Breeding for Chilli Leaf Curl Disease (ChiLVD) and South East Asian Thrips (*Thrips parvispinus*) resistance in Chilli

Name of the institute conducting research: ICAR-Indian Institute of Horticultural Research, Bangalore

Objectives of the project:

Sub project-I: Breeding for Chilli Leaf Curl Disease (ChiLVD) resistance in Chilli

1. Survey & identification of major begomoviruses and their recombinants prevalent in major chilli growing areas
2. Screening pooled germplasm at selected hot spots to identify stable sources of resistance against major begomoviruses identified
3. Development of RxS segregating populations to identify resistance loci against major begomoviruses
4. Identification of QTLs, Meta-QTLs and candidate genes associated with resistance loci and development & validation of SNP based markers
5. Pyramiding of resistance loci through MAS

Sub project-II: Breeding for South East Asian Thrips (*Thrips parvispinus*) resistance in Chilli

1. Screening chilli accessions for thrips resistance
2. Metabolite profiling of selected chilli accessions
3. Identification of rQTLs (resistant QTLs) and mQTLs (metabolite QTLs) in chilli conferring resistance against thrips
4. Incorporation of resistance loci into desirable background through MAS

Deliverables: Breeding for Chilli Leaf Curl Disease (ChiLVD) resistance in Chilli

1. Stable resistant sources for multiple begamoviruses will be identified
2. Better understanding of Genotype x Pathogen x Environment on resistance of ChLCD
3. QTLs associated with multiple begamoviruses will be identified
4. Meta QTLs (if any) conferring resistance against multiple begamoviruses
5. SNP marker assays associated with multiple begamoviruses resistance
6. Advanced stable resistant lines (with pooled resistant loci) will be developed

Deliverables: Breeding for South East Asian Thrips (*Thrips parvispinus*) resistance in Chilli

1. Resistant accessions against south east Asian thrips
2. Understanding of mechanism of south east Asian thrips resistance
3. Genetic mechanism of south east Asian thrips resistance
4. QTLs conferring resistance and associated markers for MAS
5. Advanced breeding lines with thrips resistance

Duration of the Project: 3 years

Total costs for 3-years:

210,000 USD. The participating companies will share equal funds to the project (for example if 10 APSA member companies join this project, each company will pay 21,000 USD over 3 years (USD 7000/year approximately) to participate in the project.

The participating companies shall be Indian companies or multinational companies having subsidiary in India to get utmost benefits of the project since the germplasm cannot be shipped outside of India.

Background of the research and methodology

1. Breeding for Chilli Leaf Curl Disease (ChiLVD) resistance

India is the largest producer of dry chilli with an annual estimated 1.2 MT followed by China with 0.25 MT (FAOSTAT 2020).Chilli contributes 31% among the total spice exports from India with an economic share of Rs. 8429.92 crores (Spice Board, 2021). In the present climate scenario, whitefly transmitted begomoviruses (family *Geminiviridae*) have recently become the most devastating threat to chilli production in India (Rishi et al. 2004; Kumar et al. 2015, Yadav et al., 2022). Distinct begomoviruses are associated with Chilli leaf curl disease (ChiLCD) in different locations. In India, major begomoviruses causing ChLCD are *Chilli leaf curl virus* (ChLCV), *Chilli leaf curl Ahmedabad virus*, *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Joydebpur virus* (ToLCJV) etc.Being single stranded DNA virus there exists high possibility of genetic recombination too. ChiLCD is a highly destructive plant disease that severely retards plant growth and development, producing symptoms, such as chlorosis and curling and reduction of leaf and fruit size, eventually rendering the fruit unmarketable. The destruction potential of the disease could be realized from the fact that chilli cultivation has been withdrawn by farmers in India due to nearly 100% yield loss (Kumar et al. 2015). Disease severity is manifested due to various reasons, such as climate change (erratic dry spells, relative humidity and temperature) affecting whitefly (vector) population, faulty agricultural practices and inoculum load in primary and secondary hosts present in the surrounding areas. Host plant resistance is an attractive disease management strategy against viruses because it involves in disease control, and thus is beneficial for the environment and human health. Precise molecular markers tightly associated with traits of interest

will accelerate breeding programs to develop resistant breeding lines/ F_1 hybrids to farmers for sustainable production. With this background the present sub project is aimed at mapping of chilli leaf curl disease resistant loci and development of associated SNP markers for marker assisted selection. Simultaneously resistant loci from different sources will be pooled to develop more stable resistant lines.

