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IN VITRO CALLOGENESIS AND ORGANOGENESIS IN DIFFERENT EXPLANTS OF STEVIA (*STEVIA REBAUDIANA*)

By

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ABSTRACT

Different explants of *Stevia rebaudiana* i.e. leaf, node and shoot apices were used to callus initiation and regeneration of shoots when cultured on Murashige and Skoog (MS) medium supplemented with different concentration of auxins and cytokinins. Different concentrations of 2, 4-D were used for callus initiation in leaf and nodal explants. In the case of leaf explants the best callus was observed at the concentration of 2.5 mg/l 2, 4-D (2, 4 Dichloro phenoxyacetic acid) and in nodal explants at the concentration of 1.5 mg/l 2, 4-D. Leaf calluses were subcultured in different concentrations of 2,4-D as well as BAP (Benzyl amino purine) +IAA(Indole-3- acetic acid)for further proliferation of callus and for organ formation. The combination of BAP+IAA (2+1 mg/l) was considered the best medium for organ formation. For shoot apices, different concentrations of BAP alone and in combination with IAA were tested, BAP (1mg/l) alone or BAP+IAA (2+1mg/l) gave the best response. Regenerated shoots were transferred to the rooting medium containing different levels of auxins. Medium containing 0.5 mg/l IBA (Indole-3- butyric acid) favored root formation.

Key words: *Stevia rebaudiana*, shoot apices, steviol glycosides, callogenesis, organogenesis

INTRODUCTION

Stevia is the perennial shrub that grows up to about one meter tall and has leaves 2-3 cm long. It belongs to the Aster family (Composite), which is indigenous to the Northern region of South America. Stevia is still found growing wild in the highlands of Amambay and Lguacu District (a border area between Brazil and Paraguay) (Taylor, 2005) Stevia is three hundred times sweeter than the sugar. It is non- caloric and offers a healthy, natural alternative to cooking with refined sugar or artificial sweeteners (Midmore and Rank, 2002). Stevia has a natural, non-caloric, sweet-tasting compound used around the world for its pleasant taste, as well as for its increasingly researched potential for inhibiting fat absorption and lowering blood pressure. It has been used as a dietary supplement and food additive in Latin.America, Japan, Asia and the United States for a long time (Whitaker, 1998).

For hundreds of years, indigenous people in Brazil and Paraguay have used the leaves of stevia as a sweetener. They have used stevia to sweeten teas and foods and to use it medicinally as a cardio tonic for obesity, hypertension and heart burn and to help lower uric acid levels (Chan, 2000). In addition to being a sweetener, stevia is considered (in Brazilin herbal medicine) to be hypoglycemic, hypotensive, diuretic and cardio tonic (Chang, 2005). Stevia is an exceptional aid in weight loss and weight management, because it contains no calories but reduces one craving for sweets and fatty foods. Hunger sensations are lessened when 10 or 15 drops are taken 20 minutes before meals. Other benefits of adding stevia to the daily diet include improved digestion, gastrointestinal function, smoothed upset stomachs, and quicker recovery for minor illness (Kroner, 2006).

The sweet compounds found in the Stevia leaves are Diterpene glycosides (Stevia glycosides) and are synthesized at least in the initial stages, using much the same pathway as the Gibberellic acid, an important hormone. Four major compounds and their ratios found in the stevia glycosides are stevioside (5-10%),rebaudioside A (2-4%), rebaudioside C (1- 2%),gulcoside A(0.5-1%).Two other

glycosides that may be present in plant tissue are rebaudiosides D and E, rebaudioside B has been detected but is probably. Stevia has an interesting history. One story began in the heart of South America during the mid of 1800,,when Guarani natives knew said perennial only as "kaa-he-he" .Their applications were simple and many still remain popular today. It was initially used in their medicinal portions, as well as a tea-like drink known as bitter mate. Many chewed the dried leaves simply to acquire the unique, refreshing taste (Kroner, 2006).

The synthetic–free steviosides gave the hope for some most popular foods and beverages. This research was intense, by 1990, Japan accounted for over 40% world wide stevia used. Today, it is used commercially at an enormous level with sales continue to cscalate. Knowing all these important applications of different Stevia compounds, the present study was conducted to initiate calogenesis and organogenesis from different explants of stevia to produce disease-free plants of *Stevia rebaudiana*, which would be beneficial commercially. Specifically, it deals with in vitro propagation of *Stevia rebaudiana*, including a) callus formation from different explants; b) maintenance of callus up to third subculture; and c) regeneration of plantlets from callus.

MATERIALS AND METHODS

Stevia explants (leaves, nodes, shoot tips) were obtained from Qarshi industry private limited (Hatar). The explants were thoroughly washed for five minutes under running tap water and treated with household detergent (20%) for five minutes. This was followed by second washing with tap water to make the explants free from detergent. The washed explants were surface sterilized with 10% commercial sodium hypo chlorite for 15 minutes in a beaker covered by a Petri dish lid. The explants were then washed 3-5 times with double distilled water, until the smell of sodium hypo chlorite was completely removed. Tissue culture glassware were first cleaned with detergent and dipped in Chromic acid ($K_2Cr_2O_7$ + H_2SO_4 + H_2O) for 10 hours. The treated glassware were thoroughly washed under running tap water. The last sterilization step was to place the cleaned glassware in an autoclave at 121°C for 15 minutes at 105 Kpa.

Stock solutions for culture medium, growth hormones and vitamins were prepared in double distilled water. . Analytical grade chemicals were used. Solutions were stored in amber color bottles at 4°C. The strength of stock solutions of MS macronutrients was 20x and micronutrients was 100x, while vitamins was 100x. The solutions of Auxins and Cytokinins were prepared by dissolving 1 mg of required hormone in 1 ml of double distilled water. Murashige and Skoog's (1962) basal medium was used. For each particular medium appropriate amount of all components were mixed using required amounts of the stock solutions and double distilled water. Sucrose was added to the medium at 3% concentration. The pH of the medium was adjusted to 5.5 - 5.7 using 0.1 N HCl. Bacto agar was added at concentration of 10 g/l and then medium was boiled. Appropriate volume of prepared medium was dispensed in culture tubes and medium was sterilized by autoclaving at 121^{o}C and 15Lb / inch 2 for 15 minutes. After autoclaving culture tubes were cooled down at room temperature.

Different explants such as leaf and nodes were excised and inoculated in MS medium supplemented with different concentrations of 2, 4-D for callus formation. Shoot apices were inoculated in different concentrations of BAP alone and BAP+IAA for plant formation. The cultures inoculated with explants (leaves, shoot apices, node) were grown under carefully regulated temperature and light conditions. All cultures were grown in growth incubator illuminated by 40 W white fluorescent tubes. The intensity of light was 2,000 to 3,000 lux. The temperature of incubator was 22°C+2C Duration of photoperiod is 16 and 8 hours light and dark. Callus cultures were grown for 4 - 5 weeks. At the end of this period, data on frequency of callus formation, color and compactness of calli were recorded. The best medium having maximum callusing potential and proliferation rate was selected for each explant (node, leaf, and shoot apices). Callus tissues obtained from different experiments

were sub-cultured at 4-week intervals using the same medium. Moderate organ formation from callus was also observed.

RESULTS AND DISCUSSION

Among different concentrations of 2, 4-D used, the MS medium containing 2.5 mg/l 2, 4-D gave the best result when leaf was used as the explants (Table 1). In the case of nodal explants, the best result was obtained on MS medium containing 1.5 mg/l 2, 4-D. It was observed that when the concentration of 2, 4-D was increased from 2.5 mg/l; the rate of callus formation was slightly decreased in the case of leaf explants. At 2.5 mg/l 2, 4-D, the rate of callus formation was 80% (Table-1). Callus was slight whitish green and compact. At 3mg/l 2, 4-D the rate of callus formation was 60%. The lowest rate of callus formation (30%) was observed from MS medium containing 0.5-mg/L 2, 4-D. Callus was slight granular and light green in color (Table-1). In the case of nodal explants, the highest callus formation rate was 30% at 1.5 mg/l 2, 4-D. Callus was yellowish green and less friable. The lowest callus formation rate (10%) was noticed from the medium supplemented with 0.5 mg/l 2, 4-D and the callus was light green and friable (Table-1).

During the first sub-culture, the best medium for leaf explants contained 2.5 mg/l 2, 4-D.In case of hormonal combination, the best callus proliferation was observed from the medium containing 2 mg/l BAP and 1 mg/l IAA (Table-2).For nodal explants, the best media had 1.5 mg/l 2, 4-D. During the second sub-culture, the best medium contained 2.5 mg/l 2, 4-D with callus proliferation rate of 80% and in the case of BAP+IAA (2+1mg/l), the callus proliferation rate was 70%. During the third sub-culture, different concentrations of kinetin, ranging from 1 to 5 mg/l, were used in addition to the hormonal combinations listed above and the results indicated that kinetin at 5 mg/l was the best (Table-2). Many researchers have also reported the promotive effect of 2, 4-D on callus initiation and growth. Bespalhok (1996) reported that callus initiation was observed from the floret explants on MS medium supplemented with 9.05 and 18.10 μM of 2, 4-D and 0 to 9.29 μM of kinetin. However, the maximum embryogenic callus formation occurred in a medium containing 9.05 μM of 2,4-D but without kinetin

Bras and Fisiol (1997) reported that when the explants of Stevia were inoculated on basal MS medium supplemented with different concentrations of 2, 4-D (4.52, 9.05, 18.10 μM) and kinetin (0, 0.46, 2.32, 4.65, 9.29 μM). Formation of embryogenic callus was first observed 10 days after inoculation. At 4.52 μM 2, 4-D embryogenic callus formation was limited to only one explant on the medium without kinetin.At 9.05 μM 2, 4-D without kinetin, the maximum amount of embryogenic calli was produced.

Ferreira and Handro (1987) reported the maintenance of *Stevia rebaudiana* suspension and regeneration of plants from calli derived from cell suspension. Suspension cultures composed of isolated cells and cellular aggregates were obtained in 20-30 days by using friable callus as the initial inoculums in liquid media with cytokinin and auxin combination and periodic filtering with 6 to7days interval between sub-cultures. Stock cell suspensions were plated on basal MS agar medium with cytokinin and auxin combination to form callus. Calli originating from cell suspensions when transfered to medium with kinetin and auxin were able to form buds. Swanson *et al*., (1991) reported that when the leaf explants of *Stevia rebaudiana* were cultured in MS basal medium, sucrose (30 g/l), agar (0.9% w/v) and supplemented with vitamins, auxin and cytokinin, friable calli were formed. In our study, basal MS medium supplemented with BAP in combination with IAA was used for organogenesis. Different concentrations of BAP+ IAA were used. Among different concentrations of BAP and IAA, 2 mg/l BAP in combination with 1 mg/l IAA was the best for organogenesis. The rate of organ formation was 60% but in other concentrations of BAP +IAA organ formation was comparatively less (40%) (Table-3). Swanson et al. (1992) reported that when the leaf explants of

Stevia rebaudiana were cultured in MS media with vitamins, sucrose 30 g/l, agar(0.9% w/v) and supplemented with auxin and cytokinins, friable calli were formed. Differentiation of callus tissue was then achieved by eliminating the agar and modulating the hormone concentration. Medium containing an increased auxin concentration but no cytokinin or an increased cytokinin but no auxin yielded root or shoot cultures respectively.

