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Research Article

STUDY OF MOLECULAR DIFFERENCES AMONG THE SUGARCANE VARIETIES WITH REFERENCE TO FLOWERING BEHAVIOR

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A B S T R A C T

A number of biotic and abiotic stress factors effect sugarcane yield. Some internal factors like endogenous gibberellins, protein and RNA are known to govern the flower formation. An increase in the protein and nucleic acid content during the transformation of the meristem from the vegetative to reproductive state will affect the sugarcane yield. Study was undertaken with two flowering sugarcane varieties (BO 91 and CO H 15) with a view to investigate the effect on molecular changes. Isolation of RNA, Reverse Transcription and PCR Amplification with DNA Polymerase were done and the expression of all the three genes (Acid Invertase, Sucrose Phosphate Synthase 1 and Sucrose Phosphate Synthase 2) under study was confirmed and they were 200bp (Acid Invertase, Sucrose Phosphate Synthase 1) and 600 bp (Sucrose Phosphate Synthase 2) in size.

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is a perennial grass belonging to the family Poaceae. It is an important cash crop grown predominantly in tropical and sub-tropical climates of India which is cultivated in more than 4 million hectares of land with an annual cane production of around 270 million tones. India is one of the world's largest producers of sugar. Among the states of India, Tamil Nadu holds the first place in sugarcane production with an average yield of 108 tones/hectares. A number of biotic and abiotic stress factors affect sugarcane yield. Flowering is one of the factors known to affect the productivity of cane and sugar, particularly in southern region of the country.

Flowering in sugarcane is a genetically determined trait and influenced by a number of factors such as photoperiod, temperature, moisture and nutrition (Moore and Nuss, 1987). Flowering in plants in general is a natural process which marks the culmination of vegetative growth. Flowering in sugarcane is a complex physiological process consisting of multiple stages of development, each stage having specific environmental and physiological requirements. Flowering of sugarcane is very common and profuse in peninsular India during October and November. Some internal factors like endogenous gibberellins, protein and RNA are known to govern the flower formation.

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The effect on induction of flowering has been examined in a variety of plant species (Zeevart, 1983). Although the flowering of sugarcane is essential for breeding and development of new varieties, uncontrolled flowering in commercial fields poses a serious problems for sugarcane farmers with a considerable loss of sugar as well as cane yield especially in the early flowering varieties. Besides, the harvesting time in India is generally described on the basis of crop age, i.e. 12 to 14 months. The losses due to flowering are reported to be more consistent at low latitudes (Moore and Nuss, 1987).

Studies on the flowering of sugarcane have been carried out in several sugarcane growing countries to help the breeders to combine the economic characters by crossing. However, from the production viewpoint, flowering of sugarcane crop is not desirable since it affects both yield and quality considerably. The effect of flowering on cane yield and sucrose content has been investigated in several countries (Moore and Nuss, 1987) and this varied depending on the variety, the season, the country and the latitude.

A one step quadruplex reverse transcription (RT-PCR) method employed virus- specific primers was developed for the simultaneous detection and differentiation of sugarcane mosaic disease. The optimum primer combinations and concentrations, RT temperature and time, and PCR annealing temperature and extension time were determined for RT-PCR (YujiaXie, 2009).

An increase in the protein content during transformation of the meristem from the vegetative to reproductive state has been

supported by a number of workers (Evans, 1971). In the *Impatiens balsamia,* a qualitative short-day plant, the protein content of the leaves showed an upsurge at the time of floral induction (Sawhney *et al.,* 1972). Protein patterns of mature bracts and flowers are different from those of the vegetative parts of plants of *Sinapsis alba* (Barber and Steward, 1968). No consistent qualitative change in the composition of proteins during floral induction was found in *Xanthium* (Sherwood *et al.,* 1971). On the other hand, a new protein appeared in photoperiodically-induced cotyledons of *Pharbitis nil* and this protein did not disappear even when the plants were transferred to non-inductive photoperiods (Oota and Umemura, 1970).Genetic maps for *Saccharum* species and hybrids have been developed using molecular markers. The technologies developed have found practical application in the molecular breeding, cytogenetics and systematic of sugarcane (Nair *et al.,* 2000).

MATERIALS AND METHODS

A field experiment was conducted with commercial sugarcane varieties at the main farm of Sugarcane Breeding Institute during the crop season using two early-season flowering varieties BO 91 and Co H 15. In this study, the molecular observations of Isolation of RNA and Reverse transcription – PCR were recorded in the flowered and non-flowered stalks.

