Krishan K. Verma · Xiu-Peng Song ·
Vishnu D. Rajput · Sushil Solomon ·
Yang-Rui Li · Govind P. Rao *Editors*

Agro-industrial Perspectives on Sugarcane Production under Environmental Stress

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Preface

The sugarcane crop is a major source of sweetener-sucrose with an annual value of over US$150 billion. Currently, the global production of sugar exceeds 180 million metric tons, and exports during the last decade averaged around 58 million metric tons. Besides sugar, sugarcane is utilized as a raw material for producing bio-ethanol, an alternate source of renewable energy. The fibrous by-product bagasse is used to produce bioelectricity, and press mud from processing is used in producing Bio-CNG. There are many industries that are supported by the sugarcane crop and sugar industry through diversification and utilization of its by-products and co-products. The sugar industry worldwide has experienced impressive leap in production, productivity, and diversification based on spectacular technological progress. Furthermore, with the advancement in molecular genomics, the sugarcane genome has become less mysterious. However, its complexity has been unraveled to a great extent, which may be helpful in improving its physiological efficiency and biorefinery-derived platform chemicals. Sugarcane is a complex polyploidy crop, and hence no single technique is the best for the confirmation of polygenic and phenotypic characteristics.

To better understand the application of basic omics in sugarcane regarding agronomic characters and industrial quality traits as well as responses to diverse biotic and abiotic stresses, it is important to explore the physiology, genome structure, functional integrity, and colinearity of sugarcane with other more or less similar crops/plants. Moving towards sugarcane omics, remarkable success has been achieved in gene transfer from a wide variety of plant and non-plant sources to sugarcane, accessibility of efficient transformation systems, selectable marker genes, and genetic engineering gears. Genetic engineering techniques make it possible to clone and characterize functional genes and improve commercially important traits in elite sugarcane clones, leading to the development of an ideal cultivar. However, there are limitations due to its complex genomic nature, low fertility ratio, longer production cycle, and susceptibility to several biotic and abiotic stresses.

Recent omics research in sugarcane, which encompasses genomics, transcriptomics, proteomics, and metabolomics, could be useful to achieve higher cane yields and sucrose content and biotic and abiotic stress tolerance, as well as to understand their genetic regulation and mechanisms better. A great amount of new information has been generated regarding the molecular mechanisms of sugarcane

resistance and tolerance to unfavorable environmental conditions, especially intrinsic protective mechanisms against biotic and abiotic stresses.

Written by some of the foremost experts, this book describes recent developments that support the continued use and improvement of sugarcane as a source of biomass, food, and energy. It contains detailed information on sustainable sugarcane cultivation, management of sugarcane production, and biotechnological approaches directed towards higher biomass and sugar productivity per unit area under normal and stressful environment. This compendium will be valuable to the sugarcane organizations, industry professionals, scientists, researchers, and agricultural sciences students of developing sugar-producing countries.

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Rostov-on-Don, Russia — Vishnu D. Rajput
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About the Editors

Krishan K. Verma is working as Foreign Expert at Sugarcane Research Institute, Chinese Academy of Agricultural Sciences and Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, China. His research focuses on environmental toxicology, bio-engineering modeling, plant physiology, molecular biology, their impacts on growth, physiological and molecular adaptation strategies of plants to stressful environment, and how they affect the plant structure. He has published more than 78 scientific research articles/reviews, 10 books, and 10 book chapters. He is an internationally recognized reviewer. He has been serving as an editorial board member of various peer-reviewed journals.

Xiu-Peng Song is working as associate professor (assoc. prof.) at Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, China. His ongoing research focuses on physiology and biochemistry of stress resistance, occurrence mechanisms, and comprehensive control of diseases and insect pests in sugarcane crop. He received his master's and doctorate (biochemistry and molecular biology) from Guangxi University, Nanning, Guangxi, China. He presided over four scientific research projects such as the National Natural Science Foundation China and participated in 12 national/international cooperation projects. He has also received five prestigious awards, such as Guangxi Science and Technology Progress Award. He has published more than 50 scientific publications.

Vishnu D. Rajput is working as an assoc. prof. (leading researcher), head of "Soil Heal" lab, at Sothern Federal University, Russia. His ongoing research is based on soil contaminations, i.e., potentially toxic elements and metallic nanoparticles, and investigating the bioaccumulation, bio/geo-transformations, uptake, translocation, and toxic effects of NPs on plant physiology, morphology, anatomy, the ultrastructure of cellular and subcellular organelles, cytomorphometric modifications, and DNA damage. He comprehensively detailed the state of research in environmental science in regard to "how nanoparticles/heavy metals interact with plants, soil, microbial community, and the larger environment." He has published (total of 346 scientific publications) 201 peer-reviewed highly rated full-length articles, 18 books, 70 chapters (Scopus indexed), and 44 conference articles. He is an internationally recognized reviewer (peer-reviewed 208 manuscripts for internationally reputed journals) and received an outstanding reviewing certificate by Elsevier and Springer. He is an editorial board member of various high-impacted journals.

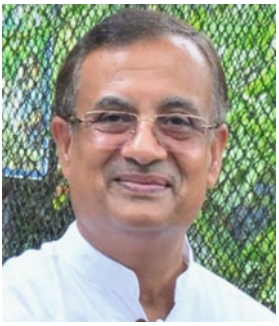
Sushil Solomon received his doctorate degree from Punjab Agricultural University, Ludhiana (India), and joined Agricultural Research Service of Indian Council of Agricultural Research (ICAR-Ministry of Agriculture) in 1977. As a director of the Indian Institute of Sugarcane Research, Lucknow, he was actively involved in the development and transfer of relevant technologies to the sugarcane farmers and industry for their sustainable development. During his 36 years of research career, he has published over 120 research papers, 22 books, and many technical reports for the benefit of global sugar industry. Dr. Solomon is the president of *Society for Sugar Research and Promotion* and vice-president of the *International Association of Professionals in Sugar and Integrated Industries* (IAPSIT) and on the advisory bodies of many national and international apex organizations. As an advisor, he has visited Brazil, Australia, China, Vietnam, Egypt, Iran, Sri Lanka, Cuba, Thailand, etc. He is editor in chief of an international journal—*Sugar Tech*, published

by Springer and has also organized many international conferences.

The Government of China bestowed on him the most prestigious honor "*Friendship Award*" in 2005 in view of his active role in promoting collaboration and partnership among sugar-producing countries. Besides, he is a recipient of many international honors and awards, including *Award of Excellence*—IAPSIT (2006), *Sinai University Peace Award*—Al Arish University, Egypt (2008), *Global Award of Excellence*—IAPSIT (2008), *Indira Gandhi Award* (2013), *Noel Deerr Gold Medal*—STAI (2014, 2016), and *Leadership Excellence Award* (2018) from Thailand Society of Sugarcane and Sugar Technologists. Dr. Solomon was appointed Vice-Chancellor of Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, for a period of 3 years in December 2016, a premier agricultural university in north India.

Yang-Rui Li (life-time professor) graduated and earned B.A. degree in the Department of Agronomy, Guangxi Agricultural University, in January 1982 and M.S. and Ph.D. degrees in the Department of Agronomy, Fujian Agricultural University, in July 1985 and January 1988, respectively. He was employed as a lecturer in the Department of Agronomy, Guangxi Agricultural University, from January 1988 to December 1989, associate professor (from March 1991 to November 1992) and professor (since December 1992) in the Department of Agronomy, Guangxi Agricultural University, vice-president of Guangxi University from April 1997 to April 1998, and president of Guangxi Academy of Agricultural Sciences from May 1998 to November 2012. He has been serving as the director of Sugarcane Research Center, Chinese Academy of Agricultural Sciences, since September 2007 and chief expert of the National Joint Research Program for Elite Sugarcane Variety Development in China since 2018. He is the president of International Association for Professionals of Sugar and Integrated Technologies (IAPSIT), vice-president of Society for Sugar Research and Promotion (SSRP), and president of Chinese

Sugarcane Industry Association for Technological Innovation (CSIATI). His research interests include sugarcane physiology, biochemistry and molecular biology, sugarcane genetics, breeding and cultivation, and chemical regulation of growth and development of sugarcane. He has published 13 books and more than 1000 papers and received 22 scientific research achievement awards from Chinese government to-date and 14 awards from international organizations, including *Lifetime Achievement Award*—SSRP (2011), *Lifetime Achievement Award*—IAPSIT (2014), and *Leadership Excellence Award* from Thailand Society of Sugarcane and Sugar Technologists (2018).



Govind P. Rao is working as Director, Institute of Agriculture & Natural Sciences, DDU Gorakhpur University, Gorakhpur, India. Earlier, he retired as principal scientist (plant pathology) at Indian Agricultural Research Institute, New Delhi, India in January 2022. He obtained his Ph.D. degree in botany (plant virology) from Gorakhpur University, India, in 1986. He did Post Doc at the University of Urbana-Champaign, Illinois, USA, with Prof. R.E. Ford in 1994 on sugarcane and maize viruses. Dr. Rao has 33 years of research experience in plant pathology, especially on plant virology and phytoplasmas. He did significant contributions in characterization of plant viruses and phytoplasmas infecting sugarcane, vegetables, ornamentals, fruits, wheat, maize, cucurbits, maize, and sorghum. He has published over 200 research publications and authored and edited nearly 10 textbooks and 16 edited books to his credit. Over 20 students have been awarded Ph.D. degree under Dr. Rao's supervision. He has been awarded several prestigious national and international awards to his credit. Dr. Rao has been presidents, secretary general, and secretary of various renowned academic societies of India and abroad. He has also been elected as fellows of several academic societies in India and abroad. He is also editor in chief of Sugar Tech journal, published from Springer-Nature. Besides, Dr. Rao has visited over 30 countries as visiting scientists, for invited talk, postdoc fellow, research training, panel discussion, and for attending workshops and conferences.

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1 Growth and Development of Sugarcane (*Saccharum* spp. Hybrid) and Its Relationship with Environmental Factors

Yang-Rui Li

Abstract

The whole duration of sugarcane (*Saccharum* spp. hybrid) production and development are usually divided into four stages such as germination, tillering, elongation, and maturation. Sugarcane growth and development are closely related to environmental factors such as temperature, sunshine, water, air, and nutrients. For commercial sugarcane production, drought, waterlogging, and frost often severely reduced cane yield. Appropriate field management such as fertilization, irrigation, drainage, and weeding at the early growth stage is very important for the yield by ensuring the rational number of plants through good germination and tillering regulation. Water supply, warm weather, and intense sunshine are also important for the elongation stage. During the processing maturation stage, cool and sunny weather with high temperature differences between day and night is beneficial to sugar accumulation in sugarcane.

Keywords

Development · Growth · Environmental factors · Sugarcane · *Saccharum* spp. hybrid

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1.1 Introduction

Sugarcane (*Saccharum* spp. hybrid) is an important member of the grass family Poaceae (Gramineae), subfamily Panicoideae, super tribe Andropogoneae, sub-tribe Saccharinae, and genus *Saccharum* (Watson et al. 1985). Sugarcane planting areas in the world are mainly distributed between the 33rd parallels of north and latitudes but focus between 25th parallels of north and latitude. Based on temperature, the sugarcane planting areas are located in places with an annual mean temperature of 17–18 °C or higher. The altitude of the sugarcane planting area reaches 1500–1600 m in Yunnan Province, China (Li 2010).

Sugarcane is a C_4 ratooning crop and well-cultivated commercially in at least 106 countries of tropical and subtropical areas, which requests hot and humid environments for growth and development (Li 2010; Verma et al. 2021a, b). Sugarcane accumulates high sucrose content in cane (Bonnett and Henry 2011; Cheavegatti-Gianotto et al. 2011). Sugarcane has significant capability for sucrose accumulation in stalks, and sucrose concentration can be as high as 0.7 M in ripen internodes (Moore 1995). Sucrose is synthesized by the carbohydrates from photosynthesis in green leaves of sugarcane plants and then transferred to sink organs, including consuming and storage sinks. In consuming sinks, sucrose is hydrolyzed to produce energy for growing roots, stems, and flowers while translocated to accumulation sink through phloem for storage purposes (Li 2010).

In a sugarcane production cycle, the first planting crop is named as plant crop, and the subsequent crop is called ratoon crop. In plant crop, from planting to harvesting, the growth and development of sugarcane plants include germination stage, seedling stage, tillering stage, grand growth stage, and maturity and ripening stage (Li 2010). Although sugarcane can produce seeds, stalk cuttings or setts are generally used in commercial production. For the breeding purpose, we need to produce sugarcane seeds. As sugarcane seeds are very tiny, whole fuzz is harvested for seed planting (Li 2010).

1.2 Germination Stage

The germination stage is from planting to the accomplishment of germination of buds and root points of seed cane setts. Under the field conditions of commercial production, the time for germination varies greatly from 7 to 110 days, depending on the environmental temperature. In sugarcane, germination implies activation and subsequent sprouting of the vegetative bud and dormant roots on the node. The germination is affected by the external as well as internal factors. The internal factors are bud health and moisture, reducing sugar content, and nutrient status in the sett. The external factors are the soil moisture, soil temperature, and aeration (Gravois et al. 2014; Li 2010; Verma et al. 2020a). During germination, a series of physiological and biochemical changes happen inside seed cane setts. The activities of various enzymes such as invertases, amylases, proteases, and oxidases are increasing, and respiration is rising, which converts the stored nutrients into simple

molecules for the growing need of roots, stem, and leaves of the young plants. In general, roots germinate earlier than buds. This stage is crucial to determine the basic plant number, which is the foundation of crop productivity. It is highly important to ensure enough strong plants for achieving high cane yield (Li 2010; Verma et al. 2020b).

1.2.1 Temperature

Temperature affects most sugarcane germination among various environmental factors. The temperatures below 20 °C or above 43.9 °C are not suitable for bud germination. The temperature for initiating bud germination is about 13 °C, good at 25–27 °C, and optimum at 30–32 °C. In a certain range, the germination gets speeding up with the increasing temperature as the enzyme activities and respiration metabolism gets rising. On the contrary, when the temperature decreases, the germination speed slows down. When the soil temperature is above 20 °C, sugarcane germination speeds up, shortens the germination stage, and improves the germination rate. The higher the temperature is, the faster the germination is. However, if the germination speed is too fast, the growth and tillering of the plants are also speeding up, excessive growth may occur, leading to lower tillering rate and weak root development. When the temperature is over 32 °C, the germination is fast, but the seedling quality is low. Over 40 °C, the germination is inhibited. When the temperature is at 13 °C or down, the buds keep dormant. When the temperature is at 0 °C, the germinating buds will be dead; the temperature is at −2 °C, the dormant buds would be destroyed. In some cases, although the temperature is not very low but keeps for long, the young shoots would become very weak and easily suffer from biotic and abiotic stresses, i.e., drought, freezing, diseases, insect pests, flooding, etc. Under a long time of low temperature, all the buds could be dead because of the stress.

The lethal temperature of the top growing point of sugarcane stalk is about −1.5 °C in Southern China, but −2.0 °C to −2.5 °C in Central China. The lateral bud (dormant bud) has stronger chilling resistance than the top growing point, and its lethal temperature is about −3 °C to −5 °C. The germination rate of the chilling injured living buds is decreased dramatically. After frost, the buds kept original healthy color are still normal, those with sugar juice or dark brown color on the surface are dead, and those with light brown color on the surface are chilling injured. The germination test could be used to determine the living state of the buds. Temperature also affects the root germination in seed canes, and the temperature for seed cane root germination is lower than that for bud. In general, the roots start germination at about 10 °C, and the best at 20–30 °C. That is why the roots grow earlier than bud under low-temperature conditions, which is beneficial to resist drought and diseases.

In Southern China, the temperature is high in autumn and spring, planting sugarcane in these seasons germinated fast, and the germination stage is about 15–20 days. The winter (December to February) planted sugarcane generally takes

70–120 days for germination as the average temperature is low to 11–16 °C. The long germination stage is unsuitable for sugarcane because the seed cane setts in soil are vulnerable to drought, diseases, and pests, especially pineapple and smut diseases. The pathogens of these diseases infect the seed cones from the two cut sides, make the tissues get rotten, and necrotize the buds and root points closed both cut ends, leading to low germination rate. That is why seed cane setts with multiple buds plus watering and plastic film mulching are recommended for winter-planted sugarcane (Li et al. 2000). Seed cane soaking, disinfection, artificially accelerating germination and covering with plastic film, and other measures can increase the temperature and moisture, shorten the germination stage, increase the seedling numbers, and strong culture seedlings.

1.2.2 Water

The germination of the seed cane root points requests higher moisture than that of buds. Insufficient water is not suitable for the germination of both roots and buds of seed cane setts. The water content in seed cane affects the hydrolytic enzymes' activities and the metabolism and transportation of organic substances. The water content in fresh seed cane is generally over 70%, basically meeting the requirement of germination and early growth of young plants. When the water content decreases to 50%, the germination rate decreases significantly; when it goes down to 40% or lower, the buds will lose the germinating ability, and it could not recover even by soaking the seed cane in water (Yang and Li 1995; Li 2010; Verma et al. 2019a).

Soil water content greatly affects seed cane germination. The appropriate soil water content for seed cane germination is 20–30% and best 25%. If the soil water content is over 40%, the germination will be inhibited, and the seed cane setts even get rotten under the long time of waterlogging or flooding conditions. So, field drainage is very important for sugarcane production (Li 2010; Li and Yang 2014). Soil drought might cause the water loss from seed canes which adversely affect the germination of buds and root points. Increasing the water content to 75–80% by soaking or keeping the soil moist (equivalent to 60–70% of field moisture keeping capacity) is recommended so that the seed canes can absorb enough water for root germination. When soil water lowers than 5%, the seed cane planted will be getting dry, leading death of most buds and young shoots. Therefore, keeping the soil moist is the key practice when sugarcane is grown in dry seasons of winter or spring. Experiments showed that leaf-removed multiple-bud seed cane setts germinated better than other seed cane treatments under spring drought conditions, which had higher emergence rate, lower dead seedling rate, higher millable stalks, finally achieving higher cane and sugar productivity (Li et al. 2000). In commercial sugarcane production, if buds germinate while seed roots keep dormant or get the day after germination, it indicates soil moisture is insufficient, and moisture supplement is necessary. Otherwise, seed canes would continuously lose water, leading to bud germination failure or death of germinated buds because of drought stress. Too

much soil water is also not good for seed cane germination, and rotten roots and dead buds would occur because of lacking oxygen.

Wang et al. (2008a, b, c) soaked sugarcane seed cane setts with three levels of ethephon (0, 100, 200 ppm) for 10 min before planting for three sugarcane varieties, ROC10, ROC22, GT17. The results showed that, under drought conditions, the plants treated with 100 and 200 mg/L ethephon had higher contents of protein and nucleic acid, the varieties GT17 and ROC10 showed lower protease activity than ROC22, the varieties ROC22 and ROC10 had higher RNA/DNA ratio in roots at 4–5 leaves stage, and the effects were higher in the treatment with 100 mg/L ethephon than in that with 200 mg/L ethephon (Wang et al. 2008a). Water stress led to the considerable amount of accumulation of spermine (Spm), spermidine (Spd), and putrescine (Put) in roots, and the varieties ROC22 and ROC10 accumulated higher polyamine content than GT17. However, they all showed lower polyamine oxidase activity in the treatments with 100 and 200 mg/L ethephon. Meanwhile, the treatments with 100 and 200 mg/L ethephon abbreviated the water potential decrease level in leaves under water stress, and the latter performed better (Wang et al. 2008b). Under water stress, the treatments with 100 and 200 ppm ethephon improved the carotenoid content in leaves of GT17; abbreviated the decreasing of chlorophyll, decreased the stomatal conductance and transpiration rate, and promoted the net photosynthesis in leaves of GT17 and ROC10; promoted the tillering bud formation in the tested three varieties, and the effect was statistically significant in ROC22 and ROC10. These results indicated that ethephon soaking seed cane could improve the drought resistance of sugarcane.

1.2.3 Air

The germination of roots and buds requests good air condition. Under good air conditions, the nutrient inversion inside seed canes acts fast, releasing enough energy and simple organic molecules to ensure normal germination and young shoot growth. In general, upland fields have good air condition, the seed roots can germinate and grow normally except the sugarcane plated in heavy clay soil, or the recovered soil is too thick, or the drainage is poor, which leads to poor air and lacking oxygen condition to inhibit the germination and seedling growth. In sugarcane production, it is important to apply deep tillage and losing soils to create good air condition and keep away from waterlogging after planting and break soil compaction after raining to improve the air condition.

1.3 Seedling Stage

This stage covers the duration from 10% emerged shoots having first true leaf to 50% seedlings having fifth true leaf. The seedling stage is the preparation time for tillering. At this stage, no plant stalk elongates, but leaf number continuously increases, and leaf area keeps enlarging; underground plants roots develop and

play roles together with seed roots, so the absorption ability gets stronger; the growth and development of roots and leaves depend on each other; the growth of seedlings becomes utterly independent from supporting by the nutrients from seed cane.

After emergence, the young shoots grow leaf sheath without blade at first, followed by a small complete leaf, the first true leaf. Since then, the following leaves have become larger and larger. When the plants have 3–4 true leaves, roots are generated from the basal node of seedlings, called plant roots stronger than seed roots. When the seedlings have 3–4 leaves, the underground parts start to generate tillering buds. If the plants grow well, they will produce more tillering buds, and oppositely, they will tiller late and less. Ensuring enough number of strong seedlings is the basis of high yield in sugarcane production (Li 2010).

Temperature, moisture, and soil nutrition are the major factors affecting the growth and development of sugarcane seedlings.

1.3.1 Temperature, Moisture, and Nutrition

The starting temperature for seedling growth is 15 °C, and the optimum is about 25 °C. In winter and spring, the seedlings grow slowly because of the relatively low temperature. Entering March and April, the ambient air temperature rises fast, and the seedlings grow faster. In early spring, the air temperature rises faster than the soil temperature, we can promote the seedling growth by appropriate control of moisture and intertillage.

The water requirement is not much at the seedling stage, and it will be good to keep 60% of the soil moisture keeping ability. If the soil water is too much, the air condition is bad, and soil temperature increases slowly, which is not suitable for the growth and development of seedling roots. Intertillage is the common practice to improve the condition for seedling growth and development. If the soil moisture is insufficient, irrigation is strongly recommended to avoid the seedling suffering from drought damage. The nutrients for the early growth of the seedling mainly come from the seed cane. The nutrition requirement of the seedling is less than 1% of the total for its whole growth duration, but it is the critical stage for sugarcane growth. The plant growth is sensitive to fertilization at this stage, and fertilization is usually carried out at the 3–4 leaves stage if the soil nutrition is poor.

1.4 Tillering Stage

This stage covers the duration from starting to ending of tillering when the plant grows less than 3 cm every 10 days, from 10% seedlings having tillers to the beginning of elongation. It is the key stage for ensuring the suitable number of stalks essential for a good yield. The lateral buds on the stem base start germinating when the mother plants have 3–4 true leaves, and the first tiller emerges at the 7–8th leaves stage and the second at the 8–9 leaves stage. The tillering reaches the peak at

the 10th leaf stage. The stalk elongation starts after the 12th leaf stage. The late tillering usually could not produce millable stalks, so it should be inhibited.

The tillering ability is closely related to variety, cultivation, and environmental conditions. The main environmental factors affecting tillering include temperature, sunshine, soil moisture, and nutrients.

1.4.1 Temperature, Sunshine, Soil Moisture, and Nutrients

Both air and soil temperatures significantly affect the tillering. The minimum air temperature for tillering is 20 °C, and the optimum is 30 °C. The practices such as plastic film mulching, sallow covered soil layer above seed cane, weeding, intertillage culture operation can promote tillering. However, if the temperature is too high, the tillering will be inhibited. Intense and long sunshine time is beneficial to the generation and growth of tillers because of increasing the air and soil temperature, which would improve the photosynthetic ability, increase organic nutrients, break the hormone balance inside sugarcane, and abbreviate the inhibition of some hormones (mainly auxin, that is, indel acetic acid, IAA) on the lateral buds on the stalk base. Appropriate plant density and weeding should be applied to provide good sunshine to the plants.

The status of soil moisture and nutrient conditions significantly affects the tillering. Sufficient soil water and nutrients promote early and rich tillering. Nitrogen (N), phosphorous (P), and potassium (K) are important to tillering, especially N and P. Insufficient soil sulfur (S), calcium (Ca), magnesium (Mg), and other micronutrients also delay and reduce the tillering. Drought or waterlogging also inhibits the generation and growth of the tillers. It is good for tillering to keep 70% of the moisture keeping compacity in the sugarcane field (Li 2010).

1.5 Elongation Stage

It is also called the grand growth stage, and it is the booming stage for sugarcane growth. This stage starts from the beginning to the end of fast stalk elongation. It is the most important stage for cane productivity formation, which duration is closely related to environmental conditions.

1.5.1 Temperature and Water

The stalk elongation likes warm and strong sunshine. The optimum temperature is about 30 °C, and the elongation is slow at 20 °C and stops below 10 °C. Water condition is highly important to sugarcane stalk elongation. The crop consumes about 50–60% of the total water required for whole life. Drought will greatly reduce the stalk growth, shorten the internode length, and finally decline the cane yield. In upland fields of Southern China, drought occurs frequently, so water management

determines the sugarcane productivity. Irrigation is very important when drought comes. In the fields without irrigation conditions, deep tillage and losing soils, sealing the ditch to store water, and mulching soil surface with the trash are the common practices for field management (Li et al. 2016; Li 2019, 2020). Sufficient water can also ensure the nutrient absorption of the plants from the soil and promote nitrogen-fixing activity inside the plants (Li et al. 2016; Li 2019, 2020).

1.6 Maturation Stage

Sugarcane maturation includes two different concepts: processing maturation and physiological maturation.

1.6.1 Processing Maturation

In general, the maturation stage means the processing maturation stage, also called the sugar accumulation stage, which starts in November in subtropical Southern China (Li 2010). During this stage, rapid sugar accumulation happens, and vegetative growth is reduced. As ripening progresses, simple sugars such as fructose glucose are converted into sucrose. Cane ripening starts from bottom to top, and therefore bottom part of the cane contains more sugars than the top portions at the early maturation stage. Sugarcane stalks account for about 75% at the harvest stage, while the leaves and tops around 25% of the total aboveground dry biomass (Li 2010). Different sugarcane varieties have different sucrose content in cane and different maturation times.

The main environmental factors affecting sugar accumulation in sugarcane are temperature and water.

1. **Temperature**
   The processing maturation requests cool temperature relatively big temperature difference between day and night. The best temperature for sugar accumulation is 13–18 °C on average for the day and 5–7 °C for the night, with about 10 °C difference between day and night. High temperature is good for growth but not suitable for sugar accumulation in sugarcane. The environment with relatively low temperature, dry air, and the big temperature difference is beneficial to ripening and accumulating sugar in the stalks of sugarcane during the late growth stage. On the contrary, wet and warm environment with the slight temperature difference between day and night is not good for sugar accumulation in sugarcane.
2. **Water**
   Rainy and wet environment is beneficial to plant growth but leads to late ripening and low sugar content. In Southern China, the rainfall in September and October is closely related to cane sucrose content in November. If the rainfall is low in September and October, the sucrose content in cane will be high in November,

and frequent raining will decrease the sucrose content in cane. So irrigation should be reduced since late September and stopped in a month before harvest. But over drought also adversely affects the sugar accumulation and leads to high colloid content in cane juice (Li 2010; Yang et al. 1998; Verma et al. 2019b). Under drought conditions, appropriate irrigation is strongly recommended to improve yield and sucrose content in cane.

3. **Nutrients**
   Over and/or late application of N fertilizer will decrease sucrose content in cane. In Guangxi, China, stopping the application of N fertilizer in May recorded the highest sucrose content in cane and sugar productivity (Ye et al. 1993). For different sugarcane varieties, those with lower N content in leaves, especially at the late growth stage, mature earlier and have higher sucrose content in cane (Li et al. 1992). Phosphorus and potassium supplements at the late growth stage are beneficial to sugar accumulation in sugarcane. Experiments showed that foliar spray of limewater and KH_2PO_4 increased the activities of Mg^{2+}-ATPase, Ca^{2+}-ATPase, NADP-malic enzyme and neutral invertase and the contents of sucrose and water in leaves and improved the sucrose content in cane while reduced the reducing sugar content in juice and increased cane yield in plant cane (Li and Yang 1994). Similar results were obtained in ratoon cane (Yang et al. 1998).

1.7 Physiological Maturation

Sugarcane plants will flow and seed under the comprehensive effect of appropriate light, temperature, and water, reaching physiological maturation. Flowering is necessary for sugarcane hybrid breeding. Sugarcane is easy to flower in lower latitude areas in the tropics, with low temperature and humidity and is not suitable for sugarcane flowering and seedling. It is common to increase the air temperature and humidity in the greenhouse (Li 2010).

1.8 Conclusion

Due to the good economic return to the growers, the area and productivity of sugarcane have constantly been rising over the last few years. Sugarcane is a warm-temperate and (semi) arid crop that grows in a warm, sunshine, and wet environment, as well as fertile, deep, and well-aerated soils. Climate variables influence the crop cycle, development, and ripening: precipitation and temperature stimulate growth, whereas dry, sunny days, and low night temperatures support developmental processes and sugar accumulation. Cold and storms or typhoons can damage the crop. In temperate regions, new modern varieties have been explored which are adapted to a shorter growth cycle.

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Impact of Climate Change on Sucrose Synthesis in Sugarcane Varieties

2

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Abstract

Sugarcane is an economically important crop, and the impact of climate change can be manifested much more in all stages like germination, tillering, grand growth, and maturity phases. Cane yield and sucrose content are the two principal traits determining commercial cane yield of sugarcane genotypes. Sucrose accumulation in sugarcane stalks is known as ripening, which is influenced by ambient air temperature and sheath moisture index of sugarcane genotypes. Early ripening genotypes are photosynthetically efficient and complete the vegetative developmental phase much faster than the mid-late cultivars by their synchronized tillering phase and low ratio of acid and neutral invertases. Prolonged lower air temperature during the maturity phase before harvest favors sucrose synthesis in sugarcane genotypes due to decreased concentration of acid invertase enzymes in stalks. The average daily temperature of 12–14 °C would be more desirable for proper ripening. However, a drastic decline in temperature below 8 °C during ripening alters the activities of sucrose synthesizing and hydrolyzing enzymes resulting in a sharp decline in sugar recovery. The impact of changing temperature regimes on sucrose accumulation emphasizes future research initiatives to develop improved models that can record the crop physiological processes that will simulate crop response to predicted changes in climate. Modeling approaches predicted that increased sucrose yield could be achieved when the decrease in stalk dry mass is not more than 10%. Impact assessment using CANEGRO model to study the effect of various combinations of temperature and CO_2 projected an enhance in fresh stalk biomass and a decrease in sucrose mass by nearly 10–70% (rainfed) and 6–37% (irrigated) in 2040–2060 compared to 1971–2000 across the agro-climatic areas in India. Therefore,

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detailed studies are required in the future to demonstrate the causes of changes in the behavior of commercial varieties and the effect of climatic variables on the enzyme balance that regulates vegetative growth and ripening.

Keywords

Ambient air temperature · Precipitation · Sucrose · Simulation models · *Saccharum* spp. · Water deficit

2.1 Introduction

Sugarcane agriculture will be adversely affected by climate change, but most significantly, changes in rainfall patterns and rainfall distribution will have a pronounced effect on overall productivity. Global appraisal reports on the impact of climate change have forecasted a decline in agricultural production (Lobell et al. 2008; Verma et al. 2021a, b). Elevated temperature will reduce crop duration by inducing early flowering and lowering the yield per unit area. The major hindrance to crop productivity in near future will be abiotic stresses like waterlogging, drought, tropical cyclones, soil moisture deficit, salinity, alkalinity, increase in temperature, water stress, etc. in more extensive areas prone to high climatic disorders (Dhillon and von Wuehlisch 2013). Stalk yield and juice sucrose content are the two essential traits determining the sugarcane genotypes' commercial cane sugar yield. Despite the crop adaptation to the conditions of high light intensity, increase in temperatures, and water deficit during the crop growth and development, significant reduction in stalk and sugar yield was observed (Neto et al. 2006; Verma et al. 2021c, d). The ability to store higher sucrose in its stalks at modest levels of water stress and low temperature has been demonstrated by earlier researchers (Van Dillewijn 1952; Alexander 1973; Clements 1980). Attempts to improve sucrose content through conventional approaches are time-consuming due to the long breeding cycle of the sugarcane crop. However, molecular techniques have made substantial efforts to enhance the upper limit of sucrose content (Groenewald and Botha 2008).

Weather plays a vital role in all growth stages of this crop; more importantly, the maturity and ripening phase requires a warm climate, clear sky with no rainfall. As a C_4 crop, elevated temperature coupled with water stress, waterlogging, or low temperature may significantly and adversely affect cane yield and sugar recovery. For most developing countries in semi-arid, arid, and tropical zones, yield levels are expected to drop considerably due to changes in total precipitation coupled with extremely high-temperature events (Srivastava and Rai 2012; Verma et al. 2020a). Although there have been several studies in the past, the emerging scenario concerning sugarcane agriculture with climate change remains speculative. With increased temperature and more sunshine hours, the photosynthetic efficiency and productivity in cooler regions may improve but can adversely affect sucrose accumulation. Recent impact study of changing climate scenario on sugarcane production has emphasized the development of heat resistant genotypes to adapt to the

future warming world (Pipitpukdee et al. 2020). In this context of changing climate, understanding the physiological basis of sucrose synthesis from the source (leaves) to the sink (the storage tissues in the stalks) is vital to re-orient breeding approaches in sugarcane to achieve maximum sugar productivity per unit area. The different stages of crop growth like germination, tillering, grand growth, and maturity phases are vulnerable to the impacts of climate change which is detrimental to the overall productivity of the crop. However, the crop is highly resilient, and the extensive genetic variability in terms of adaptation present in the varieties and germplasm offers scope for mitigating the effects of climate change through a varietal approach.

2.2 Sucrose Accumulation in Sugarcane

Sucrose synthesis and accumulation in sugarcane is a complex process, and it involves a massive network of gene interactions at various levels of the organization. Sucrose is primarily synthesized in leaves through photosynthesis. It is transported to stalk through the phloem, where it is stored or converted to hexoses (glucose and fructose) for further growth. It involves the transport of sucrose against the concentration gradient (Silva and Caputo 2012). The transported sucrose is stored in the vacuole of the cell. Through the interconversion to hexoses, the sucrose leaves the vacuole to the cytoplasm, where it gets utilized. If further growth occurs, the newly formed leaves act as a factory to produce more sucrose while the stem acts as a reservoir. Hence, the grand growth stage of sugarcane can be referred to as the critical stage of sucrose accumulation as it sets the balance among vegetative growth, sucrose synthesis, and accumulation (Bull 2000).

If the converted glucose and fructose are not utilized, they are re-converted to sucrose and stored in the stalk during maturation phase (Whittaker and Botha 1997). This is mainly because of carbon cycling between sucrose and hexoses due to reduced vegetative growth during that period. The sucrose synthesis and accumulation also depend on the cultivar, their maturity, nutrient availability, flowering, and meteorological parameters. Sucrose accumulation starts first in the basal internodes and proceeds to the apex gradually until they attain a common value. That is why the basal internodes have higher sucrose than immature and top internodes during crop growth. In contrast, younger internodes are high in hexoses and cane fiber. One of the important indices to judge ripening in sugarcane is the ratio of Brix in the top and bottom internodes approaching unity, indicating uniform maturity across all internodes of the cane.

Among crop cultivars with different maturity, early varieties tend to have more sucrose accumulation capacity as compared to mid-late varieties. This is because of their higher photosynthetic efficiency, as they utilize photosynthetic products more efficiently and complete their vegetative development as compared to mid-late varieties (Mamet and Galwey 1999; Verma et al. 2020b). Group of invertase enzymes manage the sugarcane ripening (Glasziou and Waldron 1964; Hatch and Glasziou 1963); where maximum levels of invertase acid (pH 5.1) and low levels of neutral invertase (pH 7) are linked with rapid vegetative growth and development. In

contrast, the reverse pattern is regulated with ripening (Alexander 1973). Plant growth regulators significantly alter acid and neutral invertases (Leite et al. 2009). Flowering and its intensity also affect sucrose accumulation and ripening (Silva and Caputo 2012). The flowering process is mainly characterized by loss in sucrose level, enhance fiber (%), and the formation of pith. All the factors described above, in turn, depend on each other and weather parameters. Hence, the interaction of these parameters with the corresponding climate dictates the process of sucrose accumulation and storage in the cane. These factors are more cumulative over the long period as their influence on the physiology and metabolism of the plant are not immediate (Cardozo 2012). The juice quality of sugarcane is primarily influenced by weather sequences encountered throughout the year by the crop rather than its age (Prasada-Rao 1997; Srivastava et al. 1995) at the time of harvest (Ram et al. 1973).

