Review Paper:

Genetics and molecular markers for resistance to major soil borne pathogens in chilli *(Capsicum annuum L.)*

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Abstract

Three major soil borne pathogens namely Phytophthora capsaici Leon causing root rot, bacterium Ralastonia solanacearum causing bacterial wilt and root- knot nematodes (Meloidogyne spp.) are pervasive across the major pepper growing parts of world mainly in the areas of repeated cultivation and poly houses impeding production as these pathogens are very difficult/ impossible to control. Repeated application of chemicals is in practice among the farmers and chemical management often leads to environmental pollution and the presence of pesticide residues in the fruits thus raises the concern of food safety and reduces the export potential. Recently grafting strategy using resistant root stock has been proposed mainly for protected cultivation. Resistance breeding through incorporating the resistant genes/QTLs is the best advocated strategy to circumvent these devastating soil-borne diseases which aim at developing a variety/root stock having combined and durable resistance.

Marker assisted backcross breeding is the best method which ensures precise transfer of the resistant genes/QTLs of interest from the donors to the recurrent parent with accelerated recurrent parent genome which requires thorough knowledge on the molecular marker available for fore ground selection. This review mainly emphasized on the molecular markers available for breeding peppers for combined resistance to soil borne pathogens.

Keywords: Bacterial wilt, Phytophthora root rot, Root knot nematodes, Marker assisted selection.

Introduction

Capsicum is a versatile plant being grown as a vegetable and spice crop also known as hot pepper, bell pepper, sweet pepper, bird eye pepper and paprika belonging to the nightshade (Solanaceae) family. Chilli has become an indispensable commodity in every cuisine due to its pungency, spicy taste, appealing color and flavor. Fruit biochemical compounds, carotenoids from pepper are used as natural colorants and capsaicinoids have wide applications in the food, medicine and pharmaceutical industries. The yield, quality and growth of plants are limited by many biotic and abiotic factors.

Among the biotic factors, three major soil borne diseases namely *Phytophthora* root rot caused by fungal pathogen *Phytophthora capsici* Leon., bacterial wilt caused by bacterium *Ralastonia solanacearum* and root- knots caused by nematodes belonging to *Meloidogyne* spp. are very destructive causing severe yield losses mainly in protected cultivation. These notorious pathogen propagules remain viable and active in soil for several years making the control almost impossible through any means of chemical treatments especially in the regions where repeated and protected cultivation is under taken. Many of the commercial varieties/hybrids are highly susceptible to these pathogens.

Chemical management of these diseases often leads to the presence of pesticide residues in the fruits which affect the export potential of the produce and raises the food safety issues. Vegetable grafting technology wherein the resistant root stock will be used to cultivate the high value bell peppers is highly economical, however there is no root stock is available, which is resistant to all the three diseases. Resistance breeding is the best advocated strategy to develop the verities/hybrids resistance to manage these devastating soil-borne diseases. For developing the genetic resistance without hampering the quality is the viable solution, marker assisted backcross breeding is the best method to accelerate the breeding programme.

Bacterial wilt: Bacterial wilt caused by vascular soil borne pathogen *Ralastonia solanacearum*, causes irreversible sudden death of plants and practically it is very difficult to eliminate it from the soil. The *Ralastonia solanacearum* biovars 1, 3, 3a, 3b and 4 of race 1 cause the bacterial wilt in peppers.[1] This disease is widely prevalent majorly in humid tropics of Asia. Some of the resistance sources were identified in earlier studies such as LS2341[2], Manganji[3] and PM 687[4]. The resistance of bacterial wilt is reported to be polygenic in LS2341for the bacterial strain KP9547 and a major QTL was reported named as *Bw1* and SSR marker CAMS 451 linked to this QTL indicating the use of the same in marker assisted selection.[5] This disease is severe problem mainly in humid tropics; *R.solanacearum* biovar 3 of race 1 is predominant mainly in humid areas.[6] Initially Kerala Agricultural University (KAU), India has identified a cultivar 'White Khandari' having strong resistant to bacterial wilt. This line was used in breeding programme and a

cultivar Pant C-1 was developed which also showed stable resistance. Using the Pant C-1 as a donor, KAU have developed a variety 'Ujwala' through backcross programme. Later the resistance has been transferred to wide adopted variety 'Pusa Jwala', thus produced near isogenic line and released the variety in the name of 'Anugraha'.[7]

