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ASSESSING YELLOW RUST RESISTANCE IN ADULT PAKISTANI WHEAT WITH *YR18* AND ALL-STAGE DEFEATED GENES

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ABSTRACT

Yellow rust, attributed to *Puccinia striiformis* f.sp. *tritici* Erikss (*Pst*), stands as a pivotal challenge in the context of wheat cultivation in Pakistan. The implementation of Adult Plant Resistance (APR) emerges as a robust and sustainable strategy for its effective management. In this regard, a meticulous preliminary APR study was conducted, encompassing 50 genotypes, spanning three consecutive cropping seasons in Peshawar, Pakistan. From the initial study, 29 seedling susceptible genotypes, harbouring *Yr18* and defeated all-stage resistance genes, were selected for further APR evaluation. This extended evaluation unfolded across six diverse rust-prone locations within Khyber Pakhtunkhwa (KP) Province of Pakistan from 2010 to 2013. Statistical analysis revealed significant differences ($P<0.05$) among genotypes, locations, and years x locations concerning the average coefficient of rust infection (ACI), albeit of marginal importance. Conversely, years, locations x genotypes, years x genotypes, years x locations, and years x locations x genotypes exhibited non-significance. Categorization based on ACI of 0-20 and 21-40 over years-locations were inferred to carry high and moderate levels of APR, respectively. Among the *Yr18*-based genotypes, ACI values ranged from 7 to 39, with 14 genotypes demonstrating varying degrees of APR. Additionally, 15 genotypes, carrying defeated all-stage resistance genes, showcased ACI values ranging from 12 to 34, indicative of residual resistance. Seven genotypes exhibited high APR levels (93T347, Wafaq-2001, Bakhtwar-93, 99B2278, CT00231, Kohsar-93, Shafaq-06), while an equal number demonstrated moderate levels (V-99022, V-01180, Faisalabad-83, Sindh-81, Punjab-96, Maxi-Pak, and Tandojam-83). Notably, these genotypes not only aligned with the findings of the preliminary study but also demonstrated substantial yield potential. Their inclusion in the Pakistan national wheat improvement program is deemed highly beneficial, offering a foundation for further enhancement through the integration of effective genes aimed at mitigating allo and auto rust infections across diverse regions of the country.

Keywords: Adult Plant Resistance, Wheat, Yellow Rust

INTRODUCTION

The global population is projected to reach approximately 9.1 billion by the year 2050 (Weigand, 2011), necessitating a substantial increase in food production. Against this backdrop, the demand for wheat, a cornerstone of the global food supply, reached 666 million metric tons (MMT) in 2010. If the demand-growth rate remains constant, global wheat utilization is anticipated to surge to around 880 MMT by 2050 (Weigand, 2011). To meet this escalating demand, augmenting wheat production becomes imperative, either by expanding production areas or by enhancing per-acre yield. However, the feasibility of increasing the cultivation area for wheat is constrained. Therefore, optimizing grain yield per unit area becomes pivotal, demanding the effective management of wheat diseases that significantly contribute to yield and quality losses. Among these diseases, yellow rust stands out as one of the most devastating globally (Wellings, 2011), leading to compromised yield and grain quality (Hovmøller etal., 2010). Previously confined to areas with cool and moist weather, yellow rust has expanded its geographical range

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in recent years, posing a threat to regions previously considered unfavorable for its proliferation (Hovmøller etal., 2011). The evolving nature of the pathogen renders it a formidable menace, with an estimated 88% of global wheat susceptibility and an annual production loss impact exceeding five million tons of wheat grains (Beddow etal., 2015).

Wheat, being a crucial food crop (Curtis and Halford, 2014), commands nearly half of the global wheat acreage, with Asia accounting for a significant portion. In three Asian countries (i.e. China, India, and Pakistan), wheat acreage is 62 million hectares, and 70% (43 million hectares) of this expanse is susceptible to yellow rust. Pakistan ranked as the 8th largest wheat producer globally, witnesses 80% of its farmers cultivating this vital cereal crop on nine million hectares, constituting 40% of the country's total cropped area during the winter season. Yellow rust poses a substantial public risk in Pakistan, capable of affecting 70% of the wheat landscape (Singh etal., 2005), resulting in severe economic losses through epidemic outbreaks (Duveiller etal., 2007; Afzal etal., 2008). Addressing yellow rust in wheat has traditionally relied on foliar fungicide applications and the cultivation of resistant varieties (McCallum etal., 2007). While fungicides have been employed to mitigate rust damage, various limitations, including high cost, availability, safety issues, and application methods, underscoring the appeal of genetic resistance as a preferable alternative (Oliver, 2014; Singh etal., 2016). Genetic resistance emerges as the most preferred and economical long-term strategy for yellow rust management.

