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Development of anthracnose disease resistance and heat tolerance chili through conventional breeding and molecular approaches: a review

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Abstract: Chili (*Capsicum annuum* L.) is the popular spicy vegetable crops belonging to family Solanaceae. Chili peppers are known for their pungency characteristic due to the presence of capsaicinoids that classifies them into hot or sweet pepper. Chili is used as spices, folk remedies for diseases, vegetables, and coloring agent showing a diverse role in human's life. However, its production is hampered by different biotic stress and abiotic factors. Similarly, the unavailability of high yielding varieties, high temperature, and disease incidence, particularly, anthracnose disease, are the major constraints responsible for the low production of chili pepper. The advents of molecular markers, advancement in quantitative trait loci by classical genetic analysis, and conventional breeding have shown the number of genes for many important and major traits. While the newly developed genotyping technologies and next-generation sequencing have led to the discovery of molecular basis for economic important characters in the chili genome and generate large scale data for genomic resources. Based on this background, this review summarizes progress in the development of anthracnose disease-resistant and heat-tolerant chili genotypes through conventional breeding and molecular approaches. This review would help plant breeders in understanding the phenotypic and genetic make-up of capsicum genotypes and provides opportunities for pyramiding two respected genes with the help of diversified phenotypic and molecular marker evaluation.

Introduction

Chili pepper belonging to the Solanaceae family is one of the oldest domesticated crops in the Western Hemisphere (Mexico and central South America), also domesticated throughout the world by native people, traders, and travelers. Chili cultivation began more than six thousand years ago as a self-pollinated vegetable crop and gained popularity across Asia as a spice/vegetables/cash crop. In the world, there are thirty different species of chili (*Capsicum spp.*), *Capsicum annuum* L. is the most cultivated species in South-east Asia and Australia, followed by *C. frutescens* because of favorable weather and climate condition. Inherently, the capsicum species are diploid with chromosome number 2n = 2x = 24 with a genome size of 2700 Mb and 30701 genes, although some species have chromosome number 2n = 2x = 26 and are intimately linked with others Solanaceae crops like potato, tomato, eggplant, tobacco, and petunia (Moscone *et al.*, 2007). Globally, 1988.40 thousand hectares of land were dedicated to chili cultivation with average productions of 3432.05 thousand tones and productivity 1.73 thousand tones per hectare, for the period from 2005 to 2013 (Geetha and Selvarani, 2017). Chili is consumed fresh, dried, prickled, spice, or processed into the sauce (Dahal *et al.*, 2006; Pugalendhi *et al.*, 2010; Ajjapplavara *et al.*, 2010).

Green and dry chili are important sources of energy which supplies 229 and 297 calories of energy per 100 grams, respectively. The pungent taste of chili is due to a group of closely related alkaloid constituents called capsaicinoids such as capsaicin and dihydro-capsaicin

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(Amusa *et al.*, 2004; Ridzuan *et al.*, 2019; Usman *et al.*, 2014a) and these active compounds are used in the pharmaceutical product for pain reliever against arthritis pain, inflammation, and headache and also used to treat a toothache (Vinaya *et al.*, 2009). Chili plant faced many biotic and abiotic stresses, among all the biotic factors, anthracnose is one of the most destructive fungal diseases in chili growing areas which directly affect the profitable cultivation (Saxena *et al.*, 2016). *Colletotrichum capsici*, *C. gloeosporiodes*, *C. acutatum*, *C. coccodes*, and *C. graminicola*, have been found recently able to cause anthracnose diseases (Hyde *et al.*, 2009), and these fungi bore by seed and soil, conidia and ascospores transmit through dead plant parts, water-splash, and wind and survive in or on seeds as acervuli and micro-sclerotia (Vinaya *et al.*, 2009) and also show the capability to create disease in leaves, shoots, green and ripe fruits. Among them, the lesion on fruit is very much harmful (Phoulivong *et al.*, 2010) because of the lesion on fruits decay pre and postharvest fruit quality as well as declined dry weight, capsaicin and oleoresin content (Lakshmi *et al.*, 2014), ultimately reduced pharmaceuticals quality. The disease is characterized by small dark, sunken with necrotic lesions, with concentric rings of acervuli (Voorrips *et al.*, 2004; Kim *et al.*, 2004). Due to anthracnose infestation of chili, the common phenomenon is yield loss occurred in pre-harvest and post-harvest stage (Oanh *et al.*, 2006). A 50% yield reduction occurred in Thailand, 20 to 80% in Vietnam (Don *et al.*, 2007), 10% in Korea (Byung, 2007), 50% in Malaysia (Than *et al.*, 2008) and 29.5% in India thus causing an annual loss of US$ 491.67 million (Garg *et al.*, 2014).

