Plant Archives Vol. 26, Supplement 1, 2026 pp.269-281 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-1.035

BREEDING FOR DOWNY MILDEW RESISTANCE IN CUCURBITACEOUS VEGETABLES: A REVIEW

P. Anupriya*, S. Sarada and Syama S. Thampi
Department of Vegetable Science, College of Agriculture, Vellayani, Kerala Agricultural University, Thiruvananthapuram, Kerala, 695522, India
*Corresponding author E-mail: anupriyap773@gmail.com
(Date of Receiving : 23-08-2025; Date of Acceptance : 27-10-2025)

ABSTRACT

Cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, is a devastating disease that results in substantial yield losses across many cucurbit-growing regions worldwide. Although chemical control measures have been employed, their excessive use poses environmental risks and is often ineffective against evolving pathogen strains. Developing resistant cultivars is therefore widely regarded as the most effective and sustainable long-term approach for managing cucurbit downy mildew disease. This review highlights conventional, genetic, and molecular breeding approaches for downy mildew disease resistance in cucurbits. It examines the genetic basis of resistance, with emphasis on key resistance (R) genes and quantitative trait loci (QTLs) identified through genetic mapping. Advances in molecular tools, including marker-assisted selection (MAS), genomic selection (GS), and CRISPR/Cas9 genome editing, have further accelerated the development of resistant cultivars. Additionally, the application of transcriptomics, genomics, and other omics-based approaches is discussed for elucidating resistance mechanisms and informing future breeding strategies. Collectively, these strategies pave the way for sustainable and effective management of downy mildew in cucurbits.

***Keywords*:** Cucurbit downy mildew, *Pseudoperonospora cubensis*, disease resistance, molecular breeding

Introduction

Cucurbitaceae, also known as cucurbit or the gourd family, is a large family of flowering plants in the order Cucurbitales. The family derives its name from the Latin word '*corbis',* which means bottle or basket (Labeda *et al.,* 2007). It is one of the most diverse plant families cultivated worldwide across a range of environmental conditions, comprising approximately 130 genera and 800 species (Rolnik and Olas, 2020).

The important genera belonging to Cucurbitaceae family are *Trichosanthes*, *Lagenaria*, *Luffa*, *Benincasa*, *Momordica*, *Cucumis*, *Citrullus*, *Cucurbita*, *Corallocarpus,* and *Bryonopsis* (Pandey, 1969). This plant family holds significant economic importance as a major source of human food. Numerous species including those of *Cucurbita* (pumpkins, squashes, gourds, marrows, and courgettes), *Cucumis* (melons and cucumbers), *Cucumeropsis mannii* Naud. (egusi), *Colocynthis* (watermelon), and *Sechium edule* (Jacq.) are primarily cultivated for their edible parts (Ajuru and Ajuru, 2014).

Cucurbits are mostly annuals and rarely perennials. They possess herbaceous, angular stems that typically trail along the ground or climb with the help of tendrils. Their leaves are often lobed or divided, with a reticulated, palmately veined structure and a long, hollow petiole. Both the stem and leaves contain abundant juicy sap (Rolnik and Olas, 2020). The chemical composition of the Cucurbitaceae family consists of phytochemicals. They are naturally occurring nonnutritive compounds in plants, including tannins, cardiac glycosides, terpenoids, carbohydrates, saponins, resins, carotenoids, and phytosterols (Rajasree *et al.,* 2016).

Cultivation of cucurbits is often hindered by various diseases that adversely affect plant growth and yield. Among these, downy mildew, powdery mildew, and fusarium wilt are the most significant threats (He *et al.,* 2020). Downy mildew, caused by *Pseudoperonospora cubensis* [(Berk. & Curt.) Rostow.], is particularly devastating and has been reported in over 70 countries, making it the most destructive disease of cucurbits worldwide (Savory *et al.,* 2011). Its impact is especially severe during the monsoon season, posing a major challenge to cucurbit cultivation besides other pests and diseases.

This review focuses on the impact of downy mildew on cucurbit cultivation, emphasizing the need for effective resistance breeding strategies. It highlights the identification of resistance genes, understanding the genetic basis of resistance, and the integration of conventional and modern breeding techniques. The role of modern molecular breeding techniques, including QTL mapping, marker-assisted selection (MAS), genomic selection (GS), and CRISPR/Cas9 in accelerating resistance breeding is examined. Additionally, the contribution of transcriptomics, genomics, and other omics-based approaches in understanding resistance mechanisms and shaping sustainable breeding strategies is addressed.

Downy mildew pathogen

Downy mildew of cucurbits is caused by *Pseudoperonospora cubensis*, an oomycete pathogen belonging to the class Oomycota. It is one of the most damaging foliar diseases affecting cucurbits globally, leading to significant yield reductions. A detailed account of the pathogen is discussed below.

History, Geographic distribution and Host range

Cucurbit downy mildew, caused by the oomycete *Pseudoperonospora,* has long since been known as a parasite in all parts of the world. *Pseudoperonospora* was first described by Berkeley in 1868, in herbarium plant material originated from Cuba, and hence its species was named as *cubensis*. The pathogen was first observed and described on live plants by Rostovzev in the Botanical Gardens of Moscow, Russia in 1903. Venkatanarayana and Venkatakrisniah (1953) first noticed the occurrence of downy mildew on cucumber in India, and downy mildew of watermelon in the world. It later spread to sweet melon and bottle gourd.

As early as the 19th century, downy mildew was observed on cucumbers and melons, but it did not reach economically significant levels until the mid-1980s (Colucci *et al.,* 2006). A major outbreak of downy mildew was reported on melons in France in 1984 (Pitrat and Blancard, 1988). In 1985, the disease reached epidemic levels in cucumbers grown in Central-Eastern Europe. In USA, it became a serious problem starting in 2004 (Colucci *et al*., 2006). Annual downy mildew epidemics threaten cucumber production in upto 80 countries and muskmelon production in over 50 countries, causing substantial economic losses (Lebeda and Urban, 2004; Colucci *et al.,* 2006).

P. cubensis, the causative agent of cucurbit downy mildew, is an obligate parasite with a broad geographical distribution, having been reported in over 70 countries across diverse environments, from semi-arid to tropical regions (Palti and Cohen, 1980; Bains and Jhooty, 1978). It possesses a wide host range, infecting around 20 different genera within the Cucurbitaceae family. Experimental inoculation of *P. cubensis* isolated from ridge gourd showed infection in ash gourd, bottle gourd, bitter gourd, cucumber, muskmelon, pumpkin, snake gourd, sponge gourd, and watermelon, with the exception of little gourd, which showed no signs of infection (Gurushanthappa, 1990). Further studies by Savory *et al.* (2011) expanded this host range to over 49 species in 20 genera, including 19 species in the genus *Cucumis*, as well as melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), squash (*Cucurbita* spp.), wax gourd (*Benincasa hispida*), bottle gourd (*Lagenaria siceraria*), and bitter gourd (*Momordica charantia*). This highlights the significant threat posed by *P. cubensis* to cucurbit cultivation worldwide due to its extensive adaptability and host specificity.

Taxonomy and Symptoms

P. cubensis belongs to the Kingdom Chromista, Phylum Oomycota, Order Peronosporales, Family Peronosporaceae, and Genus *Pseudoperonospora* (Lebeda and Cohen, 2011).

Downy mildew of cucurbits is a foliar disease, characterized by chlorotic lesions on the upper leaf surface, sometimes with necrotic centers. Symptom expression varies among cucurbit species and varieties, particularly in terms of lesion development, shape, and size (Savory *et al.,* 2011). Initial symptoms typically appear on the lower (abaxial) leaf surface as water-soaked lesions near the margins, while the corresponding upper (adaxial) surface exhibits chlorotic spots. These spots are confined between veins and veinlets, giving them an angular appearance. On older leaves, the abaxial spots are yellow to orange-yellow, turning purplish under humid conditions, and eventually leading to overall leaf yellowing. Necrosis begins at the margins of severely affected leaves,

ultimately causing complete leaf desiccation. Severe infections result in extensive foliage damage, reducing both flower formation and fruit development (Hashmi, 1994).

