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Review Article

Engineering Sugarcane (*Saccharum* spp) for Disease Resistance: Recent Approaches

Gulzar S Sanghera

PAU, Regional Research Station, Kapurthala, 144601, Punjab, India

Pawan K Malhotra

PAU, School of Agricultural Biotechnology, Ludhiana, 144004, Punjab, India

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****Corresponding Author:***
E-Mail: sangheragulzar@pau.edu

Abstract

Sugarcane is an important agro-industrial crop cultivated for sugar and other by-products. The future of sustainable agriculture will rely on the integration of biotechnology with traditional agricultural practices. Diseases can be caused by a variety of plant pathogens including fungi, others and their management requires the use of techniques in transgenic technology, molecular biology, and genetics. These include: genes that express proteins, peptides, or antimicrobial compounds that are directly toxic to pathogens or that reduce their growth in situ; gene products that directly inhibit pathogen virulence products or enhance plant structural defense genes, that directly or indirectly activate general plant defense responses; and resistance genes involved in the hypersensitive response and in the interactions with a virulence factors. This chapter discusses the key recent approaches to manage different pathogens within the context of recent developments in biotechnology and plant science.

Key Word: Sugarcane, Transgenic, ABC Transporters, RNA interference

Introduction

Sugarcane diseases have had a big impact on the sugar industry all over the world. Epiphytotics of major diseases, such as red rot (India), Fiji, downy mildew, and RSD have caused substantial yield losses through the last 2-3 decades [18]. Though research has led to generally good control, diseases have an ongoing influence in the industry, by affecting crop growth and the selection of resistant commercial varieties in the plant improvement program. Plant pathogens represent real threat to world agriculture. More than 70% of all major crop diseases are caused by fungi [1]. In considering the effect of a disease on sugar industry, four factors should be evaluated: the ability of the disease to reduce plant growth; the severity of the disease; the distribution of the disease and; the susceptibility of varieties being grown. Some of the factors obviously interact; resistant varieties usually show a decreased disease severity compared to susceptible, with a smaller growth reduction. The importance of diseases as a constraint in the production and productivity of sugarcane is well recognized. It is estimated that losses caused by diseases range from 10 to 15 per cent Diseases of sugarcane are caused by

different kinds of pathogens viz., fungi, bacteria, viruses and mycoplasma (**Table 1).** Among fungal diseases, red rot and smut are very important and cause severe yield losses in tropical and sub tropical India. Disease resistance in crop plants is an enigma to be unravelled, in spite of advances made in plant biology. Understanding plant-pathogen interaction precisely is still a fascinating area, which forms a basis to develop disease resistance varieties in Agriculture. Plant biotechnology has made significant strides in the past two decades or so, encompassing within its folds the spectacular developments in the plant genetic engineering [54]. Now a day, genetically engineered crops appear as the most recent technological advances to help boost food production, mainly by addressing the production constraints with minimum costs and environmental pollution (due to the indiscriminate use of pesticides and herbicides). The high level of genetic complexity in sugarcane creates challenges in the application of both conventional and molecular breeding to the genetic improvement of sugarcane. as a sugar and energy crop [56]. Recent technology developments indicate the potential to greatly increase our understanding of the sugarcane plant disease management by application of emerging technologies like tissue culture, genetic transformation [55], RNAi [60], quarm quenching [33], ABC transporters [34], microarray [68] etc. This section outlines some of the biotechnological developments that are in place and tailored to address important issues related to sugarcane improvement. The science of plant disease resistance has undergone a paradigm shift in understanding starting from the gene for gene concept to the present age of guard decoy model to decipher disease resistance genes in crop plants.

