Fine Mapping and Expression Analysis of Stripe Rust Resistance Genes derived from *Aegilops geniculata*

Shivendra Kumar*, Satinder Kaur, Parveen Chhuneja, Mitaly Bansal and Indrajeet Singh Yadav

*School of Agricultural Biotechnology Ludhiana (Punjab) 141004, India*

*Corresponding author*

**Abstract**

Wheat production in temperate area is significantly affected by rust diseases, among which stripe (or yellow) rust caused by *Puccinia striiformis* Westend f. sp. *tritici* and leaf (or brown) rust caused by *Puccinia triticina* Eriks. is major threat to production. Non-progenitor *Aegilops* species with substantial amount of variability for stripe rust resistance genes has been exploited to a limited extent. A tetraploid non-progenitor species (UUMM genome), namely *Aegilops geniculata* accession pau3549 is found to be resistant to stripe rust. A stripe rust resistant introgression line-ILT598 (BC2F8 Ae. geniculata acc. pau3549/CSS//3*WL711) was already available. In the present investigation an F5 population was derived from the cross of ILT598 with wheat cultivar WL711(NN). Inheritance studies in F5 population revealed that stripe rust resistance is controlled by a single dominant gene, temporarily designated as *YrAg*. Mapping of *YrAg* was done by using SNP based KASper marker linked with two group of linked gene on chromosome 5DS viz *Lr57-Yr40* and *Lr76-Yr70* and one marker was designed from candidate gene of *comp_121307_c0_seq4* derived from ILT598. Molecular mapping using F5 population mapped *YrAg* at a distance of 3.3cM from KASP *comp_121307_c0_seq4* towards distal end of chromosome 5D.

**Keywords**

*Aegilops geniculata*, KASper markers, Introgression, SNP, Stripe rust, Leaf rust

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**Introduction**

Bread wheat (*triticum aestivum* l. Thell, 2n=42) is a widely cultivated crop in India or worldwide and a potent source of nutrients. It occupies 17% of global crop area feeding about 40% of the world population (https://en.wikipedia.org/wiki/ international wheat production). It grows on 215 million hectares with global production stands at 739.5 million metric tons (CGAIR 2017), (FAOSTAT homepage; http://apps.fao.org) representing 19% of global cereal production with the world population is expected to grow from present 7.0 billion 9.0 billion by 2050, India is the second largest wheat producer in the world and among the Indian states, Punjab is ranked second in wheat production after
UtterPradesh. Stripe (yellow) rust (YR) caused by *Puccinia striiformis* f.sp. *Tritici*, is one of the major diseases of wheat in temperate regions also found in the tropics and subtropics. It infects leaves, leaf sheath and spikes of a wheat plant. This can infect barley, rye and more than 50 grass species also (Line 2002). The losses to wheat crop due to YR varies from 10-70 percent, depending upon the weather, races of pathogen, susceptibility of cultivar, and time of infection (Begum *et al.*, 2014).

The regular use of limited parental genotypes in the advanced wheat breeding practices and monoculture of few improved wheat varieties results in a narrow genetic base in the cultivated wheat. Breakdown of mega variety PBW343 due to evolution of virulence against gene *Yr27* is an example of pathogen evolution when a single variety is grown over large area (Prashar *et al.*, 2007). New races of pathogen may develop through mutation or recombination of nuclei via a para-sexual process. Different YR genes includes seedling resistance or all time resistance genes *Yr1, Yr2, Yr3, Yr4, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr15, Yr17, Yr27* and adult plant resistance genes: *Yr11, Yr12, Yr13, Yr14, Yr16, Yr18, Yr36*, etc. (Mclntosh *et al.*, 1995).

Fungicides are the only alternative to genetic resistance, their use increase the cost of production along-with increasing soil and water pollution and other pathogen resistance issue Thus, there is need to look for constant and diverse source of resistance. Therefore need to stack variable R genes instead of single R gene to induce the durable resistance. The germplasm of wild progenitor species consists huge reservoir of various resistance traits. This has led to inter-specific breeding for transferring preferred gene from wild progenitor into commercial cultivars (Knott 1981, Dvorak 1976, Stalker 1980, Dhaliwal *et al.*, 1993).

