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DIFFERENTIAL EXPRESSION ANALYSIS OF GIBBERELLIN-INDUCED STALK ELONGATION GENES IN SUGARCANE (*SACCHARUM* HYBRID) BY DIFFERENT TECHNOLOGIES

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ABSTRACT

The study was to investigate the molecular basis of the differential expressions of gibberellic acid (GA3)-induced stalk elongation genes in sugarcane and isolate elongation-related genes. The plants were applied with GA3 with concentration of 200 mg·L^{-1} by foliar spray in early elongation stage, while water was used as control. The total RNA was extracted in time courses (0, 6, 12, 24, and 48 h), and then two mixed RNA pools were obtained by mixing equal quantities of total RNA from the GA3 treatment and control. The differential expression of genes were analyzed with three different techniques, *i.e*., cDNA-amplified fragment length polymorphism (cDNA-AFLP), sequence-related cDNA-amplified polymorphism (cDNA-SRAP) and cDNA-amplified start codon targeted (cDNA-SCoT). There were 26,000 gene segments obtained by the three techniques with different primer combinations, and 118 different fragments were verified with reverse Northern hybridization. Among these gene segments, 110 transcript-derived fragments (TDFs) were differentially represented, of which 53 were up-regulated and 57 down-regulated by GA3. The results of BLAST analysis showed that the 110 TDFs belong to seven categories according to gene functions: energy and metabolism-related genes, genes of unknown functional proteins, unknown genes, cell wall biosynthesis and modification related genes, transcription factor-related genes, and plant resistance related genes. The results of amplification are varied for different techniques, but they resulted in the same TDFs, such as GID1, 1, 3, 4-trisphosphate 5/6-kinase, S-adenosylmethionine *etc*. The results also indicate that different technologies combined analyses will obtain more comprehensive information. Based on these results, interacting pathways of stalk elongation processes were generated. The information obtained in this study will help in identifying functional genes involved in stalk elongation of sugarcane.

KEYWORDS: cDNA-AFLP; cDNA-SRAP; cDNA-SCoT; differential expression; gibberellin; sugarcane.

INTRODUCTION

Sugarcane is the most important sugar crop in China; since 2000, the proportion of cane sugar, in relations to all types of sugars, is over 90%, and exceeded 94% in recent years (Che *et al*., 2011). China is the world's third largest sugar consumer and producer. Sugarcane is a primary economic crop to harvest stalk, and proper regulation of its stalk elongation is important in commercial production. One of the most important functions of gibberellin is to promote stem/stalk elongation and increase plant height (Lange, 1998). The effect of gibberellin on promoting stalk elongation of sugarcane has been demonstrated by many previous studies (Most and Vlitos, 1966; Moore and Buren, 1978; Kaufman *et al*., 1981; Buren *et al.*, 1979; Li and Su, 1984; Wu *et al*., 2010). It has been reported numerous times that the molecular mechanism of gibberellin promotes internode elongation in rice (Sakamoto *et al*., 2001; Ashikari and Matsuoka, 2003), pumpkin (Niki *et al*., 2001), peas (Martin *et al.*, 1996), tomato (Carrera *et al*., 1999), *Arabidopsis* (Huang *et al*., 1998) and other plants, especially significant improvements made in rice and *Arabidopsis thaliana*. The gibberellin signal transduction and biosynthesis pathways have been genetically characterized in detail in the model plants *Arabidopsis thaliana* (thale cress) and *Oryza sativa* (rice) (Yamamoto *et al*., 2010; Sun, 2010). We have studied the physiological mechanism of gibberellin-induced stalk elongation in sugarcane (Wu *et al*., 2009a, 2010b), but the molecular mechanism of gibberellin-induced stalk elongation in sugarcane still remains unknown.

The mRNA technique was established by Liang and Pardee (1992), which has been widely applied in biotechnology, especially in the screening of new genes related to some phenotypes. Since then, many approaches have been developed for analyzing differential gene expression at mRNA level (Nettuwakul *et al*., 2007; Huang *et al*., 2007; Wee *et al*., 2008; Polesani *et al*., 2008; Zamharir *et al*., 2011; Xu *et al.*, 2011). cDNA-amplified fragment length polymorphism (cDNA-AFLP) is one of the most robust and sensitive transcriptomic technologies for gene discovery and offers an attractive method to

