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Full Length Article

Achieving Near Immunity Durable-Type Resistance against Rusts in Advance Wheat Lines by Combining Race Non-Specific Resistance Genes

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Abstract

In order to incorporate slow rusting genes for rust resistance, parent lines were selected for breeding on the basis of high phenotypic uniformity and slow rusting history for race non-specific resistance. The plant material was advanced in filial generations from F1 to F5. Total 855 head rows (F6-generation) were selected from F5-generation and planted at Wheat Research Institute Faisalabad, a hot spot for wheat rusts. In primary evaluation, 112 lines were found resistant against both leaf and stripe rust and further assayed for the presence of race non-specific resistance genes through application of molecular markers. It was confirmed that the advance lines exhibiting race non-specific rust resistance possessed *Lr34/Yr18*, *Lr46/Yr29* and *Sr2/Yr30* genes alone or in combination from these lines. Results showed that 10 elite lines i.e. V-70003, V-70034, V-70039, V-70054, V-70064, V-70070, V-70076, V-70085, V-70103 and V-70104 showed the linkage of all the 3 slow rusting genes. None of these lines exhibited race specific or complete resistance but were of durable type providing resistance near immunity. All these lines are good source to be used in future breeding programs for enhancing resistance into the high yielding background wheat varieties through molecular or conventional breeding techniques, and are expected to contribute toward food security at national and global levels.

Keywords: AUDPC; Breeding; Race nonspecific resistance; Slow rusting genes; Wheat ruts

Introduction

Wheat (*Triticum aestivum* L.) along with maize and rice are strategic crops for worldwide food security. The estimated global wheat production for the year 2015–2016 was 734.2 MT which was slightly higher than the demand of 716.2 MT (FAO, 2016). The demand for wheat continues to rise at an annual rate of 1.6% and some estimates indicate that 60% more wheat will be needed by 2050 (FAO, 2016).

Wheat is mainly hit by three types of rusts namely stripe/yellow, leaf/brown and stem/black rust that reduce its produce (Roelfs *et al.*, 1992; Muhammad *et al.*, 2018). Evolution of two high temperature tolerant yellow rust races caused severe epidemics in main wheat cultivating areas worldwide since 2000 (Hovmoller *et al.*, 2008). Recent identification of various virulent races of Ug99 *i.e.*, TTKSK, TTKSF, TTKSF+, TTKSP, PTKST and 3 susceptible brown rust races CCPS, MCDS and FBPT are significant risk to wheat production worldwide necessitating integrated and collaborative management strategies of the diseases (Terefe *et al.*, 2014; Pretorius *et al.*, 2015; Patpour *et al.*, 2016; Soko, 2018).

In Pakistan, yellow and leaf rust have remained a constant risk to wheat production. The main reason behind rapid collapse of the assortments is associated to the emergence of novel virulent variant in assortment due to race specific genes of presentation. The recent and last pattern of durable resistance in wheat assortment depends upon preservative effects of accumulation of race non-specific genes (Singh *et al.*, 1998). The race non-specific leaf and yellow rust resistance appearing in several assortments is based on minor genes that have additive effects (Singh *et al.*, 2005). The economic, environmentally friendly, and easy to use method to reduce losses caused by the rusts is cultivation of resistant assortments (Kalappanavar *et al.*, 2008; Cheng and Chen, 2014; Hussain *et al.*, 2017; Mutari *et al.*, 2018). The main focus of researcher is to achieve race non-specific slow rusting resistance by combining several minor or adult plant resistance genes (Singh *et al.*, 2000; Iqbal *et al.*, 2018).

Continuous breeding results in narrow genetic variation in gene pool of wheat advance lines and also lead to problems regarding adaptation as well as biotic and abiotic stresses (Zhang *et al.*, 2005). Highest genetic variation among parentage is necessary to achieve

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transgressive segregation (Joshi *et al.,* 2004). Selection of genetically different parentage through breeding results in maximum variation in progenies. Therefore, there is an urgent need to exploit the existing elite lines to evolve high yielding lines that have extensive adoptability under changing climatic conditions (Baranwal *et al.,* 2012). Hence, the purpose of current investigation was to identify new sources of wheat advance lines having near immunity durable-type resistance against all three types *i.e.,* yellow, leaf and stem rust disease with lower AUDPC.

Materials and Methods

For genetic evaluation plant material comprised 855 wheat elite lines (F6 generation) of 45 diverse crosses based on 8–10 year wheat rust history and high yield characteristics were selected from gene pool of Wheat Research Institute Faisalabad (Table 1). The trial was sown during 2nd week of November, 2015–2016 at Wheat Research Institute Faisalabad through hand drill following augmented design with single replication split with 9 blocks having five plots per block containing 19 genotypes with one check (Morocco). Each plot comprises of 20 rows 2.5 m long and 25 cm apart. Morocco was inoculated using spraying, dusting and hypodermal needle injection methods twice during month of January and February to develop high rust inoculum pressure (Roelfs *et al.,* 1992). Disease severity percentage and filed response were observed following modified Cobb's scale for five consecutive observations after every 7 days interval when morocco became 60–70% susceptible (Table 1).The genotypes recorded to be resistant through primary evaluation (112) along with five check varieties (Inqlab-91, Faisalabad-08, Punjab 11, Galaxy-13, and Ujala-15) were subjected to further screening for rusts resistance and high yield potential at Wheat Research Institute (WRI) Faisalabad during second week of November, 2016–2017. The genotypes were planted by Norvigion in research area of Wheat Research Institute in Augmented design. Each test entry was planted in a plot (six rows of five meter length). In order to facilitate development of rust epidemics two rows of Morocco were planted around each side of experimental material. Artificial inoculation of experimental material was done in the morning from first week of January to mid-February using spraying, dusting and hypodermal needle injection method (Rao *et al.,* 1989), twice a week until a severe outbreak of disease develops (Roelfs *et al.,* 1992). The applied inoculum comprised of yellow (80E85) and mixture of brown rust (PHTTL, PGRTB, KSR/JS, TKTPR and TKTRN) races collected from Murree, Kaghan and Faisalabad.

