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DEVELOPMENT OF MOLECULAR GENETIC MARKERS FOR ACREMONIUM WILT DISEASE RESISTANCE IN GRAIN SORGHUM

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ABSTRACT

Fourteen sorghum genotypes were evaluated for the resistance and susceptibility to Acremonium strictum fungus. Two extreme cultivars; Dorado (resistant) and Giza15 (susceptible) were selected and hybridized to obtain F_1 and followed by selfing to obtain (F_2) plants during the period from 2005 to 2008 in order to select the most resistant and the most suscebtable cultivars to Acremonium strictum, to study the effect of disease stress on the two contrasting cultivars and their F_1 and F_2 generations based on their performance for seven yield- related traits and to develop some molecular markers (RAPD, ISSR and SSR) associated with the resistance and the suscebtability to acremonium wilt disease using bulked segregant analysis (BSA). Analysis of variance revealed significant differences among all studied traits. A significant positive correlation was observed between some of the studied yield-related traits. The highest significant correlation (0.999) was observed between panicle weight and grain yield. Among the 128 amplified fragments across the two parents, their F_1 plants and the F_2 bulks of the most resistant and the most susceptible groups to Acremonium strictum, results produced scorable banding patterns with 11 RAPD primers out of the 20 used ones. All the eleven primers were polymorphic and showed a moderate percentage of polymorphism (48.44%). Some primers were very informative and generated molecular markers that related to the resistance against Acremonium strictum. OP-L20 Primer generated three markers related to the resistance with molecular sizes of 952, 576 and 474 bp. Moreover, OP-B09 and OP-O19 primers also generated one marker related to the resistance with molecular sizes of 201 bp for B09 primer and 392 bp for O19 primer. Only five primers out of the 15 ISSR used primers produced amplified fragments and showed polymorphism with the studied genotypes. It produced 33 amplified fragments across the two parents, their F_1 plants and the F_2 bulks of the most resistant and the most susceptible groups to Acremonium strictum. The five primers showed a high percentage of polymorphism (80.36%). Only one primer (17898B) exhibited negative molecular marker for susceptibility trait with molecular size of 180 bp. Eight out of ten used SSR primers produced 33 amplified fragments and showed a high percentage of polymorphism (91.67%). Only three primers exhibited negative molecular marker for susceptibility trait. The molecular sizes of these fragments were 182, 215 and 222 bp for Xtxp 1, Xtxp 6 and Xtxp 8 primers, respectively. Our investigation revealed that random***

amplified polymorphic (RAPD)-Polymerase chain reaction (PCR), inter simple sequence repeat (ISSR)-PCR and simple sequence repeats (SSR)-PCR are considered good molecular techniques to obtain molecular markers for grain sorghum resistance and susceptibility to Acremonium strictum that cause acremonium wilt disease. These techniques could be used as a tool for marker-assisted selection (MAS) in sorghum breeding programs directed to predict the resistant and susceptible genotypes to Acremonium strictum.

Key words: ***Sorghum, Acremonium wilt, Acremonium strictum, Resistance, Susceptibility, Correlation, Molecular Markers RAPD, ISSR, SSR***

INTRODUCTION

Grain sorghum (*Sorghum bicolor* L. Moench) is one of the most important cereal and forage crops in Egypt and all over the world. It occupies the third position among summer cereal crops after rice and maize in terms of acreage and total grain production in Egypt. It is usually grown in the southern governorates of Egypt from Giza to Aswan where the environmental conditions are relatively favorable for its production.

Grain sorghum serves as a staple food for a considerable population of Upper Egypt owing to its nutritional value, similar to that of maize. Dual purpose sorghum varieties are cultivated to satisfy livestock feed needs in the summer. In recent years, sorghum flour has been incorporated in wheat flour at various levels when wheat is in short supply to produce bread, biscuits and snacks .Grain sorghum also serves as a main source of starch in addition to its use in industrial fermentation (Ratnadass *et al* 2003). It is considered one of the most renewable sources for liquid fuel (ethanol) production especially with petroleum shortage.

The crop is vulnerable to various destructive fungal diseases, the most important of which is acremonium wilt disease, which causes considerable losses in yield and affects drastically grain quality. Acremonium wilt is a true vascular wilt disease incited by soil borne fungus *Acremonium strictum* W. Gams (*Cephalosporium acremonium* corda). The disease was first discovered in Egypt by El-Shafey *et al* (1979).

The causal fungus occupies the wood vessels in the roots stalks and leaves of the infected plants. The main symptoms of the disease are drying up of the leaf sheaths, reddish discoloration on the stalks and along leaf veins, followed by gradual drying and death of plants. Infection may reach 50% in the susceptible cultivars (Ali *et al* 2005).

Molecular markers offer specific advantages in assessment of genetic diversity and in trait-specific crop improvement. Use of molecular markers in the applied breeding programs can facilitate appropriate choice of parents involved for crosses.

The detection of molecular markers linked to many genes of interest has been widely achieved. In this concern, RAPD as a simple and rapid procedure has gained a worldwide acceptance and application (Paran *et al* 1991). Moreover, RAPD-PCR markers were detected for salt stress in wheat and maize, as reported by Bahieldin *et al* (1994) and Abdel-Tawab *et al* (2002), respectively. SSR or microsatellites are numerous and highly polymorphic in plants (Saghai Maroof *et al* 1994, Wang *et al* 1994, Rongwen *et al* 1995 and Yang *et al* 1995). The high information content of SSR makes them excellent genetic markers for many types of investigations, including marker-assisted selection. Michelmore *et al* (1991) developed the bulked segregant analysis of F_2 plants as a simpler alternative to isogenic line analysis where the highest and lowest extremes of the F_2 population are bulked for the development of RAPD and SSR molecular markers needed for QTLs-assisted selection.