identify genes involved in plants, animals, microbes, *etc*. (Vuylsteke *et al.*, 2007). Li and Quiros (2001) developed the sequence-related amplified polymorphism (SRAP) technique for analysis of differential expression, which has been widely used in rice, cabbage, melon, etc. In 2009, Collard and Mackill (2009) developed the start codon targeted polymorphism (SCoT) technique based on the translation start codon, and successfully applied it in rice. It is important to note that the primers for SCoT marker analysis are designed based on the short conserved region surrounding the ATG translation start codon. Specific nucleotides in the primer sequence are fixed: the ATG codon (at positions +1, +2 and +3), 'G' at position +4, 'C' at position +5, and A, C and C at positions +7, +8 and +9, respectively (Collard and Mackill, 2009). We used cDNA-SCoT to analyze the gene differential expression in sugarcane under low temperature stress, and ideal results were obtained (Chen *et al.*, 2010). In recent years, many new alternative and promising marker techniques have been developed. These techniques include inter retrotransposon amplified polymorphism, retrotransposon microsatellite amplified polymorphism (Kalendar *et al.*, 1999), sequence-related amplified polymorphism (Li and Quiros, 2001), and target region amplified polymorphism (TRAP) (Hu and Vick, 2003). Coupled with the rapid growth of genomics research, there has been a trend away from random DNA markers towards gene-targeted markers (Andersen and Lubberstedt, 2003; Gupta and Rustgi, 2004). Genome sequence data offer enormous potentials for the development of new markers in diverse plant species (Holland *et al.*, 2001). There are advantages and disadvantages for each system. For example, differential display is a simple method but delivers poor consistency (Ledakis *et al.*, 1998), and the coding region is difficult to amplify. cDNA-AFLP is more consistent but the main disadvantage of this method is its complexity, including numerous time-consuming steps (*i.e*., reverse transcription, digestion, ligation and amplification), making it difficult to optimize the conditions. SRAP and SCoT, on the other hand, is a new PCR marker system that's both simple and reliable" should be "are two new simple and reliable PCR marker systems. The two techniques can be adapted to use in different crops to work on genome mapping, gene tagging, genomic and cDNA fingerprinting, and can be widely applied to different fields in different laboratories (Collard and Mackill, 2009). There have been some reports about using different molecular markers to analyze genetic diversity in plant (Mattioni *et al.*, 2002; Muthusamy, 2008; Spackman *et al.*, 2010). However, we have not seen reports on comparison of different techniques in analyzing gene differential expressions in plant. Considering the potentials of the different techniques based on different principles, the present study aim to evaluate the usefulness of expression technologies via. cDNA-AFLP, cDNA-SRAP and cDNA-SCoT, in assessing and analyzing the differential expressions of gibberellin-induced stalk elongation genes in sugarcane in order to provide references for differential gene expression.

MATERIALS & METHODS

Sugarcane (*Saccharum* hybrid cv. ROC22.) setts were planted in pots in the greenhouse at Guangxi University. The plants were sprayed with gibberellic acid (GA_3) at 200 mg/L in early elongation stage as treatment and sprayed with distilled water as control. Young stalks (the part from apical meristem to internode +1 which was wrapped by the top visible dewlap leaf) of 6 plants were taken in each treatment as sample at 0, 6, 12, 24 and 48 h after treatment, respectively. All the samples were immediately stored in a -80ºC freezer. The standard GA_3 was bought from Shanghai Dobio Biotech Co., Ltd. The related reagents, engineering bacterium JM-109, carrier PMD18-T, cDNA–AFLP, cDNA-SRAP and cDNA-SCoT, were bought from TakaRa. The reverse transcription reagent (SMART PCR cDNA bio-synthesis kit) was bought from Clontech. The primer synthesis and gene sequencing were completed by Shanghai Dobio Biotech Co., Ltd.

RNA extraction and cDNA synthesis

Total RNA was extracted from the young stem using isothiocyanate (Wu *et al.*, 2009b). The RNAs were quantified by determining ratios of OD260/28 and OD260/230, and agarose gel electrophoresis was used for analyzing its integrity. The concentration of RNA from different treatments was regulated to equal. Equal amount of RNA of the control and treatment was used to make two sample pools. The cDNA synthesis was done according to the SMART^{TM} cDNA Library Construction Kit User manual. The cDNA purification was performed using PCR Purification Kit (Qiagen).

