Gene pyramiding for stripe rust resistance in wheat (T*riticum aestivum*)

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Abstract:

Production and quality of worldwide most demanding crop, wheat (*triticum aestivum*) is getting adversely effected day by day by many biotic and abiotic stressors. One of the most devastating and alarming stressor among them is strip rust caused by *Puccinia striiformis* f. sp. tritici (Pst). Developing resistant lines by gene pyramiding is considered an inexpensive and effective approach to overcome the loss caused by stripe rust. There are many stripe rust resistant genes reported till now, but most of them have lost their effectiveness due to emergence of new virulent strains of fungi (p*uccinia*), but few of these genes are still resistant to current epidemic of *puccinia* races. Present studies was conducted to pyramid maximum stripe rust resistant genes (Yr5, Yr10, Yr15, Yr18, Yr26) in single line. Six parent for three different crosses (A, B, C) were identified by using marker assisted selection and evaluated under natural infection pressure by *Pst* races. F2 and F3 segregated populations were screened by MAS. Results revealed that the pyramiding effective or partially effective Yr genes significantly showed least coefficient of infection for stripe rust due to additive effects. Pyramided resistant F3 lines C1L2P9, C2L2P8 and C3L1P10 are highly recommended to further crossed with each other as four way cross in order to accumulate all five resistant genes in single plant for better development.

Keyword: wheat, *triticum aestivum,* gene pyramiding, stripe rust, MAS

Introduction:

Globally wheat is most widely cultivated crop plant due to its essentiality and vitality for daily life. However, there are biotic stresses alongside which renders the production per unit area directly or by affecting quality of the crop. One of the devastating biotic stress is stripe rust stimulated by *Puccinia striiformis,* causing large scale damage to wheat crop by developing yellow colored urediniospores in stripe pattern on the leaves of wheat plant which effects plant's transpiration (Bux *et al.*, 2012) and results in production of underdeveloped plants with damaged tillers and shriveled grains by absorbing essential nutrients from plant (Singh *et al.*, 2002). During the meiotic phase of life cycle of *Puccinia* genetic recombination mostly occurs, consequently new *puccinia* pathotypes emerge with more capability to invade existing resistant genes of plant. Mutation in *puccinia* genome is also responsible for evolution of new virulent pathotypes.

One of the resolution to overcome this threat is to develop genetically rust resistant wheat varieties by accumulating desired stripe rust resistant genes from diverse genotypes in single genotype of desired traits through gene pyramiding technique which is extensively used technique of transferring genes. Gene pyramiding through marker assisted selection (MAS) implicates molecular markers to achieve the goal of identification of genotypes possessing desired genes. In MAS technique various reliable molecular markers like RFLP, RAPD and SSR are applied which are reported as closely attached to desired genes to screen them accurately for estimation of genetic diversity and detailed characterization of plant genetic resources to utilize them in plant improvement programs (Jingyuan *et al.*, 2001; Murphy, 2007 & Krattinger *et al.*, 2009).

Pakistani wheat growing land (more than 65%) has high risk of susceptibility to stripe rust. Stripe rust resistance genes Yr3, Yr5, Yr10, Yr15, Yr26, YrSP and YrCV are reported still effective against widespread *puccinia* races in Pakistan while Yr18 shows moderate susceptibility (Chen, 2005; Chen, 2007; Bux *et al.*, 2011).

In our studies stripe rust resistant genes Yr5, Yr10, Yr15, Yr18 and Yr26 are utilized to be transferred in single genotype by gene pyramiding through MAS.

Material and methods:

Current study comprised of 6 wheat genotypes (Table 1). Seeds of experimental material were obtained from CIMMYT (Mexico). All material was sown in fields of Hazara University Mansehra in Randomized Complete Block Design with 30cm gap between rows, while length of row was kept about 3 meters. Plant protection agricultural measures and standard agronomic practices were applied throughout the growing period. Experimental material was screened through MAS technique for identification of rust resistant gene. Total genomic DNA extraction for this purpose was done by Langridge and Weinning protocol of DNA extraction with little modification (Weining & Langridge, 1991). Presence of desired genes was checked by amplifying specific bands by gene specific marker (Table 2). The amplification of desired gene band was done in Thermal cycler (Applied Bio System) in 25 µl reaction volume (distilled water 14µl**,** genomic DNA 2µl**,** 1µl of each primer, DNTPs 1.5µl, MgCl2 2.5 µl, Taq buffer 2.5µl, Taq polymerase 0.5µl). Resolution of PCR products was done using 1% agarose gel electrophoresis and visualized and photographed using gel documentation system**.** Band scoring as + or - was done on basis of presence and absence of gene respectively (Table 3). GNTYP1 carries a resistant gene Yr5 and Yr26 against stripe rust but shows no bands for presence of Yr18, Yr10 and Yr15**.** This genotype was crossed with GNTYP2 which was identified as carrier of Yr5, Yr10 and Yr15 (cross A)**.** GNTYP3 was successfully screened with presence of Yr5 only and crossed with GNTYP4 having stripe rust resistant genes Yr26 and Yr15 (cross B). GNTYP5 was identified as carrier of Yr10 so crossed with GNTYP6 which was possessing Yr18 (cross C). All three crosses A, B, C produced 48, 15 and 20 F2 plants respectively. Individual F2 plants were grown for DNA isolation and selection. F2 plants with screened resistant genes were allowed to grow for segregation in next generation (F3). PCR based molecular screening for all 5 desired genes was done in F3 plants as well.