2.3 Ambient Air Temperature

Air temperature is most predominant among the environmental factors that affect sucrose concentration in sugarcane. The leaf sheath moisture index and the average ambient air temperature are two of the most crucial variables involved with sugarcane ripening during the last 3 months before harvest (Clements 1962). Temperature plays a significant role in sucrose synthesis and accumulation in sugarcane. Both these variables have an inverse relationship with juice sucrose (%), which indicates that cooler evenings and lower moisture index promote high sucrose content in the cane. The combination of soil moisture and air temperature dictates the pattern of sucrose storage in the stem, with the latter having more influence over the former (Yates 1972). Several researchers described base temperatures for various phenological stages in sugarcane, which may vary according to cultivar and location (Scarpari and Beauclair 2004). A constant base temperature of 8 °C for all sugarcane processes and phenological phases was suggested by O'Callaghan et al. (1994). Cooler the air temperature during the maturing phase, the higher the sucrose content as the acid invertase concentration decreases in the stalks (Ebrahim et al. 1998b). However, this occurs only when and if there is a prolonged lower air temperature over 3–6 months before harvest. Glasziou and Waldron (1964) in their studies also proved that lower air temperatures for about 6 months before harvesting increased sucrose content to 17% from 12%. However, a drastic decline in temperature below 8 °C affects cane production and metabolic effects, which was reported by Singh et al. (1993) and Solomon et al. (1994), and they emphasized that low temperature below 8 °C leads to a drop in sucrose recovery due to inversion induced at cooler temperature. The foliar invertase activity has been found to decline significantly during winter months which plausibly helps in the movement and accumulation of sucrose (Pathak et al. 2019). Scarpari and Beauclair (2004) developed a concept of negative-degree days used to estimate the correlation between ripening and temperature, corresponding to the area between the daily minimum temperature and the base temperature. During favorable growth conditions like high air temperature and soil moisture, acid invertase level is high, and it decreases during unfavorable

conditions like nutritional or water stresses and low air temperature. It might be the result of enhanced sucrose phosphate synthase and neutral invertase activities, which consequently enhance the level of sucrose (Terauchi et al. 2000).

Along with the temperature, humidity also plays an important role in sucrose synthesis and storage. In Indian subtropical conditions, extremely low temperature prevails during the maturing phase of sugarcane, which coupled with high relative humidity has a drastic effect on cane quality and recovery. The low sugar recovery problem in coastal states of India is possibly due to humid and warm climate, which is conducive for vegetative development than sugar accumulation. Previous studies also indicated the inverse relationship between relative humidity and sucrose accumulation (Oertel 1946). Pathak et al. (2019) also observed an increase in sugar recovery in the U.P state despite the rise in the area of early maturing varieties. This was attributed to high relative humidity and low temperature during the crushing period. This study also stated that if humidity increases by more than 5% and the maximum temperature get reduced by 2–3 °C, and the minimum temperature remains the same, then it will certainly reduce the sugar recovery.

2.4 Carbon Dioxide (CO_2)

Greenhouse gases (GHGs) are among the most important causes that contribute to climate change. Carbon dioxide (CO_2) is the most important GHG, which affects the physiology and biochemistry of sugarcane crop. Furthermore, it would result in altered changes in the quantity of sugar produced. The concentration of CO_2, together with temperature, affects the crop growth and productivity of sugarcane. However, this effect is more on sugarcane productivity as compared to juice quality (Misra et al. 2019). Higher CO_2 concentration seems to positively influence sugarcane unlike with other crops (da Silva et al. 2008; Madan et al. 2014). Vu and Allen Jr (2009) have done extensive studies on the effects of elevated CO_2 on quality and production of sugarcane. They reported increased leaf area, juice volume, and leaf and stem dry weight when CO_2 concentration was doubled. These altered morphological attributes would increase photosynthesis and thereby increase sugar accumulation in the cane. A rise in temperature along with doubling of CO_2 showed an enhancement in plant dry mass, leaf area-expansion-development, and stalk juice volume by 84, 26, 50, and 124%, respectively, as compared to the cane grown at ambient temperature and CO_2 (Vu and Allen Jr 2009).

A general increase in total biomass was observed in the crop under elevated CO_2 conditions. Further, two to threefold rise in stem soluble solids was noticed by Vu and Allen Jr (2009) under the combination of high temperature and double carbon dioxide concentration, which also leads to an increase in stem diameter. Similarly, Madan et al. (2014) reported a 24% increase in fresh weight of cane stalk and fresh juice yield when CO_2 concentration was doubled from 350 ppm. This increase in juice volume and extraction % would enhance sugar recovery from the unit of cane crushed. This might be due to an increase in cell elongation (Pritchard et al. 1999) and increased photosynthesis, leading to rising XTH (Xyloglucan

endotransglucosylase/hydrolases) expression that results in more synthesis and accumulation of sugars in sugarcane. These changes ultimately enhance the sugar accumulation and improve sugar recovery under enhanced CO_2 conditions.

2.5 Soil Moisture

Soil moisture is one more factor that affects sugarcane ripening besides air temperature. Sugarcane is a water-loving crop that consumes nearly 2000 mm water on average, and this requirement increases in dry atmosphere and heavy water demand periods. Most of the water in a crop cycle is utilized during tillering and grand growth, and these are considered as critical stages of water requirement (Ramesh 2000; Verma et al. 2020a, 2021a). If the crop experiences water stress during this period, yield is drastically affected primarily because of the reduction in internode length. Less soil moisture is preferable during the maturation phase as the vegetative growth needs to be slowed down, which spares the energy for sucrose synthesis, transport, and storage. At the time of crop harvest, drought stress occurs, the level of sucrose may enhance up to 15%, with an average of 8% (Robertson and Donaldson 1998; Verma et al. 2019a, b).

In the majority of the sugarcane growing regions, the water deficit starts in May and reaches its highest in the month of September. As a result, water deficiency is closely related to sugar content, which generally increases between in the month of August and October (Cardozo et al. 2014). Biomass accumulation is severely affected when the drought is more than 120 mm, whereas, for sucrose accumulation, the value is 130 mm (Inman-Bamber 2004). However, the ideal water deficit is not defined as it depends on cumulative evapotranspiration, the specific location, and crop phenological stage (Scarpari and Beauclair 2004). They also stated that the rate of stalk elongation during the revival in plants subjected to stress is 1.6 fold compared to control plants. Boyce (1969) stated that drying off resulted in decrease in crop productivity, which, accompanied by rise in sucrose content on the basis of fresh biomass, sugar productivity similar to without stressed plants. A perfect trade-off is required between the increase in sucrose concentration and decrease in the total biomass to avoid loss in sugar yield in total. In countries like Australia, where the price is based on sucrose produced per hectare, imposing water stress during maturity by withholding irrigation would save costs for irrigation and increase sucrose content in the stalk. The exact calculations do not hold good in a ratoon crop, where the entire crop cycle is 11 months from the harvest. The precise time of drying off in ratoon crops depends on the harvest time, and number of ratoons the crop is subjected to elevations in sucrose fresh weight (FW) content under drying off occur due to modifications in the components of sucrose dry weight (DW) content and cane dry mass (Robertson and Donaldson 1998). Cardozo (2012) noted a high and positive correlation (0.95) of water stress at maturity with Brix, pol, and purity (%), while the increased negative correlation with rainfall accumulation at 120 days before harvest.

2.6 Sunlight, Photoperiod, and Flowering

These two factors act independently and in unison to control the flowering and sucrose accumulation in sugarcane. Cardozo (2012) studied the relationships between net radiation (NR), solar radiation (SR), photoperiod (N), and ripening patterns of few sugarcane cultivars. Other observations included inverse relationships between SR, NR, N, and quality parameters, i.e., total solid content present in the juice (Brix), pol and total recoverable sugar (TRS) when these traits were 3–5 months prior sampling. The radiation of solar was directly associated with the ripening of sugarcane than ambient air temperature and precipitation (Legendre 1975). At regions nearer to the equator, changes in air temperature are less and hence may not have much effect on sugarcane ripening, but in mid-latitudes, the photoperiod might be short, especially during winter months which affects photosynthetic duration and efficiency.

Flowering in sugarcane is one of the detrimental factors for sucrose accumulation in sugarcane. Flowering reduces the sucrose content in the stalks as these reserves would be used for panicle formation and its subsequent emergence. Moreover, flowering is characterized by the formation of pith and by drying the interior of the stalk from the apex. This gradually increases the fiber content in stalks and reduces the volume of juice. A wide range of environmental conditions influences this phenomenon. Araldi et al. (2010) demonstrated that the variables that influence flowering are the sensitivity of the variety to flowering, photoperiod and light density, temperature (less temperature changes may cause significant variables in flowering), minimum plant age (cultivars/genotypes that are very sensitive to flowering can be induced at 180 days), chemical products, such as different hormonal chemical products reduce flowering, which is more practical interest, humidity and cloudy days support flowering, which is less similar in summer, dry areas, altitude (lower altitudes support flowering), and fertilization approaches (more N may hinder or protect flowering).

All the above factors influence flowering, which in turn affects the sucrose storage and accumulation in sugarcane. Sugarcane flowers in short days with optimal photoperiod less than 12.5 h. In the northern hemisphere, where India is located, flowering induction starts in July to August, and flower initiation occurs from September to November. In the southern hemisphere, these factors arise between February to April and September to November.

2.7 Sugar Recovery in Relation to Climate Change

Sugarcane grows in two distinct agro-climatic zones globally, the tropical and the subtropical, between 0–10 and 10–30 latitudes, respectively. Sugarcane is grown in areas with extreme differences in temperature, rainfall, and type of soil. It is one of the reasons for getting differences in sugar recovery and cane yield in different cane growing areas of the country. Among the sugarcane cultivating countries, the maximum recovery of sugar (14%) is obtained in Queensland, Australia. The

recovery of sugar in other important cane-producing countries, i.e., Brazil, India, South Africa, USA (Hawaii, Louisiana, and Florida), Mauritius, Cuba, Puerto-Rico, and Pakistan differs from 9 to 11%. In India, the cane is grown in tropical and subtropical regions such as Maharashtra, Tamil Nadu, Gujarat, Karnataka, and Andhra Pradesh which are the main cane cultivating states in arid regions. Sugar recovery is maximum in Maharashtra, Gujarat, and Karnataka than Tamil Nadu and Andhra Pradesh in the tropics.

In the North Indian cane growing states of Uttar Pradesh, Bihar, Punjab, and Haryana, the recovery differs from 9 to 10%, with Bihar recording the lowest recovery. In these subtropical states during October–November, the optimum ripening conditions of temperature, humidity, sunshine, and photoperiod exist. Apart from January end to March, cool and dry weather conditions favor ripening and sucrose accumulation. However, because of the cold temperatures in December and January, the ripening process slows significantly (as low as 2.5 °C), high humidity because of winter rains, and subsequently less sunshine period. Due to these conditions, the overall sugar recovery of the season is affected. The coastal regions record high humidity as they have proximity to the sea. Heavy rains during South West and North East monsoon during the period of July to November coupled with ample irrigation flow from the river channels and the practice of rotating paddy with cane crop exposes the crop in these coastal areas to ill-drained situations over the large area leading to less cane productivity and drop in cane recovery. The sugar recovery tends to be low in these areas, such as 8.5–9.75%.

2.8 Response of Sugarcane Genotypes to Climatic Factors During the Ripening Phase

Sugarcane ripening is a process of physiological senescence which occurs from the basal internodes and proceeds to the top of the stalks (Alexander 1973). The factors governing the ripening are of sucrose level, decreasing sugars, and stalk humidity. Sugarcane genotypes respond differently to meteorological variables during the ripening phase. Early cultivars are those with Pol more than 12.3% at the start of crushing season, while mid and late genotypes register above this threshold from middle to end of the season (Lavanholi 2008). During the beginning of the harvest time, when the ambient air temperature and moisture content are generally excess, sugarcane cultivars rarely achieve their full ripening potential. In contrast, they are harvested at their active stage of sucrose accumulation (Legendre 1975). Early cultivars ripen sooner as they are more sensitive to climatic factors.

In contrast, the late cultivars are less sensitive, accumulating the maximum sucrose content towards the end of the crushing season resulting in differences in sugar yield. Early cultivars are considered to be physiologically efficient as they are capable of shifting from vegetative to ripening phase earlier than late cultivars. Meteorological factors such as air temperature, photoperiod, solar radiation, and soil moisture are analyzed considering the long periods (120–150 days) preceding harvest. The highest relative growth rate and sucrose accumulation have been

observed during elongation of stalk and ripening in early varieties than the late ones (Singh and Venkatramana 1983; Lingle and Irvine 1994). The environmental variables influence the invertases, the active enzymes during ripening. Ripening is delayed under high air temperature. It changes invertase balance resulting in intense growth and decreased sucrose accumulation. The decline in acid invertase activity under low temperatures could be due to the enhanced sucrose phosphate synthase (SPS) and neutral invertase enzymes with concomitant rise in sucrose levels. Studies conducted with eight Sau Paulo (SP) genotypes in Brazil have demonstrated a significant correlation existing between climatic variables and ripening in sugarcane (Cardozo 2012). Two early ripening cultivars (SP 91-1049 and SP 86-155) recorded higher Pol values between the base temperature 20 and 21 °C while the middle and late-ripening cultivars observed lower values between 18 and 19 °C. This observation explained the early ripening at higher base temperatures while late cultivars delay their growth under low temperatures by extending their development for long periods.

2.9 Pattern of Sucrose Accumulation Under Rainfed Conditions in Tropics: A Case Study in Thailand

Despite many studies on factors influencing sucrose accumulation, the ripening mechanism is poorly understood as the information on the interaction of sugarcane genotypes with locations is meager. The effect of short-term temperature fluctuations on sugar metabolism during harvest season is not known (Lingle 2004). Field experiments conducted with 17 diverse and elite sugarcane genotypes representing different agro-climatic regions in Thailand facilitated the classification of sugarcane genotypes into six groups based on the rate of sucrose accumulation and high-temperature sensitivity at over maturity (Khonghintaisong et al. 2020). Meteorological data on rainfall and maximum and minimum temperature were collected daily in two experimental sites (Khon Kaen and Udon Thani). Brix, sucrose, and commercial cane sugar (CCS) yield were recorded during 8–12 months after planting (MAP). Juice Brix and sucrose data from 8 to 10 months identified early sugar accumulating clones, while 12–15 months after planting, juice data facilitated the identification of clones sensitive to high temperatures.

Different groups include clones that accumulate sugar rapidly with increasing temperature, and CCS reduces with enhancing temperature (KK3, KKU99-01), temperature insensitive clones with rapid sugar accumulation with rising temperature (Kps01-12, MPT02-458, KK06-501), medium sugar accumulation with increasing temperature (TBy 28-0941, UT13), medium sugar and temperature insensitive (TBy 28-1211, CSB07-79, KKU99-02, MPT02-187), slow accumulation with increasing temperature (K88-92, KK06-419) and slow sucrose accumulation and temperature insensitive cultivars (UT12, CSB07-219, KKU99-03, KKU99-06). Among the 17 genotypes, KK3, Kps01-12, MPT02-458, and UT13 were identified as high CCS cultivars based on the consistency of CCS value in 12–14 MAP for both locations. This study identified Kps01-12 as high sugar and temperature insensitive

cultivar while KK3 and MPT 02-458 as early and late-ripening cultivars. No correlation could be observed between Brix and stalk diameter, leaf numbers/area expansion, and stalk height of all genotypes in both locations. However, the association between Brix and stalk diameter was negative, considering the values between the 8th and 9th month of crop age. The information generated from this study on the accumulation of sugar patterns of diverse sugarcane varieties cultivated during natural rainfed conditions served as a selection criterion for improving sugar yield in the breeding programs of Thailand.

2.10 Role of Invertases in Sucrose Accumulation

Several studies have suggested that soluble acid invertase (pH 5.2) of the immature internodes was associated with cell expansion/elongation processes leading to the growth of the stalks while its cessation with sucrose storage in the cells. Investigations by Dendsay et al. (1995) in subtropical sugarcane varieties revealed that the immature internodes of late-maturing Co 1148 showed two to three times higher acid invertase activity than the corresponding internodes of early maturing variety CoJ 64. Vacuolar invertase activity of the second top internode of several varieties was in inverse order of their maturity status. The peak activity of acid invertase coincides with the period of fastest cane growth. A comparison of 15- and 40-week-old plants of variety CoJ 64 showed that the second-lowest internode of the 15-week-old plants had Brix values as low as 4.0–6.0 and high neutral invertase activity. Upper internodes possessed higher acid invertase activity, but low and mature internodes showed low or negligible activity, indicating that the vacuolar invertases were the most active enzymes in sugarcane growth processes.

In contrast, the corresponding internode of 40-week-old plants with Brix values of >20 showed negligible neutral invertase. The maturing internode (fifth top) was comparable to the lower internode of 15-week-old plants, which may have just begun accumulating sucrose and contained high neutral invertase. In contrast, mature internode already has stored sucrose almost to its capacity and has lower invertase activity (Table 2.1).

A field experiment was conducted during 1986–1987 in India at Sugarcane Breeding Institute, Coimbatore (Venkatramana and Singh 1986) with eight sugarcane varieties (Co 7712, Co 7201, CoC 671, Co 7704, Co 6304, Co 7717, Co 62175, and Co 7224) of different maturity groups to study the role of invertase enzymes in sugar accumulation in relation to dry matter partitioning into stem tissue. The results indicated that at early stages of growth, i.e., 150 and 180 days age, varieties of different maturity groups did not vary significantly with respect to acid and neutral invertase enzymes in both top and bottom halves of early varieties Co 7712, CoC 671, and Co 7704.

Table 2.1 Activity of invertases in cane varieties at periodic intervals during ripening (Dendsay et al. 1995)

Internode	Neutral invertase activity (μg glucose/g/h)		
	October	December	January
CoJ 64			
Immature	51.31 ± 2.1	35.18 ± 1.3	21.42 ± 1.0
Maturing	62.47 ± 10.4	19.25 ± 1.7	14.16 ± 1.2
Mature	13.01 ± 0.9	11.10 ± 0.7	6.05 ± 0.3
Co 7717			
Immature	66.20 ± 2.9	52.21 ± 2.7	22.07 ± 2.6
Maturing	72.09 ± 9.0	24.05 ± 1.0	16.24 ± 1.0
Mature	22.32 ± 1.3	15.18 ± 1.0	7.07 ± 0.2
CoS 767			
Immature	99.06 ± 12.3	58.14 ± 1.8	47.07 ± 5.7
Maturing	87.47 ± 3.5	31.10 ± 2.0	23.05 ± 1.1
Mature	30.22 ± 2.2	17.00 ± 0.8	9.16 ± 0.8
Co 1148			
Immature	117.34 ± 18.5	92.58 ± 8.7	53.05 ± 1.0
Maturing	110.23 ± 8.1	48.14 ± 2.1	26.30 ± 1.0
Mature	35.34 ± 2.9	18.14 ± 0.8	14.16 ± 1.0

2.11 Effect of Cold Temperature on Sucrose Synthesis

In sugarcane, the phloem sugar transport is very sensitive to chilling temperature than photosynthesis (Ebrahim et al. 1998a, b). The sucrose synthesis and SPS enzyme activity pattern were studied in cold-resistance cultivars, i.e., *S. sinense* R. cv. Yomitanzan and *Saccharum sp*. Cv NiF4 and a cold-sensitive cultivar such as *S. officinarum* L. cv Badila exposed to 10 °C (Du and Nose 2002). The plants were grown at 30/25 °C day/night temperatures and then shifted to constant day/night temperature of 30/25 °C. Sucrose content in the leaves of the two cold-tolerant cultivars recorded a 2.5–3.5 times increase after 52 h exposure to cold temperature compared to that of control plants, while no increase could be observed in the leaves of the sensitive cultivar Badila. The other enzyme FBPase did not show any remarkable change in its activity among the three sugarcane cultivars following exposure to cold temperature. Starch content in the leaves of tolerant cultivars was maintained at high levels, whereas the leaves of the Badila cultivar showed its depletion. The possible explanation for the striking differences could be due to inhibition of both photosynthesis and phloem transport in the cold-sensitive cultivar Badila (Du et al. 1999). Whereas the cold-tolerant cultivars maintained photosynthesis and transported the excess sucrose resulting in sucrose accumulation.

2.12 Effect of Flooding on Sucrose Accumulation

Sugarcane grown on heavy-textured soil does not have a favorable environment for the normal growth and functioning of the root system due to damp soil conditions and weak internal drainage. Various sugarcane growing regions like India, Australia, Louisiana, Florida, and Japan experience frequent and heavy rains that lead to periodic flooding resulting in more productive land no suitable for sugarcane cultivation. The differences of genotypic for resistance to waterlogging situations and frequent soil flooding have been reported in earlier studies. Out of 68 clones of *Saccharum* and closely related genera subjected to flooding for the duration of 6 months (Srinivasan and Batcha 1962), *S. spontaneum* and *S. robustum* were reported as flood-tolerant. Deren et al. (1991) observed reduction in productivity as 30–100% in sugarcane clones during continuous flooding for 5 months.

Field experiments were conducted by Viator et al. (2012) to screen sugarcane clones for tolerance to periodic flooding at USDA. Two high fiber/low sugar energy canes, L79-1002 and Ho 01-12, and two low fiber high sugar clones, HoCP 96-540 and L 99-226, were studied. Periodic flooding consisted of 7 days of flood-like conditions applied each month from February to August. Flooding tolerance was demonstrated both in the plant and ratoon crops of Louisiana clones L 79-1002 and in the ratoon crops of Ho 01-12. These two clones exhibited a reduction in sucrose yields due to flooding to the extent of 23 and 24% in plant and ratoon crops, respectively. Mean performance of first and second ratoons indicated decreased sucrose yields of clones HoCP 96-540 and L 99-226 by 50 kg/ha/day in plant cane and 30 kg/ha/day due to prolonged waterlogging. Reduced cane yield observed due to continuous flooding was the causal factor for the decrease in sucrose yields observed in this study and not sucrose concentration as reported earlier (Gilbert et al. 2008). However, reports on sugarcane genotypes mention high and low sucrose levels grown under varying water table depths. Two clones, viz. L 79-1002 and Ho 01-12 registered an increase in sucrose yields by 1600 and 520 kg/ha, indicating sugarcane cultivars' differential response under flooded conditions. Sucrose increased by 21 and 13 kg/mg for L 79-1002 and Ho 01-12. Two energy canes used in this study (Ho CP 96-540, L 99-226) yielded lesser sucrose than the commercial clones.

2.13 Development of Climate-Smart Sugarcane Varieties Through Pre-breeding

Wild species from the basic gene pool possess wide adaptation strategies to the atmospheric environment and climate changes with high potential in crop improvement. Crop wild relatives (CWRs) thus form the center of unexploited genetic diversity, which may not be present in the cultivated gene pool for utilization to improve economic traits of interest, viz. resistance/tolerance against biotic and abiotic stresses, such as diseases, insect pests, water deficit, soil saline, alkalinity, chilling, temperature, and suitable agronomic adaptation with enhanced sucrose

content. Nobilization was attempted as early as in the 1900s in sugarcane. The gene introgression was carried out through backcrossing, which resulted in many inter-specific and tri-species hybrids that improved the varietal scenario in India and all sugarcane growing countries across the globe.

Pre-breeding presents a better opportunity through the introgression of favorable genes from wild germplasm into genetic background readily available for use by breeders with minimal linkage drag. In sugarcane breeding programs, the wild species, *S. spontaneum*, *S. robustum*, *S. barberi*, *S. sinense*, *Miscanthus sinensis*, and allied genera *Erianthus arundinaceus* and *E. procerus* have been used in introgression to broaden the genetic diversity of the sugarcane population and generate a new gene pool of interspecific and intraspecific hybrid derivatives. Gene pyramiding was attempted through backcross breeding at ICAR-Sugarcane Breeding Institute, Coimbatore, India.

Efforts to broaden the genetic base of sugarcane cultivars through hybridization with *S. spontaneum*, *S. barberi*, *Erianthus*, and *Sorghum* as female parents and several elite hybrids are exploited in breeding programs (Ram et al. 2007). Nair (2007) performed interspecific crosses involving cultivated and wild species of *Saccharum* (*S. officinarum*, *S. barberi*, *S. robustum*, and *S. spontaneum*). The progenies were evaluated to identify superior hybrids and for further backcrossing. To boost productivity and adaptability in new cultivars, intergeneric crosses of *Saccharum* with other associated genera such as Erianthus, Sclerostachya, and Narenga were attempted.

Earlier studies showed that hybrids from (*S. officinarum* × commercial hybrid) × commercial and (*S. officinarum* × commercial) 2☼ *S. officinarum* showed better performance concerning juice sucrose (%) and CCS/plot. Evaluation of hybrids involving *S. robustum* showed that BC_2 × double-cross hybrids were superior for CCS/plot (Ram and Hemaprabha 1992). This program resulted in developing an elite gene pool of more than 300 ISH hybrids from different stages of nobilization for utilization. Intra-population improvement program involving *S. officinarum*, *S. spontaneum*, and *S. robustum* was formulated. Many hybrid derivatives with improved quality and yield traits were developed for further introgression (Nair et al. 1998).

2.14 Improved Hybrid Derivatives for High Juice Sucrose Content

Pre-breeding activities using wild species and Co canes have been initiated at ICAR-SBI to develop new gene pools with a high frequency of valuable genes, broader adaptability, and a large genetic base. Pre-breeding strategy through backcrossing has helped identify clones combining productivity, quality, and tolerance to red rot and smut (Alarmelu et al. 2018). The study indicated that F_1 hybrids of improved *S. officinarum* × improved *S. spontaneum* mating group showed improved hybrid vigor for cane yield traits and quality. The selected hybrids, viz. 95-77, 96-77, 97-12, 97-130, 97-256, 96-259, 97-130 97-256, 96-259, 97-196, 97-66, 97-170, 97-34,

Table 2.2 Performance of climate-resilient hybrids for juice sucrose content

Clone	Sucrose (%)[a]	Improvement (%)	
		Co 86032	*S. officinarum*
13-69	20.26	4.54	12.30
13-251	19.92	2.79	10.42
13-103	19.89	2.63	10.25
13-208	19.77	2.01	9.59
13-247	19.57	0.98	8.48
13-201	19.50	0.62	8.09
Co 86032	19.38	–	–
S. officinarum	18.04	–	–

[a] 360 days after harvest

97-526, 97-72, 97-77, and 97-157 showed a significant advantage over the parents for sucrose (%) and showed a wide range for sucrose (10.29–19.07%). These clones with *S. spontaneum* base performed better in ratoon crop, and BC_1 hybrids showed an improvement of 21.9 and 14.8% for sucrose (%) at 300 and 360 days, respectively.

First stage nobilized hybrids of improved *S. officinarum* × improved *S. robustum* showed an enhancement of 12.3 and 8.5% for sucrose (%) at 300 and 360 days, respectively, over the enhanced *S. robustum* parents. BC_1 noted improvement for both sugar quality and productivity characteristics suggesting further backcrossing in this group and eight clones 98-3, 98-13, 98-176, 98-200, 98-221, 98-269, 98-270, and 98-272 with improved *S. robustum* genetic base surpassed the standards for sugar yield and quality and were identified as high-quality types. BC_1 hybrids, viz., 13-57, 13-69, 13-76, 13-103, 13-114, 13-186, 13-201, 13-208, 13-147, 13-251, and 13-253 observed juice sucrose in the range of 18.0–20.3% and performed better than the improved parents and Co 86032. These elite clones from enhanced *S. officinarum* × improved *S. robustum* crosses were identified for high sucrose (%) at 300 and 360 days. The clone 13-69 with the highest sucrose of 20.26% at 12 months of age had improved *S. robustum* base as a maternal and paternal parent. The back cross hybrids 13-69, 13-103, and 13-251 indicated an enhancement of 4.54, 2.63, and 2.79% for juice sucrose (%) at 360 days (Table 2.2), and most recombinants with higher mean Brix were obtained with improved *S. officinarum* as one of the parents in backcrosses. Two back cross hybrids, viz. 14–57 and 14–60 with *S. barberi* cytoplasm (Co 8371 × Pathri) × Co 0209) observed sucrose (%) of 18.22 and 18.17, respectively, at 300 days as compared to Co 86032 (Alarmelu et al. 2014, 2018).

2.15 Sugarcane Crop Prediction Models and Their Applications Under Changing Climate

Sugarcane crop production systems have to adapt to changing climate to warrant sustainability, and it is essential for its survival. Crop prediction models and simulations are often used to know the impact of climate change on crop production

systems and are helpful in the identification of adaptative mechanisms. Improving sugarcane productivity and sugar recovery can be realized by knowing the crop response to the varying climatic variables under climate change (Hussain et al. 2018). Improved models can capture the physiological processes occurring in the crop, which will be useful to simulate crop response to predicted changes in climate. Further research is required to explore the impact of changing temperature regimes on crop production, especially on sucrose accumulation, and the crop's physiological response to changing temperature thresholds. These experiments have to be conducted in controlled conditions to increase the precision and accuracy of prediction. The meteorological parameters over the growth period, especially in later stages of the crop, i.e., from 150 days before harvest, need to be accurately monitored, and systematic phenotyping needs to be done. In order to increase the precision of the experiment, the large number of genotypes need to be evaluated over several years so that different sets of conditions can be simulated in controlled conditions over different seasons, and varietal potential can be ascertained under particular conditions. The data thus obtained can be subjected to advanced statistical models like artificial neural networks, etc., to generate models and predict the pattern of sucrose accumulation. These artificial neural networks can be used to predict the model more accurately than classical regression models.

Crop simulation models could play a key role in the impact studies regarding decision-making and planning in the perspective of changing climate scenarios and aid in formulating robust response strategies. The crop simulation models were developed and used to stimulate plant growth for the first time in wheat during the 1980s (Porter 1984; Weir et al. 1984; Ritchie et al. 1985; Baker et al. 1985). CANEGRO-sugarcane model is the first simulation model developed to determine optimal harvest age (Inman-Bamber 1995) at the South African Sugar Association Experiment Station (SASEX). Several sugarcane specific simulation models for the climate change impact assessment (Knox et al. 2010), FAO-AZM (dos Santos and Sentelhas 2014), CANEGRO-sugarcane (Inman-Bamber 1995; Singh et al. 2010; Singels et al. 2014; Jones et al. 2015; Bhengra et al. 2016; Dias and Sentelhas 2017; Parmar et al. 2019), and QCANE (Zu et al. 2018) are in vogue for various applications. These crop models require the input of climate data from climate models and on-ground observations for climate change impact analysis (Mi et al. 2017). Some of the commonly used models developed to predict cane production, features, and performance are discussed below.

2.16 APSIM (Agricultural Production Systems sIMulator) Model

APSIM suite of crop and soil models contains modules, which is a collection of several crop models, grouped in a way specified by the user and developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Agricultural Production System Research Unit (APSRU) in 1991 (McCown et al. 1996). The APSIM-Sugarcane, thus, represents a model of sugarcane that is generic in structure to the other crop modules in APSIM. The input variables are crop-specific

characteristics defined in the form of a table (Keating et al. 1999). The model is based on uncoupled radiation use and transpiration efficiency theory and simulates the fixation of carbon from the atmosphere on a daily time step. Daily growth is split into leaf, stalk (structural and sucrose fractions), cabbage (leaf sheath and the tip of growing stalks) roots, and sucrose by various portions for individual phenological phases.

Stress factors due to water, nitrogen, and temperature are applied to leaf and stalk growth first, then to sucrose partitioning relative to previous results. APSIM-Sugarcane alters the partitioning fractions to sucrose in the stalk for different cultivars, providing an ability to simulate different sucrose content for a range of cultivars. To automatically simulate water, fertilizer, and nutrient cycling between soil and sugarcane crops, the APSIM-sugarcane model can interact with the agricultural residue, soil, and agricultural management modules. APSIM-Sugarcane has a number of characteristics that are useful in sugarcane production systems. Plant or ratoon crops can be simulated, or if a crop cycle is being simulated, a plant crop will renew as a ratoon crop. Plant production systems—several ratoons—fallow can be simulated, as well as other APSIM crop or pasture modules, in a sugarcane rotation. APSIM-sugarcane also responds to lodging via decrease in the rate of stalk death, decrease in radiation use, and decrease in the proportion of daily biomass that is partitioned as sucrose. Furthermore, it responds to a decrease in the maximum number of green leaves to capture the reported decrease in leaf appearance rate and increase in leaf senescence as all these were common in the lodged crop in sugarcane (Singh et al. 2002; Muchow et al. 1995; Robertson et al. 1996).

Peng et al. 2020 used the meteorological data from 2009 to 2017 of Guangxi Zhuang Autonomous Region in China and field observations from sugarcane plantations and worked out the sensitivity of the APSIM model parameters using an extended Fourier amplitude sensitivity test. The APSIM model was validated for cane yield and phenology of sugarcane. The good R^2 value (0.76–0.91) between observed and simulated values and good consistency index D (0.91–0.97) indicates a good model fit. They used this validated model to simulate the production potential of sugarcane in marginal lands on a surface scale basis and the distribution pattern of the production potential of sugarcane in marginal lands. Their major goal was to use an APSIM-sugarcane model and GIS spatial analysis technologies to simulate and evaluate the potential of sugarcane as an energy crop on marginal land in the Guangxi Zhuang Autonomous Region. Model prediction indicated the region's surplus ethanol production by promoting sugarcane as an energy crop in the marginal lands.

The impact of climate change on cane yield and sucrose yield was assessed in Mauritius using APSIM-sugarcane. Long-term climate data of one location that is representative of Mauritius's productivity was used to generate baseline yields that were very near to the 1954–1996 average. Long-term data was used to create climate change scenarios with doubled CO_2 levels, either with outputs from General Circulation Models (increases in temperature with differences in rainfall and radiation patterns) or with nominal increments of 2 °C and 4 °C rise in temperature with 10 and 20% increase or decrease in rainfall. Under the GCM, an increase of

temperature by 2 °C reduced sucrose yield by 32% even if effective rainfall was higher, and an increase of 4 °C temperature reduced sucrose yield by 59%. Under an incremental scenario, 2 °C rise in temperature raised the sucrose yield from 16.9 to 17.2 t/ha. The higher temperature enhanced canopy development and an earlier onset of stalk formation, whereas a 4 °C rise in temperature decreased sucrose yield by 5% t/ha. Simulations with cultivar R 570 under rainfed baseline conditions indicated that the practice of July to November harvest allowed acquiring the highest amount of sucrose under Mauritius's conditions. APSIM-sugarcane crop production model predicted a significant decline in sucrose yield under changing climate in Mauritius, which needs to be countered with irrigation, drought-resistant varieties, and harvest date. The model simulated a reduction in productivity attributed to lower water use efficiencies and higher respiratory demands of the crop (Nayamuth et al. 2002).

2.17 CANEGRO-Sugarcane Simulation Model

CANEGRO model was initially developed around the 1970s by developing equations of photosynthesis and respiration, but it was assembled into a simulation model in 1991 at SASEX. The single leaf photosynthesis, quantum efficiency, and growth respiration were added to improve the calculations in later stages (Inman-Bamber 1995). It is embedded into the Decision Support System for Agrotechnology Transfer (DSSAT) (Tsuji et al. 1994) and was widely used in Africa (Inman-Bamber and Kiker 1997), Asia (Jintrawet 1995), and America. The model contains crop development, carbon simulation, water simulation, and energy components, while direct effects of temperature on photosynthesis were not included. An empirical day of year function provides an annual sinusoidal pattern of the sucrose concentration. This is combined with stalk biomass function for both rainfed and irrigated conditions, while an additional function of cane age is included for rainfed conditions.

For mass growth, the CANEGRO-sugarcane model uses a source-sink idea, however the number of stalks is included as a state variable to explain the sink size. It used the crop's energy balance to simulate canopy development by intercepting photons for photosynthesis. The biomass is disseminated dynamically among various plant components, based on the crop's age, level of water stress, and temperature, including stalk sucrose. This model used a non-linear function of total biomass to simulate the daily partitioning of assimilate between roots and aerial parts. When thermal time since emergence exceeds a stipulated value, they used a constant fraction of aerial dry mass partitioned to stalk. The source strength was considered based on the rate of dry matter partitioning to stalk. Partitioning of dry stalk matter is regulated by sink capacity and the source to sink ratio. Existing growing conditions govern sink capacity, existing stalk mass, and varietal characteristics. The model's sucrose accumulation component is based on a framework for sucrose dispersion throughout stalks as a function of water stress and temperature.

