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Care Analysis of TF Genes in Sugarcane

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***Abstract*: Sugarcane is a commercial crop, due to high level of sugar accumulation it is a good source for bioelectricity and bioethanol. This crop is affected by both abiotic and biotic stress. Increasing vulnerability of crops to a wide range of abiotic and biotic stresses can have a marked influence on the growth and yield of major crops, especially sugarcane (*Saccharum* spp.). Abiotic stresses, such as drought, high temperature, and salinity, affect plant growth and productivity. Most common abiotic stress is water deficit; hence drought tolerant breed is an imperative for all countries that involved in major sugar cane production. This review summarizes the physiological and molecular studies on water deficit stress in sugarcane, with the aim to help formulate more effective research strategies for advancing our knowledge on genes and mechanisms underpinning plant response to water stress. It also overviews transgenic studies in sugarcane, with an emphasis on the potential strategies to develop superior sugarcane varieties that improve crop productivity in drought-prone environments.**

***Keywords*: cis acting regulatory elements, plant care, promoter, sugarcane, transcription factors.**

1. Introduction

Sugarcane a widely grown crop in both tropical and subtropical regions around the world. Largest sugarcane cultivation countries are Brazil, India, China and Thailand. Sucrose extraction is the main outcome for sugarcane cultivation in turn leads to production of sugar and ethanol. The sugarcane baggase is the residue enriched with lignocellulosic obtained from Sugarcane crop after sucrose extraction.

Sugarcane a renewable and a natural agricultural resource which provides, sugar, biofuel, fibre, fertilizer with environmental sustainability. Sugarcane used for making white sugar, brown sugar (khandsari) and jiggery (gur). It's ranked as one of the major crops for foreign exchange earnings. As stated earlier, the main byproducts are bagasse and molasses. Bagasse could be used for fuel production and is also used for making fiber board, paper and plastics. Molasses is used in distilleries for ethanol, butanol and citric acid production. Sugarcane press mud is a very good organic fertilizer. Cane green tops used as a cattle fodder. Sugar industry is as important as textile industry in India for employment of large number of people. It has health benefits like controlling low blood sugar and depression.

Sugarcane an important crop for the production of sugar and ethanol. This crop demands more water, and it is water sensitive, hence environmental stress like water deficit (abiotic stress) limit plant growth and crop productivity [20]. Hence drought is the most common abiotic stress affects sugarcane crop productivity worldwide [6]. Due to this reason production area are concentrated more in rain favorable region for sugarcane growth and development [23].

Plants involved in various stress tolerant mechanism like life cycle change, growth and development modulation that matches with water supply, plant function regulation, resource allocation for growth and stress adaptation, rapid and long-term expression of stress tolerant genes [9], [49].

Plant responses to tolerate abiotic conditions like drought, salinity and salinity paved the way towards genetic engineering to develop stress tolerant plants and water use-efficient crop productions [45]. Knowledge of genetic engineering, agronomic and molecular biology to develop and producing water stress tolerant and commercially useful sugarcane varieties [16].

Genetic improvement of modern sugarcane cultivars hits a bottleneck through conventional hybrid breeding as a result of sugarcane complex genome, heterogenous and polyploid-aneuploid nature [1]. Despite the availability of molecular tools and strategies and advancements in our understanding of stress responses, engineering crops for drought tolerance remains a major challenge. This is not only due to the complexity of the plant responses to water deficit [10], [45], but also due to the difficulty of identifying and exploiting large effect genes and alleles and the associated selection traits for developing drought tolerant varieties suitable for commercial crop production conditions [39], [3].

Polyploids are organisms having more than two genomes in their nucleus. Polyploidy is widespread in wild as well as cultivated plants and is regarded as an important mechanism of speciation and adaptation [29]. Sugarcane, which is an allopolyploid with genome contributions from *Saccharum officinarum* and *S. spontaneum*, is having high chromosome number of 2n=100 to 130 in different cultivars. The 'Saccharum complex' has species with varying ploidy level and the chromosome number ranges from 2n=20 to ~200. The high polyploidy and heterozygosity due to hybridization has restricted the classical genetic studies in sugarcane [28].

2. Polyploidy in Sugarcane

The commercial sugarcane cultivars are clonal selections

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from interspecific hybrid derivatives involving species of the genus *Saccharum L*. [38]. This genus consists of six species of which two are wild - S. *spontaneum L. and S. robustum* Brandes and Jesw. ex Grassl and four are cultivated - *S. officinarum L., S. barberi Jesw., S. sinense Roxb.* and *S. edule Hassk* [4]. [12] proposed to recognize only two species in Saccharum, first *S. spontaneum*, the putative ancestral form which has a very wide natural range, morphologically distinct from other Saccharum forms, and second, *S. officinarum*, which includes the wild species *S. robustum*, together with the land races *S. officinarum, S. edule, S. barberi and S. sinense*. The 'Saccharum complex' [4] includes the genera *Saccharum, Erianthus, Sclerostachya, Narenga* and *Miscanthus* which constitute a closely related inter breeding group involved in the origin of sugarcane [5].