Within the realm of wheat genetic resistance to yellow rust, distinctions are made between race-specific and non-race-specific resistance. Race-specific resistance, often associated with "R" genes, is effective at all plant growth stages but tends to be short-lived due to the emergence of new virulent races of the pathogen. On the contrary, non-race-specific resistance genes, referred to as APR, are predominantly displayed at the post-seedling stage, offering a more durable form of protection even over large acreages and several years. The *Yr18/Lr34* gene in wheat is a notable example, conferring race-non-specific, durable resistance to various diseases, including yellow rust (McIntosh 1992, Singh 1992a), brown rust (Dyck, 1987), powdery mildew (Spielmeyer et al. 2005; Lillemo et al. 2008), black rust (McIntosh etal., 2012), and BD-virus (Singh, 1993). Its deployment for over a century attests to its durability (Kolmer etal., 2008).

Pakistan has a history marked by devastating rust epidemics, wherein dominant cultivars with race-specific vertical resistance have contributed to cyclic patterns of "Boom and Bust" events, leading to the withdrawal of cultivars such as Yecora, Khushal-69, Tarnab-70, Chenab-70, Punjab-76, Pirsabak-85, and Inqilab-91 from commercial cultivation (Duveiller etal., 2007; Afzal etal., 2008). Given this historical context, the quest for new APR sources becomes paramount for a comprehensive understanding of APR, integral to integrated management strategies, future endeavors towards achieving durable resistance to yellow rust, and maintaining diversity in resistance mechanisms deployed in the country. This paper presents the validation results of yellow rust APR in 29 previously identified spring wheat genotypes over diverse years and locations in Pakistan.

MATERIALS AND METHODS

Description of the Study region: The experimentation was conducted across six strategically selected wheat cultivation locations situated in three distinct zones, namely Southern (Bannu, Coordinates: 32°49'N, 70°46'E), Central (Peshawar 1, Coordinates: 34° 0'N, 71°42'E; Peshawar 2, Coordinates: 34° 1'N, 71°28'E; Nowshera, Coordinates: 34° 1'N, 72° 2'), and Northern (Abbottabad, Coordinates: 34°12'N, 73°14'E; Swat, Coordinates: 34°46'N, 72°21'E). These locations strategically span diverse CIMMYT mega-environments, encompassing 1, 2B, 4, and 8 (http://wheatatlas.org/search). Positioned proximately to the Himalayan region in the northwest of Pakistan, these locales confront the severe challenge of yellow rust, as highlighted by the work of Chatrath et al. (2007). Furthermore, the geographical proximity of these areas to the Himalayan region contributes to common occurrences of over-summering, as elucidated by Hassan (1968). Additionally, the prevalence of alternate hosts, as documented by Ali et al. (2014), further accentuates the significance of these locations in the context of yellow rust dynamics. Importantly, these areas serve as the gateway for the ingress of new rust races from neighboring countries, a phenomenon extensively discussed by Singh et al. (2002, 2005).

Host material and sowing: Twenty-nine seedling-susceptible spring wheat genotypes, identified in a prior investigation executed at the Nuclear Institute for Food and Agriculture (NIFA) Peshawar Research Farm spanning 2005-07 (Shah etal., 2014), were chosen for further scrutiny. The selected genotypes, alongside the susceptible control Morocco (Afzal etal., 2008), underwent evaluation over four years from 2010 to 2013. The experimental sites encompassed Bannu, Peshawar 1, Peshawar 2, Nowshera, Abbottabad, and Swat, with each genotype being cultivated in four-row plots, each 3 meters in length and spaced 30 centimeters apart. Rigorously adhering to scientific protocols, the experimental trials were meticulously arranged in a randomized complete block (RCB) design, featuring 3-replications. To augment rust development and provide a comprehensive evaluation platform, two rows of the yellow rust susceptible wheat landrace "Local White" (Ehsan etal., 2003) were systematically planted surrounding each experimental plot at every location throughout the study period. The execution of all requisite cultural practices was diligently overseen during each cropping season, ensuring the experimental conditions mirrored real-world agricultural scenarios. Concurrently, virulence assessments for yellow rust were conducted at the same locations during the study years, utilizing Australian Avocent Near Isogenic Lines (NILs), as extensively detailed elsewhere (Ibrahim etal., 2015).