Proposed Methods/Activities

This project is proposed for three years.

Objective 1: Survey & identification of major begomoviruses and their recombinants prevalent in major chilli growing areas

Activity 1.1. Survey and collection of disease infected samples from major chilli growing areas both from susceptible & known resistant F1 hybrids

Continuous monitoring and sample collection

Activity 1.2. Whole Genome Sequencing (WGS) of begomoviruses affecting chilli in major chilli growing areas

Molecular characterization of chilli begomoviruses affecting known resistant F_1 hybrids grown at varied locations in India (sequencing of both DNA - A Component&Beta Satellite component).

Activity 1.3. Designing of virus specific primers

Objective 2: Screening pooled germplasm at selected hot spots to identify stable sources of resistance against major beomoviruses identified

Activity 2.1. Pooling & screening of germplasm in the location specific hot spots for chilli leaf curl disease incidence

Large set of germplasm of *Capsicum* sp. (both cultivated & domesticated species) will be screened at major chilli leaf curl disease hot spot locations (Guntur, Sanawad and Karnal) to identify field tolerant accessions. Data on Chilli leaf curl incidence will be recorded. Accessions showing field resistance will be selected for further screening through artificial challenge inoculation

Activity 2.2 Screening selected accessions through artificial challenge inoculation

Different virulent major begomoviruses (collected during activity 1) will be maintained on chilli plants in an insect proof net house separately. Selected accessions (from activity 1) will be challenge inoculated through white fly mediated mass inoculation as per procedure standardized at IIHR (Yadav et al., 2022). The plants exhibiting no visual symptomswill be further confirmed by grafting on susceptible root stocks and also through PCR using virus

specific primers to confirm and identify symptomless carriers. The best resistant sources against various major begomoviruses will be identified.

Objective 3: Development of RxS segregating populations to identify resistance loci against major begomoviruses

Activity 3.1. Development of mapping populations using RxS crosses

Identified resistant sources (top 4 best resistant sources) will be crossed with common highly susceptible accession to develop F1 hybrids and further F2 populations will be developed. Individual F2 plants will be selfed further to produce F2:3families.

Objective 4. Identification of QTLs, Meta-QTLs and candidate genes associated with resistant loci and development & validation of SNP markers

Activity 4.1: Phenotyping of segregating populations for begamoviruses resistance

Phenotyping F2 (200 no.) derived F3families (30 plants for each family) (developed in Activity 2.1) will be screened against begomovirusesthrough whitefly mediated mass inoculation method (at multi locations)

Activity 4.2: Identification of QTLs, Meta-QTLs and candidate genes through GBS approach

- Genomic DNA will be extracted from Individual F2 plants of different sets and Genotyping by Sequencing will be done through standard protocols (Elshire et al., 2011) and SNPs will be identified
- The Genotypic data of SNPs will be used to construct linkage map and QTLs associated with begamoviruseswill be identified using QTL IciMapping software (Lei Meng et al., 2015).
- Meta QTL analysis will be carried out using the QTLs identified in different studied populations across different begomovirusesto identify stable Meta QTLs conferring to broad spectrum resistance against begamoviruses

Activity 4.3: Development and validation of SNP based markers

- SNP markers assays (HRM assays/CAPs/dCAPs) will be developed for the candidate SNPs associated with QTL regions for begamoviruses resistance.
- Developed markers will be validated initially in F2 populations (DNA samples used for GBS) and later will be extended to larger set of F2 population, other developed F2 populations and advanced breeding populations

Objective 5: Pyramiding of resistance loci through MAS

Simultaneously, R x R crosses will be made and intercross populations will be developed and will be forwarded through single seed decent method to F4 families. Plants in F4 families will be genotyped with developed SNP markers (from objective 4) and forwarding F4 families having multiple virus resistance loci in homozygous condition will be carried out. Selected advanced breeding lines will be evaluated at multi locations (for major begomoviruses).

