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Reaction of Some Cotton (*Gossypium barbadense*) Lines against Fusarium Wilt Disease (*Fusarium oxysporum* f.sp. *vasinfectum*) and Mode of Inheritance for Resistance

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Abstract

The purpose of this study was to screen and assess ten new lines of cotton *Gossypium barbadense* and their F_1 and F_2 progenies to Fusarium Wilt Disease which caused by *Fusarium Oxysporum* f.sp *vainfectum*. The disease is known as seed borne and it can be dispersed in soil and crop residues attached to vehicles and machinery and carried in irrigation and flood water. A field and greenhouse experiments were conducted at Gezira Reseach Station Farm (GRSF) during season 2007 to 2010. Tested lines and check varieties were artificially inoculated using dipping root method in the green house experiment whereas sick plot was used to infect sown seeds in field experiments. The results showed that Domain Sakel, Huda and convential Barakat were the most susceptible genotypes, while the standard variety Barakat 90 showed a high level of resistance (33.3%) Sp-21 (44.4%), IS3(50%), SPA-9(50%) and IS10-1(44.4%) showed intermediate level of resistance (IR).The high level of resistance were recorded in F_2 generations of IM14(33%) and IMY1 (37%). There was no resistance in F_2 generation of IS3 (76%), while low resistance level was showed in F_1 generation of SPA-9 (66.9%). F_1 generations of Barakat S (42%) , IMYI (60%), IM14 (50%), SP-7 (55%), IMB-9(54%), and SP-9(58%), showed intermediate resistance as well as IS-10(42%), IMB-9(46%), and SP-21(50%) in F_2 generations. F_1 generation of IS21 also gave intermediate level of resistance (44%). Low resistance levels were showed in F_1 generations of IS3 (76%), IS10-1 (79%) and IS-10 (66.7%). In some cases modifiers or non allelic gene (s) interaction could have contributed to the resistance. It is clear that the genes for disease resistance resulted from spontaneous and induced mutations are different, more dominant and consistent. From the result above experimental lines IM14 and IMY1 could be considered as a source of resistance because the loci might be positively changed by gamma rays, they were better than Barakat 90 and Barakat S. The resistance to Fusarium Wilt in resistant parents used is controlled by two or more genes with a degree of dominance.

Keywords: mode of inheritance, *Fusarium oxyporum* f. sp. *vasinfectum* , symptoms.

Introduction

Cotton is one of the world's most important textile fibers, used in industrial fabric. Modern allotetraploid (2n:4x=52*)* *G. barrbadense* L. (Gb, AD_2 genom) and *G. hirsutum* L. (Gh, AD_1 genom) are the most extensively cultivated species worldwide (Mauricio *et al*., 2006). Although during 1980s the world cotton yield reached a record of nearly 600 kg per hectar. It was stagnated during the 1990s due to problems associated with diseases, resistance to pesticides, and disruption of production due to economic reasons (ICAC, 2006). In Sudan cotton contributes effectively to foreign exchange earning (Sudan Cotton Company, 2002/03). Many diseases are known to attack cotton and affects yield e.g. Bacterial blight, Verticilium Wilt, Boll Rot and Cotton Leaf Curl (Hillocks, 1992).

Seedling diseases are a worldwide problem, particularly wilt and root rot diseases causing seed bed losses to the farmers. The most common fungi associated with cotton diseases are *Fusarium spp, Colletotricum gossippi*, *Thielviapsis basicola*, *Rhizoctonia solani* and *Pythium spp* (Hillocks, 1992). Fusarium wilt is the most important seedling disease, caused by *Fusarium oxysporum* f.sp *vasinfectum* (*FOV*). The fungus is a soil inhibiting organism and can survive for a long period in soils, even in the absence of cotton, making it nearly difficult to be eradicate from the field. It was reported in Sudan in 1960 (Salt, 1961), the disease observed in certain localities at various level, in the Gezira Scheme namely, NewaiIa Block (Ibrahim and Khalifa, 1969). The symptoms were characterized by leaf necrosis, vascular discoloration, stunted growth and in an extreme cases leaf death occur.

