TMC MM I.2 :	Development of cotton varieties resistant to cotton leaf curl disease (ClCuD), bacterial leaf blight (BLB) and Nematodes through marker assisted breeding.

Lead Centre :	CICR, Nagpur
Cooperating centres	CICR, Coimbatore; CICR, Sirsa; CCSHAU Hisar; PAU, Ludhiana; PAU, Abhohar/Faridkot; SKRAU, Sriganganagar;

Introduction

Cotton is one of the most important fibre crops, mainly grown for its spinnable fibre. It is grown commercially in the temperate and tropical regions of 80 different countries, including the United States, India, China, Central and South America, the Middle East, and Australia (Fryxell, 1979). The genus Gossypium consists of 50 species, of which four are cultivated for their spinnable fibre and the remaining 46 are wild species distributed throughout the tropics and subtropics. Of the four cultivated species, two are diploids (2n = 26 chromosomes) viz. *G. arboreum* and *G. herbaceum* and two tetraploids (2n = 52 chromosomes) viz. *G. hirsutum* and *G. barbadense*. Tetraploid cotton, *Gossypium hirsutum* is the most widely cultivated (90%) among all Gossypium species.

In India, cotton is an important commercial crop cultivated on an area of about 121.78 lakh ha, with total production of 353 lakh bales of seed cotton and productivity of 493kg lint during 2011-12 (Anonymous 2012). India occupies top position with regard to area under cotton and ranks second in production (after China). However, productivity remains at very low among the cotton producing countries.

Stagnation in cotton yields has been a common phenomenon in most of the cotton producing countries. Steady decline in cotton yields largely account for increased incidence of pest and diseases -resulting into huge losses, built up of resistance in insects against common pesticides and narrowing of genetic variation in cultivated varieties. In fact, losses caused due to certain pests and diseases are stupendous. The cumulative losses on account of pests and diseases could be placed at more than 50%. Bt cotton could protect the cotton crop from American bollworm *Heliothis armigera* and prevent the losses. The losses caused due to diseases are alarming, however estimation of losses due to diseases were down played over the losses due to insect pests. Correct estimates of losses due to specific diseases in different parts of the country are not available. Among the

Infestation of Bacterial Leaf Blight

cotton diseases, grey mildew (*Ramularia areola*), alternaria blight (*Alternaria macrospora*) and bacterial blight (*Xanthomonas axonopodis pv. malvacearum*) cause the yield losses up to 30 per cent (Chidambaram and Kannan, 1989), 26 per cent (Chattannavar et al., 2006), 30 per cent (Ramapandu et al., 1979), respectively. These are the important diseases which appear almost every year and have seriously threatened cotton production in certain areas and reduce the yield significantly.

Bacterial blight caused by *Xanthomonas axonopodis pv. malvacearum* occurs in all cotton growing areas throughout the world and is one of the most devastating diseases of cotton causing yield losses from 5–35% (Dellanoy et al., 2005). Yield losses of 10 to 30% are not unusual in Africa or Asia (Thaxton and El-Zik, 2001, Ramapandu et al., 1979). In Australia, resistance to bacterial blight is a mandate for all commercial cotton varieties (Xiao et al., 2010).

Cotton leaf curl virus disease (CLCuD), caused by monopartite Gemini virus (Tahir et al. 2011) transmitted by white fly (Bemisia tabaci), is a major problem in Northern cotton growing region comprising states of Haryana, Punjab and Rajasthan. Since the appearance of disease in 1993, it has created havoc in North India. Estimates showed the losses to the extent of more than 50% in hot spots. In Bt cotton hybrids as well, yield losses were reported to the tune of more than 50% in three blocks i.e. Khuwan server, Fazilka and Abohar in Firozepur district of Punjab during 2009-10 and other hot spots in North Zone during 2009-10 & 2010-11(Anonymous 2010- 11).

Plant-parasitic nematodes have been identified in every state where cotton is grown. The major species are root-knot, reniform, lance, and sting nematodes. The Reniform nematode (*R. reniformis*) has been recorded to be the key nematode species on cotton in Central and Southern India while Root knot nematode (*M.incognita*) is important in Northern cotton growing areas. In India, cotton crop losses due to Reniform nematode (*R. reniformis*) has been put at 14.7%. The nematode also causes delay in boll maturity, reduction in boll size and lint quantity and also cause an increase in Wilt disease development in wilt susceptible varieties. Work done at CICR, Nagpur indicated avoidable yield losses to the extent of 8 to 10% while at CICR, RS, Coimbatore, it was showed to the extent of 9.5 to 17.4% in control plots as against the nematicide treated plots. Reduction in cotton yield due to *R. reniformis* has been estimated at 5.6% in USA. Cotton yield losses worldwide due to this pest have been estimated to be 10.7% (Sasser and Freckman, 1987).

