Genetic Characterization of Leaf Tip Necrosis and its Effect on Quantitative traits in Wheat (Triticum aestivum L.)

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Authors’ contributions
This work was carried out in collaboration among all authors. Author NKD designed the study, performed the statistical analysis and wrote the protocol. Author SU managed the review, carried further statistical studies and prepared first draft of the manuscript and Author Ashutosh managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study aimed to estimate the phenotypic variability for LTN as well as yield traits, genotypic variability for Lr34, Lr46 and Lr67 and to investigate the association between genotypic and phenotypic variability for LTN and yield traits. Two hundred fifty wheat genotypes were screened for the presence of Leaf Tip Necrosis (LTN), a phenotypic marker of wheat resistance to leaf rust infection following Randomized Block Design. Of which 77 genotypes showed variable expression of LTN. Twelve yield traits were analyzed that showed highly significant differences. All these 77 genotypes were validated for the presence of three genes using respective markers viz., csLV34 for Lr34; Xwmc44 for Lr46, and Xcfd71 for Lr67. Out of 77 genotypes, 19 genotypes showed the presence of a single gene (7 with Lr34, 5 with Lr46, and 7 with Lr67), 13 genotypes had all the 3 genes, 14 with a combination of 2 genes and 31 had not shown the presence of any gene. Wheat genotypes within the individual presence of three genes increased the LTN area but their combination, reduced the thousand grain weight, LTNA, and the plot yield. All three genes individually or in combination increased the leaf area. Lr67 alone and in combination with Lr46...
redistributed the plot yield of wheat genotypes. Interestingly, LTNA had no significant correlation with any of the traits analyzed in this study. Leaf area showed a negative correlation with days to heading, glaucousness index, and thousand grain weight (TGW). NDVI-3 (at dough stage) showed a positive correlation with plot yield and TGW but had a negative association with the leaf area. High heritability coupled with high genetic advance was observed for leaf area (99.70%, 29.52%), LTNA (99.35%), thousand grain weight (95.37%), grains per spike (93.65%, 17%), and days to headingle (88.04%).

Keywords: Wheat (Triticum aestivum L.); Leaf Tip Necrosis (LTN); quantitative traits; Adult Plant Resistance (APR).

1. INTRODUCTION

Globally, wheat is grown in about 220 Mha holding the position of highest acreage among all crops with annual production hovering around 765 million metric tonnes [1]. Wheat is staple food of India and its second largest population (1.35 million) of the world enhances the demand of wheat. Globally India is the second largest producer of wheat. Under limited area condition, the production target has been fixed at 140 million tonnes by 2050. Under stable wheat acreage and given the optimistic production target, the existing average yield has to be increased from 33 to 47 qtls/ha by 2050 [2,3]. Recent studies estimated the need of a growth requirement of about 1.1% annually. Production of wheat in India is affected by a number of diseases among which rusts and spot blotch being the most challenging ones. Among three rusts, leaf rust (Puccinia triticina) is regarded as the most damaging disease of wheat and is considered serious in almost all of the wheat growing areas. Due to coverage of diverse large acreage of wheat growing whereas breeders prefer nonspecific rust resistant genes, among them Lr34, Lr46 and Lr67 are the important genes. These genes are known to produce leaf tip necrosis (LTN) in wheat. Hence these genes have been exploited much using LTN as phenotypic marker in breeding programme.