The objectives of this study were to screen the responses of fourteen sorghum cultivars under infection fungal disease treatments, to select the most resistant and the most suscebtable cultivars to *Acremonium strictum* fungus to be hybridized in order to obtain (F_1) plants, followed by selfing to obtain (F_2) plants, to study the effect of disease stress on the two contrasting cultivars and their F_1 and F_2 generations based on their performance for seven yield- related traits and to develop some molecular markers (RAPD, ISSR and SSR) associated with the resistance and suscebtability to acremonium wilt disease in grain sorghum using bulked segregant analysis (BSA).

MATERIALS AND METHODS

MATERIALS

The present study was carried out in the research farm and laboratories of Cell Research Department (CRD), Field Crops Research Institute (FCRI), Agricultural Research Center (ARC) and the Department of Genetics, Faculty of Agriculture, Ain Shams University, Shoubra El-kheima, Cairo, Egypt, during the period from 2005 to 2008.

Selection of the most resistant and the most susceptible cultivars to *Acremonium strictum* fungus that cause acremonium wilt disease was done during the summer season of 2005 among 14 different sorghum genotypes. These genotypes are from local and exotic sources and obtained kindly from Sorghum Res. Dept., Field Crops Res. Inst., ARC. The names and sources of these cultivars are given in Table (1).

The seeds of these two sorghum cultivarss; Dorado (resistant) and Giza15 (susceptible), were sown at Giza during the summer season of 2006. Cross pollination was conducted between them in order to produce the F_1 seeds. The resulting hybrids were grown and selfed to obtain the F_2 seeds in the second season (the summer season of 2007). Sixty guarded individual plants were randomly taken from each experimental plot.

Table 1. Code numbers, names and sources of the tested fourteen sorghum cultivars under investigation.

Cod No	Cultivar name	Source	Cod No	Cultivar name	Source
1	BTX623	Texas-USA	8	ICSR 89053	ICRISAT-INDIA
2	BTX407	Texas-USA	9	ICSR 91022	ICRISAT-INDIA
3	BTX409	Texas-USA	10	ICSR 93004	ICRISAT-INDIA
4	BTX631	Texas-USA	11	Kuymne	Zambia
5	ICSR 89025	ICRISAT-INDIA	12	Shandaweel 6	Local variety (ATX 631x Dorado)
6	ICSR 89016	ICRISAT-INDIA	13	Giza 15	Local variety (G 123x G 114)
7	ICSR 89038	ICRISAT-INDIA	14	Dorado	Nebraska-USA

In the third season (the summer season of 2008), F_1, F_2 populations and the two parents were planted in a randomized complete block design with three replications. Each plot consisted of five ridges, 3 meter long spaced 0.7 m apart. Sowing was carried out in hills 20 cm apart along the ridges. Single plants from the F_1 and the two parents (Dorado and Giza15) and F_2 plants were evaluated under inoculation conditions with *Acremonium strictum* fungus. The following seven yield-related traits were recorded; plant height (cm), number of green leaves, leaf area (cm^2), panicle width (cm), panicle length (cm), panicle weight (g) and grain yield/plant (g).

METHODS

1. Field experiments

Method of inoculation was adopted as stalk inoculation technique by using the toothpick technique according to Young (1943). Two months old-field grown sorghum plants were used for inoculation. Controls were maintained in each case. One month later, the stalks were cut longitudinally and the degree of infection was determined using the rating scale adopted by IDIN- instructions manual as shown in Table (2) in order to determine the resistant and susceptible genotypes (at 2005) and to determine the resistant and the susceptible F_2 plants (at 2008).

The collected data were statistically analyzed according to Costat program and the correlations between the measured yield related-traits were estimated. The differences among means were compared using LSD new multiple range test.

Table 2. Rating scale adopted by IDIN- instructions manual.

NG	Degree of infection
0.1	Minimal reaction, indistinguishable from that to a sterile toothpick.
0.2	Discoloration centered about the wound, progressing in the superficial parts of the stalk, but not reaching either nodes.
0.5	Extensive discoloration progressing in the central part of the stalk.
0.8	Discoloration reaching one or both nodes superficially or forming a cylinder.
1.0	Most or all of one internode discolored with no penetration of nodal areas.
1.1	Slight penetration of one or both nodes.
1.2	Nearly complete penetration of one or both nodes,
1.5	Penetration of one node and slight invasion of next internode.
2.0	More than one internode but not more than two affected; infection must have spread through at least one internode.
2.5	Penetration of two nodes and slight invasion of distal internode.
3.0	Infection passed through two or more internode.
4.0	Extensive invasion of plant but not killed.
5.0	Death of plant due to stalk-rot.

NG= Numerical grade

2. Molecular markers analyses

The F_2 plants, represented by 200 individuals, were classified into six groups according to their resistance aganist acremonium wilt disease. Leaf samples of the two parents, their F_1 plants and the two extreme groups of the segregated F_2 plants (the most fifteen resistant and the most fifteen susceptible individuals) were used for further molecular analyses using bulked sergeant analysis (BSA) for the two F_2 groups.

DNA was extracted according to Junghans and Metzlatt (1990) from the two parents, the F_1 plants and the two F_2 groups. The extracted DNA was used to perform RAPD-PCR according to Williams *et al* (1990) using 20 arbitrary 10-mer primers (Operon Technologies, Inc) as presented in Table (3), ISSR-PCR according to Wang (2002) using 15 primers as shown in Table (4) and SSR-PCR using 10 primers as shown in Table (5). PCR products were analyzed on a 1.2% agarose gels and visualized using ethidium bromide under UV transilluminator [Alpha Ease FC (Alphimager^{Tm} 2200), Data were analyzed by diversity database version 2.1.1, USA].