Data Recording of Stripe and Leaf Rust

On the appearance of disease symptoms, field response and stripe and leaf rust reaction were recorded after every seven days interval through modified Cobb's scale as indicated by Peterson *et al.* (1948) (Table 1). Five observations were noted before crop maturity. The leaf and stripe rust intensity data were used to analyze the Area Under Disease Progress Curve (AUDPC) by using following method.

$$AUDPC = \sum_{i=1}^{n-1} [xi + Xi1]/2](ti + 1 - t1)$$

Where X*i* = rust severity on date *I,* t*i* = time in days between *i* and date *i* + 1 and n = number of dates on which disease was recorded (Shaner and Finney, 1980; Jeger and Viljanen-Rollinson, 2001). The acceptable range of area under disease progress curve for leaf rust is 500 and for stripe rust is 400 as stripe rust appears earlier than leaf rust and caused huge yield losses. All remaining genotypes having AUDPC values higher than 500, were discarded out of the evaluation of virulent genes through molecular markers analysis.

Molecular Marker Analysis

The putatively selected 112 wheat advance lines through primary evaluation were further assayed to molecular characterization to identify race non-specific resistance genes *Lr34/Yr18, Lr46/Yr29* and *Sr2/Yr30* using a set of 3 DNA molecular markers *viz*., X-barc-352, XWMC-44, and Xgwm-533 respectively (Suenaga *et al.,* 2003; William *et al.,* 2003; Hussain *et al.,* 2015). This present study work was carried out at Integrated Genomics Cellular Developmental and Biotechnology Laboratory, Post Graduate Agricultural Research Station (PARS) Campus, University of Agriculture Faisalabad.

DNA Extraction and Quantification

The fresh leaf samples from 30 days-old seedling were collected from the Wheat Research Institute-Faisalabad. After tagging, samples were washed with purified water and frozen immediately in liquid nitrogen (LN_2) chamber available in PARS campus University of Agriculture-Faisalabad and stored at -80°C in deep freeze for DNA extraction by using Cetyl Trimethyl Ammonium Bromide (CTAB) method (Bansal *et al.,* 2014). Leaves were crushed in CTAB buffer to release DNA from the cell. Samples were incubated in water bath at 65°C for 25–30 min. Tubes were Centrifuge at 4000 rpm for 5 min and the upper aqueous phase was transferred to new tubes. Chloroform: isoamyl alcohol (24:1 v/v) (300–500 μL) was added and vortex 4–5 times to mix the contents properly. For further purification other reagents such as RNase and NaCl were also added and centrifuged for 5 min at 14000 rpm and supernatant was transferred to fresh tubes. DNA was precipitated by adding (500 μL) of chilled isopropanol in the tubes and let it at –20°C for 25–30 min. The tubes were then centrifuged at 14000 rpm for 15 min to precipitate the DNA. The DNA pellet was washed 2–3 times with 500 μL of 70% ethanol and

Table 1: Rust reaction, Code for field response and Symptoms showing level of resistance/susceptibility of wheat advance lines

Reaction	Code	Symptoms
Immune	0	No visible infection
Resistant	R	Visible necrotic or chlorosis with or without uredia
Moderately Resistant	MR	Small uredia surrounded by necrotic areas
Mixed (Intermediate)	M	Small uredia present surrounded by necrotic areas as well as medium uredia with no necrosis but possible some distinct chlorosis
Moderately susceptible	MS	Medium uredia with no necrosis but possible some distinct chlorosis
Moderately susceptible-susceptible	MSS	Medium uredia with no necrosis but possible some distinct chlorosis as well as large uredia with little or chlorosis present
Susceptible	S	Large uredia are present with little or no chlorosis

Cobb's scale (Peterson *et al.,* 1948)

Table 2: Amplification parameters used for all primer sets linked to their specific durable resistance genes

Resistant genes	Primer	Cycle condition
Lr34/Yr18	X-barc-352	94°C 5 min., 38 cycles (94°C 30 seconds., 60°C 30 sec-1 min., 72°C 30 sec), 72°C 5 min.
Lr46/Yr29	Xwmc-44	94°C 5 min., 45 cycles (94°C 1min., 55°C 1 min., 72°C 2 min.), 72°C 10 min.
Sr2/Yr30	Xwm-533	94°C 5 min., 45 cycles (94°C 1min., 60°C 1 min., 72°C 2 min.), 72°C 10 min.

air dried before re-suspension in 20–30 μL ddH_2O. The DNA concentration was measured by spectrophotometer. An aliquot of sample was diluted in water ($1/80^{th}$ or $1/100^{th}$) and its absorbance was measured at 260 nm using a UV spectrophotometer (Zheng *et al.,* 2017).

PCR-Marker Assay

PCR amplifications were performed in a total volume of 25 μL containing 50–100 ng/μL of genomic DNA, 2.5 μL of 10X PCR buffer with 2.5 m*M* (2 μL) of Mgcl2, 0.5 of 10 m*M* $dNTP_S$, 0.5 μL each forward and reverse primer, 1U of Taq DNA polymerase and 17 μL of ddH20. Reagents were purchased from Invitrogen (USA). PCR was performed using the Eppendorf Mastercycler, Germany. The amplification parameters used for all primer sets i.e. X-barc-352, Xwmc-44 and Xgwm-533 restricted to specific durable resistance genes are presented in Table 2.

