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## ADVANCES IN CHILLI BREEDING: BALANCING TRADITIONAL METHODS AND MODERN BIOTECHNOLOGY FOR BIOTIC STRESS RESISTANCE

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### ABSTRACT

This Chilli pepper (*Capsicum annuum* L.) is a highly valued crop across the world for its utility as a spice and as a vegetable, but the cultivation of chilli is considerably hampered by biotic stresses such as various fungal, bacterial, and viral pathogens as well as insect pests. The traditional breeding methods, including introduction, mass selection, pedigree, and backcrossing, have formed the basis for developing resistant varieties, although these methods are often limited by their time-consuming nature and varying effectiveness against complex stresses. The recent advances in breeding with the application of biotechnology approaches such as MAS, genomic selection, and CRISPR/Cas9 gene editing, have opened new avenues for the improvement of resistance in a more efficient and precise manner. Complementary tools involving genetic transformation and tissue culture further complement breeding efforts despite various challenges in plant regeneration efficiency and transformation. The implementation of traditional and modern methods in chilli breeding is more effective in dealing with problems of biotic stress, thus improving crop resilience and achieving sustainable production.

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## INTRODUCTION

Hot pepper is the common term used globally for *Capsicum annuum* L.;  $2n = 2x = 24$ . It is known in different names, such as capsicum, paprika, pimento, red pepper, and bird pepper, in different parts of the world (Dasgupta & Reshmi, 2011). This crop has high economic importance throughout the world as a spice and as a vegetable. It is the second most important solanaceous crop after tomato (Bosland, 1995). From its origin in Mexico and a center of genetic diversity based in South America (Gonzalez & Bosland, 1991), *Capsicum* has been introduced to Asia by Portuguese trade routes. Some records indicate that *Capsicum* was introduced to India as early as in the sixteenth century (Singh et al., 2004). The genus *Capsicum* comprises about 30 species, but only five are widely cultivated: *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum pubescens*, and *Capsicum frutescens*. Of these, *C. annuum* is the most widely cultivated species. As a diploid, *C. annuum* has a chromosome count of  $2n=2x=24$ , an estimated genome size of about 2700 Mb, and contains approximately 30,000 genes (Moscone, 2016). While chilli pepper cultivation is economically valuable, it is also particularly vulnerable to numerous biotic stresses, including fungal, bacterial, and viral pathogens as well as insect pests. These stresses substantially affect yield and quality, causing economic setbacks for

growers. Developing varieties with biotic stress resistance has thus become a critical focus of chilli breeding programs. Although conventional phenotype-based selection methods have achieved significant progress in this area, they remain time-consuming, labor-intensive, and financially non-demanding. Biotechnological advancements have introduced novel approaches for enhancing biotic stress resistance in chilli. Techniques like marker-assisted selection (MAS), genomic selection, and CRISPR/Cas9 genome editing have revolutionized breeding, offering greater efficiency and precision. MAS and genomic selection accelerate breeding by leveraging genetic markers to predict desirable traits, while CRISPR/Cas9 facilitates direct genome modifications, enabling the introduction of specific resistance genes into the chilli genome (Barchenger et al., 2019). India is the world's largest producer of chilli peppers, and leads both in cultivation area and yield; 57% of production takes place in Andhra Pradesh, with that state also accounting for 25% of India's chilli-growing area. Asia's largest chilli market is held at Guntur district of Andhra Pradesh, which marks the significance of India in international chilli production and trade.

**Biotic stresses in chilli:** Cultivation of pepper, *Capsicum annuum* L., across different climates and regions exposes the crop to many biotic stresses, hence making it susceptible to a wide range of pathogens common among Solanaceae plants. Most of these pathogens cross-

infect species within the family, which exacerbates the challenges for chilli crops. Natural defense mechanisms in pepper plants include pathogenesis-related gene activation, hypersensitive response, and systemic acquired resistance, all of which have been known to be essential in the fight against such stresses (Ryals *et al.*, 1996; Dangel & Jones, 2001). Once a plant recognizes a pathogen, its receptors initiate complex defensive pathways, which include ROS production, protein phosphorylation, and ionic adjustments, all further enhancing SAR (Knogge, 1996). Although these are innate defenses, pathogens may bypass or counteract them, thus the need for breeding programs aimed at developing biotic stress resistance.

