



Novel sources of cauliflower for resistance to downy mildew disease, genetic analysis and design suitable breeding strategy using advanced breeding lines



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ARTICLE INFO

Article History:

Received 10 December 2024

Revised 16 May 2025

Accepted 2 June 2025

Available online xxx

Edited by Dr P. Bhattacharyya

Keywords:

Cauliflower
Downy mildew
Resistance
Marker
Breeding
Hybrids

ABSTRACT

Cauliflower is the leading cole vegetable in India and many parts of the world. The productivity of this crop is severely affected by downy mildew disease. In the present study, advanced breeding lines including resistant and susceptible checks were phenotyped followed by genotyping was carried out using linked markers. Ten plants in each line were scored and finally Disease Index (DI) was calculated. The inheritance of resistance was carried out using chi-square (χ^2) and resistant hybrids were developed using resistant advanced lines validated through linked markers. The lowest DI was recorded in advanced line DMR-2-0-7 followed by DMR-8-4-8-1. Two resistant advanced lines viz. DMR-3-0-8 and DMR-40-5-8-1 were validated by three SCAR markers (SCR15, SCJ19, UBC 359) along with the resistant check. On the basis of the cluster analysis, the advanced lines were grouped into three clusters. The dendrogram revealed significant genetic diversity among 12 advanced lines. The segregation of downy mildew susceptible and resistant plants in F2 population of DMS-27-2-1-0-3 \times DMR-40-5-8-1 segregated in 114 resistant and 36 susceptible suggesting single dominant gene. Significantly high average curd weight (1483.33 g) was obtained in Hyb 7 (DMR-7-4-5-3 \times DMR-40-5-8-1). The highest ascorbic acid (52.67 mg 100 g FW⁻¹) was obtained in Hyb 5 (DMR-7-4-5-3 \times DMR-8-4-8-1). We have emphasized development of end product to bridge the gap between availability of genetic resources and advanced molecular breeding. The study will be very useful to the cauliflower breeder in enhancing breeding efficiency for the farmers as well as consumers.

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1. Introduction

Among the cole crops, cauliflower is leading cole vegetable in India and several parts of the world. The productivity of this crop is severely affected by downy mildew disease which is caused by the *Hyaloperonospora parasitica* Constant (Pers. Fr) Fr and the pathogen affects both young seedlings and adult plants (Jensen et al., 1999a; Varalakshmi et al., 2011; Singh et al., 2022). The pathogen causes severe damage in established crops (Verma and Thakur, 1989; Saha et al., 2020) and postharvest spoilage of cauliflower curds (Lund and Wyatt, 1978; McKay et al., 1992). The disease attacks different other Brassica crops and it spreads more where cool and humid weather prevails (Vicente et al., 2012; Saha et al., 2020). Presently, it is

prevalent in almost all the states of India and many other countries wherever cruciferous crops are grown with 75–90 % seedlings mortality (Carlier et al., 2012; Verma and Singh, 2018; Saha et al., 2020; Shaw et al., 2021; Zhang et al., 2023; Wu et al., 2024). Under congenial conditions, 50–60 % loss occurs in cole crops seed production. Initially, yellowish brown or purplish spot appears on upper leaf surface with cottony mycelial growth on lower leaf surface (Fig. 1). In curds, whitish mycelial growth occur which causes dis-colouration and deformation leading to huge crop losses to the farmers (Dickson and Petzoldt, 1993; Singh et al., 2015).

A numbers of sprays are given by farmers to control the disease but these are ineffective in controlling this oomycete pathogen (Vicente et al., 2012). The indiscriminate use of toxic chemicals causes environmental pollution and health hazards (Wu et al., 2024). Fungicides provide some control against downy mildew in cauliflower, but the development and growing of resistant variety (s)/hybrid (s) offers

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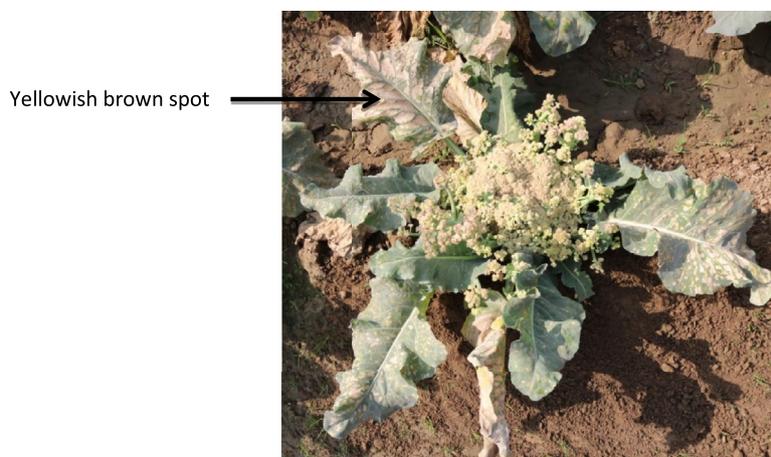


Fig. 1. Symptom of downy mildew disease in cauliflower.

a more practical and long-term solution for more effective disease management. As the use of fungicidal control has many limitations, breeding for genetic resistance presents a viable and sustainable alternative. The use of downy mildew resistant cultivars is an integral component of cost effective and safer disease management strategy. The availability of natural sources of resistance has the potential to reduce the use of fungicides and to protect environment and consumer's/farmer's health. Breeding for downy mildew resistant cultivars development is very challenging because of the obligate nature of the casual organism. Therefore, there has been a continuous effort to find new sources of resistance that can be deployed in resistant variety (s)/hybrid (s) development (Sivasithamparam et al., 2005; Stuthman et al., 2007; Zhang et al., 2009; Saha et al., 2016). Multiple sources including sister lines resistant to downy mildew have been identified in cauliflower, cabbage and other *B. oleracea* and related crops (Pandey et al., 2001; Carlsson et al., 2004; Singh et al., 2013; Vicente et al., 2012, 2015; Verma and Singh 2018; Saha et al., 2020; Singh et al., 2022; Wu et al. (2024). Depending on resistant sources and growth stages, inheritance of downy mildew resistance is reported to be monogenic in broccoli in true leaf stage (Wang et al., 2001), single recessive gene at the four to five leaf stage in broccoli (Hoser-Krauze et al., 1987), single dominant gene at maturity stage in broccoli and cauliflower (Coelho and Monteiro, 2003; Vicente et al., 2012; Singh et al., 2013; Verma and Singh, 2018; Saha et al., 2020; Singh et al., 2022), monogenic recessive in cauliflower (Hoser-Krauze et al., 1984), in Savoy cabbage, kale and Brussels sprouts as recessive gene (s) (Carlsson et al., 2004) and additive genes in cabbage, cauliflower and broccoli (Moss et al., 1988; Hoser-Krauze et al., 1995; Jensen et al., 1999b).

In the recent past, sequence characterized amplified regions (SCAR) (Farinho' et al., 2007), random amplified polymorphic DNA (RAPD) (Farinho' et al., 2004; Singh et al., 2013), and simple sequence repeats (SSR) (Saha et al., 2020) markers associated with cauliflower downy mildew resistance have been developed. A downy mildew resistant locus *BoDMR2* was mapped to 300 kb interval on chromosome 7 of cabbage using BSA-seq and linkage analysis (Wu et al., 2024). In *B. rapa*, a candidate resistant WAK gene (*BrWAK1*) was identified for major resistant quantitative trait locus by Zhang et al. (2023). Emphasis has been given to find out the gene (s) and the molecular marker (s) which can be used to identify resistance sources. To develop cauliflower variety (s)/hybrid (s) with good horticultural traits and resistance to downy mildew, it is necessary to incorporate resistance from diverse sources against prevalent pathogen isolates. In cauliflower, hybrids are very popular, and the resistant hybrids will provide more income to the farmers. Therefore, in the present study, phenotypic and genotypic responses of advanced

breeding lines of cauliflower were studied. The inheritance of resistance was identified and resistant hybrids were developed with higher yield and good quality.

2. Materials and methods

2.1. Advanced breeding lines development

Plant materials consisted of 48 advanced breeding lines and one each of susceptible (Pusa Himjyoti) and resistant check (CCm-5). The details of the advanced lines and their pedigree, generation status are presented in Table 1. The downy mildew resistant parental lines used for development of advanced lines were previously reported by Singh et al. (2013). The resistant lines were crossed with the susceptible commercial varieties, inbred lines to develop F_1 generation. In some of the combinations, backcrosses were attempted to develop BC_1F_1 generation. Selfing/sib-mating was carried out from F_1 or BC_1 generations till the lines become homozygous and uniform. The developmental scheme of one advanced breeding line (DMR-3-0-8) is given in Fig. 2. Hence, all these 48 lines were derived from different cross combinations, we called them advanced breeding lines as downy mildew resistant (DMR) and downy mildew susceptible (DMS). One each of susceptible (Pusa Himjyoti) and resistant check (CCm-5) were also tested with the advanced breeding lines. The seeds of all the lines were sown in September at the research farm of Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India. From each of the advanced lines, resistant and susceptible checks, 30 seedlings at 30 days old were transplanted in augmented design with three replications in the main field. The distance among plants and rows were kept as 60 and 60 cm, respectively. The recommended cultivation practices were followed for raising the crop as per Thamburaj and Singh (2003).

2.2. Downy mildew inoculum preparation and disease phenotyping

In the main field, the downy mildew disease in susceptible lines was confirmed by observing disease symptoms and the morphology of *H. parasitica*. The infected leaves were collected from the susceptible plants and washed using sterile water. It is then filtered through muslin cloth to remove plant parts. The spore concentration of the pathogen was determined using a haemocytometer and adjusted to spore suspension of *H. parasitica* at 5×10^3 spores ml^{-1} . Each advanced lines were inoculated using a pneumatic knapsack sprayer at 15 days after transplanting when the plants are established in field (45 days old plant). Ten plants in each line were scored on 0–9 scale and finally Disease Index (DI) was calculated as per Shuancang et al.

Table 1
Screening of advanced breeding lines of cauliflower resistance to downy mildew.