To date more than 76 YR (Xiang *et al.*, 2016) R gene have been categorized and almost half of these genes derived from their progenitor as well non-progenitor species. Evaluation of wild wheat germplasm at PAU for the past 20 years led to the identification of C, U, and M genomes from wild *Triticum* and *Aegilops species* as a potent resource for resistance to leaf and stripe rusts. *Aegilops geniculata* is a diploid (2n=42) non-progenitor species with UUMM genome. One of the accessions of *Aegilops geniculata* designated as *pau3549* was resistant to stripe rust/yellow rust (YR) and leaf rust (LR). *Ph1* mediated induced homeologous pairing resulted in transfer of 5mgl to chromosome 5D of wheat.

Initially a disomic substitution line DS5M (5D) have been developed and this line was crossed with a Chinese Spring (CS) stock carrying the *Ph1* locus (*Ph1* locus is responsible for homeologous recombination) (Chen *et al.*, 1994). F1s from this cross were further crossed with WL711, a local variety susceptible to leaf rust and stripe rust (Aghaee-Sarbarzeh *et al.*, 2002). One more backcross with WL711 and selfing of stable, leaf rust and stripe rust resistant plants with chromosome number 2n=42 were carried forward and BC2F8 WL711-*Ae.geniculata* stable introgression lines (IL) were developed. Two of these IL were IL T598 (TA5601) and IL T756 (TA5602).

Molecular marker and GISH analysis of these two IL revealed the transfer of 5mgl to chromosome 5D of wheat. IL T756 has had the smaller fragment from *Ae geniculata* [T5DL-5DS-5mgs (0.95)] and is fully resistant to leaf rust while moderately resistant to stripe rust (40MS). Linked gene *Lr57-Yr40* had already been mapped in IL T756 (Kuraparthy *et al.*, 2008). IL T598 on other hand was completely resistant (0) to leaf rust as well as stripe rust with a larger fragment from *Ae. Geniculata* [T5DL-5DS-
The present investigation was proposed with three objectives
1. Study genetics of stripe rust resistance genes in ILT598.
2. Molecular mapping of stripe rust resistance genes in ILT598.
Comparative gene expression analysis of stripe rust resistance in ILT756 (YR40) and ILT598.

Materials and Methods

The present investigation was performed at the experimental area and Molecular Biology Laboratory, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana.

Material

Stripe rust resistant Wheat-\textit{Ae. geniculata} introgression lines (IL) T598 (TA5601), ILT756 (TA5602) and susceptible cultivar WL711 NN=Non-Necrotic) were used as a parental line. F\textsubscript{4} population derived from a cross of ILT598 with WL711(NN) and ILT756 respectively (leads to crosses).

Methods

The present investigation was carried out as three different experiments.

Experiment 1

Genetics of yellow rust resistance gene (s) transferred from \textit{Ae. geniculata} in IL T598 (TA5601) (Kuraparthy. V \textit{et al.}, 2007).

Screening for Yellow rust resistance at the seedling stage

Pathogen

A single spore culture of \textit{Puccinia striformis tritici} race 110S119 and 100S84 (\textit{pst}) was used. Race 110S119 is the most dominant race against stripe rust resistance responsible for knocking down of known gene. The cultures were obtained from Regional Station, Directorate of wheat research (DWR), Flowerdale, Shimla.

Raising the seedlings and inoculation

To raise the seedling of BC2F4 population displayed as WL711-introgression line (developed through crosses of introgression line T598 with WL711 (TA4325-152). The landrace 'WL711' was used as the susceptible check (initially WL711 has \textit{Lr 11} and \textit{Lr 13} in present scenario both of the leaf rust resistance gene \textit{Lr 11} and \textit{Lr 13} became recessive (Gupta AK \textit{et al.}, 1984)).

The seedlings were raised in glasshouse maintained at 18-20\textdegree C and 100\% Relative humidity. The first leaf of seven days old seedlings was inoculated using spearhead needle with the homogenous urediniospores-talc mixture of yellow rust race 110S119 and 100S84 under above mentioned environmental condition.

Scoring of infection types (IT)

The infection types (ITs) were recorded 14 days after inoculation using the scale of Stakman \textit{et al.}, (1962) as shown in Table1. Seedlings with ITs, ranging from 0; and; were categorized as resistant and 3 as susceptible.