Terzi and Loschiavo (1990) reported that when an embryogenic single cell of Stevia, divided to form a filamentous pattern flow embryo,the basal cells of pro-embryo formed a suspensor-like structure, while the upper cells originated an globular embryo. In the same year, Merkle *et al.*, (1990) reported that when the embryos were transferred to media containing a low concentration of sucrose (30 mg/l) but no growth regulators the embryos developed roots but failed to form shoots. MS media supplemented with BAP alone or in combination with IAA was used for in vitro shoot elongation in *Stevia rebaudiana* from shoot apices explants. In our study, among different concentrations of BAP used, basal MS medium containing BAP at concentration of 1 mg/l gave the best result. The highest rate of shoot elongation (70%) was observed at1mg/lBAP, while the lowest shoot elongation rate (20%) was observed in a medium containing 0.5 mg/l BAP (Table 4). The best hormonal combination was 2 mg/lBAP plus 1 mg/l IAA with a shoot elongation rate of 60%. While in other concentration, the shoot elongation was nil (Table 4). The regenerated shoots were transferred to root induction media, i.e., MS media with full and half strengths of auxins. It is evident from the Table. 5 that MS with 0.5 mg/l IBA was the best medium because the frequency of root formation was 70% and days to root initiation was minimum as compared to other media. For initiation and multiplication of shoots, many researches used the different types of cytokinins. The results of following scientist are similar to this study. Baksha *et al.*, (2006) reported that high frequency of multiple shoot regeneration was achieved from shoot apices explants on MS medium supplemented with 2 mg/l BAP. Elongated shoot were rooted well in half strength MS medium with 0.5 mg/l auxin.

Sivaram and Mukundan (2003) reported a 70% survival rate when the explants were cultured on MS medium supplemented with BAP (8.87 μM) and IAA (5.71 μM) at hardening phase on the substrate coco peat. Rooting was observed on to a medium containing auxin. Zaerr and Mapes (1982) and Thomas Blakesely (1987) reported that media containing high level (3.0-5.0 mg/l) of BAP yielded a few of shoot and decreased with further more increase in BAP concentrations. (Shoot becomes yellow and short abnormal morphology). On the medium containing if BAP callus formation was the paired at cut end of the shoot tip. This compact morphogenic callus was whitish to light green in color. Jaiswal *et al.*, (1989) and Mathur (1993) reported that in vitro experiments involved initially the establishment of shoot tip explants in culture. As a result it occurred in the formation of multiple shoot elongated and finally developed roots for plant formation.

Table-1: Effect of different concentrations of phytohormones on callus and growth induction

Explants	Media	Conc. of hormones mg/LAre you talking about 2, 4-D?)	Days till callus initiation	No of test tubes	No of test tubes showing the callus response	%age of callus induction	Characteristic of callus	
							Colors	Types
Leaf	MS+2,4-D	0.5	20-25	10	03	30%	Light green	Slight Granular
Leaf	MS+2,4-D	1.0	20-25	10	06	60%	Light Yellowish green	Slight Granular and Compact
Leaf	MS+2,4-D	1.5	20-25	10	06	60%	Light Yellowish green	Slight Compact
Leaf	MS+2,4-D	2.0	20-25	10	07	70%	Light green	Nodular and Compact
Leaf	MS+2,4-D	2.5	20-25	10	08	80%	Slight Whitish green	Slight Compact
Leaf	MS+2,4-D	3.0	20-25	10	06	60%	Whitish green	Compact
Node	MS+2,4-D	0.5	20-25	10	01	10%	Light green	Less friable
Node	MS+2,4-D	1.0	20-25	10	02	20%	Yellowish green	Less friable
Node	MS+2,4-D	1.5	20-25	10	03	30%	//	//
Node	MS+2,4-D	2.0	20-25	10	02	20%	Light green	Friable
Node	MS+2,4-D	2.5	20-25	10	01	10%	Slight Whitish green	Slight Compact
Node	MS+2,4-D	3.0	20-25	10	01	10%	Whitish green	Slight compact and less friable
Node	MS+2,4-D	0.5	20-25	10	01	10%	Light green	Less friable
Node	MS+2,4-D	1.0	20-25	10	02	20%	Yellowish green	Less friable
Node	MS+2,4-D	1.5	20-25	10	03	30%	//	//
Node	MS+2,4-D	2.0	20-25	10	02	20%	Light green	Friable
Node	MS+2,4-D	2.5	20-25	10	01	10%	Slight Whitish green	Slight Compact
Node	MS+2,4-D	3.0	20-25	10	01	10%	Whitish green	Slight compact and less friable

Table-2: Effect of different concentrations of phytohormone and hormonal combination on callus sub-cultures

Explants	Media (mg/L)			%age of Callus Proliferation	Characteristic of Callus	
	Hormones	From	To		Color	Types
Leaf	MS+2,4-D	1	1	40%	Light yellowish green	Slight granular and Compact
Leaf	MS+2,4-D	1	2.5	60%	//	//
Leaf	MS+2,4-D	2.5	2.5	80%	Whitish and light Yellowish green	Compact
Node	MS+2,4-D	1	1	04%	Yellowish green	Less friable
Node	MS+2,4-D	1	1.5	06%	//	//
Node	MS+2,4-D	1.5	1.5	10%	Whitish green	Slight Compact and less friable
Leaf Callus	3mg/L	2 mg/L	10%	Yellowish	Compact and Nodular callus	
Leaf Callus	3mg/L	2.5 mg/L	80%	Yellowish Brown	Granular and Compact	
Leaf Callus	3mg/L	3 mg/L	50%	//	//	
Leaf Callus	2+1	2+1	70%	Yellowish	Compact and Nodular callus	
Leaf Callus	2+1	2.5 mg/L	30%	Yellowish	Compact and Nodular callus	
Leaf Callus	2+1	3 mg/L	20%	//	//	
Leaf Callus	3mg/L	2+1	70%	Yellowish and Brown	Nodular and Granular	
Leaf Callus	4mg/L	2+1	50%	//	Compact and Nodular	
Leaf Callus	2mg/L	1mg/L	20%	Light Yellowish green	Compact and Nodular	
Leaf Callus	2mg/L	2mg/L	30%	Yellowish	Nodular	
Leaf Callus	2mg/L	3mg/L	35%	//	//	
//	//	4mg/L	50%	Yellowish and Brown	Compact and Nodular	
//	//	5mg/L	80%	Yellowish	Compact and Nodular	

Table-3: Initiation of organogenesis from leaf calluses in different phytohormone combinations

Explants	Phytohormones Combination BAP+IAA mg/L	Days of initiation of Organs	No of test tubes cultured	No of test tubes in which organ formation starts	% age of organ formation	Characteristics of Callus	
						Color	Type
Leaf Callus	1+1	80	5	0	Nil	Nil	Nil
Leaf Callus	1+2	80	5	2	40	//	//
Leaf Callus	2+1	80	5	3	60	Light Yellowish to Green	Compact and Nodular
Leaf Callus	2+2	80	5	0	Nil	Nil	Nil

Table-4 Effect of different concentrations of phytohormones on shoot elongation

Explants	Phytohormones	Concentration mg/L	Days	No of test tubes cultured	No of test tubes showing response	% of shoot elongation	Color
Shoot apices	MS+BAP	0.5	25-30	5	1	20%	Light brown
Shoot Apices	MS+BAP	1.0	25-30	5	3.5	70%	//
Shoot Apices	MS+BAP	1.5	25-30	5	0	Nil	Nil
Shot Apices	MS+BAP	2.0	25-30	5	0	Nil	Nil
Shoot apices	MS+BAP+IAA	1+1	40-50	5	0	Nil	Nil
Shoot Apices	MS+BAP+IAA	1+2	40-50	5	2	40%	Yellowish Brown
Shoot Apices	MS+BAP+IAA	2+1	40-50	5	3	60%	Light Brown

Table-5 Effect of auxins on days to root initiation and frequency of root formation from regenerated shoots of *Stevia*

Treatments (µM)	Days to root initiation	No. of shoots cultured	No. of cultures showed root induction	Frequency of root formation
0.0	-	-	-	-
MS + NAA 0.5	11.6	10	1	10
MS + NAA 1.0	12.6	10	2	20
MS + NAA 2.0	14.0	10	4	40
MS + IBA 0.5	9.8	10	7	70
MS + IBA 1.0	13.4	10	5	50
MS + IBA 2.0	13.5	10	5	50
MS ½ + NAA 1.0	12.4	10	4	40
MS ½ + IBA 1.0	10.8	10	3	30

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RELATIVE RESPONSE OF SUGARCANE VARIETIES AT THE SAME IRRIGATION LEVEL

By
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ABSTRACT

An experiment was conducted during 1994-95 and 1995-96 cropping years in Bangladesh Sugarcane Research Institute (BSRI) farm at Ishurdi with a view to observe the relative response of sugarcane varieties at the same optimum irrigation level. The varieties were Isd l6, Isd 18, Isd 19, Isd 20, Isd 21 and L.Java-c. Irrigation was applied at pan ratio, PR (IW/CPE) = 0.80. In both the years, Isd 16 gave highest yield followed by Isd 18. Yield of Isd 16 was significantly higher than other varieties except Isd 18. Hence the varieties Isd 16 and Isd 18 which are known as intolerant to water stress (drought and water logging) give better response to optimum soil moisture than the [rest] varieties marked as tolerant to water stress.

Key words: Irrigation level, sugarcane varieties, tolerance to water stress, pan ratio.