Lebeda and Schwinn (1994) reported that in some species (*Cucumis, Luffa*), *P. cubensis* causes irregular, localized, yellow lesions, restricted by leaf veins, whereas in cantaloupe and watermelon, lesions were not restricted by leaf veins and are more circular and irregular. Thomas (1996) observed that as the downy mildew disease progresses, entire leaves may die within fifteen days following the initial infection as lesions expand and coalesce. The reduction in canopy due to downy mildew leads to cessation of fruit, allowing for sun scald and secondary rots, ultimately affecting crop yield and fruit quality (Keinath *et al.*, 2007). The most distinctive feature of the disease is the grayish to purplish downy coating on the undersides of leaves, which consists of sporangiophores and sporangia produced by the pathogen. This characteristic is crucial in disease diagnosis (Mirzwa-Mroz *et al.,* 2024).

Pathotypes

Different pathotypes and races of the *Pseudoperonospora* pathogen have been reported from various regions of the world. These pathotypes of *P. cubensis* infect a wide range of cucurbits, including cucumber, muskmelon, pickling melon, watermelon, pumpkin, squash, bitter gourd, bottle gourd, and sponge gourd (Cohen *et al.,* 2003).

Initial studies of variability among *P. cubensis* isolates were carried out by Thomas *et al.* (1987), who reported the existence of five pathotypes among isolates collected in Israel, Japan, and the USA. Their studies were based on pathogen compatibility reactions with species of *Cucumis, Citrullus,* and *Cucurbita*. Later, Cohen *et al.* (2003) reported a sixth pathotype, isolated in Israel. Shetty *et al.* (2002) found that pathotypes from European and North American were more closely related, and that Asian pathotypes were more distinct. Lebeda (1990) noted that the geographic origin and year of isolation played a role in the pathogenicity of *P. cubensis.* In later studies, Lebeda and Gadasova (2002) used differential hosts representing five genera of Cucurbitaceae and identified 13 distinct pathotypes in Central Europe. Lebeda and Widrlechner (2003) developed a set of 12 different hosts (representing different genera, species, and cultivars) to identify pathotypes in *P. cubensis.* This broad range of pathogen variability is typical for the Oomycetes and is also found in *Phytophthora infestans* and *Bremia lactucae* (Bouwmeester *et al.,* 2009). Recently, the genomes of three Oomycetes, *P. ramorum, P. sojae* (Tyler *et al.,* 2006), and *P. infestans* (Haas *et al.,* 2009), have been sequenced. In *P. cubensis,* the 709-bp rDNA-ITS region was sequenced, showing that it can be divided into three distinct parts: 141 bp of rDNA-ITS1, 406 bp of rDNA-ITS2, with GC contents of 41% and 46%, respectively, and the relatively conserved 5.8 S coding region. The ITS1 and ITS2 regions, which are non-coding, are commonly used in phylogenetic studies of Oomycetes because of their relatively high sequence variability and the widespread availability of polymerase chain reaction (PCR) primers. Although the rDNA-ITS sequences are nearly identical across various *P. cubensis* isolates, this consistency makes them highly effective as molecular markers for species identification (Wang *et al.,* 2008).

Disease Epidemiology and Mechanisms of Pathogen Dispersal

The downy mildew pathogen is an obligate parasite and can survive and reproduce only on living host tissue (Bains and Jhooty, 1978). *P. cubensis* reproduces asexually by forming sporangiophores, sporangia, and zoospores, which are critical for causing infection (Cohen *et al.,* 2003). In addition, *P. cubensis* can also generate oospores by sexual reproduction, but the role of oospores in the disease cycle remains unknown, as sexual reproduction is rare (Lebeda and Cohen, 2011).

Environmental conditions play a crucial role for the development of pathogen and disease intensity. The disease is most severe in tropical and subtropical regions during the monsoon season or periods with high humidity, warm temperature and frequent rainfall. It gets sporulated mostly on the underside of the leaves and results in the production of sporangia, which act as sources of primary inoculum from infected plants, mainly distributed by wind or water splash (Lebeda and Cohen, 2011). The lifespan of its sporangia is limited to a maximum of 48 hours, during which they must locate a susceptible host and successfully germinate (Cohen and Rotem, 1971). The optimal temperature range for sporangia germination is between 10°C and 20°C, while temperatures exceeding 30°C reduce the germination rate (Lebeda and Cohen, 2011).

Successful infection requires the presence of free water on the leaf surface to allow the zoospores to form germ tubes. Sporangia require at least two hours to germinate and penetrate the leaf surface (Cohen, 1981). Leaf wetness allows the infection to occur, while temperature determines the degree of disease development (Arauz *et al.,* 2010). The time between

initial infection and the appearance of visible symptoms varies depending on environmental conditions and inoculum levels, typically ranging from 4 to 12 days (Cohen, 1977). In favourable conditions, symptoms may appear as early as three days post-infection. The initial amount of inoculum also plays a crucial role. When the inoculum concentration is low (10 sporangia/cm² per leaf), symptom onset may take over a week (Cohen and Eyal, 1977). The incubation period is influenced by environmental factors, the host species, and the amount of initial inoculum. Disease development occurs most effectively at day temperatures between 25°C and 30°C and night temperatures of 10°C to 15°C (Palti and Cohen, 1980). While colonization benefited from low temperatures, symptom development increased under higher temperatures andgreater light intensity. Enhanced symptom development led to more chlorotic lesions. Hot and dry weather in the field increased the rate at which the lesions became necrotic; necrotic lesions terminated the survival of *P. cubensis,* thus ceasing sporulation (Cohen, 1981). The pathogen normally overwinters on the plant debris and in the soil (Resmi and Sreelathakumary, 2017).

Biological and chemical control for downy mildew

Biological control offers an eco-friendly approach to manage cucurbit downy mildew through the use of plant extracts, beneficial microbes, glycoproteins, and biorational products. Medicinal plants like *Glycyrrhiza glabra*, *Uvaria grandiflora*, garlic, and *Disporopsis aspersa* have shown strong antifungal activity (Mirzwa-Mroz *et al.,* 2024). Microorganisms such as *Trichoderma* spp., *Bacillus*, *Enterobacter*, *Streptomyces,* and *Lysobacter* effectively suppressed downy mildew and enhanced plant growth. Among these, *Trichoderma* species, especially *T. asperellum* and *T. atroviride* (TRS25), played a key role by reducing disease severity and inducing plant defence responses. Additionally, endophytic fungi like *Pestalotiopsis* and *Fusarium* (FO47 and FO47B10) have demonstrated both protective and therapeutic effects, while numerous bacterial genera including *Bacillus*, *Paenibacillus*, *Enterobacter*, *Streptomyces*, *Pseudomonas*, *Derxia,* and *Aneurinibacillus* have also shown significant biocontrol potential against downy mildew (Sun *et al.,* 2021).

Fungicides play an essential role in controlling cucurbit downy mildew, especially in areas that experience frequent and severe outbreaks (Ojiambo *et al.,* 2010). In Europe, effective disease management relies on combining fungicide treatments with the use of tolerant cucurbit varieties. In contrast, the USA employs a more intensive approach, requiring fungicide applications every 5–7 days for cucumber and every 7–10 days for other cucurbits to prevent outbreaks and minimize yield losses (Savory *et al.,* 2011).

Initiating a preventive fungicide spray programme early in the growing season, prior to symptom development, is crucial, as fungicides tend to be more effective when applied before infection takes hold. Commonly used active ingredients for managing cucurbit downy mildew include cyazofamid, ametoctradin, ametoctradin combined with dimethomorph, propamocarb hydrochloride, azoxystrobin, azoxystrobin with oxathiapiprolin, as well as various copper-based compounds and sulfur. Among these, fungicides containing oxathiapiprolin have shown the highest efficacy in the USA, followed by cyazofamid and propamocarb hydrochloride in reducing disease severity (Mirzwa-Mroz *et al.,* 2024). However, repeated use of the same active ingredients can accelerate the development of fungicide resistance in downy mildew pathogens. This has led to the discontinuation of strobilurin fungicides, such as azoxystrobin, for downy mildew control in the USA (Ojiambo *et al.,* 2015). Similarly, in Israel, some *P. cubensis* strains have developed resistance to propamocarb hydrochloride and dimethomorph (Lebeda and Cohen, 2011). To mitigate resistance development, mixtures of systemic and protectant fungicides have been recommended for disease management programmes, as they prolong the efficacy of systemic fungicides by delaying the buildup of resistant strains in pathogen populations.

