Iraqi J. Agric. Res. Vol.22 No.1 pp.192–199 June/2017

IDENTIFICATION OF SOME STEM AND YELLOW RUST RESISTANCE GENES IN SOME IRAQI WHEAT VARIETIES BY USING PCR MARKERS

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ABSTRACT

Some of Yellow (stripe) rust and stem rust resistance genes were characterized in the genetic matter (DNA) of nine cultivars of Iraqi wheat by using seven DNA primers. The results showed that resistance genes Yr7, Yr9,Yr26 and Yr18 were presented in all nine wheat cultivar. However, Yr 15 was not presented in any of the nine cultivars that were tested. The results indicated that resistance gene Sr26 was present in all varieties that were tested except Rabiaa, Abu Greeb and Al Rasheed whereas it was found that resistance gene Sr39 is presented in all varieties that were tested.

The identification of rust resistance genes in Iraqi wheat germplasm will help in accelerating the breeding program in the future, including Pyramiding of different resistance genes from and to different wheat cultivars.

INTRODUCTION

Wheat is the first cultivated cereal in Iraq and this important crop is affected by the two major diseases: rusts and smuts Al - Beldawi *et al* (2). Stripe and yellow rust wheat that caused by *Puccinia striiformis* f.sp. *tritici* (Pst) are currently considered an important wheat diseases especially in the recent years because the effect on yield losses. In Iraq the effect of yield losses on wheat production reach to 10-60 % if susceptible cultivars of Pst are grown Al-Maaroof *et al* (3).

yellow rust can controlled by fungicides application Sharma *et al*. (12) However, growing genetic resistance cultivars is the most effective economical, and environmental Zhou (18) friendly method to control wheat yellow rust Line and Chen (7). Deploying of resistant yellow rust genes and transferring the effective genes are the high importance priority methods in plant breeding programs Zhou *et al*. (17).

Many genetic markers and techniques have been deployed to estimate genetic diversity such as Restriction Fragment Length polymorphisms (RFLPs), Random Amplified polymorphic DNA (RAPD) , Amplified Fragment Length polymorphisms (AFLPs) and Microsatellite or Simple Sequence Repeat (SSR) Ulrich & LaReesa (13).

For detecting the presence or absence of resistance genes in wheat cultivars, different kinds of markers are used Xia *et al*. (16).

Due to the higher level of polymorphism and accurate results, SSR markers are widely applied in wheat breeding programs Cabuk *et al*. (4). However, there are still limited researches regarding the identification of wheat rust resistant genes in Iraq by using molecular markers. Thus, the aim of this study is to identify Yellow and stem rust resistant genes in Iraqi wheat varieties by using SSR markers and other PCR markers.

Plant Protection Directorate, Baghdad, Iraq.
Received:Feb./2016

Accepted:May/2017

MATERIALS AND METHODS

Seeds of different genotypes of wheat were obtained from Directorate of seed certification. The seeds were sown in small clay pots with 9 mm diameter (10 seeds / pot). The pots were placed at room temperature and periodically watered. After three weeks seedling were cut and prepared for DNA extraction.

Extraction of DNA and SSR markers

DNA of wheat genotypes was extracted from fresh leaves by CTAB (Cetyl trimethyl ammonium bromide) method Weigand et al. (15). The tested wheat genotypes were Ipaa 99, Ra biaa, Intsar, Noor, Saber Beiq, Al az, Abu Greeb, Al Lateefia and Al Rasheed.

Seven PCR markers were used for identification of wheat rust resistant genes. These markers were selected from Cabuk et al. (4), Mago et al. (8) and Mago et al. (9) respectively.The entire sequences for these primers are presented in Appendix A. The PCR reactions were carried out in a reaction volume of 20 µl containing 1unit *Taq* DNA polymerase , 250 µM dNTP, 10 mM Tris-HCL, 30 mM KCL, 1.5 mM MgCl2, 2 µl forward and reverse primers of a stock solution of 100µ M, 16 µl sterile distal water and 2 µl (100 ng) DNA template. Each sample of PCR was replicate twice so that to avoid contamination error.

