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Araştırma Makalesi - Research Article

The Efficacity Investigation for Some Markers Detecting Yellow Rust Resistance Genes in Bread Wheat Varieties

Ekmeklik Buğday Çeşitlerinde Sarı Pas Direnç Genlerini Tespit Eden Bazı Markörlerin Etkinliğinin İncelenmesi

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ABSTRACT

Yellow rust is (*Puccinia striiformis* Westend. f. sp. tritici) is among the supreme diseases causing serious losses in wheat production. The chemical fungicides are commonly used in this disease-fighting. However, chemical control is not economical and also causes environmental pollution. Therefore, the use of resistant wheat varieties in production has critical importance. The resistance against yellow rust disease is expressed with *Yr* genes. In the breeding studies, knowing which parents include resistance genes provides a great advantage in the development of new resistant varieties. This study aims to determine the efficiency of markers used to detect resistance genes against yellow rust disease. The efficiency of molecular markers (Xgwm582, RgaYr10a, Xgwm413, Xgwm11, Wmc44, Barc101, Cfa2149, Sun104, Xgwm273) that are identified for nine genes (*Yr9, Yr10, Yr15, Yr26, Yr29, Yr36, Yr48, Yr51*, and *YrCH52*) providing resistance against yellow rust disease was investigated using PCR method. Twenty bread wheat varieties were used as material. Resistance gene profiles determined using PCR-based molecular markers and data obtained from registration information and field resistance data in the literature were analysed comparatively. As a result of the analysis, the efficiency/productivity of the markers defined for different resistance genes in detecting the resistance gene profile of wheat varieties was determined. Moreover, resistance gene profiles of varieties that are known resistance states in the field and sensitive varieties were compared. Genes that are prominent in providing resistance and detected with markers were determined and the efficiency of these genes was evaluated according to their homozygous/heterozygous states. It was concluded that the efficacy of markers such as RgaYr10a, Xgwm413, Barc101, and Cfa2149, which gave positive results in all wheat varieties, was low.

Keywords- Yellow Rust, Resistance Genes, Bread Wheat, Molecular Markers, Efficacity

ÖZ

Sarı pas, (*Puccinia striiformis* Westend. f. sp. tritici) buğday üretiminde ciddi kayıplara neden olan en önemli buğday hastalıkları arasında yer almaktadır. Hastalıkla mücadelede kimyasal fungusitler yaygın olarak kullanılmaktadır. Ancak kimyasal mücadele ekonomik olmadığı gibi çevre kirliliğine de neden olmaktadır. Bu sebeple üretimde dayanıklı buğday çeşitlerin kullanılması kritik bir öneme sahiptir. Sarı pas hastalığına karşı dayanıklılık, *Yr* genleri ile ifade edilmektedir. Islah çalışmalarında kullanılacak ebeveynlerin hangi dayanıklılık genine sahip olduğunu bilmek, yeni dirençli çeşitlerin geliştirilmesinde büyük avantaj sağlamaktadır. Bu çalışma, sarı pas hastalığına karşı direnç genlerinin saptanmasında kullanılan belirteçlerin etkinliğini belirlemeyi

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amaçlamaktadır. PZR yöntemiyle sarı pas hastalığına karşı direnci sağlayan dokuz gen (*Yr9*, Y*r10*, *Yr15*, *Yr26*, *Yr29*, *Yr36*, *Yr48*, *Yr51* ve *YrCH52)* için tanımlanmış moleküler markörlerin (Xgwm582, RgaYr10a, Xgwm413, Xgwm11, Wmc44, Barc101, Cfa2149, Sun104, Xgwm273) etkinliği araştırılmıştır. Materyal olarak, yirmi ekmeklik buğday çeşidi kullanılmıştır. PCR tabanlı moleküler markörler kullanılarak belirlenen direnç gen profilleri ile tescil bilgisi ve literatürde geçen tarla dayanıklılık verileri karşılaştırmalı olarak analiz edilmiştir. Analiz sonucunda farklı direnç genleri için tanımlanmış markörlerin, buğday çeşitlerinin direnç gen profilini tespit etmedeki etkinlik durumları belirlenmiştir. Ayrıca, tarladaki dayanıklılığı bilinen çeşitlerle hassas çeşitlerin direnç gen profilleri karşılaştırılmış, direnci sağlamada öne çıkan ve markörlerle tespit edilen genler belirlenmiş ve bu genlerin homozigot/heterozigot durumlarına göre verimlilikleri değerlendirilmiştir. Tüm buğday çeşitlerinde pozitif sonuç veren RgaYr10a, Xgwm413, Barc101 ve Cfa2149 gibi markörlerin etkinliğinin düşük olduğu sonucuna varılmıştır.