The overreliance on fungicides presents several challenges, including the high cost of repeated applications, environmental concerns, and the rapid evolution of fungicide-resistant pathogen strains. These drawbacks stress the urgent need for alternative, more sustainable strategies. In this context, breeding for host plant resistance has emerged as a critical approach. Developing resistant cultivars reduces dependency on chemical inputs, minimizes environmental risks, and provides long-term protection against evolving pathogen populations. Therefore, integrating host resistance into downy mildew management programmes is essential for ensuring the sustainability and profitability of cucurbit production.

Resistance to Cucurbit Downy Mildew-Conventional, Genetic and Molecular Breeding Approaches

Genetic basis of the disease

The genetic basis of downy mildew in cucurbits has been extensively studied due to the serious threat posed by *P. cubensis*. Greater insight into this genetic foundation is essential for a successful resistance breeding programme. The nature and magnitude of gene action of the resistant germplasm determine the progress in the development of disease-resistant varieties. The gene action for resistance to downy mildew caused by *P. cubensis* in cucurbitaceous vegetables are presented in Table 1.

Table 1: Review of genetics of downy mildew resistance in cucurbits

Crop	Resistant line/cultivar	Gene action	References
Cucumber	PI 197087	One or two major genes with one or more minor genes	Burnes and Epps (1954)
Cucumber	Aojihai	Three recessive genes	Shimizu *et al.* (1963)
Cucumber	Poinsett	Single recessive gene (*dm*)	van-Vliet and Meysing (1974)
Muskmelon	MR-1	Two incompletely dominant genes (Pc-1 and Pc-2)	Thomas *et al.* (1988)
Muskmelon	PI 414723	Single dominant gene (Pc-3)	Epinat and Pitrat (1989)
Cucumber	Palmetto and Yomaki	Two pairs of dominant and recessive interaction genes	El- Hafaz *et al. (*1990)
Cucumber	Wisconsin –4783	Three recessive genes (dm-1, dm-2 and dm-3)	Doruchowski and Lakowski-Ryk (1992)
Muskmelon	PI124112F	Two incompletely dominant genes	Kenigsbuch and Cohen (1992)
Muskmelon	Phoot × Monoecious-3 and Phoot × Pusa Madhuras	Two dominant genes	Somkuwar and More (1993)
Muskmelon	Phoot × Lucknow Safeda	Two recessive genes	Somkuwar and More (1993)
Cucumber	TX302	Two pairs of dominant and recessive interaction genes	Badr and Mohamed (1998)
Muskmelon	5-4-2-1	Single dominant gene	Angelov and Krasteva (2000)
Cucumber	J-13	One or two incompletely dominant genes	Petrov *et al. (*2000)
Cucumber	Ames 2354	One to three genes	Kozik *et al.* (2013)
Cucumber	DC 70	Monogenic recessive gene	Bhutia (2015)
Ridge gourd	IIHR-52-1-30 × IIHR-17-1-7-3 and IIHR-23-8-10 × IIHR-7-5-1	Two pairs of dominant and recessive interaction genes	Varalakshmi *et al.* (2022)
Cucumber	Swarna Agethi x IIHR-438 and IIHR-431x IIHR-433	Two pairs of dominant and recessive interaction genes	Bommesh *et al.* (2024)
Bitter gourd	Priyanka x Phule Green Gold	Recessive genes by inhibitory gene action	Thampi (2024)

Conventional breeding approaches

Conventional breeding for downy mildew resistance in cucurbits involves screening diverse germplasm to identify resistant sources followed by hybridization with high-yielding susceptible varieties to combine resistance with desirable traits. Wild relatives are also utilized to introgress resistant genes. Backcrossing, pedigree selection, and recurrent selection methods are commonly employed to stabilize resistance in advanced breeding lines.

Screening of germplasm for downy mildew resistance in cucurbits

Downy mildew disease caused by *P. cubensis* is one of the most destructive pathogens affecting cucurbits, which is responsible for devastating yield losses worldwide of cucumber, squash, watermelon, cantaloupe, and other species (Thomas *et al.,* 1987). It causes yield losses of upto 66-100 percent in the most disease affected areas (Lebeda *et al.,* 2011). Various pathotypes of *P. cubensis* attacks different cucurbits such as cucumber, muskmelon, pickling melon, watermelon, pumpkin, squash, bitter gourd, bottle gourd, and sponge gourd (Cohen *et al.,* 2003). Multiple pathotypes and races of pathogen (Cohen *et al.,* 2003), environmental conditions (Palti and Cohen, 1980) and a narrow genetic base are major hurdles in identifying resistant sources in cucurbits. Host plant resistance is considered as the best alternative for controlling the disease and can be achieved by the identification of resistant sources against the pathogen (Jadhav and Sharma, 1983). Screening resistant sources against the pathogen substantially reduces excessive fungicide use and the threat of pathogen resistance. Several studies have been conducted to identify sources of resistance to downy mildew disease in cucurbits through artificial and natural screening methods.

Artificial screening

Pandey *et al.* (2005) artificially screened 148 germplasm lines of bitter gourd to identify sources of resistance to downy mildew disease. Among the different germplasm lines, NIC-12285 and VRBT-39 were moderately resistant with high degree of tolerance. Bhutia (2015) evaluated 114 genotypes of cucumber in natural and artificial condition, and among them, 10 genotypes were resistant, 18 genotypes were moderately resistant, 37 genotypes were moderately susceptible and 49 genotypes were highly susceptible to the disease. The disease severity expressed as Percent Disease Index (PDI) for the highly resistant genotypes ranged from 15 to 25%.

In a study on *Cucurbita* species, Lebeda *et al.* (2016) evaluated 97 accessions against 11 isolates of *P. cubensis* from cucumber using an artificial leaf-disc inoculation method. Out of these, 57 accessions exhibited distinct reaction patterns. Among them, 15 accessions were identified as resistant, while 12 accessions were found to be susceptible to all *P. cubensis* isolates. Artificial screening of 17 advanced lines of bottle gourd under controlled conditions against downy mildew by Bhardwaj *et al.* (2018) revealed that only one line, VRBG-12, showed resistant reaction with the lowest PDI value of 6.5. Three lines, viz. VRBG-26, VRBG-47 and VRBG-17 were moderately resistant, four lines (VRBG-11, VRBG-20, VRBG-49 and VRBG-56) were moderately susceptible and five lines (VRBG-52, VRBG-5, VRBG- 10, VRBG-66 and VRBG-53) were susceptible.

Artificial screening of 41 cucumber genotypes along with the check variety Swarna Agethi was carried out by Bommesh *et al.* (2018). They identified the resistant genotypes, *Cucumis metuliferus* and IIHR-438, which showed an average PDI of 12.8 and 14.3 respectively, which were found to be much lesser than the other genotypes and the susceptible check. Similarly, Gautam *et al.* (2020) artificially screened 50 cucumber genotypes against the downy mildew pathogen and identified the resistant genotype IC 331627 which showed PDI of 11.56 and 11.87 during the year 2017 and 2018 respectively. In another study, Pitchaimuthu *et al.* (2024) screened 12 advanced breeding lines along with susceptible check of cucumber against downy mildew disease under natural field condition and artificial inoculation through seedling assay technique. The findings revealed that three lines, namely IIHR-177-1-1-S7, IIHR-82-1-S6, and IIHR-81-1-S6, exhibited resistance with PDI <10%. These lines were also superior in yield and quality traits, compared to the check variety Swarna Agethi.

Field screening

Goswami *et al.* (2011) screened 95 muskmelon lines against downy mildew disease and revealed that four lines *viz.,* IC 274014, IC 267397, MCPS-2, and Mehna Chibber, were highly resistant to downy mildew. Call *et al.* (2012) screened 1300 cucumber genotypes for downy mildew resistance between 2005 and 2007 in North Carolina and Poland. Among them, the cultivars PI-330628, PI-605996, and PI-197088 demonstrated tolerance to the disease at both locations. In another study, Pitchaimuthu *et al.* (2012) screened 42 cucumber accessions under open-field conditions and reported that the wild species *Cucumis hardiwickii*-14 and 15, *Cucumis sativus* var. *sativus,* and SM 12735 exhibited a high level of resistance to powdery and downy mildew diseases with a PDI of 0-25 % under natural conditions.

