

## Trait phenotyping and inheritance of leaf blight resistance in wheat (*Triticum aestivum* L.) under temperate conditions of Kashmir valley

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### Abstract

A set of twenty-two advanced breeding lines of bread wheat were studied in randomized complete block design (RCBD) with four (4) replications at the experimental fields of Faculty of Agriculture, SKUAST-Kashmir, Wadura during Rabi 2019-20 and 2021-22. For each line the observations were recorded for eight (08) important agro-morphological, yield and yield related traits viz., days to 50% flowering, days to maturity, plant height (cm), number of tillers plant<sup>-1</sup>, spike length (cm), number of spikelets spike<sup>-1</sup>, 1000 grain weight (g) and grain yield (kg/ha). The analysis of variance (ANOVA) depicted that the genotypes have significant variability for traits under study. The genotypes HS675, HPW472 and VL2042 possessed better agronomic performance for yield attributing traits and were identified as high yielding wheat genotypes that could be recommended for cultivation under temperate conditions of Kashmir valley. The correlation co-efficient matrix for the traits under study revealed only 12 positive and 8 negative significant correlations out of the total 28 paired combinations of all the traits. In a separate study, a segregating F<sub>2</sub> population and F<sub>2</sub> derived F<sub>3</sub> families were developed from the cross between resistant (CIMMYT-10009) and susceptible (CIMMYT-5024) parents and subsequently evaluated for studying inheritance pattern for leaf blight resistance. The field data of F<sub>2</sub> generation and F<sub>2</sub> derived F<sub>3</sub> families segregated for resistance and fitted to two dominant gene ratio (9:7) using chi-square test. The study reports that wheat breeding line CIMMYT-10009 contains two dominant leaf blight resistance genes having complimentary effect on one another. The genes so identified are effective under temperate conditions of Kashmir valley and as such could efficiently be used in breeding programmes, for deployment of such genes over space and time and for development of gene pyramids for leaf blight resistance in wheat.

**Key words:** Wheat, Trait phenotyping, Complimentary genes, Leaf blight resistance



## 1. Introduction

Bread wheat (*Triticum aestivum* L.) is one of the important cereal crops of the family *Graminae* (*Poaceae*). In India, it is grown in low to high altitudes areas having average annual rainfall from 125 to 1125 mm across all agro-climatic zones (Anonymous, 2007). In Kashmir, it is grown at an altitude of 2000-3000 m MSL under temperate ecosystem (Singh *et al.*, 2021). Among the five different agro-climatic zones of India, NWPZ is the most productive as it provides ideal conditions for wheat growth (Mohan *et al.* 2022) Wheat can tolerate severe cold and snow during winters and can still resume its growth with the arrival of favourable weather.

For genetic improvement of wheat crop it is imperative to access the genetic variability across germplasm for various morphological, yield and yield attributing traits. Further, the fungal diseases of wheat are important production constraints in almost all wheat-growing environments of India. Wheat productivity is hampered by vagaries of climate and largely affected by new races of pathogens that evolve from time to time (Miedaner and Juroszek, 2022). The leaf blight fungus reported from India, *Alternaria triticina* is considered to be an important wheat pathogen that causes significant losses in susceptible cultivars in almost all agro climatic zones of country (Chaurasia *et al.*, 1999; Kapadiya *et al.*, 2021). Among the major diseases of wheat, foliar blight has emerged as the disease of the major concern throughout the world (Rojasara *et al.*, 2014). *Alternaria triticina* predominates in the northern states of Himachal Pradesh, Punjab and Jammu and Kashmir (Goel *et al.*, 1999). The disease is manifested by lesions on leaves that are irregular in shape and dark brown to grey in colour. Initially, the disease appears as small, oval discoloured lesions, which coalesce with disease progression, resulting in leaf death. Infection can lead to weight reduction of 46-75% individual grains with yield losses of up-to 60% (Chalkley, 2014). The disease occurs in all the five agro-climatic zones of India. The market value of the wheat seed is drastically reduced alongwith its yield due to the infection of leaf blight fungi (Singh *et al.*, 2015). Leaf blight caused by fungus *Alternaria triticina*, is ranked as one of the major disease of wheat that causes substantial damage to the crop yield and quality. In Kashmir valley, this disease occurs each year with high severity levels sometimes reaching beyond economic threshold level.