### Fungal Infections

**Anthracnose:** Anthracnose, a common fungal disease in chilli caused by *Colletotrichum* species, has a global presence and can infect a broad range of plants, including over 460 different species (Farr & Rossman, 2016). The pathogen exhibits pathotypic variation, with *C. acutatum* primarily affecting immature fruits in regions like the United States, while *C. gloeosporioides* targets ripe fruits (Harp *et al.*, 2008). Though chemical and agronomic controls are available, cost and environmental impacts make genetic resistance a preferred alternative (Setiawati *et al.*, 2011). However, identifying reliable sources of resistance within *C. annum* has been problematic, as this species has limited inherent resistance (Temiyaikul *et al.*, 2012). QTL analyses have detected loci associated with anthracnose tolerance within different species, and genetic markers with promise have been identified for resistance breeding (Kim *et al.*, 2010; Jayaram *et al.*, 2016; Suwor *et al.*, 2017).

**Powdery mildew:** Powdery mildew, caused by the fungus *Leveillula taurica*, also causes serious damage to chilli plants through defoliation of leaves and reduction of photosynthesis, which would lead to up to 80% yield loss in the affected plants (Sivaprakasam *et al.*, 1976; Gohokar & Peshney, 1981). Resistance to this disease was complex and often polygenic, involving both major genes and polygenic loci (Daubeze *et al.*, 1995; Lefebvre *et al.*, 2003). The resistance locus PMR1 was identified by Jo *et al.* (2017), who reported on interspecific hybridization using *C. baccatum* as a donor to offer promising avenues for further breeding.

### Bacterial Diseases

**Bacterial Wilt:** Bacterial wilt caused by *Ralstonia solanacearum* leads to sudden wilting in pepper plants, which drastically reduces productivity. Resistance in the breeding line IHR-B-HP 130 has been reported to follow a digenic inheritance model with complementary gene actions (Nischay *et al.*, 2021; Naveena *et al.*, 2020). Various studies have mapped QTL related to bacterial wilt resistance (Mimura *et al.*, 2009; Du *et al.*, 2019; Chae *et al.*, 2022), and molecular markers like CAMS451 have been helpful in marker-assisted selection.

**Bacterial Leaf Spot:** Bacterial leaf spot, a major disease affecting the world's plant populations, is controlled by independent dominant genes, such as Bs1, Bs2, and Bs3 (Jones & Stall, 1998). The resistance genes are race-specific, which complicates breeding programs for durable resistance (Kim & Hartmann, 1985). Marker-assisted selection has identified molecular markers to specifically breed for resistance to certain pathogen races (Pierre *et al.*, 2000).

### Viral diseases

**Chilli Leaf Curl Virus (ChiLCV):** Transmitted by whiteflies (*Bemisia tabaci*), ChiLCV is a significant threat to chilli crops, leading at times to complete crop losses (Nigam *et al.*, 2015). The genetic resistance to ChiLCV usually is determined by single dominant gene although the expression of resistance might vary with the plant's genetic background (Kumar *et al.*, 2009; Rai *et al.*, 2014; Jindal *et al.*, 2018). Wild species of chilli, *C. frutescens* and some other species of *C. annum* accessions have shown resistance, making

them important genetic material in breeding (Anandhi & Khader, 2011).

### Insect Pests and Other Biotic Threats

**Thrips:** Direct and indirect impacts of thrips infestation were observed in chilli plant damage due to virus spread and tissue damage. In the genetic resistance of the crop to thrips, Maharajaya *et al.*, 2011, concluded that this is due to dominant markers. QTL mapping detected resistance regions for thrips on chromosome 6 according to van Haperen *et al.*, 2020. Chilli lines such as BC-7-2-1 and BC-25 display notable resistance and are precious resources in breeding (Praveen *et al.*, 2021).

**Mites:** The sap-feeding mite *Polyphagotarsonemus latus* is very damaging in humid climates and reduces plant growth and yield. Though there are no genetic resistance markers for mite resistance, some varieties such as DCC-109 exhibit moderate resistance (Latha & Hunumanthraya, 2018).

