5% sodium hypochlorite solution for 5 minutes. Then sections were rinsed three times in sterilized distilled water and dried on sterilized filter papers. Potato dextrose agar was added to five Petri dishes having 9 cm diameter. Then sterilized root sections were plated at the rate of five sections per Petri plate and incubated at 25 °C for 7 days (Ekhlasi *et al.*, 2016). Seven-day-old cultured colonies were subcultured to new Petri plates. The resulting colonies were observed through a light microscope and compared with the morphological characteristics of *Fusarium oxysporum* described by Van der Maesen (1987).

### Statistical analysis

The disease incidence and severity, phenological and yield parameters data were analyzed statistically at 5% probability level with analysis

of variance (ANOVA) using the SPSS 25 software (SPSS, 2017). Principal component analysis (PCA) was developed to define the weight contribution of each trait and to evaluate the total level of genetic diversity. Correlation analysis was done to know the association of disease incidence with severity and phenology with yield-related parameters.

## Results

### Pathological parameters

#### Incidence of *Fusarium* wilt

The field chickpea genotypes screened against *Fusarium* wilt disease caused by *F. oxysporum* f. sp. *ciceris* resulted in wilting and yellowing symptoms (Fig. 1). The estimation of disease incidence from observed symptoms on the screened chickpea genotypes varied considerably and revealed a highly significant difference at ( $p < 0.01$ ) (Table 2).

The results show that the highest percentage of disease incidence was recorded on Flip 11-152C at a rate of 55.50%. In comparison, the lowest rate (14.06%) was recorded on the Flip 11-149C genotype (Table 3, Fig. 2). The overall average percentage disease incidence of the chickpea genotypes was 32.19. No genotype was spared from the disease.

Based on field results and the scale established by Iqbal *et al.* (1993), relating to the resistance of chickpea genotypes, the 41 genotypes tested can be classified into 5 resistant genotypes (Flip 11-149 C, Flip 10-354 C, Flip 11-144 C, Flip 11-143 C and Flip 11-172 C) with disease incidence ranging from 14.06% to 19.38%; 16 moderately resistant genotypes (Flip 10-380 C, Flip 11-23 C, Flip 11-176 C, Flip 10-350 C, Flip 11-52 C, Flip 11-142 C, Flip 11-35 C, Flip 11-223 C, Flip 10-358 C, Flip 11-124 C, Flip 11-37 C, Flip 11-123 C, Flip 11-24 C, Flip 11-227 C, Flip 10-368 C, Flip 11-69 C) with disease incidence ranging from 20.88% to 29.63%; 18 susceptible genotypes (Flip 10-382 C, Flip 11-68 C, Flip 11-150 C, Flip 11-159 C, Flip 11-204 C, Flip 11-122 C, Flip 10-376 C, Flip 11-186 C, Flip 11-83 C, Flip 11-

