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Identification and Screening of Leaf Rust Resistance Genes in Wheat Cultivars Through Microsatellites

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Abstract

Wheat is one of the important staple food being grown worldwide and in Pakistan. The wheat rust-causing fungi are very eco-adaptive and evolve rapidly to overcome genetic resistance of wheat varieties. Among all rust-causing fungi, *Puccinia triticina* causes leaf rust in wheat, and inflicts heavy yield losses. Deploying resistant varieties against leaf rust is the most effective, environmentally-friendly and economic way to control the disease. Therefore, new sources of genetic resistance are continually sought to develop rust resistance in wheat varieties, and nowadays rust resistant varieties have been developed through the accumulation of slow rusting or minor genes that would perform better in fields. Hence, the purpose of this study is to screen a few of the available leaf rust-resistant germplasm in field and evaluate their field response on genetic basis. For this purpose, minor genes were detected through the amplification of simple sequence repeat (SSR) in the selected wheat genotypes through screened primers. Field experiment was conducted. Molecular studies to identify the minor genes were performed. Meteorological data was recorded at the observatory laboratory of the same area. All the epidemiological factors (Temperature (maximum, minimum), Relative Humidity, and Pan Evaporation) showed significant correlation in tested 5 varieties ZARDANA89, ZARLASHTA99, RASKOH 05, BHAKKAR-2000, GA-2002 which were conducive for disease development except rainfall. PCR amplification of Xgwm118, **and** Xgwm165, showed the range of alleles in 14 genotypes. It was observed that 22 verities showed resistance, 3 were moderately resistant, 3 varieties were moderately susceptible, 8 varieties were moderately susceptible, and 5 varieties were moderately susceptible against leaf rust pathogen.

Keywords: Wheat, leaf rust, Screening, Microsatellite.

INTRODUCTION

Wheat (*Triticum aestivum*) has remarkable value as staple food for more than one third of the world population (Mancuso *et al*., 2019). It is an important source of carbohydrates, mineral resources (P, Mg, Fe, Cu and Zn) and vitamins (Shuaib *et al.*, 2007). Because of the dynamic development of wheat cultivation and production, diseases of seed plantations are increasingly being observed. Fungal diseases pose a particular threat, as they limit the leaf assimilation area and cause a significant yield loss (Saharan, 2020; Torres *et al*., 2019).

Wheat leaf rust caused by *Puccinia recondite* f. sp. *tritici* is one of the most dangerous and common fungal diseases. Currently, the most beneficial method of plant protection is conducting resistance breeding, consisting in the identification and selection of resistant cultivars Leaf rust of wheat, caused by *Puccinia triticina* is one of the most important diseases in wheat worldwide. The leaf rust pathogen can infect wheat plants in most growth stages in the cropping season. It mainly reduces the photosynthetic efficiency of wheat, resulting in the reduction of plant organic matter synthesis. Leaf rust can decrease grain quantity and quality and reduce wheat production by 40% in susceptible cultivars (Khan *et al*., 2013; Li *et al*., 2010).

Yield losses due to rusts in many wheat producing countries during last century resulted in famines in many parts of the world. The disease is mainly destructive when the upper leaves of infected plants become harshly rusted. Reduced grain filling period of wheat and small kernel size is due to heavy rust spores. Some effective resistant genes which are

race specific in seedling and in adult plants include Lr1, Lr10, Lr21, and Lr34 (Kassem *et al*., 2011).
The use of rust resistant cultivars is the most economical and efficient method to reduce the damage of the disease in a wide range of crops (Adhikari and Missaoui 2019; Hartman *et al*., 2005; Roelfs *et al*., 1992; Yu *et al*., 2018). To date, more than 100 wheat leaf rust resistance (*Lr*) genes have been discovered, and 78 of them have been officially named (McIntosh *et al*., 2017). This study was focused on screening and identification of resistant genes against leaf rust and correlation analysis with wheat cultivars.

Materials and methods

Screening of wheat genotypes against leaf rust resistance: An experiment comprising of 51 varieties were sown in the present study. The row distance (R×R=45cm) and plant distance (P×P=15-20cm) was maintained. In order to maintain crop health and vigor agronomic practices were followed to keep the crop in good condition and for proper growth.

Inoculation: Artificial inoculation of wheat plants was done by spraying urediospore suspension (30g of spore/16 liter of water). After every 10^{th} line/variety a line of highly susceptible wheat cultivar i.e. Morocco, was sown to act as rust spreader row. (Morocco is highly susceptible to all the prevalent rust races and provides a substrate for rapid multiplication and distribution of rust inoculums).

Recording of rust data: Leaf reaction**,** symbol field response and response value were recorded by the modified Cobb's scale described by Peterson *et al*. (1948). Disease severity was observed with one week interval. Rust data was recorded up to physiological maturity of the wheat. The disease severity data for leaf and stripe rusts was converted into coefficient of infection by multiplying severity with constant value for field response as described by Yadar et al. (1985), Stubbs *et al*. (1986) and Roelf *et al*. (1992).

Area Under the Disease Progress Curve (AUDPC): The area under the disease progress curve (AUDPC) is a useful measurable summary of disease intensity over time, for assessment used for many years, locations, or administration strategies. The commonly used method for estimating the AUDPC, the trapezoidal method, is to discretize the time variable (hours, days, weeks, months, or years) and calculate the average disease intensity between each pair of adjacent time points. We can consider the sample time points in a sequence {ti}, where the time interval between two time points may be consistent or may vary, and we also have related measures of the disease level {yi}. We define y (0) = y0 as the first infection or the disease level at t = 0 (i.e., the first disease severity remark in our study). A (tk), the AUDPC at t = tk, is the total accrued disease until t = tk, given by

$$A_k = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i)$$

Extraction of DNA, PCR amplification and UV light gel documentation: Fresh leaves were used for the extraction of genomic DNA of 15 different wheat genotypes according to CTAB method. Molecular markers of known sequence were used to amplify the desired DNA in the thermal cycler by the reaction of polymerase chain reaction (PCR). PCR reactions were assembled using standard protocol (Devos and Gale, 1992). The master mixture formulation for the PCR reaction was included: distilled water, $MgCl_2$, forward and reverse Specific SSR primers, template DNA, PCR buffer, dNTP and Taq DNA Polymerase. The PCR products were electrophorized on agarose gel with ethidium bromide staining and visualized under UV light gel documentation system. Data was scored from good quality photographs of each amplification reaction for the presence or absence of the leaf rust resistance genes. DNA fragments were compared with the band size described by Stepien *et al*. (2003).