Sl No	Progressive lines (Code)	Pedigree/cross combinations	Generation status	Salient characteristics
1.	DMS-1-0-6	3-5-1-1 x DC-466 x 3-5-1-1	BC1F6	Curds compact, medium, white, flower colour white
2.	DMR-2-0-7	3-5-1-1 x DC-466	F7	Curds compact, small, flower colour white
3.	DMR-3-0-8	3-5-1-1 x Improved Japanese	F6	Leaves dark green wavy, prominent midrib curd compact, medium, white
4.	DMS-4-2-4-1	309 X BR-2	F7	Curds compact, medium, creamish, flower yellow
5.	DMS-5-2-9-5-1	309 X BR-2 x 309	BC1F5	Curds loose, small, creamish
6.	DMR-6-2-2-6-2	309 X BR-2 X BR-2	BC1F5	Leaves broad, wavy, narrow tip, curds loose, small, creamish
7.	DMR-7-4-5-3	309 X Lawyana	F9	Curds compact, medium, creamish
8.	DMR-8-4-8-1	Pusa Himjyoti X BR-2	F7	Leaves broad with round tips, curds compact, small, cream yellow
9.	DMS-9-4-8-3	Pusa Himjyoti X BR-2 X Pusa Himjyoti	BC1F6	Curds loose, small, white
10.	Pusa Himjyoti (susceptible check)	Parent	Inbred line	Bluish green leaves with waxy coating, curds compact large, white
11.	DMS-11-4-0-4	Pusa Himjyoti X 401	F6	Curds compact, medium, white
12.	DMS-12-4-0-9-2	Pusa Himjyoti X 409	F8	Curds loose, small, white
13.	DMS-13-16-4-2-1	Pusa Himjyoti x BR-161	F8	Curds loose, small, creamish
14.	DMS-14-20-0-4-1	Pusa Himjyoti x BR-207	F8	Curds loose, small, creamish
15.	DMS-15-4-4-2	Pusa Himjyoti x Pusa Sharad	F8	Curds compact, large, white
16.	DMS-16-1-4-0-1	Pusa Himjyoti x BR-161 x Pusa Himjyoti	BC1F7	Curds compact, large, white
17.	DMS-17-16-4-1-2-4	Pusa Himjyoti x 161 x 161	BC1F7	Curds loose, medium, creamish
18.	DMS-18-0-12-4-1-3	Pusa Himjyoti x CCm x Pusa Sharad	DC1F7	Curds compact, medium, white
19.	DMS-19-1-4-2	Pusa Himjyoti x BR-161 x 401	DC1F7	Curds compact, medium, white
20.	DMS-20-16-2-1-4	Pusa Sharad x BR-161	F8	Curds loose, medium, creamish
21.	DMS-21-7-0-2-1	Pusa Sharad x BR-207	F8	Curds loose, medium, creamish
22.	DMR-22-2-0-7-5-4	Pusa Sharad x BR-207 x 207	BC1F7	Curds loose, small, creamish
23.	DMS-23-2-0-7-1-2-3	Pusa Sharad x BR-207 x Pusa Sharad	BC1F7	Curds compact, large, creamish
24.	DMS-24-9-0-4	Improved Japanese x BR-2 x 309	DCF7	Curd compact, large, creamish
25.	DMS-25-0-4-1-5	Improved Japanese x Pusa Himjyoti	F8	Curds loose, large, creamish
26.	DMS-26-4-23-10-1	Improved Japanese x 309	F6	Curds compact, medium, creamish
27.	DMS-27-2-1-0-3	Improved Japanese x BR-2	F7	Curds loose, large, creamish
28.	DMR-28-7-3-1-0-1	Pusa Deepali X BR-2	F8	Curds loose, small, creamish
29.	DMS-29-2-2-1	CC X DC-522	F6	Curds loose, small, creamish
30.	DMS-30-2-2-1-2	BR-2 x DC-522	F9	Curds loose, small, creamish
31.	DMR-31-0-2-6-4	BR-2 x Lawyana x DC-522	DC1F6	Curds compact, large, creamish
32.	DMS-32-7-1-2	BR-204 x Improved Japanese	F7	Curds creamish, small, compact
33.	DMS-33-4-1-0-7	BR-36 x Lawyana x DC-522	DC1F8	Curds loose, small, creamish
34.	DMR-34-1-5-7	BR-14 x Lawyana x 41-5	DC1F7	Curds loose, small, creamish
35.	DMR-35-5-1-4	AI-15 x Lawyana x 41-5	DC1F6	Curds loose, small, creamish
36.	DMS-36-1-2-5	SR-17 x Lawyana x 41-5	DC1F7	Curds loose, small, creamish
37.	DMR-37-2-1-5	CCm x BR-2	F6	Curds small, compact, yellowish
38.	DMR-38-1-7-0-3	CCm 5 x 3-5-1-1	F7	Curds compact, white medium
39.	CCm-5 (resistant check)	Parent	Inbred line	Curds compact, smalls creamish
40.	DMR-40-5-8-1	CCm-5 x DC-33-8	F8	Leaves are narrow, bluish green, curds loose, medium, white
41.	DMR-41-8-3-4-5	CCm-8 x 3-5-1-1	F8	Curds loose, small, creamish
42.	DMS-42-8-0-2	CCm-8 x HRM	F7	Curds loose, small, creamish
43.	DMS-43-2-0-2-1	476 x BR-202	F8	Curds compact small, creamish
44.	DMS-44-3-0-7	476 x BR-203-7	F6	Curds compact, medium, creamish
45.	DMS-45-5-1-1	HR-6-5-1 x BR-161	F6	Curds compact, large, white
46.	DMS-46-1-6-0-1	401 x BR-161	F7	Curds compact, small, white
47.	DMR-47-5-1-0	Kn-81 x 41-5	F7	Leaves are upright, curds loose, small, creamish
48.	DMS-48-1-8-3	Kn-81 x DC-481	F9	Curds loose, small, creamish
49.	DMR-49-1-8-5	Kn-81 x Pusa Sharad	F9	Curds loose, small, creamish
50.	DMR-50-1-8-14-2	BR-14 x Lawyana x 41-5	DC1F7	Curd loose, small, yellowish

DMS: Downy mildew susceptible; DMR: Downy mildew resistant; BC: Backcross; DC: Double cross.

(2009). During screening period, the weather parameters were mean monthly (November-December) temperature (21–15 °C), relative humidity (72–74%) which were congenial for the pathogen.

2.3. SCAR and SSR marker validation in the advance lines

Among the 48 advanced lines, 12 lines were selected based on disease reaction for genotyping using markers. The genomic DNA was isolated from the freshly collected young terminal leaves from selected advanced lines including susceptible (Pusa Himjyoti) and resistant check (CCm-5) using CTAB method (Murray and Thompson, 1980) with minor modification. The quantification of DNA was done by running samples on 0.8% agarose gels along with 1 µl of uncut lambda DNA (50 ng/µl) (Thermo Scientific, Mumbai, India).

For PCR analysis, final concentration was made of 25–50 ng µl⁻¹. Previously reported downy mildew linked SCAR, SSR and RAPD markers were used for genotyping of the selected advanced lines and checks and the details of the markers are given in Table 2. The synthesis of primers was done by Integrated DNA Technologies, Inc., Coralville, USA. All the PCR components were obtained from Thermo Fisher Scientific, USA. PCR amplifications were carried out in a total volume of 25 µl containing 25–30 ng of genomic DNA using thermal cycler Biorad, USA as per Williams et al. (1990). The amplified products of SSR were separated by electrophoresis in 3.5% agarose gel (Lonza, Switzerland) containing Ethidium Bromide (10 mg/ul). The gel was run in 1 X TAE buffer (pH 8.0) at a constant voltage of 100 V per cm for 3 h using 100 bp ladder (Gene-DireX, Taiwan) for estimation of band size. Gel pictures were taken

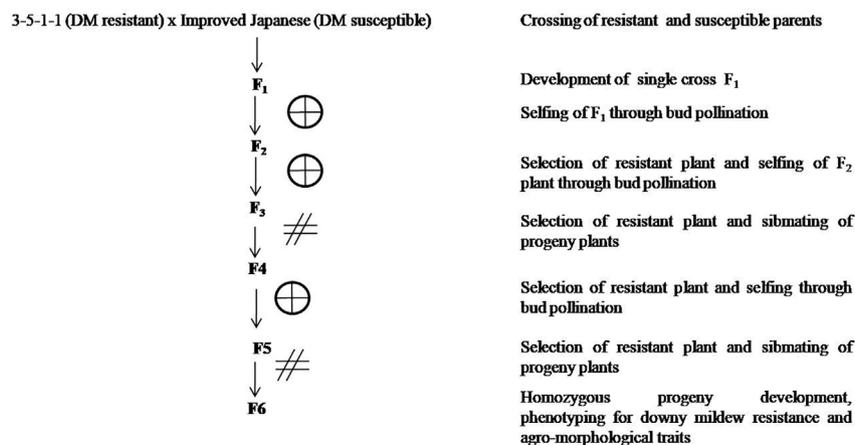


Fig. 2. Schematic representation of the advance line development.

Table 2

List of SCAR, SSR and RAPD markers and their sequence used for validation of downy mildew resistance gene.

Sl No	Primer code	Primer	Expected band size/range	Forward	Reverse	Refs.
1	SCR15 (OPR.15_920)	SCAR	920 bp	GGACAACGAGGAAAAGATG	GGACAACGAGGGACCGTGG	Farinho, 2007
2	OPM16	SCAR	750	GTAACCAGCCCTTGTAAC	GTAACCAGCCTTGAGCCCA	Janel et al., 2002
3	SCJ19 (OPJ.19_550)	SCAR	500	CCGGAACAAGTGGACCGAAAT	GGACACCCTCATCACTAG	Farinho, 2007
4	UBC359	SCAR	620 bp	AGGCAGACCTAAGGTAGACAAGTATTGTAG	AGGCAGACCTATGAGCACTCTAGAGTTATA	Janel et al., 2002
5	OI12G04	SSR	100–161	CGAACATCTTAGCCGAATC	GGTTAACCTGCGGGATATTG	Yu et al., 2009
6	OPK17_980	RAPD	980	CCCAGCTGTG		Farinho et al., 2004

using a Gel Documentation System (Alpha Innotech, California, USA).

