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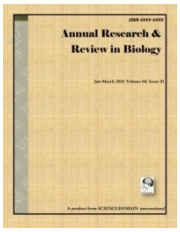

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Identification of Wheat Stripe Rust Resistance Genes in Iranian Wheat Cultivars Using Molecular Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Author ZP conducted the research and prepared the first manuscript. Author AD designed the study, provided the funding, materials and lab facilities, revised the manuscript. Authors AN, BH and EE revised the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Yellow or stripe rust is one of the most important and destructive wheat diseases all over the world. The best strategy to control this disease is genetic resistance through combining several resistance genes which results in achieving long lasting resistance. Marker assisted selection has provided a suitable means towards this strategy. The aim of this study was to identify the race specific seedling genes *Yr5* and *Yr10* and the race non-specific APR gene *Yr29* in a selection of 40 Iranian genotypes using STS and SSR markers. Therefore, genomic DNA was extracted from these genotypes, the susceptible cultivar Avocet 'S' as negative control, and the genotypes with corresponding resistance gene (positive controls). PCR was performed using YrSTS7/8, Xpsp3000 and Xwmc44 markers for *Yr5*, *Yr10* and *Yr29*, respectively. The results indicated the presence of *Yr5* in only 6 genotypes. The presence of a 260 bps band also showed that *Yr10* was present in 12 genotypes while *Yr29* was present in 13 cultivars. As all these three genes are effective against yellow rust pathogen in Iran, it will be an advantage to transfer them to promising lines and develop durable resistance.

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Keywords: *Molecular markers; polymerase chain reaction; resistance genes; wheat stripe rust.*

1. INTRODUCTION

Diseases have accompanied crops since the onset of agriculture and caused great yield losses. The annual yield loss is noticeable both in cost and labor force [1]. Of diseases, rusts are of great importance and attack different plants such as coffee, maize, oats, and are major diseases of wheat wherever it grows [2]. Wheat rusts caused by the basidiomycetes fungi from *Puccinia* family, include yellow rust, leaf rust and stem rust. Yellow or stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) is one of the most important wheat diseases in the world [3]. This disease which was first reported in 1947 in Iran [4], is also the most serious cereal disease in country.

The control strategies mainly include chemical and genetic methods. Detrimental effects emerge when chemicals are used. Therefore, breeding for resistance is a good strategy to control it which in turn affects the global agriculture economy by increasing grain yield and quality. This cost-effective and environmentally friendly strategy can be implemented through using resistant genotypes. To achieve high-level and durable resistance to stripe rust, an important task for wheat breeders and pathologists is to identify and pyramid new genes [5].

Rust resistance genes fall under two broad categories referred to as Seedling Resistance (SR) genes and Adult Plant resistance (APR) ones [6,7,8]. SR genes are detected at the seedling stage and constitute an all stage resistance phenotype [9] while APR genes are commonly detected at the post-seedling stage although some can be expressed at seedling stage under specific conditions. Seedling resistance genes are commonly race specific while APR genes in wheat appear to be race non-specific [9] and are associated with a slow rusting phenotype first described by Caldwell [10]. This type of resistance is conferred by minor genes and is durable [11]. Typically, slow rusting resistance shows longer latent periods, fewer and smaller uredinia within two weeks after inoculation compared to susceptible plants [9]. Seedling resistance genes exhibit phenotypes of major effect with varying infection types whereas most of the APR genes are partial in effect with varying levels of disease severity [9].

Stripe rust resistance genes have been identified progressively in wheat since 1966 (*Yr1*) till now (*Yr53*) bringing the total number of catalogued genes to 70 [12]. To achieve high levels of resistance, it is best to combine both seedling and APR genes in a promising line. The accumulation of four or five slow rusting genes confers near immunity to rust infection [13] and forms the basis of significant gains towards developing more durable leaf rust and stripe rust resistant wheat [9]. However, it is difficult to incorporate adult plant resistance into commercial cultivars because of its quantitative inheritance and lack of appropriate pathotypes to select for the gene combination.