Screening of stripe rust resistance under field conditions (natural temp and relative humidity)

Stripe rust infection was recorded at the adult plant stage as the percentage of leaf area covered with rust urediospores, according to the stakman rule.
Statistical analysis

The chi-square test will be used for testing goodness of fit of the data to the expected genetic Chi-square ($\chi^2$) was applied to test goodness of fit of the expected ratios to the observed ones. Following expression was used for calculating chi-square values

$$\chi^2(n-1)d.f = (O-E)/E$$

Experiment 2

Molecular mapping of yellow rust resistance genes using bulked segregant analysis.

Isolation of genomic DNA and genotyping of the Ae.geniculata derived population

Genomic DNA was isolated using CTAB method (Saghai Maroof et al., 1994) of 30 days old seedling leaf tissues were used collected from the field. DNA of all two BC$_2$F$_4$ progenies along with advanced generation of BC$_2$F$_4$ (IL) T598 * WL711 (NN) was BC$_2$F$_5$. One of the BC$_2$F$_4$ population was developed through a cross between introgression line (IL) T598 to cultivar WL711. Second BC$_2$F$_4$ = ILT598*ILT756. Extracted sample were quantified using a NanoDrop® ND-1000 and ND-8000 8-Sample Spectrophotometers instrument

KASP genotyping

For KASP genotyping primer mix was prepared as per the recommendations by LGC Genomics by adding 46 µl dH2O, 30 µl common primer (100 µM) and 12 µl of each tailed primer of 100 µ concentration. KASP genotyping assays were tested in 384-well format and each assay was set up as 4 µl reactions (2 µl template DNA (final concentration of 20–30 ng of DNA), 1.944 µl of v4 2x KASP mix (LGC Genomics, Teddington, UK), and 0.056 µl primer mix). PCR was performed on a Eppendorf Master cycler pro 384 using the following temperature profile: hot start at 95°C for 15 min, followed by ten touchdown cycles (95°C for 20 s; touchdown at 65°C with -1°C per cycle for 1 min) then followed by 30 cycles of amplification (94°C 20 s; 57°C 1 min). 384- well optically clear plates (Cat. No. E10423000, Starlab) were read on a Tecan Safire plate reader. Fluorescence was visualized at a set temperature 37°C. The fluorescence intensity scanning was then imported to KlusterCaller software (v 2.22.0.5, LGC Genomics) to check the cluster formation. If the defined genotyping clusters had not formed after the initial amplification, additional 5 to 10 amplification cycles were given in terms of add5, and the samples were scanned again. Further data analysis and scoring were performed manually using Klustercaller software.

KASP technology (a PCR based genotypic mechanism) was developed by LGC-Genomic and set a recommended standard KASP protocol. LGC-Genomic put standard with three well plate system respectively 96, 384 & 1542 well plate system.

Primer designing

We have RNA Seq data of ILs T598 and WL711 at six different time intervals 0hr, 12hr, 24hr, 48hr, 72hr and 96hr (Yadav et al., 2016) all processes done in BIOINFORMATICS LAB school of agricultural biotech ludiana punjab. One of the genes selected ID comp_121307307_c0_seq4 was selected as it was NBS-LRR encoded and high confidence gene.

Statistical analysis

Mapping software MAPDISTO were used for
computing the distance between the markers and trait (Lorieux 2007). The map was drawn using the programme MAPCHART version 2.1 developed by Voorips (2002).

**Experiment 3**

Expression analysis of candidate genes contributing towards yellow rust resistance.

**Raising the seedlings, inoculation and sample collection**

Seedlings of IL (BC$_2$F$_4$ generation) T598, IL T756, and a parental cultivar WL711(NN) were raised and first leaf of seven days old seedlings had infected with aYr race 110S119 + talc. The inoculated seedling was incubated in a dark chamber maintained at 20°C±1°C at 100% RH for 16h (Nayar et al., 1997).

Leaf samples were collected at different time interval of 0 hours, 6 hours, 12 hours, 24 hours, 48 hours 72 hours and 96 hours in three biological replicates followed by 14$^{th}$ days rust appearance.

**Total RNA extraction and c DNA conversion**

Using Trizol manual method and RNA quantification done with 1.2% Denaturing gel based running gel unit. Prime Script™ first strand cDNA Synthesis Kit based cDNA Synthesis. c-DNA quantification using NanoDrop® ND-1000 and ND-8000 8-Sample Spectrophotometers instrument

For RNA and c-DNA quantification OD value taken respectively 2.0 and 1.8 for expression analysis.