2.4. Molecular characterization and diversity analysis of advanced lines

Polymorphism was recorded as presence or absence from distinct reproducible SCAR/SSR/RAPD bands obtained from all 6 markers across all the 12 advanced lines and 2 checks. The binary data matrices were entered into the Darwin v6.0.021 software program. Cluster analysis was conducted employing 1000 bootstraps using unpaired group mean arithmetic and the Jaccard dissimilarity coefficient to assess genetic relationships among the lines (Perrier and Jacquemoud-Collet, 2006). Genetic diversity was quantified by calculating Nei's gene diversity (H_e) for each marker locus using allele frequency data derived from the binary matrix. Nei's genetic diversity reflects the probability that two randomly chosen alleles from the population are different. The formula for Nei's gene diversity (Nei, 1973) is:

$$H_e = 1 - \sum_{i=1}^k p_i^2$$

Where:

H_e is the expected heterozygosity,
 p_i is the frequency of the i^{th} allele at a locus,
 k is the total number of alleles at that locus

The genetic diversity calculations were performed using a Python script, and the mean Nei's genetic diversity values were used to interpret the overall genetic variation among the tested lines. PCA of genotyping data was done using python code (pandas, sklearn, decomposition, matplotlib.pyplot and mpl_toolkits.mplot3d).

2.5. Genetic analysis of resistance

Crosses were attempted among one highly susceptible advanced line (DMS-27-2-1-0-3) and two resistant lines (DMR3-0-8 and

DMR40-5-8-1). The resistant lines were selected based on phenotyping data and validated through linked marker. The number of resistant and susceptible plants in parents, F_{1s} (30 plants) and F_{2s} (150 plants) and backcross generation (60 plants) were counted. The goodness of fit for the observed F_2 and backcross ratio with the expected Mendelian ratio was tested using chi-square (χ^2) with a significance level of 0.05 (or 5%) (Panse and Sukhatme, 1967).

2.6. Development of hybrid combination for commercial cultivation

Five advanced lines were selected based on their desirable horticultural traits and disease resistance and crossed in half diallel combinations to develop hybrids. These hybrids were evaluated for various yield related traits like plant height, days to 50% curd maturity, marketable curd weight, curd yield, downy mildew resistance and different biochemical parameters viz. total phenolics, and ascorbic acid for their suitability to consumer acceptance.

2.7. Statistical analysis

The replicated data for yield and quality traits were analyzed through Web Agri Stat Package (Jagan et al., 2004) and the Chi square analysis were performed in WINDOWSTAT software. The genotyping data was subjected to PCA using python code (pandas, sklearn, decomposition, matplotlib.pyplot and mpl_toolkits.mplot3d).

3. Results

3.1. Disease appearance in advanced lines and phenotyping of plants

The advanced lines developed from different cross combination were found to be uniform in morphological characters after a period of selfing/sibmating and selection (Fig. 3). All the 50 lines including susceptible and resistant checks were evaluated after artificial inoculation. Thirty plants of each line were phenotyped and scored in 0



Fig. 3. A. Phenotype of advanced lines. B. DMR-3-0-8 (3-5-1-1 x Improved Japanese); C. DMR-8-4-8-1 (Pusa Himjyoti x BR-2); D. DMR-40-5-8-1 (CCm-5 x DC-33-8); E. DMR-47-5-1-0 (Kn-81 x 41-5).

to 9 scale at 30 days after inoculation. A very high infestation was noted in the susceptible advanced lines under study. The disease score, disease incidence, disease reaction of each line and group is presented in Table 3. In the resistant check CCm-5, all the plants did not show any downy mildew symptoms and scored as 0 rating. Among the advanced lines, all plants had 0 score (no symptom) in DMR-2-0-7 whereas all the plants showed 9 score in DMS-33-4-1-0-7, DMS-46-1-6-0-1. Based on the scoring data, DI was calculated and the highest DI was observed in DMS-33-4-1-0-7 (100 %) and DMS-46-1-6-0-1 (100 %) followed by DMS-19-1-4-2 (96.29 %), DMS-29-2-2-1 (95.55 %). The lowest DI was recorded in DMR-2-0-7 followed by DMR-8-4-8-1, DMR-41-8-3-4-5, DMR-40-5-8-1 and DMR-3-0-8. The advanced lines showed wide variations for DI value and these lines were categorized in five groups (Table 4). The first group consisted of twelve advanced resistant lines including resistant check CCm-5. The advanced lines in this group showed DI value ranging from 0 to 9.62. The second group showing moderately resistance to downy mildew consisted of 6 advanced lines with DI value ranging from 13.33 to 24.44. In third group, there was only one advanced line DMS-24-9-0-4 which showed DI value of 46.67. Seventeen advanced lines were placed in the fourth reaction groups with DI value ranging from 50.37 to 74.81. The remaining 14 lines including susceptible check Pusa Himjyoti were placed in the fifth reaction group because of high disease appearance. Among the 50 lines, DMS-46-1-6-0-1 and DMS-33-4-1-0-7 had 100 % DI value which belong to group 5.

3.2. Validation of linked marker and reproducibility in advanced lines

Twelve selected representative advanced lines and each of resistant and susceptible checks were subjected to marker analysis using previously reported markers associated with downy mildew resistance (Table 2). The amplification of specific band in the tested lines is given in Fig. 4 and Table 5. The presence and absence of specific band is denoted as + (validated) and – (not validated), respectively. The SCAR marker SCR15 (OPR.15_920) amplified DNA fragments of 920 bp size in resistant check (CCm-5), in three resistant advance lines (DMR-31-0-2-6-4, DMR-3-0-8, DMR-40-5-8-1), 1 moderately resistant advance line (DMR-7-4-5-3) and 1 susceptible advance line (DMS-33-4-1-0-7) and 1 highly susceptible advance line (DMS-15-4-4-2). Additionally, this marker amplified 300 bp fragment in all the fourteen lines including checks. The marker SCJ19 (OPJ.19_550) produced 500 bp fragment size in five advanced resistant lines including resistant check (DMR-31-0-2-6-4, DMR-8-4-8-1, DMR-3-0-8, DMR-40-5-8-1, CCm-5), in 1 Moderately resistant advanced line (DMR-7-4-5-3), three susceptible advanced lines (DMS-33-4-1-0-7, DMS-9-4-8-3, DMS-46-1-6-0-1), 2 highly susceptible advanced lines (DMS-10-5-2-1, DMS-15-4-4-2). The SCAR marker, UBC359 amplified 620 bp in all the advanced lines except susceptible check Pusa Himjyoti, DMR-8-4-8-1, DMS-27-2-1-0-3. Additionally, this marker amplified 360 bp size in all the advanced lines. The marker OI12G04 amplified 150 bp fragment in 12 lines including susceptible check (Pusa Himjyoti) and 170 bp fragment in advanced lines DMR-40-5-8-1 and

Table 3
Screening of advanced breeding lines of cauliflower resistance to downy mildew.

Sl. No	Code	No. of plant screened	Disease score						DI	Reaction	Group
			0	1	3	5	7	9			
1	DMS-1-0-6	30	0	0	0	12	10	8	74.81	S	4
2	DMR-2-0-7	30	30	0	0	0	0	0	0	R	1
3	DMR-3-0-8	30	25	5	0	0	0	0	1.85	R	1
4	DMS-4-2-4-1	30	0	0	0	13	10	7	73.33	S	4
5	DMS-5-2-9-5-1	30	0	0	0	17	9	4	68.15	S	4
6	DMR-6-2-2-6-2	30	14	16	0	0	0	0	5.92	R	1
7	DMR-7-4-5-3	30	8	10	2	10	0	0	24.44	MR	2
8	DMR-8-4-8-1	30	0	29	1	0	0	0	0.37	R	1
9	DMS-9-4-8-3	30	0	0	0	20	5	5	68.51	S	4
10	Pusa Himjyoti	30	0	0	0	0	0	30	100	HS	5
11	DMS-11-4-0-4	30	0	0	0	1	2	20	89.63	HS	5
12	DMS-12-4-0-9-2	30	0	0	0	6	4	20	85.15	HS	5
13	DMS-13-16-4-2-1	30	0	0	0	3	2	25	94.07	HS	5
14	DMS-14-20-0-4-1	30	0	0	4	1	20	5	74.81	S	4
15	DMS-15-4-4-2	30	0	0	0	1	8	21	92.59	HS	5
16	DMS-16-1-4-0-1	30	0	0	3	12	9	6	68.88	S	4
17	DMS-17-16-4-1-2-4	30	0	0	0	0	4	26	97.03	HS	5
18	DMS-18-0-12-4-1-3	30	0	0	0	3	7	20	90.37	HS	5
19	DMS-19-1-4-2	30	0	0	0	1	3	26	96.29	HS	5
20	DMS-20-16-2-1-4	30	0	0	3	14	7	6	67.40	S	4
21	DMS-21-7-0-2-1	30	0	0	0	13	10	7	73.33	S	4
22	DMR-22-2-0-7-5-4	30	0	17	10	3	0	0	22.96	MR	2
23	DMS-23-2-0-7-1-2-3	30	0	0	0	11	15	4	72.59	S	4
24	DMS-24-9-0-4	30	0	0	18	8	2	2	46.67	MS	3
25	DMS-25-0-4-1-5	30	0	0	0	6	4	20	88.15	HS	5
26	DMS-26-4-23-10-1	30	0	0	0	2	8	20	91.11	HS	5
27	DMS-27-2-1-0-3	30	0	0	0	20	7	3	65.18	S	1
28	DMR-28-7-3-1-0-1	30	10	18	2	0	0	0	8.89	R	1
29	DMS-29-2-2-1	30	0	0	0	1	4	25	95.55	HS	5
30	DMS-30-2-2-1-2	30	0	0	0	3	1	26	94.81	HS	5
31	DMR-31-0-2-6-4	30	4	26	0	0	0	0	9.62	R	1
32	DMS-32-7-1-2	30	0	0	1	14	9	6	70.37	S	4
33	DMS-33-4-1-0-7	30	0	0	0	0	0	30	100	HS	5
34	DMR-34-1-5-7	30	1	23	6	0	0	0	15.18	MR	2
35	DMR-35-5-1-4	30	2	24	4	0	0	0	13.33	MR	2
36	DMS-36-1-2-5	30	0	0	0	5	24	1	74.81	S	4
37	DMR-37-2-1-5	30	12	18	0	0	0	0	6.67	R	1
38	DMR-38-1-7-0-3	30	14	16	0	0	0	0	5.92	R	1
39	Ccm-5	30	26	4	0	0	0	0	1.48	R	1
40	DMR-40-5-8-1	30	25	5	0	0	0	0	1.85	R	1
41	DMR-41-8-3-4-5	30	27	2	1	0	0	0	1.85	R	1
42	DMS-42-8-0-2	30	0	0	0	25	5	0	59.26	S	4
43	DMS-43-2-0-2-1	30	0	0	0	27	3	0	57.78	S	4
44	DMS-44-3-0-7	30	0	0	0	15	7	8	50.37	S	4
45	DMS-45-5-1-1	30	0	0	0	8	18	4	74.81	S	4
46	DMS-46-1-6-0-1	30	0	0	0	0	0	30	100	HS	5
47	DMR-47-5-1-0	30	0	13	17	0	0	0	23.70	MR	2
48	DMS-48-1-8-3	30	0	0	0	23	7	0	60.74	S	4
49	DMR-49-1-8-5	30	0	16	14	0	0	0	21.48	MR	2
50	DMR-50-1-8-14-2	30	1	18	12	0	0	0	20.0	MR	2