Screening of 25 genotypes of bottle gourd revealed three genotypes *ie.,* Kaveri, Gutkha, and Sarika, showing resistance against downy mildew (Harika *et al.* 2012). Innark *et al.* (2014) evaluated 40 cucumber accessions for downy mildew resistance under natural field conditions and identified one highly resistant (PI489752), 23 moderately resistant, and six highly susceptible accessions. Screening of 38 accessions of cucumber for disease reaction showed that 12 accessions were resistant (score 0–2) and 26 accessions were susceptible (score 4–9). Among the resistant accessions, IC410617, IC527419, and IC538130 were highly resistant (score = 1) (Ranjan *et al.,* 2015). Open field screening for downy mildew was conducted by Narasannavar *et al.* (2017) in ridge gourd and identified two resistant genotypes (COHRG-9 and COHB-32), two moderately resistant lines (COHB-10 and COHB-40), and one highly susceptible line (COHB-8).

Kumar *et al.* (2018) evaluated 19 commercially grown bitter gourd varieties for their resistance to downy mildew. Among them, Unnat Kathi Gaurav, and Kathi Selection demonstrated high resistance to the disease. Three varieties (No. 4003, Bujji, and Meghanaa-2) exhibited moderate resistance, 13 varieties (VNR-28, VNR Kanhaiya, Vivek, Sagar (AG811), Nanha, Ankur Tillu, Sunil Karela, Indra Karela, Raman, Selection 05, NS1018, Katahi, and Uchha Bolder) were moderately susceptible and only one variety (VNR-22) was found to be highly

susceptible to downy mildew, making it the most vulnerable among those tested. Ten varieties and thirteen advanced breeding lines of ridge gourd were screened under natural epiphytotics by Lavanya *et al.* (2023) and reported that ten lines were moderately resistant based on PDI. Among them, two lines, IIHR-DMR-18-4-4 (PDI=23.46) and IIHR-DMR-18-65-1 (PDI=24.02) showed the lowest disease severity.

Artificial and field screening of 22 genotypes of *Momordica charantia* and *Momordica charantia* var. *muricata* was conducted to identify resistant sources to downy mildew disease by Thampi *et al.* (2024). Four genotypes (MC 48, MC 49, MC 50, and MC 53) which showed mild symptoms under artificial inoculation (PDI=11.30-23.45%) having moderate downy mildew resistance, were identified. Under field conditions, MC 48 and MC 50 were moderately resistant (PDI=11.04-11.98%).

Molecular breeding approaches for downy mildew resistance in cucurbits

Conventional breeding methods, though widely used, face several limitations. These methods are time-consuming, labour-intensive, and often influenced by environmental variability. The polygenic nature of resistance**,** limited availability of resistant germplasm and the long breeding cycles further hinder the breeding progress. Moreover, introgression of resistance traits from wild relatives can result in linkage drag, affecting other desirable traits like fruit yield or quality. These challenges make molecular breeding an essential tool for accelerating the development of resistant cultivars. Molecular approaches such as QTL mapping, bulked segregant analysis (BSA), next-generation sequencing (NGS), genome-wide association studies (GWAS), proteomics, and transcriptomics have facilitated the identification of resistance genes and their genomic locations. Marker-assisted selection (MAS) has further enhanced breeding efficiency by enabling precise trait selection at the seedling stage, reducing the breeding time.

QTL mapping

Quantitative Trait Locus (QTL) mapping is a powerful approach for identifying genes associated with downy mildew resistance in cucurbits. Several studies have successfully used QTL mapping to detect multiple resistance loci, helping breeders develop disease-resistant varieties. Various molecular markers, including Sequence Characterized Amplified Regions (SCAR), Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphisms (SNP) have been employed to locate and characterize these QTLs, providing valuable genetic resources for cucurbit breeding programmes (Mirzwa-Mroz *et al.,* 2024).

Partial resistance to downy mildew was studied by Perchepied *et al.* (2005) using a recombinant inbred line population between 'P 124112' (resistant line) and 'Vedrantais' (susceptible line) in melons. One major QTL (pcXII.1) was consistently detected and eight other *P. cubensis* resistant QTLs were identified. In cucumber, Bai *et al.* (2008) developed a mapping population with the inbred line S94 and detected four QTLs (*dm1.1*, *dm1.2*, *dm6.1*, and *dm6.2*) for downy mildew resistance, which were mapped to linkage groups 1 and 6.

PI 197088 is one among the best resistant line to *P. cubensis* (Call *et al.,* 2012). Caldwell *et al.* (2011) reported three downy mildew resistant QTL from PI 197088. Li *et al.* (2018) developed a mapping population with PI 197088 as the resistant parent. A linkage map was constructed using 141 SSR markers and five QTLs were detected on chromosomes 1, 3, 4 and 5 with downy mildew resistance contributed by PI 197088. Wang *et al.* (2018) also developed recombinant inbred lines (RIL 148) from a cross between PI 197088 (resistant) and 'Cool green' (susceptible). A high-density genetic map was constructed with 2780 single nucleotide polymorphisms from genotyping-by-sequencing and 55 SSR markers were developed and eleven QTL's mapped for downy mildew resistance. Among them, the major QTLs were dm5.1, dm5.2 and dm5.3. These experiments show that the cucumber accession PI 197088 exhibits high level of resistance.

Pang *et al.* (2013) developed an F_2 mapping population of IL52 (an introgression line) derived from a cross between cultivated cucumber and *C. hystrix,* and mapped two QTLs (dm-5.1 and dm-5.2) for downy mildew resistance on chromosome 5 using interval mapping analysis.

Yoshioka *et al.* (2014) performed QTL analysis for downy mildew resistance using recombinant inbred lines (RILs) derived from CS-PMR1 × Santou and mapped 10 QTL, of which 7 (dm1.2, dm1.3, dm3.1, dm3.2, dm5.1, dm5.2, and dm5.3) were from CS-PMR1 and 3 (dm1.1, dm6.1, and dm7.2) from Santou, exhibiting moderate resistance. QTL analysis of downy mildew resistance conducted by Szczechura *et al.* (2015) in a mapping population derived from crossing resistant PI 197085 and susceptible PI 175695 lines identified three QTLs on chromosome 5 that contribute to resistance.

A genetic linkage map was developed using 348 SSR and SNP markers with 243 F_2:3 families derived

from a cross between downy mildew resistant inbred line WI7120 (PI 330628) and susceptible line 9930. Four stable QTLs were identified for downy mildew resistance on cucumber chromosomes 2, 4, 5, and 6, respectively, with resistance inherited from WI7120 (Wang *et al.,* 2016).

A genetic linkage map developed with 66 polymorphic SSR markers led to the identification of 14 QTLs with 5-12.5% of phenotypic variance, which were assessed for downy mildew resistance in cotyledons and true leaves following inoculation. Out of these, two were major QTLs (Cot7_5.1_2 and Cot10_5.1). Major QTL Cot7_5.1_2 was located between SSR19172 and SSR07531 markers and explained 10.9% of phenotypic variation, while the other major QTL Cot10_5.1 was located between SSR03943 and SSR20859 SSR markers and explained 12.5% of the phenotypic variation (Innark *et al.,* 2020). More recently, three sub-QTLs were identified within QTL DM4.1 on chromosome 4 in Indian cucumber accession PI 197087, DM4.1.1 associated with pathogen-induced necrosis; DM4.1.2 associated with improving sporulation resistance and DM4.1.3 with recessive effects on chlorosis and sporulation (Berg *et al.,* 2020).

Bulked Segregant Analysis (BSA) and Next-Generation Sequencing (NGS)

Bulk Segregant Analysis (BSA) is one of the rapid and cost-effective genetic mapping approaches used to identify markers linked to any specific gene or genomic regions associated with specific traits in segregating populations (Michelmore *et al.,* 1991).

Zhang *et al.* (2013) reported five QTLs (dm1.1, dm5.1, dm5.2, dm5.3, and dm6.1) for downy mildew resistance in cucumber using SSR analysis combined with BSA in F_2 population using 2360 pairs of SSR primers. Win *et al.* (2017) utilized next-generation sequencing (NGS) assisted BSA to an F_2 population to map QTLs associated with downy mildew resistance in cucumber. Their study detected five QTLs dm2.2, dm4.1, dm5.1, dm5.2, and dm6.1 with dm2.2 exhibiting the most significant effect on resistance.