Timeline of activities

Activity	Year I	Year II	Year III
Objective 1: **Survey & identification of major begomoviruses and their recombinants prevalent in major chilli growing areas**			
Survey and collection of disease infected samples from major chilli growing areas both from susceptible & known resistant F1 hybrids	✓	✓	✓
Whole Genome Sequencing (WGS) of begomoviruses affecting chilli in major chilli growing areas	✓		
Designing of virus specific primers	✓		
Objective 2: **Screening pooled germplasm at selected hot spots to identify stable sources of resistance against major beomoviruses identified**			
Pooling & screening of germplasm in the location specific hot spots for chilli leaf curl disease incidence	✓		
Screening selected accessions through artificial challenge inoculation	✓		
Objective 3. **Development of RxS segregating populations to identify resistance loci against major begomoviruses**			
Development of mapping populations using RxS crosses	✓	✓	
Objective 4. **Identification of QTLs, Meta-QTLs and candidate genes associated with resistant loci and development & validation of SNP markers**			
Phenotyping of segregating populations for begamoviruses resistance		✓	
Identification of QTLs, Meta-QTLs and candidate genes through GBS approach		✓	✓
Development and validation of SNP based markers			✓
Objective 5. **Pyramiding of resistance loci through MAS**			
Developing intercross F1 hybrids (RxR combinations)	✓		
Advancing of Intercross F2 to F4 families through SSD	✓	✓	
Marker assisted selection of F4 families with homozygous resistant loci		✓	✓
Multilocation screening of advanced inbred lines		✓	✓

Work flow

Screening large set of germplasm at multiple locations (major chilli growing areas)

↓

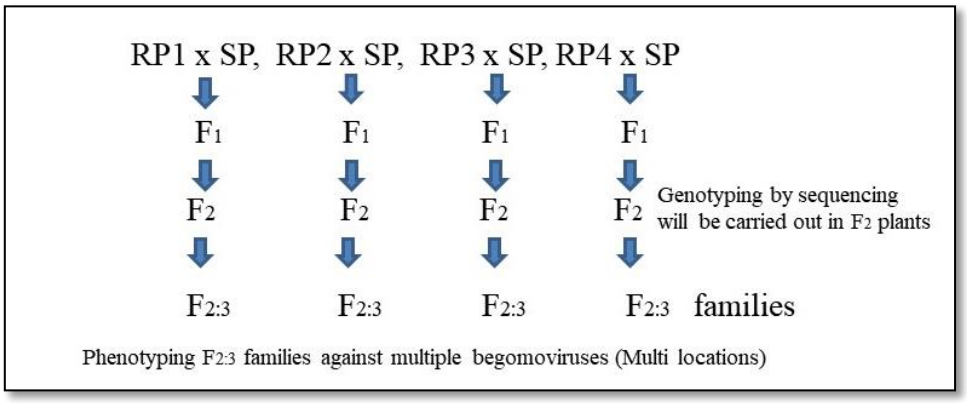
Screening selected field resistant accessions through Artificial challenge inoculation and confirmation of resistance through graft inoculation and PCR based assays

↓

Selection of best stable resistance sources

↓

Development of segregating populations (F2 and F2:3) with different resistant lines and common susceptible parent for mapping population & RxR derived populations for pyramiding *R loci (as mentioned in Fig.1)*