Statistical Analysis: LSD (Least Significant Difference) test was be conducted to determine the variation among the varieties and epidemiological factors. The Statistix 8.1 software were used for this purpose.

Results

Screening of wheat varieties/lines against leaf rust in relation to environmental conditions was observed. The results indicated that 22 wheat varieties (As-2002, Auqab2000, Chakwal50, Chakwal86, Faisalabad08, Fareed-06, Inqilab91, Kohsar95, Shafaq-06, V-04178,Abadgar-93, Bhittai , Kiran-95,Marvi-2000, Suleman96, Saleem2000, Pirsabak2004, Chenab2000, Pirsabak2005, Zarlashta99, Kohinoor 83, Kohistan 97) have shown resistance, 3 varieties (Jauhar-78, Zindad-2000, Chakwal 97) were moderately resistant, 3 varieties (Bhakkar-2000, Lasani-08, T.D-1) were moderately susceptible, 8 varieties (Bluesilver, Faisalabad83, Chenab79, SKD-1, Soghat-90, Sassi, T.J-83) were moderately susceptible and 5 varieties (Seher-06,Ufaq, Sassi, Chenab79 and Iqbal 2000) were moderately susceptible against leaf rust pathogen. .

Correlation of Environmental Factors with Leaf rust Disease: The influence of each environmental variable on leaf rust development on each variety was determined by correlation analysis. Environmental parameters having significant influence on leaf rust development was studied in detailed by plotting the data graphically. The main cause of the epidemic might be the favorable environmental conditions, and suitable environmental conditions increased the chances of disease incidence. Minimum, maximum temperature, relative humidity and rainfall and pan evaporation play a very important role in disease development. Environment plays a significant role in

development and spread of leaf rust disease and spores germination occurs at 15 to 20℃, where relative humidity ranges from 60-90%, temperature and humidity for development of mycelium of leaf rust and spores germination was very important. The fungus growth stops developing in plant tissues under temperature range from 7 to 8℃, and that's why winter wheat was less infected by leaf rust than spring wheat.

Average temperature Vs leaf rust disease: The relationship of minimum temperature with leaf rust was positive. The varieties V3 = BHAKKAR-2000, V9 = GA-2002, V41 = BLUE SILVER V42 = CHENAB 70, V43 = CHENAB 79 showed significance difference with increase in temperature range from 20-25℃, leaf rust value increases. The value of average temperature in this graph showed that the value of temperature ranges from 20℃, pathogen infection starts and gradually increases with increases in temperature value. The average temperature value at which disease increases was the 20℃ and this temperature gave significant result against leaf rust infection and infection value of leaf rust also dependent at this stage. The susceptible germplasm was conducive to environmental condition also contributes towards severe outbreak of fungal diseases. In spite of all these problems, disease resistant varieties are the only effective and durable solution to save the crop from the infection of leaf rust disease (Figure 1)

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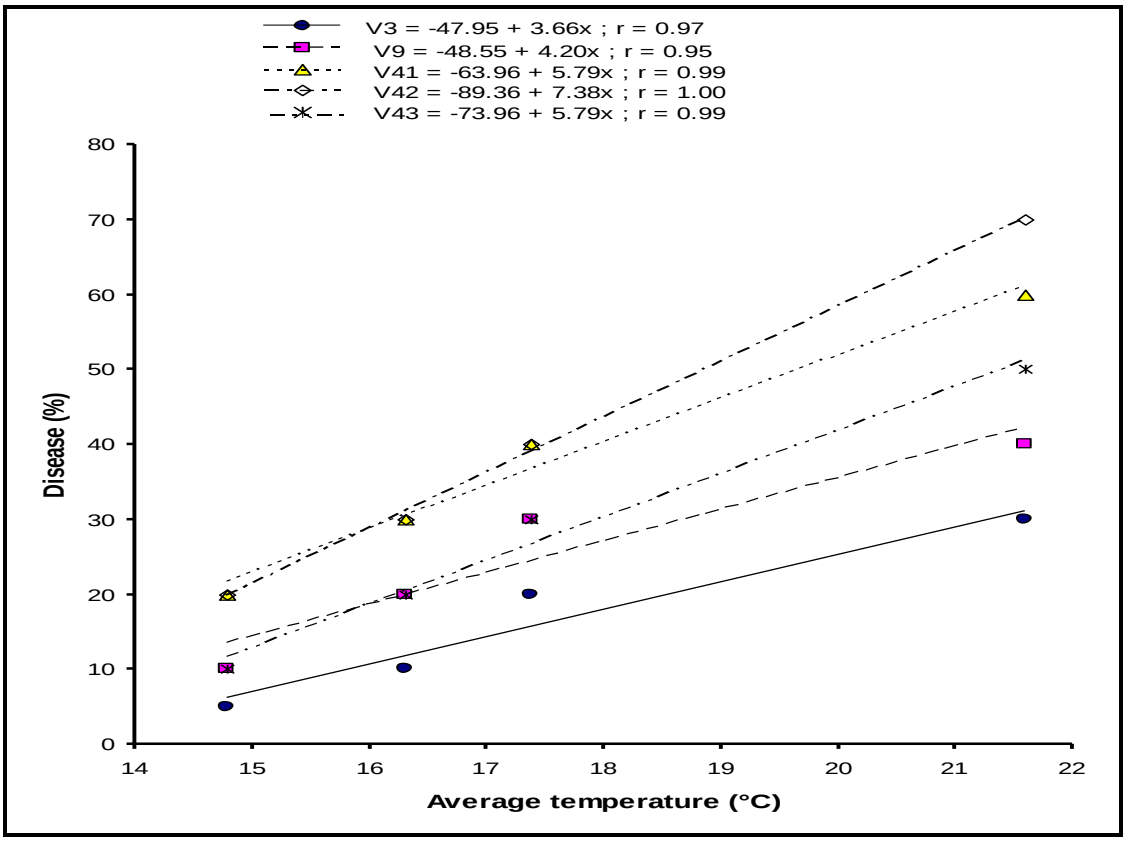

Figure 1. Relationship between average temperature and response values of leaf rust for varieties.V3 = BHAKKAR-2000, V9 = GA-2002, V41 = BLUE SILVER = SONALIKA V42 = CHENAB 70, V43 = CHENAB 79.