Table1 List of important diseases of sugarcane

Sl. No.	Name of the disease	Causal agent	Causal organism
1.	Red rot	Fungus	*Colletotrichum falcatum*
2.	Smut	Fungus	*Sporisorium scitamineum*
3.	Wilt / Top rot / Pokkah Boeng	Fungus	*Fusarium sacchari*
4.	Sett rot	Fungus	*Ceratocystis paradoxa*
5.	Rust	Fungus	*Puccinia melanocephala*
6.	Leaf spot (Eye leaf spot)	Fungus	*Helminthosporium sacchari*
7.	Leaf scald disease	Bacteria	*Xanthomonas albilineans*
8.	Ratoon stunting disease	Bacteria	*Leifsonia xyli* sub sp. *xyli*
9.	Sugarcane Mosaic Virus disease	Virus	Sugarcane Mosaic Virus (SCMV)
10.	Yellow leaf disease	Virus	Sugarcane Yellow leaf (SCYLV)
11.	Grassy shoot disease	Phytoplasma	Sugarcane Phytoplasma

[64]

Biotechnology for Biotic Stress Tolerance

Sugarcane, being a long standing crop is constantly challenged by insects, nematodes, fungi, bacteria and viruses. Plant defence responses to such perturbations are largely mediated by phytohormones through triggering

conserved defence mechanisms, each with an intricate signalling pathway leading to plant protection. It has been shown that both the ethylene and jasmonic acid signalling pathways act synergistically in plant defence. In a study to identify red-rot-related genes, enriched subtractive cDNA library prepared from the *C. falcatum* challenged stem of sugarcane variety (Co 1148) was used by [26] and at least 85 red-rot-specific clusters, unique were reported. In another study, cDNA-SSH (suppression subtractive hybridization) library was constructed and analysed to identify the up-regulated genes in sugarcane under sugarcane grassy shoot (SCGS) infection condition. Subtracted library highly represented genes potentially involved in cell rescue, defence, ageing and apoptosis. The forward SSH approach when implemented, allowed to explicate the transcriptional regulatory mechanisms of sugarcane in response to SCGS infection and isolated the R2R3-MYB (SoMYB18) gene, a potential candidate playing important roles in the regulation of secondary metabolism, signal transduction during biotic, abiotic and other environmental stresses. In an attempt towards studying the host–pathogen interaction and decipher the molecular basis of virulence of sugarcane SCGS disease, [35] isolated partial genome of first Asiatic strain of phytoplasma (SCGS) by genomic-SSH. The library yielded 83 SCGS specific fragments representing approximately 42% of the chromosome of Sugarcane grassy shoot phytoplasma, comprising approximately 85 predicted partial phytoplasmal CDS. Further, a species specific detection method was developed for early detection of SCGS infection [36].

Engineering Sugarcane for Disease Resistance

Role of Conventional Tissue Culture:

The production of quality seed through micropropagation technique is well recognized now. The sustained high production of sugar per unit area depends primarily on continuous supply of adequate quantity of good quality seed cane, which has to be genetically pure, free from diseases, pests and with no nutritional disorders. This can only be achieved by applying the tissue culture techniques. Since the plants are free from infections, so the original vigour of the newly bred variety is maintained**.** Sugarcane is a vegetatively propagated crop and normally requires 7-8 years or even more, for a newly developed variety to spread at large scale. During this period, deterioration of various yield and quality characteristics is inevitable prior to commercial use on account of systemic infections during vegetative multiplication it also helps in the rapid seed multiplication of newly released varieties which is of particular importance in sugarcane where, the normal seed multiplication rate is very low. Sugarcane production is challenged by diseases like red rot, leaf scald, ratoon stunting, grassy shoot and mosaic [67]. Tissue culture method (micro-propagation) is the only alternative approach for fast multiplication of a variety in its original form and. Micro-propagat ion is very effective in rejuvenating/reviving the well adapted promising local cultivars facing gradual decline or degenerating in yield and vigour by freeing them from diseases due to accumulation of viruses and other systemic pathogens during prolonged vegetative cultivation. In addition Moist Hot Air Treatment has not been effective against mosaic virus. The meristem culture is the only method to remove the SCMV (Sugarcane mosaic virus) as the meristematic tissue remains free from virus disease. The cells of the meristem are

genetically highly stable and, hence, the plants produced from them are generally identical to the donor plants, except for the occurrence of rare mutations [28].The genotype can be sustained over a long period in culture and this serves as alternative means of conserving the sugarcane germ plasm. Conventional tissue culture is widely adopted for commercial sugarcane seed production in many countries, Considering the above advantages, micro- propagation has an important role to play in the 'Seed Production Chain' in sugarcane.

