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Genetics and Breeding for Biotic and abiotic stress resistance in Chilli

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Introduction:

Hot pepper (*Capsicum annuum* L.) is one of the major spices cum vegetable crop belongs to the Solanaceae family originated from Mexico, Central America. Nowadays, hot pepper is cultivated all around the world, widely used in many cuisines as a spice and in pharmacy for the extraction of bioactive compounds known as capsaicinoids and the fruit is also a good source of carbohydrates, minerals, proteins, amino acids, antioxidants, phytochemicals, and vitamins. Asia is the main producer and exporter of hot pepper. Among the Asian and of the world countries, India is the largest producer of green hot pepper with the production of 3592 thousand tonnes in an area of 309 thousand hectares.

In 21st century, climate change is the most serious threat to crops, causing a negative impact on the quantity and quality of crops. Increase of CO2 along with other greenhouse gases may cause an increase of more than 2° in mean global temperature by the end of this century. Climate change in future exacerbates a rise in temperature level, which causes an adverse impact on crop growth and development. An increase in mean annual temperatures and heat waves might destroy physiological processes in plants (Jiang and Huang, 2001). The production of hot peppers in early summer is limited by high temperature, blowing of hot wind and shortage of soil moisture and high temperature and excessive moisture during rainy summer. Such conditions induce the abscission of flower buds, flowers and young fruits which are the most important factors limiting the production of hot pepper. It is also observed that hot pepper is mainly produced in the open field, summer production will be impossible in many parts of the world without heat-tolerant cultivars. Exposure of hot pepper to high temperature after anthesis adversely affects fruit growth, seed yield and seed quality of chili pepper. Drought tolerance is one of the important characteristics of hot pepper under climate

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change. Hot pepper performs well in hot and dry environments of Afghanistan with comparably lower post-harvest losses than other vegetable crops

Breeding and QTLs mapping for biotic stress resistance in chilli

Capsicum species are commonly known as peppers, one of the most important vegetable crops cultivated worldwide. The genus capsicum includes more than include 30 species, but generally five species like *C. annuum, C. chinense* and *C. frutescent, C.chinensis* and *C.baccatum* are main cultivated species grown for fresh, dried and, processed food consumption. Recently the development of NGS technology opened new opportunities for genome sequencing, and the study of the pepper genome. The Sequencing of the pepper genome was initiated by using the BAC library and estimation done by the ethidium bromide flow cytometry method. Genome of some wild and cultivated species have been sequenced and estimation like *C.annuum* (*3090 mbp), C.frutescens (3325mbp), C.chinense (3345mbp), C.baccatum (3628mbp), C.chacoense (3746mb)p C.eximum*(*3971mbp)* and the largest genome size in *C.parvifolium (5643mbp)* respectively. Genetic studies in pepper have been progressed to the identification of germplasm and genetic inheritance of various complex traits, identification of gene location, QTLs mapping and characterized molecular marker linked to various important traits for fasted pepper breeding program. Molecular mapping has been initiated in chilli for a various complex traits like fruit color, fruit size, fruit shape, pungency and other quality traits like capsaicin biosynthesize, flavor and many diseases resistance traits. QTLs have been identified for resistance to anthracnose, cucumber mosaic virus, phytophthora blight, powdery mildew, potyvirus, capsaicinoid content, fertility and number of pedicel per node etc. Recent advance has been progressed and identified some trait with linked marker like tobacco mosaic virus (L^1, L^2, L^3,L^4, L^+), CMV resistance (*cmr-1, cmr-2 locus*), potyvirus series resistance locus like *pvr-1, pvr-2,pvr-3,pvr-5, pvr-6, pvr-7, pvr-8* and bacterial leaf spot (Bs-1, Bs-2, Bs-3, Bs-4 and recent gene like Bs-5, Bs-6), anthracnose resistance (*Anr-1, Anr-2, Anr-3, Anr-4, Anr-5*) QTLs, powdery mildew *(lmr-1, lmr-2 , lmr-3*) QTLs, erect fruit habit (*up-1, up-2*) gene, carotenoid synthesis *(C-1 , C-2*) gene, purple fruit color (*im* gene), *pun locus* responsible for pungency, GMS inherited by *ms-1, ms-2,ms-12,ms-13,ms-14,* and fertility restorer gene *rf* gene for effective utilization CGMS in hybrid seed production, TSWV (*Tsw)* gene, and root knot nematode *(Me-1, Me-3, Me-4, Me-7, Mech-1*) genes mapped and linked to molecular marker for improvement of complex

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trait in chilli. Candidate gene approaches is hypothesis based method for new gene identification in chilli constructed with map-based cloning and position of gene on genomic region and validate gene by virus-induced gene silencing method. Candidate gene approaches used in *L* locus for tobamovirus, *pvr-1, pvr-2* locus for potyvirus, pun locus for pungency, and *cl* for fruit colour.

