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Research Article

Identification of two new drought specific candidate genes in sugarcane (*Saccharum* spp.)

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Abstract

Effective identification and understanding of genes contribute to improve plant drought resistance. A study was conducted to identify drought responsive candidate genes in sugarcane. Two genes *viz.,* SOD (Superoxide dismutase) and IGS (Indole 3-glycerol phosphate synthase) were used as gene specific markers. Specific primers were designed based on the sequences in Genbank databases. Mapping population developed by crossing a drought tolerant parent (Co 740) and a drought susceptible parent (Co 775) were phenotyped using physiological and sugar yield contributing parameters and were characterized into groups of varying levels of resistance and susceptibility. Parental polymorphism for SOD and IGS specific primers was established using genomic DNA from field grown drought tolerant and susceptible parents, as the presence in Co 740 (resistant) and absence in Co 775 (susceptible) respectively. Resistant and susceptible parents and six each resistant and susceptible progeny were subjected to drought imposition and RNA were isolated and RT - PCR analysis performed using these gene specific primers. A specific band of 618 bp was identified in drought tolerant parent and progeny, absent in drought susceptible parent and progeny genotyped using SOD gene. A specific band of 340 bp was identified in drought tolerant parent and progeny while it was absent in drought susceptible parent and progeny genotyped using IGS gene. These two fragments of interests were cloned in PTz57R/T vector and sequenced. SOD_{618} sequence was BLAST searched that showed 98 % homology with the drought inducible protein in *Saccharum* hybrid and IGS_{340} showed 80 % homology with the hypothetical protein expressed in rice genome. These new genes hold promise improving drought resistance of sugarcane through their use as candidate genes in marker assisted selection and in genetic transformation.

Key words: Sugar cane, drought resistance, Candidate genes

Introduction

Breeding for drought resistance is complicated as drought is a complex trait involving a battery of genes and regulatory elements and sugarcane being a complex polyploid. Though response of elite genotypes of sugarcane has been studied (Hemaprabha *et al,* 2004), a lot more on the underlying genetic factors involved in plant responses to drought stress need to be understood in order to provide a solid foundation to breed plants with improved drought tolerance (Sanchez *et al*, 2002). One of the most productive approaches to establishing the basic responses of plants to drought stress involve studying candidate gene. The candidate genes, or DNA sequences with predicted function, are used as molecular markers to associate with phenotypes expressed in segregating populations or genetic stocks (Huh *et al* .2001; Thorup *et al*. 2000). The identification of such novel genes, determination of their expression patterns in response to the stresses, and an improved understanding of their functions in stress adaptation will provide us the basis of effective engineering strategies to improve stress tolerance (Cushman & Bohnert, 2000). The present study was conducted to identify drought specific candidate genes in sugarcane (*Saccharum* spp).

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Material and methods

A highly drought tolerant sugarcane clone Co 740 and the drought susceptible clone Co 775, and six each of drought tolerant and susceptible progenies selected from a well characterized mapping population developed from the cross Co 740 x Co 775 formed the material used in the study.

Candidate genes

Two genes viz. SOD (Super oxide dismutase) and IGS (Indole-3-glycerol phosphate synthase) were used as gene specific markers. Candidate genes for drought stress responses were selected on the basis of drought stress responsive genes data from public databases (GenBank).The forward and reverse primers of the respective genes for the study were designed using primer3 software.

RNA profiling and data analysis

Leaf samples were collected from pot cultured control and drought treated tolerant and the susceptible parents and progeny. Total RNA was extracted from these samples with TRI reagent method (Chomczynski and Mackey , 1995).Single step RT PCR reaction were carried out using QUIAGEN One step RT-PCR kit. RT-PCR reactions were run for 34 cycles in PTC-100 thermocycler (MJ Perkin Elmer).The amplification conditions were as follows: 37^0 C for 30 minutes, 95^0 C for 5 minutes, followed by 35 cycles of 95^0 for 30 seconds, primer melting temperature 56^0 C (for gene specific primer SOD) ,58^0 C (for gene specific primer IGS) for 30 seconds, 72^0 C for 30 seconds follows by 72^0 C for 7 minutes. RT-PCR products were separated on 1.2% agarose gel.