Amplification

cDNA-AFLP: The AFLP template was produced by 230 ng of cDNA using the restriction enzymes *Eco*RI for 2 h and *Mse*I for 4 h. The ligation reaction was conducted with T4-DNA ligase (Clontech) for 3 h. Preamplification was performed using 2 µl of ligation product as a templates and primers of Mse-P and Eco-P in 25 L reaction volume, which was initiated at 94 ºC for 5 min, and then followed by 22 cycles at 94 ºC for 30 s, 52 ºC for 30 s, 72 ºC for 60 s, and terminated at 72 ºC for 7 min. The preamplification product was diluted for 40 times as templates for selective amplification. Selective amplification was initiated at 94 ºC for 2 min, followed by 12 cycles at 94 ºC for 30 s, 65 ºC for 30 s, -0.7 ºC / cycle, terminated at 72 ºC for 1 min; and 23 cycles 94 ºC for 30 s, 56 ºC for 30 s, 72 ºC for 60 s, terminated at 72 ºC for 7 min. Fragments were separated on a sequencing polyacrylamide gel (6% bis-acrylamide, 7 M urea, 1×TBE) and visualized by silver staining.

cDNA-SRAP: The PCR reaction system included 10×PCR buffer (+Mg^{2+}) 2 µL, 10 nM forward primer 1.0 µL, 10 nM reverse primer 1.0 µL, 2 mM dNTPs 2 µL, 10 times diluted product of first-strand cDNA 2 µL, 5 U.μL^{-1} Taq plus DNA polymerase, adding ddH_2O to 20 µL. The reaction condition of PCR was as follows: 3 min denaturation at 94°C, followed by 60 s denaturation at 94°C, 60 s annealing at 35°C (35 cycles), 60 s extension at 72°C (5 cycles), 60 s denaturation at 94°C, 60 s annealing at 56°C, 60 s extension at 72°C (35 cycles) and 7 min at 72°C. The amplification product 3 µL was placed in 1.2% agarose gel electrophoresis, examined with selected primers, and then differential fragments were detected by

electrophoresis through non-denaturing polyacrylamide gel.

cDNA-SCoT: The PCR reaction system was consisted of 13.70 µL ddH2O, 2 µL 10×PCRbuffer (+Mg^{2+}), 0.4 µL 10 mM dNTPs, 2 µL 10 times diluted product of cDNA, 1.6 µL 10 µM primer, 0.3µL 5 U.$µl^{-1}$ Taq plus DNA polymerase. The reaction of PCR was subjected to 35 cycles of amplification (3 min denaturation at 94°C, followed by 30 s for denaturation at 94°C, 60 s for annealing at 50°C, 90 s for extension at 72°C, and 7 min at 72°C). Three µL amplification products from 1.2% agarose gel electrophoresis were examined using selected primers, and then 1.2% agarose gel was used for electrophoresis to select differential fragments.

Reverse Northern blotting

Reverse Northern blotting was conducted using DIG High Prime DNA Labeling and Detection Starter Kit I (Roche, Mannheim, Germany) bought from Roche, operated according to the product instruction.

Cloning, sequencing and sequence comparison analyses of differential fragments

cDNA-AFLP: The bands corresponding to the selected TDFs were excised from gel and soaked in 30 µL of TE buffer (10 mM Tris–HCl [pH 8.0], 1 mM EDTA) at 37°C for 3 h to elute the DNA. After centrifugation at 12000 rpm for 10 min, a 4 µL aliquot was used for re-amplification of the fragments in a 20 µL of reaction mixture using the same primers as those used for preamplification. The PCR thermal cycling conditions were as follows: 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C for 30 cycles. PCR products were characterized by separation on a 1.0% agarose gel, cloned into the pMD19-T vector (Takara), and sequenced.

cDNA-SRAP: Only the DNA bands unique for GA exposed plants were removed from polyacrylamide gel following the procedure described by Thanananta *et al*., (2006) and cloned into pGEM-T plasmid (Promega USA).

cDNA-SRAP: DNA fragments from agarose gel were purified using 3S EZ-Resin DNA Gel Purification Kit (Shenzhen Smartshining Biotechnologies CO., LTD). The sequence comparisons were done with BLAST program using the NCBI website (www.ncbi.nlm.nih. gov/ BLAST/).