Relative humidity vs. leaf rust disease: The relationship of relative humidity with loose smut was positive. The varieties V3 = BHAKKAR-2000, V9 = GA-2002, V41 = BLUE SILVER V42 = CHENAB 70, V43 = CHENAB 79 showed significance response. With increase in relative humidity range from 45-60 %, leaf rust value increases. In this research it showed that the value of relative humidity that was 60% and disease incidence start at the value of relative humidity that was 65%. Research reveals that the best relative humidity for leaf rust pathogen and at this stage pathogen can stable itself for infection were the 60-90%. Different varieties showed the significant response against leaf rust and relative humidity correlation and the maximum value of relative humidity that were best and shown in graph was 63-87%. They reported that temperature range from 20 to 25°C, where relative humidity ranges from 60-90% was favorable for both development of mycelium of leaf rust and spore germination it gave significant response against leaf rust caused by *Puccinia triticina* (Figure 2).

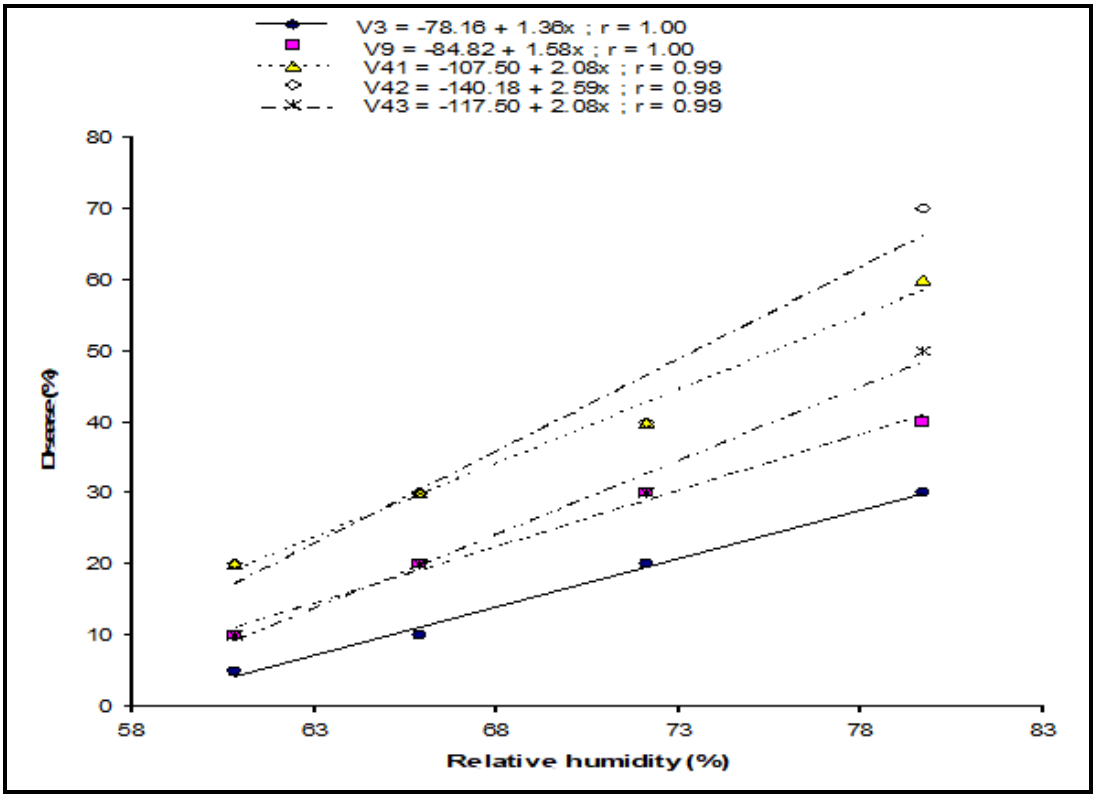

Figure 2. Relationship between Relative humidity and response values of leaf rust for varieties. V3 = BHAKKAR-2000, V9 = GA-2002, V41 = BLUE SILVER, V42 = CHENAB 70, V43 = CHENAB 79

Rainfall vs. leaf rust disease: The relationship of rainfall with leaf rust was negative. The verities (V3 = BHAKKAR-2000, V9 = GA-2002, V41 = BLUE SILVER, V42 = CHENAB 70, V43 = CHENAB 79) showed no response due to less number of rainfalls (Figure 3).