**Whiteflies:** Whiteflies weaken the plants through feeding, and they also enhance the growth of sooty mold. Some varieties of *C. annum*, like IHR 4338, have been found to be resistant to whiteflies, but the genetic enhancement in this direction is not fully explored (Yadav *et al.*, 2022).

**Root-Knot Nematodes:** The availability of genes like Me1, Me3, and Me7 controlling resistance to root-knot nematodes can be used as a breeding program for nematode resistance. Comparative mapping and marker-assisted breeding are considered as essential tools for the transfer of such resistance traits into commercial lines (Djian-Caporalino *et al.*, 2007; Wang *et al.*, 2009).

**Reduction in Yield and Quality Due to Biotic Stress in Peppers:** Pepper plants, though robust, are susceptible to several biotic stresses that have major impacts on yield and fruit quality, with the damages often being carried into post-harvest stages (Lownds *et al.*, 1994; Samira *et al.*, 2013). Unlike abiotic stresses, biotic stresses continue throughout cropping systems, causing continuous quality degradation and yield losses. Symptoms such as discoloration, curling of leaves, and insect damage affect essential tissues of the plant, whereas root and stem rot in nurseries introduce early losses, especially for exotic or rare genotypes. Pepper breeding becomes increasingly focused on fruit quality evaluation and pesticide residues to ameliorate the effects of biotic stress. For example, Golge *et al.* (2018) identified detectable pesticide residues in 12.9% of pepper samples screened from among 725 vegetables. Chillies grown in a greenhouse environment are usually optimized for their requirements but still susceptible to biotic stresses. Viral spread is facilitated by whiteflies, aphids, and thrips, whereas high humidity increases the susceptibility to fungal and bacterial infections, thereby decreasing the market value and affecting photosynthesis (Parisi *et al.*, 2020). Lesions caused by anthracnose and *Cercospora* infections are especially damaging, drastically reducing the quality of fruits. So, soil-borne wilts also present a serious yield threat. For example, the incidence of *Verticillium dahliae* varies from 6.3% to 97.8% depending on the type of pepper grown in California (Bhat *et al.*, 2003). Similarly, *Fusarium* spp. infections under optimal warm, low-moisture conditions cause yield reductions of up to 71% (Gabrekirostos & Demiyu, 2020). Moreover, *Ralstonia solanacearum* causes severe losses globally across Solanaceous crops, including peppers (Mamphogoro *et al.*, 2020; Thakur *et al.*, 2021). Although the waxy skin of peppers helps retain moisture, quality loss in storage is still a problem. A Trinidad study reported 28.6% weight loss in dry and 38.7% in humid conditions, with concomitant losses in acidity, vitamin C, and firmness (Mohammed *et al.*, 1993). These results highlight the need for post-harvest strategies to preserve pepper quality and shelf life.

**Breeding Techniques for Chilli Cultivars:** Chilli breeding focuses on the development of superior cultivars that fulfill certain agronomic, quality, and resistance requirements. This process involves a range of breeding techniques, which can be broadly categorized into

conventional and non-conventional methods. The choice of breeding method is primarily influenced by the breeding objectives and the genetic diversity of the material available.

### Conventional Breeding Techniques

**Introduction of Chilli Cultivars:** Introducing chilli from other parts of the country has also added significantly to genetic development. Chilli is domesticated around 7500 BC in South America. This crop spread its seeds and roots to every nook and corner, owing to trade and exploring people. It was carried to Europe by Columbus. And, with the help of traders who visited Europe from that region, it reached the shores of Asia and India (Srivastava & Mangal, 2019). There are also various chilli cultivars developed from germplasm introduced from Sri Lanka, like CO 3 and CO 4, and varieties like California Wonder, Yolo Wonder, and Bull Nose brought by Indian Agricultural Research Institute (IARI) which have been resistant to *Phytophthora* blight and bacterial leaf spot (Roy *et al.*, 2019; Tripathi *et al.*, 2014). Other varieties, such as NuMex Centennial, have been bred for resistance to bacterial leaf spot, root-knot nematodes, and tobacco etch virus (Cayenne Diane, 2023; Bosland, 1999). These introductions have played a crucial role in improving both yield and disease resistance in Indian chilli varieties.