The leaf tip necrosis (LTN) is one of the most popular and desirable necrotic trait among the wheat pathologists and breeders due to showing durable and potential resistance against many fungal pathogen in adult plants. LTN is kept in category of adult plant resistance (APR) due to express itself at flowering stage in crops like wheat and rice etc. LTN shows complete linkage or is pleiotropic with Lr34. Lr34 recognized as a major component of durable rust resistance was first described in a wheat line PI58548 [4]. Lr34 confers horizontal or slow rusting resistance to certain rust races and it imparts adult plant resistance (APR) to wheat cultivars and is being used as its phenotypic marker [5]. Leaf tip necrosis (LTN) is a phenotypic marker associated with resistance to spot blotch in wheat [6]. Increasing threat of spot blotch has raised a major concern for understanding the various dimensions of host resistance to breed the genotypes. Recently, Lillemo et al. [7] reported that, leaf rust resistance gene Lr34 and Lr46 are also linked with spot blotch resistance genes Sb1. LTN plays major role in selecting genotypes with multi-pathogen resistance in wheat breeding programs and, therefore, selection for LTN is a common practice among wheat breeders to select for Lr34. However, appearance of LTN under field conditions takes time and is not always reliable for predicting the presence of Lr34. Lines with Lr34/Yr18 exhibited lower leaf and stripe rust infection than lines without it. Moreover, selection for Lr34/Yr18 resulted in the elimination of lines with high yield potential [8].

The first gene to be associated with LTN was Lr34 (Ltn1), the genes Lr46/Ltn2 [9] and Lr67/Ltn3 [10,11,12] showed that these genes were also responsible for varying degree of leaf tip necrosis. The loci detected for LTN differ between locations and seasons, suggesting the high environmental dependence of this trait. Lr34 is highly environment specific, requiring optimum combinations of environmental factors for expression. The expression of LTN associated with the Lr34, Lr46 and Lr67 genes and the modified expression due to the combinations or interactions of other genes is suggested to be a result of interaction with the environment [13]. In consideration of the above conditions, this investigation was conducted to estimate the phenotypic variability for LTN as well as yield traits, genotypic variability for Lr34, Lr46 and Lr67 and to find the association between genotypic and phenotypic variability for LTN and yield traits.
2. MATERIALS AND METHODS

During the two growing seasons 2015-2016, 250 genotypes under different CIMMYT trials were screened for prominent leaf tip necrosis and identified 77 genotypes with prominent and variable types of leaf tip necrosis. These 77 genotypes were planted in randomized block design with 3 replications during 2016-2017. Data on 12 observations viz., days to heading (DH), glaucousness index (GI), NDVI at 3 stages (NDVI-1, 2 and 3), plant height (PH), grains per spike (GPS), 1000- grain weight (TGW), biomass (BM), plot yield (PY), leaf area (LA) and LTNA percentage (LTNA) were recorded. Data on 5 random plants from each genotype were collected and average data was considered. The experimental field was situated in the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi at 25°15’ North latitude and 83°03’ East longitude at an elevation of 75.5 m above the mean sea level. Each genotype was sown in two rows of one-meter-long plots keeping a row-to-row distance 25 cm and a plant-to-plant distance of 5 cm. Agronomic practices recommended for irrigated and normal fertility conditions for wheat were followed.

Flag leaves of five randomly tagged plants of each genotype were evaluated for LTNA at GS 69. For LTNA, leaves were scored of their expression on leaf on a scale of 1–5, with the % area specifying the leaf area necrosis after HR of LTNA, where 1 = no, 2 = 25%, 3 = 50%, 4 = 75% and 5 = more than 75% of flag leaf. Glaucousness or waxiness on plants was recorded visually at the time of flowering on the peduncle and flag leaf sheath on 5 randomly tagged plants. The scale used for it was 1–5 for measurement of the level of waxiness on the plant. Here, 1 denotes a very low or minimum level of waxiness appearance, 2 denotes low waxiness appearance, 3 denotes a comparatively moderate level of waxiness, 4 denotes a high level of waxiness appearance while 5 indicates a very high level of waxiness and refers to the index maximum. ANOVA was done for partitioning the total variation into variation due to treatment and replication [14]. It is worked out to test the significance of ‘F’ and ‘t’ test. Genetic parameters viz. genetic and phenotypic variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) were worked out as per Burton and De-Vane [15]. Broad sense heritability (h²) and genetic advance (GA) was computed by using the formula given by Allard [16].

