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# Determination of Virulence Genes Frequencies in Wheat Stripe Rust (*Puccinia striiformis* f. sp. *tritici*) Populations During Natural Epidemics in the Regions of Southern Aegean and Western Mediterranean in Turkey

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**Abstract:** This study was carried out to investigate virulence gene(s) frequency of wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*) populations during natural epidemics, in nine different fields, located in the regions of Southern Aegean and Western Mediterranean. Stripe rust uredospores were collected from diseased plants in 2000 and 2001. Virulence of pathogens were determined by seedling test. The results showed that virulence gene frequency on rust population varies depending on the type of genes. The genes which have high virulence frequency were more stable. In both experimental years, it was established that the virulence frequency of Yr1, Yr2, Yr3a, Yr9, Yr17, Sd and So genes ranged between 7.8 and 100% in all populations while Yr6, Yr7, Yr8 and Yr10 genes were observed in all locations.

**Key words:** *Puccinia striiformis* f. sp. *tritici*, wheat, virulence gene frequency

## INTRODUCTION

Wheat is an important cereal mainly for human consumption and is grown in all agricultural regions in Turkey. The total area cultivated in the world and Turkey are 210.0 and 9.4 million ha, respectively. Turkey is among the main wheat producing countries which is ranked seventh[1].

Wheat stripe rust (yellow rust) caused by *Puccinia* f.sp. *tritici* Eriksson, is one of the most frequent and important cereal disease in Turkey. It causes yield losses of up to 40-60% grain yield and lowers the quality of cereal products. Yield losses have been reported in different countries and were estimated around million ha in Europe per year[2-5].

In Turkey, natural epidemics occur usually in the west and occasionally in the south and are especially damaging when springs are cool and rainy. In spite of this, necessary measurements were not taken against this disease in Turkey. However, this disease has been controlled in Turkey and Europe by the use of fungicides in some wheat growing areas and also host resistance genes. Although it is possible to control stripe rust by using chemicals, fungicides are ecologically not desirable and frequent use speeds up the evolution of fungicide resistance. Another way to control the pathogen is the use of resistant varieties[6-8].

To produce healthy crops in a cheap and environmentally friendly way, knowledge is needed concerning disease resistance in the host and the ability of the pathogen to overcome the genes conferring host resistance. Based on gene-for-gene concept of Flor[9], virulence of the stripe rust population indicating the efficiency of the corresponding host resistance gene, was monitored using a set of host genotypes differing primarily by a single gene for resistance[10].

The most economic and practical control of the disease can be achieved through integrated management system, including host-plant resistance and improved agricultural practices. Generally, the most extensively used resistance genes have remained effective for only a few years, after which an increase in frequency of the corresponding virulence genes of stripe rust was observed; then the varieties with the resistance genes became increasingly more susceptible to stripe rust under field conditions[11].

*P. striiformis* is characterized by high genetic variability that makes race specific long-term resistance breeding difficult, because the pathogen can rapidly adapt and develop new virulent races. So far, European stripe rust populations have been studied on a very large scale with the objective to investigate long-distance migration and population exchanges between countries[12-14].

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Knowledge of the genetic basis of resistance to rust pathogens in wheat varieties is useful for understanding of the distribution of races of pathogen, for the choice of varieties to be grown and also for breeding for disease resistance. The success of any breeding program depends primarily upon the availability of resistance donor parent genotypes, but also on the knowledge of the composition of the pathogen population. Therefore, the aims of this study were to analysis the genetic structure of wheat stripe rust pathogen populations in the South Aegean and Mediterranean regions of Turkey during the 2001 and 2002 wheat growing seasons.

## MATERIALS AND METHODS

The materials of the study consisted of pathogen isolates and 15 different wheat cultivars with specific resistance genes and the control cultivar-Victo (Table 1). In the 2000 and 2001 wheat growing seasons, wheat cultivar- Victo was sown in one wheat field located at each location (Kas, Fethiye, Dalaman, Ortaca, Köyceğiz, Gökova, Muğla, Yatağan, Çine). Cultivar- Victo has no identified resistance gene to *P. striiformis* f.sp. *tritici* pathotypes but is resistant to leaf rust (*Puccinia triticina*) and powdery mildew (*Blumeria graminis* f. sp. *tritici*). No fungicide was applied to the fields.

