S-VII-O2

ASSOCIATION MAPPING IN SUGARCANE FOR IMPORTANT AGRONOMIC TRAITS

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The highly complex and polyploid nature of sugarcane has made the understanding of its genetics very difficult and as a result molecular markers linked to traits of interest will have immense applicability in this crop. In the present study association mapping, an emerging alternative approach to linkage mapping was used to identify the marker trait association in sugarcane for three agronomically important traits viz. number of millable canes/clump, sucrose percent in juice and resistance to stalk borer. Expressed sequence tags (EST) derived simple sequence repeats (EST-SSRs) were used as they are located in the transcribed portion of the genome, which allows for a direct association between genes and important agronomic traits. A set of 124 diverse sugarcane genotypes were phenotyped for the three traits of interest and genotyped using EST-SSR primers which generated 663 markers. Result showed high level of genetic diversity. General linear model (GLM) using TASSEL identified three putative EST- SSR markers namely U-Ctg1278_240, U-Ctg740_140 and U-Ctg867_900, which were associated with sucrose percent in juice, number of millable canes/clump and resistance to stalk borer respectively. After validation, these markers can be used for marker assisted selection (MAS) in sugarcane for varietal development.

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IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES INDUCED DURING THE EARLY EVENTS OF SUGARCANE AND *COLLETOTRICHUM FALCATUM* INTERACTION

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Red rot, caused by the fungal pathogen *Colletotrichum falcatum* is one of the devastating diseases of sugarcane in India. The pathogen infects the economically valuable stalk tissues, reduces the juice quality, finally the entire cane gets rotten and many commercial varieties like Co 1148, Co 6304, CoC 671, CoS 64 etc were removed from cultivation. Till date, the disease is managed by planting commercially available resistant varieties (Viswanathan, 2010). Inspite of its devastating effects, the red rot resistant loci or the corresponding resistant gene for C. falcatum in sugarcane is not known due to the complex polyploidy of cultivated sugarcane. Because of its multiple parentages, the commercial sugarcane varieties contain the most complex genome carrying variable chromosome numbers 2n varying between 70 and 120 (Lu et al., 1994). Several attempts have been made to understand red rot resistance mechanisms in sugarcane. As a defense response to *C. falcatum*, differential induction of chitinases and thaumatin like proteins were recorded in sugarcane varieties

varying in red rot resistance (Viswanathan et al., 2005). The upregulation of potential defense relative transcripts like putative chitinase, glycine rich protein, 14-3-3 protein and xylanase inhibitor protein in sugarcane upon *C. falcatum* inoculation was clearly established by differential display (DD) RT-PCR in the earlier work done at our Institute (Viswanathan, 2010).

In continuation to understand red rot resistance mechanisms, suppression subtractive hybridization (SSH) has been used in this study to identify the early events during sugarcane and *C. falcatum* interaction. Initial signaling events play a pivotal role in alleviating pathogen stress and provide durable resistance in the plant. A well known resistant variety Co 93009 and a highly susceptible variety CoC 671 were used for construction of SSH cDNA libraries. Stalk tissues of both the varieties were challenged with C. falcatum and inoculated sugarcane stalks were harvested at 0, 12 and 36h after pathogen inoculation. Total RNA was isolated using TRI reagent and the quality was checked in 1.2% Etbr agarose gel. Messenger RNAs were purified from total RNA to a final concentration of 1µg/µl and used for libraries. SSH cDNA libraries were constructed using Clonetech PCR – Select cDNA subtraction kit according to manufacturer's instruction. Two forward subtracted libraries enriched for differentially expressed genes in response to *C. falcatum* were constructed in sugarcane stalk tissue samples collected at 12h and 36h after pathogen inoculation. The resistant Co 93009 was used as tester and susceptible CoC 671 was used as driver.

