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Research Article

Study of Genetic Scar Marker Biotechnology of Sugarcane for Different Cultivars in Saline Environment

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A B S T R A C T

To keep pace with increasing global need of sugarcane and food security, researchers have devised various technologies to make the sugarcane production more economic and sustainable. Efforts are being made to validate and disseminate these technologies among farmers. Crop yield is affected by technological change and weather variability. Saline soil is a problem for agriculture in many parts of the world, especially in arid and semi arid regions where low precipitation, irrigation with brackish water and poor drainage interact to bring about soil salinity. Excess amount of salt in the soil adversely affects plant growth and development leading to diminished economic health and poor quality of produce, limiting the productivity of crop plants. Approximately 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity.

Keywords: Biotechnology, Sugarcane, Irrigation, Weather, Cultivated

Introduction

Knowledge of a crop's genetic architecture will become more important for increased crop production because plant genetics will be required to extend or replace extant management practices such as chemical fertilizers, pesticides and irrigation. Such knowledge will include more detailed descriptions of genome organization, the crop's gene pools and genes and pathways controlling important phenotypes. In many instances, DNA markers and genetic maps will be important tools for direct investigations of these areas and will provide vital links between plant breeding and basic plant biology. Many of the limitations of plant breeding methods have been rooted in the status of the technical infrastructure for conducting genetic analyses. Breeders and geneticists of all crops have lacked an informative and integrated genetic context to aid interpretation and conciliation of perspectives provided by seemingly different approaches to genetic improvement.

The importance of agriculture for the Indian society can hardly be over emphasized, as its role in economy, employment, food security, self-reliance and general well being does not need reiteration. India has a very well developed system for collection of crop statistics at village level and aggregating it at different administrative levels. However, the need for early and in-season crop production forecasting has been strongly felt. There has been substantial boost in food grain production over the years but it could not keep pace with rate of increase in the population of the country. Thus, increasing agricultural productivity has been main concern since the scope of increasing area under agriculture is rather limited. Fulfilling this requirement entails judicious planning based on information related to various aspects of agriculture. Thus, information on crop acreage, yield, production and conditions are important inputs for strategic planning.

Various organizations in India and abroad are engaged in

developing methodology for pre harvest forecast of crop yields using different approaches. Use of crop input and weather variables forms one class of fore-casting crop yield. The other approach uses plant vigour measured through plant characters. It can be assumed that plant characters are integrated effects of all the factors affecting crop yield.

Table 1.Members of Genus Saccharum

Species	Classi-fication	Sugar content	Chromosome number
S. spontaneum L	Wild species	Very low	2n = 36-128
S. robustum	Wild species	Very low	2n = 60-200
S. officinarum L	Noble canes	High	2n = 70-140
S. barberi Jesweit	Ancient hybrid	Low	2n = 111-120
S. sinese Roxb. Amend Jesweit	Ancient hybrid	Low	2n = 80-124
S. edule Hassk	Culti-vated species	Low-compacted inflorescence eaten as a vegetable	2n = 60-80 with aneuploid forms

Material and Method

This research attempts to study of the role played by the various weather variables affect the crop differently during different stages of development. Thus, the extent of weather influence on crop yield depends not only on the magnitude of weather variables but also on the distribution pattern of weather over the crop season which, as such, calls for the necessity of dividing the whole crop season into fine intervals. The presence of high Na^+ and Cl^- concentrations and an altered water status in the soil brings about changes in plant metabolism, membrane disorganization, generation of reactive oxygen species (ROS), metabolic toxicity, inhibition of photosynthesis and altered nutrient acquisition. Specific Ion effects may cause direct toxicity, alternatively, the insolubility or competitive absorption of ions may affect the plant's nutritional balance. These effects may be associated with enzyme activity, hormonal imbalance, or morphological modifications. Even at low salinity levels, external salt concentration is much greater than that of nutrients ions, so that a considerable concentration of ions may reach the xylem. Being the actively transpiring parts of the plant, the leaves accumulate salt to excessive level, exceeding the ability of the cells to compartmentalize these ions in the vacuole. Ions then build up rapidly in the cytoplasm and inhibit enzyme activity or they build up in the cell walls and dehydrate the cell, which leads to their premature death.

