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Screening Chickpea Genotypes against Fusarium Wilt Disease under Greenhouse Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Chickpea wilt, incited by *Fusarium oxysporum* f. sp. *ciceris*, stands as a significant vascular root disease that has economic implications. This disease can result in substantial yield losses, potentially up to 90%, during the various stages of crop growth. The study designed with the

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objective of screening chickpea genotypes against fusarium wilt. In the present investigation, 25 chickpea genotypes, including two control, WR315 (highly resistant) and JG 62 (highly susceptible), underwent screening. The screening involved artificially inoculating the pathogen responsible for Fusarium wilt under greenhouse conditions during the *rabi* seasons of 2021-22 and 2022-23. Of the genotypes screened, the genotype……(highly resistant), followed by genotype ……..and ……..In contrast the genotype …..most susceptible followed by …..and …… .

Keywords: Chickpea; Fusarium oxysporum f. sp. Ciceris; resistant, susceptible; genotypes; screening.

1. INTRODUCTION

Chickpea, also recognized by various names like gram, Bengal gram, Egyptian pea, garbanzo, or garbanzo bean is an annual, self-pollinating plant with a diploid genetic makeup (2n = 2x = 16) [1]. Its genome size is approximately 738 Mb [2]. Chickpea seeds are nutrition powerhouses, boasting high protein content and essential dietary minerals such as calcium, iron, and phosphorus. (Gupta *et al*., 2021). Furthermore, chickpea contributes to enhancing soil fertility through biological nitrogen fixation [3]. This legume holds a crucial position in the *rabi* (winter) pulse crop category on the Indian subcontinent, and its production significantly impacts the global pulse economy. Currently, approximately 15.004 million hectares of land are dedicated to chickpea cultivation, yielding a global production of 15.87 million metric tons per year, with a productivity rate of 1,057.8 kg/ha. Notably, India plays a dominant role in chickpea production, accounting for 73.78% (10.943 million hectares) of the world's total chickpea cultivation area and 73.45% (11.91 million metric tons) of global chickpea production [4].

A biotic and abiotic factors collectively contribute to the diminished productivity of chickpea [5]. A comprehensive survey carried out in 1995, encompassing 55 countries, identified the presence of 172 pathogens responsible for various diseases in chickpea. This included 67 fungi, 3 bacteria, 22 viruses and phytoplasma, and 80 nematodes [6]. Among these pathogens, "*Fusarium oxysporum* f. sp. *ciceris*, the causative agent of chickpea wilt, emerges as a prominent concern for both legume pathologists and breeders due to its detrimental impact on chickpea production" [7,8]. Remarkably, this pathogen is known to persist in the soil for extended periods, reaching up to six years, even in the absence of its host, making it both seed and soil-borne (Yadav *et al*., 2019). The primary mode of infection occurs through chlamydospores or mycelia. What's particularly intriguing is that the fungus can flourish in the roots and stem, even in seemingly healthy plants growing alongside diseased ones, with the latter harbouring a substantial quantity of the pathogen.

"Overreliance on systemic fungicides as the exclusive means of disease control has proven ineffective in completely eradicating wilt disease from afflicted areas, even with the development of wilt-resistant genotypes" [7,8]. To address this limitation, "the development of chickpea cultivars with inherent resistance to wilt has emerged as a sustainable alternative approach for disease management is crucial" [9]. Consequently, "the current focus is directed towards the creation of wilt-resistant cultivars, the conservation of genetic diversity, and the screening of genotypes against specific pathotypes. These steps are pivotal in promoting sustainable farming practices" [10]. "The extensive reliance on intensive fungicide usage as a primary agricultural management practice has proven insufficient in reducing the severity of diseases, including Fusarium wilt" (Yadav *et al.,* 2019). Hence, "exploring host plant resistance has been pursued as a financially viable strategy for managing this disease. However, the widespread deployment of resistant varieties has been hampered by undesirable agronomic traits linked to wild donor parents of chickpea, alongside the high level of pathogenic variability observed within the population of *Fusarium oxysporum* f. sp. *Ciceris"* [11].