RESULTS

Results of cDNA-AFLP analysis

A total of 11000 amplicons (size varying between 50 and 700bp) were produced by examining 186 differently expressed fragments with 169 cDNA-AFLP primers, of which 112 were up-regulated, 94 showed high expression; and 74 were down-regulated, 58 showed high expression (Figure 1). After denaturing gel electrophoresis, the differentially expressed fragments with better repeatability were recovered, and 118 different fragments were identified (Figure 1). These fragments were used for reverse Northern blotting, and the results showed that there were 56 fragments having significant differences, and 61 having no significant difference (Fig. 2). There were 38.98% of false positives in the recovery of different fragments. The differentially represented transcript-derived fragments (TDFs) were cloned, sequenced, and analyzed with Blastx tool. The results showed that the TDFs were related with GA binding to its receptor *GID1*, led to DELLA family identification of transcription factors, role of regulatory factors inhibit the gibberellin signal, cell wall biosynthesis and modification, affecting growth and differentiation of cells, cells skeleton formation, protein transportation and secretion, glycolysis for energy generation, catalyzing varieties of oxidation -reduction reactions. Some genes were unknown functional proteins since they exhibit no similarity to those in the GenBank (TABLE 1).

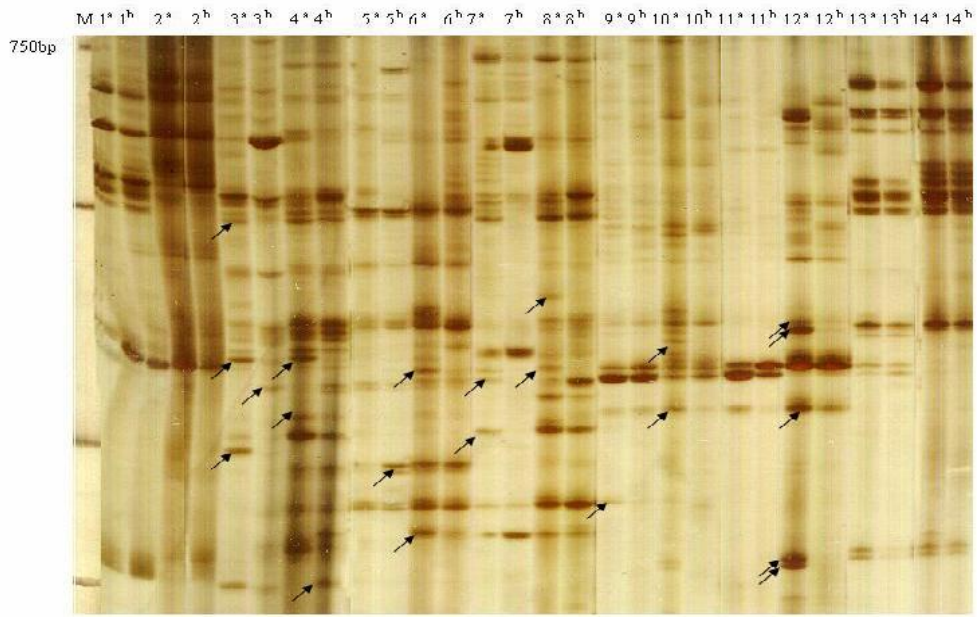

FIGURE 1: Parts of the fragments separated by gel electrophoresis after amplification of elongation gene cDNA derived from control (a) and GA_3 treated (b) sugarcane internodes analyzed using cDNA-AFLP with different primers. M: marker (DL2000); lanes 1-14 show

the primer combinations M3E2, M5E12, M6E12, M8E1, M9E3, M10E8, M11E13, M13E10, M11E7, M10E7, M10E14, M12E5, M14E9 and M15E16, respectively. a: control; b: treatment with GA_3. The arrows indicate differentially represented fragments.

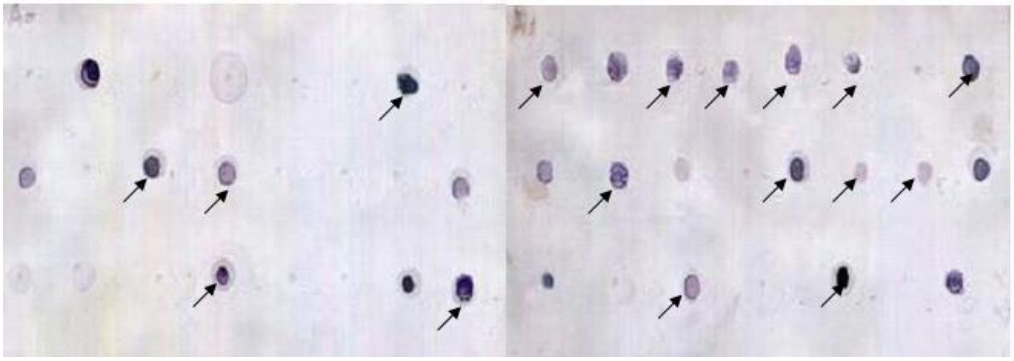

FIGURE 2: The results of reverse Northern blotting for parts of the fragments separated by gel electrophoresis after amplification of elongation gene cDNA derived from control (left) and GA_3 treated (right) sugarcane internodes . The arrows indicate differentially represented fragments.