Infestation of Root Knot Nematode

India possesses large pool of cotton genetic resources. The emphasis to explore germplasm resources with potential natural genetic resistance and application of innovative genomic tools to efficiently mobilize these useful genetic variations to breeding germplasm would facilitate faster

and desired cotton improvement.

Egg mass of Nematode

In this context, application of molecular markers in crop improvement has gained more significance. In last two decades, extensive genomic resources have been developed and the molecular marker technology has successfully been used to assess genetic diversity of germplasm resources, create genetic linkage maps and map gene(s) for desired agronomic traits (Chen et al 2007, Zhang et al 2008). Marker assisted breeding strategies could be effectively adopted in cotton as the molecular markers linked to specific trait(s) such as bacterial leaf blight and nematode resistance has already been identified. This project proposal emphasizes identification of closely linked molecular markers for CLCuD, BLB and Nematode resistance through diversified approaches; validation of inhouse identified markers and already reported (SSR and SNP haplotype) markers and its application for accelerated development of disease resistant varieties in cotton.

Cotton leaf curl virus disease (CLCuD) caused by monopartite Gemini virus is very dynamic. The reports indicates breakdown of resistance in known resistant sources (Bhatoa et al. 2009). The virus consists of a single stranded circular DNA genome and two satellite molecules, alpha and beta (Tahir et al. 2011). The beta satellite DNA molecule of the virus is found to be associated with evolution and divergence of virulent virus strains. Evidence for recombination was found for AC1 and CP ORFs and for the noncoding intergenic region (IR). The origin of replication is found to be a major recombination point in the IR. Analysis of IR suggested frequent recombination that might have major role in generation of CLCuV variability. Sanz et al. (2000) suggested that recombination occurred at the origin of replication was crucial for the emergence and evolutionary divergence, as a group, from begomoviruses causing similar diseases in other geographical regions.

1. The development of new recombinant strains leading to breakdown of resistance in resistant varieties and losses due to this disease in case of Bt cotton hybrids calls for identification of new sources of resistance.

2. The identification of molecular markers linked to leaf curl virus (CLCuV) disease resistance in cotton has potential to improve both the efficiency and the efficacy of selection in cotton breeding programs and can lead to varieties with durable resistance.

Bacterial blight caused by *Xanthomonas axonopodis pv. malvacearum* occurs in all cotton growing areas and is one of the most devastating diseases of cotton causing yield losses from 5–35% (Dellanoy et al., 2005). Yield losses of 10 to 30% are not unusual in Africa or Asia (Thaxton and El-Zik, 2001). Nineteen Xanthomonas races causing this disease have been identified of which race 18 is the most virulent and present in almost all production areas in the world (Brown, 2001). There are atleast 22 reported resistant genes in cotton that confer

Infestation of CLCuV

differing degrees of resistance to various Xcm races carrying different avirulence genes, of which gene B_{12} confers a high level of resistance to all Xcm races.

1. Earlier, the source of resistance conferring resistance to most of the virulent races was not available. Now, excellent sources of resistance for BLB have been obtained. Two exotic bacterial blight immune lines of *G. hirsutum* cotton IM216 and S295 have been identified as potential sources of resistance against bacterial blight disease (Chakrabarty, unpublished). Besides, 101-102 B highly resistant *G. hirsutum* cotton has also been identified.

2. The above identified lines are being utilized for identification of molecular marker and tagging BLBR genes. Four F_2 mapping populations have already been developed and mapping for the BLB resistant genes mapping have already been initiated at CICR, Nagpur.

3. In addition, recent published reports have indicated markers associated with BLB resistant gene B_{12}, which shall be verified with our mapping population and validated to be used for development of resistant varieties.

In India, plant-parasitic nematodes have not been considered as major pests though losses caused due to RKN and RN in major cotton growing states are well beyond 10%. The Reniform nematode (*R. reniformis*) has been recognized as the key nematode species on cotton in Central and Southern India while Root knot nematode (*M.incognita*) in Northern cotton-growing areas. The studies on nematode resistance indicate that resistant varieties could be developed using the identified resistant genotypes. Closely linked markers associated with resistant genes have also been identified that may be exploited to precisely incorporate resistance to elite varieties in India.