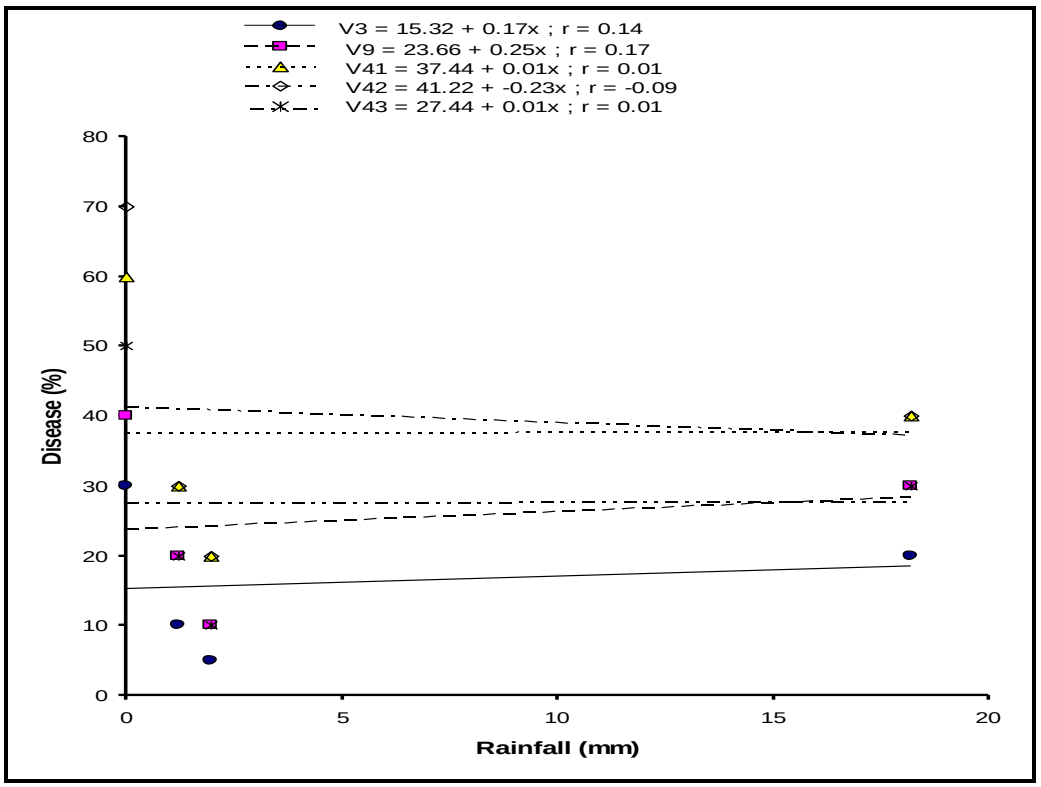

Figure 3. Relationship between Rain fall and response values of leaf rust for varieties.
V3 = BHAKKAR-2000, V9 = GA-2002, V41 = BLUE SILVER, V42 = CHENAB 70, V43 = CHENAB 79

Maximum temperature vs. leaf rust: The relationship of maximum temperature with leaf rust was positive. The varieties (ZARDANA89, ZARLASHTA99, RASKOH 05, BHAKKAR-2000, and GA-2002) showed significance results of the leaf rust disease value increases with the increase in temperature range from 15-30℃. Maximum temperature plays very important role in disease development. The correlation of environmental data with leaf rust showed that disease increased when the value of maximum temperature increased up to 30^0C and while the optimum temperature for pathogen infection is 15 to 25^0C.The highest temperature for pathogen is very important. At the range of temperature from 25-30^0C the pathogen can caused infection on wheat leaves but when temperature increases from this range pathogen activity adversely effected. The relationship between maximum temperature and leaf rust, infection of pathogen started at the temperature range of 20℃. The disease severity at this temperature 25-30℃ of CHENAB70 variety was at the highest range of 89.89% of disease. While the variety GA-2002 showed the value of disease lowest than other varieties having range from 49.25%. The temperature value at this stage was recorded 30℃ (Figure 4)

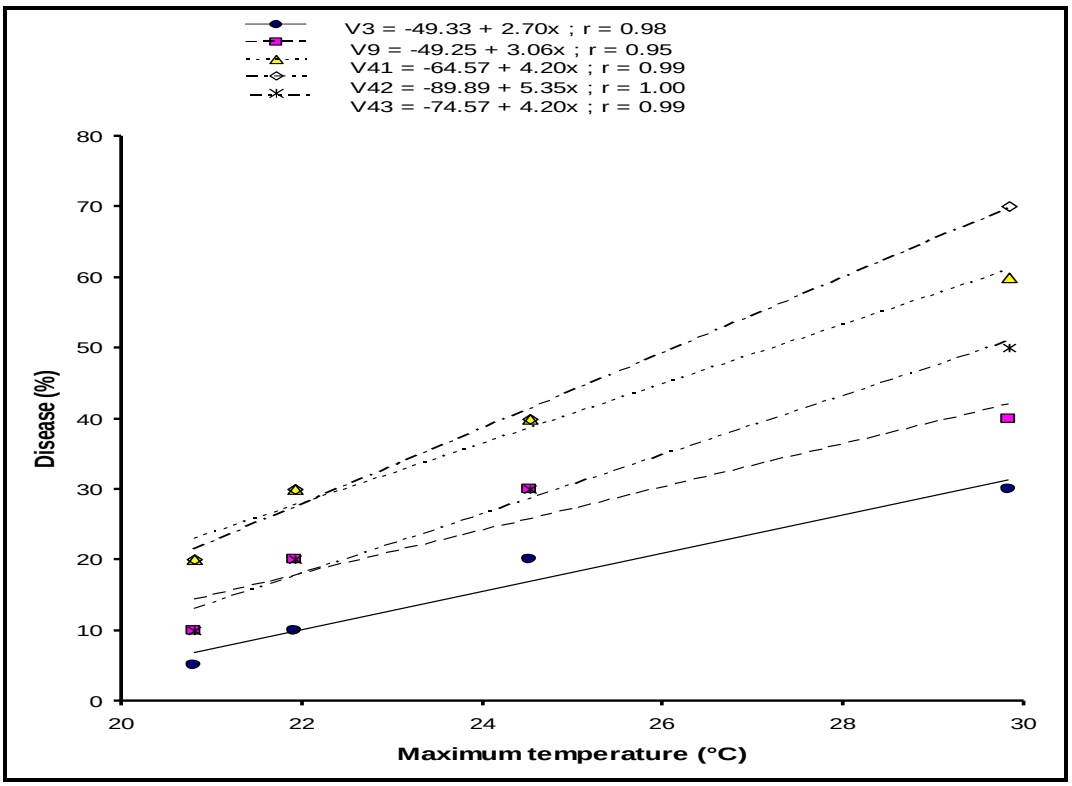

Figure. 4. Showed the relationship between maximum temperature and response values of leaf rust for varieties Where V3 = BHAKKAR-2000, V9 = GA-2002, V41 = BLUE SILVER, V42 = CHENAB 70, V43 = CHENAB 79

Minimum temperature vs. leaf rust disease: The relationship of minimum temperature with leaf rust was positive. The varieties BHAKKAR-2000, GA-2002, BLUE SILVER, CHENAB 70 and CHENAB 79 showed significance with increase in temperature range from 10-16℃ leaf rust value also increased. Minimum temperature plays very important role in disease development; results showed that disease severity with environmental correlation gave significant results. The value of minimum temperature in this graph showed that the value of temperature ranges from 11℃, pathogen infection was started and gradually increased with increases in temperature value. The minimum temperature value at which disease severity increased was the 15℃ and this temperature gave significant result against leaf rust infection. The susceptible germplasm was conducive to environmental conditions, which contributes towards severe outbreak of fungal diseases. In spite of all these problems, disease resistant varieties were the only effective and durable solution to save the crop from the infection of leaf rust disease. As the temperature decreased the fungus growth stopped at the temperature less than 7℃ and but with increased in temperature from 11℃ the pathogen started its activity (Figure 5).

