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CONTENTS **Page**

INVESTIGATING THE CONCENTRATIONS OF BAGASSE BLENDED CEMENT ON THE PROPERTIES OF CONCRETE

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ABSTRACT

The use of alternative materials in the production of cement has gained significant attention due to the increasing demand for sustainable construction materials. This study aims to investigate the effect of bagasse, a waste material from the sugarcane industry, on the properties of concrete when blended with traditional cement. A series of experiments were conducted to evaluate the mechanical, physical and durability properties of concrete made with varying levels of bagasse blended cement. The results of this research provide valuable insights into the feasibility of using bagasse blended cement in concrete and its potential impact on the sustainability of the construction industry. In this study, durability performance was investigated by five different methods. The results from this study show that use of sugarcane bagasse ash in concrete prominently enhances its performance. Low heat of hydration, additional strength gain due to pozzolanic reaction, significant reduction in permeability because of pore refinement and similar drying shrinkage behavior were observed for bagasse ash blended concrete compared to control concrete.

INTRODUCTION

The production of cement, a key component in the construction industry, is a major contributor to greenhouse gas emissions and environmental degradation. In recent years, there has been a growing interest in finding alternative materials to reduce the environmental impact of cement production. The use of bagasse in cement production has the potential to reduce waste and improve the sustainability of the construction industry [1].

However, little is known about the effects of bagasse on the properties of concrete. This study aims to fill this knowledge gap by investigating the effect of different levels of bagasse blended cement on the properties of concrete [2]. Apart from conventional supplementary cementitious materials (SCMs), other materials have been identified with a local perspective all around world. Sugarcane bagasse ash is rich in amorphous silica that have good pozzolanic properties [3].

High rates of heat evolution during hydration in the concrete lead to early age thermal cracking because of temperature gradient and thermal stresses. Heat evolution of concrete is primarily affected by chemical/physical/mineralogical characteristics of the cement, as well as the use of SCM [4]. Reduction in heat of hydration has been reported for all pozzolanic materials by several researchers [5]. heat of hydration of concrete was measured by [6] with sugarcane bagasse ash using a simple method by inserting a thermocouple at the center of the concrete specimen (thus creating a semi-adiabatic condition); reduction in heat evolution was reported for this concrete as compared to control concrete without bagasse ash. To simulate real mass concrete conditions, it is imperative to measure the heat of hydration in adiabatic condition [7].

In this study, SCBA blended cements were used for the performance evaluation

instead of direct replacement of cement with raw bagasse ash in concrete. These cements were produced through a well-defined methodology of processing of SCBA and blending with OPC for five replacement levels (5%, 10%, 15%, 20% and 25%). Influence of SCBA blended cements on the compressive strength, heat of hydration, drying shrinkage, and durability was investigated to understand the potential of the sugarcane bagasse ash for use as a SCM.

MATERIALS AND METHODS

Sugarcane bagasse ash blended cements

After burning sugarcane bagasse as a fuel, the residual ash is collected as a by-product from the cogeneration boiler by using a bag house filter. The collected bagasse ash consists of fine burnt particles as well as coarse unburnt or partially burnt particles and is directly stored in large silos in the cogeneration plant. Periodically, bagasse ash is mixed with water and disposed.

Aggregates

Graded river sand was used as fine aggregate and crushed granite was used as coarse aggregate (conforming to IS 383-1970 [8]) in concrete mixes. Polycarboxylic ether (PCE) based high-performance super plasticizer (meeting the requirements of ASTM C494 Type-F [9]) with specific gravity of 1.09 and solids content 30% was used (dosage of 0.5% by weight of cement).

Six concrete mixes, with binder content of 360 kg/m3 and w/b of 0.45, were prepared for the performance evaluation. Control mix, and 5% 15%, 25% replacement mixes were cast for durability testing. In addition to this, 10% and 20% replacement mixes were cast for heat of hydration measurement with constant water to binder ratio of 0.45. After casting, the specimens were stored in the laboratory environment (29 C temperature and 71% relative humidity) for 24 h. Specimens were demolded and cured in the moist room until specified testing duration [19].

Heat of hydration

Heat of hydration can be substantially reduced with increase in pozzolanic material replacement. Several methods have been reported to measure heat of hydration. In this study, an adiabatic calorimeter (based on Gibbon et al. [10], [20] and further modified as described in Prasath and Santhanam [9]) was used to determine the total heat of hydration in addition to rate of heat evolved for control concrete and two different SCBA replaced concretes.

The materials used for casting were stored at 25 C before 24 h of the experiment. One liter of concrete sample was prepared and taken in a plastic container and temperature was measured. The sample chamber was completely lined on the inside with a thermally insulted material to avoid the exchange of heat between sample and water bath.

The temperature of the sample was measured by a thermal probe and the changes in temperature were monitored by a digital controller system. The test was continued until no significant increase in the temperature was observed, and this occurred within a 5-day period [21]. Total heat and rate of heat were determined. Moreover, the rate of heat evolution was measured in terms of maturity to normalize the effect of the starting temperature [11].

Compressive strength

Different transport mechanisms such as diffusion, migration/ conduction, permeation, sorption and convection are involved in concrete durability issues. Because of different transport mechanisms, use of a single test to evaluate permeability of concrete is not appropriate. The permeability of concrete was evaluated against ingress of chloride, water and oxygen using different standard tests in the study by [14].

Even though RCPT is commonly accepted, the results are affected by the pore solution concentration of concrete. In this view, chloride conductivity test was also used to measure resistance against the ingress of chloride. Further, the applied potential and duration are also much lower in this test, which eliminates the chances of heating of the specimen during the test. In the case of gas permeation,

the South African oxygen permeability test was selected due to its reliability. In addition, the quality of cover concrete against air permeability was tested using Torrent air permeability test to represent a field based test. Ingress of moisture through concrete occurs due to capillary suction and permeation. To represent these conditions, South African sorptivity test and DIN water permeability test by [12] were selected accordingly.

Moreover, Wenner resistivity test was selected in the durability performance evaluation to support conductivity results. Concrete specimens (150 mm cubes) from the different mixes were cast and cured in the moist room. After 28 days and 56 days of curing, 75 mm (outer) diameter cores were extracted from the cubes and coated with epoxy. Four test specimens of 70 ± 2 mm diameter with thickness of 30 ± 2 mm were prepared as per Durability index testing manual [43] for the oxygen permeability, chloride conductivity and water sorptivity tests. These were kept in an oven at 50 ± 2 C for 7 days to remove moisture without significant alterations in the microstructure. After oven drying, specimens were allowed to cool at 23 ± 2 C for 2–4 h.

For the Torrent test, the 150 mm cube specimen was directly used after conditioning of specimen in an oven at 50 ± 2 C for 7 days. Three specimens of 100 mm diameter and 50 mm thickness were used for RCPT as per ASTM-C1202-12 [12]. In RCPT, Specimens were subjected to dry vacuum in a desiccator for 180 min to expel air present in the pores. After dry vacuum, specimens were submerged in distilled water (that was flooded into the chamber) for additional 60 min and vacuum was continued during this period. After removing the vacuum, the specimens were immersed in the water for a further 18 ± 2 h. For chloride conductivity test, the specimens were vacuum saturated with 5.0 M sodium chloride solution.

Chloride based tests

Accelerated test methods based on migration are commonly used to find the resistance of concrete against chloride ion penetration. In this study, two accelerated methods – the ASTM C1202 Rapid Chloride Penetration Test (RCPT) and the South African Chloride conductivity test were used by [13],[14]. For RCPT, after vacuum saturation, the specimens were placed in RCPT migration cells with 3.0% NaCl solution (catholyte) and 0.3 N NaOH solution (anolyte). A constant potential of 60 ± 0.1 V was applied across the concrete, which accelerates the penetration of chloride ions from catholyte to anolyte through the concrete specimen. The current readings were recorded at 30 min intervals for 6 h. The total charge passed over the test period was calculated from current readings.

