

## **Selection of Powdery Mildew Resistant Brassica Genotypes based on Disease Indexing and Microsatellite Markers**

**Abstract:** Powdery mildew disease of oilseed mustard caused by *Erysiphe cruciferarum* is a primary reason of yield reduction not only in India but also throughout the world. Identification and cultivation of resistant mustard genotypes against powdery mildew is the only way to overcome this challenge. In the present investigation, we targeted to screen 75 Brassica genotypes against powdery mildew based on disease indexing under field conditions and gene-specific molecular markers. Disease reaction on both the cotyledonary and true leaves was screened with using a modified 0-9 scale score as well as with nineteen disease linked microsatellite markers. In disease indexing under field conditions, genotypes *viz.*, L-4 and PC-5 were identified as immune, China and RP-9 were considered as highly resistant and GSC-7 and PC-6 were recognized as resistant whilst genotypes *i.e.*, RB-50, Pusa Bold, WRR-10 and GSL-1 were accredited as moderately resistant. Molecular markers based UPGMA dendrogram classified Rohini, WRR-22, PC-6, PusaBold, China, WRR-8, GSL-1, WRR-7, RH-749, L-4 and RB-50 as highly resistant mustard genotypes. In addition, disease linked marker *cnu\_m616* had the highest polymorphic information content (0.75) with greatest ability to differentiate resistant genotypes from susceptible genotypes, may be employed directly in mustard breeding programmes in future.

**Keywords:** Mustard; Powdery Mildew; Disease indexing; Gene-linked Markers, Pathogens

### **INTRODUCTION**

The genus Brassica is one of the most spirited oil seed crops being cultivated in India with high grade of genetic diversity [1-3]. The Indian mustard, yellow sarson, browns arson, raya, and toria crops are all included in the rapeseed-mustard group [4-6]. It is self-pollinated crop with some extent of cross-pollination [7-11]. It is the most important oilseed crop of India having significant economic, nutritional, and industrial applications [12-14]. Despite having significant area under farming of oilseed crops (26.20 million hectare), India is a major importer of edible oil. India imports >Rs. 0.73 trillion worth of edible oil annually [15]. During the 2018–2019 growing season, the estimated global rapeseed-mustard area, production, and yield were 36.59 Mha, 72.37 Mt, and 1980 kg $ha^{-1}$ , respectively. India contributed 19.8 and 9.8%, respectively, to global acreage and production [16].

It comprises between 38 and 46% oil [17]. The oilseed brassica characteristically comprises 27% oleic and linoleic acid, 4.7–13% linolenic acid, and 38–57% erucic acid, all of which have substantial nutritional worth and are crucial for human health [18-19]. Mustard crop is exploited in numerous submissions for instance paste, sauce, flavours, ingredients, and the most well-known spices [20-21].

Mustard is susceptible to disease attacks owing to the genetic homogeneity among all grown types [22]. Among various biotic factors responsible for severe yield loss in mustard powdery mildew causes serious damage worldwide [23-24]. Powdery mildew disease is caused by *Erysiphe cruciferarum* Opiz. ex. L. Junellac counts yield loss up to 17 % [25] in India. All the Brassica cultivars currently cultivated in India are extremely vulnerable to powdery mildew and so far, no resistance resource has been reported [26]. For prevention of losses due to plant diseases, chemical compounds have been employed but abuse in their employment has led to the development of fungicide resistance in pathogens. Fungicides also have greater repercussion on environment and human health. Therefore, use of resistant varieties seems to be a better alternative. Host plant resistance (R) genes have been broadly deployed to develop disease-resistant cultivars in mustard breeding for diminishing yield losses owing to different pathogens. Information on the resistant resources from *B. juncea* against powdery mildew are lacking. But, related species such as *B. napus*, *B. carinata*, *B. japonica*, *B. Alboglabra* [27, 9], *B. oleracea*, *B. rapa*, and *B. napus* [28] have been reported as sources of resistance against powdery mildew. This information makes it necessary to identify sources of resistance against powdery mildew in *B. juncea* as well.

DNA-based genetic markers are being used more frequently lately for quick cultivar identification, cultivar fingerprinting, and cultivar diversity analysis [29-37]. Due to their high abundance, reproducibility, simplicity, co-dominant inheritance, higher rate of polymorphism, and wider genome coverage, SSRs have emerged as the top choice among different types of DNA-based markers for plant breeders and biotechnologists to assess genetic diversity and cultivar selection [38-48].

In this context, Brassica genotype (s) resistant to powdery mildew needs to be introduced so that major yield loss due to this disease could be minimized and productivity improved without affecting quantity and quality of oil. Therefore, the present investigation was undertaken to screen resistant mustard genotype (s) against powdery mildew disease based on disease indexing and disease -linked microsatellite markers.