The stages of sugarcane crop growth can be divided into four phases (see Figure 1):

- **Germination:** The development of buds and roots, taking from 0 to 40 days
- **Tillering (formative):** Appearance of secondary and tertiary tillers, beginning approximately on the 40th day after planting and lasting up to 120 days
- **Grand growth:** Tiller growth and development with height gain and basal sugar accumulation taking up to 9 months after planting
- **Maturity:** Accumulation of photoassimilates and fast sugar synthesis, lasting until the harvesting period

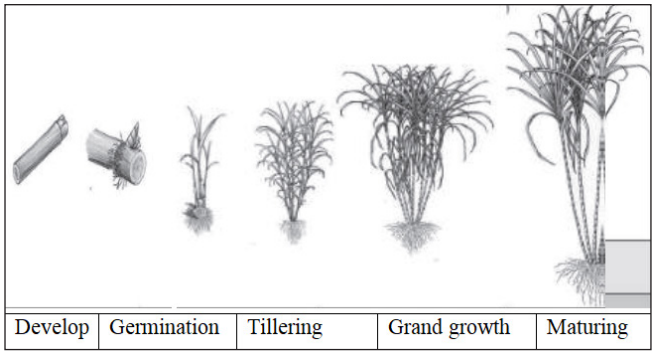

Figure 1.Sugarcane Growth Stages and their Water Requirements

Markers, Mapping and Genetics

The use of DNA markers has had an enormous impact on understanding the basic biology underlying the breeding of sexually reproduced crops. Since their introduction in the early 1980s, a variety of DNA markers have been developed, some hybridization-based and some PCR-based. The use of DNA markers has made it possible to: (a) "fingerprint" genotypes precisely, (b) verify F, hybrids, (c) estimate genetic distance and forecast heterotic groups, (d) select the best individuals for breeding, (e) to discover breaks of undesirable linkages flanking genes of interest and recover the recurrent parent's genotype, (f) undertake genetic analysis for QTL, (g) clone genes of economic importance. It is certain that DNA markers will radically alter the practice of plant breeding in the years to come. The genetic factors responsible for a part of the observed phenotypic variation for a quantitative trait are called Quantitative Trait Loci (QTL).

To be effective, the selectable marker genes should not interact with specific targets within the plant or alter signal transduction pathways in a way that changes the plant. If they create such changes it would be difficult to identify

the phenotypes associated with the Gene of Interest (GoI) or the factors affecting their expression.

Taking cognizance of this background information, the present work has been taken up with the following objectives:

- To screen a set of genetically diverse sugarcane inbred lines for their salinity tolerance levels
- To assess possible mechanisms of salinity tolerance in sugarcane
- To measure the antioxidant enzyme activity responses to salinity stress in salt-tolerant and sensitive lines of sugarcane
- To develop SCAR (Sequence Characterized Amplified Region) markers for salinity tolerance in sugarcane
- To check the validity of molecular markers identified in the course of the study
- To identify QTLs for salinity tolerance in mapping populations of sugarcane

In the current study, the salinity tolerance has been dissected into four physiological parameters namely: Chlorophyll Content (CC), Chlorophyll Fluorescence (CF), Leaf Temperature (LT) and Leaf Relative Water Content (LRWC).

A panel of 41 genotypes (Table 2), was selected and clones were derived from diverse pedigrees and do not form any pre-designed mapping population, tested in our local environment. They may further be used as a source of genetic diversity.

Table 2.Cultivars used in the Study of their Response to Salinity Stress Tolerance

S. No.	Variety	Parentage or Pedigree
1.	Co 85004	Co 6304 x Co 740
2.	Co 86032	Co 62198 x CoC 671
3.	Co 87025	Co 7704 x Co 62198
4.	Co 87044	Co 62198 x CoC 671
5.	Co 8371	Co 740 x Co 6806
6.	CoM 88121	Polybreed
7.	Co 91010	Co 312 x Co 775
8.	Co 94008	Co 7201 x Co 775
9.	Co 99004	Co 62175 x Co 86250
10.	Co 2001-13	Co 7806 PC
11.	Co 2001-15	Co 85002 x Co 775
12.	Co 0218	Co 8353 x Co 86011
13.	Co 0403	Co 8371 x Co 86011
14.	Co 06027	Polybreed
15.	CoSnk 05103	Co 740 x CoA 7602
16.	CoSnk 05104	CoC 771 PC
17	Co 86249	CoJ 64 x CoA 7601
18.	CoC 01061	69A591 GC
19.	CoOr 03151	CoC 671 x Co 1148
20.	Co 06030	Polybreed
21.	CoS 91230	Co 775 x Co 1148
22.	CoPant 90223	BO 91 GC
23.	CoH 92201	Co 7704 GC
24.	CoS 95255	Co 1158 x Co 62198
25.	CoS 94270	Co 7704 x MS 6847
26.	CoH 119	Co 7704 GC
27.	Co 98014	Co 8316 x Co 8213
28.	CoS 96268	Co 1158 x Co 62198
29.	CoPant 97222	CoPant 84212 GC
30.	CoJ 20193	LG 72115 x CoJ 82315
31.	CoS 96275	CoS 8119 x Co 62198
32.	Co 0118	Co 8347 x Co 86011
33.	Co 0238	CoLk 8102 x Co 775
34.	Co 0124	Co 89003 GC
35.	Co 0239	Co 93016 GC
36.	IJ-76-293	CoJ 64 x Co1148
37.	Co 0237	Co 93016 GC
38.	Co 05011	CoS 8436 x Co 89003
39.	CoPK 05191	Co 1158 GC
40.	Co 05009	Polybreed
41.	Co 87263	Co 312 x Co 6806

