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# Markers assisted selection for multiple stripe rust resistance genes in spring bread wheat lines

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## Abstract

Stripe rust (yellow rust) of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most important yield limiting diseases of wheat. Although, the pathogen has overcome resistance offered by more than 90 genes and QTLs still, aggregation of resistant genes in future wheat genotypes is the most practical and efficient means of tackling the rapidly evolving virulent races of *Pst*. In order to stack combination of effective stripe rust resistance genes in future wheat genotypes, 99 spring wheat lines derived from the cross of Khyber-87 × Suleman-96 were screened with five closely linked PCR-based markers with stripe rust resistance genes *Yr5, Yr10, Yr17* and *Yr9*. Out of 99 experimental lines, S19M93 and S23M41 markers revealed the presence of *Yr5* gene in 86 and 70 genotypes, respectively. While, *Xpsp3000* suggested presence of *Yr10 gene in* 66 genotypes, excluding two heterozygous lines PBS-07-60 and PBS-07-64. VENTRIUP-LN2 marker revealed that all genotypes were negative for *Yr17* gene, while iag95 marker revealed presence of rye origin *Yr9* gene in all genotypes. The presence of 1RS fragments may be related to the poor bread making quality in certain wheat backgrounds. Furthermore, coexistence of both *Xpsp3000* and iag95 markers are indicative of the potential interstitial recombination between wheat 1BS and rye 1RS chromatin or the presence of 1RS fragments on more desirable wheat chromosomes 1A or 1D. The present work will facilitate gene pyramiding approaches against stripe rust and may be useful in future wheat improvement programs.

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**Introduction**

With over 730 million tons of annual production, wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD) has become one of the most widespread and important crops on the planet. It makes the staple food for more than 40 countries and over one third of the global population (Mujeeb-Kazi *et al.,* 2004; Dubcovsky and Dvorak, 2007; Matsuoka, 2011; FAO Statistics, 2014-2015). Throughout the Subcontinent and particularly in Pakistan, wheat is the leading food grain and the country ranks seventh in terms of global wheat production. During 2014-2015, Pakistan produced approximately 25.478 million tons of wheat from 9180 thousand hectares; further the contribution of wheat to the country's GDP was 2.1% (Pakistan economic survey, 2014-15).

Food security is going to be a major challenge for the mankind and as the human population reaches to 9.6 billion by 2050; feeding this estimated population will require more than 60% increase in wheat production alone (Mujeeb-Kazi *et al.,* 2008; Foulkes *et al.,* 2011; FAO Statistics, 2014-2015). Although, over the last few decades the yield potential of wheat has increased significantly and this was only possible due to the continuous development and release of improved spring and winter wheat cultivars. Still, to be the Zero Hunger generation, improving the genetic base of wheat against biotic and abiotic stresses is crucial and to accelerate wheat improvement and meet the targets of 2050, substantial efforts of integrating genomic approaches with conventional breeding practices will have a critical role (Mujeeb-Kazi and Hettel, 1995; Davis *et al.,* 2006; Schwarzacher *et al.,* 2011).

Various plant pests, particularly fungal pathogens are the main constraints to wheat production. Therefore, breeding wheat with biotic stress resistance will comprise the core objective of most future wheat breeding and cultivar improvement programs. Further, this goal must be addressed and pursued into all policy interventions for sustainable development (Mujeeb-Kazi *et al.,* 2008). Among the various fungal diseases of wheat, stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is currently the most important yield limiting diseases of wheat. Common symptoms of the disease are appearance of necrotic spots that appear on the leaves first, and as the fungus cannot prepare its own food therefore, it relies on nutrients supply of the host. Though, moisture and temperature below 20°C favour the pathogen growth; but once the fungus has established, it can survive a temperature as high as 38°C and in optimum conditions it takes only 2-3 hours for the spore of *Pst* to germinate (Rapilly, 1979; Singh *et al.,* 2000; Chen, 2005; Luo *et al.,* 2008; Rosewarne *et al.,* 2013).