## MATERIALS AND METHODS

### Plant Material

Present study was commenced on a total of 75 mustard genotypes including RB-50, Pusa Bold, Varuna, Rohini, Kranti, RH-725, Maya, Vardan, Vasundhara, Swarn Jyoti, Pusa Jagannath, PusaJaiKisan, Albeli, Sej-2, Shraddha, DMH1, L-4, JMWR-908-1, RGN-73, NRC-HB-101, NRC-HB-506, RVM-3, RH-749, NRCDR-2, DRMRIJ-31, China, GSL-1, GSC-7, PC-5, PC-6, RP-9, Kiran, JTC-1, JM-1, JM-2, JM-3, RVM-1, RVM-2, PM-25, PM-26, PM-27, PM-28, PM-30, Pusa Vijay, JMM-927, JMM-991, RMM-10-01-01, RMM-12-01-18, RMM-12-03-18, WRR-5, WRR-6, WRR-7, WRR-8, WRR-10, WRR-11, WRR-12, WRR-13, WRR-14, WRR-15, WRR-16, WRR-17, WRR-18, WRR-19, WRR-20, WRR-21, WRR-22, WRR-25, WRR-26, WRR-27, WRR-28, WRR-29, WRR-30, WRR-31 and WRR-32 having different reaction against powdery mildew disease, acquired from All India Coordinated Research Project on Rapeseed and Mustard, Zonal Agricultural Research Station, Morena, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya (RVSKVV), Gwalior, M.P., India. All the genotypes were grown in randomized block design with three replications in *Rabi* 2021 at the experimental field of the Department of Genetics & Plant Breeding, College of Agriculture, RVSKVV, Gwalior, India. Each genotype was planted in a plot of one row of 2-meter length with an arrangement of 30 cm apart between rows and 15 cm plant to plant. The crop was enchanted with protective irrigations and recommended packages of practices right through the growing season. Five arbitrarily chosen plants from each treatment were marked for taking the observations for observation. Molecular work was conducted at Department of Plant Molecular Biology & Biotechnology, College of Agriculture, RVSKVV, Gwalior.

### Disease Assessment

The observations and screening were carried out as per the 0-9 rating [49] scale suggested by AICRP on Rapeseed and Mustard, Bharatpur at cotyledonary as well as true leaf stage. The extent of disease was rated as:

List 1 : Description of Extent of Disease

0	Immune (I)	No lesion
1	Highly resistant (HR)	Non-sporulating pinpoint size or small brown necrotic spots, less than 5% leaf area covered by lesion.

3	Resistant ( R )	Small roundish slightly sporulating larger brown necrotic spots, about 1-2 mm in diameter with a distinct margin or yellow halo, 5-10% leaf area covered by lesions
5	Moderately resistant (MR)	Moderately sporulating, non-coalescing larger brown spots about, 2-4 mm in diameter with a distinct margin or yellow halo, 11-25% leaf area covered by the spots
7	Susceptible (S)	Moderately sporulating, coalescing larger brown spots about, 4-5 mm in diameter, 26-50 % leaf area covered by the lesions
9	Highly susceptible (HS)	Profusely sporulating, rapidly coalescing brown to black spot measuring more than 6 mm diameter without margin covering more than 50% leaf area

### Severity of disease

To calculate the PDI of powdery mildew at cotyledonary stage, ten cotyledonary leaves from each replication of an accession were taken at 15 days after sowing. Leaves are evaluated individually by giving (0-9) disease rating (Table 1). At true leaf stage, average disease index on true leaves were taken at 60, 75 and 90 days after sowing in each replication by using 0-9 disease rating scale. Fifteen leaves were randomly collected from each accession and scored; disease index was calculated separately for each accession. Disease intensity was recorded at the maturity stage as per the scale. The percent disease index was calculated by the following formula.

$$Disease\ index\ (\%) = \frac{Sum\ of\ all\ numerical\ ratings}{Number\ of\ leaves\ examined \times Maximum\ Grade\ (9)} \times 100$$

### SSR Marker Analysis

#### Isolation of genomic DNA

Diluted DNA was subjected to PCR amplifications with 19 SSR primers. The genomic DNA of mustard genotypes was extracted using the modified CTAB method [50] slightly modified by Tiwari *et al.* [51] from 2-3 fresh young leaves. DNA was further purified by RNase digestion tracked by extraction with phenol: chloroform: isoamyl alcohol (25:24:1) and ethanol precipitation.

#### Polymerase chain reaction (PCR) amplification

A total of 19 powdery mildew resistance linked microsatellite markers were used in selected mustard genotypes to find out polymorphism (Table 2). The SSR primers were synthesized

by Eurofins Genomics India Pvt. Ltd. The polymerase chain reaction was performed in 10 µl reaction mixture comprising of 1X PCR buffer, 0.1 U *Taq* DNA polymerase, 1 µl dNTP (1 mM), 0.5 µl of forward and reverse primers each (10 pM) and 2 µl (10 ng/µl) of genomic DNA in a thermocycler (Bio-Rad, U.S.A.). The PCR profile started with 95 °C for 3 min followed by 35 cycles of denaturation at 95 °C for 20 sec, annealing at 52-58 °C (depending on primer) for 20 sec and extension at 72 °C for 90 sec. A final extension at 72 °C for 10 min was encompassed. The PCR products were resolved on 3% agarose gel at 65V for 2-3 h and documented using Gel Documentation System (Syngene, USA).

### **Data analysis**

Scoring of each polymorphic SSR marker was done with the help of Gel Analyzer V19.1 software. Diversity parameters *viz.*, gene diversity, allele number, major allele frequency and PIC were calculated using Power Marker v3.25 software [52]. Neis genetic distance based UPGMA tree was constructed with the help of power marker v3.25 and visualized using MEGA 6.0 software [53].

## **RESULTS AND DISCUSSION**

### **Field screening**

The field screening against powdery mildew was done based on disease intensity in the scale of 0-9. Since the growth of *E. cruciferarum* is superficial, disease symptoms can be observed easily by visual inspection. Powdery mildew first appeared on the upper surface in the lower most (oldest) leaves as small (1 to 2 cm diameter), scattered, white almost circular colonies which coalesced as the colonies grew further, eventually covering the entire leaf surface, stem, primary, secondary branches, and siliquae, progressively (Fig.1).