Recent breakthroughs in molecular markers have accelerated the process of pyramiding resistance genes and consequently making the resistance more durable. Molecular markers especially PCR based ones have been successfully used in the process of marker-assisted selection [14]. These molecular marker systems include but are not limited to simple sequence repeats (SSR), or microsatellites, and the Sequence-Tagged Site (STS).

SSR or microsatellites are useful tools for molecular genetic analysis, as they are abundant and display high levels of polymorphisms in many plant species [15,16,17,18]. This

molecular marker technology is convenient and reliable requiring low amounts of DNA and technical support, which has enabled its application to gene mapping in wheat. High-density wheat SSR genetic maps have been constructed [19,20,21], which make tagging yellow rust resistance genes in wheat cultivars possible. SSR markers have been reported for several stripe rust resistance genes, including *Xgwm501* for *Yr5* [22], *Xgwm526* for *Yr7* [23], *Xpsp3000* for *Yr10* [24], *Xgwm413* for *Yr15* [25], *csLV34* for *Yr18* [26,27], *Xgwm11* for *Yr26* [28], *Xgwm533* for *Yr30* [29,30], *cfd1* for *Yr35* [31] and *Xbarc101* for *Yr36* [32].

STS markers are relatively short sequences (200 to 500 bp) which can be specifically amplified by PCR and detected in the presence of all other genomic sequences whose location in the genome is mapped. These markers produce simple and reproducible patterns on agarose or poly-acrylamide gel. Some STS markers reported for *Yr* genes include *YrSTS(7,8)*, *YrSTS(9,10)* and *S19M93-140* for *Yr5* [33,34], *Yr10* [35] and *CYS-5* for *Yr26* [36]. In most cases, STS markers are codominant and allow the distinction of hetorozygotes from homozygotes. Wen et al. [36] also used the Resistance Gene Analog Polymorphism (RGAP) technique to develop molecular markers that were closely associated with cultivars and lines with *Yr26* (as well as *Yr24* and *YrCH42*). Five RGAP markers were identified and converted into STS markers and validated in a set of 18 near isogenic lines and 18 Chinese wheat cultivars and advanced lines [36]. Additionally, other types of DNA markers have been developed. These include RGAP and AFLP markers for the seedling resistance genes *Yr5* [37], and the APR genes, *Yr29* [38], respectively.

Despite the use of markers in validating resistance genes and breeding programmes, their application in Iran has been limited. Therefore, this study investigates the presence of yellow rust resistance genes *Yr5*, *Yr10* and *Yr29* in commercial wheat cultivars using SSR and STS molecular markers.

2. MATERIALS AND METHODS

Forty wheat genotypes were tested for the presence of the resistance genes *Yr5*, *Yr10, Yr29* (Table 3). Fresh leaves were harvested from two week old seedlings and their DNA was extracted using CTAB method [39]. Both DNA quality and quantity were measured using a spectrophotometer (Nanodrop technologies, Thermo 1000, China) and it was also quantified using 1% agarose gel electrophoresis. Working solutions of 100 ng/µl concentration were prepared and used.

To investigate the presence of *Yr5* resistance gene, the STS marker *YrSTS7/8* was used with the genotype *Triticum spelta* var. *album* as positive control. The presence of *Yr10* was studied using the SSR marker *Xpsp3000* and the cultivar Moro as positive control. The presence of *Yr29* was confirmed using the *Xwmc44* marker when the *Yr29* carrying cultivar Pavon 76 was used as positive control. The cultivar Avocet 'S' was used as negative control in all three cases.

The polymerase chain reaction was performed in 20 µl volume containing 2 µl of 100 ng/µl DNA template, 2 µl of 10x PCR buffer containing 500 mM KCl and Tris-HCl (pH 8.4, Vivantis, Malaysia), 0.5 µl of 10 mM dNTP (Vivantis, Malaysia), 10 pmol of each primer (Metabion, Germany), 0.8 µl of $MgCl_2$ (Vivantis, Malaysia), 13.5 µl of double distilled water, 0.2 µl (5U/µl) *Taq* polymerase enzyme (Vivantis, Malaysia). The sequences of the primers (STS and SSR) used for amplification have been shown in Table 1.

