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Screening the Promising Wild Emmer Wheat Genotypes Containing the Yr-15 Gene in Türkiye and Syria Through Molecular Markers

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Research Article

Keywords: Wild emmer wheat, Yr15 gene, Molecular markers, Fertile Crescent,

Posted Date: January 20th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2486157/v1

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Abstract

Background Stripe rust also known as yellow rust is a most devastating fungal disease of wheat caused by *Puccinia striiformis* f. sp. *tritici* (Pst). The possibility of the breakdown of important stripe rust resistance genes and the threat of the emergence of aggressive new races remains a concern in all wheat-growing areas around the world. The presence of *Yr15* resistant gene in the wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) confers strong broad-spectrum resistance to Pst isolates. The aim of this present study was to investigate at molecular level to confirm the presence or absence of the major resistance gene *Yr15*in tested samples.

Methods and Results A total of 140 wild emmer wheat genotypes originating from Türkiye and Syria were screened using gene specific molecular markers Y15K1_F2/uhw30_1R. Ten promising wild emmer wheat genotypes Türkiye (Diyarbakır) and Syria (Al Qunayţirah) containing the *Yr15* gene were identified.

Conclusion These findings can be beneficial in wheat breeding programs to be conducted for resistance to stripe rust. As well, this study contributes to the evolutionary process studies related to *Yr15* gene in wild emmer wheat populations of Fertile Crescent.

Introduction

Wheat is an important staple food crop of the peoples in various countries which is cultivated nearly 220 million hectares area of the world with 760 million tonnes of production (FAO, 2020). Due to its beneficial amino acid content, high commercial volume, role in crop rotation systems, socio-cultural importance, and high adaptability to different climates, wheat has been a dispensable food for all growing countries. Today, Türkiye has 17.5 million tonnes of production and more than 67 million hectare of wheat-growing area (TUIK 2021).

Various biotic and abiotic stresses affect the yield of wheat every year, specifically; the fungal pathogens that threaten the wheat cropss. Stripe rust of wheat is a serious disease caused by *Puccinia striiformis* f.sp. *tritici*, a biotrophic and heteroecious fungal pathogen. Severe losses in wheat due to this pathogen is predicted to be more than five million tonnes every year which cost is counted to be nearly one billion US Dollars (Beddow et al. 2015). Because the pathogen spread very rapidly, consequently the new emergence of Pst (*Puccinia striiformis* f.sp. *tritici*) races, studies on the development of resistant varieties have been prompted (Klymuk et al. 2018). Breeding stripe rust tolerant cultivar is one of the most efficient methods to combat this pathogen. The use of fewer fungicides due to tolerant cultivars will be environmentally friendly, also the costs for the fungicides will diminish (Turgay et al. 2022).

Currently, 83 designated, 63 provisionally designated genes and 61 quantitative trait loci (QTLs) have been described in wheat and its close relatives (Liu 2017, Jan et al. 2021). However, the changes in virulences in stripe rust pathogen and the epidemics occurring in genotypes/varieties containing a single major gene resulted in catastrophic losses (Ali et al. 2017; Chen and Khan 2017). This research explores the new resistance genes in wheat for rust. In particular, the use of wild relatives that consists of diverse valuable traits in breeding studies is getting widespread (Migicovsky and Myles 2017; McCouch et al. 2020) and of these, wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides)* is known for important disease resistance traits (Klymiuk et al. 2022; Li et al. 2020; Oliver et al. 2007; Rahman et al. 2020). The genes detected in wild emmer wheat have been used to develop new durum and bread wheat varieties (Rahman et al. 2020; Yaniv et al. 2015; Mukhtar et al. 2015; Ipek 2022). The wild emmer wheat was discovered in Israel first and then was widely found in Fertile Crescent (i.e. Israel, Jordan, Lebanon, Syria, east Türkiye, West Iran, and North Iraq) (Nevo et al. 2012) with reported various significant traits (Nevo 1983). Especially, G-25 accession of wild emmer wheat was tested on 20 different isolates from six countries and conferred a high resistance. Further studies concluded that this endurance was controlled by a gene called *Yr15*, found by Gerechter-Amitai et al. (1989).