Genome-Wide Association Studies (GWAS)

Genome-wide association studies (GWAS) test hundreds of thousands of genetic variants across many genomes to find those statistically associated with a specific trait or disease (Uffelmann *et al.,* 2021). GWAS of 97 cucumber lines identified 18 QTLs, among which six (dmG1.4, dmG4.1, dmG4.3, dmG5.2, dmG7.1, and dmG7.2) were consistently associated with stable resistance to downy mildew. Several candidate genes were proposed as potential causal genes underlying these stable and novel loci, including Csa1G575030 for dmG1.4, Csa2G060360 for dmG2.1, Csa4G064680 for dmG4.1, Csa5G606470 for dmG5.2, and Csa7G004020 for dmG7.1 (Liu *et al.,* 2020).

Proteomic analysis

Proteomic analysis of downy mildew resistant ('ZJ') and susceptible ('SDG') cucumber varieties revealed significant differences in protein expression under *P. cubensis* infection. Key enzymes involved in terpenoid backbone biosynthesis were highly accumulated in the resistant line, suggesting their role in defence. Additionally, several pathogenesis-related proteins, including PR proteins, endochitinases, and peroxidases, were identified, indicating a complex resistance network (Zhang *et al.,* 2019).

Sun *et al.* (2021) also reported significant differences in protein expression between resistant and susceptible cucumber lines in response to *P. cubensis* infection. Most differentially expressed proteins were associated with energy metabolism, cell rescue, and defence mechanisms. The resistant line showed enhanced accumulation of defence-related and energy-supplying proteins.

Transcriptome analysis

Transcriptome analysis of resistant and susceptible cucumber genotypes under *P. cubensis* infection revealed significant differences in gene expression associated with downy mildew resistance. Resistant lines exhibited early activation of key defence responses, including hormone signaling, nutrient regulation, pathogen recognition, signal transduction, reactive oxygen species (ROS) production, lignin biosynthesis, protein metabolism, and transcriptional regulation. Coexpression network and miRNA analyses further highlighted the complexity of gene regulation during infection (Burkhardt and Day, 2016; Gao *et al.,* 2021).

Importantly, five candidate genes were strongly implicated in cucumber's defence response including Csa5G139760 (acidic chitin endonuclease) involved in pathogen degradation, Csa6G080320 (LRR and transmembrane domain kinase) in immune signaling, Csa5G471600 (retroviral receptor-like protein) in pathogen recognition, and Csa5G544050 and Csa5G564290 (RNA-dependent RNA polymerases) potentially contributing to post-transcriptional gene silencing (Gao *et al.,* 2021).

Marker-assisted selection

MAS refers to the use of DNA markers tightly linked to the target loci as a substitute for phenotypic screening or to assist it. It enables more precise trait integration, reducing unintended losses and accelerating the development of improved cultivars with fewer selection cycles (Xu and Crouch, 2008), without replacing traditional breeding (Pathania *et al*., 2017).

Horejsi *et al.* (2000) identified five RAPD markers linked to the dm gene for downy mildew resistance in cucumber. The markers $G14_{800}$ and $BC519_{1100}$ were linked in repulsion and the markers $X15_{1100}$, $AS5_{800}$, and $BC526_{1000}$ were in coupling phase. These markers were utilized to develop an efficient marker-assisted selection (MAS) strategy for breeding resistant cucumber cultivars. Ding *et al.* (2007) identified another RAPD marker, SPS18-561, associated with downy mildew resistance in cucumber. Seven sequence related amplified polymorphism (SRAP) markers and one SSR marker were linked to the QTLs (dm1.1, dm1.2, dm6.1, and dm6.2) identified for downy mildew resistance in the inbred line S94 (Bai *et al.,* 2008).

A total of 143 SSR primers were employed to identify polymorphic primers using 225 F_2 populations of Swarna Agethi x IIHR-438 through BSA. SSR 3-5 and SSR 4-13 are co-segregated with disease reaction in BSA. The 'dm' genes were tagged with SSR 3-5 and SSR 4-13 markers loci, with a distance of 16.6 cM in linkage group 3 and 18.1 cM in linkage group 4, respectively. In F_2 genotyping for SSR 3-5, the segregation ratio of the marker was 1:2:1 in chi square analysis for goodness of fit and revealed that this marker linked highly with disease resistance (Bommesh, 2019).

Challenges and Future prospects in breeding for downy mildew resistance

Downy mildew disease in cucurbits, caused by *P. cubensis,* is a major threat to cucurbit production globally, leading to severe yield losses. Control is quite challenging due to the rapid evolution of new pathogen strains, that can overcome existing resistance in commercial cultivars. Moreover, relying on fungicides is not a sustainable solution, due to the increasing environmental concerns and the risk of resistance development in pathogen populations. Growing cultivars resistant to downy mildew can enhance crop yields and boost farmers income (Gautham *et al.,* 2020). Therefore, developing effective and long-lasting breeding strategies is crucial for sustainable disease management.

Conventional breeding techniques are time-consuming and labour-intensive, and there are chances of introgression of undesirable genes along with resistance genes due to linkage drag or unintended genetic changes. Therefore, to advance future research and breeding in cucurbits, it is crucial to harness the potential of advanced genomic technologies (Mirzwa-Mroz *et al.,* 2024). Recent advances in genome editing technologies, particularly CRISPR/Cas9, have revolutionised crop protection through precise and targeted genome modifications (Faizal *et al.,* 2024).

In a study conducted by Yin *et al*. (2004), a gene construct containing the PR-2d promoter linked to the *uidA* reporter gene (β-glucuronidase gene) from tobacco (*Nicotiana tabacum*) was introduced into a highly inbred line of cucumber (*C. sativus* cv. Borszczagowski) using *Agrobacterium tumefaciens*-mediated transformation. Inoculation with *P. cubensis* increased the concentrations of endogenous salicylic acid and salicylic acid glucoside in transgenic cucumber leaves, providing evidence for the role of SA in signaling plant defence responses against pathogen attack. Dong *et al.* (2023) conducted the first study that confirmed that CRISPR/Cas9 could effectively enhance resistance to downy mildew in cucumber. They employed GWAS and found a single nucleotide polymorphism (SNP) in the STAYGREEN (CsSGR) coding region at the gLTT5.1 locus related to low-temperature tolerance. Notably, CRISPR/Cas9-generated knockout mutants of *CsSGR* showed improved tolerance to multiple biotic and abiotic stresses, including *P. cubensis* infection, low temperatures, salinity, and water deficit.

Conclusion

Breeding for downy mildew resistance in cucurbitaceous vegetables in crucial for developing stable, superior resistant cultivars, which are essential for sustainable disease management. Successful breeding programmes rely on the identification of major resistance genes and a thorough understanding of the genetic basis of resistance. While traditional breeding has contributed to the development of resistant cultivars, challenges persist due to the rapid evolution of the pathogen, including the emergence of new virulent races, and the complex genetic architecture of downy mildew resistance, which often involves multiple genes and QTLs. Therefore, a multidisciplinary approach that integrates conventional and modern breeding methods is vital for reducing fungicide dependency, improving crop productivity, and ensuring sustainable management of downy mildew in cucurbitaceous crops. Therefore, a multidisciplinary approach that integrates conventional

and modern breeding techniques is vital to reduce fungicide dependency, improve crop productivity, and ensure long-term management of downy mildew. Continued research into the genetic basis of resistance will further support the development of more resilient cultivars, which is crucial for sustainable cucurbit production in the face of ongoing disease threats.

References

Ajuru, M. and Ajuru, G. (2014). Indigenous and exotic cucurbits in Nigeria. *Curr. Adv. Plant Sci. Res.*, *1*(1), 12-17.

Angelov, D., and Krasteva, L. (2000). Dominant inheritance of downy mildew resistance in melons. In: *VII Eucarpia Meeting on Cucurbit Genetics and Breeding 510,* pp. 273-276.

Arauz, L.F., Neufeld, K.N., Lloyd, A.L. and Ojiambo, P.S. (2010). Quantitative models for germination and infection of *Pseudoperonospora cubensis* in response to temperature and duration of leaf wetness. *Phytopathol.,* 100(9), 959-967.