Two different temperatures was used for the first five primers with the following program that consist of initial denaturation at 94° C for 3 min; 40/45 cycles at 94°C for 1 min, 50°C/55°C for 1 min, 72°C for 2 min; and a final extension at 72°C for 10 min while the thermal cycle for the Sr39#22r was as down below.

94oC- 3min : 1 cycle
94oC- 30sec
58oC- 30sec } 30 cycles
72oC- 40sec
20oC- 1min : 1 cycle

And the thermal cycle for Sr26 primer was 94 °C /3 min One, 94 °C /30 s; 56 °C /30 s; 72 °C /40 s Thirty, 20 °C /1 min One.

The PCR products were evaluated on 2 per cent agarose gels that used a freshly prepared 1x (Tris – Borate – Ethylenediaminetetraacetic acid (EDTA)) . Agarose gel were run in a 1xTBE buffer using horizontal submarine gel tanks at 120 v for one hour. Next, the gel was immersed for 20 minutes in 30 µl /500 ml ethidium bromide (stock concentration of 10 mg/ml) then visualized on UV transluminater.

Results and Discussion

The results of different Iraqi wheat cultivars that were tested indicate that X GWM 295 , XGWM 526 X GWM 582 and X GWM 11 show link to Yr18 , Yr7, Yr9 and Yr26 respectively . Marker X GWM 295 amplify PCR product with 250 bp in all wheat varieties that were tested. While XGWM 526, X GWM 582 marker give band in all varieties with 160 bp, 150 bp respectively. However, marker X GWM 11 give band size with 200 bp in all varieties except Rabiaa .

However, marker X GWM 413 that suppose to link to gene Yr15 according to Cabuk et al (4) did not show any band amplification in all wheat

genotypes. Whereas, marker Sr39 # 22r present in all wheat genotypes with PCR product band size 280 bp . Marker Sr26#43 give band with 207 bp in all 9 wheat genotypes that were tested except Rabiaa , Abu Greeb and Al Rasheed . The results for testing these primers were shown in (Table 1).

Table 1: The results for testing 7 marker on the 9 wheat genotypes on agarose gels

Marker name	Ipaa 99	Rabiaa	Intsar	Noor	Saber Beiq	Al az	Abu Greeb	Al Lateefia	Al Rasheed
XGWM 295	+	+	+	+	+	+	+	+	+
XGWM 526	+	+	+	+	+	+	+	+	+
XGWM 582	+	+	+	+	+	+	+	+	+
XGWM11	+	--	+	+	+	+	+	+	+
XGWM413	--	--	--	--	--	--	--	--	--
Sr39#22r	+	+	+	+	+	+	+	+	+
Sr26#43	+	--	+	+	+	+	--	+	--

