Chakravarthi Mohan *Editor*

Sugarcane Biotechnology: Challenges and Prospects

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This book is dedicated to my mentor,
Dr. N. Subramonian, Emeritus Scientist,
ICAR–Sugarcane Breeding Institute, who has
always been encouraging and supportive and
is an inspiration for young researchers.

Preface

Sugarcane is an important cash crop grown throughout the tropical and subtropical regions of the world. It serves as the major source for sugar and also used for ethanol and biomass production. The demand for sugarcane and its by-products is set to increase in recent years due to increasing population, higher demand for sugar and climate change. Moreover, sucrose yield has been unchanged for the past decades. Owing to these factors, there exists the need for sugarcane improvement through biotechnology which would inevitably improve the yield as well as the sustainability of sugar industries. With the advent of next generation sequencing technologies and genome editing tools, the realization of sugarcane improvement through biotechnology is not very far. Several transcriptomic studies have been carried out in sugarcane and whole genome sequencing is in progress. Transgenic sugarcane for several traits has been reported, the highlight being the commercialization of drought-tolerant transgenic sugarcane in Indonesia and others in pipeline. Sugarcane is being used as a platform to produce several recombinant proteins and products. Very recently, transcription activator-like effector nucleases (TALENs) have been used in sugarcane initiating genome editing approach in this complex polyploid genome.

In this volume, a collection of 11 chapters is presented by experienced researchers working on sugarcane biotechnology. This book provides exhaustive information on several recent technologies that are employed for sugarcane improvement through biotechnology. An array of topics such as genomics and transcriptomics, transgenic sugarcane for trait improvement, potential candidate promoters, new strategies for transformation, molecular farming, sugarcane as biofuel, chloroplast transformation and genome editing which are currently employed in sugarcane for trait improvement has been discussed comprehensively in this book which will serve as an encyclopaedia for graduates, postgraduates and researchers who work on sugarcane. This book will also be of great interest to plant scientists, biotechnologists, molecular biologists and breeders who work on sugarcane crop. As editor of this book, I am grateful to the contributors of various chapters for writing their chapters meticulously and enabling to produce this book on time and in a great manner. I also thank the editorial staff of Springer, New York, who were very generous

and helpful to initiate this book project. I am also grateful to the São Paulo Research Foundation (FAPESP, Proc. 2015/10855-9) for the postdoctoral research grant. Finally, special thanks to Springer, Switzerland, for publishing this book. I firmly believe that the information covered in this volume will make a sound contribution to sugarcane research.

São Carlos, SP, Brazil — Chakravarthi Mohan

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About the Editor

Dr. Chakravarthi Mohan is presently a postdoctoral fellow at the Department of Genetics and Evolution, Federal University of São Carlos, Brazil, where he aims to develop transgenic sugarcane for weevil resistance through RNAi approach. He received his Ph.D. in biotechnology in 2015 for his work on 'Isolation and characterization of constitutive and wound inducible promoters and validation of designed synthetic stem/root specific promoters for sugarcane transformation' from Bharathiar University, Coimbatore, and the study was carried out at the ICAR–Sugarcane Breeding Institute, Coimbatore, India. He has considerable expertise on sugarcane genetic engineering, transcriptome analysis and sugarcane molecular farming which is evident from his publications. He has published 13 research papers in international peer-reviewed journals and 5 book chapters. He is a life member of Indian Science Congress Association and also serves as reviewer for several international peer-reviewed journals. He has also presented his research in international and national conferences.

Chapter 1
Potential Health Benefits of Sugarcane

Chinnaraja Chinnadurai

Abstract Sugarcane is a perennial grass belonging to Poaceae family and it has been cultivated worldwide more than 90 countries because of its economical and medicinal value of high-yielding products. Refined sugar is obtained as a primary product from sugarcane juice, an eminent raw material of sugarcane. Other commercial value-added by-products such as brown sugar, molasses, and jaggery are also obtained during the process in an unrefined form. The expensive carnauba wax is produced from sugarcane wax and utilized in cosmetics and pharmaceutical applications. Sugarcane juice is widely used in traditional medicine system of several countries mainly in India, to treat several health issues such as jaundice, hemorrhage, dysuria, anuria, and other urinary diseases. In this chapter, various types of phytoconstituents and health benefits of sugarcane and its valuable products are summarized. The phytochemistry of sugarcane juice, sugarcane wax, leaves, and its products also established the occurrence of various fatty acids, alcohol, phytosterols, higher terpenoids, flavonoids, -O- and -C-glycosides, and phenolic acids. Necessity on advanced research for the production of various medicinal products from sugarcane and its phytopharmacological study has been summarized.

