PISSN : 2615-2207 Agrosainstek, 8 (2) 2024: 76-90 EISSN : 2579-843X

AGROSAINSTEK

Jurnal Ilmu dan Teknologi Pertanian

Website jurnal : http://agrosainstek.ubb.ac.id

**Review Article**

# Breeding Methods for Anthracnose Resistant Chili Pepper (*Capsicum* spp.) in the Last Decade: A Review

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Received: October 11, 2024 /Received in revised : December 22, 2024/ Accepted: December 23, 2024

## ABSTRACT

*Chili (Capsicum spp.) is one of the most economically valuable commodities cultivated worldwide. The high interest in chili can be attributed to capsaicin, which provides a spicy sensation when consumed. The level of interest in chili cultivation does not necessarily correlate with the yields obtained. The loss in production and yield can be attributed to the disruption of the Colletotrichum spp., which causes anthracnose disease. Breeding for anthracnose-resistant chili has been a focus of research for many scientists in various countries. Conventional and molecular breeding methods are employed in the production of anthracnose-resistant chilies. This study will examine a range of articles and investigate the development of anthracnose-resistant chilies. The article was searched in the Scopus database. The articles were filtered based on the publication date range of 2014 to 2024, resulting in 343 articles. Furthermore, the articles were evaluated based on predetermined criteria, resulting in the identification of 22 articles. The breeding of anthracnose-resistant chili plants employs a range of conventional and molecular techniques to identify the most suitable lines. Various techniques, including crossing and different kinds of selection, were employed and validated through the development of molecular markers. Markers and genes have been identified, including RA80f6_r1, RA80f6_g1, RA80f6_g2, and RCT1, which are responsible for anthracnose resistance. This review provides an overview of the various anthracnose-resistant chili breeding methods and it is expected to contribute to the development of durable anthracnose-resistant chili genotypes.*

***Keywords: Anthracnose; Breeding method; Chili; Conventional breeding; Molecular breeding.***

## 1. Introduction

Chili is a commodity with high economic value. Chili peppers are favoured by their capacity to produce a piquant flavour derived from the fruit. Among the various chili varieties, the 'Carolina Pepper' stands out for its exceptionally high level of spiciness, with a Scoville Heat Unit rating of 2.2 million (Lynch 2013). Apart from flavour, chili peppers are also consumed because they have nutrients in the form of vitamins A and C and various antioxidant compounds (Hudáková *et al.* 2023). The numerous advantages and high demand have resulted in chili peppers becoming one of the most economically valuable commodities.

Chili is widely cultivated across the world. To date, more than 400 varieties have been cultivated across the country (Saxena *et al.* 2016). Chili has been widely cultivated because it can be consumed as a vegetable, spice, or processed into chili powder

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DOI: https://doi.org/10.33019/agrosainstek.v8i2.796

(Oyewo *et al*. 2018; Sanabam *et al*. 2018; Rais *et al*. 2021). In the year 2022, the countries that are the major production of chili commodity are India, Bangladesh, Thailand, China, and Pakistan (Ritchie *et al*. 2023).

The consumption of chili fruit commences from the green fruit and red ripe fruit and culminates in the processing of the fruit to create chili powder. The high diversity of chilies results in a lack of consistency in their spiciness. Some varieties of chili are not particularly spicy and can be consumed directly or combined as a flavour enhancer in a dish (Welbaum 2015). The capsaicin content in chilies has been demonstrated to contain antioxidants and anticarcinogenic and immunosuppressive properties. It has been shown that capsaicin compounds that give a burning sensation can be used to relieve pain when administered in the appropriate dose and frequency (Fattori *et al*. 2016). Accordingly, the application of chilies can be optimized by their multifaceted benefits.

Anthracnose are one of the most significant diseases affecting chili cultivation. In subtropical regions, this disease has been observed to have the most significant impact on chili yield, with reported losses reaching 29.5% and a total economic loss of 491 million US dollars in India alone (Garg *et al*. 2014). A reduction in quality also results in significant financial losses. The formation of black spots caused by anthracnose can reduce the selling value of chili fruit. The spread of anthracnose can occur in various parts of the chili plant and almost in all growth phases (Saxena *et al*. 2016). Furthermore, anthracnose is a disease that can be transmitted via air, water, or seeds. As a result, the application of control measures is a challenging undertaking since the pathogen that is responsible for the disease can cause infection under several different conditions.