+Indicate band present

--Indicates band absent

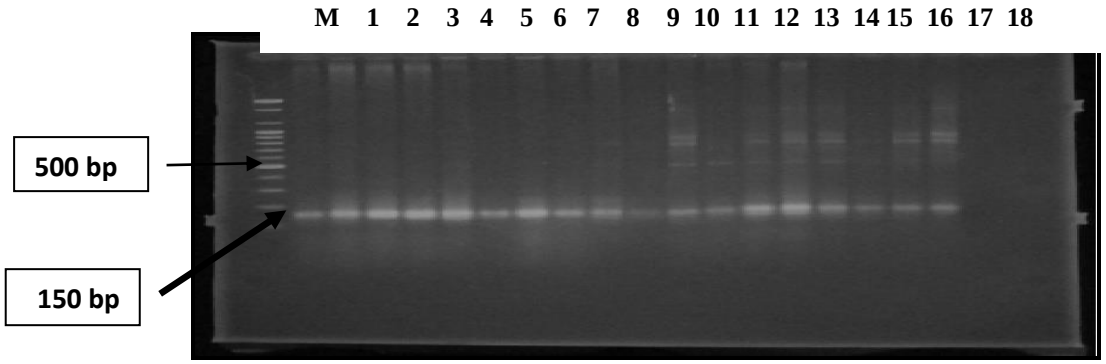

Fig. 1: Primer amplification (XGWM 582) on the 9 wheat varieties Lines:1. Ippa 99,2. Ippa 99, 3.Rabiaa,4. Rabiaa,5. Intsar, 6. Intsar, 7. Noor, 8. Noor, 9. Saber Beiq, 10. Saber Beiq, 11. Al az, 12. Alaz, 13. Abu Greeb, 14. Abu Greeb, 15. Al Lateefia, 16. Al Lateefia, 17. Al Rasheed, 18. Al Rasheed. M-100bp DNA ladder.

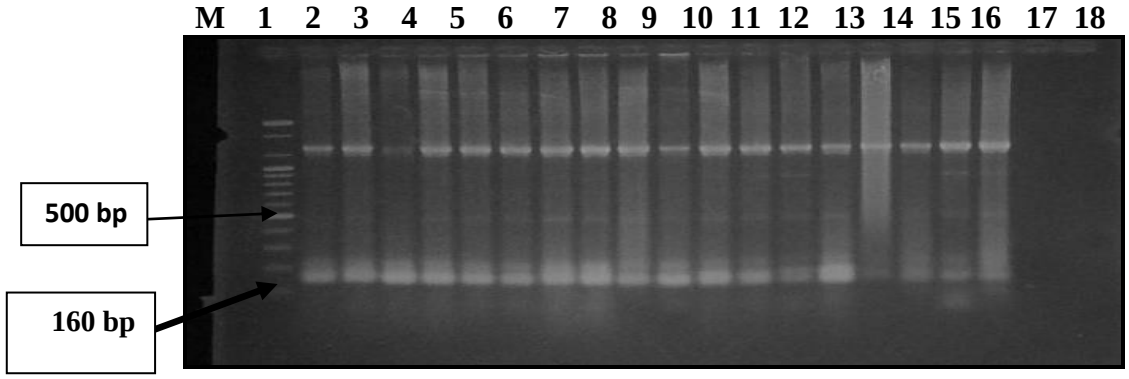

Fig. 2: Primer amplification (XGWM 526) on the 9 wheat varieties Lines:1. Ippa 99,2. Ippa 99, 3.Rabiaa,4. Rabiaa,5. Intsar, 6. Intsar, 7. Noor, 8. Noor, 9. Saber Beiq, 10. Saber Beiq, 11. Al az, 12. Alaz, 13. Abu Greeb,14. Abu Greeb, 15. Al Lateefia, 16. Al Lateefia, 17. Al Rasheed, 18. Al Rasheed. M-100bp DNA ladder.