Electrophoresis

After PCR amplification, electrophoresis was carried out on the Syngene gel documentation system USA for SSR markers. An amount of 1.5 g high resolution agarose gel was weighted in the electric balance and dissolved in 100 mL 1 X TAE buffer (acetic acid pH = 7.8; Sodium acetate 2 m*M*; EDTA 10 m*M*; Tris HCL 40 m*M*) in a conical flask. It was heated for about 2–3 min by keeping it in oven and then left to cool under running tap water and mixed gently after adding 2 μL ethidium bromide (fluorescent dye) in this solution. The prepared solution was poured slowly into the gel tank. The combs of required size and teeth were inserted in it and leave it for 10–15 min to allow polymerization of gel. After polymerization, the 1XTAE buffer was poured into the gel tank to submerge the gel to 3–6 mm depth. The first well was loaded with 1Kb ladder molecular weight marker (Promega) as a size standard. Appropriate amounts of about 8 μL of each PCR samples were loaded into the other wells. The gel tank was closed and the gel was run for 30 min by providing 50 to 100 volts current to intercalate ethidium bromide in gel. After electrophoresis, the amplified products were visualized under ultraviolet transilluminator and gel pictures were obtained using Gene Snap version 7.6.03 of Syngene gel documentation system USA.

Results

The current study was planned to achieve durable-type resistance by accumulating designated slow rusting race non-specific genes with high yield characteristics of wheat advance lines. The plant material was selected from 855 heads rows of 45 crosses planted at Wheat Research Institute-Faisalabad, during crop 2015–2016, only 112 lines were selected on the basis of grain color, shape, high phenotypic uniformity and rust reactions (Table 3).

All the 112 selected wheat elite lines were further characterized on the basis of amplification of SSR molecular markers X-Barc352, Xwmc-44 and Xgwm-533 and area under disease progress curve (Table 4). Among the tested lines, only 37 lines indicated AUDPC values less than 500 to leaf rust and 400 to stripe rust whereas, while remaining 75 lines demonstrated AUDPC values ranging from 510–1960 to both stripe and leaf/brown rust and were discarded out in this investigation. Among the 112 selected advanced lines 32 lines exhibited *Lr34/Yr18*, 22 lines showed *Lr46/Yr29*, and 30 lines indicated the combination of *Sr2/Yr3* with lower area under disease progress curve (AUDPC). Molecular marker X-barc-352 indicated association to *Lr34/Yr18* which was present on chromosomal loci 1BL. All lines demonstrated the existence of leaf rust resistance gene *Lr34/ Yr18* with the band size of 250 base pair. Only 24 advanced lines were amplified by polymerase chain reaction (PCR) in which 19 genotypes were resistant and five advanced lines *i.e.*, V-70001, V-70005, V-70006, V-70008, V-70009 and V-70010 were

Table 3: Selection of single head crosses from F6 generation of 45 crosses during 2015-2016

Sr. #	Name of crosses	Test entries	Selected entries
1	CHENAB2000/INQ.91/5/WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	19	3
2	AS-2002/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAL	19	1
3	FSD.08/6/BABAX/3/FASAN/Y//KAUZ/4/BABAX/5/LU 26/HD2179	19	4
4	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES/5/KAUZ//ALTAR	19	8
5	SH.88/PAK.81//MH.97//OTUS/TOBA97	19	3
6	SH.88/PAK.81//MH.97//CUMHURIYET/NE	19	3
7	OASIS/5*ANGRA//INQ.91///MILAN/S87230//BABAX	19	4
8	TRM//MAYA 74'S'/MON'S'/3/INQ.91/4/PBW343	19	7
9	V-87094/ERA//PAK-81/2*V-87094/3/SHAFAQ-06/4/MAYA/PVN	19	5
10	PFAU/MILAN/5/CHEN/A.SQ(TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/6/QINGHAIBRI/WBLLI//BRBT2	19	3
11	INQALAB 91*2/KUKUNA//KIRITATI///V-09014	19	3
12	AUQAB 2000*2/LAKTA-1	19	4
13	FSD.08/6/BABAX/3/FASAN/Y//KAUZ/4/BABAX/5/LU26/HD2179/7/PB.96/87094//MH.97	19	6
14	TAM200/Tui/6/PVN/CRC422/ANA/5/BOW//CROW/BUC/PVN/3/YR/YR/4/TRAP#1/7/*21NQ-91	19	2
15	INQ/AUQAB/3/SH.88/90A204//MH.97	19	1
16	SH88/WEAVER/3/DWL5023/SNB//SNB	19	1
17	SH88/WEAVER/6/LU26/HD2179/5/BABAX/3/MANGO/VEE#10//PRL /4/BABAX	19	0
18	KAUZ//ALTAR84/AOS/3/PASTOR/4/TILHI/7/CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	19	0
19	SH88/2*ATTILA/6/ACHTAR*3//KANZ/KS8585/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92	19	2
20	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETO F2001/8/OASIS/SKAUZ//4*BCN/3/2*PASTOR	19	3
21	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETOF2001/8/CROW'S'/NAC//BOW'S'	19	9
22	PFAU/SERI.1B//AMAD/3/INQALAB91*2/KUKUNA/4/WBLL1*2/KURUKU/5/PVN/YACO/3/KAUZ*2/TRAP// KAUZ	19	3
23	HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	19	0
24	PRL/2*PASTOR//PBW343*2/KUKUNA/4/CAR422/ANA//TRAP#1/3/KAUZ*2/TRAP//KAUZ	19	0
25	C80.1/3*BATAVIA//2*WBLL1/3/PBW343*2/KUKUNA/4/KAUZ / SITE	19	3
26	INQALAB 91*2/KONK//INQALAB 91*2/KUKUNA/3/INQ-91*2/TUKURU	19	1
27	WHEAR/KRONSTAD F2004/3/CROW'S'/NAC//BOW'S'	19	1
28	WHEAR/KRONSTADF2004/3/PB96/V87094//MH97	19	1
29	FRT/SA42/3/PB96/87094//MH-97	19	1
30	WHEAR/KRONSTAD F2004//KAUZ / SITE	19	7
31	PFAU/MILAN//PBW343*2/TUKURU/3/T.DICOCCON P194625/A.SQ (372)//TUI….	19	1
32	PFAU/MILAN//PBW343*2/TUKURU/3/NR381	19	1
33	CROC_1/AE.SQUARROSA(205)//KAUZ/3/ATTILA/4/BOW/PRL//BUC/3/WH576/5/AMSEL/ATTILA//INQ.91/PEW'S'	19	3
34	CROC_1/AE.SQUARROSA (205)//KAUZ/3/PASTOR/4/THELIN/5/INQ/AUQAB	19	5
35	MINO/898.97/4/INIA66/7C//MAYA/3/PCI/TRM	19	1
36	CHONTE//PBW343*2/KUKUNA/3/CHENAB2000/INQ.91	19	0
37	CHONTE//PBW343*2/KUKUNA/INQ.91*2/TUKURU/3/T.DICOCCOM/P194624/AE.SQ (409)//BCN/4/2*INQ.91/2*/….	19	1
38	PB96/87094/MH-97/3/AMSEL/ATTILA//INQ.91/PEW'S'	19	0
39	PB96/87094//MH-97/3/MILAN/S87230//BABAX	19	1
40	LU26/HD2179//TTR'S'/JUN'S'/3/HP1744//4/MILAN/S87230//BABAX	19	0
41	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/IRENA/6/LERKE/7/TAN/PEW//SARA/3/CBRD	19	5
42	PBW343*2/KUKUNA//KRONSTADF2004/3/PBW343*2/KUKUNA/4/CHENAB2000/INQ.91	19	1
43	PBW343*2/KUKUNA//KRONSTADF2004/3/PBW343*2/KUKUNA/4/CHENAB2000/INQ.91	19	1
44	ATTILA*2//CHIL/BUC*2/3/KUKUNA/4/WAXWING*2/TUKURU	19	1
45	ROLF07*2/KIRITATI/3/SW8688//PBW343*2/KUKUNA	19	2
	Total	855	112

