

## Review article

# **Genetic Analysis of Yellow Rust Resistance in Wheat**

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#### Abstract

Wheat production is affected by several biotic and abiotic stresses and fungal pathogens are the most important disease factor. Globally important fungal yellow rust diseases of wheat caused by obligate parasite biotrophic fungus named "*Puccinia striiformis f. sp. tritici*" is causing loss of quality in a grain and yield at significant level in worldwide. The obligate parasites are highly specialized, and significant variation exists in the pathogen population for virulence to specific resistance genes. Growing cultivars resistant to rust is the most sustainable, cost-effective and environmentally friendly approach preferred to use chemical pesticides for controlling yellow rust diseases. For this reason, determination and evaluation of the presence of wheat varieties resistant and susceptible to yellow rust diseases is of great importance for breeding. Genetic diversity and durability are the two most important features of the resistance for the global wheat improvement programs. Genetic analysis to understand the genetic basis of resistance is important to control of wheat yellow rust. In addition to traditional characterization of resistance using physiological methods, wheat populations also have been genetically characterized using DNA-based molecular markers related with genes to identify and select the presence or absence of genes in early generation populations that could contribute to durable resistance. This review will discuss about yellow rust disease resistance in wheat genotypes in the frame of molecular breeding efforts in combination with our previous findings and current technological developments at molecular level. This information will serve as a foundation for plant breeders and geneticists to develop durable yellow rust-resistant wheat varieties through marker-assisted breeding or gene pyramiding.

Keywords: Marker assisted selection, Molecular breeding, Puccinia striiformis, Wheat, Yellow rust

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### **INTRODUCTION**

Wheat (*Triticum aestivum*) is today the most important crop among all agricultural products and it has the most widespread cultivation area and source of food (Kashyap, 2020). Wheat provides over 20 % of the world's calorie and protein intake and is an important source of vitamins and minerals. In 2018, 773 million metric tons of wheat was produced globally on 218 million hectares of farm-land. Global harvested area of wheat peaked from 2014-2015 and decreased from 2015 to 2018. Global production of wheat peaked from 2016-2017. It started to decline from 2017 (FAO, 2019).

About 10,000 years ago, wheat (2n = 6x = 42, AABBDD) evolved by polyploidization, then spread rapidly and domesticated worldwide (Feldman and Levy, 2005). Extensive data has been obtained about the genetic factors associated with the yield, quality and resistance to biotic and abiotic stresses of wheat that cultivated most widely in the world (O'Brien et al., 2001). Wheat production is affected by several biotic and abiotic stresses causing to great economic losses and fungal pathogens are the most important disease factor among biotic stresses. Rust diseases are among the most economically important diseases affecting wheat because they cause significant yield losses worldwide. Two methods have been used for controlling yellow rust diseases: chemical and genetic control. Besides the negative effects of the use of fungicides on the environment to control the disease, the acquired resistance of the pathogen to the applied chemicals is also a disadvantage. Therefore, cultivation of genetically resistant varieties as the basic mechanism and this method eliminates the need to use fungicides and reduces the cost of production for controlling the disease.

Molecular markers frequently used in application of MAS (Marker Assisted Selection), gene cloning and pyramiding of important crop properties for the resistance of disease, are highly powerful tools (Amiri et al., 2009).

### Advanced Technology Against Yellow Rust Disease

Rust diseases caused by Puccinia species, which are among the biotic stress factors, threaten the safety of wheat production in the world and countries based on wheat (Chai et al., 2014). One of the important species causing rust disease in wheat is Yr (yellow rust) factor *Pst (Puccinia striiformis* f. sp. *tritici)*. Due to its extremely destructive feature yellow rust pathogens cause significant losses in wheat production worldwide. These fungus pathogens colonize the plant by sending their effector molecules into the host cell to suppress the plant's immunity (Feodorova-Fedotova and Bankina, 2018). Stripe rust can cause yield losses from 10 to 100% (Yuan et al., 2018). Since it is such an effective biotic stress factor worldwide, studies are carried out by many researchers to identify breeds and endurance genes and to determine which races or which are more common in which regions to develop acceptable resistance to yellow rust disease in wheat. The first study on this subject was carried out by Gassner and Straib (1932) in Germany in the 1930s. The population structure and epidemiology of the pathogen have

been tried to be revealed with the use of racial separator sets and the disease resistance improvement studies have accelerated. The primary measure taken against yellow rust disease in the world is the use of fungicide. On the other hand, the damage caused by the use of fungicide continuously in the control of the disease is undeniable and it is also very cost efficient (Wellings, 2007). The primary need for sustainable agriculture in future production activities is to develop resistant host resistance strategies to control crop diseases, so it is important to use resistant varieties in disease control (Sánchez-Martín and Keller, 2019). The use of durable varieties is not an environmental problem and it is one of the most effective and sustainable solutions that can be taken against this disease.

