

ORIGINAL PAPER

Biological Species and Effective Population Number in *Gibberella fujikuroi* Species Complex Recovered from Sorghum in Egypt

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Received: 21 February 2021 / Accepted: 03 March 2021 / Published online: 16 March 2021.

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ABSTRACT

Sorghum growing fields surveyed for the infection by *Fusarium* stalk and head rots throughout five locations in Egypt were screened depending on section *Liseola* characteristics. A number of 137 isolates from total recovered in average 87.8% isolates belonged to this section. These isolates selected from the whole number recovered from this group of fungi were subjected to the identified biologically and screened for fumonisin FB1 production. A number of 111 isolates produced fumonisin FB1 in average 81% from total isolates belonged to section *Liseola*. Four mating populations were identified, a number of 70 isolates mating population (MATP-A) *F. verticillioides*, 29 (MATP-D) *F. proliferatum*, 21 (MATP-F) *F. thapsinum* and 17 (MATP-G) *F. nygamai*. Mating population, A *F. verticillioides* consisted of 46 mating type 1 (*MAT-1*) and 24 mating type 2 (*MAT-2*), (MATP-D) *F. proliferatum* consisted of only 18 strains of (*MAT-1*) and 11 of (*MAT-2*). Whereas, (MATP-F) *F. thapsinum* consisted of 14 strains of (*MAT-1*) and 7 of (*MAT-2*) and (MATP-G) *F. nygamai* consisted of only 12 strains of (*MAT-1*) and 5 of (*MAT-2*). Isolates belong to MATP-A produced considerable amounts of fumonisin compared to those belong to MATP-D, F and G. Female fertile strains could produce higher amounts of the toxin than female sterile. Measuring the parameters of the effective population number in four biological species recovered from sorghum, it was found that the female sterility was lesser than 50% in mating population A (21.4%) and were high in MATP- D, F and G (58.6%, 61.9% and 64.7%, respectively). This caused decrease in effective population number based on female fertility (N_{ef}) overall locations in MATP- D, F and G, but it was high in MATP-A. Ration between Mating types (*Mat-1*, *Mat-2*) were differed from location to other also differed in population, it was high in all Mating populations than (N_{ef}). These results in MATP-A led to an advantage during sexual reproduction occurs. Whereas MAT-D, F and G population vegetative propagation were significant component of the fungus especially in Assiut MATP-D, based on (N_{em}) and population of Minia MATP-G, Sohag MATP-F based on (N_{ef}).

Keywords: Sorghum (*Sorghum bicolor*), *Gibberella fujikuroi* Species complex, Mating population, Effective Population Number, Fumonisin.

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INTRODUCTION

Sorghum (*Sorghum bicolor*) has spread throughout the world from its domestic origins in Africa (Nik *et al.*, 2019). In Egypt sorghum is the fourth most important cereal and is the only one of these cereals that can be easily cultivated in the 'new lands' or in very hot and arid Upper Egypt (Anonymous, 2007). *Fusarium* species in the *Gibberella fujikuroi* species complex are widely known from maize and sorghum in Egypt (Sabet *et al.*, 2006; Leslie and Summerell, 2006 and El-Shabrawy, 2007 and 2015). In many parts of the world, the only *Fusarium* species reported to occur on sorghum is *Fusarium moniliforme*. This name was retired in 2003 (Seifert *et al.*, 2003), as it now refers to

fungi in 15-50 different species. Some of the most important diseases of sorghum are stalk rot and grain mold, both of which have a *Fusarium* causal agent (Funnell-Harris *et al.*, 2016). Stalk rot incidence may be up to 90% and losses up to 50%, although year-to-year losses are usually much less (Jardine, 2006). Numerous additional *Fusarium* species from sorghum have been identified from sorghum, including *F. andiyazi*, *F. napiforme*, *F. nygamai*, *F. proliferatum*, *F. pseudonygamai*, *F. thapsinum*, and *F. verticillioides* (Klittich *et al.*, 1997; Leslie *et al.*, 2005 and Leslie & Summerell, 2006). Some of these species produce fumonisin, which are toxic to humans and domesticated animals (Desjardins, 2006).

Gibberella fujikuroi Sawada is the teleomorph for many species of conidial anamorphs in *Fusarium* section *Liseola*. This complex was divided using sexual cross fertility into nine biological species designated as mating populations A to J (Leslie and Summerell, 2006). Members of the section *Liseola* or *G. fujikuroi* species complex have a worldwide

