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DETECTION OF MOLECULAR MARKERS ASSOCIATED WITH IRON DEFFICIENCY IN SORGHUM *(Sorghum bicolor L.)* USING BULKED SEGREGANT ANALYSIS

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ABSTRACT: Two sorghum cultivars; TX 430 (iron defficiency tolerant) and IS 2219B (iron defficiency sensitive); were crossed to obtain F_1 plants that were selfed to obtain F_2 plants. Genomic DNA was extracted from the two constracting parents and their F_1 and F_2 (extreme tolerant and sensitive plants) in an attempt to find some DNA markers correlated with iron defficiency by means of bulked segregant analysis technique (BSA). Randomly ampilified polymorphic DNA of the polymerase chain reaction (RAPD – PCR) was conducted using nine 10-mer primers. Using of the primers OP–A01, OP-A02, OP-A03, OP-A04 and OP-A06; one DNA fragment amplified for the tolerant parent and the tolerant F_2 bulk. Such a DNA fragment did not appear in the sensitive parent, F_1 and sensitive F_2 bulk. These RAPD markers could be considered as reliable positive molecular markers linked with iron defficiency tolerance in sorghum i.e., OP-A01, OP-A02, OP-A03, OP-A04 and OP-A06.

Key words: **Iron, defficiency, tolerance, sorghum, DNA, RAPD-PCR.**

INTRODUCTION

Although *Sorghum bicolor L.* is a species of main economic importance among the cereal crops cultivated in countries of tropical climates, information regarding its genome organization and mapping

are limited. Moreover, sorghum is commonly used for human consumption and animal feed in many countries of the world. It is an important crop in the semiarid tropics that also receives growing attention in genetic research (Haussmann *et al.*, 2002). Iron is essential for hemoglobin construction, nitrogen fixation, photosynthesis and electron transfer reactions. It is a part of the respiratory enzymes and cytocromes. Loeppert *et al.*, 1994. Zou *et al.*, 1998 reported that iron defficiency inhibits protein synthesis in young leaves, leading to a decrease in protein content of seeds. In addition, it is observed that the nutrients mineral such as Fe and Zn are involved in the catalytic function of many enzymes (Bar-Akiva, 1984; Sijmons *et al.*, 1985). The concept of differential variety response to mineral nutrition in higher plants is well established (Ckark *et al.*, 1990 and Brown *et al.*, 1991). It is now generally accepted that most of these variations are genetically controlled (Clark and Brown 1980). In recent years and following the introduction of molecular markers in plant genetic research, considerable effort has been made to gain a better understanding of sorghum genetics and evolution and important information have been gathered. The advent of PCR-based molecular marker techniques, such as RAPDs (randomly amplified polymorphic DNAs)., has further facilitated to the analysis of sorghum genome (Williams *et al.*, 1990 and Pammi *et al.*, 1994). In addition, RAPD assay detects nucleotide sequence polymorphisms by means of PCR and a single primer of arbitrary sequence to assess genetic diversity in different crops(He *et al.*, 1992; Tao *et al.*, 1993; Menkir *et al.*, 1997; Abdel-Tawab *et al.*, 1998, 2001, 2002 and 2003; Ayana *et al.*, 2000; Dinker *et al.*, 2000; Al-Shabi *et al.*, 2002; Haussmann *et al.*, 2002; Nkongolo and Nsapata 2003; Uptmoor *et al.*, 2003, and Abeer Abdel-Bary 2004).

Michelmore *et al.*, 1991 developed the bulked segregant analysis of F_2 plants as a simple alternative method to isogenic line analysis where the highest and lowest extremes of the F_2 population are bulked for the development of RAPD molecular markers needed for QTLs assisted selection. Most of the detected linkage between marker loci and

quantitative traits were shown to be highly significant (Edwards *et al.*, 1987). The main objectives of this study were to detect the molecular genetic markers RAPD associated with iron defficiency tolerance in sorghum varieties using bulked segregant analysis (BSA) through PCR technique.