INTRODUCTION

Sugarcane plant shows a complex mechanism for its adaptability in serious soil and climatic conditions. It can withstand conditions of severe drought and some varieties may grow in waterlogged conditions as well. Varietal adaptability is due to morphological and anatomical modification of plant parts and tissue and some physiological traits associated with plant survival (Malik, 1992). Humbert (1968) reported that root growth of sugarcane is influenced by cultural practice of tillage, soil type, soil profile characteristics, soil moisture and fertilization and also [cane breeding and selection of] varieties. Amin *et al.,* (1971) reported that varieties differ in their response to changes in the intervals of irrigation, depending mainly on their root system distribution. Rahman and Alam (1985) found Isd 2/54 followed by Isd 17 to have quite a high degree of tolerance to water logging among the sugarcane clones/variety tested. Mia *et al.,* (1993) found the varieties L.Java-c, Isd 20 and Isd 21 showing higher tolerance to flood water logging, in that order. Anon (1997) found Isd 2/54 to have higher tolerance to water logging than Isd 16 and Isd 17, as well as to drought. Isd 16 yielded better in both stress conditions than Hussain *et al.,* (1995) concluded that the variety Isd 16 is a high yielding, high sugar containing, thick and tall cane intolerant to water stress. Variety Isd 2/54 is moderately high yielding, relatively thin-dwarf cane tolerant to water stress and variety Isd 20 is a high yielding, high sugar containing, thick and tall cane highly tolerant to water stress (both drought and water logging). Ali *et al.,* (1998) found Isd 16 and Isd 18 as drought intolerant whereas Isd 20 and Isd 24 as highly drought tolerant. From the above findings, it is obvious that any variety tolerant to water logging is also tolerant to drought condition, because both conditions give water stress to sugarcane (Yang, 1979). In Bangladesh sugarcane is irrigated not exceeding 20-25% of total area. Full adoption of irrigation is not done due to its high cost. Hence, identification of high yielding varieties responsive to optimum irrigation level should be a good contribution and this has been the aim of this research.

MATERIALS AND METHODS

The experiment was conducted at BSRI farm, Ishurdi, India, during 1994-95 and 1995-96 cropping seasons. The soil was calcareous loamy alluvial having bulk density of 1.42 gm/cc and field capacity (FC) of 37.00 (Vol %). The experiment was set following randomized complete block design with three replications. The varieties selected for this experiment (i.e treatments) were Isd 16, Isd 18, Isd 19, Isd 20, Isd 21 and L.Java-c.

Irrigation was given to the plot at pan ratio, PR [(IW + ER)/CPE] = 0.80, where pan ratio (PR) is the ratio of irrigation water (IW) applied plus effective rainfall (ER) between two irrigation cycles to the cumulative open pan evaporation (CPE) from USWB class A open pan. Irrigation water was calculated considering 40 cm effective root depth at the start and 100 cm during tillering stage, and assuming 50% depletion of available soil moisture allowable. Irrigation interval was determined using the following equation: CPE = (IW + ER)/PR. In each irrigation a prefixed amount of irrigation water was applied. No irrigation was needed during and after monsoon. Life irrigation with 45 mm irrigation water was applied on the day just after transplantation of 40 day's STP settlings in late November in both years. Sugarcane was planted with row-to-row spacing of 1 m and plant to plant spacing of 45 cm recommended dose of manure and fertilizer was applied. Basal dose of fertilizer and manure were applied during land preparation and before transplantation. Top dressing fertilizer was applied 4-5 days after irrigation followed by mulching. Other intercultural operations including weeding, mulching, mechanical and chemical pest control measures were taken as and when required.

RESULTS AND DISCUSSION

The effect of irrigation on the yield and yield components of sugarcane varieties in the year 1994-95 and 1995-96 cropping years are shown in table-1 and 2 respectively. From table 1 it is seen that in 1994-95 cycle there was no significant difference in plant height number of internodes, girth and also recovery of the varieties. Tiller number, number of millable cane, weight per cane and also the yield of variety under treatments differed significantly at 5% level of significance. The highest yield (86.84 t/ha) of variety Isd 16 differed significantly from the yield of other varieties except the yields of Sid 18, which was 83.42 t/ha. The data on table-2 evidence that there was no significant difference in plant height, number of internodes, plant girth and also recovery in response to irrigation in the 1995-96 cycles. However, number of tillers and of millable canes, weight per cane and yield of varieties had significant difference. Highest yield was found in the variety Isd 16 (88.84 t/ha) which was followed by the yield of variety Isd 18 (84.83 t/ha) without significant variation, but with significant difference from other varieties.

In general our varieties have attained tolerance to water stress conditions, especially caused by water logging, probably because they were selected under that condition. Water logging is a common feature in our fields and the plants suffer from internal drainage problem for long period (Anon, 1990-93; Siddique & Hossain, 1997). Regarding mortality of tiller it is seen that in both years the varieties had equimortality, but in 1995-96 cycle the mortality was higher on an average. The variation is perhaps due to the persistence of water logging for a longer period due to the higher rainfall during 1995 (Anon, 1990-93). However we may conclude that Isd 16 & Isd 18 are sensitive or responsive to irrigation as well as intolerant to water stress condition. Other varieties tested are less responsive to irrigation but tolerant to water stress condition.

Table-1 Yield and yield components of selected varieties at the same irrigation level in plant cane (1994-95), Ishurdi Pabna, Bangladesh

Treatment	Tiller (000/ha)	Millable cane (000/ha)	Mortality	Height (m)	Internode (No.)	Girth (Cm)	Weight (Kg/cane)	Recovery (%)	Yield (ton/ha)
Isdl6	164.43 a	127.71 a	22.33	2.48	20.83	2.08	0.68a	9.36	86.84 ab
Isdl8	158.71 ab	124.52 ab	21.54	2.42	22.41	2.09	0.67 ab	8.29	83.42 ab
Isd21	1 53.62 b	118.12 b	17.49	2.46	22.16	2.01	0.63 b	9071	74.41 b
Isd20	140.23 be	115.72 be	22.72	2.39	22.57	2.05	0.61 be	8.64	70.58 be
Isdl9	145.49 be	112.37 be	25.01	2.41	19.33	2.00	0.62 be	9.03	69.66 be
LJC	141.58 c	108.19 c	23.43	2.49	24.16	2.03	0.60 c	8.48	64.91 c
LSD at 5%	9.13	8.41	NS	NS	NS	NS	0.05	NS	5.12

Table-2 Yield and yield components of selected varieties at the same irrigation level in Plant cane (1995-96), Ishurdi, Pabna, Bangladesh

Treatment	Tiller (000/ha)	Millable cane (000/ha)	Mortality	Height (m)	Intern ode (No.)	Girth (Cm)	Weight (Kg/cane)	Recovery (%)	Yield (ton/ ha)
Isd 16	194.03 a	142.20 a	26.71	2.12	23.26	2.21	0.69 a	9.73	88.8 4a
Isd 18	188.31 ab	139.62 ab	25.86	2.44	19.73	2.09	0.68ab	9.33	84.8 3ab
Isd 21	183.68 b	133. 35 b	27.40	1.87	22.0	2.23	0.64 b	9.08	83.5 4b
Isd 20	181.42bc	13 1.08 be	27.75	1.93	18.06	1.97	0.62b	8.55	80.5 b
Isd 19	180.37 b	132.77 be	26.39	1.91	18.00	2.21	0.63bc	8.33	77.3 5bc
LJC	1 73.68 c	124.50 c	28.32	2.13	21.86	1.76	0.61c	8.33	75.3 9c
LSD 5%	8.35	7.85	NS	NS	NS	NS	0.05	NS	5.01 5

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GENETIC DIVERSITY OF SIX SUGARCANE VARIETIES BASED ON SSR MARKERS

By

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ABSTRACT

The investigation was conducted for analysis of genetic diversity of six sugarcane varieties (Isd 16, Isd 20, Isd 28, Isd 29, Isd 30 and Isd 31) using three SSR primers (SMC119CG, SMC319CG and SMC334BS). The three SSR primer pairs amplified a total number of 34 bands from the six varieties through 8.0% polyacrylamide gel electrophoresis. The sizes of the amplified bands in the six varieties ranged from 80 to 200bp. The highest number of alleles per locus and number of genotypes per locus (1.20) was obtained from the primer SMC334BBS followed by SMC319CG (1.15) and SMC119CG (1.00) respectively. Genetic diversity or polymorphism information content (PIC) per primer pair ranged from 0.67 to 0.83 with a mean of 0.77 for all loci across the six cultivars evaluated. Both primer pairs SMC319CG and SMC334BS showed the highest and similar PIC value 0.83 followed by SMC119CG (0.67). The most polymorphic SSR marker was associated with the highest number of bands detected. The primer pairs SMC319CG and SMC334BS were the most polymorphic marker for six varieties with PIC values of 0.83. The number of varieties with unique banding patterns distinguished by the three SSR markers ranged from 66.67 to 100%. The three SSR markers were able to discriminate 88.89% of all the cultivars evaluated with unique banding patterns. The highest linkage distance was recorded between the varieties Isd 16 and Isd 20. Genetic relationships among the varieties at the average distance of 12.5 separated the variety Isd 16 from the others. Variety Isd 16 and Isd 20 were shown to be outliers and distantly related to the rest of the varieties. The three primers were able to identify and classify six sugarcane varieties indicating genetic differences among varieties with coincidence of their field performances.

Key words: Sugarcane, genetic diversity, SSR markers.

INTRODUCTION

Genetic diversity and characterization of sugarcane germplasm as well as released varieties based on agronomical and morphological traits are being practised since initiation of sugarcane breeding. Phenotypic differences may also reveal genetic differences. Theoretically, phenotypic diversity should approximate genetic diversity. The number of genes involved in the control of phenotypic traits increases as the number of phenotypic traits being evaluated increases. Consequently, it improves the utility of phenotypic diversity in predicting genotypic diversity. Genetic relationships among varieties and populations can be measured by similarity of any number of phenotypic characters. Differences between characters are assumed to reflect the genetic divergence of the genotypes. However, characterization of varieties based on agronomic and morphological traits is subjective, labour intensive and can be influenced by genotype x environment interactions. Molecular markers are valuable tools in a plant breeding programme as well as for evolutionary and conservation studies. They are almost unlimited in number and are not influenced by environment. Simple sequence repeats (SSRs) also known as microsatellites are molecular markers based on tandem repeats of short (2-6bp) DNA sequences (Litt and Lutty, 1989). These DNA sequences are highly polymorphic even among closely related varieties due to mutation causing variation in the number of repeating units (Saghai-Maroof *et al.*, 1994). SSRs can be analyzed by a rapid, technically simple and inexpensive polymerase chain reaction (PCR) based assay that requires only small DNA quantities. Through PCR, different alleles at a locus can be detected by using conserved DNA sequences flanking the SSR as primers. SSR markers are co-dominant and can be transmitted in

simple Mendelian segregation. Lastly SSRs are abundant and uniformly distributed in plant genomes (Lagererantz *et al.*, 1993; Wang *et al.*, 1994; Akkaya *et al.*, 1995). At Bangladesh Sugarcane Research Institute, germplasm characterization based on agronomic and morphological traits has been initiated. Varieties are being released based on agronomic and morphological traits. Genetic diversity study and molecular characterization using RAPD markers started recently. Therefore, this investigation has been undertaken to study genetic diversity and to characterize six selected sugarcane varieties through fingerprinting using SSR markers .