found susceptible (Fig. 1).

SSR marker Xwmc-44 exhibited linkage to *Lr46*/*Yr29* leaf and stripe rust resistance gene located on chromosome arm 7B. Its bands showed the amplification in the range of 242 bp. Eight elite lines were resistant while 16 advanced lines like V-70011, V-70012, V-70013, V-70014, V-70016, V-70017, V-70018, V-70019, V-70020, V-70021, V-70022, V-70023, V-70024, V-70026, V-70027 and V-70028 were found susceptible with *Lr46/Yr29* and the amplification of only 24 elite lines by polymerase chain reaction has been demonstrated (Fig. 2).

PCR-based diagnostic marker XGWM-533 was linked to *Sr2*/*Yr30* stem and stripe rust resistance gene. *Sr2/Yr30* was exist on chromosomal loci 3BS. All advanced lines indicated the presence of this gene with the band size of 120 bp. Twenty four lines amplified by PCR showed that 13 lines were resistant while 11 genotypes *i.e*., V-70007, V-70015, V-70029, V-70031, V-70032, V-70035, V-70036, V-70037, V-70038, V-70040 and V-70041 were found susceptible (Fig. 3).

From this investigation it was concluded that among 112 advanced lines, only 10 lines V-70003, V-70034, V-70039, V-70054, V-70064, V-70070, V-70076, V-70085, V-70103 and V-70104 demonstrated the association of 3

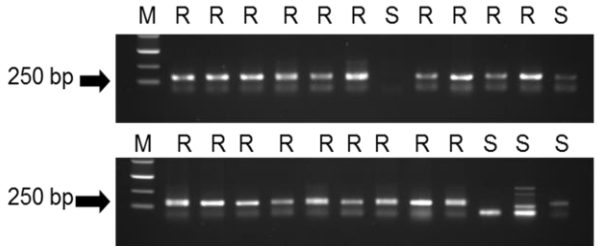

Fig. 1: PCR amplification products resolved in polyacrylamide gels using for 112 lines from elite material which produced resistant and susceptible bands by the size marker (X-barc 352)

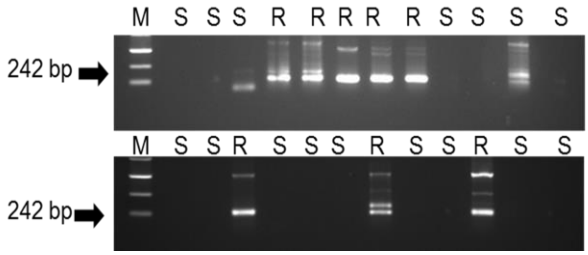

Fig. 2: PCR amplification products resolved in acrylamide gels using for 122 elite lines from elite material which produced resistant and susceptible bands by the size marker (XWMC-44)

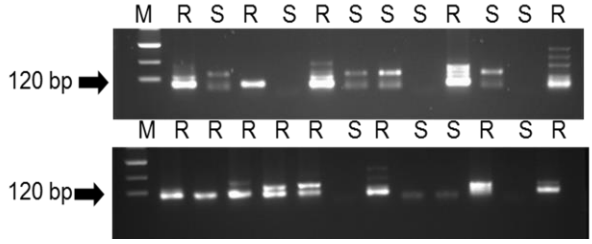

Fig. 3: PCR amplification products resolved in polyacrylamide gels when using for 112 lines of elite material which produced resistant and susceptible fragments by the size marker (Xgwm-533)

designated slow rusting/race non-specific genes.

This is very significant linkage, as it gives resistance against all 3 types of rust *i.e.*, stripe, leaf and stem rust. Similarly, 15 genotypes Viz. V-70002, V-70004, V-70012, V-70025, V-70030, V-70043, V-70046, V-70050, V-70065, V-70072, V-70084, V-70086, V-70092, V-70098 and V-70101 exhibited the linkage of *Lr34*/*Yr18* and *Sr*2/*Yr*30. Linkage of *Lr46/Yr29* and *Sr2/Yr30* was indicated in 5 lines viz. V-70014, V-70033, V-70061, V-70088, V-70096 and the association of *Lr34*/*Yr18* and *Lr46*/*Yr29* was identified in 7 lines including V-7007, V-70015, V-70047, V-70048, V-70087, V-70107 and V-70108. All these brilliant advanced lines having durable type resistance along with low values of area under disease progress curve may be used in future hybridization schemes to enhance level of resistance in the adapted wheat cultivars of Pakistan (Inqilab-91, Uqab-2000, AS-2002, Seher-2006 and Fareed-06 etc.).