On the other hand, climate change, especially high temperature, enhances the co-incidence of different abiotic and biotic stresses that distressed the growth and development of plants. Chili plants require optimum temperatures ranges of 20–30°C for proper growth and development (Berke *et al.*, 2005). There is a decline in chili production in recent years due to several factors, mainly pests, diseases, and extreme temperatures. Yáñez-López *et al.* (2012) experimented with the effect of the direct impact of high or low heat using the balanced relationship between photosynthesis and respiration, which is partially controlled by temperature. At high temperature (above 33°C), fruits setting reduced significantly while low temperature (below 18°C) enhanced parthenocarpic fruit formation. High temperature shows a direct correlation with capsaicin content (Rahman *et al.*, 2012) and also affects the yield component characters (Garruña-Hernández *et al.*, 2014). However, the cultivation of resistant varieties has shown a reduction in yield losses as well as discourage the use of heavy chemicals for controlling diseases (Agrios, 2005). Natural sources against anthracnose disease and tolerant to high temperatures are not found in cultivated chili, hence there is a need to incorporate these genes in elite genotypes (Sood *et al.*, 2009). Conventional plant breeding has been a good approach for genetic improvement of crop species regarding the resistance to biotic and abiotic stresses. However, the classical breeding program will depend on the genetic diversity, which in turn, depends on the number of parents to be crossed, their pedigree, handling of segregating generations, precision and accuracy of selections, and proper evaluation of those selections. The conventional breeding program takes many years because need to wait for data collections from different years for yield trials and different locations to know the genotypes, environmental interaction, and yield performance. In contrast, molecular studies are the most accurate, fast, economical, reproducible, and environmentally friendly methods for developing superior varieties. The most commonly used modern breeding strategies include genomic selection, genome-wide selection, marker-assisted pedigree selection, marker-assisted recurrent selection, marker-assisted backcrossing, and marker-assisted selection. Molecular breeding has to be treated as a tool to support conventional breeding but not a replacement. Integration of conventional breeding with marker-assisted selection will help to get new cultivars resistant to biotic and abiotic stress (Jiang, 2013; Collard and Mackill, 2008). For this purpose, we need to improve existing varieties that will be fitted to the high temperature as well as anthracnose disease resistance. Review condense existing status about the effect of high temperature as well as anthracnose disease on chili production as well as described the fundamental molecular mechanisms is to develop the anthracnose resistant and heat tolerant chili (*C. annuum*) hybrid through conventional breeding with marker-assisted selection.

Conventional Breeding for the Development of Anthracnose Resistance Chili

Conventional breeding is challenging, tedious, takes a longer time, unpredictable, and is greatly influenced by the environmental factors as compared to molecular breeding. Pedigree, backcrossing, recurrent selection, and hybridization are the approaches for traditional breeding to create new genotypes with a combination of one or more desirable traits. The aim of conventional breeding is to develop new genotypes with desirable characters such as high yielding, high nutritional quality, and resistance to adverse environmental factors such as heat stress, drought, salt, acidity, and different diseases hybridization between different genotypes. The most recently used conventional breeding method includes pedigree selection, backcross selection, mass selection, host resistant breeding, induced mutation, hybridization, and interspecific hybridization, as summarized in Tab. 1. Using conventional breeding techniques, countless numerous resistant genotypes have been developed (e.g., PBC80, LLS, Breck-1, Breck-2, and Jaun) that showed resistance against different *Colletotrichum* species (Mondal *et al.*, 2013; Reddy *et al.*, 2014a; Banerjee *et al.*, 2014). The Asian Vegetables Research and Development Center developed anthracnose resistant genotypes by the introduction of anthracnose resistance genes into *C. annuum* and these genotypes can be used as potentials resistant sources (AVRDC, 2003).