Recently by using these near isogenic lines (Anugraha and Pusa Jwala) and their F_2 population were used for molecular genetic studies and found resistance is recessive in nature and AFLP marker (*Eco*ACT+*Mse* CAC) polymorphic bands (103, 117 and 161bp) associated with this resistant recessive allele have been identified.[8]

Phytophthora root rot: *Phytophthora capsici* Leonian is fungal oomycete soil borne destructive pathogen which causes root rot.[9] This pathogen reproduces both sexually and asexually[10], causing a persistent problem especially in the regions of repeated chili cultivation. Several resistant sources have been identified such as 'CM334'[11,12], 'PI201232'[13] and 'PI201234'[14]. The Mexican line Criollo de Morelos-334 (CM334) was the best choice to breeders and molecular biologists due to its consistent high degree of resistance to several wide and virulent strains of *Phytophthora capsici*.[15]

Lot of work has been done so far on genetic and molecular basis of resistance for this pathogen, earlier genetic studies in CM 334 reported various genetic models varying from recessive to dominant nature.[16,17] Finally it was later concluded that resistance in CM334 is polygenic in nature with additive and epistatic effects.[11]

The molecular studies have identified several QTLs and markers associated with the resistance to *Phytophthora capsici* using CM334 as the resistant parent. Thabuis et al[12] identified QTL regions on P4, P5, P6, P11 and P12 common to both root rot and stem blight. On chromosome 5, a common QTL 'Phyto-U' was found.[14] Quirin et al[18] developed a SCAR marker for the detection of major QTL 'Phyto 5.2' on chromosome 5. Minamiyama et al[19] used 626 microsatellite markers and found a major QTL region (R^2=58.1%) on LG15, flanked by CAMS 420 marker and other seven SSR markers located very nearer to the peak of this QTL.

Kim et al[20] used CM334 × Chilsungcho population and identified four QTLs which explained 66.3% of total phenotypic variation for root rot resistance QTL major explained 41.97% variation found on chromosome 5, a RFLP marker CDI25 linked to this major QTL was converted to easily usable single nucleotide amplified polymorphism (SNAP) for marker assisted selection. Another two SSR (SSR-3 and SSR-9) and CAPs markers were also developed for the QTL on chromosome 9 which explained 7.73% of root rot resistance.

Table 1
Details of molecular markers available associated with resistant genes/QTLs for bacterial wilt and root knot nematodes