3. RESULTS AND DISCUSSION

The ANOVA depicted (Table 1) significant variation at 1 % level of significance (α=0.01) for all characters under study indicating the presence of inherent variation among genotypes. Tabular representations of parameters such as mean, range, PCV, GCV, Heritability (%), GA are presented (Table 2). The inheritance association between two variables as genotypic correlation is presented (Table 3). All the 77 genotypes of wheat expressing leaf tip necrosis were validated for the presence of 3 genes with 3 reported markers viz., ‘csLV34’ for Lr34; ‘Xwmc44’ for Lr46 and ‘Xcfd71’ for Lr67. Distribution of Lr34/Lr46/Lr67 were almost similar i.e., 30, 27 and 29 genotypes respectively. Out of 77 genotypes, 19 had only one gene (Lr34/Lr46/Lr67), 14 with combination of 2 genes, 13 had all the 3 genes (Lr34 + Lr46 + Lr67) and 31 genotypes could not show the presence of any gene. Out of 19 genotypes with single gene, 7 with Lr34, 5 with Lr46 and 7 with Lr67 were noted (Table 4).

PCR product of csLV34 for Lr34 was visible at 150bp. Lr34 was found in 30 out of 77 genotypes. Among 30 genotypes, Lr34 was alone in 7 genotypes viz., 12, 21, 25, 61, 63, 74, and 76. Lr34 was present with Lr46 in 5 genotypes viz., 18, 22, 23, 37 and 69 while with Lr67 in 4 genotypes i.e., 9, 14, 26, 73 and 75. Other 13 genotypes had all the 3 genes. When impact of Lr34 was analyzed for 4 traits by comparing t test between mean of traits of genotypes with only Lr34 and genotypes not having any LTN genes, no traits was significantly differed. The effect of Lr34 observed on leaf area was found non-significant (Table 5.1).

All genotypes were screened for the presence of Lr46 and amplicons were observed at 242 bp. Lr46 was found on 27 out of 77 genotype. Among 27 lines it was present alone in only 5 genotype viz., genotypes 1, 5, 6, 8, 71. Lr46 was present with Lr67 in 4 genotypes 3, 7, 10 and 16. When effect of Lr46 was analyzed for 4 traits using t test between mean of traits of 6 genotypes containing Lr46 and genotypes did not have any LTN genes, leaf area revealed positively significant difference (Table 5.2).
### Table 1. ANNOVA of 16 traits among 77 genotypes

| Source of variation | DF | DH | GI | SPAD 1 | SPAD 2 | SPAD 3 | GS1 | GS2 | GS3 | PH | SPL | GPS | TGW | BM | PY | LA | %LTNA |
|---------------------|----|----|----|--------|--------|--------|-----|-----|-----|----|-----|-----|-----|----|----|----|-------|
| Genotype            | 76** | 26.32** | 3.40** | 14.50** | 15.68** | 18.73** | 69.43** | 25.01** | 51.42** | 123.16** | 123.60** | 221.71** | 74.74** | 1104.36** | 535.44** | 618.61** | 65.52** |
| Replication         | 2 | 1.67 | 0.34 | 53.17 | 107.40 | 70.93 | 206.99 | 16.12 | 28.45 | 10 | 1493.84 | 326.20 | 17.08 | 0.61 |
| Error               | 152 | 1.14 | 0.192 | 53.17 | 107.40 | 70.93 | 206.99 | 16.12 | 28.45 | 10 | 1493.84 | 326.20 | 17.08 | 0.61 |
| CV                  | 1.50 | 15.67 | 5.72 | 5.32 | 6.38 | 4.92 | 5.53 | 3.58 | 5.39 | 4.57 | 2.5 | 3.95 | 9.97 | 2.33 | 5.80 |
| Mean                | 71.05 | 2.80 | 44.41 | 40.86 | 37.90 | 65.06 | 50.23 | 37.45 | 91 | 96.03 | 48.39 | 43.64 | 399.21 | 150.71 | 33.88 | 6.50 |