Discussion

PCR based DNA markers associated with genes controlling target economic traits have significant role to attain sustained wheat production. Molecular marker-trait combinations give an effective alternative to phenotyping for selecting varieties that have linkage of desirable genes in breeding populations.

Three SSR markers XGWM-533, XWMC-44 and X-barc-352 were usedfor effective marker assisted selection of *Sr2/Yr30, Lr46/Yr29* and *Lr34/Yr18* in selected wheat elite lines. This investigation exhibited among all tested genotypes only 10 advanced lines showed a tight linkage to *Sr2/Yr30, Lr46*/*Yr29* and *Lr34/Yr18* having durable type resistance under the severe disease epidemics. According to Singh and Bowden (2011) resistance near immunity can be achieved by combining 4–5 race non-specific resistance genes in a cultivar. Though, slow level of resistance can be attained by combining 2 to 3 race non-specific/minor genes in a genotype (Lagudah *et al.*, 2009). International Maize and Wheat Improvement Centre (CIMMYT) and Ayub Agriculture Research Institute (AARI) planned a technique of combining race non-specific resistance genes alone or in linkage with some other genes to control recently evolved strains of wheat rust (Rehman *et al*., 2013).

These genotypes are important source of rust protection with lower area under disease progress curve. The resistance in determined genotypes seems to be durable in nature. The race specific resistance controlled by the parent lines was vulnerable as the single line V-70001 exhibited severe disease outbreaks ranging from 50–60 percent in the disease screening plots. Combination form these parent lines against common stripe and leaf rust races proved very effective with lower disease severity in the country (Hussain *et al*., 2006).

Many new released cultivars have been restricted for general cultivation only because of disease vulnerability against novel stripe and leaf rust races (Khan *et al.*, 2002). Combining 2–3 or more genes in a wheat genotype for race nonspecific resistance has remained the main emphasis of researchers to combat the changing nature of novel virulent races (Roelfs *et al.*, 1992). To contest this problem, DNA molecular marker technique was used for improving rust resistance through combining various race non-specific resistance genes in selected wheat lines. Genotypes possessing slow rust linkage illustrated lower area under disease progress curve at adult-plant stage have durable resistance as also indicated by various researchers (Bariana *et al.*, 2001; Singh *et al.*, 2005; Singh and Bowden, 2011). Because the race non-specific resistance like partial and durable rust resistance is polygenic as observed elsewhere, therefore, it remains effective for longer time period, even if the pathogen change its virulence pattern through mutation or recombination (Dehghani and Moghaddam, 2004).

Table 4: Detail of selected elite lines showing combination of three designated slow ruster, race non-specific resistance genes and area under disease progress curve