Table 1. Sequences of STS and SSR markers used to identify yellow rust resistance genes

Gene	Marker	Marker Type	Primer Sequence	Reference
Yr5	*YrSTS(7,8)*	STS	F:GTACAATTCACCTAGAGT R:GCAAGTTTTCTCCCTATT	[33]
Yr10	*Xpsp3000*	SSR	F:GCAGACCTGTGTCATTGGTC R:GATATAGTGGCAGCAGGATACG	[40]
Yr29	*Xwmc44*	SSR	F:GGTCTTCTGGGCTTTGATCCTG R:GTTGCTAGGGACCCGTAGTGG	maswheat.ucdavis.edu

Polymerase chain reaction was performed in a BIOER thermocycler (GenPro, China) according to the conditions in Table 2. Following the amplification, 2 µl loading buffer was mixed with 5 µl PCR product that ran on 1% agarose gel in 1x TBE buffer at 85V for two hours and the bands were observed under UV light. The gels were stained using ethidium bromide and either Gene Ruler ^{TM} 50 or 100 bp DNA Ladder Plus (Fermentas, Germany) was used as a molecular weight marker.

Table 2. PCR conditions used for each primer*

Primer	Initial denaturation**	Number of cycles	Denaturation***	Annealing***	Extension***	Final Extension**
YrSTS7/8	94 (3)	30	94 (60)	60 (30)	72 (120)	72 (10)
Xpsp3000	94 (3)	30	94 (60)	60 (60)	72 (120)	72 (10)
Xwmc44	94 (3)	35	94 (45)	63 (45)	72 (90)	72 (10)

** The numbers before the parentheses indicate temperature ºC'.; ** The numbers in the parentheses indicate initial denaturation and final extension in minutes; *** The numbers in the parentheses show duration of each step in seconds.*

The products of primer *YrSTS7/8* did not separate on 1% agarose and therefore were analyzed by Poly-Acrylamide Gel Electrophoresis (PAGE) on a denaturing 6% gel at 200 V for 5 hours. Band patterns were visualized using silver staining [37] and images were captured by a scanner.

3. RESULTS AND DISCUSSION

3.1 Resistance Gene *Yr5*

Six % poly-acrylamide gel was used to separate a fragment of 439 bps in positive control; *Triticum spelta* var. *album* and a 433 bps fragment in the susceptible line; Avocet 'S' Fig. 1.

The cultivars Shiraz, Pishtaz, Kaveh, Mahdavi, and Hirmand amplified a fragment of 439 bps indicating the presence of *Yr5* while all other genotypes produced a band of 433 bps and consequently did not carry this gene Table 3. The cultivars Shiraz, Pishtaz, Kaveh, Mahdavi and Hirmand had been reported as resistant to yellow rust [41] and the results of current study suggest that *Yr5* could be one of the genes responsible for their resistance. Similarly, field assessments indicated that the cultivar Marvdasht as resistant [41]. However, this study indicates the gene *Yr5* is not present and other R gene could contribute to resistance in this cultivar. The cultivars Adl, Falat, Hamoun and Golestan were susceptible in the same study [41] and this study confirms the results.