Stripe rust resistance is classified as all stage resistance (ASR) or adult plant resistance (APR) and this is based on the expression of these genes at different stages of the plant development. Furthermore, genes conferring ASR to stripe rust pathotypes are known as major genes, while those conditioning APR are referred to as minor genes (Singh *et al.,* 2000; Bariana, 2003; Randhawa *et al.,* 2014). Major genes although; offer high levels of resistance against avirulent pathotypes but the newly emerged virulent races have defeated the resistance offered by these genes. Furthermore, minor genes provide low level of resistance at post seedling stages and pyramiding of at least three to four minor genes are recommended in commercially acceptable varieties (Rosewarne *et al.,* 2013; Randhawa *et al.,* 2015).

To date, more than 50 formally designated genes have been mapped in wheat along with another some 40 temporarily named genes or quantitative trait loci (QTL) for stripe rust resistance (McIntosh *et al.,* 2013), still new virulent races are evolving and are rendering all known sources of resistance genes as least or even ineffective. Furthermore, the very high and rapid rate of evolution in *Pst* population highlights the need for continuous search and deployment of diverse and effective sources of stripe rust resistance genes. Moreover, the success of resistance breeding program therefore, heavily relies on the availability of novel sources of resistance and

their subsequent utilization as pyramided genes in a single genotype (Ali *et al.,* 2016). Though precise knowledge of the pathotype genetic diversity is equally important for positive outcomes in varietal development and cultivar release programs (Mujeeb-Kazi *et al.,* 1989; Xu *et al.,* 2014; Randhawa *et al.,* 2015).

Advancements in the field of molecular biology have provided new insights of the genomic constitution of crop plants, and phenotypes may now be selected based on association of molecular marker(s) with linked traits. Nonetheless, identification of molecular markers that are closely linked with quality or resistance genes has steadily progressed and in the last decade and a wide range of high-throughput, reliable and cost-effective genotyping facilities such as SSR, ESTs and EST-SSRs along diversity arrays technology (DArT), genotyping-by-sequencing (GBS) and development of SNP chips have emerged (Jaccoud *et al.* 2001; Akbari *et al.,* 2006; Poland *et al.,* 2012; Rosewarne *et al.,* 2013; Xu *et al.,* 2014). All these technologies individually or in combination may be used to fine map the genes of interest and estimate more precise and objective quantification of the true genetic variability present within wheat genotypes.

This current investigation was planned to acquire information about the presence/absence of multiple stripe rust resistant genes in 99 spring wheat genotypes derived from the cross of Khyber-87 × Suleman-96.

The knowledge of diverse stripe resistant genes will be extremely important for marker-assisted selection (MAS) breeding programs as well as in stacking the resistance genes together in one genotype to safeguard future wheat cultivars of Pakistan against the losses of stripe rust.

**Materials and methods**

*Plant material*

Table 1 enlists 99 experimental spring wheat genotypes and 10 control checks used in the current study. Experimental lines were selected from F4 single head rows populations derived from the cross of Khyber-87 × Suleman-96 while positive checks (line with stripe rust resistance genes) included the Australian wheat varieties in Avocet background and included Avocet-*Yr5,* Avocet-*Yr9,* Avocet-*Yr10* and -Avocet-*Yr17* lines, while the negative control included Avocet 'S' lines.

*DNA isolation, quantification and gel electrophoresis*

Total genomic DNA was isolated from the seedlings of all lines with standard CTAB method modified from Doyle and Doyle (1987) and treated with 1µl of 10mg/ml RNase A solution (Fermentas, Life Sciences, USA) for 1 hours at 37°C to get rid of RNA. Both quality and quantity of the extracted genomic DNA was measured on 1.5% agarose gels (BioPLUS Fine Research Chemicals, USA) prepared in 1x TBE buffer. The DNA samples were loaded into gels after mixing it with appropriate amount of loading dye and run on 7V/cm for 45 minutes. Gels were visualized using VU gel documentation system (Biodiagnostics) after staining with ethidium bromide (0.5µg/ml final concentration), size as well as intensity of the DNA was compared with a standard 100bp lambda DNA ladder for quantification. DNA samples were diluted to 25ng/µl for PCR amplification and stored at -20°C freezers.