Inoculation and yellow rust assessment: The inoculation process involved utilizing inoculum from two local yellow rust (YR) races, namely 70E16-*v27* (possessing virulences *Vr2*, *Vr6*+, *Vr7*, *Vr8*, *VrA*, *VrA*+, *VrSu*, *VrMichigan*, and *Vr27*) and 70E0-*v27* (*Vr2*, *Vr6*+, *Vr7*, *VrA*, *VrA*+, *VrSu*, *VrMichigan*, and *Vr27*). These races were originally employed in seedling tests as documented by Shah et al. (2014) and were subsequently applied in the field experiments. The procedures outlined by Roelfs et al. (1992) and Khanna et al. (2004) were meticulously followed to ensure standardization and consistency in the inoculation process. At each experimental location during the designated test years, both the local white and the test genotypes underwent inoculation at the heading stage. This was achieved by uniformly spraying a suspension containing 0.1g spores (0.05g of each race) per 1-liter sterile distilled H_2O, supplemented with 2-3 drops of Tween 20, using an ultra-low volume turbo sprayer after the sunset. Yellow rust severity was systematically recorded on flag leaves at the peak stage of epidemic development, employing a modified Cobb's scale (Peterson etal., 1948) ranging from 0 to 100%, where 0% denoted no apparent symptoms and 100% represented the maximum severity

The host response to infection was assessed by the criteria established by Roelfs et al. (1992). For yellow rust, 'R' indicated resistance, characterized by minute uredinia surrounded by necrotic leaf tissue. 'MR' denoted moderately resistant, signifying smaller to moderate-sized uredinia surrounded by necrotic or chlorotic leaf tissue. 'MS' represented moderately susceptible, indicating moderate-sized uredinia without necrotic or chlorotic leaf tissues, while 'S' indicated susceptibility, denoted by large uredinia without necrotic or chlorotic leaf tissue. The coefficient of yellow rust infection (CI) values for each genotype were computed following the methodology outlined by Pathan and Park (2006). Severity values were multiplied by predefined factors (i.e. 0.10, 0.25, 0.50, 0.75, or 1.00) corresponding to host response ratings of resistant, moderately resistant, intermediate, moderately susceptible, and susceptible, respectively. Due to germination issues and minimal disease levels in 2010 (3 locations) and 2012 (2 locations), these instances were excluded from the study.

STATISTICAL ANALYSES

The Average Coefficient of Infection (ACI) data underwent thorough analysis employing the GLM Procedure (SAS Institute, Inc. 2010). Analysis of Variance was systematically generated to evaluate the significance of factors such as genotypes, locations, years, and their respective two and three-way interactions. Subsequently, to facilitate a comprehensive understanding of the ACI data, Duncan's multiple range test was implemented for grouping genotypes, locations, and years based on their statistical distinctions. For a detailed exploration of the data distribution, essential basic statistics, including minimum, maximum, and standard deviation, were computed for the Average Coefficient of Yellow Rust Infection across different years and locations. This statistical analysis was conducted utilizing the MEANS Procedure within the SAS 9.2 framework. Furthermore, to elucidate the interrelationships within the dataset, Pearson correlation analysis was executed for the coefficient of infection across the 19 location-year combinations. This correlation analysis was performed through the CORR Procedure of SAS 9.2.