SNPs identification through GBS in F2 populations

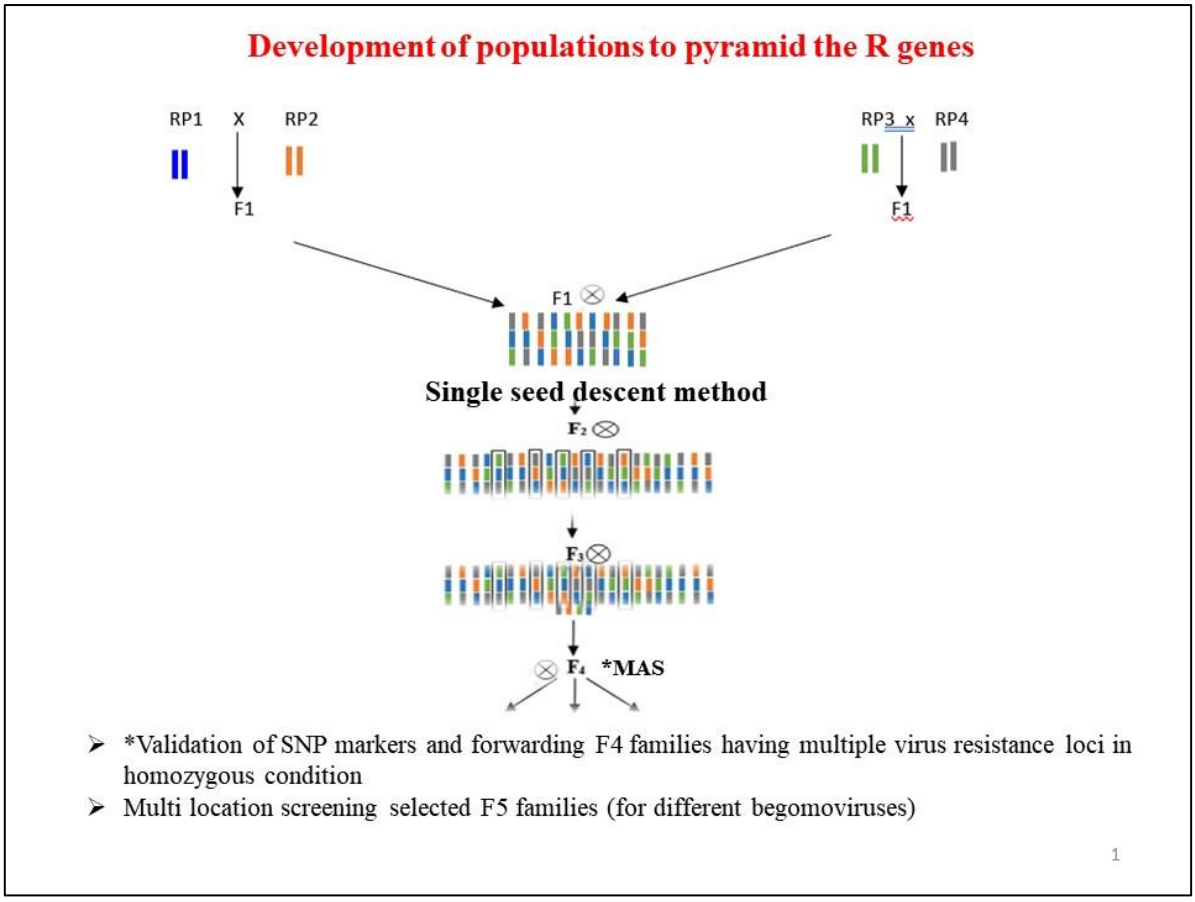
↓

Identification of QTLs, Meta-QTLs and candidate genes associated with multiple begomoviruses

↓

Development of SNP based markers and validation in various populations

Work plan of pooling resistant loci from different resistant sources will be taken up simultaneously



References

Elshire, RJ, J. C. Glaubitz , Q. Sun, J. A. Poland, K. Kawamoto, E. S Buckler et al. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One* 6(5):e19379

Kumar RV, Singh AK, Singh AK, Yadav T, Basu S, Kushwaha N (2015) Complexity of begomo virus and beta satellite populations associated with chilli leaf curl disease in India. J Gen Virol96:3143-3158.

Lei, Meng., Huihui, Li., Luyan, Zhang., Jiankang, Wang. "QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations." Crop Journal, 3 (2015).:269-283.

Rishi N (2004) Current status of begomoviruses in the Indian subcontinent. Indian Phytopathol57:396-407.

Yadav RK, Krishna Reddy M, Ashwathappa KV, Manish Kumar, Naresh Ponnam and Madhavi Reddy K (2022)Screening of *Capsicum* germplasm and inheritance of resistance to chilli leaf curl virus. *Indian Phytopathology* **75**, 1129–1136.