The *Brassica* genotypes were classified based on categorizations of reactions against powdery mildew fungal disease (Table 3). For powdery mildew infestation, genotypes *viz.*, L-4 and PC-5 were found to be immune, whilst China and RP-9 genotypes investigated to be highly resistant, whereas genotypes namely GSC-7 and PC-6 considered to be resistant whilst genotypes *i.e.*, RB-50, Pusa Bold, WRR-10 and GSL-1 were found to be moderately resistant. However, rest of the genotypes were exhibited their susceptibility to disease (Fig. 1; Table 4). According to our findings, genotypes *viz.*, Varuna, Kranti, Maya, Albeli, Shraddha, DHM-1, WR-908-1, RGN-73, NRC HB-101, NRC DR-2, DRMR IJ-31, Kiran, JTC-1, JM-2, RVM-1, PM-25, PM-26, PM-27, PM-30, RMM-12-01-18, RMM-12-03-18, WRR-5, WRR-6, WRR-7, WRR-8, WRR-9, WRR-11, WRR-12, WRR-13, WRR-14, WRR-15, WRR-16,

WRR-17, WRR-18, WRR-19, WRR-20, WRR-21, WRR-22, WRR-25, WRR-26, WRR-27, WRR-28, WRR-29, WRR-30, WRR-31, WRR-32 and Shraddha were considered susceptible while genotypes namely Rohini, RH-725, Vardan, Vasundhara, Swarn Jyoti, Pusa Jagannath, Pusa Jay Kisan, Sej-2, NRC-HB-506, RVM-3, RH-749, JM-3, PM-28, Pusa Vijay, JMM-927, JMM-991 and RMM-10-01-01 were investigated highly susceptible.

Our findings are in accordance with recent study conducted by Nanjundan *et al.* [26] in which resistance source against *E. cruciferarum* have been identified. A total of 1,020 Indian mustard accessions were evaluated against *E. cruciferarum* PMN isolate, at Wellington, The Nilgiris, Tamil Nadu, India under natural hot spot conditions. The study identified one accession viz., RDV 29 with complete resistance against *E. cruciferarum* PMN isolate for the first time, which was consistent in five independent evaluations. Genetic investigation of F<sub>1</sub>, F<sub>2</sub> and backcross populations acquired from the cross RDV29 (highly resistant) x RSEJ775 (highly susceptible) for two seasons showed that the resistance is administrated by two genes through gene dosage and semi-dominant effect. However, according to Khalik [54]) the *B. juncea* plant's physical characteristics, such as the numbers of non-glandular trichomes on plants, may also be connected to the resistance to powdery mildew. A larger density of non-glandular trichomes may increase the risk of fungal infections because they are able to catch airborne particles like fungus spores [55]. Unless they have an anti-fungal hydrolase, non-glandular trichomes are good homes for pathogens [56]. This hydrolase prevents or lessens fungal infection by hydrolyzing fungi's cell walls.

Powdery mildew infection risk and disease severity have both increased in India as a result of the widespread usage of one or more highly sensitive cultivars of *Brassica* species. The successful management of powdery mildew of *Brassica* in India is possible through deployment of cultivar resistance. Genetic engineering is a promising technique to fight infection since host resistance is a significant factor in the spread of disease. *B. juncea* transgenic lines with BjNPR1 have shown promising results in terms of increased *E. cruciferarum* resistance in Australia [57].

### **Molecular screening**

The field screening does not reflect the comprehensive screening of the genotypes due to the expression of many morphological traits is masked *via* various environmental factors [58-60]. The genotypes having similar disease symptoms may exhibit differences at DNA level [39]. Therefore, in the present investigation, powdery mildew linked microsatellite markers were

also employed for the screening Brassica genotypes at molecular level. In the present study, 19 powdery mildew linked microsatellite markers were deployed for screening of 75 Brassica genotypes. Among employed 19 microsatellite markers, only five markers displayed consistent polymorphism among Brassica genotypes and were selected for further assessment.

Total 25 alleles were identified with 5.0 as an average allele per locus (Table 4). The genetic diversity ranged between 0.711 to 0.785 with a mean worth of 0.749. The maximum genetic diversity was investigated for the marker *cnu\_m616* and minimum for MF-4. The major allele frequency varied between 0.266 to 0.40 with an average value of 0.330. Maximum major allele frequency shown by marker MF-4 and minimum by the marker *cnu\_m616*. Whereas PIC values arrayed between 0.662 to 0.750 with a mean worth of 0.707. Maximum PIC value was evidenced for the markers *cnu\_m616* and minimum for MF-4 respectively.

PIC value defines the discriminatory power of a marker and is the representative of allelic diversity and frequency among the genotypes [7, 8, 14, 31,32, 34,35,36,37,40]. The occurrence of multiple bands from the same locus could be explained by the presence of cryptic site upstream, downstream and between the primer binding sites. Our study was found in accordance with previous study in which gene diversity ranged from 0.20 to 0.70 for markers Ni2F11 and BrgMS713 respectively, with an average value of 0.53 per marker [36]. Genetic diversity of *Brassica spp.* genotypes was studied by Baghel *et al.* [7] using SSR markers in which highest PIC value (0.6851) was reported for the marker OI10-CO 5. In other investigation, Sharma *et al.* [36]) reported higher PIC values than the average PIC value (0.44) in the reference set of genotypes using highly polymorphic 48 SSR markers that can be used in trait mapping studies in mustard.