Table 3. Names of the twenty used RAPD primers and their nucleotide sequences.

Primer name	Sequence	Primer name	Sequence
OP-A 19	5´ CAAACGTCGG 3`	OP-I15	5` TGCGGCTGAG 3`
OP-A10	5` GTGATCGCAG 3`	OP- B12	5` CCTTGACGCA 3`
OP-C 05	5` GATGACCGCC 3`	OP-L 20	5` TGGTGGACCA 3`
OP-C 08	5` TGTCTGGGTG 3`	OP-O20	5` ACACACGCTG3`
OP-C 15	5` GACGGATCAG 3`	OP-O19	5`GGTGCACGTTGG3`
OP-D 01	5` ACCGCGAAGC 3`	OP- D02	5` GGACCCAACC 3`
OP-D 07	5` TTGGCACGGG 3`	OP-O 15	5` TGGCGTCCTT 3`
OP-E 06	5` AAGAGAGGGG 3`	OP-O 20	5` ACACACGCTG 3`
OP-F 04	5` GGTGATCAGG 3`	OP- C12	5` TGTCATCCCC 3`
OP-I 17	5` AGCCTGAGCC 3`	OP- B09	5` TGGGGGACTC 3`

Table 4. Names and sequences of the fifteen used ISSR primers

Primer name	Sequence	Primer name	Sequence
814A	5'-TCTCTCTCTCTCTCTTG-3'	HBO9	5'-GTGTGTGTGTGTGG-3'
844A	5'-TCTCTCTCTCTCTCTAC-3'	HB1O	5'-GAGAGAGAGAGACC-3'
844B	5'-TCTCTCTCTCTCTCTGC-3'	HB11	5'-GTGTGTGTGTGTCC-3'
17898A	5'-CACACACACACAAC-3'	HB12	5'-CACCACCACGC-3'
17898B	5'-CACACACACACAGT-3'	HB13	5'-GAGGAGGAGGC-3'
17899A	5'-CACACACACACAAG-3'	HB14	5'-CTCCTCCTCGC-3'
17899B	5'-CACACACACACAGG-3'	HB15	5'-GTGGTGGTGGC-3'
HBO8	5'- GAGAGAGAGAGAGG-3'		

Table 5. SSR primer-pairs codes, their sequences and their required annealing temperature

Primers	Forward Sequences	Reverse Sequences	Suit Ann. temp
Xtxp1	TTGGCTTTTGTGGAGCTG	ACCCAGCAGCAGTACACTAC	55
Xtxp6	ATCGGATCCGTCAGATC	TCTAGGGAGGTTGCCAC	50
Xtxp8	ATATGGAAGGAAGAAGCCGG	AACACAACATGCACGCATG	50
Xtxpl0	ATACTATCAAGAGGGGAGC	AGTACTAGCCACACGTCAC	55
Xtxpl7	CGGACCAACGACGATTATC	ACTCGTCTCACTGCAATACTG	55
Xtxpl9	CTTTAATCGGTTCCAGAC	CTTCCACCTCCGTACTC	60
Xtxp37	AACCTAAGAGGCCTATTTAACC	ACGGCGACTATGTAACTCATAG	60
Xtxp75	CGATGCCTCGAAAAAAAAACG	CCGATCAGAGCGTGGCAGG	55
Xtxpl15	TTGTTTCGGTGACCAC	TATCTTTAAATTGCCTTTGTT	60
Xtxp231	GGAAATCCAGGATAGGGT	AGGCAAAGGGTCATCA	55

RESULTS AND DISCUSSION

1.Evaluation of acremonium wilt disease resistance

1.1. Field experiments

Evaluation of the resistance for the fourteen tested sorghum cultivars (Table 1) to *Acremonium strictum* was done and the results showed that Dorado cultivar was the most resistant cultivar, while Giza 15 cultivar was the most susceptible one.

The degree of infection was determined for the segregated F_2 plants using the rating scale adopted by IDIN- instructions manual (Table 2) and the frequency distribution of the symptoms was recorded as shown in Table (6).

The segregated F_2 individual plants were classified into six groups (Table 6). About 75% of the plant were resistant or highly and moderate resistant, whereas about 25% were susceptible or moderate.

The first appearance of acremonium wilt disease symptoms was appeared after the first half of the growing season as a pale green discoloration of the basal internodes with narrow yellowish to reddish streaks extending longitudinally on one side of the stalk and further developed to involve the whole internodes (Fig. 1A).

When these stalks were cut lengthwise, reddish to dark brown vascular bundles could be observed (Fig. 1B). Drying up of the leaf sheath and purple stripping along the leaf veins were the main symptoms of acremonium wilt disease (Fig. 1C).

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In severe cases of infection, no head formation was took place, and when formed, it was small with shrunken grains (Fig. 1D). In the late stages of growth, the plants began to dry and its color became dark brown and shrunken. These findings were in agreements with those of El-Shafey *et al* (1979). Natural *el al* (1982) reported acremonium wilt at that time as a new sorghum disease in the USA and distinguished it from the other diseases by large patches of dead tissues developed along the axis of a leaf. More recently, similar symptoms were recorded in a comparative study on some graminaceous plant diseases. The disease probably developed in susceptible sorghum plants wherever the environment favors infection (Khalefa 2000). Saba *et al* (2008) reported that H. 301 and H. 302 hybrids were resistant to acremonium wilt disease.

1.2. Yield–related traits associated with acremonium wilt disease resistance

The mean values of seven yield–related traits are shown in Table (7).