Cloning and sequencing

The desired fragments resulted from RT-PCR reaction were ligated with a cloning vector, pTZ57R/T (2886 bp size) with the aid of TA clone ^{TM} PCR Cloning Kit(MBI Fermentas, USA).Transformation of our sequence of interest was confirmed by performing colony PCR using the gene specific primers SOD and IGS respectively. The amplified PCR products were electrophorised on 1.2% agarose gel. The recombinant plasmids with our sequence of interest were completely sequenced by performing single pass sequencing using ABI PRISM 377 DNA sequencer,using Big Dye Terminator Cycle Sequencing Kit. M13 primer was used for sequencing, utilizing the sequencing facility of First Base, Selangor Darul Ehsan, Malaysia.

Results and Discussion

Polymorphism between the drought tolerant and susceptible parents for both gene specific markers viz. SOD and IGS was observed. Following the establishment of parental polymorphism the study was extended to six progeny each with drought tolerance and susceptibility selected from the mapping population (data not shown) . A specific band of 618 bp (Fig.1) was identified in drought tolerant parent and six tolerant progenies through RNA profiled using SOD gene. A specific band of 340 bp (Fig.2) was identified in drought tolerant parent and six tolerant progenies, with IGS primers. These specific bands were absent in the drought susceptible parent and six susceptible progenies with both gene specific primers.

These two fragments of interests were cloned in pTZ57R/T vector and sequenced. SOD_{618} sequence is given below. BLAST search of the sequences resulted to show 98% homology with the drought inducible protein in Saccharum hybrid.(Fig 3). IGS_{340} (sequence given below) showed 80% homology with the hypothetical protein expressed in rice genome Fig 3.

SoD gene (reverse) sequence

TGGGGTCACATCTAGGCGCGTATAGATCTGA
TTCTTAGCGTCCCTATCGTAACGCGATATACC
ATGTCGACTATCCTGTGGTATATCGCCTCATG
AGTGCGTAACTGACAATCTCAAGGCAATGTA
GTGCAGTGGAAACGCAGTGTCCTTGTGCGTT
CCGTGCTATAGCGAATGTCTACGAACGCCAG
AGAGAGAACAGGGCTGGCCCCGTGTCACTCT
ACTGGCCTGAGATCGTTTCTGTCCATATCGTC
CATGTAACTAAGATCTGTGGAATGGAGATTG
CGCTTAGCATGGGACCTTAAAGGCGTTCCCC
TAAGGTTACGAGGGGATGTTGCACCGACATC
CGGCAGGTCTGAATCAAAAAAAACAGTCGTC
GGACGACGACGACGACGAAGGAGGACCACG
ACGACCTACTACTGCGGAGGCGGCGGCGATG
ATGGCGGCCGCCTCGACGAGCTGCTGGTTGT
GCTGCTCCTCCTCCTCCTCCTTCTAGTTGTCG
TAGTACTCGTCCTCGGCGGACAACACCTCCT
CCATCGCGGTGTTCTCCTACTCCGCGGCCGCC
TCGTCCCCGCCGCCGCCGGCGGGCTGCTGCA
CCTCCTCCTCCTCCTCCTTCTTGTGGTGGAAC
AAGTGGGAAGTAAGTAATC

SoD gene (forward) sequence

ACCACGGTGTACGGAGGTGGTGTCCACGGGC
GAGGACGAGTACGACAAGTACAAGAAGGAG
GAGAAGGAGCACAAGCACAAGCAGCACCTC

GGCGAGGCCGGCGCCATCGCCGCCGGCGCCT
TCGCACTCGTACGTAATTATCA

IGS Gene (Forward) sequence
CCTCGCGAATGCATCTAGATCCAACAAGCTC
GATTCCTTCGCCGAGGCGGAGAGGGACGCGG
CATGGCGTGCCGCGATGCAGATGGAGATGGA
CGCCGTCGAGCGGAACAAGACGTGGGAGCT
GGCCGATCTTCCAGCCGGCCACCACGCCATC
TCTCTCAAGTGGGTCTTCAAGCTCAAGAAGG
ACGAGACCGGAGAGGTGATCAAGCACAAGG
CGCGTCTGGTGGCGCGCGGCTTCGTCCAGCA
AGAAGGAATCGAGCTTGTTGGAATCGGATCC
CGGGCCCGTCGACTGCAGAGGCCTGCATGCA
AGCTTTCCCTATAGTGAGTCGTATTAGAGCTT
GGCGTAATCATGGTCATAGCTGTTTCCTGTGT
GAAATTGTTATCCGCTCACAATTCCACACAA
CATACGAGCCGGAAGCATAAAGTGTAAAGCC
TGGGGTGCCTAATGAGTGAGCTAACTCACAT
TAATTGCGTTGCGCTCACTGCCCGCTTTCCAG
TCGGGAAACCTGTCGTGCCAGCTGCATTAAT
GAATCGGCCAACGCGCGGGGAGAGGCGGTTT
GCGTATTGGGCGCTCTTCCGCTTCCTCGCTCA
CTGACTCGCTGCGCTCGGTCGTTCGGCTGCG
GCGAGCGGTATCAGCTCACTCAAAGGCGGTA
ATACGGTTATCCACAGAATCAGGGGATAACG
CAGGAAAGAACATGTGAGCAAAAGGCCAGC
AAAAGGCCAGGA