In Sudan, the cotton variety improvement program, mainly addresses the higher yield, earliness, disease and insect resistance, breeding that having different balances of fiber characteristics to meet the textile and spinning technology needs, breeding for vertical up grading of productivity via the Integrated Crop Management (ICM) and maintaining the existing cultivars. However, in pima cottons a single dominant gene and one or more minor genes that may provide transgressive segregation have been reported for *FOC* resistance (Ulloa *et al*., 2005, 2006 in press). As a result a number of more than 50 varieties and improved lines were being released; of these only 7 varieties are currently grown either commercially or in limited propagation plots, covering wide range of fiber characteristic namely, Barakt, Barkat 90, Shambat B, Nour, Barc (67) B. Acrain and Albar (57)12.

Recently, nine varieties were released. However of which only four varieties are currently grown commercially namely, Hamid, Abdin, Wagar and Burhan (Abdelramn and Elfadil, 2012). For efficient and successful breeding programs, behavior of resistance should be determined. It is necessary to breed for fusarium wilt to keep pace with the improvement program in order that fusarium wilt resistance will be included in the newer varieties or at least to be available to the plant breeders in near future. This study reports on field and greenhouse evaluations of the susceptibility and resistance of new lines of *G. barbadense* to *Fusarium oxysporum* f. sp. *vasinfectum*, race 5 and the mode of inheritance of resistance to it.

Materials and Methods

Ten new cotton lines (Barakat S, IMP-9, SP-7, IM14, IMY1, SP-21, IS10-1, IS3, IS-10 and SPA-9) were obtained from Cotton Research Program of Agricultural Research Corporation (ARC). The progenies were grown at the Gezira Research Station Farm (GRSF) Wad Madani during 2007-2010 to produce F_1 seeds. All F_1 seeds were selfed to produce F_2 seeds. Varieties, Barakat 90 and X1730A were used as resistant check and

Huda and Barakat were used as susceptible check.

Fuarium oxysporum f. sp *vasinfectum* (*FOV*) was isolated from the contaminated soil at Gezira Research Station Farm (GRSF) called(plot 4) which was artificially inoculated with the pathogen (*FOV*) since 1962 and determined as a sick plot, then stored on the Potatoes Dextrose Agar medium (PDA) for further uses.

Greenhouse experiment:

Pathogenicity test was done using dipping root method (Ibrahim and Nerenberg, 1993). From the 7 days old culture conidial suspension was prepared and adjusted using a hemacytometer to the required dilution (10^6 conidial/ ml). Nine seeds from each previous lines and check cultivars were sown on three pots (20 cm in diameter) contained 2 kg of sand-loam soil sterilized at160 Cº for 3 hrs. Two weeks after sowing, seedlings were removed from the pots and were artificially inoculated by dipping in the previous inoculum for five min. Inoculated plants were transferred to other similar pots. The remaining seedlings were removed and dipped in sterile water and employed as negative control checks.

All treatments were kept at 25 ± 2Cº and 40% Relative Humidity (R.H). Each treatment was replicated three times. Observations were taken daily. The final infection count was made three weeks after inoculation. Infection response was expressed by the disease level (%) based on incidence and severity of disease symptoms

*Severity grades were used from 0 - 3

Grade	Designation of D.S
0	No symptoms appeared
1	Vein clearing appeared partially
2	Yellow discoloration extend up to half of the leaf
3	Yellow discoloration extend throughout the leaf

$$\text{Disease level \%} = \frac{\sum (ni \times i) \times 100}{n \times k}$$

Where:

i= minimum score of inoculated plant.

K= maximum score of inoculated plant

i = 1; k = 3.

n=the total number of plants scored.

ni= number of plants which scoring minimum scale (After Ibrahim, G.(2005). 72 th Meeting of the Pest and Diseases Committee – ARC- Wad Medani – Sudan).

Field experiment: The previous lines and check cultivars were naturally infected by sowing at sick plot (plot 4). Each cultivar or line was sown at single row, and 7 meter length and 50 cm between rows. Treatments were replicated 3 times in a randomized block design. Plants were fertilized by 2 N and irrigated every 2 weeks. Level of infection was based on percent infected plants. The disease count was made daily after symptoms appeared till five week later.

Results and Discussion

All lines and varieties tested gave comparable result in field experiment with greenhouse experiment for the fusarium wilt grades (Table1), but the progress of the disease is not satisfactory and this could be due to the heterogeneity of the pathogen distribution in the soil. The greenhouse tests clearly indicated that the highest level of infection (88.9%) and (95%) was obtained with varieties Domain Sakel (DS) and convential Barakat, respectively. These results confirm the previous work which done by Prof .Ibrahim and Niredeg (1993). Also, Huda gave a high level of infection to Fusarium Wilt (88.9%). This result contrast with that obtained by Ibrahim, (1987). The standard variety Barakat 90 showed a high level of resistance (33.3%). This result agreed with Fadlalla and Ibrahim, (2004).

