Response of Released Chickpea Cultivars to some *Fusarium oxysporium* f.sp *ciceris* Isolates in Sudan

Abstract

Chickpea seeds in Sudan is an economically important, as a cash crop that generates income for farmers and rural communities, and as a significant source of protein for poor people. It is used increasingly as a substitute for animal protein This study was conducted to screen eight chickpea cultivars viz Salawa, Burgeig, Wadhamid, Jebelmarra, Hawatta, Shendi, Atmour, and Mattama using eighteen (18) isolates of *Fusarium oxysporum* f.sp *ciceris* (*FOC*) isolated from infected plants of chickpea displaying the characteristic symptoms of *Fusarium* wilt disease in winter season from different locations in ~~The~~ Sudan. ~~Pot~~ A pot experiment was carried out to assess disease intensity in ~~term~~ terms of disease incidence (DI) and disease severity (DS). After seven weeks from inoculation 19 out of 144 isolated-cultivar combinations do not ~~showed~~ show disease symptoms. The cultivar Burgeig was found to be immune to all *Fusarium* wilt isolates in the second and third week after inoculation. After seven weeks from inoculation, the least DI and DS were registered in Burgeig, whereas the highest ones were observed ~~in cultivar~~in cultivar Shendi. The remaining cultivars showed different ~~response~~ responses to FOC isolates. Regarding disease development, the high jump ~~of~~ in incidence and severity occurred between the third and fourth week after inoculation. The FOC isolate S9 seems to be more virulent and aggressive compared to the others.

Key words: ~~Fusaruim~~ Fusarium wilt, *Cicer arietinum*, Screening, Sudan

1. Introduction

Chickpea (*Cicer arietinum* L.) is an important food ~~legumes~~ legume in ~~the~~ most countries of the world with ~~the~~ a productivity of about 913 kg ha^{-1} (FAO, 2012). The cultivated chickpea originated in south-eastern Turkey (van der Maesen, 1984). In Sudan, it is a cash crop that ~~generate~~ generates income for farmers in rural ~~communities and~~communities and as a significant source of protein for people. The production fluctuates widely and farmers face ~~a~~ debilitating ~~constrains~~ constraints such as the ~~wide spread~~widespread incidence of diseases, the destructive

activities of pests, parasitic weeds, and limited access to quality high-yielding cultivars. The ICARDA has demonstrated high-yield varieties of ~~chickpea~~ chickpeas to farmers and other stakeholders in the Gezira region of Sudan and other areas throughout the River Nile State. In the Gezira, the varieties Salwa and Burgaig have performed extremely well, generating (ICARDA, 2014). More than 60 pathogens have been reported so far to infect ~~chickpea~~ chickpeas in different parts of the world, but only a few of them have the potential to devastate the crop, The important diseases are *ascochyta* blight, dry root rot, black root rot, *phytophthora* root rot, *pythium* root, and seed rot and *Fusarium* wilt (Nene et al., 1991). *Fusarium* wilt (*Fusarium oxyspoum* f.sp. *ciceris*) is a major constraint to chickpea cultivation ~~through~~ throughout the world (Nikam *et al*., 2011). The yield losses attribute ~~vary~~varies about (10-15%), but the disease span completely ~~destroy~~ destroys the crop ~~under~~ in the unfavorable environment (Cherif *et al*., 2007).

The use of resistant cultivars is the most effective and practical ~~mean~~ means to control *Fusarium* wilt (Mahmood *et al*., 2011). However, the efficiency of resistant ~~cultivar~~ cultivars in managing a disease can be seriously limited by pathogenic variability occurring in pathogen populations, including the existence of pathogenic races and pathotypes (Jimenez-Gasco *et al*., 2004). There are eight races of *F*. *oxysporum* f.sp. *ciceris* which are identified by the reaction on a set of differential chickpea cultivars (Jimenez~~s~~-Gasco and Jimenez~~s~~-Diaz, 2003). This study ~~aim~~ aims to screen the released Sudanese chickpea cultivars using some *Fusarium oxysporum* f.sp.*ciceris* isolates.