RESULTS

A comprehensive assessment was conducted on twenty-nine wheat genotypes, derived from a prior study, alongside a susceptible check named "Morocco," to characterize their adult plant resistance to yellow rust. The phenotypic evaluations spanned six locations over a four-year period, employing the Average Coefficient of Infection

(ACI) as a key parameter to derive means and variances. The ensuing analysis encompassed observations, minimum and maximum values, standard deviations, and the significance of both years and locations, providing a detailed overview summarized in Table 1. In the field tests conducted across 19 environments (location-year combinations), a substantial yellow rust epidemic was evident, as illustrated by the ACI values of the susceptible check "Morocco," ranging between 80-90, with an overall mean of 85 (Table 2). Notably, despite deliberate inoculation, ACI values displayed considerable variability, ranging from 4 to 43 across different years and locations (Table 2). Specific observations revealed higher ACI means during 2011 and 2013 at Peshawar-1 and Peshawar-2, with certain locations displaying elevated ACI levels, notably Swat (Table 2).

Table 1. Temporal and spatial statistics of average coefficients of infection (ACI) for yellow rust across multiple locations in northwest Pakistan (2010-2013).

Years	ACI for yellow rust				Year means
	No of observations	Minimum	Maximum	Standard deviation	
2010	90	0	90	21	29.00 a
2011	180	0	90	22	25.00 a
2012	120	0	80	22	24.00 a
2013	180	0	90	18	15.00 a
Locations	No of observations	Minimum	Maximum	Standard deviation	Location means
Peshawar-1	120	0	90	23	28.00 abc
Peshawar-2	120	0	90	24	26.00 abc
Nowshara	120	0	90	18	14.00 bc
Bannu	60	0	80	16	10.00 c
Abbottabad	60	3	90	17	33.00 ab
Swat	90	0	80	17	40.00 a

The maximum Average Coefficient of Infection (ACI) was observed at Swat (40), demonstrating a significant contrast with Bannu (10) and Nowshera (14). Conversely, Peshawar-1 (28), Peshawar-2 (26), and Nowshera (14) exhibited comparable ACI values (Table 1). In terms of yearly variations, the highest ACI occurred in 2010, followed by 2011, 2012, and 2013, ranging from 15 to 29, with no statistical significance (Table 1). Except for specific instances, 11, 18, and 15 genotypes exhibited elevated ACI levels equal to or exceeding 40 at Peshawar-1 (range: 40-80), Peshawar-2 (40-80), and Abbottabad (40-72) in 2011, surpassing other location-year combinations (Table 2). In 2013, ACI levels greater than or equal to 40 were observed in 15 and 7 genotypes at Peshawar-1 (40-52) and Peshawar-2 (40-56), respectively. During 2012, a lower number of genotypes were diseased, with 21 exhibiting no rust infection in Nowshera. The correlation analysis revealed positive and statistically significant ($P \leq 0.05$) associations among ACI values across the four years and six locations, although the correlations remained moderate. In 14 cases, the correlation was positive but non-significant and small (Table 3).

The analysis of variance for ACI unveiled the significant effects of genotypes, locations, and the years × locations interaction ($P \leq 0.05$) (Table 4), while interactions such as years, locations × cultivars, years × cultivars, years × locations, and years × locations × cultivars were deemed non-significant. The greatest proportion of variability for ACI was attributed to the years × locations × genotypes interactions (24%), followed by locations × genotypes interactions (12%), years × genotypes interaction (6%), genotypes (6%), year × locations interactions (2%), and each of locations and years contributing around 1%. An exploration off high APR, defined by ACI values ranging from 0 to 20, revealed that 19 genotypes exhibited such resistance in 10 to 16 environments (location-year combinations) These genotypes, including Bakhtawar-93 (16 times), 93T347 (15), Wafaq-2001 (15), CT-00231 (14), 91BT010-84 (14), Kohsar-93 (13), Parwaz-94 (13), Nowshera-96 (13), Kaghan-93 (13), Pak-81 (13), 99B2278) (13), 7_03 (13), 99B2237 (12), Sarsabz (11), Zardana-89 (11), Faisalabad-83 (11), Sariab-92 (11), 99B4012 (11) and V-00183 (10) (Table 2). In certain environments (year-location combinations), these 19 genotypes also displayed 4-7 times moderate levels of APR (21-40). Low-level APR having 41-60 ACI range was displayed by 14 genotypes (Bakhtawar-93, Wafaq-2001, CT-00231, Nowshera-96, Kaghan-93, Kohsar-93, Pak-81, 7_03, 99B2237, 99B4012, Sariab-92, Zardana-89, Sarsabz and Faisalabad-83) for 26 times while Faisalabad-83, 99B2237 and 99B4012 displayed susceptibility for four times over 19 year-location combinations (Table 1).