**Mass Selection:** Mass selection is a common phenotypic method employed to improve desirable traits in chilli populations. This approach involves selecting the best-performing plants based solely on observable characteristics, particularly in populations with high genetic variability. It is especially effective when aiming to improve traits with high heritability. The method works best when the target trait can be fully expressed in the environment, and off-types are removed before flowering to maintain the desired genetic composition. For example, in India, Arka Basant was developed through mass selection from the Hungarian cultivar 'Soroksari.' It is resistant to leaf curl virus (LCV) and thrips with a yield of 15 t/ha and suitability for both kharif and rabi seasons (Ashok *et al.*, 2020). Similarly, Arka Mohini and Arka Gaurav have been developed through mass selection and are resistant to powdery mildew, anthracnose, and bacterial wilt.

**Pureline Selection:** Pureline selection is the most common method used for landraces and locally adapted varieties. It involves the selection of superior plants from a diverse population, followed by self-pollination and harvest of seeds from these selected plants. The subsequent generations are tested to fix the desirable traits while maintaining the genetic uniformity. Several varieties have been developed in India using pureline selection. Some of the varieties developed include G1, G2, G3, K1, and Co1. The Indian Institute of Horticultural Research (IIHR) developed Arka Lohit, a very pungent variety resistant to bacterial spot and chilli veinal mottle virus (Choudhary & Gupta, 2015). For example, Arka Abhir is a line out of Dyavanur Dubba, resistant to bacterial wilt and produces around 20 t/ha in 160-180 days (Singh & Yadav, 2017).

**Pedigree Approach:** In the pedigree approach, after hybridization, it takes superior plants from the segregating populations with great detailed records of the genealogical history of the each plant. It has a widespread application for betterment of chilli cultivar by advancing generations one generation after another. Andhra Jyoti, Pusa Jwala, and Punjab Lal cultivars have been bred with this technology (Fehr, 1987). For example, the Arka Suphal variety has tolerance for powdery mildew and viral infection since it came from a cross Pant C1 × IIHR 517 A. Similarly, Pusa Jwala, developed by the Indian Agricultural Research Institute (IARI), is resistant to Cucumber Mosaic Virus (CMV) and bacterial leaf spot, while Pusa Sadabahar shows tolerance to powdery mildew and chilli veinal mottle virus.

**Single Seed Descent:** The single seed descent (SSD) method is a rapid generation advancement technique that involves selecting a single seed from each plant, without prior selection, and advancing it to the next generation. This technique shortens the reproduction cycle and is commonly applied to construct inbred lines for hybridization

purposes. SSD is very suitable for the fixation of recessive characters, as attested by Villalon, 1986, who worked with SSD to stabilize genes pertaining to potyvirus tolerance in peppers. Likewise, Moreira *et al.* (2009) used SSD to achieve gene fixation for resistance bacterial leaf spot while maintaining relatively high yields.

**Backcross Method:** Backcross breeding is a very useful tool for the transfer of specific traits such as disease resistance or stress tolerance from wild relatives or primitive cultivars to elite cultivars. This is a commonly used method in transferring disease resistance genes from wild relatives to cultivated forms. In chilli, backcrossing has been applied to introduce genes for resistance to tobacco mosaic virus (TMV), bacterial leaf spot, and other pathogens. For instance, the trait of virus resistance in *Capsicum chinense* has been transferred successfully to *Capsicum frutescens* through backcrossing (Greenleaf, 1986). This approach has played a key role in increasing the resistance of modern varieties against diseases.

**Recurrent Selection:** Recurrent selection is a breeding technique that mates two different populations to enhance quantitative traits such as yield or resistance to disease. It is very effective for traits with low heritability and greatly enhances genetic diversity. Palloix *et al.* (1990) applied recurrent selection to improve two lines of chilli resistant to *Verticillium dahliae* and *Phytophthora capsici*, showing its potential in enhancing disease resistance.

**Heterosis Breeding:** Heterosis, or hybrid vigor, is the phenomenon where the offspring of two genetically diverse parents exhibit superior traits. Hybrid breeding is widely used in chilli cultivation, particularly for increasing yield and disease resistance. Hybrid progeny often perform better than their parents, showing higher yield potential and resistance to various stresses. In India, heterotic hybrid varieties are supported by private sector companies, which have invested more in research and seed production. Hybrids like F<sub>1</sub> bell pepper are gaining popularity with higher yields and resistance to pests and diseases (Singh *et al.*, 2014). Availability of male sterility has also helped in the development of high-yielding hybrid varieties, which have further added to the growth of hybrid chilli market in India.