Different virulent *Colletotrichum* spp., the causal agent of chili anthracnose, has been reported in different parts of the world (Montri, 2009; Voorrips *et al.*, 2004; Mahmodi *et al.*, 2014). Among all the *Colletotrichum* species, *Colletotrichum acutatum* is the major and dangerous among the anthracnose pathogens, as reported by Hasyim *et al.* (2014). Ripe and unripe chili fruits were inoculated with different isolates of *Colletotrichum* and identified resistance lines

TABLE 1

Traditional breeding for the development of anthracnose resistant chili genotypes

S/N	Anthracnose resistant genotypes	Pathogens	Breeding method	Country/region	Reference
1	Ten anthracnose resistant homozygous lines, *Capsicum annuum* obtained from the cross between Kulai 907 × AVPP0805, Kulai 907 × AVPP9813, Chili Bangi 3 × AVPP0805 and Chili Bangi 3 × AVPP9813	*Colletotrichum truncatum Colletotrichum fructicola Colletotrichum sojae*	Pedigree selection	Malaysia	Ridzuan *et al.*, 2018
2	F_2 and BC_1s population, PBC80 (*Capsicum baccatum*)	*Colletotrichum truncatum Colletotrichum acutatum*	F2 and backcross selection	Thailand	Mahasuk *et al.*, 2013
3	PBC932 (*Capsicum chinense*)	*Colletotrichum acutatum*	Backcross selection and Interspecific hybridization	Brazil	Pereira *et al.*, 2011
4	Acchar lanka, CA-4, Pant C-1, Punjab Lal, Bhut Jolokia and BS-35	*Colletotrichum spp.*	Host resistant breeding	India	Mishra *et al.*, 2019
5	Hybrid (CA-UGCE09-3XPP9852-115 and CA-UGKI09-6x PP9852-115) Hybrid (PP9852-115XPP0537-7504)	Cercospora Leaf spot and Virus	Mass selection	Uganda	Nsabiyera *et al.*, 2013
6	UENF 1718 and UENF 1797 (*C. baccatum var. pendulum*)	*Colletotrichum gloeosporioides*	Host resistant breeding	Brazil	Silva *et al.*, 2014
7	Progressive lines (205, 210 and 215)	*Colletotrichum spp.*	Host resistant breeding	North and northeast Thailand	Suwor *et al.*, 2015
8	RPE41 and MPU29	*Colletotrichum spp.*	Germplasm screened for the identification of resistance	Brazil	Souza *et al.*, 2019
9	Hybrid (*Capsicum annuum* cv Bangchang and *Capsicum chinense* PBC932)	*Colletotrichum capcisi*	Hybridization, F2 populations	Brazil	Mahasuk *et al.*, 2009a
10	UENF 1381 (*Capsicum annuum*)	*Colletotrichum gloeosporioides*	Host resistant screening	Brazil	Bento *et al.*, 2017
11	Daepoong-cho' (local Korean variety) and AR (anthracnose-resistant breeding line derived from *Capsicum chinense* Jacq. 'PBC 932')	*Colletotrichum capsici*	Hybridization, allelism test	Korea	Kim *et al.*, 2008

(UENF 1718 and UENF 1797), and these lines showed resistance against the species of *Colletotrichum baccatum* var. pendulum (Pandi *et al.*, 2018) and suggested that different genes are responsible for the nature of resistance (Kim *et al.*, 2010; Rodrigues *et al.*, 2016). Also, identified anthracnose resistant genotypes (*C. annuum*) against *C. acutatum* shown that this resistance can be used as potential genetic resources in the chili breeding program. Despite widespread research, chili cultivars that are resistant to the *Colletotrichum* spp. have not yet been developed and commercialized. However, the success of the breeding program is to develop durable resistant varieties and is hampered due to the association of multiple *Colletotrichum* species in anthracnose infection (Than *et al.*, 2008; Saxena *et al.*, 2016; Agrios, 2005) and along with the differential capabilities of the pathogenic virulence (Montri, 2009).