Genetic Transformation

Different kinds of plant pathogens viz. fungi, bacteria, virus and phytoplasmas infect sugarcane. At least 150 diseases were recorded in sugarcane in different countries. In India more than 50 diseases were recorded [2]. Among them red rot, smut, wilt, sett rot, grassy shoot, ratoon stunting, leaf scald and mosaic are the major diseases seriously affecting sugarcane production. For the past 100 years, new varieties were developed with higher yield with high sugar through breeding approaches. However combining higher sugar yield with disease resistance and favourable agronomic traits is difficult. Many sugarcane varieties can be cited here. Best example is CoC 671 an early maturing high sugar variety that revolutionized the sugar industry in Peninsular India could not be sustained due to its susceptibility to red rot. Other examples: CoJ 64 and CoC 92061 susceptibility to red rot, CoSe 95071 resistant to red rot but highly susceptible to smut. Since the genome is complex polyploidy in these varieties backcrossing may not be useful for the introduction of disease resistant genes. Disease control via genetic engineering strategies may have particular value where resistant germplasm is not available in the target species for breeding, where pathogen adaptation can quickly overcome resistance genes and where there is little information on sources of resistance and bioassays are difficult or very time consuming to carry out. Several genes for resistance to diseases have already been isolated and shown to be effective in field trials with transgenic plants of other crop species.

Chitin is a significant component in the cell walls of large groups of fungi except members of Oomycetes. It is made up of molecules of N-acetylglucosamine, which are the building blocks linked together by 1,4-ß-glycosidic bonds. Chitinases are enzymes that cleave the bond between the C1 and C4 of two consecutive N-acetylglucosamines of chitin. Chitinases and other microbial hydrolytic have been shown to be involved in a variety of functions such as cell wall digestion, germination of spores, assimilation of chitin and mycoparasitism. Thus chitinases have the potential as effective antifungal agents. Many *Trichoderma* and *Gliocladium* spp. isolates used in biocontrol kill the host by direct hyphal contact, causing the affected cells to collapse or disintegrate; vegetative hyphae of all species have been found susceptible. Cloning of genes encoding for lytic enzymes, characterizing their products, and elucidating their individual roles in the mycoparasitic activity has opened the way to improving the biocontrol capacity of different fungi (Table 2). Also the genes coding for these lytic enzymes were expressed in plants to resist invading pathogens. Cloning of genes encoding for endochitinase and N-acetylglucanase from *Trichoderma* spp. was reported by previous workers [39]. Lytic enzymes of fluorescent Pseudomonas strains and *Trichoderma harzianum* strain T5 were inhibitory to conidial germination, germ tube elongation

and hyphal growth red rot pathogen. Electrolytic leakage studies indicated that the lytic enzymes produced by these strains caused cell wall degradation and brought about loss of electrolytes from the mycelium. The lytic enzymes produced by *T. harzianum* T5 and *P. fluorescens* FP7 showed more antifungal activity [66]. Detailed studies were conducted on inactivation of *C. falcatum* toxin by bacterial and fungal antagonists. The results clearly proved that *P. fluorescens* VPT4 and *T. harzianum* T5 have completely arrested biological activity of the toxin and altered spectral properties of the toxin [41]. Further characterization of the inactivating protein revealed it as a 97 kDa protein [42].