**Uredospores sampling:** Uredospores sampling was done once at the end of April and beginning of May, from the all experimental fields when epidemic was prevalent. Initially, several foci of about 1 $m^2$ in which plants were severely diseased were observed. Ten sampling points about 10 m apart, were defined along two parallel transects in the field and 20 sporulating leaves were collected at each point. At the end of epidemics, the whole fields were heavily diseased, where in 12 to 20 sporulating leaves were collected in each field. From each site, samples were collected randomly so that all areas of each population could be covered. For transportation, sporulating leaves were kept in plastic bags at 4°C. In the laboratory, leaves were taped on plates keeping sporulating side up and placed in a plastic bag with 100% humidity at 8°C. After 12 h, spores were collected from sporulating leaves from each location, desiccated and stored in liquid nitrogen.

**Isolation and multiplication of the pathogen isolates:** Method of isolation and multiplication of pathogen was similar to Villareal *et al.*[15]. Spores were taken out of liquid nitrogen, heat shocked (40°C, 5 min) and used to inoculate 7-day-old seedlings of cultivar Victo. Four pots containing twelve seedlings were sown in 10 cm wide square pots, in a row along one of the pot edges and protected from airborne contamination in isolated compartments. When seedlings were 1-cm high, growth was reduced by adding 15 mL of maleic hydrazide acid (0.25 g $L^{-1}$) per pot.

Inoculations were performed by using settling tower. When plants 12 days old, 8 pots of seedlings were placed horizontally in a settling tower, 45 cm wide and 1 m high. In order to obtain very low lesion density on the leaves, 1 mg of *P. striiformis* f.sp. *tritici* spores were diluted 25 times in talc and 5 mg of the mixture was projected in the settling tower.

The inoculated seedlings were placed in a dew chamber at 8°C for 16 h and then in a climate chamber (d: 17 h at 15°C; night: 7 h at 10°C) in individual booths. After 10 days, latent lesions were observed for each successful infection. One leaf per pot, bearing a single lesion, was then chosen and the remaining leaves were removed. Pots were then replaced in individual booths within the climate chamber. After 7 days, selected lesions were sporulating and spores were collected in 2.2 mL microtubes before multiplication. With this sort of process, totally 491 isolates were sampled from all fields.

To multiply, 20 Victo seedlings were grown in a 7 cm wide plastic square pot for each isolate, protected from airborne spores and treated with 20 mL of maleic hydrazide acid (0.25 g $L^{-1}$) when 1 cm high. Collected spores in 2.2 microtubes were mixed with talc (1:3 v/v) and applied manually along each leaf with a cotton swab. Inoculated plants were then incubated in a dew chamber at 8°C for 16 h and then in a climate chamber (d: 16 h, 17°C; night: 8 h, 14°C). During the whole multiplication procedure, plants were kept separated from each other in individual booths. Eighteen days after inoculation, spores were collected, dried in a desiccator at 4°C for 3 days and stored in microtubes at -80°C. For each isolate, two independent multiplication procedures were carried out, from which collected spores were stored apart.

**Virulence test:** Virulence spectra of the 491 isolates were determined using the European and world sets of 15 differential cultivars with specific resistance genes and control cultivar-Victo. It is possible to detect the virulence matching to resistance genes via this differential set. The list of the wheat lines used as differentials in the determination of the virulence test of the isolates and of the presence of resistance genes is presented in Table 1.

Twelve milligrams of uredospores of each isolate was mixed with talc (1:3 w:w) and sprayed at the two-leaf-stage onto six seedlings of each variety. After inoculation, seedlings were placed in a dark climatic room for 24 h at 8°C and 100% relative humidity and then transferred into

Table 1: List of the wheat lines used as differentials in the determination of virulence of isolates

| Line | Resistance gene (s) | Line | Gene (s) |
|---|---|---|---|
| Chinese 166 | Yr1 | Moro | Yr10 |
| Kalyonsona | Yr2 | Maris Huntsman | Yr13 |
| Nord Desprez | Yr3a | Hobbit | Yr 14 |
| Triticum spelta album | Yr5 | Hussar | Yr17 |
| Austerlitz | Yr6 | Strubes Dikkopf | Sd |
| Lee | Yr7 | Cartens V | CV |
| Compare | Yr8 | Swon 92 X Omar | So |
| KavkazXFedaration | Yr9 | Victo | Control |