TABLE 1: cDNA-AFLP analysis of TDFs regulated with GA_3 in young stalk of sugarcane

TDFs	Size (bp)	Homologous gene	Function category	E value
TDFs1	186	*Zea mays* contig61137		2.00E-78
TDFs2	139	Ras-related protein	Affecting growth and differentiation of cells, cells skeleton formation, protein transportation and secretion, etc.	7.00E-55
TDFs3	153	Glyceroldehyde-3-phosphate dehydrogenase GAPC2	Regulating glycolysis for energy generation	3.00E-65
TDFs4	156	Hypothetical protein		5.00E-33
TDFs5	112	Oxidoreductase	Catalyzing varieties of oxidation -reduction reactions	3.00E-43
TDFs6	100	*Saccharum officinarum* clone SCCCCL3001H07		4.00E-32
TDFs7	173	Hypothetical protein		4.00E-50
TDFs8	164	Hypothetical protein		3.00E-40
TDFs9	206	GID1-like gibberellin receptor (GID1 gene)	Binding GA to its receptor *GID1*, led to DELLA family identification of transcription factors, regulating the factors that inhibit the gibberellin signal	3.00E-91
TDFs10	193	Oxidoreductase	Catalyzing varieties of oxidation - reduction reactions	4.00E-16

Results of cDNA- SRAP analysis

Seven hundred cDNA-SRAP primer pair combinations generated 15000 cDNA fragments (size varying between 50 and 750 bp). In order to ensure reliability of differential expression, only bands with significant differential expression were further characterized. Based on this standard, 134 different cDNA fragments were selected (FIGURE 3).

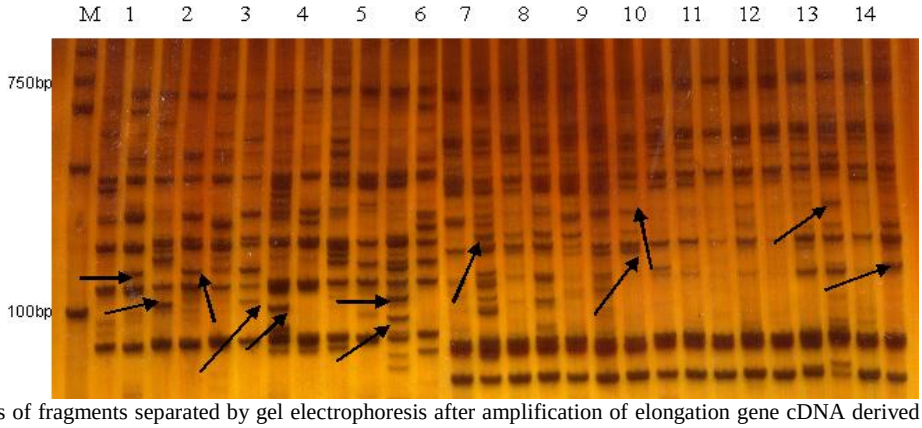

FIGURE 3: Parts of fragments separated by gel electrophoresis after amplification of elongation gene cDNA derived from control and GA_3 treated sugarcane internodes analyzed using cDNA-SRAP with different primers. M: marker (DL2000), numbers 1-14 show the primer combinations em21me15, em21me17, em21me19, em21me20, em22me16, em22me18, em22me19, em22me22, em23me15, em23me23, em23me24, em23me25, em24me15 and em24me16, respectively, and the templates labeled with odd (1, 3, 5, 7, 9, 11, 13)

and even (2, 4, 6, 8, 10, 12, 14) numbers are the control and treatment with GA_3, respectively. The arrows indicate differentially represented fragments.

These fragments were used for reverse Northern blotting, and the results (FIGURE 4) showed that there were 24 fragments having significant differences, as differentially represented TDFs and 33.82% of false positive differences in the recovery of different fragments. Twenty-four differentially represented TDFs were cloned and sequenced, and then BLAST analysis was performed with Blastx tool. The analytical results showed that 14 TDFs had relatively high similarity to those in the database of GenBank, which included gibberellin receptor and the key enzymes of polyamines and ethylene synthesis precursor, and energy and metabolism signal transduction (TABLE 2).