In Australia, [63] initially demonstrated that coat protein constructs SC were expressed in a sugarcane protoplast cell system, and these constructs were subsequently used to transform sugarcane callus. The lines generated from the transformed and selected callus resisted mechanical challenge inoculation. From USA [46] reported that sugarcane plants containing a coat protein construct derived from SrMV-H resisted subsequent infection by SrMV strains H, I and M but not SCMV strains A, D or E. The mechanism virus resistance in these cases did not appear to be protein mediated but appeared to be RNA mediated, which corresponds with the observations recorded in other transgenic plant potyvirus interactions [61]. The transgenic sugarcane plants did not contain virions, and extracts prepared from these plants were not infectious when back inoculated onto maize [31]. Other PDR genes considered for developing SCMV resistance are nuclear protein b (Nib), known as the 'replicase' or RNA-dependent RNA polymerase [62]. [71] reported that transgenic sugarcane containing an untranslatable SCYLV coat protein gene had increased resistance to SCYLV. Gene silencing seems to be a universal mechanism for plant resistance to viral infection as suggested by [5]. Posttranscriptional gene silencing has already been achieved in sugarcane by transformation with an untranslatable piece of sorghum mosaic virus. Albicidins, a family of phytotoxins produced by the LSD pathogen *X. albilineans*, are known to cause white pencil like symptoms in sugarcane by inhibition of DNA replication in the plastids of young leaves. Several potentially useful albicidin resistance genes have been cloned from different bacteria, including albA gene from *K. oxytoca* which encodes an albicidin binding protein and albD gene from enzyme detoxification of albicidin in *Pantoea dispersa*. The albD protein irreversibly inactivates albicidin toxin and is likely to be an albicidin hydrolase. These two genes have been introduced separately into leaf scald susceptible sugarcane cultivar Q63 and Q87 by co-bambardment with a gene. Both albA and AlbD genes can confer resistance to LSD, but albD is more effective than albA [70]. Analysis of transgenic sugarcane lines expressing the albD gene for hydrolysis of albicidin toxins show a clear correlation between the level of the resistance gene product and resistance to leaf scald disease following challenge by *X. albilineans*. A peptide from fish has been found to possess antibacterial activity against the bacterial pathogens *X. albilineans* and *X. campestris* pv *vasculorum*.

Table 2. Engineering sugarcane for disease resistance

Disease	Gene(s)	Method	Reference
SCMV	SCMV-CP	Microprojectile	[31]
Sugarcane leaf scald	albD	Microprojectile	[70]
SrMV	SrMV-CP	Microprojectile	[30]
Puccinia melanocephala Glucanase	chitanase & ap24	Agrobacterium	[22]
SCYLV	SCYLV-CP	Microprojectile	[24]
Fiji leaf gall	FDVS9 ORF 1	Microprojectile	[44]

Mode of Action of Plant Disease Resistance Genes

Several other researchers grouped transgenic plants into five general categories [45,52]

The expression of gene products that are directly toxic to pathogens or that reduce their growth. These include pathogenesis-related proteins (PR proteins) such as hydrolytic enzymes (chitinases, glucanases), antifungal proteins (osmotin- and thaumatin-like), antimicrobial peptides (thionins, defensins, lectin), ribosome inactivating proteins (RIP), and phytoalexins (Fig. 1).

- The expression of gene products that destroy or neutralize a component of the pathogen arsenal such as polygalacturonase, oxalic acid, and lipase.
- The expression of gene products that can potentially enhance the structural defenses in the plant. These include elevated levels of peroxidase and lignin.
- The expression of gene products releasing signals that can regulate plant defenses. This includes the production of specific elicitors, hydrogen peroxide (H_2O_2), salicylic acid (SA), and ethylene (C_2H_4).
- The expression of resistance gene (R) products involved in the hypersensitive response (HR) and in interactions with avirulence (Avr) factors.