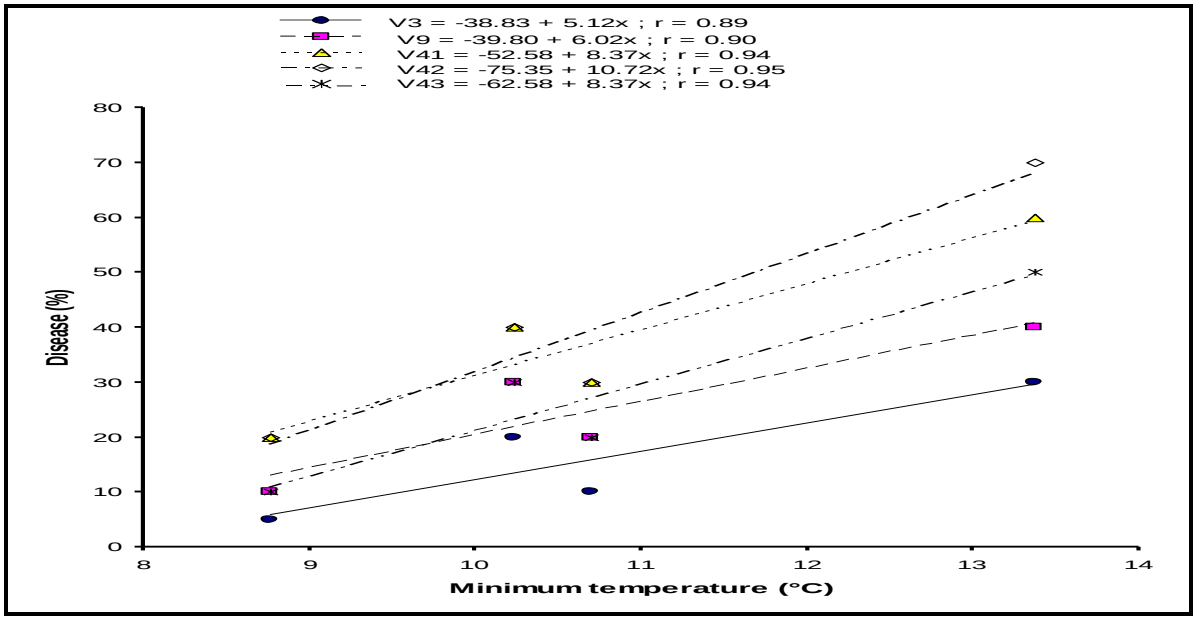

Figure.5. Showed the relationship between minimum temperature and response values of leaf rust for varieties Where V3 = BHAKKAR-2000, V9 = GA-2002, V41 = BLUE SILVER, V42 = CHENAB 70, V43 = CHENAB 79

Molecular studies: The resistant genes were found in many wheat varieties and absent in some varieties. The presence and absence of the resistant genes was showed by the **+ve** signs and **–ve** signs (Table 1).

Table 1. Wheat germplasm showing presence and absence of resistant gene

S/N	**Variety**	***Lr34***	***LR46***	***LR67***
1	Auqab 2000	-	-	+
2	Chakwal 86	+	+	+
3	Chakwal 97	+	-	+
4	GA-2002	-	+	-
5	Lasani-08	+	-	-
6	Mehran-89	-	-	-
7	SkD-1	+	-	+
8	T.D-1	+	+	+
9	Zindad-2000	+	-	-
10	Saleem 2000	+	+	+
11	Pirsabak2005	-	-	-
12	Blue silver	+	-	-
13	Chenab 70	+	-	-
14	Jauhar-78	+	-	+

List of 14 wheat germplasm showing presence and absence of *Lr34, LR46* and *LR67:* Three wheat genotypes Chakwal 86, Chakwal 97, T.D-1, Saleem 2000 have *Lr34, LR46* and *LR67* resistant gene present in them. While the resistant genes were absent in Mehran-89 Pirsabak2005 varieties. A diagnostic band of 1000bp and 500bp was amplified showing the presence of *Lr*34, *Lr*46, and *Lr*67 gene (Figures 6 & 7) respectively. The polymorphic survey revealed that out of the 15 varieties, the marker for *Lr*46 and *Lr*67 were identified as a fragment of 1000bp and 500bp in some varieties namely: AUQAB 2000, CHAKWAL 86, CHAKWAL 97, GA-2002, LASANI-08, BLUE SILVER, T.D-1, ZINDAD-2000, SALEEM 2000, CHENAB 70, JAUHAR-78, and KARAWAN-2. The *Lr*34, *Lr*46, and *Lr*67 gene was amplified from the DNA samples of resistant genotypes like AUQAB 2000, CHAKWAL 86, CHAKWAL 97 and was absent in the susceptible genotypes, SKD-1, PIRSABAK2005 and MEHRAN-89. The genotypes ZINDAD-2000 and JAUHAR-78 were also found to have gene and were moderately resistant to leaf rust. The three primers showed their result in detecting the resistant genes in the wheat varieties. The bands showed the presence of resistant gene (Figure 6 and 7) The 2, 3 bands were also seen in some wheat varieties. The identification of *Lr*34, *Lr*46, and *Lr*67 genes in Pakistan wheat germplasm were determined. By introducing these resistant genes will help in accelerating the breeding program in future, including pyramiding of different wheat resistant genes in wheat cultivar