**Transcriptome analysis and identification of candidate gene**

Introgression line T598 and susceptible cultivar WL711 have been sequenced for RNA-Seq (cDNA) at six different time interval 0hr, 12hr, 24hr, 48hr, 72hr and 96hr (all RNA Seq data were generated from RNA which sample was inoculated with the 77-5 race of leaf rust) in another project. The reference Transcriptome Shotgun Assembly of WL711 submitted at GenBank (AC. GEWU00000000) was used as reference. The differentially expressed genes were investigated through using bioinformatic tools in SOAB-ludhiana and final candidate gene were investigated based on FPKM value (table: 7 and fig. 3) of expression over bar graph.

All five sequences related to comp121307_c0_seq4 gene sequence (candidate sequence out of 5 isolated sequence) were aligned and the SNPs identification had accomplished. Based on the SNPs site primer for KASP genotyping and QRT-PCR were designed. All primer designing has done with Vector-NTI Advanced software.

**Real-time quantitative RT-PCR**

Expression analysis using real-time quantitative PCR conducted on the cDNA samples of IL T598, IL T756 and cultivar WL711 for six different time interval in triplicate The PCR amplification efficiency was determined for each primer combination by the slope of the standard curve obtained by plotting the fluorescence versus concentration of the individual cDNA of sample ILT598, ILT756, and cultivar WL711.

**Real-time quantitative PCR- assay**

Done all the QRT PCR reaction with LightCycler96 well plate qRT PCR (Roche). A total of 10 microliters of PCR reaction was performed at the School of Agricultural Biotechnology in wheat genomics lab. QRT-
PCR reaction per performed with cDNA and two type of primer one was constitutive primer (Tubulin primer) and second was target primer (121307_c0_seq4) within the single white PCR Plate of 96-well, segmented, semi-skirted. Simultaneously a negative-template control (NTC) negative control was performed in same 96 PCR well plates. The pcr profile were used as initial denaturation at 94°C/4 min, denaturation at 94°C/1min, annealing at 56°C/1min extension 72°C/1min, and final elongation 94°C/7min.

Results and Discussion

Inheritance of stripe rust resistance in ILT598 and WL711 against YR race 110s119 and 110s84 and list of Resistance, susceptible and homozygous in table: 1 & 2

The segregation of 250 progenies in F5 generation fit into 1.75HR: 0.5 Sepr:1.75HS progenies with chi square value 4.4 indicating a single gene for YR resistance in ILT598. Since ILT598 is resistant at SS while WL711 is susceptible the YR resistance of ILT598 is due to seedling resistance gene also known as all-time resistance gene. Thus YR resistance of ILT598 is due to single resistance gene effective at all stage of plant growth. The gene was temporarily designated as YrAg.

Mapping of stripe rust resistance gene

Selection of markers for mapping of YR resistance

Linked LR and YR and genes have already been reported on chromosome 5DS Lr57/Yr40 from Ae. geniculata (Kuraparthy et al., 2007) in ILT756, a sister line of T598. Another linked gene Lr76/Yr70 from Ae. Umbellulata have also been mapped on chromosome 5DS (Bansal et al., 2017). The SNP markers for both pair of genes reported by Kuraparthy et al., (2016) and Bansal et al., (2017) were selected initially with the hypothesis that ILT598 also has YR gene on the chromosome 5DS. Thus 41SNP markers have been selected of which 9 markers reported to be linked with Lr57-Yr40 and 33 markers have been linked with Lr76-Yr70 (Table: 3). Another new marker has been designed from candidate gene sequence derived from RNA sequence data of IL-T598 (candidate gene sequence from RNA extracted after inoculation with leaf rust pathogen) in table: 4.

Selection of candidate gene

Possible candidate R-gene transcripts were selected, which have homologs on chromosome 5DS, as resistance gene in another IL-T756 was previously mapped short arm of chromosome 5D (Kuraparthy et al., 2008). Finally we identified six R-genes mapping to chromosome 5DS, filtered on the basis of maximum percent identity >99% and >98% query coverage. FPKM (Fragments PerKilobase of transcript per Million mapped reads) values of these mapped transcripts were extracted for both WL711 and ILT598 at 6 time intervals and were averaged for replicates. One R-gene (named comp_121307_c0_seq4) transcript demonstrating consistent expression in term of FPKM values was selected.