** denotes values significant at α=0.01

DF- Degree of Freedom, DH-days to heading, GI-Glaucousness Index, SPAD-Soil Plant Analysis Development (at 3 stages), GS-green seeker (at 3 stages), PH-plant height, SPL- Spikes per line, GPS-grains per spike, TGW-thousand grain weight, BM-biomass, PY-plot yield, LA-leaf area, %LTNA-percent leaf tip necrosis area, CV-Coefficient of Variation

### Table 2. Estimates of Mean, Range, Broad range heritability, Genetic Advance, Phenotypic and Genotypic coefficient of variation of traits of 77 genotypes

| Source of variation | DH | GI | SPAD 1 | SPAD 2 | SPAD 3 | GS1 | GS2 | GS3 | PH | SPL | GPS | TGW | BM | PY | LA | %LTNA |
|---------------------|----|----|--------|--------|--------|-----|-----|-----|----|-----|-----|-----|----|----|----|-------|
| Mean                | 71.05 | 2.80 | 44.41 | 40.86 | 37.90 | 65.06 | 54.23 | 37.45 | 91 | 96.03 | 48.39 | 43.64 | 399.21 | 150.71 | 33.88 | 6.50 |
| Range Max           | 78.33 | 5 | 51.87 | 47.13 | 46.13 | 76 | 65.67 | 49.67 | 105.55 | 111.67 | 67.33 | 55.83 | 458.33 | 190.83 | 64.44 | 20.86 |
| Range Min           | 67 | 1 | 39.33 | 30.33 | 31.27 | 52.67 | 48.33 | 31 | 77.98 | 55.33 | 29.67 | 34.33 | 351.67 | 123.67 | 12.13 | 0.45 |
| Heritability (%)    | 88.04 | 84.78 | 29.38 | 43.56 | 42.27 | 65.91 | 37.26 | 39.35 | 77.90 | 54.58 | 93.65 | 53.36 | 31.50 | 99.70 | 99.35 |
| Genetic Advance     | 5.6 | 1.96 | 1.83 | 2.60 | 2.77 | 7.43 | 2.91 | 4.35 | 11.14 | 8.64 | 16.95 | 9.96 | 25.51 | 11.76 | 29.52 | 9.59 |
| Phenotypic CoV (%)  | 4.35 | 40.11 | 6.81 | 7.08 | 8.41 | 6.98 | 14.32 | 7.63 | 8.01 | 18.15 | 11.62 | 5.79 | 12.03 | 42.42 | 72.01 |
| Genotypic CoV (%)   | 4.08 | 36.93 | 3.69 | 4.68 | 5.46 | 6.83 | 4.26 | 8.99 | 6.73 | 5.91 | 17.57 | 11.35 | 4.23 | 6.75 | 42.36 | 71.78 |

DH-days to heading, GI-Glaucousness Index, SPAD-Soil Plant Analysis Development (at 3 stages), GS-green seeker (at 3 stages), PH-plant height, SPL- Spikes per line, GPS-grains per spike, TGW-thousand grain weight, BM-biomass, PY-plot yield, LA-leaf area, %LTNA-percent leaf tip necrosis area, GA- Genetic Advance, PCV-Phenotypic Coefficient of Variation, GCV-Genotypic Coefficient of Variation
Table 3. Genotypic correlation coefficients between tested parameters among 77 genotypes