Although, application of chemical fungicides can provide immediate control, but such methods are not economically viable. Development of leaf blight resistant cultivars is as such considered the most effective and viable strategy to minimize such yield losses. After extensive breeding efforts at SKUAST-Kashmir, sustainable leaf blight resistant cultivars in wheat could not so far be developed and even at national level till date, only few genetic studies for leaf blight in wheat have been reported The progress in respect of breeding varieties resistant to this disease would depend upon the knowledge of various resistance sources in germplasm and the study of inheritance pattern of such resistance genes (Sokhi *et al.*, 1973, Nelwadker *et al.*, 2017; Navathe *et al.*, 2022). Although, the leaf blight can effectively be managed through chemical sprays , but this not only adds to the cost of cultivation but also has its adverse effects on the environment. Screening of germplasm for identification of effective sources of resistance to leaf blight remains a prime objective of wheat breeding programmes of areas usually experiencing high disease epidemic.

A reliable fungal disease screening technique could be efficiently employed for screening of the wheat germplasm for identification of sources of resistance. Proper inoculum development in the field depending on the type of disease and the type of resistance the disease screening methodology involves field or polyhouse tests either at seedling or at adult plant stages. Although the methodology followed for screening of germplasm for resistance to many diseases has been established and employed in many resistance breeding programmes. The resistance to disease has always a comparative estimate, as such check varieties for resistance and susceptibility play an important role in assessing the disease pressure and degree of resistance. The environmental conditions for disease development and choice of experimentation are crucial parameters for generation of selection pressure and selection for resistance under field conditions (Singh and Rajaram, 2002).

The identification of novel sources of resistance to leaf blight and investigation of genetic basis of resistance to leaf blight would as such form a fundamental piece of work. The present investigation is aimed at evaluation of wheat germplasm lines for morphological, yield and yield



related traits and to investigate the inheritance pattern of leaf blight resistance gene (s) in wheat.

## 2. Materials and methods

### 2.1 Experimental materials, design and layout

The experimental trials were conducted for evaluation of a set of wheat breeding lines for trait phenotyping (agro-morphological, yield and yield related traits) in the Research Field of Division of Genetics and Plant Breeding, Faculty of Agriculture, Wadura during *Rabi* seasons 2019-20 & 2020-21. The material constituted twenty two (22) advanced wheat breeding lines procured from IIW&BR, Karnal which were laid out in Randomized Complete Block Design (RCBD) with four replications. All the recommended package and practices were followed to raise a healthy crop. Five competitive plants were randomly tagged in each entry and each replication for observing all the quantitative characters (except for days to 50 percent flowering and maturity, which were taken overall basis) and mean of each character was used for statistical analysis.

### 2.2 Experimental methodology and Statistical analysis

The breeding line CIMMYT-10009 (QUAIU #2/BAVIS #1) was continuously identified as highly resistant line since last few years in leaf blight screening tests conducted at our location. As such it was important to investigate the genetic basis of such resistance. The F<sub>2</sub> population of cross CIMMYT-5024 (FRANCOLIN #1/3/PBW343\*2/KUKUNA\*2//YANAC/4/KINGBIRD #1//INQALAB 91\*2/TUKURU) X CIMMYT-10009 (QUAIU #2/BAVIS #1) was advanced to F<sub>3</sub> families following single plant harvest. Field screening of wheat parents, F<sub>1</sub>, F<sub>2</sub> plants and F<sub>2</sub> derived F<sub>3</sub> families against leaf blight was done based on the natural disease intensity in field and no artificial inoculation was adopted for disease spread. The disease intensity was recorded by using 0-5 disease rating scale proposed by Conn *et al.*, (1990). The lines were scored only when infector rows and susceptible checks recorded maximum susceptibility for leaf blight. From the field screening of the populations observed values of resistant and susceptible plants from each generation were recorded.