This gene is located on chromosome arm 1BS, reported using different molecular techniques (McIntoshand Park 1995; Sun et al. 1997; Yaniv et al. 2015). Since the *Yr15* gene has a wide broad-spectrum resistance even against the *Warrior* races (PstS7; *1,2,3,4,-,6,7,-,9,-,-,17,-,25,-,32, Sp, AvS, Amb* effective on these resistance genes) threatening considerable wheat growing areas around the world (Sharma-Poudyal et al. 2013, Ali et al. 2017; Chen and Kang 2017; Klymuk et al. 2018), *Yr15* as genetics source started to be attracted by the breeders in recent years (Yaniv et al. 2015; Mukhtar et al. 2015; Ipek 2022).

Many of the diverse wild emmer wheat and all other relatives in the first tier of gene pools that constitute a modern wheat originated from Türkiye (Harlan, 1995; Van Zeitz and De Roller, 1995; Karagöz et al. 2010). However the studies of wild emmer wheat populations have been intensively done in the south Fertile Crescent (Israel, Lebanon, Jordan, and Syria). Therefore, this study has been conducted with materials- mostly from the northern Fertile Crescent (southeast Türkiye) and from Syria. A total of 140 genotypes were screened with molecular markers to check the presence of the *Yr15* resistant genes.

Material And Method

1. *Plant materials and isolation of DNA*

The wild emmer wheat accessions were obtained from the Türkiye Seed Gene Bank of Field Crops Central Research Institute, Ankara, Türkiye (TGB), and the National Small Grains Collection (USDA), USA. The obtained seed from these gene banks were sown in the first year for taxonomic verification.

The seeds were selected and separated morphologically by spike color, awns, and single spikes was obtained from these selected seeds. These single spikes collected isolated from the accessions grown for 2 years and 140 genotypes composing the materials of this study were acquired (Table.1and Table.2). Of 140 genotypes, 31 from TGB and 109 from USDA were obtained.

Table1. Wild emmer wheat genotypes obtained from Green Global, USDA screened for *Yr15* resistant gene

USDA Genotypes No	Origin	USDA Genotypes No	Origin	USDA Genotypes No	Origin	USDA Genotypes No	Origin
1	Türkiye/Diyarbakır	23-1	Türkiye/Diyarbakır	51	Türkiye/Diyarbakır	86	Syria Al Qunayţirah
2	Türkiye/Kahramanmaraş	24	Türkiye/Diyarbakır	52	Türkiye/Şanlıurfa	87	Syria Al Qunayţirah
3	Türkiye/Diyarbakır	24-1	Türkiye/Diyarbakır	53	Türkiye/Şanlıurfa	88	Syria Al Qunayţirah
3-1	Türkiye/Diyarbakır	25	Türkiye/Diyarbakır	54	Türkiye/Şanlıurfa	89	Syria Al Qunayţirah
4	Türkiye/Diyarbakır	25-1	Türkiye/Diyarbakır	55	Türkiye/Şanlıurfa	90	Syria Al Qunayţirah
5	Türkiye/Diyarbakır	26	Türkiye/Diyarbakır	58	Türkiye/Şanlıurfa	91	Syria Al Qunayţirah
7	Türkiye/Diyarbakır	27	Türkiye/Diyarbakır	60	Türkiye/Şanlıurfa	92	Syria Al Qunayţirah
9	Türkiye/Diyarbakır	28	Türkiye/Diyarbakır	61	Türkiye/Şanlıurfa	93-2	Syria Al Qunayţirah
10	Türkiye/Diyarbakır	28-2	Türkiye/Diyarbakır	62	Türkiye/Şanlıurfa	95	Syria As Suwaydā’
11	Türkiye/Diyarbakır	29	Türkiye/Diyarbakır	63	Türkiye/Mardin	96-1	Syria Darā
11-3	Türkiye/Diyarbakır	30	Türkiye/Diyarbakır	64	Syria Al Qunayţirah	96-2	Syria Darā
12	Türkiye/Diyarbakır	31	Türkiye/Diyarbakır	65-1	Syria Al Qunayţirah	97	Syria Darā
13	Türkiye/Diyarbakır	32	Türkiye/Diyarbakır	66	Syria Al Qunayţirah	99-1	Syria AsS
14	Türkiye/Diyarbakır	33	Türkiye/Diyarbakır	68	Syria Al Qunayţirah	100	Syria Dimaş
14-2	Türkiye/Diyarbakır	34	Türkiye/Diyarbakır	69	Syria Al Qunayţirah	101	Syria Halep
15	Türkiye/Diyarbakır	35	Türkiye/Diyarbakır	70	Syria Al Qunayţirah	103	Türkiye/Diyarbakır
15-1	Türkiye/Diyarbakır	36	Türkiye/Diyarbakır	71	Syria Al Qunayţirah	103-2	Türkiye/Diyarbakır
16	Türkiye/Diyarbakır	37	Türkiye/Diyarbakır	72	Syria Al Qunayţirah	104	Türkiye/Diyarbakır
17	Türkiye/Diyarbakır	38	Türkiye/Diyarbakır	75	Syria Al Qunayţirah	105	Türkiye/Diyarbakır
18	Türkiye/Diyarbakır	40	Türkiye/Diyarbakır	76	Syria Al Qunayţirah	105-2	Türkiye/Diyarbakır
19	Türkiye/Diyarbakır	41	Türkiye/Şanlıurfa	77	Syria Al Qunayţirah	106-1	Türkiye/Diyarbakır
19-2	Türkiye/Diyarbakır	42	Türkiye/Şanlıurfa	78	Syria Al Qunayţirah	106-2	Türkiye/Diyarbakır
20	Türkiye/Diyarbakır	43	Türkiye/Şanlıurfa	81	Syria Al Qunayţirah	107	Türkiye/Şanlıurfa
21	Türkiye/Diyarbakır	44	Türkiye/Şanlıurfa	82	Syria Al Qunayţirah	108	Türkiye/Diyarbakır
21-1	Türkiye/Diyarbakır	45	Türkiye/Şanlıurfa	83-1	Syria Al Qunayţirah	110	Türkiye/Şanlıurfa
21-3	Türkiye/Diyarbakır	46	Türkiye/Diyarbakır	83-5	Syria Al Qunayţirah		
22	Türkiye/Diyarbakır	48	Türkiye/Diyarbakır	84-1	Syria Al Qunayţirah		