*Molecular markers and polymerase chain reaction (PCR)*

Five PCR primers (also referred to as markers) were used in the current study. These markers have been previously mentioned as closely linked to stripe rust resistance genes *Yr5, Yr9, Yr10* and *Yr17.* Sequences of primer pairs were obtained from published reports and ordered form Biobasic (INC, Canada). These primer sequences along with the annealing temperature, expected product size and original source are given as Table 2.

DNA was amplified in a 96 wells Veriti Thermal cycler (Applied Biosystems) in a final volume of 20µl. The PCR master mixture contained 2µl 10x PCR Buffer [10mM Tris HCl (PH 8.2), 50mM KCl], 2.4µl of 25mM $MgCl_2$, 0.4µl of 0.2mM Deoxynucleotide

triphosphate (dNTPs), 1µl 10µM forward and reverse primer, 0.2µl of 1U Taq Polymerase and 2µl of template DNA. The PCR was programmed as; 94°C for 5 minutes followed by 30 cycles at 94°C for 45 seconds, 58-65°C for 45 seconds, 72°C for 1 minute, and then final extension of 15 minutes at 72°C. Amplification and polymorphism of the PCR products was confirmed by 1.5% agarose gel as described. Expected PCR amplified fragments for all five primers were scored as present (1) or absent (0) and given in Table 1.

**Results and discussion**

At least 49 chromosomal regions containing QTLs for stripe rust resistance have been documented and with the availability of latest and improved genotyping facilities at lower cost more regions are expected to be added (Murphy *et al.,* 2009; Rosewarne *et al.,* 2013).