MATERIALS AND METHODS

The experiment was carried out at the Biotechnology Laboratory, Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh in the cropping season 2006-2007.

Plant materials and sample collection

Six BSRI released sugarcane varieties (Isd 16, Isd 20, Isd 28, Isd 29, Isd 30 and Isd 31) were used for DNA isolation. The fresh tops from the 8 month old field grown sugarcane were collected and outer leaf sheaths were removed leaving the inner spindle to get the meristem cylinder. Then the meristem cylinder (spindle base) was cut into small pieces (about 5 mm long with 3 mm dia) with sterile scissors and the required weight (0.2 g) using a fine balance. Care was taken during sampling to avoid injury of the meristem cylinder after removal of the outer leaf sheaths.

DNA isolation

A modified method of Aljanbi *et al.* (1999) was used to isolate total genomic DNA. Cut pieces of meristem cylinder (about 5 mm long with 3 mm dia) weighing 0.2 g were taken in a small mortar (dia 6.5 cm and 3.5 cm depth) homogenized with pestle in 800 µL of extraction buffer (200mM Tris HCl, pH 8.0; 50mM EDTA.H_2O, pH 8.0; 2.2M NaCl; 2% CTAB; 0.06% Sodium Sulfite) until finely shredded within 40-50 second. The grinded sample was taken into a 2 mL eppendorf tube to which was added 150 µL of each 5% SDS, 10% PVP, 20% CTAB. It was mixed well and incubated at 65°C in a water bath for 40 minutes. During incubation 3-4 times inversion was done. After incubation the samples were cooled to room temperature and equal volume of Phenol Chloroform Isoamyl Alcohol (25 t 24 t 1) was added mixed well by inversion and centrifuged at 10,000 rpm at room temperature for 30 minutes. Then the aqueous phase (about 800 µL) was recovered and transferred to a fresh ice-cold 2 mL eppendorf tube and an equal volume of ice-cold isopropanol was added followed by 120 µL of 6M NaCl. The samples were incubated at -20°C for at least 1h and centrifuged at 10,000 rpm at room temperature for 20 minutes. The upper layer of solution was discarded carefully by using an adjustable micropipette and 70% ice-cold ethanol about 2.5 times of the solution was added. It was centrifuged again at 10,000 rpm at room temperature for 10 minutes. After pellet formation the solution was discarded from the tube carefully so that the DNA pellet remains constant and undisturbed. Then 70% ethanol was added to the slant of the tube. The ethanol was discarded from the tube carefully so that the DNA pellet remained constant and undisturbed. DNA pellet was dried for at least 30 minutes by putting the tubes upside down on a filter paper. Then the DNA pellet was re-suspended in 50 µL of TE buffer (10mM Tris HCl, pH 8.0; 1mM EDTA.H_2O pH 8.0) and stored at -20°C for lateral use.

Primers used

Three selected sugarcane SSR primers (markers) developed by International Sugarcane Microsatellite Consortium, NSW, Australia were used to amplify simple sequence repeats of genomic DNA from six sugarcane varieties. These primers were SMC119CG, SMC319CG and SMC334BS (Table-1). Primers were evaluated on the basis of intensity or resolution of bands, repeatability of markers, consistency within individuals, and potential to differentiate varieties (polymorphism).

PCR amplification and electrophoresis

PCR amplification was done in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf) following the PCR profile of 94ºC for 3 minutes (initial denaturation) followed by 25 cycles of 45 seconds denaturation at 94ºC, 30 second annealing at 57ºC and elongation or extension at 73ºC for 30 seconds. After the last cycle, a final step of 3 minutes at 73ºC was added to allow complete extension of all amplified fragments. After completion of cycling programme, reactions were held at 4ºC. PCR reactions were performed on each DNA sample in a 10 µL reaction mixture containing 1.0 µL of 10x Ampli Taq polymerase buffer (PCR buffer), 0.3 µL of 50mM $MgCl_2$, 0.8 µL of 2.5mM DNTPs, 1.0 µL each of Primer Forward and Reverse from 2.0µM stock, 0.2 µL of 5U/µl Ampli Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India), 3.0 µL of 5ng/µL genomic DNA and a suitable amount (2.7 µL) of sterile deionized water. After amplification 2.0 µL loading dye was added to the amplification product and stored at 4ºC for separation using polyacrylamide gel electrophoresis. In each well 1.5 µL of PCR product of each DNA sample for each primer was loaded in 8% polyacrylamide gel (acrylamide and biacrylamide of SRL, India). Electrophoresis was performed at 50V for 2.5 hours. DNA ladder 100bp (Bangalore Genei Pvt. Ltd., India) was run alongside the reactions. The gel after electrophoresis was silver stained and dried at room temperature. DNA bands were observed on white light box and photographed by digital camera.

SSR data analysis

SSR data were analyzed for Percentage of Polymorphic Loci (*P*), Average Number of Alleles per Locus (*A*), Average Number of Alleles per Polymorphic Loci (*Ap*), Average Number of Genotypes per Locus (*G*), and Gene Diversity-Polymorphic Information Content (PIC). Cluster analysis and Dendrograms were constructed following electrophoresis, and the size of amplification products was estimated by comparing the migration of each amplified fragment with that of a known size fragments of molecular weight marker: 100bp DNA ladder and pBR322HaeIII. All distinct bands or fragment (SSR marker) were thereby given identification numbers according to their position on the gel and scored visually on the basis of their presence (1) or absence (0), separately for each individual variety and each primer. The scores obtained using all primers in the SSR analysis were then combined to create a single data matrix. Linkage distances were computed from frequencies of polymorphic markers to estimate genetic diversity and relationship between six sugarcane varieties using the Unweighted Pair-Group Method of Arithmetic Means (UPGMA) (Sneath and Sokal, 1973) using computer program "Statistica".

RESULTS AND DISCUSSION

The investigation was conducted to study the genetic diversity of six sugarcane varieties using three selected SSR primers according to Muyco (2002). He selected six SSR primers from the list of 259 primers developed by the International Sugarcane Microsatellite Consortium, NSW, Australia to study genetic diversity of 81 sugarcane varieties. From the primer list and according to the selection of Muyco (2002), three primers (SMC119CG, SMC319CG and SMC334BS) were selected for this investigation. These three primers were highly efficient for genetic diversity analysis of sugarcane (Muyco, 2002).

SSR primers with corresponding bands scored, their size range, number of polymorphic bands, percentage of polymorphic bands and number of bands per variety in six sugarcane varieties are presented in the table-2. The sizes of the amplified bands in the six sugarcane varieties ranged from 80 to 200bp. SSR primer pair SMC119CG revealed band sizes that ranged from 89bp to 124bp; from 150bp to 200bp for primer SMC319CG and band sizes ranging from 80bp to 184bp for SMC334BS. However, Muyco (2002) identified band sizes that ranged from 102bp to 152bp for the primer pair SMC119CG. This was perhaps due to the sample differences from this investigation. Yang *et al*.

(1994) pointed out that the range in allele sizes can be influenced by the large number of samples screened. All three SSR primer pairs used amplified a total of 34 bands from the six varieties of sugarcane using the Thermal Cycler (Genius, Techne) and 8% polyacrylamide gel electrophoresis (PAGE). Representative electrophoregrams according to primer pairs SMC119CG, SMC319CG and SMC334BS are shown in Figures 1, 2 and 3 respectively. For each primer pair, the number of bands varied from 7 to 15. The primer pair SMC319CG amplified the highest number of bands (15) followed by SMC334BS (12) and SMNC119CG (7). Due to the polyploidy nature of sugarcane, the SSR markers revealed multiple bands per locus. In Mauritius Sugar Industry Research Institute (MSIRI), Mauritius, the number of alleles generated per primer pair ranged from 9 to 20 using 5 primer pairs on 96 sugarcane cultivars (Janno, 2000). At Centre for Plant Conservation Genetics (CPCG), Southern Cross University (SCU) in NSW, Australia 3 to 12 alleles per primer pair were recorded across five sugarcane genotypes using 91 primer pairs (Cordeiro *et al.*, 2000). The highest number of polymorphic bands (11) was scored from the primer pair SMC319CG followed by SMC334BS (9) and SMC119CG (7). However, the same percentage (100.0%) of polymorphic bands was recorded from the primer pair SMC119CG which produced the lowest number of total bands (7) followed by primer pair SMC334BS (75.0%) and SMC319CG (73.3%). The highest number of bands (2.50) per variety was amplified from the primer pair SMC319CG followed by SMC334BS (2.00) and the lowest number of bands per variety was recorded from the primer SMC119CG (1.17). Among the three primers two primers (SMC319CG and SMC334BS) were able to distinguish 100% of varieties while the primer SMC119CG distinguished 66.67% of varieties.

The average number of alleles per locus, average number of alleles per polymorphic locus, average number of genotypes per locus and polymorphism information content (PIC) data for three primers are presented in table-3. The average number of alleles per locus and average number of genotypes per locus for the three primers were similar. The highest number of alleles per locus and the number of genotypes per locus (1.20) was obtained from the primer SMC334BBS followed by SMC319CG (1.15) and SMC119CG (1.00) respectively. The average number of alleles per polymorphic locus was recorded. The highest (1.36) was from primer SMC319CG followed by primer SMC334BS (1.33) and the lowest (1.00) was recorded from primer SMC119CG. Genetic diversity or polymorphism information content (PIC) per primer pair ranged from 0.67 to 0.83 with a mean of 0.77 for all loci across the six varieties evaluated. The primer pairs SMC319CG and SMC334BS showed the highest PIC values (0.83) followed by SMC119CG (0.67). The most polymorphic SSR marker was associated with the highest number of bands detected. The primer pairs SMC319CG and SMC334BS were the most polymorphic markers across the six varieties with PIC value of 0.83. The PIC values are dependent on the genetic diversity of the varieties under study. A high proportion of closely related genotypes would have the effect of lowering the PIC values (Garland *et al*., 1999). Mean PIC value among the six varieties was 0.76 indicating a high level of variability present in the varieties based on the three SSR primers. Comparable results were reported by Cordeiro *et al*., (2000) on sugarcane SSRs.

Genetic relationships among the varieties at the average distance of 12.5 showed two major clusters (C_1 and C_2) presented in Figure 4. At the linkage distance of 11.0 the cluster C_2 produced sub-cluster SC_1 and SC_2. Sub-cluster SC_2 produced sub-cluster SC_3 and SC_4 at the linkage distance of 10.0. Sub-cluster SC_4 again produced sub-cluster SC_5 and SC_6 at the linkage distance 8.5. Finally, sub-cluster SC_6 divided into two sub-clusters of variety Isd 28 and Isd 29 at the linkage distance of 7.0. The major cluster C_1 separated the variety Isd 16 from the other varieties. The variety Isd 16 is one of the best and sustainable varieties among the released varieties of BSRI. The results of DNA polymorphism of the six varieties bear the genetic diversity of variety Isd 16 from other varieties investigated. Variety Isd 16 and Isd 20 were shown to be outliers in the dendrogram and distantly related to the rest of the cultivars based on their genetic distances. The result agreed with the findings of Shahnawaz (2006). He also analyzed DNA polymorphism of the four varieties/accessions and found that the variety Isd 16 separated from the other varieties in a major cluster and the remaining three to another cluster. The variety Isd 20 is another variety having superior performances against

biotic and abiotic stresses than the other varieties. It lies in the sub-cluster SC_1 and is separated from the other varieties at the linkage distance of 11.0. This result also coincided with the field performances of the varieties.