Table 2. Multi-locational assessment of wheat genotypes for efficacy and adult plant yellow rust resistance levels in Northwest Pakistan (2010-2013)

Wheat Genotypes	Peshawar 1				Peshawar 2				Nowshera				Bannu		Abbottabad		Swat			ACI means over years-locations
	Yearly ACI				Yearly ACI				Yearly ACI				Yearly ACI		Yearly ACI		Yearly ACI			
	10	11	12	13	10	11	12	13	10	11	12	13	11	13	11	13	11	12	13	
93T347	0	9	0	20	0	21	0	28	0	21	0	0.2	4	8	33	6	0	0	8	7 b
Bakhtawar-93	0	9	0	17	0	53	0	14	0	11	0	0.4	0	12	26	24	12	16	0	9 b
Wafaq-2001	0	27	0	48	0	24	0	16	0	12	8	4	0	4	17	28	0	0	12	10 b
91BT010-84	40	24	8	24	0	21	0	12	15	15	0	8	0	8	12	21	12	0	16	12 b
Parwaz-94	0	31	0	24	0	0	4	20	0	3	0	21	16	8	40	16	0	40	24	12 b
7_03	0	18	0	36	0	21	16	24	21	12	0	3	0	8	52	32	15	0	8	12 b
Mairag-08	0	21	24	20	0	21	12	8	7.5	12	0	5	8	12	36	32	0	24	12	13 b
Pak-81	0	24	0	20	3	43	0	20	17	14	0	22	0	8	40	12	30	36	6	13 b
CT-00231	0	12	8	28	0	41	16	28	0	19	0	4.4	12	16	36	30	20	20	20	15 b
Kaghan-93	0	36	8	24	8	47	16	16	21	12	0	6	0	12	48	16	0	24	12	15 b
Kohsar-93	0	18	0	32	0	49	24	24	12	14	0	20	0	20	32	16	20	28	16	16 b
Zardana-89	0	28	24	28	0	60	8	24	12	3	0	12	0	16	24	16	12	32	32	17 b
Shafaq-06	32	27	0	40	3	40	0	20	0	24	8	20	0	20	25	24	36	16	24	18 b
Nowshera-96	0	20	12	44	0	30	18	32	3	6	0	14	20	12	46	24	12	40	16	18 b
Sariab-92	0	48	4	36	27	33	0	20	9.5	15	4	22	12	12	56	28	39	20	12	20 b
99B4012	12	52	12	44	0	80	0	8	17	31	0	46	0	16	27	3	42	32	16	20 b
V-99022	48	30	40	40	0	39	32	20	6	24	0	18	8	8	40	40	43	16	4	22 b
V-01180	16	36	32	32	12	33	12	52	25	28	0	12	0	8	56	13	48	36	8	22 b
Kohistan-97	0	40	0	48	18	36	16	52	3	11	0	33	12	32	40	28	30	40	4	22 b
Faisalabad-83	20	55	32	32	12	58	0	40	9	35	0	15	0	12	64	16	27	16	12	23 b
Sarsabz	0	50	16	52	3	55	56	32	14	15	0	15	8	10	30	32	36	16	16	23 b
99B2237	21	68	80	44	6	42	18	20	12	21	8	20	0	12	41	3	12	32	20	24 b
Sind-81	0	42	32	44	30	49	0	36	38	15	4	27	6	44	39	28	33	20	2	25 b
Punjab-96	40	64	0	40	30	42	14	44	9	12	0	33	12	24	52	24	37	20	12	25 b
Zargoon-79	44	32	40	48	3	44	24	35	18	12	0	18	8	36	33	32	12	48	28	26 b
Maxi-Pak	40	44	12	52	24	55	56	44	22	32	0	33	3	40	56	16	48	24	8	30 b
Pirsabak-91	56	36	16	44	24	80	80	12	14	17	4	8	25	8	72	20	47	60	12	32 b
WL-711	0	80	0	52	64	41	80	56	18	20	12	52	4	36	27	44	21	40	10	34 b
Tandojam-83	40	80	80	52	48	43	28	52	28	15	4	64	12	32	44	40	40	24	20	39 b
Morocco	90	90	80	90	90	90	80	90	90	90	80	80	80	80	90	80	80	80	80	85 a
Means	17	39	19	39	14	43	20	30	15	19	4	21	8	19	41	25	25	27	16	
Standard deviation	23	21	24	14	21	19	25	18	17	15	14	18	15	16	16	14	19	17	14	