**Distant Hybridization:** Distant hybridization is crossing species or subspecies in the same genus for novel genetic traits. Wild relatives of domesticated crops represent potential reservoirs of genes that determine important traits such as resistance to disease and insects that can be exploited in breeding programs. However, effective hybridization often depends on overcoming different reproductive barriers. These pre-zygotic as well as post-zygotic barriers sometimes lead to the abortion of an embryo or an endosperm, as well as hybrid sterility. To increase the likelihood of successful distant hybridization, pre-zygotic barriers, such as compatibility studies, must be done. For example, interspecific hybridization between *Capsicum annuum* and its wild relatives like *Capsicum baccatum* and *Capsicum pubescens* has been investigated for the transfer of resistance genes against diseases such as anthracnose, bacterial wilt, and yellow mosaic virus. Although there is low or no cross-compatibility between *C. annuum* and these species (Yoon *et al.*, 2004), the wild relatives possess some useful resistance genes. The application of distant hybridization was aimed at expanding the genetic variability that allows resistance to certain diseases in a broad range of traits; thus, a good potential exists. This potential does not come out into chilli breeding much due to its complications by overcoming barriers to hybridization. A study demonstrated *Capsicum chinense* as Bhut Jolokia or Ghost pepper being used as a donor for resistance against anthracnose Dwivedi *et al.* 2021 and leaf curl virus Kumar *et al.*, 2011.

**Special Breeding Methods for Chilli Improvement:** Non-conventional breeding techniques are vital in generating new genetic variability, especially where natural genetic variation for the specific traits is inadequate. This approach is crucial in addressing genetic bottlenecks to create allelic variation that could be used for developing traits such as disease resistance, pest tolerance, and better agronomic performance. However, the results obtained are often

unpredictable, hence requiring much more resources and planning, coupled with a selection process that is extremely strict.

**Mutation Breeding:** Mutation breeding is a cutting-edge technique that induces genetic variation by changing the DNA's base sequence through nucleotide additions or deletions. It develops new alleles for specific traits, providing an alternative when traditional genetic variation is not available. Use of mutation breeding in chilli began with the application of Raghavan and Venkatasubban's X ray to the dry seeds in 1940 that led to the selection of changed plant phenotypes. Mutation breeding has henceforth been used in greater measure to create variability pertaining to fruit color, resistance to diseases, and some agronomic traits. For instance, Bhargava and Umalkar (1989) induced mutants with different colors in the pericarp through chemical mutagens and ionizing radiation. They selected the stable mutants in the succeeding generations. Venkataiah *et al.* (2005) reported the occurrence of mutants resistant to streptomycin from chemical mutagenesis of *Capsicum praetermissum*. In another research study, Nascimento *et al.* (2015) applied ethyl methane sulfonate (EMS) to produce pepper mutants of different fruit shapes and identified optimum exposure times that allowed the achievement of the desired frequency of mutations. Mutation breeding is a significant resource that can be exploited in genetic diversity increase for producing varieties with improved characters. However, as with other non-conventional methods, it requires careful selection and rigorous evaluation to ensure the stability and heritability of beneficial mutations.

**Innovative Approaches to Improve Chilli:** Recent breakthroughs in biotechnological approaches like plant tissue culture, genetic transformation, and gene editing offer powerful tools to be used in conjunction with conventional breeding approaches for *Capsicum* improvement.

**Tissue Culture:** The idea of plant cell totipotency discovered by Haberlandt in 1902 allows regeneration of whole plants from cells in vitro, which acts as a basis for improving *Capsicum*. However, chilli is highly recalcitrant to tissue culture with very poor regeneration capacity and abnormalities like rosette shoot differentiation (Benson, 2000). Although it was found that the overall transformation efficiency of model crops, such as tobacco and tomato, was more, considerable success in chilli could be reported with idealistic conditions (Ochoa-Alejo & Ireta-Moreno, 1990; Venkataiah *et al.*, 2003).

