

Screening of Chickpea (*Cicer arietinum*) Genotypes to Three Identified Races of *Fusarium oxysporum* f. sp. *ciceris* in Sudan

Omyma Elmahi Mohamed^{1*}, Amel Adam Mohamed²

¹Plant Pathology Research Institute, Agricultural Research Corporation, Sudan; ²Chickpea Breeding Program, Agricultural Research Corporation, Sudan

ABSTRACT

Fusarium wilt is the main biotic stress that limited chickpea production in Sudan. Twenty chickpea genotypes were screened for resistance against *Fusarium oxysporum* f. sp. *ciceris* (Foc) the causal agent of *Fusarium* wilt of chickpea. The study was done in pot experiment at Gezira Research Station greenhouse. The genotypes were screened against three identified races of Foc in Sudan namely race 0, 2 and unidentified race to know their reaction against the pathogen. Race 0 is the most prevalent and widely spread in Sudan, while the unidentified race is limited to Gezira State. The variety Hawta (Iccv-92318) showed resistant reaction to the three tested Foc races while Shendi (ILC-1335) and Jabel Marra (ILC-915) varieties showed susceptible reaction to race 0 and highly susceptible reaction to the other two races. The other genotypes showed susceptible reactions to the unidentified race and variable reactions to races 2 and 0. This evaluation has helped to identify new sources of resistance to wilt disease for use in chickpea breeding program and for proper chickpea cultivation in the different areas of Sudan. It also will be useful in preventing the spread of the pathogen from infested areas to uninfected ones.

Keywords: *Cicer arietinum*; *Fusarium oxysporum*; Resistance; Screening; Sudan

INTRODUCTION

Chickpea (*Cicer arietinum* L) is a cool season food legume and an important cash crop in Sudan contributing in the sustainability and profitability of production systems. The crop was traditionally grown on residual moisture of the flood in northern Sudan [1] and recently it is introduced to the central parts of the country as an irrigated crop. *Fusarium* wilt disease caused by the fungus *Fusarium oxysporum* Schlechtend. *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo & K. Sato (Foc) is one of the most important vascular wilt diseases of the crop. The disease emerged as a devastating and economically important constraint that affects chickpea production in Sudan. The fungus could survive on crop residues in soil for more than 6 years thus the practical and cost-efficient method for management is the use of resistant cultivars which represent a key component in integrated disease management (IDM) programs [2].

A field survey was conducted during seasons 2011-2012 in the main chickpea production areas of Sudan to determine the importance and distribution of wilt and root rot disease [3] and to investigate the genetic diversity of Sudanese Foc isolates [4]. These studies revealed that management of chickpea wilt and root rot disease should be done through integrated crop management approach by using high yielding, wilt/root rot resistant cultivars with recommended seeding rates, irrigation frequency, sowing dates and appropriate cropping sequences [3].

The Sudanese isolates collected from six different chickpea-growing states in the Sudan were characterized using four random amplified polymorphic DNA (RAPD), three simple sequence repeats (SSR), five sequence-characterized amplified region (SCAR) primers and three specific Foc genome primers. Based on the similarity coefficient, the results indicated that the isolates from the Sudan were grouped and identified as 0, 2 and unknown races. This study identified a new race of Foc (race 0) in the Sudan [4]. Race 0 is widely distributed in central Sudan, while the unidentified race is limited to Gezira State. This clearly

*Correspondence to: Omyma Elmahi Mohamed, Plant Pathology Research Institute, Agricultural Research Corporation, Sudan, Tel: +249122974114; E-mail: omymahiaw@gmail.com

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indicates that race 0 is the most prevalent and widely spread. Race 2 is prevalent in Northern State, River Nile State and northern parts of Gezira State.

Adequate characterization of the resistance of chickpea lines and cultivars to specific races of *F. oxysporum* f. sp. *ciceris* is essential for resistance deployment. In the absence of agronomical and/or commercially suitable resistant cultivars, prediction of disease risk potential in a geographic area based on assessment of pathogen race and inoculum density thresholds in soil and susceptibility of cultivars can be of use for the management of Fusarium wilt in chickpea [5]. Thus the current study was carried out to assess the reaction of the released chickpea cultivars and some other promising genotypes in Sudan to the recently identified races of Foc.