|       | GI   | SP1  | SP2  | SP3  | GS1  | GS2  | GS3  | PH   | SPL  | GPS  | TGW  | BM   | PY   | LA   | %LTNA |
|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-------|
| DH    | 0.31**| 0.17 | 0.15 | -0.03| 0.19 | 0.34**| 0.48**| 0.12 | -0.14| -0.02| 0.28*| -0.08| 0.13 | -0.46**| 0.1   |
| GI    | 0.18 | 0.40**| 0.2  | -0.2 | -0.34**| 0.19 | -0.03| 0.05 | 0    | 0.32**| -0.02| 0    | -0.36**| -0.1  |
| SP1   | 0.25*| 0.14 | 0.17 | 0.07 | 0.14 | 0.2  | -0.04| 0.12 | 0.05 | -0.08| -0.11| -0.31**| 0.11  |
| SP2   | 0.25*| 0.14 | 0.07 | 0.14 | 0.2  | -0.04| 0.12 | 0.05 | -0.08| -0.11| -0.31**| 0.11  |
| SP3   | -0.28*| 0.16 | -0.07| 0.07 | 0.15 | -0.05| 0.11 | -0.08| -0.12| 0.08 | -0.17| 0.08  |
| GS1   | -0.17| 0.11 | -0.08| 0.15 | -0.09| 0.11 | -0.09| 0.15 | 0.15 | 0.15 | 0.15  |
| GS2   | 0.42**| 0.11 | -0.08| 0.09 | 0.15 | -0.09| 0.11 | -0.09| 0.05 | 0.18 | -0.23*| 0.23* | -0.04 | 0.11  | 0.09  | 0.05  |
| GS3   | -0.09| 0.30**| 0.11 | 0.22*| 0.02 | 0.21*| -0.34**| 0.14 | -0.18 |
| PH    | +    | 0.18 | -0.23*| 0.23*| 0.01 | 0.03**| 0.27* | 0.14 | -0.18 |
| SPL   | 0.12 | 0.01 | 0.13  | 0.08 | 0    | 0.02 | 0.02 | 0    | -0.1 |
| GPS   | -0.32**| 0.08 | 0.06 | 0.03 | 0.1 | 0.44**| 0.01 | 0    | 0.02 |
| TGW   | 0.03 | 0.06 | 0.26* | 0.03 | 0.01 | 0.01 | 0.01 | 0    | 0.02 |
| BM    | 0.44**| 0.01 | 0.01 | 0.01 | 0    | 0    | 0.04 | -0.19 |
| PY    | -0.19| -0.04| 0.04  | 0.04 | 0    | 0    | 0.04 | -0.11 |
| LA    | -0.11| 0.09 | -0.09 | -0.11| 0    | 0.02 | 0.02 | 0    | 0.01 |

* and ** denote α=0.05 and 0.01 level of significance respectively.

DF- Degree of Freedom, DH-days to heading, G.I.-Glaucousness Index, SPAD-Soil Plant Analysis Development (at 3 stages), GS-green seeker (at 3 stages), PH-plant height, SPL-Spikes per line, GPS-grains per spike, TGW-thousand grain weight, BM-biomass, PY-plot yield, LA-leaf area, %LTNA-percent leaf tip necrosis area, CV-Coefficient of Variation.
The presence of all 3 genes reduced the LTN% followed by mean of LTN percentage was compared and significantly. genotypes with containing yield reduced significantly in those lines Lr67 between mean of traits of 7 genotypes containing Lr67 4, 13, 15, 20, 40, 50 and 72. When impact of 29 genotypes it was present alone in genotypes 216bp. Xcfd71 was found in 29 genotypes. Among 29 genotypes it was present alone in genotypes 4, 13, 15, 20, 40, 50 and 72. When impact of Lr67 was analyzed for 4 traits by comparing t test between mean of traits of 7 genotypes containing Lr67 and genotypes absent for LTN genes, plot yield reduced significantly in those lines containing Lr67 (Table 5.3). Leaf area of genotypes with Lr67 was increased non-significantly. Mean of LTN percentage was compared and maximum LTNA was observed for Lr67 (7.811%) followed by Lr46 (7.348%) and Lr34 (6.47%). The presence of all 3 genes reduced the LTN% (4.7055%) when compared with absence of genes (6.011) and comparing the genotypes having single gene (Table 5.4).