The phylogenetic tree classified genotypes Rohini, WRR-22, PC-6, Pusa Bold, China, WRR-8, GSL-1, WRR-7, RH-749, L-4 and RB-50 in a separate group (Fig. 2). Grouping of these genotypes in the separate group may be due the amplification of similar alleles with all the polymorphic markers. Our result is an accordance with investigation of Ghosh *et al.* [61] in which the UPGMA based dendrogram resulted in two major clusters. Cluster I contain a wild type genotype B1D (*S. alba*), genetically dissimilar from all other varieties. Cluster II divided into two sub-clusters cluster IIA and cluster IIB. The phylogenetic tree of *Brassica spp.* genotypes was also constructed by Baghel *et al.* [7] using SSR markers and divided 48 genotypes into three major groups, first main cluster contained 17 genotypes, second chief cluster had 24 genotypes and third core group included 7 genotypes. Sharma *et al.* [36] also

reported 59 leafy mustard accessions were grouped into two distinct groups based on UNJ dendrogram. Genotype IC597868 formed a separate cluster *i.e.*, cluster I while, cluster II was comprising of 58 mustard genotypes. Cluster II was further sub divided into two subclusters IIA and IIB. Subcluster IIA consisted of three accessions including IC597901, IC597886 and 5IC597949, whilst the rest of the 55 accessions grouped into subcluster IIB.

According to the field screening results, genotypes that showed resistance against powdery mildew disease having possibilities to have resistant gene in these genotypes and power of polymorphic markers to separate them accordingly. Results indicate the linkage of polymorphic markers with powdery mildew resistance in *Brassica species*. However, to confirm their association it is important to perform further study using mapping populations. According to the Brassica Microsatellite Information Exchange (BMIE), there are still not enough SSR markers in Brassica that have been mapped completely. The density of the current genetic maps would increase due to the polymorphic SSRs, and breeders would benefit from having a source of polymorphic DNA markers for high-throughput mapping, marker-assisted selection, and improvement of *Brassica species*.

## CONCLUSION

Powdery mildew disease of oilseed mustard is one of the primary causes of yield penalty not only in India but also throughout the world. Most of the mustard cultivars currently grown in India are extremely vulnerable to powdery mildew and so far, no resistance resource has been reported. Disease indexing classified mustard genotypes *viz.*, L-4, PC-5 as immune against *Erysiphe cruciferarum*. Disease linked microsatellite markers showing consistent polymorphism among 75 mustard genotypes identified Rohini, WRR-22, PC-6, Pusa Bold, China, WRR-8, GSL-1, WRR-7, RH-749, L-4 and RB-50 genotypes as highly resistant genotypes and confirmed the results obtained from disease indexing. In addition, disease linked marker *cnu\_m616* having greatest aptitude to distinguish resistant genotype (s) from susceptible genotypes, could be employed straight in mustard breeding programmes.

## REFERENCES

1. Tandayu E., Borpatragohain P, Mauleon R., Kretschmar T. Genome-wide association reveals trait loci for seed glucosinolate accumulation in Indian mustard (*Brassica juncea* L.). *Plants*, 2022; 11(3): 364.
2. Tripathi M K, Tomar SS, Tiwari VK, Awasthi D, Gupta J C Heterosis in Indian mustard [*Brassica juncea* (L) Czern and Coss]. *Prog. Res.* 2015;10 (Special-VI):3376-3379.

3. Shrivastava A, Tripathi M K, Solanki R S, Tiwari S, Tripathi N, Singh J, Yadav R. Genetic correlation and path coefficient analysis of yield attributing parameters in Indian mustard. *Curr. J. Appl. Sci. Technol.* 2023a; 42 (7), 42-58. DOI: [10.9734/CJAST/2023/v42i74079](https://doi.org/10.9734/CJAST/2023/v42i74079)
4. Shrivastava A, Tripathi MK, Singh P, Tiwari S, Tripathi N, Tiwari P N, Parihar P, Singh J, Chauhan S. Disease indexing of Indian mustard genotypes against *Alternaria* blight disease. *The Pharma Innovation Journal*; 2023b; 12(4): 08-13.
5. Barfa D, Tripathi MK, Kandalkar VS, Gupta JC, Kumar G. (). Heterosis and combining ability analysis for seed yield in Indian mustard [*Brassica Juncea* (L) Czern & Coss]. *Eco. Env. & Cons.* 2017;23.Suppl 75-83.
6. Shyam C, Tripathi M K, Tiwari S, Ahuja A, Tripathi N, Gupta N. *In vitro* regeneration from callus and cell suspension cultures in Indian mustard [*Brassica juncea* (Linn.) Czern & Coss] *International Journal of Agricultural Technology.* 2021a;17(3):1095-1112.
7. Baghel R., Sharma A.K., Tiwari S., Tripathi M.K., Tripathi N. Genetic diversity analysis of Indian mustard (*Brassica spp.*) germplasm lines using SSR molecular markers. *Int J Curr Microbiol App Sci.* 2020; 9 (12): 137-143.
8. Rajpoot NS, Tripathi MK, Tiwari S, Tomar RS, Kandalkar VS. Characterization of Indian mustard germplasm on the basis of morphological traits and SSR markers. *Curr J Appl Sci Technol.* 2020; 39:300-311.
9. Kumar S, Saharan GS, Singh D. (): Inheritance of resistance in inter and intraspecific crosses of *Brassica juncea* and *Brassica carinata* to *Albugo candida* and *Erysiphe cruciferarum*. *Journal of Mycology and Plant Pathology (India)*, 2002; 32: 59-63.
10. Shyam C, Tripathi MK, Tiwari S, Tripathi N. Genetic components, and diversity analysis in Indian mustard [*Brassica juncea* (Linn.) Czern & Coss] based on different morpho-physiological traits. *Current Journal of Applied Science and Technology.* 2021b;40 (20):34-57. DOI: [10.9734/CJAST/2021/v40i2031462](https://doi.org/10.9734/CJAST/2021/v40i2031462)
11. Shrivastava A, Tripathi MK, Tiwari S, Singh P, Tripathi N, Tiwari PN., Parihar P, Singh J, Chauhan S. Screening of Indian mustard genotypes against white rust disease based on disease indexing. *Biological Forum – An International Journal*, 2023c; 15(4): 268-272
12. Thakur A K, Singh KH, Parmar N, Sharma D, Mishra DC, Singh L., ... & Yadav S. Population structure and genetic diversity as revealed by SSR markers in Ethiopian