CANEGRO-sugarcane model was validated in East Uttar Pradesh, India (Singh et al. 2010). The model simulates the stalk fresh mass, sucrose yield, and stalk height within ±15% of range compared to the observed values. CANEGRO-sugarcane model was used to assess the impact of climate change on sugarcane in various combinations of elevated CO_2 concentrations and temperature (Sonkar et al. 2020), along with dynamically downscaled bias-corrected regional climate model (RCM) data using RegCM4 under RCP45 scenario (2040–2060) to project the forthcoming change in sugarcane stalk fresh mass and sucrose mass. The results showed an elevated temperature, precipitation, and solar radiation in the future projections at the study location. The sugarcane stalk fresh mass (SFM) and sucrose mass (SM) were found to be sensitive (3–25% decrease) for increased temperature (1–4 °C), however, higher values (2–14% increase) were observed for both SFM and SM under raised CO_2 levels (450–850 ppm). The combined effect of elevated temperature and CO_2 had a favorable impact on SFM but a damaging impact on SM. Their study anticipated the increase of SFM by 7–47% (irrigated) and 3–39% (rainfed) in 2040–2060 relative to 1971–2000 in varied agro-climatic zones of the region. Similarly, SM was projected to decrease by 6–37% (irrigated) and 9–69% (rainfed).

CANEGRO model, along with the climate scenarios from the regional climate model CCAM (Conformal Cubic Atmospheric Model), was used to evaluate the potential impacts of climate change on sugarcane production systems in two selected locations in the Mekong River Basin in Thailand, and it signposted positive influences of climate scenarios on fresh sugarcane yield while less sugar yield per ton of cane yield (Jintrawet And Prammanee 2005).

The climate change effects on sugarcane yield, irrigation needs, and water use efficiency in southern Brazil, using CANEGRO based on downscaled outputs of two general circulation models (PRECIS and CSIRO) show the sensitivity of simulated cane yield to CO_2 concentration and air temperature (Marin et al. 2013).

2.18 QCANE Sugarcane Simulation Model

The Bureau of Sugar Experiment Stations (BSES) in Queensland, Australia, developed the QCANE model (Liu and Kingston 1994). The major goal of QCANE was to research sugar accumulation and develop strategies to increase it. Therefore, strong emphasis was applied to photosynthesis, partitioning of photosynthate, and respiration. The temperature, growth stage, and growth rate were considered to allocate the photosynthate to determine stalk sucrose. QCANE had the lowest error in simulating leaf area index (LAI) and biomass compared to CANEGRO and APSIM-sugarcane (Keating et al. 1995). The seasonal changes in LAI and biomass followed the observed data in validation studies closely. The model performance in simulating sucrose yield was found promising in a diverse range of environments from subtropical to tropical regions (Liu and Kingston 1994).

2.19 Ricardian Model for Impact Analysis of Sugarcane Production Under Dryland and Irrigated Conditions

Developing countries are more vulnerable to climate change because of their low capital investments and being technologically less equipped. Most of them are in hot climates that are likely to get hotter. Sugarcane cultivation is expected to be significantly influenced by climate change, and it will largely affect the contribution of the sugar industry to total GDP and the country's overall economy. A case study was conducted to analyze the economic impact of climate change in the South African sugarcane farming system using an empirical modeling approach (Deressa et al. 2005). Ricardian model accounts for changes in environmental factors to simulate the response of land value or net revenue response using a regression approach. The model measures the marginal contribution of these environmental factors to net farm income capitalized in land value. South African sugarcane farming is ideally suited for this study as employment within the sugar industry is 85,000 jobs, direct and indirect employment is estimated at 350,000 people, and approximately one million people depend on the sugar industry (SASA 2001).

The crop modeling method known as the production function approach is based on empirical or experimental analysis of the relationship between environmental factors and yield (Chang 2002). This method could predict accurate yield responses regarding the relationship between climatic variables and yield. Sugarcane growth models (Kiker et al. 2002) to simulate sucrose yields and growth factors indicated that climatic factors (temperature and rainfall) affect different sites differently across the sugarcane-producing areas. District-wise weather (temperature and rainfall) and geographic variables (latitude and altitude) data were collected from the experiment stations. Altitude was included to account for solar energy in that location, while control variables like soil type were also included as they influence cane yield and vary across the districts.

This Ricardian technique uses a non-linear (quadratic) model using net revenue per hectare as the dependent variable for each district. The climatic and other control factors were regressed on net revenues. Climate factors, altitude, soil, irrigation dummies, and the temporal trend all have a substantial impact on net revenue from sugarcane growing, according to the results of regression research. Most of the climate variables' linear, quadratic, and interaction factors (temperature and rainfall) had statistically significant coefficients. Temperature and rainfall significantly affected net revenue per hectare across seasons. The results further indicated that net revenue per hectare in the dryland farming areas decreased at a higher rate than in the irrigated regions. The drop in net revenue per hectare in both regions is well explained by the negative time trend parameter values and the reasons being unfavorable price trends and patterns of technological change.

Among the geographic variables, altitude was negatively related to net revenue per hectare, explaining the cooler temperature prevailing at higher altitudes facilitating a longer production period before maturity for the sugarcane crop. The sandy soil type positively affected sugarcane production compared to the shallow

and high lime content soils. Sandy-loam soils with better drainage give a better sugarcane crop than shallow and high lime soils.

A regression model was used to simulate the impact of changing temperature and rainfall in net revenue per hectare of sugarcane (Kumar and Parikh 1998). Based on the combined analysis for both dryland and irrigated regions for the period starting from 1976–1977 to 1997–1998, the change in the net revenue per hectare (response variable) was estimated for warming of 20 °C rise in average temperature and 7% increase in average rainfall levels is simulated utilizing estimated regression coefficients. The reduction in average net revenue per hectare was 27% under dryland farming compared to 26% under irrigation, with only a marginal difference. Pooled analysis based on South African sugarcane farming indicated a negative impact for both regions suggesting that irrigation did not serve as an effective adaptation strategy to combat damage caused by climate change. An increase in net revenue with a rise in harvesting temperature observed in this study needs to be noted with caution because high temperature is not recommended as it initiates growth and reduces sucrose. Low temperature allows for sucrose accumulation during ripening, but very low temperature, below 10 °C rupture cells and cause irreparable damage (Humbert 1968). The key findings highlighted the need for cost-effective approaches of regulating yield-decreasing factors corelated with the temperature increase, particularly during the winter growing season as well as the availability of sugarcane varieties that are relatively unaffected by rising temperature during ripening and harvesting (Deressa et al. 2005).

2.20 Predicting Sucrose Yield Through Modeling Approaches

Comparative evaluation of three sugarcane simulation models with respect to their prediction of sucrose yield highlighted the strengths and limitations in these modeling approaches (O'Leary 1999). It was pointed out that the improvements in these models for predicting sucrose yields lie in the visualization of the effects of stress (temperature, water, and nitrogen) on the partitioning of photosynthate to stored sucrose, the differential response of sugarcane genotypes to stress, and the differences in terms of radiation-use efficiency and transpiration efficiency across crop cycles (Plant and ratoon crop). A novel approach employing a source-sink concept is suggested that involves the volume of stalks as a state variable to define the sink size. Inclusion of reducing sugars as an additional variable to permit the hydrolysis and re-synthesis of sucrose has also been suggested as an improvement measure in these models. This innovative idea is likely to give a better knowledge of the growth and management of sugarcane for its sucrose yield and juice purity, especially at various stages of sucrose accumulation.

2.21 Future Directions

Environmental variables play a predominant role in the process of sucrose accumulation in sugarcane genotypes. The genotype greatly determines sugar yield and its component traits but can significantly influence the environment. Despite substantial research in the past, the pattern of sucrose accumulation and the factors associated with sugar accumulation are relatively less understood as the information on sugarcane genotypes and environments is meager. Genotype interaction with the environment during the onset of ripening needs to be studied extensively to maximize the genetic improvement for sugar productivity. Recent developments in plant molecular biology have helped us identify the key regulatory steps in the pathway of sucrose synthesis. The temperature fluctuation during the harvest season is very high, and the effect of short-term temperature on sucrose metabolism in sugarcane stalks is unknown. Studies on the partitioning of dry matter at different harvest times provided the basis for understanding the underlying mechanism of ripening in sugarcane. More investigations need to be conducted to illustrate the effects of climatic factors on crop physiology, particularly enzymatic balance that regulates the processes of vegetative growth and sugar accumulation, and studies that ascertain the causes of changes in the behavior of sugarcane genotypes. Therefore, in the context of changing climate scenario, selection of parents for ripening behavior demands greater attention from sugarcane breeders as there is significant variety × harvest interaction effect, particularly for sucrose content and consequently sugar yields.

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3 Impact of Salinity Stress on Sugarcane Yield and Quality: Management Approaches for Higher Cane Sugar Productivity

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Abstract

Salinity stress is one major environmental stress that adversely affects cane yield. It interferes with cane growth, development, and crop production. Na^+, Ca^{2+}, and Mg^{2+}, Cl^-, $SO_4{}^{2-}$, $HCO_3{}^-$ ions are the primary sources contributing to the soil salinity. Globally, about 33% of irrigated land and 20% of cultivated land area are salinity-affected. Additionally, salt-affected soil is disseminated at a faster rate annually due to many reasons. Under the changing climate scenario, frequent low precipitation and elevated temperature coupled with high evaporation rate, irrigation with saline water, and faulty agricultural practices lead to twin soil salinity and waterlogging problems, which in tern distressing the cane productivity. Effects of salinity on plant phenotype are characterized by reduced cane germination and cane height (stunted growth) of the crop, reduced leaf area, and finally, a significant reduction in cane yield and sugar content of the crop. When the plant is exposed to salinity stress, there are many changes in physiological traits, such as reduction in plant's ability to absorb water and minerals, partial stomata closure, and ionic toxicity injuries to the plant cell, which ultimately leads to a decrease in the photosynthetic rate, that may be the prime factor responsible for reducing cane growth and development. Many positive changes occur in cell organelles during salinity stress. Changes in cell structure, membrane regulation system, and restoration of plant cell REDOX potential by osmotic adjustment are significant in managing the salinity stress in sugarcane. Complex nature of salinity response hinders many metabolic activities due to the accumulation of

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many by-products and reactive oxygen species. An increase in ion level of the juice due to salinity can decrease the efficiency of the stalk for sucrose storage, and salinity stress can also decrease the cane photosynthetic rate and translocation of sucrose from leaves to stem. Several management and omics approaches have been successfully employed in sugarcane crops to ensure sustainable cane productivity during salinity stress conditions.

Keywords

Biomass · Cane production · Growth · Management strategies · Salinity stress · Sugarcane

3.1 Introduction

Sugarcane (*Saccharum officinarum* L.) belongs to the Poaceae family and represents the high level of tolerance to salinity stress at different crop phases (Verma et al. 2021a). Being glycophytic, sodium ions toxicity in cane means the major ionic stress that enforces ionic imbalance, hyperosmotic and hyperionic stress, thereby upsetting the whole metabolic activities. Proline is typical plant amino acid that accumulates, enhancing the saline and water deficit resistance in plants (Zhang et al. 2020; Bray et al. 2000; Verma et al. 2019a, 2020). It is recognized to be associated in attenuating cytosolic acidosis related to many plant stresses. It is non-toxic, protects the plant during stress, and acts as an osmoregulatory substance in sugarcane that can preserve cell structure and tolerate adverse environmental stresses. Research showed that an increased proline in transgenic events of sugarcane imparts the tolerance mechanism in sugarcane crops (Ferreira et al. 2017).

Abiotic stress-tolerant sugarcane varieties can be developed by employing genomics and biotechnological tools. Transcriptome analysis helps find some useful transcripts and genes and helps trace the key biological pathways associated with stress tolerance and their network. Differential gene expression profiling through the transcriptome approach helps elucidate the mechanism of stress tolerance and differential gene expression profiles in sugarcane. In the recent past, efforts have been made to introgress the major tolerant genes from wild species into cultivated sugarcane species. Several genes confirming salinity tolerance were transferred into the sugarcane cultivars using the transgenic methodology. In this manner, the *EaGly III* gene to enhance the salinity tolerance was overexpressed in sugarcane using particle bombardment, which was evident by observing the morphological and physiological parameters (Augustine et al. 2015a, b, c).

Additionally, modified agronomic practices would significantly reduce the impact of salinity on cane yield and quality. Due to the high production capacity for bioenergy and biomass and tolerance to this crop's salinity, researchers' interest has increased. The knowledge of crop physiological responses to salinity is important for further selection of the parental line/donors in distant hybridization programs and finding out desired clones with tolerance to salinity. This chapter deals with the

impact of saline stress on cane production, quality and their management strategies for higher cane productivity. This chapter would help several sugarcane researchers and policymakers address the salinity problem in sugarcane cultivation for sustainable crop production.

3.2 Impact of Salinity on Sugarcane Production and Quality of Juice

Abiotic stresses reduce the crops productivity, depending on the variety of plants species/cultivars, stress duration, and severity. In different tropical and subtropical parts of the globe, plant productivity is limited due to the enhanced saline stress. Salinity and water deficit are complex stress, and the identification of tolerances clones against these stresses is an important step to breed the clones suitable under drought and salinity stress (Verma et al. 2021b, c, d). The timing and severity of salt stress may differ considerably, severely affecting plant performance and crop output. The reduction in leaf area, slow crop growth of cane, succulent crop canopy, and stunted crop are among the major features under such a stressed environment. The plant phenotypic effects of salinity are categorized as stunted crop growth, leaf area, and biomass reduction (Singh et al. 2015). High salinity affects the photosynthesis rate by the closure of stomata, reduction in transpiration, and causes injury in the plant cells due to ionic toxicity.

Sugarcane is a moderately sensitive crop to salinity that can tolerate a threshold limit of 1.7 dS m^{-1}. The soils with high water content and nutrients are ideal for sugarcane crops to realize maximum cane yield. Cultivating sugarcane in salt-affected soils results in a drastic loss in cane development and production losses of 50% or more compared to normal soils (Suprasanna et al. 2009; Kumar et al. 2014; Almeida Moreira and Ricardo 2017; Verma et al. 2021e). Cane yield in saline soil or irrigation water declines significantly by reducing stalk population and stalk weight (Lingle and Wiegand 1997). Lingle and Wiegand (1997) have also observed that each dS m^{-1} enhance in root zone salinity decreases stalk population by 0.6 stalk m^{-1} and individual stalk weight by 0.15 kg, reducing stalk yield by 13.7 t ha^{-1}. The main possible reason for reduced cane yield under salinity stress could be attributed to the photosynthetic parameters greatly affected by increased salinity levels (Shabala and Cuin 2008) through changes in the CO_2 uptake and its assimilation by the leaves. This is mainly linked with stomatal oscillation. The anatomical changes induced by salinity at leaf level are smaller leaves, reduced frequency of stomata, and changes in the mesophyll area of leaves. All these traits indicated a close association with each other, and hence all of them play an important role in reducing final yield and productivity. Various studies have reported a more significant impact of salinity on the shoot than root growth (Rozeff 1999; Plaut et al. 2000; Zeng and Shannon 2000).

The findings also reported that salinity stress provoked some crucial changes in photosynthetic and anatomical characteristics, important in determining the cane yield (Plaut et al. 2000; Verma et al. 2019b). Loss in the transport of water and

ion-conducting tissues caused by decreased area of xylem and phloem cells which offered most resistance to the flow of water. Therefore, one of the essential consequences of salt stress, its impact on the mesophyll cell, which reduces the photosynthetic rate in plants (Longstreth and Nobel 1979; Bliss et al. 2019). Salinity is one factor that directly or indirectly influences leaf area index (LAI) and/or leaf photosynthesis (Vasantha et al. 2010; Hussain and Reigosa 2015). Among the specific leaf parameters, leaf area expansion and photosynthesis are interconnected with other physiological characteristics, i.e., intercellular CO_2 concentration (C*i*), stomatal conductance (gs), and photo-assimilate enzyme activities. The excess salts adversely affect cane development and productivity. Sugarcane output may have decreased under saline conditions due to declining crop growth and productivity characteristics. During salinity, the excess salts are taken up by the root zone of the crop and accumulated in the aerial portion, which subsequently reduces crop growth and cane yield (Akhtar et al. 2003). The varietal difference under various salinity levels was reported by Thakur et al. (2010), and similar observations were also monitored by Gomathi and Thandapani (2014).

The effect of salinity on juice sucrose in sugarcane varied in commercial hybrids, and it can be estimated before the harvest of the crop. Lingle and Wiegand (1997) observed that osmolality in cane juice was unaffected by soil salinity (0.5 to 4.0 dS m^{-1}). However, level of various solutes in the cane juice get changed, indicating that the cane stalk has a certain ability to accumulate more solutes in juice and as a result of minerals in juice enhanced. In contrast, the level of sucrose and other dissolved solids reduced, either by displacement or reduced the import rate. Also, it was observed with each dS m^{-1} increase in ECe, there is a decrease of Brix and sucrose (%) in juice by 0.5–0.6% and a decrease in purity by 1.0–1.3% (Lingle and Wiegand 1997). However, in large scale, several years of field trials, Thomas et al. (1981) demonstrated that the saline irrigation water did not consistently reduce the cane juice Brix, Pol, and juice quality. An increase in ion content of the juice due to salinity can decrease the efficiency of the stalk for sucrose storage, and saline stress can also decrease the cane leaf gas exchange and translocation of sucrose from the leaves to stem (Lingle et al. 2000).

The osmotic component of NaCl found to have the influence on sucrose transport to stalks, followed by increased sucrolytic activity in cane internodes (Wahid 2004). The differential response of sugarcane varieties with respect to soil salinity and acidity has been observed. At the early crop stage, germination and early growth stages of the crop become more sensitive than the later stages of the crop. Additionally, the salinity effect is more in ratoon crops than plant crops. It has been observed that the sugarcane crop is highly susceptible to a threshold of EC of <2 of dS m^{-1}, and different soil types, rate of transpiration, and solar radiation may further alter the salinity tolerance in sugarcane. The crop can show a yield decrease of up to 50% or more with soil salinity of an EC of 10.4 dS m^{-1} (Simões et al. 2016; Courtney et al. 2010).

3.3 Salinity and Jaggery Quality

Sugarcane is the main commercial crops, and it is used mainly for the production of raw and refined sugar, jaggery, and other by-products. Jaggery is an available alternative to sugarcane growers, and nearly 26% of the sugarcane produced is diverted for jaggery production (Vasantha et al. 2009). The jaggery's best quality depends on the quality of cane juice, which is further determined by the sugarcane cultivars and the environmental variables in which the cane is cultivated. Among the tolerant sugarcane genotypes, noticeable variations in jaggery quality as indicated by net rendement value and color were witnessed. Under high soil salinity, the tolerant cane varieties such as Co 85019, Co 94008, and Co 97008 produced jaggery with a low quality, color, and taste, while the genotypes Co 94012 (Fig. 3.1) and Co 99004 yielded good quality jaggery even during saline conditions. Under the salinity stress, Na^+ and Cl^- content enhanced only marginally, and cane productivity and juice quality were not affected (Vasantha et al. 2009).

In the context with a sizeable area of sugarcane occupied during sodic soils, there is a need to identify cultivars that perform better under such conditions. Cane varieties such as Co 94012 and Co 99004 produced better jaggery quality subjected to salinity stress. Level of Na^+ in juice is considered an essential new criteria than salinity resistance per se in assessing suitable cultivars solely for jaggery making purposes (Vasantha et al. 2009).

Fig. 3.1 Jaggery from salinity tolerant genotypes of sugarcane (Source: Vasantha et al. 2009)

Fig. 3.2 List of cane parameters affected under salinity stress conditions

Plants are innately fortified with defensive action of mechanisms to scavenge highly produced toxic metabolites and reactive oxygen species (ROS), including ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), and superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), glutathione reductase (GR), and glyoxalase pathway enzymes. Methylglyoxal is a highly harmful metabolite accumulated due to abiotic stresses in plants, which in excess is capable of complete cellular destruction with inducing advanced glycation end products, oxidation of fatty acids, and commotion of membrane structures or functions (Conde et al. 2011). Living organisms have evolved the glyoxalase system to detoxify methylglyoxal into non-toxic D-Lactate by the consecutive action of Gly I and Gly II disbursing glutathione as a cofactor (Kumar et al. 2014; Amtmann et al. 2005; Brijesh et al. 2021). Overexpression of these glyoxalase genes separately or in combination showed resistance during different abiotic stresses like salt, toxic ions, osmotic, oxidative, and cytotoxic compounds like methylglyoxal in several crops.

Several management strategies can mitigate the salinity stress impact on crops and improve plant growth efficiency. These strategies include both crop management practices and molecular approaches. Among the molecular approaches, genetic modification, tissue culture techniques, molecular markers linked to salinity tolerance, transcriptome sequencing, microarray techniques, and plant transformation techniques are key to develop salinity tolerant genotypes (Fig. 3.2). Employing suitable agronomic practices, including soil reclamation methods, saline irrigation

management, priming of sugarcane seed at the initial stage, and proper drainage facility, would be beneficial for salinity management in the salt-affected area.

3.4 Molecular Marker for Salinity Tolerance in Sugarcane

Molecular markers are powerful tools to identify the genetic diversity associated with salinity tolerance in sugarcane. The markers linked to salinity tolerance can be used to trace the particular genetic loci for salinity tolerance. These identified genetic loci will provide an opportunity for sugarcane breeders to introgress the salinity tolerance lines into cultivated sugarcane. Many PCR-based molecular markers have been exploited in sugarcane to access the genetic diversity and agronomic traits, including salinity tolerance in sugarcane clones (Azevedo et al. 2011). Additionally, tracing the genetic loci linked to salinity would also help in developing the desired strategy and understanding the molecular mechanism on salinity tolerance in sugarcane crop (Hasegawa et al. 2000). DNA markers can be used to identify and classify salt-tolerant sugarcane genotypes. Using PCR-based markers for the RAPD amplification of particular DNA sequences is a basic step for identifying the salinity tolerant genes. Many TRAP markers were developed from the EST database to identify candidate genes (Hu et al. 2008; Farsangi et al. 2018). Characterization of susceptible and tolerance lines of sugarcane was carried out using RAPD markers in tissue cultures derived from embryonic calli treated with ethyl-methane sulphonate (EMS) (Yadav et al. 2006).

The salinity resistant lines were separated from susceptible based on the RAPD polymorphism profile (Gadakh et al. 2017). Similarly, 15 ISSR markers were effectively used to access the genetic diversity of sugarcane varieties for salinity tolerance. The salt-tolerant and susceptible clones were differentiated based on the similarity index among the studied lines (Markad et al. 2014). Recently, 18 sugarcane clones were characterized using five TRAP markers for salinity tolerance (Farsangi et al. 2018). This study revealed the limited variation among the entries tested under salinity stress with the similarity coefficient of 0.72. Molecular markers are considered as powerful tools for crop improvement programs, from germplasm characterization to identification of genetic loci for salinity tolerance in sugarcane.

3.4.1 QTL for the Salinity Tolerance in Sugarcane

Quantitative trait locus (QTLs) are the segment of DNA associated with specific phenotypic traits, and they may be clusters of genes or segments of the genome. Salinity stress tolerance is a complex mechanism, therefore identified desired QTLs for salinity tolerance have a significant role in understanding the stress response and producing the salinity stress-tolerant sugarcane. Unlike map-based cloning, recently using new approaches such as microarray-based differential expressed genes, salinity tolerance genes have been linked to QTLs. Several salt stress tolerance QTLs have been reported in various crops. With the help of these molecular markers, it is

convenient to tag the quantitative traits loci and their further evaluation. Despite the undented efforts to understand the salinity tolerance in sugarcane, the information on markers and QTLs associated with salt tolerance is limited due to the complexity of the genome and lack of information. However, several successful efforts were made to identify the salt-tolerant QTLs in sorghum crops (Tang et al. 2015; Wang et al. 2017).

3.5 Transcriptome Approach to Develop Salinity Tolerance in Sugarcane

The sum of the total transcript expressed in tissue during a specific time helps detect the pathway and regulatory proteins for salinity tolerance. RNA sequencing has been used to study the plant transcriptome, and therefore, analysis of gene expression is fundamental to transcriptome study. Several non-coding RNAs, such as miRNAs, small RNAs, si-RNAs, and long non-coding RNAs play significant roles in regulating key genes in sugarcane during abiotic stresses (Khraiwesh et al. 2010). MicroRNAs are small (20–24 nts) non-coding RNAs derived from long hairpin-like structures. In cell cytoplasm, these RNAs assemble as RNA-induced silencing complex (RISC), directing towards the target miRNAs, which get degraded or repressed. The expression of these miRNAs varies according to environmental conditions or abiotic stress (Sunkar et al. 2012). RNA sequencing was used in six sugarcane varieties to study the gene expression. It generated 72,269 unigenes; of which 35,456 had shown similarity to viridiplantae and the high percentage of unigenes did not show similarity to the database of viridiplantae, this finding highlights the possibilities and efforts of discovering new genes in sugarcane (Dharshini et al. 2016; Meena et al. 2020; Ferreira et al. 2016; Brenes et al. 2020). Understanding gene expression and its products during salinity stress in sugarcane help in targeting the key pathway involved in response to salinity tolerance. Eighty-nine conserved miRNAs have recently been identified in sugarcane tolerance to salinity stress using high-throughput sequencing of small RNA in five sugarcane genotypes (Mariana et al. 2013). These findings will help develop the molecular markers for salinity resistance and will be helpful in enhancing the sugarcane breeding programs towards abiotic stress.

3.6 Tissue Culture Technique for In Vitro Selection of Salinity Tolerant Sugarcane

In coming years, the tissue culture approaches have been beneficial for developing stress-resistance plants. Tissue culture techniques are ideal to get the desired variant under in vitro conditions. Many salinity tolerant variants in sugarcane calluses using embryogenic calli were identified. Similarly, in sugarcane, several somaclonal variants tolerant to salinity stress are identified using in vitro conditions. A researcher in Florida has identified the salt-tolerant variant from embryogenic calli

in sugarcane variety CP48-103 using different salinity levels such as 0.2–0.8% of NaCl (Mahmoud et al. 2011; Tanimoto 1969). Some molecular factors are being used for genetic engineering of stress-tolerant plant-like overexpression of specific transcription factors, expression and characterization of molecular chaperon including novel boiling stable homo-oligomeric sp1 protein, overproduction of osmoprotectant of water channel protein and ion transporter expression, and characterization of dehydrin protein. Among these are in vitro propagation, characterization, and identification of molecular markers, despite being used in genetic engineering for specific traits. Mutation induction that can enhance genetic diversity, followed by in vitro or in vivo selection has been broadly used and resulted in advance cultivars that are resistance stresses in a variety of crops. Physical and chemical mutations in plants use physical mutagens such as x-ray radiation or gamma rays, as well as chemical mutagens such as colchicine and EMS, to produce mutants. Because the mutations are random, the new genotypes produced by mutation induction are extremely different. In the in vitro selection procedure, particular select agents can be used to select mutants.

3.7 Genetic Engineering for Salinity Tolerance in Sugarcane

The sugarcane genome is more complex due to its polyploidy nature, and it limits the genetic improvement through traditional methodology in sugarcane breeding. Therefore, the creation of genetic variability through mutation, and genetic transformation are seen as available options to incorporate the salinity tolerant traits in potential sugarcane variety otherwise susceptible to salinity stress. In vitro culture of sugarcane has the great potential to generate somaclonal variants from regenerated plants. However, during the micropropagation, the effect of epigenetic variation of somaclonal variants was overcome systematically in micro-propagated sampling. The range of important traits, including herbicide resistance, salinity and drought tolerance, and resistance to major insects and diseases, are the many successful examples of the transgenic approach in sugarcane.

Sugarcane can be genetically engineered through micro-projectile bombardment, electroporation, or *Agrobacterium*-mediated transformation methods. Genetic improvement for salinity stress resistance in sugarcane plants has been achieved either by transferring a single or multiple or pyramiding genes. High-throughput sequencing of small RNA transcriptome reveals salinity stress-regulated mRNAs and their targets in sugarcane (Bottino et al. 2013).

A number of proteins associated with lignification, pathogenic disease, and environmental stresses in plants are found in the dirigent and dirigent-like family of proteins. The expressed dirigent-like gene designated (*ScDir*) (JQ622282) protein had enhanced the host cell's resistance to PEG and NaCl and recorded significantly higher expression in sugarcane stems than that in the roots, leaves, and buds (Jin-long et al. 2012).

Under H_2O_2, PEG, or NaCl stress, the *ScDir* transcript levels enhanced in sugarcane plants. *ScDir* expression was dramatically increased in response to PEG

stress, with the greatest level found at 12 h after stress condition. Both the elevated expressions in sugarcane and the ScDir-hosted cell performance suggest that the *ScDir* gene is implicated in the response to limited water supply, salinity, and oxidation. The real-time qPCR demonstrated that the ScDir gene transcription is more stem-specific (Jin-long et al. 2012). Brindha et al. (2021) also reported the tissue-specific gene expression of the salt overly sensitive (SOS) genes in the tolerant genotype (Co 85019) and susceptible genotype (Co 97010).

Genome research has mostly been limited to model plants that meet specific requirements, i.e., small genome size, short generation time, small size to enable growth in confined space, and the accessibility of gene manipulation tools. Several studies have highlighted the importance of the undiscovered protein genes, which make up a major fraction of most genomes. *Scdr1* is a stress-resistance protein that protects cells and the entire plant. *Scdr1* could be employed in biotechnological approaches to develop sugarcane genotypes that are more resistant to water and salt stress.

A novel sugarcane drought-responsive 1 (*Scdr1*) gene isolated from sugarcane was overexpressed in transgenic tobacco plants. These transgenic tobacco lines showed resistance to water, saline, and oxidative stresses by modulating the physiological and biochemical parameters such as enhanced photosynthetic responses, content of water, mass, germination frequency, photosynthetic pigments, and decreased ROS accumulation. Leaf gas exchange responses, i.e., rate of transpiration (E), photosynthetic CO_2 assimilation rate (P_N), stomatal conductance (gs), and internal leaf CO_2 level (Ci) were compared with wild-type plants (Begcy et al. 2012). The remarkable achievement was made with the overexpression of Arabidopsis Vacuolar Pyrophosphatase (AVP1) in sugarcane. The transgenic lines exhibited effectiveness subjected to salinity and limited water supply with improved production of newly develop leaves and increased growth after the restoration of control conditions (Kumar et al. 2014). This was also achieved by including intronic fragments in the AVP1 gene, and in turn, higher expression of AVP1 was recorded in sugarcane transgenic lines compared to control. Transcriptional regulator of the ethylene-responsive factor SodERF3 from sugarcane (*S. officinarum* L. cv Ja60-5) cDNA encodes a 201-amino acid DNA-binding protein induced by ethylene as well under salt stress and wound conditions. Transgenic tobacco lines overexpressed with SodERF3 displayed increased resistance to water and osmotic stresses (Trujillo et al. 2008).

Heat shock proteins (HSPs) play an important function in plant stress tolerance. HSP70 gene isolated from *E. arundinaceus* and driven by Port Uvi2.3 promoter was introduced in sugarcane variety (Co 86032) through *Agrobacterium*-mediated approach. The results indicated that EaHSP70 played a vital role in sugarcane acclimation to water and saline stress condition by enhancing the cell membrane thermo-stability and upregulation of stress-responsive genes. This study identified HSP70 as a potential candidate for genetic engineering of sugarcane for developing stress-resistance strategies (Al-Whaibi 2011; Augustine et al. 2015a).

Augustine et al. (2015b) introduced the pea DNA Helicase45 (PDH45) driven by Port Ubi 2.3 promoter into sugarcane variety, i.e., Co 86032 through the *Agrobacterium*-mediated application. The analysis of V_o and V_1 plants for resistance to soil moisture stress exhibited significantly excess cell membrane thermostability, transgene expression, photosynthetic pigments, relative water content (RWC), and photosynthesis. Further, pyramiding of PDH45 gene with *EaDREB2* increased resistance capacity to water and salinity stress (Augustine et al. 2015c).

A new gene, BcZAT12 from *Brassica carinata,* was constitutively expressed in sugarcane. The transgenics were analyzed for agronomic performance and revealed that growth, development, and vigor, RWC, P_N, E, gs, chlorophylls, proline, and glycine betaine level were increased in the stress-resistance transgenic plants compared to normal plants. The SoMYB18 gene isolated from *S. officinarum* was transferred into tobacco. Compared to un-transformed tobacco plants, SoMYB18-expressing plants exhibited notably enhanced resistance efficiency to salinity and water deficit condition through modulation of activities of SOD and CAT in transgenic plants, as well as proline accumulation and chlorophyll level were considerably excess and lower MDA during salt stress (Shingote et al. 2015).

Methylglyoxal (MG) is a highly cytotoxic metabolite accumulated due to abiotic stresses in plants. It can complete the cellular destruction laidback by inducing advanced glycation end products (AGEs), oxidation of fatty acids, and commotion of membrane structures or functions. This MG is detoxified by the consecutive action of Glyoxalase I (Gly I) and Glyoxalase II (Gly II) in the presence of glutathione (GSH) as a cofactor and by the action of single gene Glyoxalase III (Gly III) without any cofactor. These genes were differentially modulated in *Saccharum* and *Erianthus* expression during environmental stresses (Manoj et al. 2019). Overexpression of Gly III from *E. arundinaceus* in sugarcane significantly enhanced P_N, gs, and E during salinity stress. Additionally, transgenic events overexpressing the *EaGly III*gene also showed improved PAR and Fv/Fm ratio compared to WT (Manoj et al. 2021).

Sugarcane breeding strategy to improve salt tolerance is ineffective due to difficulty in hybridization and risk of transfer of other undesirable traits. Hence to avoid this problem, a transgenic approach is preferred, which deals with the specific gene(s) of interest. Sugarcane plants can cope with salinity stress by inducing various metabolic changes such as the production of antioxidative enzymes, osmolytes, and up-regulating several genes like ion transporters, transcriptional factors, ion channels, and various signalling pathways associated with salt resistance. Many genes are known to confer salinity tolerance when transferred in plants through a genetic engineering approach. A list of several such transgenes has been given in Table 3.1.