Molecular Breeding for Anthracnose Resistant Chili

In recent decades, different types of molecular markers have been widely used to measure the genetic variation among pathogen population such as simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP) and inter-simple sequence repeat (ISSR). Several studies have been conducted for the development of anthracnose resistant genotypes with the help of a molecular marker, as illustrated in Tab. 2. Similarly, QTL analysis of the identification of resistance to *Colletotrichum spp*. was summarized in Tab. 3.

Conventional Breeding for Heat Tolerance Chili

Assessment of heat stress tolerance is a major requirement, and also plays important criteria to recognize heat-tolerant

TABLE 2

Chili anthracnose-resistant traits linked with molecular markers

S/N	Resistant genotypes	Pathogen Strains	Molecular marker	Marker Name	Reference
1	Progressive lines (*Capsicum annuum*) derived from PBC80 (*Capsicum chinense*, resistant to anthracnose)	*Colletotrichum acutatum* and *Colletotrichum capsici*	Simple Sequence Repeat (SSR), Sequence Characterized Amplified Region (SCAR)	HpmsE032	Suwor *et al.*, 2015
2	Hybrid, KULAI 907(Malaysian elite genotypes, Susceptible) x AVPP0805 (Developed by AVRDC, Resistant)	*Colletotrichum truncatum*	Simple Sequence Repeat (SSR)	Hpms 2-24	Ridzuan *et al.*, 2018
3	*Capsicum annuum* x *Capsicum chinense*	*Colletotrichum acutatum*	Simple Sequence Repeat (SSR), Sequence Characterized Amplified Region (SCAR) and Cleaved Amplified Polymorphic Sequence (CAPS)	HpmsE057, HpmsE116, HpmsE126, ES382, Gpms161, Gp20068, ES64, Epms745, Gp20068, ES118, ES181, InDel, C2_At4g03400	Sun *et al.*, 2015
4	*Capsicum annuum* x *Capsicum chinense*, *Capsicum annuum* x *Capsicum baccatum*, *Capsicum annuum* x *Capsicum baccatum*	*Colletotrichum truncatum* *Colletotrichum truncatum* *Colletotrichum truncatum*, *Colletotrichum acutatum*	Simple Sequence Repeat (SSR), Sequence Characterized Amplified Region (SCAR)	HpmsE032, HpmsE143, InDel, CaR12.2M1, CcR9M1	Lee *et al.*, 2011; Wang, 2011; Suwor *et al.*, 2015
5	*Capsicum annuum* x *Capsicum baccatum*	*Colletotrichum truncatum*, *Colletotrichum acutatum*	Simple Sequence Repeat (SSR)	Hpms2-24, HpmsE143, HpmsE092, HpmsE032, HpmsE063	Lee *et al.*, 2010
6	*Capsicum annuum* x *Capsicum chinense*	*Colletotrichum gloeosporioides*, *Colletotrichum truncatum*	Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR)	CA-MS6, CA-MS25, B2, CA-MS22, B1, G1, H1, CA-MS23, CA-MS12, D1,	Voorrips *et al.*, 2004
7	F_1, F_2, and BC_1F_1 populations (Punjab Lal x Arka Lohit, susceptible cultivar)	*C. truncatum*	Sequence-Tagged Site (STS)	CtR-431, CtR-594	Mishra *et al.*, 2019

TABLE 3

Genes conferring resistances to anthracnose in Capsicum, identified in previous studies