Isolation of total RNA

500mg of shoot/plant material was ground in liquid nitrogen. The ground plant material was added to the 30ml polypropylene tube containing 5ml of denaturing solution (solution D). 0.5ml of 2M Sodium acetate (pH4.0) was added and mixed by inverting the tube.5ml of water saturated phenol was added and mixed by inverting the tube twice.1ml of chloroform:isoamyl alcohol (24:1) was added and mixed by inverting. Then the tube was shaken and incubated on ice for 15min. After that it was centrifuged at 10,000g for 20mins at 4^0C. The aqueous phase containing total RNA (Ω5ml) was transferred carefully to a fresh poly propylene tube .5ml of isopropanol was added, mixed well and incubated at -20^0C for at least 1hr to precipitate RNA. Then it was centrifuged at 10,000g for 20 min at 4^0C and the supernatant was discarded. The pellet containing total RNA was dissolved in 1.5ml of solution D and 0.5ml of that suspension was taken in three microfuge tubes. Equal volume of isoproponal was added to each tube, mixed and incubated at -20^0C for 1hr. Centrifuged at 10,000g for 10min at 4^0C and the supernatant was discarded. The RNA pellet was resuspended in 200µl of 75% ethanol and kept at room temperature for 15min. Centrifuged at 10,000g for 5min at 4^0C and the supernatant was discarded. Vacuum dried the RNA pellet for 15min and dissolved in 50 to 250µl of RNase free water. Kept overnight at 4^0C for dissolving pellet.

Reverse Transcription

In a 0.2/0.5ml tube, 10µM gene specific primer (A-Acid Invertase, SPS1-Sucrose phosphate synthase, SPS2) and RNA was combined and the volume was adjusted to 10µl with diethyl pyrocarbonate (DEPC) treated water.

Primer	-1µl
RNA (10pg to 5µg)	-xµl
DEPC treated water to	-10µl

Denaturated the RNA and primer by incubating at 65^0C for 5min and then kept on ice. Vortexed the 5xcDNA synthesis buffer for 5 seconds just prior to use. A master reaction mix was prepared on ice and vortexed gently.

5x cDNA synthesis buffer	-4µl
0.1M DTT	-1µl
RNaseinhibitor (40U/µl)	-1µl
DEPC treated water	-1µl
10Mm dNTP mix	-2µl
Reverse transcriptase (15U/µl)	-1µl

Reduced the amount of enzyme to 0.5µl per reaction. The amount of RNA is less than 1ng and replaced the volume with DEPC treated water. Add 10µl from step 2 above to the reaction tube on ice(step 4). The sample was transferred to a thermal cycler preheated to the appropriate cDNA synthesis temperature and incubated as follows.

Oligo dT_{20} Primer	: 30 to 60mins at 50^0C (50-60^0C)
Gene specific primer	: 30 to 60mins at 50^0C (50-65^0C)
Random hexamer primer	: 10min at 25^0C, followed by 20 to 50min at 50^0C

The reaction was terminated by incubating at 85^0C for 5 minutes. Add 1µl of RNase H and incubate at 37^0C for 20min (optional). cDNA synthesis reactions can be stored at -20^0C used for PCR immediately.

PCR amplification with DNA polymerase

Only 10% of the cDNA synthesis reaction (2µl) for PCR and proceeded for the reaction using Taq DNA polymerase. The following components were added to the thin walled PCR tube.

If using Platinum Taq DNA polymerase (GIBCO BRL enzyme):

10x PCR buffer minus Mg	-5.0µl
50mM$Mgcl_2$	-1.5µl
10mMdNTP mix	-1.0µl
10µM anti-sense Primer	-1.0µl
10µM sense Primer	-1.0µl
Platinum Taq DNA polymerase (5U/µl)	-0.4µl
cDNA (from cDNA synthesis reaction)	-2.0µl
DEPC treated water	-38.1µl
Final volume	-50.0µl

Gently mixed, incubated at 94^0C for 2min, then performed 20 to 40 cycles of PCR with optimized conditions for the sample (1min/kb extension time at 68 to 72^0C). 10µl of the amplified sample was analyzed by agarose gel electrophoresis.

RESULT AND DISCUSSION

Total RNA was isolated and c DNA was synthesized using the standard protocol (Fig.1&2).