Table 3.1 Genes used for salinity resistance in sugarcane

Name of gene	Abiotic stress	Methodology	References
miR159-MYB protein *miR169-HAP12*-CAAT-Box TFs	Saline	Transcriptomics	Bottino et al. (2013); Hu et al. (2012)
Sugarcane drought-responsive(*Scdr1*)	Water, saline, and oxidative	Transcriptomics	Begcy et al. (2012)
Sugarcane dirigent protein gene(*ScDir*)	Drought, saline, oxidative	Transcriptomics	Jin-long et al. (2012); Cho et al. (2006)
Arabidopsis Vacuolar Pyrophosphatase (*AVP1*)	Water and saline	Transgenic	Kumar et al. (2014)
Erianthus arundinaceus DREB2 (*EaDREB2*) and EaHSP70 and, pea DNA helicase45(*PDH45*)	Water and saline	Transgenic	Augustine et al. (2015a, b, c)
Sugarcane ethylene-responsive factor (*SodERF3*)	Water and salt	Transgenic	Trujillo et al. (2008)
BcZAT12	Water and salinity	Transgenic	Saravanan et al. (2018)
Sugarcane MYB(*SoMYB18*)	Salinity and dehydration	Transcription factor	Shingote et al. (2015)
Glyoxalase III	Salinity	Transgenic	Manoj et al. (2021)

3.8 Management of Sugarcane Production Under Saline Conditions

Rozeff (1999) has cited the importance of Crawley's (1902) work regarding the regular irrigations combined with intermittent leaching for maintenance of soil from the continuous accumulation of salts in low rainfall areas of Hawaiian sugarcane field. Management of salinity mainly depends upon the depth of the water table (WT), i.e., water table lesser than 100 cm often causes more upward movement of salts, causing severe implications on sugarcane crops. Sundara and Vasantha (2004) have discussed an integrated approach for the management of sugarcane during salinity for better yield, and their approach includes (1) a higher seed rate of 25% is to compensate for germination reduction and proper establishment, (2) modified trench method of plant in NaCl contaminated soils, and saltwater irrigated areas have recorded enhanced productivity of around 15% (Fig. 3.3), (3) organic manures, viz. press mud (10–15 t/ha), farmyard manure (25 t/ha), and bioearth enhance the accessibility of essential nutrient, viz. Zn, Fe, Ca, Mg, and Mn. In calcareous soil, the organic manures decrease the soil pH, electrical conductivity (EC), and exchangeable sodium (%) rendering the soil most suitable for growing sugarcane,

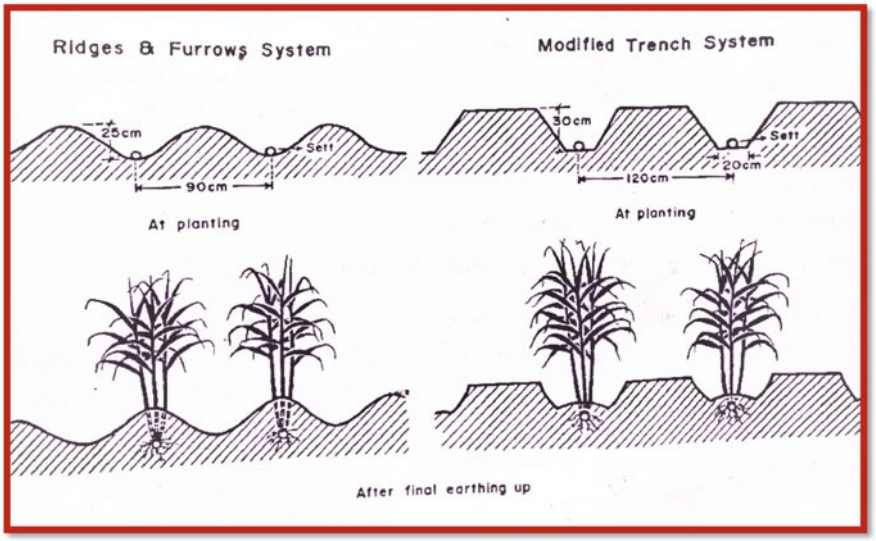

Fig. 3.3 Modified trench method of plant in saline soils and saltwater irrigated areas (Adapted from Sundara and Vasantha 2004)

(4) application of gypsum 3–6 ton/ha is sufficient for most of the soil, and the gypsum requirement varies accordingly with pH, (5) good quality of irrigation water during critical stages (up to 150 DAP) will benefit the crop growth, (6) growing of green manures and additional nutrient application, (7) crop rotation with salt-tolerant crops, viz. cotton, mustard, (8) growing tolerant varieties, viz. Co 0403, C0 0218, Co 99004, Co 2001-13, Co 94012, Co 85019, etc. (Vasantha et al. 2017; Kumar et al. 2017).

Several sugarcane clones have been reported as saline tolerant (Hemaprabha 2008). Recently, Ram (2017) has documented various genetic stocks for evolving climate-resilient (drought, salinity, and waterlogging stress tolerance) sugarcane varieties for future sugarcane agriculture.

3.9 Conclusion

Salinity resistance is a complex trait, and the responses of plants to saline stress are variable at physiological, molecular, metabolic, cellular, and whole-plant levels. Improvement of sugarcane for salinity tolerance through conventional breeding and agronomic practices was adopted since its nobilization. However, considering the present climate change scenario and increasing future demand for cane, there is a great need to use the recent molecular tools and techniques to develop salt-tolerant sugarcane varieties. Successful genetic manipulation of sugarcane using modern techniques such as molecular marker-assisted selection, multi-omics technologies

such as transcriptomics, proteomics, and genomic approach, genome-editing, and genetic transformation has excellent potential for genetic improvement of cane. The transgenic approach has improved the possibility of transferring candidate genes for salinity tolerance. Many salt-tolerant molecular markers such as ISSR, RAPDS, SSRs, and QTLs have been successfully identified and widely used in several crops, including sugarcane, to improve the adaptability of the cane against salinity stress and other abiotic stresses. Omics technologies like transcriptomics, proteomics, and genomics have been successfully employed in sugarcane to ensure cane productivity in a sustainable way under the changing climatic conditions.

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Potential Parents for Developing Climate-Resilient Sugarcane Varieties in India: A Breeding Perspective

4

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Abstract

Under the changing climatic conditions due to aberrations of weather parameters, there is a change in the microclimate *vis-a-vis* change in the pest and disease scenario of crops that are widely cultivated. Sugarcane, being a long-duration crop grown on a larger scale across many states in India, often encounters the vagaries of weather conditions over the seasons. Several strategies and workable solutions are available to mitigate the climate-induced stresses in crops in general and sugarcane in particular. Evolving climate-resilient sugarcane genetic stock or parental clone is one of the focused breeding objectives with the ultimate purpose of evolving climate-resilient commercial sugarcane cultivars. Sugarcane breeders worldwide emphasize evolving sugarcane cultivars that can withstand different stresses posed by biotic and abiotic factors. Red rot, which was once considered an important disease in the subtropical region of India, is now a major disease in the tropical region of the country as well and phasing many high sugared and high-yielding varieties. Several sugarcane varieties especially those from sub-tropical India and basic species clones of *Saccharum* and related genera like *Erianthus* have been recognized as stable parents with respect to resistance to the predominant isolates/pathotypes of *Colletotrichum falcatum* prevailing in India. In India, sugarcane is also affected by another significant disease, i.e., smut caused by *Sporisorium scitamineum*, and the disease is much more pronounced in the ratoon crop than in the plant crop. Sources of resistance to smut were identified in *S. officinarum* clones and could be efficiently utilized in the commercial breeding program. Very few diseases like pokkah boeng, rust, sugarcane grassy shoot and viral syndromes may assume greater importance if the macro and micro-climates are altered drastically. Besides the fungal diseases, yellow

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leaf disease (YLD) has inflicted more damage than other viral diseases. Incorporation of coat protein genes through transgenic or RNAi technology is being viewed as practical strategy to control YLD. Occurrence of drought, erratic rainfall necessitates developing drought tolerant varieties. Co, Co allied and other varieties and inter specific hybrids can be used as drought tolerant parents. Sugarcane grown under arid and semiarid region is subjected to salinity. Among the species clones, *S. spontaneum, S. robustum* and Narenga were flood tolerant while *S. robustum* is better at high temperature. Thermo-insensitive genotypes identified from varietal evaluation trials performed equally in both the extremes of temperature. To breed varieties with for higher winter ratooning ability, it is suggested to have one of the parents of subtropical origin. Among the different crossing methods, the bi-parental crossing is the most effective method in commercial sugarcane improvement. Development of pre-breeding materials or genetic stock with greater tolerance intensity by utilizing the wild species or intergeneric hybrids followed by bi-parental crossing between parents of diverse origin, where one parent with resistance to different biotic and abiotic stresses is the way forward to evolve climate-resilient sugarcane varieties.

Keywords

Breeding · Climate change · Diseases · Environmental stress · Sugarcane · Stress resistance

4.1 Introduction

Energy is an absolute requisite for maintaining the structural organization of any organism. This energy provides the dynamic drive for performing important biological processes like cellular biosynthesis and transport to take care of its characteristic structure and organization by being in the homeostatic state, which is a steady and metastable condition. The sudden changeovers from optimal to suboptimal condition disrupt this stable condition leading to adverse effects on the physiology of a plant.

The stress encountered by the sugarcane plant is often classified as biotic stress caused by pests and diseases creating biological slur during its lifetime and abiotic stress imposed by the environmental factors causing physical or chemical pressure (Verma et al. 2020a, 2021a). Extreme weather events like heatwaves, droughts, heavy rains, and floods due to global climate change are inevitable and unpredictable. The changes in precipitation and rate of evapotranspiration, frequency of droughts, floods, and cyclones will negatively impact agricultural production in India. The winter precipitation is expected to undergo drastic changes hence it will increase the demand for water for Rabi crops, i.e., grown in winter season (Jain 2012). Kharif crops (grown in rainy season) production will also need to deal with heavy floods and drought (Shah 2009). Increased temperature will favor the expansion of weeds and their shift to the upper latitudes. As a result, environmental stress

on crops may increase, making them more susceptible to insects, pathogens, and weeds. The excessive weed growth will hamper the yield of crops which can have negative impact on the national income. India is very sensitive to global climate change in terms of its effect on the water system for irrigation needs (Mall et al. 2006; Verma et al. 2019a, 2021b, c). Sugarcane, a long-duration crop, faces extreme weather conditions of all seasons. It is challenging and impracticable to provide an environment conducive to different phases of crop growth and maturity.

The changing climate in a particular locality causes aberrations in weather parameters resulted in change in the occurrence of pests and disease outbreaks faced by sugarcane. It has been noticed that the majority of dominant diseases in a region have abated and minor ones are becoming an area of prime concern. Red rot is now devastating the varieties in the tropical region. Similar is the case with smut disease. Minor diseases like pokkah boeng, rust, sugarcane grassy shoot and viral diseases are assuming greater importance. Among the viral diseases, yellow leaf disease (YLD), which was identified first in India in 1999 caused by the sugarcane yellow leaf virus, has caused more damage than any other virus in India since the increase in temperature during the maturity stage of plants was conducive for the development of this disease (Viswanathan 2002).

Interestingly, it has been observed that temperature has a significant correlation with YLD severity. Hence, the traits to be given importance within the context of vagaries of weather and changing biotic stresses in sugarcane breeding are resistance/tolerance to different stresses caused by biotic and abiotic factors. Mitigating these conditions is important to realize higher yield in a stressed environment.

The sugarcane breeding program is a highly networked activity in India. A standard facility called National Hybridization Garden (NHG) at Sugarcane Breeding Institute, Coimbatore has been established to impact crosses to produce hybrid seeds because of profuse flowering and good seed set in sugarcane reported in Coimbatore. However, the flowering of sugarcane with some level of seed set was also observed in other places like Pusa in Samastipur (Bihar) developing BO (Bihar–Orissa) clones, Seorahi and Shahjahanpur (Uttar Pradesh) developing CoS, CoSe and UP clones, Mandya and Hebbal in Karnataka developing KMS and KHS clones and Sirugamani in Tamil Nadu developing TNAU (SC)Si (Tamil Nadu Agricultural University Sugarcane, Sirugamani) varieties, and Amboli in Maharashtra (VSI clones). The flowering and seed set observed at these places provide a little opportunity for the breeders to breed local specific varieties for these states. Hence for evolving new improved varieties, sugarcane breeders throughout the country utilize the genetic variability present within the NHG available at ICAR-SBI, Coimbatore, to spot the source of resistance to different stresses like red rot, smut, stalk borer, drought, salinity, waterlogging, coldness, and heat and winter ratooning ability (Durai et al. 2015). The current effort was made to collate the knowledge available on the source of resistance/tolerance to different biotic and abiotic stresses and their potential utilization in the sugarcane improvement program.

4.2 Biotic Stresses

4.2.1 Red Rot

Varietal failure and degeneration in sugarcane in India are mainly caused by red rot. Many varieties like Co 210 from Bihar, Co 213 from eastern Uttar Pradesh, Co 312 and Co 313 from Punjab, Co 419, Co 997 and Co 62175 from Andhra Pradesh, Co 658, Co 6304, and CoC 671 from Tamil Nadu, Co 419, Co 7805, and Co 997 from Kerala, and CoJ 64 from Punjab and Haryana were eliminated from commercial cultivation because of the epidemics of this disease. Viswanathan (2010) and Viswanathan et al. (2021) reported that varieties like CoS 8436, CoSe 95422, CoSe 92423, BO 138, CoSi 6, CoV 94101, 91V 83, Co 7805, etc. released during the last decade have been became susceptible to the red rot pathogen.

4.2.1.1 Inheritance of Red Rot Resistance

Red rot resistance in sugarcane is governed by a few genes with additive effects (Chaudhary et al. 1986). The inheritance of red rot resistance in sugarcane is indiscriminate, where crosses between susceptible parents sometimes produce resistant progenies (Chona and Srivastava 1960). The inheritance pattern of red rot resistance varied from cross to cross. The proportion of resistant progenies obtained from the crosses involving both the resistant parents was high. In contrast, when one of the parents was susceptible, relatively a good number of progenies were resistant, and when both the parents were susceptible, most of the progenies were susceptible (Babu et al. 2010). Different segregation ratios of resistance to susceptibility such as 1:3, 3:1, 1:5, 4:1, and 2:1 were reported. The role of the masking gene (M) was also reported (Chaudhary et al. 1986; Ram et al. 2001; Alarmelu et al. 2010). Susceptible × susceptible cross had given some resistant progenies and vice versa. The segregation ratio does not fit into the expected Mendelian ratio due to the high heterozygous nature of parents as well as peculiar cytological behavior in this crop. A few gene controls of red rot inheritance have been postulated by different authors (Azab and Chillon 1952; Babu et al. 2010; Alarmelu et al. 2010). Both additive and dominance variance with high heritability were found equally important in governing red rot resistance (Ram et al. 2005a, b), indicating the negligible influence of the environment.

4.2.1.2 Sources of Red Rot Resistance

Among the various red rot disease management strategies, the development of resistant varieties is important. It is vital to use resistant parents to develop resistant varieties. For this, sugarcane breeders need a source of resistance for red rot disease (Sreenivasan and Alexander 1971; Natarajan et al. 2001). The large number of clones with red rot resistance are available in *S. spontaneum* germplasm at ICAR-SBI, Coimbatore (Alexander et al. 1983). Remarkably few clones of *S. officinarum*, *S. barberi*, *S. sinense*, and *S. robustum* showed resistance to red rot (Alexander 1987; Malathi and Viswanathan 2012), of course, the number of red rot-resistant clones in

S. barberi was relatively more as compared to other cultivated species (Alexander and Rao 1976).

In a recent study, 417 clones, including the parents from 20 sugarcane breeding centers in India, 18 foreign hybrids from Barbados, Canal Point, Java, Natal, Queensland, and SauPaulo, and 39 interspecific hybrids were screened for their extent of resistance against important isolates of red rot pathogen collected from the tropical and subtropical regions of India. Among the parents studied, 83 exhibited either R or MR reactions to all the studied isolates from the tropical and subtropical region. Only one parent, viz. CoS 07231, developed by the U.P Council of Sugarcane Research, Shahjahanpur was found to be resistant to both the tropical and the subtropical pathotypes. Thirty-eight parents, viz. BO 109, BO 120, BO 130, BO 96, CoP 9206, and CoP 9302 from Pusa, Co 0121, Co 0240, Co 06036, Co 8353, Co 87271 Co 97015 HR 83–65, ISH 101, ISH 228, and ISH 267 from ICAR-SBI, CoH 12 and CoH 14 from Uchani, CoJ 72, CoJ 80 and CoJ 84191 from Jalandhar, CoLk 94184 from Lucknow, CoPant 01215, CoPant 88220, CoPant 90224 and CoPant 94213 from Pantnagar, CoS 8432, CoS 93278, CoS 94270, CoS 95270, CoS 96260, CoS 97264, UP 22, UP 39 and UP 5 from Shahjahanpur and CoSe 92423 and CoSe 95427 from Seorahi were showing stable resistance behavior of moderately resistant to tropical and subtropical pathotypes. Only one parent of exotic origin showing MR reaction to both isolates was CP 61–23 from Canal Point. Similar to this study, Viswanathan et al. (2018) reported that among the 281 ISH clones evaluated, 35 including ISH 7, ISH 100, ISH 135, ISH 146, ISH 286, ISH 314, ISH 421, ISH 425, ISH 177, ISH 241, ISH 243, ISH 263, ISH 265, and ISH 268 had a diverse genetic background and were found resistant to three pathotypes of subtropical origin.

Similarly, UP 12 and UP 15 were reported to show a moderately resistant reaction to all the stains of red rot pathogen (Singh and Singh 1989). Parents exhibiting resistance to tropical and subtropical isolates of red rot pathogen may have greater utility in the breeding program. Sources of red rot resistance present in the NHG maintained at ICAR-Sugarcane Breeding Institute, Coimbatore, India, are given in Table 4.1.

Parents showing consistent resistance to various isolates of red rot pathogen were from sugarcane breeding centers located in the subtropical region of the country. The parent HR 83-65 developed through a specific program aiming at horizontal resistance was the only parent from the tropical region showing firm resistance to CF06, CF12 and CF08 of *C. falcatum*. The present-day commercial varieties are selected from interspecific hybrids of *S. officinarum*, *S. spontaneum*, *S. sinense*, *S. barberi*, and *S. robustum*. Among these species, *S. spontaneum* has been used for genes of hardiness that impart the sugarcane genotypes resistant to various adverse climatic conditions and pest and diseases and biomass. Conscious selection for such traits in the subtropical belt might have resulted in the retention of more of *S. spontaneum* alleles. While studying the Indian varieties using the AFLP technique, Selvi et al. (2006) proved that genotypes grown in subtropical India where the crops face extremities of climatic conditions like high and low temperature, and salinity retained more of *S. spontaneum* genome than their counterparts grown in tropical

Table 4.1 Sources of red rot resistance available in the national breeding gene pool for sugarcane improvement in India (Durai et al. 2021)

States of breeding centers	Parents resistance to subtropical isolates	Parents resistance to tropical isolates
Anakapalle, Andhra Pradesh	CoA 8401, 69 A 591, 72 A 66, CoA 7701, CoA 8402	–
Buralikson, Assam	CoBln 03174, CoBln 03176, CoBln 05502, CoBln 94063, CoBln 03175, CoBln 05501	CoBln 03176, CoBln 05502
Co canes, Coimbatore—Tamil Nadu and Karnal—Haryana	Co 62174, Co 86011, Co 87045, Co 87263, Co 87268, Co 87272, Co 87273, Co 88039, Co 89010, Co 89029, Co 91002, Co 91019, Co 93009, Co 99006, Co 0116, Co 0424, Co 06032, Co 06035, Co 06037, NB 940545, Co 312, Co 976, Co 1305, Co 62399, Co 7201, Co 7204, Co 7704, Co 7910, Co 8013, Co 8208, Co 8316, Co 8338, Co 8340, Co 8353, Co 85019, Co 85033, Co 85246, Co 86250, Co 87021, Co 87267, Co 87269, Co 87271, Co 89036, Co 90006, Co 91010, Co 92008, Co 92020, Co 97015, Co 98016, Co 98017, Co 0120, Co 0121, Co 0238, Co 0240, Co 0331, Co 05010, Co 06036, HR 83-65	Co 87273, Co 89010,C0 89029, Co 93009, Co 87271, HR 83-65, Co 87045, Co 87272, Co 0121, Co 0240, Co 06036, Co 97015, Co 06032, Co 06035, Co 06037, Co 87263, Co 8353, Co 05011, NB 94-545
Cuddalore, Tamil Nadu	C 81615, CoC 774, CoC 775, CoC 778, CoC 773, CoC 779, CoC 8201	C 81615
Jalandhar, Punjab	CoJ 86, CoJ 89, CoJ 46, CoJ 58, CoJ 72, CoJ 77, CoJ 80, CoJ 83536, CoJ 84191, CoJ 84291, CoJ 85, CoJ 87	CoJ 72, CoJ 980, CoJ 84191
Lucknow, Uttar Pradesh	CoLk 9412, CoLk 96029, CoLk 9618, LG 01116, LG 99001, LG 991, LG 99112, CoLk 94184, CoLk 97022, CoLk 97154, LG 641	CoLk 94184, LG 0120, CoLK 9618, LG 01116, LG 99001, LG 991 and LG 01014
Navsar, Gujarat	CoN 85134	CoN 85134
Padegaon, Maharashtra	CoM 6615, CoM 6806, CoM 7712, MS 68/47, CoM 7704, CoM 88121, CoM 9206	CoM 6806 and MS 68/47
Pantnagar, Uttarakhand	CoPant 01215, CoPant 84213, CoPant 88220, CoPant 90224, CoPant 94213, CoPant 96219	CoPant 01215, CoPant 88220, CoPant 90224, CoPant 94213, CoPant 90223, CoPant 92226
Perumalapalle, Andhra Pradesh	CoT 8201	–

(continued)

Table 4.1 (continued)

States of breeding centers	Parents resistance to subtropical isolates	Parents resistance to tropical isolates
Powarkheda, Madhya Pradesh	CoJaw 70	–
Pusa, Bihar	BO 102, BO 110, BO 128, BO 47, BO 78, BO 89, BO 91, BO 97, CoP 9301, BO 109, BO 120, BO 130, BO 32, BO 68, BO 92, BO 96, CoP 9206, CoP 9302	BO 108, BO 109, BO 120, BO 130, BO 96, CoP 9206, Co 9302, BO 128, BO 47, BO 89, BO 91, BO 97, CoP 9301
Rudrur, Telangana	79 R 207, 87 R 40, 97 R 401	79 R 207
Sankeshwar, Karnataka	CoSnk 03–044, CoSnk 05103	CoSnk 03-044, CoSnk 05103
Shahjahanpur, Uttar Pradesh	CoS 07231, CoS 633, CoS 796, CoS 8119, CoS 88216, CoS 91269, CoS 95255, CoS 96275, CoS 97261, CoS 99259, UP 40, UP 48, UP 9529, UP 9530, CoS 8315, CoS 8432, CoS 92263, CoS 93278, CoS 94270, CoS 95270, CoS 96260, CoS 97264, UP 0097, UP 1, UP 22, UP 39 and UP 5	CoS 07231, CoS 8432, CoS 93278, CoS 94270, CoS 95270, CoS 96260, CoS 97264, UP 22, UP 39, UP 5, CoS 88216, CoS 91269, CoS 95255, CoS 99259, UP 40, UP 9529, UP 9530, CoS 90269 and S 4396/03
Seorahi, Uttar Pradesh	CoSe 95436, CoSe 92423, CoSe 95427, CoSe 96436, CoSe 98231	CoSe 92423, CoSe 95427 and CoSe 95436
Thiruvalla, Kerala	Thirumadhuram, CoTl 85119, Madhumathi, Madhuri, Madhurima	–
Uchani, Haryana	CoH 102, CoH 106, CoH 13, CoH 76, CoH 99, CoH 104, CoH 112, CoH 12, CoH 14, CoH 15, CoH 92	CoH 12, CoH 14, CoH 106, CoH 13
Vuyyuru, Andhra Pradesh	CoV 06356, CoV 09356	CoV 09356
ISH clones	ISH 110, ISH 136, ISH 176, ISH 287, ISH 100, ISH 101, ISH 135, ISH 156, ISH 2, ISH 228, ISH 267, ISH 28, ISH 306, ISH 307	ISH 101, ISH 228, ISH 267, ISH 139, ISH 110, ISH 176, ISH 287, ISH 111and ISH 12
Foreign clones	Q 65, SP 80–3250, SP 83–5073, CP 52–256, CP 61–23, CP 63–326, SP 80–185	CP 61-23

India. Comparatively higher amounts of *S. spontaneum* alleles in the subtropical varieties may have contributed to the higher resistance level. Hence, it is essential to introduce diverse and novel genes of *S. spontaneum* in the sugarcane genome to develop resistant varieties.

S. spontaneum has been utilized by breeders for developing superior sugarcane varieties. However, only two accessions of *S. spontaneum*, viz. Coimbatore and Java

Table 4.2 Status of red rot resistance in ISH and IGH clones of sugarcane developed by ICAR-SBI, Coimbatore (Viswanathan et al. 2018)

Class of germplasm	No of genotypes evaluated	R and MR	MS	S and HS
Improved ISH clones	216	8	35	173
Cytoplasmic clones crossed with commercial varieties	462	155	89	221
Cytoplasmic diverse and back cross clones	131	53	18	60
Erianthus-sugarcane hybrid derivatives	30	15	5	10

forms, have been utilized to develop commercial varieties. Utilizing the unutilized *S. spontaneum* and other clones of *Saccharum* complex, viz. Baragua, H.M. Black, Saipan-G, Seleri, 28 NG 4, 28 NG 266, 57 NG 77 of *S. officinarum* and Lalri *of S. barberi* may yield a higher proportion of resistant progenies. Improvement of red rot resistance of susceptible clones by incorporation of the resistant gene from *S. spontaneum* was demonstrated by testing the progenies of the crosses involving Co 7201, a susceptible parent and IND 82-319, IND 82-254, SES 147B, SES 148, and SES 137 B which gave higher number of resistance progenies. Among the crosses involving various CD clones with different *S. spontaneum* cytoplasm BC 27 × CoT 8201 showed a high level of red rot resistance (52.9 %) followed by CD 11 × CoC 8001 (34.6%) and CD 04-79 × CoC 8001 (33.3%). Screening of the derivatives involving *S. officinarum* × *Erianthus arundinaceus* and *S. spontaneum* × *Erianthus arundinaceus* hybrids revealed that all the five progenies of GU 01-572 × BO 99 were resistant while GU 00-858 × Co 96011 gave 38.9% resistant types out of 18 progenies. Among the 1081 half-sib progenies of 33 crosses, resistance progenies were more within the crosses 987032 × Co 93009, (87.5%), 987042 × Co 7301 (84.2%), and RS 93-2182 × Co 930009 (81.3%) and there is no susceptible progenies in the crosses 987042 × Co 87301 and RS 93-2182 × Co 93009. After evaluating the 462 CYM hybrids, it was observed that 86% of the clones derived from CYM 07-649 × Co 89029 were found to be resistant. Other CYM clones yielding a higher number of progenies resistant to red rot were CYM 07-986, CYM 08-314, CYM 07-941, CYM 07-649, and CYM 07-871 (Viswanathan et al. 2018). The reaction of parents/progenies to the red rot pathogen under different category parental classes is given in Table 4.2.

Singh et al. (2019) identified Co 62198, Co 89003, Co 0238, CoS 8436, CoS 95255, CoS 96360, Co 08272, and CoSe 92423 as resistant parents having the excellent general combining ability. The combinations involving these parents produced mostly resistant progenies in the range of 40–100%. Virk et al. (1985) stated that Co 7314 and Co 7704 as good general combiners which transmit resistance into 80.0–84.6% progenies. In another study, Co 7201 was found as an excellent general combiner and could transmit its resistant behavior to most of its progenies (Alarmelu et al. 2010). Parents exhibiting resistance to tropical and subtropical isolates of red rot pathogen may have greater utility in the breeding program.

4.2.2 Smut

Next to red rot, sugarcane is affected by another disease called smut. This disease is more pronounced in the ratoon crop than in the plant crop. Smut was of concern only in Asia until the 1950s and some incidence in Argentina. Later, it spread to South, Central, East, and West Africa, Hawaii, the Caribbean, the mainland USA, Central America, Southern Brazil, Morocco, Iran, and Australia. Breeding for disease resistance is continuous and complex progress because of the rapid emergence of new pathotypes which evolve along with the host genome, thereby overpowering the resistant varieties. This makes the cultivators unable to harvest the benefit of high-yielding varieties in many developing countries (Sundar et al. 2012).

4.2.2.1 Inheritance of Smut Resistance

Kandasamy et al. (1980) suggested that a few significant genes might control resistance to smut. However, in other studies, quantitative genes influenced smut resistance (Walker 1980; Wu et al. 1983). Resistance to smut is a trait of moderate heritability, and a high frequency of progenies with smut resistance was produced in the crosses (R × R) where both parents were resistant while the resistant behavior of other types of crosses was erratic (Chao 1988).

4.2.2.2 Sources of Smut Resistance

Characteristics of host plants thought to be involved in resistance are bud anatomy, bud scale fungi toxic substances, and plant physiology. Chao et al. (1990) reported that resistant parents enhance the percentage of resistant progenies in sugarcane cultivars and breeding lines. Alexander (1987) reported that as many as 95 *S. officinarum* clones from the world germplasm collections were resistant to smut. Naidu and Sreenivasan (1987) evaluated five species of *Saccharum* and found that *S. officinarum* (97out of 428 clones) and *S. spontaneum* (137 out of 324 clones) had the highest and *S. sinense* (15 clones), *S. barberi* (9 clones), and *S. robustum* (3 clones) showed a lowest level of resistance against smut pathogen. Among the six groups of *Saccharum* complex, viz., *S. officinarum*, *S. barberi*, *S. sinense*, *S. robustum*, *S. spontaneum*, and *Erianthus* spp., *Erianthus* spp. section *Ripidium* showed the highest level of resistance while *S. officinarum* and *S. robustum* showed the lowest level of resistance against smut pathogen (Burner et al. 1993). In a study, a total of 79 backcross progenies (BC1 and BC2) of *E. arundinaceus* were studied for their smut resistance behavior in the artificial inoculation method. Seven BC1 and three BC2 lines of *E. arundinaceus* were found to show moderate to higher resistance levels, and they could serve as an elite source of resistance against smut (Shen et al. 2014). Among the 30 Japanese wild sugarcane (*S. spontaneum*) accessions and five cultivars, JW 90, Iriomote 8, Iriomote 15, Iriomote 28, and T16 were found resistant, and the cultivar Ni F8 was found moderately resistant to the only one race of smut pathogen prevalent in Japan (Sakaigaichia et al. 2018). Unlike the red rot resistance source, a large number of clones of *S. officinarum*, many accessions of *S. spontaneum* and *S. robustum* showed resistance to smut (Sreenivasan and Alexander 1971; Alexander et al. 1985). Apart from these hybrids, sources of resistance

Table 4.3 Source of resistance available in *Saccharum species* for smut pathogen (Sinha 2016)

Species	Source of resistant
S. officinarum	Ardjoena, Swela Green Sport, Balghat Thin, BetecLupog, Big Tanna, Striped Aubin, BandjerMasimHitam, Bois Rogue, Branchue, Bravo de Perico, Caira, Cavengerie, Fiji 15, Fotiogo, HaakKwatChe, HitamBroewang, Hawaii Original M 26, Horne Java, Hebbal, Javari Kabbu, KaludaiBoothan, Keong, Khajuria, Kham, Laukona-15, La Purple, Local red, Loethers, Mauritius-131, Ohia-1, Oidang, Badangsche, Pilimai-60, Poona, Port Mackey Black, Preanger Striped, RatgrosVentre, Red Ribbon, Rood Djapara, SS 60-1, Stripped Tip, Tahiti-3, Tamarin, Tanna, Timor Riet, ToloFua Lau-1, TomohonZwart, Tonga Tabu-6, UB-1, Vellai, NC-17, NC-24 Dark Purple, NC-25 Purple, NC-32 Sport, NC-33, 37 NG 7, 51 NG 9, 57 NG 45, IJ 76-314, IK 76-2, IM 76-245, IS 76-117, 77 NG-28.
S. barberi	Baroukha, Dhaurkinara, Hemja, Kansarkhatuia, Mankia, Sararoo
S. sinense	Reha, Kalkya, Kavangire, Maneira (IMP 1648), Mecikrum, Archi, Cayana, Merthizel, Oshima, Rounda, Tekcha-Chiki-Island, Tekcha-Chung-Island, Kukuya No.1, Uba-Del-Natal, Uba-Naquin, Uba-Reunion

against smut pathogen available in different species clones of *Saccharum* in India are given in Table 4.3.

The accessions collected from India appear to have a moderate level of resistance, whereas those from Indonesia and the Philippines were reported to get infected more than 50% (Sundar et al. 2012). Recently, in India, because of not considering the smut susceptibility/resistance during the selection process of parents, the frequency of smut susceptible clones has become high (Premachandran 2012). Smut-resistant parents present in NHG, ICAR-SBI, Coimbatore for Indian sugarcane breeders are C 79218, Co 62198, Co 6806, Co 7704, Co 8381, Co 85002, Co 85053, Co 85246, Co 86002, Co 86010, Co 84012, Co 976, CoH 110, CoSnk 05-103, Co 1148, Co 1307, Co 312, Co 356, Co 453, Co 62174, Co 7527, Co 7706, Co 7910, Co 8013, Co 8316, Co 8338, Co 8339, Co 8340, Co 8347, Co 8353, Co 8371, Co85019, Co 85033, Co 85036, Co 86249, Co 87012, Co 87021, Co 87025, Co 88013, Co 89010, Co 89036, Co 90006, Co 90018, Co 91002, Co 91010, Co 91019, Co 92002, Co 92006, Co 92008, Co 92020, Co 93003, Co 93009, Co 94003, Co 94008, Co 94012, Co 95005, Co 95021, Co 976, Co 98006, Co 99004, CoM 0265, CoN 03131, and CoN 03132.

Out of 75 breeding materials screened against the smut disease, 38 genotypes, viz. LG 12201, LG 13001, LG 13002, LG 13009, LG 15169, LG 15016, LG 15026, LG 15166, LG 15185, LG 15196, LG 15245, LG 15256, LG 15259, LG 15262, LG15265, LG 15267, LG 16067, LG 16070, LG 16098, LG 16138, LG 16140, LG 16169, LG 16170, LG 16178, LG 16181, LG 16294, LG 17127, LG 17137, LG 17156, CoLk 14201, Co 14034, CoPb 14185, Co 15025, Co 16030, CoPant 16222, CoJ 64, CoLk 7701, Co 7717 and Co 419 were rated as resistant (Singh et al. 2020). Similarly, in Australia, resistant varieties like Q99, Q133, Q146, Q149, Q151, Q171A, Q177A, Q199A, Q200A, Q212A, Q219A, KQ228A, Q232A, Q235A, KQ236A, Q238A, MQ239A, Q240A, Q241A, Q245A, Q246A, Q247A, BN73-3416, BN81-1394, Cassius, CP74-2005 and Florida are recommended for all areas of sugarcane cultivation (https://sugarresearch.com.au/sugar_files/2017/02/Control-

of-sugarcane-smut-IS13006.pdf). YZ03-258, YZ01-1413, YT96-86, and LC05-136 are the smut-resistant cultivars from China (Su et al. 2016). Eight varieties of Pakistan, viz. S2006-US-469, S2006-US-272, S2005-US-54, S2008-AUS-130, S2006-US-658, S2008-AUS-190, S2008-AUS-107, and S2009-SA-169) were found resistant to smut (Mansoor et al. 2016). Through the conscious effort of increasing the frequency of smut-resistant parents, it is possible to get better smut-resistant varieties.

Apart from resistant varieties, smut can also be managed through the application of Si, which enhanced the level of smut resistance, where smut incidence decreased from 22.58% to 11.57% in the sugarcane variety ROC22 and from 46.67 to 27.75% in Badila. Further, the smut incidence was found negatively correlated with the quantity of Si applied. The Si present in the sugarcane roots, leaves, and stems regulates biochemical processes like secondary metabolism, ROS metabolism, and pathogenesis-related protein activity (Deng et al. 2020).

4.2.3 Yellow Leaf Disease

Yellow leaf disease (YLD) is reported in more than 30 countries worldwide. Viswanathan (2015) reported the reduction of 37.23% in cane diameter, 5.03% in length of internodes, and 19.45% in juice yield due to the incidence of YLD in endemic states of India like Tamil Nadu, Karnataka, and Andhra Pradesh. Parameshari et al. (2018) reported that in the case of *Saccharum* spp., 86% of *S. robustum* were resistant to YLD, followed by *S. sinense* (80%), *S. officinarum* (78%), and *S. barberi* (76%) in the world collection of sugarcane germplasm in Kannur, India. Similarly, Comstock et al. (2001) reported the occurrence of YLD in the world germplasm collection of sugarcane at Miami, Florida, which was highest in *S. officinarum* (75.8%) followed by *S. robustum* (62.5%), *S. sinense* (46.2%), *S. barberi* (13.6%), and *S. spontaneum* (7.0%). SCYLV resistance was observed to be a dominant trait since a cross between *S. robustum* (resistant parent) and *S. officinarum* (susceptible parent) produced 85% resistant progenies (Table 4.4).

Viswanathan (2012) identified BO 91 Co 475, Co 527, Co 951, Co 62175, Co 62197, Co 622, Co 678, Co 7202, Co 7318, Co 7527, Co 87025, Co 92002, Co 92020, Co 98014, Co 0120, CoC 92061, CoH 110, CoJaw 270, CoLK 8102 CoM 6806, CoM 0265, CoSnk 03754, Q63, ISH 69, ISH 100, and ISH 176 as resistant to

Table 4.4 Identified source of resistance for YLD

Resistant source	Reference	Country
BO 91, Co 678, Co 976, CoPant 97222, CoJ 89, CoP 9302, ISH 76	Parameshari et al. (2018)	India
CC01-746, CC 01-678, CC 01-1228, CC 99-2282, CC 01-1940, and CC 93-7711	Garcés-Obando et al. (2018)	Colombia
CoA 84081, BO 91, CoP 9302, CoN 05071, CoN 98061, ISH 2, 19, 22, 25, 26, 27, 30, 31, 48, 49, 57, 63, 67, 102, 106, 113, 117	Chinnaraja (2014)	India

YLD. After evaluating them for five crop seasons, about 357 Co canes and 98 ISH clones were reported to be resistant to YLD. Among these, BO 91 and CoP 9302 were selected from Pusa, Co 678, Co 976, and ISH 176 selected from Coimbatore, CoJ 89 selected from Jalandhar, and CoPant 97222 selected from Pantnagar were found to be symptomless. However, their true resistance is to be confirmed by artificial inoculation using viruliferous aphids.

