APPROACHES FOR IMPROVEMENT OF RESISTANCE TO POWDERY MILDEW IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Powdery mildew disease caused by *Golovinomyces cichoracearum* (DC) V.P. Heluta var. *Cichoracearum* has become a serious problem in sunflower cultivation in India since the last decade. Initially, the disease was confined to *rabi* crop (October-March) at flowering and post-flowering stages but in the recent past, the pathogen attack is witnessed during all seasons and all stages of crop growth necessitating resistance deployment strategies. A screening method and scoring scale were developed for reliable identification of genotypes resistant to the disease. Screening of germplasm, breeding lines and wild *Helianthus* species resulted in identification of two interspecific derivatives namely HIR-1734-2 (EC-633077) and RES-834-3 (EC-633089), two exotic lines namely PI 642072 (EC-595333) and USDA-25 (EC-537925) and ten *Helianthus* species namely *H. argophyllus, H. agrestis, H. debilis, H. praecox, H. angustifolius, H. atrorubens, H. rigidus, H. salicifolius, H. pauciflorus* and *H. resinosus* tolerant to the disease. Based on consistent reaction in different accessions and across seasons, four accessions namely RES-834-3, PI 642072, *H. debilis* and *H. praecox* along with the highly susceptible line PS 2023 were studied extensively for host-pathogen interactions, biochemical profiling of defense related enzymes and transcript profiling in control and post-infected samples which indicated different mechanisms of tolerance. Development of mapping populations (RILs, BC_1F_1) involving the resistant donors are in various stages towards mapping of genes conferring resistance to powdery mildew in sunflower.

Keywords: Sunflower, powdery mildew, *Golovinomyces cichoracearum*, differential transcripts, host-pathogen relationships

INTRODUCTION

In India, powdery mildew disease was sporadically observed before 2006, but during the year 2006-07 it was reported in high intensity (80%) on *rabi* crop (October-March) in some areas around Bengaluru and Raichur which increased over the years (Anonymous, 2007). Polycyclic nature and short life cycle of the pathogen under conditions of high humidity resulted in rapid spread of the disease to all the sunflower growing states (South, Central and North India) and seasons (rainy, spring and summer) in India (Sujatha et al., 2015). The disease begins during the post-flowering stage as minute discoloured specks on leaves from which powdery mass radiates on all the sides. All the aerial parts of the host are covered with white powdery mass containing mycelia and conidia of the fungus. At present, the disease is seen regularly in all sunflower growing areas of the country in moderate to severe form. A field experiment on yield loss assessment of powdery mildew in sunflower was conducted and the results revealed that, at 30% and 64% of disease severity levels the seed yields were reduced by 20.5% and 52.6%, respectively (Anonymous, 2014) necessitating research for development of appropriate

management strategies. Yield reduction is mainly due to the reduced photosynthetic activity, physiological changes and increased rate of senescence.