Plant Material		Genotypic Markers			Units of AUDPC	
V. Code	Name of genotypes	*Lr34/Yr18* (X-barc-352)	*Lr46/Yr29* (XWMC-44)	*Sr2/Yr30* (Xgwm-533)	LR	YR
70002	CHENAB2000/INQ.91/5/WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ PB-36259-0A-0A-0A-9A-0A	+	−	+	308	400
70003	CHENAB2000/INQ.91/5/WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ PB-36259-0A-0A-0A-12A-0A	+	+	+	192.5	175
70004	AS2002/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAL PB-36109-0A-0A-0A-7A-0A	+	−	+	122.5	105
70007	FSD.08/6/BABAX/3/FASAN/Y//KAUZ/4/BABAX/5/LU 26/HD2179 PB-36121-0A-0A-0A-8A-0A	+	+	−	345	332.5
70012	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES/5/KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES PB-36189-0A-0A-0A-5A-0A	+	−	+	192.5	394
70014	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES/5/KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES PB-36189-0A-0A-0A-15A-0A	−	+	+	161	196
70015	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES/5/KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES PB-36189-0A-0A-0A-17A-0A	+	+	−	172	175
70025	OASIS/5*ANGRA//INQ.91///MILAN/S87230//BABAX PB-36286-0A-0A-0A-8A-0A	+	−	+	87.5	397
70030	TRM//MAYA74'S'/MON'S'/3/INQ.91/4/PBW 343 PB-36360-0A-0A-0A-11A-0A	+	−	+	275	310
70033	TRM//MAYA74'S'/MON'S'/3/INQ.91/4/PBW 343 PB-36360-0A-0A-0A-19A-0A	−	+	+	145	178
70034	V-87094/ERA//PAK81/2*V87094/3SHAFAQ06/4/MAYA/PVN PB-36369-0A-0A-0A-11A-0A	+	+	+	245	120
70039	PFAU/MILAN/5/CHEN/A.SQ (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/6/QINGHAIBRI/WBLLI//BRBT2 PB-36377-0A-0A-0A-3A-0A	+	+	+	378	365
70043	INQ.91*2/KUKUNA//KIRITATI///V-09014 PB-36447-0A-0A-0A-14A-0A	+	−	+	297.5	135
70046	AUQ.2000*2/LAKTA-1 PB.37077-0A-0A-0A-8A-0A	+	−	+	70	399.5
70047	AUQ.2000*2/LAKTA-1 PB.37077-0A-0A-0A-14A-0A	+	+	−	322	395
70048	AUQ.2000*2/LAKTA-1 PB.37077-0A-0A-0A-19A-0A	+	+	−	398	122.5
70050	FSD.08/6/BABAX/3/FASAN/Y//KAUZ/4/BABAX/5/LU 26/HD2179/7/PB.96/87094//MH.97 PB.37082-0A-0A-0A-9A-0A	+	−	+	73.5	80.5
70054	FSD.08/6/BABAX/3/FASAN/Y//KAUZ/4/BABAX/5/LU26/HD217 9/7/PB.96/87094//MH.97 PB.37082-0A-0A-0A-19A-0A	+	+	+	375	349
70061	SH88/2*ATTILA/6/ACHTAR*3//KANZ/KS8585/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92 PB No. 36821-0A-0A-0K-8A-0A	−	+	+	175	389.5
70064	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETOF2001/8/OASIS/SKAUZ//4*BCN/3/2*PASTOR PB No. 36829-0A-0A-0K-15A-0A	+	+	+	140	122.5
70065	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETOF2001/8/CROW'S'/NAC//BOW'S' PB No. 36830-0A-0A-0K-1A-0A	+	−	+	211	189
70070	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETOF2001/8/CROW'S'/NAC//BOW'S' PB No. 36830-0A-0A-0K-12A-0A	+	+	+	218	154
70072	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PICUS/6/TROST/7/TACUPETOF2001/8/CROW'S'/NAC//BOW'S' PB No. 36830-0A-0A-0K-14A-0A	+	−	+	210	245
70076	PFAU/SERI.1B//AMAD/3/INQALAB91*2/KUKUNA/4/WBLL1*2/KURUKU/5/PVN/YACO/3/KAUZ*2/TRAP// KAUZ PB No. 36836-0A-0A-0K-10A-0A	+	+	+	297	170
70084	WHEAR/KRONSTADF2004//KAUZ / SITE PB No. 36880-0A-0A-0K-1A-0A	+	−	+	143.5	210
70085	WHEAR/KRONSTADF2004//KAUZ / SITE PB No. 36880-0A-0A-0K-11A-0A	+	+	+	168	122.5
70086	WHEAR/KRONSTADF2004//KAUZ / SITE PB No. 36880-0A-0A-0K-12A-0A	+	−	+	195	161
70087	WHEAR/KRONSTADF2004//KAUZ / SITE PB No. 36880-0A-0A-0K-13A-0A	+	+	−	105	84
70088	WHEAR/KRONSTADF2004//KAUZ / SITE PB No. 36880-0A-0A-0K-15A-0A	−	+	+	410	392
70092	PFAU/MILAN//PBW343*2/TUKURU/3/NR381 PB No. 36885-0A-0A-0K-13A-0A	+	−	+	134	122.5
70096	CROC_1/AE.SQUARROSA(205)//KAUZ/3/PASTOR/4/THELIN/5/INQ/AUQAB PB No. 36893-0A-0A-0K-3A-0A	−	+	+	94.5	95
70098	CROC_1/AE.SQUARROSA(205)//KAUZ/3/PASTOR/4/THELIN/5/INQ/AUQAB PB No. 36893-0A-0A-0K-5A-0A	+	−	+	500	345

Table 4: Continued

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70101	MINO/898.97/4/INIA66/7C//MAYA/3/PCI/TRM PB No. 36894-0A-0A-0K-15A-0A	+	−	+	157.5	297.5
70103	CNDO/R143//ENTE/MEXI_2/3/ AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/IRENA/6/LERKE/7/ TAN/PEW//SARA/3/CBRD PB No. 36976-0A-0A-0K-4A-0A	+	+	+	140	52.5
70104	CNDO/R143//ENTE/MEXI_2/3/ AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/IRENA/6/LERKE/7/ TAN/PEW//SARA/3/CBRD PB No. 36976-0A-0A-0K-10A-0A	+	+	+	272	140
70107	CNDO/R143//ENTE/MEXI_2/3/ AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/IRENA/6/LERKE/7/ TAN/PEW//SARA/3/CBRD PB No. 36976-0A-0A-0K-18A-0A	+	+	−	136.5	375.5
70108	PBW343*2/KUKUNA//KRONSTADF2004/3/PBW343*2/KUKUNA/4/CHENAB2000/INQ.91 PB No. 36978-0A-0A-0K-8A-0A	+	+	−	498	112

+ Sign shows the presence of rust resistance genes in wheat genotypes while
- Sign shows absence of rust resistance genes in wheat genotypes

Thus, in present study, Genotypes having low rust intensity and AUDPC could be considered as durable lines carrying high level of rust resistance to *Sr2*, *Lr46*, and *Lr34* virulences, that might be used in future hybridization schemes to protect crop stability. For its relative ease, productivity and specificity, many researchers have examined the robustness of these molecular markers to identify the occurrence of stripe and leaf rust resistance in wheat germplasm (Lagudah *et al.*, 2006; Dakouri *et al.*, 2013; Mustafa *et al.*, 2013).

When molecular study were corresponded with field data, two sets of observations were noted; firstly elite lines which indicated the presence of *Sr2, Lr34* and *Lr46* matched well to field investigation i.e. response of elite lines in the field for avirulent pattern. The advanced lines which demonstrated the presence of fragment size with 250 base pair for gene *Lr34* showed slow rusting response (Priyumvada *et al*., 2009). Whereas, lines which do not exhibit the existence of 250 base pair fragment size for this gene were vulnerable against leaf rust severity under field conditions. Thus, the lack of *Lr34* as indicated by molecular study corresponded well with the phenotypic data of field expression (Lagudah *et al*., 2006).