distribution (Leslie, 1995). To distinguish species in the section *Liseola*, mating study methods was used or crosses (sexual cross fertility) with standard tester strain, since each anamorph belongs to one or more sexually compatible mating populations. Classifying isolates of *Fusarium* by mating study would place the isolates in biological species (Leslie, 1991). In mating study of *G. fujikuroi* species complex, the female parent is the tester strain for the mating population and the male parent is the isolate to be identified. Members of *G. fujikuroi* species complex are generally heterothallic in which sexual cross requires interaction between two morphologically indistinguishable spores but different in their mating types allele or *MAT* allele. Mating type in these fungi is controlled by a single locus with two alleles termed *MAT-1* and *MAT-2* (Nelson 1996; Leslie and Summerell, 2006). For crosses to occur, one isolate must carry *MAT-1* allele and the other *MAT-2*. Being fertile or having the ability to mate among isolates of *Fusarium* in section *Liseola* could provide information on genetic diversity, population genetics and can be important for plant pathological purposes (Zakaria *et al.*, 2011). In strains of *Gibberella fujikuroi*, female fertility is often lost, and populations are composed of hermaphrodites and female-sterile (FS) strains. The multiple types and roles of the male gametes are probably an important factor in explaining these data, especially since these propagules may function both in vegetative propagation and sexual reproduction (Leslie, 1995). The reduction in the frequency of hermaphrodites by either mutation or selection during vegetative propagation may be further accelerated by genetic drift during sexual reproduction. It is expressed as the effective population number (N_e), which is used to estimate the effects of genetic drift during sexual reproduction and inbreeding by comparing field populations to an idealized population using the equations adopted by (Crow, 1954; Crow & Denniston, 1988; and Leslie and Klein, 1996).

The aim of this investigation was to identify the biological species (Mating populations), mating types and female fertility of *G. fujikuroi* species complex on *Fusarium* spp. section *Liseola* recovered from sorghum head and stalk. Also, to study the effects of mating type and male/ female/ hermaphroditism on inbreeding and variance effective population numbers of *G. fujikuroi* species complex recovered from sorghum collected from Fayoum, Beni-Suef,

Minia, Assiut and Sohag farmer's fields. Chosen such locations for this investigation to develop theory and assess the role that the loss of female fertility plays in shifting species from sexual to asexual reproductive modes which may explain the appearance of *Fusarium* stalk rot infection in some hot spot locations under study. Also, study aim to evaluate data from different biological species of the *Gibberella fujikuroi* species complex.

MATERIALS AND METHODS

Isolates collection and conditions:

Fusarium spp. affected sorghum stalk and grain samples were obtained from different localities, namely Fayoum, Beni-Suef, Minia, Assiut and Sohag. Head and stalk samples were collected from farmer's fields of 3 districts from the five governorates at harvest time during 2019-2020 growing season. Heads were shelled and mixed together and 500gm sub-samples were taken at random for fungal isolation with stalk. Isolations were done on dichloran chloramphenicol peptone agar (DCPA), this medium was developed for selective isolation of *Fusarium* spp. (Andrews and Pitt, 1986). Obtained isolates were purified using single spore technique and preliminary identified microscopically on PDA medium according to Nelson *et al.* (1983), Burgess *et al.* (1994), Summerell *et al.* (2003) and Leslie and Summerell (2006). Isolates were preserved in 15% glycerol at -70°C until use.

Determination of fumonisin (FB1) produced by *Fusarium* isolates:

Recovered *Fusarium* spp. section *Liseola* isolates were subject to screened for FB1 production, they were grown on coarsely cracked yellow maize grains as described by Nelson *et al.* (1993). Quantitative determination was carried out following the method adopted by Dupuy *et al.* (1993). Quantification of FB1 was performed according to A.O.A.C (1990) by scanning the TLC plates with a spectrophotodensitometer (No. CS930; Shimadzu Corp., Kyoto, Japan) set at 600 nm to identifying sample peak area comparing with the FB1 standard concentration area peaks. Sample concentrations were calculated by the following equation:

$$\mu\text{g/kg} = (\text{B.Y.S.V})/(\text{Z.X.W})$$

Whereas: B= average area of peak in identified sample.

Y= concentration of FB1 standard ($\mu\text{g} / \text{ml}$).

S= μl spotted FB1.

V= final dilution of extracted sample (μl).

Z= average area of FB1 peaks in standard aliquots.

X= μ l of spotted sample extract.

W= weight (g) sample represents final extract.

Biological identification of mating populations, mating types and female fertility in *G. fujikuroi* species complex:

Fusarium spp. isolates section *Liseola* were crossed with standard female-fertile tester isolates. Sexual crosses were made on carrot agar as described by Klittich and Leslie (1988) and Leslie (1995, 1996 and 1999). Fertility was determined by the presence of perithecia exuding cirrus of ascospores 2-4 weeks after fertilization. Positive crosses were repeated twice, and negative crosses were repeated three times. Field isolates were tested as both male and female parents in crosses with the standard testers after the *MAT* allele in the field isolate was identified whether it *MAT-1* or *MAT-2*. Strains fertility was scored in crosses in which the field isolate was the male and the standard tester strain was the female parent. Female fertility was scored in crosses in which the roles were reversed, and the field isolate was the female parent, and the standard tester strain was the male which is known as reciprocal crosses. This was done by crossing the recovered toxigenic *Fusarium* isolates (field strains), with standard testers of both mating types from four mating populations, i.e., *Fusarium verticillioides* (*MAT-1A* 81, *MAT-2 A* 78), *F. proliferatum* (*MAT-1 D54*, *MAT-2 D53*), *F. thapsinum* (*MAT-1 F* 94, *MAT-2 F* 93) and *F. nygamai* (*MAT-1 G5111*, *MAT-2 G5112*) that were kindly provided by Prof. J.F. Leslie, Kansas State University.