Measurement of Na^+ and K^+ ions

Na^+ and K^+ were determined by flame photometry using 60 mg of dry weight of seedlings made to ash at 800°C.

Steps in Sample Preparation for flame photometry

- For 60 mg of the sample 5 ml of HCl and 2 to 3 drops of perchloric acid were added. The samples were mixed and kept at 60-70°C for total evaporation
- To the above treated sample, 2 ml of HCl and 2 ml of HNO_3 were added and the samples were kept at 60-70°C for total evaporation
- To the above treated sample 1 ml of HCl was added and the samples were kept at 60-70°C for total evaporation
- Finally, the samples were dissolved in 2 ml of double-distilled water. The samples must be transparent at this stage and it can be subjected to flame photometry for ion quantification

Result and Discussion

For measuring ions such as Na^+, K^+, Ca^{+2} and Cl^- in short term salt treatments; 50 mg of dry weight of seedlings was

used. Ions were extracted by boiling the dried seedlings in distilled water and incubated in a boiling water bath for an hour. Ion contents were estimated by using a Metrohm Ion Analyzer. Specific electrodes were used for estimations by following the instructions from the manual provided by the company. Standard solutions of Na^+, K^+, Ca^{+2} and Cl supplied by the company were used for calibration.

At 75 mM NaCl, accumulation of Na^+ in sensitive lines was in the range of that of the moderately tolerant and highly tolerant lines. Most of the sensitive lines showed higher accumulation of K^+ at 75mM NaCl than did the moderately and highly tolerant lines. Also, K^+/ Na^+ ratios were higher than the other two categories at 0mM and 75mM NaCl in these five salinity sensitive lines. In two of the lines viz. IJ-76-293 and Katha-Coimbatore this ratio decreased indicating relatively less accumulation of K^+ than Na^+ at the higher salinity level. In rest of these lines, increasing salinity levels resulted in relatively increased accumulation of K^+. Among the moderately tolerant inbred lines, Na^+ and K^+ levels varied considerably across the different salinity levels. Most of the lines in this category showed a positive relationship between salt concentration and uptake of Na^+ and K^+. Of these lines S. sinense had conspicuously reduced K^+/Na^+, ratios with increased salinity levels. Other line that exhibited similar trends were S. officinarum, while the rest of the lines accumulated more K^+ than Na^+ with increasing salt stress.

Table 3.Sensitive/ Tolerated Salinity Tolerance Varieties of Sugarcane under Validation of SCAR Markers

Salinity tolerance	**S. robustum**	**IJ-76-293, IM-76-232, 51 NG 6, 51 NG 27**
	S. barberi	Katha-Coimbatore, Kewali -14-G, Khatuia-124, Kuswar Ottur, Lalri, Nargori, Pathri
	S. sinense	Khakai, Pansahi, Reha, Uba-Seedling
	S. officinarum	IJ-76-442
	S. robustum	IJ-76-470, 28 NG 251, 57 NG 201, 57 NG 231, 77 NG 34, 77 NG 136, 77 NG 160, 77 NG 167, 77 NG 170, 77 NG 221, 77 NG 237

Of the five tolerant lines, S robustum accumulated the highest levels of Na^+ as well as K^+. The K^+/Na^+ ratio was quite low in his line, however, it increased gradually with increases in salinity levels of the media. In the tolerant lines, K^+/Na^+ ratio increased substantially, significantly, at the higher media salinity levels. Thus, the inbred lines in this study differed markedly in their ion uptake behavior in response to increased salinity especially in terms of the ratio of K^+ over Na^+. Plants depend upon the maintenance of low cytoplasmic Na^+ and Cl concentrations and a high K^+/Na^+ ratio under salt stress, because K^+ counteracts the inhibitory effects of Na^+ and Li^+. Most plant cells maintain cytosolic K^+ concentrations in the range of 100-200 mM and Na^+ values in the low mM range (1- 10 mM) up to a maximum of 100 mM. In contrast to K^+, Na^+ is not essential for, but facilitates volume regulation and growth in most plants. However, at high concentrations, Na^+ limits growth. In the present studies, sensitive lines showed more accumulation of Na^+ when compared to K^+.