Breeders are directing their efforts towards genetic resistance with the goal of deploy cultivars that can resist Fusarium wilt more effectively, thereby decreasing the necessity for chemical interventions. The implementation of sustainable management practices and the utilization of resistant varieties offer potential solutions for achieving effective disease control and improving chickpea productivity over the long run. Given the challenges at hand, the present study was undertaken to screen chickpea genotypes that result in resistant

reaction to wilt disease under controlled greenhouse conditions.

2. MATERIALS AND METHODS

Screening against wilt pathogen *Fusarium oxysporum* f.sp. *ciceri* was carried out using pot culture (i.e. sick soil) at ICAR-IARI Regional Research Centre, Dharwad during *rabi* 2021-22 and 2022-23 under greenhouse condition.

2.1 Isolation, Purification and Identification of *Fusarium oxysporum* f. sp. cicero

2.1.1 Isolation of pathogen

The pathogen *Fusarium oxysporum* f. sp. *ciceris* was isolated from infected chickpea plants using the tissue segment method, following the procedure detailed by Rangaswami and Mahadevan in 1999. Plants displaying symptoms of wilt were gathered and thoroughly washed with running tap water to remove soil particles. Subsequently, small tissue fragments, approximately 5 mm in size, were carefully excised from the root sections displaying vascular discoloration, ensuring that both healthy and diseased portions were included. To prevent contamination, the tissue fragments were surface-sterilized by immersing them in a 1% sodium hypochlorite solution for 60 seconds and then rinsed twice with sterilized double-distilled water to eliminate any traces of sodium hypochlorite. Subsequently, 4-5 of these tissue fragments were placed onto Petri plates containing Potato Dextrose Agar (PDA) under sterile conditions. The plates were then incubated at a controlled temperature of 27±2°C for 3 to 4 days until early fungal mycelial growth became visible.

2.1.2 Purification and identification of wilt pathogen

A pure culture of *Fusarium oxysporum* f. sp. *ciceri* was identified based on its morphological characteristics, as described by Booth [12]. To prepare a spore suspension of the isolated pathogen, *Fusarium oxysporum* f. sp. *ciceris*, spores were dissolved in sterile distilled water. A milliliter of this spore suspension was evenly spread across 2% agar plates, allowing excess suspension to drain off. Under microscopic observation, spores were monitored, and single spore was carefully identified and marked on the reverse side of the Petri plates using a marker. For further propagation, the marked agar was excised and transferred onto Potato Dextrose Agar (PDA) plates. These plates were then incubated at a temperature of 27±2°C. Following incubation, the resulting pure culture of the fungus was transferred to slants. Characteristics such as colony color, mycelial growth, pigmentation, and sporulation were examined in accordance with the guidelines provided in Booth's monographs on Fusarium, as detailed in Booth's work from [13]. To confirm the pathogenicity of the isolated pathogen, disease development was demonstrated by inoculating susceptible plants with it. For long-term preservation, the pathogen was sub-cultured on a monthly basis and stored at 4°C in a refrigerator.

2.2 Screening of Genotypes under Controlled Conditions

In the screening process, a total of 25 chickpea genotypes were evaluated for their resistance to *Fusarium oxysporum* f.sp. *ciceri*. This evaluation was conducted in a controlled environment, specifically a greenhouse. To ensure the consistency and reliability of the results, both genotype WR315 (highly resistant) and the susceptible genotype JG62 were included in the experimental setup. The experiment involved the use of plastic pots filled with sterilized soil to create a standardized testing environment.