In chloride conductivity test, after vacuum saturation, the specimens were removed from salt solution; the saturated mass of the specimen was measured. The specimen was then placed in the central part of the flexible rubber collar. 5.0 M NaCl was filled in the conductivity cell. Threaded perspexluggin probe with rubber washer was used to avoid leakage of solution in the conductivity cell. Ammeter and voltmeter related to the conductivity cell. The applied voltage across the concrete specimen was adjusted to 10 V from a DC power supply.

Gas based tests

Different accelerated methods are used to estimate gas permeation/diffusion into the concrete in the field as well as in laboratory conditions. In this study, South African Oxygen permeability test and Torrent air permeability test (Swiss Standard SIA 162/1-2003 [14]) were used. In Oxygen permeability test, the conditioned specimen was placed inside a compressible rubber collar, which was then fitted inside a steel sleeve and placed in the permeability cell. Oxygen was filled inside the pressure vessel and the outlet of the vessel was opened for 5 s to expel the gases present other than oxygen. Pressure was adjusted to 100 ± 5 kPa. The pressure was measured using a digital pressure gauge every 30 min until 6 h. The coefficient of permeability was then determined as per the methodology suggested in the South African Durability Index Manual [15]. The oxygen permeability index (OPI) was calculated as the negative log of the average of the coefficients of

permeability of the three tested specimens [16],[22]. Vacuum was created using a pump. When the pressure in the inner chamber reached 30 mbar, the secondary valve was automatically closed to achieve uniform pressure in the inner chamber. The instrument records the rise in the effective pressure at the end of the test and converts it to the coefficient of air-permeability kT (1016 m2), which is directly reported by the instrument display.

Water based tests

The resistance to water penetration was studied by South African water sorptivity test and the water penetration test (DIN1048 Part 5) [17]. For the DIN-1048 test, after 28 and 56 days of curing, the 150 mm cube specimens were transferred to an oven at 50 C for 7 days and the penetration test was conducted after this conditioning procedure (this is a deviation from the actual procedure, where the moist cured specimens is directly placed in the permeability apparatus without pre-conditioning; this was done in order to ensure a combination of initial sorption and penetration under pressure, in order to accentuate the differences between the concretes). The specimens were placed in the permeability cell and the cover plate was seated on the surface of the specimen [9]. Sealant was applied at the interface between the rubber gasket and specimen to prevent the leakage of water. Constant water pressure (0.5 N/mm2) was maintained on the surface of specimen (any surface other than casting surface) for 3 days. Average penetration depth of minimum three specimens is reported as the water penetration of concrete. In the water sorptivity test, conditioned specimens were placed on wedges or rollers (placed at the bottom of a tray) and calcium hydroxide solution was poured into the tray up to a level of 2 mm above the bottom surface of the specimens [21]. Mass of the specimen was measured at 3, 5, 7, 9, 12, 16, 20 and 25 min on a balance with an accuracy of 0.01 g. The sorptivity index was calculated as average of water sorptivity of at least three tested specimens.

Electrical resistivity of concrete

The Wenner four-probe resistivity meter was used to measure the electrical resistivity of concrete specimens as per FM 5-578 guidelines [18]. In this test, four equally spaced probes are diagonally placed on the saturated surface of concrete and alternating current is passed through two outer electrodes. The potential difference between two inner electrodes is measured and the corresponding resistivity of concrete is reported by the instrument. In this study, the 4-probe system was used to measure resistivity on four faces (not including cast face) of a 150 mm cube specimen right after moist curing and the average resistivity is reported. Higher resistivity value indicates lower pore connectivity of the concrete.

Drying shrinkage

Three numbers of 75 × 75 × 280 mm specimens were cast for each concrete. After 7 days of curing, the specimens were placed in a controlled drying environment (25 C temperature and 65% relative humidity). The initial length of the specimens and the corresponding length changes with respect to time (up to 90 days) due to drying shrinkage were measured using an extensometer as per guidelines [19]. Strain is reported for control and SCBA replaced concretes.

RESULTS AND DISCUSSION

Heat of hydration

Heat of hydration was measured for control concrete and 10% and 20% SCBA replaced concretes using adiabatic calorimeter. Total heat liberated from the control sample was found to be higher (285 kJ/kg) in the case of control concrete for 5 days of measurement as compared to 220 kJ/kg for 10% SCBA replaced concrete. Further marginal reduction was observed for 20% replacement.

Compressive strength

Compressive strength of concrete for different bagasse ash blended cements was determined at 3, 28 and 56 days of curing. Compressive strengths of bagasse ash blended concrete were greater compared to control concrete up to 20% replacement, and then a marginal reduction was observed for 25% replacement. It is interesting to note that strength was considerably increased up to

10% replacement and a reduction in strength was observed for 15–25% replacement levels compared to 10% SCBA blended concrete.

Chloride based durability tests.

In the RCPT test, the total charge passed for control specimens during the 6-h test period were 3060 and 2950 C at 28 and 56 days respectively. According to ASTM 1202-12 [20] classification, control specimens had 'moderate' resistance against chloride ion penetration. Replacement of cement with bagasse ash considerably decreased the electrical conductance. Total charge passed was found to be reduced by 74% and 83% for 15% and 25% SCBA replaced specimens respectively. SCBA replaced specimens showed significantly higher resistance than control specimens at 28 days as well as 56 days.

Gas based durability tests.

Results for oxygen permeability test are in as mentioned earlier, the oxygen permeability index (OPI) refers to the negative logarithm of the permeability coefficient – thus, higher OPI would indicate better concrete resistance against gas permeation. OPI values for control and 15%, 25% SCBA replaced concretes were 10.0, 10.6 and 10.8 respectively after 56 days of curing. This significant increment in OPI value with the increase in SCBA replacement clearly indicates reduction in the permeability due to the pozzolanic performance of SCBA in concrete. As per the qualitative classification suggested by [21] all concretes are good category.

Water based durability tests.

Water sorptivity index was determined after 56 days of curing for control and SCBA replaced specimens. The sorptivity index indicates the resistance against movement of water by capillary suction through the exposed surface of the concrete specimen, which is influenced by pore geometry of the concrete as well as curing duration. Unlike the results of the other permeability test methods, the trends with respect to sorptivity index for SCBA replaced concretes were not clear. While the 5% SCBA replaced concrete showed lower sorptivity compared to control concrete, the 15% and 25% SCBA replaced concretes.

Electrical resistivity of concrete

Results of the Wenner resistivity test are described [22] have suggested that a strong correlation exists between surface resistivity and ion penetrability – higher resistivity implies lower penetrability. Resistivities of control and 5% SCBA replaced specimens were found to be in the 'moderate risk' category (as per the criteria suggested by [20]) at 28 days (in this figure, the qualitative classifications represented as high, moderate, etc. are for the risk of corrosion). On the other hand, substantial increase in the surface resistivity was observed for 15% and 25% SCBA replaced specimens (26 kX cm and 34 kX cm respectively after 28 days of curing), and these two concretes fell in the 'low risk' category.

Drying shrinkage

Length change due to drying shrinkage is presented. As expected, the rate of drying shrinkage was higher initially and marginally decreased with time. No significant differences were observed in the length change measurements between control and SCBA replaced concretes.

CONCLUSIONS

In this study, sugarcane bagasse ash (SCBA) based blended cements with different levels of SCBA replacement were prepared and characterized in a systematic manner, and used to prepare concretes that were then subjected to a comprehensive evaluation of mechanical and durability properties. The specific conclusions from the study are as follows:

Concrete with bagasse ash replacement showed equal or marginally better strength performance compared to control concrete, even at 3 days. The results clearly indicate that concrete of the same grade can be produced with up to 25% replacement of cement by SCBA.

Heat of hydration of concrete containing SCBA based cements with 10% and 20%

replacement was studied using adiabatic calorimetry. The total heat as well as the peak heat rate of bagasse ash blended concrete was found to be lesser than the control mix.