**Genetic Transformation:** Genetic transformation, most commonly done through a vector of *Agrobacterium tumefaciens*, has the potential for conferring useful traits from various species. However, because chilli plants regenerate relatively slowly, cotyledons and leaf tissues have successfully been transformed (Liu *et al.*, 1990). Other methods are emerging, like the tissue culture-free *Agrobacterium*-mediated system developed by Kumar *et al.* (2009) for improvement of efficiency in *C. annuum*. Biolistics have also been examined, though efficient, standard transformation systems for chilli have not yet been established.

**Marker-Assisted Selection (MAS):** Marker-assisted selection has advanced the selection of complex traits, allowing breeders to target specific genes for desirable characteristics like disease resistance. In chilli, genetic mapping has identified numerous QTLs tied to significant economic traits, paving the way for combining molecular markers with traditional breeding for targeted improvements.

**Gene Editing – CRISPR/Cas9:** CRISPR/Cas9 technology has introduced a targeted approach for enhancing disease resistance in chilli. Joshi (2019) applied cytidine base editing on the NAC72 gene and was able to significantly enhance anthracnose resistance with high efficiency. Similarly, Mishra *et al.* (2021) used a single transcript unit CRISPR/Cas9 system to modify the CaERF28 gene, which enhanced disease resistance in *C. annuum*. Researchers such as Roy *et al.* (2019) have recommended multiplexed gRNAs which target several regions of virus genomic sequences and thus, this

method significantly reduces the manifestations of chilli leaf curl virus. This is probably a promising approach to maintain sustainable resistance against the chilli crops.

**Future Prospects:** Biotechnological innovations, such as doubled haploid production and efficient transformation methods, are necessary to advance biotic stress resistance breeding in chilli. Availability of the *Capsicum* genome sequence and powerful genome-editing technologies opens significant scope for targeted genetic improvement. The breeding of resistant or tolerance chilli varieties toward pest and diseases can be easy if a priority is attached to develop DNA markers in MAS/GS. This also applies to the understanding of complex traits through epigenetic regulation of biotic and abiotic stress responses, making the breed more resilient. A concerted global effort for collecting, cataloguing, and systemically evaluating *Capsicum* germplasm for diverse levels of stress tolerance, biochemistry, and agronomy traits would significantly strengthen genetic resources for chilli crop improvement. Genome-wide association studies, using a large collection of accessions of *Capsicum* with contrasting recombination histories and phenotypes, could identify minor genes and QTLs, and high-resolution mapping should enable precision breeding for traits of interest. Advantages in chilli breeding though seen, some important areas await further research. For instance, furthering the practice of male sterility to enhance hybrid seed production and utilization may improve the profitability of the seed industry although diversifying cytoplasmic male sterility source is critical for avoiding its diseases vulnerability risks. Furthermore, optimization of genomic selection to detect individuals that possess desirable breeding values may allow the creation of elite parent lines to strengthen hybrid breeding programs that target higher yield and stress tolerance. These approaches can be used in the advancement of chilli breeding that will meet industry demands and improve crop resilience against biotic stresses.

## CONCLUSION

Biotic stress resistance breeding in chilli is essential to sustain agricultural productivity and meeting the growing demands across various sectors, including spice, cosmetics, and pharmaceuticals. The economic significance of chilli, attributed to its unique bioactive compounds like capsaicinoids and carotenoids, highlights the necessity of breeding efforts to overcome biotic challenges posed by evolving pests, diseases, and climate variability. Thus, advanced breeding programs coupled with genomic tools like MAS accelerate the development of strong varieties that are disease-resistant and can improve genetic diversity in chilli. In contrast, wild *Capsicum* species can still be considered good for new alleles discovery, and novel approaches like doubled haploid production, genetic transformation, etc, help offer new hope in breeding with higher efficiency. Future research directions should include the expansion of molecular resources, the conduct of genome-wide association studies (GWAS), and rigorous phenotyping across diverse germplasm to identify superior genotypes with enhanced resistance traits. Development of these approaches will further assist in breeding chilli cultivars that are not only resistant to biotic stresses but also satisfy market demands and help to support sustainable production systems and economic security for growers worldwide.

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