A detailed account on sources of resistance available in Indian breeding gene pool, which includes genotypes from different states of the country, inbreeds, interspecific hybrids, intergeneric hybrids, and world collection of sugarcane germplasm and exotic clones from Natal, Indo American clones, Australia, Barbados, Brazil, Colombia, Fiji, Mauritius, Puerto Rico, Taiwan, and USA was given by Chinnaraja (2014).

4.2.4 Rust

Among the foliar fungal diseases affecting sugarcane, rust is an important disease reported worldwide in more than 60 countries. As reported by Chu et al. (1982), the genes of rust susceptibility were transmitted to modern sugarcane varieties mainly from some accessions of *S. officinarum*. Selfed progenies of the sugarcane variety R570 were used to investigate the inheritance of rust resistance in sugarcane. Phenotyping for rust resistance/susceptibility was done in both field trials and under controlled conditions in the greenhouse. The resistance and susceptible segregation ratio obtained in the experiments was 3:1, which clearly indicated that brown rust resistance in the selfed progenies of R 570 was controlled by a major dominant gene called Bru 1. This gene showed resistance to all the rust pathogen isolates collected from varied geographic locations (Daugrois et al. 1996; Asnaghi et al. 2001). Another major resistance gene known as Bru 2 controlling sporulation of brown rust fungi was also reported (Raboin et al. 2006; Costet et al. 2012). Heritability for rust resistance was reported to be intermediate (Tai et al. 1981; Gonzales et al. 1987).

Comstock et al. (1992) reported high narrow sense and broad-sense heritability of 0.84 and 0.73, respectively, which was determined by the regression analysis of the rust grades of progenies and that of parents. Similarly, Hogarth et al. (1993) reported narrow sense heritability value of 0.84 and broad-sense heritability value of 0.73 for rust resistance. Costet et al. (2012) analyzed 380 recent varieties and other genetic/breeding materials from more than 30 breeding locations worldwide, with 22 molecular markers reported to be genetically linked to Bru1. From this studies, 17 genotypes, viz. B 41227, Co 214, MEX 73 523, MQ 76 53, N 53 216, NCO 334, R 84 693, Q 127, Q 136, R 570, R 572, R 573, R 575, R 577, H 72-8597, R 579, and R 83 1592 were identified as the stable resistant source for rust disease. Breeding methods that can be employed to improve the resistance against the important sugarcane diseases are given in Table 4.5.

Table 4.5 Strategies for improvement of sugarcane to tolerate different biotic stresses (Premachandran 2012)

Disease	Breeding strategies
Red rot	Use of resistant parents through conventional testing and transgenics using antifungal genes
Smut	Use of resistant parents in breeding and avoid susceptible clones as parents
Rust	Breeding resistant varieties using resistant parents, rejection of susceptible clones as a parent, and using marker aided selection using molecular markers
YLD	Transgenic with coat protein gene, identify resistance source and use in breeding, RNAi technology
Mosaic virus	Incorporation of coat protein gene through transgenic or RNAi technology

4.3 Abiotic Stress

4.3.1 Drought Tolerance

Water stress remains an ever-growing problem; it is the major limiting factor in crop production worldwide. With its longer crop duration, sugarcane faces many abiotic stresses that affect the metabolism, growth, and development of the crop. These abiotic stresses also affect the chemical composition, accumulation and synthesis of sugar, availability of seed and also aggravate other stresses making the crop susceptible (Shrivastava et al. 2016; Verma et al. 2021a, c). In India, drought coverage is 2.97 lakh ha while 2.5 lakh ha is under waterlogged condition, which is one of the causes of low cane productivity and production (Misra et al. 2020). The occurrence of drought and erratic rainfall necessitates identifying drought-tolerant sugarcane genotypes (Verma et al. 2020b). Sugarcane has complex ploidy status, and the trait drought tolerance as such in any crop is a complex trait with low genetic variance and developing the drought-tolerant varieties becomes a challenging task for the sugarcane breeders.

In this context, an innovative biotechnological approach like molecular marker techniques helps us understand the plant's responses to drought at the molecular and whole plant level and identify the genes for this complex trait. The molecular and biotechnological intervention has been initiated at ICAR-SBI, Coimbatore, India but it may go a long way in developing commercial varieties with drought tolerance or multiple stress tolerance. Hybridization between commercial clones and wild species and selection of progenies showing high yield, high sugar combined with drought tolerance is the practical and short-term approach for developing drought-tolerant variety. Drought-tolerant genotypes identified by the different sugarcane workers and their unique features are presented in Table 4.6.

Apart from the above results obtained by the different scientists working on the drought tolerance of sugarcane, the qualities expected from the drought-tolerant sugarcane as obtained from other studies are listed below.

Table 4.6 Characteristics of drought-tolerant sugarcane varieties

Drought-tolerant genotypes	Special features	Reference
Co 06022, Co 99004, and Co 06015	Possessed better *Fv/Fm*, SPAD value, CSI, SOD, POX, proline, and RNase activity	Devi et al. (2018)
RB073028, RB867515, and RB72454	Greater stalk length and diameter with higher dry stalk mass under higher water tension condition	Silvério et al. (2017)
Co 85019, Co 740, Co 97008, Co 775, CoV 92102, Co 92002, Co 88025 and Co 2000-10	Accumulated more proline under drought	Hemaprabha et al. (2013)
Co 740, ISH 100, NS 83/247, Co 85019, Co 997, and Co 99008	They did not show an appreciable reduction in drought conditions for the component characters	Hemaprabha et al. (2013)
Co 98014, Co 05011, Co 0238, and Co 12029	Maintaining better Pn rate, higher WUE, RWC, chlorophyll content, etc., or inherent capabilities to withstand water deficit at formative phase of growth	Pooja et al. (2021)
CoPb 11211, ISH148, ISH07, and ISH135	They did not show appreciable reduction in drought conditions for the component characters	Sanghera and Bhatt (2018)
Co 98014, Co 0118, CoPk 05191, Co 0238, and Co 05011	Maintaining of water potential and cellular integrity, SCMR value, increase in proline accumulation, higher activity of antioxidant enzymes, and fewer fluctuations in NR activity under stress condition	Kumar et al. (2021)
CP92-675, HoCP01-523, TCP89-3505, and TCP87-3388	Showed lower chlorophyll degradation and higher capacity to preserve water in the leaves during the initial growth	Silva et al. (2010)
Co 1163, Co 419, CoJn 94-8, Co 7704, Madumathii, CoJ 83, Co 8213, Co 86002, Co 7602, CoSNK 03044, CoSNK 03632, Co 403, Co 86250, CoM265, Co 94012, CP 5268, CoSNK 05104, Co 85002, Co 62175, CoSNK 05103, Co 92005, Co 85004, Co 740, Co 99008, Co 1148, Co 86032, CoC 671, Co 7405, Co 88025, Ms 68 47, Co 7424, Madhuri, Co 86249, Co 2001-15, Co 93009, Co 99004, CoT 8201, ISH 100, Co 94008	Less reduction in RWC and chlorophyll content under stress condition and show wilting symptom after 8 days of withholding irrigation	Dapanage and Bhat (2017)
CoVC 99263	Better root length, dry root weight, and dry cane weight under moisture stress condition	Meena et al. (2013)
Co 97008, Co 95017, and Co 87023	High net assimilation rate, relative growth rate SLA and leaf area index, osmotic potential, chlorophyll index, epicuticular wax content photosynthesis	Sajitha (2008)

(continued)

Table 4.6 (continued)

Drought-tolerant genotypes	Special features	Reference
Co 285, Co 1148	Greater stomatal resistance, less membrane	Venkatramana et al. (1986)
93 R 98	Higher expression of yield component traits under water deficit condition	Mukunda Rao et al. (2001)

- Drought-resistant varieties close their stomata earlier and, on rewatering, open their stomata earlier than drought susceptible varieties (Naidu and Bhagyalakshmi 1967).
- Smith et al. (2005) and Verma et al. (2020c) stated that drought tolerance was higher in genotypes that developed a deep root system and suggested that this characteristic feature of roots be used as a selection criterion for identifying drought-tolerant varieties.
- Naidu et al. (1989) identified the formative phase of sugarcane (60–150 days of crop maturity) as the most critical stage of water requirement; any shortage of water during this stage would result in the reduction of growth, dry matter accumulation, cane yield, and juice quality.
- Selecting genotypes that give higher productivity because of higher stalk number, stalk height, and stalk weight even under moderate water deficit situations could also be used as a criterion (Silva et al. 2008).
- The Si application improves the plant growth and development under stress, accompanied by up-regulation of photosynthesis, stomatal conductance, transpiration rate, photosynthetic pigments, relative water content, and biochemical activities, i.e., CAT, POD, and SOD (Verma et al. 2019).

4.3.1.1 Source of Drought Tolerance

Once the component traits and sources of drought resistance are identified, these will be utilized in the breeding program. As of now, there is no directed breeding for drought tolerance. High-yielding commercial varieties identified for each agro-climatic zones of the country are usually tested under the local climatic conditions in normal and moisture stress conditions to ascertain the drought tolerance potential of that variety. Varieties such as Co 86032, Co 0212, which combine desired traits like high yield, high sugar, and tolerance to red rot, have been identified as drought tolerant.

The drought-tolerant parents available in NHG are 80 R 41, Co 6304, Co 6806, Co 7219, Co 7704, Co 7717, Co 7910, Co 8209, CoM 88121, Co 8371, Co 85002, Co 85246,Co 86032, Co 87012, Co 87021, Co 87023, Co 87025, Co 87252, Co 88028, Co 89010, Co 1148, Co 1158, Co 2000-10, Co 312, Co 453, Co 617, Co 6806, Co 740, Co 8208, Co 91018, Co 91019, Co 92002, Co 94005, Co 94008, Co 9502, 1Co 98013, Co 99004, CoC 90063, CoM0265, CoSnk 03-632, CoV 92102, Co 85019, Co 86010, Co 86011, Co 86036, Co 86249, Co 86250, Co 87263, Co

Table 4.7 Source of tolerance to drought stress in *Saccharum species* clones (Sinha 2016)

Species	Source of resistant
S. officinarum	Gungera, 57NG 73, IJ 76-412, IJ 76-564, Coaledonia ribbon
S. barberi	Nargori, Lalri, Manga Sic, MatnaShaj, ParariaShaj
S. robustum	NG 77-79, 57NG 19, NG 77-146, NG 77-23, 57 NG 27, NG 77-38
S. sinense	Mcilkrum, Reha, Lalkhadi, Kalkya, Kheli
S. spontaneum	TS 76-216, US 56-20-1, Taiwan 96, Pamba, Ponape 1, SES 32A, IND 90-805, IND 90-796, IND 85-503, Tabongo, IND 84-351

89003, Co 91010, ISH 100 and ISH 135. Hemaprabha et al. (2006) reported that sugarcane varieties Co740,Co 6304,Co 6806, Co7201, and Co775were found to be useful as parents in drought resistance breeding program, and the derivatives of Co 740, Co 775, Co 6304, Co 6806, Co 7201, and CoC 671 were able to withstand drought situations. Other Co and Co allied canes like Co 7336, Co 7805, Co 8367, Co 8213, CoC 85061, Co 91017, Co 92003, Co 92006, Co 95014, Co 95020, and CoLk 8001 and ISH clones like ISH 9, ISH 23, ISH 41, ISH 58, ISH 100, ISH 110, ISH 118, ISH 175 available in NHG can also be used as drought-tolerant parents. The drought-tolerant varieties reported in different studies, viz. Co 740, Co 997, Co 1103, Co 1107, Co 8338, Co 87263, Co 87016, Co 91010, Co 92020, Co 93009, Co 94012, Co 94019, Co 95003, Co 95005, Co 97009, Co 98014, Co 99004, Co 2000-12, Co 0212, Co 0218, CoLk 8003, CoS 96269, CoS 97261, CoS 767, CoA 7602, CoM 7054, CoM 7125, CoSi 94071, CoSi 94072, CoC 671, CoA 92081, CoA 03081, BO 89, BO 90, BO 109, BO 104, BO 9, 81 A 99, 93R 44, and 92 R 277 (Singh 1989; Vasantha et al. 2005; Ram 2008) may be used.

Genetic stocks for different abiotic stresses were identified at ICAR-SBI Regional Centre, Karnal, based on their performance in stress and normal environments for sugar yield (Ram 2016). Clones such as Co 6806, Co 7717, Co 95021, Co 97015, DhaurAlig, ISH-007, ISH-135, ISH-148, ISH-261, ISH-273, Co 1148 were identified as genetic stocks for water stress. Four clones, viz. Co 6806, DhaurAlig, ISH-007, and ISH-135 were identified as genetic stocks for three abiotic stresses (water stress, waterlogging, and salinity). These clones along with species clones presented in Table 4.7 may be utilized in the hybridization as parents in future breeding programs to incorporate tolerance to various abiotic stresses.

4.3.1.2 Use of Molecular Markers and Transgenic Technology for Drought Tolerance in Sugarcane Improvement

Utilizing the two methods of transformation, agrobacterium-mediated and particle gun methods, transgenics carrying different genes relating to drought tolerance, viz. the key enzymes of structural genes for osmolyte biosynthesis, detoxifying enzymes regulatory genes have been developed in rice, wheat, maize, sugarcane, tobacco, Arabidopsis, groundnut, tomato, and potato (Satbir et al. 2009). Candidate genes analysis is generally used to find genes expressed differentially in drought-tolerant/susceptible varieties. Candidate genes analysis was carried out on a set of drought-tolerant clones of *S. spontaneum*, *S. barberi*, *S. sinense*, *S. robustum*, and *Erianthus*

species and drought susceptible *S. officinarum* accessions to identify sugarcane specific drought-responsive genes. Species-specific markers were identified and validated in drought-tolerant and susceptible clones. In the drought-tolerant hybrids (Co 2000-10, Co 92002, Co 86010, Co 86032, Co 740), all the 26 genes/alleles were present, while in the drought susceptible hybrids (Co 8021, Co 8368, Co 419, Co 775) only three ABA-dependent genes, viz. ABF 3, CDPK 18, and TPS 2 were present, and the remaining 23 genes were absent (Priji and Hemaprabha 2015).

4.3.2 Salinity

Salinity is one of the important abiotic stresses affecting crop productivity and the quality of the produce. Sugarcane grown under arid and semiarid regions is frequently subjected to salinity. The various stages of the sugarcane crop show high sensitivity to salinity. However, sugarcane genotypes differ in their capacity to tolerate salinity (Mahajan et al. 2013). Evaluation of germplasm for the source of salinity tolerance and breeding for salinity tolerance are being used to develop salt-tolerant plants besides biotechnological approaches (Saif-Ur-Rasheed et al. 2001).

Several promising genotypes, viz. Co 86011, Co 7717, Co 7219, Co 8208, Co 85004, CoC 671, Co 6806, Co 94008, Co 85019, Co 94012, Co 97008, and Co 99004 identified as tolerant types were found suitable for salt-affected soils. Co 453 and CoJ 13 are typically salinity tolerant types (Hemaprabha 2015). Saline tolerant varieties identified are BO 91, BO 99, BO 102, BO 104, BO 108, BO 109, CoJ 88, CoS 767, Co 1148, Co 7717, Co 8145, Co 8347, Co 8371, Co 85004, Co 86032, Co 89010, Co 94008, Co 94012, Co 97008, Co 99004, (Sundara 1994: Singh et al. 2007), Co 6806, Co 89035, Co 93026, Co 95021, Co 97014, Co 97015, Co 98015, and Co 98016 (Ram et al. 2003). Other salinity tolerant parents available in NHG are Co 6806, Co 7219, Co 85019, Co 6806, Co 8208, Co 85019, Co 86011, CoLk 8102, CoM 0265, Co 92013, CoSnk 05-103, ISH 135, and ISH 175. Among the 346 *S. officinarum* clones evaluated, 113 were tolerant, while 59 *S. robustum* clones 15 were tolerant. Similarly, among 39 *S. barberi* accessions evaluated, 12 were tolerant to drought, and among 155 IND clones, 21 were tolerant. Resistant parents available in different species of *Saccharum* to salinity are given in Table 4.8.

Table 4.8 Source of tolerance to salinity stress in *Saccharum species* clones (Sinha 2016)

Species	Source of resistant
S. barberi	Katha-Coimbatore, Kewali-14-G, Khatuia-124, KuswarOttur, Lalri, Nargori, Pathri
S. sinense	Khakai, Pansahi, Reha, Uba-Seedling
S. officinarum	IJ-76-442
S. robustum	IJ-76-470, 28 NG 251, 57 NG 201, 57 NG 231, 77 NG 34, 77 NG 136, 77 NG 160, 77 NG 167, 77 NG 170, 77 NG 221, 77 NG 237

4.3.3 Waterlogging Tolerance

Waterlogging limits sugarcane productivity in many major sugarcane growing parts of the world. Factors like competition with other crops have compelled sugarcane to be grown in such lands. In India, around 10 to 30% of the sugarcane acreage is under waterlogged conditions that may increase due to climate change. The extent of damage due to waterlogging in sugarcane was correlated to different factors like genetic potential of the varieties to tolerate the stress, the stage at which stress occurs, and the extent of the duration of the waterlogged condition (Gomathi et al. 2015).

Many parental clones in NHG like Co 6806, Co 7717, Co 8371, Co 98016, Co 99006, BO 91, BO 99, Bo 110, Bo 128, Co 8231, Co 8371, UP 9529, UP 9530, CoSe 96436, CoS 97264, ISH 135, ISH 175 were screened as waterlogging tolerant. Nair and Govindaraj (2007) reported Co 85286, Co 87033, Co 97014, Co 98016, CoLk 8102, CoS 94267, ISH 7, ISH 148, ISH 175 as tolerant to waterlogging. The varieties which were identified as waterlogging resistance in the recent years include BO 130, Co 95021, Co 97015, Co 0118, Co 0232, Co 0233, Co 0238, CoP 9103, CoP 9104, CoBln 9103, CoS 8118, CoS 96436, CoTl 88322, UP 9529, UP 9530, CoSe 01424, and CoSe 04432 (Premachandran 2002; Ram et al. 2003; Singh et al. 2005). Among 125 genotypes tested for their ability to withstand waterlogging conditions, 19 exotic hybrids, and 30 Indian hybrids were tolerant. Varieties like Co 62175, Co 8231, Co 8232, Co 8145, CoSi 86071, CoSi 776, and Co 8371 were well adapted to excess moisture stress conditions. Gomathi et al. (2015) identified 93A 4, 93A 11, 93A 145, and 93A 21 from Anakaplle; Co 8371 from Kolhapur; Bo 91, Co 87263 and Co 87268 from Bihar; Co T1 8201 and Co T1 88322, Co 99006 from Kerala as tolerant varieties under waterlogging conditions. To withstand waterlogged conditions, two clones of *S. spontaneum*, viz. SES 334 and SES 340 and a hybrid Co 285 having negatively geotropic roots and adventitious roots to withstand waterlogged conditions are recommended as donors in the breeding program for waterlogging tolerance (Srinivasan and Rao 1960).

Among the species clones, *S. officinarum* clones did not survive/withstand waterlogged conditions and were highly susceptible, while the accessions of *S. spontaneum* (SES220), *S. robustum* (28NG219A), and Narenga were flood-tolerant (Srinivasan and Batcha 1962). Clones with profuse fibrous floating roots and negative geotropic roots with aerenchyma were found to be resistant to waterlogging (Srinivasan and Rao 1960; Srinivasan and Batcha 1962). The parents with these traits may be utilized in the breeding program to develop varieties for low-lying areas and submerged conditions.

4.3.4 High-Temperature Tolerance

The capacity to tolerate increased atmospheric temperature becomes a necessary feature of the varieties of coming years to tolerate the higher temperature. Further high temperature during the ripening period acting singly or in combination with

suboptimal photoperiodism during flowering was responsible for low viability of fuzz. Significant differences among the varieties to tolerate high temperature were observed (Hemaprabha et al. 2012). Co 95018, Co 93006, Co 86032, Co 95007, Co 99006, CoSnk 03632, Co 85004, Co 2000-13, Co 95020, Co 97009, Co 95003, Co 2001-15, Co 91001, Co 94019, Co 95012, Co 95005, Co 91010 Co 6304, Co 99012, Co 93001, Co 0112, and Co 7914 were identified as thermo-insensitive genotypes as they performed equally well in the extremes of temperature. Further, they reported that genotypes developed from the sugarcane hybrids CoC 671, Co 7201, Co 740, Co 7717, and Co 658 and *S. robustum* performed better at high temperature. Among the five clones, viz. Co 06022, Co 0315, Co 8021, Co 86032, and Co 99004 studied, Co 99004 was highly thermo-tolerant and can be used for developing varieties with high-temperature tolerance. The formative phase of the sugarcane crop is highly sensitive to high temperatures compared to the grand growth phase. Indicators for heat tolerance like chlorophyll content, chlorophyll stability index, antioxidant enzymes, enzymes of sucrose metabolism, soluble sugar content, proline content, total phenolics, and leaf gas exchange can be used as heat tolerance index for screening the genotypes (Kokila and Gomathi 2018).

4.3.5 Winter Ratooning Ability

Out of 2.7 million ha cane area in the subtropical India, 50% area is under ratoon crop, which is harvested during October to December when the temperature is around 3.1–10.4 °C against the optimum requirement of 26–32 °C for better sprouting (Ram et al. 2017). This is one of the major abiotic constraints reducing the production potential of ratoon crops in subtropical India (Jain et al. 2007). At this juncture, identifying varieties with desirable economic traits coupled with better winter ratooning ability is prioritized in the subtropical sugarcane breeding programs. Progenies of crosses involving *S. spontaneum*, *S. barberi*, and *E. bengalensis* showed excellent winter sprouting. The result reporting thin stalk and low sucrose varieties is more tolerant to low temperature (Irvine 1978), was not repeated by Ram et al. (2017). The crosses like CoPant 84212 × Co 89003, CoH 110 × Co 8213, and Co 8353 × Co 1148, where at least one of the parents is of subtropical origin, produced a higher number of selections for winter ratooning ability. However, selection percentage was higher for spring harvested seedlings in the crosses like CoS 8436 × Co 89003, CoH 110 × Co 1148, CoS 94257 × CoT 8201, Co 8353 × Co 62198, Co 8371 × CoT 8201, Co 8353 × Co 88021, and Co 86002 × Co 775.

Ram et al. (2005a, b) reported that no progeny among the tropical × tropical crosses was selected for the winter rationing ability. The crosses where both parents were from tropical origin did not give selections that could withstand the severity of winter occurring subtropical region of the country. It indicated that in order to have progenies that have winter hardiness, the cross combinations should be decided so that one of the parents is of subtropical origin. The parents like NCo 310, Co 1148, and Co 453 were frost resistant, whereas Co 312 and Co 1158 were susceptible to

Table 4.9 Genetic stocks identified for different abiotic stress occurring in subtropical India (Ram 2016)

Stress	Genetics stocks
Water stress, waterlogging, and salinity	Co 6806, DhaurAlig., ISH-007, ISH-135
Water stress and waterlogging	Co 6806, Co 7717, DhaurAlig., ISH-007, ISH-135, ISH-261
Water stress and salinity	Co 6806, Co 95021, DhaurAlig., ISH-007, ISH-135, ISH-148
Waterlogging and salinity	Co 6415, Co 6806, Co 87033, Co 93026, Co 97014, Co 98016, CoS 94267, DhaurAlig., ISH-007, ISH-135, ISH-175
Water stress	Co 6806, Co 7717, Co 95021, Co 97015, DhaurAlig., ISH-007, ISH-135, ISH-148, ISH-261, ISH-273, Co 1148
Waterlogging	Co 6415, Co 6806, Co 7717, Co 87033, Co 93026, Co 97014, Co 97017, Co 98016, CoS 94267, BO 91, DhaurAlig., PaRariaShaj., ISH-007, ISH-135, ISH-175, ISH-261
Salinity	Co 6415, Co 6806, Co 87033, Co 89035, Co 93026, Co 95021, Co 97014, Co 97015, Co 98015, Co 98016, CoLk 8102, CoS 94267, DhaurAlig., PaRariaShaj., ISH-007, ISH-135, ISH-148, ISH-175

Table 4.10 Parents tolerance/resistance to biotic and abiotic stresses in NHG

Traits of interest	Source
Rust	Co 86249
Wilt	Co 86032, Co 356, Co 1148
Cold tolerance	Q 63, Co 8339, Co 1148
Winter ratooning ability	Co 06036, Co 06035, CoP 9302, CoS 109, CoS 95422, CoS 94270, and BO 130, Q 65
Stalk borer tolerance	CoS 96268 and CoS 96269
Top borer tolerance	Co 89029, Co 453, Co 7717, Co 7219, Co 1305
Scale insect	Co 7706

frost. The clones like Co 97009, Co 0238, and CoS 93230 with good sprouting in winter months (Ram and Sahi 2007) can be used in the sugarcane improvement program. Genetics stocks identified for different abiotic stresses prevailing in the subtropical India are presented in Table 4.9.

The parental clones identified for different minor biotic and abiotic stresses affecting sugarcane are presented in Table 4.10.

Apart from these parents, varieties recommended for major abiotic stresses prevailing in India are presented in Table 4.11.

Table 4.11 Varieties recommended for cultivation in different abiotic stress conditions prevailing in India (Hemaprabha 2015)

Type of stress	Varieties
Drought	Co 997, Co 1103, Co 1107, CoLk 8003, Co 87263, Co 87016, BO 89, BO 90, BO 99, BO109, BO104, BO109, CoS 767 Co 98014, Co 740, Co 997, CoA 7602, MS 7054, CoM 7125, CoSi 94071, CoSi 94072, Co 94012, CoC 671, Co 91010, Co 92002, Co 92020,Co 93009, Co 97009, Co 99004, Co 8338, Co 91017, Co 200012, Co 0212, Co 0218, Co M 0265, 81A99, CoA 92081, CoA 92081, CoA 03081
Salinity	BO 91, BO99, BO 102, BO 104, BO108, BO109, CoS 767, Co 1148, Co 8347, Co 8371, CoC 671, Co 89010, Co 94008, Co 94012, Co 97008, Co 99004
Tolerance to cold/frost	Co 97009, Co 0238, Co 08339, and CoS 93230
Waterlogging	Bo 99, BO 110, BO 128, Co 8231, Co 8371, Co 98007, Co 99006, Co 0118, Co 0232, Co 0233, Co 0238, CoP 9103, CoP 9104, CoBln 9103, CoS 8118, CoTl 88322, CoSe 96436, UP 9529 and UP 9530

4.4 Conclusion

Hybridization between parents of diverse origins is the most effective way for developing new cultivars in sugarcane. However, most of the crosses were made among a few superior sugarcane parents resulting in narrowing down the genetic base of recently released varieties, leading to sudden breakdown of the stress resistance/tolerance behaviour of the new varieties developed. Hence, an understanding of the genetic diversity of parental clones is essential. Sugarcane breeding in India has a history of being region-specific for parents' choice and selection and evaluation of the progenies that resulted in the evolution of the different sets of varieties having genes for resistance or tolerance to different biotic and abiotic stresses prevailing in the location of origin for tropical and subtropical regions. The higher success rate from early generation recombinants indicated that genetic stocks with novel basic species clones could also be successful parents in widening the genetic base of sugarcane varieties of the future. Therefore, new holistic strategies need to be formulated to utilize the untapped genetic potential of basic species clones of *Saccharum*. The parental clones identified for red rot resistance, smut resistance, drought tolerance, salinity tolerance, waterlogging tolerance, low-temperature tolerance, winter ratooning ability, and high-temperature tolerance will be helpful for the breeders throughout the country to include them in the sugarcane improvement program.

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Bioactive Silicon: Approach to Enhance Sugarcane Yield Under Stress Environment

5

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Abstract

Sugarcane is a silicon (Si) accumulative plant with Si content ranged from 1–2% and more. The level of Si absorption by sugarcane depends on the variety and soil properties. The sugarcane cultivation results in massive removal of the plant-available Si from the soil. Silicon soil amendments mostly affect soil properties. Si fertilizers provide Si nutrition to plants, and Si-based biostimulators which affect the plant immune system. Numerous investigations suggest that the primary function of Si in the plant is protection against biotic and abiotic stresses. Silicon-induced mechanism includes reinforcing the plant stress defense such as mechanical reinforcement of the epidermal tissue via Si accumulation in the cuticular layer and stem nodes, physiological responses enhancing the stability of cell organelles, such as chloroplasts, mitochondria, ribosome, and others. Biochemical activities comprise activation of stress ferments and mitigation of oxidative destruction and molecular functions increasing the stability of chlorophyll, DNA, and RNA and immobilizing inorganic pollutants, i.e., heavy metals. These processes are universal for a variety of crops, including sugarcane, and

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plants alleviate abiotic and biotic stresses. Although the mechanisms underlying the stimulant effect of Si on the plant defense systems are briefly discussed, they remain poorly understood. The hypothesis about the direct impact of active forms of Si on the synthesis of enzymes or stress proteins has been discussed.

Keywords

Biomass · Biochemical · Physiological · Sugarcane · Silicon · Soil

5.1 Introduction

5.1.1 Silicon in Sugarcane

Sugarcane (*Saccharum* species hybrid) is a silicon (Si) accumulating plant. The total content of Si in sugarcane depends on numerous factors, including the plant variety, soil type, application of mineral fertilizers, and others (Table 5.1). The distribution and deposition of Si in sugarcane tissues are well documented (Deren et al. 1993; Thangavelu 2005; Keeping 2017; de Tombeur et al. 2020).

Sugarcane can remove 86–795 kg Si ha^{-1} $year^{-1}$ (Keeping 2017; Camargo and Keeping 2021). Data on changing the soil plant-available Si under sugarcane cultivation is limited. Kennedy et al. (2021) analyzed plant-available forms of Si in the soils on which sugarcane has been cultivated since the middle of the last century. Soils samples were collected at 0–25 cm depth in several regions of North Queensland for the analysis of water- and acid-extractable forms of Si (Matichenkov and Snyder 1996; Borges et al. 2016; Matichenkov et al. 2017). The water-extractable Si from fresh soil samples characterizes the actual concentration of

Table 5.1 Total Si content in sugarcane leaves of different varieties

Total Si (g kg^{-1})	Soil	Variety	Country	Reference
7.0	Nitisol	R570	Guadeloupe	de Tombeur et al. (2020)
14.7	Andosol	R579	Guadeloupe	de Tombeur et al. (2020)
21.0	Vertisol	B80689	Guadeloupe	de Tombeur et al. (2020)
18.1	–	Co419	India	Thangavelu (2005)
18.4	–	Co617	India	Thangavelu (2005)
18.1	–	Co678	India	Thangavelu (2005)
17.9	–	Co740	India	Thangavelu (2005)
17.5	–	Co853	India	Thangavelu (2005)
19.1	–	Co1148	India	Thangavelu (2005)
18.1	–	Co62101	India	Thangavelu (2005)
10.2	Histosol	CP 72-1210	USA	Deren et al. (1993)
9.1	Histosol	CP 90-1172	USA	Deren et al. (1993)
7.8	Histosol	CP 90-1430	USA	Deren et al. (1993)
6.4	Histosol	CP 90-1638	USA	Deren et al. (1993)
1.3	Inceptisol	N12	South Africa	Keeping (2017)

Table 5.2 Content of plant-available Si in the surface layer (0–25 cm) of virgin and cultivated soils in several regions of North Queensland, Australia

	Water-extractable Si (mg kg^{-1})		Acid-extractable Si (mg kg^{-1})	
Region	Virgin soil	Cultivated soil	Virgin soil	Cultivated soil
Ayr	12.4	3.6	350	230
Atherton	24.5	1.8	240	82
Narada	20.1	0.8	210	45
Ravenshoe	12.5	1.4	185	51
Evelyn	10.4	2.4	195	70
Ingham	15.8	1.1	158	39
LSD05	0.4	0.2	15	4

monosilicic acid, which plants can absorb (Bocharnikova and Matichenkov 2012). The 0.1 M HCl^{-} extraction method characterized amorphous Si (phytoliths, Si films) that can readily pass into solution and be taken up by cultivated plants during the growing period (NIAES 1987). The data showed that sugarcane cultivation reduced the water-extractable Si 3.4–25 times and the acid-extractable Si 1.5–4 times (Table 5.2). Reduction in plant-available Si was shown to promote the soil degradation processes (Matichenkov and Calvert 2002). Long-term cultivation of sugarcane reduces the soil supply of plant-available Si. Therefore, the application of Si-rich materials is necessary to restore the plant-available Si supply.

Liebig (1840) conducted the first greenhouse trial of Si fertilizer in 1840 in Germany. He suggested the theory of plant mineral nutrition and designated four essential elements for plant growth: N, P, K, and Si. However, today Si is considered only as a beneficial element. Arnon and Stout (1939) suggested that an essential element should meet three criteria: (1) plant inability to complete its life cycle without the element; (2) specificity of action and the impossibility of replacement by any other element; and (3) direct involvement in plant metabolic processes. Silicon essentiality has been recognized for diatoms that accumulate SiO_2 across the plasma membrane (Raven 1983). Silicon is also regarded as an essential trace element for the growth and development of animals that involve Si in forming bone and cartilage (Carlisle 1984). For higher plants, Si essentiality has not been proven yet. A proof of the Si essentiality is problematic because of several technical difficulties. Therefore, further research is needed on the direct role of Si in physiological processes.

5.2 Status of Plant-Available Silicon in Sugarcane

Si has been recognized as an "agronomically essential" element for sugarcane production (Fox and Silva 1978) because sugarcane contains more than 1.50% Si in its shoot on a dry weight basis (Hodson et al. 2005; Keeping et al. 2017; Verma et al. 2020b, c, 2021c). Hence, it is noticed that intensive sugarcane cultivation may

Table 5.3 Quantum of silicon removed by sugarcane crop

Crop	Si removal (kg ha^{-1})	Source
Sugarcane	379	Samuels (1969)
	408	Ross et al. (1974)
	300	Meyer and Keeping (2001)
	500–700	Anderson et al. (1991)
	200–500	Camargo et al. (2010a, b)
	300–700	Savant et al. (1999)
	86–795	Keeping (2017); Camargo and Keeping (2021)

Source: Majumdar and Prakash (2020a, b)

deplete the plant-available silicon (PAS) content in the soil. The perusal of the data presented in Table 5.3 summarized the content of Si annually removed by sugarcane crop globally. At the same time, with rigorous weathering, commonly noticed in ultisols and oxisols, silica to sesquioxide ratio decreases, and therefore, the soil becomes deficient in Si (Foy 1992; Juo and Sanchez 1986). Therefore, desilication triggered by the natural weathering process and plants uptake might be well-thought-out as a significant factor for the decline in PAS content in tropical soils across the world.

The dissolved silicon (DSi) and the adsorbed silicon (AdSi) in soil commonly constitute the PAS content in the soil. The DSi is measured through calcium chloride ($CaCl_2.2H_2O$) extraction, whereas acetic acid (CH_3COOH) is used for estimation of AdSi in soil (Höhn et al. 2008; Korndorfer et al. 2001; Prakash and Majumdar 2019). Few studies critically revealed that AdSi controls PAS when soils are rich in iron and aluminum oxides (Philippini et al. 2006; Hiemstra et al. 2007). Consequently, quantification of PAS is crucial to recognize the Si mass balance for a particular region.

In order to generate data on the distribution of PAS content in sugarcane fields for the first time in India, a research was performed by selecting four agro-climatic zones of Karnataka, viz. southern dry zone (SDZ), southern transition zone (STZ), coastal zone (CZ), and central dry zone (CDZ) (Majumdar 2019). Soil samples were collected from the one-decade-old intensively cultivated sugarcane field at three depths: 0–30, 30–60, and 60–90 cm from all four zones. This study indicated that the DSi content of SDZ, STZ, CZ, and CDZ profile soil samples ranged from 23.00–39.39, 56.35–82.23, 45.29–87.77, and 29.00–35.38 mg kg^{-1} in sugarcane crop, respectively (Table 5.4). Higher content of DSi was recorded in STZ (72.55 mg kg^{-1}) followed by CZ (63.93 mg kg^{-1}) (Table 5.4). The DSi content increased with an increase in depth in STZ and CDZ, whereas a reverse trend was noticed in SDZ and CZ. Hence, the study revealed that SDZ and CDZ were medium in DSi content (between 20 and 40 mg kg^{-1} Si), and on the other hand, STZ and CZ had high DSi content (more than 40 mg kg^{-1} Si) (Haysom and Chapman 1975; Matichenkov and Bocharnikova 2008).