IGS Gene (Reverse)
CTCACTATAGGGAAAGCTTGCATGCAGGCCT
CTGCAGTCGACGGGCCCGGGATCCGATTCAA
CAAGCTCGATTCCTTCTTGCTGGACGAAGCC
GCGCGCCACCGACGCGCCTTGTGCTTGATCA
CCTCTCCGGTCTCGTCCTTCTTGAGCTTGAAG
CCCACTTGAGAGAGATGGCGTGGTGGCCGGC
TGGAAGATCGGCCAGCTCCCACGTCTTGTTC
CGCTCGACGGCGTCCATCTCCATCTGCATCGC
GGCACGCCATGCCGCGTCCCTCTCCGCCTCG
GCGAAGGAATCGAGCTTGTTGGATCTAGATG
CATTCGCGAGGTACCGAGCTCGAATTCACTG
GCCGTCGTTTTACAACGTCGTGACTGGGAAA
ACCCTGGCGTTACCCAACTTAATCGCCTTGCA
GCACATCCCCCTTTCGCCAGCTGGCGTAATA
GCGAAGAGGCCCGCACCGATCGCCCTTCCCA
ACAGTTGCGCAGCCTGAATGGCGAATGGAAA
TTGTAAGCGTTAATATTTTGTTAAATTCGCGT
TAAATTTTTGTTAAATCAGCTCATTTTTTAAC
CAATAGGCCGAAATCGGCAAAATCCCTTATA
AATCAAAAGAATAGACCGAGATAGGGTTGA
GTGTTGTTCCAGTTTGGAACAAGAGTCCACT
ATTAAAGAACGTGGACTCCAACGTCAAAGGG
CGAAAAACCGTCTATCAGGCGATGGCCCACT
ACGT

The two new sugarcane specific genes hold promise in improving drought resistance in crop plants. SOD gene is reported to help the plant during rehydration when the superoxide anions increase in the cells and tissues. The establishment of a reactive oxygen scavenging system involving the enzyme superoxide dismutase countered the superoxide activity. The ability of plants to resist cellular damage can be enhanced by over expressing the enzymes of antioxidant pathways in transgenic alfalfa that showed stress tolerance under field conditions (McKersie *et al.* 1996). The phytohormone auxin plays a prominent role by acting as a versatile signal in regulating and coordinating plant growth at all stages and during drought and other stresses (Spoel *et al*., 2008) During drought, auxin related gene expression has established the activation of genes including IGS, which is a IAA biosynthesis gene (Aloni *et al,* 2003). In transgenic plants gene specific primer analysis has provided powerful tools for the discovery of stress responsive genes. Based on the similarities between responses at plant, tissue, or single cell level, it is hypothesized that a common mechanism of reactive oxygen species enhanced auxin responsiveness underlies the stress induced re orientation of growth, and are directly relevant in terms of understanding the whole plant behavior (Pasternak *et al* .2005).These results pinpoint towards the role of reactive oxygen species and auxins and plant response mechanism in view of the depleting water resource and global warming. In light of these results, the use of these genes as candidate genes in marker assisted selection and in genetic transformation hold promise in the genetic improvement of drought tolerance apart form studying the underlying genetic mechanism of drought tolerance. Further validation across an array of drought tolerant and susceptible genotypes of sugarcane is essential to enable their routine use in drought screening in order to identify tolerant types and to enhance drought tolerance of the existing sugarcane varieties.

References

Aloni,R., Schwalm,K., Langhans, M and Ullrich, C.2003.Gradual shifts in sites of free-auxin production during leaf-primordium development and their role in vascular differentiation and leaf morphogenesis in Arabidopsis.Planta.216:841-853.

Chomczynski, P and Mackey, K .1995. Modification of the TRI ReagentDNA/Protein Isolation procedure for isolation of RNA from polysaccharide - and proteoglycan - rich sources. Biotechniques, 19, 942–945.