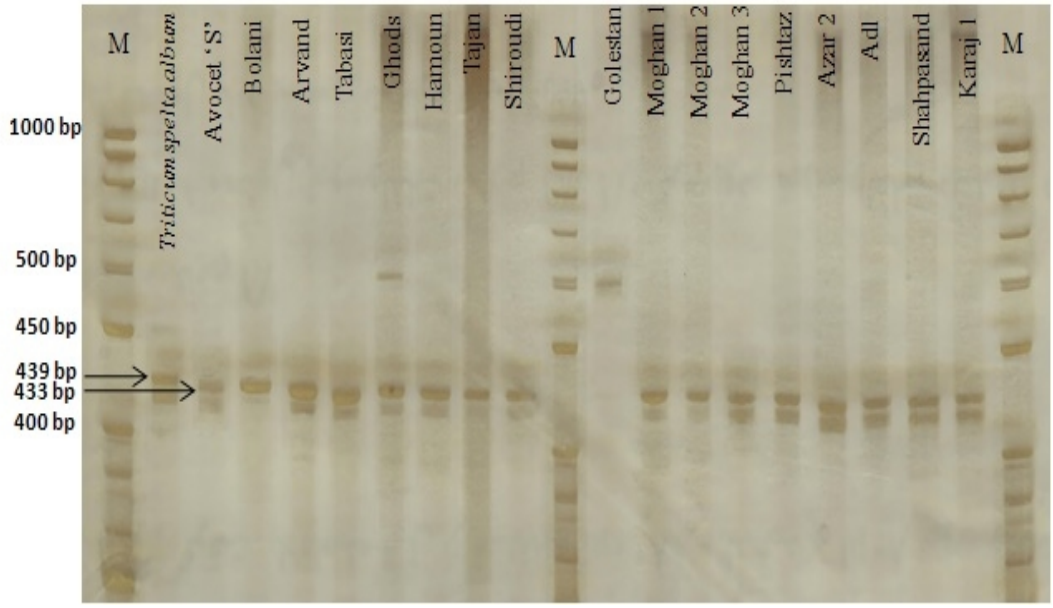

Fig. 1. Band pattern of *YrSTS7/8* marker for the yellow rust resistance *Yr5*. M; 50bp weight marker. The first two lanes after marker (from left to right) present positive and negative genotypes, respectively while the rest show some other genotypes used in the experiment

Most of these cultivars have originated from CIMMYT germplasm with the exception of Marvdasht, Adl and Hamoun which have been derived from national germplasm through hybridization and selection. Therefore CIMMYT-deriverd cultivars are resistant while both field and molecular studies indicate the lack of *Yr5* or other effective resistance genes in cultivars with local origin.

The gene *Yr5* was originally derived from *Triticum spelta* var *album* by Macer [42]. Using classic genetic analyses, it was identified to be dominant and named as *Yr5* [42], which was also confirmed using several crosses tested against north American races of *Puccinia striiformis* f. sp. *tritici* [7,8,43]. The chromosomal location of this gene was determined using cytogenetic approaches on 2BL and 21-cM away from the centromere [42,44].

By investigating two codominant STS primers; *YrSTS7/8* and *YrSTS9/10* in 114 BC_7F_3 lines, Chen et al. [33] concluded that these markers are completely linked to *Yr5*. In another study, Zhang et al. [45] applied the *YrSTS9/10* marker in a number of isogenic lines (NILs), and found allelic relation between *Yr5* and *Yr7*. The *Yr7* resistance gene was also named by Macer [42] and transferred from tetraploid species *T. turgidum* to the hexaploid cultivar Thatcher and subsequently to the cultivar Lee. Mapping showed that *Yr7* was located on chromosome 2B [46].

Based on epidemiological studies, *Yr5* is effective against all rust virulent races in North America [11,33,47] and Iran [48]. This gene is known to show high levels of resistance to stripe rust in China [47,49] and Turkey [50]. Also, in surveys of resistance genes in the Caucasian region and middle Asia [51] and Pakistan [11], *Yr5* and *Yr15* were identified to be

effective against all *Pst* races. The fact that *Yr5* is effective in Iran and its surrounding countries makes it a good candidate for wheat breeding programs.

Table 3. Absence/presence of three yellow rust resistance genes in a collection of Iranian commercial wheat cultivars

Genotype	YR-R gene			Genotype	YR-R gene		
	Yr5	*Yr10*	*Yr29*		*Yr5*	*Yr10*	*Yr29*
Ghods	-	-	-	Atrak	-	+	-
Pishtaz	+	-	-	Shiroudi	-	-	-
Shiraz	+	+	+	Adl	-	-	-
Omid	-	-	-	Shah Pasand	-	-	-
Karkhe	-	-	+	Azar 2	-	+	-
Mahdavi	+	-	+	Kaveh	+	-	-
Tajan	-	+	-	B.C. Shahi	-	-	-
Tabasi	-	-	-	Hamoun	-	-	-
Star	-	-	-	Alborz	-	+	-
Sholeh	-	-	-	Karaj 1	-	-	-
Arvand	-	-	+	Karaj 2	-	-	+
Hirmand	+	+	-	Karaj 3	-	-	-
Azadi	-	+	-	Marvdasht	-	-	-
Moghan 1	-	-	-	Kavir	-	-	-
Moghan 2	-	+	-	Sardari	-	-	-
Moghan 3	-	-	-	Nik Nejad	-	-	+
Fallat	-	+	-	B.C. Roushan	-	-	-
Sabalan	-	+	-	Inia	-	-	-
Golestan	-	-	-	Bayat	-	-	+
Bolani	-	-	-	Darab 2	-	+	+

+/ –, indicates the presence/absence of corresponding genes.