- mustard (*Brassica carinata* A. Braun): a potential edible and industrially important oilseed crop. *Genetic Resources and Crop Evolution*, 2021; 68, 321-333.
13. Shyam C, Tripathi MK, Tiwari S, Tripathi N, Solanki RS, Sapre S, Ahuja A, Tiwari S. *In vitro* production of somaclones with decreased erucic acid content in Indian mustard [*Brassica juncea* (Linn.) Czern & Coss. *Plants*. 2021c; 10:1297. Available: <https://doi.org/10.3390/plants10071297>
  14. Shrivastava A, Tripathi MK, Tiwari S, Tripathi N, Tiwari P N, Bimal SS, Rajpoot P, Chauhan S. Evaluation of genetic diversity in Indian mustard (*Brassica juncea* var. *rugosa*) employing SSR molecular markers. *Plant Cell Biotechnology and Molecular Biology*, 2023d; 24(3-4): 10-21.
  15. Ministry of Agriculture and Farmers Welfare, Government of India. Pocket book of agricultural statistics. Directorate of Economics and Statistics, Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Government of India; New Delhi, India: 2017; p. 194.
  16. USDA. Foreign Agriculture Service. United States Department of Agriculture. 2020; Available online at: <https://www.fas.usda.gov/regions/india>.
  17. Shyam C, Tripathi MK. Biochemical studies in Indian mustard [*Brassica juncea* (Linn) Czern & Coss] for fatty acid profiling. *Int. J. Chem. Stud.* 2019;7(4):338-343.
  18. Shyam C, Tripathi MK, Tripathi N, Tiwari S, Sikarwar R S. Genetic variations in fatty acids and oil compositions among 188 Indian mustard *Brassica juncea* (Linn.) Czern & Coss genotypes. *Curr J Appl Sci Technol.* 2022a;40(46):9-28 DOI: [10.9734/CJAST/2021/v40i4631629](https://doi.org/10.9734/CJAST/2021/v40i4631629)
  19. Shyam C., Tripathi M. K., Tripathi N, Tiwari S, and Sikarwar R. S. Analysis of genetic differences in fatty acids and oil contents among *Brassica juncea* (Linn.) Czern & Coss genotypes. In book: *Research Developments in Science and Technology*, 2022b;1:127-149, DOI: [10.9734/bpi/rdst/v1/6010F](https://doi.org/10.9734/bpi/rdst/v1/6010F)
  20. Shyam C, Tripathi M K, Tiwari S, Ahuja A, Tripathi N, Gupta N. Plant regeneration in Indian mustard [*Brassica juncea* (Linn.) Czern & Coss]: Experimental investigation. In book: *Current Topics in Agricultural Sciences*. 2021c;3:120-135. DOI: [10.9734/bpi/ctas/v3/2118C](https://doi.org/10.9734/bpi/ctas/v3/2118C)
  21. Shyam C, Tripathi M K, Tiwari S, Tripathi N and Sikarwar RS. Morpho-physiological

- variations and genetic components analysis in *Brassica juncea* (Linn.) Czern & Coss. In book: Research Developments in Science and Technology, 2022d;1:98-126, DOI: 10.9734/bpi/rdst/v1/6009F
22. Inturrisi F, Bayer PE, Cantila, AY, Timaz S, Edwards D, Bately, J. *In silico* integration of disease resistance QTL, genes, and markers with the *Brassica juncea* physical map. Mol Breeding 2022; 42: 37. <https://doi.org/10.1007/s11032-022-01309-5>.
  23. Bisht KS, Rana M, Gairola K. Sharma BC, Tewari AK, Awasthi R.P. Screening of *Brassica* germplasm for resistance to major diseases of rapeseed-mustard. The Bioscan 2015; 10 (4):2111-2119
  24. Mir ZA, Ali S, Tyagi A, Yadav P, Chandrashekar N, El-Sheikh MA, Alansi S, Grover A. Comparative Analysis of Powdery Mildew Disease resistance and susceptibility in *Brassica* Coenospecies. Agronomy. 2023; 13(4):1033.
  25. Dange SRS, Patel RL, Patel SI, Patel KK. Effect of planting time on the appearance and severity of White Rust and Powdery mildew diseases of mustard. Indian J.Agric. Res. 2003; 37 (2): 154-156.
  26. Nanjundan J, Manjunatha C, Radhamani J, Thakur A K, Yadav R, Kumar A. Identification of new source of resistance to powdery mildew of Indian mustard and studying its inheritance. The Plant Pathology Journal, 2020; 36(2): 111.
  27. Mohitkar SA, Mate GD, Pillai T. Screening of mustard varieties against powdery mildew caused by *Erysiphe cruciferarum*. 3rd Global Conference - Plant Pathology for Food Security; Udaipur, Rajasthan, India. Indian Society of Mycology and Plant Pathology at Maharana Pratap University of Agriculture and Technology, editor. 2012; pp. 1–224.
  28. Li Q, Li J, Sun JL, Ma, XF, Wang TT, Berkey R. Multiple evolutionary events involved in maintaining homologs of resistance to powdery mildew 8 in *Brassica napus*. Frontiers in Plant Science, 2016;7: 1065.
  29. Thakur A K, Singh K H, Singh L, Verma V, Singh D. Morphological and molecular descriptors for first Indian mustard hybrid, NRCHB 506. Indian Journal of Biotechnology 2016; 15(1):116-119.
  30. Tripathi N, Khare D. Molecular approaches for genetic improvement of seed quality and characterization of genetic diversity in soybean: a critical review. Biotechnol Lett. 2016; 38(10):1645-1654.

