Characterization of elite bread wheat (*Triticum aestivum* L.) germplasm for Powdery mildew *Blumeria graminis* f.sp. *tritici* resistance

ABSTRACT

Aims: In the current investigation, an effort was made to confirm the resistance with gene linked markers in the genotypes showing phenotypic resistance to powdery mildew in the field conditions under epiphytotic conditions during 2021-2022.

Place and Duration of Study: Department of Genetics and Plant Breeding, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, between December during 2021-2022.

Methodology: Twenty genotypes VL2041, HPW349, HS507, VL907, HD3349 PBW876B, DBW313, HD2967, HI1628, HI8827(d), MACS5057, PBW874, PBW874, PBW873, WH1270(I) , WH1406, PBW868, WH1407, DBW368, along with two control : PBW343(C) and HS562(C) were taken.

Results: Out of 13 SSR markers used, four markers viz., Xgwm312, Cfd26, Xgwm174 and Xgwm182 were amplified to confirm the resistance in genotypes showing phenotypic resistance to powdery mildew.

Conclusion: Molecular marker characterization revealed that Xgwm312 confirmed the resistance to 9 genotypes (HS562, VL2041, PBW874, DBW313, DBW368, WH1407, HD3349, PBW873, and HD3043). Cfd26 confirmed the resistance in five genotypes (HS562, VL2041, DBW313, HS5079 and HD3043). Xgwm174 confirmed the resistance in seven genotypes HS562, VL2041, WH1406, DBW368, WH1407, HPW349, and PBW873 and the marker Xgwm182 confirmed the resistance in 7 genotypes (HS562, DBW187 (I) (C), WH1406, VL907, HS507, WH1407, HPW349 out of 20 genotypes.

Keywords: AUDPC, Molecular marker, powdery mildew, wheat germplasm

1. INTRODUCTION

Wheat (*Triticum aestivum L.*) is foremost and basic cereal crop consumed by approximately 2.5 billion of global population. Centre of origin of wheat is south-western Asia. Wheat is one of main food crop among domesticated food crop of major civilizations of Europe, West Asia and North Africa for 8000 years **(Giraldo *et al.,* 2019).** The total area of wheat in the world is around 220.83 mha with a production of 769.31mt. The normal world productivity is 3483.72 kg/ha **(United States Department of Agriculture, 2019-2020).** United States Department of Agriculture estimates that the world major wheat producing countries 2020-2021 are European Union, China, India, Russia, United State OF America, Canada, Australia, Pakistan, Ukraine, Turkey, Argentina, Iran, Kazakhstan**.**

Powdery mildew of wheat is the fourth most devastating disease after the three rusts and is caused by *Blumeria graminis* f.sp*. tritici* **Em. Marchal**. The disease is a significant constraint on wheat production **(Friebe *et al.,* 1994; Shi *et al.,* 1998)**. It occurs everywhere wheat is grown. It is favoured by mild temperature i.e., 15-22^0 C with 85-100 percent relative humidity as well as increased application of nitrogen fertilizers. Powdery mildew is an endemic disease widespread in many parts of the world that have been recognized as a devastating disease problem **(Bennett, 1984)**. It occurs rapidly in the cooler regions of China, Japan, and other areas in Asia, in North and East Africa, northern Europe and eastern North America **(Roelfs, 1977; McIntosh, 1997**. In India, the disease had been important in the northern and southern hill zone and foothills of the country. However, with the cultivation of the post green revolution high yielding, semi dwarf varieties responsive to high fertilization and extension of the area under irrigation the disease has started causing

immense losses in the Northern hills zone (NHZ) and Northern-western plain zone (NWPZ), the grain bowl of the country. However, it started appearing sporadically in the plains and foothills of the country also.

2. MATERIAL AND METHODS

2.1 Experimental site and crop management

The field experiments were conducted during the winter crop-growing season (Rabi) 2020–21 and 2021–22 at Norman E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology (GBPUAT) Pantnagar, U.S. Nagar, India. Pantnagar is in the Tarai region of the Indo-Gangetic plains and falls in the humid subtropical zone and is located between 29°N latitude and 79.3°E longitude with an altitude of 243.84 m above mean sea level. Agronomic practices recommended for normal fertility (120 kg N: 60 kg P_2O_5: 40 kg K_2O) were followed. The full dose of P_2O_5 and K_2O was applied at the time of sowing. Nitrogen was given as split application; 1/3 at sowing, 1/3 at first irrigation (21 d after sowing) and 1/3 at the time of second irrigation (40 days after sowing). Other standard crop management practices were used to raise a healthy crop.