Badr, L.A.A. and Mohamed, F.G. (1998). Inheritance and nature of resistance to downy mildew disease in cucumber (*Cucumis sativus* L.). *Ann. Agric. Sci., 36*(4), 2517-2544.

Bai, Z., Yuan, X., Cai, R., Liu, L., He, H., Zhou, H. and Pan, J. (2008). QTL analysis of downy mildew resistance in cucumber. *Prog. Nat. Sci.,* 18, 706-710.

Bains, S.S. and Jhooty, J.S. (1978). Relationship between mineral nutrition of muskmelon and development of downy mildew caused by *Pseudoperonospora cubensis*. *Plant soil*, *49*(1), 85-90.

Berg, J.A., Hermans, F.W., Beenders, F., Lou, L., Vriezen, W.H., Visser, R.G., Bai, Y. and Schouten, H.J. (2020). Analysis of QTL DM4. 1 for downy mildew resistance in cucumber reveals multiple subQTL: a novel RLK as candidate gene for the most important subQTL. *Front. Plant Sci.,* 11, 569876.

Bhardwaj, D.R., Gautam, K.K., Saha, S., Nagendran, K., Pandey, K.K., Singh, A.K., Singh, P.M. and Singh, B. (2018). Mining the source of resistance for downy mildew and gummy stem blight in bottle gourd (*Lagenaria siceraria*) accessions. *Indian J. Agric. Sci*., 88(5), 746-750.

Bhutia, T.L. (2015). Genetics of yield traits and downy mildew resistance in cucumber (*Cucumis sativus L.*) *Ph.D. Thesis,* Indian Agricultural Research Institute, New Delhi.

Bommesh, J.C. (2019). Genetics for downy mildew resistance in slicing cucumber. *Ph.D. Thesis,* Indian Horticultural Research Institute, Hesaraghatta, Bangalore.

Bommesh, J.C., Pitchaimuthu, M., Ravishankar, K.V., Devaraju, H.M. and Sriram, S. (2024). Genetic analysis of downy mildew resistance in Indian slicing cucumber (*Cucumis sativus* L.). DOI: https://doi.org/10.21203/rs.3.rs-3897107/v1

Bommesh, J.C., Pitchaimuthu, M., Sadashiva, A.T., Sriram, S., Varalakshmi, B. and Ravishankar, K.V. (2018). Identification and confirmation of downy mildew (*Pseudoperonospora cubensis* Berk. & Curt.) resistance sources in cucumber (*Cucumis sativus* L.). *Indian Phytopathol.,* 71(3), 337-348.

Bouwmeester, K., van Poppel P.M.J.A. and Govers, F. (2009). Genome biology cracks enigmas of oomycete plant pathogens. *Annu. Plant Rev.*,34, 102-133.

Burkhardt, A. and Day, B. (2016). Transcriptome and small RNAome dynamics during a resistant and susceptible interaction between cucumber and downy mildew. *Plant Genome,9*(1), 1-19.

Burnes, W.C. and Epps, W.M. (1954). An unreported type of resistance of cucumber downy mildew. *Pl. Dis. Rep.,* 38(9), 620.

Caldwell, D., Chan, E., De Vries, J., Joobeur, T., King, J., Reina, A. and Shetty, N. (2011). *Methods and compositions for identifying downy mildew resistant cucumber plants*. United States patent US 2011/0126309 A1

Call, A.D., Criswell, A.D., Wehner, T.C., Klosinska, U. and Kozik, E.U. (2012). Screening cucumber for resistance to downy mildew caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov. *Crop Sci*., 52(2), 577-592.

Cohen, Y. and Eyal, H. (1977). Growth and differentiation of sporangia andsporangiophores of *Pseudoperonospora cubensis* on cucumber cotyledons under variouscombinations of light and temperature. *Physiol. Plant Pathol.,* 10(2), 93–103.

Cohen, Y. and Rotem, J. (1971). Dispersal and viability of sporangia of *Pseudoperonospora cubensis. Trans. Br. Mycol. Soc.,* 57(1), 67-74.

Cohen, Y. (1977). The combined effects of temperature, leaf wetness, and inoculum concentration on infection of cucumbers with *Pseudoperonospora cubensis. Can. J. Bot.,* 55(11), 1478-1487.

Cohen, Y. (1981). Downy mildew of cucurbits. In: Spencer, D.M. (ed). *The Downy Mildews.* New York, USA: Academic Press, pp. 341-354.

Cohen, Y., Meron, I., Mor, N. and Zuriel, S. (2003). A new pathotype of *Pseudoperonospora cubensis* causing downy mildew in cucurbits in Israel. *Phytoparasitica,* 31(5), 458-466.

Colucci, S.J., Wehner, T.C. and Holmes, G.J. (2006). The downy mildew epidemic of 2004 and 2005 in the Eastern United States. In: Holmes, G.J. (ed). *Proceedings of Cucurbitaceae 2006.* Raleigh, NC: Universal Press, pp. 403–410.

Ding, G., Qin, Z., Zhou, X. and Fan, J. (2007). RAPD and SCAR markers linked to downy mildew resistance genes in cucumber. *Acta Bot.,* 27, 1747-1751.

Dong, S., Li, C., Tian, H., Wang, W., Yang, X., Beckles, D.M., Liu, X., Guan, J., Gu, X., Sun, J. and Miao, H. (2023). Natural variation in STAYGREEN contributes to low‐temperature tolerance in cucumber. *J. Integr. Plant Biol.*, 65(12), 2552-2568.

Doruchowski, R.W. and Lakowska-Ryk, E. (1992). Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis* Berk and Curt) in *Cucumis sativus.* In: *Proc. 5th Eucarpia Cucurbitaceae Symp.,* pp. 66-69.

El-Hafez, A.A.A., Shehata, S.A., El-Din, S.A.B., El-Doweny, H.H. and Awad, M.M.W. (1990). Inheritance of downy mildew disease and its nature of resistance in cucumber. *Ann. Agric. Sci.* 28(3), 1681-1697.

Epinat, C. and Pitrat M. (1989). Inheritance of resistance of three lines of muskmelon (*Cucumis melo* L.) to downy mildew (*Pseudoperonopsora cubensis*). In: Thomas, C.E.

(ed) *Proceedings of Cucurbitaceae 89: Evaluation and Enhancement of Cucurbit Germplasm.* US Department of Agricultural Research Service, Charleston, SC, pp. 133-135.

Faizal, A., Nugroho, S., Sembada, A.A., Theda, Y., Komariyah, T. and Esyanti, R.R. (2024). Genome editing in future crop protection: utilizing CRISPR/Cas9 to improve crop resistance against diseases, pests, and weeds. *Discov. Agric.,* 2, 104.

Gao, X., Guo, P., Wang, Z., Chen, C. and Ren, Z. (2021). Transcriptome profiling reveals response genes for downy mildew resistance in cucumber. *Planta,* 253(5), 112.

Gautam, D., Nath, R., Gaikwad, A.B., Bhat, K.V., Mondal, B., Akhtar, J., Jat, G.S., Iquebal, A.M., Tiwari, B. and Archak, S. (2020). Identification of new resistant sources against downy mildew disease from a selected set of cucumber germplasm and its wild relatives. *Indian J. Genet. Plant Breed.,* 80(4), 427-431.

Goswami, S., Thind, T. S., Raheja, S. and Vashisht, V. K. (2011). Screening of muskmelon lines against downy mildew caused by *Pseudoperonospora cubensis* under field condition. *Indian Phytopathol.,* 64(4), 374-375.

Gurushanthappa, H. (1990). Studies on downy mildew of cucurbits with special reference to ridge gourd (*Luffa acutangula* Roxb.) caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rost. *M.Sc. (Agri.) Thesis,* University of Agricultural Sciences, Dharwad.

Haas, B.J., Kamoun, S., Zody, M.C., Jiang, R.H., Handsaker, R.E., Cano, L.M., Grabherr, M., Kodira, C.D., Raffaele, S., Torto-Alalibo, T. and Bozkurt, T.O. (2009). Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans. Nature,* 461(7262), 393-398.