When data of years 2018 and 2020 were analyzed together with the year as a component in total variation and subjected to ANOVA, non-significant effect was obtained for G, ED and G x T (Table 4).

Table 4.Analysis of Variance for Chlorophyll Fluorescence

S. No.	Effect	F-Value		
		2018	2020	2018-2020
1.	Block (B)	7.86**	53.02**	25.56**
2.	Year (Y)	-	-	14.73**
3.	Treatment (T)	19.54**	58.27**	37.37**
4.	Genotype (G)	3.07**	2.57**	1.02^{ns}
5.	Evaluation Date (ED)	7.62**	103.81**	1.42^{ns}
6.	G*T	2.43**	0.94^{ns}	1.04^{ns}
7.	Y*T	-	-	22.30**
8.	G*T*ED	6.30**	1.72**	1.34**

* = P<0.05, ** = P<0.001, ns = non significant

Table 5.Analysis of Variance for Chlorophyll Content

S. No.	Effect	F-Value		
		2018	2020	2018-2020
1.	Block (B)	1.32**	40.76**	2.40**
2.	Year (Y)	-	-	33.83**
3.	Treatment (T)	3.77**	161.89**	13.98**
4.	Genotype (G)	3.36**	13.14**	7.58**
5.	Evaluation Date (ED)	10.05**	426.89**	147.53**
6.	G*T	0.21^{ns}	1.14^{ns}	0.77^{ns}
7.	Y*T	-	-	4.52**
8.	G*T*ED	0.88^{ns}	2.81**	2.16**

* = P<0.05, ** = P<0.001, ns = non significant

The one-way interactions in variance components on

chlorophyll content (SPAD index) were significant in year 2018. However, the interaction effect of G x T and G x T x ED was non-significant. The G x T x ED interaction affected the SPAD index significantly in year 2020 (Table 5). Significant Y x T and G x T x ED interactions were observed in 2018-2020 collective analysis.

The main effect of treatment was not significant for leaf temperature in year 2018. Significant G x T x E interactions were observed. In year 2020 the genotype and evaluation date as main effects were not significant. G x T and G x T x ED interactions were not observed. Y x T significantly affected the leaf temperature in the combined analysis of 2018-2020 (Table 6).

Table 6.Analysis of Variance for Leaf Temperature

S. No.	Effect	F-Value		
		2018	2020	2018-2020
1.	Block (B)	2.28**	125.33**	3.23**
2.	Year (Y)	-	-	4.43**
3.	Treatment (T)	0.44^{ns}	11.77**	3.98*
4.	Genotype (G)	2.24**	1.11^{ns}	1.00^{ns}
5.	Evaluation Date (ED)	13.67**	0.14^{ns}	1.23^{ns}
6.	G*T	0.68^{ns}	0.23^{ns}	0.42^{ns}
7.	Y*T	-	-	2.66*
8.	G*T*ED	2.25**	0.59^{ns}	0.74^{ns}

* = P<0.05, ** = P<0.001, ns = non significant

Collecting leaf disks was a time consuming process. Therefore, measurements for LRWC were made only on one block in both treatments. Consequently, ANOVA could not be conducted on LRWC.

Conclusion

Molecular markers are a promising tool to help us understand the genetic control of salt tolerance as well as to follow the introduction of important genomic regions for tolerance into susceptible genotypes by Marker Assisted Selection (MAS). The expression of salt stress induced genes is an essential part of tolerance mechanisms, but for many genes with salt stress-responsive expression, no direct function in tolerance was clearly demonstrated. Consequently, mapping the location of such candidate genes near or within QTLs involved in tolerance and adaptation to saline conditions could give some information on their role. QTL mapping and subsequent marker-assisted backcrossing to improve salt tolerance of pearl millet cultivars is a promising aspect for the crop improvement in future for this 'orphan' crop with a dearth of markers.

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