2.2 Resistance evaluation for Powdery mildew in the field condition

Genetic resistance is the primary means to manage powdery mildew. **Bennett (1984), Ecker and Lein (1994)** have reviewed the use of several important resistance genes and their development in Western Europe and North America. Genes for resistance have been identified in at least 30 loci in wheat **(Shi *et al*., 1998; Xie *et al.,* 2003; Peusha *et al.,* 2000; Rong *et al*., 2000 Liu *et al.,* 2001 and McIntosh *et al.,* 2001).** These genes often act only against specific races of the pathogen causing a hypersensitive resistance reaction in the wheat plant. A major concern is that only a few genes have been used widely in cultivar development. Resistance may be lost when new strains of the fungus develop. For example, increased virulence towards *Pm17,* a widely used gene from Amigo wheat **(Heun *et al.,* 1990).** However, the genetics of *B. graminis f. sp. tritici* is complex. Higher frequencies of virulence were also found in the powdery mildew population due to *Pm* genes not known to be widely developed **(Niewoehner and Leath, 1998).**

Resistance to powdery mildew is also accomplished by a combination of factors that slow the rate of disease progress so that plants mature before significant damage occurs. This is known as slow-mildewing or partial resistance and is race-nonspecific. Plants are susceptible as seedlings but are less susceptible in the adult stage so that this is a form of adult plant resistance. Several genes usually control partial resistance. Genes for avirulence in the fungus may be expressed differently depending on the host genotype. An isolate of *B. graminis f. sp. tritici* may also grow rapidly on one genotype but much more slowly on another genotypes, so that the response of the host- parasite interaction is a partial resistance **(Martin and Ellingboe, 1976).**

Wild relatives of wheat have been exploited as source of new resistance genes **(Bennett, 1984).** Wild emmer *Triticum turgidum* var. *dicoccides* is a source of genes, some of which are expressed in both seedling and adult plants and some of which are expressed only in adult plants. Some wild emmer also possesses genes for partial resistance **(Moseman *et al*., 1984).**

2.3 Disease assessment

Bennett and Westcott, 1982 described that the powdery mildew was assessed on a 0 to 9 scale, with 0 indicating no disease and 9 indicating disease in more than 90% of the plant tissue. James, 1971 reported that standard area diagrams can be used to rate the severity of the condition on this scale.

The evaluation of resistance in the field is generally based on a visual assessment of the progress of the disease from the lower portion of the canopy. The most effective system consists of using a scale (0-9) developed as a modification of Saari and Prescott's severity scale **(Saari and Prescott, 1975).** Disease intensity at the adult plant stage was recorded according to the 0-9 Saari Prescott scale. Using this scale, 0 indicates Free from infection whereas 9 **-** Highly susceptible (Severe infection on all leaves, spike also infected to some degree).

The area under the disease progress curve (AUDPC) can be calculated using the percentage severity estimates corresponding to three to four recordings as shown below.

$$\mathbf{AUDPC} = \sum_{i=1}^{K} \frac{(Yi + (Yi+1))(Ti - (Ti+1))}{2}$$

Where,

Yi = severity on the i^{th} date, ti = i^{th} day and k = number of dates on which the disease recorded. The lines that showed AUDPC (<100) were considered highly resistant and the lines that showed AUDPC (>300) were considered highly susceptible **(Batheja *et al.,* 2022).**

Table 1: Saari and Prescott (0-9) scale for appraising Powdery Mildew Severity

Scale	Category	Description
0	No infection	Free from infection
1	Resistant	Few isolated lesions on the lower most leaves
3	Moderately resistant	leaf infection of the lower third of the plant, lower most leaves infected at moderate to severe levels
5	Moderately susceptible	Severe infection on lower leaves, moderate infections extending to the mid-point of the plant with upper leaves free. Infection does not extend beyond mid-point of the plant
7	Susceptible	lesions severe on lower and middle leaves with infection extending to the leaf below the flag leaf or with trace infections on the flag leaf
9	Highly susceptible	Severe infection on all leaves and spike infected to some degree. Spike infections are scored as modified scale of the percentage of the total areas covered
E	Escape	Free from infection but represents an escape
N	Necrosis	Used to indicate no scoring possible due to necrosis as a result of other diseases or factors