IM14 which was originated from Barakat 90 physical mutation (gamma rays), expressed the best resistance (22.2%) compared to others and this it could be considered as source of resistance. The result indicated that it's still resistant and the Fusarium Wilt remained with unchanged locus resistance.

Table (1): Disease resistance rating of pot experiment

Line/ variety	parent	D.R	F1S	D.R	F2S	D.R
Barakat S	33.3	HR	66.9	LR	44.4	IR
IMB-9	37.5	HR	66.7	LR	50	IR
SP-7	37.5	HR	66.7	LR	44.4	IR
IM14	22.2	HR	44.4	IR	33.3	HR
IMY1	33.3	HR	44.4	IR	33.3	HR
SP-21	44.4	IR	62.5	LR	44.4	IR
IS10-1	44.4	IR	62.5	LR	50.0	IR
IS3	50.0	IR	77.8	LR	88.9	NR
IS-10	37.5	HR	62.4	LR	44.4	IR
SPA-9	50.0	IR	66.7	LR	77.8	LR
Domain Sakel	88.9	NR	-	-	-	-
Barakat90	33.3	HR	-	-	-	-
Huda	88.9	NR	-	-	-	-
X1730A	37.5	HR	-	-	-	-
Barakat	95	NR	-	-	-	-

IMY1 which gave also high degree of resistance (33.3%) than previous lines and less than IM14. It is also considered as a source of resistance since it was obtained from Barakat 90, treated by gama rays and affected by partial treatment but still remained resistant. Sp-21 (44.4%), IS3 (50%), SPA-9(50%) and IS10-1(44.4%) showed intermediate resistance (IR). This may be due to gene expression. Barakat S (33.3%), IMB-9(37.5%), SP-7 (37.5%), IS-10 (37.5%) and X1730A (37.5%) were high level of resistant, (HR). A possible explanation might be that the high level of resistance to race 5 masked the genetic resistance due to their mechanical defense by tylosis and their toxins or due to differences between the strains within the race. However, an inoculum of 10^6 conidia / ml for race 5 reported by Ibrahim and Nirderge (1994) as well as in this work may be not necessarily enough for race 5. Moreover, the positive reactions of these new lines by race 5 which was observed in the early stages of the disease development and further disease progress was restricted. Whereas - the very mild disease symptoms may suggest that the infection is largely prevented by hardiness of the roots.

The F_1 generation of Barakat S, IMB-9, SP-7, SP-21, IS10-1, IS3, IS-10 and SPA-9 gave low levels of resistance, while F_1 generations of IM14 and IMY1 gave intermediate level of resistance. F_2 generations of Barakat S, IMB-9, SP-7, SP-21, IS10-1, showed intermediate levels of resistance. Obviously, the high levels of resistance were recorded in F_2 generations of IM14 and IMY1. There was no resistance in F_2 generation of IS3, while low resistance was showed in F_2 generation of SPA-9.

However, incidence of the fusarium wilt was low and inconstant to reveal true differences between entries (Table 2). Varieties Domain Sakel (88.9%), Convential Barakat (91%), and Huda (96%) were remained susceptible to the disease. Whereas Barakat 90 (22%) gave a high level of resistance. This result confirm the previous work which done by Ibrahim and Fadlalla (1994) who reported that, Barakat 90 was resistant variety. Also X1730A (33.3%) gave a high level of resistance. These results clearly indicated that the disease incidence was low and inconsistent when compared to greenhouse results. Inoculum concentration in the field might have been suboptimal.