The causal agent of anthracnose is a fungus of the genus *Colletotrichum* spp. Table 1 illustrates the diversity of *Colletotrichum* spp. that has been observed to cause infection in various countries. Some *Colletotrichum* spp has been documented to cause disease in multiple countries. This suggests that *Colletotrichum* spp. exhibits a high degree of adaptability and can thrive in a range of environmental conditions. Anthracnose is predominantly prevalent in tropical and subtropical regions, mainly due to the conducive moisture conditions that facilitate the proliferation of the disease (Than *et al*., 2008). Factors contributing to the high level of damage caused by anthracnose are humidity, intensity and frequency of rainfall, which results in wet plant parts and provides an environment conducive to the growth of Colletotrichum fungi. Environmental conditions with a temperature of 27$^{o}$C and humidity reaching 80% are optimal for the growth and spread of anthracnose (Hema *et al*. 2023). Therefore, in the current climate of favourable environmental conditions in a number of countries, the spread of anthracnose and the consequent loss of yield continue and are likely to persist.

High destructive of anthracnose is a serious impact to the cultivation of chili. The breeding for anthracnose resistance become crucial, as one of the controls is using fungicide and that can be ineficient depending on environmental conditions. Repetead and intensive application of fungicides, especially *sterol demethylation inhibitor* (DMI) fungicides such as tebuconazole, has resulted in the emergence of fungicide resistance in *Colletotrichum* species responsible for anthracnose in chili (Wei *et al*. 2020; Chen *et al*. 2022). The consequences of fungicide resistance might worsen issues in the future. Resistant varieties offer a more sustainable strategy for disease management.

Several factors must be considered when breeding anthracnose-resistant chilies. The process often employs both conventional and molecular methods. The former has been used for some time but has several disadvantages, including lengthy periods and significant environmental impact. The pedigree method in chili breeding takes up to eight growing seasons to produce superior candidate varieties (Kirana *et al*. 2023). Molecular methods are typically employed in the form of molecular markers, quantitative trait locus (QTL) analysis, or the identification of various genes that influence the expression of anthracnose resistance. Mishra *et al*., (2021) using CRISPR/Cas9 technique to study CaERF28 gene expression and Nanda *et al*., (2016) using SSR markers to confirm resistance against *C. capsici* and *C. gloeosporioides*. Choosing the right method can help the breeding process of anthracnose-resistant chili peppers run faster and as expected. The selection of an appropriate methodology can facilitate the acceleration of the breeding process for anthracnose-resistant chili peppers, thereby ensuring the attainment of the desired outcomes.

The resistance mechanism represents a crucial factor to be considered in the context of chili breeding activities to develop varieties resistant to anthracnose. Plants have evolved a range of resistance mechanisms to cope with pathogen attacks. Constitutive resistance traits are typically expressed from the outset, in contrast to antimicrobial compounds that are produced in response to pathogen attack (Rojas *et al*. 2014). The process of induced resistance is initiated when the plant detects the presence of pathogens, commonly referred to as pathogen-associated molecular patterns (PAMPs). This detection also occurs when the plant encounters proteins produced by the pathogen that are directed against its host (Boller and He 2009; Göhre *et al*. 2012). These mechanisms may allow breeders to enhance the resistance of chili peppers to anthracnose.

There are several review regarding breeding of chili pepper for anthracnose resistance traits have been published (Saxena *et al*. 2016; Chowdhury *et al*. 2020). Simultaneously, review articles addressing breeding method are also published although they do not using PRISMA methodology and not mention resistance menchanism. Consequently, it is essential to conduct a review that emphasizes using PRISMA as their methodology to ensure the quality of paper for the review. Besides that, it is important to mention about the resistance mechanism for anthracnose resistance in chili as an adaptation strategy againts *Colletotrichum* spp.

## 2. Method

The methodology applied in this systematic literature review is derived from the Scopus database. Keywords are employed to search the database, using Boolean operators "AND" and "OR" to combine search keywords. In the Scopus database, a search was conducted using the keywords "Capsicum annuum OR chili pepper AND Anthracnose OR Colletotrichum AND Resistant OR Disease Resistance AND Plant Breeding OR Breeding Method OR Genetic Improvement OR Selection Process OR Molecular Breeding OR Marker Assisted Selection" in accordance with additional criteria pertaining to the subject areas of agriculture and biological sciences. Only articles were considered within the specified time span of 2014 to 2024 for analysis. A total of 343 articles were retrieved in response to the search query. The entire article was evaluated based on the title and abstract, identifying 98 articles. Based on these results, additional criteria were applied to narrow the selection, including full-text availability, use of English, lack of relationship with anthracnose-resistant chili breeding methods, and absence of artificial anthracnose inoculation testing. Applying these criteria led to the selecting of 22 articles, as illustrated in Figure 1. During the writing process, supplementary searches were conducted to obtain articles to support the discussion.