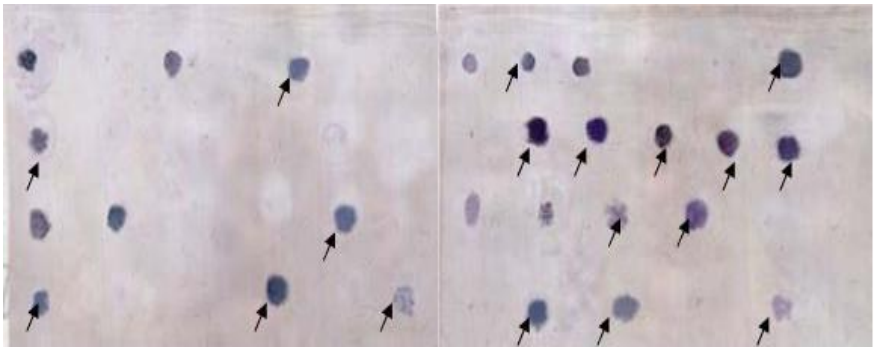

FIGURE 4: The results of reverse Northern blotting for parts of the fragments separated by gel electrophoresis after amplification of elongation gene cDNA derived from control (left) and GA_3 treated (right) sugarcane internodes . The arrows indicate differentially represented fragments.

TABLE 2: Functional analysis of parts of S-TDFs regulated with GA_3 in young stalk of sugarcane

S-TDFs	Size (bp)	Homologous gene	Function category	E value
S-TDFs 1	148	S-adenosylmethionine decarboxylase	Key enzyme catalyzing S-Adenosyl-methionine to be decarboxylated-SAM in polyamine synthesis	6e-47
S-TDFs 2	147	1, 3, 4-trisphosphate 5/6-kinase family protein	Possibly participate in the stress signal transduction of ABA-independent pathway, therefore, possibly interact with CSN and participate in the plant light morphogenesis	1e-58
S-TDFs 3	147	*Zea mays* PCO131983 mRNA sequence		1e-93
S-TDFs 4	144	ZM_BFb0178O20 mRNA		1e-93
S-TDFs 5	116	Hypothetical protein		2e-33
S-TDFs 6	167	Methylthioribose kinase	Catalyze Met- synthesizing	1e-40
S-TDFs 7	76	Ubiquinol-cytochrome C Reductase complex ubiquinone binding protein		3e-21
S-TDFs 8	85	Solanum lycopersicum C11HBa0054I23		2e-09
S-TDFs 9	206	Photosystem I reaction center	Photosynthesis	1e-36
S-TDFs 10	104	Ribosomal protein	Ribosomal proteins	1e-43
S-TDFs 11	181	*Zea mays* PCO131983 mRNA sequence		7e-73
S-TDFs 12	182	ZM_BFb0309D13 mRNA		6e-78
S-TDFs 13	119	1, 3, 4-trisphosphate 5/6-kinase family protein	Possibly participating in the stress signal transduction of ABA-Independent pathway, therefore, possibly interacting with CSN and participates in the plant light morphogenesis	2e-46
S-TDFs 14	370	GID1-like gibberellin receptor	Recognized by the DELLA family transcription regulation factors that suppress the gibberellin signal function	0.0

Results of cDNA- SCoT analysis

Total RNA extraction from sugarcane young stalk was used to synthesize first- and second- strands cDNA. The first-strand cDNA, first-strand cDNA purification products, second-strand cDNA and second-strand cDNA purification products were used as template in PCR for amplification. The optimizing reaction system created includes the template of second-strand cDNA purification products diluted for 10 times, and 35 cycles of amplification. Forty six cDNA- SCoT single primers produced about 700 amplicons (size varying between 50 and 1200 bp) of which 96 different cDNA fragments were selected (FIGURE 5). After reverse Northern blotting analysis, 56 differentially represented TDFs were selected

for cloning and sequencing analysis, and the results (FIGURE 6) showed that there were 30 fragments having significant differences, 26 having no significant difference. There were 23.20% of false positive differences in the recovery of different fragments. Thirty differentially represented TDFs were cloned and sequenced, and then BLAST analysis was performed with Blastx tool. The analytical results showed that some TDFs had high similarity to those in the database of GenBank, which included gibberellin receptor, the key enzymes of polyamines and ethylene synthesis precursor, protein kinase, zinc finger protein, energy and metabolism (TABLE 3).

