Journal of Scientific Research and Reports

Volume 31, Issue 10, Page 218-224, 2025; Article no.JSRR.145451
ISSN: 2320-0227

Molecular Characterization of Recombinant Inbred Lines (RILs) in Cauliflower (*Brassica oleracea* var. botrytis L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jsrr/2025/v31i103564

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/145451

Original Research Article

Received: 24/07/2025
Published: 04/10/2025

ABSTRACT

Cauliflower is an important vegetable crop grown commercially in India. Information on molecular markers along with linkage map construction is a prerequisite for molecular breeding. The present investigation was carried at the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India, to study the variability of 66 recombinant inbred lines (RILs) developed in downy mildew resistance programme using 119 SSR markers. Among these, SSR markers, six were found to be polymorphic between the parents and finally five markers were polymorphic among the RILs. The cluster analysis using the UPGMA method showed six clusters at

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Cite as: *B. Rajasekhar Reddy, Pritam Kalia, and Partha Saha. 2025. "Molecular Characterization of Recombinant Inbred Lines (RILs) in Cauliflower (Brassica Oleracea Var. Botrytis L.)". Journal of Scientific Research and Reports 31 (10):218–224. https://doi.org/10.9734/jsrr/2025/v31i103564.*

a 0.38 Jaccard dissimilarity coefficient. The clustering of RILs allocated 12 RILs outside cluster of both parents revealing differentiation of the RILs studied. The cluster I had maximum number of RILs (37) while cluster VI had only one RIL. It is concluded that these five markers *i.e*., Na12G12, Ol11G11, FIT0043, BoSF 376, and BoSF 2079 can further be used to link different trait in cauliflower.

Keywords: Cauliflower; RILs; SSR; diversity; vegetable crop.

1. INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is an important vegetable crop which belongs to the family Brassicaceae and is grown commercially in India (Saha et al., 2025). Due to its high nutritional value, such as vitamins, minerals, dietary fibre, and desirable glucosinolate, it provides significant health benefits (Neelavathi et al., 2015; Zhang et al., 2025). There are different groups of cauliflower based on their time of maturity, temperature requirement, and morphological characteristics. Generation of superior inbred lines is the prior requirement for commercial hybrid seed production of the crop, and development of recombinant inbred lines (RILs) with desirable traits can be used in heterosis breeding. Mapping of QTLs using recombinant inbred lines (RILs) populations is most widely adopted (Bernardo 2008). A RIL population can be used for evaluation of multiple morphological traits across locations and years (Chu et al., 2018). Genetic variability among inbred lines is very important to select suitable parents to develop superior hybrids. Open pollinated cauliflower varieties can be developed using different inbred lines followed by hybridization and selection. The nature and magnitude of variability present in the available germplasm is required for selecting effective breeding methods (Ukkund et al., 2007). The variability among the existing germplasm/breeding lines must be broadened using other sources for selecting superior recombinant inbred lines (RILs). While selecting genetically diverse cauliflower lines, morpho-agronomic traits and molecular markers information should be considered for their genetic variability. These morphological descriptors are evaluated at different growth stages of the plant and are considered in identifying lines for traits of economic interest. But such descriptors have many limitations and affected by both genotype and environment. In addition, these descriptors are unable to distinguish RILs that have the same genealogy (Priolli et al., 2002).

Work to generate marker information and linkage map construction was carried out in *Brassica oleracea* L. for use by breeders worldwide (Carlier et al., 2011; Farinho et al., 2004; Farinho et al., 2007). Several molecular markers were used to determine diversity in cauliflower collections (Vanlalneihi et al., 2019; Rana et al., 2023).The molecular markers are very powerful tools to characterize germplasm of many crops which shows differences at the DNA level and are unaffected by stage of growth, environment, and other management practices (Gopikrishna 2023). Among molecular markers, simple sequence repeats (SSR) are used widely for the characterization of cultivars, DNA fingerprinting and detection of clonal variation (Moreira et al., 2013; Vieira et al., 2016). This marker is one of the most efficient tool for genetic diversity studies with more genetic information due to PCR based technology and co-dominant in nature (Nadeem et al., 2017; dos Oliveira et al., 2021). Therefore, the present study was undertaken to characterize the 66 RILs obtained in the cauliflower breeding programme.