Objectives

1. Identification of molecular markers for BLB, CLCuD and nematode (Reniform and Root-knot nematodes) resistance.
2. Validation of identified markers using mapping populations and known genetic stocks.
3. Identification of markers for background selection.
4. MAB to transfer the resistance trait into elite varieties (minimum two generations per year) using markers.
5. Confirmation of resistant trait in the homozygous lines.

Technical programme

S.No	Activities	Cooperating Centres
1	Identification of genetically diverse parental lines for CLCuD and nematode (Reniform and Root-knot nematodes) resistance/ suceptiblity.	CICR, Nagpur; CICR, Sirsa; CICR, Coimbatore; CCSHAU, Hisar PAU, Abhohar/ Faridkot; SKRAU, Sriganganagar
2	Development of mapping population for CLCuD and Nematode (Reniform and Root-knot nematodes)	CICR, Nagpur; CICR, Sirsa; HAU, Hisar; PAU, (Ludhiana/Abhohar/Faridkot) SKRAU, Sriganganagar
3	Extensive phenotyping of the mapping population under epiphytotic condition.	CICR, Nagpur; CICR, Sirsa; CICR, Coimbatore; HAU, Hisar PAU, (Ludhiana/Abhohar/Faridkot) SKRAU, Sriganganagar
4	Survey for parental polymorphism using SSR and SNPs markers (also genotyping by sequencing) and identification of informative markers.	CICR, Nagpur; CICR, Sirsa; PAU, Ludhiana
5	Genotyping of mapping population with informative markers.	CICR, Nagpur; CICR, Sirsa PAU, Ludhiana; CICR, Coimbatore
6	Data analysis and identification of markers associated with BLB, CLCuD and nematode (Reniform and Root-knot nematodes) resistance.	CICR, Nagpur; CICR,Sirsa; PAU, Ludhiana
7	Validation of identified markers using different mapping populations and known genetic stocks.	CICR, Nagpur; CICR,Sirsa; PAU, Ludhiana
8	Identification of markers for background selection.	CICR, Nagpur
9	Effecting crosses of donor parent (resistant) with the identified recurrent elite varieties (generation of F1 & Bc1)	CICR, Nagpur; CICR, Sirsa; HAU, Hisar; PAU, Ludhiana
10	Marker assisted selection for resistant trait using foreground and background selection (minimum two generations per year).	CICR, Nagpur; CICR, Sirsa; HAU, Hisar; PAU, Ludhiana
11	Confirmation of resistant trait in Bc_2S_1/ Bc_3S_1, the homozygous individuals and seed multiplication for trials.	CICR, Nagpur; CICR, Sirsa; HAU, Hisar; PAU, Ludhiana

Time frame

Sr. No.	Activity	Time frame											
		2012		2013		2014		2015		2016		2017	
		I	II	I	II	I	II	I	II	I	II	I	II
1.	Identification of genetically diverse parental lines for CLCuD and nematode (Reniform and Root-knot nematodes) resistance/ suceptiblity.												
2	Development of mapping population for CLCuD and Nematode (Reniform and Root-knot nematodes)												
3	Extensive phenotyping of the mapping population under epiphytotic condition.												
4	Survey for parental polymorphism using SSR and SNPs markers (also genotyping by sequencing) and identification of informative markers.												
5	Genotyping of mapping population with informative markers.												
6	Data analysis and identification of markers associated with BLB, CLCuD and nematode (Reniform and Root-knot nematodes) resistance.												
7	Validation of identified markers using different mapping populations and known genetic stocks.												
8	Identification of markers for background selection.												
9	Effecting crosses of donor parent (resistant) with the identified recurrent elite varieties (generation of F1 & Bc1)												
10	Marker assisted selection for resistant trait using foreground and background selection (minimum two generations per year).												
11	Confirmation of resistant trait in Bc2S1/ Bc3 S1, the homozygous individuals and seed multiplication for trials.												

Output

- Identification of genetically diverse resistant & susceptible genotypes.
- Development of F2 mapping populations for CLCuD & Nematodes
- Phenotyping of the mapping populations
- Identification of informative markers for genotyping of mapping population
- Genotyping with informative markers.
- Identification of markers associated with BLB, CLCuD and nematode (Reniform and Root-knot nematodes) resistance.
- Validation of markers for trait association.
- Identification of markers for background selection
- Generation of F1 & Bc1 populations
- Transfer of resistant trait in elite cultivar
- Identification of homozygous individuals for desired trait.