Genotyping of F5 population

The selected 42 selected markers from chromosome 5DS were amplified on parental lines ILT598 and WL711, of which nine were found to be polymorphic including one marker linked to Lr57/Yr40, seven markers linked to Lr76/Yr70 and one marker from selected candidate gene from 5DS. These nine markers were further amplified on the 250 F5-progenies. The entire 9 marker found to be associated with YrAg gene leading to formation of linkage map of 7.72cM with KASP marker comp_121307_c0_seq4 being
closest at a distance of 3.3cM (Fig 1). All the markers were scored as co-dominant markers with “A” given to susceptible WL711 allele, “B” given to resistant ILT598 allele and “H” given to progenies amplifying both the resistant and susceptible alleles in fig 2. Segregation of three alleles in F$_5$ population is given in Table: 5.

**Validation of candidate gene using qRT-PCR**

The real-time PCR has been performed to validate the time a specific level of transcription of a leaf rust resistance and stripe rust in the fig. Resistance genes respectively. Samples were collected from two resisters IL T598 and T756 and one susceptible cultivar WL711 at six different time intervals after the inoculation with respective pathogen. Real time PCR primers have been designed from candidate gene *comp_121307_c0_seq4*.

**Expression analysis report for validation of comp_121307_c0_seq4 using QRT-PCR**

qRT-PCR was done for real-time expression analysis, among leaf samples of at six different time interval (0, 12, 24, 48, 72 and 96 hr after leaf rust and stripe rust inoculations). Samples were collected in the month of November 2017 in RNA later solution. Validation of the candidate reference gene *comp_1231307_c0_seq4* qRT-PCR was done in School of Agricultural Biotechnology (SOAB) Ludhiana (Wheat Molecular Biotechnology Lab). For a successful qRT-real-time experiment, housekeeping gene alpha-Tubulin was used as internal control among the most commonly used reference gene for wheat (Teneaet al., 2011).

**Expression profile for comp_121307_c0_seq4**

The expression of candidate *comp_121307_c0_seq4* gene relative to an alpha-tubulin gene in stressed plants was determined using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). Using $2^{-\Delta\Delta CT}$ method, the fold change in candidate $121307_c0_seq4$ gene expression for different time interval transcript normalized to tubulin gene and related to the mock plants was determined. Relative changes depict in terms of threshold cycle (CQ) value. It has been used for detection of expression level. Cq value is the amplification cycle number over which fluorescent signal reached above the baseline. Baseline level has been standardized to mean. A CQ value will be inversely proportional to the level of expression (Zhi et al., 2016). The expression profile of wheat developmental stage was investigated by candidate gene *comp_121307_c0_seq4*, which depicts the level of expression for all three parental line IL T598, IL T756 and WL711. The expression profile reveals the concept of the candidature of gene for leaf rust and stripe rust resistance.

**Expression analysis of comp_121307_c0_seq4 gene after leaf rust inoculations**

Relative gene expression was calculated in three ILT598, ILT756 and WL711 at six different time interval as shown in Table: 9. In IL-T598, IL-T756 and susceptible cv.WL711 the transcripts of gene *comp_121307_c0_seq4* were detectable at all six different time intervals. Resulted fold expression were shoot up to a higher level in IL T598 and maximum 5.4041 at 24hr, subsequently hypersensitive response decreases simultaneously relatively fold expression fall down to 1.652. Similarly for IL T756 relative fold expression were observed maximum 2.10 at 12hr, further
declined 1.22 at 24hr and 0.732 at 48hr. In WL711 relative fold expression has been observed 0.335 almost throughout the all six different time interval. The elevated pattern in expression of comp_121307_c0_seq4 was noticed at 24hr for IL T598, at 12hr for IL T756 shown in fig 4. The results showed a higher expression of leaf rust resistance gene from ILT598 as compared to ILT756 indicating a different R gene controlling the resistance in both the introgression lines.

Inheritance of stripe rust resistance in ILT598 and WL711 against YR race 110s119 and 110s84 and list of Resistance, susceptible and homozygous in table: 1 & 2.