SCREENING AND IDENTIFICATION OF RELIABLE SOURCES OF RESISTANCE TO POWDERY MILDEW

It is reported that three genera namely *Golovinomyces cichoracearum* f.sp. *helianthi* (syn *Erysiphe cichoracearum* DC ex Meret; *Oidium asteris punicei* Peck), *Leveillula taurica* (=*Leveillula compositarum*) and *Podosphaera xanthii* Castagne Braun & Shishkoff (=*Sphaerotheca fuliginea* auct p.p.) are the causative agents of powdery mildew in sunflower; of which, *G. cichoracearum* is of the most common occurrence in all the continents (Saliman et al., 1982; Gulya et al., 1991; Chen et al., 2008). Classical identification methods based on microscopical analysis and spore trapping are labour intensive and require considerable experience in differentiating the morphologies of the powdery mildews (Grote et al., 2002). Hence, morphological characteristics supported by molecular analysis of the powdery mildew isolates collected from different geographical locations in India using the powdery mildew specific ITS universal primer pair (Bardin et al., 1999) and also primers that are specific to the ITS regions of *P. xanthii*, *G. cichoracearum* and *L. taurica* indicated that disease infection is caused by *G. cichoracearum* (Prathap Reddy et al., 2013). Reliable sources of resistance are not available in the released cultivars and the parental lines of hybrids. Hence, wild *Helianthus* species, backcross inbred lines, interspecific derivatives, core germplasm set, inbred lines and few exotic accessions were screened under field conditions by simulating the conditions followed by rescreening under artificial inoculation conditions (Fig. 1). Sources of resistance were identified in five annual wild species namely *H. argophyllus, H. agrestis, H. debilis, H. niveus, H. praecox* and six perennials namely *H. angustifolius, H. atrorubens, H. rigidus, H. salcifolius*, *H. pauciflorus* and *H. resinosus*, two interspecific derivatives (HIR-1734-2/EC-633077, RES-834-3/ EC-633089) and two exotic lines accessions (PI 642072/EC-595333/TX16R, USDA-25/EC-537925). The species, *H. strumosus* was highly susceptible and harboured the pathogen throughout the year. Seven different methods described earlier (Karuna, 2010) were tested; of which, dusting of spores from infected leaves on to the healthy leaves of the test plants proved to be the most convenient and effective method of infection. Artificial screening showed low infestation of powdery mildew on ID-25 (RES-834-3) and other accessions (TX16R, EC-537925) with negligible conidial count (2500 conidia/cm^2) when compared to 1,30,000 conidia/cm^2 in the control (Prathap Reddy et al., 2013). Based on the differential response of the accessions derived from diverse genetic backgrounds, a 0-9 scale for obtaining reliable estimate of the disease has been devised based on the percentage of leaf area as well as the spread of the disease on the plant on different leaves (Prathap Reddy et al., 2013; Sujatha et al., 2015). Crosses were effected with the resistant and susceptible lines and plant-pathogen interaction studies in lines with contrasting reaction were done to understand the mechanism of resistance in different sources.

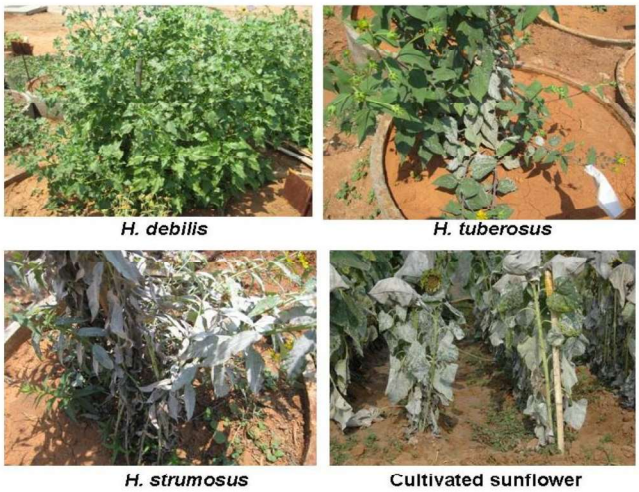

Fig. 1. Reaction of wild sunflowers to powdery mildew

HOST-PATHOGEN INTERACTIONS AND DIFFERENTIAL EXPRESSION OF GENES

The infection process of *G. cichoracearum* was studied in sunflower which included immune/resistant (*H. debilis, H. praecox*), tolerant (RES-834-3, TX16R) and susceptible (Morden, PS 2023A) genotypes both in controlled environment and field conditions. Inoculation was done by dusting the conidia on leaf blades of plants using camel hair brush. At 8, 12, 16, 20, 24, 36, 48, 72 and 96 hours following inoculation, leaves were sampled, cleared and stained. Powdery mildew infection in susceptible (2023B) line was within 8 hrs while spread and infection was slow in TX16R. There was no conidial germination and hyphal growth even after 4 days in *H. debilis* and *H. praecox.* Biochemical analysis of the accumulation of reactive oxygen intermediates (ROIs) such as superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) was done using ROI-specific dyes such as nitroblue tetrazolium (NBT) and 3,3-diaminobenzidine (DAB). This study provided a distinct accumulation pattern during host-pathogen interaction and it was observed that the level accumulation of ROIs was higher in resistant than susceptible genotypes. It is presumed that a higher inducible level of ROIs during infection in resistant lines is responsible for the arrest of the pathogen.