2. MATERIALS AND METHODS

The plant materials comprised of 66 recombinant inbred lines (RILs) plants which were randomly selected for this study. These RILs were derived from a cross between Pusa Himjyoti (downy mildew susceptible) and BR-2 (downy mildew resistant) parents and were previously screened for downy mildew disease. These lines were developed by selfing upto F_7 generation and are maintained in the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India. After establishment of transplanted seedlings to the main field, young leaves were collected in liquid nitrogen from three randomly selected plants of each 66 RIL and two parents. Plant genomic DNA was extracted using cetyl trimethyl ammonium bromide (CTAB) method according to Elias et al. (2004). For DNA quantification, 1% agarose gel is prepared, and λ DNA (50 ng/µl) is used as a standard. The gel picture of the DNA quantification showed the presence of DNA in parents and RIL population.

SSR markers of Na, Ol, Ra, CB, BRA, FIT, and the BoSF series were used for polymorphism survey among parents. PCR was performed in a 20 µl reaction mixture containing 2 µl of template DNA (35 ng/µl), 1.5 µl of dNTP mix (0.2 mM each of dATP, dGTP, dTTP, dCTP), 1.5 µl of each of two primers, 2 µl of 10X PCR buffer (10mM Tris-HCl, 50 mM KCl, pH 8.3), and 0.2 µl Taq polymerase (5U/µl). PCR products were resolved on 3% agarose gel for 3 hours. Gels were prepared and run in 1X TAE buffer and visualization of fragments was done using EtBr staining. Position and size of alleles was measured with the help of a 50 bp ladder. The markers that distinguished the two parents were selected for genotyping of RILs. The data of the polymorphic markers among the parents and RILs was analyzed by using NTSYS software for the construction of dendogram.

3. RESULTS AND DISCUSSION

For genetic improvement of any traits, the variability present in the gene pool is essential (Kumar et al., 2011; Vanlalneihi et al., 2019; Nandi et al., 2020). The identification of a superior line is important for better classification of cultivar. Genetic diversity among the germplasm, advanced lines is desirable for obtaining progenies with desirable characters in any hybridization programme. Traditional diversity study in cauliflower genotypes/breeding lines or recombinant inbred lines using morphological description are laborious, less informative (Cansian and Echeverrigaray, 2000; Singh et al., 2022; Rana et al., 2023). Therefore, diversity study using molecular markers is the best approach. In the present study, SSR markers were used to assess the genetic diversity among 66 recombinant inbred lines (RILs) of cauliflower. Out of 119 SSR markers, six were found to be polymorphic between the parents Pusa Himjyoti and BR-2. The representative gel picture of polymorphism surveys of 12 markers are given in Fig. 1. The low level of polymorphism (5.04 %) generated by SSR markers in the present study contradict the previous study of Lowe et al. (2004). In previous study, Louarn et al. (2007) used SSR markers for characterization of 59 cultivars of *Brassica oleracea* with polymorphism information content (PIC) value of of more than 0.5. Zhao et al. (2014) studied the genetic diversity of 57 *Brassica oleracea* genotypes using 14 simple SSR markers with genetic similarity of 0.74 and 0.83 for compact and loose curd cauliflower accessions, respectively. Out of six markers, five markers viz. Na12G12, Ol11G11, FIT0043, BoSF 376 and BoSF 2079 showed polymorphism among the RILs population along with parents. In the present study our objective was to see the diversity among the RILs, therefore we used SSR markers available with us without targeting any linked markers. The gel picture was scored according to the presence of respective fragments of the difference polymorphic markers. In case of marker Na12G12, it amplified 2 fragments of different sizes one at 160 bp in the parent Pusa Himjyoti and another at a 150 bp size in BR-2 parent. The marker Ol11G11 amplified 2 fragments at 160 bp in Pusa Himjyoti parent and 140 bp in BR-2 parent. The marker FIT0043 amplified two fragments at 180 bp in Pusa Himjyoti parent and 140 bp in BR-2 parent. It is interesting to mention that the marker BoSF 376 showed 3 fragment positions at 280 bp, 250 bp and 200 bp in parent Pusa Himjyoti whereas it amplified 2 fragments (280 bp and 200 bp) in parent BR-2. The marker BoSF 2079 amplified 2 fragments at 140 bp in Pusa Himjyoti and 140 bp in BR-2. After polymorphism survey between the parents, the polymorphic markers were run among the 66 RILs. The amplification of the marker Na12G12 in the 66 RILs along with both the parents showed consistency in polymorphism between the parents with respective fragment size of the marker. Among the 66 RILs, 160 bp fragment amplified in 16 RILs corresponding to parent Pusa Himjyoti and 150 bp fragment in 34 RILs corresponding to BR-2 parent and 16 plant did not show any amplification. In case of marker Ol11G11, 160 bp fragment was amplified in parent Pusa Himjyoti and 36 RILs, whereas 140 bp fragment amplified in parent BR-2 and 13 RILs. For this marker, 4 RILs showed both 160 bp and 140 fragment which are heterozygote in nature and 13 RILs did not show any amplification. In case of marker FIT0043, parent Pusa Himjyoti and 40 RILs produced 180 bp fragment whereas parent BR-2 and 16 RILs produced 140 bp frangement. 3 RILs amplified both 180 bp and 140 bp fragment and 7 RILs did not amplify any fragment. The segregation of the markers showed distortion in the RILs population as all the RILs population were seleted randomly. In previous study, distortion in segregation of AFLP markers was observed in tomato recombinant inbred lines by Pratta et al. (2011). In a study of Rathod et al. (2018), about 8 markers showed distorted segregation in recombinant inbred line population derived from a soja and max soybean cross.Explanations of such result were reported by Kearsey and Pooni (1996) which could be due to lethal factors