2. Materials and Methods

2.1 Isolation of the pathogen:

Eighteen ~~isolate~~ isolates of *Fusarium oxysoprum* f.sp *ciceris* were isolated from infected plants of ~~chickpea~~ chickpeas displaying the characteristic symptoms of *Fusarium* wilt disease in winter (2013) from different locations in ~~centeral~~ central Sudan (El- Madina Arab, Ganeb, Abugota, El-Moaileg, and Agricultural Research Corporation-Madani) and in ~~Northeren~~ Northern Sudan from Hudeiba Research Station, (three isolates from each location).

The roots and ~~stem~~ stems of infected plants were washed in running tap water to remove soil before isolation to avoid contamination. The roots were cut into small bits of ~~the~~ size (5-10 mm),

These bits were then surface sterilized with 0.1 percent mercuric chloride for 2 minutes and washed with three changes of sterilized water to remove traces of mercuric chloride. Each bit was blot dried and four bits each were placed on the solidified potato dextrose agar (PDA) plates. These plates were then incubated at 27 C^0 for seven days. The fungal growth was transferred to the plates of PDA.

~~*Fusarium* species~~*Fusarium* species were maintained on PDA slants and were stored at 4°C till use (Hend *et al*., 2012).

2.2 Chickpea genotypes:

In order to evaluate the varietal response of different chickpea cultivars to *F. oxysporum* f. sp. *ciceris* (Foc), a pot experiment ~~was~~ was conducted at Department of Crop Sciences nursery, Kordofan University El-obied-Sudan, in the month of November 2013. Eight chickpea cultivars viz., Wad-Hamed, Mattama, Burgaig, Hawata, Shandi, Gebel Marra and Atmour obtained from Agricultural ~~Resaerch~~ Research Corporation, Plant Breeding-Hudeiba Research Station, El-Damer, Sudan. Screened for the source of resistance against eighteen isolates of *Fusarium oxysporum* f.sp *ciceris* the causal agent of chickpea wilt disease, isolated from the most important chickpea regions in ~~Centeral~~ Central and ~~Northeren~~ Northern Sudan El-Madina Arab,Ganeb, Abugota, El-Moaileg, Agricultural Research Corporation-Madani and Hodeiba Research Station (three isolates from each location).

Treatments were arranged in factorial experiments in a complete block design. The treatment consisted of 3 replicates with one pot per replication and three plants per sack.

2.3 Preparation of the host plant

Soil prepared from sand and clay soil at the ratio of 1:1 the soil was placed into 30x40 inch plastic sacks. Seeds of each variety were surface sterilized and four seeds were sown in each sack.

2.4 Preparation of the pathogen inocula:

Ten ml of sterilized water ~~were~~ was added to each culture of the pathogen isolates, and the surface of the culture was scraped with a glass spatula to dislodge the chlamydospores. The spore

suspensions were transferred to 100 ml sterilized flasks. The ~~Concentratin~~Concentration of the ~~suspentions~~ suspensions ~~were~~ was determined with a ~~haemocytometer~~hemocytometer. A high suspension ~~of 9~~of 9×10^{-2} ~~spore~~^{2} spore ml $^{-1}$ was prepared from each isolate ready for soil treatment. Half ml of the ~~spores~~spore's suspension was injected gently beside each ~~one week old~~one-week-old seedling using a sterilized insulin syringe (Fisher and Toussoun, 1983).

Inoculated plants were kept in nursery with three replicates adopting factorial design.

2.5 Disease assessment

Disease reactions were assessed by the incidence and severity of symptoms at 7-day intervals. Severity of symptoms in individual plants

of a microplot was assessed on a 0-to-4 rating scale based on the percentage of foliage with yellowing or necrosis in acropetal pro-gression (0 = 0%, 1 = 1 to 33%, 2 = 34 to 66%, 3 = 67 to 100%,and 4 = dead plant). Incidence of foliar symptoms, I(0-to-1 scale) (Landa .,2001).(Navas-Cortes *et al* ., 1998).

Calculation of disease incidence:

The plants displaying the typical symptoms of the *Fusarium* wilt disease were considered infected. Percentage of the disease incidence was calculated using the following formula:

$$wilt\ incidence = \frac{No\ of\ plants\ wilted}{Total\ No\ of\ plants} \times 100$$

Calculation of disease severity:

The disease severity was assessed by visual estimation adopting the scale presented in Table 1.