The statistical analysis of the data collected on individual morphological traits was carried out on the basis of mean performance over replications. Analysis of variance for

all the characters for testing variation among genotypes was carried out as per the procedure suggested by Verma *et.al* (1987). For disease screening, Chi Square Test was applied to test the goodness of fit for Mendelian ratios, after comparing observed and calculated/ expected values for each class separately for F<sub>2</sub> plants and F<sub>2</sub> derived F<sub>3</sub> families.

$$\chi^2 = \sum (O - E)^2 / E$$

Where, O = the frequencies of observed values

E = the frequencies of observe expected values

Σ = the sum of

To accept or reject the null hypothesis total chi-square values were compared with tabulated values at appropriate degrees of freedom.

## 3. Results and Discussion

The plant breeders improve plants by selecting genetic resources with key characteristics, cross them and select the best recombinants in subsequent generations. Breeders also look beyond modern varieties to develop a valuable trait combination, such as improvement in yield or resistance to a disease or pest etc. The lack of genetic diversity in wheat and associated vulnerability to pests and diseases may sometimes cause crop failures. The existence of genetically diverse genotypes maximizes the chances of finding resistant genotypes, which in turn may help to avoid these catastrophic outcomes (Anonymous, 2019). The present investigation identifies the promising genotypes with good yield attributing traits, genotypes with effective sources of resistance and determines the inheritance pattern of resistance genes effective against leaf blight disease of wheat.

### A. Trait phenotyping of genotypes

#### Mean performance for morphological traits

The average days to maturity (Table 1) was 235.48, variance 24.90 and coefficient of variance was found to be 1.82 percent. For 1000 grain weight the genotypes recorded an average value of 65.85 with variance 44.48 and coefficient of variance was found to be 10.84 percent. The mean value of grain yield kg per ha was observed as 2488.90 with variance of 495573.76 and coefficient of variance was found to be 28.04 percent. On the basis of mean performance the significant early maturity over checks was depicted by genotypes viz., HS668 (229 days),



VL2036 (230.75), VL2041 (231.25 days), HS507 (232 days) and VL2039 (231.50 days) while the genotypes HS 675 (3091.20 Kg/ ha), HPW472 (3062.70 Kg/ ha), VL2042 (2989.47 Kg/ha), HS677 (2802.90 Kg/ ha), UP3064 (2763.78 Kg/ ha), HS 676 (2735.34 Kg/ ha) and HS 562 (2683.94 Kg/ ha) showed significantly higher yields over four check varieties. The frequency distribution (Figure1) of genotypes for different morphological and yield attributing traits depicted normal distribution for all

the traits under study. The grain yield in wheat depends on physiological characters like days to 50% flowering and days to maturity (Lutfullah, 2017), while grain yield may also vary with plant height, resistance to biotic and abiotic stresses and the growing conditions of the crop (Mukhtar *et al.*, 2015; Oudjehih, 2018). In the present study the genotypes HPW471 and HS675 have been identified as early flowering, late maturing and highest yielding genotypes.