Following preliminary light microscopic and biochemical analysis, the host-pathogen interactions were studied by transcriptome profiling. Leaves of the resistant species- *H. debilis, H. praecox, H. niveus,* tolerant genotypes- TX16R, USDA-25 and the susceptible genotype - PS 2023B were dusted with the powdery mildew conidia from infected leaves of the susceptible accession (PS 2023B). Infected leaves were fixed at 0 (no infection), 24, 48 and 72 hours post infection (hpi) and subjected to transcriptome profiling. Libraries were prepared using TruSeq RNA library prep kit (Illumina) and were sequenced (PE-2x100) on HiSeq to obtain 80 million reads per sample. Following filtration of organelle genome and non-coding RNA sequences, the cleaned reads were aligned to the reference genome of *H. annuus* cv. Ha-412-HO with a gene model downloaded from Genomics of Sunflower database using Tophat2 tool. Results showed that in each of the donors, the mechanism of resistance varied as evident for the upregulation and downregulation of genes following infection. Maximum number of genes upregulated in response to the pathogen infection was observed in TX16R and *H. praecox* (Table 1).

Table 1: Total up and down regulated genes in transcript level [P value <= 0.01 and FPKM >= 1] found using Cuffdiff analysis

Samples	Up Regulated	Down Regulated
2023_B_Control vs 2023_B_Pool (24,48,72 hpi)	779	335
TX16R_Control vs TX16R_Pool (24,48,72 hpi)	4,464	211
ID25_Control vs ID25_Pool (24,48,72 hpi)	441	723
*H. niveus*_1452_Control vs *H. niveus*_1452_Pool (24,48,72 hpi)	909	263
*H. praecox*_1823_Control vs *H. praecox*_1823_Pool (24,48,72 hpi)	3,818	186
*H. debilis*_Control vs *H. debilis*_Pool (24,48,72 hpi)	308	468
2023_B_Control vs TX16R_Pool (24,48,72 hpi)	677	803
2023_B_Control vs ID25_Pool (24,48,72 hpi)	892	435
2023_B_Control vs *H. niveus*_1452_Pool (24,48,72 hpi)	1,252	797
2023_B_Control vs *H. praecox*_1823_Pool (24,48,72 hpi)	3,824	204
2023_B_Control vs *H. debilis*_Pool (24,48,72 hpi)	677	803

Analysis was done to check the genes which are commonly upregulated and downregulated in the susceptible versus resistant donors, among the resistant lines, and the tolerant lines, which are presented in Fig. 2 and 3, respectively. Only two genes were commonly upregulated in the susceptible and resistant genotypes while no genes were commonly downregulated between the two groups. The tolerant genotypes (TX16R and ID-25) had 14 and 19 genes in common that were upregulated and downregulated, respectively. Venn diagrams showed more common genes between *H. praecox* and *H. niveus* than those between *H. debilis* and *H. niveus.*

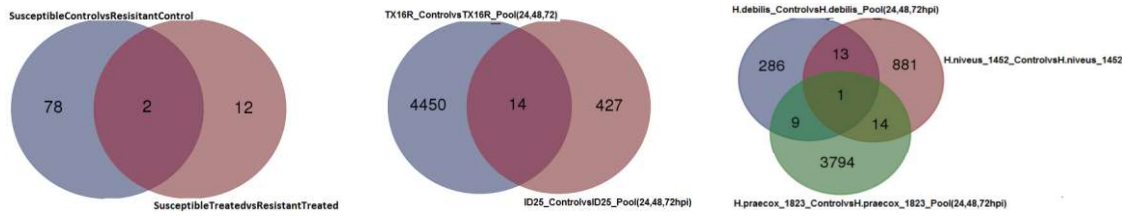

Fig. 2 Venn diagram showing commonly upregulated genes in different groups

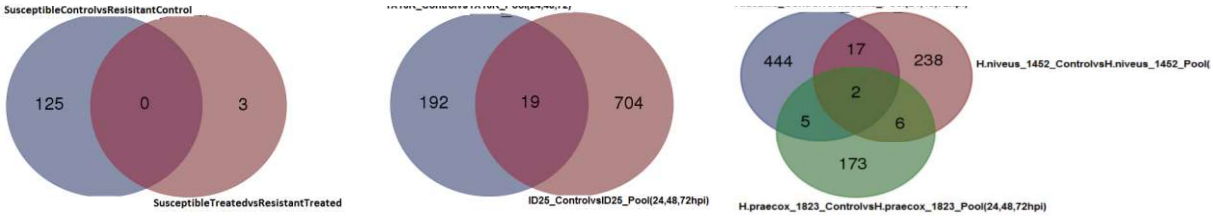

Fig. 3 Venn diagram showing commonly downregulated genes in different groups

Pathway enrichment was performed using Reactome database (Fig. 4). Pathway analysis indicated that the MAPK/MAPK6/MAPK4 signaling cascades are involved in *H. praecox;* Vesicle-mediated transport and membrane trafficking, regulation of HSF1-mediated heat shock response in *H. debilis,* mRNA splicing in TX16R, purine catabolism and detoxification of ROS in the susceptible genotype (PS 2023B).