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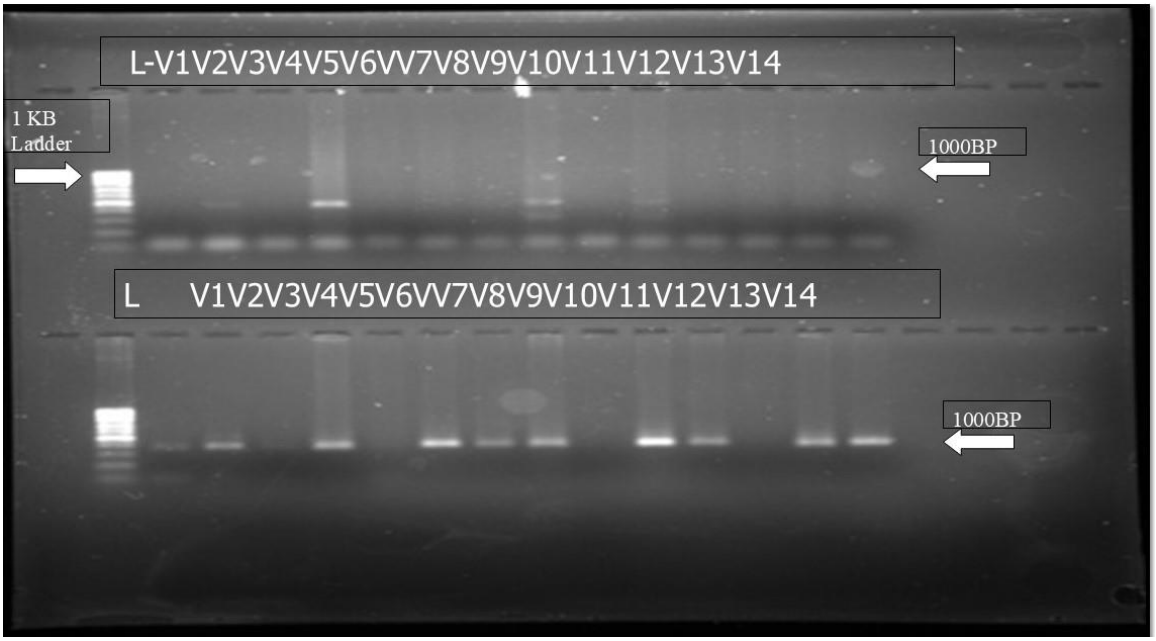

Figure.6.Wheat germplasm showing presence and absence of *Lr34, LR46* and *LR67:* Primer Xgwm118, CSLV34

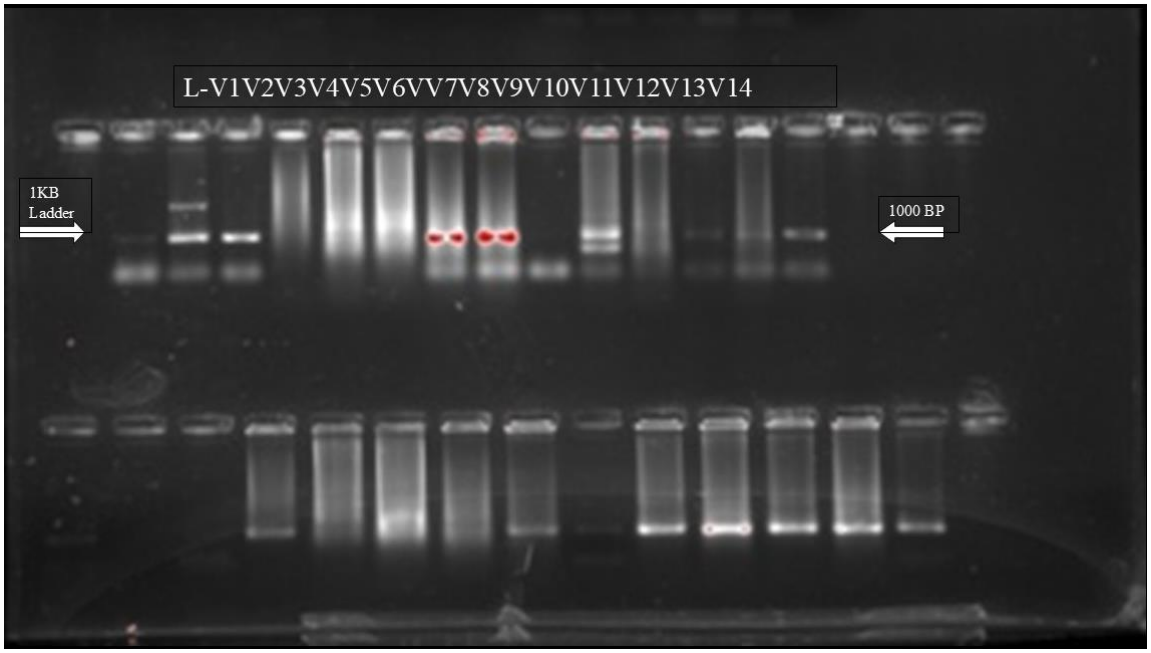

Figure 7. Wheat germplasm showing presence and absence of *Lr34, LR46* and *LR67:* Primer Xgwm165

Table 2. AUDPC calculations (original order)

Sr.#	**Parentage/Pedigree**	**1st**	**2nd**	**3rd**	**4th**	**AUDPC_1**	**AUDPC_2**	**AUDPC_3**	**AUDPC**
1	AS-2002=WD-97603	5	5	15	20	35	70	122.5	227.5
2	AUQAB 2000	0	0	0	0	0	0	0	0
3	BHAKKAR-2000	10	5	20	30	52.5	87.5	175	315
4	CHAKWAL-50	0	0	0	0	0	0	0	0
5	CHAKWAL 86	0	0	0	0	0	0	0	0
6	CHAKWAL 97	10	10	25	30	70	122.5	192.5	385
7	FAISALABAD-08	5	5	20	30	35	87.5	175	297.5
8	FAREED-06	0	0	0	0	0	0	0	0
9	GA-2002	20	10	30	40	105	140	245	490
10	INQILAB 91	0	0	0	0	0	0	0	0
11	KOHSAR 95	0	0	0	0	0	0	0	0
12	LASANI-08	5	10	15	20	52.5	87.5	122.5	262.5
13	MANTHAR	5	5	10	20	35	52.5	105	192.5