Table (2): Disease incidence rating in field experiment:

Line/ variety	parent	D.R	F_1S	D.R	F_2s	D.R
Barakat S	33	HR	42	IR	39	HR
IMB-9	28	HR	76	LR	37	HR
SP-7	22	HR	64	IR	37	HR
IM14	33	HR	60	IR	37	HR
IMY1	28	HR	79	LR	66	IR
SP-21	22	HR	50	IR	33	HR
IS10-1	33	HR	55	IR	37	HR
IS3	38	HR	66.7	LR	42	IR
IS-10	39	HR	54	IR	46	IR
SPA-9	44	IR	58	IR	50	IR
Domain Sakel	96	NR	-	-	-	-
Barakat90	88.9	NR	-	-	-	-
Huda	22	HR	-	-	-	-
X1730A	33.3	HR	-	-	-	-
Barakat	91	NR	-	-	-	-

D.R: Degree of Resistance- HR: Highly Resistant IR: Intermediate Resistant LR: Low Resistant R: No Resistant

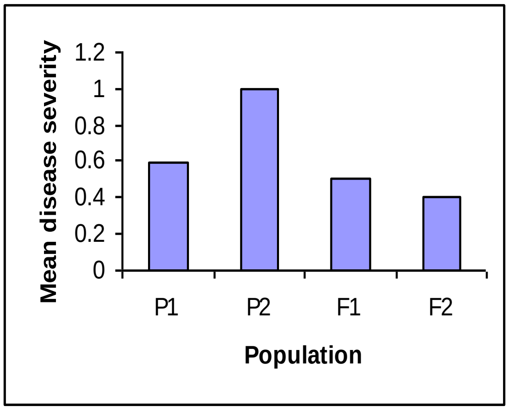
At low inoculums density the Fusarium remained fail to invade the vascular system unless reached the optimum concentration. However, typical disease symptoms appeared on plants grown in soil containing 5000 propagules $g^{-1,}$ Garber (1979). The propagules in field experiments were uncounted due to contamination of the soil by other microorganism. Generally, the temperature considered as a main factor to cause wilting and Fusarium *oxysporum* f. sp. *vasinfectum* need not than 25± C. During the field experiment the temperature was not constant and this was reflected on infection percents. This claim on lines with Hillocks, (1992) who reported that if temperature remains below 23 °C a little disease occurred, then the plants may recover from the disease and regrow, also the field experiments showed IMY14 is a promising resistant line and it can be used as a source of resistance.

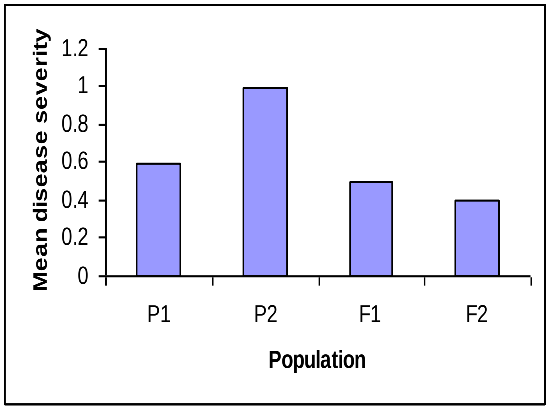
Regarding the cross of IMY1 and IM14 with Domain Sakel, result indicated that the genetic control of fusarium wilt resistance in these two parents is the same. This is due to the decreased disease severity (0.4) in the $_{F2}$ generation (Figures1&2) this resistance is expected to be controlled by two or more dominant genes. They are better than Barakat90 and Barakt S (Figures 3&4).

Likewise, the trend of disease severity in the $F_1$and F_2 generations for both crosses involving Barakat 90 and Barakat S with Domain Sakel (DS) is the same indicating that the genetic control of fusarium wilt resistance is the same. Moreover, due to the increased diseased severity (0.7) in the F_2 control of its resistance is expected to be by two or more genes.

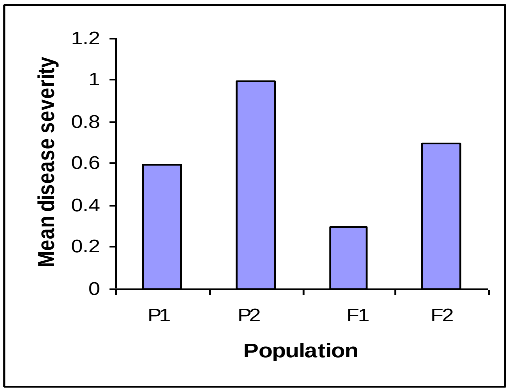
It is apparent that based on the high resistance obtained in the F_1 generation, there is a considerable degree of dominance is apparent for the control of this disease resistance. Also new resistance genes combinations might have occurred as a result of the induce mutation.