Because *Yr5* is a race specific seedling resistance gene, it should be used in combination with other effective genes and/or with race non-specific adult-plant resistance genes. Such combination could provide durable resistance [37]. Recent advances in molecular characterization of plant resistance genes have provided the opportunities to develop direct markers to combine major race-specific resistance with APR genes [52]. Therefore, the *YrSTS7/8* marker can be useful to transfer *Yr5* in combination with other resistance genes into commercial cultivars.

3.2 Resistance Gene *Yr10*

By using *Xpsp3000* primer pairs for *Yr10*, PCR produced a fragment of 260 bps in the positive control; Moro and 240 bps in the negative control; Avocet 'S' Fig. 2. Results indicated that a band of 260 bps was amplified in 10 cultivars including Tajan, Shiraz, Sabalan, Moghan 2, Azadi, Azar 2, Alborz, Atrak and Hirmand while a 240 bps fragment was amplified in 19 varieties that include Bolani, Arvand, Hamoun, Golestan, Adl, Karaj 1, Karaj 2, Karaj 3, Kavir, Kaveh, Marvdasht, Moghan 1, Pishtaz, Sardari and Omid. The remaining 6 genotypes produced no band (Table 3). These results were similar to those reported by Bariana et al. [40] who indicated varieties with *Yr10* amplify a 258-260 bps fragment and those lacking this gene amplify 240 bps band.

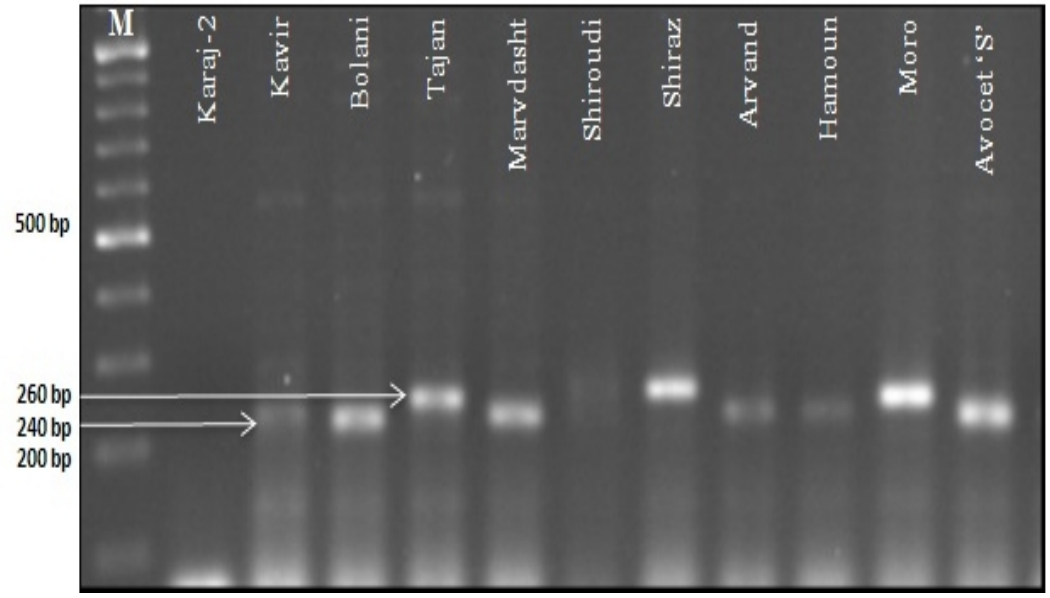

Fig. 2. *Xpsp3000* marker band pattern for the yellow rust resistance *Yr10*. M; 100 bp molecular weight marker. The first two lanes (from right to left) present negative and positive genotypes, respectively while the rest show band patterns of some other genotypes used in the experiment

