Research Paper
Impact Factor - **2.262**
Peer Reviewed Journal

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

RESISTANCE IN CHILLI (*CAPSICUM ANNUUM* L.) TO YELLOW MITE (*POLYPHAGOTARSONEMUS LATUS* BANKS) LINKED THROUGH SSR MARKERS

Dr.Girish. R*   Dr. Srinivasa. N   Dr. Mohan Rao. A****

**Asst. Professor, Krishi Vigyan Kendra, Mudigere, Chikkamagaluru.*
***Asso. Professors, Department of Agricultural Entomology, UAS, GKVK, Bangalore.*

Abstract
Murda complex is considered as one of the major limiting factor in chilli production infested by mites in Karnataka. So it is essential to select genotypes with the desired resistant allele combination using DNA markers. Twenty SSR primers were used to screen the mite resistant and sensitive parents. Six chilli entries namely S 49 and Aparna (highly resistant), BVC 47 (resistant), BVC 53 and Capsicum frutescens (moderately resistant) and Byadgi kaddi (highly susceptible) were screened for primary linkage map based on 20 SSR primers. None of these primers showed polymorphism to designate them for mite resistance; however five primers namely Hpms E 140, Hpms E 142, Hpms E 143, Hpms E 144 and Hpms E 148 sparsely differentiated two or more alleles in few of the entries.

Introduction: Pungent peppers commonly known as chilli in India, belong to the species *Capsicum annuum* L. (2n=24) which is the most widely cultivated species in the world. It is one of the important condiment crops grown on a large scale in India especially in the South. Capsaicin, an alkaloid responsible for pungency in chillies has medicinal properties and it prevents heart attack by dilating the blood vessels (Gill, 1988). The description of genus capsicum has been disputeuted and partly questionable these days. Capsicum plants are ancestors of bell peppers and other domesticated peppers today. There are currently about 27-30 known and verified wild capsicum species. The three domesticated *Capsicum annuum* are *Capsicum annuum* var *annuum, Capsicum chinensis, Capsicum frutescens*.

Chilli crop has many insect and non-insect pests, yellow mite *Polyphagotarsonemus latus* (Banks) is most destructive (Butani, 1976) causing nearly 25 per cent loss in yield (Ahmed *et al*., 1987). The yield loss due to this pest may be more than 90 per cent (Borah, 1987) and at times complete devastation of the crop might occur. *P. latus,* a member of the family Tarsonemidae commonly known as yellow mite, broad mite *etc*. is an important mite pest of chilli with wide host range (Gibson and Valencia, 1978).

The genetic analysis in chilli was studied in the past using only morpho physiological traits such as plant height, harvest index, capsaicin content and phenols content. Such traits are limited in number and are often influenced by the environment, thus making them unsuitable for correct assessment of this analysis. This limitation can largely be overcome by the use of molecular tools, which are unlimited in number and are not influenced by the environment. Therefore in recent years, considerable emphasis has been placed on the development and use of molecular marker in all major crops.(Liu *et al*, 1996; Hirata *et al*, 2006). PCR based markers have been successfully developed such as RFLPs, RAPDs and SSRs (Bradshaw *et al*, 1994; Jung *et al*, 1999). These markers have been successfully exploited in many crops including chilli for genetic diversity analysis as well as genotyping. Among these markers of choice due to several desirable attributes including their abundance, multiallelic and co dominant nature, cross species transferability etc. (Gupta and varshney, 2000). Therefore SSR markers are useful for a variety of application in plant genetics, genetic linkage mapping, plant breeding and marker aided selection thereby speeding up the breeding process. The aim of this study was to evaluate SSR markers for their link to the mite resistance trait for marker aided selection through the use of large segregating population.

Material Methods

Plant materials: For mapping, plant samples from F2 plants which was reported to be most tolerant to the mite attack and most sensitive.

Phenotypic data: A mite tolerability index based on the intensity of mite infestation was developed by the acarology lab in Department of Entomology, UAS, Bangalore. Scoring of all F2 plants between 0 (Mite free) to 4 (entire plant curled) for their resistance to the natural mite attack.