2. Breeding for South East Asian thrips resistance

Recently, *Thrips parvispinus* (Karny), which is regarded as an invasive has caused colossal loss to chilli growers in Andhra Pradesh, Telangana and Karnataka (Prasannakumar*et al*., 2021). Tyagi *et al.* (2015) reported the first instance of *T. Parvispinus* in India on papaya from Bangalore. Later it was reported on many alternate hosts such as beans, eggplant, papaya, pepper, potatoes, shallots, strawberries, coriander, cotton (Prasannakumar*et al.*, 2021; Sridhar *et al*., 2021). Thrips severely damages the plant by rasping and sucking the sap from blooms and foliage resulting in crinkling, curling and drying of the plant (Kalshoven 1981). *T. parvispinus* can cause 70–100% damage in the Southern Indian states *viz*., Andhra Pradesh, Karnataka and Telangana, which are known for cultivating chilli widely (Sridhar *et al*., 2021and Prasannakumar*et al*., 2021). The first instant of causing significant loss on chilli was recorded in Chilakaluripeta and Prattipadu mandals of Andhra Pradesh in January 2021 (Loday*aet al*., 2021). Subsequently, spread of this invasive thrips was also identified in all the chilli growing districts of Telangana and Karnataka states (Anitha*et al*.,2021and Loday*aet al*., 2021). Recently, farmersincurred huge crop losses (>6000 crores in Telangana state alone) due to outbreak of *T. parvispinus*. Upon survey, it was confirmed for the first time *T. parvispinus,* an invasive to India responsible for causing huge yield loses. Farmers tried all the management options available with them including the expert's suggestions but no satisfactory results observed. In general, thrips are not easy to control, therefore, farmers spray insecticides indiscriminately but due to their polyphagous nature, high reproductive rate, concealed behavior of feeding (hiding inside the flowers) and facultative parthenogenetic mode of reproduction all management efforts go in vain (Brodsgaard, 1989; Jensen, 2000; Herron and James, 2005). Hence, heavy crop losses on chilli were observed by the chilli growers during 2021(Prasannakumar*et al*., 2021). After thorough surveys, it was also opinioned that there was no resistance source for *T. parvispinus*. Success of any management programme also depends on host plant resistance which plays a crucial role in imparting resistance to the pests. Considering the present status of *T.parvispinus* at national and global level,the sub project was aimed at identification of resistant sources and mapping genomic regions associated with resistance and develop SNP based marker assays

Objectives:

6. Screening chilli accessions for thrips resistance
7. Metabolite profiling of selected chilli accessions
8. Identification ofrQTLs (resistant QTLs) and mQTLs (metabolite QTLs) in chilli conferring resistance against thrips.
9. Incorporation of resistance loci into desirable background through MAS

Proposed Methods/Activities

This project will take place over a 3 year period

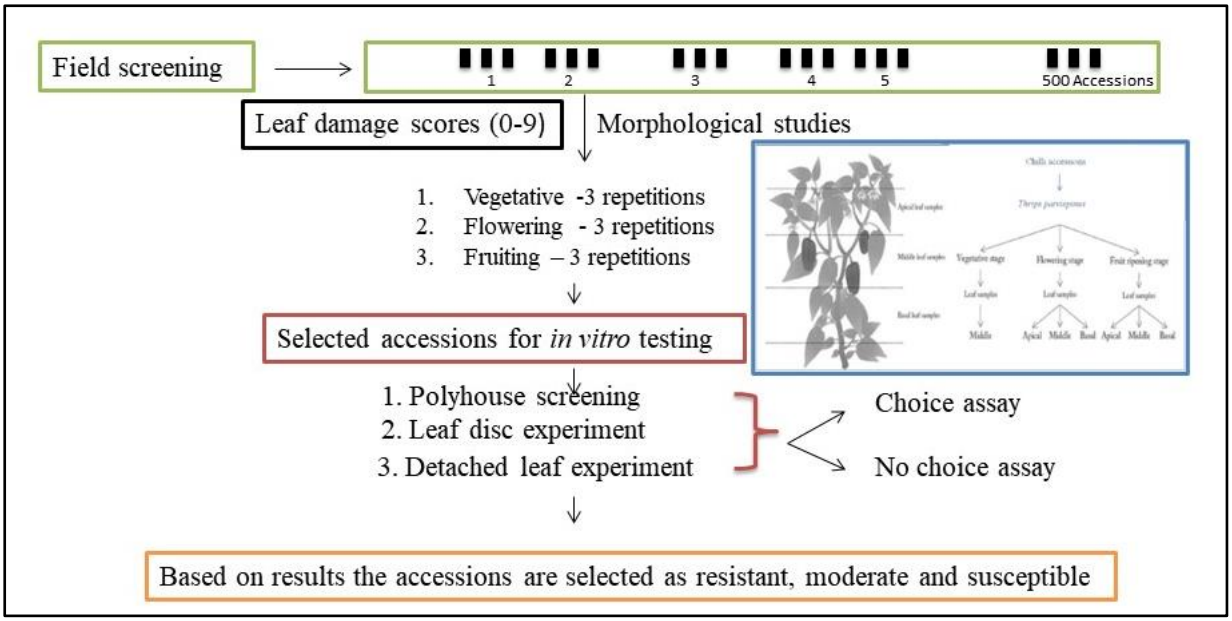
Objective 1: **Screening chilli accessions for thrips resistance**