Table 1: The Adopted Disease Severity Scale for *Fusarium* wilt Disease.

Scale	**Designation of Disease Severity**
0	No infection* on leaf
1	1-33% of the leaf were infected
2	34-66% of the leaf were infected
3	67-100% of the leaf were infected
4	Dead plant

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*infection: Displayed the typical *Fusarium* wilt disease symptoms.

2.6 Statistical analysis

Statistical analysis for factorial experiments in completely randomize design using MSTATC program.

3. Results and Discussion

Fusarium wilt disease cause yellowing and drying of leaves from the base to upward and finally death of plants (plate 1).

The study was conducted to screen eight (8) chickpea cultivars viz Salawa, Burgeig, Wadhamid, Jebelmarra, Hawatta, Shendi, Atmour, and Mattama using eighteen (18) isolates of *Fusarium oxysporum* f.sp *ciceris* (*FOC*).

The overall development of disease incidence in the eight cultivars presented in Figure 1

~~The~~ In the second week after inoculation: highly significant differences were obtained among cultivars and isolates. Burgeig was found to be immune to all *Fusarium* wilt isolates in this week, while the other 7 cultivars were susceptible. The highest infection (11.6) was recorded in cultivar Shendi which was infected by fifteen (15) *FOC* isolates. Regarding isolates, the highest infection (13.88) was recorded in Isolate S7, whereas the lowest one (1.38) was registered in Isolate S11 and S17.

The third week after inoculation: shows highly significant differences among cultivars and isolates in this week. The ~~cultivars~~ cultivar Burgieg is still immune to all Foc isolates, and ~~cultivars~~ cultivar Shendi scored 17.49. In addition, Shendi was infected by all isolates except S10 and S13. Other chickpea cultivars scored less than 10% disease incidence. The most virulent isolate was S9 which scored 19.33 whereas the lowest one was S8 with 2.75 disease incidence.

The fourth week after inoculation: all chickpea cultivars were affected by the causal fungus isolates in the fourth week after the inoculation. Analysis of variance revealed highly significant differences among cultivars and isolates. The highest disease incidence (46.79) was scored by the cultivar Shendi and the lowest one (6.42) was scored by the cultivar Burgeig. ~~Its~~ It's worthily notice that Burgeig immunity to some isolates ~~break~~ breaks after three weeks from inoculation. The largest disease incidence (30.54) was recorded in isolate S9 and the smallest one (9.67) was obtained in isolate S16.

~~The~~ In the fifth week after inoculation: highly significant differences were observed among cultivars and isolates. The lowest disease incidence was 16.40 % attained by the cultivar Burgieg and the highest one was 73.72% attained by the cultivar Shendi. The Isolates S9 and S16 cause the highest (58.38) and the lowest (26.38) disease incidence, respectively.

The sixth week after inoculation: analysis of variance showed highly significant differences between cultivars and isolates. In this week, the cultivar Burgieg scored the lowest disease incidence (16.98 %) while Shendi scored the highest disease incidence (76.07 %). Concerning the main effect of isolates, the highest disease incidence (58.4) was registered in S9, and the lowest one (32) was registered in S16.

~~The~~ In the seventh week after inoculation: the lowest disease incidence (16.98%) was registered in cultivar Burgieg whereas the highest one (76.07) was still registered in cultivar Shendi. ~~The~~

Isolate S9 ~~cause~~ causes the highest disease incidence (59.7) and ~~the~~ Isolate S16 ~~cause~~ causes the lowest one (34.7).

~~The~~ In the eighth week after inoculation: highly significant differences were obtained among cultivars and isolates. Burgeig ~~seem~~ seems to be more resistant to most *FOC* isolates. Interestingly, the lowest disease incidence (17.58) was registered in this cultivar. Whereas the cultivar Shendi was infected by all *FOC* isolates. Moreover, the highest infection (77.82 %) ~~in~~ this week was recorded in its canopy. The most virulent Isolate was S9, ~~it~~ which gave 59.75 disease incidence, while the lessen virulent *FOC* ~~isolates~~ isolate was S2. It gave 36.7 disease incidence.

The overall development of disease severity in the eight cultivars is presented in Figure 2.

