# SELECTION OF RESISTANCE TO ROOT-ROT DISEASE CAUSED BY *Fusarium Graminearum* IN WHEAT CULTIVARS BY DNA MARKERS

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**ABSTRACT**

*Resistance to Fusarium graminearum in ten wheat cultivars was investigated under artificial inoculums in two field experiments during 2005/2006 and 2006/2007 winter seasons at the Farm of College of Agriculture and Veterinary Medicine, Al-Qassim University, Saudi Arabia. The results indicated that wheat cultivars showed different characteristic response when inoculated with F. graminearum. In general, Sids 1 and Giza-164 cultivars produced the highest grain yield. While, Bani Swef 3 and Bani Swef 1 cultivars produced the lowest grain yield. Also, the latter cultivars gave the lowest harvest index. Wheat cultivars, Sama, Giza-164 and Sids 1, were the tallest plants. Moreover, there were significant differences in grain yield components among wheat cultivars. The results, also, indicated that Shandawel, Giza 168, Yocra Rojo, Sakha 61, Sakha 8 and Bani Swef 1 cultivars were the most susceptible cultivars to root rot disease. While, Sama and Bani Swef 3 were consistently moderate cultivars. On the other hand, Sids 1 and Giza-164 wheat cultivars were the most resistant cultivars. Specific-PCR assay was performed on DNA extracts of stem bases of wheat cultivars.*

*Specific primer SCAR-85 revealed the presence of F. graminearum in wheat cultivars, except for Sids 1 and Giza-164 cultivars. These cultivars were the most resistant ones. Moreover, it was shown that Sids 1 cultivar had the dehydrin gene and showed the most resistant cultivar to root rot disease, whereas, Giza 164 was the most resistant cultivar to root rot disease, but did not amplify the dehydrin gene. The work, presented in this paper, illustrated that SSR marker for amplifying dehydrin gene, in conjunction with SCAR marker for detection of Fusarium infection, could be a valuable and versatile tool for diagnosis and screening of wheat breeding material for resistance to F. graminearum under drought stress.*

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is grown over a wide area in the world than any other major crop. It is the principal food for nearly one-third of the world population (Wiese, 1987). *F. graminearum* infected wheat plants and caused pre-and postemergence damping- off on seedlings, crown and foot rot and head blight (Ibrahim, 1997 and Schisler *et al.*, 2002). Fusarium head blight (FHB) is a major problem in cereal production worldwide, with wheat and barley losses of more than $ 3 billion estimated for a 6- year period in the 1990s in the United States alone (Browne *et al.*, 2005). Also, wheat production is limited by the availability of water resources. Plants have developed different strategies to face water deficit and over the past few years much attention has been focused on the identification of genes induced in response to environmental stress (Zhu, 2002). Inheritance studies, especially QTL analysis, in several crop plants have revealed apparent co-segregation of dehydrin genes (*HVA*) with phenotypes associated with dehydrative stress, such as drought (Sivamani *et al.*, 2000). Water stress may, also, cause the development of certain diseases associated with plant stress. One such disease is foot rot (also, referred to as root and crown rots) disease of wheat. Complex communities of fungal pathogens have been reported in association with foot rot disease of small grain cereals and grasses worldwide (Smiley and Patterson, 1996 and Schilling *et al.*, 1996). These pathogens include several *Fusarium* species, such as *F. culmorum*, *F. graminearum* and *F. avenaceum* (Schilling *et al.*, 1996), as well as other pathogenic fungi, including *Bipolaris sorokiniana* (Duczek, 1993), *Microdochium nivale* (Rossi *et al.*, 1995) and *Rhizoctonia solani* (Fouly *et al.*, 1996).

Diagnostic methods for identifying Fusarium species are based on cultural and morphological characteristics observed on selective media (Burgess *et al.*, 1994). Considerable expertise is required to differentiate and identify closely related Fusarium species, especially *F. graminearum* and *F. culmorum*. Recently, the utilization of molecular markers for species specific detection assays has become very popular (Henson and French, 1993). Based on polymerase chain reaction (PCR), highly sensitive diagnostic assays have been successfully implemented for the identification and detection of the major fungi involved in Fusarium ear blight of wheat (Doohan *et al.*, 1998 and Nicholson and Parry, 1996). Wigleswoth *et al.* (1994) isolated a distinct fragment of randomly amplified polymorphic DNA(RAPD) of *Peronospora tabacina*, representing a repetitive sequence. Using specific primers, the amplification of this sequence enabled the detection of minute amounts of fungal DNA in plant tissues (Wiglesworth *et al.*, 1994). This approach of sequence-characterization RAPD fragments (SCARs) was first applied by Paran and Michelmore (1993) to detect downy mildew resistance genes in lettuce.