S/N	Chili genotypes	QTLs/Genes name	Pathogen pathotypes	Reference
1	*Capsicum annuum* 'SP26' (susceptible recurrent parent) and *Capsicum baccatum* 'PBC81' (resistant donor)	QTLs (CaR12.2 and CcR9)	*Colletotrichum acutatum* and *Colletotrichum capsici*	Lee *et al.*, 2010
2	*Capsicum chinense* accession (PBC932) was studied in a BC1 population derived from a hybrid with *Capsicum annuum* line 77013 (susceptible)	QLTs (AnRGO5, AnRGT5, AnRGD5, AnRRO5, AnRRT5, AnRRD5)	*Colletotrichum acutatum*	Sun *et al.*, 2015
3	*Capsicum baccatum* (PBC80), *Capsicum chinense* (PBC932)	Genes (co4 and Co5)	*Colletotrichum capsici* and *Colletotrichum acutatum*	Mahasuk *et al.*, 2009b
4	126 F_2 population (*Capsicum baccatum* var. pendulum (resistant) and *Capsicum baccatum* 'Golden-aji' (susceptible)	Major QTLs-(An8.1, An9.1) Minor QTLs-(An7.3, An7.4, An4.1, An3.1, An3.2)	*Colletotrichum acutatum*	Kim *et al.*, 2010

genotypes with high yield. Under high temperatures, plants followed different mechanisms involved in long-term adaptation and short-term avoidance, among which the plant produced heat shock proteins for adaptation (Srivastava *et al.*, 2012). Plant adaptation can be sustained by appropriate breeding methods involved in phenotypic and genotypic evaluation (Chapman *et al.*, 2012). Hybrid chili production has increased on commercial bases like other Solanaceae vegetables such as tomatoes and eggplant. Chili plants are inspected under heat stress in their lifecycle from seedling to ripening; they react differently at different temperatures (Schramm *et al.*, 2006; Pagamas and Nawata, 2008). Researches have been done to find out suitable heat-tolerant chili genotypes, which are summarized in Tab. 4. Researchers suggested that heat-tolerant genotypes can be used as a potential genetic material in chili breeding programs to develop new genotypes. Hot pepper traits are most suitable for the development of heat-tolerant chili genotypes to get the desired quality and quantity like yield, fruit traits, disease, pungency nature, and other antioxidant contents (Padilha *et al.*, 2015). In the case of sweet peppers, leaf proline content helps to overcome plant heat stress because at high-temperature condition sensitive varieties produced lower amounts of proline in leaf than heat tolerant (Saha *et al.*, 2010).

Molecular Breeding for the Development of Heat-Tolerant Chili Genotypes

Heat stress conditions are recognized by the induction of stress proteins or genes that protect the organism from cellular damage called heat shock proteins (HSPs) expression and exert a significant role in plant tolerance to heat stress (Usman *et al.*, 2017; Usman *et al.*, 2018). HSPs can be classified into five categories such as HSP100 (or ClpB), HSP90 (HtpG), HSP70 (or DnaK), HSP60 (or GroEL) and HSP 20 (or small HSP, sHSP) and they are involved in regulating different development stages of plants (Prasinos *et al.*, 2005; Swindell *et al.*, 2007). During infection, Hsp70s are involved in the regulation of the viral infection cycle as well as in the host stress response (Gorovits *et al.*, 2013 and Jungkunz *et al.*, 2011). Heat-inducible genes are responsible for the regulation of heat shock protein (HSP) metabolism (synthesis and breakdown), which confers tolerance against high temperature and also helps to protect intracellular protein structure from denaturation and conserve their strength and role through protein folding thus acting as molecular chaperones (Chang *et al.*, 2007). The selection of constitutive QTL is a vital requirement for fruitful MAS for developing heat tolerance. Plant breeders are interested in working on the manipulation of genes for the improvement of genotypes that can survive at extreme temperatures (Farnham and Bjorkman, 2011). Chili plant has diverse innate than other Solanaceae crops like tomatoes, that criteria give opportunities for altering or rearrangements of the gene at the molecular level. Heat stress creates artificial imbalance and massive membrane disruption (Usman *et al.*, 2014b and Gajanayake *et al.*, 2011) of plant physio-biochemical function, for that need to development of heat-tolerant genotypes, this is the prime requirement and established a linkage between phenotypic and genotypic study to identify QLT for crop improvement. Introgression of the LeUCP gene (responsible for stress tolerance) in tomato and confirmed by real-time PCR and Southern blot hybridization and the transgenic plants, showed improved bio-molecular state and suggested that this gene might help to control biotic and abiotic stress in many aspects (Chen *et al.*, 2013). Perseverance and excellence finding is the optimal condition for abiotic stress in the breeding program, and some characters are controlled by polygene that can be easily misinterpreted phenotypically and need observation for the accurate response of plants in a given situation (Farnham and Bjorkman, 2011). Drought and extreme temperatures are especially considered as crucial stress factors with high potential impact on crop yield and in this situation, the defense can be attained by quick alterations in gene manifestation (Bita and Gerats, 2013). In chili producing country, due to global warming, heat-tolerant chili genotypes development is the primmest requirement for sustainable agricultural production