S. spontaneum is a highly polymorphic wild grass widely distributed in the tropics and sub tropics, with wide eco-geographical distribution and wide range of chromosome numbers from 2n = 40 to 128 [26], [38].

Gene redundancy due to polyploidy provides a selective advantage for a wider geographical adaptation, increased vigour, sucrose and fiber content of sugarcane crop. There is increased global demand for alternative fuel sources and sugarcane is gaining importance as a biofuel crop with its high biomass production potential, besides being a major sugar crop. Now a day's data base helps us to identify the key genes associated with drought tolerance and growth maintenance under water deficit condition in sugar cane crops [45].

Over expression of abiotic-stress-inducible genes in wild-type sugarcane in response to abiotic stress. The approach is the use of transcription factors which regulate drought response in transgenic plants has successfully produced.

Drought-inducible genes identified through the recent microarray analyses in *Arabidopsis* can be classified into two groups [37]. The first group has proteins that most probably function in abiotic stress tolerance. The molecules such as chaperones, late embryogenesis abundant (LEA) proteins, osmotin, antifreeze proteins, mRNA-binding proteins, key enzymes for osmolyte biosynthesis, water channel proteins, sugar and proline transporters, detoxification enzymes, and various proteases. The second group has regulatory proteins, i.e. protein factors involved in further regulation of signal transduction and stress-responsive gene expression. These include various transcription factors, protein kinases, protein phosphatases, enzymes involved in phospholipid metabolism, and other signaling molecules such as calmodulin-binding protein. Many transcription factor genes were stress inducible, suggesting that various transcriptional regulatory mechanisms may function in regulating drought, cold, or high salinity stress signal transduction pathways. These transcription factors could govern expression of stress-inducible genes either cooperatively or independently and may constitute gene networks in *Arabidopsis*.

Transcription factors have also proven quite useful in improving stress tolerance in transgenic plants, through influencing expression of a number of stress-related target genes [37].

Transcriptome analyses based on microarrays have provided powerful tools for discovery of stress-responsive genes not only in *Arabidopsis* but also in various crop plants and tree species. Transgenic plants generated to express antisense or RNAi constructs, as well as T-DNA- or transposon-tagged mutants, were used to analyse the function of these stress-responsive genes based upon phenotypes resulting from loss of function. Moreover, transgenic overexpressors were very useful not only for functional analyses of stress-inducible genes but also for demonstrating improved stress tolerance in these plants generated by gene transfer. Introduction of *Arabidopsis* stress-related genes proved valuable for improving drought-stress tolerance in transgenic crops and trees, as well as serving as key tools for the discovery of stress-related genes in those systems by a comparative genomics approach [37].

3. Stress Response Mechanism in Plants

Plants develop highly efficient and remarkable mechanisms to survive under frequent and extreme environmental stress conditions. Exposure of plants to various stress factors is associated with coordinated changes in gene expression at the transcriptional level and hence transcription factors, such as those belonging to the MYB family play a central role in triggering the right responses.

Extensive studies have identified other stress-responsive transcription factors belonging to the NAC, AP2/ERF, MYB, and WRKY families that mediate plant response and tolerance to abiotic stress. These suggest that transcriptional regulation of stress-responsive genes is an essential step to determine the mechanisms underlying plant stress responses and tolerance to abiotic stress, and that these transcription factors may be important targets for development of crops with enhanced abiotic stress tolerance

The most important proteins in regulatory control, metabolism and biotic, abiotic stress are MYB proteins. MYB protein in *Arabidopsis* helps us in prediction of plant biology. MYB transcription factor plays key role in regulating stress responses and also in the activation of drought-related genes, helps us in improving the drought stress tolerance in crops. Even though MYB TF involved in regulatory roles in plant growth and abiotic stress response, knowledge about the MYB gene involvement in plants for salinity and drought resistance are largely unknown. Studies have suggested that MYB proteins are also involved in regulating plant responses to abiotic stress [19], [46], [48].

4. *MYB* Domain Proteins Act as DNA-binding Transcription Factors

In plants the MYB family represents one of the large, functionally diverse classes of proteins. In general, most of the MYB proteins function as transcription factors and are characterized by the presence of variable numbers of N-terminus conserved MYB repeats (R), mainly associated with DNA-binding and protein-protein interactions. The variable C-terminal region is responsible for modulating the regulatory activity of the protein. Several members of this family have been identified in *Arabidopsis*, rice, maize, and soybean and

shown to be involved in regulating various cellular processes, including cell cycle and cell morphogenesis, biotic and abiotic stress responses [21].