MATERIALS AND METHODS

Materials

Two sorghum varieties, one is considered as the most tolerant for iron defficiency stress (TX 430-Egypt), and the other representing as most sensitive one (IS 2219 B-Egypt) were chosen after a study done by Al-Shabi *et al.*, 2002. These two cultivars were grown in the field and crossed to obtain the F_1 grains and the F_1 grains were sown in the field and selfed to obtain the F_2 grains. The two sorghum cultivars, F_1 and F_2 grains were sown in a sand culture experiment in plastic pots which were filled with pre-washed fine sand (with 0 mg/L iron concentration). Plants were irrigated with Hoagland and Arnon solution Table 1-a and b every three days. Upon iron defficiency symptoms appearance (45 days after planting) four vegetative traits related to iron defficiency, i.e., leaf length, leaf width, total number of leaves/plant and shoot weight/plant were measured for 257 F_2 plants. These previous traits can be used as a parameter to evaluate the effect of iron defficiency on sorghum plants (Al-Shabi *et al.*, 2002). The two parents, their F_1 and the two extremes for iron defficiency performance in the F_2 were taken for further molecular genetic analysis using bulked segregant analysis technique according to Michelmore *et al.*, 1991.All field and laboratory procedures wrer done at the Farm and Bio.tec-Hnological Lab., Fac. Agric.Ain shams Univ. during 2001 2004.

Methods

Randomly amplified polymorphic DNA (RAPD) analysis were conducted for the two parents (TX 430 and IS 2219 B), their F_1 hybrid and the more sensitive for iron defficiency as well as the more tolerant F_2 groups to detect molecular markers for iron defficiency in sorghum. Total genomic DNA was extracted according to the method of Dellaporta *et al.*, 1993. PCR was performed in 30 Ml volumes tubes

according to Williams *et al.*, 1990. The amplification was carried out in a Hybrid PCR Express Programmed for 47 cycles as follows: 94 C°/2 min (1 cycle) ;94C°/1 min., 35 C°/1.5 min., 72C°/2 min. (45 cycles); 72C°/5 min.(1 cycle); 4C° infinitive. Nine 10-mer primers were used for RAPD analysis These primer sequences, are listed in Table 2.

The electrophoretic run was performed and DNA fragment bands were detected and photographed and then scanned with Bio-Rad densitometer modle G20 at a wave length of 577 nm. Software data analysis for Bio-Rad model 620 USA densitometer and computer were used.

RESULTS AND DISCUSSION

Bulked segregant analysis (BSA) developed by Michelmore *et al.*, 1991 was adopted in this study to develop RAPD markers for identification of iron-defficiency tolerance in sorghum. The DNA bulks of the two extremes of F_2 genotypes, F_1 and their parents (TX 430 and IS 2219 B) were analysed against nine 10-mer random primers: Only five out of these nine primers developed molecular markers for iron defficiency in sorghum. These five primers named; OP-A01, OP-A02, OP-A03, OP-A04 and OP-A06. The total number of bands which developed through each of five primers were 8, 8, 8, 13 and 7, respectively. Banding patterns of the five primers were illustrated in Figure 1 and scored as present (+) or absent (-) as shown in Table 3. In addition, as shown in Table 3, bands of high and low molecular sizes were scored. All of 44 bands were detected with the five primers. Five RAPD markers were detected with molecular size of 1980 bp, 333 bp, 1343 bp, 1691 bp and 2355 using the five primers respectively (Table 4). In present investigation, primers OP-A01, OP-A04 and OP-A06 developed one band which appeared in the tolerant parent and in the tolerant F_2 bulk, while it was absent in the sensitive parent, F_1 and F_2 sensitive bulk. In run of primers OP-A02 and Op-A03, one molecular band was detected also for each which appeared in the tolerant parent, F_1 and in the tolerant F_2 bulk, while it was absent in sensitive parent and sensitive F_2 bulk.(Figure 1 and Tables 3 and 4). These five RAPD

markers could be considered as reliable positive molecular markers associated with iron defficiency tolerance. No negative markers linked with iron defficiency were detected in this study. Regarding the detection of molecular markers associated with iron defficiency, a few numbers of studies were reported in the literature. By means of RAPD–PCR technique, Al-Shabi *et al.*, 2002 reported that five random 10-mer primers out of nine (A01, A03, A04, A07, A10) succeeded in differentiating the most sensitive from the most tolerant sorghum cultivars for iron and salt stresses. The result of the present investigation agreed partially with the findings of Al-Shabi *et al.*, 2002. Primers A01, A03 and A04 exhibited one positive molecular marker linked with iron defficiency. On the other hand, many reports detected RAPD markers for salt or drought tolerance, identify varieties and to assess genetic diversity in different crops (He *et al.*, 1992; Menkir *et al.*, 1997; Abdel–Tawab *et al.*, 1998, 2001, 2002 and 2003; Nkongola and Nsapta 2003 and Abeer Abdel–Bary, 2004).