Although cultivars such as Kaveh, Marvdasht, Moghan 1 and Pishtaz have been reported to be resistant to yellow rust [41], the presence of the 240 bps fragment indicates that these genotypes do not carry *Yr10* or lack the corresponding band which could be as a result of recombination. However a distance of 1.2 cM has been reported to exist between *Xpsp3000* marker and *Yr10*, which makes the chance of recombination very low. Therefore it is highly likely that these genotypes do not carry *Yr10*. Yet, another effective gene could be responsible for resistance in these genotypes. Conversely, Arvand and Azar 2 have been reported as susceptible [41], while the results of this study indicate the presence of *Yr10* in them. Such result could indicate that this gene cannot be expressed in these two cultivars and need further investigation. As stated previously for *Yr5*, *Yr10* is mainly present in CIMMYT-derived cultivars.

The dominant gene *Yr10* was first identified in PI178383 line and was located on the short arm of chromosome 1B. This gene is race specific and has been reported to be effective against all races in China [53], Iran [48], Pakistan and USA [11]. Some reports suggest that this gene is linked to some genes responsible for morphologic traits [54]. Its close linkage with glum brown color (*Rg1*) is used to identify it at mature stage [54]. However, this gene expresses at the final stage of plant growth and makes it inappropriate for early selection of resistance to yellow rust. Close association between *Xpsp3000* marker and Gliadin gene (*Gli-B1*) has also been reported [55; 56]. *Gli-B1* is one of the storage protein genes in wheat endosperm which improves plant resistance to abiotic stresses. This gene has been mapped in different loci on 1 and 6 groups of wheat chromosomes [21]. Bariana et al. [40] verified close association between *Yr10* and *Gli-B1* by genetic analysis of the cultivar Moro. Regarding these facts, the *Xpsp3000* marker is suitable for identifying resistant genotypes at different plant growth stages [24].

3.3 Resistance Gene *Yr29*

The *Xwmc44* primer pair produced two different bands classifying the genotypes into three groups. The first group included 13 varieties with a fragment of approximately 270 bps similar to that observed in the resistant control Pavon 76 Fig. 3. Shiraz, Bayat, Darab 2, Niknejad, Arvand, Karaj 2, and Mahdavi that previously had been reported as resistant [41], belonged to this group Table 3. The *Lr46/Yr29* presence in Iranian wheat genotypes can be tracked back into CIMMYT germplasm which is distributed annually to wheat producing countries.