Gene/QTL	Marker	Sequences	Chromosome location	Population where they are polymorphic
For bacterial wilt resistance				
Bacterial wilt resistant recessive allele[8]	AFLP	*Eco*ACT+*Mse*CAC	Not mapped	Anugraha (*bwr*) ×Pusa Jwala
Bw1[5] (Explained 33% resistance)	SSR	CAMS 451 (mapped in the center of QTL) 5'-TGCATTGGTGGGCTAACATA-3' 5-GCTCTTGACACAACCCCAAT-3'	Linkage group 11	Double Haploid population of Calfornia Wonder x LS2341
For root knot nematodes resistance (*Me* genes linked markers)				
Me3-Me4[29]	SCAR_B94	5'-GCTTATCATGGCTAGTAGGG-3' 5'-CGGACCATACTGGGACGATC-3'	The resistance genes are clustered in a 28 cM interval on chromosome P9	PM687× Yolo Wonder
Me1 & Me7[29]	SCAR_CD	5'-GAAGCTTATGTGGTAMCC-3' 5'-GCAAAGTAATTATATGCAAGAGT-3'		DLL × PM702 (PM702-inbred line from CM334)
Me3, Me4 & Me7[29]	SSCP_B54	5'-CGGTGGCTGTTACGCTC-3' 5'-GCATGTCTTTCTTTACC-3'		
Me3, Me4 & Me7[29]	SSCP_B322	5'-GATTCCATAACCTGGAAATTTCTGG-3' 5'-CGAACCCGGTCTATTTTC-3'		
Me7[29]	CAPS_F4R4 (*Tru91*)	5'-AGAACAATAGAATCTCTCTTG-3' 5'-CTTCAGGAACCCCTCAGC-3'		
N gene allelic to *Me 7*[30,31]	SCAR	5'-AATTCAGAAAAAGACTTGGAAGG-3' 5'-TAAAGGGATTCATTTTATGCATAC-3'	Mapped on Chr 9 at 7cM from *Me1* and 2cM from *Me3*	Carolina Wonder × 20080-5-29

Table 2
Details of molecular markers available associated with *Phytophthora* root rot resistant QTLs

QTL	R^2 (%)	Marker	Sequence (5'-3')	Chr	Mapped population
Meta QTL Pc5.1 (Mallard et al[21])					
Phyto QTL (CDI25-CDI78)[20]	41.97	P5-SNAP	P5-SNAP-CM-F 5'-TCATGAGGTTGCTATTAAGATTGGTCCTGTTATATA-3' P5-SNAP-Chil-F 5'-GAGGTTGCTATTAAGATTGGTCCTGTTATCCG-3' P5-SNAP-R 5'-CATAGAAAGGGATATCATCTGGTACATGCAGAAA-3'	Chr 5	CM334 × Chilsungcho
Ph051-5.2 Ph051-5.4 Ph051-5.6[22]	20.0-48.2	SA 133_4 (co-dominant SCAR)	5'-GAATCACAAGGAAAAGAAAACAAG-3' 5'-TGAAAGGAGTCTCTGAATCCATAA-3'	Chr 5	YCM334 × Tean
QTL on LG15[19]	58.1	SSR markers	CAMS 420 (marker at peak of QTL) (Minamiya et al[33])	Chr 5	Mangangy × CM334
			CAMS 362, CAMS 051, CAMS 163,CAMS 211,CAMS 319,CAMS 134,CAMS 839 (Minamiya et al[33]) CAMS 072 (markers spanning within 21cM of the QTL) (Minamiya et al[33])	Chr 5	Mangangy × CM334
***Meta QTL Pc5.2* (Mallard et al[21])**					
Phyto QTL (CT211A-CDI128)[20]	7.73	SSR-9	5'-CAAGCACCTACAAATGCAAAAT-3' 5'-CCGGATGAGAAAACTTGCTACT-3'	Chr 9	CM334 ×Chilsungcho

Recently Mallard et al[21] used INRA pepper maps (Yolo Wonder × CM334, H3 × Vania and Perennial × Yolo Wonder) and identified a major QTL *Pc 5.1* on chromosome 5 explaining 55 to 70% of resistance. Further they have performed meta analyses of the independent QTL studies done so far for the *Phytophthora capsici* resistance in peppers and identified a three major meta QTLs viz. *Meta Pc5.1*, *Meta Pc5.2* and *Meta Pc5.3* all on the chromosome 5. *Meta Pc5.1* which is a key QTL showing broad spectrum resistance (as it was retrived from four resistant accessions CM334, Vania, Perennial and AC2258) included the *Pc 5.1* QTL reported by Mallard et al[21], three QTLs were detected by Troung et al[22], *Phyt-1*QTL was detected by Sugita et al[23] and the QTL was detected by Minamiyama et al[19]. *Meta Pc5.2* included *Pc5.2* reported by Mallard et al[21], Phyto-U was detected by Ogundiwin et al[14] and QTL between CDI78 to CDI25 (for which SNAP was developed). The *Meta Pc 5.3* included *Pc5.3*[21], three QTLs were detected by Truong et al.[22]