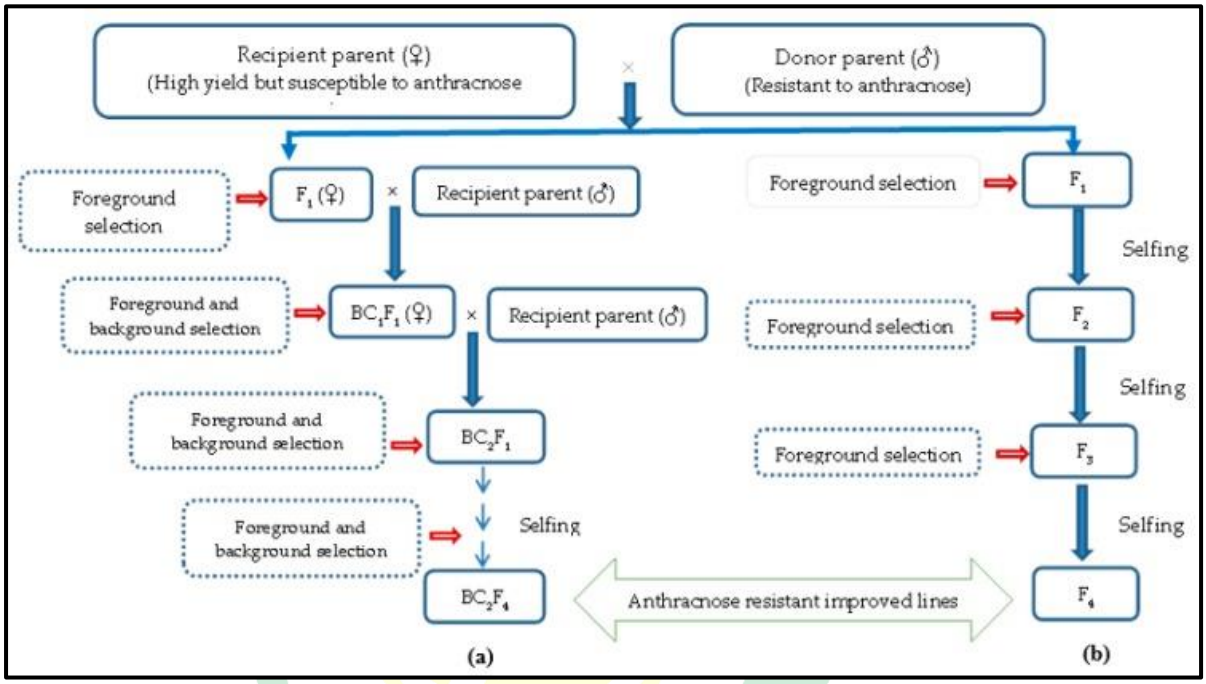

Fig 1: Diagram showing the development of anthracnose-resistant varieties through (**a**) marker-assisted backcrossing and (**b**) pedigree selection

QTLs mapping achievement for various traits in chilli:

Traits	Locus Name	Genetic inheritance	References
Putative Acyltransferase gene AT3 (Presence/Absence of Pungency)	*Pun1*	Gene, Dominant	(Lee *et al*. 2005; Stewart *et al*. 2005; Stewart et al. 2007)
Fasciculate	*fa*	Gene, Recessive	(Elitzur *et al*. 2009)
Male sterility (Genic)	*Camf1*	Gene, Dominant	Chen *et al*. 2012
Pungency	*Cap7.1*,	QTL	Ben Chaim *et al*. 2006