2.4 PCR analysis and electrophoresis

The molecular marker analysis was carried out at Wheat Grain Quality and Molecular Breeding Laboratory, Department of Genetics and Plant Breeding GBPUAT, Pantnagar. Genomic DNA isolation was done by using the CTAB method modified from Doyle and Doyle (1990). Polymerase chain reaction (PCR) was performed using a microsatellite marker *Xgwm312* linked to PM QTL located on chromosome 2A. For conducting PCR, 20 µl amplification reaction contained buffer (10X) with 10 mM Tris-HCl (pH 9.0), 15 mM MgCl2, 50mMKCl and 0.01% gelatin; 2.5 mM of each dNTP (deoxyribonucleotide); 40 ng of each primer; 50 ng genomic DNA; and 1U Taq DNA polymerase (Genei Merck, Bangalore, India) were used. Amplifications were performed in a Paqlab™ Gradient Thermal cycler (Sigma-SVI Biosolutions Pvt. Ltd., New Delhi, India) programmed at 94°C for 4 min, followed by 40 cycles at 94°C for 1 min, at annealing temperature for 1 min and 72°C for 1 min, the final extension step was kept at 72°C for 10 min. Total PCR product was analysed on 3.5% metaphor agarose gels stained with ethidium bromide and visualized on the Alphaimager™ gel documentation system. Analysis of the presence and absence of the desired allele was done manually for the respective gels.

3. RESULTS AND DISCUSSION

Identification of germplasm genotypes for resistance to powdery mildew using molecular markers helps in the use of resistance genotypes in breeding programme as donors with certainty as screening based on phenotypic performance is subjected to disease pressure in natural conditions. Therefore, in the current investigation an effort was made to confirm the resistance with gene linked markers in the genotypes showing phenotypic resistance to powdery mildew in the field conditions under epiphytotic conditions.

Twenty genotypes VL2041, HPW349, HS507, VL907, HD3349 PBW876B, DBW313, HD2967, HI1628, HI8827(d), MACS5057, PBW874, PBW874, PBW873, WH1270(I), WH1406, PBW868, WH1407, DBW368, PBW343(C)

and HS562(C) which have shown phenotypic resistant were subjected for validation of resistance with gene linked molecular markers.

Out of 13 SSR markers used, four markers *viz., Xgwm312, Cfd26, Xgwm174 and Xgwm182* were amplified to confirm the resistance in genotypes showing phenotypic resistance to powdery mildew.

Molecular marker characterization revealed that *Xgwm312* confirmed the resistance to 9 genotypes (HS562(C), VL2041, PBW874, DBW313, DBW368, WH1407, HD3349, PBW873, HD3043). *Cfd26* confirmed the resistance in five genotypes (HS562(C), VL2041, DBW313, HS507 and HD3043). *Xgwm174* confirmed the resistance in seven genotypes HS562(C), VL2041, WH1406, DBW368, WH1407, HPW349, and PBW873 and the marker *Xgwm182* confirmed the resistance in 8 genotypes (HS562(C), DBW187 (I) , WH1406, VL907, HS507, WH1407, HPW349 out of 20 genotypes **.**

The genotype, HS562(C) confirmed for resistance to powdery mildew by all four markers-*Xgwm312, Cfd26, Xgwm174 and Xgwm182.* Similarly in the genotype, VL2041 the resistance was confirmed by three markers - *Xgwm312, Cfd26 and Xgwm174* and in DBW313 by two markers i.e*., Xgwm312 and Cfd26* and in DBW368 resistance for powdery mildew was confirmed by two markers - *Xgwm312* and *Xgwm174*. The genotype (s) in which the resistance for powdery mildew has been confirmed by more than two molecular markers could be used as donor (s) in breeding programme for developing the powdery mildew resistant wheat genotypes.