Wheat production is estimated to feed a population of nine billion by about 70% by 2050, along with a rapidly growing world population (Ray et al., 2013). To meet this challenge in breeding, genetic development of new wheat varieties can be accelerated by the utilization of advanced genomic technologies (Edae et al., 2015). Therefore, the use of new breeding technologies seems absolutely necessary to achieve the goal in less time, with less labor and cost. Molecular markers linked resistance genes are so functional to develop disease resistant varieties in breeding studies. The economical use of PCR based markers in breeding is the most important reason for the routine use of such molecular markers in most wheat breeding programs. In addition, high-throughput genotyping is an important requirement in large-scale population studies. With advanced sequencing technologies such as NGS (Next Generation Sequencing) radically changing yields and greatly reducing the cost of DNA sequencing, it makes it possible for routine screening of breeding materials (Elshire et al., 2011). GBS (Genotyping by Sequencing) is a convenient method because it sequences a subset of a complex genome reducing genome complexity, analyzing a large number of samples quickly and being cost-effective. As a valuable platform for breeding and genomic studies, GBS can discover and genotype SNP simultaneously (He et al., 2014). SNPs are the most abundant type of sequence variations in plant genomes and since SNPs are the most abundant type of sequence variations in plant genomes (Batley et al., 2007) they could meet the expectation in several studies which includes the analysis of numerous markers such as genetic mapping, genomic selection, QTL (Quantitative Trait Locus) screening, association mapping, population structure and genetic variation analysis (Kumar et al., 2012). Crops such as maize have been sequenced in a comprehensive manner and SSRs in this species have mostly displaced by SNPs. Thus, it is believed that SNPs can take the place of other molecular markers in the near future with the wide spreading utilization of next generation sequencing technologies (Semagn et al., 2013). Furthermore, SNPs are effective tools for high-throughput detection due to their priorities such as locus specificity, co-dominant inheritance, relatively low genotyping error rates, low cost requirements and simple documentation (Schlotterer, 2004). Miedaner et al. (2019) used GBS at DArT (Diversity Arrays Technology) based on dominant silico-DArTs and SNP-marker information in their study analyzing the genetic structure of resistance to yellow rust and stem rust using 12,550 mapped markers.

The newly developed next-generation sequencing techniques enable the discovery of a large number of SNP markers (Appels et al., 2018). SNP markers increase the selection speed and efficiency in visualizable wheat breeding programs by converting them into KASP (Kompetitive Allele Specific PCR) markers to create a high-throughput genotyping platform for the MAS of target genes and transform these functional markers into KASP assays. Rasheed et al. (2016) developed and validated KASP assays for genes that involve agriculturally important traits in bread wheat including adaptability, grain yield, quality, and biotic and abiotic stress resistances. Seventy KASP analyses developed from databases have been verified for their reliability in the application.

*Sr26* stem rust resistance gene (Qureshi et al., 2018a), *Yr34* and *Yr48* stripe rust resistance genes have also been specified via KASP assay and it was showed that *Yr34* and *Yr48* are the same gene and suggested that *Yr48* should be considered a synonym of *Yr34* (Qureshi et al., 2018b). Also, Pakeerathan et al. (2019) performed BSA (Bulked Segregant Analysis) method (Michelmore et al., 1991) using the iSelect 90 K Infinium SNP array with *Yr82* on the long arm of chromosome 3B, and the RIL population was screened for stripe rust in field conditions and analyzed by GBS to reveal genotyping results. With this study, it was shown that two markers (sunKASP\_300 and KASP\_8775) flanked to *Yr82* at a distance of 2 cM, *Yr82*-related these KASP markers that are polymorphic among 84% of Australian cultivars showed usability of *Yr82* for MAS. *Yrcen* gene which is recessive resistant was identified by Mu et al. (2019) via construction of genetic map with the help of genotyping the segregating populations based on two marker platforms. KASP markers were converted from polymorphic SNPs next to *Yrcen* with an interval of 1.7 cM. Thus, more precise and closer markers for resistance gene *Yrcen* were revealed.