Table 5.4 Vertical distribution of dissolved silicon and adsorbed silicon content in soil of sugarcane fields in four different climatic zones of Karnataka, India (Majumdar 2019; Majumdar and Prakash 2021)

	Dissolved Si (mg kg^{-1})			Zone	Adsorbed Si (mg kg^{-1})			Zone
ACZ	0–30	30–60	60–90	mean	0–30	30–60	60–90	mean
SDZ	39.39	23.00	30.05	30.81	103.31	113.59	123.06	113.32
STZ	79.06	82.23	56.35	72.55	67.49	80.30	106.97	84.92
CZ	87.77	58.73	45.29	63.93	45.83	51.31	51.12	49.42
CDZ	29.00	34.70	35.38	33.03	95.63	86.62	82.82	88.36
Depth mean	58.80	49.67	41.77	–	78.06	82.95	90.99	–

ACZ agro-climatic zone, *SDZ* southern dry zone, *STZ* southern transition zone, *CZ* coastal zone, *CDZ* central dry zone

The content of AdSi in CZ, SDZ, STZ, and CDZ profile soil samples ranged from 103.31–123.06, 67.49–106.97, 45.83–51.31, and 82.62–95.63 mg kg^{-1} in sugarcane crop, respectively (Table 5.4). A higher concentration of AdSi was noticed in SDZ followed by CDZ (Table 5.4). The lowest AdSi concentration was recorded in the CZ. The AdSi concentration increased with an increase in depth in SDZ and STZ. This study indicated that irrespective of the zone, the AdSi content was higher than DSi content which could be attributed to the dissolution of soluble, exchangeable, and specifically adsorbed Si; type of the extractant and pH of the extractant used for the estimation (Narayanaswamy and Prakash 2009). It is known that the Si extracting power of the soil increases with the lower pH of the extractant. In this study, 0.5 M CH_3COOH was used as an extractant, which extracted a higher amount of AdSi than DSi by using 0.1 M $CaCl_2.2H_2O$. This suggested that the acetic acid provided access to physio and chemisorbed Si.

5.3 Classification of Si-Rich Materials in Agriculture

Davy (1814) first noted silicon as a plant nutrient. He supposed that Si accumulation in the epidermal tissue creates mechanical protection against insects and diseases. Then, based on the plant elemental composition, Liebig (1840) concluded that Si fertilizer is essential. He conducted the first greenhouse experiment with sodium silicate on sugar beet. In addition to an increased weight of the root crop, Liebig (1840) recorded enhanced sugar content. Liebig's findings promoted field trials with sodium silicate as Si fertilizer. Lowes (1856) demonstrated the Grass Park experiment at the Rothamsted Station in England which further demonstrated the effect of sodium silicate on grass productivity (Rothamsted Experimental Station Guide the Classical Experiment 1991).

In 1870, the great Russian chemist D.I. Mendeleev suggested to use amorphous silicon dioxide as Si fertilizer (Mendeleev 1870). The first patent on using Si-Ca slag as a fertilizer was obtained by Zippicotte and Zippicotte (1881). Maxwell (1898)

conducted the first soil test for plant-available Si in the Hawaiian Islands. Among the first studies of the Si role in plant physiology were the works of French and German scientists: Pierre (1866), Jodin (1883), Kreuzhage and Wilf (1884) (Epstein 1999; Sommer 1926). Grob (1896) investigated the anatomy of epidermal tissue and confirmed the Davy's hypothesis about the Si role in the plant defense system against diseases and insect attacks.

In 1915–1917, Japanese scientist I. Onodera started studied Si fertilizers after he visited the universities of Konigsberg and Cambridge (Onodera 1917). His works initiated research on the Si role for rice. In Japan, numerous experiments have resulted in obligatory using Si fertilizers in rice cultivation (Miyake and Adachi 1922; Suzuki 1934; Yoshida 1965). In 1955, Japanese Ministry of Agriculture, Forestry and Fisheries recommended to use Si fertilizer (calcium silicate slags) in rice cultivation for the following reasons (Ma and Takahashi 2002).

- Rice, the most important crop in Japan, characterized by high accumulation of Si.
- A high-density cultivation system is commonly used for rice with heavy nitrogen fertilizer application in Japan.
- Silicon-deficient soils such as degraded paddy soils are widely distributed.
- The iron industry provides cheaper silicate fertilizers like slag.
- Return of the main Si source rice straw to the paddy soil is a gradually decreasing practice mainly because of labor shortage.

In the first quarter of the twentieth century in the USA, the benefits from Si fertilization of acid soils attracted attention. Industrial by-products like slag and ash were used as Si fertilizers and liming materials (MacIntire et al. 1925; Schollenberger 1920). In 1936, Ayres conducted the first field trial of Si fertilizers on sugarcane in Hawaii. Further investigations were continued in Florida, where today, Si fertilizers are used successfully for rice, sugarcane, and grasses (Anderson 1991; Savant et al. 1997). At present, approximately 3.5 million tons of Si materials are used in the world annually (http://www.slg.jp/e/slag/product/hiryo.html; http://www.euroslag.org/products/statistics/). Three main groups can be distinguished among the currently used Si-based agrochemicals.

- Silicon soil amendments mainly affect soil properties (adsorption capacity, pH, structure, and others) and are commonly applied at rates more than 500 kg ha^{-1}. Considering high application rates, these materials also contribute to plant Si nutrition. This group includes calcium silicate slag, zeolite, diatomite, and others (Chaiyaraksa and Tumtong 2019; Matichenkov et al. 2020; Verma et al. 2020b, c).
- The primary purpose of silicon fertilizers is to provide plant Si nutrition. Their application rates range between 50 and 500 kg ha^{-1}. Amorphous silicon dioxide (microsilica, fumed silica), silicon gel, and sodium or potassium silicate are recognized as fertilizer (Ma and Takahashi 2002; Rao et al. 2017).

- Silicon biostimulator is a class of Si-based agrochemicals that are foliar applied at rates less than 10 kg ha^{-1} (Gugała et al. 2019; Quinonez et al. 2020; Artyszak et al. 2021).

5.4 Silicon and Pest Management in Sugarcane

One recent novel approach suggested to manage stem borers in sugarcane agro-ecosystems is the application of Si fertilizers as a nutritional soil amendment. This scenario is classified as nutritionally combined pest management as it encompasses improving crop resistance by increasing crop vigor (Reynolds et al. 2016; Alhousari and Greger 2018). Si is the second most abundant element in the Earth's crust and is considered a major nutritional element that may positively affect the growth and development of crops. Higher plants absorb Si in the form of monosilicic acid [$Si(OH)_4$]. After transportation via roots to vegetative shoots, silicon becomes concentrated in cell walls as silica gel (Ma and Yamaji 2006; Verma et al. 2021d).

Silicon may act mechanically and biochemically in plant defense against arthropod pests. Silicon depositions under leaf cuticles provide a mechanical barrier that leads to increasing rigidity and abrasiveness of plant tissues and may decrease palatability and digestibility to arthropod pests and eventually, food intake becomes reduced (Reynolds et al. 2016). Observations indicated that silicon fertilization boosts levels of defense-related genes, moreover increasing the activities of plant defense enzymes leading to enhanced accumulation of protective compounds such as phenolics and phytoalexins (Reynolds et al. 2016). Silicon fertilization in accumulating plants such as sugarcane proved to provide satisfactory results against arthropods pests (stem borers, spittlebugs, and mites) in several countries (Keeping et al. 2013; Korndörfer et al. 2011; Nikpay and Soleyman Nejadian 2014; Nikpay et al. 2015; Nikpay 2016; Nikpay and Laane 2017, 2020; Atencio et al. 2019; Rahardjo et al. 2020). The main target pest in the sugarcane agro-ecosystem is stem borers, and they are managed efficiently by applying silicon fertilizers. The standard type of silicon prevalently used in sugarcane is solid silicon formulations in calcium silicate (Nikpay and Goebel 2015; Reynolds et al. 2016). Nikpay et al. (2015) applied calcium silicate to protect three sugarcane varieties, CP69-1062, SP70-1143, and IRC99-01, under field conditions. Silicon fertilizer was sprinkled in the furrow and mixed thoroughly in the soil to a depth of 35 cm. The results showed that by applying calcium silicate fertilizer, the percentage of stalk damage, internode bored, and borer exit holes, length of borer tunnel, and the number of live borer per stalks reduced significantly in comparison with control (Fig. 5.1).

Silicon can be incorporated successfully with other environmentally sound practices such as beneficial parasitoids. Nikpay (2016) evaluated the potential efficacy of silicon for improving biological control of Scelionid parasitoid, *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae) on susceptible variety CP69-1062. The results of this study indicated that the application of silicon as a soil amendment plus half release of parasitoids provided a significant reduction of percentage stalk damage and percentage of bored internodes caused by *Sesamia* spp.

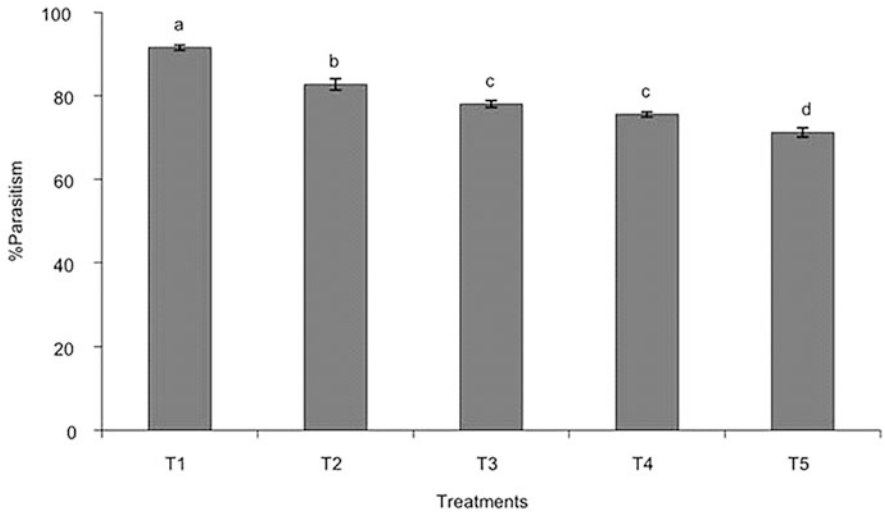

Fig. 5.1 Silicon treatment enhances biological control activity in sugarcane. Mean (%) parasitism of *T. busseolae* on stalk borers ± SE for all treatments such as T1—calcium silicate (1200 kg ha^{-1}) and 2500 *T. busseolae*, T2—5000 *T. busseolae*, T3—2500 *T. busseolae*, T4—1250 *T. busseolae* and T5—untreated control. Same letters are not significantly different ($p < 0.05$) (Nikpay 2016)

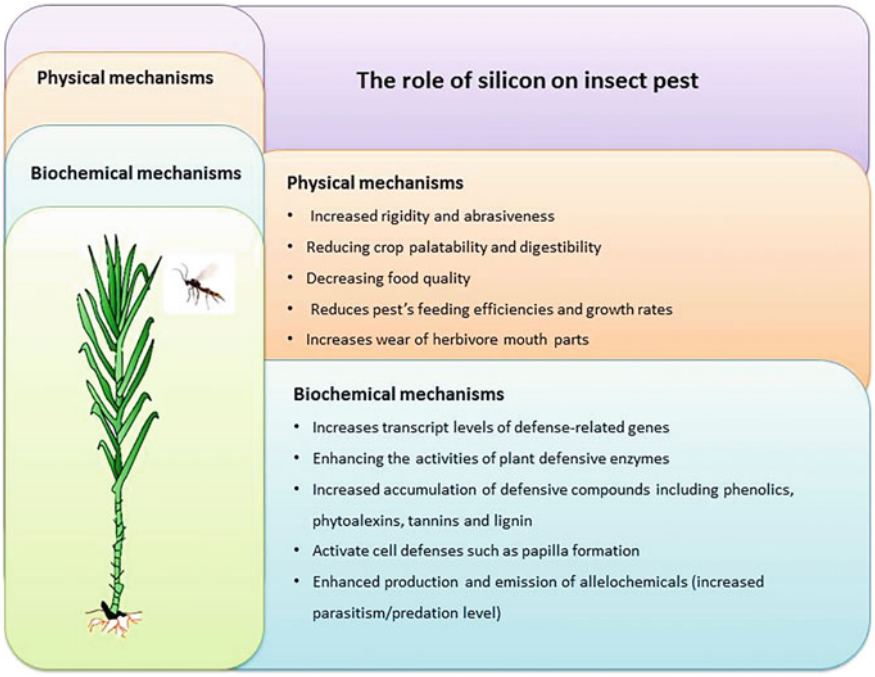

Fig. 5.2 Role of silicon on insect pests species (Reynolds et al. 2016)

stem borers. Moreover, the cane quality characteristics, including Brix (%), pol (%), and purity, increased compared to control. Interestingly, the parasitism rate was higher in silicon with parasitoid treatment than in check plots (Fig. 5.2).

Another aspect of silicon fertilization is its effects on the tri-trophic level. Silicon properties may affect beneficial arthropods (parasitoids and predators) on insect pests. Silicon may alter the emissions of the herbivore-induced plant volatiles (HIPVs) emissions, which can affect the attraction of enemies to treated plants (Reynolds et al. 2016). Nikpay et al. (2017) investigated the efficacy of three silicon formulations on the rate of parasitism on five sugarcane commercial varieties. The parasitism rate on treated and untreated sugarcane varieties was recorded for two consecutive years. The results showed significant differences between Si treatments and control in all sugarcane tested varieties. The results of the mentioned experiment confirm that silicon fertilization may positively enhance biological control effectiveness.

5.5 Effect of Silicon Fertilization in Water Stress and Salinity Stress Amelioration in Sugarcane

Water stress is known as one of the most harmful abiotic stress, which affects yield productivity across the world (Wang et al. 2003; Rampino et al. 2006). Sugarcane is considered a quite high-water demanding crop, and its growth and productivity are positively correlated with the presence of water in the soil (Lakshmanan and Robinson 2014; Verma et al. 2021a, b, c, d). Several studies highlighted the negative influence of water deficit in sugarcane (Boaretto et al. 2014; Silva et al. 2008; Oliveira et al. 2011), leading up to 80% of its productivity loss (Ramesh 2000; Basnayake et al. 2012; Gentile et al. 2015; Verma et al. 2020a, b, c). The most conspicuous responses of sugarcane concerning water stress are stomatal closure, inhibition of stalk and leaf growth, leaf rolling, the decline in leaf area (Inman-Bamber et al. 2012), reduction of water potential, photosynthetic activity, electrolyte leakage (Medeiros et al. 2013; Ferreira et al. 2017) and interruption of cell division and cell elongation (Machado et al. 2009). Moreover, tillering and stem elongation are the two most important phases which are highly susceptible to water stress conditions in sugarcane (Inman-Bamber and Smith 2005; Machado et al. 2009; Verma et al. 2019a).

The external Si fertilization can be well-thought-out as a viable substitute to improve the tolerance of sugarcane under water deficit conditions with the improvement of antioxidant enzymes and photosynthetic capacity (Verma et al. 2019a, b, 2020a). The encouraging effect of different sources of Si in mitigation of water stress in sugarcane is presented briefly in Table 5.5. Under moderate water stress conditions, the application of Si increased the dry biomass of sugarcane up to 34% compared to control (Oliveira et al. 2010). Bokhtiar et al. (2012) noticed the more significant deposition of silica in the epidermal layers of sugarcane plants treated with calcium silicate, which leads to a decline in water loss by cuticle transpiration. Studies also indicated that Si supplementation positively impacts the increase in

Table 5.5 The positive impact of Si fertilization in water stress amelioration in sugarcane (Majumdar and Prakash 2020a, b)

Type of stress	Sources of Si	Country	References
Water stress	Calcium silicate	Brazil	Oliveira et al. (2010)
	Calcium silicate	China	Bokhtiar et al. (2012)
	Potassium silicate	China	Shi et al. (2016)
	Calcium magnesium silicate	Brazil	Camargo et al. (2017)
	Calcium magnesium silicate	Brazil	Camargo et al. (2019)
	Calcium magnesium silicate	Brazil	Bezerra et al. (2019)
	Calcium metasilicate	China	Verma et al. (2019a, b, 2020)
	Sorbitol stabilized sodium and potassium metasilicate	Brazil	Teixeira et al. (2020a, b)
	Calcium metasilicate	China	Verma et al. (2021a, b, c)

stalk and sugar yield of sugarcane cultivars under water stress conditions (Camargo et al. 2017, 2019). Bezerra et al. (2019) observed that application of Si increased proline content and antioxidant enzymes such as superoxide dismutase and ascorbate peroxidase in sugarcane cultivars grown under water deficit conditions. Therefore, it is said that Si fertilization may be considered as an eco-friendly alternative solution for improving the productivity of sugarcane under water stress conditions.

In addition to water stress, salinity is also another significant abiotic stress which is highly ruthless and limits the productivity of crops worldwide (Rasool et al. 2013). It has been predicted that more than 50% of the arable land will be salinized by 2050 (Jamil et al. 2011). Sugarcane is moderately sensitive to salinity with a threshold value for yield reduction at 1.7 dS m^{-1} (Maas and Grieve 1990; Shannon 1997). Limited research has been documented in the existing literature to explore the role of Si in ameliorating salinity in sugarcane. However, studies indicated that the response of Si fertilization was more significant in the salt-sensitive genotype of sugarcane compared to the salt-tolerant genotype (Ashraf et al. 2009). Likewise, Si fertilization resulted in a significant increase in yield and associated attributes of sugarcane under salt stress conditions (Ashraf et al. 2010a). Moreover, the application of Si has also been shown to advance the juice quality of sugarcane when grown under salt stress conditions (Ashraf et al. 2010a, b). However, a further detailed investigation is necessary to determine the exact mechanism by which Si ameliorates salinity in sugarcane.

5.6 Silicon-Mediated Mechanisms Responsible for Increasing Plant Resistance to Stress

The effects of Si on the plant are versatile. Silicon impacts the yield of agricultural plants directly and indirectly through the soil (Ma and Takahashi 2002). The indirect Si-induced effects on cultivated plants are described in numerous reviews (Kim et al.

2017; Etesami and Jeong 2018; Zhu et al. 2019; Lesharadevi et al. 2021). Silicon is well known to be taken up by plants in the form of monomers of silicic acid [$Si(OH)_4$] (Ma and Takahashi 2002). In the plant, monosilicic acid accumulates and polymerizes in the epidermal tissues (bark, leaves, roots) or is transformed into various phytoliths (Mann and Perry 1986). A double cuticular layer is formed in the epidermal tissues, which mechanically strengthens and protects plants against diseases and insect pests (Ma and Takahashi 2002). Many authors declared the same mechanism in sugarcane (Kvedaras and Keeping 2007; Keeping et al. 2009; Majumdar and Prakash 2020a; Rahardjo et al. 2020; Verma et al. 2021d).

Plant supplementation with Si leads to an increase in the weight, volume, total and adsorbing surfaces of roots (Dakora and Nelwamondo 2003). Silicon fertilizers improve root respiration (Matichenkov 1996) and enhance the resistance to nematodes and other root pests due to the Si accumulated in the epidermal tissues of roots (Zhan et al. 2018). Silicon materials directly or indirectly affect insect herbivores (Reynolds et al. 2009). The direct effect relies on finely ground diatomite or silica nanoparticles to kill insects due to dehydration (Quarles 1992; Benelli 2018; Plumier et al. 2019). Indirect effects may result from delayed or reduced insect penetration and increased plant tolerance to abiotic stresses, for example, water stress, thus resulting in enhanced plant resistance to insect attack (Yin et al. 2019; Reynolds et al. 2009). Keeping et al. found that enhanced sugarcane resistance to borer *Eldana saccharina* was due to Si deposition mainly at the internode and root band.

Great attention was paid to study the Si-assisted stability of plant organelles (mitochondria, ribosome, and nucleus), cells, and molecules (pigments, DNA, RNA) (Bocharnikova et al. 2014; Kim et al. 2017; Wang et al. 2017; Zhang et al. 2018; Verma et al. 2020). However, most studies only report the effect of Si nutrition. There are very few hypotheses about underlying chemical or biochemical mechanisms. There is an assumption that Si impacts the biochemical properties of plant cells via element transport regulation. Silicon promotes active root-to-leaf transport of essential macro-and microelements (Pilon et al. 2013; Tubana et al. 2016; Teixeira et al. 2020a, b) but hinders the transport of toxic elements (heavy metals and metalloids) or excessive accumulation of nutrients (Imtiaz et al. 2016; Wei et al. 2021).

Presently, the mechanisms of enhancing plant defense by reducing destructive oxidative processes caused by various stresses are widely discussed (Manivannan and Ahn 2017; Verma et al. 2021). Any stress causes oxidative damage by increasing the generation of reactive oxygen species (ROS) (Noguchi and Niki 2019). ROS includes oxygen ($^1O_2$), superoxide (O^-_2), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) (Xie et al. 2019). The activities of antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (ASP), glutathione reductase (GR), and guaiacol peroxidase (GPX) play a key role in neutralizing ROS and alleviating oxidative injury (Yang and Lee 2015; Caverzan et al. 2016). Silicon reportedly enhanced the activity of ROS scavengers in many plant species, including sugarcane (Kim et al. 2017; Verma et al. 2021). Considering that any stress

stimulates ROS synthesis, Si supplementation could be a universal way to enhance plant stress resistance.

Although the mechanisms underlying the stimulant effect of Si on the plant defense system are widely discussed, they remain poorly investigated. We hypothesize that active forms of Si can participate in the synthesis of enzymes or stress proteins directly, and this process may include the following steps.

Step 1: *Initiation*—Stress activates the plant signaling system resulting in Si transport to the stressed site (Matichenkov et al. 1999; Bosnic et al. 2018; Minden et al. 2020).

Step 2: *Silicon uptake*—Soil- or foliar-applied monosilicic acid penetrates through the root plasmalemma (cell "sluice") or leaf epidermal tissue inside the cell and forms polysilicic acids. Monosilicic and polysilicic acids move within the plant (Matichenkov et al. 2008; Frazao et al. 2020; Wei et al. 2021).

Step 3: *Silicon distribution*—Silicon compounds partly translocate into the epidermal layer, root caps, cell walls, and other organs and tissues form Si-containing structures like phytoliths. Some Si compounds return into the cell to form Si gel, the basis for further low-temperature synthesis of organic compounds. Another part of Si can be stored "in reserve" as polysilicic acid or gel within the cell or intercellular space (Deng et al. 2020; Wei et al. 2021).

Step 4: *Synthesis of organic compounds on a polysilicic acid matrix at non-stress conditions*—Inside the cell, newly formed Si gel can absorb any organic molecule (Banerjee et al. 2001). The organic molecule adsorption on the Si gel surface must involve specific surface alterations with the formation of a special matrix that "remembers" the structure of the adsorbed molecule (Fig. 5.3). After a "printing" and moving out of replicating organic molecule, modified Si gel-plate provides a catalytic synthesis of copies of a former molecule (Banerjee et al. 2001). This process is widely used in organic chemistry and pharmacology (Mendes et al. 2012; Ji et al. 2016; Maurya et al. 2016).

Step 5: *Silicon-mediated synthesis of protective compounds at stressful conditions*—Stress activates the plant signaling system initiating additional synthesis of the stress proteins and antioxidants. Simultaneously, stressed plant forwards demand for further Si uptake from the environment and translocation of the stored Si to stress-exposed site. After receiving the information about stress, cell nucleus finds an adequate response, thus modulating the additional synthesis of defense-related compounds such as stress proteins, antioxidant enzymes, and low molecular antioxidants (Fig. 5.3). Then the molecules synthesized in response to stress are transported to damaged targets. However, at solid stress, the synthesis rate and quantity of synthesizing compounds may be insufficient owing to the necessity to solve other problems vitally crucial for the plant. As a result of escalating energy and time deficiencies, the process of synthesis of "routine" compounds essential for cell functioning slows down or even ceases. We suppose that some protective compounds are translocated to the newly formed Si gel, printed as former molecules. Then, former molecules move to the stressed zone leaving their prints on the Si gel surface, thus facilitating the

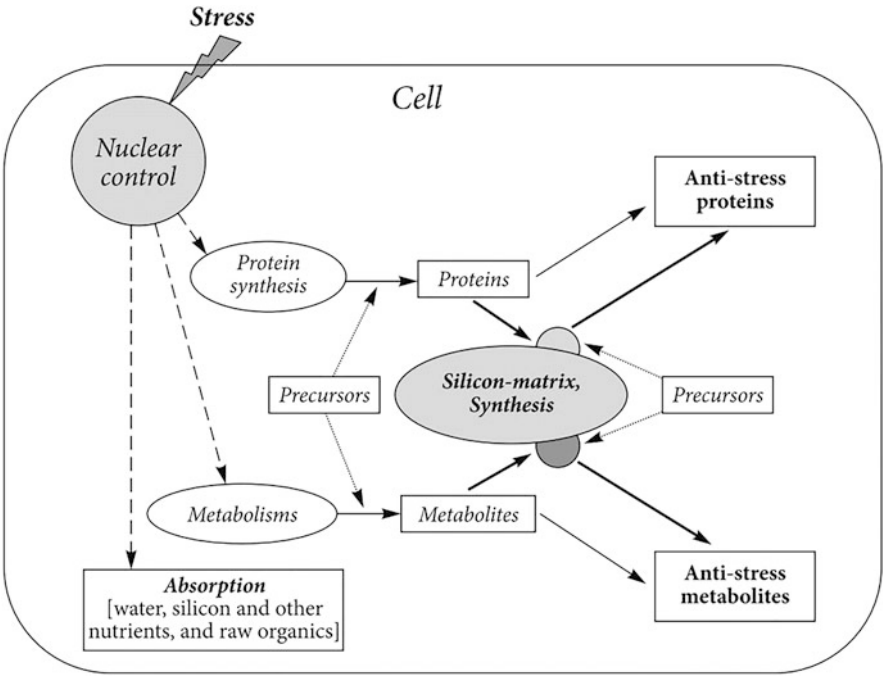

Fig. 5.3 Scheme of Si gel-mediated synthesis of organic molecules in the plant cell

synthesis of the same molecules. So, the Si gel matrix provides the formation of defense-related compounds before stress without the direct participation of the genetic apparatus. This hypothesis is possible from a chemical and biological point of view but requires direct evidence.

5.7 Conclusion

Globally, environmental stresses have a negative impact on plant performance and production. Several studies have found that the application of Si benefits the development of a variety of plants, particularly when they are exposed to environmental challenges. Silicon has been shown to improve stress resistance capacity by controlling various physiological, biochemical, and molecular processes. Furthermore, we observed that the beneficial effect of exogenous applied Si depends on stress severity, which differs from plant to plant, application methods, and cultivation strategies used for experiments such as soil or soilless culture. However, various factors and regulatory mechanisms have not been examined in detail and thus need further exploration.

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Anatomy of Tolerance Mechanisms in Sugarcane Crop to Abiotic Stresses

6

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Abstract

Plants respond and adapt to various environmental conditions through morphological, anatomical, and physiological adaptations at the cellular and plant level. These morphological, anatomical, and physiological adaptations help the plant to cope up with the environmental variations and the stress created by those variations. Among these adaptations, morphological and physiological adaptive traits are the most well-studied traits in most crops, including model crops. Drought and salinity stresses are the major abiotic stress factors affecting yield loss worldwide. Sugarcane with 12–18 months of crop cycle is not flexible enough to avoid unfavorable environmental conditions and faces all climatic variability throughout the year. In sugarcane development, about 80% of the sugar accumulates during tillering and grand growth period. Abiotic stresses during these growth stages critically affect sugarcane yield. Both leaf and root anatomical plasticity in crops play an important role in imparting tolerance to various abiotic stresses such as drought, salinity, oxidative stress, high and low temperature. An increase in the leaf cuticle thickness and increase in leaf epidermal thickness are reported to be the anatomical traits in drought-tolerant sugarcane varieties. Intact bulliform cells, bulliform cell area, chloroplast content, and chloroplast ultrastructure, especially the length, width, and width/length of chloroplasts, are reported to be effective indexes for drought-resistant sugarcane variety. Roots are the actual site that requires the highest plasticity during drought combined with high temperature to ensure continuous water movement through

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the soil-plant-atmosphere continuum. The efficiency of soil water uptake by the root system determines the rate of transpiration and above-ground performance. Increased root length, reduced cortical layer, increased protoxylem poles, increased metaxylem vessels, and reduced metaxylem diameter, which provides better hydraulic resistance, are some of the adaptive root traits reported in sugarcane under drought conditions. This chapter provides an overview of these leaf and root anatomical traits conferring tolerance to various abiotic stresses in sugarcane.

Keywords

Anatomy · Leaf-root · Environmental variables · Stress resistance · Sugarcane

6.1 Introduction

Sugarcane (*Saccharum officinarum* L.) (Poaceae) is an economically important crop used for approximately 80% of sugar production globally. Due to the high biomass production, sugarcane is also increasingly used as a source of bioenergy crop. However, a lack of water often limits sugarcane production, specifically at the critical growth stages such as formative and grand growth stages (Naik 2001; Silva et al. 2008; Tammisola 2010; Verma et al. 2019a, b, 2021a, b, c). In India, sugarcane cultivation is experiencing drought in tropical and subtropical regions and depends on supplementary irrigation for growth.

Abiotic stress is a recurrent problem in sugarcane that affects the quantity and quality of its yield. It is estimated that about 2.94 lakh ha is affected by drought in India, and about 2.5 lakh ha is affected by waterlogging (Misra et al. 2020), while nearly 9 mha sugarcane area is reported to be affected by salinity (Brindha et al. 2019). These abiotic stresses disturb the metabolism, growth, and development of sugarcane crop and finally leads to yield loss (Shrivastava and Srivastava 2016; Verma et al. 2020a, b, 2021d). Drought stress is one of the most destructive among all abiotic stresses since sugarcane is known to be a water-loving crop (Zingaretti et al. 2012; Lakshmanan and Robinson 2013; Verma et al. 2021c). Drought stress simultaneously affects several morphological and physiological traits in sugarcane, thereby causing the reduction in overall growth and crop productivity (Yardanov et al. 2003). Sugarcane needs a lot of water during the tillering and grand growth phase (Ramesh 2000). Plants have evolved to adapt to any stress conditions through various morphological, anatomical, and physiological mechanisms. Understanding these mechanisms will not only provide clues towards the crop adaptation to various stress conditions, but it will also help us develop improved tolerant genotypes (Chandler and Bartels 2008; Verma et al. 2019a).

The structural adaptations through leaf and root anatomical features help the plant respond and adapt to limited resources (Matsuda and Rayan 1990). The structural transformations in the leaf are more crucial for plants to survive under drought conditions, which help the plant to protect the photosynthetic machinery and

minimize water loss under drought. Adaptive anatomical features of leaves are directly linked to CO_2 assimilation rates and photosynthetic efficiency (Terashima et al. 2001). Some leaf traits such as leaf area are reported to contribute to yields in sugarcane directly. Leaf area is another essential characteristic to maximize solar radiation interception and is directly associated with carbon fixation (Sinclair et al. 2004). However, the root is the first organ to sense and respond to water dehydration in soil (Ferreira et al. 2017). Due to their functions in nutrient and water uptake, the anatomical adaptations among root traits also play an important role in determining sustainable yield under stress.

6.2 Leaf Anatomy and Drought Tolerance

Any stress condition impacts the internal structure, reflecting the poor physiological performance of a crop (Pan et al. 2011). The leaf is the first organ to reflect physiological performance, and leaf anatomy and physiology directly correlate with plant drought resistance (Wang et al. 2006). Table 6.1 summarizes the leaf anatomical features studied so far and reported to be important markers for drought resistance in sugarcane.

6.3 Stomatal Density and Size

Stomata with a pair of specialized guard cells surrounding a central pore provide access to the mesophyll cells (Grantz et al. 1987). Stomata play a crucial role in regulating water use and carbon uptake; hence stomatal structures are most extensively studied for plant water use efficiency and drought tolerance (Grantz et al. 1987; Bertolino et al. 2019; Hetherington and Woodward 2003).

Stomatal conductance is regulated in plants through substantial crosstalk between guard cell turgor pressure and stomatal pore aperture movement (Grantz et al. 1987; Kollist et al. 2014). Under reduced soil moisture, high temperature, or light intensity, the guard cell turgor pressure decreases, which results in reduced stomatal aperture and conductance (Schroeder et al. 2001; Mustilli et al. 2002; Tombesi et al. 2015; Bartlett et al. 2016; McAdam and Brodribb 2016). By reducing the stomatal aperture and conductance, plants improve water conservation, but often at the expense of reduced photosynthesis (Flexas and Medrano 2002).

Although the stomatal aperture is significant for stomatal conductance and photosynthesis under water-limiting conditions, stomatal density and stomatal size play an important role (Bertolino et al. 2019; Verma et al. 2020). Stomatal density and size have shown correlation to drought resistance in sugarcane (Zhang et al. 2015). Due to reduced stomatal size, loss in stomatal conductance has been linked to higher water conservation under water deficit conditions (Zhang et al. 2003). Verma et al. (2020) have reported reductions in the stomatal density and stomatal aperture size in sugarcane plant leaves under drought to reduce water loss. Further Si application has enhanced stomatal density and aperture size under drought stress.

Table 6.1 Impact of leaf anatomical mechanism under abiotic stress conditions

Characteristics	Leaf anatomy under stressed condition	References
Lamina thickness	Reduces significantly during the water-deficient condition	Taratima et al. (2020)
Cell wall and cuticle thickness (ab and ad)	Getting thickened or thickness increased during stress in comparison with control	Zhang et al. (2015); Taratima et al. (2020), Malik (1986); Meneses Rodriguez (1985); Xu (1986); Mo and Zhou (1984)
Major vascular bundle of the midrib	Higher lignification degree of thick-walled cells	Zhu et al. (2010)
Vertical length	Increases during stress	Zhu et al. (2010); Taratima et al. (2020)
Horizontal length	Increases during stress	Zhu et al. (2010); Taratima et al. (2020)
First and second vessel diameter (metaxylem)	Increases during stress	Taratima et al. (2020)
Vessel cell wall thickness (protoxylem)	Reduces during stress	Taratima et al. (2020)
Phloem vertical length	Increases during stress	Hölttä et al. (2009); McDowell and Sevanto (2010), Taratima et al. (2020)
Phloem horizontal length	Increases during stress	Hölttä et al. (2009); McDowell and Sevanto (2010), Taratima et al. (2019, 2020)
Bundle sheath extension length	Increase during stress	Taratima et al. (2019, 2020)
Major vascular bundle of the lamina	Increase during stress	Taratima et al. (2020)
Vertical length	Increase during stress	Taratima et al. (2020)
Horizontal length	Increase during stress	Taratima et al. (2020)
First metaxylem diameter	Reduces during stress	Taratima et al. (2020); da Cruz Maciel et al. (2015); Passioura (1982); Melo et al. (2007)
Second metaxylem diameter	Reduces during stress	Taratima et al. (2020); da Cruz Maciel et al. (2015); Passioura (1982); Melo et al. (2007)
Protoxylem cell wall thickness	Increase during stress	Taratima et al. (2020); da Cruz Maciel et al. (2015)
Phloem vertical length	Reduces during stress	da Cruz Maciel et al. (2015); Taratima et al. (2019, 2020)
Phloem horizontal length	Reduces during stress	Taratima et al. (2020)
Bulliform cell vertical length/ horizontal length	Thicker leaf cuticle, reduces widened vesicles in bulliform cells	Mo and Zhou (1984); Meneses Rodriguez (1985); Malik (1986); Xu (1986)

(continued)

Table 6.1 (continued)

Characteristics	Leaf anatomy under stressed condition	References
Stomata per unit area	Reduction during stress	Mo and Zhou (1984); Meneses Rodriguez (1985); Malik (1986); Xu (1986); Zhang et al. (2015)
Stomatal width (ab)	Reduces during stress	Meneses Rodriguez (1985); Malik (1986); Taratima et al. (2020)
Stomatal length (ab)	Increases during stress	Taratima et al. (2020); Verma et al. (2020)
Stomatal width (ad)	Reduces during stress	Taratima et al. (2020); Verma et al. (2020)
Stomatal length (ad)	Increases during stress	Taratima et al. (2020); Verma et al. (2020)
Inter-stomatal cell width, length (ad)	Reduces during stress	Taratima et al. (2020)
Inter-stomatal cell width (ab)	Increases	Taratima et al. (2020)
Inter-stomatal cell, length (ab)	Reduces	Taratima et al. (2020)
Short-cell width (ad and ab)	Increases during stress	Taratima et al. (2019); Taratima et al. (2020)
Short-cell length (ad)	Increases during stress	Taratima et al. (2020)
Short-cell length (ab)	Reduces during stress	Taratima et al. (2020)
Long-cell length (ad and ab)	Reduces during stress	Taratima et al. (2020)
Stomatal density (ad and ab)	Reduction in stomatal density	Taratima et al. (2020); Verma et al. (2020)

ab abaxial, *ad* adaxial

Smaller stomata can reduce the total leaf pore area, and smaller cells permit faster aperture response (Franks and Beerling 2009; Drake et al. 2013; Lawson and Blatt 2014). The more rapid stomatal response has shown maximum Water Use Efficiency (WUE) under fluctuating light conditions than prolonged water stress (Drake et al. 2013; McAusland et al. 2016; Kardiman and Ræbild 2018). Along with the stomatal size, the shape of guard cells and subsidiary cells are also proposed to affect stomatal functioning for water use efficiency and drought tolerance (Lawson and Vialet-Chabrand 2019).