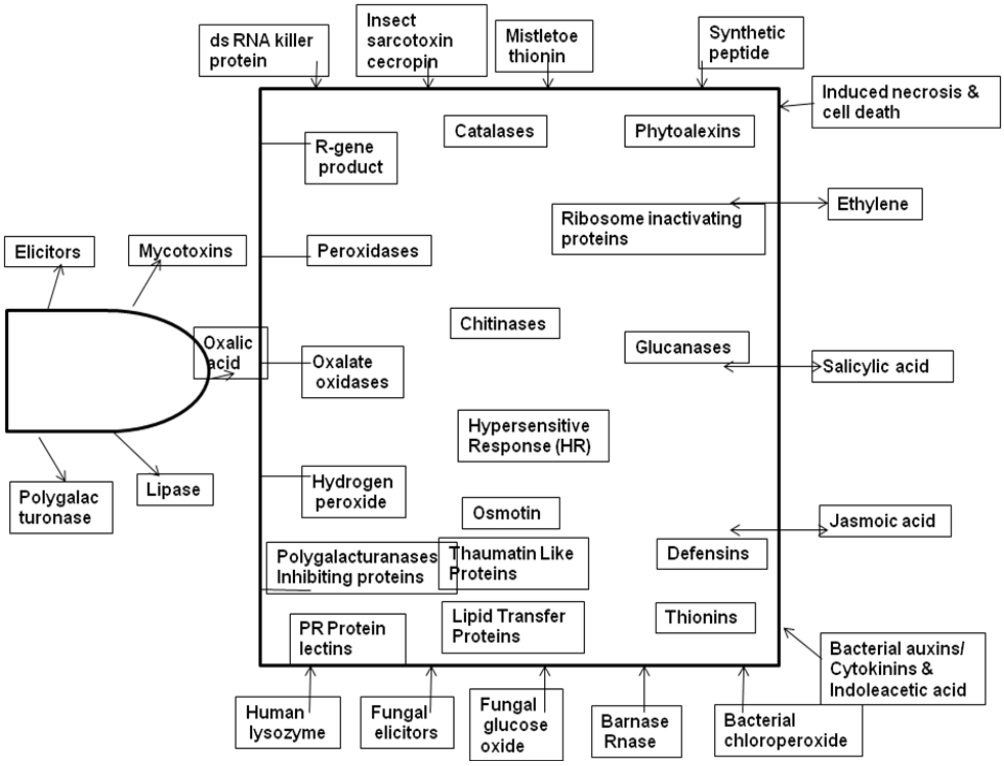

Fig 1 Transgenic plants with enhanced disease resistance have been engineered to express gene products to control attack fungal virulence products (from hypha on left), enhanced expression of plant-derived gene products (inside of cell) or gene products from non plant sources (outside of cell).

Role of ABC Transporters molecules

ATP binding cassette (ABC) transporters are the major classes of membrane transporters found at the cell periphery [40]. These fascinating molecular machines use the energy of ATP to catalyze the transport of a variety of hydrophobic compounds (peptides, carbohydrates, lipids, heavy metal chelates, inorganic acids, steroids, and xenobiotics) across biological membranes against a concentration gradient [29,51]. The energy needed for transport of these compounds is generated by ATP hydrolysis; these molecules are considered as a primary active transport system and truly regarded as "molecular gatekeeper of a cell". These transporters compose one of the largest protein families present in a wide variety of organisms, ranging from bacteria to man [29]. In plant system, ABC transporters have been localized to the tonoplast, mitochondria, chloroplasts, and plasma membrane [38,25]. These transporters have the potency to secrete antifungal compounds back into the environment. By this inherent property, ABC transporters prevent the accumulation of fungitoxic concentrations at their target sites inside mycelia cells and hence prevent or reduce their toxic action. Thus, indicating their association with the acquisition of multiple drug resistance by pathogenic organisms [50,58], as well as the occurrence of multidrug resistant tumours [21,32]. This ability of some ABC transporters to excrete xenobiotics [37,72] in plants has been reported to be involved in responses to detoxification of herbicides, fungicides as well as in transport of plant defense

compounds [13,17]. The identification of transport substrates, which are critical for defense of the plant against pathogen, will be a next major step in understanding more details of the interaction between plants and pathogens.

Role of RNAi Technology

Micro RNAs (miRNAs) are small single stranded, noncoding, naturally occurring, highly conserved families of transcripts (18-25 nt in length). Several miRNAs are either up regulated or down regulated by abiotic stresses, suggesting that they may be involved in stress-responsive gene expression and stress adaptation [65]. The involvement of miRNAs in abiotic stress has been studied in plants in response to dehydration or NaCl by using expression analysis, suggesting stress specific regulation of expression of miRNA [49] in sugarcane. In response to long term (15 days) isoosmotic (-0.7 MPa) NaCl or PEG stress, no change in mature transcript level of miR159 over the control was detected. However, under the short-term (up to 24 h) salt stress, transcript level of the mature miRNA increased to 112% of the control at 16 h treatment. The mature transcript level of miR159 was higher under all the PEG induced osmotic stress treatments as compared to the control, and it progressively increased with stress exposure period (1.3 fold at 8 h treatment). This indicated that expression of miR159 gene was more responsive to osmotic stress than ionic stress. The authors studied expression of one of the predicted target MYB under the same stress (NaCl or PEG) conditions to study the changes in target gene expression in response to over or under expression of miR159. The results on the expression of specific miR159 and its targets could be useful in developing appropriate markers for selection of tolerant cultivars in sugarcane.