DI: Disease Index.

resistant check CCm-5. The marker OPK17_980 amplified 450 bp in all the tested lines but did not amplify the resistant specific 980 bp fragment. The marker OPM16 amplified 750 bp fragment in only two lines DMS-10-5-2-1 and DMS-14-20-0-4-1. It amplified 175 bp fragment in all the tested lines.

3.3. Diversity analysis of advance lines including checks and PCA

On the basis of the cluster analysis, the advanced lines were grouped into three clusters (Fig. 5). The dendrogram revealed significant genetic diversity among 12 advanced lines. The cluster 1 consisted of two susceptible advanced lines (DMS-46-1-6-0-1 and DMS-9-4-8-3). Cluster 2 was divided into 2 subgroups 2a and 2b which indicated further genetic differentiation within this cluster. Cluster subgroup 2a was consisted of two susceptible advanced lines (DMS-10-5-2-1, DMS-33-4-1-0-7) and one resistant advanced line DMR-31-0-2-6-4 and subgroup 2b consisted of resistant

advanced line DMR-8-4-8-1 and susceptible check Pusa Himjyoti. Cluster 3, the largest group, showed considerable genetic variability. Subgroup 3a consisted of one susceptible advanced line DMS-15-4-4-2 and one resistant advanced line DMR-27-2-1-0-3 whereas subgroup 3b consisted of three resistant advanced lines DMR-40-5-8-1, DMR-3-0-8 and DMR-28-7-3-1-0-1 and resistant check CCm-5. The solitary position of DMR-7-4-5-3 showed distinct cluster.

The 3D Principal Component Analysis (PCA) plot was generated to assess the genetic relationships among the advanced breeding lines and checks based on molecular marker data (Fig. 6). The resistant check (CCm-5) and resistant advanced lines (DMR-3-0-8, DMR-40-5-8-1, DMR-28-7-3-1-0-1) were grouped closely in the 3D space. The susceptible check (Pusa Himjyoti) and advanced susceptible lines (e.g., DMS-10-5-2-1, DMS-33-4-1-0-7, DMS-9-4-8-3) also clustered together. Some other lines, such as DMR-27-2-1-0-3, DMS-15-4-4-2, and DMR-31-0-2-6-4, occupied

Table 4
Grouping of advanced lines based on DI value after challenged inoculation.

Group	Number of lines	DI vale range (%)	Categorization
1	12*	0–9.62	R
2	6	13.33–24.44	MR
3	1	46.67	MS
4	17	50.37–74.81	S
5	14**	85.15–100	HS

* Including resistant parent check (Ccm);

** including susceptible parent check (Pusa Himjyoti)

DI: Disease Index; R: Resistant; MR: Moderate resistant; MS: Moderate susceptible; S: Susceptible; HS: Highly resistant.

intermediate positions in the plot. DMR-7-4-5-3 appeared as a solitary in the dendrogram.

The first three principal components (PC1, PC2, and PC3) accounted for 29.55 %, 21.75 %, and 15.49 % of the total variance, respectively (Fig. 7). The average Nei’s genetic diversity across all polymorphic markers was calculated to be 0.2495.

3.4. Genetic analysis of resistance

Based on the validation of markers in advanced lines, one susceptible advanced line (DMS-27-2-1-0-3) and two resistant advanced lines (DMR-3-0-8 and DMR-40-5-8-1) were crossed to develop F₁, F₂, BC₁ and BC₂ populations and the genetics of resistance was studied. There was no sterility in crosses of susceptible and resistant parents and all the crosses were successful. All the F₁s (S × R) plants were resistant to downy mildew disease (Table 6). The goodness of fit was used to calculate χ^2 for 3:1 ratio with 5 % probability value. In the cross of DMS-27-2-1-0-3 × DMR-3-0-8, the F₂ population segregated into 111 (DMR): 39 (DMS) which were in close agreement with the expected 3:1 ratio, with high degree of confidence (P = 0.40). The backcross generations with the susceptible parent (DMS-27-2-1-0-3) showed segregation of resistant (34) and susceptible (36) plants. The segregation of downy mildew susceptible and resistant plants was also studied in F₂ population of DMS-27-2-1-0-3 × DMR-40-5-8-1 and the perusal of data revealed that plants segregated in 114 (DMR) and 36 (DMS) with very high confidence (P = 0.67). The backcross with susceptible parent (DMR-

14-20-0-4-1) showed the expected ratio of 1 (DMR):1 (DMS) (Table 5).

3.5. Development and evaluation of resistant hybrids for commercial cultivation

A total of five resistant and morphologically superior advanced lines were selected and 10 hybrid combinations were developed in half diallel cross (Fig. 8). These hybrids were evaluated for 2 consecutive years for yield and quality parameters in replicated trial and pooled data is presented in Table 7. There was variation in days to 50 % curd maturity. Among the hybrid combinations, the Hyb 4 (DMR 31-0-2-6-4 × DMR-40-5-8-1) was earliest which took only 55 days for curd maturity from transplanting. The hybrid Hyb 5 (DMR-7-4-5-3 × DMR-8-4-8-1) took 64.33 days for marketable curd maturity. During the experiment, the hybrids were evaluated for marketable curd weight, total curd yield hectare⁻¹. Significantly, high average curd weight (1483.33 g) was obtained in Hyb7 (DMR-7-4-5-3 × DMR-40-5-8-1). Total curd yield hectare⁻¹ (54.94 t ha⁻¹) was found to be highest in Hyb 7 (DMR-7-4-5-3 × DMR-40-5-8-1) and lowest yield (30.62 t ha⁻¹) was obtained in Hyb 6 (DMR-7-4-5-3 × DMR-3-0-8). The ascorbic acid and phenolics content of curd were significantly influenced by the hybrid combinations. The highest ascorbic acid (52.67 mg100 g FW⁻¹) was obtained in Hyb 5 (DMR-7-4-5-3 × DMR-8-4-8-1) followed by Hyb 1 (DMR 31-0-2-6-4 × DMR-7-4-5-3) (52 mg 100 g FW⁻¹). The phenolics content in curd varied from 26.86 to 42.45 mg100 g FW⁻¹.

4. Discussion

Downy mildew is one of the devastating diseases in cauliflower and other Brassica crops (Shaw et al., 2021; Wu et al., 2024). Various control measures such as mechanical, chemicals and cultural methods are time consuming, expensive, health hazardous and environment polluter. Identification of resistant sources to disease having diverse genetic backgrounds is essential in broadening genetic base of disease resistance. Therefore, natural resistance source plays a major role in developing commercial resistant variety (s) and/ or hybrid (s). Identification of resistant breeding lines to downy mildew disease could improve the efforts to select the genotypes that are resistant. Field trials alone do not allow the selection of resistant varieties, but comparisons of field, micro-plot and green house trials may

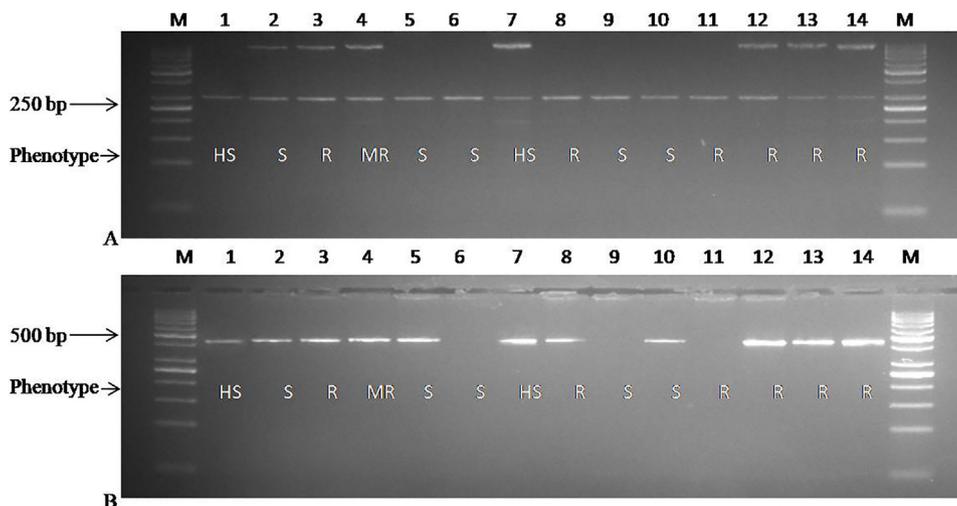


Fig. 4. PCR with amplification in 12 advanced lines and 2 parents with the A) SCAR marker SCR15 and B) SCJ19; M: 100 bp ladder; 1: DMS-10-5-2-1; 2: DMS-33-4-1-0-7; 3: DMR-31-0-2-6-4; 4: DMR-7-4-5-3; 5: DMS-9-4-8-3; 6: Pusa Himjyoti; 7: DMS-15-4-4-2; 8: DMR-8-4-8-1; 9: DMS-27-2-1-0-3; 10: DMS-46-1-6-0-1; 11: DMR-28-7-3-1-0-1; 12: DMR-3-0-8; 13: DMR-40-5-8-1; 14: Ccm-5.