In the 2nd set of observations, both the field and molecular studies failed to correspond with each other such as some elite lines showed the existence of *Lr34* in the molecular study but under field conditions remained vulnerable against leaf rust severity. This difference in the both data sets results might be due to the emergence of new pathotype, or random mutations, deletion or suppression can also be the viable reason of failure of the genotypes to survive with the avirulent response (Dakouri *et al.*, 2013). Some elite lines failed to exhibit the presence *Lr34* chromosomal loci when evolved through molecular markers application even in the field conditions those genotypes demonstrated slow level of resistance against leaf rust. Similarly, Mclntosh (1992) identified the existence of *Lr34* in common wheat genotype Cappelle Desprez on the basis of phenotypic expression associated with stripe and leaf rust resistance but in stark contrast was not confirmed by application of specific DNA molecular markers (Lagudah *et al*., 2009). Besides, existence of some other avirulent genes might also be the viable cause of crop resistance to stripe and leaf rust as is the case of *Lr67* gene which is tightly linked in various characteristics to this gene (Spielmeyer *et al*., 2013). Correspondingly, polymerase chain reaction do not amplify the specific fragments during amplification might be one more possibility of variation between molecular marker and field data (Ali *et al*., 2007).

Conclusion

It was concluded that V-70003, V-70034, V-70039, V-70054, V-70064, V-70070, V-70076, V-70085, V-70103 and V-70104 were found most prominent crosses yielded lines exhibited the combination of three slow rusting genes (*Lr34/Yr18, Lr46/Yr29* and *Sr2/Yr30*) with lower AUDPC units. All these outstanding lines may be used in future breeding programs to transfer its durable resistance character to the adapted wheat varieties of Pakistan (Inqilab-91, AS-02, Seher-06 and Fareed-06 etc) and also after testing under different environmental conditions could be used for their direct release as variety.

References

Ali, S., S.J.A. Shah, I.H. Khalil, H. Raman, K. Maqbool and W. Ullah, 2007. Partial resistance to yellow rust in introduced winter wheat germplasm at the north of Pakistan. *Aust. J. Crop Sci*., 3: 37–43

Awan, S.I., S.D. Ahmad, L.A.J. Mur and M.S. Ahmed, 2017. Marker-assisted selection for durable rust resistance in a widely adopted wheat cultivar "Inqilab-91". *Intl. J. Agric. Biol.*, 19: 1319–1324

Bansal, U.K., A.G. Kazi, B. Singh, R.A. Hare and H.S. Bariana, 2014. Mapping of durable stripe rust resistance in a durum wheat cultivar Wollaroi. *Mol. Breed*., 33: 51–59

Baranwal, D.K., V.K. Mishra, M.K. Vishwakarma, P.S. Yadav and B. Arun, 2012. Studies on genetic variability, correlation and path analysis for yield and yield contributing traits in wheat (*T. aestivum* L. em Thell.). *Plant Arch.*, 12: 99–104

Bariana, H.S., M.J. Hayden, N.U. Ahmed, J.A. Bell, P.J. Sharp and R.A. McIntosh, 2001. Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. *Aust. J. Agric. Res*., 52: 1247–1255

Cheng, P. and X.M. Chen, 2014. Virulence and molecular analyses support asexual reproduction of *Puccinia striiformis* f. spp. *tritici* in the U.S. Pacific Northwest. *Phytopathology*, 104: 1208–1220

Dakouri, A., B.D. McCallum, N. Radovanovic and S. Cloutier, 2013. Molecular and phenotypic characterization of seedling and adult plant leaf rust resistance in a world wheat collection. *Mol. Breed*., 32: 663–677

Dehghani, H. and M. Moghaddam, 2004. Genetic analysis of the latent period of stripe rust in wheat seedlings. *J. Phytopathol.*, 152: 325–330

FAO, 2016. *What is conservation Agriculture*? CA website. (http://www.fao.org/ag/ca/la.html), FAO, Rome, Italy

Hovmoller, M.S., H. Amor, E.A. Yahyaoui and F.J. Annemarie, 2008. Rapid global spread of two aggressive strains of a wheat rust fungus. *Mol. Ecol.*, 17: 3818–3826

Hussain, M., M.A. Khan, Y. Ali, M.M. Javaid, B. Iqbal, M. Nasir and F. Muhammad, 2017. Wheat breeding for durable rust resistance and high yield potential in historical prospective and current status. *Adv. Zool. Bot*., 5: 55–63

Hussain, M., M.A. Khan, M. Hussain, N. Javed and I. Khaliq, 2015. Application of phenotypic and molecular markers to combine genes for durable resistance against rust virulences and high yield potential in wheat. *Intl. J. Agric. Biol.*, 17: 421–430

Hussain, M., N. Ayub, S.M. Khan, M.A. Khan, F. Muhammad and N. Ahmad, 2006. Pyramiding rust resistance and high yield in bread wheat. *Pak. J. Phytopathol.*, 18: 11–21

Jeger, M.J. and S.L.H. Viljanen-Rollinson, 2001. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. *Theor. Appl. Genet*., 102: 32–40

Joshi, A.K., S. Kumar, R. Chand and G. Ortiz- Ferrara, 2004. Inheritance of Resistance to Spot Blotch Caused by *Bipolaris sorokiniana* in Spring Wheat. *Plant Breed*., 123: 213–219

Kalappanavar, I.K., R.K. Patidar and K. Srikant, 2008. Management strategies of leaf rust of wheat caused by *Puccinia recondita* f. Spp. *Tritici* rob. Ex. Desm. *Karnat. J. Agric. Sci.*, 21: 61–64

Khan, M.A., M. Hussain, M. Hussain and M. Hussain, 2002. Wheat leaf rust (*Puccinia recondita*) occurrence and shifts in its virulence in Punjab and NWFP. *Pak. J. Phytopathol*., 14: 1–6

Lagudah, E.S., S.G. Krattinger, S. Herrera-Foessel, R.P. Singh, J. Huerta-Espino, W. Spielmeyer, G. Brown-Guedira, L.L. Selter and B. Keller, 2009. Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. *Theor. Appl. Genet.*, 119: 889–898

Lagudah, E.S., H. McFadden, R.P. Singh, J. Huerta-Espino, H.S. Bariana and W. Spielmeyer, 2006. Molecular genetic characterisation of the *Lr34/Yr18* slow rusting resistance gene region in wheat. *Theor. Appl. Genet*., 114: 21–30