The results of the present investigation revealed that the three SSR primers were able to identify and classify the six sugarcane varieties indicating genetic diversity among the varieties with coincidence of their field performances.

Table-1 Parameters of primers sequences of three sugarcane SSR primers from the International Sugarcane Microsatellite Consortium, NSW, Australia

Primer Code	Sequence (5′-3′)	G+C Content (%)
SMC119CG	Forward: -TTC ATC TCT AGC CTA CCC CAA-	47.61
	Reverse: -AGC AGC CAT TTA CCC AGG A-	52.63
SMC319CG	Forward: -CCT TTC ATC CAC CGA GGA CAA-	52.38
	Reverse: -GGT TCA CCG AAG CAA GAG AAC-	52.38
SMC334BS	Forward: -CAA TTC TGA CCG TGC AAA GAT-	42.85
	Reverse: -CGA TGA GCT TGA TTG CGA ATG-	47.61

Table- 2 SSR primers with corresponding bands scored, their size range, number of polymorphic bands, percentage of polymorphic bands and number of band per variety together with variety distinguished in six sugarcane varieties

Primer codes	Size ranges (bp)	Total bands scored	Polymorphic bands	Polymorphic bands (%)	Band per variety	Variety distinguished (%)
SMC119CG	89-124	7	7	100.0	1.17	66.67
SMC319CG	150-200	15	11	73.3	2.50	100.00
SMC334BS	80-184	12	9	75.0	2.00	100.00
Total		34	27	-	-	-

Table-3 SSR primers with corresponding average number of alleles per locus, average number of alleles per polymorphic locus, average number of genotypes per locus together with Polymorphism Information Content (PIC)

Primer Codes	Avg. alleles per locus	Avg. alleles per polymorphic locus	Avg. genotypes per locus	PIC (Polymorphism Information Content)
SMC119CG	1.00	1.00	1.00	0.67
SMC319CG	1.15	1.36	1.15	0.83
SMC334BS	1.20	1.33	1.20	0.83

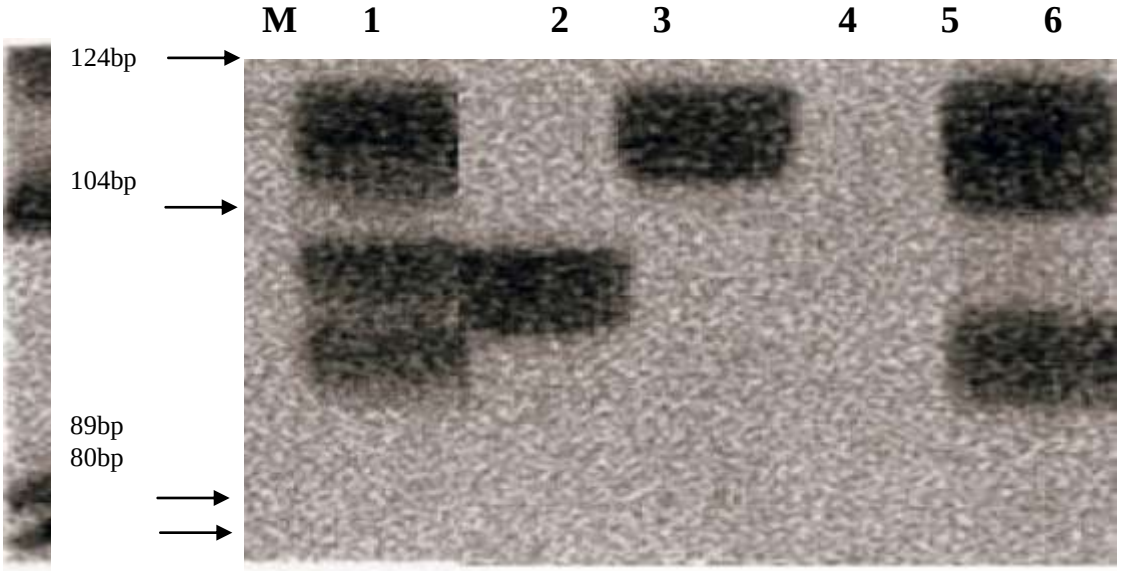

Fig.1 DNA Fingerprinting of BSRI released six varieties of sugarcane based on SSR primer pair SMC119CG through PAGE (M= marker pBR322HaeIII, Lane 1= Isd 16, Lane 2= Isd 20, Lane 3= Isd 28, Lane 4= Isd 29, Lane 5= Isd 30, Lane 6= Isd 31)

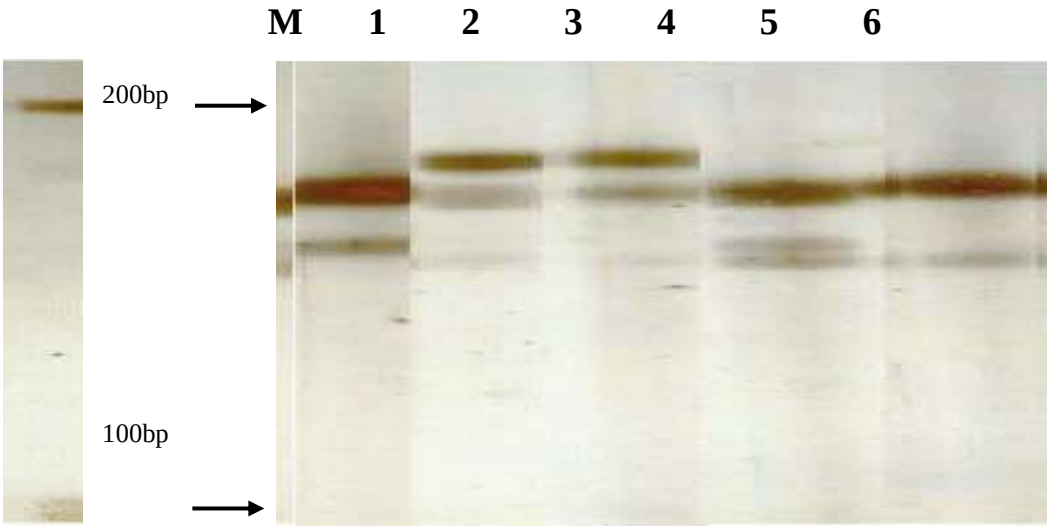

Fig.2 DNA Fingerprinting of BSRI released six varieties of sugarcane based on SSR primer pair SMC319CG through PAGE (M= marker 100bp ladder, Lane 1= Isd 16, Lane 2= Isd 20, Lane 3= Isd 28, Lane 4= Isd 29, Lane 5= Isd 30, Lane 6= Isd 31)

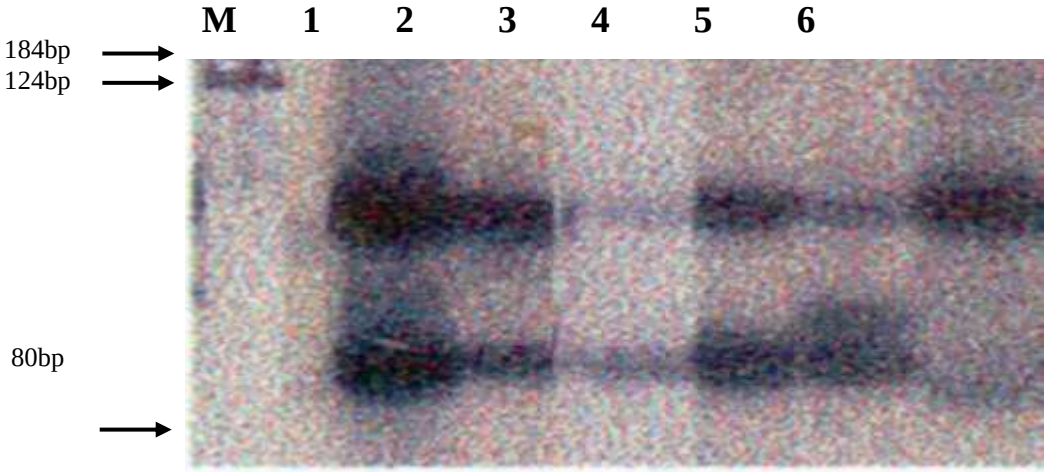

Fig.3 DNA Fingerprinting of BSRI released six varieties of sugarcane based on SSR primer pair SMC334BS through PAGE (M= marker pBR322HaeIII, Lane 1= Isd 16, Lane 2= Isd 20, Lane 3= Isd 28, Lane 4= Isd 29, Lane 5= Isd 30, Lane 6= Isd 31)

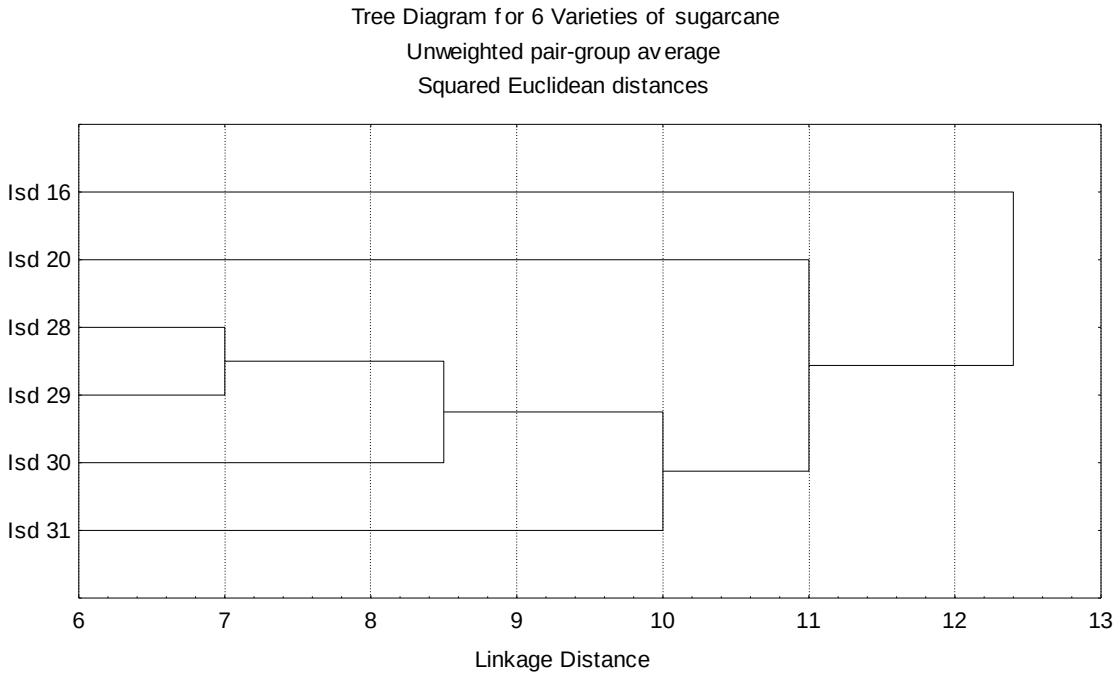

Fig.4 Cluster analysis by unweighted pair group method of arithmetic means (UPGMA) of six BSRI released sugarcane varieties based on three SSR markers