In the process of preparing a culture for the growth of the *Fusarium oxysporum* f. sp. *ciceri* fungus, a substrate consisting of sand and corn meal in a 90:10 ratio was used. This substrate was placed in conical flasks and subjected to sterilization on two consecutive days to ensure the removal of any contaminants. A fresh culture of *F. oxysporum* f. sp. *ciceri*, aged seven days, in the form of a 5 mm disc, was introduced into each flask. These flasks were then incubated for a period of 20 days to allow the fungus to reach full growth. During the incubation period, the culture was regularly mixed to ensure uniform growth. Subsequently, the giant culture was used for inoculation in each pot, with an inoculum density of 8 percent. In each pot, five seeds were sown. As a control, a pot with healthy plants without any added inoculum was maintained. Before sowing the seeds in individual pots, the seeds of each chickpea genotype were sterilized using a 1% sodium hypochlorite solution for one minute and then rinsed with double-distilled water. This entire treatment process was replicated four times, and observations were

recorded to assess the percentage of disease incidence and classify the different genotypes based on their resistance or susceptibility to the fungus.

The observations on the disease incidence was recorded on mortality per cent the per cent wilt incidence of each test entry was calculated by the following formula given by Wheeler [14].

$$\text{Per cent Disease Incidence} = \frac{\text{Number of wilted plants in a quadrat}}{\text{Total number of plants assessed}} \times 100$$

The percent disease was recorded and grouped by using following grade system given by Haware and Nene, 1982 with slight modification.

List 1. Disease Reaction category according to grade system given by Haware and Nene, [15]

Sl. No.	Disease Reaction category	Disease incidence (%)
1.	Resistant	0 – 10
2.	Moderately resistant	10.1 – 20
3	Moderately susceptible	20.1 – 30
4	Susceptible	30.1 – 50
5.	Highly susceptible	50.1 – 100

Based on their disease reaction, genotypes were categorized into immune, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible genotypes

3. RESULTS AND DISCUSSION

3.1 Screening of the Chickpea Varieties/Lines for Resistance to *Fusarium oxysporum* f.sp. *ciceri* in Sick Soil at Greenhouse Condition

The total of 25 genotypes were screened in the greenhouse condition against *Fusarium oxysporum* f.sp. *ciceri,* were none of the genotypes were immune. WR315, Pusa 212, BG 618-11, BG 618 12, BG718-154, BG718-231, BG718-50, BG 618-30 genotypes showed resistant reaction. Three genotypes was moderately resistant *i.e.* BGD103, BGD111-1 and JG -11. ICC14395, BGD-1536, BG-256 and JAKI 9218 genotypes showed moderately susceptible reaction. Two genotypes BGD-225 and KAK-2 were showing susceptible reaction. Eight genotypes were showing highly susceptible reaction *viz* JG62, BGD-227, BGD-199, ICCV 191101, ICCV 191102, ICCV 191105, ICCV 191108, ICCV 191113 (Tables 1 & 2.).

The assessment of a wide range of chickpea genotypes in the context of Fusarium wilt yielded encouraging findings, as numerous genotypes displayed resistance to the disease. These resilient genotypes offer substantial promise for integration into breeding initiatives focused on the creation of chickpea varieties that are resistant or tolerant to Fusarium wilt. Notably, the disease's advancement was notably sluggish in these resistant lines, in stark contrast to the rapid decline observed in susceptible lines when exposed to the pathogen. This stark divergence in disease progression underscores the critical importance of identifying and prioritizing these resistant genotypes in order to effectively combat the destructive effects of Fusarium wilt.

In a study by Kumar et al. in [16], 101 genotypes were assessed. Out of these, 57 displayed resistance, 28 exhibited tolerance, and 16 were susceptible at the seedling stage. At the reproductive stage, 31 genotypes were identified as resistant, 26 as tolerant, and 44 as susceptible to the pathogen. Similarly, in the research conducted by Thaware et al. in [17], 50 chickpea entries were evaluated for their reactions to *F. oxysporum* f. sp. *ciceris*. Among these entries, six were highly resistant, 31 were resistant, eight were moderately resistant, two were moderately susceptible, and three were highly susceptible. In a separate study by Patil et al. in [18], seven isolates of *Fusarium oxysporum* f. sp. *ciceris* were examined in chickpea.

Among these isolates, I-19 and I-28 were determined to be resistant, while I-20, I-13, and I-1 were classified as moderately resistant. In contrast, I-4 and I-80 were found to be susceptible to the pathogen.