DNA extraction protocol (CTAB method)

Genomic DNA was extracted from one month old transplanted chilli plants as suggested in the CTAB method with minor modifications. The following was the protocol used for extracting DNA from leaf samples.Extraction buffer (1M Tris HCl, 0.5M EDTA, 5M NaCl, 2% CTAB) proportionate to quantify 200 mg of the lyophilized ground tissue in a sterile centrifuge tube was added and contents were transferred to 2ml centrifuge tubes.The extract was incubated at 60^0C in a water bath for

45 minutes with intermittent shaking. Equal volume of 24:1 chloroform-isoamyl alcohol was added to the extract after cooling to room temperature and mixed vigorously for 5 to 10 minutes until a homogenous suspension was obtained. The suspension was centrifuged at 12000rpm for 5 to 7 minutes. The supernatant was transferred to fresh 2ml centrifuge tube and then equal volume of 24:1 chloroform-isoamyl alcohol was added and mixed thoroughly. The suspension was centrifuged at 12000rpm for 5 minutes. The supernatant was transferred into fresh 2ml centrifuge tube and equal volume of cold isopropanol was added and then incubated for 1 hour or overnight at -20^{0}C. The suspension was centrifuged at 5000 rpm for 5 minutes and supernatant was discarded by retaining the pellet. The pellet was washed with 70% alcohol and centrifuged at 5000 rpm for 5 minutes. The supernatant was discarded; 50-100µl of TE buffer was added to dissolve the pellet. The quality and quantity of DNA was assessed using 0.8% agarose gel.

PCR amplification: PCR was carried out with 20 SSR primers in a total reaction volume of 15µl mixture containing 20ng of template DNA, 1unit of Taq polymerase, 1.5µl of reaction buffer, 0.2µl $MgCl_2$ (50mM), 2.5µl dNTP (1mM), 1µl of species specific primer1 (10pmol) and 1µl of primer2 (10pmol).

Amplification conditions were:

Initial denaturation:	94^{0}C for 3 minutes	
	94^{0}C for 30 seconds	35 cycles
	55^{0}C for 30 seconds	Annealing temperature 55^{0}C
	72^{0}C for 1 minute	
Final extension:	72^{0}C for 10 minutes	

Visualization of PCR products: Visualized on 2% agarose gel

Agarose gel electrophoresis: 2g agarose was added to 100ml of 1X TAE buffer and mixture was heated in a microwave oven until the agarose completely dissolved with care to avoid over boiling. The agarose solution was cooled to 40^{0}C and 5µl of ethidium bromide stock (10mg/ml) was added and mixed thoroughly. The solution was then poured on gel casting tray with combs. Five µl of gel loading dye was mixed with 5 µl of PCR product and loaded onto the gel. 100bp ladder was loaded along side. Electrophoresis was run at 100V, 50mA for 45 to 90 minutes. The gel was visualized under UV transilluminator and captured in a gel documentation system (BioRad).

SSR primers used for polymorphism - Hpms E 072, Hpms E 074, Hpms E 078, Hpms E 081, Hpms E 083, Hpms E 084, Hpms E 090, Hpms E 100, Hpms E 101, Hpms E 122, Hpms E 125, Hpms E 135, Hpms E 136, Hpms E 137, Hpms E 140, Hpms E 142, Hpms E 143, Hpms E 144, Hpms E 148, Hpms E 058.

Scoring and Data Analysis

the amplified PCR products were scored for further analysis. During scoring, only the intense and clearly resolved amplification products that were reproducible in multiple runs were considered for linkage analysis. Polymorphisms were scored for the presence and absence of bands on polacrylamide gels. Using 1kb + ladder the product sizes were found to vary from 100 to 300bps. The bands resembled the mite tolerant parental DNA, they were designed as homozygous, similarly when the bands resembled mite sensitive parental DNA, they were designed as heterozygous. When the bands showed similarity with both the parental DNA, they were designed as heterozygous. When no bands were obtained after PCR amplification with specific SSR primer from plant DNA, they were scored null.

Results

Genetic variation in chilli entries evidenced by Simple Sequence Repeat (SSR) primers for parental polymorphism. Chilli entries Aparna, S 49, BVC 47, BVC 53, *Capsicum frutescens* and *Byadgi kaddi* were screened with 20 SSR primers to identify for polymorphic primer combinations, if any (Table 1, Plate 1).