23	Türkiye/Diyarbakır	50	Türkiye/Diyarbakır	84-2	Syria Al Qunayţirah

Table2. Wild emmer wheat genotypes obtained from Gene Bank of Field Crops Central Research Institute, Türkiye (TGB) screened for *Yr15* resistance gene.

TGB Genotypes No	Origin	TGB Genotypes No	Origin
76	Türkiye/Diyarbakır	89	Türkiye
77	Türkiye/Diyarbakır	90	Türkiye
78	Türkiye/Diyarbakır	91	Türkiye
79	Türkiye/Diyarbakır	91-1	Türkiye
80	Türkiye/Diyarbakır	92	Türkiye
81	Türkiye/Diyarbakır	92-1	Türkiye
81-2	Türkiye/Diyarbakır	92-2	Türkiye
82	Türkiye/Diyarbakır	94	Türkiye
83	Türkiye/Diyarbakır	94-1	Türkiye
84	Türkiye/Diyarbakır	95	Türkiye
84-1	Türkiye/Diyarbakır	96	Türkiye
85	Türkiye/Diyarbakır	97	Türkiye
86	Türkiye	98	Türkiye
87	Türkiye	98-2	Türkiye
88	Türkiye	99	Türkiye
88-2	Türkiye		

Leaf tissue samples were collected from 140 genotypes frozen in liquid nitrogen and stored at -80°C. DNA was extracted according to the method described by Rogers and Bendich (1985). The quality and quantity of extracted DNA were checked by using 1% of agarose gel and compared to it with 50ng and 100ng lamda DNA for dilution preparations. 1% of agarose gel showed clear bands of extracted DNA of wild emmer wheat (Figure1).

2. *PCR analysis*

DNA samples of 140 genotypes were used for the detection and to check the presence of *Yr15* resistance gene s using gene-specific primer sets (Y15K1_F2 5-GGAGATAGAGCACATTACAGAC-3 and uhw30_1R 5-TTTCGCATCCCACCCTACTG-3) of Yr15 (Klymiuk et al. 2018). Amplification of PCR was done by using the MyTaq^{TM} 'Red DNA Polymerase' (Cat. No. BIO-21108) Bioline. A total of 10µL of PCR reaction mixture was prepared including 2µl of 5x Taq Red Reaction Buffer, 0.4µl of 10 µM each (F/R) primers, 0.05 µl of MyTaq Red DNA Polymerase, 1µl of DNA Template (80-100ng) and remaining water to complete the total volume of PCR reaction. Successful amplification of *Yr15* resistant gene was done by using the PCR thermocycler conditions according to the manufacturing company of MyTaq^{TM} 'Red DNA Polymerase including initial denaturation at 95 °C for 3min, followed by 35 cycles of 95°C for 15sec, 56°C for 15sec and 72°C for 10sec and there was no final extension. The amplified product was confirmed by gel electrophoresis in 2% of agarose with the expected 993 bp band size of *Yr15*.