Keywords Medicine • Molasses • Pharmacological properties • Phytochemical profile • Sugarcane juice

1.1 Introduction

Sugarcane is a tall perennial true grass belonging to the genus *Saccharum* and tribe Andropogoneae. It originated in Southeast Asia and is now cultivated in tropical and subtropical countries throughout the world for sugar and by-products. The genus *Saccharum* contains five important species, viz., *Saccharum officinarum, Saccharum sinense, Saccharum barberi, Saccharum robustum*, and *Saccharum spontaneum*.

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The cultivation of *S. officinarum* and its hybrids is mostly used for the production of sugar and ethanol and other industrial applications in more than 90 countries around the world. The stems and the by-products of the sugar industry are also used for feeding livestock. *S. officinarum* was originally grown in Southeast Asia and Western India. Around 327 B.C. it was an important crop in the Indian subcontinent. It was introduced to Egypt around 647 A.D. and about one century later, to Spain (755 A.D.). Since then, the cultivation of sugarcane extended to nearly all tropical and subtropical regions around the world. Portuguese and Spaniards introduced sugarcane to the New World early in the sixteenth century. *S. officinarum* L. more recently is utilized as a replacement of fossil fuel for motor vehicles.

Worldwide, sugarcane inhabits 20.42 million ha area with a total production of 1900 million metric tons (FAO 2014). Sugarcane area and productivity differ widely from country to country. Brazil occupies the highest sugarcane-growing area (5.343 million ha) followed by India, China, Thailand, Pakistan, and Mexico. Sugarcane is a best example for renewable natural agricultural resource since it provides sugar, besides biofuel, fiber, fertilizer, and a myriad of by-products/coproducts with ecological sustainability. White sugar, brown sugar (Khandsari), jaggery (Gur), and ethanol are obtained from sugarcane juice and bagasse and molasses are the main by-products of the sugar industry. Molasses are the chief by-products used as main raw material for the production of alcohol. Excess bagasse is now being used as raw material in the paper industry. In addition, cogeneration of power using bagasse as fuel is considered feasible in most sugar mills.

Sugarcane holds potential health benefits and generally most of them are not aware of it. Sugarcane can be edible in the form of either pieces of stem or juice. Sugarcane juice extracted from the cane is nutritious and refreshing. It contains about 15% natural sugar that helps to rehydrate the human body and gives instant energy. Sugarcane juice is rich in minerals such as phosphorus, potassium, calcium, iron, and magnesium and vitamins such as vitamin A, B1, B2, B3, B5, B6, C, and E. About 100 mL of sugarcane juice contains 39 calories of energy and 9 g of carbohydrates.

1.2 Health Benefits of Sugarcane

Sugarcane juice is used to cure several types of human diseases in different parts of the world. It has been used in Ayurveda and Unani systems of medicine in India since time immemorial either as single drug or in combination with other plant products. Sugarcane extracts were established with a wide range of biological effects such as immunostimulation (El-Abasy et al. 2002), anti-thrombosis activity, anti-inflammatory activity, vaccine adjuvant, modulation of acetylcholine release (Barocci et al. 1999), and anti-stress effects. Sugarcane juice has broad biological effects on raising innate immunity to infections (Lo et al. 2005).