**Table 1.** List of bread wheat genotypes used in the current study.

| Serial | Wheat genotype/Accession | Species | S19M93 | S23M41 | *Xpsp3000* | VENTRIUP- LN2 | iag95 |
|---|---|---|---|---|---|---|---|
| 1. | PBP-07-1 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 2. | PBP-07-2 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 3. | PBP-07-3 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 4. | PBP-07-4 | *T. aestivum* | 1 | 1 | D | 0 | 1 |
| 5. | PBP-07-5 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 6. | PBP-07-6 | *T. aestivum* | 1 | 0 | 0 | 0 | 1 |
| 7. | PBP-07-7 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 8. | PBP-07-8 | *T. aestivum* | 1 | 0 | 0 | 0 | 1 |
| 9. | PBP-07-9 | *T. aestivum* | 0 | 0 | D | 0 | 1 |
| 10. | PBP-07-10 | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 11. | PBP-07-11 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 12. | PBP-07-12 | *T. aestivum* | 0 | 1 | 0 | 0 | 1 |
| 13. | PBP-07-13 | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 14. | PBP-07-14 | *T. aestivum* | 1 | 0 | 0 | 0 | 1 |
| 15. | PBP-07-15 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 16. | PBP-07-16 | *T. aestivum* | 0 | 1 | 0 | 0 | 1 |
| 17. | PBP-07-17 | *T. aestivum* | 1 | 1 | D | 0 | 1 |
| 18. | PBP-07-18 | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 19. | PBP-07-19 | *T. aestivum* | 1 | 1 | D | 0 | 1 |
| 20. | PBP-07-20 | *T. aestivum* | 1 | 0 | 1 | 0 | 1 |
| 21. | PBP-07-21 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 22. | PBP-07-22 | *T. aestivum* | 0 | 0 | 1 | 0 | 1 |
| 23. | PBP-07-23 | *T. aestivum* | 0 | 0 | 1 | 0 | 1 |
| 24. | PBP-07-24 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 25. | PBP-07-25 | *T. aestivum* | 1 | 1 | D | 0 | 1 |
| 26. | PBP-07-26 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 27. | PBP-07-27 | *T. aestivum* | 0 | 0 | 1 | 0 | 1 |
| 28. | PBP-07-28 | *T. aestivum* | 1 | 0 | 1 | 0 | 1 |
| 29. | PBP-07-29 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 30. | PBP-07-30 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 31. | PBP-07-31 | *T. aestivum* | 1 | 1 | D | 0 | 1 |
| 32. | PBP-07-32 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 33. | PBP-07-33 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 34. | PBP-07-34 | *T. aestivum* | 1 | 1 | D | 0 | 1 |
| 35. | PBP-07-35 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 36. | PBP-07-36 | *T. aestivum* | 0 | 0 | 1 | 0 | 1 |
| 37. | PBP-07-37 | *T. aestivum* | 1 | 1 | I | 0 | 1 |
| 38. | PBP-07-38 | *T. aestivum* | 1 | 0 | 0 | 0 | 1 |
| 39. | PBP-07-39 | *T. aestivum* | 0 | 0 | D | 0 | 1 |
| 40. | PBP-07-40 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 41. | PBP-07-41 | *T. aestivum* | 1 | 1 | D | 0 | 1 |
| 42. | PBP-07-42 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 43. | PBP-07-43 | *T. aestivum* | 1 | 1 | D | 0 | 1 |
| 44. | PBP-07-44 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 45. | PBP-07-45 | *T. aestivum* | 1 | 1 | D | 0 | 1 |
| 46. | PBP-07-46 | *T. aestivum* | 1 | 0 | D | 0 | 1 |
| 47. | PBP-07-47 | *T. aestivum* | 1 | 0 | I | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 48. | PBP-07-48 | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 49. | PBP-07-49 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 50. | PBP-07-50 | *T. aestivum* | 1 | 0 | 1 | 0 | 1 |
| 51. | PBP-07-51 | *T. aestivum* | 1 | 0 | D | 0 | 1 |
| 52. | PBP-07-52 | *T. aestivum* | 1 | 0 | 0 | 0 | 1 |
| 53. | PBP-07-53 | *T. aestivum* | 1 | 0 | 1 | 0 | 1 |