MATERIALS AND METHODS

Twenty chickpea genotypes (8 released varieties + 12 genotypes) (Table 1) were screened to determine their reaction to the identified races 0, 2 and X (unidentified) [4] under greenhouse conditions. Inoculums from pure single spore cultures of each of the three races were multiplied on PDA media. With a 3-mm cork borer, 2 agar plugs were used to inoculate and increased in 180 g Sorghum Sand Mixture (SSM =135 g sorghum + 45 g sand + 100 ml water) autoclaved at 121°C for 20 minutes in 500-ml flasks (21- 1). The inoculated flasks were incubated at 25°C and a 12-h photoperiod using fluorescent lamps for two weeks. The infested sorghum sand mixture was mixed thoroughly with 4 kg sterilized soil (clay loam/sand, 1: 1, w/w). Surface sterilized ten seeds of each genotype were sown in a separate pot filled with infested SSM. The pots were arranged in a completely randomized design with 2 replicates for each race-genotype combination and were kept in the greenhouse under natural conditions. The plants were observed daily for symptoms development. Disease reactions were assessed and scored four and six weeks after inoculation. The level of resistance and susceptibility of each tested genotype was determined by using the rating scale of Iqbal et al., [6] and Suliman [7] with some modifications where R=0-20% wilted plants=1, MR=21-40% wilted plants=2, S=41-80% wilted plants=3 and HS = ≥ 80% wilted plants=4. Wilted plants were checked for vascular discoloration symptoms and re-isolation to confirm that the disease is caused by Foc.

The data was analyzed using MSTSC Program and the Duncan Multiple Range Test was used for mean separation.

Table 1: List of chickpea screened genotypes.

S. No	Accession No.	Name	No	Accession No.
Released cultivars			9	Elixir
1	ILC 915	Jabel Marra	10	FLIP 93-93
2	ILC 1335	Shendi	11	ILC 1929
3	Iccv-92318	Hwata	12	FLIP 97-263c
4	ICCV-2	Wad Hamid	13	ILC 464

5	Iccv-91302	Burgeig	14	FLIP 84-79c
6	FLIPc 82-89	Salwa	15	FLIP 81-71c
7	FLIP 77-91c	Matama	16	FLIP 97-530
8	Iccv-89509	Atmor	17	FLIP 03-104c
			18	ILC 3279
			19	FLIP 84-48c
			20	FLIP 85-17c

RESULTS

There were significant differences between genotypes for the different studied races (Table 2). Figure 1 illustrated the variable virulence of the three races on the tested chickpea genotypes. Race 0 isolates showed progressive yellowing of the plants and wilting within 45 days after inoculation, whereas race 2 isolates exhibited wilting of the plants after one month from inoculation. Race X (unidentified) showed wilting of the plants within 45 days after inoculation without yellowing. Symptoms of yellowing and wilting plants as compared with healthy plants were exhibited in Figure 2. Jabel Marra, Shendi, Elixir and FLIP 84-48c were the only genotypes susceptible to race 0. All other genotypes were either resistant or moderately resistant to the same race. All genotypes except of Hwata variety were susceptible to the unidentified race (X). However, Jabel Marra and Shendi varieties were highly susceptible to this race. The genotypes Jabel Marra, Shendi, Wad Hamid, Salwa, Matama, Atmoor, ILC 464, FLIP 84-79C, FLIP-03-104c and FLIP 84-48c were susceptible or highly susceptible to isolates of race 2. Hwata variety reaction to the three tested races was either resistant or moderately resistant (Table 2 and Figures 1 and 2).