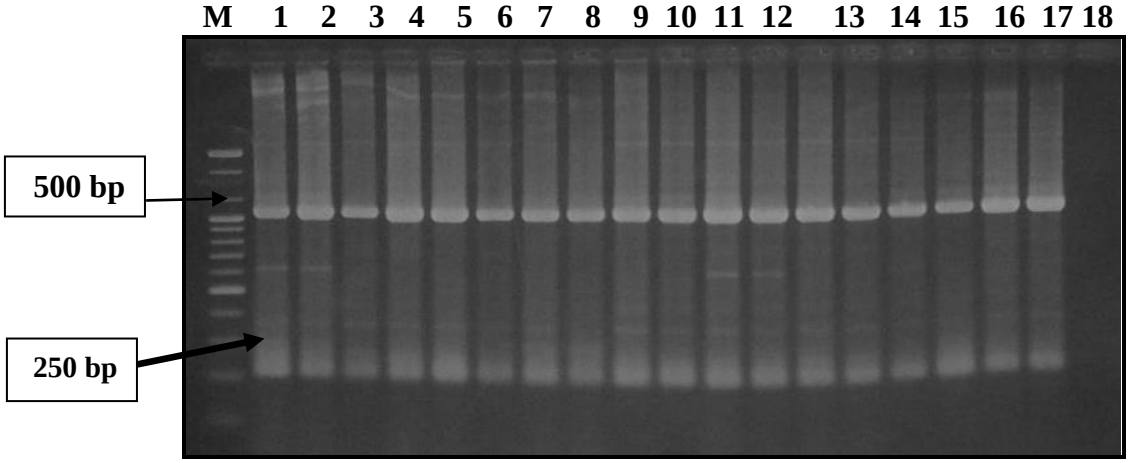

Fig.: 3 Primer amplification (XGWM 295) on the 9 wheat varieties Lines:1. Ippa 99,2. Ippa 99, 3.Rabiaa, 4. Rabiaa, 5. Intsar, 6. Intsar, 7. Noor, 8. Noor, 9. Saber Beiq, 10. Saber Beiq, 11. Al az, 12. Alaz, 13. Abu Greeb, 14. Abu Greeb, 15. Al Lateefia, 16. Al Lateefia, 17. Al Rasheed, 18. Al Rasheed. M-100bp DNA ladder.

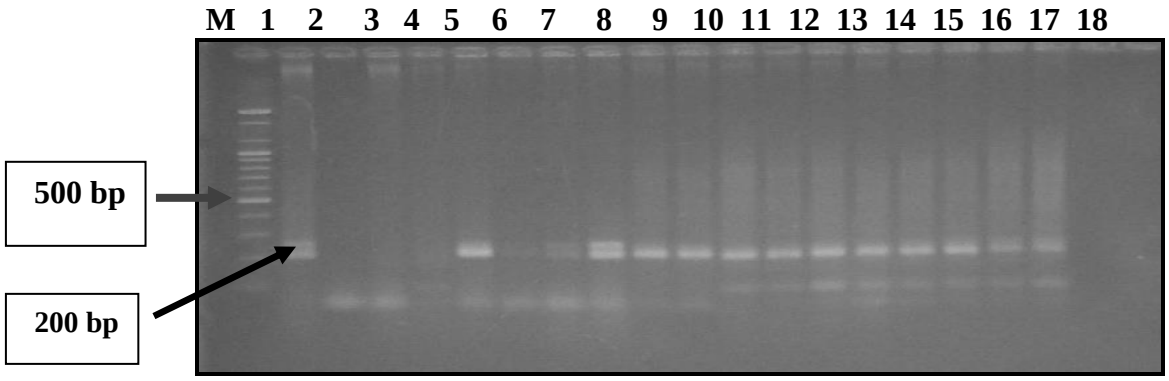

Fig. 4: Primer amplification (XGWM 11) on the 9 wheat varieties Lines:1. Ippa 99,2. Ippa 99, 3.Rabiaa,4. Rabiaa,5. Intsar, 6. Intsar, 7. Noor, 8. Noor, 9. Saber Beiq, 10. Saber Beiq, 11. Al az, 12. Alaz, 13. Abu Greeb, 14. Abu Greeb, 15. Al Lateefia, 16. Al Lateefia, 17. Al Rasheed, 18. Al Rasheed. M-100bp DNA ladder.