Jaundice patients and people having liver-related disorders have been encouraged to consume sugarcane extract in traditional system of medicine in curing diseases. Sugarcane juice is also used as aphrodisiac, laxative, demulcent, antiseptic, and tonic (Xu et al. 2005). According to the Unani system of medicine in India, sugarcane juice is considered beneficial for the liver by regulating the bilirubin levels and it is recommended that consumption of large amount of sugarcane juice helps for an immediate relief from jaundice. These assumptions have also been supported by modern pharmacological studies, which revealed that sugarcane contains various bioactivities like anti-inflammatory, analgesic, antihyperglycemic, diuretic, and hepatoprotective effects. Although apigenin, tricin, and luteoline glycosides like orientin, vitexin, schaftoside, and swertisin were reported as the main constituents in sugarcane juice, various policosanols and steroids were also reported in different parts of *S. officinarum.* Based on these bioactivities and chemical constituents of sugarcane, great attention has been given for the investigation of some lead molecules of this cheapest crop for various diseases.

Sugarcane juice regulates natural immunity of host cells against different microbial infections such as viral, bacterial, and protozoan having effects on the levels of macrophages, neutrophils, and natural killer cells (El-Abasy et al. 2002, 2003; Lo et al. 2005). A wide range of biological activities are observed with by-products of sugarcane juice including antioxidant activities (Tanaki et al. 2003), prophylactic activities, and other physiological functions (Takara et al. 2002).

Sugarcane juice is a rich source of antioxidants. Free radicals have been concerned in the etiology of several human ailments and many antioxidants are being considered as potential therapeutic agents (Sies 1996; Spiteller 2001). The mechanism involved in many human diseases such as hepatotoxicities, hepatocarcinogenesis, diabetes, malaria, acute myocardial infarction, and skin cancer includes lipid peroxidation as a main source of membrane damage (Yoshikava et al. 2000). Antioxidants are molecules capable of terminating the chain reaction of free radicals before vital molecules are damaged. Supplementation of these antioxidants became an attractive therapeutic strategy for reducing the risk of diseases caused by free radicals (Brash and Harve 2002). Recent studies on the role of phenolic compounds from foods and beverages against free radical-mediated diseases became more significant due to the finding of association between lipid peroxidation of LDL and arthrosclerosis. Antioxidant properties of phenolic compounds can be attributed to a wide range of pharmacological activities. These compounds in general act by quenching free radicals, inhibiting the activation of pro-carcinogens, or binding carcinogens to macromolecules. The phenolic and flavonoid contents of sugarcane juice were found with equal proportion of antioxidant effects (Krishnaswamy 1996).

The polyphenols in sugarcane juice also induce metabolism and help keep weight gain during pregnancy and its low glycemic index helps to maintain energy levels. A glass of sugarcane juice with a dash of ginger helps to reduce morning sickness of pregnant women. Small doses of sugarcane juice more than twice a day are recommended for morning sickness, a common complaint among pregnant women. Since sugarcane juice is a rich source of calcium, magnesium, and iron, regular consumption can help boost immunity and keep mineral deficiency at bay during pregnancy. Constipation is also an issue with pregnancy. The juice can also be used to boost digestion and treat constipation due to the presence of potassium. It helps in proper functioning of digestive system and prevents stomach infections.

Sugarcane juice has been recommended for its diuretic property (Karthikeyan and Simipillai 2010; Cáceres et al. 1987). Regular use of sugarcane juice leads to clear urinary flow since it aids kidneys to perform their function properly. With addition of lime juice and coconut water, sugarcane juice helps in reducing burning sensation which is commonly associated with urinary tract infections, sexually transmitted diseases, kidney stones, and prostatitis.

Intake of sugarcane juice is recommended for diabetic patients. It comprises natural sugar which has low glycemic index that prevents steep rise in blood glucose levels in diabetics. Noni fruit juice was mixed with sugarcane juice and kukui nuts (*Aleurites moluccana* (L.) Wild, Euphorbiaceae) to be used as purgative, or diluted with spring water to treat diabetes and high blood pressure or prevent intoxication from kava (McClatchey 2002; Chun 1994). However, type 2 diabetes patients are recommended to consume it in moderate levels after doctor consultation.

Cancer cannot survive in an alkaline environment. Sugarcane juice comprises high concentration of calcium, magnesium, potassium, iron, and manganese since it is proven that regular consumption of sugarcane juice is effectively fighting against cancer, especially prostate and breast cancer.