**Table.1**

| S.No | STAGE               | ILT598 | WL711 |
|------|---------------------|--------|-------|
| 1    | SEEDLING STAGE (SS) | 1      | 3-3+  |
| 2    | ADULT               | 0-TR   | 60S-80S |

**Table.2**

| S.No | RESISTANCE (0-20MR) | (0-60S) | SUSCEPTIBLE (20S-60S) | HOMOZYGOUS (TR-40S) |
|------|---------------------|---------|----------------------|---------------------|
| 1    | 120                 | 109     | 19                   |

**Table.3** KASP markers specific to alien introgression on chromosome 5DS

| S. No. | Source of SNP markers | Non-progenitor involved in introgression/genome introgressed on chromosome 5DS | No. of markers applied to the F2 population | Number of polymorphic markers | Polymorphic KASP markers |
|--------|-----------------------|--------------------------------------------------------------------------------|---------------------------------------------|-------------------------------|--------------------------|
| 1      | Lr57/Yr40 (IL-T756)  (Tiwari et al., 2015) | *Aegilops geniculata/UUMM* | 9                                           | 1                             | KASP3                    |
| 2      | Lr76/Yr70 (Bansal et al., 2017) | *Aegilops umbellulata/UU* | 33                                          | 7                             | KASP178 (Plate3), KASP71, KASP119, KASP217, KASP221, KASP228, KASP117 |
| 3      | Gene ID comp_121307_c0_seq4 (IL-T598) | *Aegilops geniculata/UUMM* | 1                                           | 1                             | KASP1                    |
|        | Total                |                                    | 42                                          | 9                             |                          |
Table 4 Sequence of different KASP markers used for mapping of YR resistance gene in IL-T598 in present study KASP_121307307_c0_seq4 information

| S. No | Primer             | Sequence                                                      |
|-------|--------------------|---------------------------------------------------------------|
| 1     | Lr57_cds_kasp1_FAM | GAAGGTGACCAAGTCATTGTTTTTGGACCTgGCATGGAATAAGC                  |
| 2     | Lr57_cds_kasp1_HEX | GAAGGTGGAGGTCAACGGATTTTGGACCTgGCATGGAATAAGT                   |
| 3     | Lr57_cds_kasp1_COM | AGATTCCTGAGCCTTTGACTTCGG                                      |

Table 5 Segregation of different alleles in selected polymorphic KASP markers in F5 population derived from T598X WL711

| Marker                  | F5 Population | Chi Square value |
|-------------------------|---------------|------------------|
|                         | B  | H  | A  |                |
| Lr76/Yr70_KASP1        | 94 | 34 | 122| 3.86           |
| Lr57/Yr40_KASP3        | 126| 0  | 125| 35.9           |
| Lr76/Yr70_KASP117      | 92 | 35 | 122| 4.68           |
| Lr76/Yr70_KASP119      | 94 | 29 | 121| 3.5            |
| Lr76/Yr70_KASP178      | 96 | 32 | 121| 2.9            |
| Lr76/Yr70_KASP221      | 96 | 31 | 123| 3.33           |
| Lr76/Yr70_KASP228      | 94 | 32 | 117| 2.59           |
| Lr76/Yr70_KASP71       | 94 | 32 | 117| 2.59           |
| Lr76/Yr70_KASP217      | 93 | 26 | 127| 6.21           |
| COMP_121307_CO_SEQ4    | 90 | 33 | 117| 3.81           |

All the selected KASP markers segregated in expected ratio of 1.75HR:0.5segr.:1.5HS, except marker Lr57/Yr40_KASP3 which showed segregation distortion with no heterozygous progenies (table 5).

Expression analysis of comp_121307_c0_seq4 gene after stripe rust inoculation

The transcripts of comp_121307_c0_seq4 gene were detectable at all six different time intervals. There was increase in fold expression in IL T598 and maximum 1.077 at 24hr. (Table: 10) subsequently hypersensitive response decreases simultaneously relatively fold expression fall down to 0.246 at 48 hr. In IL T756 relative fold expression were observed maximum 10.65 at 12hr, further declined 1.14 at 24hr and 0.695 at 48hr. In susceptible cultivar WL711 relative fold expression has been observed 0.335 almost throughout the all six different time interval.

The elevated pattern of increase in expression of comp_121307_c0_seq4 was noticed at 24hr for IL T598, for IL T756 it was maximum at 12hr and in case of WL711 gene expression at baseline shown in fig 5. qRT-PCR results showed that might be different alleles of stripe rust resistance gene will be responsible for abrupt fold change (10.65) in T756 than in T598.gene). In T598 the expression of leaf rust and stripe rust transcript increases to maximum 24 hr after inoculation while in IL T756 maximum transcript were raised after 12 hr of inoculation indicating a separate mechanism of expression in both the introgression lines derived from same source.
**Table 6.** Details of six candidate genes identified from RNA sample taken at six different time intervals in IL-T598