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DATE PALM CULTIVATION IN BANGLADESH: FARMERS' PRACTICES

By
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ABSTRACT

Studies were conducted during 2006-07 cropping season at Keshabpur upzilla (Jessore) to investigate the present practices of date palm plant cultivation and juice extraction, marketing of both juice and gur, per plant gross margins, constraints, economic potentiality etc. Studies reveal that farmers' do not apply fertilizer, irrigation and follow no methodical cultivation practices in the study area. It was also found that during last ten years farmers cut 5 plants per year, whereas planted 1.5 plants only. A farmer earned Tk. 750-850 per plant per tapping season (about three months) by selling juice. Cost of date palm gur (molasses locally called 'gur') production was recorded Tk. 330.0 per plant of which 62 per cent was fuel cost. It was also observed that some farmers' used juice for gur production and found that approximately 23.1 kg gur plant $^{-1}$ was obtained when entire juice was used for gur production. Gross return and gross margin were recorded as Tk. 688.4 and Tk. 358.4 plant $^{-1}$ respectively. Return per Taka investment (variable cost basis) was found Tk. 2.08. Farmers sold approximately 30 per cent of their gur to direct village consumers. Constraints identified were very low or no juice secretion from some plants, fuel scarcity for gur making, lack of gachee (expert manpower for date palm cutting and juice tapping), increased wages for gachee for juice collection, low price of gur during season, poor marketing and storage facilities for both gur and juice, lack of improved production technologies for date palm cultivation etc. These constraints need to be resolved to increase date palm cultivation and gur production in order to meet increasing demand of sugar and gur in Bangladesh.

Key words: Date palm, farmers' practice, grosses margin, gur, juice, marketing.

INTRODUCTION

Date palm is an important food-cum-cottage industrial plant in Bangladesh. It grows in homesteads, road sides, embankment sides, fallow lands, orchard or crop lands in unplanned way. It is mainly grown in greater districts of Jessore, Khulna, Noakhali, Patuakhali and Rajshahi. Demand of sugar and gur are being increased with population increase and urbanization, while sugarcane acreage is being gradually decreased and/or pushed to low lying marginal lands due to higher demand for cereals, vegetables etc and utilization of crop lands for houses, roads, industries etc. Therefore, there is little or no scope to increase both cane yield and sugarcane acreage to meet higher demand of sugar and gur for ever increasing population in Bangladesh. According to FAO recommendation, per capita at least 13 kg sugar is required for human balanced diet, and as such present requirement of sugar for 140 million populations in Bangladesh is about 1.8 million tons. Present production of sugar and gur in the country is about 0.2 million tons and 0.5 million tons respectively, and as such shortfall of sugar/gur is about 1.1 million tons. Present shortfall of sugar/gur can not be met through sugarcane cultivation alone. Date palm gur may be an alternative source of sugar to supplement the increased demand for sugar/gur (Anon, 2003-04).

Idris (1983) stated that 70 million date palm can be grown in road/rail/embankment without reducing the acreage of other crops and 0.7 million 5-6 member rural families can survive from the earning of this plant alone. He also estimated that if date palm is grown in a planned way it is possible to run two new sugar mills and sugarcane field can be released for the cultivation of other crops. Emran (1993) stated that a gachee (labourer who cut date palm plant to trap/extract juice to make gur) might earn Tk. 1500 to Tk. 2000 from juice tapping of date palm. Date palm acts an alternative source of sugarcane gur to supplement increased demand of sugar, and have a positive impact on sugar

industries in Bangladesh. During juice tapping season, 200-250 litres juice is obtained from each plant and gets 25-30 kg gur/molasses (Asaduzzaman *et al.*, 1986). Chowdhury and Satter (1993) reported that last five years total cash income from date palm varied from Tk. 810 to Tk. 5740 per farm with a mean of Tk. 2823 per farm. Miah and Alam (2001) revealed that juice secretion, degree. Brix of juice and gur recovery per cent may be enhanced through application of appropriate agronomic management practices such as irrigation, NPK fertilizer application and trash mulching to date palm plants. According to BBS report date palm and palmyra palm is grown in around 10755 to 10767 hectares of land and total estimated juice production is 3.34 to 3.48 million tons and @ 10 percent gur recovery 0.334 to 0.348 million tons of gur is produced per year in Bangladesh.

Date palm gur is very sweet, popular for its aroma and palatable to eat. It has higher demand in the cities of Bangladesh. It is grown in the country with no inputs and minimum care and this sugar plant has established her to accrue an economic benefit and income generating tools to the rural livelihoods. The study was designed to investigate the present practices of date palm plant cultivation and juice extraction, marketing of both juice and gur, per plant gross return and margins, constraints, economic potentiality etc.

METHODOLOGY

The present study was conducted during 2006-07 cropping season at Keshabpur upzilla (Jessore). Random sampling technique was followed to select growers for data collection from the list of Datepalm growers'. Data were collected from 60 sample growers using both structured and open ended questionnaire. Simple tabular analysis was done for the investigation. Use of juice and gur for home purposes were considered in calculating gross return. To examine profitability of date palm cultivation Gross Margin (GM) analysis was done since growers are more interested to know their return over variable costs rather than profit (through subtracting fixed cost from GM).

$GR = Ag_1 \times Pg_1 + Ag_2 \times Pg_2 + Sj \times Pj$

Where,

GR	=	Gross Return
Ag_1	=	Amount of gur prepared from total fresh juice (first night/time collected)
Pg_1	=	Price of gur kg $^{-1}$ made from fresh juice
Ag_2	=	Amount of gur made from second night/time collected juice
Pg_2	=	Price of gur kg $^{-1}$ made from second night/time collected juice
Sj	=	Sale of juice
Pj	=	Price of juice (per litre)

RESULTS AND DISCUSSION

The results of the investigation have been shown in tables 1-5.

Agronomic practices of date palm plant

No methodical cultivation of date palm plant was found in the area. Even, they didn't have any technical knowledge regarding date palm cultivation, date palm cutting, juice collection and gur preparation and preservation. Growers' did not apply any fertilizer and irrigation to date palm plant. New plantation of date palm seedling was found to be rare. Date palm gardens are being sold and rice and vegetables are being cultivating in those lands. Results shows that during last ten years growers cut 5 plants whereas planted 1.5 plants per year. They did not get any extension services regarding date palm plant cultivation either from DAE (Departments of Agricultural Extension) or other organizations.

Collection of date palm juice

Growers believe a date palm plant become mature after 6-7 years of plantation. Tapping of juice is seasonal and it is started from the month of November and continues upto mid March until day and night temperature became above 32^0C and 25^0C respectively. First night collected juice is the sweetest and the best. Good quality both solid gur (locally called 'patali'), semi-solid gur are prepared from this first night collected juice. Juice collected in the second night is relatively poor quality compared to 1st night collected juice and prepared relatively poor quality gur. After these two nights, plants are allowed to remain at rest for 2-3 nights and the same process again begins. During the tapping period (100-120 d), 170 - 220 litres of juice were obtained per plant.

Date palm juice marketing

Date palm juice is sold in villages, market places, hat/bagar etc in the study area. At evening, growers bring fresh juice, pure water and glass/mag to sell it to the mobile consumers at the market. Fresh juice charged for Tk. 2 to 3 depending on the size of mag/glass. By selling fresh juice a farmer could earn Tk 30 to 35 plant^{-1} day^{-1}. Besides, growers who do not have date palm plant usually bought fresh juice for making pita (solid preparation by rice powder) and paiesh (semi-solid rice preparation using date palm juice/gur) and it is a very old age practice in village level of the study area. Growers stated that Tk. 750-850 per date palm plant could be earned during the tapping season (about three months).

Cost and Return from date palm plant

Table-2 shows that cost of date palm gur production was Tk. 330.0 plant $^{-1}$ per season of which about 62, 18 and 14 per cent were for fuel, gachee and labour cost respectively. Higher standard deviation indicating variation of cost among respondents. It can be seen from the Table-3 that if growers use juice only for gur production he could get 23.1 kg gur plant $^{-1}$ of which 13.7 kg from first night collected juice and 10.0 kg from second night collected juice. Per plant gross return was Tk. 688.4, cost of gur production was Tk. 330.0 and gross margin was Tk. 358.4. Return per Taka investment (variable cost basis) was Tk. 2.08 (Table 4).

Marketing of date palm gur

In the study area three types of date palm gur viz semi-liquid, solid and patali were produced. The marketing channels of date palm gur are shown in the Figure 1. Growers sold 30 per cent gur direct to village consumers. They also sold gur to consumers at local/village market or upzilla/central market. Middle man (locally called Bapari) normally purchased gur from growers at local and/or upzilla market. But sometimes they purchased directly from growers' house. Bapari then sold it to stockist (locally called Aratder) then via wholesaler, retailer to consumer.

Constraints

It was also observed from the investigation that growers learnt entire process of juice collection to gur making from their ancestor and/or through trail and error methods and had no technical know-how about date plam plant cutting , juice tapping (collection), gur making and preservation. Fuel scarcity for making gur from date palm plant was one of the main constraints of growers. However, increased wages of gachee for date palm cutting and juice collection, lack of expert manpower for date palm cutting (gachee) and juice tapping, lack of improved production technologies, low price of gur during season, poor marketing and storage facilities of gur and juice were also identified as serious constraints (Table-5). It is seen from the table-5 that all growers reported that plant to plant great variation in juice secretion is the main constraints to increase date palm plant cultivation.