Out of all genotypes 13 showed the presence of all the 3 genes together while 28 genotypes did not have any of these genes tested by 3 linked markers. Average values of the four characters viz; leaf area, LTNA percentage, thousand grain weight and plot yield were analyzed using t test (Table 5.4). All the 3 genes increased the leaf area significantly and reduced thousand grain weight, LTN percentage and plot yield non-significantly. Five groups of genotypes viz, with Lr34, Lr46, Lr67, genotypes containing all three genes and genotypes absent for these genes

Table 4. Markers distribution indicating presence and absence of gene(s) in genotypes

| Genotypes | Markers | No. of genes | Genotypes | Markers | No. of genes |
|-----------|---------|--------------|-----------|---------|--------------|
| csLV34 | wmc44 | Xcfd71 | csLV34 | wmc44 | Xcfd71 |
| 1 | - | + | - | 1 | 39 | + | + | + | 3 |
| 2 | - | - | - | 0 | 40 | - | - | + | 1 |
| 3 | - | + | + | 2 | 41 | - | - | - | 0 |
| 4 | - | + | - | 1 | 42 | - | - | - | 0 |
| 5 | - | + | - | 1 | 43 | - | - | - | 0 |
| 6 | - | + | - | 1 | 44 | - | - | - | 0 |
| 7 | - | + | + | 2 | 45 | - | - | - | 0 |
| 8 | - | + | - | 1 | 46 | - | - | - | 0 |
| 9 | + | - | - | 2 | 47 | - | - | - | 0 |
| 10 | - | + | + | 2 | 48 | - | - | - | 0 |
| 11 | - | - | - | 0 | 49 | - | - | - | 0 |
| 12 | + | - | - | 1 | 50 | - | - | + | 1 |
| 13 | - | - | + | 1 | 51 | - | - | - | 0 |
| 14 | + | - | - | 2 | 52 | - | - | - | 0 |
| 15 | - | - | + | 1 | 53 | - | - | - | 0 |
| 16 | - | + | - | 2 | 54 | - | - | - | 0 |
| 17 | + | + | + | 3 | 55 | - | - | - | 0 |
| 18 | + | + | - | 2 | 56 | - | - | - | 0 |
| 19 | + | + | + | 3 | 57 | - | - | - | 0 |
| 20 | - | - | - | 0 | 58 | - | - | - | 0 |
| 21 | + | - | - | 1 | 59 | - | - | - | 0 |
| 22 | + | - | - | 2 | 60 | - | - | - | 0 |
| 23 | + | + | - | 2 | 61 | + | - | - | 1 |
| 24 | - | - | - | 0 | 62 | - | - | - | 0 |
| 25 | - | - | + | 1 | 63 | + | - | - | 1 |
| 26 | + | + | + | 2 | 64 | - | - | - | 0 |
| 27 | + | + | + | 3 | 65 | - | - | - | 0 |
| 28 | + | + | - | 3 | 66 | - | - | - | 0 |
| 29 | + | + | + | 3 | 67 | - | - | - | 0 |
| 30 | - | + | + | 3 | 68 | - | - | - | 0 |
| 31 | - | + | + | 3 | 69 | + | - | - | 2 |
| 32 | + | + | + | 3 | 70 | - | - | - | 0 |
| 33 | + | + | + | 3 | 71 | - | - | - | 0 |
| 34 | + | + | + | 3 | 72 | - | - | + | 1 |
| 35 | + | + | + | 3 | 73 | + | - | + | 2 |
| 36 | - | - | - | 0 | 74 | + | - | - | 1 |
| 37 | + | - | + | 2 | 75 | + | - | + | 2 |
| 38 | + | - | + | 3 | 76 | - | - | - | 1 |
| 39 | - | - | - | 0 | 77 | - | - | - | 0 |

The amplicons of Xcfd71 were observed at 216bp. Lr67 was found in 29 genotypes. Among 29 genotypes it was present alone in genotypes 4, 13, 15, 20, 40, 50 and 72. When impact of Lr67 was analyzed for 4 traits by comparing t test between mean of traits of 7 genotypes containing Lr67 and genotypes absent for LTN genes, plot yield reduced significantly in those lines containing Lr67 (Table 5.3). Leaf area of genotypes with Lr67 was increased non-significantly.
Table 5.1. Comparison between the genotypes having *Lr34* and genotypes with no genes for LTN