Anahtar Kelimeler- Sarı Pas, Direnç Genleri, Ekmeklik Buğday, Moleküler Markörler, Verimlilik

I. INTRODUCTION

Wheat is among the top three grains in crop production in the World [1, 2]. However, wheat production is limited by biotic and abiotic factors. Wheat yellow rust disease (*Puccinia striiformis* Westend. f. sp. tritici) is one of the most important diseases affecting wheat production negatively. Because the disease limits the area of leaf photosynthesis, significant yield and quality losses occur. Environmental conditions, pathogen virulence, and host genotype affect the severity of yield loss [3]. Bhardwaj et al. [4] reported that the yield loss caused by yellow rust disease can reach over 50%.

Although fungicides are widely used in the disease control, disease-resistant genotypes in crop production should be preferred as an environmentally friendly practice [5-7]. Various breeding methods are applied in the development of resistant varieties. The Cobb scale, which provides information about the host response and the type of infection, is generally used to assess the disease reaction in the traditional breeding method. [8]. However, marker assisted selection (MAS), which is significantly shortened the process of obtaining new resistant varieties, can be used effectively in breeding studies. Molecular markers can determine the linkage of resistance genes with gene and/or gene regions. Plant varieties carrying two or more genes in the homozygous and/or heterozygous states are powerful tools to facilitate the identification of these genes in the MAS studies [13]. It can give information about homozygous/heterozygous states. Homozygous/heterozygous distinction with molecular markers is of great importance in the early selection of plants. Varieties carrying homozygous genes are very valuable [14]. This provides a great advantage in accelerating breeding studies [15]. Simple sequence repeats (SSR) markers are often preferred in the detection of resistance genes by marker-assisted selection. Because SSRs provide highly informative markers because they are co-dominant and generally highly polymorphic [10]. Resistance genes in yellow rust disease are expressed as *Yr* genes. More than 78 yellow rust resistance genes have been identified worldwide [11]. Identification of *Yr* genes with molecular markers will help to identify parents to be selected as a source of resistance the development of gene-specific DNA markers is important in the identification of resistance genes in varieties [12]. In this study, the efficacy of nine markers defining yellow rust resistance genes was tested on twenty wheat varieties.

II. MATERIAL AND METHOD

A. Plant Material

Seeds of twenty commercial wheat varieties and disease reaction data (Table 1) used in the study were provided by Transitional Zone Agricultural Research Institute (Eskisehir, Turkey). Seeds were germinated in flowerpots under laboratory conditions for DNA isolation.

Table 1. Wheat Varieties, Place of Registration and Disease Reaction

No	Variety	Place of Registration	Disease Reaction*
1	Altay 2000	Transitional Zone Agricultural Research Institute	R
2	Sertak 52	Transitional Zone Agricultural Research Institute	Unknown
3	Kırgız 95	Transitional Zone Agricultural Research Institute	S
4	Bolal 2973	Transitional Zone Agricultural Research Institute	MR
5	Demir 2000	Field Crops Central Research Institute	R
6	Kutluk 94	Transitional Zone Agricultural Research Institute	R
7	Kıraç 66	Transitional Zone Agricultural Research Institute	MS
8	Harmankaya 99	Transitional Zone Agricultural Research Institute	MS
9	Müfitbey	Transitional Zone Agricultural Research Institute	R
10	Nacibey	Transitional Zone Agricultural Research Institute	R
11	Pehlivan	Trakya Agricultural Research Institute	MR
12	Tosunbey	Field Crops Central Research Institute	MR
13	Alpu 01	Transitional Zone Agricultural Research Institute	R
14	Soyer 02	Transitional Zone Agricultural Research Institute	R
15	Yayla 305	Transitional Zone Agricultural Research Institute	Unknown
16	4-11	Field Crops Central Research Institute	Unknown
17	Sönmez 01	Transitional Zone Agricultural Research Institute	R
18	Bezostaja-1	Transitional Zone Agricultural Research Institute	MR
19	Sultan95	Transitional Zone Agricultural Research Institute	R
20	PI178383	Turkey	Unknown

* Disease reactions are the data of the Institute where the varieties are registered. The modified Cobb Scales; R (resistant), MR (moderately resistant), MS (moderately susceptible, S (susceptible) [8, 16].