linkage.Although characterization of germplasm or breeding lines through morphological descriptors is effective in differentiating genotypes but has lack of polymorphism, affected by environment, depend on the stage of plant development, and the type of character (Costa et al., 2009). In addition, it is important to mention that the cauliflower RILs used in this study have the same genealogy. To investigate more thoroughly about genetic variability among RILs, we used the information revealed by SSR analysis. The narrow genetic difference among the RILs by the SSR markers may have occurred because the lines are closely related and derived from same parentage.

The cluster analysis using the UPGMA (Unweighted paired group method of analysis) method showed six clusters (Table 1 and Fig. 2) at 0.38 Jaccard dissimilarity coefficient. The cluster I had maximum number of RILs (37) with parent Pusa Himjyoti. Cluster III had 17 RILs with parent BR-2. A total of 12 RILs formed different cluster outside the cluster of the two parents, contributing to differentiation of the RILs studied. We expected better differentiation among RILs using SSR marker analysis, but only 12 RILs (RIL25, RIL56, RIL60, RIL26, RIL27, RIL37, RIL49, RIL28, RIL29, RIL30, RIL31 and RIL 59) were separated from the respective parents using the UPGMA method. The RIL 59 formed a separate cluster (VI). In the genetic diversity study of the chilli, both morphological and molecular markers data was able to distinguish different accessions and excluded duplicates (Costa et al., 2009). Giancola et al. (2002) reported that the combination of SSR markers and morphological descriptors is the best technique for differentiating soybean lines. The same result was observed in the present study, because the molecular analysis was able to distinguish the RILs. In the present study, we only considered SSR data for the differentiation of RILs population. In future study morphological observations needs to be consisiderd for the diversity of the RILs as reported by Bermejoet al. (2010); Moreira et al. (2013).

Table 1. Grouping of Recombinant Inbred Lines (RILs) and parents according to UPGMA method

Clusters	Parents/RILs
Cluster I	Pusa Himjyoti, RIL41, RIL62, RIL24, RIL34, RIL42, RIL65, RIL52, RIL53, RIL45, RIL1, RIL8, RIL10, RIL2, RIL3, RIL4, RIL6, RIL7, RIL11, RIL13, RIL14, RIL15, RIL18, RIL19, RIL20, RIL21, RIL22, RIL23, RIL16, RIL38, RIL5, RIL12, RIL9, RIL17, RIL48, RIL57, RIL43, RIL55
Cluster II	RIL25, RIL56, RIL60, RIL26, RIL27, RIL37, RIL49
Cluster III	BR2, RIL44, RIL47, RIL61, RIL32, RIL40, RIL51, RIL58, RIL54, RIL36, RIL39, RIL46, RIL63, RIL66, RRIL64, RIL50, RIL33, RIL35
Cluster IV	RIL28, RIL29
Cluster V	RIL30, RIL31
Cluster VI	RIL59