Table 2: Infection types of *Puccinia striiformis* f. sp. *tritici* isolates on wheat differentials lines[16]

| Description of infection type | Code symbol[a] | Index value |
|---|---|---|
| No data | | |
| No visible infection | 0 | 0 |
| Necrotic/chlorotic flecks- no sporulation | VR | 1 |
| Necrotic/chlorotic stripes- no sporulation | R | 2 |
| Necrotic/chlorotic stripes- trace of sporulation | MR | 3 |
| Necrotic/chlorotic stripes- light sporulation | LM | 4 |
| Necrotic/chlorotic stripes- intermediate sporulation | M | 5 |
| Necrotic/chlorotic stripes- moderate sporulation | HM | 6 |
| Necrotic/chlorotic stripes- abundant sporulation | MS | 7 |
| Chlorosis behind sporulating area- abundant sporulation | S | 8 |
| Necrosis/chlorosis abundant sporulation | VS | 9 |

[a]H= high, L= light, M= moderate, R= resistant, S= susceptible, V= very

a greenhouse at 20°C for 10 day. Daylight was supplemented to 16 h with high-pressure sodium vapor lamps. Fifteen and seventeen days after inoculation, based on the presence of necrosis and chlorosis and the intensity of sporulation, plants were evaluated individually on a scale from 1 to 9 (Table 2).

The reaction types were evaluated according to infection types as described by Mc Neal *et al.*[16] (Table 2). The reactions to infection were summarized by combining the infection type into three groups with resistant (R)=1-3; moderate resistant (MR)= 4-6; and susceptible (S)=7-9. The virulence reactions were defined as infection types 7 to 9. For the identification of the infection, the infection assessment was transformed into binary codes generated from the infection types (virulent or avirulent) on the differential cultivars. Infection types 7–9 (susceptible) were transformed to 1, while the rest (R and MR) was transformed to 0.

## RESULTS AND DISCUSSION

In Turkey the stripe rust epidemic began in early spring and ran from south towards the northern and eastern regions in 2001, although appeared late in growing season in year 2000. According to the progress of the epidemic, Dalaman, Köyceğiz Fethiye and Kaş locations showed heavier infection while rest of the locations showed lower level of infection at a similar sampling date. As far as possible, the sampling was done at the beginning and at the end of the epidemic.

The virulence gene frequencies for the all samples collected from nine different locations in two years (2000 and 2001) are given in Table 3 and 4, respectively. In both the experimental years, the frequency of Yr2, Yr3a, Yr17 and Sd genes were found to be close to 100% in all the populations. It was also reported that Yr17 virulence gene frequency closed to 100% in northern European countries[15]. Based on these results, these genes seem to be fixed in all the populations in Turkey. On the other hand, frequency of Yr6, Yr7, Yr8 and Yr10 genes was 0%. Thus, no fungal isolates having Yr6, Yr7, Yr8 and Yr10 virulence genes were observed in these populations.

Table 3: Virulence genes frequencies (%) observed in samples of wheat stripe rust populations from 9 different locations in the South Aegean and West Mediterranean Regions of Turkey in 2000

| | Locations | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Resistance gene (s) | Kas | Fethiye | Dalaman | Ortaca | Köyceğiz | Gökova | Muğla | Yatağan | Çine |
| Yr1 | 7.8 | 18.6 | 78.6 | 65.2 | 71.3 | 52.8 | 26.4 | 22.4 | 64.2 |
| Yr2 | 96.4 | 98.2 | 100.0 | 100.0 | 100.0 | 96.6 | 98.2 | 100.0 | 98.2 |
| Yr3a | 100.0 | 100.0 | 94.6 | 100.0 | 93.6 | 68.2 | 100.0 | 100.0 | 100.0 |
| Yr5 | 0.0 | 0.0 | 4.6 | 6.2 | 0.0 | 0.0 | 0.0 | 0.0 | 4.2 |
| Yr6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Yr7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Yr8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Yr9 | 65.2 | 72.4 | 86.2 | 95.2 | 78.4 | 84.2 | 76.4 | 45.2 | 76.2 |
| Yr10 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Yr13 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 14.2 | 12.3 | 0.0 |
| Yr14 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.2 | 0.0 | 0.0 | 0.0 |
| Yr17 | 100.0 | 100.0 | 100.0 | 96.4 | 94.4 | 96.8 | 98.2 | 98.5 | 100.0 |
| Sd | 100.0 | 100.0 | 97.0 | 96.0 | 100.0 | 100.0 | 98.0 | 100.0 | 100.0 |
| CV | 45.2 | 75.4 | 38.4 | 46.2 | 58.4 | 45.6 | 0.0 | 0.0 | 65.4 |
| So | 65.2 | 42.4 | 72.6 | 46.5 | 32.2 | 56.6 | 68.4 | 36.2 | 75.4 |
| Control (Victo) | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| No* | 24.0 | 23.0 | 31.0 | 28.0 | 25.0 | 27.0 | 31.0 | 26.0 | 34.0 |