3.1 Field evaluation of wheat germplasm accessions under epiphytotic conditions

The observations on disease severity on the check entries recorded by using Sari and Prescott scale (0-9) revealed that in the field experiments conducted during 2020-2021 and 2021-2022 Rabi seasons check PBW343 and HS562. Out of forty-eight genotypes, forty-four showing lower AUDPC value (<100) can be further used for the development of powdery mildew resistant genotypes. The check HS562(C) observed to be highly resistant (HR) with the A-value of 24.5 and the check PBW343(C) is observed to be highly susceptible (HS) with A-value of 225.

In the field experiment, during 2021-2022, out of forty eight genotypes, forty four genotypes that showed phenotypically resistance to powdery mildew of wheat were also confirmed resistant by AUDPC value (<100). Among forty-eight genotypes the highest AUDPC value i.e. 115.5 was calculated in genotype DDW48 (d) (I) whereas, the lowest A-value i.e. 17.5 was recorded in three genotypes i.e. HD2967, PBW873 and DBW368.

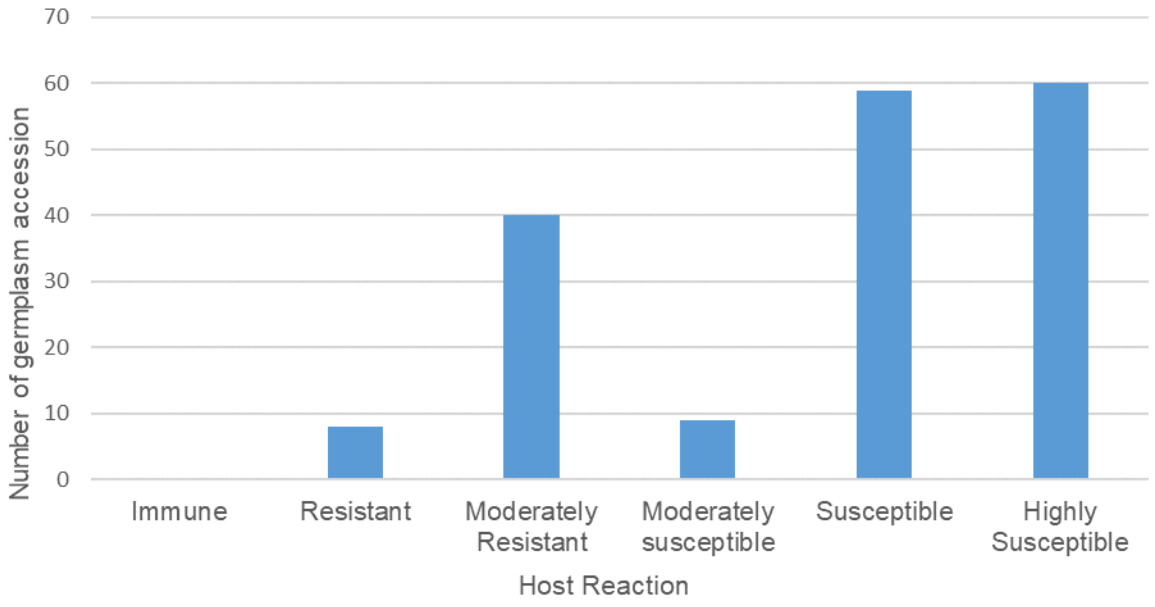


Fig.1. The frequency distribution of 176 germplasm accessions showing the pathological rating in the field-based study against the powdery mildew reaction in 2020-2021

3.2 Molecular screening for the confirmation of PM resistance

Molecular screening of 20 genotypes of wheat for Powdery mildew resistance was carried out using the specific SSR markers, *Xgwm312* linked to the QTL on the chromosome 2A for resistance along with the checks, PBW343(C) and HS562(C). The marker *Fcd26, Xgwm174, Xgwm182, Xgwm192 and the marker Cfd57* was linked to the QTL on the chromosome 5D. The marker *Xgwm165* was linked with the chromosome 4D. The marker *Xgwm1061* and the marker *Xgwm46* was linked to the chromosome 7A and 7B respectively. The marker *Xgwm372* was linked QTL chromosome 2A. The marker *Xwmc415* was linked to the QTL chromosome 5B and the marker *Xwmc445* was linked to the QTL chromosome 2D. The marker *Cfd57* was linked to the QTL chromosome 5D and the *Xwmc818* was linked to the QTL chromosome 1A.