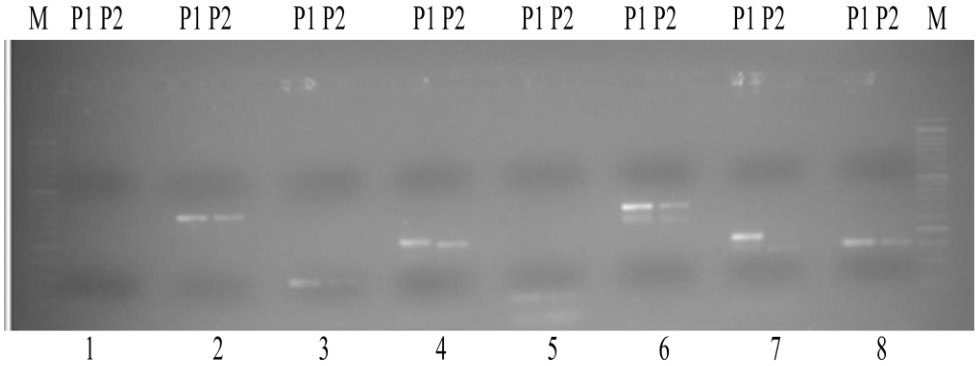

Fig. 1. Parental polymorphism survey using SSR markers (M: 50 bp ladder; P1: Pusa Himjyoti; P2: BR-2; Lane1: FIT0019; 2: FIT0024; 3: FIT0034; 4: FIT0036; 5: FIT0040; 6: FIT0041; 7: FIT0043; 8: FIT0050

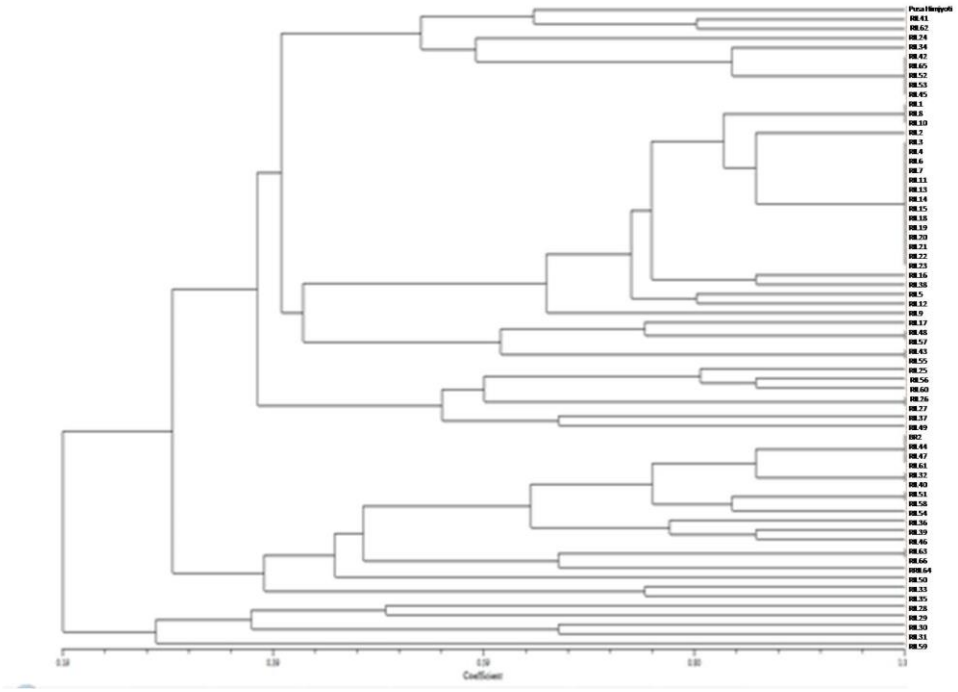

Fig. 2. Dendrogram obtained with UPGMA from the Jaccard dissimilarity matrix of66Recombinant Inbred Lines (RILs), parents Pusa Himjyoti and BR-2

4. CONCLUSION

Parental screening of Pusa Himjyoti and BR-2 using 119 SSR markers showed six polymorphic markers. The SSR marker has been found to be a useful and robust tool for detecting genetic diversity of RILs. Five markers was found to be more informative that can further be used to link different traits in cauliflower.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text to image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENT

We would like to thank the Indian Council of Agricultural Research, New Delhi, India and ICAR-Indian Agricultural Research, New Delhi, India for financial support under inhouse project CRSCIARISIL2014017249.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interestsor non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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