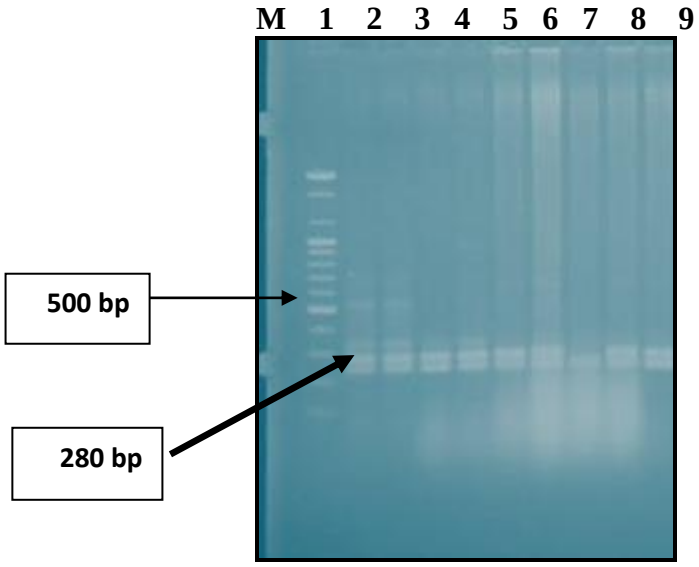

Fig. 5: Primer amplification Sr 39 # 22r on the 9 wheat varieties Lines:1. Ippa 99, 2.Rabiaa, 3. Intsar, 4. Noor, 5. Saber Beiq, 6. Al az, 7. Abu Greeb, 8. Al Lateefia, 9. Al Rasheed. M-100bp DNA ladder.

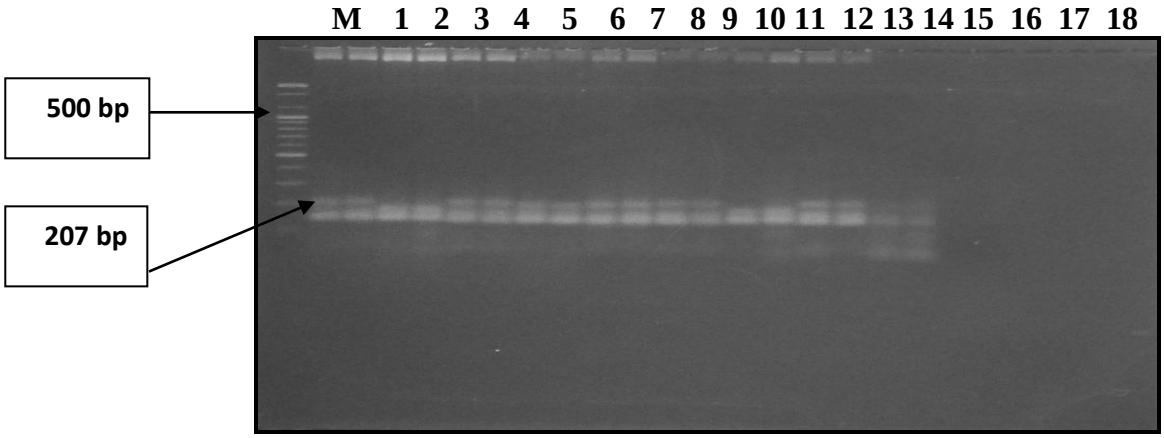

Fig. 6: Primer amplification Sr 26# 43 on the 9 wheat varieties Lines:1. Ippa 99,2. Ippa 99, 3.Rabiaa,4. Rabiaa,5. Intsar, 6.Intsar, 7. Noor, 8. Noor, 9. Saber Beiq, 10. Saber Beiq, 11. Al az, 12. Alaz, 13. Abu Greeb,14. Abu Greeb, 15. Al Lateefia,16. Al Lateefia, 17. Al Rasheed, 18. Al Rasheed M-100bp DNA ladder.

In this study, Yr 18, Yr7, Yr9 and Yr 26 were identified in all Iraqi wheat cultivars that were tested. These identified genes have an important role in rust diseases and it frequently associated with other rust genes. However, Yr 15 did