Concerning the two parents and the F_1 plants, Giza 15 (the susceptible parent) showed marked decrease in the mean values for all studied traits comparing with the resistant parent (Dorado) and the F_1 plants. Dorado and the F_1 plants exhibited nearly equal mean values for all studied traits, except panicle weight and grain yield traits, which were lower in the F_1 plants than the resistant parent. Concerning the F_2 plants, the most resistant and the most susceptible groups exhibited significant differences for all studied traits, which were lower in the most susceptible group than the most resistant one, except plant height and number of green leaves traits. Moreover, the most susceptible group showed zero values for both panicle weight and grain yield traits. These results indicated that there were clear differences between the resistant parent and the susceptible parent as well as between the most resistant F_2 group and the most susceptible F_2 group which were segregated from their contrasting parents.

Table 6. Category and frequency for the infestation with *Acremonium strictum* fungus that cause acremonium wilt disease for the 200 segregated F_2 plants.

No.	Category	Frequency		Level of infection
		Numerical	Percentage	
1	0.0-0.5	25	12.5	Highly resistant
2	0.6-1.0	55	27.5	Resistant
3	1.1-1.5	71	35.5	Moderately resistant
4	1.6-3.0	48	24	Moderately susceptible
5	3.1-4.0	1	0.5	Susceptible
6	4.1-5.0	0	0	Highly Susceptible
Total		**200**	**100**	

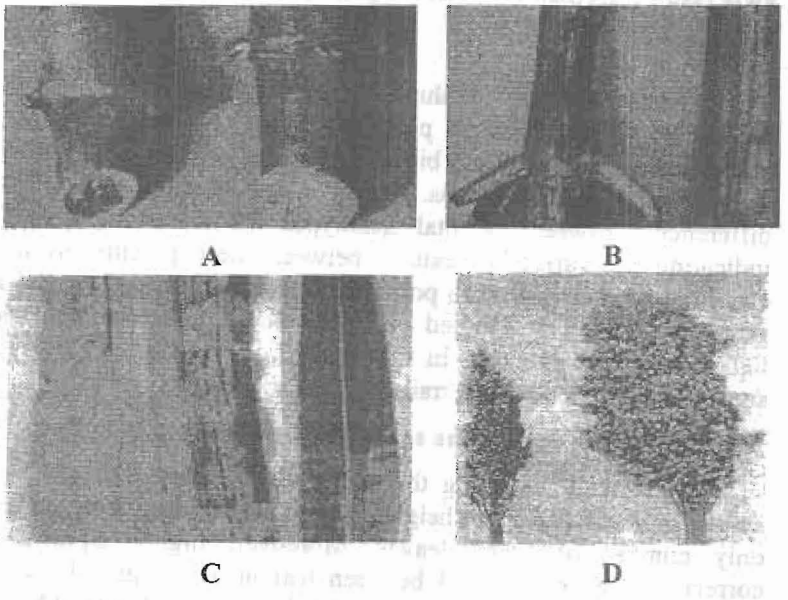

Fig. 1. Some symptoms on grain sorghum plant, which artificially infected with *Acremonium strictum* fungus (left). A healthy plant was shown on the right side in each picture.

Table 7. Mean values of the seven yield-related traits associated with the resistance against *Acremonium strictum* fungus in the two parents, their F_1 and F_2 plants.

Genotype	PH	NL	LA	PWI	GY	PW	PL
Giza 15 (P s)	130.00	12.47	6986.88	20.80	38.89	47.15	15.90
Dorado (P r)	252.33	19.60	11829.03	24.17	81.14	92.15	19.97
F_1	255.57	19.71	12388.90	24.60	71.26	81.80	19.08
F_2 (Resistant)	186.83	14.00	6969.03	23.80	60.87	71.24	15.97
F_2 (Susceptible)	204.77	18.53	3633.50	19.27	0	0	14.00
LSD 5%	43.07	3.95	2221.99	1.73	9.45	9.55	1.21
1%	61.26	5.61	3160.53	2.46	13.44	13.59	1.72

PH =Plant height (cm), NL= Number of green leaves, LA= Leaf area (cm^2), PWI= Panicle width (cm), PL= Panicle length (cm), PW= Panicle weight (g) and GY= Grain yield/plant (g).

Many authors evaluated two contrasting parents and their segregated F_2 population plants to detect some molecular markers associated with abiotic and biotic stresses as well as yield component and quality traits in these plants. However, their results reflected significant differences between parental genotypes for the studied trait(s) which indicating the variability existed between these parents. Moreover, they classified the segregated F_2 population plants to the highest and the lowest groups based on the studied trait(s) to develop molecular markers using bulked sergeant analysis. In this respect, Younis *et al* (2007) evaluated some salt tolerance-related traits in grain sorghum.

1.3. Correlation among the seven yield-related traits in F_2

Correlations among the seven yield-related traits in F_2 plants are shown in Table (8). Plant height showed highly significant correlation with only number of green leaves. Moreover, highly significant positive correlations were observed between leaf area and panicle width, panicle length, panicle weight and grain yield/plant (0,784, 0.890, 0.842 and 0.847, respectively), between panicle width and both of panicle weight and grain yield/plant (0.895 and 0.894, respectively), as well as, between panicle length and both of panicle weight and grain yield/plant (0.858 and 0.873, respectively). On the other hand, the highest significant correlation (0.999***) was observed between panicle weight and grain yield. These results indicated that leaf area affect grain yield/plant which was not found with plant height, as shown in Table (8).

Table 8. Correlations among the seven yield-related traits in sorghum F_2 plants.