Seedlings face a heightened susceptibility to Fusarium wilt owing to their underdeveloped root systems and limited defense capabilities against pathogens. As plants mature and reach the reproductive stage, their root systems become more robust, affording a measure of protection against initial infections. Nevertheless, the pathogen may persist in the soil, and when plants allocate more resources to reproduction, their defenses against Fusarium wilt could become compromised. The consistent findings from these studies underscore the importance of identifying and utilizing chickpea genotypes that are resistant or tolerant to Fusarium wilt in

breeding programs aimed at developing resistant varieties. Understanding the diverse responses to the disease across different genotypes and growth stages is vital for crafting effective disease management strategies and promoting sustainable chickpea cultivation. The presence of varying levels of resistance among distinct genotypes underscores the potential for selecting promising candidates to breed Fusarium wilt-resistant varieties, thereby contributing to improved disease control and the sustainable growth of chickpeas [19].

Table 1. Screening of chickpea greenhouse in greenhouse against *Fusarium oxysporum f.sp. cicero*

Sl. No.	Genotype	Wilt % 2021-22	Disease reaction	Wilt % 2022-23	Disease reaction
1	WR315	0	R	6.25	R
2	JG62	81.25	HS	100	HS
3	ICC14395	37.5	MS	43.75	MS
4	Pusa 212	6.25	R	6.25	R
5	BGD-225	21.25	MS	37.5	S
6	BGD-227	56.25	HS	68.75	HS
7	BGD-199	62.5	HS	75	HS
8	BGD-1536	27.5	MS	27.5	MS
9	BGD -103	12.5	MR	18.75	MR
10	BG-256	27.5	MS	27.5	MS
11	JAKI 9218	31.25	MS	27.5	MR
12	ICCV 191101	68.75	HS	75	HS
13	ICCV 191102	62.5	HS	68.75	HS
14	ICCV 191105	81.25	HS	68.75	S
15	ICCV 191108	68.75	HS	93.75	HS
16	ICCV 191113	56.25	HS	56.25	S
17	JG -11	12.5	MR	12.5	MR
18	BGD111-1	18.75	MR	18.75	MR
19	BG 618-30	0	R	6.25	R
20	BG 618-11	6.25	R	6.25	R
21	BG 618-12	6.25	R	6.25	R
22	BG718-231	6.25	R	0	R
23	BG718-154	6.25	R	6.25	R
24	BG718-50	0	R	6.25	R
25	KAK-2	37.5	S	73.75	S

Table 2. Grouping of genotypes based on their reaction to *Fusarium oxysporum f.sp. cicero*

Grade	Per cent infection	Reaction	Genotypes
0	0	Immune	Nil
1	0.1-10	Resistant	Pusa 212, BG 618-11, WR315BG 618 12, BG718-154, BG718-231, BG718-50, BG 618-30
2	10.1-20	Moderately Resistant	BGD103, BGD111-1, JG -11
3	20.1-30	Moderately Susceptible	ICC14395, BGD-1536, BG-256, JAKI 9218
4	30.1-50	Susceptible	BGD-225, KAK-2
5	> 50	Highly susceptible	JG62, BGD-227, BGD-199, ICCV 191101, ICCV 191102, ICCV 191105 , ICCV 191108, ICCV 191113

4. CONCLUSION

Fusarium wilt continues to pose a significant threat as a destructive vascular disease in chickpeas. Among tested genotypes, certain ones demonstrated notable levels of resistance and moderate resistance to *F. oxysporum* f. sp. *ciceris*, making them promising candidates as valuable sources of disease resistance for future chickpea improvement programs. Furthermore, those genotypes displaying resistance are well-suited for direct cultivation in regions prone to wilt outbreaks and can play a pivotal role in breeding initiatives as crucial contributors of disease resistance traits. The incorporation of these resistant genotypes as donors in breeding programs warrants further exploration into the inheritance patterns of their disease resistance characteristics. To ensure comprehensive disease management, it is advisable to consistently screen a wide array of genotypes under both field and greenhouse conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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