31. Bhawar PC, Tiwari S, Tripathi MK, Tomar RS, Sikarwar RS. Screening of groundnut germplasm for foliar fungal diseases and population structure analysis using gene based SSR markers. *Current Journal of Applied Science and Technology*. 2020;39(2): 75-84.
32. Mishra N, Tripathi MK, Tiwari S, Tripathi N, Trivedi HK. Morphological and Molecular Screening of Soybean Genotypes against yellow mosaic virus disease. *Legume Research*; 2020;DOI: 10.18805/LR-4240.
33. Thakur AK, Parmar N, Singh KH, Nanjundan J. Current achievements and future prospects of genetic engineering in Indian mustard (*Brassica juncea* L. Czern & Coss.). *Planta* 2020; 252:56.
34. Verma K, Tripathi MK, Tiwari S, Tripathi N. Analysis of genetic diversity among *Brassica juncea* genotypes using morpho-physiological and SSR markers. *Int J Curr Microbiol App Sci*. 2021a; 10(01):1108-1117.
35. Rajpoot N S, Tripathi M K., Tiwari S, Tomar R S, Tripathi N, Sikarwar R. S. Morphological and molecular characterization of Indian mustard germplasm lines. *Research Developments in Science and Technology* 2022;4: 151-165. <https://doi.org/10.9734/bpi/rdst/v4/2307B>
36. Sharma D, Nanjundan J, Singh L, Singh SP, Parmar N, Sujith Kumar M S, ... & Thakur, A K. Genetic diversity in leafy mustard (*Brassica juncea* var. *rugosa*) as revealed by agro-morphological traits and SSR markers. *Physiology and Molecular Biology of Plants*, 2020;26, 2005-2018.
37. Shyam C, Tripathi MK, Tiwari S, Tripathi N, Ahuja A. Molecular characterization and identification of *Brassica* genotype(s) for low and high erucic acid content using SSR markers. *Global J Biosci Biotechnol*. 2020;9(2):56-66.
38. Asati R, Tripathi MK, Tiwari S, Yadav RK, Tripathi N. Molecular breeding and drought tolerance in chickpea. *Life* 2022; 12: 1846. <https://doi.org/10.3390/life12111846>.
39. Sharma D, Nanjundan J, Singh L, Parmar N, Singh KH, Verma KS, Thakur AK. Genetic diversity and population structure analysis in Indian Mustard germplasm using phenotypic traits and SSR markers. *Plant Mol. Biol. Rep.* 2022, 40:579–594.
40. Shyam C, Tripathi MK, Tripathi N, Tiwari S, Sikarwar R S. Identification of low and high erucic acid containing genotype(s) in Indian mustard employing molecular markers. In book: *Recent Progress in Plant and Soil Research*, 2022c; 5: 18-36

DOI: [10.9734/bpi/rppsr/v5/15384D](https://doi.org/10.9734/bpi/rppsr/v5/15384D)

41. Tripathi N., Tripathi M.K., Tiwari S., Payasi D.K. Molecular breeding to overcome biotic stresses in soybean: update. *Plants (Basel)*. 2022;11(15):1967.
42. Yadav PK, Singh AK, Tripathi MK, Tiwari S, Yadav SK, Tripathi N. Morpho-physiological and molecular characterization of maize (*Zea Mays* L.) genotypes for drought tolerance. *European Journal of Applied Sciences*. 2022a; 10(6):65-87.
43. Yadava D K, Yadav R, Vishwakarma H, Yashpal, Yadav S, Saini N, Vasudev S. Genetic diversity characterization and population structure in *Brassica juncea*. In: Kole C., Mohapatra T. (eds) *The Brassica juncea Genome. Compendium of Plant Genomes*. 2022b; pp 73-84, Springer, Cham. [https://doi.org/10.1007/978-3-030-91507-0\\_5](https://doi.org/10.1007/978-3-030-91507-0_5)
44. Ningwal R, Tripathi MK, Tiwari S, Asati R. Yadav RK, Tripathi N, and Yasin, M. Identification of polymorphic SSR marker and diversity analysis in a set of desi chickpea genotypes, *Biological Forum-An International Journal*. 2023;15(3): 45-51.
45. Rajpoot P, Tripathi MK, Tiwari S., Bimal, S S, Tripathi N, Parihar P, Pandya R K, C. Tara Satyavathi. Characterization of pearl millet [*Pennisetum glaucum* (L.) R br.] Genotypes against blast disease employing disease scoring and gene specific SSR markers. *SCI*, 2023;3(3);16-30.
46. Tripathi MK, Tripathi N, Tiwari S, Mishra N, Sharma A, Tiwari S, Singh S. Identification of Indian soybean (*Glycine max* [L.] Merr.) genotypes for drought tolerance and genetic diversity analysis using SSR markers *SCI* 2023;3(3);2023;31-46.
47. Yadav RK, Tripathi MK, Tiwari S, Tripathi N, Asati R, Patel V, Sikarwar RS, Payasi DK. Breeding and genomic approaches towards development of Fusarium wilt resistance in chickpea. *Life* 2023a;13(4):988.
48. Yadav S , Tiwari S , Tripathi MK, Tripathi N , Gupta N , . Tiwari S. Evaluation of high oleic acid content in a set of 96 genotypes of *Arachis hypogaea* L *Scientist*, 2023;2 :132-143
49. Anonymous. AICRP on RM ICAR, Directorate of Rapeseed and Mustard, Bharatpur (Raj.). Proceedings 26<sup>th</sup> Annual group meeting held at BAU, Ranchi (Jharkhand) Aug.3-5, 2019;1- 10.
50. Murray MG, Thompson W. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, 1980; 8(19): 4321-4326.