Activity 1.1: Screening germplasm for thrips resistance under field conditions

About 500 accessions at different ontogenetic stages will be screened to check the level of resistance against thrips. Field screening of accessions will be done with the aid of damaging symptoms and scoring the damage from 0 to 5 (0 being most resistant and 5 being most susceptible). Data on Trichome type, Trichome density , Leaf area index, Leaf cuticle thickness (µm),Leaf color, Leaf angle (°), Flower color and Fruit malformation will be recorded.

Activity 1.2: Screening of selected accessions through challenge inoculation

A prominent resistance lines will be selected for artificial challenge inoculation in polyhouse, *in vitro* screening using leaf disc method and detached leaf method. Determination of feeding damagewillbe based on area fed to characterize different levels of resistance as per Maharijaya (2013).



Schematic diagram showing the work flow of identification of resistant accessions against SE Asian thrips

Objective 2. Metabolite profiling of selected chilli accessions

The selected accessions based on the screening data (from objective 1) will be used for metabolite profiling. Gas chromatography – Mass spectrometry and Liquid chromatography – Mass spectrometry will be used to identify the metabolites related to resistance. The following metabolites will be quantified as per the standard procedure in selected accessions

Sugar profiling - LC-MS(Azeez *et al.*, 2016)
Phenolic acids - LC-MS(Azeez *et al.*, 2016)
Flavonoids - LC-MS(Azeez *et al.*, 2016)
Epicuticular wax profiling - GC-MS(Bisht *et al*., 2014)
Glandular trichome component profiling - GC-MS (Bisht *et al*., 2014)
GC- MS profiling of leaf volatile aroma (Bisht *et al*., 2014)

Secondary metabolite data and level of resistance data will be correlated and candidate metabolites associated with thrips resistance will be identified.

***Objective 3.* Identification of rQTLs (resistant QTLs) and mQTLs (metabolite QTLs) in chilli conferring resistance against thrips.**

Activity 3.1 Development and phenotyping of RxS segregating populations

The selected resistant and susceptible accessions will be used for the development of 250 F_2 mapping population.

Activity 3.2 Identification of QTLs and mQTLs conferring resistance

Bulks of F_2 population will be genotyped with parental polymorphic SSR markers to identify rQTLs (resistance Quantitative Trait Loci) through BSA QTLSeq approach. Similarly mQTLs (metabolite Quantitative Trait Loci) will be identified by using GC-MS and LC-MS data and molecular data.

Activity 3.3 Development and validation of SNP markers associated with QTLs

Validation of rQTLs and mQTLs linked molecular markers across the F_2segregating population.

Objective 4. Incorporation of resistance loci into desirable background through MAS

Activity 4.1: Development of backcross populations

Backcrossing of F1 (R x S (elite line)) with recurrent parent wo generations to produce BC2F1 populations (large set of population will be generated)

Activity 4.2: Advancing of BC2F1 to BC2F4 families through SSD

BC2F1 will be forwarded to BC2F4 families through single seed descent method

Activity 4.3: Validation of SNP markers and forwarding BC1F4 families with resistant loci in homozygous condition

BC2F4 families will be subjected to SNP marker assays and families with positive resistant loci will be selected and forwarded

Activity 4.4 : Screening of advanced lines for thrips resistance at multi locations

Selected advanced breeding lines will be screened for thrips resistance, yield and fruit quality traits at multi locations