Table 5
Reproducibility of markers and their validation with phenotypic data of downy mildew resistance and susceptibility of advanced lines.

	Progressive lines	Phenotypic data	Genotypic data of markers					
			SCR15	SCJ19	UBC 359	O112G04	OPK17_980	OPM16
1	DMS-10-5-2-1:	HS	-	+(Recom)	+(Recom)	+(150 bp)	+(450 bp)	+(Recom)
2	DMS-33-4-1-0-7:	S	+(Recom)	+(Recom)	+(Recom)	+(150 bp)	+(450 bp)	-
3	DMR-31-0-2-6-4:	R	+	+	+	+(150 bp)	+(450 bp)	-
4	DMR-7-4-5-3:	MR	+	+	+	+(150 bp)	+(450 bp)	-
5	DMS-9-4-8-3:	S	-	+(Recom)	+(Recom)	+(150 bp)	+(450 bp)	-
6	Pusa Himjyoti	S	-	-	-	+(150 bp)	+(450 bp)	+(Recom)
7	DMS-15-4-4-2:	HS	+(Recom)	+(Recom)	+(Recom)	+(150 bp)	+(450 bp)	-
8	DMR-8-4-8-1:	R	-(Recom)	+	-(Recom)	+(150 bp)	+(450 bp)	-
9	DMS-27-2-1-0-3:	S	-(Recom)	-(Recom)	-(Recom)	+(150 bp)	+(450 bp)	-
10	DMS-46-1-6-0-1:	S	-	+(Recom)	+(Recom)	+(150 bp)	+(450 bp)	-
11	DMR-28-7-3-1-0-1:	R	-(Recom)	-(Recom)	+	+(150 bp)	+(450 bp)	-
12	DMR-3-0-8:	R	+	+	+	+(150 bp)	+(450 bp)	-
13	DMR-40-5-8-1:	R	+	+	+	+(170 bp)	+(450 bp)	-
14	Ccm-5	R	+	+	+	+(170 bp)	+(450 bp)	-

+ presence of band (validated); - absence of band (not validated); recombinant in terms of opposite phenotypic reaction; DMS: Downy mildew susceptible; DMR: Downy mildew resistant.

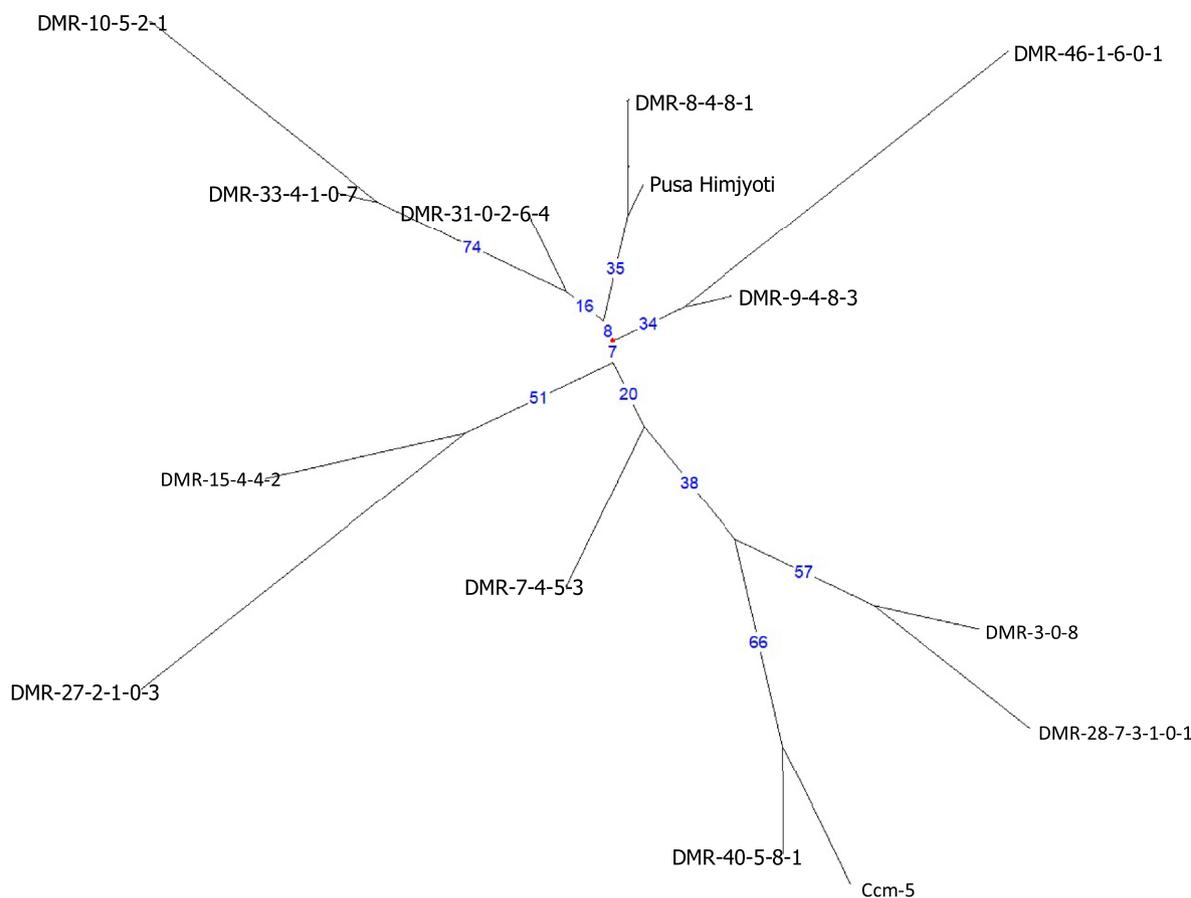


Fig. 5. PCR with amplification in 12 advanced lines and 2 parents with the SCAR marker SCR15 and SCJ19.

help to identify the different reliable sources of resistance (Shew et al., 1987).

In the present study, the occurrence of downy mildew in the susceptible advanced lines indicates a heavy inoculum pressure of the pathogen due to favourable climatic conditions during November to December, as observed by Sen et al. (1991); Singh et al. (2013); Saha et al. (2020) and Singh et al. (2022). In the present study, the reactions of 48 advanced breeding lines and two checks were categorized

into 5 groups, based on their different disease responses to the isolate. In the first group, 12 advanced lines was showing resistance due to the selection of plants from progeny of resistant parent BR-2, 3-5-1-1, CCm, CCm-5 and the gene from resistant parent inherited over the period of selection. In the second group, 7 advanced lines showed moderate resistance which could be due to selection of plants from the progeny of moderate resistant parental lines as reported by Singh et al. (2013). However, there was one line DMS-

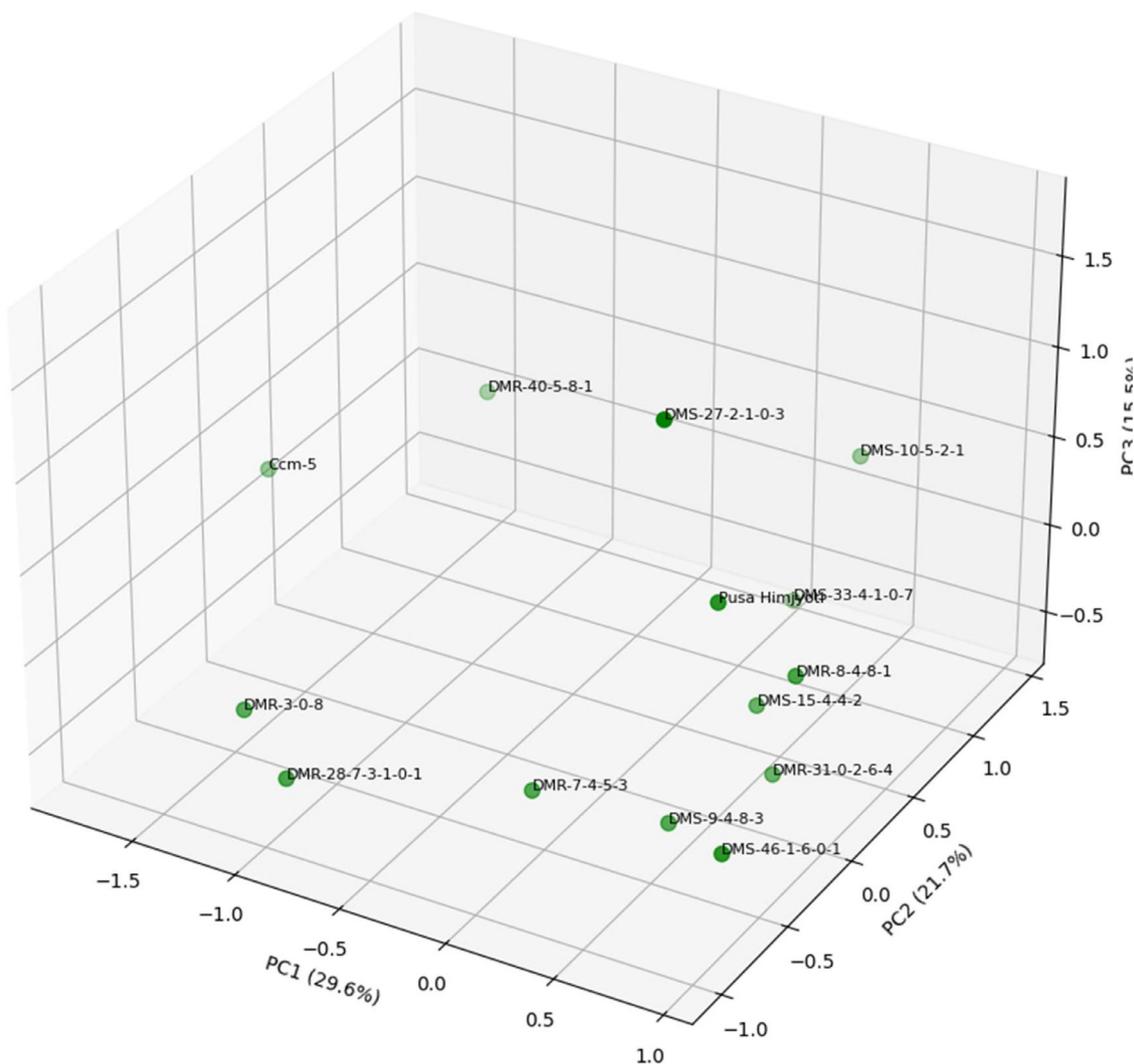


Fig. 6. 3D PCA plot (PC1 vs PC2 vs PC3) PCR with amplification in 12 advanced lines and 2 parents with markers.