51. Tiwari S, Tomar RS, Tripathi MK, Ahuja A. Modified protocol for plant genomic DNA isolation. *Indian Research Journal of Genetics and Biotechnology*, 2017; 9(4): 478-485.
52. Liu K, Muse SV. Power Marker integrated analysis environment for genetic marker data. *Bioinformatics*, 2005;21: 2128-2129.
53. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology Evolution*, 2007;24(8): 1596-1599.
54. Khalik KA. Morphological studies on trichomes of *Brassicaceae* in Egypt and taxonomic significance. *Acta Bot Croat.* 2005;64: 57–73.
55. Roda A, Nyrop J, English-Loeb G. Leaf pubescence mediates the abundance of non-prey food and the density of the predatory mite *Typhlodromus pyri*. *Exp Appl Acarol.* 2003; 29:193–211.
56. Kim KW. Plant trichomes as microbial habitats and infection sites. *Eur J Plant Pathol.* 2019; 154:157–169.
57. Ali S, Mir ZA, Tyagi A, Mehari H, Meena RP, Bhat JA, Yadav P, Papolu PL., Rawat S, Grover A. Overexpression of NPR1 in *Brassica juncea* confers broad spectrum resistance to fungal pathogens. *Front Plant Sci.* 2017;8:1693.
58. Parihar P, Singh P, Pandya R K, Tiwari S, Tripathi M K. (). Screening of pearl millet promising hybrids and varieties against blast (*Pyricularia grisea*) by disease indexing. *The Pharma Innov. J.*, 2022; 11(6): 664-667.
59. Parmar A, Tripathi MK, Tiwari S, Tripathi N, Parihar P and Pandya R K. (). Characterization of pearl millet [*Pennisetum glaucum* (L.) R Br.] genotypes against downey mildew disease employing disease indexing and ISSR markers. *Octa J. Biosci.* 2022;10 (2):134-142.
60. Verma R, Tripathi M K, Tiwari S, Pandya R K, Tripathi N, Parihar, P. Screening of pearl millet [*Pennisetum glaucum* (L.) R. Br.] genotypes against blast disease on the basis of disease indexing and gene specific SSR markers. *Int J Curr Microbiol Appl Sci.* 2021b; 10(02):1108-1117.
61. Ghosh S, Mazumder M, Mondal B, Mukherjee A, De A, Bose R. Morphological and SSR marker-based genetic diversity analysis of Indian mustard (*Brassica juncea* L.) differing in *Alternaria brassicicola* tolerance. *Euphytica*, 2019; 215: 1-19.

Table 1 Disease intensity scale for calculation of the percentage disease index

S. No.	Rating Scale For leaf	Leaf area covered (%)	Disease Reaction
1	0	0	immune
2	1	<5%	Highly Resistant (HR)
3	3	5-10%	Resistant (R)
4	5	11-25%	Moderately Resistant (MR)
5	7	26-50%	Susceptible (S)
6	9	>50%	Highly Susceptible (HS)

Table 2 Details of 19 Powdery mildew disease linked microsatellite markers used in present investigation

S. No.	Marker Name	Forward sequence (5'-3')	Reverse sequence (5'-3')
1	BoGMS0570	TACAATCTTCTTCGCTGCT	AAACCTGAAACTCCCTCAA
2	BoGMS0811	GCACTGTCAAATCACTCAA	ACTTCTCCCAATCTCTGTCTC
3	BoGMS0826	GCAATGATGAAGTTAGGAGAA	AACCAGCGAGAGAGAGGT
4	BoGMS0369	AGGACAGCATCGGTATGA	CTGGATAGTTTCTCTTCTTGG
5	BoGMS0687	GACAACACAACAGACGCA	GCATTTCCTTACTTCCA
6	BoGMS0977	TTGTCTTCCTTCTCTAAAC	CAGCAATAACCATCTCTCA
7	BoGMS0812	GCTGGCACATAGTTGTAATG	CTCATCTCTCTGCTGGA
8	BoGMS0112	ACTGGTCCGAATAGGTGAG	CGATAAATGGTTCAATGTCC
9	BoGMS1166	TAATGGAAACGCACCAAG	AAACAATCTTAGCAGAGGAGG
10	cnu_m073	TGGCATTGACAGAGCTAGTA	TTTATTTAGTTTCATACCCT
11	cnu_m046	GCTAAAGGTTTAGTCCAAATAGGATTC	GCAAAATGATGCCCCATAAA
12	cnu_m098	TGCGACCCAAGTAGGTGAAAC	TGTCTCTCGCTCATTCACCAA
13	cnu_m616	TCGGTACGAAACTCAATGTTC	GCGTCAGTCTGAACAAAACG
14	cnu_m617	GGAATGCTGCTGGAAGAATC	TTCAATTTGTCCCTGCATTC
15	nia_m105	GACGAAGGAGCGTATGAAA	AATGCAATCTCAACAAAGGT
16	nia_m110	GACAGTGGTTGAAAAAGCCA	CATTCAGTGAGGTTTCACCA
17	nia_m113	CAAAAAGTTGCGGTCAATCT	CCTCCAAAGCTCAATCACTG
18	nia_m138	GTTTTAAATGCCGCGTTG	GGGATCAAGAGATGTGGGA
19	MF-2	GGTTCCGTCGTTCCCATCGC	CATAATAATTAGATAAATCTGTCC

Table 3 Categorizations of reactions of *Brassica* genotypes against powdery mildew disease

