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Genotypic characterization for Yr18 gene of different wheat cultivars using STS marker (csLV34)

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Abstract
Wheat (*Triticum aestivum*) (2n=42) is one of the edible grains of human food that belongs to the family Poaceae. It is a polyploidy, and is cultivated all over the world. India is the largest producer of wheat after China. Stripe (Yellow) Rust caused by *Puccinia striiformis f.sp. tritici*, is one of the most severe fungal diseases, threaten bread wheat cultivars (*Triticum aestivum*) in India and many other countries, worldwide. It is a good air traveller and can spread over long distances under favourable climatic conditions. This disease can be managed through genetic resistance. Twenty-one elite wheat cultivars were grown to check the Yr18 gene presence using STS marker. PCR profiles revealed co-dominant pattern with distinct fragment of 150 bp in 10 cultivars, showing presence of Yr18 gene and 229 bp band in 11 wheat cultivars exhibiting absence of Yr18 gene.

Keywords: Yellow rust, genotypic characterization, STS marker, csLV34

Introduction
Wheat is the most important crop of India, it covers 38 percent of total arable land (Schaminee *et al.*, 2021) [6] and ranks third after rice and maize (Asseng *et al.*, 2011) [20] with an annual production more than 600 million tonnes. Wheat crop is affected by number of the diseases Rusts (Black rust, Yellow rust, Brown rust), Loose Smut, Karnal Bunt etc which leads to reduction in grain yield and quality. Among these different wheat diseases, cereal rusts are the most important threat to wheat productivity. Stripe rust (*Puccinia striiformis* f.sp. *tritici*) was considered as the most extensive disease (Zheng *et al.*, 2020)) [8] which belongs to the division Basidiomycota under the class Urediniomycetes and causes significant reduction in yield and result in total losses of the production (Ali *et al.*, 2017) [1]. *Puccinia striiformis* f.sp. *tritici* is spread by wind for thousands of kilo-meters from initial infection sites across numerous continents and oceans in the form of clonally produced dikaryotic urediniospores, it is obligate parasite and attacks all the above ground parts of the wheat plant and is characterised by pustules (powdery masses) of the yellow spores, that form stripes on the leaf surface. Wheat carrying Yr18 have been growing over a considerable acerage for more than 50 years in different wheat growing areas, worldwide. Two loci, Lr34/Yr18 complex on chromosome 7DS, and the Lr46/Yr29 complex on 1BL, expressed resistance to both leaf and yellow rusts. The Yr18/Lr34 locus, in particular is of great importance, where it was contributed to achieve durable resistance against the two rust pathogens (Lagudah *et al.*, 2009) [4]. Lr34/Yr18 complex alone may cause a high amount of disease, but when combined with other genes, it may provide effective management. The pathotypes with virulence for Yr9 and Yr27 currently predominate in Africa and Asia, whereas, Yr 17 and Yr 32 were prevalent in Europe. (Ali *et al.*, 2017) [1].

Materials and Methods
Molecular marker analysis
21 wheat germplasms (HD2501, HD2329, HD2864, WH533, HD2967, HD2307, WH542, SHARBATI SONARA, SONARA64, HD2687, WH711, HD3086, HD2285, PBW443, PBW824, WL410, HD2824, HD2177. PBW343, WH1105) were used, that were obtained from Department of Plant Breeding and Genetics, PAU, Ludhiana.

DNA Extraction
Young leaves were collected in an aluminium foil and kept in ice, till their transfer to laboratory. These leaves were stored in -80 °C freezer until isolation of DNA. Leaves were crushed to fine powder using Liquid nitrogen and autoclaved pre-chilled pestle and mortars.

Fine tissue powder was transferred immediately to a 2ml autoclaved Oakridge tube to avoid moisture absorption. Then, about 700 µl pre warmed (65 °C) CTAB extraction buffer was added to homogenised leaf material in an Oakridge tube. The samples were then thoroughly mixed by inverting and rotating the tubes. The homogenised samples were incubated at 65 °C for 60 minutes in water bath with intermittent shaking of the tubes for mixing. After incubation, 800µl of chloroform: Isoamyl alcohol (24:1) was added in each tube. Then the tubes were swirled, till it made a dark green emulsion. After that, the tubes were centrifuged for 15 minutes at 10000 rpm in centrifuge at 4 °C. The supernatant was then transferred into a new tube and later, two-third of the chilled isopropyl alcohol was added and the tubes were inverted gently several times. DNA formed white cotton like precipitate and good quality DNA floated at top. Then the tubes were kept in the incubator at -20 °C overnight. Next day, the tubes were centrifuged for 15 minutes at 10000 rpm at 4 °C and the pellet was visible at the end of the tube. Then the isopropyl alcohol was discarded and 2 washing were given with 70% ethanol. After adding ethanol to the tubes, the tubes were centrifuged at 4000 rpm for 5 minutes. Later, the ethanol was also discarded, to obtain the DNA pellet. Then these tubes were turned down on the blotting paper and air dried (at room temperature) for few hours. Then 200 µl of 1X TE (Tris EDTA buffer-10 ml Tris HCl, 1mM EDTA, pH 8.0) was added. The tubes were left at room temperature for few hours for the DNA to dissolve in 1 X TE buffer. The DNA was stored at -20 °C until further use.