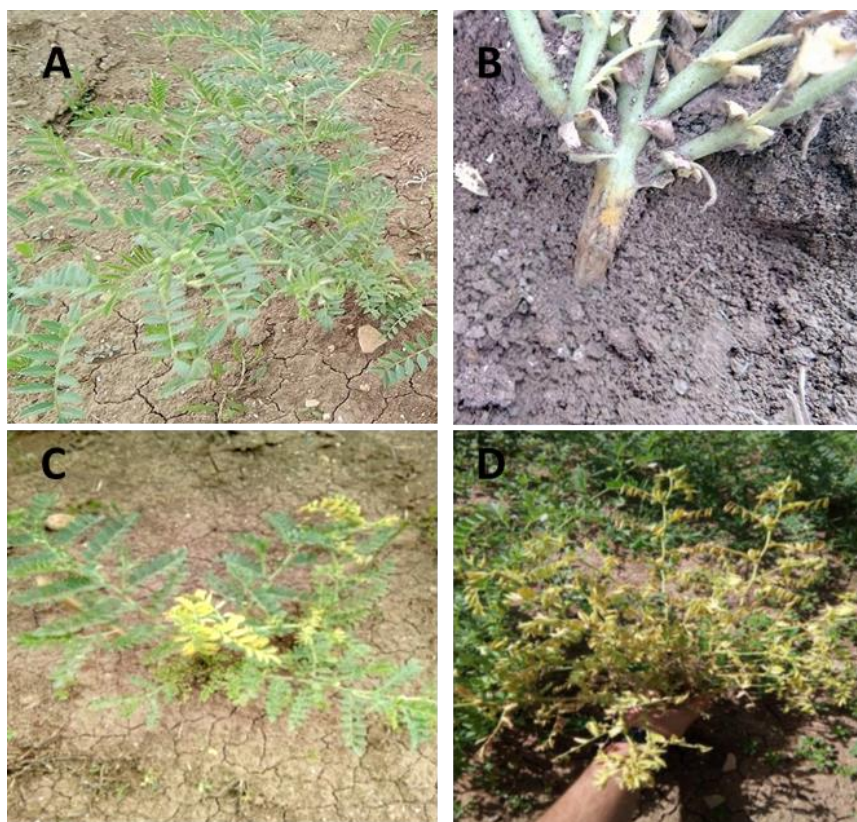
49 C, Flip 11-116 C, Flip 11-77 C, ILC482, Flip 11-90 C, Flip 10-357 C, Flip 11-48 C, Flip 11-121 C, Flip 11-115 C) with DI range from 31.12% to 49.69% and finally only 2 very sensitive genotypes (Flip 11-82 C and Flip 11-152 C) with the incidence of 52.89% to 55.5% (Table 3).

### Severity of *Fusarium* wilt

The results revealed that severity increased over time, and plants that were infected and showed symptoms deteriorated their sanitary state, and sometimes death ensued. The levels of disease severity varied between every two consecutive periods of recording, translated statistically by a highly significant difference at ( $p < 0.01$ ) (Table 2). The percentage of disease severity of chickpea genotypes ranged from 4.00 as the mean of the highest disease severity recorded for the Flip 11-121C genotype to 1.35 as the lowest severity

recorded for the Flip 11-172C genotype (Table 3).

Regarding the ISM and according to the scale of El-Aoufir (2001), we can classify the disease as moderately severe on 8 genotypes (Flip 11-172 C, Flip 11-176 C, Flip 11-124 C, Flip 11-149 C, Flip 11-142 C, Flip 10-354 C, Flip 11-23 C, Flip 10-380 C), as serious disease on 22 genotypes (Flip 11-52C, Flip 10-368C, Flip 11-24 C, Flip 10-358 C, Flip 11-144 C, Flip 11-143 C, Flip 10-382 C, Flip 11-186 C, Flip 11-69 C, Flip 10-357 C, Flip 11-150 C, Flip 11-227 C, Flip 10-350 C, Flip 11-123 C, Flip 11-204 C, Flip 11-223 C, Flip 11-49 C, Flip 11-68 C, Flip 10-376 C, Flip 11-77 C, Flip 11-35 C and Flip 11-159 C) and very serious disease on 11 genotypes (Flip 11-37 C, Flip 11-122 C, Flip 11-83 C, Flip 11-48 C, Flip 11-90 C, Flip 11-116 C, Flip 11-115 C, Flip 11-152 C, ILC482, Flip 11-82 C and Flip 11-121 C) (Table 3).



**Figure 1** Symptoms observed with *Fusarium* wilt disease on chickpeas plants. A: Healthy plant; B: Collar necrosis; C: Yellowing plant; D: Wilting plant.

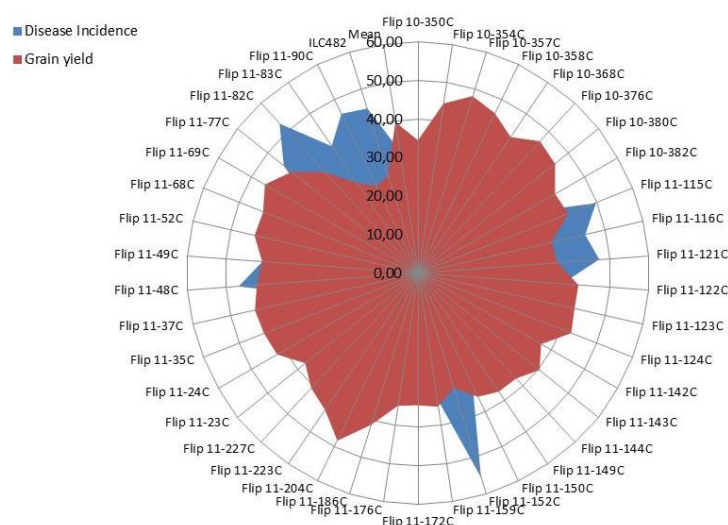
**Table 2** Analysis of variance of disease parameters according to chickpeas genotypes.