Studies established that sugarcane juice protects against tooth decay and bad breath due to its high mineral content. Deficiency of nutrients in the body can easily be recovered by including sugarcane juice in our diet. Febrile disorder is quite common in infants and children resulting in fevers, which can lead to seizures and loss of proteins in the body. Sugarcane juice helps in compensating the lost protein and helps in recovery.

Alpha hydroxy acids help fight acne, reduce blemishes, prevent ageing, and keep the skin hydrated. One of the most effective alpha hydroxy acids is glycolic acid and is present in sugarcane and considered as one of its few natural sources. Even though sugarcane juice has many advantages, it is also important to consume the juice as soon as it is extracted because it tends to get oxidized within 15 min. As it is rich with medicinal values, sugarcane juice is considered as a miracle drink.

1.3 Phytochemical Profile of Sugarcane and Its By-products

1.3.1 Sugarcane Leaves

Sugarcane leaves are naturally coated with waxes which are considered as an important source of various policosanols and D-003. In addition, various flavones -*O*- and -*C*- glycosides were isolated from methanolic extracts of sugarcane leaves through HPLC microfractionation techniques.

1.3.2 Sugarcane Wax

Sugarcane wax deposits on the surface of stalks and leaves seem whitish to dark yellow in color and are extracted from the sugarcane filter residue, the so-called bagasse, during sugar production and utilized for industrial, cosmetic, and pharmaceutical applications (Hoepfner and Botha 2004). It is one of the important commercial sources of long-chain fatty alcohols, acids, esters, aldehydes, and ketones. Apart from that policosanols and D-003, some steroids and terpenoids have also been isolated as by-products from sugarcane wax. Policosanols range from 2.5 to 80% and are a blend of long-chain primary aliphatic alcohols. Octacosanol constitutes 50–80% of the total policosanols (Awika and Rooney 2004). Other active components of sugarcane wax are long-chain aliphatic fatty acids that occur at lower concentrations. The blend of these acids is known as D-003 (Mas 2004). Several phytosterols, steroids, and higher terpenoids were also reported (Georges et al. 2006; Bryce et al. 1967) apart from the major constituents of fatty acid and fatty alcohol in sugarcane wax (Goswami et al. 1984). The quantity of wax derived from sugarcane is between the range of 0.1 and 0.3% and it differs from variety to variety (Laguna Granja et al. 1999). The sugarcane wax is considered as a possible substitute for the expensive carnauba wax.

1.3.3 Sugarcane Juice

Sugarcane juice is extracted by grinding the sugarcane stems for the production of white/brown sugar, jaggery, and molasses. Sugarcane juice holds water (70–75%), sucrose (13–15%), and fiber (10–15%). Several color components with chlorogenic acid, cinnamic acid, and flavones were identified from sugarcane juice during 1971 (Farber et al. 1971). Further, all the colored components were categorized into four major classes: plant pigments, polyphenolic compounds, caramels, and degradation products of sugars condensed with amino derivatives.

The presence of phenolic acids such as hydroxycinnamic acid, sinapic acid, and caffeic acid, along with flavones such as apigenin, luteolin, and tricin, was also identified in high-performance liquid chromatography with diode array detection (HPLC-DAD) analysis of phenolic compounds from sugarcane juice. In that, tricin derivatives were obtained with highest concentration (Maurício Duarte-Almeida et al. 2006). Further, detailed chromatographic and spectroscopic studies established the presence of various -*O*- and -*C*- glycosides of the above-mentioned flavones (Vila et al. 2008). Apart from that few minor flavones swertisin, tricin-7-*O*-neohesperoside-4′-*O*-rhamnoside, tricin-7-*O*-methylglucuronate-4′-*O*-rhamnoside, and tricin-7-*O*-methylglucuronide (Colombo et al. 2009) and some novel acylated flavone glycosides, such as tricin-7-*O*-β-(6′-methoxycinnamic)-glucoside, luteolin-8-*C*-rhamnosyl glucoside, and tricin-4′-*O*-(erthroguaicylglyceryl)-ether, were isolated, along with orientin, from sugarcane juice (Duarte-Almeida et al. 2007).