Effective population number (*Ne*) in *G. fujikuroi* species complex:

The effective population number is the reduction in the frequency of hermaphrodites by either mutation or selection during vegetative propagation, may be further accelerated by genetic drift during sexual reproduction. It used to estimate the effects of genetic drift during sexual reproduction and inbreeding by compare field populations to an idealized population. Two commonly used effective population

numbers are defined: the inbreeding effective population number *Ne(f)*, which is based on the probability of identity due to common ancestry (differences in female sterile / hermaphrodite) which is expressed as a percentage of the count, based on female fertility, and the effective population number *Ne(mt)* which expressed as a percentage of the count, based on mating type. Estimates of effective population number were made by using the equations of Leslie and Klein (1996) shown below:

a- Based on mating type:

$$Ne_{(mt)} = (4NmNf) / (Nm + Nf)$$

Where, *Nm* = *MAT-1* and *Nf* = *MAT-2*.

b- Based on female sterile/ hermaphrodite polymorphism:

$$Ne(f) = (4N^2Nh) / (N + Nh)^2$$

Where, *N* = female sterile & *Nh* = hermaphrodite.

RESULTS

The preliminary microscopic and morphological identification to recovered *Fusarium* spp. from the surveyed locations under study indicated that representing isolates belonging to section *Liseola* obtained were 137 isolates, 87.8% from 156 recovered *Fusarium* spp., chosen by microscopic inspection (Table, 1). The other recovered *Fusarium* spp. were *Fusarium oxysporum*, *F. solani* and *F. semitectum* which isolated in low frequencies. Recovered isolates of *Fusarium* section *Liseola* were screened quantitatively for their ability to produce fumonisin (FB1) using Thin Layer Chromatography (TLC) analysis. Data showed that only 111 isolates were able to produce FB1 mycotoxin from 137 belonging to section *Liseola*, the average percentage number of the toxigenic strains from the total isolates was 81%. Isolates recovered from Fayoum, Beni Suef, Minia were highly in percentage number of the toxigenic strains also isolates belonging to section *Liseola* comparable to isolates collected from the other locations. Whereas *Fusarium* isolates obtained from Assiut and Sohag were low in percentage of the toxigenic strains.

Table (1): Percentage of *Fusarium* trains producing fumonisin recovered from sorghum.

Location	Total No. of <i>Fusarium</i> spp.	Total No. of section <i>Liseola</i>	% of section <i>Liseola</i>	No. of isolates FB1 producing	% of Toxigenic isolates
Fayoum	33	30	90.9	28	93.3
Beni-Suef	28	27	96.4	23	85.2
Minia	25	24	96	20	83.3
Assiut	36	28	77.7	21	75
Sohag	34	28	82.3	19	67.8
Total	156	137	87.8	111	81

Identification of biological species and female fertility:

Results of crossing between the unknown *Fusarium* isolates and both of mating types (*MAT-1* and *MAT-2*) from the references strain (testers) of the known mating populations, i.e., *Fusarium verticillioides* (*MAT-1* A 81, *MAT-2* A 78), *F. proliferatum* (*MAT-1* D54, *MAT-2* D53), *F. thapsinum* (*MAT-1* F 94, *MAT-2* F 93) and *F. nygamai* (*MAT-1* G5111, *MAT-2* G5112) as mentioned under Materials and Methods are given in Table (2). Results revealed that the four mating populations were biologically identified among the 137 isolates of *Fusarium* under study belonged to section *Liseola*. A number of 70 isolates mating population (*MATP-A*) *F. verticillioides*, 29 (*MATP-D*) *F. proliferatum*, 21 (*MATP-F*) *F. thapsinum* and 17 (*MATP-G*) *F. nygamai*. Mating population, A *F. verticillioides* consisted of 46 mating type 1 (*MAT-1*) and 24 mating type 2 (*MAT-2*), (*MATP-D*) *F. proliferatum* consisted of only 18 strains of (*MAT-1*) and 11 of (*MAT-2*). Whereas (*MATP-F*) *F. thapsinum* consisted of 14 strains of (*MAT-1*) and 7 of (*MAT-2*) and (*MATP-G*) *F. nygamai* consisted of only 12 strains of (*MAT-1*) and 5 of (*MAT-2*) as shown in (Table 2). Also results showed that both mating type alleles were scored for strains in all species recovered. *F. verticillioides*, *F. proliferatum* and *F. thapsinum* were the most frequently recovered strains from sorghum plants, *F. thapsinum* and *F. proliferatum* were introduced primarily with grains than stalks.