14	MIRAJ-08	10	5	30	30	52.5	122.5	210	385
15	SEHER-06	5	10	30	30	52.5	140	210	402.5
16	SHAFAQ-06	0	0	0	0	0	0	0	0
17	V-04178 = AARI-11	0	0	0	0	0	0	0	0
18	UFAQ	30	20	40	40	175	210	280	665
19	ABADGAR-93	0	0	0	0	0	0	0	0
20	ANMOLE-91	30	40	60	80	245	350	490	1085
21	BHITTAI	0	0	0	0	0	0	0	0
22	KIRAN-95	0	0	0	0	0	0	0	0
23	KHIRMAN	30	20	50	70	175	245	420	840
24	MARVI-2000	0	0	0	0	0	0	0	0
25	MEHRAN-89	40	20	50	60	210	245	385	840
26	SKD-1	30	20	40	60	175	210	350	735
27	SOGHAT-90=PVN	20	30	40	50	175	245	315	735
28	SASSI	20	30	40	60	175	245	350	770
29	T.J-83	30	20	40	50	175	210	315	700
30	T.D-1	5	10	20	40	52.5	105	210	367.5
31	ZINDAD-2000	10	5	20	40	52.5	87.5	210	350
32	SULEMAN 96	0	0	0	0	0	0	0	0
33	SALEEM 2000	0	0	0	0	0	0	0	0
34	PIRSABAK 2004	0	0	0	0	0	0	0	0
35	ZARGOON 79	20	30	40	60	175	245	350	770
36	PIRSABAK 2005	0	0	0	0	0	0	0	0
37	ZARDANA 89	30	40	50	70	245	315	420	980
38	ZARLASHTA 99	0	0	0	0	0	0	0	0
39	RASKOH 05	30	40	60	80	245	350	490	1085
40	SARIAB 92	20	30	40	60	175	245	350	770
41	BLUE SILVER = SONALIKA	30	20	40	60	175	210	350	735
42	CHENAB 70	30	20	40	70	175	210	385	770
43	CHENAB 79	20	10	30	50	105	140	280	525
44	CHENAB-2000	0	0	0	0	0	0	0	0
45	FAISALABAD 83	30	20	40	40	175	210	280	665
46	FAISALABAD 85	5	10	20	40	52.5	105	210	367.5
47	IQBAL2000	20	15	30	40	122.5	157.5	245	525
48	JAUHAR-78	10	20	30	40	105	175	245	525
49	KARAWAN-2	10	5	20	30	52.5	87.5	175	315
50	KOHINOOR 83	0	0	0	0	0	0	0	0
51	KOHISTAN 97	0	0	0	0	0	0	0	0

it was showed that the varieties RASKOH 05, ZARDANA 89, ANMOLE-9 were the most susceptible against leaf rust. The value of area under disease progress curve (AUDPC) was also calculated that was given the value 1085 of variety RASKOH 05 that was highest. The lowest value of (AUDPC) showed by the variety AARI-11 and that value was zero (Table 2).

Discussion

The effect of environment on the soft spring wheat cultivars infected by leaf rust and expressed the results that at the range of temperature from 25-30^{0}C the pathogen can caused infection on wheat leaves but when temperature increases from this range pathogen activity adversely effected (Khan, 1997). The relationship between maximum temperature and leaf rust, infection of pathogen started at the temperature range of 20℃. The disease severity at this temperature 25-30℃ of CHENAB70 variety was at the highest range of 89.89% of disease which showing the similar results of environmental effect on leaf rust and the procedure mention above followed by scientist.

An experiment in which he said that Simple Sequence Repeat (SSR) having loci of multicellular genomes. It showed that these loci were very polymorphic due to alter in the number of repeating units between the single varieties. Each microsatellite locus can easily be amplified by using a polymerase chain reaction (PCR) knowing the DNA series flanking the repeat area specifically (Dourar *et al*., 2001). Captioned results reflecting with my findings of the research findings.

The rust is the major wheat disease in Pakistan and other countries of the world. When several rust resistance genes were introduced into a single line the disease is more effectively controlled. A molecular survey was organized to screen twenty five Pakistani wheat varieties for the presence of leaf rust resistance gene Lr10using specific primer (Hussain *et al*., 2011). As use of primer is one of the essential methods to find the resistance gene availability in the wheat I

have also used the primers and results showing the similar interpretation if compared with findings.

leaf rust is the wheat germplasm from the Central Asia was characterized by using DNA markers attached to the Yr18/ Lr34 dual rust resistance gene. Interpretation of the results showing that the some germplasm of the wheat are resistant against the leaf rust due to resistance gene (Alma *et al*., 2012) with having similarities with results of the screening as resistance were shown by germplasm.

The 38 varieties of wheat were taken by using specific molecular marker for 6 significant Lr genes containingLr10, Lr13, Lr21, Lr24, Lr26, Lr27 and Lr31in the presence of these genes in 18, 6, 0, 0, 6, 3 and 5 varieties respectively. Thirty one commercial wheat varieties bear more than one Lr genes. The molecular marker with cM distance, less than 1 showed a valuable prediction for effective genes using Gel electrophoresis image. The molecular markers showed efficiency to verify four effective genes Lr10, Lr21, Lr24, and Lr27in local germplasm (Mustafa *et al*., 2013). Gel imaging is an important step to find the results of the markers used for observing the efficiency of the effective genes in the germplasm that either that is resistant or not, finding of the results are interpreted similar as my findings.

The experiments of virulence of leaf rust of wheat and found the resistant genes like *Lr* 27 and others against leaf rust in wheat line which was a helpful step for breeders to find minor as well as major gene and their combine control against the pathogen to check the sustainability as if combined it was more sustainable (Wu *et al*., 2020). Results of my studies are showing similarity with scientist.

Release of resistance mechanism in the wheat is in progress though out the world as well as in Pakistan, which resulting in the such progress about wheat varieties (Ahmad *et al*., 2021) As per my results resistant germplasm has also been observed so that further processes can be done as per interpret of the mentioned scientist.

Research was carried out the process of testing line or varieties and found the resistance genes in one line/germplasm, it was found at various localities of Pakistan some advanced lines have not shown any symptoms of the leaf rust in the field study even disease inoculum was already used to infect the wheat plants. However moderate resistance was also observed in few lines including V-14154 (Ahmad *et al*., 2021). Susceptible germplasms were also observed in the trial at some localities. As results interpreted my results also showed the similarities with scientist all type of line were observed like resistant and other as mentioned in the text.

Conclusion:

It was concluded from screening that among all wheat germplasm, two varieties/lines were found moderately resistant of varying degree (HR, R, and MR) and rest of varieties susceptible and highly susceptible.

Authors Contribution

All the authors contributed while writing the manuscript

Authors Conflict

The authors have no conflict of interest.

References

Adhikari, L., & Missaoui, A. M. (2019). Quantitative trait loci mapping of leaf rust resistance in tetraploid alfalfa. Physiol. Mol. Plant Pathol. **10**(6):238 245.

Ahmad, J., Tabassum, M. I., Ahmad, N., Nadeem, M., Shamim, S., & Asghar, S. (2021). Subhani-21: a tower yielding and rust resistant wheat variety for irrigated areas of punjab-pakistan. J Agric Res.;**59**(4):335–45.