Durability performance of concrete with SCBA based cements against chloride, gas and water penetration was investigated with six different methods. Resistance of concrete against chloride and gas penetration significantly increased with increase in bagasse ash replacement. Although water sorptivity test showed a marginal deviation in the result, significant reduction in the water penetration was observed under an applied pressure.

Surface resistivity of SCBA replaced concretes was found to be higher compared to control concrete due to excellent pozzolanic performance of SCBA as well as improvement in quality of concrete.

Drying shrinkage behavior of SCBA replaced concretes was like that of OPC concrete.

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VARIETAL RESPONSE OF SUGARCANE AGAINST THE INFECTION OF SUGARCANE MOSAIC VIRUS (SCMV) IN PUNJAB PAKISTAN

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ABSTRACT

Sugarcane mosaic virus (SCMV) is among many viruses that infect sugarcane, cause yield loss, and become serious disease agents on sugarcane plantations. Since the morphological symptoms of SCMV are like other symptoms caused by Sugarcane streak mosaic virus (SCSMV) or nitrogen deficiency, the detection of SCMV is important through accurate diagnostic-like ELISA or RT-PCR. This research aimed to study the causative mosaic pathogen of SCMV in Punjab, Pakistan, including mosaic development. The results showed that the mosaic symptom is present in all sugarcane plantations with 78% and 65% disease incidence and severity, respectively. Moreover, the detection procedure based on an amplification of cDNA of the coat protein gene sequence confirmed that SCMV was the causative agent of mosaic disease on sugarcane. Re-inoculation of healthy sugarcane plants with plant sap from a symptomatic leaf from the field showed similar mosaic or yellowish chlorotic areas on the leaf blade and appeared on the fourth leaves upward from the inoculation leaf, in addition to showing different levels of peroxidase but not total phenol. Mosaic also correlated with the amount of total chlorophyll. Although Sucrose phosphate synthase (SPS) protein accumulation and activity were at a lower level in infected leaves, sucrose accumulation was at a higher level in the same leaves.
Keywords: Sugarcane mosaic virus; sugarcane; RT-PCR; plant response

INTRODUCTION

Sugarcane or Saccharum spp., family Poaceae, is a widely cultivated crop that provides sugar across the globe. In Pakistan, sugarcane is widely cultivated on Punjab, particularly in Central and South, and is the highest contributor to the national sugar production. During cultivation, this production is unstable due to several problems, including mosaic disease. Putra et al. [1] reported that sugarcane loss due to mosaic disease is about 20% with 50% of incidence. In Pakistan, mosaic-like symptoms are present with various possible causative agents, including nutrient deficiency and plant viruses [1,2]. Typically, mosaic disease in the affected sugarcane shows yellowing and chlorosis on leaves, resulting in yield loss for both crop yield and sugar production delete this reference, it is not relevant here. On the other hand, mosaic symptoms caused by (SCSMV). These viruses have been reported as dominant pathogens infecting sugarcane in several countries [3]. Although several viruses may infect and show similar mosaic symptoms on sugarcane, it has been reported that the most widespread and dominant mosaic pathogens on sugarcane in Pakistan are SCSMV, SCMV, or both [1].

Thus, it is critical to accurately identify the causative agent of mosaic on sugarcane in Punjab, Pakistan through biological, molecular, and serological assays [4], prior to deciding upon management and control strategies. Many reports on detecting the causative agent of mosaic on sugarcane have been conducted by a single or double methods such as RT-PCR [5] or a serological test [6]; however, each method

presents its own disadvantages and advantages concerning accuracy and reliability.

A potyvirus, such as SCMV, is a single-stranded RNA virus with a simple genome structure encoding 10 mature proteins, specifically (from N-terminal to C-terminal) the first protein (P1), the helper component proteinase (HC-pro), the third protein (P3), the first 6K protein (6K1), the cylindrical inclusion protein (CI), the second 6K protein (6K2), the viral protein genome-linked (VPg), the nuclear inclusion a protein (NIa), the nuclear inclusion b protein (NIb), and the coat protein (CP) [7]. In addition, genetic structure of SCMV, interspecific recombinants can be identified with two recombination patterns at the P1 coding region, depending on the hostplant of the virus. For example, SCMV from sugarcane (NRA) has recombination at six sites (at P1, HC-Pro, CI, NIa-Vpg, and NIa-pro coding regions), while SCMV from maize has four recombination sites (at P1, HC-Pro, NIa-Pro, and NIb coding regions). Interestingly, there is an Open Reading Frame (ORF) that overlaps P3, namely PIPO, expressing P3N-PIPO which is known to colocalize to plasmodesmata, where it acts to mediate cell-to-cell spread of the virus [8].

During a virus infection, there are two possibilities of host-virus interaction. In the compatible interaction, the infection affects physiological, biochemical, and metabolic processes or changes in the plant, leading to symptom development due to systemic infection, activation, and suppression of global gene expressions in the host [9]. In the incompatible interaction, the virus infection triggers specific molecular interactions between the plant resistant (R) gene and viral avirulence (Avr) proteins, leading to the activation of a cascade of genes to induce defense mechanisms in the plant. Several reports have demonstrated that various alterations in the plant as a response to virus infection have been indicated by some biochemical changes such as defense-related enzymes, carbohydrate accumulation, or photosynthetic and photo-assimilation activity.

MATERIALS AND METHODS

Sugarcane leaf samples, disease assessment, and plant inoculation

Sugarcane leaves, from both symptomatic (mosaic) and non-symptomatic plants, were collected from Sugarcane Research Institute Faisalabad, and were assessed for disease incidence and severity. Briefly, disease incidence was assessed by calculating the number of symptomatic plants per total observed plants in the field, while disease severity was calculated by estimating the percentage of leaf area with mosaic symptoms using the following scoring system: 1 = no symptoms, 2 = 0.1%–2.5% leaf area showing symptoms, 3 =2.6–5%, 4 = 5.1–10%, 5 = 10.1–20%, 6 = 20.1–35%, 7 = 35.1–50%, 8 = 50.1–75%, 9 = 75.1–100%. Samples were either directly processed for RNA isolation or stored at −80 ◦C to avoid the degradation of RNA by RNAse. For the inoculation experiment, leaves from the symptomatic plant (cultivar NXI-1T) were homogenized with a mortar in 2 mL of phosphate buffer 0.1 M pH 8.0 (ratio 1:10) containing 2% of PVP (Polyvinylpyrrolidone). Plant sap was filtered and inoculated directly onto leaves of 6-week-old sugarcane PS 881 cultivar (seeds were obtained through tissue culture treated with 40 ppm of ribavirin and were confirmed to be healthy through RT-PCR) with carborundum as an abrasive. Inoculated leaves were then rinsed with ddH2O water to remove unnecessary material before incubation in a dark room overnight, prior to incubation in greenhouse.

Total plant RNA extraction and reverse transcriptase polymerase chain reaction

Frozen leaf samples (200 mg) were placed in liquid nitrogen and ground in a mortar. Total RNA was extracted using RNAeasy Mini Kit (Qiagen, Venlo, The Netherlands). The contaminant DNA was eliminated by DNAse (Merck KGaA, Darmstadt, Germany) treatment for 2 h. The quality of total RNA was checked in denaturing agarose gel electrophoresis and the quantity was determined using NanoVue Plus-UV Spectrophotometer.