| Leaf area | Thousand grain weight | LTN | Plot yield |
|-----------|----------------------|-----|------------|
| Only *Lr34* | No gene for LTN | Only *Lr34* | No gene for LTN | Only *Lr34* | No gene for LTN | Only *Lr34* | No gene for LTN |
| Mean | 44.847 | 24.705 | Mean | 44.772 | 45.656 | Mean | 6.47 | 6.011 | Mean | 146 | 155.766 |
| Variance | 235.887 | 85.153 | Variance | 13.36 | 32.522 | Variance | 14.77 | 20.435 | Variance | 81.45 | 196.72 |
| SD | 15.358 | 9.227 | SD | 3.655 | 5.7 | SD | 3.844 | 4.52 | SD | 9.02 | 14.025 |
| N | 4 | 28 | N | 4 | 28 | N | 4 | 28 | n | 4 | 28 |
| t_{cal} | 2.5578 | t_{table} | 2.571 | t_{cal} | 0.2182 | t_{table} | 2.776 | t_{table} | 2.571 |

Table 5.2. Comparison between the genotypes having *Lr46* and genotypes with no genes for LTN

| Leaf area | Thousand grain weight | LTN | Plot yield |
|-----------|----------------------|-----|------------|
| Only *Lr46* | No gene for LTN | Only *Lr46* | No gene for LTN | Only *Lr46* | No gene for LTN | Only *Lr46* | No gene for LTN |
| Mean | 40.984 | 24.705 | Mean | 43.784 | 45.656 | Mean | 7.348 | 6.011 | Mean | 160.6 | 155.766 |
| Variance | 161.165 | 85.1537 | Variance | 8.33 | 32.522 | Variance | 5.346 | 20.435 | Variance | 176.34 | 196.72 |
| SD | 12.69 | 9.2279 | SD | 2.88 | 5.7 | SD | 2.3122 | 4.52 | SD | 13.16 | 14.025 |
| N | 5 | 28 | N | 5 | 28 | N | 5 | 28 | n | 5 | 28 |
| t_{cal} | 2.7409 | t_{cal} | -1.1137 | t_{cal} | 0.9967 | t_{cal} | 2.201 | t_{cal} | 2.447 |
| t_{table} | 2.571 | t_{table} | 2.201 | t_{table} | 2.201 | t_{table} | 2.447 |

Table 5.3. Comparison between the genotypes having only *Lr67* and genotypes with no genes for LTN

| Leaf area | Thousand grain weight | LTN | Plot Yield |
|-----------|----------------------|-----|------------|
| Only *Lr67* | No gene for LTN | Only *Lr67* | No gene for LTN | Only *Lr67* | No gene for LTN | Only *Lr67* | No gene for LTN |
| Mean | 34.744 | 24.705 | Mean | 43.75 | 45.656 | Mean | 7.811 | 6.011 | Mean | 145.16 | 155.766 |
| Variance | 168.96 | 85.153 | Variance | 33.04 | 32.522 | Variance | 17.087 | 20.435 | Variance | 117.06 | 196.72 |
| SD | 12.99 | 9.2279 | SD | 5.748 | 5.7 | SD | 4.133 | 4.52 | SD | 10.81 | 14.025 |
| N | 7 | 28 | N | 7 | 28 | N | 7 | 28 | n | 7 | 28 |
| t_{cal} | 1.9256 | t_{cal} | -0.7844 | t_{cal} | 1.011 | t_{cal} | 2.228 | t_{cal} | -2.1853 |
| t_{table} | 2.306 | t_{table} | 2.262 | t_{table} | 2.228 | t_{table} | 2.179 |
Table 5.4. Comparison between the genotypes having all the three LTN genes \((Lr34+Lr46+Lr67)\) and genotypes with no genes for LTN