Any stomatal damage affects carbon uptake, leading to the loss of photosynthetic machinery and reduced crop yield. Several authors have reported an increase in stomatal density and a decrease in size as an adaptive character during drought stress (Nawazish et al. 2006; Taratima et al. 2019). Few authors have also reported anatomical features such as more veins and lesser stomata per unit area in leaf to be closely related with sugarcane drought resistance (Mo and Zhou 1984; Meneses Rodriguez 1985; Malik 1986; Xu 1986).

6.4 Enlargement of Bulliform and Epidermal Cells

Bulliform cells are the water-storing epidermal cells present in the upper surface of leaves and play an essential role in regulating the rate of transpiration. Under moisture stress, bulliform cells assist in leaf rolling to avoid water loss through transpiration. Leaf rolling and reduced transpiration are related to plants' drought resistance (Baranova 1987). The inefficiency of bulliform cells in leaf rolling and reduction in bulliform cell area under drought is considered as a susceptible character in sugarcane (Zhang et al. 2015; Taratima et al. 2019). With the water loss from the leaf, the perimeter/area ratio in bulliform cells is reported to reduce under drought. It is also noticed that the smaller ratio of perimeter and area is better for material and energy conversion (Wang et al. 2009; Zhang et al. 2015; Taratima et al. 2019).

Other important anatomical modifications reported in sugarcane under drought stress are enlargement of bulliform cells and epidermal cells, widened vesicles in bulliform cells, and bulliform cells with thin cell walls (Nawazish et al. 2006; Taratima et al. 2019). Under drought stress conditions, the pit of sclerenchyma cell walls is also reported to increase (Bosabalidis and Kofidis 2002).

6.5 Thickening of Leaf Lamina and Cuticle Layer

In sugarcane, the thickening of adaxial and abaxial cuticles covering the epidermis happens under both drought and salinity (Mo and Zhou 1984; Taratima et al. 2019). Along with the cuticle layer, increased lignification of cells around the vascular bundle is found in drought-resistant sugarcane varieties (Zhu et al. 2010). Strong lignification around the vascular bundle protects the conducting tissues under drought stress.

6.6 Other Anatomical Features

The size of bundle-sheath cells and vascular bundles gets modified under drought stress in sugarcane (Wu et al. 2011). Under moisture, increase in the vascular bundle size improves water and food transportation efficiency (Bosabalidis and Kofidis 2002). The number of vessels per unit area in sugarcane roots and stems is positively correlated with drought resistance (Tan 1988). Under severe drought stress, plasmolysis of chloroplasts is reported in sugarcane (Zhang et al. 2015). Movement of chloroplasts towards the center of the cell, change in shape, and increase in starch content are shown in susceptible sugarcane genotypes (Zhang et al. 2015). Reduced length, width, and width/length of chloroplasts are effective indexes for drought and salinity (Wu et al. 2011).

6.7 Root Anatomical Traits

Roots are the organs to detect moisture stress, and the physiological and molecular signals to induce resistance are sent by the roots (Atkinson and Urwin 2012). These root system signals help the plant adapt through various biological mechanisms to maintain optimal growth and yield under stress conditions (Sieburth and Lee 2010). Roots not only initiate the molecular signaling, but also modify the root architecture and anatomical traits, which contributes to enhance above-ground performance. Root System Architecture (RSA) plays a vital role in the agronomic performance of a crop. The adaptive plasticity in root anatomical helps to maintain photosynthesis and stomatal regulation, resulting in better yield under stress conditions (Chimungu et al. 2014a, b, 2015; Kadam et al. 2015).

In sugarcane, the relationship between root and shoot growth under diverse conditions has shown a positive correlation, and the efficient root traits also determine to stalk dry weight (Glover 1967; Smith et al. 1999; Ferreira et al. 2017). Sugarcane root system is highly divergent, comprising of highly branched sett roots (roots originating from the sett), shoot roots (main roots originating directly from the shoot), and deep rope roots formed by the agglomeration of shoot roots (Lynch 2013; Valarmathi et al. 2020). Sett roots arise from root eyes of setts within 24 h after planting that are required essentially for settling development and eventually degrades after 30–40 days. Shoot roots are stable, thicker, and fleshier permanent roots that provide strong anchorage developed from shoot bases 5–7 days after planting. These roots penetrate deeper soil beyond 1.5 m providing access to deep soil water reserves. The development of these root types strongly contributes to the performance of the above-ground parts (Gregory 2006). In sugarcane, extensive root systems support physiological and morphological traits of the above-ground parts under early drought stress (Khonghintaisong et al. 2018; Smith et al. 2005). Among the Root System Architecture (RSA), deep rooting is an extensively studied and reported root trait under stress conditions. Tolerant sugarcane genotypes have a long root system compared to susceptible genotypes under both drought and salinity stress conditions (Kumar et al. 2017; Khonghintaisong et al. 2018; Ogbaga et al. 2020). The genotypes with deep and extensive root systems are selected as water stress-tolerant genotypes (Smith et al. 2005). Long roots result in better water uptake, a desirable trait to extract deep soil moisture when water is limiting (Tardieu et al. 1992; Blum 2005; Tardieu 2012). At the cellular level, increased biosynthesis of lignin has one of the most crucial reactions under water-limiting conditions. The increased biosynthesis of lignin leads to cell-wall thickening of the vascular tissues, endodermis, and exodermis (Enstone et al. 2002; Naseer et al. 2012).

Anatomically monocot roots are characterized by the presence of two highly suberized layers called endodermis and pericycle. These two cell layers play a significant role in selective absorption as well as mineral and water uptake (Vásquez 2003). The pericycle is the meristematic layer, the source of lateral roots and surrounds the vascular bundle or stele (Richards and Passioura 1981). The major challenge for the plant under moisture stress is to protect the root water-conducting tissues from hydraulic pressure. Another challenge is to protect the meristematic

layer pericycle for the growth of lateral roots. These two modifications are achieved either by lignifying the cells surrounding the vascular cylinder or by reducing the diameter of the xylem vessels. Only three authors have so far worked on the anatomical structures of sugarcane roots under drought conditions (Queiroz-Voltan et al. 1998; Chaves et al. 2009; da Cruz Maciel et al. 2015). The anatomical features studied in sugarcane are described in detail in separate sections.

6.8 Reduced Xylem Diameter

It is reported that continuous drought intensifies the imbalance between water transport and transpiration (through stomata and cuticles). This imbalance develops highly negative water potential and increases xylem tension, leading to bubble formation or cavitation of the vessel elements. Cavitation interrupts the flow through the xylem elements and may reduce the stomatal conductance, rate of photosynthesis, and, consequently, growth (Tyree and Sperry 1989). Under moisture stress conditions, this is the first symptom that directly affects the hydraulic system. To avoid this problem, the major adaptive root plasticity in the root system is making the hydraulic system more resistant and preventing cavitation (Kadam et al. 2015). Studies have demonstrated that the adaptive plasticity of xylem elements is the key to improve water use efficiency. The efficiency of the xylem hydraulic conductance shows direct relation to drought resistance and sustained yield. Reduced metaxylem diameter is very common in plants under water stress, and reductions in diameter of the metaxylem elements result in greater resistance to water flow (Passioura 1982; Melo et al. 2007). The tolerant sugarcane genotype RB867515 showed reduced vessel diameter under drought conditions (da Cruz Maciel et al. 2015). Several studies have also reported early stomatal closure as an adaptive mechanism that prevents xylem cavitation (Tardieu and Davies 1993; Plaut et al. 2012). Two major anatomical root traits have been reported to increase hydraulic root resistance: reduced xylem diameter and increased xylem number (Richards and Passioura 1981; Plaut et al. 2012).

6.9 Increased Exodermal Layer

Exodermis is the unicellular cell layer below the outermost epidermal layer in roots. Both epidermis and exodermis serve as apoplasmic barriers to transport water and ions to the inner vascular cylinder (Enstone et al. 2002; Enstone and Peterson 2005). The increased exodermal layer acts as a barrier for oxygen and water movement (Colmer 2003). On the other hand, a thin exodermis allows free radical oxygen and water movement. The rhizosphere, with better-aerated conditions, protects the roots against phytotoxins (Armstrong et al. 2000; Soukup et al. 2002). The low oxygen levels also stimulate ethylene synthesis, which inhibits root elongation. da Cruz Maciel et al. (2015) showed that roots of susceptible sugarcane genotypes had the highest number of exodermis.

6.10 Thin-Walled Exodermis

The deposition of suberin in the cell wall of the exodermis makes the layer thicker. The suberin layer acts as a barrier and prevents the radial loss of oxygen to the rhizosphere. In contrast, the barrier increases the longitudinal diffusion of oxygen in the aerenchyma (Soukup et al. 2002). Similar to the condition in increased exodermal layer, a thick-walled exodermis reduces the aeration in roots. It is also shown that the suberized exodermis reduces the flow of water and minerals from epidermis to cortex and the vascular cylinder (Prado 2005). A drought-tolerant sugarcane genotype RB867515 with thin-walled exodermis has been shown to facilitate water movement and maintain productivity under reduced moisture (Prado 2005; Ferreira et al. 2007; da Cruz Maciel et al. 2015).

6.11 Reduced Cortical Layer

The cell layer forms the cortex in between the exodermis and the stele. The reduced cortical layer is an adaptive trait in roots under drought conditions. It has been shown in several crops that reduced cortical cell layer reduces the metabolic costs of root growth and maintenance. Reduction in the cortical layer reduces the root volume, which has more metabolic demand than the stele region (Lynch 2013; Chimungu et al. 2014a, b). Reduced root volume decreases the metabolic demand under resource-limiting conditions. The drought tolerance is improved by reducing the metabolism cost, enabling continuous root growth and deeper soil exploration. Deeper soil exploration gives better water acquisition from the deeper soil reserves for better yield under water stress (Chimungu et al. 2014a, b; da Cruz Maciel et al. 2015).

6.12 Cortical Lysigenous Aerenchyma

The phytohormone ethylene triggers the formation of lysigenous aerenchyma in plants subjected to abiotic stress conditions (Bouranis et al. 2007). Aerenchyma develops intercellular spaces in the cortical layer. The reduced cortical layer filled with aerenchyma is found to be an adaptive character under drought as well as waterlogging conditions. The presence of aerenchyma is reported to have two important roles under drought conditions such as (1) it prevents the sudden shrinking of cortical cells due to the change in hydric potential and (2) the air spaces in the aerenchyma layer help in avoiding excess loss of water from the compact cortical layer (Melo et al. 2007). Aerenchyma cells facilitate better O_2 diffusion, which helps to maintain aerobic respiration and cellular metabolism in roots (Vasellati et al. 2001; Bouranis et al. 2007; Melo et al. 2007). The presence of aerenchyma in the roots of sugarcane genotypes tolerant to drought has been reported (da Cruz Maciel et al. 2015).

6.13 Endodermis with U-Thickening

The endodermis is the outermost safety layer surrounding the stele and functions as an apoplasmic layer in regulating the movement of water, ions, and hormones into and out of the vascular system. In sugarcane under drought conditions, the anticlinal and inner periclinal walls of endodermal layers were found to be thickened (da Cruz Maciel et al. 2015). This is called U-thickening, which is more in the tolerant genotype than the susceptible sugarcane genotypes (da Cruz Maciel et al. 2015; Valarmathi unpublished data). Endodermal thickening is reported to play an important role in the conduction of water and photosynthates under both salinity and drought stress conditions. The thickening of endodermal cells helps to protect the vascular cylinder from damage due to hydraulic resistance and also prevents excess water loss from the stele region. Increased lignification of root endodermal cell wall is found to be one of the major salinity tolerance strategies in the roots of halophytes (Barzegargolchini et al. 2017).

6.14 Sclerification of Pericycle

As already mentioned, the pericycle is the meristematic layer, which is the source of lateral roots and surrounds the vascular bundle or stele (Richards and Passioura 1981). A common feature of the roots of monocots is the sclerification of the pericycle under drought and salinity stress (da Cruz Maciel et al. 2015). The sclerification of the pericycle helps to protect the vascular cylinder and increases the hydraulic resistance, while it reduces the morphogenic ability of this layer to form lateral roots (Ferri et al. 2000; Raven et al. 2008). In sugarcane, sclerified pericycle is reported intolerant genotypes under drought conditions (da Cruz Maciel et al. 2015). Sclerification of pericycle may prevent the cellular damage during stress, once the cessation of stress if the pericycle is intact, new roots will arise.

6.15 Conclusion

Sugarcane is an economically important crop for sugar and bioenergy production. Abiotic stress factors such as drought, salinity, high temperature, and waterlogging impact sugarcane productivity. Drought and salinity stresses are considered as one of the most deleterious stresses affecting sugarcane yield losses. Developing a tolerant genotype is essential to sustain sugarcane production under extreme environmental conditions. Studying the physiological, anatomical, and molecular changes during stress is essential to develop a tolerant genotype with a holistic approach. Very limited studies have been carried out to understand the anatomical tolerance mechanisms in sugarcane. However, the details given in this chapter show that anatomical feature of sugarcane leaf and root responds to stress conditions, and they also help in imparting tolerance to sugarcane crops. These traits can be used as a marker trait to identify the most stress-resistant genotype.

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7 Interaction of Plant Growth-Promoting Rhizobacteria with Sugarcane Plants for Alleviating Abiotic Stresses and Improving Crop Yields

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Abstract

Abiotic stresses are a severe threat to crop productivity as well as the quality of crop produces. When the sugarcane plant is challenged with abiotic stresses, plant physiological and biochemical processes are adversely affected. Affected plant processes result in reduced crop growth and yield. Sugarcane takes a long duration to mature and harvest, and it is huge biomass generating crop. The sugarcane crop has different growth phases but tillering and formative stages are most sensitive to the abiotic stresses. Abiotic stresses are drought, salinity, soil-contaminated with heavy metals, scarce minerals in the soil, waterlogging/flooding, improper temperature and light, low oxygen and ozone, etc. It is well known that plant roots play an important role in the absorption of water and minerals from the soil, and roots are badly affected under abiotic stress conditions. Plant growth-promoting rhizobacteria (PGPRs) are potential abiotic stress managers. Application of PGPRs is environmentally friendly, low cost, and viable approach and being used worldwide. Plant growth-promoting bacteria for alleviating abiotic stresses produce exopolysaccharide, ACC deaminase enzymes, antioxidants/osmolytes, volatile compounds, etc. Some PGPRs like *Azospirillum* spp., *Pseudomonas* spp., and *Bacillus* spp. are identified as tolerant to drought and salinity. Some PGPRs are reported for metals detoxification and absorption. Interactions of plant growth-promoting rhizobacteria with sugarcane plants play an important role in adaptation, maintenance, and survival under abiotic stresses.

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Keywords

Abiotic stress · Drought · Heavy metals · PGPR · Alleviation · Salinity · Sugarcane

7.1 Introduction

Sugarcane is a commercial cum industrial crop cultivated on more than 25 million hectares worldwide. It takes 12–18 months to mature and is exposed to biotic and abiotic stresses for a longer duration. The crop has a strong root system and a better photosynthetic C_4 system. However, crop suffers from several biotic and abiotic stresses. Biotic stress caused by pest and diseases is very damaging to the sugarcane crop. The expected long duration of the crop requires a quantum amount of irrigation water and chemical fertilizers, which increase the cost of sugarcane production. Besides biotic stresses, abiotic stresses are also major constraints in crop productivity worldwide, and the area under abiotic stress is increasing day by day. Abiotic stresses include water stress (drought/flooding), salinity, heavy metals, nutritional deficiency, and improper temperature and light. To cope with these abiotic stresses, the sugarcane growers adopt several adaptations and mitigation strategies (Verma et al. 2020a, e, 2021b, c).

Applying plant growth-promoting rhizobacteria (PGPRs) is one of the potential strategies to mitigate the adverse impact of abiotic stress (Verma et al. 2020c). The bacteria associated with the plant roots region are called rhizobacteria (Hiltner 1904) and assist plants by a plethora of mechanisms. These PGPRs are beneficial and directly or indirectly assist in plant growth promotion. These PGPRs colonize mainly the rhizosphere region of plant roots and the endo-rhizosphere region. These PGPRs impart abiotic stress tolerance in plants by producing ACC deaminase enzyme, abscisic acid, antioxidative enzyme, osmoprotectants, exopolysaccharides, defense-related proteins, various enzymes, and volatile compounds; expression stress-related genes and proteins and biosorption/immobilization/detoxification of heavy metals. Li et al. (2017) isolated different species of *Pseudomonas* from the sugarcane rhizosphere and characterized them for beneficial plant growth-promoting activities like ACC deaminase, IAA production, and disease management. Keeping the potential of PGPRs concerning alleviating abiotic stresses, different mechanisms are discussed in this chapter for alleviating abiotic stresses using PGPRs in sugarcane and other crops.

7.2 Sugarcane Crop

Sugarcane (*Saccharum* spp. hybrid) is a crop of the tropical region. However, it is also cultivated in the subtropical regions of the world. The sugarcane crop takes 12–18 months to ripen. It is cultivated in more than 120 countries of the world (Shukla et al. 2017; Verma et al. 2019). The crop provides raw materials for the

sugar and alcohol industry, biofuel and biogas production, paper industry, and cosmetics. Among the sugarcane-producing countries, the largest sugarcane area under cultivation is in Brazil (10 mha), followed by India. In India, sugarcane is cultivated on around 5.0 mha of land. The average cane productivity of the country is around 81 tonnes per hectare.

Sugarcane crops can be cultivated in almost any soil texture, but water-holding soils and rich organic carbon content (0.6% OC) are most suited. The crop productivity varies from state to state because of soil quality and fertility, varietal adaptations, agronomic interventions, and climatic conditions. These conditions greatly influence the process of sugarcane ripening and, subsequently, sucrose recovery. Improved sugarcane varieties have more genetic potential to produce vigorous growth and resistance/tolerance to biotic and abiotic stresses (Kingston 2013; Verma et al. 2021b; Shukla et al. 2022). Agronomic interventions boost crop potential and maximize crop yield. Climatic conditions have very pronounced and significant effects on the sugarcane crop, from sett germination to cane ripening. A long dry, warm growing season followed by cool and frost-free weather is considered ideal for sugarcane production (Jaiswal et al. 2021). Long dry days support better germination and tillering, whereas warm-season supports stem elongation. The cool and frost-free season is best for cane ripening and harvesting. Temperature and light intensity affect juice quality parameters. In addition to this, many other abiotic stresses like scarce minerals in the soil (iron, zinc, copper), water stress (drought or flooding), salinity, heavy metals (cadmium, lead, nickel) exert considerable influence on crop growth and development as well as crop yield (Verma et al. 2020b, d, 2021d).

7.3 Abiotic Stresses

The environmental stresses other than biotic factors influence growth attributing traits, and crop yield is called abiotic stresses. Abiotic stresses may be inadequate availability of minerals in the soil (iron, zinc, copper), water deficit or excess condition (drought or flooding), salinity, heavy metals (cadmium, lead, nickel), improper temperature, and light. Among these abiotic stresses, drought, salinity, and heavy metals are predominant and of economic importance (Verma et al. 2019, 2020a, e). Nutritional deficiency symptoms are widespread in ratoon crops and may cause economic damage. The stresses caused by drought, salinity, and heavy metals significantly affect root architecture, stem elongation, photosynthetic traits, and juice quality. Drought is predominant abiotic stress worldwide (Verma et al. 2021a). It has been recorded that almost one-third of total world agricultural land is under drought conditions. In the future, it will be more, nearly 50% of total world agricultural land is expected to be by 2050. Approximately 40% of land in India is drought affected, and 6.3 mha of land is flood affected. It affects almost 40% population of the country. The crop cultivated under water deficit conditions suffers from several disadvantages like poor germination, gaps in the crop field, poor crop growth, late maturity, poor juice quality, and crop becoming prone to several insect pests and

diseases. In severe drought conditions, sucrose synthesis, transportation, and accumulation are badly affected.

Similarly, salinity is the cause of concern. It has been speculated that huge land area is under saline condition, causing imbalance and reduction in crop growth and performance of the crop (Cicek and Cakirlar 2002; Cuartero et al. 2006; Beck et al. 2007; Dimkpa et al. 2009; Sandhya et al. 2010; Ahemad 2012; Gupta et al. 2012; Ali et al. 2013; Islam et al. 2016; Sah et al. 2016; Egamberdieva et al. 2017; Etesami 2018). About 7–8 mha of land in India have been affected by salinity and alkalinity. Almost all the states have salinity and alkalinity, but it is most common in Uttar Pradesh, Gujarat, West Bengal, Rajasthan, Punjab, Maharashtra, and Haryana. Salinity and alkalinity impose serious problems in sugarcane's normal growth and development. Soil contamination with heavy metals is nowadays cause of concern for agricultural soils because of their negative effect on crop production, human health, and the environment. Soil health is deteriorating at a greater rate, and the population of beneficial microbes is severely affected.

7.4 Plant Growth-Promoting Rhizobacteria (PGPRs)

Microbes are small living entities on Earth and are found everywhere, from cold regions to hot springs. Microbial diversity and population structure also vary from place to place. It has been recorded that fertile soil is rich in the diverse microbial population. Among soil microbes, some of them have beneficial interactions with plants, and others may be pathogenic to them. The beneficial microbes may have free-living interaction, associative and or symbiotic relationships (Shukla et al. 2021). Soil is further designated based on root influence; the soil directly under the influence of a plant's root system is called the rhizosphere, and away from root influences is called bulk soil (Sharma et al. 2019). The soil microbes have direct or indirect support for growth and development of plants. The plant growth-promoting rhizobacteria perform many mechanisms and processes for promoting plant growth and protecting from adverse biotic and abiotic stresses (Shukla et al. 2020b; Xia et al. 2020). Direct plant growth-promoting mechanisms used by PGPRs are phytohormone production (auxins, cytokinins, gibberellins, abscisic acid, and ethylene), biological nitrogen fixation, phosphorus solubilization, mineralization, potassium solubilization, and by way of biofertilizers.

In contrast, indirect mechanisms used by PGPRs are the production of siderophores, detoxification or immobilization of toxic metals, production of antibiotics, production of lytic enzymes (chitinases, glucanases), and plant defense mechanisms activation, which is called biocontrol potential (Shukla et al. 2020a, b). In addition to these mechanisms used by plant growth-promoting rhizobacteria, some potential PGPRs have a key role in alleviating abiotic stresses like salinity, drought, and heavy metals by the ACC deaminase enzyme production, production of abscisic acid, antioxidative enzyme production, osmoprotectants production, exopolysaccharides production, defense-related proteins, and enzymes production,

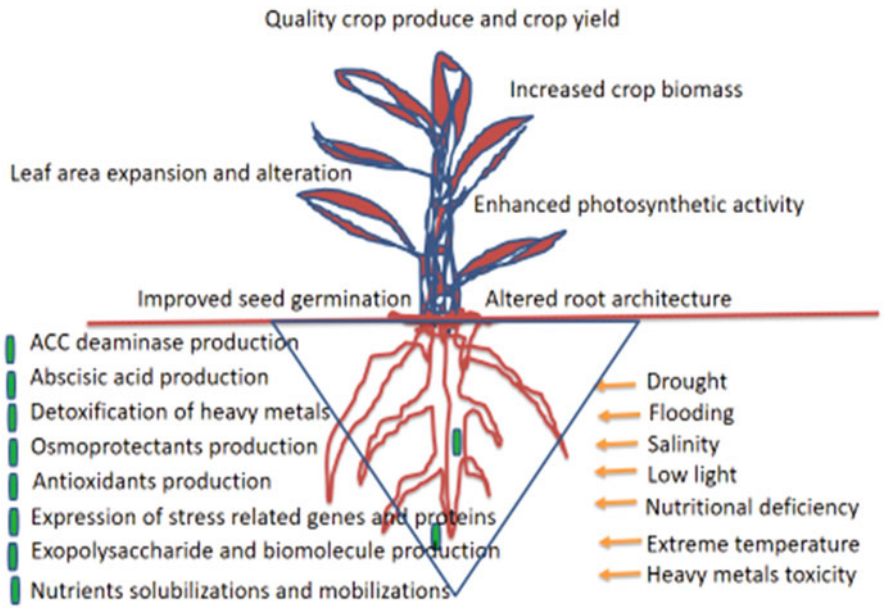

Fig. 7.1 Mechanisms of PGPRs for alleviating abiotic stresses

expression stresses related genes and proteins, production of volatile compounds, and biosorption/immobilization/detoxification of heavy metals.

The mechanisms used by PGPRs in alleviating plant abiotic stress are illustrated in Fig. 7.1 and Table 7.1. Besides this, plants themselves adopt defense mechanisms to mitigate abiotic stresses by decreasing sodium accumulation and enhanced potassium concentration under saline conditions, reduced photosynthesis under drought conditions, and increased reactive oxygen species and deposition of excess metals in vacuoles under metal stress conditions.

7.5 Mechanisms of PGPRs for Alleviating Abiotic Stresses

7.5.1 ACC Deaminase Enzyme Production

Ethylene at low concentration assists in seed germination, root elongation, nodule formation, and flower initiation. Still, at high concentration, it restricts the growth of plants by leaf defoliation and senescence and root growth inhibition. Mechanisms of the increased level of ethylene can be understood easily; when a plant is challenged with any stress by drought, salinity, toxic metals, etc., the plant starts to produce 1-aminocyclopropane-1-carboxylate (ACC). ACC molecule works as a precursor for ethylene production, and relatively increased ethylene level becomes toxic to crop plants. Leaf senescence can be noticed on the plants. Once a higher level of ethylene is accumulated in plant tissues, recovery for the growth and development of the plant

Table 7.1 Example of some potential PGPRs in alleviating abiotic stresses on various crops

Abiotic stresses	Some potential PGPRs	Mechanisms	Crop	References
Drought	*Azospirillum* spp., *Pseudomonas* spp., *Klebsiella pneumonia*, *Bacillus cereus* AR156	Production of abscisic acid and gibberellins, antioxidants	Maize, tomato, rice, wheat	Cohen et al. (2009); Sandhya et al. (2010); Juan et al. (2012)
Salinity	*Bacillus* spp., *Azospirillum* spp.	Production of ACC deaminase and ROS scavenging enzymes	Potato, sugarcane, tomato, rice	Gururani et al. (2013); Moutia et al. (2010); Cuartero et al. (2006)
Heavy metals toxicity	*Bacillus thuringiensis* GDB-1, Copper resistant bacteria, *Bacillus* spp., *B. cereus*, *B. sphaericus*, *B. subtilis*, *Burkholderia* spp., *Pseudomonas* spp.	Bacterial bioremediation, detoxification, bioaccumulation	Alnus, lentil, tomato, mustard	Babu et al. (2013); Islam et al. (2016); Syed and Chinthala (2015); Costa and Duta (2001); Dong et al. (2006); Dourado et al. (2013); Jing et al. (2007); Madhaiyan et al. (2007); Sheng et al. (2008); Singh et al. (2010)
Nutritional deficiency	*Bacillus cereus*, *B. macrolides*, *B. pumilus*, *Pseudomonas* spp.	Nutrient mobilizations, fixation, and production of gibberellins	Red pepper, sugarcane, sunflower	Joo et al. (2004); Muthukumarasamy et al. (2017); Belimov et al. (2014); Pourbabaee et al. (2018); Sah et al. (2016)
Multi stresses	*Bacillus xiamenensis* PM14, *Trichoderma harzianum* T6, *Pseudomonas fluorescens* PSB28, *Gluconacetobacter diazotrophicus* NB73, *Bacillus licheniformis* K11, *Azospirillum brasilense* Cd1843, *Bacillus subtilis* SYST2	Performing plant growth and plant protection characteristics	Sugarcane, pepper, carnation	Xia et al. (2020), Shukla et al. (2020a, b), Lim and Kim (2013), Li et al. (2005); Tahir et al. (2017); Dourado et al. (2014); Shukla et al. (2019); Zhang et al. (2017)

is very difficult (Juan et al. 2012; Kasim et al. 2013; Kaushal et al. 2016a, b; Ngumbai and Kloepper 2016). In that situation, crop produce and biomass loss will happen. Some potential microbial cultures can synthesize the ACC deaminase enzyme, transforming 1-aminocyclopropane-1-carboxylate into ammonia and

α-ketobutyrate. This transformation of ACC by the ACC deaminase enzyme minimizes the level of ethylene in the plants. The ACC deaminase enzyme-producing bacteria are mostly found in the rhizosphere region and become beneficial to the growing plant when the crop is stressed by salinity, drought, toxic metals, and other abiotic stresses (Mayak et al. 2004; Li et al. 2005; Madhaiyan et al. 2007; Moutia et al. 2010; Juan et al. 2012; Lim and Kim 2013; Kasim et al. 2013; Glick 2014; Vejan et al. 2016; Kaushal et al. 2016a, b; Ngumbai and Kloepper 2016). Besides this, transformed chemicals are not toxic to growing crops, and in such a way, the level of ethylene can be managed by applying PGPRs. Several *Pseudomonas*, *Azospirillum*, *Azotobacter* have been reported and identified for ACC deaminase production (Ahemad and Kibret 2014; Pérez-Montaño et al. 2014; Ruzzi and Aroca 2015).

7.5.2 Abscisic Acid Production

Abscisic acid is a stress phytohormone, and this plays a major role in stomata opening and the growth and development of crop plants. When the plant is in drought condition means water deficit condition, abscisic acid phytohormone biosynthesis occurs in the plants, which causes partial stomatal opening that conserve water level and its requirements. Increased level of abscisic acid results in drop down of fruits and leaves and also plant senescence. Plant growth-promoting rhizobacteria have been identified for reducing the level of abscisic acid at the stress condition, mainly drought conditions. This reduction of abscisic acid level indirectly increases plant growth and development (Belimov et al. 2001, 2014; Pospisilova 2003; Cohen et al. 2009; Goswami et al. 2014; Zhou et al. 2016; Pourbabaee et al. 2018). The PGPRs are identified for reducing ABA concentration, and the PGPRs strains are *Pseudomonas putida*, *Brevibacterium halotolerans*, *Azotobacter brasilense*, and archeobacteria. Bharti et al. (2016) reported that inoculation of *Dietzia natronolimnaea*, halotolerant bacteria in wheat crop, has been involved in the ABA signaling pathway and salt overly sensitive pathway.

7.5.3 Bioremediation of Heavy Toxic Metals

Soil is a storehouse for all materials which may be degradable or non-degradable, toxic or non-toxic, and so on. Toxic metals are a serious concern in the present scenario because they negatively impact crop growth and development, human health, and the environment. The soil has become contaminated with many toxic metals like cadmium, lead, nickel, iron, zinc, aluminium, and copper. Plants are exposed to them, and vegetable crops are most sensitive to them. Green leafy vegetables are prone to them and easily absorb metals. The appearance of yellowing at the tip, stunted growth, and root browning are common symptoms of metal toxicity. It mainly happens when water contaminated with toxic metals is used for irrigation purposes or is unknowingly flooded in crop fields. Effluents discharged

from industries are a rich source of metals. Plants become loaded with them and, when consumed, cause detrimental effects on the human body. The microbial population is also adversely affected. However, some microbes are a potential source to minimize metal concentration by removing, destroying, scavenging, absorbing, neutralizing, and immobilizing (Shaw et al. 2004; Jiang et al. 2008; Sheng et al. 2008; Singh et al. 2010; Rajkumar et al. 2010; Dourado et al. 2013; Babu et al. 2013; Nemati and Bostani 2014; Syed and Chinthala 2015; Kamran et al. 2016). Some plant growth-promoting microbes reduce toxic metal concentration by neutralizing negatively charged functional groups available at the cell wall of the microbes for the positive-charged metal toxic ions. This mechanism is called metal bioabsorption. Some PGPRs strains can produce low molecular weight biomolecules, which could assist in chelating toxic metals and immobilizing them so that plants cannot absorb them. Toxic metal-chelating molecules are produced by several bacterial species, *Serratia*, *Streptomyces*, *Azospirillum*, *Nocardia*, and *Pantoea* (Verma et al. 2020c; Eid et al. 2021).

7.5.4 Osmoprotectants/Antioxidants Production

Osmoprotectants are low molecular weight chemical compounds. When the plant is challenged to any abiotic stresses like drought, salinity, and metals, osmoprotectants are produced to minimize their adverse effects. Osmoprotectants produced by the plants accumulate in the vacuoles of the cytoplasm. Osmoprotectants are grouped in different groups based on their chemical relationship. Proline belongs to amino acids; glycine betaine belongs to quaternary ammonium compounds, mannitol, d-mannitol, trehalose, and fructans belongs to sugars and also polyols. The commonly occurring osmoprotectants produced by the plants are glycine betaine, proline, and mannitol. They can easily dissolve in water and maintain the osmotic pressure of the plant cell, which has been disturbed during abiotic stress. These osmoprotectants are not toxic to plant cells even at higher concentrations. These low molecular weight organic compounds increase osmotic pressure in the cytoplasm and thereby assist in balancing the water uptake and solutes/minerals. Besides the balancing osmotic pressure of the cytoplasm, they also work as scavengers of reactive oxygen species (ROS) produced inside plant cells and stabilize proteins available in the cell membrane during oxidative damage caused by reactive oxygen species.

Similarly, when the plant is under osmotic stress caused by salinity conditions, production of some antioxidative enzymes takes place, which helps maintain or minimize reactive oxygen species levels. The antioxidative enzymes may be superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). Under the saline situations, enhanced content of malondialdehyde (MDA) and phenols has been reported (Gururani et al. 2013; Islam et al. 2016; Dong et al. 2006). Some plant growth-promoting rhizobacteria are used to alleviate/minimize osmotic stress on the crop plants. Earlier studies reported bacterial strains like *Pseudomonas fluorescens*, *P. migulae*, *P. putida*, *P. chlororaphis*, *P. exterminatus*, *Rhizophagus irregularis*,

Variovorax paradoxus are effective in salt stress management in tomato plants (Ali et al. 2014; Eid et al. 2021). For drought management, PGPRs like *Azospirillum brasilense*, *Bacillus cereus*, *Bacillus polymyxa*, *Citrobacter freundii*, and *Burkholderia seminalis* are effective in tomato crops. Similarly, some strains of these PGPRs are also effective in managing heavy metals toxicity in tomato crops (Khanna et al. 2019; Verma et al. 2020c).

7.5.5 Expression of Stress-Related Genes and Proteins

Heat shock proteins (HSPs) is a group of conserved proteins family. They are found in the cytoplasm as well as the intermembrane space of chloroplasts. Extreme temperature influences the growth and development of crop plants. High temperature affects seed germination, chlorophyll biosynthesis, metabolites production, and the vigour of the crop. Specific genes and proteins are expressed during high temperatures to overcome adverse effects in the plants. Some PGPRs are identified for association with genes and proteins expression, such as sulfatase substrates. This protein family regulates the number of cellular processes like phytohormone production and signaling pathways. Similarly, the carbohydrate kinase protein family is associated with sugar accumulation. In addition, the phosphodiesterase protein family is involved in the DNA protein crosslink repair pathway in plants.