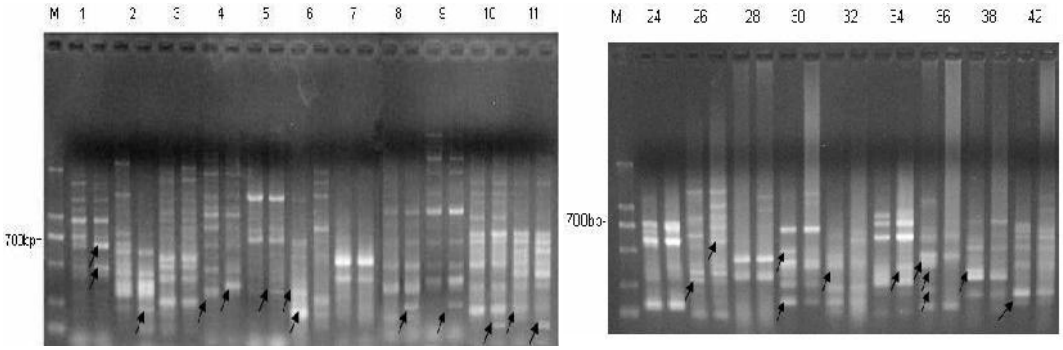

FIGURE 5: Parts of the fragments separated by gel electrophoresis after amplification of elongation gene cDNA derived from control and GA_3 treated sugarcane internodes analyzed using cDNA-ScoT with different primers. M: marker (DMarker II); 1–11; 24–42: different primers and the templates numbered with odd and even numbers are the control and treatment with GA_3, respectively. The arrows indicate differentially represented fragments.

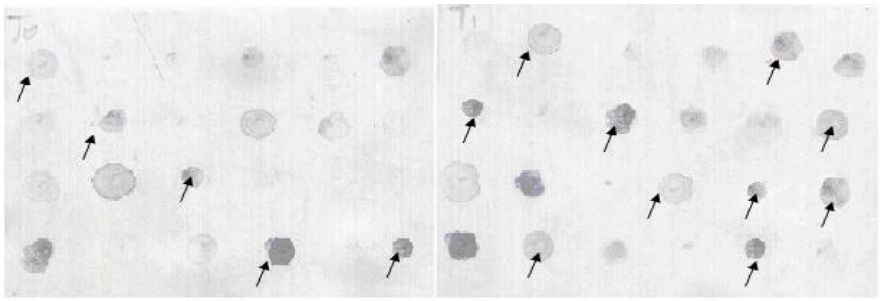

FIGURE 6: The results of reverse Northern blotting for parts of the fragments separated by gel electrophoresis after amplification of elongation gene cDNA derived from control (left) and GA_3 treated (right) sugarcane internodes . The arrows indicate differentially represented fragments.

TABLE 3: cDNA-SCoT analysis of parts of TDFs regulated with GA_3 in young stalk of sugarcane

ST-TDFs	Size (bp)	Homologous gene	Function category	E value
ST-TDFS1	435	*Hordeum vulgaresubsp* FLbaf60d04		2e-117
ST-TDFs2	961	Serine/threonine-protein kinase receptor	Transforming growth factor-βs family members that function with a wide range of effects on cells.	0.0
ST-TDFs3	98	Transmembrane BAX inhibitor motif-containing protein	Cell apoptosis.	3e-33
ST-TDFs4	52	Ribosomal RNA gene	Constituting an integral part of ribosome.	1e-93
ST-TDFs5	592	Zinc finger CCCH type domain-containing protein	Variety of functions in different organizations and different plant development stages.	0.0
ST-TDFs6	128	Ribosomal RNA gene	Constituting an integral part of the ribosome.	4e-54
ST-TDFs7	369	*Sorghum bicolor* mitochondrion, complete genome	Mitochondria controlled variety of life activities, deciding plant growth and development.	0.0
ST-TDFs8	890	Sorghum bicolor cultivar BTx623 chloroplast, complete genome	Coding the proteins closely related to photosynthesis and some ribosomal protein.	0.0
ST-TDFs9	223	Sorghum bicolor cultivar BTx623 chloroplast	Coding the proteins closely related to photosynthesis and some ribosomal protein.	3e-112
ST-TDFs10	164	Hypothetical protein		2e-62
ST-TDFs11	340	*Zea mays* ZM_BFc0129I19		2e-140
ST-TDFs12	170	Hypothetical protein		1e-70
ST-TDFs13	632	S-adenosylmethionine synthetase	Biochemical reactions involved in more than 40 kinds of main plant transfer methyl, propyl and the transfer of sulfur transfer reaction of ammonia and other important physiological processes.	0.0
ST-TDFs14	786	*Zea mays* ZM_BFc0091N05		0.0
ST-TDFs15	367	Zea mays SHL1(LOC1-		4e-137