Table 1. Mean values of agro morphological yield and yield related traits in wheat

Genotype	Days to 50% flowering	DTM	Plant height (cm)	Number of tillers plant <sup>-1</sup>	Spike length (cm)	Spikelets spike <sup>-1</sup>	1000 Grain weight (g)	Grain yield (kg/ha)
SKW356	170.00*	234.75	93.85*	6.50*	12.15	17.25*	66.25*	2410.40
VL2042	170.75*	239.25	95.88*	5.25	14.15*	19.75*	67.00*	2989.47*
HPW471	168.75*	238.75	94.65*	4.25	12.90	18.00*	67.50*	2292.93
HS675	167.25*	239.00	87.48	5.25	15.07*	21.00*	69.75*	3091.20*
HPW472	168.50*	241.75	90.68*	5.50*	14.90*	19.75*	67.25*	3062.70*
VL2039	169.25*	231.50*	89.63	5.50*	11.10	15.75	66.25*	1884.12
HS677	168.25*	237.00	91.53*	6.50*	13.55*	19.25*	65.50*	2802.90*
HS676	171.00*	237.25	97.38*	5.50*	12.28	19.00*	70.25*	2735.34*
UP3064	172.25	237.25	98.80*	6.50*	13.25*	18.25*	65.00*	2763.78*
HS678	172.50	240.00	100.40*	6.25*	12.63	16.50	63.50*	2355.44
HPW470	170.00*	234.75	93.90*	6.00*	12.70	16.25	66.00*	2209.30
HPW469	170.50*	237.00	93.90*	5.75*	12.40	17.00	68.00*	2231.04
VL2041	172.00	231.25*	97.20*	5.25	12.05	16.00	61.50	2152.88
VL2040	170.50*	235.50	91.85*	5.50*	11.18	16.00	64.25*	1894.88
HS507	170.25*	232.00*	88.48	4.75	10.30	14.50	67.25*	1616.33
HS562	171.75	238.00	93.70*	5.00	12.93	17.75*	71.25*	2683.94*
HS668	169.75*	229.00*	87.53	6.25*	12.65	17.50*	67.00*	2551.79
VL2036	170.25*	230.75*	91.90*	5.75*	13.23*	16.75	65.00*	2619.72
SW-1	169.50	234.00	90.18	5.25	13.12	16.75	62.50*	2507.64
SW-2	171.00	232.75	90.95	5.25	13.60	17.75	64.75	2680.98
HPW349	171.75	233.25	88.55	5.25	12.62	17.50	61.75	2623.50
VL907	170.75	235.75	87.53	6.25	12.85	17.50	61.25	2595.60
SED	1.36	3.38	3.77	0.60	1.12	1.53	2.73	384.42
SEM	0.28	0.72	0.80	0.12	0.23	0.32	0.58	81.96
CD	0.48	1.23	1.37	0.20	0.39	0.55	0.99	141.05