1.3.4 Sugarcane Products

Brown sugar, molasses, syrups, and non-centrifugal sugar are the several important by-products of sugarcane (Balasundaram et al. 2006). Apart from some identified compounds of sugarcane juice, three new flavonoid glycosides, tricin7-(2′-rhamnosyl)-α-galacturonide, orientin-7,3′-dimethyl ether, and iso-orientin-7,3′-*O*-dimethyl ether, were isolated from mill syrups (Mabry et al. 1984). Along with the already stated isoorientin-7 and 3′-*O*-dimethyl ether, a novel O-glycoside and dehydroconiferylalcohol-9′-*O*-β-D-glucopyranoside were also isolated from sugarcane molasses and have been validated as antibacterial compounds (Takara et al. 2007). Through liquid chromatography-mass spectrometry (LC-MS) analysis of aqueous and dichloromethane extracts of brown sugars, the presence of various phenolic acids and eight major volatile constituents has been described.

1.4 Pharmacological Properties of Sugarcane and Its By-products

Various phytochemicals including phenolic compounds, plant sterols, and policosanols are present in sugarcane and help in defense against pest and diseases. Several studies have proven the biological activities of sugarcane products including antioxidant activity, cholesterol-lowering properties, and other potential health benefits.

1.4.1 Antithrombotic Activity

Antithrombotic activity was examined with policosanols and D-003 for their platelet aggregation and in rats. Plasma level of 6 keto-PGF1-α (a stable metabolite of prostacyclin PGI) was significantly increased with oral administration of D-003 at a single dose of 200 mg/kg and policosanols at a concentration of 25 mg/kg in rats, compared to control. In addition, D-003 significantly reduced the thromboxane plasma levels and weight of venous thrombus in collagen-stimulated whole blood of rats (Molina et al. 2002). Also, the pharmacokinetic study established that the effect of D-003 was detected after 30 min of dosing and the maximal effect exhibited after 1–2 h of treatment (Molina et al. 2000).

1.4.2 Diuretic Activity

Intragastric application of ethanol extracts (50%) of fresh leaves to rats at a dose of 40 mL/kg was found with diuretic activity, while its decoction was not found with any diuretic activity (Ribeiro Rde et al. 1986; Cáceres et al. 1987).

1.4.3 Analgesic and Antihepatotoxic Activity

Ethanol extracts (95%) from sugarcane leaves and shoots were recorded with analgesic activity in mice with intragastric application at a dose of 1 g/kg. The ethanol extract of sugarcane shoots was found active only against the tail-flick method while leaf extracts were active against benzoyl peroxide-induced writhing and tail-flick response (Costa et al. 1989).

Intraperitoneal application of aqueous extract of dried stems to mice, at a dose of 25 mg/kg, was found active against chloroform-induced hepatotoxicity (Jin et al. 1981).

1.4.4 Antihypercholesterolemic Effect

Oral administration of sugarcane policosanols (5–200 mg/kg) on normocholesterolemic New Zealand rabbits revealed a significant decrease in the level of total cholesterol and low-density lipoprotein cholesterol (LDL-C) in a dose-dependent manner. It also reduced the level of serum triglyceride, but it was not found as dose dependent. However, the high-density lipoprotein levels remained unchanged (Arruzazabala et al. 1994).

Policosanols also prevented atherosclerosis in male New Zealand rabbits fed on a cholesterol-rich diet for 60 days at doses of 25 or 200 mg/kg. Interestingly, hypercholesterolemia was not found in policosanol-treated rabbits and the intima thickness was also found significantly less compared to control animals (Arruzazabala et al. 2000).

1.4.5 Antihyperglycemic Activity

Intragastrical application of ethanol extract of leaves at a dose of 1 g/kg and 60 mg/animal, respectively, produced weak activity against alloxan-induced hyperglycemia (Arruzazabala et al. 1994). Further, intraperitoneal application of juice of dried stems exhibited hypoglycemic activity at a dose of 200 mg/kg (Takahashi et al. 1985).