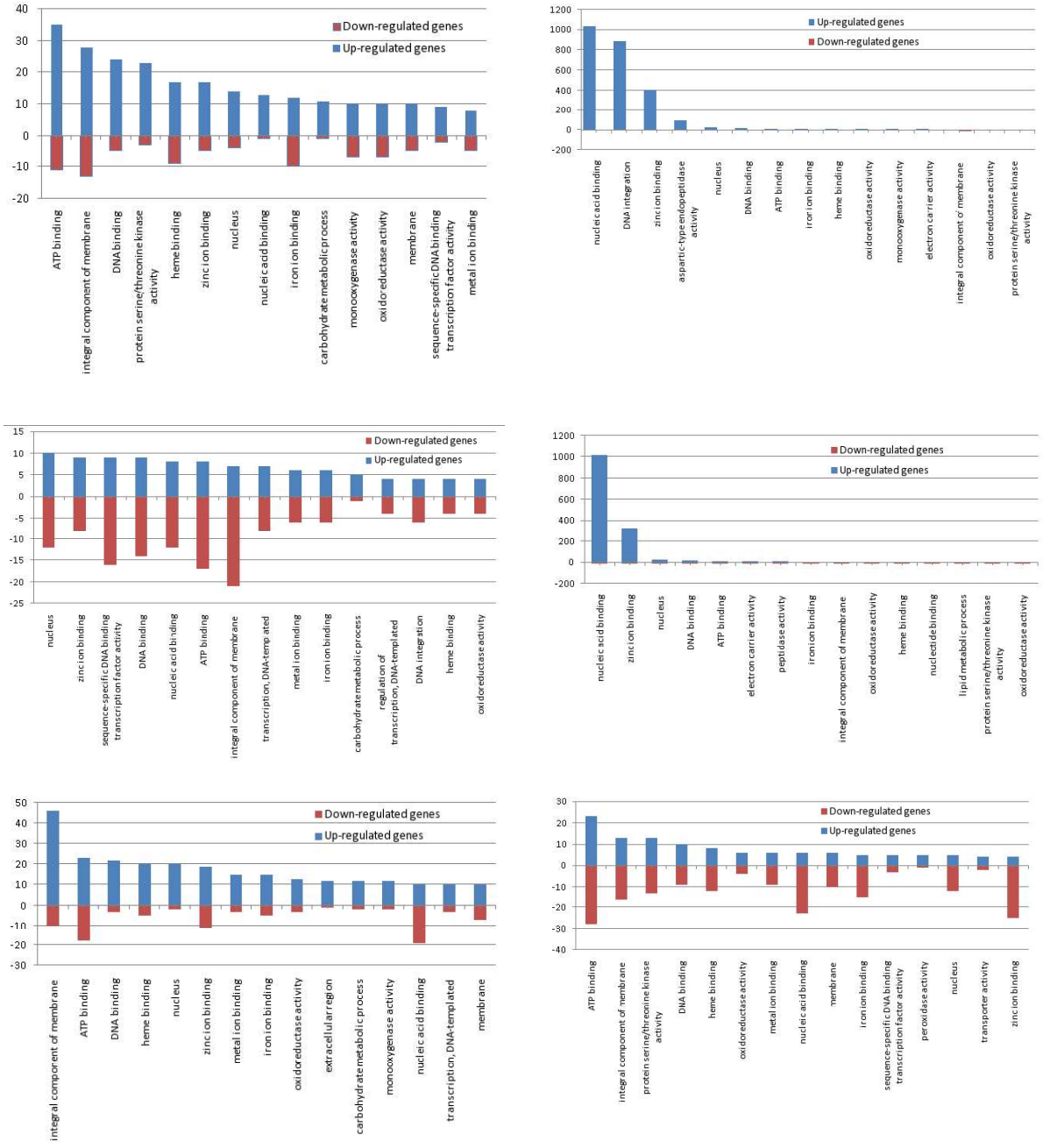

Fig. 4 Ontological analysis of differential expressed genes in PS 2023B, *H. praecox*, *H. debilis,* TX16R, *H. niveus* and ID-25 (in control vs infected)

The transcriptome data was explored for WRKY, Kinases and MAPK in the up and down regulated genes across all the pair-wise combinations. In ABSTRACT, there were 412 genes related to Kinases, 3 MAPK genes and 19 WRKY related genes from both up and down regulation. Work on validation of the key genes for their role in conferring resistance to powdery mildew in sunflower is underway.