The ease with which many sugarcane genotypes can now be transformed, together with the identification of the sequences of thousands of genes that this plant expresses raises the possibility of altering the expression of specific genes in the plant and identifying the effects this modification has on the plant's phenotype. The development of RNAi and anti-sense technology allows for gene down-regulation [57]. In sugarcane commercial fields flowering is an undesirable characteristic. At the Cane Technology Center (CTC) in Piracicaba, Brazil, the anti-sense expression of a single gene found in the Sucest database, and involved in flower development has produced non-flowering plants of a sugarcane variety that, otherwise, flowers heavily every year. Down-regulation and over-expression of genes involved in carbohydrate metabolism is also the topic of many globally, with the aim of increasing the content of sucrose and other metabolites. An initial data mining of the large sugarcane EST database for genes involved in carbohydrate metabolism allowed the identification of more than 150 expressed sequences representing these genes. Six of them were selected for individual down regulation in transgenic sugarcane plants, trying to shunt sugar in the direction of sucrose accumulation. Preliminary results indicated that for at least three of these genes, down regulation increased up to 30% the sugar accumulated by transgenic sugarcane plants with no interference in their phenotype. The results of expression studies mentioned above will certainly help to identify other target genes to control sugar metabolism as well as biotic and abiotic stresses.

Quorum Quenching: A New Hope

Disease outbreaks caused by phyto-pathogenic bacteria are considered to be major constraints to the development of the agriculture sector. So far, the strategies advocated for the management of bacterial diseases in plants (use of antibiotics and disinfectants) have shown limited success in the prevention or cure of bacterial diseases [1]. Moreover, the frequent use of such chemicals in therapeutic doses has led to the build-up resistance among bacterial population as these do not directly interfere with the bacterial communication network yielding uneven management of such diseases.

Therefore, there is an urgent need to develop alternative ways to interrupt the virulence factor leading to serious expression of disease by cell-to-cell communication system. This phenomenon of cell to cell communication in bacteria is known as quorum sensing [23] which relies on the production and sensing of one or more secreted low-molecular-mass signal molecules. This mechanism involves the diffusion or secretion of different compounds from bacteria as their populations grow. The compounds such as butyrolactones and "auto-inducing peptides" (autoinducers) and N-acyl homoserine lactones, quinolones, or cyclic dipeptides in gram-positive and gram-negative bacteria, respectively [27,20] either diffuse away from the cell and/or interact with inter or intra cells by attaching or activating specific cell surface receptors. Once the events in QS are detected by bacterial community it leads to the induction of genes that control a variety of biological functions, including the production of antimicrobial substances and protection against the host's defense mechanisms [53]. The knowledge of the molecular mechanisms underlying these QS signaling systems and their co-ordination to control virulence factors enlighten us with a completely new perspective to manage the potential action of pathogenic bacterial by disrupting QS mechanisms (i.e. quorum quenching, QQ).

The QQ techniques comprise various steps like the inhibition of signal molecule biosynthesis, the application of quorum sensing antagonists (including natural occurring as well as synthetic halogenated furanones, undefined exudates of higher plants and algae, the chemical inactivation of

quorum sensing signals by oxidised halogen antimicrobials, signal molecule biodegradation by bacterial lactonases and by bacterial and eukaryotic acylases and the application of quorum sensing agonists . Several QS quenchers have been identified. Transgenic plants that express one of these bacterial quorum quenchers have been constructed and appeared to be more resistant to sensing pathogens than their wild-type parents [19].In light of recent research advancements in QS mechanisms, [34] reviewed the possibilities of phyto-bacterial disease managements especially by focusing on the principle of QS and its intervention QQ, their molecular aspects and potential implications of QQ to save agricultural crops from important bacterial intruders.