The objectives of this study were to (1) evaluate ten wheat cultivars for their productivity and resistance to root rot disease, (2) develop SCAR marker for the detection of *F. graminearum* in such wheat cultivars and (3) investigate the presence of the dehydrin gene as a marker of drought tolerance in wheat cultivars.

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## MATERIALS AND METHODS

**Field trials:**

Field experiments were carried out at the Farm of College of Agriculture and Veterinary Medicine, Al-Qassim University, Saudi Arabia, during 2005/2006 and 2006/2007 winter seasons. Ten cultivars of wheat (Sama, Yocra Rojo, Sids 1, Sakha 8, Sakha 61, Giza 164, Giza 168, Bani Swef 1, Bani Swef 3 and Shandawel) were sown on December 1st and 15th 2005 and 2006, respectively, with a seeding rate of 140 kg/ha and inoculated with *F. graminearum*. A randomized complete block design, with three replicates, was used. The plot size was 4x3 $m^2$ with row to row spacing of 25 cm. The recommended fertilizer requirements of wheat, as NPK, were 200, 200 and 100 kg/ha, respectively, for a growing season of 120 days of wheat, according to Bashour and Al-Jaloud (1984).

At harvesting time, ten plants were randomly chosen to measure plant height, 1000- grain weight and the number of grains per spike. Also, harvest index and grain yield per square meter were recorded.

**Disease evaluation:**

Disease rating was conducted at the jointing stage of wheat plants. Five random plants from the four middle rows (total of twenty plants) of each treatment were sampled for disease evaluation.

Disease severity was estimated, according to the method recommended by Piccini *et al.* (2000). Plants were gently removed from the soil and washed thoroughly in tap water. The disease was rated on the subcrown internodes on a 0 to 4 scale, where : 0= no visible symptoms, 1= 1 to 25% discoloration, 2= 26-50% discoloration, 3= 51-75% discoloration and 4= 76% or more of the subcrown internodes discolored. Disease index (DI) was calculated, according to the following formula:

$$DI = \frac{\{(X1 \times 1) + (X2 \times 2) + (X3 \times 3) + (X4 \times 4)\}}{(X0 + X1 + X2 + X3 + X4)}$$

Where, (X0 + X 1 + X 2 +X 3 and X 4 ) were the number of plants having disease rating of 0, 1, 2, 3 and 4, respectively.

**DNA extraction :**

Total genomic DNA of wheat cultivars was extracted, using the method described by Saghai-Maroof *et al.* (1984) for dehydrin gene.

DNA of Fusarium was extracted from 2- cm stem base sections of freeze- dried plant samples, using the protocol of Doohan *et al.* (1998). Dried plant materials were ground for 10 min. in a mixer/mill 800), using steel ball bearings and DNA was extracted in 9 ml CTAB (hexadecyltrimethylammonium bromide) buffer (CTAB 8 g, sarkosyl 10 g, sorbitol 25 g, Na Cl 47 g, EDTA 8 g, polyvinyl polyplrolidone (PVPP) 10g in IL H2O) at 65 °C , for 2 h, together with 15 µl proteinase k (10 mg/ml). Following incubation, an equal volume of chloroform/octanol (24:1) was added to the tubes mixed and centrifuged at 2600 g for 15 min. The aqueous phase was removed to a fresh tube and an equal volume of ice-cold isopropanol was added, followed by centrifugation, as above, to precipitate the DNA. The pellet was washed in 70% ethanol and dissolved in TE buffer (10 mM Tris-Hcl pH 8.0, 0.1mM EDTA).

**Specific PCR amplification for *F. graminearum*:**

Specific-PCR was performed, using SCARs primers, which were synthesized by Pharmacia Biotech, Roosendaal, Netherlands. The primer sequences for *F. graminearum* was (SCAR-85 F/R: GCAGGGTTTGAATCCGAGAC/GAATGGAGCTA CCAACGGC) (Schiling *et al.*, 1996). PCR amplification was conducted in 25- µl reactions, containing 1x Taq polymerase buffer (50 mM KCl, 10 mM Tris, pH 7.5, 1.5 mM Mg Cl2) and one unit of Taq polymerase (Pharmacia Biotech, Germany) supplemented with 0.01% gelatin, 0.2 mM of each dNTP (Pharmacia Biotech, Germany), 25 pmol of each forward and reverse primer and 50 ng of total genomic DNA. Cycling profiles, consisted of 30 cycles for 1 min at 94 °C, 1min. at 61 °C and 2 min. at 72 °C. At the beginning of the cycling profile, reactions were held for 2 min. at 94 °C to denature the genomic DNA templates, and the final cycle was extended to 5 min. at 72 °C. After completion of PCR, samples were immediately cooled to 10 °C and stored at 4 °C until gel separation. A gel-loading solution (5ul) was added, and 10 µl of the total product volume was resolved in 1.5 agarose in 1x TAE buffer for 2 h aside with a 100-bp ladder (Pharmacia Biotech, Germany), as the size standard. Gels were stained in ethedium bromide and images were recorded.