DISCUSSION

Chickpea production was affected by wilt diseases in all States of Sudan with varying incidence levels which was affected by chickpea cultivars, soil types, and agronomic and cropping system practices. Large number of germplasm lines were introduced to Sudan and by extensive screening and selection methods between breeders and pathologists at River Nile State, a number of lines emerged and tested [8]. Selections were for adaptation, high and stable yield, resistance to wilt/root-rot disease, earliness and large seed size. The evaluation justified the release of ten cultivars with varying degrees of resistance to wilt and root rot disease. Actually, the effectiveness of resistant cultivars is limited by the occurrence of different pathotypes and races of the fungus as the crop was entered to the central States of the country.

The screening of the chickpea genotypes against the identified races (0, 2 and X), demonstrated that the already released cultivars (Shendi and Jabel Marra) were susceptible to the three identified races of the pathogen. If we need to save Shendi and Jabel Marra as high yielding and good quality chickpea cultivars, incorporation of the resistant genes of Fusarium wilt pathogen

to those cultivars becomes indispensable. Hawta variety is a promising variety resistant to the three races and could be introduced in demonstration fields to chickpea farmers. Hwata and Burgeig varieties were identified as the top seed yielding varieties that adapted to favorable conditions of Sudan [9]. Furthermore, Jabel Marra variety gave high seed yield and recognized as optional under poor soil at high terrace [10]. The released varieties Wad Hamid, Burgeig, Salwa, Matama and Atmor have fluctuating reactions to the three races. All the tested cultivars and lines are susceptible to the unidentified race found in Sudan and this indicates that it is the most aggressive race in Sudan (Figure 1). To overcome this situation in areas where this race is prevailing, a large chickpea germplasm should be screened for resistance to this race. Similar results of variation in chickpea resistance were reported in Sudan, India, Pakistan, Italy, Syria and Tunisia [11-18]. Also the greenhouse

screening of chickpea should be extended to field testing to confirm resistance in the field. Therefore, the stage of plant growth at which infection occurs is crucial as the symptoms of the disease started very late after 45 days from sowing in case of race 0 and the unidentified one. Race 0 is widely distributed in central states of Sudan, while the unidentified race is limited to Gezira State. This clearly indicates that race 0 is the most prevalent and widely spread. Therefore, some measures should be taken to prevent its spread to other new areas.

Sources of resistance against *F. oxysporum* f. sp. *ciceris* have been identified [19,20] and exploited in several chickpea breeding programs consequently. Fair number of resistant chickpea germplasm lines operative against specific races of the pathogen has been developed globally.

Table 2: Reaction of chickpea genotypes to Race 0, Race 2 and the unidentified race of *Fusarium oxysporum* f. sp. *ciceris*.

No.	Genotypes	Grade	Reaction to R 0	Grade	Reaction to R X	Grade	Reaction to R 2
1	JabelMarra	3.33 b	S	4.00 AM	HS	3.67 a	HS
2	Shendi	2.67 c	S	4.00 AM	HS	3.33 ab	S
3	Hwata	1.67 d	MR	1.00 c	R	2.00 cd	MR
4	Wad Hamid	1.33 de	R	3.00 b	S	3.67 a	HS
5	Burgeig	1.33 de	R	3.00 b	S	1.33 d	R
6	Salwa	1.33 de	R	3.00 b	S	3.67 a	HS
7	Matama	1.33 de	R	3.00 b	S	3.67 a	HS
8	Atmor	1.67 c	MR	4.00 AM	HS	3.67 a	HS
9	Elixir	3.00 bc	S	3.33 b	S	1.33 d	R
10	FLIP 93-93	1.00 e	R	3.33 b	S	1.67 d	MR
11	ILC 1929	1.33 de	R	3.33 b	S	1.67 d	MR
12	FLIP 97-263c	1.67 d	MR	3.33 b	S	1.67 d	MR
13	ILC 464	1.67 d	MR	3.33 b	S	2.67 bc	S
14	FLIP 84-79c	1.67 d	MR	3.33 b	S	2.67 bc	S
15	FLIP 81-71c	1.67 d	MR	3.33 b	S	2.00 cd	MR
16	FLIP 97-530	1.00 e	R	3.33 b	S	1.67 d	MR
17	FLIP 03-104c	1.00 e	R	3.33 b	S	3.00 ab	S
18	ILC 3279	1.00 e	R	3.33 b	S	1.67 d	MR
19	FLIP 84-48c	4.00 AM	HS	3.33 b	S	3.00 ab	S
20	FLIP 85-17c	1.33 de	R	3.33 b	S	1.67 d	MR