		00285536)		
ST-TDFs16	252	GID1-like gibberellin receptor	Binding GA to its receptor GID1 led to DELLA family identification of transcription factors, regulating the factors inhibiting gibberellin signal.	3e-122

DISCUSSION

The emergence of differential gene expression technology provides a strong arm for studying genes. At present, the main technologies for analyzing differential gene expression include differential hybridization, subtractive hybridization of cDNA (SHD), mRNA differential display (DD), restriction fragment differential display PCR (RFDD-PCR), suppression subtractive hybridization (SSH), represential display analysis (RDA), reciprocal subtraction differential RNA display, serial analysis of gene expression (SAGE), electronic subtraction, DNA microarray, DNA amplified fragment length polymorphism (cDNA-AFLP), cDNA-SRAP, cDNA array technologies, RNA Seq, etc. Along with the development of molecular biology, differential gene expression technologies have been developed and applied to many biological problems including the gene expression induced by GA_3 (Chen *et al*., 1995; Knaap and Kende 1995; Knaap *et al*., 1997; Fabian *et al*., 2000; Mo *et al*., 2000; Van der Knaap *et al.*, 2000; Lou *et al*., 2001; Yu 2005). There have been many reports of these technologies related to principle, method, peculiarity, *etc*., and analyses on their advantages and shortcomings (Money *et al*., 1996; Habu *et al.*, 1997; Lu and Cao 2002; Jiang *et al.*, 2003; Breyne *et al.*, 2003; Tong, 2006; Wu *et al.*, 2009c; Hu *et al*., 2010).

In this study, we used cDNA-AFLP, cDNA-SRAP and cDNA-SCoT to analyze differential expressions of gibberellin-induced stalk elongation genes in sugarcane. The results in the present study indicate that cDNA-AFLP is highly reproducible, reliable, accurate and efficient, but it has the shortcomings of many operating steps, so it is time consuming, expensive, difficult to operate, and needs strict template. On the other hand, the cDNA-SRAP's advantages are easy to operate, produce abundant bands, able to test many samples simultaneously, able to test low levels of gene expression, but it has disadvantages, such as having too many primers (more than 1000 primer combinations), time consuming and low repeatability. CDNA-SCoT uses single primers, has less primers (46 single primers), and is easy to operate with low cost and low rate of false positives, but it produces less bands. The results in the present study are in accordance with the reports from other plants and prokaryotes (Tong, 2006; Nettuwakul *et al.*, 2007; Hu *et al.*, 2010). We can conclude that different technologies have their own advantages and disadvantages, and it is difficult to obtain complete information using any single technology, and more convincing, reliable and comprehensive information could be obtained by combining application of different technologies.

The gene expression in plants induced by GA_3 includes cell division cycle related genes (Fabian *et al*., 2000) and cell wall related genes (Chen *et al.*, 1995; Knaap and Kende, 1995). In this study, high level of differential gene expression was detected with cDNA-AFLP, cDNA-SRAP and cDNA-SCoT analyses. This is in agreement with previous studies on gene expression in others plants induced by GA_3 (Chen *et al.*, 1995; Knaap and Kende 1995; Knaap *et al.*, 1997; Wang *et al*., 1997; Fabian *et al.*, 2000; Mo *et al.*, 2000; Van der Knaap *et al.*, 2000; Lou *et al*., 2001; Yu, 2005; Zhao *et al*., 2010). In this study, isolation and identification of some genes are the same compared with previous studies, such as peroxidase (Yu 2005), ras related proteins (Yu, 2005), ATP synthase (Lou *et al*., 2000), ribosomal protein (Yu, 2005), GA receptor GID1 (Zhao *et al.*, 2010), and so on. It indicates that different plants might have similar gene expression that was regulated by GA. These technologies provided valuable references for studying plant resistance, heterosis and crop breeding.

CONCLUSION

The differential expression of gibberellic acid (GA3)-induced stalk elongation genes were analyzed with three different techniques, *i.e*., cDNA-amplified fragment length polymorphism (cDNA-AFLP), sequence-related cDNA-amplified polymorphism (cDNA-SRAP) and cDNA-amplified start codon targeted (cDNA-SCoT). The results are varied from different techniques, more comprehensive information has been obtained by combined analyses, however, they resulted in the same important TDFs, such as GID1, 1,3,4-trisphosphate 5/6-kinase, S-adenosyl methionine *etc*. The information obtained is helpful in identifying functional genes involved in stalk elongation of sugarcane.

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