Reciprocal crosses were done to scoring female sterility and hermaphrodite individuals in

recovered strains by use the standards once as male and once as female between the same isolate and tester. Data in Table (2) reveal that mating population A *F. verticillioides* 55 isolates were shown to be female-fertile or hermaphrodites, but 15 isolates functioned as male only. While, mating population D, 16 isolates as female sterile and 13 ones were hermaphrodites. Whereas (*MATP-F*) *F. thapsinum* consisted of 13 strains female sterile and 7 of (*MAT-2*) and (*MATP-G*) *F. nygamai* consisted of only 12 strains of (*MAT-1*) and 5 of (*MAT-2*). Results shown in Table (2) indicate that fumonisin FB1 production was differed according to mating populations and locations, it was observed that *Fusarium* isolates from Fayoum, Beni Suef, Minia have the potential to produce the highest average levels of fumonisin FB1 comparable to the isolates from the other locations. Whereas, *Fusarium* isolates obtained from Assiut, Sohag were low in the average levels of fumonisin FB1 producing. Results also showed that the strains of the mating populations A (*F. verticillioides*) and D (*F. proliferatum*) recovered in this study were higher in their efficiency to produce FB1 comparable to (*MATP-F*) *F. thapsinum* and (*MATP-G*) *F. nygamai*. It was found also a correlation between the mating type and female fertility in relation to the amount of toxin production. The female fertile *MAT-1* strains were able to produce high levels of FB1 when compared with the female sterile *MAT-1* or *MAT-2* (Table 2)

Table (2): Biological identification of mating population (*MAT-P*), mating type (*MAT*-allele), female fertility (F.F) and fumonisin (FB1 µg/g) for *Fusarium* strains recovered from sorghum obtained from different locations.

No.	Locations	Isolate	Source	<i>MAT-P</i>	<i>MAT</i> allele	F.F	FB1 µg/g
1	Fayoum	F100	Stalk	A	<i>MAT-2</i>	Yes	30
2	„	F101	Stalk	A	<i>MAT-2</i>	Yes	70
3	„	F102	Stalk	A	<i>MAT-1</i>	Yes	810
4	„	F103	Stalk	A	<i>MAT-2</i>	Yes	400
5	„	F104	Stalk	A	<i>MAT-1</i>	Yes	212
6	„	F105	Stalk	A	<i>MAT-2</i>	Yes	65
7	„	F106	Stalk	A	<i>MAT-1</i>	Yes	998
8	„	F107	Stalk	A	<i>MAT-1</i>	Yes	890
9	„	F108	Stalk	A	<i>MAT-1</i>	No	544
10	„	F109	Grain	A	<i>MAT-2</i>	No	69
11	„	F110	Grain	A	<i>MAT-1</i>	Yes	180
12	„	F111	Grain	A	<i>MAT-1</i>	No	50
13	„	F112	Stalk	D	<i>MAT-1</i>	Yes	175
14	„	F113	Stalk	D	<i>MAT-2</i>	No	60
15	„	F114	heads	D	<i>MAT-1</i>	No	114
16	„	F115	heads	D	<i>MAT-2</i>	Yes	18

17	„	F116	heads	D	MAT -1	Yes	120
18	„	F117	heads	D	MAT -1	No	70
19	„	F118	heads	D	MAT -1	Yes	60
20	„	F119	heads	D	MAT -1	Yes	120
21	„	F120	heads	D	MAT -2	No	98
22	„	F121	heads	F	MAT -1	Yes	65
23	„	F122	heads	F	MAT -1	No	35
24	„	F123	heads	F	MAT -2	No	16
25	„	F124	heads	F	MAT -1	No	10
26	„	F125	heads	F	MAT -2	Yes	ND
27	„	F126	Stalk	G	MAT -1	No	ND
28	„	F127	Stalk	G	MAT -1	No	10
29	„	F128	Stalk	G	MAT -2	Yes	14
30	„	F129	Stalk	G	MAT -1	Yes	50
31	Beni-Suef	B100	Stalk	A	MAT -2	Yes	145
32	„	B101	Stalk	A	MAT -2	Yes	136
33	„	B102	Stalk	A	MAT -1	Yes	134
34	„	B103	Stalk	A	MAT -1	Yes	473
35	„	B104	Stalk	A	MAT -1	Yes	160
36	„	B105	Stalk	A	MAT -2	Yes	142
37	„	B106	Stalk	A	MAT -2	No	90
38	„	B107	Stalk	A	MAT -1	Yes	36
39	„	B108	Stalk	A	MAT -1	Yes	255
40	„	B109	heads	A	MAT -1	Yes	145
41	„	B110	Stalk	A	MAT -2	Yes	120
42	„	B111	Stalk	A	MAT -1	Yes	150
43	„	B112	heads	A	MAT -2	No	178
44	„	B113	Stalk	A	MAT -1	Yes	230
45	„	B114	Stalk	A	MAT -1	Yes	195
46	„	B115	heads	D	MAT -1	No	20
47	„	B116	heads	D	MAT -2	No	15
48	„	B117	heads	D	MAT -2	Yes	10
49	„	B118	heads	D	MAT -1	Yes	120
50	„	B119	heads	D	MAT -1	No	95
51	„	B120	heads	F	MAT -1	No	24
52	„	B121	heads	F	MAT -2	Yes	ND
53	„	B122	heads	F	MAT -1	No	15
54	„	B123	heads	F	MAT -1	Yes	ND
55	„	B124	Stalk	G	MAT -2	Yes	ND
56	„	B125	Stalk	G	MAT -1	Yes	60
57	„	B126	Stalk	G	MAT -1	No	ND
58	Minia	M100	Stalk	A	MAT -1	No	250
59	„	M101	Stalk	A	MAT -1	Yes	550
60	„	M102	Stalk	A	MAT -1	Yes	115
61	„	M103	Stalk	A	MAT -2	No	25
62	„	M104	Stalk	A	MAT -1	Yes	240
63	„	M105	Stalk	A	MAT -1	Yes	180
64	„	M106	Stalk	A	MAT -1	Yes	165
65	„	M107	Stalk	A	MAT -1	Yes	450
66	„	M108	heads	A	MAT -1	Yes	130
67	„	M109	Stalk	A	MAT -1	Yes	350
68	„	M110	heads	A	MAT -2	Yes	115
69	„	M111	Stalk	A	MAT -1	Yes	160
70	„	M112	Heads	D	MAT -2	No	ND
71	„	M113	Heads	D	MAT -1	Yes	95
72	„	M114	Heads	D	MAT -2	Yes	105
73	„	M115	Heads	F	MAT -1	Yes	ND
74	„	M116	Heads	F	MAT -1	No	40
75	„	M117	Heads	F	MAT -2	Yes	15
76	„	M118	Heads	F	MAT -2	No	40
77	„	M119	Heads	F	MAT -1	No	20
78	„	M120	Stalk	G	MAT -1	Yes	30