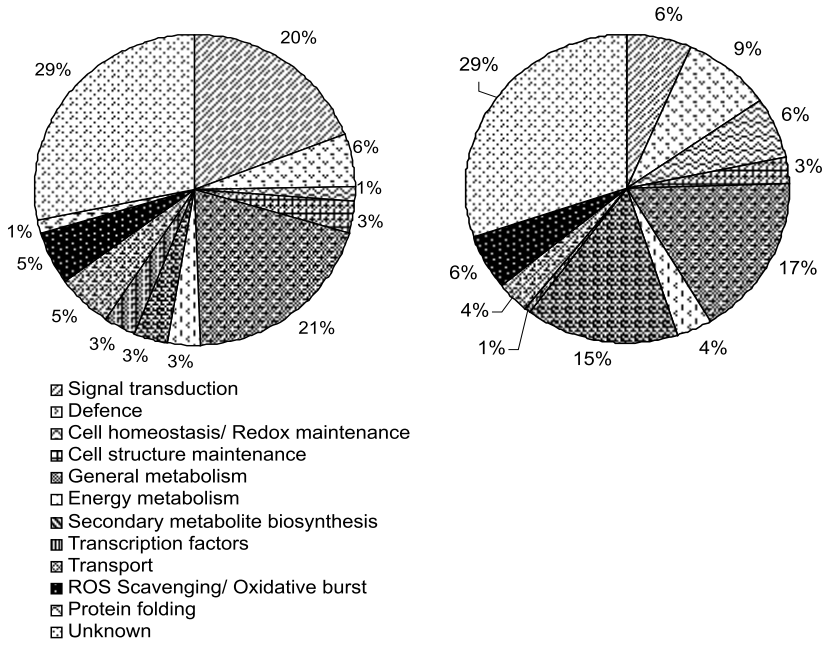

Fig .1. Functional classification of ESTs with significant protein homologies in NCBI and TIGR databases

At the end of subtraction and cloning, a total of 530 clones were sequenced from 5' end with M13 universal primer in a big dye sequencing terminator. The ESTs were blasted against NCBI and TIGR databases for homology search. The differentially expressed ESTs are classified into various functional categories based on homology search (Fig .1). A total of 317 annotatable ESTs were obtained from both the libraries, 146 corresponded to sugarcane response triggered at 12h after *C. falcatum* inoculation and 171 corresponded to sugarcane response triggered at 36h after *C. falcatum* inoculation.

This study identified key transcripts likely to be involved in red rot resistance in sugarcane. Signaling plays an important role in defense response. In both the libraries, several signal transduction genes like lipoxygenase 4, leucine rich repeat protein and oryzain γ chain precursor were commonly expressed. Mitogen activated protein (MAP) kinase 7 was expressed in 12h response library and MAP kinase 4 was expressed in 36h response library. There were 14 transcripts of lipoxygenase 4 in the 12h response library. Lipoxygenase is the key enzyme responsible for jasmonic acid biosynthesis. The high abundance of lipoxygenase 4 suggests the role for jasmonic acid signaling to trigger defense response in sugarcane against *C. falcatum.* Apart from this, calmodulin like protein, protein kinase domain containing protein, Ca binding EF hand protein and so on are specifically induced in 12h response library.

Defense response transcripts like HAD superfamily protein involved in N-acetyl glucosamine (NAG) catabolism and asparaginyl endopeptidase REP-2 were induced in 12h response library. NAG is the structural constituent of fungal cell walls. Rapid response to degrade the fungal cell wall is one of the primary defense responses. HAD superfamily protein might be involved in degrading the fungal cell walls during the initial entry of pathogen. β-1, 3 glucanase, a well known anti-fungal protein, chalcone synthase 8 and avr9/cf9 rapidly elicited protein were induced in 36h response library. As a consequence of defense response, reactive oxygen species (ROS) accumulate in the plant cells. To scavenge the deleterious effects of ROS in plant cells, the induction of L-ascorbate peroxidase 6 was evidenced in the 12h response and the induction of class III peroxidase was noticed in the 36h response. This suggests the importance of ROS scavenging right from the beginning of pathogen invasion. During the initial events of signal transduction and mounting defense response against the pathogen, the cell homeostasis has to be maintained in a balance. This is accomplished by proton exporting ATPase's induction in 12h response and 36h response induction include glutathione S- transferase, high affinity potassium transporter and major facilitator superfamily antiporter.