Source of variability	Sum of squares (Type III)	df	Mean squares	F	Significance
Disease incidence	39617.411	40	990.435	3.973	0.000
Index of severity	157.455	40	3.936	5.492	0.000
Disease index	61255.739	40	1531.393	5.071	0.000

**Table 3** Results of growth yield and *Fusarium* wilt disease parameters according to chickpeas genotypes.

Genotype	Emergence rate	Branching	Flowering	Height	Number of pods	Hundred-grain weight	Grain yield (q)	Disease incidence	Index of severity	Disease index
Flip 10-350 C	81.25	3.13	132	57.03	57.5	25.59	3.45	21.31	2.53	13.49
Flip 10-354 C	91.25	4.13	140	51.83	77.88	28.64	4.45	15.63	1.85	7.41
Flip 10-357 C	95	3.25	137.5	57.24	71.44	27.37	4.81	46.65	2.43	35.08
Flip 10-358 C	78.75	3.38	136.5	58.62	57.81	29	4.61	25.85	2.2	15.59
Flip 10-368 C	88.75	3.75	135.5	65.79	76	31.89	4.27	28.23	2.07	15.81
Flip 10-376 C	81.25	3.5	134.5	64.45	64	29.63	4.66	38.11	2.72	27.84
Flip 10-380 C	96.25	3.13	137	68.36	81.69	27.79	4.55	20.88	1.91	10.9
Flip 10-382 C	91.25	3.5	138.5	64.89	83.94	32.61	4.11	31.12	2.37	23.04
Flip 11-115 C	86.25	3.38	138	61.7	59.06	26.38	4.2	49.69	3.71	46.93
Flip 11-116 C	76.25	3.25	134	58.9	63.38	22.52	3.56	44.58	3.61	39.45
Flip 11-121 C	82.5	3.63	135.5	60.47	63.5	27.17	3.64	47.22	4	46.04
Flip 11-122 C	73.75	3.38	136	56.95	61.38	29.03	4.18	37.56	3.13	32.56
Flip 11-123 C	90	4.38	132.5	58.59	72.56	30.27	4.17	26.52	2.59	18.21
Flip 11-124 C	97.5	3.5	134.5	62.23	53.31	24.38	4.28	26.4	1.55	10.11
Flip 11-142 C	86.25	4.25	138.5	58.08	72.69	18.64	3.7	23.69	1.68	9.61
Flip 11-143 C	91.25	3.5	133	51.4	57	18.64	4.04	19.1	2.28	10.94
Flip 11-144 C	83.75	3.63	135.5	61.16	64.38	22.04	3.74	16.88	2.26	9.51
Flip 11-149 C	90	3.63	134	58.68	90.25	26.53	3.73	14.06	1.64	5.83
Flip 11-150 C	96.25	3.38	133	59.77	80.19	23	3.57	32.75	2.45	24.34
Flip 11-152 C	80	3.38	132	50.27	40.38	20.45	3.12	55.5	3.75	53.46
Flip 11-159 C	87.5	3.5	137.5	59.01	65.31	20.29	3.51	32.76	2.87	30.94
Flip 11-172 C	86.25	3.5	137.5	61.48	72.19	26.06	3.44	19.38	1.35	6.26
Flip 11-176 C	92.5	3.5	137.5	51.93	76.5	27.04	3.49	21.31	1.54	8.4
Flip 11-186 C	92.5	3.88	137	63.49	80.5	23.4	4.12	38.97	2.37	27.42
Flip 11-204 C	95	3.88	137.5	58.47	57.5	27.44	4.83	34.4	2.6	22.23
Flip 11-223 C	88.75	3.88	137	55.02	74.44	29.24	4.3	25.63	2.67	17.79
Flip 11-227 C	83.75	3.5	138.5	60.12	55.38	29.06	4.08	27.11	2.48	15.35
Flip 11-23 C	81.25	3.25	138	50.41	60.5	30.01	3.75	21.06	1.87	9.37
Flip 11-24 C	80	3.5	130.5	57.67	79.13	28.44	4.23	26.96	2.14	14.53
Flip 11-35 C	81.25	3.88	135	57.14	74.31	29.8	4.3	25.09	2.87	18.12
Flip 11-37 C	76.25	3.38	136.5	58.27	76.13	29.3	4.35	26.44	3.02	19.33
Flip 11-48 C	81.25	3.75	134	51.72	61.25	26.99	4.2	46.77	3.48	45.73
Flip 11-49 C	83.75	4.25	136.5	66.18	72.63	24.67	4.06	40.49	2.7	27.38
Flip 11-52 C	85	3.88	137.5	61.65	85.19	24.35	4.36	22.83	2.05	12.49
Flip 11-68 C	76.25	3.38	138	50.14	63	28.3	4.33	31.13	2.71	23.38
Flip 11-69 C	93.75	3.75	132.5	55.41	83.88	23.95	4.6	29.63	2.38	21.02
Flip 11-77 C	90	3.5	134.5	52.36	62.44	18.64	4.21	44.61	2.74	40.15
Flip 11-82 C	78.75	3.88	137	53.26	64.13	22.59	3.57	52.89	3.84	50.6
Flip 11-83 C	86.25	3.75	134.5	51.75	61.13	28.06	2.83	39.86	3.2	31.66
Flip 11-90 C	85	3.63	136	54.73	60.88	29.39	2.51	45.9	3.57	43.81
ILC482	82.5	4.13	134.5	61.97	64.69	23.6	2.65	44.7	3.75	42.65