BSR-seq (Bulked Segregant Analysis-RNA-Seq) technique is another efficient strategy for genelinked molecular marker identification, which revealing the combination of the BSA and RNA-seq (Trick et al., 2012). This technique enables fast and high-throughput localization of resistance genes in large genome involving crops such as wheat. Molecular characterization of wheat disease resistance genes, such as *Yr15* (Ramirez-Gonzalez et al., 2015), *YrZH22* (Wang et al., 2017), *YrMM58* and *YrHY1* (Wang et al., 2018a), *Yr26* (Wu et al., 2018) have been carried out via this method. Also, with the developments in the field of plant biotechnology, plant breeding studies are more directed towards these fields. In addition to stamina improvement programs that are carried out quickly with marker assisted selection, CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats-Cas9) (Jinek et al., 2012), TALENs (Transcription Activator-Like Effector Nuclease) (Christian et al. 2010) and zinc finger nuclease. It is thought that studies in this area will accelerate by the successful combination of genome regulatory technologies such as zinc finger nuclease (Kim et al., 1996; Miller et al., 2007). Although CRISPR/Cas9-based knockouts of *TaMLO* (Wang et al., 2014) and *TaEDR1* (Zhang et al., 2017) are irrelevant, both of them provide resistance to powdery mildew wheat disease. Also, *TaGW2* and *TaLpx-1* genes were conducted in allohexaploid wheat (Wang et al., 2018b). Although genome editing studies have been initiated for resistance to biotic stress factors in many plants, no studies against yellow rust disease in wheat have been encountered so far.

## **DNA Markers and Identification of Rust Resistance**

Traditional breeding methods based on phenotype are used in developing durable varieties, and this method requires extensive workforce and requires long processes. Today, molecular breeding approach is considered as an important alternative to traditional breeding methods in order to increase efficiency in breeding studies and make more reliable and faster selection (Collard and Mackill, 2008). Identification of new or effective resistance genes in different germplasms of wheat cultivars is essential for providing genetic resistance. Moreover, due to evolution and new virulent races of fungus have appeared, increasing the need to develop durable resistance for yellow rust (Todorovska et.al., 2009). Hence, using combinations of resistance genes is the best method for achieving adequate genetic control of yellow rust diseases affecting wheat (Roelfs, 1989).

Researches have been carried out to identify wheat lines that are resistant to these destructive fungal diseases and to benefit from these sources. But virulent pathotypes continue to be a significant problem to wheat yield. Field evaluation is expensive, time-consuming and highly affected by environmental conditions. The advent of relatively inexpensive, high throughput molecular marker platforms makes MAS (Marker-Assisted Selection) a viable approach to tracking resistance genes. In MAS, DNA markers are used to select for desirable traits. Therefore, establishing a marker-trait relationship is the first step in developing MAS protocols for any given trait. The two requirements to build the marker-trait association are to have accurate phenotypic information and reliable marker data (Elbasyoni et. al., 2019).

MAS method is an essential tool for wheat breeding programs, especially in Canada, USA, Australia, many EU countries, and international wheat and corn research centers (e.g. CIMMYT: International Maize and Wheat Improvement Center), especially for new genotypes of characters with single gene inheritance (Gupta et al., 2010; Randhawa et al., 2013). Gene pyramidization can also be done by conventional breeding methods, but it is not possible to choose phenotypically for plants with more than one gene. Many studies show that the combination of multiple genes against breeds of a pathogen can provide a durable resistance.

The application areas of molecular markers in plant systems are to make indirect selection and increase the use of conventional plant breeding by using the markers depending on the feature to be determined. Molecular markers; to be more reliable than other systems, the plant can be used at every stage of development, is not affected by the environment and it has been widely used in recent years due to its wide variation. Molecular markers distinguish genotypes from each other using nucleic acid sequence differences. Also, when compared with classical morphological or biochemical markers, these

markers have more DNA polymorphism (Kloppers and Pretorius, 1997; Liu et al., 2000; Joshi and Nayak, 2010).

Many gene regions that provide resistance to the breeds of yellow rust disease factor (*Pst*) in wheat have been identified and more than 70 resistance genes have been characterized as a result of molecular studies (McIntosh et al., 2015). Many of the *Yr* genes which provide resistance to yellow rust disease are characterized by race (Goutam et al., 2015). Following the determination of the relevant gene regions, it led to the initiation of many studies on the development of the marker systems associated with these genes and the selection based on it (Enjalbert et al., 2002; Bahri et al., 2009; Chen et al., 2009; Wang et al., 2010). Marker systems developed for this purpose; RFLP (*Yr28*) (Singh et al., 2000), SSR (*Yr10, Yr15, Yr26, YrH52, YrSN104, Yr50, Yr64, Yr65*) (Peng et al., 2000; Wang et al., 2002; Asad et al., 2012; Liu et al., 2013; Cheng et al., 2014), STS / CAPS (*Yr17, YrMoro*) (Robert et al., 1999; Helguera et al., 2003), STS (*Yr61*) (Zhou et al., 2014a), DArt (*Yr51*) (Randhawa et al., 2014) and RGAP / SSR (*Yr59*) (Zhou et al., 2014b).