**Simple sequence repeats (SSR) marker for dehydrin gene**

SSR primer pairs for amplification of dehydrin gene were as follows: *HVDHN*1 forward primer, 5'-GAATTCTCATGAGGGATGCTTC-3'; and reverse primer 5'-CAACTGAAACTCATGGCCAT-3'. These primers were designed on the basis of the published sequence (Becker and Heun, 1995). Amplification was carried out in 25 µL reaction volumes, containing 1X Taq polymerase buffer (50 mM KCl, 10 mM Tris, pH 7.5, 1.5 mM MgCl2) and one unit of Taq polymerase (Pharmacia Biotech, Germany) supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 25 pmol primer, and 50 ng of total genomic DNA. Amplification was performed in a thermal cycler (Thermolyne Amplitron) programmed for one cycle of 30 s at 94°C; and 40 cycles of 1 min.at 94°C, 1 min at 55°C, and one min.at 72°C, followed by five min. at 72°C.

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**Table 2: Number of grains per spike, spike length and 1000-grain weight of wheat cultivars during 2005/2006 and 2006/2007 seasons.**

| Cultivars | No. of grain/spike | | Spike length (cm) | | 1000-grain weight (g) | |
|---|---|---|---|---|---|---|
| | 2005/2006 | 2006/2007 | 2005/2006 | 2006/2007 | 2005/2006 | 2006/2007 |
| Sama | 36.6 bc | 35.5 ed | 7.5 abc | 6.5 e | 26.8 c | 31.6 abc |
| Yocra Rojo | 31.5 dc | 31.4 e | 6.5 bc | 9.0 c | 31.3 ab | 38.0 a |
| Sids 1 | 43.7 b | 53.0 a | 9.5 a | 13.0 a | 32.6 ab | 31.8 abc |
| Sakha 8 | 36.1 bc | 41.7 bcd | 7.0 abc | 10.0 c | 34.6 a | 33.6 ab |
| Sakha 61 | 39.9 bc | 38.5 cde | 9.0 ab | 11.5 b | 32.6 ab | 24.9 bc |
| Giza 164 | 54.6 a | 53.5 a | 9.5 a | 9.7 c | 32.2 ab | 30.2 abc |
| Giza 168 | 54.8 a | 49.0 ab | 7.5 abc | 7.8 d | 31.4 ab | 29.0 bc |
| Bani Swef 1 | 22.5 d | 47.1 abc | 4.0 d | 8.0 d | 25.9 cd | 26.9 bc |
| Bani Swef 3 | 23.6 d | 43.9 bcd | 4.0 d | 7.5 d | 23.3 d | 22.6 c |
| Shandawel | 29.7 cd | 43.1 bcd | 6.0 cd | 9.5 c | 29.0 bc | 27.5 bc |

-Data are expressed as means.
-Means within the same column and followed by the same letter (s) are not significant according to L.S..D..05..

**DNA markers for detection of *F. graminearum*:**

RAPD maker needs to be converted into SCAR markers (Cao *et al.*, 1999). Therefore, specific-PCR analysis, using SCAR – 85 primers, was employed to determine if any of wheat cultivars was infected by *F. graminearum* (Fig. 1). Amplification of SCAR-85 did not detect *F. graminearum* in Sids 1 and Giza-164 wheat cultivars. These cultivars, also, were resistant to infection with *F. graminearum*, as compared with the other cultivars (Table 1). On the other hand, specific-PCR assays detected *F. graminearum* in Sama, Yocra Rojo, Sakha 61, Sakha 8, Giza-168, Bani Swef 1, Bani Swef 3 and Shandawel cultivars. These cultivars had susceptible disease scores. Therefore, the specific-PCR assay was very reliable in identifying *F. graminearum* directly in extracts of infected plant tissues (Schiling *et al.*, 1996). Previous results, reported by Doohan *et al.* (1998) who had indicated that a linear relationship was observed between visual disease assessment and *F. culmorum* PCR. Similar to the present PCR assay, a simple and highly selective PCR test was developed by Nicholson and Parry (1996) to detect *Rhizoctonia cereals* in wheat. To consider routine usage of the PCR assay for detection of *Fusarium* spp. in wheat, the present test would have to be further optimized. However, the PCR assay could be applied to monitor the growth of pathogens over various development stages of the host plant. The PCR assay, also, could be valuable when testing advanced plant breeding materials for root rot resistance. The search for molecular markers, ,for disease resistance, has become a high profile activity in many laboratories (McIntosh *et al.*, 1995). Also, Xiang Ma *et al.* (2006) suggested that missing chromosome arms in wheat cultivars might carry genes that contribute to resistance to *F. graminearum* and susceptibility factors, or resistance suppressors might be on these missing chromosome arms.