| 54. | PBP-07-54 | *T. aestivum* | 1 | 0 | D | 0 | 1 |
| 55. | PBP-07-55 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 56. | PBP-07-56 | *T. aestivum* | 1 | 0 | I | 0 | 1 |
| 57. | PBP-07-57 | *T. aestivum* | 1 | 0 | 0 | 0 | 1 |
| 58. | PBP-07-58 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 59. | PBP-07-59 | *T. aestivum* | 1 | 0 | 1 | 0 | 1 |
| 60. | PBP-07-60 | *T. aestivum* | 0 | 0 | U | 0 | 1 |
| 61. | PBP-07-61 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 62. | PBP-07-62 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 63. | PBP-07-63 | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 64. | PBP-07-64 | *T. aestivum* | 1 | 0 | U | 0 | 1 |
| 65. | PBP-07-65 | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 66. | PBP-07-66 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 67. | PBP-07-67 | *T. aestivum* | 0 | 1 | 1 | 0 | 1 |
| 68. | PBP-07-68 | *T. aestivum* | 1 | 0 | 0 | 0 | 1 |
| 69. | PBP-07-69 | *T. aestivum* | 1 | 0 | 1 | 0 | 1 |
| 70. | PBP-07-70 | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 71. | PBP-07-71 | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 72. | PBP-07-72 | *T. aestivum* | 1 | 0 | 1 | 0 | 1 |
| 73. | PBP-07-73 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 74. | PBP-07-74 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 75. | PBP-07-75 | *T. aestivum* | 0 | 1 | 0 | 0 | 1 |
| 76. | PBP-07-76 | *T. aestivum* | 0 | 1 | 0 | 0 | 1 |
| 77. | PBP-07-77 | *T. aestivum* | 0 | 1 | 0 | 0 | 1 |
| 78. | PBP-07-78 | *T. aestivum* | 0 | 1 | 0 | 0 | 1 |
| 79. | PBP-07-79 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 80. | PBP-07-80 | *T. aestivum* | 0 | 1 | 0 | 0 | 1 |
| 81. | PBP-07-81 | *T. aestivum* | 0 | 1 | 0 | 0 | 1 |
| 82. | PBP-07-82 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 83. | PBP-07-83 | *T. aestivum* | 1 | 0 | 0 | 0 | 1 |
| 84. | PBP-07-84 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 85. | PBP-07-29 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 86. | PBP-07-30 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 87. | PBP-07-31 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 88. | PBP-07-32 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 89. | PBP-07-33 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 90. | PBP-07-34 | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 91. | PBP-07-35 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 92. | PBP-07-36 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 93. | PBP-07-37 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 94. | PBP-07-38 | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 95. | PBP-07-39 | *T. aestivum* | 1 | 0 | 0 | 0 | 1 |
| 96. | PBP-07-40 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| 97. | PBP-07-41 | *T. aestivum* | 0 | 1 | 0 | 0 | 1 |
| 98. | PBP-07-42 | *T. aestivum* | 1 | 1 | 0 | 0 | 1 |
| 99. | PBP-07-43 | *T. aestivum* | 1 | 1 | 1 | 0 | 1 |
| | | Control resistant and susceptible lines | | | | | |
| 1. | Avocet-*Yr5* | *T. aestivum* | 1 | 1 | 0 | 0 | 0 |
| 2. | Avocet- *Yr5* | *T. aestivum* | 1 | 1 | 0 | 0 | 0 |
| 3. | Avocet-*Yr9* | *T. aestivum* | 0 | 0 | 1 | 0 | 0 |
| 4. | Avocet-*Yr10* | *T. aestivum* | 0 | 0 | 0 | 0 | 1 |
| 5. | Avocet-*Yr17* | *T. aestivum* | 0 | 0 | 0 | 1 | 0 |
| 6. | Avocet 'S' | *T. aestivum* | 0 | 0 | 0 | 0 | 0 |
| 7. | Avocet 'S' | *T. aestivum* | 0 | 0 | 0 | 0 | 0 |
| 8. | Avocet 'S' | *T. aestivum* | 0 | 0 | 0 | 0 | 0 |
| 9. | Avocet 'S' | *T. aestivum* | 0 | 0 | 0 | 0 | 0 |
| 10. | Avocet 'S' | *T. aestivum* | 0 | 0 | 0 | 0 | 0 |