Traits	PH	NL	LA	PWI	PL	PW
NL	0.828***					
LA	0.625*	0.526*				
PWI	0.490	0.243	0.784***			
PL	0.586*	0.372	0.890***	0.735**		
PW	0.428	0.117	0.842***	0.895***	0.858***	
GY	0.458	0.143	0.847***	0.894***	0.873***	0.999***

PH =Plant height (cm), NL= Number of green leaves, LA= Leaf area (cm^2), PWI= Panicle width (cm), PL= Panicle length (cm), PW= Panicle weight (g) and GY= Grain yield/plant (g). *significant at ($p<0.05$), ** significant at ($p<0.01$), and * significant at ($p< 0.001$) according to LSD analysis.**

The results reflected that plant height and number of green leaves had no correlations with the other studied traits, which indicated that these two traits had no association with the resistance against acremonium wilt disease. The mean values of the F_2 groups for the seven yield–related traits (Table 7) also indicated that plant height and number of green leaves had no association with the resistance for this disease where their mean values were higher in the most susceptible F_2 group than in the resistant F_2 group, while the mean values of the other five traits were lower in the most susceptible F_2 group than in the most resistant one.

2. Development of molecular markers associated with the resistance against acremonium wilt disease in sorghum

2.1. RAPD-PCR markers

Table (9) summarizes the number of generated fragments using eleven RAPD primers, which produce 128 amplified fragments across the two parents; Dorado (resistant) and Giza 15 (susceptible), their F_1 plants and the F_2 bulks of the most resistant and the most susceptible groups to *Acremonium strictum* fungus.

The results produced scorable amplified fragments with eleven primers out of the 20 used primers (Table 3). All primers were polymorphic and showed a moderate percentage of polymorphism (48.44%). The lowest number of polymorphic RAPD amplified fragments was detected by OP-A19 primer that generated only three polymorphic fragments with 21.43% of polymorphism, while the largest number was ten, which was generated by OP-D02 primer and gave the highest percentage of polymorphism (71.43%). Some primers were very informative and generated molecular markers that related to the resistance against *Acremonium strictum* in

Table 9. Total number of amplified fragments (monomorphic and polymorphic), polymorphism percentages and RAPD-markers generated for the resistance against *Acremonium strictum* fungus using eleven RAPD primers

Primer name	Total bands	Monomo-rphic bands	Polymo-rphic bands	Polymo-rphism %	RAPD- markers	
					resistant	susceptible
OP-A10	9	4	5	55.56	-	-
OP-A19	14	11	3	21.43	-	-
OP-	14	5	9	64.29	3	-
OP-B09	11	6	5	45.45	1	-
OP-D02	14	4	10	71.43	-	-
OP-O15	10	7	3	30.00	-	-
OP-O19	13	4	9	69.23	1	-
OP-O20	11	5	6	54.55	-	-
OP-I15	10	7	3	30.00	-	-
OP-B12	11	6	5	45.45	-	-
OP-L12	11	6	5	45.45	-	-
Total	128	65	63		5	-
Average	11.64	5.91	5.73	48.44%		

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sorghum. OP-L20 primer generated three markers related to the resistance against *Acremonium strictum*. Moreover, OP-B09 and OP-O19 primers, also generated one marker related to the resistance against *Acremonium strictum*. The molecular sizes of these five fragments were 952, 576 and 474 bp for L20 primer, 201 bp for B09 primer and 392 bp for O19 primer, as shown in Fig. (2). These five positive RAPD fragments could be considered as reliable markers for the resistance trait in grain sorghum. However, no marker was generated related to the susceptibility to *Acremonium strictum* fungus.

In conclusion, our investigation revealed that RAPD-PCR analysis is considered as a good molecular technique to obtain molecular markers associated with the resistance against *Acremonium strictum* fungus in sorghum. Moreover, RAPD molecular markers were more prominent, especially when applied to F_2 bulked plants. This technique could be used as a tool for marker-assisted selection (MAS) in sorghum breeding programs directed to predict the resistant genotypes against *Acremonium strictum*.

Tao *et al* (1993) reported that molecular markers such as randomly amplified polymorphic DNA (RAPD) was used to determine the frequency of DNA polymorphism in grain sorghum (*Sorghum bicolor* (L.) Moench). Hallden *et al* (1994) reported that RAPD markers are easier and quicker to