Role of Array Technologies

The first transcriptomics tools developed for sugarcane were macro-arrays, prepared using plasmid DNA or DNA fragments spotted on to nylon membranes followed by cross-linking. These have been used to investigate differences between immature and mature leaf as well as immature and mature internodal tissue [8,9], the effect of exposure of young sugarcane plantlets to cold [47], tissue profiling of

transcriptionally active transposable elements [14], expression profiling of genes with established roles in sucrose accumulation through stem development and across various genotypes [69], identification of methyl jasmonate-responsive genes in sugarcane leaves [16], identification of genes responsive to the application of ethanol on sugarcane leaves [7], and, most recently, expression profiling of sink activities after source perturbation by shading [43] and identification of new ABA- and MEJA-activated sugarcane bZIP transcription factors [59]. A feature of their continued use, despite the advent of new technologies, is their convenience, especially if assaying a smaller number of genes, low cost and adaptability, particularly since all the groups using macro-arrays also have access to EST clone collections. Researchers were able to identify 132 sugarcane cDNA clones differentially expressed in immature and maturing internodal tissue, using a 400 clone nylon macroarray derived from reciprocal subtractive hybridization libraries [8]. Most of the genes with putative identities were involved in stress responses, regulatory processes, carbohydrate metabolism and cell wall metabolism but not specifically in sucrose metabolism. A slightly later study profiling immature leaf, mature leaf, immature stem and mature stem using a 1,000 clone macroarray detected 61 transcripts that accumulated more highly in leaf tissue and 25 transcripts that had higher expression in the stem. Sucrose synthase 2 was the only differentially expressed sucrose metabolism-related transcript and, as reported previously, it was preferentially expressed in immature leaf [9]. The use of larger arrays (2 x 768 random EST targets), combined with a clearly defined and short cold treatment, resulted in the identification of 14 of 34 identified cold- inducible ESTs that were homologous to previously described cold- or drought-inducible genes [47].

The advent of micro-arrays represented a quantum leap in expression profiling. The ability to deposit larger numbers of probes robotically on to a glass slide resulted in the first truly large-scale expression profiling analyses and also offered the first opportunity to simultaneously assay two samples using the one microarray. These were first used in sugarcane by [11,10] to profile the expression differences between immature stem (meristem and internodes 1-3), maturing stem (internode 8) and mature stem (internode 20) of sugarcane, using the GeneChip® Sugar Cane Genome Array produced by Affymetrix assaying up to 4715 non-redundant random ESTs. It enabled to distinguish transcript expression differences clearly between various members of the cellulose synthase subunit family and the allied cellulose synthase-like gene family and also identified many co-expressed transcripts with involvement in cell wall synthesis and degradation as well as lignification. The study elucidated carbohydrate metabolism-related transcripts and found that putative sugar transporters were highly expressed in maturing stem and that coordinated expression of enzymes involved in sucrose synthesis and cleavage was also evident. Another study concentrated on transcripts associated with stem maturation, finding that transcripts associated with fibre metabolism, defence and stress mechanisms, especially putative dirigent proteins, were the most highly expressed transcripts in maturing stem. Defence signalling was further explored in roots by [6] who used these arrays to profile the transcriptional response of sugarcane roots to methyl jasmonate. They identified several transcripts induced by exogenous

application of methyl jasmonate, including PR-10, lipoxygenase and dirigent as well as enzymes involved the metabolism of phenolics and oxidative stress.

This microarray was further customised with additional clones, mainly derived sugarcane kinase genes and used to study signal transduction-related responses to phytohormones and environmental challenges in sugarcane including methyl jasmonate, abscisic acid, insect (*Diatraea saccharalis*), and endophytic bacteria (Gluconacetobacter and Herbaspirillum) elicited responses. Adopting an outliers searching method, 179 genes with strikingly different expression levels were identified as differentially expressed in at least one of the treatments analysed. A customed 3,598 clone microarrays was used to profile the effect of elevated CO_2 on sugarcane leaves which indicated that increased CO_2 concentration resulted in a 50% increase in biomass, altered photosynthesis and an increase in cellulose content. A total of 33 genes were found responsive to high CO_2 treatment including those involved in photosynthesis and carbohydrate metabolism genes. This may be directly related to the increased biomass phenotype observed.