**Figure 2** Mean of Disease Incidence and grains yield according to chickpeas genotypes.

The different situations observed in the severity of the disease ranging from moderately severe to severe disease, do not depend solely on the behaviour of the genotype. Still, other parameters can play a significant role in its progression. We can cite the density of the primary inoculum of the pathogen in the soil, the climatic conditions, and the chickpea cultivar susceptibility.

### Disease intensity index (DII)

The result revealed a highly significant difference at ( $p < 0.01$ ) regarding the disease intensity index among the genotypes tested. The overall mean percentage of the disease index of chickpea genotypes was (24.02%). Thus, the highest disease index (53.46%) was recorded for Flip 11-152C genotype, and the lowest severity (5.83%) was recorded on Flip 11-149C genotype with a general DI means of the experiment equal to 32.64% (Table 3).

Since the two previous parameters studied, the incidence and disease severity, are positively correlated with the majority of genotypes, the Disease Intensity index will mathematically follow the same trend. The results show whether the incidence of the disease increases, the severity index increases, and vice versa, which results in a gradual rise in the disease index (Table 3). In a previous study, Debbi (2010)

confirmed the existence of a single physiological race of *F. oxysporum* f.sp. *ciceris* in the experimental plot, and the behavior of genotypes seems to be much more linked to intrinsic characteristics and other environmental parameters than to the pathogen severity.

### Agronomical parameters

The result of the range of parameters suggested that there were considerable differences observed in all of the traits under investigation and especially for emergence rate (ER), Number of ramifications (Ram/Pl), Height of plant (PH), Number of pods per plant (Pods/Pl), Weight of 100 grains (WHG) and Grain yield (GrY). However, the number of days to reach 50% of flowering (FP) revealed an insignificant effect between the genotypes studied at the level of 5% (Table 4).

The highest grain yield was produced by the most resistant genotype Flip 11-204 C (4.83 qx/ha), and the lowest by the highly susceptible genotype Flip 11-90 C (2.51 qx/ha). Regarding the Hundred grain weight, the highest HGW was produced by Flip 10-382 C (32,61 g), moderately susceptible, and the lowest by the most resistant genotype Flip 11-143 C (18,64 g) (Table 3, Figure 2).