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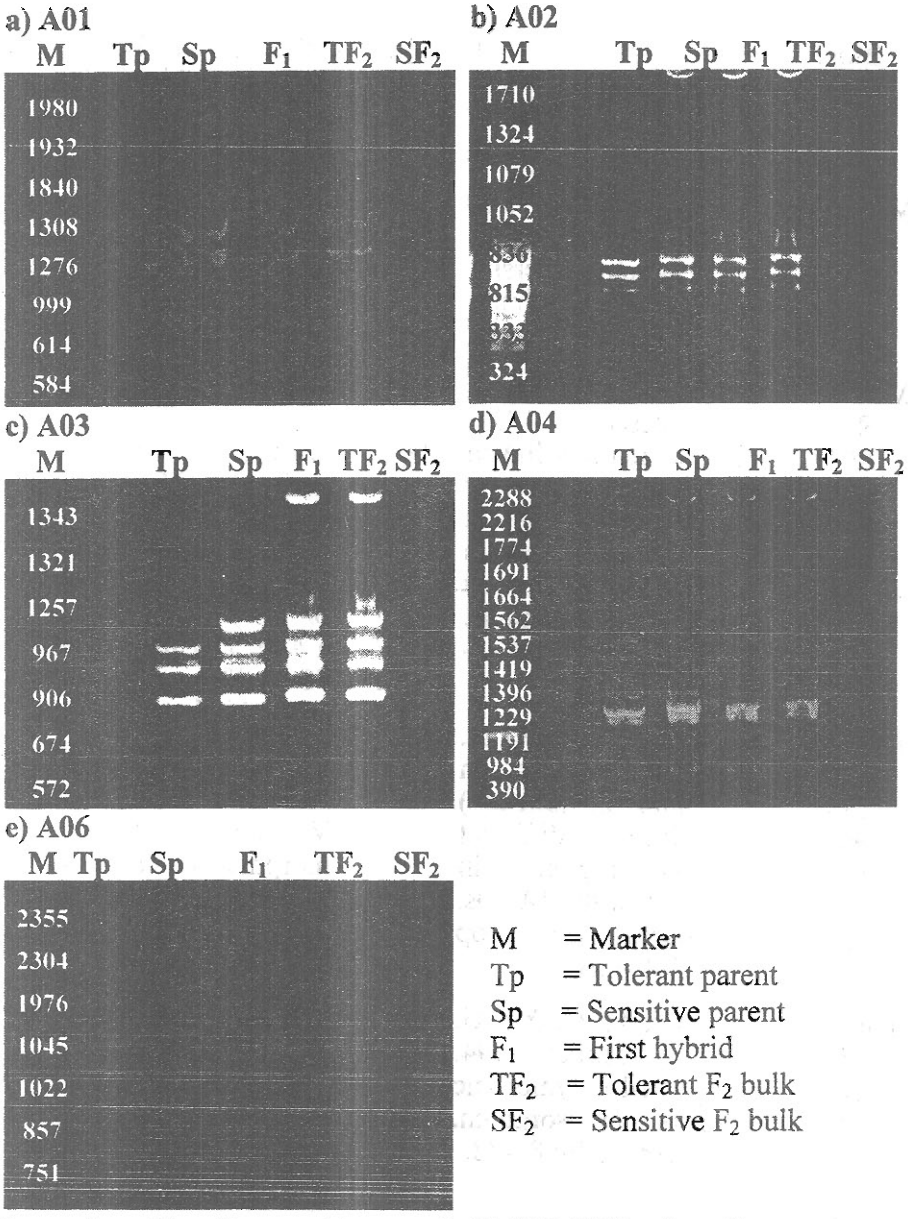

Figure 1 : Banding patterns of RAPD-PCR for five primers sorghum parents, F_1 and bulked tolerant and sensitive F_2 using primers for iron defficiency.

Table 1 : The composition of macronutrient (a) and micronutrient (b) solution according to Hoagland and Arnon nutrient solution.

a.

Salt	Concentration of ion expressed as mg/L						g/l
	Cations			Anions			
	Ca^{++}	Mg^{++}	K^{+}	NO_3^-	$SO_4^=$	H_2PO_4	
$Ca(NO_3)_2 \cdot H_2O$	10	--	--	10	--	--	1.180
KNO_3	--	--	5	5	--	--	0.505
$MgSO_4 \cdot 7H_2O$	--	4	--	--	4	--	0.492
KH_2PO_4	--	--	1	--	--	1	0.136
Total	10	4	6	15	4	1	

b.

Boric acid (H_3BO_3)	2.85
Manganese chloride ($Mncl_2 \cdot 4H_2O$)	1.81
Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)	0.22
Copper sulphate ($CuSO_4 \cdot 5H_2O$)	0.08
Ammonium molebdate ($NH_4)_2O \cdot 4Mo_3$	0.02
Ferric EDTA	6.92

Table 2: The nucleotide sequence of the primers used in this study .