B. DNA Extraction, PCR Amplification, Electrophoresis and Gel Visualization

Total genomic DNA was extracted from plant leaves ground with liquid nitrogen using the cetyltrimethyl ammonium bromide (CTAB) method as described by Doyle and Doyle methods [17].

PCR amplification for microsatellite primers was carried out in a total reaction volume of 25 µl containing 10 ng of template DNA, 1X Taq polymerase reaction buffer, 2 mm $MgCl_2$, 0.1mm each of dNTPs (dATP, dCTP, dGTP, and dTTP), 0.2 mM primer and 1 U of Taq DNA polymerase (Fermentas). Amplifications were performed in a Techne TC Plus thermocycler (Techne Inc.) programmed as follows: 3 min denaturation at 94 ºC and 35 cycles of 1 min. each denaturation at 94 ºC, 1 min annealing at 50-60 ºC for SSR amplification, and a 2 min extension at 72 ºC, followed by a final extension at 72 ºC for 7 min. Primer sequence information was obtained from the Grain Genes database (Table 2) [18].

Table 2. List Of Primers Along with Their Gene, Sequence, Expected Product Size

Primer	Linked *Yr* Gene	Sequence (F/R)	Expected product size	References
Xgwm582	*Yr9*	AAGCACTACGAAAATATGAC TCTTAAGGGGTGTTATCATA	150	[19]
RgaYr10a	*Yr10*	ATCAAGAGCCGCATCAAGG CCAAAGCCAACAATAGAGACC	233	[20]
Xgwm413	*Yr15*	TGCTTGTCTAGATTGCTTGGG GATCGTCTCGTCCTTGGCA	96	[19, 21]
Xgwm11	*Yr26*	GGATAGTCAGACAATTCTTGTG GTGAATTGTGTCTTGTATGCTTCC	200	[19]
Wmc44	*Yr29*	GGTCTTCTGGGCTTTGATCCTG TGTTGCTAGGGACCCGTAGTGG	242	[21, 22, 23]
Barc101	*Yr36*	GCTCCTCTCACGATCACGCAAAG GCGAGTCGATCACACTATGAGCCAATG	123 (116,138,160,165)	[22]
Cfa2149	*Yr48*	CTTGGAGCTCGGGTAGTAGC AAGGCAGCTCAATCGGAGTA	231	[22]
Sun104	*Yr51*	TGCTATGTGCGTGATGATGA TTACATGCTCCAGCGACTTG	225(+)	[21, 24]
Xgwm273	*YrCH52*	ATTGGACGGACAGATGCTTT AGCAGTGAGGAAGGGGATC	170, 180, 190, 200	[19]

Amplification products were separated on 1.3% agarose gel containing ethidium bromide (0.5 µg/ml) using 100 bp DNA ladder (Solis Bio Dyne, Estonia). Gels were visualized under UV light and digitally

photographed with Gel Logic 212 Pro imaging system (Carestream, USA). The polymorphism information content (PIC) was calculated according to the formula:

$$PIC = 1 - \sum_i^n pi^2 \quad (1)$$

III. RESULTS AND DISCUSSION

Rust diseases in wheat cause destructive results all over the world. It was reported that yellow rust disease caused a widespread epidemic from 1936 to 1963 in Turkey. In addition, it caused regional epidemics from 1975 to 1984, a yield loss of up to 62.5% in 1991, serious yield losses in Central Anatolia in 1998, and epidemics in Central Anatolia and Transition Regions from 2009 to 2010 [25, 26]. Fungicides are widely used in the fight against yellow rust. In 2019, according to the data from the Turkish Statistical Institute (2021), a total of 51,297 tons of pesticides were used in Turkey. The amount of fungicides is 19,698 tons in these used pesticides. However, because pesticide use causes damage to the environment, it is recommended to use resistant varieties in sustainable agriculture and breeding [3, 5, 27]. Molecular markers defined for resistance genes are used to identify resistant varieties for breeding studies. Resistance to yellow rust disease in wheat is defined by *Yr* genes called. Many genes or DNA sites have been identified to provide resistance against the different yellow rust disease races in studies carried out until today. More than seventy resistance genes have been characterized using molecular studies [28]. It has been reported that DNA amplification fragments obtained using PCR markers identifying these genes may be a specific indicator of disease resistance [29, 30, 31, 32, 33]. Determination of resistance gene regions in wheat contributed greatly to the development of markers and the rapid identification of resistant varieties using molecular methods. Resistance to rust diseases emerges in two forms race-specific and non-race-specific. The race-specific resistance is controlled by a single gene. When a racial change in the pathogen organism, the resistance is lost. When non-racial resistance is provided by multiple genes, stronger resistance to race changes occurs [34]. As a result of the amplification of microsatellite loci by PCR method and their execution in gel electrophoresis, bands formed by spreading according to molecular weights of heterozygous and homozygous individuals can be visualized on the gel. While PCR products of heterozygous individuals are seen as two bands on the gel, PCR products of homozygous individuals are displayed as a single band [13, 35].

In this study, the effectiveness of molecular markers used in detecting the presence of *Yr* resistance genes in bread wheat varieties was evaluated. It was analysed comparatively PCR results (Figure 1) obtained using molecular markers with the literature information including the field observations. Identified markers for nine *Yr* genes (*Yr9, Yr10, Yr15, Yr26, Yr29, Yr36, Yr48, Yr51, YrCH52*) were tested on twenty bread wheat varieties.

Nine SSRs markers were used to study variation at *Yr* loci in twenty wheat lines. DNA markers Xgwm582, *Yr10* marker Xgwm413, Xgwm11, Wmc44, Barc101, Cfa2149, Sun104, and Xgwm273 gave the expected results (Table 2).PR (Polymorphism rates) and PIC (Polymorphism Information Content) values were calculated for all primers. Because four primers (RgaYr10a, Xgwm413, Barc101, and Cfa2149) include only a monomorphic band (homozygote character), their PR and PIC values were obtained as zero. PR values of the other five primers (Xgwm582, Xgwm11, Wmc44, Sun104, and Xgwm273) include different two bants (heterozygote character) were less than 50% and found inefficient. PIC values for these primers were determined respectively Xgwm582 (0,09), Xgwm11 (0,36), Wmc44 (0,22), Sun104 (0,31) and Xgwm273 (0,27).