Table 1. Spread of *Colletotrichum* in various countries

| No | *Spesies* | Country | Reference |
|---|---|---|---|
| 1 | *C. acutatum* | India, Indonesia, Korea, Sri Lanka, Taiwan, Thailand, United States, and Vietnam. | (Damm *et al*., 2009, 2012; Harp *et al*., 2008; Ranathunge *et al*., 2012; Saxena *et al*., 2014; Than, *et al*., 2008; Weir *et al*., 2012) |
| 2 | *C. capsici* | India, Indonesia, Mexico, Papua New Guinea, Taiwan, Thailand, United States, and Vietnam. | (Harp *et al*. 2008; Than *et al*. 2008; Damm *et al*. 2009; Ranathunge *et al*. 2012; Weir *et al*. 2012; Saxena *et al*. 2014) |
| 3 | *C. gloeosporioides* | Indonesia, Korea, Papua New Guinea, Taiwan, Thailand, United States, and Vietnam. | (Harp *et al*. 2008; Than *et al*. 2008; Damm *et al*. 2009; Weir *et al*. 2012) |
| 4 | *C. nymphaeae* | Indonesia, Korea, and Zimbabwe. | (Damm *et al*. 2009) |
| 5 | *C. coccodes* | Korea, New Zealand, Thailand, and United States. | (Than *et al*. 2008; Damm *et al*. 2009; Damm *et al*. 2012; Wei *et al*. 2012; Liu *et al*. 2013) |
| 6 | *C. nigrum* | New Zealand and Vietnam. | (Liu *et al*. 2013) |

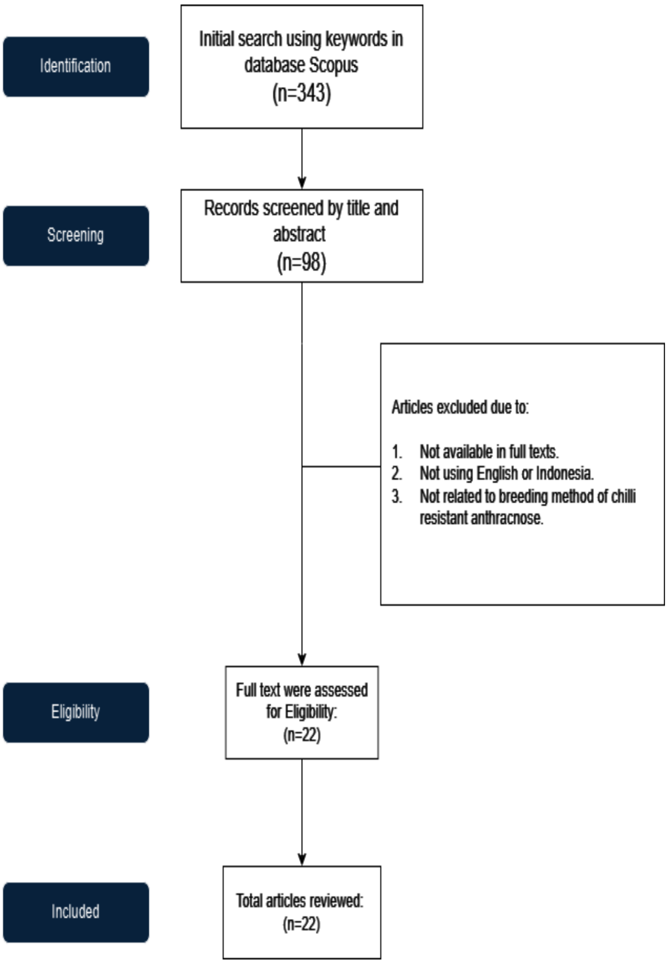

Figure 1. Review design based on Prisma Diagram

## 3. Result

The selected articles were subjected to a rigorous analysis and subsequently organized into a comprehensive table. A total of 22 articles were analyzed, and it was found that six articles employed conventional breeding methods (Table 1). Eleven articles utilized molecular methods, and four articles employed a combination of both methods (Table 2). The articles employing conventional methods yielded results in the form of multiple candidate elders exhibiting resistance to anthracnose. In contrast, the molecular methods yielded a range of gene analyses pertinent to anthracnose resistance. Of the 22 articles included in the analysis, four employed a combination of conventional and molecular methods. These studies involved the formation of conventional populations and the confirmation of resistance genes through molecular techniques. The majority of research was conducted in Brazil, India, and South Korea, which are notable for their high consumption of chili. Additionally, studies were conducted in China, Malaysia, Thailand, Taiwan, and Indonesia.