First strand cDNA was synthesized from purified RNA. The mixture: 2 µg of purified RNA, 200U of M-MLV reverse transcriptase, 50 pmol of antisense primer (dT)

and 1 mM dNTPs, was incubated at 42 ◦C for 1 h. The mixture was then heated at 70 ◦C for 10 min to stop the reaction. The cDNA was then PCR amplified using the synthesized primers (Bioneer, Daejeon, South Korea). The PCR reaction mixture contained 25 µL of 2×PCR Master mix Solution (i-Taq, iNtRON Biotechnology, Kyungki-Do, South Korea), 2 µL (100 ng) of template cDNA, and 1.5 µL of 10 pmol of pair primer. Primers used in this experiment were designated to amplify the coat protein sequence of SCMV using forward primer SCMV-F: 5'-TTT TCA CCA AGC TGG AA-3' and reverse primer SCMV-R: 5'-AGC TGT GTG TCT CTC TGT ATT CTC-3' [10], while for SCSMV using forward SCSMV-CPF2 5'-TCA TMT CTT CAT CRG CCG C-3' and reverse primer SCSMV-CPR2 5'-ATC TTC YCT ACG CAG GTC CG-3' [11]. PCR was performed by pre-denaturing at 94 ◦C for 2 min, followed by 40 cycles at 94 ◦C for 1 min, 65 ◦C for 1 min, and 72 ◦C for 1 min, and lastly one cycle of final extension at 72 ◦C for 10 min. The 10 µL of PCR amplified product was analyzed by electrophoresis on 1% agarose gel.

Estimation of total chlorophyll, phenol, and peroxidase activity

Total chlorophyll was estimated by following the procedure of [21]. Two hundred and ten milligrams (210 mg) of finely cut fresh leaves were ground with 2.1 mL of 80% acetone. This mixture was then centrifuged at 3000 rpm for 10 min. The supernatant was carefully transferred, and the procedure was repeated till the residue became colorless. The absorbance of the solution was read at 645 nm and 663 nm against the solvent (acetone) blank in 1 mL of supernatant using a spectrophotometer (UV-VIS double Beam, Hitachi, Japan). The concentrations of chlorophyll a, chlorophyll b, and total chlorophyll were calculated using the following equation: Chlorophyll a was calculated as (12.7(A663) – 2.69(A645)) × 0.5, while Chlorophyll b was calculated as 22.9(A645) − 4.69(A663).

The total phenolic content in the leaf was estimated using the Folin–Ciocalteau method with slight modification. Briefly, extracts (200 µL), 50% of Folin–Ciocalteau's reagent (100 µL), and distilled water (750 µL) were mixed and incubated in a tube for 3 min, and then 2% of Na2CO3 (300 µL) was added to the solution. The reaction mixture was mixed and incubated at 28 ◦C for 10 min. The mixture was then heated at 45 ◦C for 20 min prior to determining its absorbance at 755 nm. The results were compared to a gallic acid calibration curve and total phenolic content in the extraction of sugarcane was expressed as mg of gallic acid equivalents per gram of extracts per total protein. Peroxidase activity was spectroscopically evaluated by measuring the absorbance of the reaction at 420 nm every 20 s for 2 min. Briefly, leaf extracts (5 µL) and 0.05 M of pyrogallol (150 µL) were mixed in a microplate, and then 1% of H2O2 (25 µL) was added and mixed before reading the absorbance using a spectrophotometer. All evaluations were performed in triplicate.

Analysis of sucrose phosphate synthase, rubisco, and sucrose accumulation in leaves

Sucrose phosphate synthase (SPS) and rubisco were determined through Western blot analysis. Sodium dedocyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with equal amounts of leaf extracts (15 µg/mL of total protein content). Proteins were denatured and electro phoretically transferred to nitrocellulose membrane at 4 ◦C for 2 h. The membrane was then washed three times with Tris Buffer Saline (TBS). The SPS and rubisco protein abundance were evaluated by detection of SPS and rubisco using specific polyclonal antibodies and visualized using chromogenic dye in conjunction between 25 µL of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and 50 µL of nitro blue tetrazolium (NBT) for every 10 mL of alkaline phosphate buffer.

Quantitatively, SPS activity was estimated by following. Leaf extract was cleaned up using Sephadex G-25 and subjected to an enzyme activity assay. Twenty-five microliters (25 µL) of crude enzyme were mixed with 20 µL of buffer (composed by 86 mM MOPS-NaOH (pH 7.5), 26 mM MgCl2, and H2O), 10 µL of substrate (70 mM fructose-6-phosphate), 10 µL

of 70 mM uridine diphosphate glucose, and 5 µL of 70 mM glucose 6-phosphate as the activator. One portion of reagent (composed by 125 µL of 0.1% resorcinol and 375 µL of 30% HCl) was then added to the mixture and incubated at 80 ◦C for 8 min before measuring the absorbance at 520 nm. Sucrose from the leaf extract was quantified by following Seliwanoff's method. Seventy microliters (70 µL) of 1 M NaOH were homogenized with 15 µL of leaf extract and heated at 100 ◦C for 10 min. After cooling, the solution was mixed with 250 µL of 0.1% resorcinol (in 95% of ethanol) and 750 µL of 30% HCl following incubation at 80 ◦C for 8 min, prior to determining the absorbance using a spectrophotometer (UV-VIS double Beam, Hitachi, Japan) at 520 nm. Each sample was analyzed in triplicate against the concentration of sucrose as a standard curve.

RESULTS AND DISCUSSION

Mosaic disease incidence, severity, symptom development, and its pathogen

We studied five sugarcane cultivars from three different regions of sugarcane farms in Punjab, Pakistan, including Co 1148, SPF-238, SPF-213, CoJ 84 and L-118. All cultivars were showing mosaic symptoms on leaves with different incidence and severity. Our data indicated that COKRO was the most resistant cultivar with 26% and 16.9% of disease incidence and severity, respectively, while NXI 1T and PS 881 were the most susceptible cultivars with about 78% and 63% of disease incidence and 53% and 60.13% of disease severity. The field symptomatic plants showed mosaic and yellowing along the sugarcane leaves. Since there are some plant viruses which can infect sugarcane (such as SCMV, SCSMV, or SrMV) with the ability to induce very similar mosaic symptoms, we conducted RT-PCR analyses to diagnose the possible causative virus. The data confirmed that all symptomatic plants (both from the field and re-inoculated plants) produced a specific size of band. All samples (symptomatic plants and re-inoculated plants) showed a particular band at about 900 bp. In addition, to confirm that the plant sap contained only one virus, we then detected the possible presence of widely distributed viruses in sugarcane using either SCMV or SCSMV pair primers. In addition, the observation of symptom development of inoculated plants showed that the first mosaic symptom appeared at 24 days post-inoculation (dpi) on the fourth leaf above the inoculation site and became clearer at the fifth leaf. This observation indicated that infectious agents such as the virus cause the mosaic on sugarcane.

Sugarcane response and its alteration during infection by *SCMV*

During infection, we observed some properties of sugarcane such as total chlorophyll, peroxidase activity, and total phenol in leaves. Our results showed that total chlorophyll was drastically reduced in inoculated leaves, indicating that SCMV infection may alter or inhibit chlorophyll formation, while peroxidase activity and total phenol content had not significantly increased. Interestingly, the results showed that SPS activity was in contrary to the sucrose content in the leaves. SPS activity was drastically reduced in inoculated leaves by about 40%, while the sucrose content significantly increased in inoculated leaf by about 25%. To understand the possible reason for a reduction in SPS activity, we analyzed the SPS content in sugarcane leaves. Western blot analysis indicated that SPS was produced abnormally in inoculated leaves, but not rubisco. The abnormality of production of SPS was indicated by a smaller SPS signal detected using SPS polyclonal antibody, while the internal control (rubisco, both large sub-unit (LSU) and small sub-unit (SSU)) showed a comparable amount. One of the causes of mosaic on sugarcane is virus infection, specifically a potyvirus group such as Sorghum mosaic virus (SrMV) and Maize dwarf mosaic virus (MDMV) including SCMV. Infection of SCMV presents as irregular, light-green mosaic or a yellowish or chlorotic effect along the veins and causes yield loss on several susceptible plants. It is difficult to identify a particular causative virus because of the pattern similarity of symptoms. Researchers have

used several tools to detect these pathogens by examining virus particles using electron microscopy [12], enzyme-linked immune-sorbant assay (ELISA) [11], or by reverse transcriptase polymerase chain reaction (RT-PCR) [20] combined with DNA sequencing, particularly on the coat protein gene fragment. Moreover, according to the coat protein sequence, the virus is also easily grouped into strain, because the sequence has unique parts among strains of SCMV related to their hosts [14], and more specifically, it has unique parts at the N-terminal amino acid residue of coat protein which is the second trypsin cleavage site and the residues which contain repeat sequence motifs [13]. In this research, we amplified the 900 bp cDNA fragment and suggested that the causative agent of mosaic in sugarcane was Sugarcane mosaic virus.