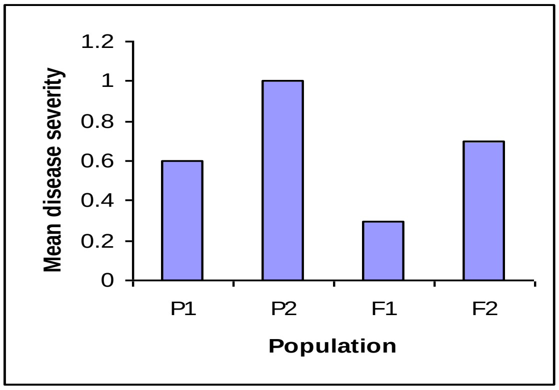
Accordingly, these lines may be considered as a new source of resistance to fusarium wilt. From the results of these crosses, it could be concluded that the resistance to fusarium wilt disease in the resistant parents used is controlled by two or more genes with a degree of dominance. In some cases modifiers or non allelic gene (s) interaction could have contributed to the resistance. It is clear that the gene for disease resistance resulting from spontaneous and induced mutations are different, more dominant and consistent.





Figures (1, 2): Mean disease severity in parental F_1 and F_2 population in the cross of IMY1 (P_1) × Domain Sakel (P_2) (left) and IM14 (P_1)× Domain Sakel (P_2) (right).





Figures (3, 4): Mean disease severity in parental F_1 and F_2 population in the cross of Barakat 90 (P_1) × Domain Sakel (P_2) (left) and Barakat S(P_1)× Domain Sakel (P_2) (right).

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تفاعل بعض سلالات القطن المصري ضد مرض الذبول الفيوزيرمى

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المستخلص:

الهدف من هذه الدراسة مسح و تقييم عشرة خطوط جديدة من القطن طويل التيلة و الأجيال الأولى و الثانية منها لمرض الذبول الفيوزيرمى والذى يسببه *Fusarium Oxysporum* f.sp *vainfectum* الذى يكمن في البذرة و ينتشر أيضآ بواسطة التربة و بقايا االنبات التي تحمل بالآلة و مياه الرى والفيضان . أجريت تجربة البيت المحمي و الحقل بمحطة بحوث الجزيرة خلال المواسم 2007– 2010. الخطوط و الأصناف المختبرة أعديت إصطناعيآ بإستعمال طريقة غمس الجذور في تجربة البيت المحمي و القطاع الموبؤ بالفطر المستعمل لإصابة البذرة المنزرعة فى تجارب الحقل. أوضحت النتائج أن الأصناف دومين ساكل, هدى و بركات التقليدية أكثر عرضة للإصابة, بينما الصنف بركات 90 أظهر مستوى عالي من المقاومة (33%). SP-21(44.4%), IS3 (50%), SPA-9 (50%), IS10-1 (44.4%) أظهرت مستوى متوسط من المقاومة. أعلى مستوى من المقاومة سجل في الأجيال الثانية للخطوط (37% IMY1 ,(33%) IM14. لم توجد مقاومة في الأجيال الثانية ل (76%) IS3 بينما أدنى مستوى مقاومة وضح في الجيل الأول ل (66.9% SPA-9. الأجيال الأولى -SP(55%) (55%), IM14(50%), IMY1 (60%),(42%) S Barakat ,IMB-9(57%)SP-9(58%) 7أظهرت مستوى متوسط من المقاومة كمثيلاتها IMP-9 (46%) (42%) IS-10, SP-21(50%) في الأجيال الثانية. الجيل الأول ل IS21 أعطى أيضآ مستوى متوسط من المقاومة (44%). أدنى مستوى مقاومة ظهر في الأجيال الأولى ل ,(79%) IS3(76%), IS10-1 IS-10 (66.7%) في بعض الأحيان قد يكون هنالك تداخل لبعض الجينات غير الأليلية التي تسهم في المقاومة أو المحورات الموجودة داخل التركيب الوراثي للنبات.واضح أن الجينات المقاومة للمرض الناتجة من الطفرات الذاتية وكذلك التي أحدثت إصطناعيآ كانت مختلفة وأكثر سيادة وثباتآ. من نتائج هذه التجارب السلالات التجريبية IM114 و IMY1 يمكن إعتبارها مصادر للمقاومة لأن المعاملة بأشعة قاما أعطت نتيجة إيجابية أفضل من بركات 90 وبركات S. توارث المقاومة لمرض الذبول الفيوزيرمي في الآباء المقاومة المستخدمة في هذه الدراسة يتحكم فيه جينان أو أكثر مع سيادة معتبرة.