In the context of conventional breeding activities, a total of six articles were identified that described various methods of crossing between elders, selection, and collection based on exploration results. Regarding molecular breeding activities, a total of 11 articles were found that conducted a range of analyses with the objective of identifying QTL or genes responsible for resistance. In addition, four articles were identified that combined conventional and molecular methods, employing population formation techniques whereby the population was utilized in molecular analysis. Molecular analysis gives information on resistance genes at different phases of fruit maturity.

## 4. Discussion

### *Conventional Breeding Methods for anthracnose-resistant chili peppers*

Conventional breeding methods are commonly used in the breeding of anthracnose resistant chili peppers. Conventional methods often have various difficulties and challenges, such as long time, high environmental influences, and things that are difficult to predict (Chowdhury *et al*., 2020). Some of the techniques used in conventional methods include pedigree, backcrossing, recurrent selection and artificial hybridization. The goal of conventional methods is to improve the characteristics of a variety. These traits can be in the form of increased yield, nutrition, or resistance to a pathogen.

The techniques used in conventional methods are mostly pedigree selection, backcross selection and mutation induction. Some varieties breed by conventional methods include PBC80, LLS, Breck-1, Breck-2 and Braun, which show different resistance to *Colletotrichum* (Garg *et al*. 2013; Reddy *et al*. 2014). Techniques that have produced anthracnose-resistant chili varieties continue to be developed and carried out to form new varieties with resistance to other *Colletotrichum* species. Mass selection is one of the efficient techniques for breeding resistance because it involves selecting the best-performing plants-based resistance on the traits. Some researchers have reported the performance based on the resistance performance againts inoculation of *Colletotrichum* (Souza *et al*. 2019; Chowdhury *et al*. 2023). On the other hand, pedigree selection gives clearer information because of the tracking of ancestry of each plant and selecting based on known parentage. Giacomin *et al.* (2020) reported the inheritance of resistance genes againt anthracnose, the crossing are between parents *C. annuum* GBUEL104 ($P_1$) and GBUEL103 ($P_2$) and their ancestry was evaluated to see the inheritance of the genes.

Table 2. Breeding methods for anthracnose-resistant chili peppers

| Genotype | *Colletotrichum* Variety | Method | Fruit Development Stage | Result | Reference |
|---|---|---|---|---|---|
| - Unemat Pedro<br>- Unemat Malagueta Pantaneira | *C. scovillei* | Mass selection of Unemat 44 elders | Mature green fruit | The results showed smaller lesion size in the Unemat Pedro variety (1,83 $mm^2$), Unemat Malagueta Pantaneira (1,74 $mm^2$) compared to susceptible varieties (114,22 $mm^2$) | (Amorim *et al.* 2024) |
| Crossing between<br>- CP-3<br>- DP-37<br>- DP-57<br>- AP-25<br>- BP-23<br>- Putra Chili 1<br>- Putra Chili 4<br>- Putra Chili 7<br>- Putra Chili 9<br>- Putra Chili 10 | - *C. fructicola*<br>- *C. sojae* | Crossing between Genotypes | - Mature green fruit<br>- Ripe fruit | In the green maturity phase all $F_1$ strains had resistance to all inoculants except DP-57 x Putra Chili 7.<br>In the Red Ripe phase, 10 elders and 14 $F_1$ strains were found to have resistance to C. *fructiola*.<br>Resistance to *C. sojae* was found in 17 $F_1$ strains out of a total of 25 $F_1$ strains. | (Chowdhury *et al.* 2023) |
| Crossing of GBUEL104 x GBUEL103 | *C. scovillei* | Crossing between genotypes | - Mature green fruit<br>- Ripe fruit | In the $F_1$ generation, resistance to *C. scovillei* was found in the mature green and red phases.<br>In the $F_2$ generation, resistance only occurred in the mature green phase. | (Giacomin *et al.* 2020) |
| *Capsicum chinense* var. *pimenta-de-cheiro* | *C. brevisporum* | Mass selection | - Mature green fruit<br>- Ripe fruit | Five strains were found to have resistance to anthracnose and 14 strains had moderate resistance to anthracnose. | (Souza *et al.* 2019) |
| 59 local accecsion from Gene Bank Universidade Estadual de Londrina (UEL). | *C. gloeosporioides* | Exploration of genetic resources | - Mature green fruit<br>- Ripe fruit | There are 10 accessions that have high resistance in the green mature phase.<br>In the ripe fruit phase, 25 accessions were found to have high resistance to anthracnose attack. | (V.Y. Baba et al. 2019) |