Results And Discussion

One hundred-forty (140) extracted DNA samples were tested to check the presence of *Yr15* resistant gene. The first two-lane after the ladder was positive control and the further 1st arrow showed TGB DNA 84-1 and the 2nd arrow showed USDA DNA 3 in 2% of agarose gel. Thirty-one out of 140 DNA samples were obtained from TGB and only one sample TGB genotypes number 84-1obtained from Türkiye/Diyarbakır showed *Yr15* resistant gene with 993 bp amplicon (Figure2) (Table2).

One hundred nine (109) out of 140 DNA samples were obtained from USDA and only nine samples (USDA 3, 21-1,30, 82, 83-1, 83-5, 84-1, 93-2, 106-1) was showed the positive amplification of *Yr15* resistant gene with 993 bp amplicon (Figure3a, b and c). Samples from USDA genotypes number 3, 21-1, 30, 106-1 obtained from Türkiye/Diyarbakır and genotypes number 82, 83-1, 83-5, 84-1, 93-2 obtained from Syria Al Qunayţirah (Table-1).

In this study, we screened a total of 140 genotypes for the presence of *Yr15* resistant gene using gene-specific primers. The materials showed successful amplification of the expected fragment size (993 bp) in the PCR. The obtained result shows that 7.1% of the screening wild emmer wheat genotypes were positive for *Yr15* resistant gene and the rest of the 92.9 % showed no amplification for *Yr15* resistant gene. In total, 10 out of 140

genotypes was detected in *Yr15* resistant gene and 5 of the10 genotypes with *Yr15* resistant gene were determined from Türkiye/Diyarbakır and the rest of the 5 genotypes with *Yr15* resistant gene were determined in Syria Al Qunayţirah (Table1and Table2).

Molecular study showed that wild emmer wheat is known as a species that is about 360,000 years old that was found somewhere in Fertile Crescent (Dvorak and Akhunov 2005). The first yellow rust resistance *Yr15* gene was discovered in1989 in accession G25 (G25) a wild emmer accession G-25 by Gerechter-Amitai. The wild emmer wheat was probably domesticated in western Fertile Crescent including southeastern Türkiye (Ozkan et al. 2002; Luo et al. 2007). İntrogression of wild emmer disease-resistant genes to wheat and the development of resistant wheat varieties have an important role in today's wheat breeding studies (Perugini et al. 2008). It is also used as a valuable resource against many important diseases of wheat as well as yellow rust disease (Grama and Gerechter-Amitai 1974; Moseman et al. 1984; Moseman et al. 1985; Nevo et al. 1991).

He et al. (2020) conducted a study to find the Yr15 resistant gene from 361 wild emmer wheat accessions, 19 were originating from Türkiye, one from Lebanon, and one from Syria and rest of the materials were originating from Israel that screened using three pairs of gene-specific primers for *Yr15* gene. As a result of the study, only 13.6% of 361 wild emmer wheat accessions were positive for the *Yr15* gene-specific markers, and accessions from Lebanon, Syria, and Türkiye populations were *Yr15* negative. According to Klymiuk et al. (2018), Yr15 was detected in 18% of the accessions from Israel, Lebanon, Jordan, and Syria but was not detected in Türkiye and Iran. They used as a material 19 wild emmer wheat originating from Türkiye and Iran, and 162 wild emmer wheat originating from Israel, Lebanon, Jordan, or Syria.

According to the current studies, the presence of *Yr15* resistant gene was found in populations of Israel, Lebanon, Jordan, and Syria. However, while a majority of the materials in those studies belonged to Israel accessions, the number of materials from Türkiye, Iran and Iraq was limited. Most of the materials in this study is from Türkiye and there is no study done with a great number of material from Türkiye and Syria. Moreover, no previous record has been found that *Yr15* resistant genes were detected from these regions. In conclusion, more genotypes needed to be screened in these regions, and as a result of these screenings, the results could be compared with sequence information related to Israel accessions, which detected *Yr15* gene before. Therefore, this comparison will contribute to the evolutionary process of wild emmer wheat populations in Fertile Crescent.