| S.No. | Ids                | Chr  | % identity | Alignment length | Q start      | Q end        | End point   |
|-------|--------------------|------|------------|------------------|--------------|--------------|-------------|
| 1     | Comp_44675_c0_seq1 | chr5D | 100        | 482              | 555065344    | 555065825    | 891         |
| 2     | Comp_92855_c0_seq2 | chr5D | 100        | 1839             | 555050581    | 555048743    | 3397        |
| 3     | Comp_110328_c0_seq1| chr5D | 99.889     | 2700             | 555046066    | 555048765    | 4968        |
| 4     | Comp_113501_c0_seq1| chr5D | 99.92      | 2493             | 193130396    | 193132887    | 4591        |
| 5     | Comp_114926_c1_seq1| chr5D | 99.599     | 2493             | 193130396    | 193132887    | 4547        |
| 6     | Comp_121307_c0_seq4| chr5D | 99.517     | 2279             | 28483925     | 28481647     | 4148        |

**Table 7.** Mean FPKM value of comp121307_c0_seq4 of all 6 different time intervals

| S.No. | Time interval | IL T598 | WL711 |
|-------|---------------|---------|-------|
| 1     | 0hr           | 0.17667 | 0.38  |
| 2     | 12hr          | 0.845   | 0.09333 |
| 3     | 24hr          | 0.58    | 0.25667 |
| 4     | 48hr          | 0.055   | 0.66333 |
| 5     | 72hr          | 0.375   | 0.30667 |
| 6     | 96hr          | 2.09    | 1.23  |

**Table 8.** Primer information for RT-PCR

| S.No | primer               | Gene sequence                  | Tm value (°C) |
|------|----------------------|--------------------------------|---------------|
| 1    | Alpha tubulin F      | 5’AGGAGGATGCAGCCACAAC3’         | 58            |
|      | Alpha-tubulin R      | 5’AGGGCCAGAGCCAGTTCCA3’         |               |
| 2    | Lr57_cds_RT_F        | 5’AGATTCCTGAGCCTTGTACTTCGG3’    | 58            |
|      | Lr57_cds_RT_R        | 5’TAAGCTGTCGGAAGATTGCCTAC3’     |               |

**Table 9.** Relative change in fold expression of candidate comp_121307_c0_seq4 for leaf rust infected transcript

| ILS/Time interval | ILT598 | ILT756 | WL711 |
|------------------|--------|--------|-------|
| 0HR              | 1      | 1      | 1     |
| 12HR             | 1.153672 | 2.109344 | 0.332754 |
| 24HR             | 5.404151 | 1.220928 | 0.029429 |
| 48HR             | 1.652991 | 0.732692 | 0.335372 |
| 72HR             | 0.059284 | 0.011861 | 0.000744 |
| 96HR             | 0.007882 | 0.000829 | 0.000196 |
Table 10 Relative change in fold expression of candidate comp_121307_c0_seq4 for Stripe rust infected transcript

| ILS/Time interval | ILT598  | ILT756  | WL711  |
|-------------------|---------|---------|--------|
| 0HR               | 1       | 1       | 1      |
| 12HR              | 0.9338787 | 10.656887 | 0.613007 |
| 24HR              | 1.0779788 | 1.1426095 | 0.108814 |
| 48HR              | 0.2467669 | 0.6954796 | 0.215301 |
| 72HR              | 0.1178811 | 0.2168354 | 0.015147 |
| 96HR              | 0.0772041 | 0.2431148 | 0.066347 |

Fig.1 Partial linkage map of homoeologous group 5 carrying an introgressed segment with stripe rust resistance gene (YrAg) from Ae. geniculata acc pau 3549 based on F5 population derived from a cross wheat-Ae. geniculata ILT598*WL711

Fig.2 Kompetitive allele specific polymorphic (KASP) marker quantification kasp_121307_c0_seq4 investigate the resistant and susceptible parents and selected progenies of F5 population derived from Ae. Geniculata, blue susceptible, red resistant progenies and green is homozygous which is highlighted in figure
Fig. 3 Comp121307_c0_seq4 FPKM value analysis

Fig. 4 Gene folds expression (for leaf rust inoculated) at six different time interval in three parental lines IL T598, IL T756, and WL711

Fig. 5 Gene folds expression (for stripe rust inoculated) at six different time interval in three parental lines IL T598, IL T756, and WL11
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