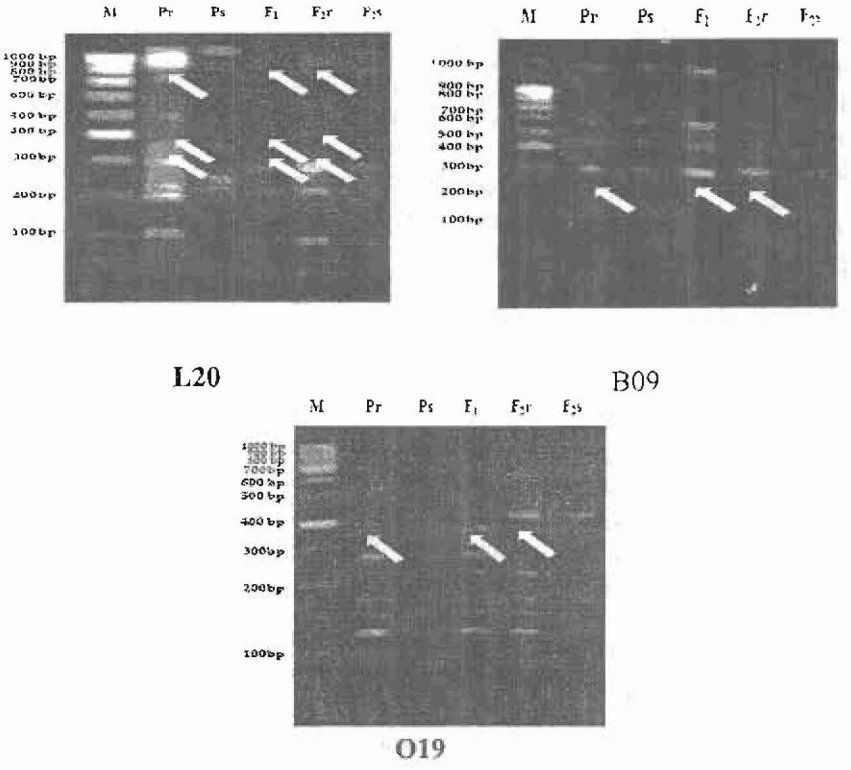


Fig. 2. Molecular markers associated with the resistance against *Acremonium strictum* fungus in sorghum generated by three RAPD primers (L20, B09 and O19) for the resistant parent (Pr), susceptible parent (Ps), the F_1 plants, the resistant F_2 bulk (F_2r) and the susceptible F_2 bulk (F_2s).

use. These markers may be preferred in applications where the relationships between closely related breeding lines are of interest.

Azzam *et al* (2007) found several molecular markers associated with pod rot resistance and susceptibility in peanut mutants and their parental cultivar by the RAPD primers.

Our results also agreed with those of Michelmore *et al* (1991), Pammi *et al* (1994) and Demeke *et al* (1997) in grain sorghum.

2.2. ISSR-PCR markers

DNA isolated from the two contrasting parents, their subsequent F_1 plants and the F_2 bulks of the resistant and susceptible groups plants and were tested against 15 preselected primers (Table 4). Only five primers, out of the 15 used, produced amplified fragments and showed polymorphism

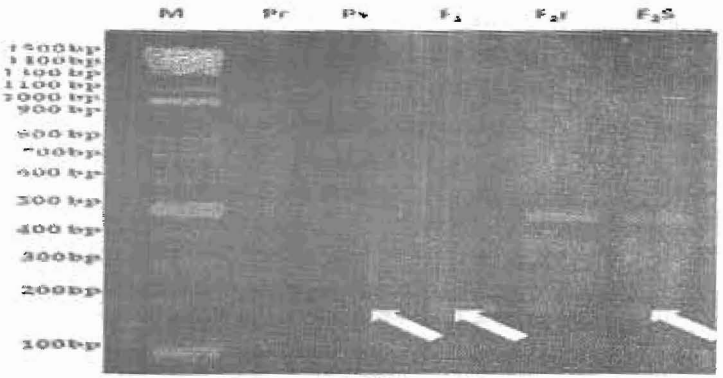
with the studied genotypes. Table (10) summarizes the number of generated fragments using five ISSR-primers, which produced 33 amplified fragments across the two parents, their F_1 plants and the F_2 bulks of the most resistant and the most susceptible groups to *Acremonium strictum*.

These five primers showed a high percentage of polymorphism (80.36%). The lowest number of polymorphic ISSR fragments was detected by HB-08 and HB-11primers (only four fragments with a 57.14% of polymorphism), while the largest number was seven and revealed a 87.5% of polymorphism, which was generated by HB-10 primer. Moreover, 17899B and 17898B primers showed a 100% of polymorphism, which did not produce any monomorphic fragments. No marker was related to the resistance against *Acremonium strictum* fungus, while 17898B primer exhibited a negative molecular marker for the susceptibility trait, which was found only in the susceptible parent (Giza 15), the F_1 plants and the susceptible F_2 bulk plants. The molecular size of this fragment was 180 bp and this negative ISSR marker could be considered as a reliable marker for the susceptibility trait in sorghum, as shown in Fig (3).

Table 10. Total number of amplified fragments (monomorphic and polymorphic), polymorphism percentages and ISSR-marker generated for the susceptibility to *Acremonium strictum* fungus using five ISSR primers

Primer name	Total bands	Mono-morphic bands	Poly-morphic bands	Poly-morphism %	ISSR- markers	
					resistant	susceptible
17899 B	6	-	6	100	-	-
17898B	5	-	5	100	-	1
HB-08	7	3	4	57.14	-	-
HB-10	8	1	7	87.5	-	-
HB-11	7	3	4	57.14	-	-
Total	33	7	26		-	1
Average	6.6	2.3	5.2	80.36%		

ISSR markers closely linked to important agronomic traits have greatly contributed to crop improvement. In chickpea, ISSR markers, $UBC855_{500}$ generated by primer $(AG)_8YT$ and $UBC825_{1200}$ using primer $(AG)_8T$, were linked to the gene conferring resistance to race 4 of *Fusarium* wilt (Ratnaparkhe *et al* 1998).



17898B

Fig. 3. A molecular marker associated with the susceptibility to *Acremonium strictum* fungus in sorghum generated by the ISSR primer; 17898B, for the resistant parent (Pr), the susceptible parent (Ps), the F_1 plants, the resistant F_2 bulk (F_2r) and the susceptible F_2 bulk (F_2s).

In conclusion, our investigation revealed that ISSR-PCR analysis is considered unsatisfactory molecular technique to obtain molecular markers for the resistance and susceptibility trait of grain sorghum.

Michelmore *et al* (1991), who stated that ISSR-directed approach in combination with bulked segregant analysis (BSA) has a wide application in plant and animal genome mapping.

2.3. SSR-PCR markers

DNA isolated from the two contrasting parents, their F_1 plants and the F_2 bulks of the most resistant and susceptible groups plants were tested against ten preselected primers as represented in Table (5).