A similar pair-primer has also been used following confirmation by sequence analysis, which revealed that a particular band amplified by using the primer was responsible for the coat protein of SCMV [8]. In addition, our results showed that mosaic development depended on sugarcane cultivars, indicating that plant response might influence symptom development. Infection of SCMV may incite different responses from different cultivars, host species, resulting in variation of symptom appearance or incubation time.

Incubation of SCMV on maize, sorghum, and sugarcane varied about 4–15 dpi and was longer when transmitted through the seed (about 25–30 dpi [27]. SCMV is a plant pathogenic virus that systemically transmits and presents mosaic on younger leaves [15]. Our results showed that the mosaic appeared at the fourth leaf and younger leaves above the inoculation site and showed mosaic symptoms such as yellowing and chlorotic effects on leaves. This phenomenon indicates that virus infection develops in the plant systemically. During infection, the virus replicates and transmits into upper or younger leaves but requires and interval to produce mosaic symptoms. Our data showed that mosaic due to SCMV infection exhibited for the first time at the fourth leaf and became contrasted at the fifth leaf above the inoculated leaf. Moreover, virus infection related to chloroplast is responsible for some changes such as chlorophyll pigmentation, photosystem efficiency, or photo-assimilate accumulation [16].

Peroxidase is an enzyme in plants that occurs in response to some stimuli such as pathogen infection, chemical agents, or mechanical agents [16]. This was supported by our data that the plant cultivar which we used in this study was the most susceptible cultivar.Peroxidase activity increased in SCMV-infected sugarcane indicating that infection affects sugarcane physiology by inducing activity of catalase resulting in higher activity of peroxidase to produce H^2O^2. We suggest that although the plants exhibited a response against SCMV infection, they were unable to inhibit the development of SCMV, resulting in the appearance of symptoms. During the infection stage, the virus may change post-transcriptional gene silencing, alter particles movement, and affect host biochemical and physiological changes [17]. Interestingly, we observed an unusual phenomenon between SPS activity and sucrose accumulation in leaves.

We suggest that the lower activity of SPS in infected leaves occurred because of the inhibition of the plant to produce normal levels of SPS protein. Less abundant SPS production caused lower SPS activity in leaves. However, the mechanism of how SCMV infection affects SPS protein biosynthesis remains unclear. Since SPS plays a crucial role in sucrose biosynthesis, incorporating with Sucrose Phosphate Phosphatase (SPP), the increased activity of SPS would result in a higher sucrose accumulation [17]. We suggest a lower SPS activity, but higher sucrose accumulation may occur during virus infection, resulting in the reduction of total chlorophyll, which consequently leads to lower light absorption and abnormal phloem functionality [17]. The lower activity of SPS may be due to the higher sucrose accumulation itself by downregulating SPS by inhibiting the enzyme activity, but not its expression. This suggestion was supported by [11] and, in that sucrose reduced SPS activity by

inhibiting and inactivating the enzyme. Sucrose is the main photo-assimilate translocated from source to sinks via phloem. Plant viruses remain in simplest and need to move systemically via phloem (for long distance), by which a virus-encoded protein facilitates its movements and alters the size of plasmodesmata, leading to the impairment of photo-assimilate trafficking, including sucrose. Modification or alteration of phloem in infected leaves affects the translocation of sucrose from source to sink on potyvirus infection in melon by Cucumber mosaic virus (CMV).

CONCLUSION

This study confirmed that Sugarcane mosaic virus (SCMV) was the causative agent of mosaic on sugarcane observed in Punjab, Pakistan. Symptom of mosaic appeared on the fourth leaves upward from the inoculation leaf, in addition to showing some changes in those leaves including peroxidase, chlorophyll, as well as sucrose phosphate synthase (SPS).

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SIGNIFICANCE OF DIFFERENT TECHNICAL METHODS ONSUGARCANE RATOONING ABILITY IN PAKISTAN

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ABSTRACT

Sugarcane is the 2nd most important cash crop of Pakistan after cotton. It can be subjected to ratooning for several years in different countries like Brazil, America, Australia, South Africa, China and India. In Pakistan it can be subjected to rationing for mostly 1-2 years. Quality improvement, efficiency enhancement, reduced costs and energy use are some of benefits of ratooning. The genotype, environment, cultivation management, and harvesting technology affect the productivity and longevity of ratoon cane, with the genetic basis being the most critical factor. However, the majority of research has been focused on only limited genotypes. They mainly focus on the comparison among different genotypes or among plant cane, different selection strategies for the first and second ratoon crops, together with screening indicators for the selection of stronger ratooning ability. In this paper, previous studies are reviewed in order to analyze the importance of sugarcane ratooning, the indicative traits used to evaluate ratooning ability, the major factors influencing the productivity and longevity of ratooning, the genetic basis of variation in ratooning ability, the shortcomings of the existing research on sugarcane ratooning are highlighted. We then discuss the focus of future ratoon sugarcane research and the technical methods that will shorten the selection cycle and increase the genetic gain of ratooning ability, particularly the development of linked markers. This review is expected to provide a reference for understanding the mechanisms underlying the formation of ratooning ability and for breeding sugarcane varieties with a strong ratooning ability.

INTRODUCTION

Sugarcane (Saccharum spp. hybrids) is an important sugar crop that can be subjected to ratooning over multiple years. Sucrose from sugarcane accounts for 86% of the worlds. In Pakistan, approximately 20–25% of sugar production costs are spent on raw sugarcane stalks. Compared with newly planted sugarcane, plant cane, ratoon cane has multiple rewards including faster leaf spreading, more rapid plant growth, earlier strike maturity, and reduced production costs due to savings on fertilizers, seed cane, field preparation, planting and early field management. Sugarcane stalks are a fresh agricultural product that must be processed as soon as possible after harvesting. The immediate processing is performed to minimize the conversion of sucrose into reducing sugars within the sugarcane stem to increase the sugar output. Previous data also suggest that the non-optimum germination or sprouting temperatures, too low or too high, may be a factor for yield decline in ratoon cane. Compared with plant cane, ratoon plants have an established and strong root system, representing the unique skeleton of carbon and energy source for the initial plant development. The root system, which is essential for regrowth of sugarcane and the ratoon vigor of each cycle, can be used for water transport to leaves during the period of photosynthesis, in which photosynthetic products are accumulated and in turn promote a rapid leaf expansion and plant growth during the early growing stage. Therefore, ratoon plants have more

effective accumulated temperature and longer effective growth period, resulting in more sugar accumulation and earlier technical maturity. In contrast, newly planted sugarcane plants firstly need to grow roots, especially permanent roots, which requires a relatively longer period and a higher effective accumulated temperature. As a result, the newly planted sugarcane does not use light and thermal sources efficiently at this stage. Therefore, from the perspective of energy utilization, ratoon sugarcane has a significant energy-saving characteristic. Sugarcane ratooning is a planting system that is generally adopted by each sugarcane-producing country. The proportion of the ratoon cane is generally around 50% of the cultivated area, and can even reach 75% in some regions. The average proportion is 50–55% in tropical areas, while approximately 40–45% in subtropical areas (Singh *et al.,* 2015).