*Note:* 1 indicate presence of marker loci, 0 indicate absence of marker loci
D: indicates no amplification due to unknown reason; U: indicates to presence of more than one band

Similarly, strains of *Pst* may be controlled using several available effective fungicides. Despite of these effective stripe rust resistance genes it is still very difficult to tackle the disease as the pathogen undergoes rapid mutation and adapt themselves better with the newly applied sources of resistance (Sharma, 2000; Chen *et al.,* 2010; Xu *et al.,* 2014). Further, the resistance within wheat genotypes against *Pst* decreases if the same cultivar or genotype is exposed to the same strain for several successive generations. The persistence of fungicides in the environment is hazardous to both human and wildlife refuge and therefore, utilization of stripe rust resistant genotypes offers a durable and cost effective means of controlling the disease. Therefore, continuous supply of diverse sources of resistance and the release of new varieties is necessary to overcome virulence of the *Pst* strains (Wang *et al.,* 2002; Chen, 2005; Rosewarne *et al.,* 2013).

**Table 2.** PCR primers used for screening of stripe rust resistance genotypes.

| Primer name | Sequence | Annealing temp (°C) | Expected product (bp) | Reference |
|---|---|---|---|---|
| S19M93 | F: TAATTGGGACCGAGAGACG<br>R: TTCTTGCAGCTCCAAAACCT | 62 | 100 | Smith *et al.*, 2007 |
| S23M41 | F: TCAACGGAACCTCCAATTTC<br>R: AGGTAGGTGTTCCAGCTTGC | 58 | 275 | Smith *et al.*, 2007 |
| *Xpsp3000* | F: GCAGACCTGTGTCATTGGTC<br>R:GATATAGTGGCAGCAGCAGGATAC | 55 | 240-286 | Bryan *et al.,* 1997 |
| Iag95 | F: CTCTGTGGATAGTTACTTGATCGA<br>R:CCTAGAACATGCATGGCTGTTACA | 55 | 1100 | Mago *et al.*, 2002 |
| VENTRIUP-LN2 | F:AGGGGCTACTGACCAAGGCT<br>R:TGCAGCTACAGCAGTATGTACACAAAA | 65 | 252 | Helguera *et al.,* 2003 |

The current study aimed at analyzing the presence of stripe resistant genes *Yr5, Yr9*, *Yr10* and *Yr17* in 99 spring wheat lines obtained from a cross between Khyber-87 and Suleman-96 by using five primers pairs S19M93, S23M41, *Xpsp3000*, VENTRIUP-LN2 and iag95 (Table 1). These markers have been previously reported as polymorphic, and have shown a strong correlation for the presence of a marker band to the existence of the respective resistance gene (Wang *et al.,* 2002; Mago *et al.,* 2002; Helguera *et al.,* 2003; Smith *et al.,* 2007; Murphy *et al.,* 2009). For all the PCR markers, initial screening was carried out using control resistance and susceptible lines along a representative sample of the 10 randomly selected experimental lines and presence/absence of the PCR amplicons was compared with the control checks. Our results correlated with the published reports at least for the size of expected PCR product and provided sufficient information for choosing specific resistant genes and an opportunity to stack the resistant genes against *Pst* pathotypes in a single wheat line.

S19M93 marker gave the expected 100bp product in 86 experimental lines as well as in the control resistant line, Avocet-*Yr5*. Similarly, the susceptible line Avocet-S and 10 experimental lines gave a negative PCR result, while in 4 lines this PCR product was not clearly resolved (Fig. 1).

The STS marker S23M41 produced the expected product of 275bp from 70 experimental and control resistant lines, while 30 experimental lines and the control susceptible Avocet-S line did not produce this marker band (Table 1). Both S19M93 and S23M41 markers are closely linked to*Yr5* gene and these markers have been reported as co-segregating with *Yr5* gene (Smith *et al.*, 2007). The efficiency of both markers was confirmed by absence of the respective PCR amplicon from the negative control lines Avocet-S and both these markers may be used in MAS breeding for *Yr5* gene.

*Similarly, Xpsp3000* is a codominant SSR marker residing on wheat 1BS (Bryan *et al.*, 1997; Reddy *et al.*, 2008; Sharma *et al.*, 2009) and here it amplified polymorphic loci where, the lower band of approximately 240bp is correlated to the presence of *Y10* gene (Wang *et al.*, 2002; Murphy *et al.*, 2009). Among the 99 spring wheat genotypes 66 given positive PCR results. Surprisingly, 14 lines did not give any band while 2 lines PBP-07-60 and PBP-07-64 produced double bands, indicating to the heterozygosity of these lines and further selfing is recommended to isolate pure lines (Fig. 1).