Root-knot nematodes: Root-knot nematodes are polyphagous soil-living pests that exist in areas with hot climates or short winters which are uncontrollable.[24] This pathogen is major problem in protected cultivation of crops. They belong to the genus *Meloidogyne*. More than 70 known species of *Meloidogyne* were known and four of them (*M. javanica*, *M. arenaria*, *M. incognita*, *M. hapla*) are major pests worldwide.[25] Among these species, *M. incognita* race 2 is the most common root-knot nematode found in India. The disruption of water transport and diversion of nutrients to the nematode caused stunted growth and chlorosis, thereby poorer yield or death of plants.

Several resistant sources have been identified in *Capsicum spp.*[26] In India, several germplasms have been screened, S-343, Surjamukhi, Punjab Tej, Japani Longi, Sel 15, JCA-288, Sel 6, Perennial, C00226 were reported to be resistant to nematodes, efforts being under way to combine nematode and leaf curl virus resistance at Punjab Agricultural University.[27] Of all these, PI322719, PI201234 and CM 334 are the highly stable resistant sources across the different *spp* of *Meloidogyne.* Different genetic resistant studies have shown that resistance is dominant in nature. Nine independent resistance genes (*N, Me1, Me2, Me3, Me4, Me5, Me7, Mech1 and Mech2*) were reported.[28] Some genes such as *Me4, Me2, Mech1 and Mech2* are specific to certain *Meloidogyne spp.* or populations, however *Me1, Me3, Me7* are effective against a wide range of *Meloidoyne spp.*, including *M. arenaria*, *M. javanica* and *M. incognita*.[29]

Comparative mapping indicated that the six resistance genes (*Me1, Me3, Me4, Me7, Mech1* and *Mech2*) are clustered in a 28 cM interval on chromosome P9 and *Me* genes linked markers (SCAR, SSCP and CAPs) were developed for

marker assisted selection.[29] A SCAR marker linked to *N* gene was developed[30] and further studies showed that this N gene colocalized in the *Me* gene cluster on chr 9 was identified by Djian-Caporalino et al[29] and allelic to *Me7* gene[31], thus indicating the use of this *N* gene linked SCAR marker in marker assisted selection for *Me* genes.

Breeding strategy for combining resistance: Recent study shows that the publications on reporting the development of molecular markers and QTL studies for different traits in crops have increased enormously during past two decades showing that the research community is spending a lot of time and money in only generating information with little impact or not much focus on their extent of application in plant breeding.[32] This observation is very much relevant in the commercial crop chilli as evidenced by the above review of literature which focused mainly on identification of resistant sources, markers and QTLs for soil borne diseases that have not been translated into product so far.

The review shows that resistance in the 'Anugraha' for bacterial wilt and in 'CM334' for *Phytophthora* root rot and root knot nematodes is stable and these can be effectively used as donors in breeding programme. The genetic molecular study done so far showed that the bacterial wilt resistance is recessive in nature in 'Anugraha' and AFLP marker associated with it is available[8] (table 1). For root-knot nematode, the resistant dominant *Me* genes are linked in a cluster on chromosome 9 with and molecular markers linked to these genes are reported using resistance derived from 'CM334'[29] (table 1). The complex polygenic resistance for *Phytophthora* root rot resistance also has been simplified through identification of *Meta QTLs* (Key QTLs) and linked markers to these QTL regions are available[21] (table 2). The available literature can be translated into product form through marker assisted breeding which will have more practical importance. The pyramided lines with these soil-borne diseases resistance can also be exploited as common root stock for cultivation of different commercial varieties/hybrids of high value commercial bell pepper varieties/hybrids for protected cultivation, which solves the problem of introgression of these genes/QTLs into different genetic backgrounds.

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