Harika, M., Kasti, V.D., Kulkarni, M.S., Mulge, R., Mastiholi, A.B., Shirol, A.M. and Shantappa, T. (2012). Reaction of bottle gourd genotypes against fruit fly and downy mildew under natural epiphytotic conditions. *Karnataka J. Agric. Sci*., 25(2), 296–297.

Hashmi, A.A. (1994). Horticulture insect pest management of vegetables compiled by Elina Bashir. *National Book Foundation of Pakistan*, p. 633.

He, Y., Wei, M., Yan, Y., Yu, C., Cheng, S., Sun, Y., Zhu, X., Wei, L., Wang, H. and Miao, L. (2022). Research advances in genetic mechanisms of major cucumber diseases resistance. *Front. Plant Sci.*, *13*, 862486.

Horejsi, T., Jack, E.S. and Thomas, C. (2000). Linkage of random amplified polymorphic DNA markers to downy mildew resistance in cucumber (*Cucumis sativus* L). *Euphytica,* 115(2), 105-113.

Innark, P., Panyanitikoon, H., Khanobdee, C., Samipak, S. and Jantasuriyarat, C. (2020). QTL identification for downy mildew resistance in cucumber using genetic linkage map based on SSR markers. *J. Genet.,* 99, 1-9.

Innark, P., Ratanachana, T., Khanobdee, C., Samipak, S. and Jantasuriyarat, C. (2014). Downy mildew resistant/susceptible cucumber germplasm (*Cucumis sativus* L.) genetic diversity assessment using ISSR markers. *Crop Prot.,* 60, 56-61.

Jadhav, V.M.R. and Sharma, B.L. (1983). Field reaction of mungbean (*Vigna radiata* L.) Wilczek varieties to *Cercospora* leaf spot in north Madhya Pradesh. *Legume Res*., 6(2), 99–100.

Keinath, A.P., Holmes, G.J., Everts, K.L., Egel, D.S. and Langston, D.B. (2007). Evaluation of combinations of chlorothalonil with azoxystrobin, harpin, and disease forecasting for control of downy mildew and gummy stem blight on melon. *Crop Prot.,* 26(2), 83–88.

Kenigsbuch, D. and Cohen, Y. (1992). Inheritance of resistance to downy mildew in *Cucumis melo* PI 124112 and commonality of resistance genes with PI 124111F. *Plant Dis.,*76(6), 615-617.

Kozik, E.U., Klosinska, U., Call, A.D. and Wehner, T.C. (2013). Heritability and genetic variance estimates for resistance to downy mildew in cucumber accession Ames 2354. *Crop Sci., 53*(1), 177-182.

Kumar, V., Amrotin. and Khare, C.P. (2018). Response of bitter gourd varieties against multi-disease resistance. *Trends Biosci.,* 11(5), 671-675.

Lavanya, H.N., Varalakshmi, B., Pitchaimuthu, M., Sriram, S., Venugopalan, R. and Sandeep Kumar, G.M. (2023). Screening of ridge gourd genotypes for resistance to downy mildew (*Pseudoperonospora cubensis*). *Australasian Plant Pathol., 52*(5), 439-449.

Lebeda, A. (1990). Biology and ecology of cucurbit downy mildew. In: Lebeda, A. (ed). *Cucurbit downy mildew*. Czechoslovak Academy of Sciences, Prague, pp. 13-46.

Lebeda, A. and Schwinn, F.J. (1994). The downy mildews—An overview of recent research progress. *J. Plant Dis. Prot.,*101(3), 225–254.

Lebeda, A. and Urban, J. (2004). Distribution, harmfulness and pathogenic variability of cucurbit downy mildew in the Czech Republic. *Acta Fytotech. Zootech.,* 7, 170–173.

Lebeda, A. and Widrlechner, M.P. (2003). A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *J. Plant Dis. Protect.,*110(4), 337-349.

Lebeda, A., and Cohen, Y. (2011). Cucurbit downy mildew (*Pseudoperonospora cubensis*)-biology, ecology, epidemiology, host-pathogen interaction and control. *European J. Plant Pathol.*, *129*(2), 157-192.

Lebeda, A., Kristkova, E., Rohackova, J., Sedlakova, B., Widrlechner, M.P. and Paris, H.S. (2016). Race-specific response of *Cucurbita* germplasm to *Pseudoperonospora cubensis*. *Euphytica,* 212(1), 145-156.

Lebeda, A., Pavelkova, J., Urban, J., and Sedlakova, B. (2011). Distribution, host range and disease severity of *Pseudoperonospora cubensis* on cucurbits in the Czech Republic. *J. Phytopathol.,*159(9), 589-596.

Lebeda, A., Widrlechner, M.P., Staub, J., Ezura, H., Zalapa, J. and Krístkova, H. (2007). Cucurbits (Cucurbitaceae; *Cucumis* spp., *Cucurbita* spp., *Citrullus* spp.). *Genetic resources, chromosome engineering, and crop improvement.* Taylor and Francis Publishing, Boca, FL.

Lebeda, A. and Gadasova, J. (2002). Pathogenic variation of *Pseudoperonospora cubensis* in the Czech Republic and some other European countries. In: Nishimura S., Ezura H., Matsuda T. and Tazuke, A. (eds). *Proceedings of the 2nd International Symposium on Cucurbits*. http://www.actahort.org/books/588.

Li, L., He, H., Zou, Z. and Li, Y. (2018). QTL analysis for downy mildew resistance in cucumber inbred line PI 197088. *Plant Dis.,* 102(7), 1240-1245

Liu, X., Lu, H., Liu, P., Miao, H., Bai, Y., Gu, X. and Zhang, S. (2020). Identification of novel loci and candidate genes for cucumber downy mildew resistance using GWAS. *Plants,*9(12), 1659.

Michelmore, R.W., Paran, I. and Kesseli, R. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci.,*88(21), 9828-9832.

Mirzwa-Mroz, E., Zieniuk, B., Yin, Z. and Pawełkowicz, M. (2024). Genetic Insights and Molecular Breeding Approaches for Downy Mildew Resistance in Cucumber (*Cucumis sativus* L.): Current Progress and Future Prospects. *Int. J. Mol. Sci., 25*(23), 12726.

Narasannavar, A., Devappa, V., Fhakrudin, M.P., Lingaiah, H.B., Anjanappa, M. and Sriram, S. (2017). Selection parameters for downy mildew disease, fruit yield and related traits in ridge gourd (*Luffa acutangula* (Roxb.) L.). *Electro. J. Plant Breed.,* 8(2), 601-608.

Ojiambo, P.S., Gent, D.H., Quesada-Ocampo, L.M., Hausbeck, M.K. and Holmes, G.J. (2015). Epidemiology and population biology of *Pseudoperonospora cubensis*: A model system for management of downy mildews. *Annu. Rev. Phytopathol.,* 53(1), 223-246.

Ojiambo, P.S., Paul, P.A. and Holmes, G.J. (2010). A quantitative review of fungicide efficacy for managing downy mildew in cucurbits. *Phytopathol.,* 100(10), 1066-1076.

Palti, J. and Cohen, Y. (1980). Downy mildew of cucurbits (*Pseudoperonospora cubensis*): The fungus and its hosts, distribution, epidemiology and control. *Phytoparasitica*, *8*(2), 109-147.

Pandey, B.P. (1969). *Taxonomy of angiosperms*. S. Chand and company Ltd, New Delhi, India.

Pandey, K.K., Ram, D., Pandey, P.K. and Rai, M. (2005). Artificial screening technique and sources of resistance to downy mildew disease in bitter gourd. *Int. J. Veg. Sci.,* 32(1), 107-108.

Pang, X., Zhou, X., Wan, H. and Chen, J. (2013). QTL mapping of downy mildew resistance in an introgression line derived from interspecific hybridization between cucumber and *Cucumis hystrix. J. Phytopathol.,* 161(7), 536-543.

Pathania, A., Rialch, N. and Sharma, P. N. (2017). Marker-assisted selection in disease resistance breeding. *Current Developments in Biotechnology and Bioengineering*, Elsevier, pp. 187–213.

Perchepied, L., Bardin, M., Dogimont, C. and Pitrat, M. (2005). Relationship between loci conferring downy mildew and powdery mildew resistance in melon assessed by quantitative trait loci mapping. *Phytopathol.,* 95(5), 556-565.