Markers	**Genotypes**

Cfd26	HS562(C) ,VL2041, DBW313, HD3043, HS507, HS562(C)
Xgwm312	VL2041, DBW313, DBW368, WH1407, PBW873, HD3043
Xgwm174	HS562(C), VL2041, DBW368, WH1407, PBW873, HPW349
Xgwm182	HS562(C) , WH1407, HPW349, HS507

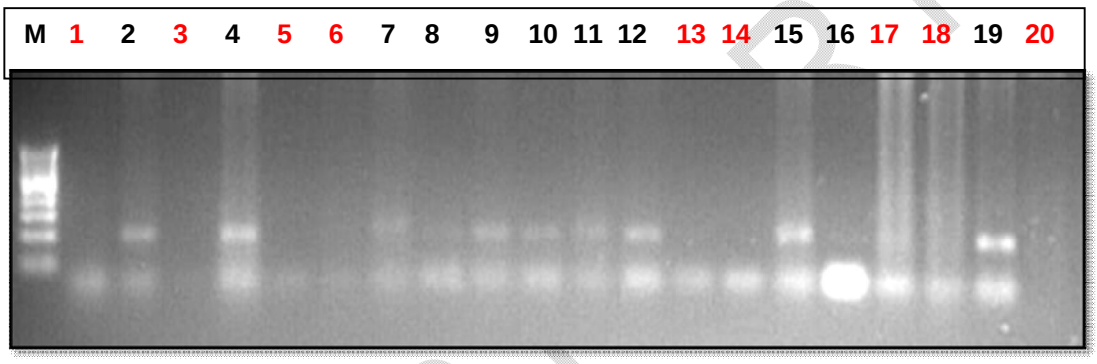
Discussion

Several biotic and abiotic stresses constrain wheat production. Among biotic stresses, rust, spot blotch powdery mildew is the immense importance in the Indo-Gangetic Plains of India, which accounts for major acreage under wheat. Due to widespread prevalence and severe intensities, powdery mildew is of increasing concern in India and the eastern part of South Asia.

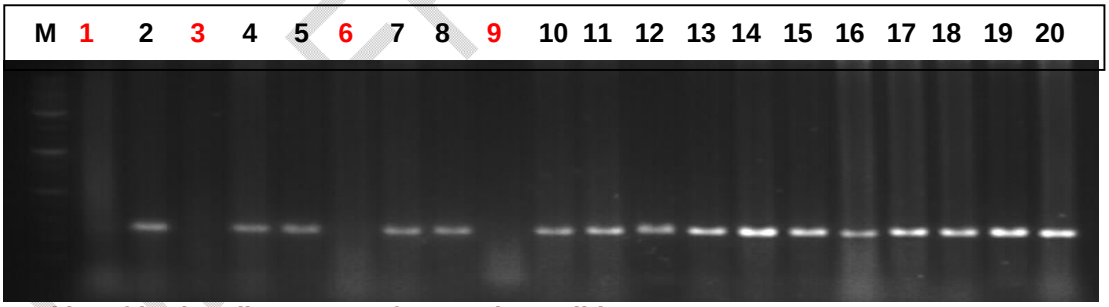
Identification of germplasm genotypes for resistance to powdery mildew using molecular markers helps in the use of resistance genotypes in breeding programme as donors with certainty as screening based on phenotypic performance is subjected to disease pressure in natural conditions. Therefore, in the current investigation an effort was made to confirm the resistance with gene linked markers in the genotypes showing phenotypic resistance to powdery mildew in the field conditions under epiphytotic conditions.

According to **Xu *et al*., (2011)** Zheng 9754's resistance to powdery mildew was produced by a single dominant gene on chromosome 2AL that has been tentatively given the name *PmHNK54* and is flanked by the SSR markers *Xgwm312*.

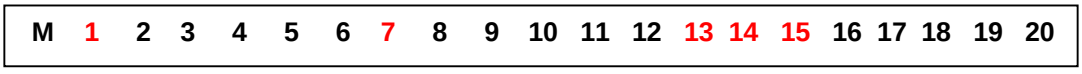
Sun *et al.,* (2006) confirmed that microsatellite markers, *Xgwm174 and Cfd26* were found to be linked to *PmY201* with genetic distances of 5.2, 7.7 cm respectively, and SSR marker*, Xgwm182* was found to be linked to *PmY212* with distances of 7.2 cm.