Table 2. Breeding methods for anthracnose-resistant chili peppers (continued)

| Genotype | *Colletotrichum* Variety | Method | Fruit Development Stage | Result | Reference |
|---|---|---|---|---|---|
| 37 Local Accessions of Capsicum spp in the collection of Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). | *C. gloeosporioides* | Exploration of genetic resources | - Mature green fruit<br>- Ripe fruit | There were 8 accessions derived from *Capsicum baccatum* (UENF 1624, UENF 1628, UENF 1714, UENF 1718, UENF 1732, UENF 1733 and UENF 1737) and two accessions derived from *Capsicum chinense* (UENF 1764 and UENF 1770) that showed resistance in the green mature phase.<br>Accessions (UENF 1554, UENF 1703, UENF 1706 and UENF 1751) derived from *C. chinense* showed resistance in the fipe fruit mature phase. | (Silva *et al*. 2014) |
| Crossing between *C. annum* AVPP0207 dan *C. baccatum* PBC80 | *C. acutatum* | Crosses and confirmation using molecular markers | Mature green fruit | Marker SCAR-Indel$_{90}$ dan SSR-HpmsE032$_{231}$ identifying anthracnose resistance of crosses of both parents. | (Suwor *et al*. 2017) |
| Crossing of *C.baccatum* PBC80 and SB1 | *C. capsici* | Crossing and confirmation with SSR markers | Ripe fruit | The F$_2$ population showed smaller lesion sizes than the susceptible. Four primers were polymorphic. The marker are HpmsE 081, HpmsE 097, HpmsE 116, HpmsE 139 | (Nanda *et al*. 2016) |
| 41 Genotypes derived from pedigree selection. Cross between PBC398x80 and PBC758x80 | - *C. acutatum*<br>- *C. capsici* | Pedigree Selection Results and confirmed with SSR and SCAR markers | - Mature green fruit<br>- Ripe fruit | Three lines (205, 210, 215) derived from PBC80 and one line (101) derived from PBC932 showed resistance to anthracnose.<br>The markers that validate such resistance are SSR markers – HpmsE032 | (Suwor *et al*. 2015) |
| Crossing between 77013 (*C. annum*) and PBC932 (*C. chinense*) | *C. acutatum* | Interspecific crosses and establishment of backcross populations for QTL analysis | - Mature green fruit<br>- Ripe fruit | Resistance to *C. acutatum* in the PBC932 population was shown to be caused by a major QTL on chromosome P5 and had dominant expression. Indel and HpmsE116 markers can describe anthracnose resistance. | (Chunying *et al*. 2015) |

Table 2. Breeding methods for anthracnose-resistant chili peppers (continued)

| Genotype | *Colletotrichum* Variety | Method | Fruit Development Stage | Result | Reference |
|---|---|---|---|---|---|
| 17 Chilli variety from Beijing Vegetable Research Center | *C. capsici* | Varieties are from Beijing Vegetable Research Center.<br>RNA sequence was conducted to confirm anthracnose resistance. | Ripe fruit | The B158 variety was found to be resistant with the smallest lesion size based on the artificial inoculation test.<br>Based on transcriptional analysis, it was found that ARR-B, AP2-EREBP, bHLH, WRKY, and NAC are transcription factors that affect the expression of anthracnose resistance genes. | (Wang *et al*. 2024) |
| 197 Accession (*C. chinense*) collection from Republic of Korea National Agro Biodiversity Center | - *C. acutatum*<br>- *C. scovillei* | Exploration of genetic resources<br>Molecular and confirmation of anthracnose resistance through SNP markers | - Mature green fruit<br>- Ripe fruit | 6 accessions were suspected to have anthracnose resistance based on artificial inoculation tests.<br>Based on SNP markers, it was found that the gene encoding protein kinase on chromosome two affects anthracnose resistance. | (Ro *et al*., 2023) |
| Backcross population derived from crossing: *C. annum* 'CM334' and *C. baccatum PBC1* | *C. acutatum* | Backcross populations that have been developed for linkage map formation and QTL analysis | Ripe fruit | Population obtained SILs (Subtitutions Inbred Line) and AB (Advanced Backcross Line) which describes 94.8% of the *C. baccatum* genome.<br>The identified QTL also helped in identifying resistance to anthracnose. | (Jo *et al*. 2023) |
| Two chili genotype Punjab Lal and Arka Lohit | *C. truncatum* | Expression analysis and genome wide profiling on $C_2H_2ZF$ in a population of two chili genotypes | Ripe fruit | 79 $C_2H_2ZF$ transcription factors were obtained in the test genotypes. Eight $C_2H_2ZF$ genes showed increased expression in anthracnose-resistant genotypes. | (Sharma *et al*. 2021) |
| 3738 accessions of *Capsicum*. spp originating from 112 countries | *C. acutatum* | Resistance testing on test accessions by artificial inoculation<br>Confirmation of resistance to anthracnose based on High Resolution Melting analysis | Green ripe fruit | 261 accessions showed resistance to anthracnose based on artificial inoculation without wounding.<br>Based on inoculation with wounding, 12 accessions showed 25% higher resistance than inoculation without wounding.<br>HRM analysis showed results in the form of allelic diversity | (Ro *et al*., 2021) |