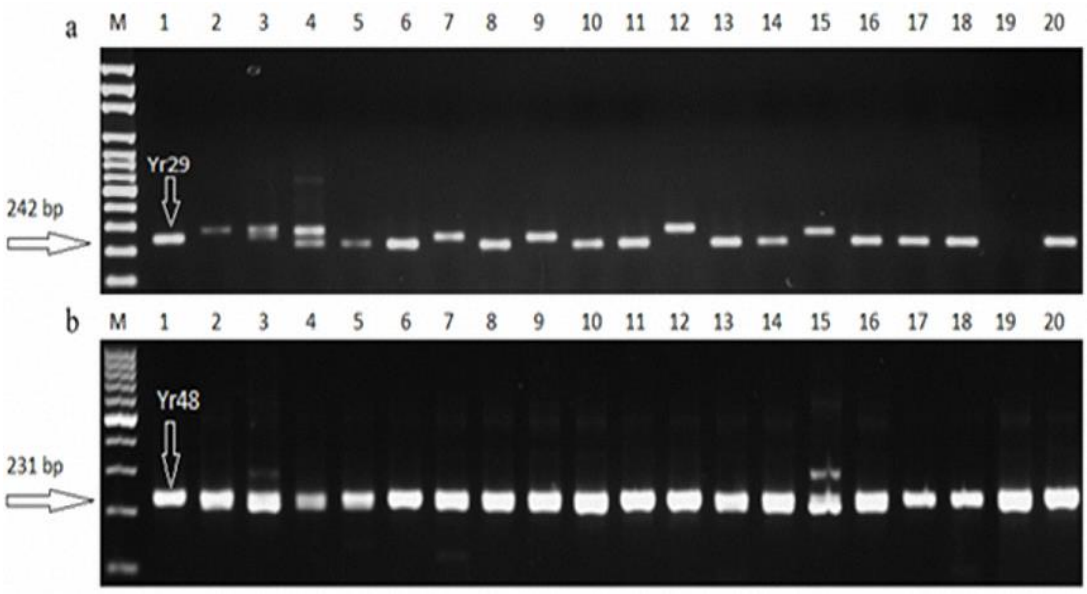

Figure 1. PCR Amplified Products of (A) Marker (100 Bp DNA Ladder) Wmc44 for *Yr29* (242 Bp) And (B) Marker Cfa2149 for Detecting *Yr48* (231 Bp) Gene in Wheat Varieties.

The disease reaction may differ depending on the genotype-environment interaction. This reaction is under the influence of pathogen virulence and external factors. Some pure lines may be resistant in some environments and susceptible in others [36].

Ay (2013) determined the disease rates of some wheat varieties. While he evaluated the obtained results, he also considered the relative humidity reported between the years 2009-2010. Since the relative humidity average in 2010 is more than in 2009, he has asserted that 2010 has more favourable conditions for the rust epidemic. Depending on this result, Ay (2013) reported that Kırgız-95 and Bolal-2973 showed moderately sensitive and sensitive reactions in 2010 compared to 2009, respectively [31]. We observed that Kırgız-95 and Bolal-2973 varieties have a positive band product of 242 bp in our screening with the Wmc44 marker.

It was observed that the tested markers (RgaYr10a, Xgwm413, Barc101, Cfa2149) for *Yr10*, *Yr15*, *Yr36*, and *Yr48* of these genes yielded products in all varieties (Table 3). As a result, it is possible to talk about the presence of resistance genes in all of the varieties of wheat. However, the presence of these genes in varieties that react sensitively under field conditions can be explained by the fact that the resistance is controlled by more than one gene or gene pair or the markers lose their effectiveness [34].

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Table 3. PCR Amplification Results and Disease Reactions in the Literature

Wheat varieties / Gene/ markers	*Yr9*	*Yr10*	*Yr15*	*Yr26*	*Yr29*	*Yr36*	*Yr48*	*Yr51*	*Yr52*	2009 Field Resistance *	2010 Field Resistance
Altay 2000	+	+	+		+	+	+			Immune	Immune
Sertak 52	+	+	+			+	+			Unknown	Unknown
Kırgız 95	+	+	+	+		+	+			Resistant	M. Susceptible
Bolal 2973	+	+	+			+	+			Immune	Susceptible
Demir 2000	+	+	+		+	+	+			Resistant	Immune
Kutluk94	+	+	+		+	+	+			Immune	M. Resistant
Kıraç 66	+	+	+			+	+			Immune	Immune
Harmankaya 99	+	+	+		+	+	+			M. Resistant	Immune
Müfitbey	+	+	+			+	+	+	+	Immune	Immune
Nacibey	+	+	+		+	+	+	+	+	Unknown	Unknown
Pehlivan	+	+	+	+	+	+	+			M. Resistant	Immune
Tosunbey	+	+	+	+		+	+			Immune	Immune
Alpu 01	+	+	+		+	+	+	-		Immune	Immune
Soyer 02	+	+	+		+	+	+	+		Resistant	Immune
Yayla 305	+	+	+	+		+	+			Immune	Immune
4-11	+	+	+		+	+	+			Unknown	Unknown
Sönmez 01	+	+	+	+	+	+	+			Resistant	Immune
Bezostaja-1	+	+	+	+	+	+	+			Resistant	Immune
Sultan95		+	+	+		+	+	+	+	Resistant	Immune
PI178383	+	+	+	+	+	+	+	+	+	Unknown	Unknown

* 2009-2010 Filed Resistance [31], Immune (I), Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS), Susceptible (S) [27].

It was procured yields bythe*Yr9* gene marker in all wheat varieties used in the study apart from Sultan 95 variety. It obtained a monomorphic band at 150 bp for primer Xgwm582. The product size for this marker is consistent with the results obtained by Çabuk et al. (2011). The same result was obtained for the PI178383 variety used in their studies [19]. The results of both studies confirm each other.