79	„	M121	Stalk	G	MAT-2	No	ND
80	„	M122	Stalk	G	MAT-1	No	20
81	„	M123	Stalk	G	MAT-1	No	ND
82	Assiut	A100	Stalk	A	MAT-2	No	ND
83	„	A101	Stalk	A	MAT-1	Yes	280
84	„	A102	Stalk	A	MAT-1	No	16
85	„	A103	Stalk	A	MAT-1	Yes	110
86	„	A104	Stalk	A	MAT-2	Yes	55
87	„	A105	Stalk	A	MAT-1	Yes	145
88	„	A106	Stalk	A	MAT-1	Yes	160
89	„	A107	Stalk	A	MAT-1	Yes	360
90	„	A108	Stalk	A	MAT-2	Yes	30
91	„	A109	Stalk	A	MAT-2	No	ND
92	„	A110	Stalk	A	MAT-1	Yes	275
93	„	A111	Stalk	A	MAT-1	Yes	40
94	„	A112	Stalk	A	MAT-2	Yes	ND
95	„	A113	Stalk	A	MAT-2	No	175
96	„	A114	Stalk	A	MAT-1	Yes	195
97	„	A115	Stalk	A	MAT-1	Yes	430
98	„	A116	Stalk	A	MAT-1	No	56
99	„	A117	Heads	D	MAT-1	No	267
100	„	A118	Heads	D	MAT-1	Yes	100
101	„	A119	Heads	D	MAT-2	Yes	ND
102	„	A120	Heads	D	MAT-1	No	24
103	„	A121	Heads	D	MAT-1	No	ND
104	„	A122	Heads	F	MAT-1	No	30
105	„	A123	Heads	F	MAT-1	No	ND
106	„	A124	Heads	F	MAT-2	Yes	15
107	„	A125	Stalk	G	MAT-1	No	ND
108	„	A126	Stalk	G	MAT-1	No	25
109	„	A127	Stalk	G	MAT-2	Yes	8
110	Sohag	S100	Stalk	A	MAT-2	No	ND
111	„	S101	Stalk	A	MAT-1	Yes	45
112	„	S102	Stalk	A	MAT-1	Yes	473
113	„	S103	Stalk	A	MAT-1	Yes	134
114	„	S104	Stalk	A	MAT-1	Yes	ND
115	„	S105	Stalk	A	MAT-2	Yes	ND
116	„	S106	Stalk	A	MAT-2	Yes	35
117	„	S107	Stalk	A	MAT-2	No	8
118	„	S108	Stalk	A	MAT-1	Yes	230
119	„	S109	Stalk	A	MAT-1	Yes	160
120	„	S110	Stalk	A	MAT-1	No	ND
121	„	S111	Stalk	A	MAT-1	Yes	36
122	„	S112	Stalk	A	MAT-1	Yes	150
123	„	S113	Stalk	A	MAT-2	Yes	40
124	„	S114	Heads	D	MAT-2	Yes	ND
125	„	S115	Heads	D	MAT-1	Yes	ND
126	„	S116	Heads	D	MAT-2	No	ND
127	„	S117	Heads	D	MAT-1	Yes	160
128	„	S118	Heads	D	MAT-1	No	39
129	„	S119	Heads	D	MAT-1	No	23
130	„	S120	Heads	D	MAT-2	No	78
131	„	S121	Heads	F	MAT-1	Yes	25
132	„	S122	Heads	F	MAT-1	No	ND
133	„	S123	Heads	F	MAT-2	No	10
134	„	S124	Heads	F	MAT-1	No	5
135	„	S125	Stalk	G	MAT-2	No	14
136	„	S126	Stalk	G	MAT-1	Yes	ND
137	„	S127	Stalk	G	MAT-1	No	12

(MAT-P) = Mating population, A (*F. verticillioides*), D (*F. proliferatum*), F (*F. thapsinum*), G (*F. nygamai*). MAT allele= (Mating type 1/2). F.F = Female fertility (Yes, female fertile strains and No, female sterile strains). FB1 $\mu\text{g/g}$ (levels of fumonisin B1 quantitatively, ND, not detected).