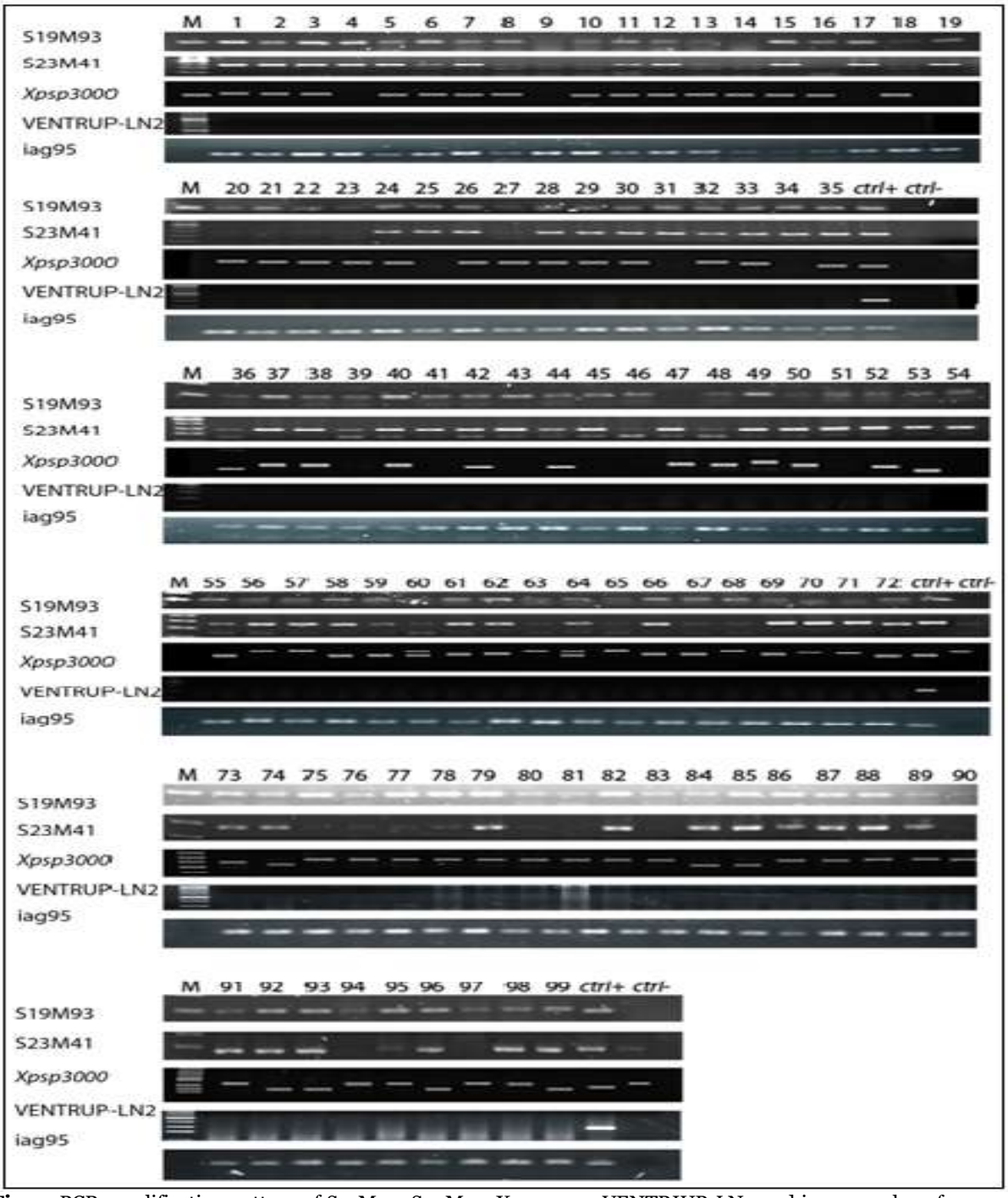


**Fig. 1.** PCR amplification pattern of S19M93, S23M41, Xpsp3000, VENTRIUP-LN2 and iag95 markers from 99 experimental and control lines (Table 1). *Ctrl+* represent the control resistant line, while *Ctrl-* indicates to susceptible line. On the left hand side of the agarose gel (1.5%), M indicating to a 100bp lambda DNA ladder (Fermentas, Life Sciences).

The microsatellite marker *Xpsp3000* may also be used to see if there is any interstitial recombination between the wheat 1BS and rye 1RS chromatin. Although, homoeologous recombination between the 1BS and 1RS is rare without exploiting the *Ph* system, but recently such recombination has been reported in lines that carried *Thinoprym intermedium* segments translocated onto wheat chromosome 4D (Ali *et al*., 2016).