CONCLUSION

In the paradox of decreasing sugarcane land in Bangladesh it is very difficult to meet up increasing demand of sugar and gur through bringing more area under sugarcane cultivation. So, it is important to pay more attention on date palm cultivation in homestead, embankment, ponds, road sides, marginal lands and ail (demarcation mark) of different crop fields that remain fallow. This will reduce pressure on sugarcane cultivation resulting release of sugarcane lands for other crops cultivation and will increase income of rural people and thus contribute to reduce poverty and uplift meant of nutritional status of mass rural people. It is also very important to undertake systematic research to develop modern varieties, production technologies for improved cultivation practices, juice tapping, gur and juice marketing and preservation to mitigate the problems facing by growers. Appropriate steps should be taken to disseminate information regarding benefits of date palm cultivation and to encourage more growers to practice date palm plant plantation through collaborative approaches among themselves and between government departments.

Table-1 Agronomic cultural practices of date palm cultivation and growers practices

Agronomic Cultural Practices	Growers' Practices
Fertilizer application	Not applied
Irrigation application	Not applied
Methodical cultivation	Not followed
New seedlings plantation	Rare
Technical knowledge regarding date palm cultivation	No
Training/ Extension services received	No

Source: Field Survey, 2006-07

Table-2 Average cost of gur production in 2006-07 cropping year per date palm plant

Particulars	Cost (Tk $^{-1}$ plant)	Standard deviation	Percent of total cost
Gachee (juice tapping)	60	23	18
Fuel for gur production	205	40	62
Labour	45	23	14
Pot and others	20	15	6
Total	330.0	32	100

Source: Field Survey, 2006-07
Total plants of sample growers = 850

Table-3 Average return from gur production per date palm plant

Particulars	Amount (Kg)	Tk. Kg^{-1}	Gross Return
Gur from first night collected fresh juice	13.7	32.0	438.4
Gur from second night collected juice	10.0	25.0	250.0
Total	23.12		688.4

Source: Field Survey, 2006-07

Table-4 Total return, Variable cost and Gross margin per plant

Items	In Taka
Total return	688.40
Variable cost (producing cost)	330.00
Gross margin	358.40
Return per Taka investment (variable cost basis)	2.08

Source: Field Survey, 2006-07

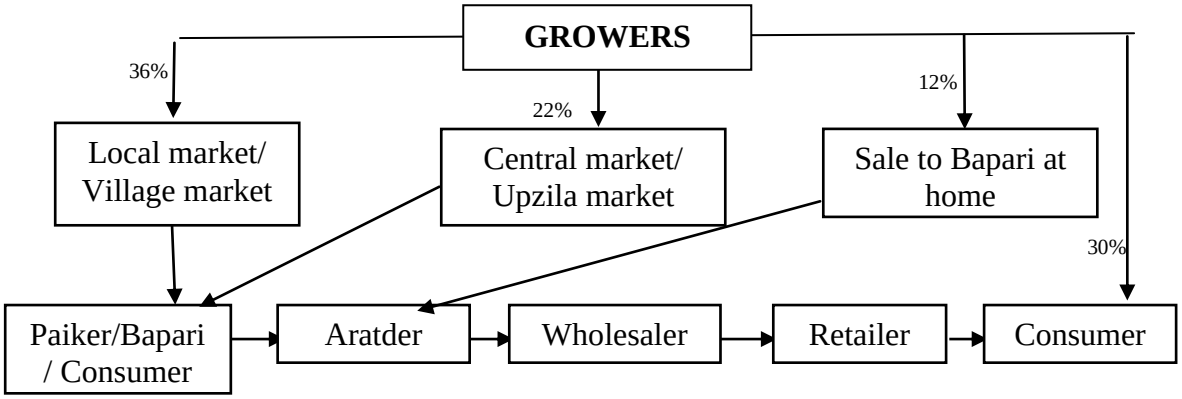

Figure-1 Marketing channel of date palm gur

Table-5 Constraints of date palm cultivation and gur making

Constraints	Respondent	Percentage
Fuel scarcity for making gur	60	100
Increased cost of gachee	60	100
No or very low juice extraction by some plants	60	100
Lack of expert manpower for date palm cutting (Gachee) and juice secretion	52	86
Low price of gur during tapping season	47	78
Lack of improved production technologies	45	75
Poor marketing and storage facilities of gur and juice	30	50

Source: Field Survey, 2006-07

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SUGAR INDUSTRY ABSTRACTS

By
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AGRICULTURAL ENGINEERING

A review of the cane haulage problems at hippo valley estates

S. Chidoma
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Hippo Valley Estates, a sugar producer in the south-eastern Lowveld of Zimbabwe experienced some problems with its cane haulage system from 2000 to 2005. These manifested themselves as erratic cane supplies, 'no-cane' mill stops, increased haulage costs, long burn-to-crush delays, and distortions to season length. These problems had serious operational and cost implications for the Estate, hence the need to review the system. The review incorporated a number of research methods. These included time and motion studies to measure the time spent by the various machines in loading and hauling the cane, as well as tracking the times spent by cane consignments at the various stages of the cane supply chain. Questionnaires were used to evaluate cane haulage operators' assessments of the factors they felt contributed to their failure to achieve set targets. The study confirmed the existence of the cane haulage problems. The causes of these were the use of old equipment, high downtime, slow reaction to breakdowns, poor coordination and communication between the mill, cane haulage and cane harvesting fronts, zone and mill delays, low stack weights, use of many cutting fronts and poor roads. Several recommendations were made to address the identified problems. These included the timely replacement of old equipment, improved servicing of broken down vehicles, improved synchronisation and communication, and the use of fewer consolidated cutting and harvesting fronts. There is also need to reconfigure the estates' cane haulage fleet to tractors with greater horsepower designed for cane haulage for increased payload and operational and cost effectiveness.

Mechanisation of sugarcane production in India

R.N.S. Yadav
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In India most of the cultural practices associated with sugarcane production are undertaken by using traditional tools, equipment and machinery. Mechanisation of sugarcane cultivation is evolving as a shift occurs from traditional practices to modern cultivation methods. These include appropriate mechanisation of tillage, planting, weeding and inter-row cultivation, plant protection, harvesting, loading, transport and other post-harvest operations including ratooning. The advantages include enhanced productivity, timeliness of operation, work quality, and utilisation of inputs and resources such as seed, fertiliser and chemicals, along with reductions in total cultivation costs and human drudgery. In India, the cost of sugarcane cultivation is US$800–1000 per hectare. About 25–30 percent of the cost is for manual labour which may equate to $US220 per hectare. In comparison to traditional practices, there is a cost saving of about 30 to 60% under mechanised farming systems. Mechanised sugarcane cultivation can reduce the cost of wages incurred for the various cultural operations and has economic benefits as well as timeliness and crop husbandry. Feedback studies conducted under the National Agricultural Technology Project on Sugarcane Mechanisation (ICAR) indicate that sugarcane growers are slowly adopting modern sugarcane machinery for selected operations such as tillage and planting, either on ownership or custom hire basis. Under Indian conditions, overall productivity of sugarcane can be increased by 10–15% through appropriate

mechanisation. However, access to the equipment by growers is a constraint. The commercialisation of a suitable design of sugarcane combine harvester is also urgently needed.

AGRICULTURAL AGRONOMY

Electrical resistivity measurements for fast and precise large scale characterisation of the agricultural land of cameroon sugar society (SOSUCAM)

T. Viremouneix, L. Guiard, M. Dabas and B. Tsogo Zamba
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A good knowledge of soil properties and their agricultural suitability is a major prequisite for managing agriculture in a planned, sustainable, and environmentally friendly manner. To optimise and extend its agricultural area, Cameroon Sugar Society (SOSUCAM) has thus decided to use an innovative technology based on a survey of the electrical resistivity of soils. In the first step of the project, the feasibility and interpretation of the measurements were tested on the Oxisols of SOSUCAM by an electrical survey conducted at two depths (0.5 and 1 m) on a 40 ha sugarcane field. Specialised software was then used to interpolate the measurements. Soil samples were taken and analysed simultaneously, and sugarcane was monitored in specific areas through measurements of growth, tillering and yield. Areas with homogenous resistivity and common properties have been identified using the map produced. The electrical values were correlated with the field.s physical properties such as its stoniness, depth and clay %. We have also observed different sugarcane growth patterns depending on resistivity values. In view of these good results, SOSUCAM has decided to extend this experiment to 26 000 ha of sugarcane fields and fallow land, without any particular reference to soil type, through the adaptation of a specific and patented technology, named ARP06© (for Automatic Resistivity Profiling). This technology, which has an adequate system of electrodes configuration, makes it possible to prospect continuously at different depths and get data to elaborate, through interpolation, resistivity maps. Resistivity surveying should thus be a quick and reliable technique to map the spatial variability of soil properties on large sugarcane areas and to define the latter.s agricultural potential. Furthermore, it should provide other important elements in field planning, beyond logistic constraints: delineating homogenous zones should improve the use of results of physical and chemical analyses and to identify zones where better practices and operations can be adopted.

The effect of a novel system of vinasse application on sugarcane growth and yield in China

Yang-Rui Li, Qiu-Zhen Zhu, Wei-Zhan Wang and Sushil Solomon
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A practical, feasible method of vinasse application for upland sugarcane plantation areas of Guangxi was developed, which does not impede cane sugar productivity and soil health. This method comprised pre-emergence liquid-line application of vinasse in furrows through valve controlled pipes, followed by covering with plastic film. Vinasse was applied @ 45 t/ha, 75 t/ha and 105 t/ha, mixed with water and experimentation was done at six locations in China. This method showed improvement in tillering, stalk elongation and sugar productivity. However, the response varied with the rate of vinasse application. Application of vinasse at 45 t/ha, 75 t/ha and 105 t/ha improved cane yield as compared to non-fertiliser control by 13.24%, 17.55% and 14.92%, respectively. The field data suggest that the vinasse treatments were also good for sugar accumulation as compared with the conventional fertilisation application. Vinasse has excellent nutritional qualities, and is a very good complete organic fertiliser for sugarcane. It produced significant increases in cane tonnage, and the benefit-cost analysis favoured its large-scale application under upland conditions. The application of 75 t/ha vinasse showed the best economic benefit in this study.

SUGARCANE BREEDING

Estimating genetic parameters and the efficacy of molecular breeding for resistance to the stalk borer

M.K. Butterfield, M.G. Keeping and C. Sewpersad
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Breeding for resistance to the stalk borer *Eldana saccharina* is one of the goals of the SASRI variety improvement program being addressed through an integrated approach involving conventional and molecular breeding. The objectives of this study were: to evaluate a mass screening method to estimate eldana resistance at a family level; to estimate narrow-sense heritability of eldana resistance, as well as family repeatability of resistance; and to attempt an initial verification of the efficacy of molecular breeding for resistance. Seedlings from 36 bi-parental crosses were planted at high density and inoculated with eldana larvae at 6 months of age. Phenotypic data for eldana resistance of the parents of the crosses were available, and the parents had been characterised for eight previously identified marker loci that ascribe 37% of the phenotypic variation in resistance. After one month, plots were harvested, eldana damage was measured and subjected to appropriate analysis. Mean stalk length damaged was highly significant between families. Variance components estimated by REML gave a family repeatability of 0.90, indicating that the mass-screening method was highly effective in estimating eldana resistance on a family level. Heritability calculated from phenotypic mid-parentoffspring regression was estimated at 0.57 explaining 12.6% of the variation in resistance at the family level. Heritability estimated from molecular marker prediction was 0.82, explaining 19.2% of the variation in progeny resistance. Molecular markers have apparently been effective in breeding for eldana resistance. Using sets of markers exhibiting stronger association with resistance may further increase the efficiency of molecular breeding.