TOWARDS MAPPING GENE(S) FOR RESISTANCE TO POWDERY MILDEW

Among the identified sources of resistance to powdery mildew, PI 642072 (TX16R) was selected as resistance source for mapping gene(s) that confer resistance to powdery mildew in sunflower. The F_1s were made by crossing PS 2023A (highly susceptible) and TX16R (resistant to powdery mildew) and further selfed to develop F_2 population. The F_1s showed resistance reaction to powdery mildew infection suggesting dominance nature. Variation for resistance to powdery mildew in F_2 population appeared to be quantitative (did not fit into Mendelian ratios) (Fig. 5) The F_2 population was advanced further by single seed descent method in order to develop recombinant inbred line (F_7-RIL) population for use in mapping of powdery mildew resistance in TX16R.

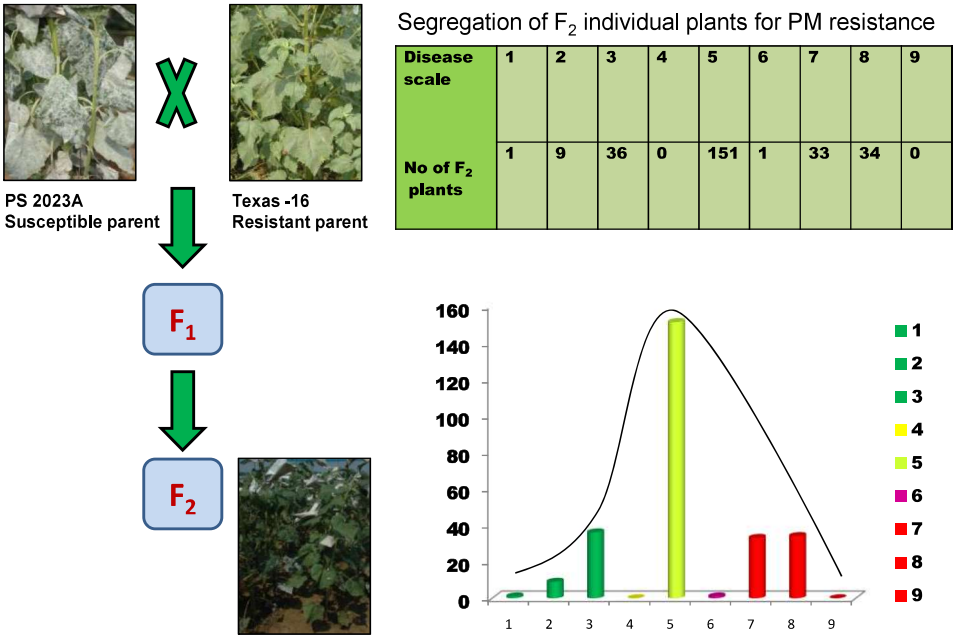

Fig. 5 Inheritance of resistance to powdery mildew in F_2 population produced from the cross: PS2033A x TX16R

Furthermore, interspecific crosses of cultivated sunflower with annual diploid species (*H. argophyllus, H. debilis* and *H. praecox*) were also made. The F_1s were confirmed for hybridity using SSR markers (ORS925, ORS505 and ORS898) and characterized for their reaction to powdery mildew. The F_1s involving *H. debilis* and *H. praecox* were highly resistant suggesting the dominance nature of resistance to powdery mildew in these sources. Development of backcross inbred line (BIL) populations is in progress towards mapping of powdery mildew resistance from wild sources. Till date, about 2100 sunflower specific SSR markers are available in public domain (Tang et al., 2002). The transcriptome data generated for the six genotypes has been mined for SSRs and SNPs and the additional markers would be used for trait mapping.

Thus, based on the importance and severity of the disease which is increasing over years and across seasons, the future line of research priorities would include determination of genetics

resistance to powdery mildew in different resistant donors including wild *Helianthus* species, introgression of resistance from the identified resistant donors into promising parental lines and molecular mapping of genes, which would enable marker-assisted selection (MAS) for resistance to powdery mildew in sunflower breeding.

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