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	Cap7.2		
Resistance to Cucumber mosaic virus (CMV)	*Cmr1*	Dominant	Kang *et al*. 2010
Resistance to potyvirus- tobacco etch virus (TEV), potato virus Y (PVY), potyvirus E (PVE)	*pvr1*	Recessive	(Murphy *et al*. 1998; Kang *et al*. 2005)
Resistance to tobamo-viruses	*L3*	Gene, Dominant	(Berzal-Herranz *et al*. 1995; Tomita et al. 2008)
Resistance to tobamo-viruses	*L4*	Gene, Dominant	(Yang *et al*. 2009)
Tomato spotted wilt virus (TSWV)	*Tsw*	Gene, Dominant	(Jahn *et al*. 2000; Moury *et al*. 2000)
Resistance to Xanthomonas campestris (Bacterial spot)	*Bs2*	Dominant	(Tai *et al*. 1999b; Mazourek *et al*. 2009)
Resistance to Xanthomonas campestris (Bacterial spot)	*Bs3*	Dominant	(Pierre *et al*. 2000; Jordan *et al*. 2006)
Resistance to Root-knot nematodes (Meloidogyne spp.)	*Me*	Dominant	(Djian-Caporalino *et al*. 2001; DjianCaporalino *et al*. 2007)
Resistance to Root-knot nematodes (*Meloidogyne chitwoodi*)	*Mech*	Multiple genes, Independent dominant	Djian-Caporalino *et al*. (2004)
Resistance to potyvirus—pepper veinal mottle virus (PVMV)	*pvr1*	Recessive	(Murphy *et al*. 1998; Kang *et al*. 2005)
Resistance to potyvirus—pepper veinal mottle virus (PVMV)	*Pvr 4*	Dominant	(Grube *et al*. 2010)

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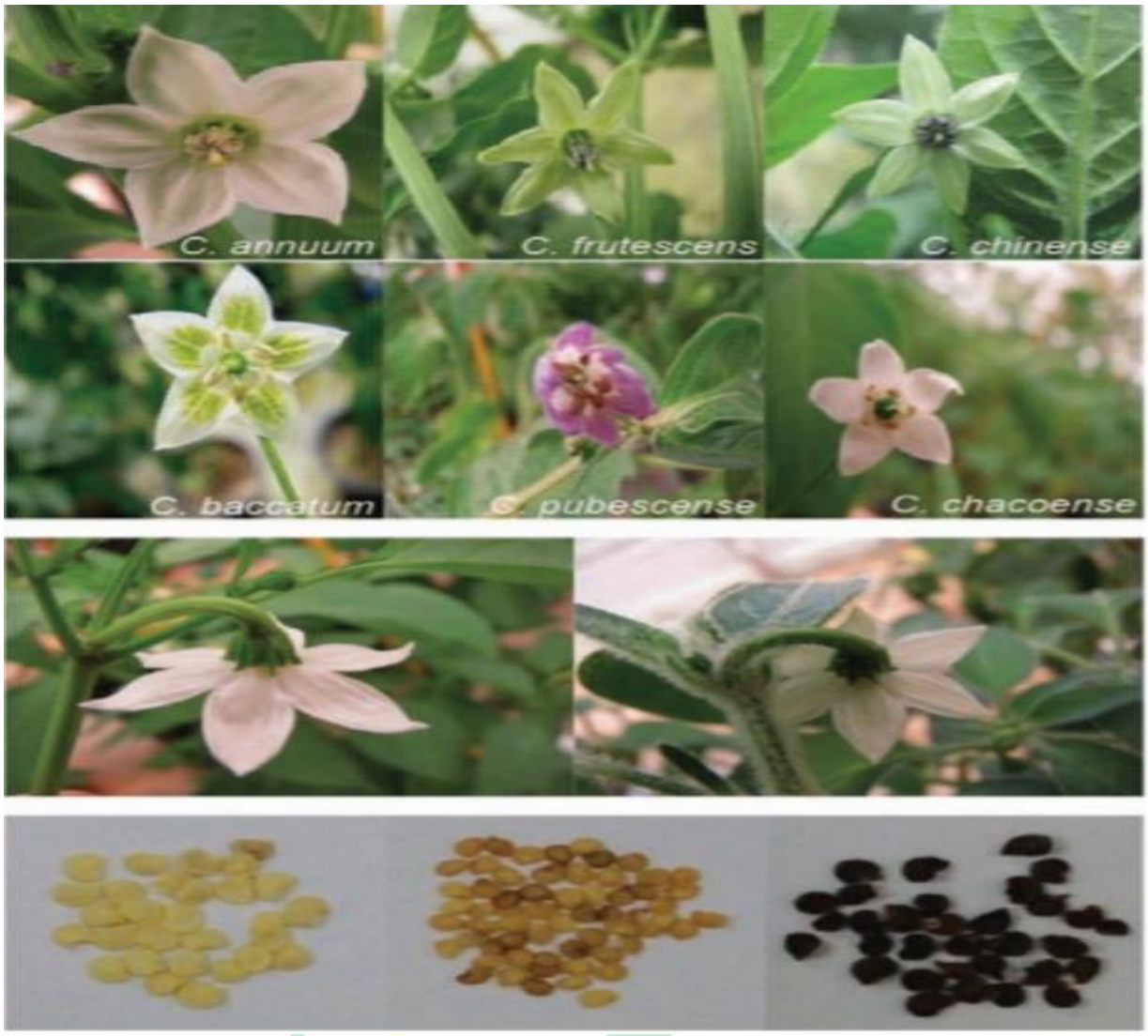