Cushman, J.C and Bohnert, H.J .2000. Genomic approaches to plant stress tolerance.Curr Opin Plant Biol 3: 117-124.

Hemaprabha, G**.,** R. Nagarajan and S. Alarmelu. 2004. Response of sugarcane genotypes to water deficit stress. Sugar Tech 6(3): 165-168.

Huh, J., Kang, B, Nahm, S., Ha,K. Lee, M.H and Kim, B.D. 2001. A candidate gene approach identified phytoene synthase as the locus for mature fruit color in red pepper (*Capsicum* spp).Theor.Appl.Genet.102:524-530.

McKersie, B.D., Bowley, S.R. Harjanto, E. and Leprince, O.1996.Water-deficit tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase. .Plant Physiol.124:153-162.

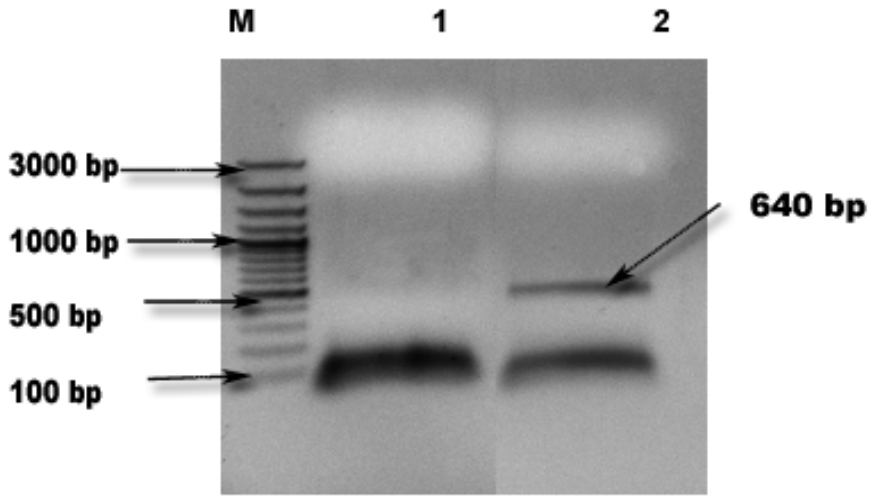
Pasternak T. G., Potters, R., Caubergs and M.A.K. Jansen .2005.Complementary interactions between oxidative stress and auxins control plant growth responses at plant, organ, and cell level. J Expl Bot. 56: 1991 -2001.

Sanchez, A.C., Subudhi, P.K., Rosenow, D.T.,and Ngugen, H.T. 2002.Mapping QTLs associated with drought resistance in sorghum (Sorghum bicolor L.Moench). Plant Mol.Bio.48:713-726.

Spoel, S.H. and Dong, X.2008.Making sense of hormone crosstalk during plant immune response .Cell Host Microbe.3: 348-351.

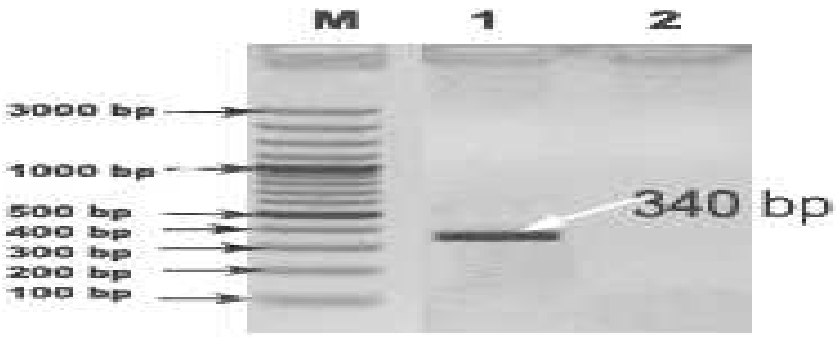
Thorup, T.A., Tanyolac. B., Livingstone, K.D., Papovsky, S., Paran, I and Jahn M. 2000. Candidate gene analysis of organ pigmentation loci in the Solanaceae.Proc.Natl.Acad.Sci.U.S.A.97:11192-11197.

Fig 1. SOD gene



M- 100 bp DNA ladder
1- Co 775
2- Co 740

Fig 2. IGS



M- 100 bp DNA ladder
1- Drought resistant parent
2- Drought susceptible parent

Fig. 3.Tree Diagram showing the homology relation of Sequence of interest