Less than 10 times high APR expression was displayed by ten genotypes over 19 location-year combinations, included Kohistan-97 (9 times), Tandojam-83 (9), V-01180 (9), Pirsabak-91 (9), V-99022 (9), Punjab-96 (8), Sind-81 (7), WL-711 (7), Zargoon-79 (7) and Maxi-Pak (5) (Table 1). In certain year-location combinations, these 10 genotypes also displayed moderate levels of APR (21-40) with an occurrence frequency of 4 to 7. Low-level APR was displayed by these ten genotypes 39 times while Punjab-96, WL-711, Pirsabak-91, Faisalabad-83, and Tandojam-83 displayed susceptibility ten times over 19 year-location combinations (Table 1). Multi-year-locations combined analysis underscored the resilience of certain genotypes to yellow rust (Table 2) where YR coefficients for all genotypes were statistically non-significantly different except susceptible check Morocco which had a maximum ACI value of 85. Based on overall analysis, 16 genotypes with ACI range 0-20 were regarded as possessing high APR in the descending order and included Sariab-92 (20), 99B4012 (20), V00183(18), Nowshera-96 (18), Zardana-89 (17), Kohsar-93 (16), Kaghan-93 (15), CT00231 (15), Pak-81 (13), 99B2278 (13), 7_03 (12), Parwaz-94 (12), 91BT010-84 (12), Wafaq-2001 (10), Bakhtwar-93 (9), 93T347 (7). Similarly, 13 genotypes with ACI range 21-40 were regarded as possessing moderate APR in the descending order and included Tandojam-83 (39), WL-711 (34), Pirsabak-91 (32), Maxi-Pak (30), Zargoon-79 (26), Punjab-96 (25), Sindh-81(25), 99B2237 (24), Sarsabz (23), Faisalabad-83 (23), V99022 (22), V01180 (22) and Kohistan-97 (22).

Table 3. Pearson correlation matrix for 19 location-year combinations of average coefficients of infection (ACI) in northwest Pakistan for yellow rust.

Locations		Locations-Years																		
		Peshawar 1				Peshawar 2				Nowshara				Bannu		Abbottabad		Swat		
	Years	10	11	12	13	10	11	12	13	10	11	12	13	11	13	11	13	11	12	13
Peshawar 1	10	1	0.4	0.5	0.6	0.5	0.4	0.5	0.4	0.5	0.6	0.5	0.4	0.6	0.5	0.5	0.4	0.6	0.4	0.5
	11	0.4	1	0.5	0.7	0.8	0.4	0.5	0.6	0.5	0.5	0.5	0.8	0.4	0.5	0.4	0.4	0.5	0.4	0.4
	12	0.5	0.5	1	0.5	0.4	0.3	0.2	0.4	0.5	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.5
	13	0.6	0.7	0.5	1	0.7	0.5	0.6	0.7	0.6	0.6	0.7	0.7	0.6	0.7	0.4	0.6	0.6	0.5	0.5
Peshawar 2	10	0.5	0.8	0.4	0.7	1	0.4	0.6	0.8	0.7	0.6	0.7	0.8	0.6	0.8	0.5	0.6	0.6	0.5	0.4
	11	0.4	0.43	0.35	0.51	0.43	1	0.4	0.2	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.2	0.6	0.5	0.3
	12	0.5	0.5	0.2	0.6	0.6	0.4	1	0.5	0.4	0.4	0.4	0.4	0.5	0.5	0.4	0.5	0.5	0.5	0.3
	13	0.4	0.6	0.4	0.7	0.8	0.2	0.5	1	0.6	0.6	0.6	0.7	0.6	0.7	0.5	0.6	0.5	0.4	0.4
Nowshara	10	0.5	0.5	0.5	0.6	0.7	0.5	0.4	0.6	1	0.7	0.8	0.6	0.7	0.7	0.5	0.6	0.6	0.5	0.6
	11	0.6	0.5	0.4	0.6	0.6	0.5	0.4	0.6	0.7	1	0.8	0.5	0.6	0.6	0.5	0.5	0.7	0.4	0.6
	12	0.5	0.5	0.4	0.7	0.7	0.4	0.4	0.6	0.8	0.8	1	0.6	0.8	0.7	0.5	0.7	0.5	0.5	0.8
	13	0.4	0.8	0.4	0.7	0.8	0.4	0.4	0.7	0.6	0.5	0.6	1	0.5	0.7	0.3	0.5	0.6	0.5	0.5
Bann	11	0.6	0.4	0.4	0.6	0.6	0.4	0.5	0.6	0.7	0.6	0.8	0.5	1	0.6	0.6	0.7	0.5	0.6	0.7
	13	0.5	0.5	0.4	0.7	0.8	0.4	0.5	0.7	0.7	0.6	0.7	0.7	0.6	1	0.4	0.6	0.5	0.5	0.6
Abbo ttaba	11	0.5	0.4	0.3	0.4	0.5	0.4	0.4	0.5	0.5	0.5	0.5	0.3	0.6	0.4	1	0.3	0.6	0.5	0.3
	13	0.4	0.4	0.3	0.63	0.6	0.21	0.5	0.6	0.6	0.5	0.7	0.5	0.7	0.6	0.3	1	0.4	0.3	0.5
Swat	11	0.6	0.5	0.3	0.6	0.6	0.6	0.5	0.5	0.6	0.7	0.5	0.6	0.5	0.5	0.6	0.4	1	0.4	0.3
	12	0.4	0.4	0.3	0.5	0.5	0.5	0.5	0.4	0.5	0.4	0.5	0.5	0.6	0.5	0.5	0.3	0.4	1	0.5
	13	0.5	0.4	0.5	0.5	0.4	0.3	0.3	0.4	0.6	0.6	0.8	0.5	0.7	0.6	0.3	0.5	0.3	0.57	1