**SSR marker for dehydrin gene:**

Dehydrins (DHNs) are one of the typical families of proteins that occur in plants as a consequence of dehydration and osmotic stress (Sivamani *et al.*, 2000). The dehydrin gene was amplified from Sama, Yocra Rojieo, Sids 1, Sakha 8, Sakha 61, Giza 168, Bani Swef 1, Bani Swef 3 and Shandawel cultivars, using *HVDHN*1 primers. On the other hand, SSR analysis, using *HVDHN*1 primers, did not detect dehydrin gene in Giza 164 cultivar (Fig. 2). Brini *et al.* (2007) concluded that the observed differential phosphorylation pattern of DHN-5, in the resistant and sensitive wheat varieties, could be used as a basis for a molecular screen of tolerance/sensitivity to drought and salt stresses in wheat germplasm. Moreover, transgenic rice plants, over expressing the barley dehydrin gene *HVA*1, showed enhanced tolerance to water and salt stresses (Xu *et al.*, 1996).

In the present study, it was shown that Sids 1 cultivar had the dehydrin gene and showed the most resistance to root rot disease. Whereas, Giza 164 was the most resistantce to foot rot disease and did not amplify the dehydrin gene. Therefore, SSR markers, for amplifying dehydrin gene in conjunction with SCAR marker for detection of Fusarium infection, could be valuable when testing wheat genotypes foot rot disease resistance under drought stress.

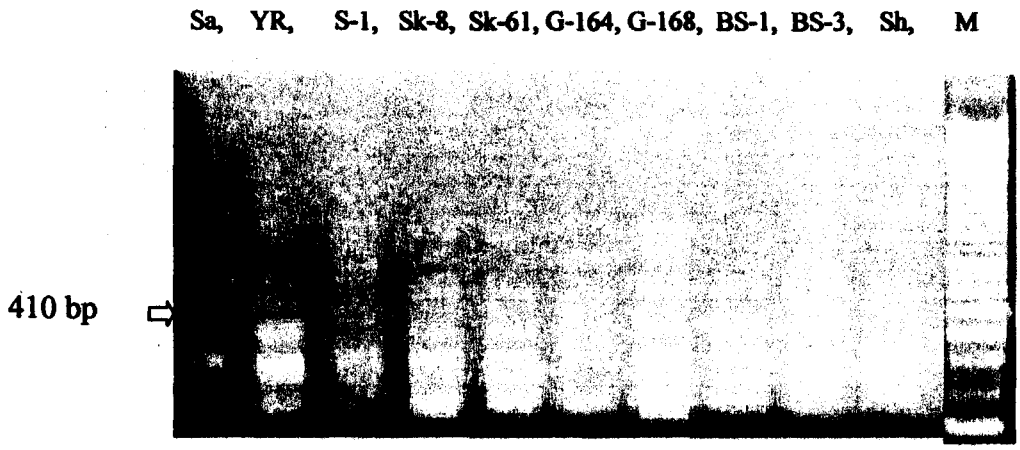


**Fig. 1: Detection of *F. graminearum* in wheat cultivars, using specific primer (SCAR 85). Cultivars of wheat , Sa = Sama, YR = Yocra Rojo, S-1 = Sids 1, Sk-8 = Sakha 8, Sk-61 = Sakha 61, G-164 = Giza 164, G-168 = Giza 168, BS-1= Bani Swef 1, BS-3 = Bani Swef 3 and Sh = Shandawel. M line is kbp DNA marker. The arrow points to a unique fragment of approximately 410 bp present in *F. graminearum*.**