The second week after inoculation: highly significant differences were obtained among cultivars and a significant differences were obtained between the isolates. Burgeig found to be immune to all *Fusarium* wilt isolates in this week, while the other 7 cultivars were susceptible. The highest disease ~~sevirity~~ severity (0.2) was recorded in the cultivar Atmour. Regarding ~~isolates~~ isolates, the highest infection (0.25) was recorded in Isolate S4, whereas the lowest one (0.02) was registered in Isolates S1, S11, and S17. The third week after inoculation: Analysis of variance showed non-significant differences among cultivars and isolates. The fourth week after inoculation: all chickpea cultivars were affected by the causal fungus isolates in the fourth week after the inoculation. Analysis of variance revealed highly significant differences only among cultivars. The highest disease ~~severity (~~severity (0.79) was scored by the cultivar Shendi and the lowest ~~one (~~one (0.11) was scored by the cultivar Burgeig. ~~The~~ In the fifth week after inoculation: highly significant differences were observed among cultivars and isolates. The lowest disease severity was .036% attained by the cultivar Burgieg and the highest one was 1.81% attained by the cultivar Shendi. The Isolates S9 cause the highest (1.39) and the lowest disease severity (0.67) attained by S17. The sixth week after inoculation: in this week, the cultivar Burgieg scored the lowest disease severity (0.6) while Shendi scored the highest disease severity (2.74). Concerning the main effect of isolates, the highest disease severity (2.15) was registered in S18 and the lowest one (1.04) was registered in S16.

~~The~~ In the eighth week after inoculation: highly significant differences were obtained among cultivars and isolates. Burgeig ~~seem~~ seems to be more resistant to most *FOC* isolates. Interestingly, the lowest disease severity (0.68) was registered in this cultivar. Whereas the cultivar Shendi was infected by all *FOC* isolates. Moreover, the highest infection (3.06 %) ~~in~~ this week was recorded in its canopy. The most virulent Isolate was S9, it gave 2.39 disease severity, while the lessen virulent *FOC* ~~isolates~~ isolate was S16. It gave 1.35 disease severity.

Effect of cultivars x FOC isolates on disease severity: ~~non significant~~nonsignificant cultivar x FOC isolates interaction was detected in all weeks except week six.

Figure 3 shows that all cultivars exhibit immunity (severity = 0.00) against a few FOC isolates except Jebelmarra and Shendi. The highest severity (4.00) reported in cultivar Jebelmarra with S9 and S18.

In this study and after seven weeks from inoculation 19 out of 144 isolated-cultivar combinations do not showed disease symptoms. Navas-Cortes et al. (2000), Sibtain et al.(2001) and Chaudhry et al. (2006) observed ~~conciderable~~considerable variation in response of chickpea genotypes when inoculated by FOC races. This might be due to the fact that ~~the~~ the races of FOC differ ~~in~~ in pathogencity and virulence, depending on the ~~susceptibility~~ susceptibility of the cultivar (Olivares et al. 2022b). Other ~~factors~~ factors favoring the development of FOC are high ~~t emperature~~ temperature, amount of ~~i noculums~~ inoculums and excess ~~soil~~ soil water (Navas-Cortes et al ., 2000; Olivares et al. 2021; Olivares 2022; Olivares et al. 2022a). Moreover, Shinde et al. (2010) ~~concluded~~ concluded that both the resistance and wilt is ~~polygenic~~ polygenic and ~~that~~ that ~~may~~ may have genes ~~with~~ with ~~secondary~~ secondary ~~effects~~ effects which modify the response to the disease. According to disease incidence, based on the main effect at the end of the experiment (the seventh week after inoculation), cultivars could be divided ~~in~~ into three groups viz, < 30% incidence which ~~include~~ includes only Burgeig (17.58%), 30%< and > 60%, include (Wadhamid (33.35%), Mattamma (42.70%), Hawatta (43.32%) Salawa (46.27%) and Atmour (49.74%), >60% incidence which include Shendi (77.82%). The results of Burgeig and Shendi is in accordance with Ahmed and Adam (2014). Concerning disease incidence progress (Figure 1) for the different cultivars, it ~~is appear~~appears that ~~the~~ a great change in incidence occurred between the third and fourth week after inoculation. Then incidence ~~progress~~ progresses slightly in all cultivars. The slow and fast

development of disease incidence was observed in Burgeig and Shendi, respectively. Kumar *et al*. (2013) reported that ~~development~~ development of ~~disease~~ disease is slow in ~~resistant~~ resistant lines and fast in ~~susceptible~~ susceptible lines. Furthermore, he suggested field screening at the reproductive stage for genotypes ~~exhibit~~ exhibiting ~~resistant~~ resistance at the early growth stage and became susceptible at the reproductive stage.