All the traits were subjected to principal component analysis (PCA) for estimation of the

weight contribution of each trait and evaluation of the total level of genetic diversity. Three components gave Eigenvalues > 1.0. Thus, they were important in considering genetic variability amongst all the genotypes. Three components (PC1-PC3) contributed 66.96% genetic variability (Table 5). The importance of this technique has been reported for selecting field chickpea lines of high yield and *Fusarium* wilt disease and explained 60% of genetic variability by this technique.

The PC1 explained 35.86% of the total variability. All parameters of the disease development are linked to it: the disease index, the disease severity, and the disease incidence, and they are negatively highly correlated with grain yield and the number of pods. The PC2 explained 18.36% of the total contribution toward variability. The PC3 contributed 12.75% of the variability. All vegetative parameters of plant development are linked: emergence rate, branching, height, and hundred-grain weight, and

it is negatively correlated to genotype. It is linked to genotype and the number of pod parameters and negatively correlates with grain yield.

Disease incidence is positively correlated with genotypes tested and time to reach 50% flowering and negatively correlated with the number of pods per plant and grain yield. Genotypes which flower early are more exposed to attacks by *Fusarium oxysporum*. Conversely, the incidence of the disease is negatively correlated with the number of pods per plant, which results in a drop in yield. Moreover, the study of Pearson Correlations reveals that the severity index is positively correlated with the incidence of the disease. In other words, the higher the incidence, the more aggressively the affected plants are attacked by the pathogen. In addition, the disease index is negatively correlated with the number of pods per plant and grain yield. Thus, the highest severity rates negatively affected the pods quantitatively and qualitatively (Table 6).

**Table 4** Analysis of variance of chickpea genotypes growth and yield at harvest.

Source of variability	Sum of squares (Type III)	df	Mean squares	F	Significance
Emergence rate	12512.805	40	312.820	1.559	0.022
Branching of plant	30.372	40	0.759	2.321	0.000
Flowering period	1536.488	40	38.412	1.277	0.132
Height of plant	7311.724	40	182.793	4.231	0.000
Number of pods	35607.378	40	890.184	6.559	0.000
Hundred grain weight	4252.705	40	106.318	5.140	0.000
Grain yield	113.023	40	2.826	3.812	0.000

**Table 5** Principal Component Analysis (PCA) of traits among chickpea genotypes. Eigenvalues, percentages, and variability explained by the first three components.

Parameter	PC1	PC2	PC3
Genotype	0.280	-0.505**	0.609**
Height	-0.189	0.649**	0.255
Number of pods	-0.523*	0.175	0.561**
Hundred grain weight	-0.083	0.577**	0.361*
Grain yield	-0.204	0.543**	-0.364*
Disease incidence	0.871**	0.293*	0.073
Index of severity	0.875**	0.068	0.020
Disease index	0.953**	0.209	0.049
Eigen value	3.585	1.835	1.275
Percentage variability	35.855	18.355	12.753
Cumulative variability	35.855	54.209	66.962



**Table 6** The Pearson correlations recorded between agronomical and pathological parameters.

Parameters	Genotype	Emergence rate	Flowering	Branching	Height	Number of pods	Hundred-grain weight	Grain yield	Disease Incidence	Index of severity	Disease index
Genotype	1										
Emergence rate	-0.077	1									
Flowering	-0.027	0.265**	1								
Branching	0.150**	0.127*	0.214**	1							
Height	-0.210**	0.247**	0.276**	0.104	1						
Number of pods	0.002	0.172**	0.122*	0.181**	0.203**	1					
Hundred-grain weight	-0.110*	0.083	0.197**	0.007	0.189**	0.124*	1				
Grain yield	-0.228**	0.036	0.177**	-0.02	0.125*	0.113*	0.123*	1			
Disease incidence	0.140*	-0.042	0.276**	0.08	0.025	-0.291**	0.024	-0.018	1		
Index of severity	0.177**	-0.265**	-0.09	0.041	-0.127*	-0.380**	-0.007	-0.151**	0.629**	1	
Disease index	0.159**	-0.111*	0.152**	0.059	-0.062	-0.336**	-0.015	-0.08	0.933**	0.821**	1

In conclusion, the productive performances of the genotypes are weakened by the conjugation of the impact of the incidence with the severity of the disease.