Table 2. Breeding methods for anthracnose-resistant chili peppers (continued)

| Genotype | *Colletotrichum* Variety | Method | Fruit Development Stage | Result | Reference |
|---|---|---|---|---|---|
| *C. annum* Arka Lohit | *C. truncatum* | Crispr-Cas9 are used for over expres the CaERF28 gene. | Ripe fruit | By utilizing the CRIPR/Cas9 method, it was found that the CaERF28 gene does have an effect on anthracnose resistance in Arka Lohit chili. | (Mishra *et al*. 2021) |
| 97 $F_6$ *Recombinant Inbred* line derived from crossing PBC80 x CA1316 | *C. scovillei* | Linkage map generation and QTL analysis by identifying SNPs | - Mature green fruit<br>- Ripe Fruit | Based on QTL analysis, there are three genes (RA80f6_r1, RA80f6_g1, RA80f6_g2) with 36% known function in anthracnose resistance. | (Kethom and Mongkolporn 2021) |
| Interspecific Cross and Backcross between *C.annum* 77013 x *C.chinense* PBC932 | *C. scovillei* | Population backcross generation, linkage map and QTL analysis | Mature green fruit | A major QTL of 164 kb was obtained and five genes were predicted. The candidate gene CA05g17730 is thought to have the highest influence on anthracnose resistance in the mature green phase. | (Zhao *et al*. 2020) |
| 15 genotypes which consist of eight variety of *C. annum* and seven variety of *C. frutescens.* | *C. capsici* | Microsatellite molecular marker testing and artificial inoculation | Ripe Fruit | GPMS29 marker can describe a strong relationship to anthracnose resistance | (Nugroho *et al*. 2019) |
| Two chilli genotypes Punjab Lal dan Arka Lohit | *C. truncatum* | $F_1$, $F_2$, and backcross populations and confirmation with marker | Ripe fruit | It was found that resistance to anthracnose was derived from the monogenic-dominant *RCt1* gene. The *RCt1* gene can be identified based on two markers (CtR-431 and CtR-594). | (Mishra, *et al*., 2019) |
| 49 genotypes of chilly from Indian Institute of Vegetable Research | - *C. truncatum*<br>- *C. gloeosporioides* | Resistance testing based on artificial inoculation in the laboratory and in the field. | - Mature green fruit<br>- Ripe fruit | Six genotypes were obtained that showed resistance to anthracnose, namely Punjab Lal, Bhut Jolokia, BS-35, Pant C-1, CA-4, Acchar Lanka. | (Mishra *et al*, 2019) |
| A population derived from a cross of *Capsicum annuum* cv. 'Bangchang' x *C. chinense* 'PBC932' And cross of *C. baccatum* 'PBC80' x 'CA1316'. | *C. scovillei* | Establishment of $F_2$ population and identification of resistance through QTL analysis | - Mature green fruit<br>- Ripe fruit | The resistance of F1 from PBC80 was caused by three major QTL and represented 80% of the variation in resistance compared to F1 from PBC932. | (Mahasuk *et al*. 2016) |

One of the key advantages of using conventional breeding methods are the using of naturally occuring genetic variantions within existing chili pepper. Several studies have identified potential sources of of anhracnose resistance such as *C. baccatum* (Son *et al.* 2021). Naturally variations allowing breeders to improve resistance without introducing foreign genes, ensuring more natural and locally adapted solutions. Conventional method are also allows for the pyramiding of different resistance genes, which can provide more durable and broad-spectrum resistance (Lee *et al.* 2010). Conventional breeding is well-established, straightforward process and reliable approach for enhancing disease resistance.

Conventional methods have produced a wide range of anthracnose resistant varieties. The results obtained will diminish as the state of genetic diversity changes. A decrease in genetic diversity can occur if the breeding process does not pay attention to diversity, resulting in genetic erosion. (Fu, 2015). Unintended consequences, such as the loss of genetic variation in the population, can also result from repeated selection (Hajjar and Hodgkin 2007). The high possibility of adverse effects has led to a shift away from conventional breeding methods. This has led to various researchers preferring molecular breeding methods in assembling of new superior plants.