CV%	18.98	8.93	22.09
SE±	0.197	0.168	0.317

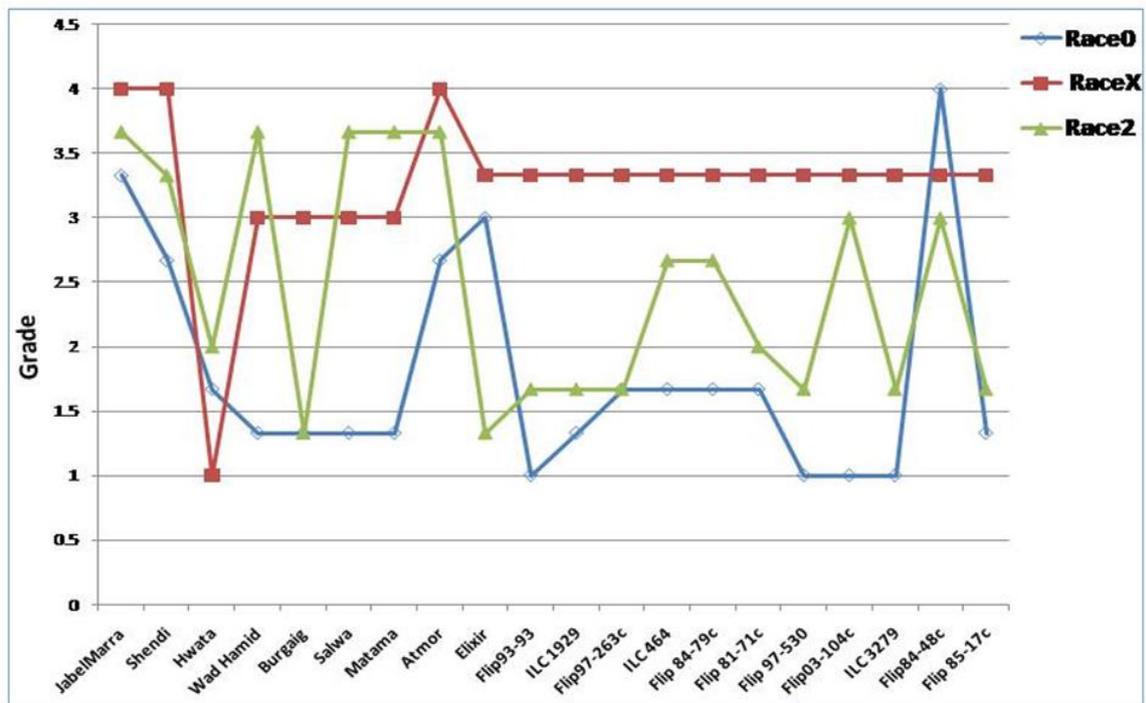


Figure 1: Reaction of the tested genotypes to Race 0, Race 2 and the unidentified race of *Fusarium oxysporum* f. sp. *ciceris*



Figure 2: Symptoms of yellowing and wilting of plants as compared with healthy plants. (1= Healthy plants, 2= yellowing of the lower leaves, 3= Wilted plants).

CONCLUSION

In conclusion, there should be a breeding program to replace the highly susceptible varieties by resistant one for the identified races of Foc. The resistant Hwata variety should be shown in the disease infected areas to provide high and stable yield. Certified

seeds of the promising resistant genotypes should be produced and distributed to farmers for commercial production to replace the wilt susceptible chickpea varieties. There is a need to conduct surveys to understand the presence of different pathotypes of Foc since knowledge on variability of pathogen is essential in breeding for durable resistance and would overcome

the challenge of breakdown of resistance due to development of new pathotypes.

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