Out of 32 short listed chilli entries based on the population density of yellow mite they encouraged six genotypes were selected and analysed for parental polymorphism. These six genotypes were Aparna and S 49 (<5 mites/6 leaves); BVC 47 (<10 mites/6 leaves); BVC 53 and *Capsicum frutescens* (< 20 mites/ 6 leaves) and *Byadgi kaddi* (>50 mites/6 leaves). Twenty SSR primers were used against these six chilli genotypes from resistant to susceptible reactions to ascertain the polymorphism. In all the six entries studied, out of 20 SSR polymorphic primers 15 primers showed only one allele with no distinct variation in their genomic region. However, five primers namely Hpms E 140, Hpms E 142, Hpms E 143, Hpms E 144 and Hpms E 148 sparsely differentiated two or more alleles in few of the entries (Plate 1), while primer Hpms E 58 did

not align with any of these entries. Thus few more primers along with those primers which differentiated two or more alleles in the present study may be utilized for further genotypic studies to identify the polymorphism, if any.

The primers, showed few polymorphic bands which might be due to the heterozygosity in the plant material used. Ghosh *et al*. (2010) who screened 88 SSR markers in jute for yellow mite damage observed only 2 markers, HK 64 and J-170 to show 100% selection efficiency. By increasing the number of markers, a marker dense saturated linkage map of chilli genome may be constructed, which would help to identify the location of genes responsible for yellow mite resistance trait in chilli crop.

Not much is known about the plant defense responses to small arthropods that pierce single plant cells and feed on intracellular fluids, such as broad mites (Grinberg et al, 2005). One of the most significant advances to occur in the last decade for the development of improved crops is the use of molecular markers to identify and track genes of interest (Tanksley and mccouch, 1997). Many disease resistance gene linked to SSR markers have already been identified in different plants such as southern corn rust (Liu et al, 2003). Mite resistance is typically monogenic (Herron & rophail, 1993), although some cases of polygenic resistance have been reported. Polygenic resistance is not associated with a typical mechanism of resistance but only refers to the number of genes involved in resistance (Lindhout, 2002).

Most of the pest and disease resistant traits are controlled by many genes (Schiff et al, 2001). They could be genetically complex quantitative traits, involving many genes and environmental factors. In our study the map presented here provides a good starting point for the production of the saturated linkage map of the chilli genome based on SSR markers. The linkage analysis in future help to isolate the mite resistant gene in chilli.

References

1. Ahmed, k., Hanumantha Rao,V.and Purnachandra rao., 2001, Resistance in chilli cultivars to yellow mite, *Polyphagotarsonemus latus* (Banks). *Indian J. Agric. Res.*, **35**(2): 95-99.
2. Borah, D. C., 1987, Bio-ecology of *Polyphagotarsonemus latus* (Banks) and *Scirtothrips dorsalis* (Hood) infesting chilli and their natural enemies. Ph. D. Thesis, University of Agricultural Sciences, Dharwad, p. 234.
3. Bradshaw, H. D., Vil#lar, M., Watson, B. D. and Stettler, R. F. 1994, Molecular genetics of growth and development in populous. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS and RAPD markers. Theoretical and Applied Genetics. 89: 167-178.
4. Gill, H. S., 1988, Improved technologies for chilli production. *Indian cocoa, arecanut and spice Journal*, **12**(4):118.
5. Gibson, R. W. and Valencia, L., 1978, A survey of potato species for resistance to the mite *Polyphagotarsonemus latus*, with reference to the protection of *Solanum berthaultii* and *S. tarjensii* by glandular hairs. *Potato Res*., **21:** 217-223.
6. Ghosh, A., Sharmin, S., Islam, S., Pahloan, M. U. and Khan, H., 2010, SSR markers linked to mite *Polyphagotarsonemus latus* (Banks) resistance in Jute (*Corchorus olitorius* L.). *Genet. Plant Breeding*. **46**(2): 64-74.
7. Grienberg, M., Perl-treves, R., Palevsky, E., Shomer, I. and Soroker, V. 2005, Interaction between cucumber plants and broad mite, Polyphagotarsonemus latus from damage to defense gene expression. *Entomologia experimentalis et applicata*, **115**:135-144
8. Hirata, M., Cai, H., Inoue, M., Yuyama, N., Miura, Y., Komatsu, T. and Fujimori, M. 2006, Development of simple sequence repeat markers and construction of an SSR based linkage map in Italian ryegrass (*Lolium multiforum* Lam.). *Theoretical and Applied Genetics*. **113**:270-279.
9. Jung, G., Skroch, P. W., Nienhuts, J and Marita, J. M. 1999, Confirmation of QTL associated with common bacterial blight resistance in four different genetic backgrounds in common bean. *Crop science*, **39**: 1448-1455.
10. Liu, Z. W., Biyashew, R. M. and Saghai-maroof, M. A. 1996, Development of simple sequence repeat DNA markers and their integration into a barley linkage map. *Theoretical and applied genetics*. **93**:869-876.
11. Schiff, C. L., Wilson I. W. and Somerville, S. C. 2001, Polygenic powdery mildew disease resistance in Arabidopsis thaliana: quantitative trait analysis of the accession warschau-1. *Plant pathology*, **50**:690-701.
12. Tankskley, S. D. and Mccouh, S.R. 1997, Seed bank and molecular maps: unlocking genetic potential from the wild. *Science*, **277**: 10.63-1066.