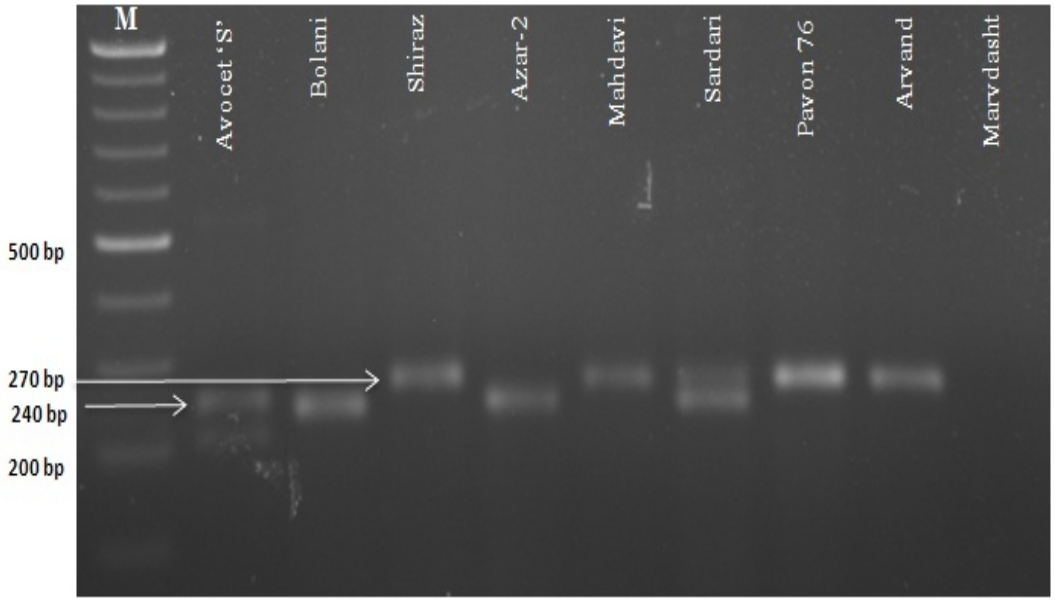

Fig. 3. *Xwmc44* marker band pattern for the yellow rust resistance gene *Yr29* in hexaploid wheat genotypes. M; 100bp molecular weight marker. Of genotypes, Avocet 'S' (first lane from left) and Pavon 76 (seventh lane from left) are negative and positive controls, respectively

The second group consists of Karaj 1, Kavir, Omid, Bolani, Kaveh, Hirmand, Atrak, Moghan 2, Shiruodi, Tajan, Azar 2, Sardari and the susceptible genotype Avocet 'S' which amplified a fragment of 240 bps. These cultivars had been reported as susceptible in field tests [41] and their band was similar to that amplified in Chinese Spring [57]. The last group included Hamoun, Chenab, Golestan, Moghan 1, Pishtaz, Adl, Falat, Marvdasht and Alborz which no band amplified in them. Despite the repetition of PCR, there was no band in these cultivars which implies the need for further investigation of mutation or the absence of similar repeat as primer.

The slow rusting and tightly linked genes *Lr46* and *Yr29* were identified in the cultivar Pavon 76 and were located on chromosome 1B using monosomic series of Lal Bahadur [58]. William et al. [38] established the precise genomic location of gene *Lr46* using molecular approaches and determined its association with adult plant resistance to stripe rust which was designated as *Yr29*.

Strong parallels between the dual adult plant leaf and stripe rust resistance gene(s) *Lr46/Yr29* and *Lr34/Yr18* have been documented. The genes *Lr46/Yr29* have responses similar to those of *Lr34/Yr18* because neither group provides complete immunity to disease. Plants with *Yr29* also show higher rates of fungal colonies abortion without any chlorotic or necrotic effects and decrease the colony size [59]. Cosegregation of *Lr46/Yr29* with *Ltn2*, a second gene for leaf tip necrosis [60] and adult plant powdery mildew partial resistance, *Pm39* [61] bear resemblance to the corresponding phenotypes of *Ltn1* and *Pm38* with the *Lr34/Yr18* gene. Comparatively weaker phenotypes are associated with *Lr46/Yr29* allele than those with *Lr34/Yr18*.

The SSR marker *Xwmc44* located on 1BL chromosome [McIntosh et al., 2001; Catalogue of gene symbols for wheat: http://grain.jouy.inra.fr/ggpages/wgc] and with a distance of 5.6 cM to *Lr46* [62] and 3.6 cM to *Yr29* [60] is useful to investigate these race non-specific genes which are effective at adult plant stage [38].

4. CONCLUSION

To sum up, the cultivars Shiraz, Mahdavi, Hirmand and Darab 2 have been resistant in Iran and current study showed that they carry at least two of the resistance genes *Yr5*, *Yr10* and *Yr29*. In a similar study, Kadkhodaei et al. [63] indicated that the cultivars Atrak, Tajan, Niknejad, Inia, Darab 2, Moghan and Hirmand carry *Lr34* and consequently *Yr18*. Given the fact that these genotypes are resistant, and also drought and salt tolerant and have optimum yield, their cultivation could continue in the arid and semi-arid regions of Iran. As the seedling and race specific genes *Yr5* and *Yr10*, and the race non-specific APR gene *Yr29* are effective against *Pts* pathotypes, they can also be used to develop durable resistance. Their combination is expected to extend the useful life of resistance. However, it would be also a great advantage to transfer *Yr5*, *Yr10*, *Yr18* and *Yr29* to promising lines.

It would be also of great interest to evaluate the presence of seedling genes such as *Yr15* and *Lr67/Yr46* [64; 65], *Sr2/Yr30* [66] in Iranian wheat genotypes. These genes can also be utilized along with the above-mentioned resistance genes to attain long lasting resistance against stripe rust.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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