not present in any of wheat cultivar that were tested. Yr7 gene which constantly associated with stem rust resistance gene Sr9 and Yr9 and also associated with leaf rust resistance gene Lr26 and Sr31 gene and powdery mildew resistance gene Pm8 can provide durable resistance Mclntosh *et al.* (10). It was found that Yr7 , Yr9 , and Yr18 confer resistance for wheat plant race that controlled by single major resistance gene Coram *et al.*, (5). However, Yr 18 gene is considered an effective gene for rust resistance and confer yellow rust resistance at seedling and adult plant stages Morgounov *et al.* (11). Yr26 which is identified in all tested Iraqi wheat cultivars and it was effective resistance gene against many of *P. Striiformis* because it can provide durable resistance with combination of other genes Xia *et al.* (17). Many Yr genes have been widely deployed in wheat genotypes by its association with leaf rust resistance or by its phenotyping effect on stripe rust response and considered to have durable stripe resistance Singh (13). cultivars with this single resistance gene often become susceptible soon after they released because of the rapid evolution of new races of pathogen Welling *et al.* (16).

Yr 9 that show virulence against rust pathogens was found in many Iraqi wheat cultivars Al- Maaroof *et al.* (1).

Although stem rust disease are not presently have potential effect on wheat crop in Iraq, the rapid worldwide spread of stem rust pathogens races especially Ug99 obligate needs for controlling the destructive diseases before it spread.

For Sr39 marker, Sr39 gene was identified in all 9 wheat varieties. Thus, this marker can be useful in breeding program to distinguishing resistant genes. It was found that this gene have 3% recombination with Lr34 Kerber *et al.* (6).

Wherese, Sr26 gene were presented in all tested cultivars except Rabiaa , Abu Greeb and Al Rasheed. This gene has been considered an effective resistance genes against Ug99 and has been used as source of resistance in Australia Mclntosh *et al.* (10).

In conclusion, this study has demonstrated that six markers, X GWM 295 , XGWM 526 X GWM 582 , X GWM 11, Sr39 # 22r and Sr26#43 can potentially be useful for identifying rust resistance genes in wheat and it can be more useful tools in wheat breeding for yellow and stem rust resistance genes. Further research might be more useful by screening more wheat varieties and using more efficient markers to detect resistant gene in wheat genome.

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Appendix1

Primer sequences for the 7 markers that were used in this study

Primer name	Primer sequence
XGWM295 F	GTGAAGCAGACCCACAACAC
R	GACGGCTGCGACGTAGAG
XGWM582 F	AAGCACTACGAAAATATGAC
R	TCTTAAGGGGTGTTATCATA
XGWM526 F	CAATAGTTCTGTGAGAGCTGCG
R	CCAACCCAAATACACATTCTCA
XGWM413 F	TGCTTGTCTAGATTGCTTGGG
R	GATCGTCTCGTCCTTGGCA
XGWM11 F	GGATAGTCAGACAATTCTTGTG
R	GTGAATTGTGTCTTGTATGCTTCC
Sr 39 # 22r F	AGAGAAGATAAGCAGTAAACATG
R	TGCTGTCATGAGAGGAACTCTG
Sr26#43 F	AATCGTCCACATTGGCTTCT
R	CGCAACAAAATCATGCACTA

تشخيص بعض جينات المقاومة لصدأ الساق والصدأ الاصفر في بعض أصناف الحنطة العراقية باستخدام واسمات المسلسل البسيط

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الملخص

تم تشخيص بعض جينات المقاومة لمرض صدأ الساق والصدأ الأصفر (المخطط) في المادة الوراثية (**DNA**) لتسعة أصناف من الحنطة العراقية باستخدام سبع بادئات. أظهرت النتائج وجود جينات مقاومة الصدأ الأصفر **Yr7** , **Yr9** , **Yr26** و**Yr18** في الأصناف المختبرة وعدم وجود الجين **Yr15** في أي من هذه الأصناف. كما بينت النتائج وجود جين مقاومة صدأ الساق **Sr26** في الأصناف المختبرة كافة عدا الاصناف (ربيعة وأبو غريب والرشيد) بينما وجد جين المقاومة **Sr39** في الاصناف جميعها التي تم اختبارها. إن تشخيص هذه الجينات سيساعد كثيراً في برامج تربية النبات المستقبلية بما فيها تقانات نقل جينات المقاومة من وإلى أصناف الحنطة المختلفة.

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