Similarly, based on the main effect at the end of the experiment (the seventh week after inoculation) the chickpea cultivars could be divided as follows:

(i) 1 ≥ severity, represented by Burgeig (0.68),(ii) 1< severity ≤ 3, this include Wadhamid (1.33), Mattama (1.63), Hawatta (1.72), Salawa (1.87), Jebemarra (1.94) and Atmour (1.98). (iii) more than three ~~severity~~severities, which include Shendi (3.06).

Regarding FOC isolates, no significance differences were observed among the eighteen them after seven weeks from inoculation for disease incidence and severity, but ~~the~~ isolate S9 seems to be ~~the~~ more virulent and aggressive compared to other FOC isolates.

4. **Conclusion**:

In this study it could be concluded that the cultivars Burgeig and Shendi were the best and worst one respectively. The high jump of incidence and severity occurred between third and fourth week after inoculation. The FOC isolate S9 seems to be more virulent and aggressive compared to the other FOC isolates. Generally in this study the release chickpea cultivar, Burgaig was found to be the most resistant cultivated variety to *Fusarium oxysporium* f.sp *ciceris.* Further studies should be carried out in future to confirm ~~this results~~these results.

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Healthy plant

A

B

C

Plate 1. Healthy plant and Disease development symptoms from A to C.

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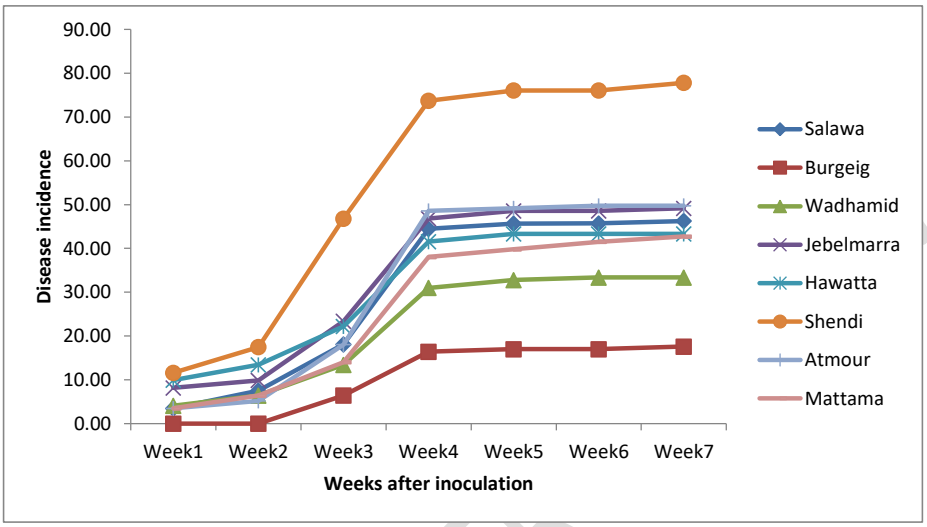