Can flowering in sugarcane be optimised by use of differential declinations for the initiation and development phases?

N. Berding, R.S. Pendrigh and V. Dunne
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Managed initiation of flowering in the BSES Meringa photoperiod facilities (PFs) has resulted in about 70% of stalks delivering panicles from about 90% of clones treated. These results are excellent compared to natural flowering, but the infrastructure investment (AUD 620 000 per facility, present value) begs the question whether managed flowering can be further optimised. Early South African research hinted that the initiation and development phases of flowering in sugarcane differed in optimal declinations. Our objective initially was to determine responses to differential declinations applied to the whole flowering process and, from this, to study their effect when applied to the initiation and development phases. In 2004, declinations of 30, 45, and 60 s/d were applied to two populations of 89 and 128 clones. In 2005, these were repeated using 108 and 60 clones. In 2006, 116 and 64 clones were subjected to these in the initiation phase, followed by a common declination of 45 s/d when the photoperiod reached about 12 h 30 min. All treatments had a commencing day length of 12 h 55 min, with the lowest declination (30 s/d) commencing on 5 April. No treatments were exposed to temperatures $\geq 32°C$ in the initiation phase. Flowering in the 2006 PF experiments was excellent. However, declination treatments did not differ significantly, although the clones x declinations interaction was highly significant. A subsequent experiment will explore the effect of differential declinations applied to the developmental phase. A sub-set of 141 of 180 treated clones for which flowering data were available in two field sites yielded 71% flowered stalks compared to

14% for the field. The opportunity to increase usable flowering some 30% towards total flowering strongly encourages attempts to optimise fractions of the whole process to achieve this.

MOLECULAR BIOLOGY

Micropropagation by direct somatic embryogenesis: is disease elimination also a possibility?

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The purpose of the SASRI Quarantine facility is to intercept diseased sugarcane cultivars. There are several *in vitro* techniques available for cleaning up diseased sugarcane material. However, it would be advantageous for the plant breeding program if disease-containing cultivars, imported to enlarge the germplasm base, could be simultaneously cleaned up and rapidly multiplied. In addition, it would be beneficial to export local germplasm as micropropagated, disease-free plantlets. NovaCane® is a process developed by SASRI for the rapid micropropagation of sugarcane using direct somatic embryogenesis. This study investigates the application of the NovaCane® technique to clean up disease-infected material, and therefore its usefulness in the SASRI Quarantine facility. Transverse sections (2 mm) of immature leaf whorls from healthy and diseased stalks were placed on the following media: (1) embryo production – MS basal salts and vitamins, sucrose (20 g/L), casein hydrolysate (0.5 g/L), 2,4-D (0.6 mg/L), agar (8 g/L), pH5.8 for 5 weeks; (2) embryo germination – medium as above, without auxin, for a further 5 weeks. The following cultivars (infected with respective diseases) were cultured: N14 (Ratoon Stunting Disease – RSD), N27 (Sugarcane leaf yellows phytoplasma – SCYP), N30 (*Sugarcane yellow leaf virus* – ScYLV), N32 (*Sugarcane mosaic virus* – SCMV and ScYLV), NCo376 (SCMV) and L76 (leaf scald). The disease status of a sample of regenerated plants was evaluated using molecular diagnosis two months after hardening-off. No plants were regenerated from diseased cultivar L76. Tissue blots confirmed that RSD had been eliminated from cultivar N14. Similarly, ScYLV was not detected by RT-PCR in previously infected cultivars N32 and N30. SCYP was present in 25% of the NovaCane®-generated N27 plants tested. SCMV was eradicated from 92% and 25% of N32 and NCo376 plants tested, respectively. NovaCane® could be used to micropropagate plant material in the Quarantine facility, provided the appropriate screening process is carried out to verify the disease-free status of regenerated plants.

Photosynthesis and the regulatory role of sucrose and hexose in sugarcane leaves

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In crops other than sugarcane, there is good evidence that the size and activity of carbon sinks influence source photosynthetic activity via regulation of photosynthesisrelated enzymes, an effect that is partly mediated through coarse regulation of gene expression. The existence in sugarcane of a robust sugar-dependent relationship between leaf and sink tissues could represent a potentially fundamental limiting factor for sucrose accumulation in the stalk and, consequently, play a major role in overall sucrose yield. Previous work in our laboratories has demonstrated that increased culm sink demand through partial shading resulted in increased photosynthetic rates that correlated with a reduction in hexose levels in the leaves. In an extension of that study, we have examined source regulation in cold-girdled and detached leaves (second and third fully-expanded) of pot grown *Saccharum* spp. hybrid *cv.* N19 (N19) with the aim of elucidating the mechanisms that determine carbon partitioning in sugarcane. Coldgirdled leaves (at 5oC) showed increased sucrose and hexose levels and a decline in photosynthetic rates over the duration of the 5 d treatment. Excised leaves, preincubated in darkness for 3 h, had increased photosynthetic rates on transfer back to light, relative to control plants maintained in the light. Tissue sucrose accumulation was reduced by darkness, but

accumulated again upon transfer to the light. However, after the dark period, hexose levels remained significantly lower for the remainder of the incubation time; possibly indicating that photosynthesis was up-regulated by lack of hexose accumulation. When the excised leaves were fed or pre-fed sucrose via the transpiration stream, dark-treated leaves exhibited reduced photosynthetic rates, which were associated with increased sucrose and hexose concentrations within the leaf tissue. The observed down-regulation of photosynthesis by sugar accumulation has provided a starting point for future identification of gene transcripts that have putative roles in mediating the source-sink relationship.

SUGARCANE PATHOLOGY

Laboratory, glasshouse and field assessments of fungicides to control *Ustilago scitaminea*, causal organism of sugarcane smut

K. McFarlane, D. Moodley, G. Chinnasamy and S.A. McFarlane
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Sugarcane smut, caused by *Ustilago scitaminea H.* & P. Sydow, is prevalent in the northern sugarcane growing areas of South Africa. Bayleton 25% WP [triadimefon (triazole)] has been used effectively to limit smut development in susceptible varieties for many years and has been particularly useful in protecting germinating cane from smut infection after hot water treatment. Bayer withdrew the product from southern Africa recently and an alternative fungicide for smut control was required. Agar plate spore germination assays were used to evaluate the efficacy of a range of anti-fungal formulations against *U. scitaminea*. Those that prevented the germination of smut spores were then tested for phytotoxicity to cane in glasshouse experiments. Three fungicides inhibited smut spore germination on agar plates at all concentrations tested and had no obvious effect on the germination of seedcane in glasshouse experiments. These three formulations were included in an inoculated field trial at the Pongola Research Station. One formulation from Syngenta (a.i. fludioxonil:mefenoxam:azoxy-strobin) provided acceptable smut control and gave consistently good yields using all methods of application.

Intensive surveys to estimate the incidence and effects of ratoon stunting disease (rsd) in the south african sugar industry

S.A. McFarlane and D.S. Subramoney
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Efforts have been made over the past 30 years to educate growers on the effects of RSD (*Leifsonia xyli* subsp. *xyli*) on sugarcane yield and the importance of managing the disease using standard practices. Intensive random surveys were conducted from 2000 to 2006 to determine the incidence and severity of RSD throughout the South African sugar industry. For these surveys, more than 230 000 stalks were collected from 2576 fields and examined microscopically for the RSD bacterium. These data and data from yield loss trials were used to estimate the current economic significance of RSD. The results were compared with the outcomes of earlier surveys to assess the progress made in reducing the impact of RSD in the industry. In 1984, it was estimated that total losses due to RSD amounted to 3.0% of the annual crop; this decreased to between 2.0 and 2.5% in 1991. In the most recent surveys, 15% of the fields that were sampled tested positive for RSD while 2.5% of the stalks were infected. Based on these data, total losses were estimated to be 1.1% of the annual crop. In the northern irrigated areas of the industry where RSD incidence is high, losses were in the region of 2.6% of the annual crop compared to 0.2% further south. Strategies to reduce RSD levels, particularly in the northern parts of the industry, are being implemented. Seedcane schemes are being introduced and growers are being encouraged to establish on-farm nurseries to meet their seedcane requirements, while the practice of planting break crops after crop eradication is being adopted by an

increasing number of growers. The RSD Diagnostic Service at SASRI continues to provide a valuable service to the industry.

COPRODUCTS

Benchmarks for a strategy in the energy generation of the sugarcane industry: a really clean source of energy

Oscar A. Almazán and MigueL A. Otero
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An historical analysis of the development of the energy use in the sugarcane industry is presented. The study shows how the search for less energy consumption was the driving force that led the sugar industry through its qualitative technological development up to date. In addition, the main goal to achieve as much energy as bagasse and cane residues, which are renewable resources, can assist the environment, opening new ways and means to succeed in the effort for less steam consumed per tonne of sugar, higher combustion efficiency, less equipment and investment. In addition, more surplus bagasse and electricity produced from it leads to an important reduction of fossil fuel usage, in the sugar industry and in the electricity grid. The results obtained in different countries are reported in detail giving the improvements in 'green' electricity generated per tonne of milled cane, and its potential effect on climatic change.

Fodder yeast production: A new approach for distillery vinasse treatment

M.A. Otero Rambla, G. Saura Laria, J.A. Martínez Valdivielso and O.A. Almazán Del Olmo
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Distillery vinasse is one of the most contaminating industrial wastes and, for a full development of an ethanol industry, this problem has to be solved. The study was conducted at lab scale in a yeast factory using raw materials from industry. Through this technology, the organic load of vinasse was reduced by 75% when the process was carried out batch wise or slightly over 60% in continuous mode. Important amounts of high quality proteins with relatively high yields (about 60 cubic metres of vinasse per tonne of yeast on a dry matter basis) can be obtained in parallel with wastewater treatment. Distillery vinasse can be utilised as the sole source of carbon in fodder yeast production. Addition of growth enhancer QZ-350 at a ratio of 1 to 3 kg/t of yeast, substitutes 1.1 tonnes of molasses. If protein production is the main target, continuous culture is the indicated choice. However, if environmental issues prevail, batch mode is preferred since it leads to a higher depletion of organic matter (potential pollution) in the propagation medium.

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