Fig 2: Wild species of chilli used for resistance breeding against various biotic and abiotic stress resistant breeding programme

Breeding and QTL mapping for cold and hot resistance in chilli

The cold resistance should be governed by a polygenic and the broad spectrum genetics of inheritance in poorly understand in hot pepper. Plants respond with changes in their pattern of gene expression and protein product when exposed to low temperature. Most appear to be involved tolerance to the cold stress and expression of some of these are regulated by C-repeat binding factor/dehydration-responsive element-binding (CBF/DERB-1). To struggle with abiotic challenges, plants are evolving with a complex mechanism of perception and reactions. Abiotic stresses are recognized by many signaling cascades which later activates ion channels, kinase cascades and sometimes producing reactive oxygen species and by some

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other means such as accumulation of hormones, i.e., salicylic acid (SA), ethylene (ET), Jasmonic acid (JA) and abscisic acid (ABA). The late embryogenesis abundant (*LEA*) gene from barley is effective in increasing cold tolerance when introduced to rice plants. Many structural and transcriptional factor-encoding genes that get induced by cold stress have been identified in Capsicum species including *EREBP (CaEREBPC1 to C4), WRKY (CaWRKY1*), and *bZIP (CaBZ1*) genes. EBR (2, 4-epibrassinolide) enhances salicylic acid & jasmonic acid cycle and suppresses the ethylene biosynthesis pathway in cold stress. RNA-seq analyses of peppers treated with heat, cold, salinity and osmotic stress at six different time points will provide useful information for basic studies of various stimuli to facilitate the development of stress-resistant pepper cultivars.

Mapping of Quantitative trait loci (QTLs) controlling cold resistance is a major breeding objective. QTLs controlling to sensitivity to chilling have been identified in maize (Hund et al 2005), Sorghum seedling (Knoll and Ejeta *et al*., 2008), and cultivated tomato (Goodstal *et al*., 2005). A *CBF* gene (*CFB-2*) also mapped as freezing tolerant QTL mapped in Arabidopsis and controlling gene (Alonso Blanco *et al*., 2005). Presently, hot pepper breeding is mainly focused on the development of cold-tolerant cultivars due to the continues rise in global mean temperature necessitating constant requirement of cold-tolerant sources and genomic information related to the cold that enhanced breeding for dissecting QTLs or gene for cold tolerance in chilli.

Heat tolerance appears to be polygenically controlled, because of which genetic basis of heat stress tolerance in plants is poorly understood (Wahid *et al.* 2007; Ainsworth and Ort 2010; Collins *et al.* 2008). Hence QTL mapping is found to be an effective way to identify the genes responsible for heat tolerance (Janick *et al*. 2011), many efforts have been made to identify quantitative trait loci (QTL) for heat stress in segregating mapping populations. Jha *et al.* (2014) showed QTLs associated with heat tolerance in various plants. Similarly heat shock proteins which are produced in plants under heat stress, play an important role in heat resistance in plants. They function as molecular chaperones and can bind partially denatured proteins, thereby prevent deleterious protein conformations and eliminate non-native aggregations formed during stress (Morimoto 1998; Boston *et al.* 1996; Vierling 1991). Zhu *et al.* (2011) indicated that the expression of *CaHSP24* small heat shock protein gene in hot pepper was induced by heat stress. Two SSR markers (Hsp70-u2 and AGi42) are tightly

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linked to the Hsp genes in pepper (Magaji *et al.* 2016; Ince *et al.* 2010). Sharma *et al.* (2017) observed that fine mapping using a recombinant inbred line population where many recombinant events as well as replicated phenotypic evaluation might give better resolution of the QTL region and candidate gene for heat tolerance as compared to the mapping which was based on F_2 populations. Wen *et al.* (2019) demonstrated that combination of conventional QTL mapping, QTL-seq analysis and RNA-seq can rapidly identify heat-tolerance QTLs and high-temperature stress-responsive genes. Transcriptome Analysis through RNA-Seq proved to be an effective approach for the identification of heat stress-related genes and also to investigate the underlying heat stress response mechanism in hot pepper genotypes (Li *et al.* 2015). Kang *et al.* (2020) also inferred RNA-seq analyses of peppers treated with heat, cold, salinity, and osmotic stress at six different time points will provide useful information for basic studies of various stimuli to facilitate the development of stress-resistant pepper cultivars.

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