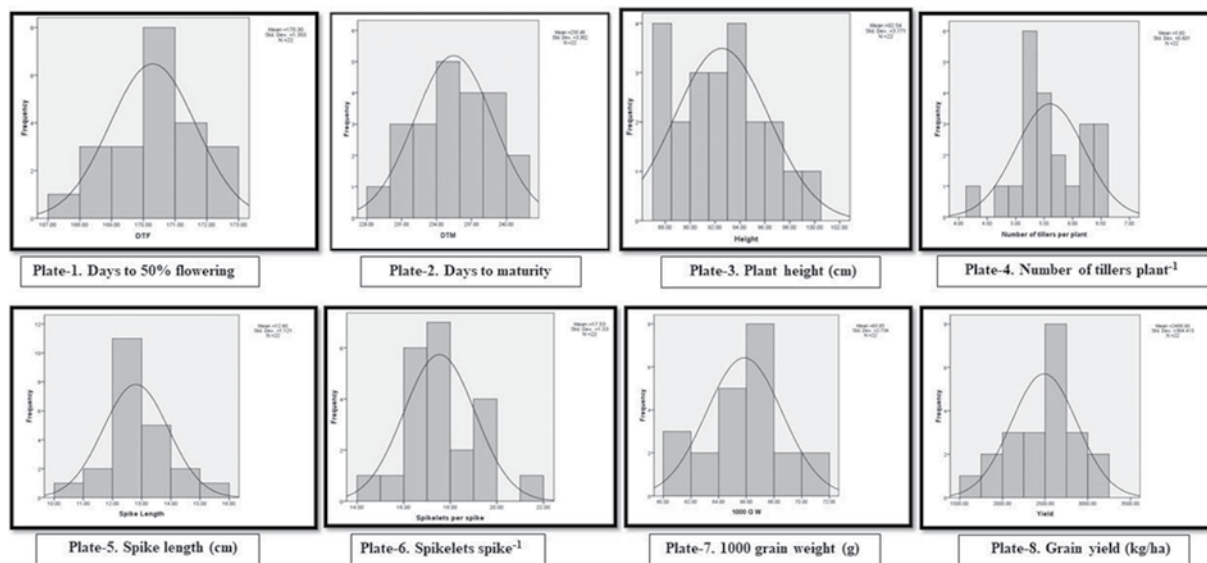


Fig. 1. Expression of morphological (plate-1 to plate-3), yield and yield attributing (plate-4 to plate-8 in traits in wheat genotypes

*Genetic parameters of variability*

Genetic diversity among plant species provides opportunities for improving the plant characteristics. Its assessment is necessary to tackle the threats of environmental fluctuations and for the effective exploitation of genetic resources in plant breeding programs. Different parameters of genetic variability have been estimated (Table 2) and are described. The highest phenotypic coefficient of variance (PCV) was recorded for grain yield (24.86) whereas, lowest values were recorded for days to 50% flowering (1.1). The highest genotypic

coefficient of variance (GCV) was recorded for number of tillers plant<sup>-1</sup> (6.91) and grain yield (6.47), whereas, lowest values were recorded for days to 50% flowering (0.29).

*Analysis of variance*

The analysis of variance (ANOVA) depicted highly significant differences among the genotypes for all the traits under study except for days to maturity (Table 3). This confirms the presence of considerable amount of variability among the genotypes for the traits under study.

Table 2. Descriptive statistics for morphological and yield related traits in advanced wheat breeding lines

Character	Mean	Variance	Std. error	PCV	GCV	Coefficient of variance
Days to 50% flowering	170.30	7.84	0.87	1.1	0.29	1.70
Days to maturity	235.48	24.90	1.29	1.91	1.10	1.82
Plant Height (cm)	92.54	56.40	2.06	6.5	1.73	7.37
No. of tillers plant <sup>-1</sup>	5.60	0.96	0.27	15.66	6.91	16.45
Spike length (cm)	12.80	5.29	0.69	15.42	1.74	17.93
Spikelets spike <sup>-1</sup>	17.53	7.84	0.83	13.97	3.78	15.72
1000 grain weight (g)	65.85	44.48	2.15	9.93	3.56	10.84
Grain yield (kg/ha)	2488.90	495573.76	210.44	24.86	6.47	28.04



Table 3: Analysis of variance for morphological and yield related traits in advanced wheat breeding lines

Source of variation	Degree of freedom	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of tillers plant <sup>-1</sup>	Spike length (cm)	Spikelets Spike <sup>-1</sup>	1000 grain weight (g)	Grain yield (kg/ha)
Replication	3	0.68	17.16	260.02	0.40	7.78	2.13	10.40	3670.70
Genotype	21	7.34*	45.75	56.91*	1.44*	5.03*	9.36*	29.89*	591101.05*
Error	63	8.38	18.37	46.56	0.84	5.26	7.60	50.92	487154.36
Total	87								

(\* indicates significance at 5% level of significance)

Table 4: Correlation coefficient for morphological and yield related traits in advanced wheat breeding lines

Trait	Days to 50% Flowering	Days to maturity	Plant Height	No. of tillers	Spike length	Spikelets spike <sup>-1</sup>	1000 grain weight	Grain yield
Days to 50% flowering	1	-0.182	0.410**	0.100	-0.330**	-0.412**	-0.193	-0.254*
DTM		1	-0.009	0.003	0.602**	0.607**	0.348**	0.570**
Plant height			1	0.214*	-0.299**	-0.280**	-0.325**	-0.236*
No. of tillers				1	-0.063	-0.105	-0.239*	-0.030
Spike length					1	0.819**	0.469**	0.887**
Spikelets spike <sup>-1</sup>						1	0.482**	0.921**
1000 grain weight							1	0.477**
Grain yield								1