The *Aegilops ventricosa* origin *Yr17* gene was checked with the help of VENRIUP-LN2 primers (Table 2). The marker produced the characteristic 259bp amplicons from the control resistant line only (Table 1). None of the 99 experimental lines gave positive PCR amplification but only the positive control Avocet-*Yr17*, and indicates the absence of this gene in these lines (Table 1). The VENTRIUP- LN2 marker is associated with *Triticum ventricosum* chromosome 2NS translocated to the short arm of bread wheat chromosome 2AS (Helguera *et al*., 2003). Therefore, the results obtained here are not surprising, and indicates that most of the line applied in this current study lack this alien chromatin. Further, it also highlights the importance of aggregating this gene in future wheat lines for better resistance against *Puccinia striiformis*.

The iag95 marker is tightly linked to *Yr9* gene as well as applied for the detection of 1RS chromatin of rye in MAS breeding (Mago *et al.,* 2002; Pretorious *et al*., 2012). The results obtained showed that all 99 lines except control susceptible carried rye chromatin and this indicates to the high frequency of 1RS chromatin in Pakistani wheat germplasm, which is segregating knowingly or unknowingly. The rye 1RS of is perhaps the most successful introgression carrying several other resistant and quality genes along with *Yr9* gene. However, the same arm also carries rye gene, which negatively affects bread-making quality (Schwarzacher *et al*., 1992; Anugrahwati *et al*., 2008; Sharma *et al.,* 2009). It is interesting to note, that iag95 produces characteristic amplicon from lines that carry 1RS chromatin (Mago *et al*., 2002) while *Xpsp3000* amplifies DNA from the wheat 1BS only and segregates with several wheat quality genes (Bryan *et al*., 1997; Reddy *et al.,* 2009; Sharma *et al*., 2009). Presence of both markers band in lines used here indicates to a possible recombination between the 1BS and 1RS and requires further investigation. A recent study that analysed 100 Pakistani wheat varieties for the presence of rye origin *Yr9* resistance gene reported the frequency *Yr9* gene to be only 12% (Tabassum *et al*., 2010). However, in our analysis 100% lines tested positive for the presence of *Yr9* gene (Table R1). One reason is, that both parents of our lines carried the 1RS chromatin either in homozygous or in heterozygous conditions (results not shown) and the subsequent lines incorporated this chromatin. Further, the exact genes involved in the success of 1RS chromatin are not clear, but there is linkage disequilibrium and the 1RS fragments are preferentially transmitted (Rubio *et al*., 1999; Landjeva *et al*., 2006). Nonetheless, primer contamination with rye DNA is another possibility; also most of the Pakistani wheat lines and varieties have CIMMYT (Mexico) origin, where the 1RS is still the most frequent translocation. Furthermore, coexistence of both *Xpsp3000* and iag95 markers may also be associated with rye 1RS fragments that are present on wheat chromosomes other than 1B/1R such as 1A/1R or 1D/1R and the latter are more desirable as such lines will carry the major *Gli-1* locus of wheat origin (Reddy *et al*., 2008; Sharma *et al*., 2009; Murphy *et al.,* 2009).

Intense breeding and selection over the last few decades has significantly reduced the genetic base of wheat, still new genes have been discovered and introgressed in the form of chromosomal segments to increase the genetic base of wheat against both biotic and abiotic stresses (Mujeeb-Kazi *et al.,* 1989; Schwarzacher *et al.,* 2011). Utilization of multiple resistant genes pyramided or "stacked together" in one genotype offers the most attractive strategy of wheat diseases control (Ali *et al*., 2016). The resistance capabilities, of each resistant gene although, varies significantly among different varieties (William *et al.,* 2006; Bariana *et al.,* 2007; Murphy *et al.,* 2009), and therefore the current study

is very important by identifying multiple stripe rust resistant genes in wheat breeding lines and emphasize to stack these multiple resistance genes in a single line. Such natural resistance is a cost effective, enduring and environmentally safe to tackle the new races of *Pst* and will be immensely important to safeguard the future wheat varieties against the deadly pathogen.

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