In bold, significant values (except diagonal) at the level of significance alpha=0.050 (two tailed test)

Table 4. Analysis of variance (ANOVA) for coefficient of infection (CI) of yellow rust in 29 seedling susceptible wheat genotypes across four years and six locations in northwest Pakistan.

Source of Variation	DF	Sum of Squares	Mean Square	F Value	Probability	Variation (%)
Genotypes	29	621052.631	21415.608	2.56	<.0001	6
Locations	5	113827.487	22765.497	2.73	0.019	1.09
Years	3	19173.684	6391.228	0.77	0.5138	<1
Locations*Genotypes	145	1235958.045	8523.849	1.02	0.4273	12
Years*Genotypes	87	639380.869	7349.205	0.88	0.7685	6
Years*Locations	11	196736.609	17885.146	2.14	0.0161	2
Years*Locations*Genotypes	319	2545021.127	7978.123	0.96	0.6754	24
Error	600	5010639.54	8351.07			
Total	1199	10393519.52				

DISCUSSION

The utilization of genetic resistance stands as the paramount and economically sound strategy for the enduring management of yellow rust. Adult plant resistance holds greater significance in the context of yellow rust management than all-stage resistance, as elucidated by scholarly works (Ellis etal., 2014; Muleta etal., 2017). Among the diverse methodologies available, field trials are acknowledged as robust tools for identifying sources of APR, reflecting the authentic conditions to which selected materials will be ultimately exposed. Adult plant resistance is conventionally studied under field conditions, particularly when genotypes exhibit susceptibility at the seedling stage (Singh etal., 2001). In the present study, 29 previously identified APR genotypes, characterized by seedling susceptibility, were subjected to rigorous multi-year and multi-location testing to validate adult plant yellow rust resistance. The coefficient of infection was employed as a measure for the classification of APR (Pathan and Park, 2006).

The combined analysis of variance (ANOVA) for the average coefficient of yellow rust infection discerned significant variations among genotypes and environments (locations and years × locations). Notably, genotype by environment interactions, encompassing locations × genotypes, years × genotypes, and years × locations × genotypes, remained non-significant. The year × location interaction effects underscored that each location and year represents a distinct environment, implying that cultivar resistance responds to environmental changes rather than a specific location or year effects. Yellow rust susceptibility is known to be influenced by weather conditions (Coakley, 1978; de Vallavieille-Pope etal., 1995) and variations in pathogen virulence (Bahri, 2008). A parallel study at the same locations confirmed variability in yellow rust virulence (Ibrahim etal., 2015). Furthermore, the interplay of genotypes × locations × years emerged as comparatively more significant, albeit non-significant for APR for yellow rust resistance over four years and six locations, indicating the overall consistency of genotypes in expressing resistance across diverse environmental conditions. Similar observations have been reported previously in several other crops (Toledo etal., 2006; Adeniyan etal., 2014). The correlation among the 19 location-year combinations remained moderate in most instances, suggesting both environmental and genetic influences on the expressed phenotypes (Mihalyov etal., 2017).