Assessment of Quantity of DNA

Quantity of the DNA was checked by agarose gel electrophoresis, 0.50g of agarose was dissolved in 50ml of 1X TAE electrophoresis buffer. DNA samples for loading were prepared by adding loading dye (10X loading dye consisting of 0.4% Bromophenol blue, 0.4% Xylene cyanol, 50% glycerol in sterile water) to the DNA so that the final concentration of the loading dye is 1X. Using Gel Doc the DNA was checked under the UV light. The intensity of the fluorescence of each sample was compared with that of the standard DNA ladder and the DNA concentration of each sample was ascertained.

Molecular marker and polymerase chain reaction (PCR)

SSR marker csLV34 was linked in the current study. This marker has been previously mentioned as closely linked to leaf and stripe rust resistance gene Lr34/Yr18. Sequences of primer pairs were obtained from Lakshay enterprises (PAU, Ludhiana). These primer sequences along with the annealing temperature, expected product size and original source. DNA was amplified in a 96 wells PCR in a final volume of 25µl. the PCR master mixture contained 12.5 µl of sterile distilled water, 2.5 µl 10x buffer (with $MgCl_2$), 2 µl Deoxynucleotide triphosphate (dNTPs), 2.5µl (2.5pmoles/µl) forward and reverse primer each, 1µl Taq DNA polymerase and 2µl of DNA template. The PCR was programmed as; 94 °C for 5 minutes followed by 35 cycles at 94 °C for 30 seconds, 58-60 °C for 30 seconds, 72 °C for 50 seconds, and then final extension for 5 minutes at 72 °C. Amplification and polymorphism of the PCR products was confirmed by 1% agarose gel as described.

Sequences of primer pairs

Sr. No.	Primer	Forward Primer sequence	Reverse Primer Sequence	Expected basepair +Lr34 -Lr34	
1	csLV34	5'GTTGGTTAAGACTGGTGATGG3'	5'TGCTTCGTATTGCTGAATAGT3'	150	229

Result and Discussion

Molecular characterization of cultivars

Molecular characterization STS (Sequence Tagged Sites) marker (csLV34F/csLV34R) was used for a specific gene Yr18 in selected wheat germplasms. STS marker showed polymorphism for Yr18 in wheat cultivars. Results revealed that STS marker csLV34 amplified two alleles, a band of 150 bp size that has been reported to be tightly linked with resistant gene Yr18 and other fragment of 229 bp size, not associated with resistant gene. Out of 21 wheat germplasms HD2501, HD2864, WH533, HD2687, HD2135, WH711, PBW443, PBW824, HD2177, WH1105 showed 150bp size of the band. Whereas, HD2329, HD2967, HD2307, WH542, SHARBATI SONARA, SONARA64, HD3086, HD2285, WL410, HD2824, PBW343 showed 229 bp size of the bands. Amplification products from the csLV34 locus, have proven to be diagnostic for Lr34/Yr18, across a wide range of wheat genotypes (Lagudah *et al.*, 2006) [5]. The results are in agreement with previous studies which also confirmed the presence of adult plant durable rust resistance gene (Lr34/Yr18) in wheat cultivars by STS marker (Gupta *et al.*, 2018; Shahin *et al* 2018) [3, 7].

Molecular characterization of cultivars

Sr. No.	Wheat Cultivars	Molecular Characterization csLV34	
		150 bp (+)	229 bp (-)
1	HD2501	+	
2	HD2329		-
3	HD2864	+	
4	WH533	+	
5	HD2967		-
6	HD2307		-
7	WH542		-
8	SHARBATI SONARA		-
9	SONARA64		-
10	HD2687	+	
11	HD2135	+	

12	WH711	+	
13	HD3086		-
14	HD2285		-
15	PBW443	+	
16	PBW824	+	
17	WL410		-
18	HD2824		-
19	HD2177	+	
20	PBW343		-
21	WH1105	+	

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