Mclntosh, R.A., 1992. Close genetic linkage of genes conferring adult-plant resistance to leaf rust and stripe rust in wheat. *Plant Pathol.*, 41: 523–527

Muhammad, S., A. Ahmad, F.S. Awan, A.I. Khan, M. Qasim, A. Rehman, A. Rehman, M.A. Javed, I. Manzoor and M. Sajjad, 2018. Genome wide association analysis for leaf rust resistance in spring wheat (*Triticum aestivum*) germplasm. *Intl. J. Agric. Biol*., 20: 2387–2394

Mustafa, G., M.M. Alam, S.U. Khan, M. Naveed and A.S. Mumtaz, 2013. Leaf rust resistance in semi dwarf wheat cultivars: a conspectus of post green revolution period in Pakistan. *Pak. J. Bot*., 45: 415–422

Mutari, B., S.M. Udupa, P. Mavindidze and C.S. Mutengwa, 2018. Detection of rust resistance in selected Zimbabwean and ICARDA bread wheat (*Triticum aestivum*) germplasm using conventional and molecular techniques. *South Afr. J. Plant Soil*, 35: 101–110

Patpour, M., M. Hovmoller, A. Justesen, M. Newcomb, P. Olivera, Y. Jin, L. Szabo, D. Hodson, A. Shahin, R. Wanyera and I. Habarurema, 2016. Emergence of virulence to SrTmp in the Ug99 race group of wheat stem rust, *Puccinia graminis* f. spp. *tritici*, in Africa. *Plant Dis.*, 100: 522

Peterson, R.F., A.B. Campbell and A.E. Hannah, 1948. A diagrammatic scale for estimating rust severity on leaves and stems of cereals. *Can. J. Genet. Cytol*., 26: 496–500

Pretorius, Z., B. Visser, T. Terefe, L. Herselman, R. Prins, T. Soko, J. Siwale, B. Mutari, T.I. Selinga and D.P. Hodson, 2015. Races of *Puccinia triticina* detected on wheat in Zimbabwe, Zambia and Malawi and regional germplasm responses. *Aust. Plant Pathol.*, 44: 217–224

Priyumvada, R.T., M.S. Saharan, R. Chatrath, P. Siwatch and B. Mishra, 2009. STS Marker based tracking of slow rusting *Lr34* gene in Indian wheat genotypes. *Ind. J. Biotechnol*., 8: 207–213

Rao, S.K.V., J.P. Snow and G.T. Berggren, 1989. Effect of growth stages and initial inoculum level on leaf rust development and yield losses caused by *Puccinia recondita* f. spp. *tritici. J. Phytopathol.*, 127: 200–210

Rehman, A.U., M. Sajjad, S.H. Khan and N. Ahmad, 2013. Prospects of wheat breeding for durable resistance against brown, yellow and black rust fungi. *Intl. J. Agric. Biol*., 15: 1209–1220

Roelfs, A.P., R.P. Singh and E.E. Saari, 1992. *Rust Diseases of Wheat: Concepts and Methods of Disease Management*. CIMMYT, Mexico

Shaner, G. and R.E. Finney, 1980. New sources of slow leaf rusting resistance in wheat. *Phytopathology*, 70: 1183–1186

Soko, T., 2018. Stem rust resistance and yield performance of irrigated Zimbabwean spring wheat. *Doctoral Dissertation.* Depart. Plant Sci. (Plant Pathol. Plant Breed.), Fac. Nat. Agric. Sci., Univ. Free State. Bloemfontein, Republ. South Africa

Singh, R.P., J. Huerta-Espino and H.M. William, 2005. Genetics and breeding for durable resistance to leaf and stripe rusts in wheat. *Turk. J. Agric.*, 29: 121–127

Singh, R.P., J. Huerta-Espino and S. Rajaram, 2000. Achieving near-immunity to leaf rust and stripe rust in wheat by combining slow rusting resistance genes. *Acta Phytopathol. Entomol.*, 35: 133–139

Singh, R.P., A. Mujeeb-Kazi and J. Huerta-Espino, 1998. *Lr46*: a gene conferring slow rusting resistance to leaf rust in wheat. *Phytopathology*, 88: 890–894

Singh, S. and R.L. Bowden, 2011. Molecular mapping of adult-plant race-specific leaf rust resistance gene Lr12 in bread wheat. *Mol. Breed*., 28: 137–142

Spielmeyer, W., R. Mago, C. Wellings and M. Ayliffe, 2013. Lr67 and Lr34 rust resistance genes have much in common, they confer broad spectrum resistance to multiple pathogens in wheat. *BMC Plant Biol*., 13: 96

Suenaga, K., R.P. Singh, J. Huerta-Espino and H.M. William, 2003. Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. *Phytopathology*, 93: 881–890

Terefe, T.G., B. Visser, L. Herselman, T. Selinga and Z.A. Pretorius, 2014. First report of *Puccinia triticina* (leaf rust) race FBPT on wheat in South Africa. *Plant Dis*., 98: 1001

William, H.M., R.P. Singh, J. Huerta-Espino, S. Ortiz-Islas and D. Hoisington, 2003. Molecular Marker mapping of leaf rust resistance gene Lr46 and its association with stripe rust gene *Yr29* in wheat. *Phytopathology*, 93: 153–159

Zhang, P., S. Dreisigacker, A.E. Melchinger, J.C. Reif, A.M. Kazi, M.V. Ginkel, D. Hoisington and M.L. Warburton, 2005. Quantifying Novel Sequence Variation and Selective Advantage in Synthetic Hexaploid Wheats and Their Backcross-derived Lines Using SSR Markers. *Mol. Breed*., 15: 1–10

Zheng, L., J. Zhao, X. Liang, G. Zhan, S. Jiang and Z. Kang, 2017. Identification of a novel Alternaria alternata strain able to hyperparasitize *Puccinia striiformis* f. spp. *tritici*, the causal agent of wheat stripe rust. *Front. Microbiol.*, 8: 71

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