*: Number of tested isolates

Table 4: Virulence genes frequencies (%) observed in samples of wheat stripe rust populations from 9 different locations in the South Aegean and West Mediterranean Regions of Turkey in 2001.

| | Locations | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Resistance gene (s) | Kas | Fethiye | Dalaman | Ortaca | Köyceğiz | Gökova | Muğla | Yatağan | Çine |
| Yr1 | 22.8 | 28.4 | 48.6 | 45.2 | 41.4 | 32.6 | 46.4 | 42.4 | 62.2 |
| Yr2 | 98.2 | 100.0 | 100.0 | 100.0 | 100.0 | 98.2 | 100.0 | 100.0 | 96.4 |
| Yr3a | 100.0 | 100.0 | 98.6 | 100.0 | 100.0 | 84.2 | 100.0 | 100.0 | 100.0 |
| Yr5 | 0.0 | 0.0 | 9.6 | 4.2 | 0.0 | 0.0 | 0.0 | 0.0 | 7.2 |
| Yr6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Yr7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Yr8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Yr9 | 78.6 | 66.4 | 82.4 | 87.4 | 68.8 | 94.4 | 76.4 | 56.8 | 71.2 |
| Yr10 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Yr13 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 8.6 | 10.6 | 0.0 |
| Yr14 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 12.6 | 0.0 | 0.0 | 0.0 |
| Yr17 | 100.0 | 100.0 | 100.0 | 100.0 | 98.4 | 98.8 | 100.0 | 96.8 | 100.0 |
| Sd | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| CV | 65.2 | 65.4 | 44.4 | 58.4 | 65.4 | 74.6 | 0.0 | 0.0 | 72.4 |
| So | 58.4 | 62.2 | 78.8 | 58.5 | 28.2 | 52.2 | 64.6 | 46.2 | 68.4 |
| Control (Victo) | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| No* | 26.0 | 28.0 | 32.0 | 31.0 | 25.0 | 26.0 | 24.0 | 23.0 | 27.0 |

*: Number of tested isolates

Our results also revealed some variation among samples with respect to gene frequency. For example, the differences were most evident for samples collected in Dalaman and Ortaca locations for Yr5 gene. Frequency of Yr5 virulence gene changed between 4.2 and 9.6% in these two locations in both the experimental years. Yr13 gene was observed only in Muğla and Yatağan populations with virulence gene frequency in the range of 8.6 and 14.2% in two years. Yr14 gene was noticed only in Gökova populations, with frequency of 6.2- 12.6% in two years. Virulence frequency of Yr14 doubled in the year 2001 as compared to year 2000. It was also found that the virulence frequency of Yr5 changed between 4.2 to 9.2% at isolates from Dalaman, Ortaca and Çine locations during both the years. In Dalaman, Fethiye, Çine and Gökova fields, low efficiency was observed by near isogenic line carrying the resistance gene of Yr9 and CV while rest of the lines with other resistance genes showed significant resistance to the pathogen in almost all the locations. Based on our studies, it can be concluded that the severity of stripe rust on wheat was variable during different years and regions. The values of disease severity obtained from the first year study were higher than that of the second year in many cases. Thus the disease severity and virulence genes frequency were probably affected by environmental conditions and to some extent by spore collecting years.

Distribution of major resistance genes and their decreasing efficiency to existing stripe rust population, suggest that there is an urgent need to search for novel sources of resistance that can be used in wheat breeding. Among bread wheat cultivars , Cumhuriyet-75, widely cultivated in the southern Aegean region and Western Mediterranean Region of Turkey for more than 20 years, still expresses high resistance to stripe rust in both field and greenhouse experiment, despite the pathogen showing increasing virulence otherwise (personal observation). Cumhurriyet-75 may have as yet unidentified genes that confer durable resistance

Our capacity to develop durable and efficient control methods against crop diseases is largely based on knowledge of the pathogen population structure and its potential for adaptation to new cultivars. Growing resistant varieties is the fundamental aspect of integrated management of wheat stripe rust. But, the breakdown of resistance in wheat cultivars to stripe rust is an important problem confronting the application of resistant varieties. To overcome this problem, preference needs to be given to use various cultivars with different resistance genes or cultivar mixtures in same region. The efficacy of host resistance management strategies like cultivar mixtures or multilines is largely influenced by the local diversity of the pathogen. Therefore, studies towards genetic diversity of pathogens in different locations including one such effort done by us is the important step in host resistance management efforts.

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