(\* and \*\* indicate significance at 5% and 1% level of significance, respectively)

### Correlation coefficient analysis

#### Significant positive correlation

The days to 50% flowering was significantly and positively correlated with plant height (Table 4) whereas, days to maturity was significantly and positively correlated with 1000 grain weight and grain yield. The plant height was significantly and positively correlated with number of effective tillers whereas, spike length was positively and significantly correlated with days to maturity, spikelets spike<sup>-1</sup>, 1000 grain weight and grain yield. The spikelets spike<sup>-1</sup> was significantly and positively correlated with days to maturity, 1000 grain weight was significantly and positively correlated with grain yield.

#### Significantly negative correlation

The significant negative correlation was observed for days to 50% flowering with spike length, spikelets spike<sup>-1</sup> and grain yield, for plant height with spike length, spikelets spike<sup>-1</sup>, 1000 grain weight and grain yield and for number of tillers with 1000 grain weight.

#### Positive and non-significant correlation

The positive and non-significant correlation was observed for days to 50% flowering with number of tillers and for number of tillers with days to maturity.

#### Negative and non-significant correlation

The negative but non-significant correlation were recorded in case of days to 50% flowering with days to maturity and 1000 grain weight; plant height with days to maturity



and number of tillers with spike length, spikelets spike<sup>-1</sup> and grain yield.

The grain yield of wheat is determined by three important components i.e., productive spike per unit area, grains per spike and grain weight (Collaku, 1994). Grain yield was found to have positive correlation with grain filling period, number of productive tillers per plant, spike length, number of spikelets per spike, number of grains per spike, 1000 grain weight, biomass yield per plot, hectolitre weight and harvest index at both phenotypic and genotypic level (Birhanu *et al.*, 2017). Singh *et al.* (1995) found that grain yield in wheat was positively and significantly correlated with number of productive tillers and flag leaf area. Grain yield in wheat is a function of number of spikes per unit area, number of grains per spike and grain weight (Sofield *et al.*, 1977 and Verma *et al.*, 2019). Grain yield was found to be positively correlated with plant height, number of leaves and chaff weight (Ibrahim, 1994). Biological yield had significant positive correlation with seed yield per plant followed by harvest index, days to maturity, test weight, spike length and number of productive tillers per plant (Tripathi *et al.*, 2011). Tammam *et al.* (2000) found that grain yield per plant was positively correlated with number of spikes per plant and 1000-grain weight at genotypic level. Shahid *et al.* (2002) found that spike length was positively and significantly correlated with grain yield at genotypic level. Singh and Dewivedi (2002) found that grain yield per plant was positively and significantly correlated with number of spikes bearing tillers per plant both at genotypic and phenotypic level.

### *B. Inheritance of leaf blight resistance in wheat*

The F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> generations of the cross (CIMMYT-5024 X CIMMYT-10009) were screened for leaf blight resistance. All F<sub>1</sub> plants were resistant to leaf blight whereas, in F<sub>2</sub> & F<sub>3</sub> generations leaf blight scores were recorded plant wise and family wise, respectively. To study the inheritance pattern of leaf blight resistance chi-square test was applied to test the goodness of fit for Mendelian ratios. The F<sub>2</sub> generation constituted 217 plants that segregated (Table 5) into two phenotypic classes viz., resistant and susceptible. In F<sub>2</sub> 134 plants were classified as resistant and 83 plants were found susceptible. Chi-square test was applied to test the goodness of fit for Mendelian gene ratios in F<sub>2</sub> generation. After calculating chi-square values on observed and