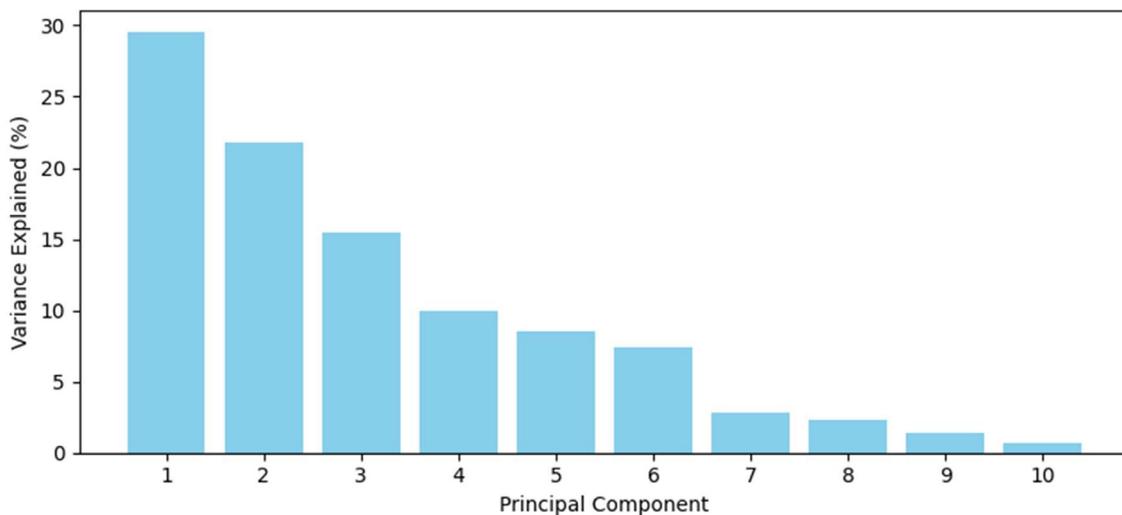


Fig. 7. Percent variance explained by each of the ten principal components.

Table 6
Downy mildew disease reaction and segregation of downy mildew resistance in advanced parental lines, F₁, and F₂ and back cross generations.

Parents/generation	Observed plants		Expected ratio	χ^2	P value at 5 %
	R	S			
Cross DMS-27-2-1-0-3 × DMR-3-0-8					
DMS-27-2-1-0-3	0	30			
DMR-3-0-8	30	0			
F ₁	30	0			
F ₂	111	39	3:1	0.71	0.40
B ₁	34	36	1:1	0.3	0.53
B ₂	60	0			
DMS-27-2-1-0-3 × DMR-40-5-8-1					
DMS-27-2-1-0-3	0	30			
DMR-40-5-8-1	30	0			
F ₁	30	0			
F ₂	114	36	3:1	0.18	0.67
B ₁	28	32	1:1	1.11	0.29
B ₂	60	3			

DMS: Downy mildew susceptible; DMR: Downy mildew resistant; P: Probability.

24-9-0-4 showed moderate susceptible reaction though it was selected from the cross involving resistant parent BR-2. The advanced line DMR-33-4-1-0-7 selected from the backcross of resistant parent 3-5-1-1 was found to be susceptible. This may be because of phenotyping or environmental error. Besides, the host reaction is typically defined in the context of a gain or loss of resistance (Parker et al., 1996; Vogel and Somerville, 2000; Chisholm et al., 2006) and pathogens often possess multiple virulence factors (called effectors),

each contributing incrementally to the disease phenotype. Besides, during the screening process, the partial role of escape factor cannot be ruled out. It is evident from the observations that most of the advanced lines appears to be susceptible to downy mildew disease due to true genetic factor i.e. selection of susceptible progeny which retained a high disease score. This is substantiated by the congenial weather for pathogen which requires mild temperatures (Achar, 1998) and prolonged periods of leaf-surface wetness (Verma and Thakur, 1989). Further, 19 new resistant or moderately resistant advanced lines ($0 \leq DI \leq 25$) from the tested advance progenies provided excellent opportunities to be used in the breeding programme. Interestingly, certain advanced lines selected from the cross of resistant parent BR-2 and 3-5-1-1, CCm, CCm-5 maintained the same level of resistance against downy mildew as reported earlier by Mahajan et al. (1995) and Singh et al. (2013; 2022).

The process of phenotypic selection takes long time and also ineffective due to influence of environment. Therefore, DNA marker technology has made it possible to identify resistant lines without phenotypic screening (Tanksley et al., 1995; Saha et al., 2014). Here, we used previously reported linked markers to validate the resistant lines. The use of these markers has advantage over SNPs as SNPs are very costly, labour intensive than SSRs. Besides, SCARs are highly reproducible and can be scored easily, rapid and easy to perform (Weng et al. 1998; Yuskianti and Shiraishi, 2010). Hence, 12 advanced lines, resistant and susceptible checks were selected for validation by six previously reported linked markers. Among the SCAR markers, none of them could show 100 % accuracy in distinguishing resistant and susceptible advanced lines. Two resistant lines viz. DMR-3-0-8



Fig. 8. Hybrids developed from the cross of downy mildew resistant advanced lines.

Table 7
Evaluation of hybrids developed from crossing of advanced lines.

Hybrids	Days to 50 % Curd maturity from transplanting	Marketable Curd Weight (g)	Yield (t ha ⁻¹)	Ascorbic Acid (mg/ 100 g FW ⁻¹)	Total phenols (mg 100 g FW ⁻¹)
Hyb 1 (DMR 31-0-2-6-4 × DMR-7-4-5-3)	60.00	983.33	36.42	52	29.47
Hyb 2 (DMR 31-0-2-6-4 × DMR-8-4-8-1)	59.00	1183.33	43.83	39	34.46
Hyb 3 (DMR 31-0-2-6-4 × DMR-3-0-8)	60.67	1246.67	46.17	42.33	40.64
Hyb 4 (DMR 31-0-2-6-4 × DMR-40-5-8-1)	55.00	926.67	34.32	46.28	26.86
Hyb 5 (DMR-7-4-5-3 × DMR-8-4-8-1)	64.33	1163.33	43.09	52.67	37.47
Hyb 6 (DMR-7-4-5-3 × DMR-3-0-8)	60.33	826.83	30.62	38.0	21.30
Hyb 7 (DMR-7-4-5-3 × DMR-40-5-8-1)	59.67	1483.33	54.94	45.33	42.45
Hyb 8 (DMR-8-4-8-1 × DMR-3-0-8)	59.00	960.00	35.56	31.33	28.59
Hyb 9 (DMR-8-4-8-1 × DMR-40-5-8-1)	61.33	1116.67	41.36	39.0	36.35
Hyb 10 (DMR-3-0-8 × DMR-40-5-8-1)	60.33	1040.00	38.52	33.33	31.55
CD at 0.05	2.31	185.58	6.87	3.46	1.79
CV %	2.25	9.89	9.89	4.81	3.17

DMS: Downy mildew susceptible; DMR: Downy mildew resistant; CD: Critical difference; CV: Coefficient of variation.

and DMR-40-5-8-1 were validated by three SCAR markers (SCR15, SCJ19 and UBC 359) along with the resistant check (CCm-5). Therefore, these two lines derived from 3 to 5-1-1 and CCm-5 can be used as potential source of downy mildew resistance in cauliflower breeding. Besides these, three SCAR markers will accelerate marker assisted breeding against downy mildew. However, other markers like SSR and RAPD were not effective in validating the resistant lines. The variation of results in our study compared to previous study of Janel et al. (2002); Farinho et al. (2004); Farinho (2007) may be due to the use of different genetic background. Besides, application of molecular markers is case specific especially for highly complex pathosystem of *Brassica oleracea*. Therefore, further research on high throughput genome sequencing such as whole genome or BSA-Seq or RNA-Seq can be carried out for identifying genomic regions associated with resistance. Besides, linked SNPs can be mapped to resistance loci and SNP based KASP markers could then be developed for precise selection.

The cluster analysis was carried out which enables researchers to group breeding lines based on their genetic similarities and dissimilarities. It helps in the selection of diverse and valuable breeding materials in crossing programme which can create more diverse breeding populations (Salgotra and Chauhan, 2023). The clustering pattern developed by Darwin software helps to assess the genotypes clustering more clearly (Umamaheswari et al. 2010). The dendrogram obtained from cluster analysis revealed significant genetic diversity among the 12 advanced lines and 2 checks. The Cluster 1 showed a close genetic relationship between DMS-46-1-6-0-1 and DMS-9-4-8-3, suggesting that they have undergone similar selective pressures towards susceptibility. Cluster 2 was divided into subgroups 2a and 2b which indicates further genetic differentiation within this cluster. The subgroup 2a have two susceptible advanced lines except one resistant advanced line DMR-31-0-2-6-4 and they are more closely related to each other as depicted by the markers. The grouping of one resistant advanced line (DMR-8-4-8-1) with susceptible check Pusa Himjyoti indicates the presence of same parentage in this line. During the process of advanced line development, there was a bias for plants with low natural incidence in this intermediate progenies, the partial role of the escape factor cannot be ruled out for the lines DMR-31-0-2-6-4 and DMR-8-4-8-1 as reported by Singh et al. (2022). Cluster 3, the largest, displays considerable genetic variability. Subgroup 3a suggests a moderate level of genetic similarity, while subgroup 3b includes accessions with more diverse genetic backgrounds. The solitary position of moderate resistant line DMR-7-4-5-3 on a separate branch highlights its distinct genetic profile compared to the other advanced lines. It is interesting to mention that the resistant check (CCm-5) and susceptible check (Pusa Himjyoti) formed distinct clusters depicting diverse genetic background.