In light of the promising results obtained from this study, genotypes found to have the *Yr15* resistance gene can be considered as new genetic resources in breeding programs for the management of stripe rust disease. Breeding for stripe rust resistance will continue to be based on the current knowledge of stripe rust's variability, a commitment to research and commercial development of new and effective resistance combinations, and their use as the best way to control the disease.

Declarations

Acknowledgment

Dr. Ayten SALANTUR and Dr. Fatih ÖLMEZ are thanked for their help in this study. The Gene Bank of Central Research Institute for Field Crops and National Small Grains Collection (USDA), USA. provided the seed of the accession used.

Conflict of interest

The author declares no competing financial and nonfinancial interests

Ethical approval None.

Research involving human and animal participants This article does not contain any studies with human or animal subjects.

Informed consent

The author did the study and she has written and reviewed the manuscript and approved its submission to Molecular Biology Reports

Data Avability Stament

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

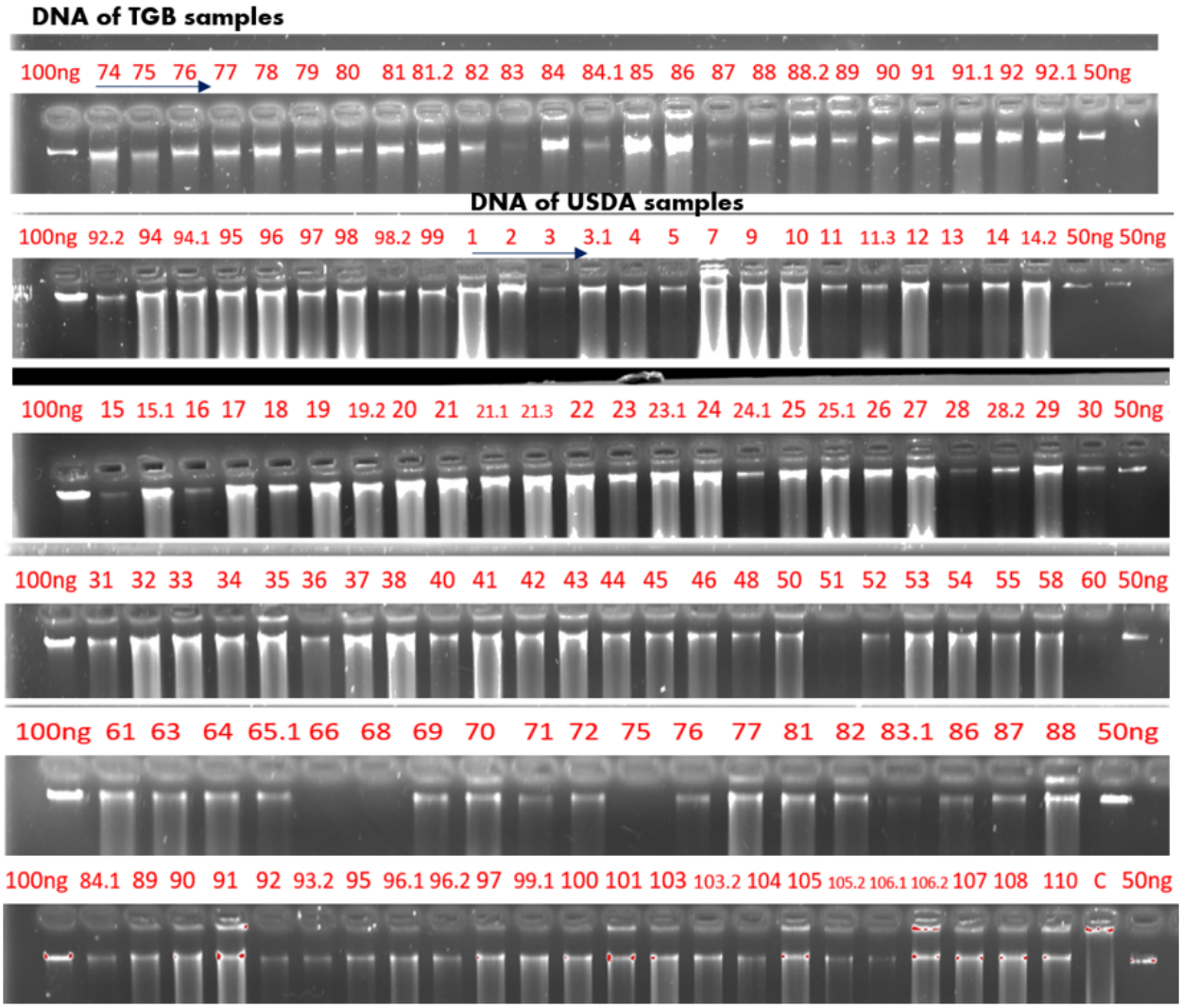


Figure 1

Quality and quantity of extracted DNA result from 140 wild emmer wheat genotypes

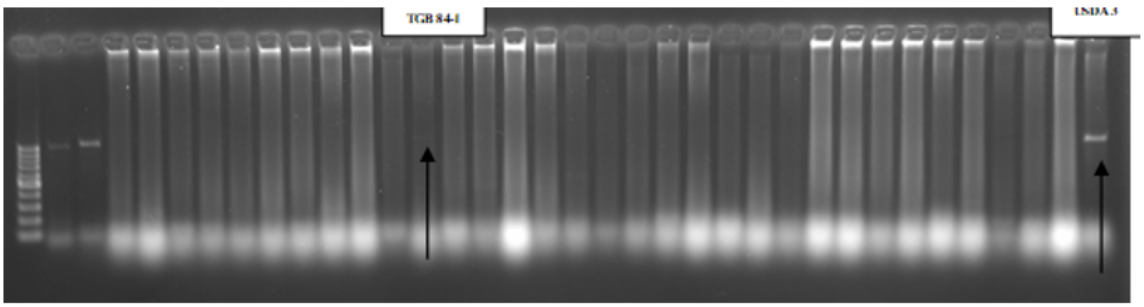


Figure 2

2% of agarose gel showed the positive amplification of *Yr15* resistant gene

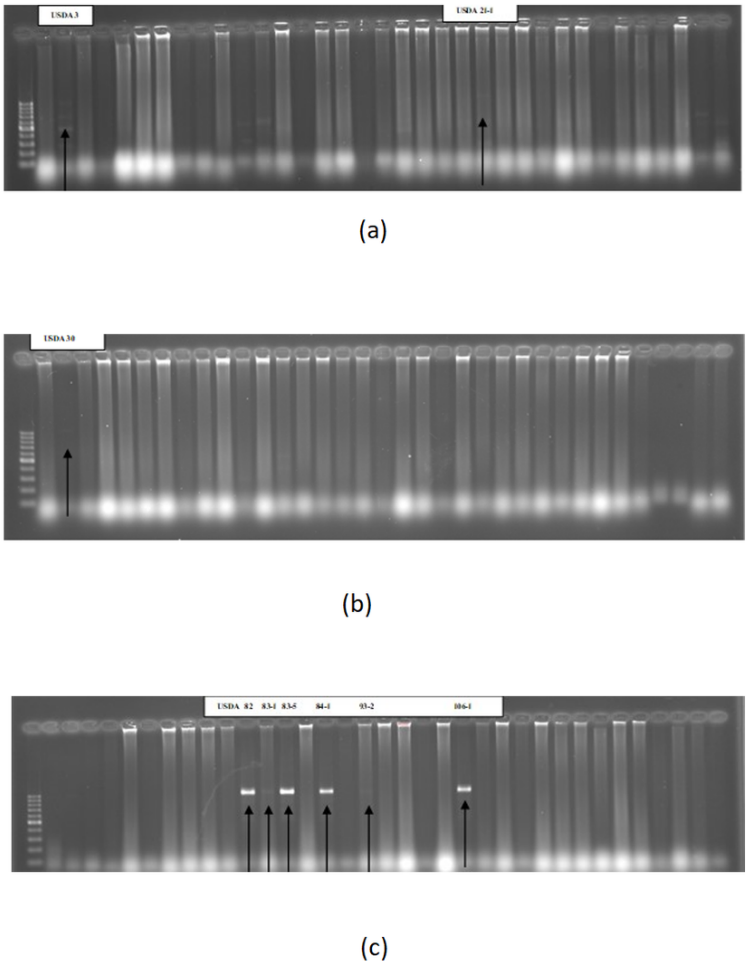


Figure 3

3a. 2% of agarose gel showed the positive amplification of *Yr15*resistant gene

3b. 2% of agarose gel showed the positive amplification of *Yr15* resistant gene

3c. 2% of agarose gel showed the positive amplification of *Yr15* resistant gene