[27] elucidated the stress tolerance mechanism of ScMYBAS1 gene at the transcriptional level. They isolated and characterized the promoter (PScMYBAS1, 1,033 bp) flanking the 50 ScMYBAS1 coding region from the sugarcane genome. Deletion analysis of the promoter, PScMYBAS1, suggested that the 303-bp promoter region was required for basal expression. Also, the expression levels of this gene a various abiotic stress conditions like dehydration, salt, drought, hormones were studied using GUS expression analysis. The results of the study widened the understanding of the regulation of ScMYBAS1 expression and provided a new stress-inducible promoter system in transgenic plants.

[13] made a detailed study on various types of transcription factors that are involved in abiotic and biotic stress response in sugarcane. It was found that a significant role was played by the TFs belonging to MYB, WRKY, NAC, AP2/ERF which provides an important clue for engineering sugarcane to develop stress tolerant varieties.

Expression analysis of various ScMYB genes involved in various stress tolerance were done [36], [8], [15], and [31]. In all these studies, it was found that various MYB genes are involved in protecting sugarcane from stress. The promoter analysis and overexpression study has to be widened so as to get a deep insight into the actual functioning of the MYB family genes. It was also understood that MYB is the largest family of TFs involved in almost all the process of the plant, right from growth and development to reproduction.

5. CAREs

Cis-regulatory elements (CARE), which are sequences controlling gene expression at all developmental stages. It consists of promoters, enhancers, insulators and silencers. Knowledge about gene promoters, cis sequences and their cooperating factors allows uniform expression systems and highly predictable results. Many elements present in plant genome control the gene expression level through interactions with DNA or regulatory proteins at every stage of implementation of genetic information. Regulators are classified in terms of their structure as cis sequences and Trans factors. Cis regulatory sequences are linear nucleotide fragments of non-coding DNA. Their localization and orientation in relation to genes and activity is various [44]. Plant regulatory sequences are located directly in the transcribed DNA strand: promoters, enhancers, silencers and insulators; or may be added during post-transcriptional modifications: 5′cap, poly-A tail, signal sequences [43]. Specific regulatory proteins, called Trans elements, interact with cis sequences and other proteins to form active complexes. Organization of all eukaryotic genomes is similar and most of regulatory elements are universal. However, substantial differences occur among elements assigned to particular tissues e.g. tissue/organ-specific promoters [41], [44].

6. Role of cis Sequences

Cis elements directly involved gene regulation. The sequence are not constant so they may be found on hypothetical localization and analyzing interacting proteins [35]. Cis sequence may vary in copy numbers, variable distances and orientation depending on the gene [44]. Cis sequences are classified in to 2 types i.e., enhancers and silencers. Currently cis regulatory research concentrates on the elements localization, orientation and cooperation which lead to findings of more efficient novel applications. Finally, to create methods free from gene coding sequences and purely based on promoter organization [34].

Gene expression plays an important role, and it describes the function of a gene. Enormous amount of data been generated by genome sequence project, which in turn helps in identification and location of gene structure, gene expression and the elements that regulates the function. Knowledge on Plant promoter is important to know about the gene control expression and is possible nowadays with the help of Plant CARE database and currently available analysis tools [30].

7. Plant Care Database

Plant Care database consist of cis-acting regulatory elements, enhancers and repressors. Database could be researched on TF sites, motif sequence, function, species, cell type, gene, TF and Literature references [17]. It was first introduced by [30]. Results could be obtained from the internal database and also access details from another database like EMBL, GenBank, TRANSFAC and MEDLINE. Plant Care database has various programs to identify regulatory elements in silico of transcriptome data. To identify over-represented motifs in upstream regions various methods like motif sampler, Gibbs sampling are used. [17]. Plant Prom is another database which gives a detailed annotation of the promoter regions of plant genes. Though various databases are available like TRANSFAC, TRRD, ooTFD, COMPEL, PLACE (10) and RegSite, the most commonly used database is PLANT Care, especially for polyploidy genomes like sugarcane, sorghum, etc.

Scientist states by means of transcriptome expression analysis i.e., micro arrays product co-expressed gene sets could also be co regulated. By concentration on over expressed oligonucleotides, regulatory elements could be found that are shared by promoter sequences of genes from a given cluster [11].

8. RACE

Rapid Amplification of cDNA Ends (RACE) is a technique used for amplification of cDNA isolated from any organism. Using this technique, a full length RNA transcript is converted to cDNA by RT-PCR and then the amplified cDNA is analyzed from either the 5’ end (5’ RACE) or 3’ end (3’ RACE). Information regarding the transcription start site and the location of *cis*-acting regulatory elements (CAREs) in the upstream region of a gene can be identified using RACE with accuracy. The regions beyond the 3’ ends can be analyzed

taking the poly-A tail of the mRNA as a support but with respect to the 5' ends, the upstream promoter regions are difficult to be analyzed. To overcome this difficulty, a linker is attached to the 5' end and is used as a reference for designing primer. Using this primer, PCR is carried out in a reverse manner to amplify the upstream regions. The PCR is then carried out in a usual manner to amplify the number of fragments. The amplified fragments are sequenced and analyzed for the locations of CARE elements like MYB, WRKY, TATA box, etc. [7], [33].