F**ield screening of stripe rust:**

Field screening of plants for stripe rust was also accomplished side by side with marker assisted selection to validate the effectiveness of gene pyramiding. F2 and F3 progenies of three crosses were screened separately against stripe rust at the adult plant stage in the field. Screening was done while relying on natural infection and susceptible genotype tatara was fully loaded with stripe rust. In this studies the Coefficient of Infection (CI) for stripe rust was calculated according to Akhtar et al (Akhtar *et al.*, 2002). According to this method percent severity of rust is multiplied with response values in order to calculate coefficient of infection (Table 4).

Results and discussion:

STS-7/STS-8 provided a sharp bands of 478bp for carrier lines of Yr5**.** Banding pattern of 250bp identified the lines with presence of Yr18 by primer X-BARC352 while by using *STSBQ74* 400bp band confers presence of Yr26. Yr10 was revealed by a band of 286bp through application of *xpsp3000*. *Xbarc8* amplified a band of 190bp in carrier lines of gene Yr15 (Figure 1). A, B and C crosses developed hybrids which were allowed to self-fertilize to produce F2 generation. 48 F2 plants of cross A were selected and screened for presence of Yr5, Yr26, Yr10 and Yr15 genes. 11 plants revealed with combination of three different desired genes. Only 1 plant C1L4P3 showed results for all four genes to be transferred in single plant. This single plant was allowed to self-fertilize for segregation and then obtained seeds were grown, 20 F3 plants were successfully grown and screened, only plant C1L2P9 was pyramided with all 4 genes (Yr5, Yr10, Yr26 and Yr15). In cross B 15 F2 plants were screened among them 3 plants showed the presence of all three desired genes, seeds from these three lines were further grown to achieve F3 generation. Molecular screening of 17 F3 lines showed presence of all three (Yr5, Yr15 and Yr26) desired genes in only one line C2L2P8. Cross C produced 20 F2 plants and molecular screening identified 3 plants with presence of both the desired genes, further self-fertilization of these 3 plants and molecular screening of their progeny of 20 F3 plants revealed single line C3L1P10 with successful pyramiding of desired genes (Yr10 and Yr18). These three developed lines were subjected to field screening to evaluate the validity of pyramided genes and their combination. Gene pyramiding can also produce

transgenic plants with different resistance to insects (jacksn et al., 2003, Gahan et al., 2005). Current studies contrasts to previous work of scientists revealed certain similarities and differences which may be arose due to diverse and different environmental conditions and different breeding material. Resistant lines against soybean mosaic virus have been produced by gene pyramiding through MAS Field screening of developed lines during generation advancement under natural disease pressure revealed that combination of resistant genes showed more resistance with less coefficient of infection (Table 5, Figure 2). Gene pyramiding is an effective and inexpensive method of producing resistant plants by using efficient selection of a genotype carrying different combination of genes for disease resistance. The pyramided lines with maximum rust resistant genes can be grown as more yielding and more resistant