Hpms E 072 Hpms E 074 Hpms E 078

Hpms E 081 Hpms E 083 Hpms E 084

Hpms E 090 Hpms E 100 Hpms E 101

Plate 1. Amplified PCR product of chilli entries with SSR primers

L – 100bp DNA ladder; 1 – Aparna ; 2- S 49; 3- BVC 47; 4-BVC – 53; 5 – Capsicum frutescens; 6 – Byadgi kaddi

Table 1.Reaction of chilli entries to yellow mite, *Polyphagotarsonemus latus* (Banks)

Sl. No.	Entries	Mean No. of mites/6 leaves			Mean damage score	Percentage of plants infested by mites
		Eggs	Active stages	Total		
1	Aparna	0.40	0.80	0.90	0.00	5.56
2	S 49	0.50	0.60	1.10	0.00	0.00
3	BVC 47	4.50	2.60	6.80	0.40	11.11
4	BVC 53	10.40	6.60	16.50	0.80	61.11
5	*Capsicum frutescens*	11.20	6.80	18.00	1.60	19.44
6	CA 2	17.10	10.40	27.50	1.00	83.33
7	KDC 2	18.40	13.30	27.50	1.20	72.22
8	CA 9	17.80	12.20	28.70	1.20	69.44
9	PMR 21	20.80	11.00	30.30	1.20	77.78
10	KNG 2	20.30	13.50	30.50	1.40	88.89
11	KDC 1	18.10	12.50	30.60	1.40	88.89
12	G 4	22.90	13.60	30.90	1.60	63.89
13	PBC 631	24.60	12.10	31.00	1.80	69.44
14	CMS 1B	20.90	12.20	31.30	2.40	91.67
15	LCA 273	22.60	12.30	31.40	1.60	66.67
16	PBC 142	20.70	13.90	32.90	2.40	58.33
17	CA 960	25.20	12.60	33.10	1.80	75.00
18	CMS 7B	21.10	15.70	34.00	1.60	69.44
19	*Phule jyothi*	25.20	12.90	34.60	1.80	63.89
20	LCA 334	21.70	14.50	34.70	1.40	91.67
21	KNG1	23.80	14.80	35.20	3.00	77.78
22	Vamsi	26.90	13.20	35.50	2.00	61.11
23	LCA 336	24.30	14.10	35.80	2.20	88.89
24	CA10	23.90	16.00	36.90	2.20	86.11
25	Koira	25.90	14.60	37.50	1.40	80.56
26	AVNPC 131	26.20	15.20	38.00	1.40	86.11
27	CMS 5B	25.10	21.00	38.10	1.40	94.44
28	ICPN 14	22.50	15.60	38.20	2.00	88.89
29	*Pusa sadabahar*	29.20	14.60	40.30	2.80	38.89
30	*Punjab gucchedhar*	33.60	15.00	46.10	1.90	91.67
31	*Oothgod local*	38.50	21.10	59.60	2.40	91.67
32	*Bydagi kaddi*	65.20	91.60	87.20	2.90	94.44
F test		*	*	*	*	-
SEM±		7.37	14.99	9.76	0.13	-
CD at P=0.05		16.06	32.65	21.27	0.28	-

**** significant at 5% probability***