Using this technique, promoter regions of many plant genes that are involved in stress tolerance is analyzed. For instance, in *Solanum sp*., miRNA components that are involved in cold stress response have been analyzed by [40] has studied how miRNA39 was regulated in sugarcane during the cold stress response. He identified MYB and ABA elements in the upstream regions got over regulated during the cold stress. This result was further confirmed using whole mount in-situ hybridization method (WISH).

In *Clarika sp*., the shift in the spot position in the flowers were found to be caused by two genes *CgbHLH1 and CgCPC1* which were governed by the *MYB* and *bHLH* regulatory elements [14].

[25], Cloned a full length cDNA encoding CIN gene by RT-PCR and RACE-PCR from sugarcane. A promoter sequence SoCIN1 gene was isolated and analyzed using PlantCARE and PLACE software. The results showed many CARE elements involved in stress tolerance. The expression level of the SoCIN1 gene was predicted in many tissues and was found that the expression level was higher in the immature leaves and maturing leaves than the matured leaves and internodes at elongation stage and processing maturing stage.

The promoter region of pathogen specific expressive gene *Xa13* in *Oryzae* was analyzed using RACE techniques which was further confirmed by Agrobacterium mediated gene transfer technique. The location of TATA box and other CAREs identified using RACE, agrobacterium technique and insilico analysis coincided well [50].

Distinct microRNA targets were identified in sugarcane *Saccharum officinarum* which were involved in abiotic stress response [51]. 5' RACE was performed to amplify the CARE regions and sequenced. The sequence was then compared with Sorghum database and homologous sequences between the two species were identified that were involved in stress response. Thus, it was concluded that the two genome share evolutionary relationship which was further confirmed by phylogenetic analysis. A dataset was created which included the miRNA targets of both Sugarcane and Sorghum which can be used as a reference set for polyploidy genome analysis.

[2] in his work designed synthetic promoters for WRKY family of TFs which were involved in wound and pathogen response. The cis-regions of the genes were amplified by RACE and based on the sequences obtained synthetic promoters were constructed which were expressed in tobacco using CaMV promoter. The results obtained were then used for overexpression analysis of these promoter regions which was confirmed by PCR and GUS assay.

A knockoff of the novel sugar transporter gene (ScERD6) was developed by RACE-PCR from sugarcane by [22]. The promoter sequence was analyzed and cloned by using genome walking and RACE-PCR, which included CAAT-boxes and TATA boxes, as well as specific acting elements such as cis-regulatory elements involved in hormone response, seed development, environmental stresses, ACES, G-box, I-box, L-box, and rbcS-CMA7 light response element, etc. It was found that ScERD6 expression in stem was different from that in leaf and root of sugarcane. This finding predicted an important role of this gene in reproductive and floral development.

Promoter regions of dehydration response and cold stress response genes that belong to DREB family of TFs were analyzed in *Saccharum spontaneum* [18]. DRE element and AP2/ERFBP superfamily promoters were active in the upstream regions of the genes involved in cold stress and dehydration stress response. The results obtained were compared with Arabidopsis, rice and sorghum and phylogenetic analysis was also done. Structural analysis of these proteins showed that all the proteins expressed during the stress response had WLG motif in common.

Pathogenesis expression related proteins were studied in *Medicago* species by [32]. TF binding sites in the promoter regions of MtPR5, MtPR10, and MsPR10 genes were analyzed and found that WRKY, MYB and ERF domains were found in the upstream regions of these genes. The *Medicago* promoters analyzed in this experiment were all responsive to this varied suite of significant alfalfa pathogens and could be used to engineer efficient and effective disease resistance in alfalfa.

Several studies are available in many plants which aim at analyzing the cis domains of genes that get expressed at specific situations. For such type of studies, commonly used methods will be isolating the RNA, running RACE-PCR and analyzing the sequences using Plant CARE databases. The results can be further confirmed using phylogenetic analysis. Such results can be used in overexpression studies using GUS which can help in developing disease resistant and stress tolerant varieties.

9. Conclusion

Taking into consideration all the above mentioned information, combination of these techniques will definitely give a comprehensive perception about the expression of polyploidy genes like sugarcane. Based on the results obtained, it can be further extended to develop transgenic sugarcane which will have stress tolerant characteristics. This is an essential quality for cash crops like sugarcane because in tropical countries where regular stress conditions prevail, such transgenic varieties will prevent crop loss as well as economic loss.

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