Microarrays are very powerful tools for the dissection of differential transcript expression but two-color microarray experiments nearly always use different clone sets attached to the substrate and, in any case, are designed as either direct comparison between pairs of samples, closed loops or use a common reference sample [12]. This makes individual microarrays difficult to incorporate into any later experiments, particularly those generated in other laboratories. In addition, standard microarrays prepared using DNA fragments exhibit problems with hybridization kinetics and often show great variability in spot fluorescence intensity for the same transcript represented by DNA fragments of varying lengths. The variability of hybridization kinetics can be lessened by the use of oligonucleotide arrays since all of the probes attached to the substrate are of equal length.

Oligonucleotide array design can also assist in the ability to discriminate closely related members of multi-gene families and the use of a one-colour system allows for the building of an array bank, either within a laboratory or in a public database e.g. Gene Expression Omnibus (GEO-http://www.ncbi.nlm.nih.gov/geo/) or Array Express (http://www.ebi.ac.uk/microarray-as/ae/) [4, 48]. Oligonucleotide array technology consists of either multiple short oligonucleotides representing one transcript or several long oligonucleotides per transcript synthesized in situ on a glass or quartz substrate. These arrays offer the opportunity to use all transcript sequence data that is publically available since there is no requirement to physically possess the DNA fragments. The first company to offer short oligonucleotide technology was Affymetrix, Inc. Their expression arrays routinely represent each transcript using at least one probe set of 11 probe pairs each, with each probe being 25 nucleotides long. Each probe pair contains one perfect match oligonucleotide and one with a central mismatch, the latter acting as a control for non-specific binding and the probe set is preferentially complementary to regions of 3' untranslated region sequence. Affymetrix offers a standard suite of arrays designed to assay each of five monocot crops (rice, barley, wheat, maize and sugarcane) but the number of sugarcane genes represented are relatively low (around 7,000). Alternatively, custom arrays can be designed by

Affymetrix or other companies such as Agilent Technologies or Roche NimbleGen to represent larger numbers of transcripts. These arrays were also used by [43] to profile leaves whose sugar content had been manipulated by cold-girdling. Numerous differentially expressed transcripts involved in photosynthesis, assimilate partitioning, cell wall synthesis, phosphate metabolism and stress were identified. The expression profiles generated can be considered to be foundation sets since, due to the consistency of array design and the use of one-color technology, these expression profiles could easily be reanalysed in the context of other expression profiles generated using these arrays.

Even though oligonucleotide array technologies have solved many of the well-documented problems encountered with microarrays, there is still the problem of a limited dynamic range, usually only detecting more abundant transcripts to the detriment of low-copy transcripts. More importantly, array technologies only report the transcription levels of the transcripts that are included in the array, possibly missing out on key information for identifying critical pathways that underpin cellular function.

Future Outlook

Sugarcane is one of the important industrial crops because of its economic importance and by-products. More than half of the world's sugar is derived from sugarcane. Conventional breeding methods have greatly contributed to sugarcane improvement; however limitations such as complex genome, narrow genetic base, susceptibility to biotic and abiotic stresses and long duration to breed elite cultivars still impose a big challenge to sugarcane scientists. Sugarcane diseases representing fungal, bacterial, viral and mycoplasma imposes a serious threat to the cultivation of this crop. With the advent of recent techniques, it is now important that conventional breeding can be integrated with genomic/biotechnological tools RNAi, ABC transporters, quarm quenching, genetic transformation etc., so that maximum advantage can be taken of new opportunities to manage the sugarcane diseases. Advancements in plant biotechnology, especially genomics have paved the way for a detailed understanding of the mechanisms underlying various biotic and abiotic stress responses. At present, both micro and macro arrays are being explored for identification of genes expressed specifically in stems, disease resistance genes and those involved in carbohydrate metabolism. Further, gene silencing is being used in transgenic research aimed at down-regulation of endogenous genes in sugarcane. Some of the important challenges include gene discovery, transgenics and controlled transgene expression, sucrose metabolism and photosynthesis. Ideally, this will involve transgene testing and/or fine mapping, both of which are resource hungry endeavours. What is clear is that genomics cannot be used alone for the improvement of sugarcane with respect to diseases but is an essential component of a system biology approach.

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