*Molecular breeding methods for anthracnose-resistant chili peppers*

The use of molecular methods has been widely adopted in plant breeding. Molecular methods can help identify variations that cannot be seen or limitations that exist in conventional breeding (Kage *et al.* 2016; Pan *et al.* 2021). Variations in chili resistance to anthracnose are currently quite diverse in several chili cultivars. This is achieved through a screening process based on molecular methods. The use of molecular markers such as simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP) and inter-simple sequence repeat (ISSR) helps to identify gene variations for anthracnose resistance in chili.

Molecular breeding for anthracnose resistant chili continues to develop. Genes responsible for different mechanisms of chili resistance to anthracnose have been found. The identification of these genes is achieved through various methods, such as sequence or gene function analysis. Some of the genes or QTL found are CaR12.2, CcR9, AnRGO5, AnRGT5, AnRGD5, AnRRO5, AnRRT5, AnRRD5, $Co_4$ dan $Co_5$ (Lee *et al.*, 2010; Mahasuk *et al.*, 2009; Sun *et al.*, 2015). These genes influence the resistance characteristics of some Colletotrichum species.

Resistance caused by genes or QTLs occurs at various phases of fruit maturity. Lee *et al.* (2010) showed the presence of different QTLs at the fruit ripening stage for anthracnose resistance. The difference in resistance may be caused by the expression of traits that cause resistance. One of the resistance mechanisms is the hypersensitive reaction (HR), which is the result of the spread of the pathogen from the site of infection. (Kethom *et al.* 2023). Therefore, the fruit ripening phase is one of the important aspects that indicate chili pepper resistance to anthracnose.

Several genes possessed by *colletorichum* as the cause of anthracnose have been discovered. Fu *et al.* (2022) discovered various genes responsible for the character of NADPH Oxidase (Nox). These genes affect the formation of parts of the Colletotrichum fungus such as mycelial formation, conidia, conidial germination, and apportionment. In addition to genes, molecular markers are used to identify different variations of *Colletotrichum*. The use of SSR markers can help identify the types of Colletotrichum that cause anthracnose in chili peppers (Chen *et al.* 2021). Therefore, molecular technology can help the breeding process of anthracnose-resistant chilies that are more specific to the gene.

Molecular breeding has an advantage as it is precisions and speed. Utilizing molecular markers, breeders can identify and select specific genes associated with anthracnose resistance early in the breeding process (Ridzuan *et al.* 2018). Molecular method are allow breeders to enhance the genetic traits withour relying to traditional crossbreading. Molecular method such as using markers can help to map and give a better understanding of the genetic mechanisms of the target traits (Li *et al.* 2021). Besides that, molecular tools such as high-throughput sequencing and the development of marker systems have further enhanced, facilitating the genomic selection and other advanced breeding strategies.

Molecular method not only brings an advantage but also give some limitation in the breeding process. One of the key limitations in molecular for breeding disease resistance is the complexity of the traits, which often controlled by multiple genes (Esse *et al.* 2020). Resources intensive process could be happen in molecular markers for disease resistance genes, the marker may not always be able to transfer across different genetic bakcgrounds and limiting their borader applicability (Bueren *et al.* 2010). Furthermore, the durability of resistance conferred by molecular breeding aproaches may be limited as pathogens can evolve to overcome single or few resistance genes.

### *Anthracnose Resistance on chilli*

One of the key factor breeding anthracnose resistant chili is the method used for inoculation and evaluate the resistance. Inoculation test can be done not only in fruits, but also other parts of the plant or even the whole plants. Each method has their own advantages or disadvantages. Naturally, the infection of *Colletotrichum* spp. through spore attachment to the cuticle, appressoria formation and direct infection (Mongkolporn and Taylor 2018).

The resistance of chili to anthracnose is tested by artificial inoculation with isolates of *Colletotrichum* spp. Research that was conducted by Hodiyah *et al*. (2024) and Oo *et al*. (2017) using this method to test the level of pathogenicity and resistance in chili peppers to anthracnose. Artificial inoculation can be done by micro injection or spray method. Mahasuk *et al*., (2013) compared resistance based on the inoculation method and showed that two types of genes affect resistance. Inoculation with the injection method had a higher hypersensitive reaction process compared to the spray method. The damage that occurred in the injection method was higher than in the spray method. This may be due to differences in the germination rate of *Colletotrichum* fungal spores that cause damage to the host. The difference in methods causes damage only at the beginning of inoculation, but still activates the same resistance mechanism.

Fruit ripening is an essential component of anthracnose resistance testing. Tests are generally conducted at the green and red ripening stages. The resistance mechanism produced at each stage has a different mechanism against *Colletotrichum*. The resistance present at each stage of chili ripening may be different and controlled by the expression of different resistance genes (V.Y. Baba et al. 2019). Mongkolporn *et al*., (2010) found differences in resistance responses to anthracnose based on maturity phase in 33 isolates of Colletotrichum spp. in 10 chili cultivars. *Colletotrichum gloeosporioides* can infected almost all stages of fruit maturity (Katoch *et al*. 2017), in contrast to *Colletorichum truncatum* makes damage in the mature phase of red fruit (Saxena *et al*. 2014). Therefore, differences in the time of infection can cause different resistance to anthracnose in chili peppers.