Only eight primers, out of the ten used, produced amplified fragments, while six primers showed polymorphism with the studied genotypes. The eight primers produced 33 amplified fragments and showed a high percentage of polymorphism (91.67%). Only one polymorphic SSR fragment was detected with Xtxp75 primer, while only one monomorphic SSR fragment was detected with Xtxp17 and Xtxp 115 primers. Xtxp1, Xtxp 6, Xtxp 8, Xtxp10 and Xtxp 37 primers revealed a 100% of polymorphism. No detected marker was related to the resistance against *Acremonium strictum* fungus, while only three primers exhibited three negative molecular markers for the susceptibility trait, as shown in Table (11) and Fig. (4). They were found only in the susceptible parent (Giza 15), the F_1 plants and the susceptible F_2 bulk plants, while they were absent in the resistant parent (Dorado) and the resistant F_2 bulk plants. The molecular

sizes of these fragments were 182, 215 and 222 bp for Xtxp 1, Xtxp 6 and Xtxp 8 primers, respectively. These three negative SSR markers could be considered as reliable markers for the susceptibility trait in sorghum.

In conclusion, our investigation revealed that SSR-PCR analysis could be used as a tool for marker-assisted selection (MAS) in sorghum breeding programs directed to predict the susceptible genotypes to *Acremonium strictum* fungus. SSR-markers were positively generated for different crop plants. Our results also agreed with those of Abdel-Tawab *et al* (2001) who confirmed that SSR profiling is a helpful PCR- based technique for fingerprinting of sorghum inbred lines and for molecular mapping.

However, the results indicated that RAPD-PCR is considered as a good molecular technique to obtain molecular markers associated with the resistance against *Acremonium strictum* fungus in sorghum. ISSR and SSR techniques are better than RAPD technique to obtain molecular markers for the susceptibility to acremonium wilt disease in sorghum. Moreover, RAPD molecular markers were more prominent, especially when applied to the F_2 bulked plants. This technique could be used as a tool for marker-assisted selection (MAS) in sorghum breeding programs directed to predict resistant genotypes against *Acremonium strictum* fungus.

Table 11. Total number of amplified fragments (monomorphic and polymorphic), polymorphism percentages and SSR-markers generated for the susceptibility to *Acremonium strictum* fungus using eight ISSR primers

Primer name	Total bands	Mono-morphic bands	Poly-morphic bands	Poly-morphism %	ISSR- markers	
					resistant	susceptible
Xtxp1	2	-	2	100	-	1
Xtxp6	2	-	2	100	-	1
Xtxp8	2	-	2	100	-	1
Xtxp10	2	-	2	100	-	-
Xtxp17	1	1	-	0	-	-
Xtxp37	2	-	2	100	-	-
Xtxp75	2	1	1	50	-	-
Xtxp115	1	1	-	0	-	-
Total	14	3	11			3
Average	1.75	1.0	1.83	**91.67**		

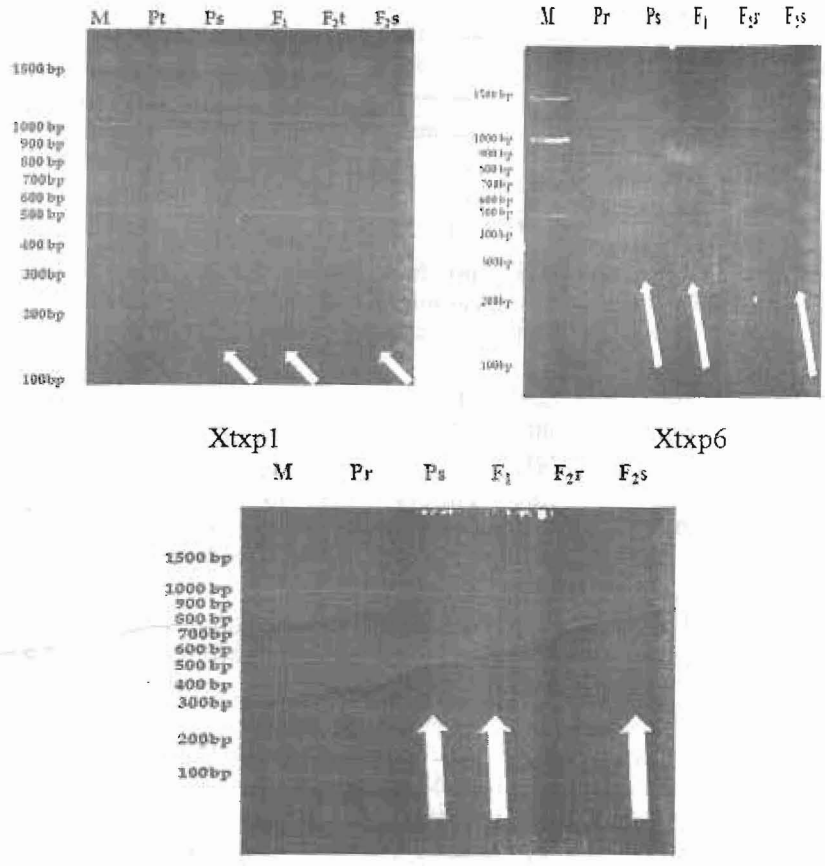


Fig. 4. Molecular markers associated with the susceptibility to *Acremonium strictum* fungus in sorghum generated by the SSR primers; Xtxp1, Xtxp6 and Xtxp8, for the resistant parent (Pr), the susceptible parent (Ps), the F_1 plants, the resistant F_2 bulk (F_2r) and the susceptible F_2 bulk (F_2s).