Figure 1. Disease incidence progress in chickpea cultivars

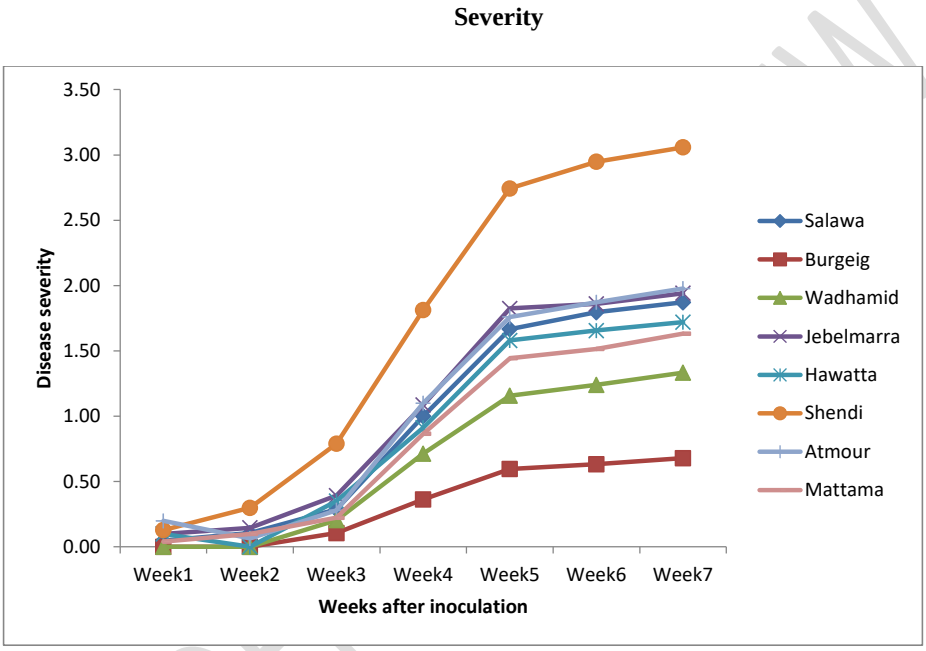

Figure 2. Disease severity progress in chickpea cultivars

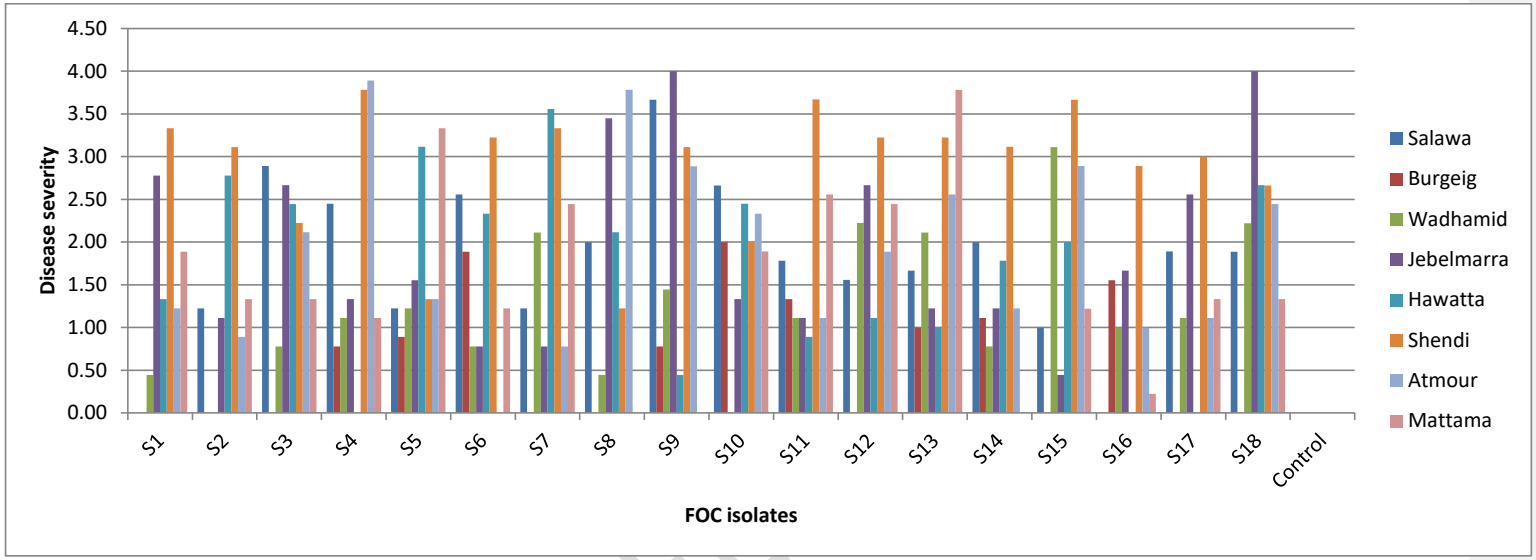

Fig. 3. Effect of chickpea cultivar x FOC isolates interaction on disease severity.

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