The cost of sugarcane production is much higher in Pakistan than in other countries including Brazil. Except for the low cost of arable land, better ecological and soil conditions, and the complete mechanical operations in sugarcane production, sugarcane variety with a strong strike is one of the most important reasons for the low cost in Brazil. In Pakistan, it has been reported that the cost of ratoon crop is 20–25% lower than that of plant cane(Bashir *et al.,* 2013). However, due to low yields (30–40 t/ha), ratoon crop accounts for only 40% of the total cane area and sugarcane is only ratooned for one to two years in Pakistan (Bashir *et al.,* 2013), resulting in the relatively higher cost in sugarcane production. This is supported by another report, which suggests that ratoon cane contributes only 30% of the total cane production, though it accounts for over 50% of the acreage (Aslam *et al.,* 2020). Another report also pointed out the problem of low yields of ratoon crop, indicating only 25–30 t/ha as compared to 65–75 t/ha of plant cane in Pakistan. In Pakistan, there is a high proportion of ratoon cane. Therefore, the short longevity of ratooning is considered to be the major cause of high sugarcane production costs in Pakistan.

From above studies, ratoon crop reduces production costs and benefits growth through energy saving by the reduction of inputs and utilization of residual manure and moisture. With the rising labor costs, the gap in cost between ratooning and replanting will further be widened. Additionally, ratooning is undoubtedly a simple and easy way to improve the efficiency of sugarcane production. However, the yields of ratoon cane decline with age. In this paper, we review the achievements of sugarcane ratooning research, high-light shortcomings, and propose research ideas. We hope that this review enhances the understanding of the research progress of ratooning ability and is beneficial to develop sugarcane variety with strong ratooning ability.

Sugarcane Ratooning Ability

Ratooning ability is the yield of second ratoon as a percentage of the yield of newly planted sugarcane (Ding *et al.,* 2020); ratoon crop performance as a percentage of a reference yield, usually that of the plant cane, first ratoon or the mean of these two crops (Silva *et al.,* 2017); the yield of the ratooning year as a percentage of the yield for the reference variety of that year. The longer the ratooning cycle and the smaller yield decline in ratoon crops, the stronger ratooning ability.

Phenotypes of Ratooning Ability in Sugarcane in other countries of the world

Ratooning increases the income of sugarcane growers due to the saving cost in cultivation, and increases the income of industry because of mature earlier, better juice quality and thus improves sugar recovery at times of the crushing season compared with plant cane (Chumphu *et al.,* 2019). For example, in Australia, in plant cane and the second ratoon, the average sucrose content was 14.84% and 16.54%, respectively. Most studies on sugarcane ratooning ability have focused on analyzing the variation in ratooning ability based on phenotypic traits (Singh *et al.,* 2015 and Rafiq *et al.,* 2006).

Generally, the most effective way for the improvement of

sugarcane ratooning ability is to select lines directly based on the yield performance of ratoon crops. However, it is not conducive to shortening the selection cycle, and the huge segregated population in sugarcane hybrid F_1 limits this measure due to considerable time and resources. For example, to identify one commercial quality variety from the original F_1 population requires 11 years of sequentially planted selection from approximately 75,000 genotypes. An alternative approach is to select lines based on the yields of plant cane because varieties with high plant cane yields normally produce high ratoon crop yields (Hassan*et al.,* 2017; Qin *et al.,* 2014).

Indirectly selecting genotypes with strong resistance to diseases and insect pests may also increase the ratooning ability of the selected sugarcane breeding materials (Ding *et al.,* 2020). In some cases, the ratooning ability has been indirectly evaluated by assessing the biomass or light utilization efficiency of sugarcane, and assessing drought tolerance in those arid or semi-arid cultivated regions is also suggested (Qin *et al.,* 2014). Ratooning ability is a trait that a commercial quality variety must have. Indicative traits of a strong ratooning ability include both morphological indicators of sugarcane root residue/stubble and traits that directly contribute to cane yield and sugar output, such as a high number of stalks, high viability of buds, large number of viable buds, large number of viable roots, high cane yield, high sugar output (Singh *et al.,* 2015;Ding *et al.,* 2020; Qin *et al.,* 2014, Hassan *et al.,* 2017; Ramburana *et al.,* 2013) and high tillering rate in plant cane. Additionally, a higher stubble germination rate and the larger shoot number were observed in the ratoon crops, which result in high stalk number and higher cane yields than those in plant-cane. A similar observation was obtained by other reports (Aslam *et al.,* 2020; Ramburana *et al.,* 2013). It is also believed that the ratooning ability of sugarcane is mainly identified by four important factors, namely, root traits, the total number of strikes or shoot population, stalk number, and cane yield. Good performance on the four aspects above in its plant cane and the ratoon crops is necessary for the selection of varieties with a strong ratooning ability.. The morphological characteristics of sugarcane stubble are closely related to the ratooning ability of the sugarcane (Bashir *et al.,* 2013). In addition, sugarcane varieties with strong ratooning ability have a low stubble mortality rate and a short inter-nodal length of underground stems, together with the obviously larger total number of underground buds and the effective tillers (Bashir *et al.,* 2013). Generally, if there is an increased number of effective tillers formed by the lower buds of the main stems, and there is an increased total number of effective tillers on the main stems, then the variety likely has strong ratooning ability (Bashir *et al.,* 2013).

There was a significant interaction effect between varieties and growing seasons for all yield and qualitative traits except for the purity of sugarcane juice. Based on an investigation of later crop, Olaoye found that single stalk weight, cane yield, total soluble solids (Brix), and sucrose percentage, were highly heritable traits that displayed the potential to obtain high genetic gain. Additionally, a study on the genetic relationships among sugarcane traits in a large population indicates that stalk number was the primary determinant of cane yield and thus became more important trait in determining cane yield in the ratoon crops, much higher than those of stalk diameter and stalk length (Ramburana *et al.,* 2013). Research also indicated that, for varieties with poor ratooning ability, the ratoon crops had a much lower cane yield than the plant cane (Bashir *et al.,* 2013) or a sharp decline in cane yield in the first ratoon compared with plant cane (Silva *et al.,* 2017). Meanwhile, the yield decrease was only observed in varieties with strong ratooning ability in the second ratoon crop. In brief, for the selection of ratooning ability, direct indicators are the stubble morphology, stalk number, and the germination and tillering rates in the plant cane and the ratooncrops, while indirect indicators included disease resistance especially smut, pest

resistance, biomass, light use efficiency, and hormone content during stubble bud germination. The number of indicators used in selection may vary, but researchers have the same or a similar opinion on those indicators. In addition, more attention should be paid to the selection of the experimental location, mostly due to the reason that the effect of the location on ratooning ability is visible.

Factors influencing longevity and productivity of ratoon sugarcane

The ratooning ability or good ratooning potential is an essential pre-requisite or the most critical factor for good ratoon (Aslam *et al.,* 2020). The genotype, cultivation management, and environment contribute to the ratoon crop in descending order (Aslam *et al.,* 2020). The ratoon crop yields decline typically with age. Studies have also shown that, in subtropical regions, a major bottleneck for improving ratoon productivity is the poor germination rate of buds in the stubble remaining after winter harvesting (Singh *et al.,* 2015). The trait of stalk number has the greatest impact on sugarcane yield. Therefore, the ratooning ability is one of the most important target traits in sugarcane breeding and has always been valued by breeders (Singh *et al.,* 2015; Rafiq *et al.,* 2006).From both the perspective of reducing production costs and improving the productivity of the ratoon crops, breeding and growing varieties with a strong ratooning ability is the most important prerequisite for extending the number of ratooning years and increasing the yield of the ratoon crops.

Furthermore, in sugarcane-producing areas with low temperatures, frost, drought, pests, diseases (especially smut), stem borers, or extensive management, the ratooning ability of sugarcane varieties is particularly important for extending the number of ratooning years and increasing the yield of ratoon crops.

Variation in ratooning ability between different sugarcane genotypes

Sugarcane genotypes with higher proportions of the genetic background of *Saccharum spontaneum* display stronger ratooning ability (Burnera *et al.,* 2017) because the characteristics of a species can be affected by kinship (Huang *et al.,* 2018), i.e., hereditary basis. Sugarcane 'nobilization' breeding aimed at bringing the genes controlling vigor, vitality, stress resistance, and strong ratooning ability from wild species into original cultivated species, i.e., 'noble' S. officinarum. A wild species S. spontaneum, the mostly used and studied, was the first species to naturally hybridize with S. officinarum (Liu, 2018). Meanwhile, the ratooning ability was negatively correlated with single stalk weight and commercial cane sugar (CCS). Therefore, strengthening CCS through selection without considering the ratooning ability is not conducive to pyramiding the genotypes with strong ratooning ability (Liu, 2018).