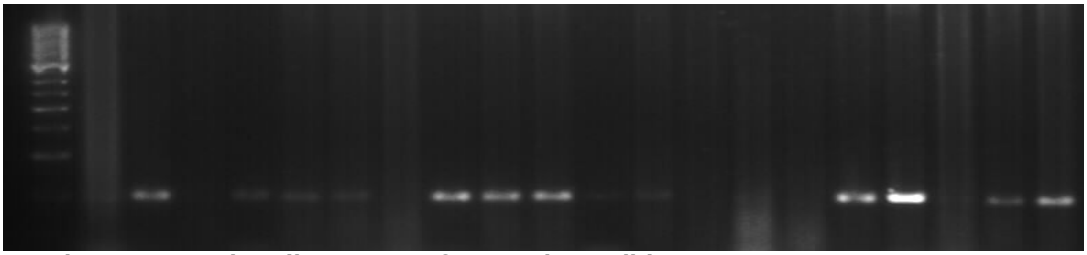


a) *Xgwm312* banding pattern for powdery mildew
M: 1kb marker ladder, 1 to 20 genotypes showing resistance to powdery mildew.

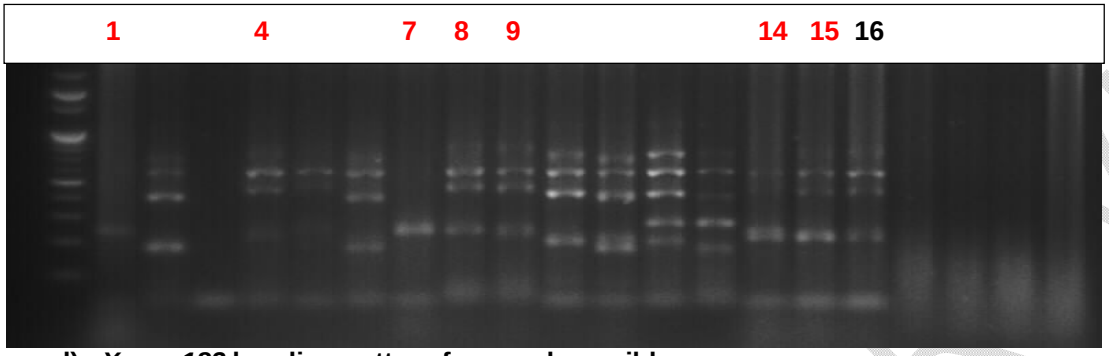


b) *Cfd26* banding pattern for powdery mildew
M: 1kb marker ladder, 1 to 20 genotypes showing resistance to powdery mildew.





c) *Xgwm174* banding pattern for powdery mildew
M: 1kb marker ladder, 1 to 20 genotypes showing resistance to powdery mildew.



d) *Xgwm182* banding pattern for powdery mildew
M: 1kb marker ladder, 1 to 20 genotypes showing resistance to powdery mildew
Plate 1. Confirmation of major resistance genotypes in powdery mildew

4. CONCLUSION

Molecular marker characterization revealed that *Xgwm312* confirmed the resistance to 9 genotypes (HS562, VL2041, PBW874, DBW313, DBW368, WH1407, HD3349, PBW873, and HD3043)**.** *Cfd26* confirmed the resistance in five genotypes (HS562, VL2041, DBW313, HS5079 and HD3043). *Xgwm174* confirmed the resistance in seven genotypes HS562, VL2041, WH1406, DBW368, WH1407, HPW349, and PBW873 and the marker *Xgwm182* confirmed the resistance in 7 genotypes (HS562, DBW187 (I) (C), WH1406, VL907, HS507, WH1407, HPW349 out of 20 genotypes.

The result concluded that, the genotypes which came out to be resistant through genotypic and phenotypic way can be further used by breeders for the development of powdery mildew resistant varieties.

CONSENT (WHERE EVER APPLICABLE)

"Written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

REFERENCES

Batheja, A., Rana, V., Basandrai, A. K., and Sarial, A. K. 2022. Evaluation of wheat genotypes for adult plant resistance against powdery mildew caused by Blumeria graminis tritici. Himachal j. agric. Res., 47(2): 149-155.

Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. Plant Pathol., 33(3): 279-300.