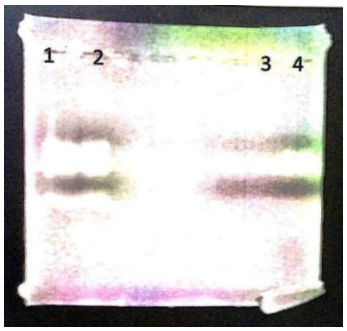

Fig 1 Isolation of RNA
1-BO 91 (R1) **2**-BO 91 (R2)
3-Co H 15 (R1) **4**-Co H 15 (R2)

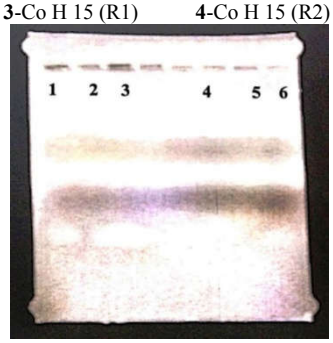

Fig 2 cDNA Synthesis

1,2,3-BO 91
4,5,6-Co H 15
1,4 - Acid invertase
2,5 - Sucrose phosphate synthase 1
3,6 - Sucrose phosphate synthase 2

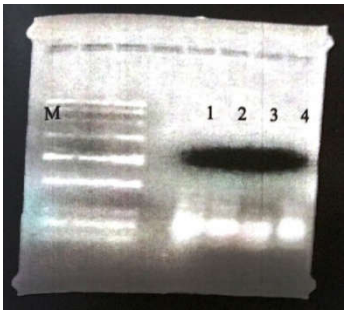

Fig 3 Reverse transcriptase-PCR

M - Marker
1,3 - BO 91
2,4 - Co H 15
1,2 - Acid invertase
3,4 - Sucrose phosphate synthase 1

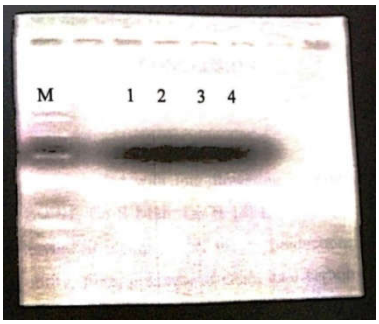

Fig 4 Reverse transcriptase-PCR

M - Marker
1,3 - BO 91
2,4 - Co H 15
1,2,3,4 - Sucrose phosphate synthase 2

Using cDNA of two varities (BO 91 and Co H 15) RT-PCR was performed to analyze the expression Acid Invertase, Sucrose Phosphate Synthase 1 and Sucrose Phosphate Synthase 2. RT-PCR products were resolved on the gel and the products were compared. From this analysis, expression of all the three genes under study was confirmed and they were 200bp (Acid Invertase, Sucrose Phosphate Synthase 1) and 600 bp (Sucrose Phosphate Synthase 2) in size (Fig.3 & 4).

CONCLUSION

The gene is expressed and the intensity of bands was feeble with the molecular mass in the two sugarcane flowering varieties. The expression of all the three genes under study was confirmed. This indicates there will be molecular changes in the selected two sugarcane varieties and will affect the yield of sugarcane.

References

1. Barber, J.T and F.C. Steward. 1986. The proteins of Tulipa and their relation to morphologenesis develop. Biol. (17):326-349.
2. Evans, I.T.1971. Floral induction and Florigen concept. Ann. Rev. Plant Physiol. (22): 365-394.
3. Moore, P. H and K.J. Nuss. 1987. Flower synchronization in sugarcane improvement through Breeding. Ed. D. J. Heinz Elsevi., Amsterdam. 273-311.
4. Nair, N.V., A. Selvi, T.V. Sreenevasan and K.N. Puspalatha. 2000. Mloecular approaches in sugarcane varietal improvement. Proceeding of sugarcane tech association of India (62):104-114.
5. Oota.Y and K. Umemura. 1970. Specific RNA produced in photo periodically induced cotyledons of Pharbitis nil seedlings. In cellular and molecular aspects of floral induction. Edited by G. Bernier. p. 224-242.
6. Sawhney, S., N. Sawhney and K.K. Nanda. 1972. Partial substitution by long days of short days required for floral induction in *Impatiens balsamia.* Plant and Cell Physiology (13):6-10.
7. Sherwood. S. B., J.O. Evans. and C. Ross. 1971. Gel electrophoresis studies of proteins from leabes of photoperiodically induced and vegetative cocklebur plants. Plant and Cell Physiol. (12): 111-116.
8. YujieXie. 2009. Simultaneous detection and identification of four sugarcane viruses by one- step RT-PCR. Laboratory of Plant Virology, South China Agricultural University, Guangzhou, China.
9. Zeevart. J.A.D. 1962. DNA multiplication as a requirement for expression of floral stimulus in Pharmitis nil. *Plant Physiol*. (37): 296-304.

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