Bowden, W. M. (1959).The Taxonomy and Nomenclature of the Wheats, Barleys and Ryes and their Wild Relatives. A review paper on the systematics of wheat, barley and rye. Canadian Journal of Botany, **37**: 657-84.

Brennan. J. P., & Murray, G. M. (1988). Australian wheat diseases assessing their economic importance. Agricultural Science New Series **2**: 26-35.

Devos, K. M., & Gale, M. D. (1992). The Random Amplified Polymorphic DNA markers in wheat. Theorical Applied Genetics, **8**(4): 567-572.

Dourar, N., Mahinur, S., & Akkaya. (2001). Optimization of PCR Amplification of Wheat Simple Sequence Repeat DNA Markers.Turk .J.Biol.**25** (2001) 153-158.

Eversmeyer, M. G., & Browder, L. E. (1974). Effect of leaf and stem rust in 1973 Kansas wheat yields. Plant Dis. Rep. **5**(8): 469-471.

G.O.P. (2011). Pakistan Statistical Year Book. Federal Bureau of Statistics, Statistical Division, Islamabad.

Hartman, G. L., Miles, M. R., & Frederick, R. D. (2005). Breeding for resistance to soybean rust. Plant Dis. **8**(9):664-666.

Hussain,W., Inamullah., Ahmad, H., Iqbal, M. S., Abbassi, F. M., Rabnawaz., Ahmad, W., Liaqat., & Hussain, S. (2011). Identification of leaf rust resistant gene Lr10 in Pakistani wheat germplasm.Afri. J. Biot. Vol. **10**(43), 8578-8584

Hussain, M., Hassan, S. F., & Kirmani, M. A. S. (1980). Virulence in Puccinia recondite Rob. ExDesm.F. Sp. triticina in Pakistan Proceeding of fifth European and Mediterranean cereal rusts conference, Bari, Itlay, 179-184.

Kassem, M. A. El.Ahmed., Hakim, M. S., Al. Saleh, A., Khalifeh, M. E. L., & Nachit, M. (2011). Identifying leaf rust resistance gene *Lr*19 in durum Wheat using simple sequence repeat (SSR) marker. Afri. J. of Biotec. **10**: 8716-8719.

Khan, M. H., Bukhari, A., Dar, Z. A., & Rizvi, S. M. (2013). Status and strategies in breeding for rust resistance in wheat. Agric. Sci. **04**:292-301.

Kokhmetova, A., Gulzat, Y., Alex, M., & Francis, O. (2012). The Screening of Wheat Germplasm for Resistance to Stripe and Leaf Rust in Kazakhstan Using Molecular Markers. J. of Life Sciences **6,** 353-362

Kolmer J. A. (1996). Genetics of resistance to leaf rust. Ann. Rev. Phytopathology, **3**(4): 435-455.

Li, Z. F., Xia, X. C., He, Z. H., Li, X., Zhang, L. J., Wang, H. Y., Meng, Q. F., Yang, W. X., Li, G. Q., & Liu, D. Q. (2010). Seedling and slow rusting resistance to leaf rust in Chinese wheat cultivars. Plant Dis. **9**(4):45-53.

Mancuso, T., Verduna, T., Blanc, S., Di Vita, G., & Brun, F. (2019). Environmental sustainability and economic matters of commercial types of common wheat. Agric Econ (Zemědělská Ekon).;**65**(4):194–202.

McIntosh, R. A., Dubcovsky, J., Rogers, W. J., Morris, C., & Xia, X. C. (2017). Catalogue of gene symbols for wheat: 2017

McIntosh. R. A., Yamazaki. Y., Dubcovsky. J., Rogers, W. J., Morris, C., Appels, R., & Devos, K. M. (2010). Catalogue of gene symbols for wheat: In KOMUGI Integrated Wheat Science Database.

Mustafa, G., Alam, M. M., Khan, S., Naveed, M., & Mumtaz, A. S. (2013). Leaf rust resistance in semi dwarf wheat cultivars a Conspectus of post green revolution period in Pakistan. Pak. J. Bot., **45**(SI): 415-422.

Peterson, R., Campbell, A. B., & Hanna. (1948). A diagramatic scale for estimating rust severity on leaves and stems of cereals. Can. J. Res. Sec. C.**2**(6): 496-500.

Roelf, A. P., Singh, R. P., & Saar, E. E. (1992). Rust diseases of wheat: Concept and methods of disease management 'Mexico' D.F. CIMMYT, 81.

Saharan, M. S. (2020). Current status of resistant source to Fusarium head blight disease of wheat: a review. Indian Phytopathol.;**73**(1):3–9.

Shuaib, M., Ali, A., Ali, Z., Ahmad, W., & Khan, T. (2007). Characterization of wheat varieties by seed storage protein electrophoresis. Afr. J. Biotechnol. 6: 497-500.

Stepien L., Golka, L., & Chelkowski, J. (2003). Leaf rust resistance genes of wheat: identification in cultivars and resistance sources. J. Appl. Genet., **4**(4): 139-149.

Stubbs, R. W., Prescott, J. M., Suari, E. E., & Dubin, H. J. (1986). Cereal Disease Methodology Manual Centro International de Maize by trig (CIMMVT), Mexico.

Torres, A. M., Palacios, S. A., Yerkovich, N., Palazzini, J. M., Battilani, P., & Leslie, J. F. (2019). Fusarium head blight and mycotoxins in wheat: prevention and control strategies across the food chain. World Mycotoxin J.;**12**(4):333–55.

Wu, H., Kang, Z., Li, X., Li, Y., Li, Y., & Wang S. (2020) Identification of wheat leaf rustresistance genes in Chinese wheat cultivars and the improved. Plant Dis.;**104**(10):2669–80.

Yadar, B., Ram, B., Sethi, S. K., & Luthra, O. P. (1992). Genetics of field resistance and transgressive segregation to leaf rust of wheat (*Triticum aestivum* L.) Cereal Research Communication 1: 41-48.

Yu, X. H., Kong, H. Y., Meiyalaghan, V., Casonato, S., Chng, S. F., Jones, E. E., Butler, R. C., Pickering, R., & Johnston, P. A. (2018). Genetic mapping of a barley leaf rust resistance gene *Rph26* introgressed from *Hordeum bulbosum*. Theor. Appl. Genet. **13**(1):2567-2580

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