Effective population number (N_e) in *G. fujikuroi* species complex:

Effective population number was estimated after the *MAT* allele in the field isolate was identified and fertility was scored by tested field isolates as both male and female parents in crosses with the standard testers. From data of mating populations, mating types and female fertility shown in Table (2), and by the aid of using the equations of the effective population number (N_e) for differences based on mating

type $N_e(mt)$ and female fertility $N_e(f)$, the relative rarity of sexual reproduction that may permit female sterile strains to accumulate to a level such that local population could completely lose sexuality and appear as asexual (imperfect) species which could be illustrated in Tables (3, 4). Results in Table (3) indicate that mating populations All over the locations under study had female sterility (FS) in frequencies < 50%, in mating population A and > 50 in D, F and G mating population individuals.

Table (3): The effective population number (N_e) in *Gibberella fujikuroi* species complex from infected sorghum stalk and head overall locations.

Biological species	Mating type <i>Mat-1/Mat-2</i>	$N(fs):N(h)$	% (F_s)count.	N_e	
				$N_e(mt)$	$N_e(f)$
A	46/24	15/55	21.4	90.1	98.6
D	18/11	16/13	58.6	94.2	82.8
F	14/7	13/8	61.9	88.8	79.9
G	12/5	11/6	64.7	83.1	77.1

$N(fs)$ = number of female sterile strains; $N(h)$ = number of female fertile strains; F_s %= percentage of female sterile per total count; (N_e) effective population number= ($N_e(mt)$) inbreeding effective population number based on mating types and expressed as percent of actual count; $N_e(f)$ inbreeding effective population number based on number of males and hermaphrodites and expressed as percent of actual count).

Table (4): The effective population number (N_e) in *Gibberella fujikuroi* species complex in relation to locations under study.

Location	Mating population MAT-P	Mating type <i>Mat-1/Mat-2</i>	$N(fs): N(h)$	F_s %	N_e	
					$N_e(mt)$	$N_e(f)$
Fayoum	MAT-PA	7/5	3/9	25.0	97.2	97.9
	MAT-PD	6/3	5/4	55.5	88.8	85.2
	MAT-PF	3/2	3/2	60.0	96.0	81.6
	MAT-PG	3/1	2/2	50.0	75.0	88.9
Beni-Suef	MAT-PA	9/6	2/13	13.3	96.0	99.5
	MAT-PD	3/2	3/2	60.0	96.0	81.6
	MAT-PF	3/1	2/2	50.0	75.0	88.9
	MAT-PG	2/1	2/1	66.6	88.9	75.0
Minia	MAT-PA	10/2	2/10	16.7	55.5	99.1
	MAT-PD	1/2	2/1	66.6	88.8	75.0
	MAT-PF	3/2	3/2	60.0	96.0	81.6
	MAT-PG	3/1	3/1	75.0	75.0	64.0
Assiut	MAT-PA	11/6	5/12	29.4	91.3	97.0
	MAT-PD	4/1	3/2	60.0	64.0	81.6
	MAT-PF	2/1	2/1	66.6	88.9	75.0
	MAT-PG	2/1	2/1	66.6	88.9	75.0
Sohag	MAT-PA	9/5	3/11	21.4	91.8	98.6
	MAT-PD	4/3	4/3	57.1	97.9	84.0
	MAT-PF	3/1	3/1	75.0	75.0	64.0
	MAT-PG	2/1	2/1	66.6	88.9	75.0

$N(fs)$ = number of female sterile strains; $N(h)$ = number of female fertile strains her maphrodites; F_s %= percentage of female sterile per total count; (N_e) effective population number = (inbreeding effective population number $N_e(mt)$) based on mating types and expressed as percent of actual count., inbreeding effective population number $N_e(f)$ based on number of males and hermaphrodites and expressed as percent of actual count).

where, % FS were found 21.4% in MATP-A, 58.6% in MATP-D, 61.9% in MATP-F and 64.7% in MATP-G. Population size of the effective population number in MAT-A were high based on mating type (90.1%) also based on $Ne(f)$ 98.6% followed by MATP-D based on mating type (94.2%) also based on $Ne(f)$ 82.8%. whereas MATP-F based on mating type (88.8%) also based on $Ne(f)$ 79.9% followed by MATP-G based on mating type (83.1%) also based on $Ne(f)$ 77.1%.