PI178383 variety stands out as the variety that yields products for all markers among the varieties used in the study. The yellow rust resistance locus *Yr10* on chromosome 1B originates from PI178383 which is a local variety obtained by selection from Şemdinli's region and is known to carry three minor genes.

It was known that wheat varieties containing genes determined by *Yr9*, *Yr10*, *Yr15*, *Yr26*, *Yr36*, and *Yr48* markers (Table 3) showed generally resistant reactions over the years [31]. It was strikingly that Tosunbey and Yayla305 varieties, which are known to be resistant in the field according to different years, contain the *Yr29* gene as well as some other *Yr* genes. Müfitbey is a variety known to be resistant throughout the years in the field and remarks among the other wheat varieties used in the study. According to the obtained results, we think that the Müfitbey, Nacibey, and PI178383 varieties may be used as a parent in the breeding studies aimed at yellow rust resistance.

Pathogenicity of wheat yellow rust disease; varies from year to year and from region to region, depending on the susceptibility of the varieties, the race of the pathogen, and environmental conditions, especially humidity and precipitation [11]. There are differences in the disease reactions depending on the years in Kırgız95 and Bolal2973 varieties. While both varieties showed resistant (immune) reactions in 2009 year, they showed sensitive reactions in the next year (Table 1, 3). In our study, while the Xgwm11 marker associated with the *Yr26* gene didn't form bands in the sensitively reacted Bolal variety, produced a band product in the Kırgız variety that has got a moderately sensitive reaction. This argument can be explained by the race change of the pathogen and the loss of effectiveness of the marker. Therefore, it is important to investigate the presence of more *Yr* genes, to detect markers that lose their effectiveness, and to take this into account in resistance breeding studies against possible epidemics.

Yr29 gene was detected in Altay2000, Demir2000, Kutluk94, Harmankaya99, Nacibey, Pehlivan, Alpu01, Soyer02, 4-11, Sönmez01, Bezostaja-1, and PI178383 varieties. When the studies about these wheat varieties and their characteristics consider, it is seen that they are resistant in the field. The absence of this gene in susceptible varieties has indicated that the *Yr29* gene is an important gene in controlling resistance and that the Wmc44 marker defining this gene is effective. When the Sun104 marker activity for the *Yr51* gene was evaluated, the detection of

this gene only in resistant-reacting varieties reveals that the gene and its marker can still be used effectively. Similarly, the *YrCH52* gene was detected only in resistant-reacting varieties, and it appeared that the activity of its marker (Xgwm273) continues.

PI178383 variety is frequently used as a resistant variety in breeding studies against yellow rust disease [19, 37]. This variety obtained positive band products for all markers as a result of the study. As a result, we can assert that the PI178383 variety acts as a control group in terms of resistance. We observed that PI178383, Müfitbey, and Nacibey varieties that have the resistant reaction formed positive band products for most markers (Table2-3). We predict that these varieties will be beneficial to use in aquaculture and breeding studies against yellow rust epidemics in the long term. We recommend including Wmc44, Sun104, and Xgwm273 markers, respectively, in screening the *Yr29*, *Yr51*, and YrCH52 genes to control the resistance status of the wheat varieties.

As a result, the co-existence of *Yr29*, *Yr51*, and *YrCH52* genes apart from other genes which are mutual in PI178383, Müfitbey, and Nacibey varieties which show effective resistance in the field, also reveals the effectiveness of the markers that define these genes. Our results showed that PI178383, Müfitbey, and Nacibey varieties will be beneficial to be used in breeding studies against yellow rust epidemics in the long term. We think that *Yr29*, *Yr51*, and *YrCH52* genes will have a critical role in determining resistance in genotypes in marker-assisted selection studies against yellow rust disease. Therefore, we suggest that these genes should be given priority when determining parent candidates in breeding studies.

Knowing and confirming the genes which establish resistance in varieties is very important. The obtained results from this study are very valuable in terms of creating new strategies against racial changes and accelerating breeding studies. If we evaluate results from a general point of view, we understand that the studies at the DNA level to be made with molecular markers should be used together with the field conditions. In this way, it may be possible to prevent possible pandemics by following the race change of the pathogen.

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