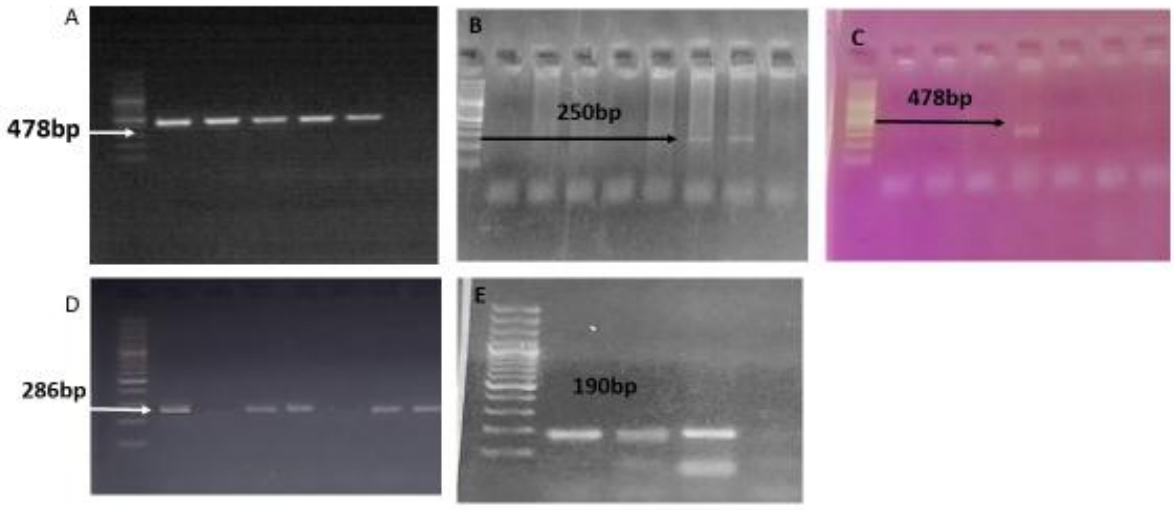

Figure 1: Amplification of rust resistant genes A: Yr5, B: Yr18, C: Yr26, D: Yr10, E: Yr15

Figure 2: A: Parents with more disease severity, B: Pyramided resistant lines

Table 1: Six genotypes used in gene pyramiding

Genotypes (with experimental code)		
YR24/3*AOC (GNTYP1)	(CX96.1.3.12)	1055 (GNTYP2)
1121 (GNTYP3)		AOC-YR*3/3/ALTAR84/AE.SQ//OPATA (CGSS00Y00204T-099M-3Y) (GNTYP4)
1076 (GNTYP5)		AOC-YR*3//LALBMONO1*4/PVN (CGSS01Y00011T-099B-37Y) (GNTYP6)

Table 2: Detail of primers for screening of genotypes

Gene / marker	Forward (1) and reverse (2) primer Sequence	Band size	Annealing Temp	Reference
Yr5 STS-7/STS-8	(1) GTACAATTCACCTAGAGT (2) GCAAGTTTTCTCCCTATT	478bp	45°C	(Chen *et al.*, 2003)
YR10 xpsp3000	(1) GCAGACCTGTGTCATTGGTC (2) GATATAGTGGCAGCAGGATA	286bp	51°C	(Wang *et al.*, 2002)
YR15 Xbarc8	(1) GCGGGAATCATGCATAGGAA (2) GCGGGGGCGAAACATACACA	190bp	55°C	(Murphy *et al.*, 2009)
Yr18 X-BARC 352	(1) GATCCACCTTCCTCTCTCTC (2) GATTATACTGGTGCCGAAAC	250bp	55°C	(Lagudah *et al.*, 2009)

YR26 STS BQ 74	(1) TGGATGAACCAACGATAGT (2) TGGGAAACACTTGACTGC	420bp	47°C	(Zhang *et al.*, 2013)

Table 3: Molecular screening for presence/absence of stripe rust resistant genes

Exp. Code	Genotypes	Yr5	Yr10	Yr15	Yr18	Yr26
GNTYP1	YR24/3*AOC (CX96.1.3.12)	+	-	-	-	+
GNTYP2	1055	+	+	+	-	-
GNTYP3	1121	+	-	-	-	-
GNTYP4	AOC-YR*3/3/ALTAR 84/AE.SQ//OPATA (CGSS00Y00204T-099M-3Y)	-	-	+	-	+
GNTYP5	1076	-	+	-	-	-
GNTYP6	AOC-YR*3//LALBMONO1*4/PVN (CGSS01Y00011T-099B-37Y)	-	-	-	+	-

Table 4: Coefficient of infection

Reactions	Host response	Response value
No disease	O	0.0
Resistant	R	0.2
Resistant to moderately resistant	RMR	0.3
Moderately resistant	MR	0.4
Moderately resistant to moderately susceptible	MRMS	0.6
Moderately susceptible	MS	0.8
Moderately susceptible to susceptible	MSS	0.9
Susceptible	S	1.0

Table 5: Coefficient of infection of 6 parents and their developed lines

Crosses		Research Code	Resistant genes	Disease severity%	Host response	Coefficient of infection
A	Parent 1	GNTYP1	Yr5,Yr26	30	MRMS	18
	Parent 2	GNTYP2	Yr5,Yr10,Yr15	10	R	2
	Developed line (F3)	C1L2P9	Yr5,Yr10,Yr15, Yr26	5	R	1
B	Parent 1	GNTYP3	Yr5	25	MRMS	15
	Parent 2	GNTYP4	Yr15,Yr26	10	R	2
	Developed line (F3)	C2L2P8	Yr5,Yr15,Yr26	10	R	2
C	Parent 1	GNTYP5	Yr10	50	MRMS	30
	Parent 2	GNTYP6	Yr18	10	R	2
	Developed line(F3)	C3L1P10	Yr10,Yr18	15	R	3

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