|                | Leaf area | Thousand grain weight | LTN | Plot yield |
|----------------|-----------|-----------------------|-----|------------|
|                | \(Lr34+Lr46+Lr67\) | No gene for LTN | \(Lr34+Lr46+Lr67\) | No gene for LTN | \(Lr34+Lr46+Lr67\) | No gene for LTN |
| Mean           | 44.67     | 24.705                | Mean | 41.852     | 45.656 | Mean | 4.705    | 6.011     | Mean | 148.69 | 155.766 |
| Variance       | 33.84     | 85.153                | Variance | 24.898    | 32.522 | Variance | 9.689 | 20.435    | Variance | 135.055 | 196.72  |
| SD             | 5.817     | 9.227                | SD  | 4.989     | 5.7    | SD  | 3.112    | 4.52      | SD  | 11.621 | 14.025  |
| \(n\)          | 11        | 28                    | \(n\) | 11        | 28     | \(n\) | 11       | 28        | \(n\) | 11      | 28      |
| \(t_{cal}\)   | 8.0739    | \(t_{cal}\)          | \(-2.0955\)| \(t_{cal}\) | \(-1.028\) | \(t_{cal}\) | \(-1.6093\) | \(t_{cal}\) | \(-1.6093\) | \(t_{cal}\) | \(-1.6093\) |
| \(t_{table}\) | 2.045     | 2.08                  | \(t_{table}\) | 2.052    | \(t_{table}\) | 2.074 |
were categorized on the basis of marker data and the mean values of four traits i.e., Leaf area, TGW, LTN percentage and Plot yield were analyzed using t test. Maximum but non-significant effect of Lr34 was observed on leaf area. Lr46 was found to increase the leaf area significantly. Lr34 and Lr67 were observed to reduce plot yield significantly. Effect of Lr46 was found as non-significant increase over leaf area. Presence of all the 3 genes reduced the LTNA (4.7055%) as compared with absence of these genes (6.011 %) which can be attributed due to complementary effect of all the genes.

It was interesting to note that all the genes increased the leaf area individually as well as when they come together but they reduced thousand grain weight in all the situations. It can be explained as each gene increased LTN percentage, negatively decreased the photosyntheticefficiency. Lr46 increases yield non-significantly when alone while other two were found to reduce plot yield. It was evident that these genes separately increased LTN percentage but when they accumulate together reduction in LTN percentage was observed. Rosewarne et al. [17] also came to the same conclusion that different Ltn gene combination will be more effective and significant.

Heritability studies provide valid information about the traits that are transmitted from parents to offspring and to the successive generations. Highest recorded heritability were for the traits LA (99.70 %), % LTNA (99.35 %), TGW (95.37 %) and GPS (93.65 %). These observations were supplemented by the findings of Kumar and Sharma [18] and Firouzian [19]. Furthermore, the second highest ranges of heritability were for the traits DH (88.04 %) and GI (84.78 %). Gupta and Verma [20] founded similar reports. These yield attributing traits can be picked up for carrying out selection following their percentage magnitude. Higher heritability indicating more feasible for selection studies since variation is primarily due to genetic difference. Highest genetic advance was noted for the trait viz., leaf area (29.52%) followed by the biomass (25.41%).

Highly significant correlation was found between the traits days to headings and glaucousness index and thousand grain weight. Significant positive correlation found between biomass and plot yield. Plant height showed positive correlation with thousand-grain weight and negatively correlated with grains per spike. Ehdaie and Waines [21] supported this result. Spikes per line showed positive significant correlation with the plot yield and biomass.

4. CONCLUSION

The above study demonstrates the variation occurring in leaf tip necrosis, a phenotypic marker conferring Adult Plant Resistance. Wheat genotypes within the individual presence of three genes increased the LTN area but their combination, reduced the thousand grain weight, LTNA, and the plot yield. All three genes individually or in combination increased the leaf area. Lr67 alone and in combination with Lr46 reduced the plot yield of wheat genotypes. Interestingly, LTNA had no significant correlation with any of the traits analyzed in this study. The expression of LTN depends on the number of genes either single or in combination involved thus making it a quantitative character. These genes either complement or supplement provides a scope for future investigation. Later, incorporating a set number of genes in various combinations may confer varying levels of adult plant resistance.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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