## Discussion

The success of any breeding program depends on the stable performance of any traits within the genotypes. The selection of landraces is mainly based on the commercial characteristics of the grain, while disease resistance is not often taken into account (Zaccardelli *et al.*, 2012). Deploying *Fusarium* wilt-resistant chickpea cultivars is one of the sustainable strategies breeders adopt as part of integrated disease management.

The present study for the screening of chickpea germplasm resistant to *Fusarium* wilt disease under natural conditions of infection in the field revealed that the disease incidence on 41 chickpea genotypes varies considerably from 14.05% to 55.50%. Similar results were recorded by Ayana *et al.* (2019), where disease incidence ranged between 27 to 73% from the Desi-type chickpea. Mirzapour *et al.* (2014) evaluated 18 genotypes/cultivars against chickpea wilt and observed disease incidence of 0% - 46.6% at the seedling stage, and it varied from 0-100% at the reproductive stage. Benzohra-Belaidi (2016) screened 13 chickpea genotype accessions to evaluate the resistance to two races of *F. oxysporum* f. sp. *ciceris*, the causal pathogen of chickpea wilt and reported that 3 chickpea

genotypes (Flip 4107, Kadri and Flip 97-555) had an important resistance and other 10 genotypes (PPC25, Bouazza, INRAA199, P505, Col15-24, Col15-07, ILC1929, ILC482, Flip9393, Flip3701c) were susceptible to *Fusarium* wilt. Thaware *et al.* (2017) observed that all 50 chickpea entries exhibited different reactions against *F. oxysporum* f. sp. *ciceris*. However, six test entries were found highly resistant, thirty-one were resistant, eight were moderately resistant, two were moderately susceptible, and three were highly susceptible, which matched our findings.

The disease severity of *Fusarium* observed increases over time, the sanitary state of infected plants deteriorates over time, and sometimes death ensues. The disease severity recorded ranged from 4.00 for dead plants on the Flip 11-121 C genotype to 1.35 recorded with yellowed or withered leaves of the Flip 11-172 C genotype. The result of this study is in line with the findings of Maitlo *et al.* (2016) reported that the degree of disease severity of *Fusarium* wilt of chickpea increases from seedling to flowering stage, and the highest severity was recorded at the podding stage. Ayana *et al.* (2019) noted that in the fourth week, the highest severity (50.38%) was recorded for the variety Dube and the lowest severity (35.91%) for Fetenech.

Regarding the results of disease incidence, we found that five genotypes can be classified as resistant; 16 genotypes were moderately resistant, 18 were susceptible, and only two genotypes were highly susceptible. The resistance source of

*Fusarium* wilt in chickpea germplasm is not uncommon, and several other workers have also reported the occurrence against a high level of resistance of *Fusarium* wilt (Tariq Mahmud, 2015; Shivalingappa *et al.*, 2018). Zewdie and Bedasa (2019) revealed that considerable variations were recorded for resistance in desi and Kabuli chickpea against *Fusarium* wilt diseases. Kabuli germplasm proved to be a better resistance source than the desi material.

Parasappa *et al.* (2017) observed a high level of resistance in advanced genotypes compared to landraces. Our results are also consistent with those reported by Nazir *et al.* (2012) screened 178 chickpea lines against *Fusarium* wilt and observed that none of the test lines was immune. However, the number of resistance sources identified against wilt across the globe shows continuous changes in the genetic makeup of the pathogen, which warrants an ongoing search for host resistance.

Flowering time is an important trait in increasing the profitability of chickpea crops, and early flowering allows plants to escape biotic and abiotic stresses (Mallu *et al.*, 2014). Our results show a strong positive correlation between the incidence of the disease and the duration of the flowering period; the longer the period, the more the disease can take hold and progress. While a strong negative correlation was observed between the number of pods per plant and the severity index, and the incidence of the disease. While grain yield is only negatively correlated with severity index, indicating that the combination of these two pathological parameters affects performance and its components. Differences in disease severity among the other landraces studied here are unlikely to be associated with different degrees of genetic resistance to *Foc5*. Still, they are more likely from the effect of different responses to specific functions involved in wilt stress. These may include iron uptake, nutrients, and water deficiency responses (Blum, 2017).