Primer Code	Base Sequences
OP-A01	5′CAGGCCCTTC3′
OP-A02	5′TGCCGAGCTG3′
OP-A03	5′AGTCAGCCAC3′
OP-A04	5′ATTCGGGCTG3′
OP-A05	5′AGGGGTCTTG3′
OP-A06	5′GGTCCCTGAC3′
OP-A07	5′GAAACGGGTG3′
OP-A09	5′GGGTAACGCC3′
OP-A10	5′GTGATCGCAG3′

Table 3 : Banding patterns for RAPD-PCR of the two sorghum parents, F_1 and F_2 for iron defficiency sensitivity using the five primers OP-A01, OP-A02, OP-A03, OP-A04 and OP-A06.

a. A01

Band no.	(bp)	TP	SP	F_1	TF_2	SF_2
1	1980					
2	1932	-	+	-	-	-
3	1840	-	-	+	-	-
4	1308	+	+	-	+	-
5	1276	-	-	+	-	-
6	999	-	-	+	+	-
7	614	+	+	-	-	-
8	584	-	-	+	+	-

b. A02

Band no.	(bp)	TP	SP	F_1	TF_2	SF_2
1	1710	-	-	-	+	-
2	1324	-	-	-	+	-
3	1079	-	-	+	+	-
4	1052	-	-	-	+	-
5	836	-	-	+	-	-
6	815	-	+	-	+	-
7	333					
8	324	-	+	-	-	-

c. A03

Band no.	(bp)	TP	SP	F_1	TF_2	SF_2
1	1343					
2	1321	-	+	-	-	-
3	1257	+	-	+	+	-
4	967	-	+	-	-	-
5	906	-	+	+	-	-
6	674	+	+	+	-	-
7	572	+	-	-	-	-
8	470	-	-	+	-	-

Table 3 : Continued.

d. A04

Band no.	(bp)	TP	SP	F_1	TF_2	SF_2
1	2288	+	+	-	-	-
2	2216	+	-	-	-	-
3	1774	-	+	-	-	-
4	1691					
5	1664	-	+	-	-	-
6	1562	-	-	-	+	-
7	1537	+	+	-	-	-
8	1419	-	-	-	+	-
9	1396	-	-	-	+	-
10	1229	-	+	-	-	-
11	1191	+	-	-	-	-
12	984	-	+	-	-	-
13	390	-	-	+	+	-

e. A06

Band no.	(bp)	TP	SP	F_1	TF_2	SF_2
1	2355					
2	2304	-	+	-	-	-
3	1976	+	-	-	-	-
4	1045	-	+	+	+	-
5	1022	+	-	-	-	-
6	857	-	+	+	-	-
7	751	-	-	-	+	-

Table 4: List of RAPD positive markers for iron deficiency tolerance detected with the 5 primers .

Primer code	(bp)	TP	SP	F_1	TF_2	SF_2
A01	1980					
A02	333					
A03	1343					
A04	1691					
A06	2355					

+ = presence of band, - = absence of band, TP = tolerant parent, SP = sensitive parent, F_1 = first generation, TF_2 = tolerant F_2 and SF_2 = sensitive F_2.

تحديد المعلمات الوراثية الجزيئية المرتبطة بنقص الحديد فى السورجم (*Sorghum bicolor L.*) باستخدام اختبار تحليل الانعزالات المتفارقة

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أجرى هذا البحث بغرض تحديد الدلالات الجزيئية المرتبطة بتحمل نقص عنصر الحديد فى السورجم . تم أختبار مستخلص الـ DNA للطرز الوراثية لكل من الأب الحساس IS 2219B والأب المتحمل TX 430 وكذلك لنباتات الجيل الأول والجيل الثانى (النباتات المتحملة والحساسة لنقص الحديد) الناتجة من التهجين بينهما . وقد تم استخدام تقنية تفاعل البلمرة المتسلسل RAPD – PCR مع ٩ بادئات عشوائية وذلك من خلال أختبار تحليل ضم الانعزالات المتفارقة. وقد أظهر كل بادئ من الخمسة بادئات : OP-A01 , OP-A02 , OP-A03 , OP-A04 and OP-A06 حزمة واحدة مميزة لتحمل نقص الحديد حيث ظهرت هذه الحزمة فى كل من الأب المتحمل TX 430 وفى مجموعة نباتات الجيل الثانى المتحمل بينما غابت هذه الحزمة فى كل من الأب الحساس IS 2219B وفى مجموعة نباتات الجيل الثانى الحساسة لنقص عنصر الحديد وبذلك يمكن اعتبار هذه الحزم من الكشافات الجزيئية الموجبة والمرتبطة بتحمل نقص عنصر الحديد فى السورجم .