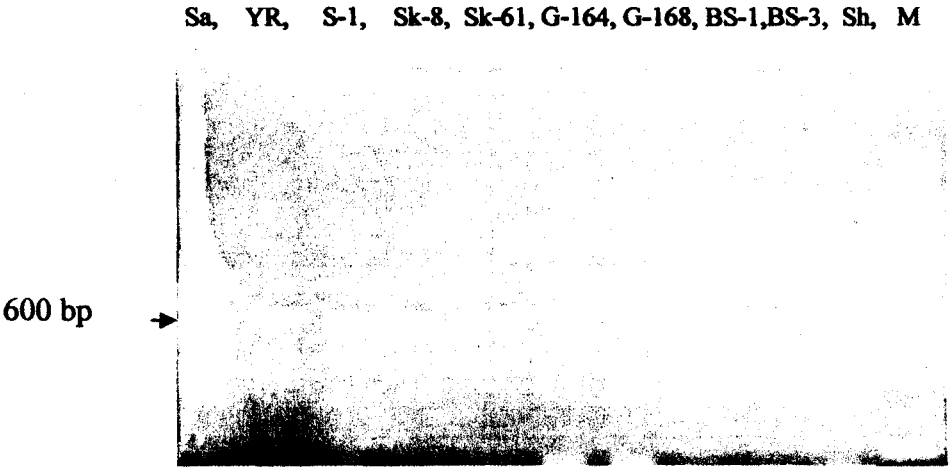


**Fig. 2: Detection of dehydrin gene in wheat cultivars, using SSR primer (*HVDHN1*). Cultivars of wheat , Sa = Sama, YR = Yocra Rojo, S-1 = Sids 1, Sk-8 = Sakha 8, Sk-61 = Sakha 61, G-164 = Giza 164, G-168 = Giza 168, BS-1= Bani Swef 1, BS-3 = Bani Swef 3 and Sh = Shandawel. M line is kbp DNA marker.**

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## الملخص العربي

# الانتخاب لمقاومة مرض عفن الجذور المتسبب عن الفطر *Fusarium graminearum* في أصناف القمح باستخدام الدلائل الجزيئية ( DNA markers )

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تم اختبار درجة المقاومة لمرض عفن الجذور في القمح المتسبب عن الفطر *Fusarium graminearum* وذلك لعشرة أصناف من القمح تحت ظروف العدوى الصناعية بالفطر في تجربتين حقليتين خلال موسمي الزراعة الشتوية ٢٠٠٥/٢٠٠٦ و ٢٠٠٦/٢٠٠٧م. ومن النتائج اتضح أن هذه الأصناف تختلف معنويا عن بعضها في ناتج المحصول ومكوناته. وعموما كانت الأصناف " سدس ١ وجيزة١٦٤ " من أكثر الأصناف التي أعطت أعلى محصول للحبوب بينما كان الصنفان " بني سويف ٣ وبني سويف ١ " اقل الأصناف في محصول الحبوب ومعامل الحصاد. و كانت الأصناف "صاما و سدس ١ وجيزة ١٦٤ " أطول الأصناف. و أيضا كانت هذه الأصناف تختلف معنويا عن بعضها في مكونات محصول الحبوب. وتوضح النتائج أن الأصناف " شاندويل وجيزة ١٦٨ وايكورواوجو وسخا ٦١ وسخا ٨ وبني سويف١" هي أكثر الأصناف قابلية للإصابة. بينما كان الصنفان" صاما وبني سويف ٣" تعتبر متوسطة القابلية للإصابة. وعلي الجانب الآخر اتضح أن الصنفين" سدس ١ وجيزة١٦٤" من أكثر الأصناف مقاومة للإصابة .

وقد استخدمت طريقة Specific-PCR على الحامض النووي DNA المستخلص من قواعد سيقان أصناف القمح تحت الاختبار، وقد تم اكتشاف وجود الإصابة بمرض عفن الجذور الفيوزاريومى في جميع الأصناف المدروسة ماعدا الصنفين" سدس ١ وجيزة ١٦٤" وذلك باستخدام الدليل الجزيئي SCAR-85 حيث أنهما من أكثر الأصناف مقاومة. و اتضح أيضا أن الصنف "سدس ١" يحتوي على جين dehydrin بالإضافة إلي المقاومة لمرض عفن الجذور، بينما كان الصنف " جيزة ١٦٤ " أكثر الأصناف مقاومة للمرض و لكنه لا يحتوي على جين dehydrin. ولذلك يوضح هذا البحث أهمية استخدام الدلائل الجزيئية للكشف عن جين dehydrin مع استخدام طريقة Specific-PCR في الكشف عن المرض في تقويم الأصناف المرباة للمقاومة لمرض عفن الجذور المتسبب عن الفطر *Fusarium graminearum* تحت ظروف الجفاف.