Chemical Components in Capsicum Related to Anthracnose Resistance and Heat Tolerance

Chili has multipurpose uses due to chemical, medicinal, and nutritional properties for its pigment, flavor, and hotness. There are two types of chili according to taste, one is

TABLE 4

Traditional breeding for the development of heat-tolerant chili genotypes

S/N	Heat tolerant Genotypes	Minimum temp.	Maximum temp.	Heat tolerance	Reference
1	Backcross progenies, BC_1F_3	30°C	42°C	Excellent sources for heat-tolerant chili development	Usman *et al.*, 2015
2	Tomato inbreed lines (HT019, C11, C41, C51, C71 and WP10)	20–25°C	55°C	Good to initiate a breeding program to develop heat-tolerant tomato variety	Islam *et al.*, 2014
3	Chilly Chili, Medusa, Thai Hot, Explosive Ember, and Treasures Red	10°C	45°C	Excellent for the development of reproductive temperature tolerance and vegetative temperature tolerance	Gajanayake *et al.*, 2011
4	Pepsi-17-2, DCL524, VR-16, MS-12, and S-217621	34°C	40°C	Genotypes better for fruit set under high temperature	Kaur *et al.*, 2016

Capsicum species with pungent, called hot pepper, and that without pungency is called sweet pepper (Nadeem *et al.*, 2011). Chili fruits contain oleoresin, capsanthin, capsorubin, zeaxanthin, cryptoxanthin, and lutein as a pigment, indicating the presence of carotenoids (Wesolowska *et al.*, 2011). Capsaicin and dihydrocapsaicin are collectively known as capsaicinoids, non-volatile, alkaloids substances, accumulated in the placenta of chili fruits where seeds are usually attached, responsible for pungency (Gahungu *et al.*, 2011). Capsaicinoids have multipurpose uses like antioxidant, antimicrobial, and anticarcinogen, and increase metabolism, inhibitors for fat accumulation, bio-pesticides, and anti-inflammatory effects (Materska and Perucka, 2005). For antimicrobial activity associated with phenol group-containing substances, capsaicinoids increase the resistance to anthracnose and degree of heat tolerances. Capsaicinoids and pungency are directly correlated with each other (Usman *et al.*, 2014b), and genotypes might have a specific pungency level and also have commercial value. There is a positive correlation between the capsaicin content in Capsicum and prevalence of the anthracnose disease and heat stress (Tenaya *et al.*, 2001 and Azad, 1991) and agreed that a higher capsaicin level in red chili (*C. annuum*) was associated with greater resistance to anthracnose. Capsaicin is considered a biopesticide that has antimicrobial properties and has no toxic effect, as described by Walter (1995) and Gudeva *et al.* (2013). Phenol and some enzymes enhance the anthracnose diseases resistant in chili peppers (Prasath and Ponnuswami, 2008). Different polyphenols play a vital role in defense mechanisms as phytoalexins, which are responsible for biotic (fungal, bacteria and virus) and abiotic (extreme heat, drought, and salt) stresses in healthy chili peppers fruits against *C. gloeosporioides* namely N-caffeoyl putrescine and caffeoyl O-hexoside.