Data recorded from the twelve advanced and two parental lines were subjected to PCA to understand the basic constitution in the experiment as well as the relationship between lines and the various markers parameters. The first three PCs explained 66.79 % of the total genetic variation captured by the marker dataset confirming a robust representation of the underlying diversity. The PCA visualization supported the clustering results, showing a clear separation between resistant and susceptible genotypes. The resistant check (CCm-5) and multiple resistant advanced lines (DMR-3-0-8, MR-40-5-8-1, DMR-28-7-3-1-0-1) were grouped closely in the 3D space, reflecting their shared genetic background. The susceptible check (Pusa Himjyoti) and advanced susceptible lines (e.g., DMS-10-5-2-1, DMS-33-4-1-0-7, DMS-9-4-8-3) also clustered together, indicating distinct divergence from the resistant group. Intermediate positions of DMR-27-2-1-0-3, DMS-15-4-4-2, and DMR-31-0-2-6-4 in the plot was consistent with their grouping in the dendrogram and suggestive of mixed or partially introgressed genetic backgrounds. DMR-7-4-5-3, which appeared as a solitary cluster in the dendrogram, also occupied a peripheral location in the PCA plot, further confirming its distinctiveness. The lower value of average Nei's genetic diversity across all polymorphic markers indicating a moderate level of genetic variation among the advanced breeding lines and checks. The genetic diversity seemed to be low on the basis of these markers due to less number of linked markers available.

Contradictory reports were described by previous workers where presence of single dominant genes for downy mildew resistance in *Brassica oleracea* (Jensen et al., 1999a), broccoli (Farnham et al., 2002; Coelho and Monteiro, 2003), and different cauliflowers including mid-group (Mahajan et al., 1995; Saha et al., 2020), late group (Verma and Singh, 2018) and European type (Vicente et al., 2012) have been mentioned. However, single recessive or two to three genes with recessive epistatic interactions were also reported in Indian cauliflower group III (Mahajan et al., 1995) and more than one recessive gene in *B. oleracea* (Carlsson et al., 2004). These reports indicate that the nature of the inheritance varies with genotype, study region and pathogen race (s). The stable performance of parents and F₁ generation for disease resistance during crossing for developing different generations showed consistency of downy mildew resistance in parents and F₁s. Individual plants in all generations were scored for downy mildew resistance and classified as downy mildew resistant (DMR) and downy mildew susceptible (DMS) plants. For any monogenic trait, the segregation of plants in F₂ generation should be 3:1 ratio (resistant: susceptible). In a recent study by Singh et al. (2022) where single gene has been reported in the background of 3-5-1-1 and similar genetics of resistance we found in the present resistant advanced lines where CCm-5 is one of the parents. In this study, we report the presence of single dominant gene in the background of

DMR-3-0-8 and DMR-40-5-8-1 where 3-5-1-1 and CCm-5 which are one of the resistant parents, respectively. This reveals that despite the diverse resistance sources in the Indian cauliflower germplasm, the resistance is monogenic dominant and *Ppa3* gene potentially involved in downy mildew resistance in all the tested resistant sources (DMR-3-0-8 and DMR-40-5-8-1). Sharma et al. (1972) reported similar nature of resistance in 'BR-161', 'BR-207' and 'BR-2' due to their common parentage (S. No. 15 × MGS-2-3). But the allelic relationship can't be overruled as these two lines were validated by three SCAR markers which were actually linked with *Pp523as* reported by Farinho et al. (2007) and *Dm* gene by Jannel (2002). Besides, there may be possibility that some gene (s) have regulatory role on growth, defense mechanism, disease resistance and quality that may be simultaneously enhanced through selective breeding (Derbyshire et al., 2024). Contradictory observation was reported as the presence of the same gene for downy mildew resistance in the Indian cauliflower germplasm (Singh et al., 2022). The pattern of F₁ population in this study confirms the dominance nature of downy mildew resistance gene which has great potential in resistant hybrid development.

The F₁ hybrids have tremendous potential in cauliflower due to their uniform maturity, earliness, high yield, better curd quality with respect to compactness and colour, resistance to biotic and abiotic stress (Sharma et al., 2004; Kucera et al., 2006; Dey et al., 2014). Although, Indian cauliflower have self-incompatibility (SI) genetic mechanism which have been exploited for developing F₁ hybrids Pusa Kartik Sankar (early group) and Pusa Hybrid-2 (mid-group), but this genetic mechanism has limitation of maintenance and chances of instability due to breaking of SI in the changing climate scenario (Sharma et al., 2004; Selvakumar et al., 2007). Cultural, chemical and biological control of downy mildew may not be very effective, economical and durable. Therefore, host-plant resistance is widely recognized as the least expensive, easiest, safest and most effective method of disease management (Agrios, 2005). For sustainable cauliflower production, development of variety (s)/hybrid (s) with durable resistance against broad-spectrum pathogens is the best strategy for a long term and reliable solution (Shaw et al., 2021; Singh et al., 2022; Singh et al., 2023; Karigar et al., 2023). Hybrid breeding for disease resistance helps to assemble desirable combinations of resistant gene (s) and other economic trait (s). Conventional plant breeding takes considerable time (5–10 years) or more to develop resistant variety (s)/hybrid (s) making the process expensive, time consuming and requires artificial screening. In this study, based on a combination of the F₁, the resistance to downy mildew along with other desirable horticultural traits was developed. There are many reports available regarding identification of resistance source, genetics study, QTLs mapping and marker development in cauliflower. It is for the first time from where our study has reported utilization of resistance source in commercial hybrid development utilizing molecular markers. Some of the developed hybrids were best performer in terms of yield, quality along with disease resistance. We have also emphasized development of end product to bridge the gap between availability of genetic resources and advanced molecular breeding.

In conclusion, we have identified strong downy mildew resistant sources for diversifying the breeding programme. The involvement of single dominant gene for resistance could be utilized for straight use as donors in hybrid breeding. The study will be very useful to the cauliflower breeder in enhancing breeding efficiency for the farmers as well as consumers.

Declaration of competing interest

The authors declare that we have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this article

CRediT authorship contribution statement

Partha Saha: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Pritam Kalita:** Writing – review & editing, Supervision, Resources, Conceptualization. **Munish Sharma:** Writing – review & editing, Methodology, Investigation. **Pratibha Sharma:** Writing – review & editing, Resources, Methodology. **Chandrika Ghoshal:** Writing – review & editing, Investigation, Formal analysis. **Akriti Verma:** Writing – review & editing, Formal analysis. **N.D. Saha:** Writing – review & editing, Formal analysis. **B.S. Tomar:** Writing – review & editing, Supervision, Project administration.

Acknowledgments

We acknowledge our sincere gratitude to the Head, Division of Vegetable Science, Head, Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi for the financial support, laboratory facility and guidance while undertaking this research.

References

- Achar, P.N., 1998. Effects of temperature on germination of *Peronospora parasitica* conidia and infection of *Brassica oleracea*. J. Phytopathol. 146, 137–141 s.
- Carlier, J.D., Alabac, C.A., Coelho, P.S., Monteiro, A.A., Leitão, J.M., 2012. The downy mildew resistance locus *Pp523* is located on chromosome C8 of *Brassica oleracea* L. Plant Breed. 131, 170–175. <https://doi.org/10.1111/j.1439-0523.2011.01904.x>.
- Carlsson, M., Bothmer, R., Merker, A., 2004. Screening and evaluation of resistance to downy mildew (*Peronospora parasitica*) and clubroot (*Plasmiodiophora brassicae*) in genetic resources of *Brassica oleracea*. Hereditas 141 (3), 293–300 Lund.
- Chisholm, S.T., Coaker, G., Day, B., Staskawicz, B.J., 2006. Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124 (4), 803–814. <https://doi.org/10.1016/j.cell.2006.02.008> PMID: 16497589.
- Coelho, P.S., Monteiro, A., 2003. Inheritance of downy mildew resistance in mature broccoli plants. Euphytica 131, 65–69. <https://doi.org/10.1023/A:1023008619400>.
- Dickson, M.H., Petzoldt, R., 1993. Plant age and isolate source affect expression of downy mildew resistance in broccoli. HortScience 28, 730–731.
- Derbyshire, M.C., Newman, T.E., Thomas, J., Batley, J., Edwards, D., 2024. The complex relationship between disease resistance and yield in crops. Plant Biotechnol. J. 22, 2612–2623. <https://doi.org/10.1111/pbi.14373>.
- Farinho, M., Coelho, P., Carlier, J., Svetleva, D., Monteiro, A., Leitão, J., 2004. Mapping of a locus for adult plant resistance to downy mildew in broccoli (*Brassica oleracea* con var. *italica*). Theor. Appl. Genet. 109, 1392–1398.
- Farinho, M., Coelho, P., Monteiro, A., Leitão, J., 2007. SCAR and CAPS markers flanking the *Brassica oleracea* L. *Pp523* downy mildew resistance locus demarcate a genomic region syntenic to the top arm end of *Arabidopsis thaliana* L. chromosome 1. Euphytica 157, 215–221.
- Farnham, M.W., Wang, M., Thomas, C.E., 2002. A single dominant gene for downy mildew resistance in broccoli. Euphytica 128 (3), 405–407.
- Hoser-Krauze, J., Lakowska-Ryk, E., Antosik, J., 1984. Resistance of cauliflower and broccoli (*B. oleracea* L. *botrytis* L.) seedlings to downy mildew, *Peronospora parasitica*. Crucif. Newsl. 9, 92–93.
- Hoser-Krauze, J., Lakowska-Ryk, E., Antosik, J., 1987. The inheritance of broccoli (*Brassica oleracea* L. var. *botrytis*) leaf resistance to downy mildew- *Peronospora parasitica* (pers.) ex. Fr. Genet. Pol. 28, 377–380.
- Janel, L.G., Farnham, M.W., Wang, M., 2002. Development of sequence characterized amplified region markers linked to downy mildew resistance in Broccoli. J. Am. Soc. Hortic. Sci. 127 (4), 597–601.
- Jensen, B.D., Hockenull, J., Munk, L., 1999a. Seedling and adult plant resistance to downy mildew (*Peronospora parasitica*) in cauliflower (*Brassica oleracea* con var. *botrytis*). Plant Pathol. 48, 604–612.
- Jensen, B.D., Værbak, S., Munk, L., Andersen, S.B., 1999b. Characterization and inheritance of partial resistance to downy mildew, *Peronospora parasitica*, in breeding material of broccoli, *Brassica oleracea* con var. *botrytis* var. *italica*. Plant Breed. 118, 549–554.
- Karigar, G.P., Singh, S., Mangal, M., Saroha, S., Saini, N., Ray, M., Behera, T.K., 2023. Dietary micronutrient content, heterosis and combining ability for breeding mineral-rich hybrids in early and mid-maturity groups of Indian cauliflower. Sci. Hortic. 312, 111848. <https://doi.org/10.1016/j.scienta.2023.111848>.
- Lund, B.M., Wyatt, G.M., 1978. Post harvest spoilage of cauliflower by downy mildew, *Peronospora parasitica*. Plant Pathol. 27, 143–144. <https://doi.org/10.1111/j.1365-3059.1978.tb01100.x>.
- McKay, A.G., Floyd, R.M., Boyd, C.J., 1992. Phosphonic acid controls downy mildew (*Peronospora parasitica*) in cauliflower curds. Aust. J. Exp. Agric. 32, 127–129. <https://doi.org/10.1071/EA9920127>.
- Mahajan, V., Gill, H.S., More, T.A., 1995. Inheritance of downy mildew resistance in Indian cauliflower (group III). Euphytica 86, 1–3. <https://doi.org/10.1007/BF00035932>.