Timeline of activities

Activity	**Year I**	**Year II**	**Year III**
Objective 1: **Screening chilli accessions for thrips resistance**			
Screening germplasm for thrips resistance under field conditions	✓		
Screening of selected accessions through challenge inoculation	✓		
Objective 2. Metabolite profiling of selected chilli accessions			
Profiling metabolites in resistance and susceptible accessions	✓		
Identification of candidate meatbolites associated with resistance	✓		
Objective 3. **Identification of rQTLs (resistant QTLs) and mQTLs (metabolite QTLs) in chilli conferring resistance against thrips.**			
Development and phenotyping of RxS segregating populations	✓	✓	
Identification of QTLs and mQTLs conferring resistance		✓	
Development and validation of SNP markers associated with QTLs		✓	✓
Objective 4. Incorporation of resistance loci into desirable background through MAS			
Development of backcross F1 populations	✓		
Advancing of BC1F1 to BC1F4 families through SSD	✓		✓
Validation of SNP markers and forwarding BC1F4 families with resistant loci in homozygous condition		✓	✓
Screening of advanced lines for thrips resistance at multi locations			✓

References

Anitha K.D., Bhasker, K and Suresh, V. 2021. A new invasive chilli thrips (*Thrips parvispinus*) in Telangana State. *Insect Environment*. 24(4): 520-522.

Azeez, S., Karunakaran, G., Tripathi, P.C., Shivashankara, K.S and Roy, T.K. 2016. Evaluation of antioxidant activity, total phenolics and phytochemical content of selected varieties of karonda fruits (*Carissa carandas*). *Indian Journal of Agricultural Sciences*. 86(6): 815-822.

Bisht, H., Bhagat, D and Bhatnagar, M.K. 2014. Metabolic profiling of tomatoes with pest infestation using GC-MS and NMR spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 6(3): 550-556.

Brødsgaard, H.F., 1989. Coloured sticky traps for Frankliniella occidentalis (Pergande)(Thysanoptera, Thripidae) in glasshouses. *Journal of applied entomology*. *107*(1-5): 136-140.

Herron, G.A. and James, T.M., 2005. Monitoring insecticide resistance in Australian Frankliniella occidentalis Pergande (Thysanoptera: Thripidae) detects fipronil and spinosad resistance. *Australian Journal of Entomology*. *44*(3): 299-303.

Jensen, S.E., 2000. Mechanisms associated with methiocarb resistance in Frankliniella occidentalis (Thysanoptera: Thripidae). *Journal of economic entomology*. *93*(2): 464-471.

Kalshoven, L.G.E. 1981. Pests of crops in Indonesia. *Jakarta: IchtiarBaru*. 1-5

Lodaya, J.P., Suthar, M., Patel, H.C., Sisodiya, D.B., Acharya, R.R., Raval, A.T and Mohapatra, A.R. 2022. Status of invasive species of thrips, *Thrips parvispinus* (Karny) infesting chilli grown in middle Gujarat. *Omega*. 5: 6.

Maharijaya, A., Vosman, B., Steenhuis, B.G., Harpenas, A., Purwito, A., Visser, R.G and Voorrips, R.E. 2011. Screening of pepper accessions for resistance against two thrips species (*Frankliniella occidentalis* and *Thrips parvispinus*). *Euphytica*. 177(3): 401-410.

Prasannakumar, N.R., Venkataravanappa, V., Rachana, R.R., Sridhar, V., Govindappa, M.R., Basavarajappa, M.P., Hemalatha, K.J., Aswathnarayana, D.S., Reddy, M.K and Samuel, D.K., 2021. Status of the outbreak of *Thrips parvispinus* (Karny) on chilli in Karnataka. *Pest Management in Horticultural Ecosystems*. 27(2): 286-290.

Sridhar, V., Rachana, N.R., Prasannakumar, V., Venkataravanappa, K., Sireesha, D., Anitha Kumari, K., Madhavireddy, Onkara S., Naik, P., Sree, C and Krishnareddy, M. 2021. "Dominance of invasive species, *Thrips parvispinus* (Karny) over the existing chilli thrips, *Scirtothrips dorsalis*. H on chilli in the southern states of India with a note on its host range: A likely case of species displacement. *Pest Management in Horticultural Ecosystems*. 27(2): 132-136.