The green ripe phase of chili has higher resistance than the red ripe phase. The interaction that occurs between the fruit cuticle and *Colletotrichum* affects the infection of anthracnose on the host. In the green ripe phase, anthracnose infection is lower than in the red ripe phase because the cuticle is more vulnerable to pathogen penetration (Giacomin *et al*. 2020). Baba *et al*. (2019) showed longer signs and damage in the green ripe stage than in the red ripe stage. Environmental factors can also affect the growth of pathogens in either the green or red ripening stages (Bento *et al*. 2017). Such as high humidity can easily exacerbate the damage and cause anthracnose fruit loss. It is known that the resistance shown at a certain stage of ripening is due to a combination of resistance mechanisms and environmental conditions that suppress anthracnose growth.

The defense mechanism for anthracnose itself in chili are complex and different resistance againt other *Colletotrichum* spp. Three genotypes with trait resistance such as PBC932 (*C. chinense*), PBC80 and PBC81 (*C. baccatum*) are reportedly having hypersensitive reaction (HR) (Kim *et al*. 2004; Mahasuk *et al*. 2009). The resistance varieties having active mechanisms like cuticle thickening and Program cell death as a form hypersensitive reaction. Beside that, infected cell could be thickening their cell wall with high levels of reactive oxygen species (Ranathunge *et al*. 2012). Molecular mechanisms was also reported by evaluate of the expression related genes. Mishra *et al*. (2017) reported several genes related to resistance in Bhut Jolokia (*C. truncatum* resistance) such as PDF1.2, lipoxygenase Lox3, PR2, PR5, and transcription factors (WRKY33 and CaMYB). Another genoypes with different molecular expression mechanism can be found in cv. Nokkwang (*Capsicum annum*) with *Colletotrichum siamense*. Six defense-related were identified such as Cytochrome P450 (PepCYP), Thionin-like gene (PepThi), Defensin gene (J1-1) (Oh *et al*. 1999), Thaumatin-like gene (PepTLP), MADS-box gene (PepMADS) (Oh *et al*. 2003) and Esterase gene (PepEST) (Moon *et al*. 2005).

Secondary metabolite also support the resistance mechanism againts anthracnose on chili. Genotypes GBUEL104 (*C. annum*) with infection of *C. siamense* having leads high level of caffeic acid and Chlorogenic acid depended on the fruit development stage and the time of post-inoculation (Baba *et al*. 2019). Son *et al*. (2021) reported has been identified NLR (nucleotide-binding and leucine-rich repeat) Proteins that suggest these proteins may regulate secondary metabolites that enchanhing immunity of chili againts anthracnose.

## 5. Conclusion

This review examines the conventional and molecular methods employed in the breeding of chili plants with enhanced resistance to anthracnose. This review focuses on the results obtained from the various methods that have been carried out. A variety of conventional methods, including crossing and selection techniques, are commonly employed in the formation of populations. Mass and pedigree selection are typically regarded as efficient methods for selection activities. In molecular methods, population formation by backcross is frequently employed due to its efficacy in introducing a particular character without influencing other traits. The combination of methods employed in the article review is limited to population formation and is corroborated by the identification of genes that confer resistance to anthracnose. There is an opportunity to conduct further research using mutation methods to increase diversity, particularly in relation to the character of resistance to anthracnose. A review of several articles revealed that most research focused on general resistance, as indicated by the Disease Index Rating, which was subsequently confirmed by the presence of genes through molecular analysis. Further research opportunities exist, particularly with regard to the mechanism of resistance, which may manifest as secondary metabolites or pathways. The diversity of anthracnose-causing agents, including Colletotrichum, underscores the continued urgency of anthracnose-resistant chili breeding. The review findings indicate that chili breeding with the combinations of conventional dan molecular breeding method are promising for research activity and can be pursued through various methods to produce candidate genotypes with enhanced resistance to anthracnose.

## 6. Acknowledgments

The authors would like to The Republic of Indonesia under PMDSU Research Grant financial year 2024. This research was also the part of SATREPS project "The project for Breeding Innovation for Chilli Pepper and Tomato to Accelerate Sustainable Vegetable Production in Tropical Regions".

## 7. Declaration of Conflicting Interests

The authors have declared no potential conflicts of interest concerning the study, authorship, and/or publication of this article.

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