Petrov, L., Boodert, K., Sheck, L., Baider, A., Rubin, E., Cohen, Y., Katzir, N. and Paris, H. S. (2000). Resistance to downy mildew, *Pseudoperonospora cubensis,* in cucumbers. *Acta Hortic.,* 510, 203-210.

Pitchaimuthu, M., Kumar, G.S., Ravishankar, K.V., Hegde, R. and Chaithra, L.T. (2024). Screening for resistance to downy mildew disease [*Pseudoperonospora cubensis* (Berk. and Curt.) Rostov.] in Cucumber (*Cucumis sativus* L.). *J. Hortic. Sci.,19*(1).

Pitchaimuthu, M., Souravi, K., Ganeshan, G., Kumar, G.S. and Pushpalatha, R. (2012). Identification of sources of resistance to powdery and downy mildew diseases in Cucumber [*Cucumis sativus* (L.)]. *Pest Manag. Hortic. Ecosyst.,* 18(1), 105-107.

Pitrat, M. and Blancard, D. (1988). Some aspects of resistance of muskmelon to downy mildew. *Cucurbitaceae 88. Proceedings of the Eucarpia meeting on cucurbit genetics and breeding,* Avignon-Montfavet, France, 31 May - 2 June 1988, pp. 57–62.

Rajasree, R.S., Sibi, P.I., Francis, F. and William, H. (2016). Phytochemicals of Cucurbitaceae family—A review. *Int. J. Pharm. Phytochem. Res*, *8*(1), 113-123.

Ranjan, P., Gangopadhay, K.K., Bag, M.K., Roy, A., Srivastava, R., Bhardwaj, R. and Dutta, M. (2015). Evaluation of cucumber (*Cucumis sativus* L.) germplasm for agronomic traits and disease resistance and estimation of genetic variability. *Indian J. Agric. Sci.,*85(2), 234-239.

Rolnik, A. and Olas, B. (2020). Vegetables from the Cucurbitaceae family and their products: Positive effect on human health. *Nutr.*, *78*, 110788.

Savory, E.A., Granke, L.L., Quesada-Ocampo, L.M., Varbanova, M., Hausbeck, M.K. and Day, B. (2011). The cucurbit downy mildew pathogen *Pseudoperonospora cubensis. Mol. Plant Pathol.*, *12*(3), 217-226.

Shetty, N. V., Wehner, T.C., Thomas, C.E., Doruchowski, R.W. and Shetty, K.V. (2002). Evidence for downy mildew races in cucumber tested in Asia, Europe, and North America. *Sci. Hortic.*, *94*(3-4), 231-239.

Shimizu, S., Kanazawa, K. and Kate, A. (1963). Studies on the breeding of cucumber for resistance to downy mildew. *Hortic. Res. J.,* 3, 65-81.

Somkuwar, R.G. and More, T.A. (1993). Downy mildew (*Pseudoperonospora cubensis* B and C) resistance in melon (*Cucumis melo* L.). *Cucurbit Genet. Coop. Rep*., 16, 40-41.

Sun, C., Song, X., Zheng, J., Li, X., Feng, Z. and Yan, L. (2021). Comparative proteomic profiles of resistant/susceptible cucumber leaves in response to downy mildew infection. *Hortic. Plant J.,* 7(4), 327-340.

Szczechura, W., Staniaszek, M., Klosinska, U. and Kozik, E.U. (2015). Molecular analysis of new sources of resistance to *Pseudoperonospora cubensis* (Berk. Curt.) Rostovzev in cucumber. *Russ. J. Genet.,* 51(10), 974-979.

Thampi, S.S. (2024). Breeding for downy mildew resistance in bitter gourd (*Momordica charantia* L.). *Ph.D. Thesis,* Kerala Agricultural University, Thrissur.

Thampi, S.S., Sarada, S., Joy, M., and Radhika, N.S. (2024). Assessment of bitter gourd (*Momordica charantia* L.) genotypes for resistance against downy mildew. *Indian Phytopathol.*, *77*(2), 533-538.

Thomas, C.E. (1996). Downy mildew. In: *Compendium of Cucurbit Diseases (ed. Zitter T.A.),* Ithaca, NY: Cornell University Press, pp. 25–27.

Thomas, C.E., Cohen, Y., Jordain, McCreight, J.D. and Cohen, S. (1988). Inheritance of resistance to downy mildew in *Cucumis melo. Plant Dis.,*72(1), 33-35.

Thomas, C.E., Inaba, T. and Cohen, Y. (1987). Physiological specialization in *Pseudoperonospora cubensis. Phytopathol.* 77(12), 1621-1624.

Tyler, B.M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R.H., Aerts, A., Arredondo, F.D., Baxter, L., Bensasson, D., Beynon, J.L. and Chapman, J. (2006). *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Sci.,*313(5791), 1261-1266.

Uffelmann, E., Huang, Q.Q., Munung, N.S., De Vries, J., Okada, Y., Martin, A.R., Martin, H.C., Lappalainen, T. and Posthuma, D. (2021). Genome-wide association studies. *Nat. Rev. Methods Primers*, 1(1), 59.

van-Vliet, G.J.A. and Meysing, W.D. (1974). Inheritance of resistance to *Pseudoperonospora cubensis* in cucumber (*Cucumis staivus* L.). *Euphytica,*23(2), 251-255.

Varalakshmi, B., Pitchaimuthu, M., Rao, E.S. and Chowdappa, P. (2022). Inheritance of Resistance to Downy Mildew [*Pseudoperonospora cubensis* (Berk. and Curt.) Rostovzev.] in Ridge Gourd [*Luffa acutangula* (Roxb.) L.]. *Indian J. Plant Genet. Resour., 35*(1), 21-26.

Venkatanarayana, S.V. and Venkatakrishniah, N.S. (1953). New host of the downy mildew of cucurbits in India. *Curr. Sci.,* 22, 183-184.

Wang, N., Ma, Y., Yang, C., Dai, G. and Wang, Z. (2008). rDNA-ITS sequence analysis of pathogens of cucumber downy mildew and cucumber powdery mildew. *Front. Agric. China,* 2(3), 317-320.

Wang, Y., VandenLangenberg, K., Wehner, T.C., Kraan, P.A., Suelmann, J., Zheng, X., Owens, K. and Weng, Y. (2016). QTL mapping for downy mildew resistance in cucumber inbred line WI7120 (PI 330628). *Theor. Appl. Genet.,*129(8), 1493-1505.

Wang, Y., Vanden-Langenberg, K., Wen, C., Wehner, T.C. and Weng, Y. (2018). QTL mapping of downy and powdery mildew resistances in PI 197088 cucumber with genotyping-by-sequencing in RIL population. *Theor. Appl. Genet*., 131(3), 597-611.

Win, K.T., Vegas, J., Zhang, C., Song, K. and Lee, S. (2017). QTL mapping for downy mildew resistance in cucumber via bulked segregant analysis using next-generation sequencing and conventional methods. *Theor. Appl. Genet.,* 130, 199-211.

Xu, Y. and Crouch, J.H. (2008). Marker-assisted selection in plant breeding: From publications to practice. *Crop Sci.,* 48(2), 391-407.

Yin, Z., Hennig, J., Szwacka, M. and Malepszy, S. (2004). Tobacco PR-2d promoter is induced in transgenic cucumber in response to biotic and abiotic stimuli. *J. Plant Physiol.,*161(5), 621-629.

Yoshioka, Y., Sakata, Y., Sugiyama, M. and Fukino, N. (2014). Identification of quantitative trait loci for downy mildew resistance in cucumber (*Cucumis sativus* L.). *Euphytica,*198(2), 265-276.

Zhang, P., Zhu, Y., Luo, X. and Zhou, S. (2019). Comparative proteomic analysis provides insights into the complex responses to *Pseudoperonospora cubensis* infection of cucumber (*Cucumis sativus* L.). *Sci. Rep.,* 9(1), 9433.

Zhang, S.P., Liu, M.M., Miao, H., Zhang, S.Q., Yang, Y.H., Xie, B.Y., Wehner, T.C. and Gu, X.F. (2013). Chromosomal mapping and QTL analysis of resistance to downy mildew in *Cucumis sativus. Plant Dis.,* 97(2), 245-251.