Secondary metabolism plays a vital role in plant cells as a defense response which synthesizes secondary metabolites which are lethal for pathogens. S-adenosyl methionine (SAM) synthetase, involved in polyamine biosynthesis was expressed in 12h response library and dehydroquinate dehydratase involved in phenyl propanoid pathway was induced in the other library. Dirigent protein which is widely known for conifer defense and which is involved in dictating stereochemistry of lignin biosynthesis was found to be expressed in both the libraries but the transcript level was 15 in 36 h response libraries. The expression of a dirigent protein in response to *C. falcatum* challenge in sugarcane is a novel outcome which interlinks the architecture and molecular evolution of sugarcane and conifers. Apart from these, many transcripts involved in general metabolism, energy metabolism, transport and several transcription factors were expressed in both the libraries. In conclusion, this study identified key transcripts, several of which are not reported in sugarcane resistance response. Functional validation and gene silencing studies of key enzymes/transcripts makes them a suitable candidate for transgenic approaches or breeding programmes. Our studies are in progress to functionally validate candidate transcripts through qRT-PCR and to standardize gene silencing strategies.

References

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S-VII-O4

HOW *ERIANTHUS ARUNDINACEUS* ADAPT TO DIFFERENT MOISTURE REGIMES?

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Erianthus is a cane forming wild species, related to the genus *Saccharum*. Among the four species of *Erianthus*, *E.arundinaceus* has a wider distribution in India, China, Myanmar, Thailand and Indonesia. This genus grows in mountain ranges in both the Indian subcontinent and 'Indochina'. It is well known for high fiber, high biomass, tolerance to drought and water logging, pest and disease resistance with multi ratooning ability.

The present study was aimed at understanding the possible mechanisms involved in drought tolerance in *Erianthus* species. We have taken up this study at two levels. As a first step we have attempted to understand how *E. arundinaceus* adjust the membrane stability and the expression pattern of two important genes ie, HSP70 and DREB2 in comparison with the commercial sugarcane variety Co 86032 when subjected to varying soil moisture regimes. Single budded setts of an *E. arundinaceus* clone IK76-81 and a commercial sugarcane variety ie.Co 86032 were planted in pots and irrigation was withheld at the 120th day (formative phase) for a period of 10 days and the soil moisture stress was released on the 11th day with normal irrigation. Membrane stability and the relative expression of HSP70 and DREB2 genes through real time PCR were studied on the 0th, 5th, 10th and 15th days. Membrane stability index in *Erianthus* was negatively correlated with soil moisture levels, whereas in Co 86032 there was no appreciable variation. Likewise, there was a significant increase in relative expression of HSP and DREB in *Erianthus* with increasing soil moisture stress and reduced expression with the release of the water stress, in comparison with Co 86032. In the second set of experiments the variation in membrane stability with varying soil moisture stress was studied in different *Erianthus* species under field condition along with few physiological parameters such as photosynthetic rate, stomatal conductance, and transpiration rate and leaf temperature. Though all the *Erianthus* species showed adjustments in membrane stability in relation to different soil moisture levels, the extent of modulations varied between accessions.

From the present study it can be concluded that the membrane stability increases with the increase in soil moisture stress and decreases with the decrease in soil moisture stress in *Erianthus* perhaps a first report on the modulations of the membrane stability with varying soil moisture stresses. The increased relative expression of HSP70 and DREB2 in *Erianthus* points to some of the underlying regulatory mechanism behind enhanced moisture stress tolerance compared to cultivated sugarcane varieties. Taken together our experiments shed light to the adaptive behavior of *Erianthus* under