The results showed that the probability of the asexual stage was high in MATP-F, G more than A and D which could be gave a new recombination differed than exist in the population on contrary MATP-F and G which could be selective vegetative reproduction.

Regarding to the effective population number among locations, data in Table (4) reveal that the female sterility (FS) percentage was < 50 % overall locations in MATP-A but it was high in Assiut and Fayoum (29.4 %, 25%) and low in other locations (from 21.4 to 13.3 %). The effective population number (Ne) based on the mating type $Ne(mt)$ was low 55.5% in Minia, where MAT-1 (10 strains) MAT-2 (2 strains). But the Ne based on the FS/hermaphrodites $Ne(f)$ was high in allover location.

Concerning to the effective population number in MATP-D, F and G the female sterility (FS) was high than 50% in allover location it was ranged between (% 50 - 66.6%). Also, the effective population number (Ne) according to the measured parameters based on the ratio between *MAT-1* to *MAT-2* differed from location to location, where low Ne (mt) was found in MATP-D at Assiut 64% but higher in Sohag and Beni-Suef (97.9% and 96%, respectively) whereas, Ne based on FS/hermaphrodites was low in population recovered from Minia MATP-G and Sohag MATP-F where it achieved 64% (Table, 4).

DISCUSSION

Sorghum is the fourth most important cereal crop in Egypt (after maize, wheat and rice), and is the only one of these cereals that can be easily cultivated in the "new lands" or in very hot and arid Upper Egypt (Anonymous, 2007). Isolation and biologically identification done throughout the present study revealed that the common and prevalent fungal isolates that attack sorghum are belonging to *Fusarium* species in the *Gibberella fujikuroi* species complex which are widely known from sorghum in Egypt. A common

perception is that cause stalk; head and root rot and produce mycotoxins such as fumonisins and moniliformin (Leslie and Summerell, 2006; Palmero *et al.* 2012). This study concentrated on *Fusarium* spp. in particular those belonging to section *Liseola* which are known broadly as *Gibberella fujikuroi* species complex which are common on maize and sorghum in Egypt (Leslie *et al.*, 1990 and El-Shabrawi *et al.*, 2007). Results indicated that four mating populations (MATP) were identified *i.e.*, MATP-A (*Fusarium verticillioides*), MATP-D (*F. proliferatum*), MATP-F (*F. thapsinum*) and MATP-G (*F. nygamai*) from *Gibberella fujikuroi* species complex. These populations were found in sorghum overall localities under study, this finding agrees with several investigators (Leslie *et al.*, 1990; Onyike and Nelson, 1992; Leslie, 2002; Leslie and Summerell, 2006; Tarekegn *et al.*, 2006; Lincy *et al.*, 2011 and Sharma *et al.*, 2011;). They found that *Fusarium verticillioides*, *F. proliferatum*, *F. thapsinum* were most frequently recovered from sorghum plants. Also, Leslie *et al.* (2005) and Del Palacio *et al.*, (2016) reported that *F. nygamai* was recovered from sorghum plants while, Abdel-Hafez *et al.* (2014) isolated *F. nygamai* from sorghum in Egypt. Results in this study indicated that *F. verticillioides*, *F. proliferatum*, *F. thapsinum* were the most frequent species on sorghum in Egypt. This was in the contrary with the work done by Nik *et al.* (2019) who reported that the relative frequencies of *F. proliferatum*, *F. thapsinum*, and *F. verticillioides* were approximately the same, in contrast to the frequencies of these species recovered from sorghum in Kansas, where *F. thapsinum* was the most frequent, whereas *F. proliferatum* was the next in this effect and isolates of *F. verticillioides* were relatively rare. Results showed that *F. thapsinum* was the most dominant on sorghum grains head, also *F. proliferatum* but *F. verticillioides* and *F. nygamai* were more frequent on stalk. This is in agreement with that found by Salleh *et al.* (1995) who stated that sorghum is usually planted as a second crop after harvesting maize or rice. Thus, the increased frequencies of *F. proliferatum* and *F. verticillioides* might be due to inoculum carryover from a previous crop. *F. proliferatum* is common on both rice and maize, and *F. verticillioides* is common on maize, where these fungi may be hosted either as endophytes or as disease-causing agents (Leslie and Summerell, 2006; Munkvold and White, 2016 and Cartwright *et al.*, 2018). In sorghum,

both *F. verticillioides* and *F. proliferatum* can cause stalk and root rot (Jardine and Leslie, 1992 and Palmero *et al.*, 2012), and *F. proliferatum* also can cause grain mold (Tesfaendrias *et al.*, 2011 and Sharma *et al.*, 2011). Also, many investigators reported that *F. thapsinum* is known to be an important causal of stalk rot and grain mold of sorghum (Jardine and Leslie, 1992; Onyike and Nelson, 1992; Klittich *et al.*, 1997; Leslie *et al.*, 2005 and Little *et al.*, 2012).