Limitations of Existing Research

It is precisely because of this highly heterozygous genetic background that the offspring of sugarcane hybrids are widely segregated and the probability of aggregation of excellent traits is extremely low (1/100,000–1/300,000). Therefore, for a long time, sugarcane cross breeding had to rely on large segregating populations. In Pakistan, a very low number of seedlings being planted in the field, a commercial cultivar with high yield, disease resistance, especially primary diseases including smut resistance, and strong ratooning ability has not been yet identified or developed. Approximately 95–97% of planted seedlings are discarded after observation in the first year, without ratooning. Therefore, in Pakistan, the problem of the short ratooning longevity of the leading sugarcane varieties needs to be solved.

Sugarcane cross breeding relies on a huge, segregated population. There is still a lack of effective and high-throughput selection technology suitable for early segregating generations and large populations. Although the selection of ratooning ability based on phenotype is intuitive and effective in general, it is still difficult to identify and select varieties with strong ratooning ability, disease resistance, and high yield simultaneously.

CONCLUSIONS

Ratooning can largely reduce production costs compared with replanting sugarcane. Labor costs increase yearly, and the cost difference between ratooning and replanting sugarcane widens. In this paper, previous studies on sugarcane ratooning ability were reviewed in terms of the definition, phenotypic traits and major influencing factors. In addition, the limitations of existing research on ratooning ability were highlighted. We do hope that this review can provide a reference for understanding the limitations underlying sugarcane ratooning ability, and for breeding sugarcane varieties with strong ratooning ability.

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SUGAR INDUSTRY ABSTRACTS

Evaluating a legume break crop and its residual effect on increasing sugarcane production and reducing nitrogen application

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Proceedings of the International Society of Sugar Cane Technologists, volume 30, 1109–1116, 2019.

The use of the leguminous plant “velvet bean” (*Mucuna pruriens*) as a rotation crop in sugarcane production at sugar mills is being adopted in Ecuador. Therefore, it is important to evaluate the use of this legume by incorporating it either as green or dry plant biomass into the soil to increase yield and improve chemical soil conditions. The experiment carried out at CINCAE Experimental Station started with planting a velvet bean crop in plots in early January 2016 to incorporate into the soil in May as green and dry (burnt with glyphosate) biomass. Approximately, 4.0 t/ha of dry matter was incorporated in the soil 35 days before sugarcane planting. In the first year (plant cane), the application of different levels of N: 25, 50 and 75%, of the full rate of N fertilizer (125 kg N/ha) to velvet bean plots (independent of the type of biomass incorporation) did not show differences in sugarcane yields among N rates. However, they increased significantly (*P<0.05*) the cane yield by 27% compared with the control treatments (zero and 125 kg N/ha, both without incorporated legume biomass). In first ratoon crop, the residual effect of legume with fertilizer N (not depending on N fertilizer rates) enhanced cane yield between 13 and 40% (P<0.05) compared to full rate of N (125 kg N/ha) and the zero N application, respectively. These responses highlight that planting velvet bean as a break crop improved cane yields and its residual effect can remain until the second-year crop. During the two experimental years, the application of N fertilizer (average of the N levels) over plots that had incorporated legume biomass increased N uptake by 27 and 13% related to the 100% of full N rate application in plant cane and first ratoon, respectively. This response could be a result of the combination of the high N availability from the N fertilizer and by the N mineralization of the incorporated legume biomass. Further, this study points out the possibility of reducing by 25% the N fertilizer (from the 100% full N rate) during the two-year sugarcane crop (plant cane and first ratoon). Nevertheless, to achieve a new equilibrium between the reduction of synthetic N fertilizer and the incorporation of legume biomass more research is needed.

Selection of varieties for humid environments of the sugarcane area of Colombia

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Proceedings of the International Society of Sugar Cane Technologists, volume 30, 591–598, 2019

In the Colombian sugarcane area three mega zones have been identified in terms of precipitation, evapotranspiration and permeability (semi-dry, humid and piedmont). The humid zone represents 27% of the total area cultivated and has excess moisture and poorly drained soils. In Cenicaña, the development and selection of cultivars is a continuous process, with in the last stage the clones planted in multilocation trials with the objective of evaluating stability and predicting behaviour in other areas homologous to the initial evaluation sites. A trial was planted in seven locations in order to evaluate the performance of five elite clones of the 2001 series compared to the commercial control CC 85-92. A randomized complete-block

design with three replications was used. The stability of the clones and the prediction of environments with greater production potential was determined with a graphical analysis of the GGE biplot, and sucrose contents, cane yield and sugar yield were analyzed. Significant statistical differences among locations, varieties and the interaction of variety x mills were found. Sucrose content was very sensitive to changes in the environment and explained 57%, 4.2% and 28% of the total variance, respectively. The first two components of the interaction explained between 72% and 88% of the variance of the clone x location interaction. CC 01-1940 showed a high cane yield in semi-dry environments, with a low sucrose content and a high degree of lodging. The additive genetic component was positively correlated with the higher rainfall areas and two groups of environments were delineated, one with high rainfall and the second with transitional environments. CC 01-1940 in humid environments produced higher sucrose contents with equal tonnages and in some cases higher than CC 85-92, and also showed genetic plasticity for sugar yield and higher income.

Spatial mapping of trash recovery costs

DG Duft, FM Okuno, ACS Luciano, TF Cardoso, A Bonomi and MRLV Leal

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 720–723, 2019

Trash, both in the field and in the factory, has now become an important sugarcane co-product. Due to the social and environmental pressure to stop trash burning in sugarcane fields and the evaluation of the generation of renewable and decentralized electric energy, the use of trash in the boilers became a viable opportunity to generate income in the sugar mills. In addition, this potentially eliminates some problems that excess trash in the field could cause in reducing the yield of sugarcane. Trash-recovery alternatives have different characteristics and costs. Trash recovery by baling is often linked only to the distance to the mill. However, the recovery cost is tied also to the amount of trash available in the field and the amount that can be collected without causing agricultural problems; for this reason, different fields within a mill catchment will have different recovery costs. This work aimed to create a cost-based model listing some essential variables and applying them spatially in a case study to identify which fields around a mill have a more attractive cost of recovery and which areas deserve different attention. The result showed that the proximity of the sugar factory is not the fundamental variable and that good management of recovery areas can lead to lower costs.

Analysis of milling operation with electro-hydraulic individual drives in the Ferrari Sugar Mill

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The Ferrari Sugar Mill tandem in Brazil is fully automated with variable speed for all the drives involved. The mills' rollers are individually moved by electro-hydraulic drives (including the fourth roller in mill one), thus offering a great advantage to automate the mill in a very accurate way and to also measure the speed and torque in each of the rollers driven. The speeds and the torque of each roller of five four rollers mills tandem were registered continuously, providing information on the power consumed in each roller, in each mill and in the whole tandem. The laboratory results were analysed at the same time and the power consumption in the mills against the tonnage of fibre extracted was reported. Special attention was given to the possibility of changing the speed ratio of the fourth roller in relation to the top roller in a continuous way and measure the torques and power consumption, looking for the additional advantages of the corresponding individual drive. The operational results of the milling section of the

factory were good, considering the size, number of mills and the crushing rate. Also, the power consumption in the mills was excellent for these conditions. The advantage of using the individual drive for the fourth roller in mill number one was clearly observed during the season and the factory decided to drive all the fourth rollers individually in the short future.