Yellow rust disease is frequently seen in Turkey as well as in the world and affects wheat agriculture negatively and significantly. For this reason, the project (TUBITAK Project, No:105G075) was realized which of title is "Development of Molecular Markers Genetically Linked Yellow Rust Resistance in Winter Type Bread Wheat" that was supported by The Scientific and Technological Research Council of Turkey in the period of 2006-2010 to prepare of wheat germplasm and to cross in order to obtain mapping population for yellow rust resistance, to perform disease screening tests after inoculation in greenhouse or field and to identify of PCR based molecular markers related with yellow rust resistant genes. In this project, İzgi2001, Sönmez2001, Aytın98, ES14 and Harmankaya99 varieties developed by AARI (Anatolia Agricultural Research Institute) and PI178383, a local variety, were used as plant material. Rust inoculations of these varieties were carried out by CRIFC (Central Research Institute of Field Crops), and as a result of the evaluations, resistant cultivars were identified as İzgi2001, Sönmez2001, PI178383, and Aytin98, ES14, Harmankaya99 were sensitive varieties. Accordingly, by combining between durable and sensitive varieties by AARI, combinations of PI178383 x Harmankaya99, İzgi2001 x ES14, Sönmez2001 x Aytın98, PI178383 x Aytın98 and İzgi2001 x Aytın98 and their  $F_2$  individuals were obtained. Greenhouse and field trials were carried out by CRIFC in order to determine seedling and adult plant resistance of yellow rust disease to reveal the molecular markers that show genetic linkage with yellow rust disease resistance. BSA was performed by using different type of molecular markers (366 SSRs, 190 EST-SSRs, 58 ISSRs, 96 RGAPs, 18 SRAPs, 34 AFLPs, 124 RAPDs, 17 STSs and 209 ESTs) and as a result of the scans performed, although they are specific to different combinations, 3 SSRs - Xgwm382 (Akfirat et al., 2010), Xgwm311 (Figure 1B, 1D), Xwmc658 (Akfirat et al., 2013), 2 EST-SSRs - bu099658 (Hasancebi et al., 2014), PK54 (Figure 1A, 1C) (Ercan et al., 2010) and 1 AFLP - P-GAC/M-ACG markers (Balta et al., 2014) which show a genetic linkage

with yellow rust disease resistance were identified. In order to determine whether these markers, which are specific to combinations, can be used in larger germplasms, scans were performed in populations representing suitable gene pools and it was determined that the obtained markers were 60% usable to identify the yellow rust resistance.



**Figure 1.** Fragment analysis by fluorescence-based capillary electrophoresis of different molecular markers: *bu099658* marker (Hasancebi et al., 2014); RP- Resistance Parent (İzgi01), SP- Susceptible Parent (ES14). (A), *Xgwm382* marker (Akfirat et al., 2010); RP (İzgi2001), SP (ES14), RB- Resistance Bulk, SB- Susceptible Bulk. (B), *Pk54* marker (Ercan et al., 2010); RP (PI178383), SP (Harmankaya99) (C), *Xgwm311* marker (Akfirat et al., 2013); RP (İzgi2001), SP (ES14) (D). The appropriate permissions for figures have been obtained from the copyright holders of this work.

As a final result of this project, these markers have the potential to be used in plant breeding programs in relation to yellow rust disease in the country by contributing to the selection of sensitive and tolerant wheat genotypes in a short time in effective populations.

## **Conclusion and Future Perspectives**

*Puccinia striiformis* f. sp. *tritici* that causes yellow rust disease, which continues to be one of the most important fungal biotic stress factors that limit wheat production in the world. For this reason, studies on yellow rust disease continue increasingly and population change at global level is constantly observed by international organizations or working groups. As a result of the studies and evaluations carried out on the subject, it is clearly seen how many different *Pst* breeds exist in many and their distribution throughout the world. The use of new breeding technologies integrated in traditional plant

breeding efforts seems absolutely necessary to achieve the goal in less time, with less labor and cost. Gene pyramiding through MAS and the use of different molecular approaches is essential for ensuring the sustainability of long-term resistance in wheat cultivars and for controlling these diseases. Although the complexity of the wheat genome and the draft status of the genomic reference, it is possible to proceed in the development of effective genetic approaches such as NGS in wheat. The development of gene-specific markers and KASP experiments, the development of germplasm with adult plant resistance based on small genes, the introduction and use of germplasm and the establishment of collaborative platforms and trainings on this subject are important steps in the fight against plant diseases.

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