Total recovered *Fusarium* spp. was 156 isolates, a number of 137 strains were belonging to section *Liseola*. These isolates were screened for fumonisin FB1 production and results revealed that 111 isolates produced different levels of FB1. It was found that isolates recovered from Fayoum, Beni Suef, Minia were highly in percentage number of the toxigenic strains. Also, Isolates belong to MATP-A and D were able to produce considerable amounts of FB1 if compared with isolates of other MATP-F and G. The same findings were found by Thiel *et al.* (1991) who stated that *F. verticillioides* is considered the most dangerous strain in section *Liseola* as a toxigenic agent threatening man and animal health. Dealing with the toxigenic isolates of *Fusarium* in the present study showed clear correlation between the levels of fumonisin produced, location from which these isolates were recovered from, mating population and the female fertility in the population. Findings stated by Nelson *et al.* (1991) are consistent with these results. They found that the potential exists for production of fumonisin by such strains in agricultural commodities and other substrates are widespread in geographic areas. Alike findings were obtained throughout this study, Leslie *et al.* (1992a) reported that members of the biological species A (MAT-A) could produce an average of 1,786 ppm of the toxin, much higher than members of the 'D' population which averaged 636 ppm when grown on maize grains. The amounts produced by *F. thapsinum* usually are quite small (Leslie *et al.*, 2005; Desjardins, 2006; Leslie and Summerell, 2006). It could also be emphasized by Leslie *et al.* (1992b) that the female fertile strains produced considerable amounts of FB1 comparable to that produced by the female sterile ones of the same species.

Concerning to the effective population number (N_e) based on FS / hermaphroditism $N_e(f)$ overall locations, results indicated that the female sterility in MATP-A was very low < 50%. On the other hand, it was very high in MATP-D, F and G which rise $N_e(f)$ value in

MATP-A (98.6%) than other population which was low in MATP-G where, it was 79.9% because of the low percentage of FS proportion in MATP-A (21.4%) than that in MATP-D, F and G the corresponding values were 58.6%, 61.9% and 64.7%, respectively. Regarding to the effective population number based on the mating types ($MAT-1/MAT-2$) ratios it was found differences in mating populations (A, D, F and G) from 1:1, resulting in large decreases in the inbreeding effective population number to 83.1 % of the count for MAT-G and higher to 94.2 % in MATP-D and 90.1% in MATP-A. Generally, the effective population number based on the mating types larger than N_e based on female fertility. This finding is in agreement with that reported by Leslie and Klein (1996). Results in this study suggest that sexual reproduction is an important part of the life cycle of *F. verticillioides* recovered from sorghum in Egypt, as maintaining high levels of female fertility is disadvantageous, at least in the short term, if a more efficient asexual means of reproduction is available. The level of female fertility in *F. proliferatum* usually is lower than in *F. verticillioides*. The values observed in this study suggest that this species reproduces extensively via asexual reproduction in Egypt. Sexual reproduction is not of primary importance for *F. thapsinum* and *F. nygamai* at any location, as assessed by $N_e(f)$, since the number of female fertile strains always is low. This finding is in agreement with that reported by Nik *et al.* (2019) to population recovered from sorghum in Thailand where, $N_e(f)$ for this Thai population, 42% of the count, which is somewhat higher than the 32% previously reported (Leslie and Klein, 1996), and strengthen the current view of *F. thapsinum* as a species that maintains just enough sexual reproduction to avoid losing sexual reproductive capacity altogether.

It could be concluded from results derived from this study that the effective population number was differed from location to another, strains within the mating population (MATP-A) all over the surveyed locations have higher amount of fumonisin also have the advantage to sexual reproduction occurs and the new recombination would be found. This advantage was higher in all locations expect Minia. Generally, strains of MATP- D, f and G were higher in female sterility where it was > 50%. Furthermore, Sohag individuals MATP-F and MATP-G in Minia achieved 75% FS that have disadvantage to sexual reproduction occurrence

because of decrease $N_e(f)$ to 64%. The selection for or against sexually and asexually produced spore types was considered and showed that a stable polymorphism for Fs strains and hermaphrodites can occur (Nauta and Hoekstra, 1992). Analysis of fungal population, the determination of FS levels done by Leslie and Klein (1996) provides extensive information. They reported that if sexual reproduction is always required as part of the life cycle, FS strains would not be expected at a rate above a few percent because the FS alleles are selected against during sexual reproduction. If sexual reproduction is never necessary, then the population eventually should become all FS. As well as found in this study at Sohag and Minia for F, G population. In previous study on *Fusarium* population on maize, results revealed that *F. proliferatum* have advantage to asexual reproduction occurs than *F. verticillioides* which reproduced sexually (El-Shabrawy, 2015) suggests that sorghum in upper Egypt was dominant than maize so, *F. proliferatum* the main pathogen to sorghum has no necessary to sexual reproduction than *F. verticillioides* population which has an advantage to sexual reproduction occurs to caritas a new recombination for competition with MATP-D, F and G the main pathogens of sorghum in Egypt.

CONCLUSION

The hot spot location of sorghum stalk rot found have high frequency of hermaphrodites gave the population an advantage to sexual reproduction occurs and the new recombinations were found. Depending on this finding the hybrids cultivated in these locations must be followed and improved against these new strains.

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