Severity (%)	Disease reaction	Number of Brassica genotypes			Categorized Brassica genotypes based on pooled
		R1	R2	R3	
0	Immune	2	2	2	L-4, PC-5
<5	Highly Resistant	2	2	2	CHINA, RP-9
5.0-10	Resistant	2	2	2	GSC-7, PC-6
10.1-25	Moderately Resistant	4	4	4	RB-50, Pusa Bold, WRR-10, GSL-1
25.1-50	Susceptible	47	47	47	Varuna, Kranti, Maya, Albeli, Shraddha, DMH 1, JMWR-908-1, RGN-73, NRC-HB-101, NRC DR-2, DRMR IJ-31, KIRAN, JTC-1, JM-2, RVM-1, PM-25, PM-26, PM-27, PM-30, RMM-12-01-18, R M M -12-03-18, WRR-5, WRR-6, WRR-7, WRR-8, WRR-9, WRR-11, WRR-12, WRR-13, WRR-14, WRR-15, WRR-16, WRR-17, WRR-18, WRR-19, WRR-20, WRR-21, WRR-22, WRR-25, WRR-26, WRR-27, WRR-28, WRR-29, WRR-30, WRR-31, WRR-32, Shraddha
>50.1	Highly Susceptible	18	18	18	Rohini, RH- 725, Vardan, Vasundhara, Swarn Jyoti, Pusa Jagannath, Pusa JaiKisan, Sej-2, , NRC-HB- 506, RVM-3, RH-749, JM-3, PM-28, Pusa Vijay,JMM-927, JMM-991, RMM-10-01-01

Table 4 Details of 5 disease linked microsatellite markers exhibited consistent polymorphism among 75 mustard genotypes

S. No.	Marker	Allele No	Major Allele Frequency	Gene Diversity	PIC
1	cnu_m616	5	0.266	0.785	0.750
2	nia_m105	5	0.293	0.759	0.717
3	MF-7	5	0.333	0.731	0.682
4	MF-4	5	0.4	0.711	0.662
5	CCMP6	5	0.36	0.761	0.724
<b>Mean</b>		<b>5</b>	<b>0.330</b>	<b>0.749</b>	<b>0.707</b>
<b>SD</b>		<b>0</b>	<b>0.052</b>	<b>0.028</b>	<b>0.035</b>

UNDER PEER REVIEW



Fig. 1 Categorizations of reactions of Powdery Mildew (*Erysiphe cruciferarum*) in *Brassica* genotypes

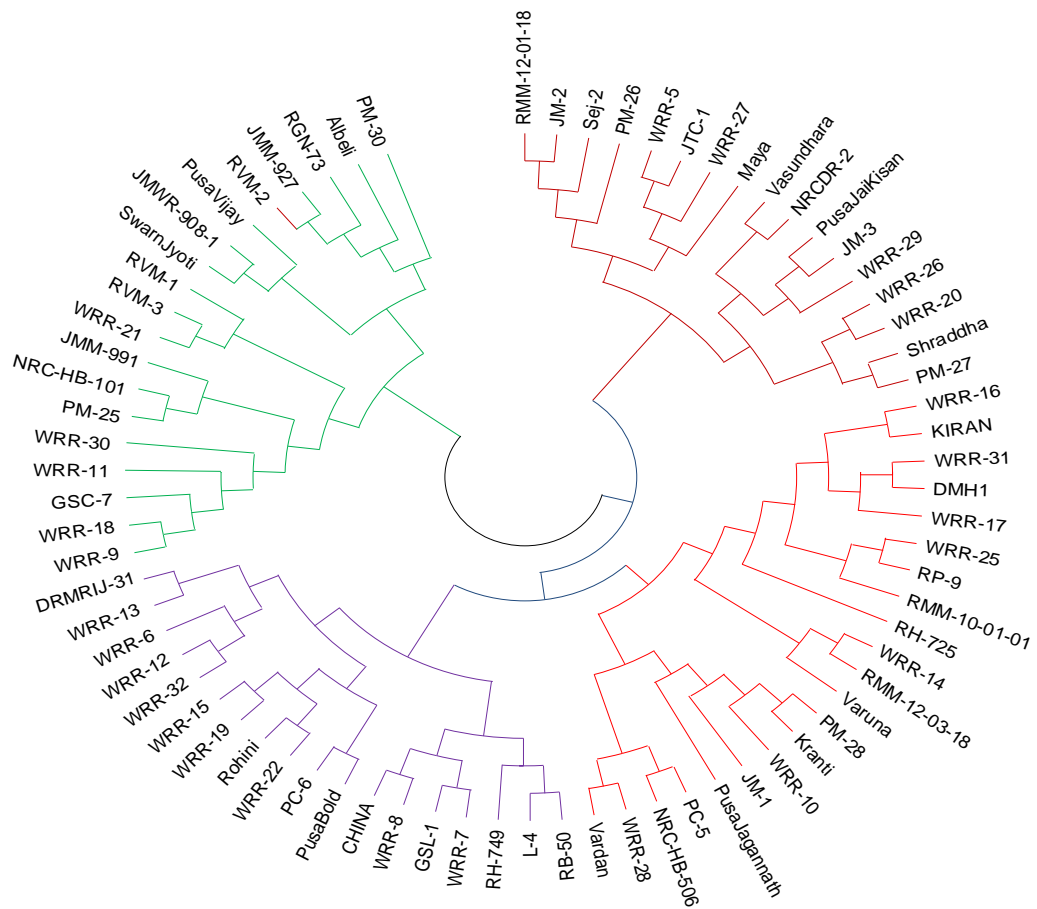


Fig.2 Neighbour Joining Tree of 75 mustard genotypes based on disease linked polymorphic microsatellite markers

UNDER