- Moss, N.A., Crute, I.R., Lucas, J.A., Gordon, P.L., 1988. Requirements for analysis of host-species specificity in *Peronospora parasitica* (downy mildew). *Crucif. Newsl.* 13, 114–116.
- Pandey, K.K., Pandey, P.K., Singh, B., Kallou, G., Kapoor, K.S., 2001. Sources of resistance to downy mildew (*Peronospora parasitica*) disease in the Asiatic group of cauliflower. *Veg. Sci.* 28, 55–57.
- Panse, V.G., Sukhatme, P.V., 1967. *Statistical Methods For Agricultural Workers*. ICAR Publication, New Delhi.
- Parker, J.E., Holub, E.B., Frost, L.N., Falk, A., Gunn, N.D., Daniels, M.J., 1996. Characterization of *eds1*, a mutation in Arabidopsis suppressing resistance to *Peronospora parasitica* specified by several different RPP genes. *Plant Cell* 8 (11), 2033–2046. <https://doi.org/10.1105/tpc.8.11.2033>.
- Perrier, X., Jacquemoud-Collet, J.P., 2006. DARwin software. <http://darwin.cirad.fr/Darwin/shew>.
- Saha, P., Ghoshal, C., Ray, S., Saha, N.D., Srivastava, M., Kalia, P., Tomar, B.S., 2020. Genetic analysis of downy mildew resistance and identification of molecular markers linked to resistance gene *Ppa207* on chromosome 2 in cauliflower. *Euphytica* 216 (11), 1–13.
- Saha, P., Kalia, P., Sharma, M., Singh, D., 2016. New source of black rot disease resistance in *Brassica oleracea* and genetic analysis of resistance. *Euphytica* 207, 35–48. <https://doi.org/10.1007/s10681-015-1524-y>.
- Salgotra, R.K., Chauhan, B.S., 2023. Genetic diversity, conservation, and utilization of plant genetic resources. *Genes* 14 (1), 174. <https://doi.org/10.3390/genes14010174> (Basel).
- Sen, B., Trivedi, B.M., Singh, R., 1991. Disease situation in northwestern plains of India. *Indian Phytopathol.* 45, 263 abstract.
- Shaw, R.K., Shen, Y., Zhao, Z., Sheng, X., Wang, J., Yu, H., Gu, H., 2021. Molecular Breeding Strategy and Challenges Towards Improvement of Downy Mildew Resistance in Cauliflower (*Brassica oleracea* var. *botrytis* L.). *Front. Plant Sci.* 12, 667757. <https://doi.org/10.3389/fpls.2021.667757>.
- Shew, B.B., Wynne, J.C., Beute, M.K., 1987. Field, micro plot and greenhouse evaluations for resistance to *Sclerotium rolfsii* in peanut. *Plant Dis.* 71, 188–191.
- Shuancang, Y., Zhang, F., Yu, R., Zou, Y., Qi, J., Zhao, X., Yu, Y., Zhang, D., Li, L., 2009. Genetic mapping and localization of a major QTL for seedling resistance to downy mildew in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). *Mol. Breed.* 23, 573–590.
- Singh, J., Sharma, A., Lata, H., Thakur, A., Kumar, N., 2023. Genetic diversity for curd yield and its attributes in late cauliflower. *Indian J. Hortic.* 80 (2), 136–142.
- Singh, S., Sharma, S.R., Kalia, P., Sharma, P., Kumar, V., Kumar, R., Meena, B.L., Kumar, S., Sharma, T.R., 2013. Screening of cauliflower (*Brassica oleracea* L. var. *botrytis* L.) germplasm for resistance to downy mildew [*Hyaloperonospora parasitica* Constant (Pers.:Fr.) Fr.] and designing appropriate multiple resistance breeding strategies. *J. Hortic. Sci. Biotechnol.* 88 (1), 103–109.
- Singh, S., Kalia, P., Sharma, S.R., 2022. Sharma New sources of downy mildew resistance in cauliflower and their prospects in resistance breeding. *Eur. J. Plant Pathol.* <https://doi.org/10.1007/s10658-022-02517-7>.
- Singh, S., Sharma, S.R., Kalia, P., Deshmukh, R., Katara, J., Sharma, P., 2015. Identification of putative DNA markers for disease resistance breeding in Indian cauliflower (*Brassica oleracea* var. *botrytis* L.). *Indian J. Biotechnol.* 14, 455–460.
- Sivasithamparam, K., Barbetti, M.J., Li, H., 2005. Recurring challenges from a necrotrophic fungal plant pathogen: a case study with *Leptosphaeria maculans* (causal agent of blackleg disease in brassicas) in Western Australia. *Ann. Bot.* 96, 363–377.
- Stuthman, D.D., Leonard, K.J., Miller-Garvin, J., 2007. Breeding crops for durable resistance to disease. *Adv. Agro* 95, 319–367.
- Thamburaj, S., Singh, N., 2003. *Vegetables, Tuber Crops and Spices*. Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research, New Delhi, pp. 76–97.
- Umamaheswari, D., Paramathma, M., Manivannan, N., 2010. Molecular genetic diversity analysis in seed sources of *Jatropha (Jatropha curcas* L) using ISSR markers. *Electron. J. Plant Breed.* 1 (3), 268–278.
- Varalakshmi, B., Giriya, G., Pushpalatha, A., Chethana, B.S., 2011. Inheritance of downy mildew resistance in cauliflower (Group I). *Veg. Sci.* 38 (1), 115–116.
- Verma, A., Singh, Y., 2018. Inheritance of downy mildew resistance and its relationship with biochemical traits in cauliflower (*Brassica oleracea* L. var. *botrytis*). *Crop Prot.* 106, 132–138. <https://doi.org/10.1016/j.cropro.2017.12.024>.
- Verma, T.S., Thakur, P.C., 1989. Comparative field resistance of cabbage collection to downy mildew at seedling stage. *Indian J. Plant Prot.* 17, 79–80.
- Vicente, J.G., Gunn, N.D., Bailey, L., Pink, D.A.C., Holub, E.B., 2012. Genetics of resistance to downy mildew in *Brassica oleracea* and breeding towards durable disease control for UK vegetable production. *Plant Pathol.* 61 (3), 600–609.
- Vogel, J., Sommerville, S., 2000. Isolation and characterization of powdery mildew-resistant Arabidopsis mutants. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1897–1902.
- Wang, M., Farnham, M.W., Thomas, C.E., 2001. Inheritance of true leaf stage downy mildew resistance in broccoli. *J. Am. Soc. Hortic. Sci.* 126 (6), 727–729 2001.
- Williams, J.G.K., Kubelik, A.R., Livak, J.K., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary markers are useful as genetic markers. *Nucleic Acids Res.* 18, 6531–6535.
- Wu, Y., Zhang, B., Yang, L., Zhuang, M., Lv, H., Wang, Y., Ji, J., Hou, X., Zhang, Y., 2024. Fine mapping and identification of the downy mildew resistance gene *BoDMR2* in Cabbage (*Brassica oleracea* L. var. *capitata*). *BMC Plant Biol.* 24, 987. <https://doi.org/10.1186/s12870-024-05685-2>.
- Yuskianti, V., Shiraiishi, S., 2010. Sequence characterized amplified region (SCAR) markers in sengon (*Paraseriathes falcataria* (L.) Nielsen. HAYATI J. Biosci. 17 (4), 167–172. <https://doi.org/10.4308/hjb.17.4.167>.
- Zhang, N.W.W., Pelgrom, K., Niks, R.E., Visser, R.G.F., Jeuken, M.J.W., 2009. Three combined quantitative trait loci from non-host *Lactuca saligna* are sufficient to provide complete resistance of lettuce against *Bremia lactucae*. *Mol. Plant-Microbe Interact.* 22, 1160–1168.
- Zhang, B., Su, T., Xin, X., Li, P., Wang, J., Wang, W., Yu, Y., Zhao, X., Zhang, D., Li, D., Zhang, F., Yu, S., 2023. Wall-associated kinase BrWAK1 confers resistance to downy mildew in *Brassica rapa*. *Plant Biotechnol. J.* 21, 2125–2139.