Notable variations in disease levels were recorded during 2011 and 2013, with Peshawar-1, Peshawar-2, and Abbottabad exhibiting high disease levels in comparison to other location-years. Conversely, the least disease level was recorded at Nowshara. Beyond pathogen variability, potential reasons for the inconsistency of ACI may encompass specific sensitivities of different genotypes to overall disease severity levels and environmental effects on the expression of resistance, given the varying impacts of temperature and precipitation on plant diseases (Garrett etal., 2006; Chakraborty, 2011).

The study identified 19 and 10 genotypes demonstrating high APR to yellow rust in 10-16 and <10 environments (location-years), respectively. In other environments, the APR of these genotypes varied, exhibiting either moderate, low, or susceptible levels. The observed genotype variations in APR levels against yellow rust suggest potential differences in the number and/or size of APR genes conferring this variability in resistance (Bariana etal., 2001; Singh etal., 2001; Herrera-Foessel etal., 2007). In light of the combined analysis, sixteen genotypes were classified as having high APR (0-20 ACI), carrying known yellow rust resistance genes, including 93T347 (*Yr18*+), Bakhtwar-93 (*Yr5*, *Yr18*, *Yr26*, *Yr27*), Wafaq-2001 (*Yr5*, *Yr10*, *Yr18*, *Yr26*), and others. Similarly, 13 genotypes demonstrated moderate APR (21-40 ACI), harboring known yellow rust resistance genes, such as V-99022 (*Yr18*, *Yr27*), V01180 (*Yr18*+), Kohistan-97(*Yr5*, *Yr7*, *Yr9*, *Yr10*, *Yr26*), and others (Shah, 2010; Shah etal., 2011; Bahri etal., 2011; Iqbal etal., 2016).

In the present study, it is observed that genotypes harboring various genes have not afforded adequate protection against yellow rust at specific locations. A race analysis conducted under controlled conditions on the 2010 sampled population from northwest Pakistan, revealed virulence for *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr27*, *YrSu*, and *YrAv* (Ali etal., 2014). Concurrently, a parallel field study conducted during the same location years, as documented by Ibrahim et al. (2015), identified virulence for *Yr1*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr18*, *Yr24*, *Yr26*, *Yr27*, *Yr32*, *YrSP*, *Jupateco R*, and *Avocet S*. Significantly, the susceptibility of the majority of yellow rust resistance genes in the region is noteworthy. Even *Yr18*, previously acknowledged as durable (Morgounov etal., 2012), has been recently categorized as moderately susceptible (Baboev etal., 2014).

Race non-specific APR genes, including *Yr18*, exhibit a limited degree of resistance when considered in isolation. However, their efficacy is significantly enhanced when strategically combined with other minor genes, as elucidated by Singh et al. (2000a). The current inventory of yellow rust (YR) resistance genes comprises more than 78 entries, underscoring the diverse genetic landscape influencing resistance (McIntosh etal., 2016). Noteworthy among these genes are *Yr18*, documented by Singh et al. (2012), along with *Yr29* and *Yr46*, as reported by Singh et al. (2013) and Herrera-Foessel et al. (2014), respectively. These genes confer pleiotropic, race-non-specific APR to YR. The synergistic effect achieved through the pyramiding of *Yr29* and *Yr46* holds significant promise, particularly in the context of Pakistani conditions. This combination is anticipated to elevate the levels of APR in the studied genotypes. Such an enhancement is of particular significance given the observed potential yields ranging from 5167 to 8349 kg/ha in genotypes exhibiting high and moderate levels of APR, as detailed by Rasheed et al. (2016). This strategic approach underscores the potential for optimizing yield outcomes through the judicious deployment of genetically diverse resistance mechanisms.

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