Our results were not in a harmony with those of Pradeep *et al* (2002) and Wang (2002) who stated that, coupled with the separation of amplification products on a agarose gels, ISSR marker amplification can reveal a much larger number of fragments per primer than RAPD marker. They concluded that ISSR technique provides a quick, reliable and highly informative system for DNA fingerprinting. Karp *et al* (1997) reported that ISSR markers are universal, easy, useful to develop gene tagging and useful to find markers linked to the gene of interest. ISSR marker has been proposed as a new source of genetic marker which overcomes the technical

limitations of RFLP and RAPD markers. In addition, Ratnaparkhe *et al* (1998) stated that ISSR-directed approach in combination with bulked segregant analysis (BSA) has a wide application in plant and animal genome mapping. It can be extremely useful in identifying the markers at clusters of disease resistance genes, filling large gaps in linkage maps, developing the sequence-tagged microsatellite sites and providing marker enrichment at desired region.

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استنباط كشافات وراثية جزئية للمقاومة لمرض عفن الساق فى الذرة الرفيعة

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أجريت هذه الدراسة في المعامل و المزارع التجريبية في كلاً من قسم بحوث الخلية – معهد المحاصيل الحقلية – مركز البحوث الزراعية ، الجيزة و قسم الوراثة ، كلية الزراعة ، جامعة عين شمس ، شبرا الخيمة . بهدف تقييم ١٤ صنف من الذرة الرفيعة للمقاومة لمرض عفن الساق باستخدام العدوى الصناعية واختيار أفضل صنف و أقل صنف في المقاومة لمرض عفن الساق وعمل التهجينات بينهما للحصول علي نباتات F_1 وعمل التلقيح الذاتي للحصول علي نباتات F_2 و دراسة الصفات المرتبطة بصفة المقاومة لمرض عفن الساق مثل (طول النبات (سم) ، عدد الاوراق/ نبات ، مساحة الاوراق (سم^2) ، عرض القنديل (سم) ، طول القنديل/نبات (سم) ، وزن القنديل/نبات (جرام)، وزن البذور/نبات (جرام) ، ودراسة الارتباط بينهما واستنباط بعض الكشافات الوراثية الجزئية باستخدام تقنيات : RAPD و ISSR وSSR للمساعدة في الانتخاب لصفة المقاومة و الحساسية لمرض عفن الساق. ويمكن تلخيص أهم النتائج المتحصل عليها كالتالي : أظهر الصنف دورادو مقاومة لمرض عفن الساق بينما الصنف المصري جيزة ١٥ كان شديد الحساسية لمرض عفن الساق لذا فقد اعتبرا أكثر الأصناف تباينا في المقاومة و الحساسية لمرض عفن الساق فى الدراسة. تمت دراسة سبعة صفات مرتبطة بصفة المقاومة لمرض عفن الساق في نباتات الذرة الرفيعة للابوين والجيل الهجينى الأول والجيل الأنعزالي الاول المجمع (المقاوم والحساس) بأستخدام العدوى الصناعية بالفطر. أظهر تحليل

التباين وجود اختلافات معنوية بين كل الصفات المدروسة. كما أظهرت نتائج الارتباط بين كل الصفات المدروسة خلال نباتات الجيل الانعزالي الاول وجود ارتباط موجب بين كثير من الصفات المدروسة. ووجد ان اعلى ارتباط معنوى كان بين وزن القنديل ومحصول الحبوب (**٠.٩٩٩). وقد نجح لحد عشر بادئ لل RAPD من السعشرون المستخدمة في تكوين تضاعف للحزم للخمسة عشائر تحت الدراسة: الصنف دورادو (المقاوم) والصنف جيزة ١٥ (الحساس) والجيل الهجينى الأول والجيل الانعزالي الأول المجمع (المقاوم والحساس). وكانت نسبة الصور المظهرية المتعددة ٤٨.٤٤ %. كما نجحت بعض البادئات فى اعطاء كشافات وراثية جزئية مرتبطة بصفة المقاومة لمرض عفن الساق في الذرة الرفيعة. نجح البادئ OP-L20 في استنباط ثلاث كشافات للمقاومة. أما البادئان OP-O19،B09 فقد نجح كلا منها فى استنباط كشاف واحد مرتبط بصفة المقاومة لمرض عفن الساق. نجحت خمس بادئات من الخمسة عشر المستخدمة من بادئات الـ ISSR في تكوين تضاعف للحزم حيث أظهرت ٣٣ حزمة متضاعفة للخمسة عشائر تحت الدراسة. وكانت نسبة الصور المظهرية المتعددة ٨٠.٣٦ % . وقد نجح البادئ 17898 B في استنباط كشاف وراثي مرتبط بالحساسية تجاه المرض فى الذرة الرفيعة. نجحت ثمانى بادئات من العشرة المستخدمة من بادئات الـ SSR في تكوين تضاعف للحزم حيث أظهرت ١٤ حزمة متضاعفة وكانت نسبة الصور المظهرية المتعددة ٩١.٦٧ % . وقد نجح كل من البادئ Xtxp8, Xtxp6 ,Xtxp1 في استنباط كشاف وراثي واحد مرتبط بالحساسية تجاة المرض فى الذرة الرفيعة. أظهرت النتائج إمكان استخدام الكشافات الجزيئية باستخدام كل من تقنية الـ RAPD و الـ ISSR و الـ SSR فى تحديد اصناف الذرة الرفيعة المقاومة و الحساسة لمرض عفن الساق بحيث يمكن الكشف المبكر بين اعداد كبيرة من الاصناف او السلالات والتركيز على الاعداد المبشرة القليلة منها للمساعدة فى برامج التربية الخاصة بالمقاومة و الحساسية لمرض عفن الساق فى الذرة الرفيعة.