expected gene frequencies and comparing T-calculated and T-tabulated values the F<sub>2</sub> generation fitted in the ratio of 9 (resistant): 7 (susceptible) plants. The 9:7 ratio explains two dominant genes with complimentary effect on one another actually govern leaf blight resistance in resistant parent. The F<sub>2</sub> segregation ratio (9:7), was confirmed from the pattern of segregation in F<sub>2</sub> derived F<sub>3</sub> families. After calculating chi-square values on observed and expected gene frequencies and comparing T-calculated and T-tabulated values it was concluded that F<sub>3</sub> generation also segregates in the ratio of 9:7, confirming the presence of two dominant genes with complimentary effect for leaf blight resistance in resistant breeding line CIMMYT-10009. The study thus reports that the leaf blight resistance in breeding line CIMMYT-10009 is governed by two dominant genes which are complimentary to one another. Sokhi *et al.* (1973) studied inheritance of resistance to *Alternaria* blight of wheat and found that resistance to leaf blight was governed by a pair of recessive genes in the varieties, Agra Local, NP 52 and NP 4 and by two pairs of recessive genes in varieties NP 824 and NP 809. In variety Kenya 184. P.2. A.I.F. (E 581) leaf blight resistance was controlled by one pair of dominant genes. Srivastava *et al.* (1981) studied the genetics of seedling resistance to *Alternaria* leaf blight of wheat and concluded that the resistance is governed by recessive genes. Sinha *et al.* (2006) reported *Alternaria* leaf blight susceptibility of wheat cultivars HD 4502, DWL 5023, Raj 1555 and HD 4530 to be controlled by a dominant gene and that of cultivar Meghdoot by two dominant complementary genes. The resistant parents carry recessive alleles of the genes present in susceptible cultivars. They could conclude that all the res-genes in the resistant parents are identical in nature. Goel *et al.* (1999) while discussing status and identification of donor lines for leaf blight resistance in wheat revealed scarcity of *Alternaria* leaf blight donors and importance of taking up such studies in India. Nelwadker *et al.*, (2017) has laid emphasis on screening of wheat germplasm for identification of sources of resistance to diseases, while Navathe *et al.*, (2022) have given overall scenario of wheat and barley diseases and how identification of novel sources of resistance to different diseases could be useful in designing wheat and barley breeding programmes in future.



Table 5. Segregation for leaf blight resistance in cross CIMMYT-5024 X CIMMYT-10009 under field tests

Cross	F <sub>1</sub> generation			F <sub>2</sub> generation			F <sub>3</sub> generation					
	F <sub>1</sub> plants	Resistant	Susceptible	F <sub>2</sub> plants	Total	Expected ratio	χ <sup>2</sup>	Resistant	Susceptible	Total	Expected ratio	χ <sup>2</sup>
CIMMYT-5024 X CIMMYT-10009	Resistant	134	83	217	217	9:7	2.69	124	93	217	9:7	0.348

## Conclusion

The study reports significant variability among genotypes for the traits under study that means there is great scope for making direct selection for these traits. The genotypes HS675, VL2042 and HPW472 show better yield performance under temperate conditions of Kashmir valley, which could be exploited for their use in future wheat breeding programmes. The study also reports two dominant genes conferring leaf blight resistance in breeding line CIMMYT-10009. Such resistance could efficiently be utilized for improvement in leaf blight resistance and further its use could be advocated in future wheat improvement programmes of the area.

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## Compliance with ethical standards

This article does not contain any studies involving human or animal participants performed by any of the authors.

**Conflict of interest** (Reply in Yes and No.):

The authors declare no conflict of interest.

## Author contributions:

AD, MAK, RRM: Conceptualized the methodology for the study; MAB<sup>2</sup>, FJW: contributed in experimental design and statistical analysis while MAK, AD, RRM MAB<sup>1</sup>: were involved in writing, review and editing of the manuscript.

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