Bennett, F.G. 1984. Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. Plant Pathol., 33(3): 279-300.

Bennett, F.G. 1984. Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. Plant Pathol., 33(3): 279-300.

Bennett, F.G. and Westcott, B. 1982. Field assessment of resistance to powdery mildew in mature wheat plants. Plant Pathol., 31(3): 261-268.

Ecker, U. and Lein, K.A. 1994. Use and significance of resistance genes against powdery mildew (Erysiphe graminis) in German wheat varieties in the years 1980 to 1993. Phlanzenschutz-Nach, 47: 91-128.

Friebe, B., Heun, M., Tuleen, N., Zeller, F.J. and Gill, B.S. 1994. Cytogenetically monitored transfer of powdery mildew resistance from rye into wheat. Crop Sci., 34(3): 621-625.

Giraldo, P., Benavente, E., Manzano-Agugliaro, F. and Gimenez, E. 2019. Worldwide research trends on wheat and barley: A bibliometric comparative analysis. Agron., 9(7): 352.

Heun, M., Friebe, B. and Bushuk, W. 1990. Chromosomal location of the powdery mildew resistance gene of Amigo wheat. Phytopathol., 80(10): 1129-1133.

James, W.C. 1971. Importance of foliage diseases of winter wheat in Ontario in 1969 and 1970. Can. Plant Dis. Survey. 51: 24-31.

Liu, S., Griffey, C. A., and Maroof, M. S. 2001. Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. Crop Sci., 41(4):1268-1275.

Martin, T. J., and Ellingboe, A. H. 1976. Differences between compatible parasite/host genotypes involving the Pm4 locus of wheat and the corresponding genes in Erysiphe graminis f. sp. tritici. Phytopathol., 66:1435-1438.

McIntosh, R. A. 1997. Breeding wheat for resistance to biotic stresses. In Wheat: prospects for global improvement Springer, Dordrecht. (71-86 pp.).

McKinney, H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by helminthosporium. J. Agric. Res., 26(195):213-250.

Moseman, J. G., Nevo, E., Morshidy, M. E., and Zohary, D. 1984. Resistance of Triticum dicoccoides to infection with Erysiphe graminis tritici. Euphytica, 33(1): 41-47.

Niewoehner, A. S. and Leath, S. 1998. Virulence of Blumeria graminis f. sp. tritici on winter wheat in the eastern United States. Plant Dis., 82(1): 64-68.

Peusha, H., Enno, T., and Priilinn, O. 2000. Chromosomal location of powdery mildew resistance genes and cytogenetic analysis of meiosis in common wheat cultivar Meri. Hereditas, 132(1): 29-34.

Rong, J. K., Millet, E., Manisterski, J., and Feldman, M. 2000. A new powdery mildew resistance gene: introgression from wild emmer into common wheat and RFLP-based mapping. Euphytica, 115(2): 121-126.

Saari, E. E., and Prescott, J. M. 1975. Scale for appraising the foliar intensity of wheat diseases. Plant Dis. Reporter.7: 49- 57

Shi, A.N., Leath, S. and Murphy, J.P. 1998. A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. Phytopathol., 88(2): 144-147.

Sun, X. L., Liu, D., Zhang, H. Q., Huo, N. X., Zhou, R.H., and Jia, J.Z. 2006. Identification and mapping of two new genes conferring resistance to powdery mildew from Aegilops tauschii (Coss.) Schmal. J. Integr. Plant Biol., 48(10): 1204-1209.

Xie, C., Sun, Q., Ni, Z., Yang, T., Nevo, E., and Fahima, T. 2003. Chromosomal location of a Triticum dicoccoides derived powdery mildew resistance gene in common wheat by using microsatellite markers. Theor. Appl. Genet., 106(2): 341-345.

Xu, W., Li, C., Hu, L., Wang, H., Dong, H., Zhang, J., and Zan, X. 2011. Identification and molecular mapping of PmHNK54: a novel powdery mildew resistance gene in common wheat. Plant Breed., 130(6): 603-607.
Note: Authors are also encouraged to add other database's unique identifier (like PUBMED

DEFINITIONS, ACRONYMS, ABBREVIATIONS

Term: QTL - A quantitative trait locus (QTL) is a region of DNA associated with a specific phenotype or trait that varies within a population.