On the other hand, Feruloyl O-glucoside, kaempferol O-pentosyldihexoside, and dihydroxy flavone O-hexoside are the polyphenol was found in infected *C. annuum* fruits as phytoanticipin (metabolites with the defensive role) (Park *et al.*, 2012). The capsaicin level in chili predicts the chili genotypes are susceptible or not, meaning that the determination of the level of anthracnose resistance not only provides information on a molecular level but also chemical content as well.

Bottlenecks of Conventional and Molecular Tools for the Development of Anthracnose and Heat Tolerance Chili Genotypes

The development of anthracnose resistant and heat tolerant chili hybrids are the primmest requirement in this present situation. But development procedure has faced a lot of problems. Different plant breeding methods are used to obtain resistant or tolerant chili genotypes against biotic and abiotic stress, namely introduction, hybridization, selection, mutation, and genetic engineering (Pujar *et al.*, 2017). Desirable genes that confer resistance or tolerance to biotic and abiotic stresses under varying environmental conditions are not found in cultivated cultivars but present in the wild cultivars. Hybridization is the most popular breeding method for crop improvement through the use of valuable genes from wild species incorporated into elite cultivars through distant hybridization (Manzur *et al.*, 2015). Pyramiding the anthracnose resistant and heat tolerant genes in chili hybrids through hybridization or crossing techniques has faced some problems such as the occurrence of self and cross incompatibility, pre fertilization problems or inhibition of pollen germination by inhibitors, post-fertilization problems due to embryo collapse (Yoon *et al.*, 2006). Through distant hybridization, many undesirable genes will be transferred while transferring desirable genes due to undesirable linkage that leads to a low success rate. These barriers slow the hybrids development procedure and can be significantly overcome by proper management and supervision during the crossing, provide supporter pollination, use of bridge species, embryo rescue, and protoplast fusion (Pujar *et al.*, 2017). On the other hand, *Colletotrichum spp*. has pathogenic variability and also has several species or biotypes with a single host, conventionally and phenotypically identification of pathogen association is very tuff and sometimes difficult based on conidial morphology, pathogenicity, and biochemical test (Saxena *et al.*, 2014). Plant heat tolerance involves plant physiology, plant biology, biochemical and molecular reaction because heat tolerance is not a single trait. The development of new varieties is expensive and time-consuming, sometimes it takes 10–30 years. At this point, molecular physiology of the plant biotic and abiotic stress response can speed up the identification of connecting genes subsequently QTL documentation (Driedonks *et al.*, 2016).

Conclusion and Prospects

Plant breeders have applied different classical and modern breeding methods for the development of disease-resistant and heat-tolerant chili varieties by maintaining desirable genetic differences through controlled crosses between individual plants and conveyed to the next generation through the phenotypic selection process. Integration of conventional breeding with marker-assisted selection is the most efficient technique for the introgression of resistant genes into new plants and help to select multiple resistant genes in a single genotype. Understanding the mechanism of monogenic and polygenic disease resistance, as well as biotic and abiotic stress responses of plants, are important for the development of resistant varieties. Several researchers have investigated some characters like canopy temperature depression and membrane thermo-stability for heat tolerance criteria through conventional breeding and may be an effective indicator for heat tolerance test. PCR-based markers will be useful for the characterization of the locus, isolation of desirable genes for resistance to anthracnose as well as heat-tolerant in the chili breeding program. While numerous genetic factors have been effectively developed in chili breeding program to boost up biotic and abiotic stress-resistant and their function in diverse genomic circumstances is still to be examined. This review would help to understand the molecular basis of chili hybrid development with traditional field practices and pyramid multiple genes into a single one for sustainable production.

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