Impact of tops and green leaves on sugarcane processing: laboratory testing

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In Réunion, changes in harvesting practices have led to increased amounts of sugarcane tops and leaves delivered to factories. To anticipate the changes in sugar recovery processing, laboratory trials were undertaken. Samples with known quantities of tops or green leaves were prepared and cane processing was simulated at laboratory scale: juice extraction, clarification and evaporation with operating parameters similar to those in the factory. Juice and syrup were collected and analyzed for sugar quality parameters (brix, pol, purity), as well as parameters that impact on sugar recovery or processing quality: ashes and reducing sugars were monitored to estimate the sucrose loss to molasses, while calcium, phosphate and oxalate were monitored to evaluate the risk of fouling in evaporator. Results highlight a degradation of juice composition with increasing quantities of tops and leaves (decrease in purity and pH, and an increase in non-sucrose components), an increase in lime consumption, and an increase in color. An increase in residual calcium in syrup resulting from increasing lime consumption was observed showing that part of the calcium did not react with phosphate thus increasing the risk of evaporators fouling. The mixed juice, clear juice and syrup qualities mentioned above decline in the same proportion and the composition of the juice did not get worse with juice treatment. Results from this study will enable technologists to better manage the effects of cane tops and green leaves on sugar recovery and quality and provide useful information for decision-making.

Effects of plant-growth-promoting rhizobacteria on diseases, pest insects and agronomic traits of sugarcane

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Proceedings of the International Society of Sugar Cane Technologists, volume 30, 1736–1742, 2019

Sugarcane is an industrially important crop. It is affected by numerous pathogens including fungi, bacteria, nematodes and viruses. Biological control, being ecofriendly, has been widely used throughout the world. Amongst the multiple biocontrol agents, plant-growth-promoting rhizobacteria (PGPR) have been found to be effective against multiple sugarcane pathogens. The effective rhizobacteria (15-20%) have been found in the rhizosphere and endosphere of sugarcane. The strains have shown competency in the root rhizosphere as well as the endosphere determined by their stability based on molecular markers developed specifically for the different strains. The effective bioformulation based on these strains have significantly reduced the incidence of sugarcane red rot (*Colletotrichum falcatum*), pokkah boeng (*Fusarium verticilloides*) and borers. The strains have produced several antifungal metabolites, viz siderophores, hydrolytic enzymes and antibiotics. A significant correlation ($p<0.01$-0.05) between the antifungal metabolites production, ISR elicitors, root colonization and disease suppression has been detected in numerous studies. The strains have also shown efficacy under field conditions on highly susceptible to moderate susceptible varieties. This suitable bioformulation could be registered as biopesticide to control the sugarcane diseases.

TRAP markers allow the identification of transgenic lines that are genetically close to their parental genotype

MF Perera, SN Ovejero, J Racedo, AS Noguera, MI Cuenya and AP Castagnaro

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 103–109, 2019

Molecular markers could be used to screen for somaclonal variation during the process of genetic transformation. We aimed to test Target Region Amplified Polymorphism (TRAP) marker systems to identify and quickly predict similarity to the parental line of different transgenic lines. DNA of transgenic lines, wild-type genotypes and three additional sugarcane clones was genotyped with seven to nine TRAP primer combinations. Amplification products were separated by electrophoresis on polyacrylamide denaturing gels in a 4300 DNA Analyzer (Li-COR), images were analyzed, and bands were transformed into a 0 or 1 matrix. Similarity was calculated using the Jaccard coefficient and dendrograms were generated using UPGMA analysis. TRAP was initially used to determine whether the close growth resemblance between six herbicide-tolerant (HT) lines and their parental cultivar RA 87-3 was also true at the genetic level. The genetic characterization confirmed the preliminary phenotypic evaluations since transformed lines exhibited none or only minor genetic changes whereas lines with growth aberrations also included in the analysis showed a significant degree of polymorphism. The incorporation of other genotypes as controls allowed us to internally evaluate the accuracy of the survey ensuring that a significant number of polymorphic bands were analyzed. These markers were routinely applied to evaluate transgenic lines of LCP 85-384, TUCCP 77-42, TUC 95-10 and TUC 03-12 at early stages of the characterization process. Our results showed that the use of TRAP markers to genetically characterize promising transgenic lines is a rapid and recommendable first approach to identify transformed plants that are genetically close to their parental genotype as they could be applied at the early stages of evaluation to select for the most valuable lines to carry out field tests.

AEGIS, an extended information system to support agroecological transition for sugarcane industries

S Auzoux, E Scopel, M Christina, C Poser and J-C Soulié

Proceedings of the International Society of Sugar Cane Technologists, volume 30, 186–192, 2019

Faced with increasing environmental, economic and social challenges, sugarcane industries are adopting agroecological approaches to design and evaluate systems that use natural resources more efficiently, mobilize plant biodiversity and adopt agroecological practices. In order to set up this agroecological transition, stakeholders of sugarcane industries need to: (i) access and analyze raw data; (ii) capitalize and share knowledge through professional networks; (iii) define performance and impact indicators; and (iv) engage in learning processes to acquire new skills based on successful experiments. CIRAD developed AEGIS (AgroEcological Global Information System), a platform to support digital agriculture and successful agroecological transition. AEGIS can provide standardized, harmonized and organized data that come from various sugarcane agroecosystems. Data stored in AEGIS are collected at different spatial and temporal scales, from different experiment designs and protocols, and in different contexts (agronomy, ecology, sociology, and economy). AEGIS meets the expectations of stakeholders through the development of generic statistical analysis tools and the implementation of ex-ante and ex-post data processing methodologies. It provides datasets for simulation of crop models and complex visualization tools to facilitate the interpretation of data and to highlight

indicators, patterns and correlations inaccessible from raw data. AEGIS uses ontologies, metadata standards and web services, which ensure the semantic and technical interoperability of the various components of the information system. These features allowed development of a common language for sharing and exchanging contextualized information between stakeholders, whatever their fields of activity. By integrating dashboards, statistical analysis tools, data processing tools (data mining), simulation and visualization tools (artificial intelligence), our platform is a complete steering and decision support tool in the context of the agroecological transition.

INTERNATIONAL EVENTS CALENDAR

2023 CONFERENCES & MEETINGS

February 11-14, 2023	10th International Conference on Sugar and Integrated Industries (ICSII 2023), Luxor, Egypt
February 16-25, 2023	International Society of Sugarcane Technologists (ISSCT) XXXI Congress at Hyderabad India
Feb 27 - Mar 02, 2023	American Society of Sugar Beet Technologists (ASSBT) 2023 Biennial Meeting, Savannah, GA. USA
Mar 31 - Apr 02, 2023	8th International Symposium of International Society of Rare Sugars Takamatsu, Kagawa, Japan
April 17-20, 2023	Geneva Sugar and Biofuels Conference, Fairmont Grand Hotel Geneva, Switzerland
April 18-21, 2023	44th Australian Society of Sugarcane Technologists (ASSCT) Conference, Cairns Australia
May 07-11, 2023	Sugar Industry Technologists Annual Technical Meeting, New Orleans, USA
June 12-14, 2023	33rd ICUMSA Session, Friedrich-Wilhelm-Raiffeisen-Platz 1, 1020 Wien Vienna, Austria
June 13-15, 2023	American Society of Sugar Cane Technologists (ASSCT) Annual Joint Meeting Westin Savannah Harbor Savannah, GA. USA
June 23-26, 2023	25th Carbo Solutions International Sugar Conference Casablanca Morocco
July 5-7, 2023	2nd International Conference on Cane and Sugar 2023, Aswin Grand Convention Hotel Talad Bangkhen, Lak Si, Bangkok Thailand
August 4-8, 2023	38th International Sweetener Symposium Meritage Resort, Bordeaux Way, Napa, CA USA
August 15-17, 2023	95th South African Sugar Technologists' Association (SASTA) Congress Durban, South Africa
September 18-20, 2023	12th Congress ATALAC, Heredia Costa Rica
October 03-05, 2023	16th Annual Sugar & Ethanol Asia Conference Bangkok, Thailand
November 21-22, 2023	32nd International Sugar Organization (ISO) INTERNATIONAL SEMINAR, London United Kingdom

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