Crop yields are often affected by adverse factors such as low seed germination rates and high disease incidence (Rossini, 2008). Since the most important environmental and agronomic factors affecting yields are the length of crop

cycles and growth patterns, it is essential to consider these in future breeding programs. The long cultural cycle with winter sowing is potentially more productive.

The disease-free, resistant lines and moderately resistant ones can be utilized in a resistance breeding program towards incorporating resistance genes in releasing cultivars or hybrids. Before such transfer of their resistance to a commercial cultivar, the genetic basis of resistance (vertical or horizontal) must be determined against the virulence of *F. oxysporum* f. sp. *ciceris*. Furthermore, the genotypes that showed resistance are most suitable for exploitation in breeding programs or direct sowing in wilt-prone areas.

## Conclusion

The results of a two-year experiment revealed the presence of large variability between the genotypes tested regarding the resistance against the vascular wilt disease of chickpeas and yield in grains, indicating significant potential for many genotypes. The genotypes Flip 10-354 C (15,63% and 44,53 q/ha), Flip 10-380 C (20,88% and 45,53 q/ha), Flip 11-143 C (19,10% and 40,38 q/ha), and Flip 11-52 C (22,83% and 43,59 q/ha) with an exciting grain yield greater than four qx/ha conjugated with resistance or moderate resistance to *Fusarium* wilt, should be assessed under a broader range of agro-climatic conditions in potential chickpea areas. Subsequently, it could be selected and gradually introduced into the chickpea production circuit. Concerning susceptible genotypes to the disease, such as Flip 11-152 C (55,50% and 31,2 q/ha) and Flip 11-77 C (44,61% and 42,1 q/ha), it could be used as check controls in future experiments while the resistant and low-yielding genotypes such as Flip 10-350 C (3,45% and 21,31 q/ha), it could be exploited for selection as direct sources of resistance and crossed with high-yielding but disease-susceptible genotypes.

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### Conflict of interests

The authors declare that they have no conflicting interests.

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## غربالگری ژرم پلاسما نخود مقاوم به بیماری پژمردگی فوزاریوم در شرایط طبیعی

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**چکیده:** ازجمله بهترین راه‌های کنترل بیماری پژمردگی نخود ناشی از *Fusarium oxysporum* f.sp. *ciceris* (Padwick) استفاده از ژنوتیپ‌های مقاوم است. براین اساس، مقاومت چهل و یک ژنوتیپ مختلف نخود در دو فصل رشد در شرایط آلودگی مزرعه مورد ارزیابی قرار گرفت. آزمایش‌های بعدی نشان داد که بیشتر ژنوتیپ‌های نخود علائم معمولی زردی و پژمردگی مرتبط با بیماری پژمردگی را نشان می‌دهند. کمی‌سازی بروز بیماری در مراحل مختلف، تنوع قابل‌توجهی را در بین ژنوتیپ‌های نخود از ۲۸/۱۳ تا ۶۶/۱۵ درصد نشان داد. از بین ژنوتیپ‌های مورد آزمایش، پنج ژنوتیپ مقاوم و شانزده ژنوتیپ نسبتاً مقاوم و هجده ژنوتیپ حساس بودند. اما تنها دو ژنوتیپ بسیار حساس به پژمردگی فوزاریوم مشاهده شد. نتایج نشان می‌دهد که شدت بیماری در طول زمان افزایش می‌یابد و با بروز بیماری هم‌بستگی دارد و بالعکس. علاوه بر این، عملکرد دانه به‌طور منفی با بروز بیماری تحت تأثیر قرار گرفت. با این‌حال، این بیماری بر وزن صد دانه تأثیری نداشت. ژنوتیپ‌هایی که با مقاومت در برابر پژمردگی و همراه با عملکرد تولیدی مشخص می‌شوند را می‌توان استفاده کرد یا در برنامه‌های اصلاحی برای توسعه واریته‌های مقاوم به پژمردگی فوزاریوم ادغام کرد.

**واژگان کلیدی:** نخود، *Cicer arietinum*، *Fusarium oxysporum*، مقاومت ژنوتیپ