7.5.6 Expolysaccharide and Biomolecules Production

Microbes have the potential to synthesize diverse groups of chemical compounds. These can be intracellular or extracellular. Among them, polysaccharides production is one of them. Multifunctional polysaccharides are produced by microbes consisting of carbohydrate and non-carbohydrate sub-constituents. Plant growth-promoting rhizobacteria produce exopolysaccharide (EPS) under stress conditions caused by either drought or salinity. These exopolysaccharides protect plant root desiccation, uptake of ions, provide nutrients to plants and also develop a friendly environment for microbial augmentation. Sodium-ion uptake is also regulated in the plants by the production of EPS. Exopolysaccharide-producing bacteria are reported for maintaining the growth of plants even under severe dried sandy soils. Species of PGPRs like *Azospirillum* and *Pseudomonas* are examples of exopolysaccharide production (Jones et al. 2004; Bais et al. 2006; Musilova et al. 2016). Some PGPRs also produce secondary metabolites that improve the stress tolerance of the crops. Polyamines, spermidine, lumichrome, riboflavin, lipo-chitooligosaccharides, and thuricin 17 (Th 17) are well documented by the microbial production. This results in biomass increase, altered root architecture, leaf area expansion and alteration, and enhanced photosynthetic activity (Subramanian and Smith 2015; Dakora et al. 2015; Tahir et al. 2017).

7.5.7 Nutrients Solubilization and Mobilization

The soil is rich in all the minerals, nutrients, and ions. During the green revolution in the twentieth century or 1970s onwards, intensive chemical inputs like chemical fertilizers and pesticides were applied to boost crop production, mainly wheat and rice crop. Surplus crop production has been recorded worldwide, and their side effect is noticed with deficiency of several macros and micronutrients. Soil health and quality have deteriorated, and the diversity of beneficial microbes in soil is also severely affected. Microbial application is also considered an alternative approach in place of chemical fertilizers and has started their use as biofertilizers and biocontrol agents. Several bacterial genera are identified for enhanced nutrient uptake, such as nitrogen fixation, phosphate, potassium solubilization and mobilization, zinc and manganese solubilization, and even silica solubilization bacteria (Beattie 2015; Pii et al. 2015). Worldwide, several microbial inoculants have been developed, bearing potential strains of *Pseudomonas*, *Rhizobium*, *Azospirillum*, *Azotobacter*, *Acetobacter*, *Trichoderma*, *and Bacillus*, *Enterobacter*, *Azoarcus*, *Herbaspirillum*, and many more. These microbial inoculants performed on a broad spectrum of crops saved up to 50% of chemical fertilizers and increased crop yield (Bashan and de Bashan 2015; Shakeri et al. 2016; Niu et al. 2016). These beneficial microbial inoculants are mostly compatible with each other and synergistically affect the crop plants (Dakora and Phillips 2002; Mehnaz 2016; Sharma et al. 2019). The best biofertilizers extensively studied and exploited in crops are nitrogen-fixing bacteria in legume crops and phosphate solubilizing bacteria.

7.6 Conclusion

Abiotic stresses are major constraints in agricultural productivity, food quality as well as food security. Several methods and processes are being used to minimize the deleterious effects of drought, salinity, heavy metal, and so on. Breeding for the development of drought, salinity, and metal tolerance agricultural crops is a very tedious, cumbersome, and time-consuming task. Several strains are identified, and microbial inoculants have been developed and are being used. Microbial technology, including PGPRs is viable and effective when it has the merit of microbial culture and is rigorously validated at farmers' fields. The application of PGPR in sugarcane production is an effective alternative with eco-environmental impact for increasing the efficiency of mineral fertilizers such as phosphate while giving high cost-effective harvests. Appropriate combinations of PGPR, ambient environmental variables, and plant genotypes could be used to promote sugarcane plant growth and development. Further research remains to be done to develop suitable inoculants and production systems that reduce the amount of synthetic fertilizers and insecticides used to boost soil fertility and crop yield.

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Morpho-Physiological, Biochemical, and Ultrastructural Modifications on Sugarcane to Prolonged Water Deficit

8

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Abstract

Water stress occurs in most farming regions that lack proper irrigation systems and get insufficient moisture. Using biotechnological approaches, researchers could better understand the physiological and biochemical mechanisms that support a plant's response to water stress, allowing them to produce drought-tolerant plants. Plants use a variety of mechanisms to cope with insufficient water supply, including variations in the expression of genes and the buildup of organic compounds to survive and grow effectively. According to biochemical investigations on the drought-tolerance mechanism, harmless micro compounds of suitable solute accumulate during a water shortage. The main goal of this chapter is to compile research innovations on stress-responsive genes and functional machinery subjected to water stress by discussing agronomic, physiological, ultrastructural modifications, and omic aspects of drought in sugarcane crops.

Keywords

Biomass · Photosynthetic responses · Biochemical aspects · Drought-tolerance · Sugarcane

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8.1 Introduction

Climate change has been recognized as a severe problem in recent decades, affecting crop yield, human and animal health. Abrupt changes in temperature, floods, and drought are expected to become more common due to climatic fluctuation and modification. Water losses are expected to grow as the global temperature rises, owing to the high evapotranspiration rate, thereby increasing the water stress (Raza et al. 2019; Verma et al. 2020b, 2021c). Furthermore, with the projected human population of over ten billion by 2050, there will be an increased need for food, energy, and habitation (Rojas-Downing et al. 2017). Environmental stressors limit plant development and agricultural productivity. Insufficient water availability is one of the most severe environmental stress, reducing crop output globally (Verma et al. 2020b, 2021c). Sugarcane, a major source for sugar crystals and bioethanol production, its growth is susceptible to lack of sufficient water supply (Verma et al. 2021d). The sugarcane productivity can be reduced by about 80% due to lack of irrigation water (Basnayake et al. 2012; Gentile et al. 2015; Ferreira et al. 2017; Verma et al. 2021a). As a result, farming areas are dependent on favorable precipitation patterns or alternate sources of water supply for the proper development of sugarcane (Walter et al. 2013; Verma et al. 2021b).

Numerous sugarcane crop development projects have invested in water use-efficient (WUE) resistance cultivars, and WUE crop production techniques as the stress frequency (long/short term) and severity have increased. The better understanding of the functional mechanisms obtained from the morphogenic, physiological, and molecular aspects in variety of plants such as sugarcane is having a significant impact on the development of biotechnological approaches for developing stress resistance and agro-industrially important sugarcane cultivars (Augustine et al. 2015; Ramiro et al. 2016; Khan et al. 2016; Verma et al. 2021b). Plants have evolved stress-resistance techniques, i.e., variation in the plant life cycle, growth/development, regulation of total plant activities to stabilize the distribution of resources for growth as well as stress resilience, and transformation of stress signal perception for long- and short-term periods of stress resistance (Hirayama and Shinozaki 2010; Hu and Xiong 2014; You and Chan 2015). The increasing volume of research has aided in identifying critical genes linked with stress resistance and growth in a variety of plant cultivars (Hu and Xiong 2014; Augustine et al. 2015; Ramiro et al. 2016; Li et al. 2016). Crop production can be improved by using biotechnological and molecular techniques in water-stressed regions. Despite advances in the understanding of stress responses and the availability of omic approaches, developing drought-tolerant crops remains a serious issue (Wang et al. 2003, 2016; Hu and Xiong 2014).

Sugarcane has become an important agro-economic crop in tropical and subtropical areas due to the multiple valuable goods. The enhancement in sugar productivity and processing would enhance the supply of sugar and the socio-economic status of farmers and improve the security of bioenergy produced from sugarcane. This chapter discusses recent advancements in sugarcane water stress-response systems from morphological, physiological, biochemical, anatomical, and molecular aspects.

8.2 Water Deficit

Insufficient water supply is a severe problem for the plants as it is essential for their survival. Water availability can influence plant growth and productivity, and it decreases plants' survival, development, and production by disrupting the water status of plants (Verma et al. 2020b, 2021d). Sugarcane has high water-uptake efficiency among photosynthetically C_4 plant species. C_4 plants may close their stomata partially throughout the day to reduce evapotranspiration while maintaining leaf gas exchange response (Verma et al. 2020a). Sufficient water will promote fast growth, elongation of the main stem, and internode development during the vegetative period. Inadequate water supply will stifle the growth and development of sugarcane and reduce its production (Ferreira et al. 2017). Sugarcane acts as a major source for the production of sugar, bioethanol, sustainable bioenergy, and feed, thus developing new water-resistant sugarcane cultivars will be the main priority.

The understanding of the physio-biochemical and omic mechanisms of water deficiency in sugarcane would be the most promising strategy for creating biotechnological approaches (Ferreira et al. 2017). To survive and develop effectively in the face of water stress, plants use a variety of tactics, including variation in the expression of genes (Shinozaki and Yamaguchi-Shinozaki 2006) and the uptake of specific compounds, i.e., proline, sugar, alcohol, and glycine betaine (GB) (Rhodes and Hanson 1993; Ingram and Bartels 1996). Stress increases the concentration of abscisic acid (ABA), which has an effective action mechanism in signal transduction and gene expression, resulting in changes in stress adaptation strategies (Bray 1997; Shinozaki and Yamaguchi-Shinozaki 1997; Li et al. 2016). In sugarcane, changes in stress-responsive genes are linked with sucrose buildup, as well as genes encoding amino acid metabolic enzymes. (Iskandar et al. 2011; Sugiharto et al. 2002). Furthermore, GB is a suitable solute that is hypothesized to function as an osmoprotectant in some plants to make them more resistant to drought conditions. Understanding the molecular and physiological mechanisms of water stress is very crucial in designing biotechnology methods to develop drought-tolerant sugarcane.

8.3 Effect of Agronomic, Physiological, and Molecular Aspects in Sugarcane During Water Stress

Drought can reduce the potential yield of crops by 60%. Germination, tillering, grand growth, and maturity are the four important phases of sugarcane development (Verma et al. 2020b). Due to the excess water requirement, tillering, proper growth, and productivity are the crucial stages of drought sensitivity in sugarcane (Fig. 8.1) (Ramesh 2000; Verma et al. 2020b). The association between water content and photosynthetic activities may be employed during these stages to identify and differentiate stress-resistance sugarcane genotypes/cultivars (Endres et al. 2010).

Plants must retain their stomata open to absorb CO_2 (Verma et al. 2020b). However, this strategy necessitates a higher rate of transpiration, which can be a

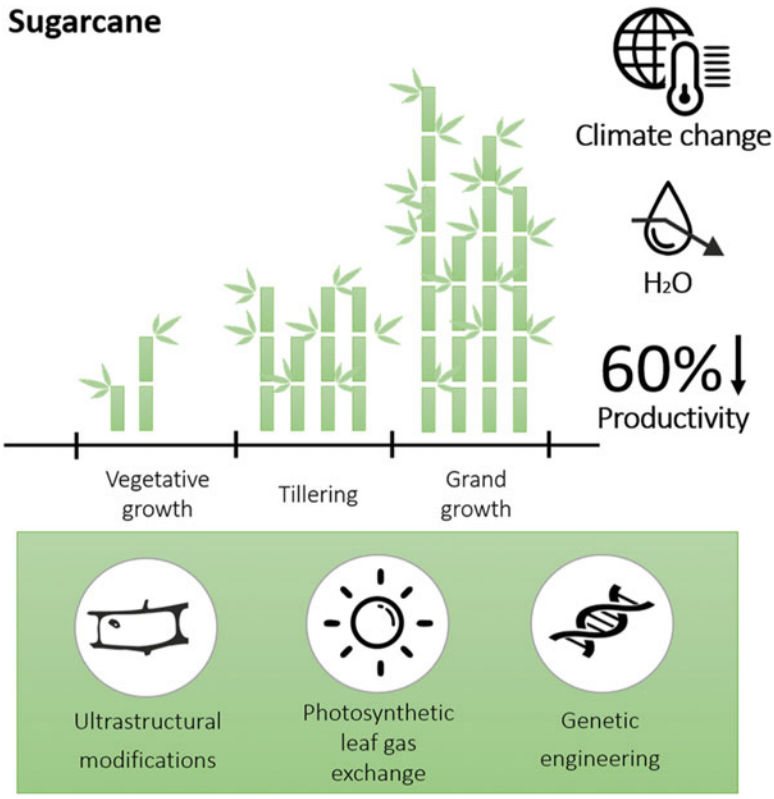

Fig. 8.1 Schematic presentation of drought stress impact on sugarcane plants under changing climatic environment

limiting factor in some regions due to drought (Molina 2002; Azevedo et al. 2011). Sugarcane C_4 metabolism can undoubtedly help it to grow in hot, dry climates by minimizing photorespiration and water loss. Water restrictions on sugarcane fields that sustain longer can significantly influence economic growth and quality (Verma et al. 2021c). Several studies reported that photosynthesis in C_4 plants is very sensitive to shortage of water (Ghannoum 2009). Furthermore, these plants have less restoration efficiency, which means if the plant's restoration potential is reached, their photosynthetic metabolic pathways are affected (Ripley et al. 2010). The ability of Brazilian sugarcane cultivars to restore photosynthetic parameters was reduced at the beginning of the stress, resulting in damage to the photosynthetic machinery as demonstrated by low photosynthetic efficiency (Graça et al. 2010).

Plants modify their metabolism to cope with water shortage (Li et al. 2016). The roots are the first organ to detect stress and signal the rest of the plant organs to these alterations. Hydraulic fluctuation stimulates plants to send signaling molecules through roots to produce variations in stomata under moisture stress (Buckley 2005). According to research carried out at the molecular level, sugarcane plants

express various genes in response to drought (Iskandar et al. 2011; Rodrigues et al. 2011; Li et al. 2016). In terms of stress responses, hormone-regulated signaling pathways, particularly those linked to enhanced ABA production, are drought-responsive (Pinheiro and Chaves 2011).

Specific genes resembled ABA-regulated proteins and genes actively or passively associated with its formation in sugarcane genotypes exposed to shortage of water and decrease in stomatal conductance (gs) (Rodrigues et al. 2011). The amount of water in the soil appears to have more significant influence on gs than the amount of water in the plant (Davies et al. 2002; Li et al. 2016; Verma et al. 2020b). Under moderate and severe stress, sugarcane plants showed a drop in soil water content, which resulted in alterations in photosynthetic responses, leaf relative water content, chlorophyll fluorescence yield, and enhancement in leaf canopy temperature (Rodrigues et al. 2009, 2011; Li et al. 2016; Verma et al. 2021c). Cultivars were chosen and categorized as consequences of the physiological parameter observed by relative analysis utilizing cultivars with known drought resistance or sensitive potential.

Relative water content (RWC) is a plant water adjustment indicator because it measures how much relative water the plant needs to achieve complete artificial hydration (González and González-Vilar 2003). It measures the amount of water in tissues and cells, which is essential for plant's metabolic activities (Silva et al. 2007). Plant water content regulates physiological processes, and differences in RWC appear to directly impact the entire photosynthetic machinery in sugarcane (Graça et al. 2010). In sugarcane, a 10–20% reduction in RWC inhibited the photosynthetic machinery of resistant and susceptible cultivars exposed to moisture stress (Graça et al. 2010; Verma et al. 2020b, 2021c). To select drought-resistance genotypes, proline accumulation and photosynthetic capacity were used as efficient indices in sugarcane (Cha-um and Kirdmane 2008). In response to salinity and drought, sugarcane plants appear to enhance the production of osmoprotectant proline. In the same study, stress decreased the activity of photosystem II, gs, and E (Cha-um and Kirdmane 2008; Li et al. 2016; Verma et al. 2020b, 2021c). The photosynthetic rate of plants under drought stress depends on the species and frequency of stress. Sugarcane genotypes exposed to a limited water condition with no watering exhibited lower photosynthetic efficiency under moderate stress. Under continuous water supply, resistant plants indicated a better photosynthetic CO_2 assimilation rate than susceptible plants (Graça et al. 2010).

In addition to the losses caused by water deprivation, stressed plants may experience secondary stress, like oxidative stress, resulting from the initial stressful circumstances. Reactive oxygen species (ROS) build up spontaneously (Miller et al. 2010). When plants close their stomata and decrease internal CO_2 concentration due to the lack of water, ROS generation appears to drive processes that reduce oxidative stress, suggesting that it can play a role in water deficit resistance capacity (Arora et al. 2002). Nonetheless, there were differences between resistant and susceptible genotypes when drought-stressed plants were used to measure photochemical efficiency (PS-II). The resistant cultivars showed higher utilization of the photosynthetic apparatus. Unlike susceptible plants, tolerant plants can balance the

oxidative process at the control level of photochemical efficiency (Li et al. 2016; Verma et al. 2020b). The finding hypothesized that resistant plants, unlike sensitive cultivars, can sustain the normal level of photochemical efficiency in the oxidative process. To maintain the temperature of the leaves below the ambient air temperature and ensure the proper functioning of the photosynthesis (P_N), vast volumes of water must be transpired throughout the plant (Machado and Paulsen 2001; Li et al. 2016). Leaf rolling is stated as a sensitive trait in sugarcane plants. Still, it could be understood as part of the acclimatization process, in which plants reduce their specific leaf area to avoid rather than tolerate water shortages (Inman-Bamber and Smith 2005).

According to Graça et al. (2010), the increase in leaf temperature in water-stressed sugarcane plants was driven by a decrease in the rate of transpiration, which has been induced by stomatal closure. Higher water status assists the stomatal aperture and maintains leaf cooling intolerant plants (Silva et al. 2007). Sugarcane plants react to water shortages in different ways. The tolerant cultivar had a lower TRA, which caused stomatal closure and, as a result, a rise in leaf canopy temperature. The increase in leaf temperature in the resistance cultivar became significant only when the RWC was decreased in stressed plants (Graça et al. 2010). Stomatal closure appears to be associated with soil water resources than the potential of leaf water, according to signaling between roots and leaves (Inman-Bamber and Smith 2005; Smit and Singels 2006; Li et al. 2016).

Drought-tolerant genotypes have been identified using physiological indicators like RWC, photochemical efficiency, gs, and P_N (Buckley 2005; Shao et al. 2008; Tezara et al. 2008). Identifying physiological variables and genes may be utilized as a reference point for generating new hybrids of sugarcane (Hotta et al. 2010). Several physio-biochemical approaches utilized in breeding projects to choose genotypes that are susceptible and resistant to water shortage have shown interest. They have also demonstrated broad applicability, owing to the inexpensive cost of a few techniques, i.e., RWC (Silva et al. 2007; Azevedo et al. 2011). The more common drought symptoms in sugarcane include curling leaves, stomatal closure, stem elongation, leaf area expansion, and leaf chlorosis (Inman-Bamber and Smith 2005; Inman-Bamber et al. 2012; Verma et al. 2021c). Furthermore, drought disrupts cell division and the elongation process, with stem and leaf elongation being the most severely affected morphological activities (Machado et al. 2009; Li et al. 2016). Water deficit condition affects root development as well (Smit and Singels 2006), but to a lesser extent than above-ground biomass.

Photosynthetic efficiency declines under mild water stress conditions due to stomatal constraints (Li et al. 2016; Verma et al. 2020a, b, 2021c). The more specific initial adaptation is to establish stem and leaf suppression when plants are subjected to dryness (Inman-Bamber and Smith 2005). Non-stomatal constraints caused by water stress have also been described as a source of photosynthetic suppression in sugarcane plants (Ribeiro et al. 2013). It is worth noting that sugar accumulation in the leaves affects the photosynthetic rate (McCormick et al. 2008). Water stress causes several physio-biochemical aspects in plants, such as changes in the expression of genes. ABA-dependent and independent regulatory mechanisms triggered

the shift in gene expression. Furthermore, two clusters of drought-inducible genes in Arabidopsis were identified using microarray analysis. Genes encoding proteins involved in abiotic stress resistance make up the first category (Shinozaki and Yamaguchi-Shinozaki 2006). In molecular studies of sugarcane responses to water deficit, the presence of an inducible stress protein known as SoDIP22 in stress-resistance genotypes was observed (Sugiharto et al. 2002).

Water deficiency alters metabolic reactions, resulting in creating a diverse range of secondary metabolites. Drought produces highly reactive or toxic ROS in plants, causing loss to cellular components, i.e., proteins, lipids, glucose, and DNA. Various functions, i.e., cell cycle and programmed cell death, are also regulated by ROS (Sawitri 2012). Plants exposed to drought produce more ROS, including free radicals and non-radical forms. Plants have evolved excellent antioxidant machinery that can scavenge and detoxify ROS to survive drought stress conditions (Gill and Tuteja 2010). Plants have an enzymatic and non-enzymatic antioxidative defensive apparatus that scavenges ROS to protect plant cells from oxidative stress. Enzymatic activities like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) can work synergistically to scavenge ROS, as can non-enzymatic antioxidative components like ascorbic acid, decreased glutathione, phenolic, alkaloids, and amino acids contents. Depending on the variety of sugarcane plants and degree of stress, water scarcity causes change in SOD, CAT, APX, and GR activities (Verma et al. 2020b; Li et al. 2016).

Compared to drought-sensitive cultivars, stress-resistance cultivars reported an increase in CAT and APX activity in the initial stages of stress. In contrast, GR content reached the highest at the end of stress (Cia et al. 2012). Most sugarcane cultivars showed increased SOD, CAT, and APX when subjected to water deficit (dos Santos and Silva 2015). As a result, the ROS-scavenging enzymatic activities could be employed as a marker for sugarcane resistance to water stress. To defend themselves from oxidative damage caused by ROS, many plants accumulate non-enzymatic antioxidant defense systems in response to water deficiency stress. Ascorbic acid is an antioxidant that helps to reduce the injury caused by ROS. Ascorbic acid can provide electrons in various processes while scavenging superoxide and hydroxyl radicals' interaction with cell membranes (Gill and Tuteja 2010).

Additionally, glutathione is an important antioxidant that can help to reduce the damage caused by ROS. Glutathione is a metabolite that can be diminished and has a range of activities, including influencing plant responses to environmental circumstances (Gill et al. 2013). Although ROS-scavenging antioxidative enzymes have been found to promote plant stress resistance in numerous transgenic plants (Gill and Tuteja 2010), their application in the development of stress-resistant sugarcane is still limited. According to research at the agronomical, metabolic, and cellular levels, complementary solutes appear to play a significant role in plant's adaptation strategies to salt and moisture stress conditions. Sugar and sugar alcohols have long been recognized as osmoprotectants that protect membranes while scavenging ROS. Sugar buildup, i.e., trehalose, fructans, and sucrose, acts as an osmoprotectant in plants during water stress conditions (Singh et al. 2015).

Sugarcane can sustain more sucrose in the stem cells' storage parenchyma, creating an osmotic gradient and acting as an osmoprotectant.

There was variation in stress-responsive genes and sucrose production when water stressed, but the response mechanism to water deficiency was diverse. Numerous genes, including those that encode asparagine synthase (AS), proline biosynthesis (OAT), and sugar transporters, were positively associated with sucrose content in mature sugarcane culms. The proline biosynthesis pathway (P5CS) and the bZIP transcription factor (TF1) were poorly related. Proline content increased when sugarcane was restricted to water, but it was negatively associated with sucrose content, showing that proline has no osmoprotective action in sugarcane (Iskandar et al. 2011). Although the function of proline in plant osmotolerance is debatable, research of transgenic sugarcane overexpressing the heterologous P5CS gene showed that proline concentration increased during water stress. Enhanced proline levels did not affect osmotic adjustment, and proline can protect sugarcane from oxidative damage caused by water scarcity. Proline accumulation appears to be a component of the antioxidant defense machinery rather than osmotic adjustment (Molinari et al. 2004).

Glycine betaine (GB), an amphoteric quaternary amine, is compatible solute that protects plants from stressful conditions (Rhodes and Hanson 1993; Sakamoto and Murata 2002). GB protects protein against water stress dissociation and allows cells to alter the osmotic potential in their cytoplasm to balance optimum water levels (Sakamoto and Murata 2002). When a plant system is exposed to moisture or salinity stress, GB helps to keep the membrane intact and function correctly by stabilizing the macromolecule structure.

ABA is the major regulatory signaling molecule (Tanaka et al. 2005; An et al. 2016). Li et al. (2016) observed a sustained decrease in gs, E, and upregulation in ABA level in sugarcane subjected to drought. Endogenous and exogenous ABA can promote stomatal closure in plants via multiple signaling pathways (Neill et al. 2008), which involve various intermediate molecules such as secondary metabolites and ions (An et al. 2016; Li et al. 2016). Furthermore, some authors have proposed that H_2O_2 is important for ABA signaling and activating the antioxidative gene expression (Guan et al. 2000; Jiang and Zhang 2001, 2002).

One of the ABA-responsive genes associated with sugarcane water stress response, SoNCED, a 9-cis-epoxycarotenoid dioxygenase that regulates a rate-limiting phase in ABA production and is activated in leaves and roots under stress, boosting ABA accumulation (Li et al. 2013, 2016). In bundle sheath cells, SoDip22 (sucrose-phosphate synthase) is associated with regulation of water uptake (Sugiharto et al. 1997). ScCAT1 (catalase) is a gene that defends against ROS caused by abiotic stressors (Su et al. 2014). These findings suggest that sugarcane shares ABA-controlled mechanisms for stress adaptation to resistance. This understanding could aid in developing genotypes that perform better in water-stressed situations.

The most visible indication of oxidative stress in plants is lipid peroxidation induced by ROS (Huang et al. 2012). Oxygen molecules produced by PS-II are involved in the most prevalent lipid peroxidation process (PS-II). These compounds

are absorbed into plastid membranes and converted into LOOH (lipid hydroperoxide) by lipoxygenases (LOX), making the membrane prone to fragmentation and triggering a chain reaction of stress situations (Skorzynska-Polit 2007). New radicals can be activated and propagated due to the fragmentation process. One of the by-products of this process is malondialdehyde (MDA), which alters cell membrane properties like fluidity, transport of ions, and function of enzymes (Sharma et al. 2012). During the initial growth stage of immature sugarcane plants during severe stress conditions, a high amount of H_2O_2 was found, along with increased lipid peroxidation (Boaretto et al. 2014). Lipid peroxidation could be a helpful indicator for detecting water stress-resistant capacity in sugarcane plants (Abbas et al. 2014).

The enzymes 11-pyrroline-5-carboxylate (P5C) synthetase (P5CS) and P5C reductase catalyze proline biosynthesis from glutamate (P5CR). Pro can also be made from ornithine transformed to P5C/GSA by the enzyme ornithine-d-aminotransferase (OAT) (Liang et al. 2013; Bhaskara et al. 2015). Plants accumulate free amino acids in response to stress conditions (Pagariya et al. 2012), which raises osmotic pressure and functions as osmoregulatory (Molinari et al. 2004; Boaretto et al. 2014). Overall, water stress appears to link the response of the antioxidative system to sugarcane. The ROS-scavenging enzyme activities in sugarcane could be employed to diagnose drought resistance.

cDNA arrays were employed by Rocha et al. (2007), Rodrigues et al. (2011), and Li et al. (2016) to investigate the profile of gene expression in sugarcane leaves under various water stress circumstances. Despite the changes in experimental circumstances, the expression pattern of several genes associated with cellular metabolism, signal transduction, transport, hormone production, and stress responses was strikingly comparable. However, the expression patterns of several genes differed dramatically, possibly reflecting the severity of the stress that the test plants were exposed to. Rodrigues et al. (2009) used microarray, including ESTs from leaf libraries developed by the SUCEST project, to compare two genotypes, categorized as drought stress-resistance (SP83-5073) and susceptible (SP90-1638), in an attempt to identify an association between stress resistance and expression of genes. Along with the length and severity of stress, both genotypes show a rise in the differentially expressed genes.

The authors hypothesized that the gene expression profile supported these morpho-physiological findings because susceptible plants initiate metabolic variations before resistance plants. 93% of differentially expressed genes of the resistant cultivars were upregulated under severe moisture stress conditions. However, the differentially expressed genes (36%) were repressed in stress-sensitive plants, i.e., stress and photosynthetic apparatus responsive genes (Li et al. 2016). The microtranscriptome (miRNA transcriptome) is altered in various cultivars and developmental stages to deal with varying stress levels, according to studies on sugarcane miRNA expression during drought conditions (Ferreira et al. 2012; Gentile et al. 2013, 2015; Thiebaut et al. 2014). Skirycz et al. (2011) reported that mild stress levels favor growth, photosynthetic, and metabolic activities during stress, resulting in a novel paradigm for discovering resistance alleles. When the expression patterns of these field-grown plants were compared to those of glasshouse

plants, significant differences were found (Ferreira et al. 2012). As a result, research on wild plants will likely provide differential genes expression patterns as compared to plants maintained in the glasshouse.

There was a lot of overlap between the two datasets when differentially expressed genes from cultivars with variable Brix (sugar) content were compared to those under stress (Rocha et al. 2007; Papini-Terzi et al. 2009). Iskandar et al. (2011) revealed evidence that sucrose buildup also induces the expression of genes in sugarcane that are not activated by the shortage of water. As a result, subsets of common and stress-specific genes complicate these occurrences. Even though sugarcane transcriptome responses to drought vary mainly depending on the genetic background of the test clones and the stress applied, Iskandar et al. (2011) discovered a positive relationship between the expression of stress-induced genes and the expression of a sequence similar to dehydrin. Dehydrin proteins are a category of late embryogenesis abundant (LEA) proteins that protect sugarcane's cell membranes and organelles from dehydration (Wahid and Close 2007). The expression of this gene is raised as the stress becomes more severe (Rocha et al. 2007), and there is no significant variation in the expression of genes in response to sucrose accumulation (Papini-Terzi et al. 2009; Iskandar et al. 2011). As a result, it could be used as a molecular marker for drought responses in sugarcane studies (Ferreira et al. 2012; Gentile et al. 2013).

Understanding the gene activity in the roots of stressed plants can provide more insights to create research techniques to increase crop yield. Vantini et al. (2015) revealed differentially expressed genes in resistance and susceptible cultivars in root tissues throughout specific time intervals. Genes encoding proteins with protective roles were activated in the tolerant variety at the initiation of the stress. Genes encoding an ABA-response protein, a trehalose phosphatase synthase, and serine/threonine kinase receptors indicated increased expression in the resistance cultivars, indicating that the two sugarcane genotypes have different drought protection and adaptative strategies.

In summary, targeted gene expression studies have led to the discovery of genes associated with sugarcane stress responses, but it remains difficult to link their functionality to resistance capacity. No well-characterized sugarcane genetic lines or mutants are available to establish the gene functions found by transcriptomic analysis (da Silva et al. 2013; Thiebaut et al. 2014; Yang et al. 2014). The increasing use of transcriptomic approaches has been significantly linked to real-time quantitative PCR (qRT-PCR) as a technique for validating data (Czechowski et al. 2005; Gutierrez et al. 2008). Appropriate internal controls are crucial for real-time reliability (Bustin 2000, 2002). Despite its widespread use, the qRT-PCR data normalization parameters are still a source of debate (Gutierrez et al. 2008). Recent research has integrated qRT-PCR assays with statistical techniques to find the optimal sugarcane reference genes (Guo et al. 2014; Ling et al. 2014). Silva et al. (2014) demonstrated the effectiveness of six candidate genes in two sugarcane cultivars subjected to a water shortage. Under moisture stress, the GAPDH, α-tubulin, and histone H1 genes were the most influential for standardizing gene expression data in sugarcane roots. Ling et al. (2014) investigated the stability of 13 possible putative

reference genes in various sugarcane samples, including five different plant organs exposed to environmental stresses and hormone application. Guo et al. (2014) found similar observations in sugarcane plants under stressed conditions, utilizing GAPDH and eEF-1a as standardized genes.

8.4 Genetic Engineering for Sugarcane Improvement

The overexpression of target genes has increased sugarcane tolerance to water stress. This method also enables the identification and validation of gene function, even for functionally redundant genes (Kondou et al. 2010; Abdeeva et al. 2012; Li et al. 2016). Despite the great economic value of sugarcane equipped with water stress resistance, only a few examples of transgenic research have made significant progress. The chosen gene has been associated with all of their moisture stress responses or known to confer moisture stress resistance in other species (Reis et al. 2014; Augustine et al. 2015; Ramiro et al. 2016). Plants having drought-induced regulatory genes could be developed to resist water deficit conditions (Reis et al. 2014). The first transcription factors (TFs) linked with the regulation of genes in response to environmental variables were the DREB genes (Moran et al. 1994). Drought resistance was improved in sugarcane by overexpressing AtDREB2A CA (Constitutively Active), as evidenced by maximum RWC, P_N, sucrose content, and sprouting of buds with no harmful impact on biomass accumulation (Reis et al. 2014).

Drought-tolerance processes can be studied by manipulating genes that regulate osmotic pressure when there is water shortage (Nelson 1994; Raza et al. 2016). The Arabidopsis H^+-PPase (AVP1) gene for a vacuolar membrane protein increases vacuolar solute concentration by bringing H^+ into the vacuoles from the cytoplasm. AVP1 overexpression in transgenic sugarcane plants enhances stress resistance capacity such as moisture and salinity stress by boosting RWC, osmotic and turgor potential, and root traits (Kumar et al. 2014; Raza et al. 2016). Constitutive promoters are primarily used for sugarcane transformation. Plant transformation via the 35S gene promoter of the cauliflower mosaic virus (CaMV) (Porto et al. 2014) resulted in high transgenic expression levels (Dutt et al. 2014). Additional sequences, such as repeated 35S elements, could be added (Dhadi et al. 2009). Recent research has identified ubiquitin promoters as a promising candidate for constitutive transgene expression in sugarcane plants (Lakshmanan et al. 2005), owing to their significantly higher level of transgene expression than other promoters, i.e., the CaMV 35S, the rice actin Act1 (McElroy et al. 1991), and the synthetic Emu (Last et al. 1991).

However, the number of helpful conditional promoters in sugarcane is limited (Chakravarthi et al. 2016). The two main methods for developing transgenic sugarcane plants are direct transformation using microprojectile (biolistics) (Bower and Birch 1992) and indirect transformation via *Agrobacterium tumefaciens* (Arencibia et al. 1998). Biolistics is a simple approach for sugarcane transformation because of its convenience and ability to work with a wide range of tissues and cultivars (Lakshmanan et al. 2005; Altpeter and Sandhu 2010). However, it has certain

drawbacks, such as low repeatability and the need to integrate a high number of transgene copies (Zhangsun et al. 2007).

The relationship between food supply and energy production has been a major concern for more economists in various nations, not just for sugarcane but also for other biofuel crops such as soybeans, corn, and sugar beet. The climatic change could exacerbate the detrimental consequences of water scarcity on agriculture. Understanding the expression of gene patterns of resistance and susceptible plants can benefit additional techniques to help the selection of cultivars. As a result, crop development must withstand extended periods of drought, and agricultural production must be maintained and expanded in light of future food needs and the competitiveness of the biofuel and ethanol industry.

To extend sugarcane plantations, new drought-tolerant cultivars must be developed and cultivars with additional traits such as the ability to grow in nutrient deficient soil. Sugarcane also accumulated a significant level of sucrose in immature tissues after being genetically manipulated to inhibit a gene associated negatively with bioenergetics metabolism (Groenewald and Botha 2008). Glycine betaine is an osmoprotectant produced by a variety of microbes, plants, and animals in different environmental situations (Rhodes and Hanson 1993). Glycine betaine is predominantly produced from choline via two-step procedures involving choline dehydrogenation and betaine aldehyde oxygenation. In higher plants, choline is transformed to betaine aldehyde by choline monooxygenase (CMO), which is converted to GB by betaine aldehyde dehydrogenase (BADH) (Sakamoto and Murata 2001, 2002).

The gene implicated in the biochemical pathway may be exploited to raise or decrease the metabolism produced by overexpressing the responsible genes for metabolism. Enzymes involved in the biochemical pathways have been discovered as possible target for changing the content in non-accumulator plants using metabolic engineering. As a result, genes encoding enzymes associated with the GB synthesis pathway have been cloned from a range of GB-accumulating bacteria and plants (Landfald and Strøm 1986; Andresen et al. 1988). The genes responsible for GB synthesis from microorganisms in *Solanum lycopersicum*, *Solanum tuberosum*, *Oryza sativa*, and *Zea mays* have been a prominent objective in genetic engineering of moisture stress resistance plants that are otherwise unable to accumulate GB (Sakamoto and Murata 2000; Quan et al. 2004). One strategy for enhancing GB content in transgenic plants is to introduce the relevant genes under the transcriptional control of a strong DNA promoter to ensure high-level expression. It is an indicator of stress resistance in sugarcane (Smith et al. 2005; Jangpromma et al. 2012). The improved root system has a better water absorption mechanism to utilize limited water from deep soil. These findings suggest that increased GB content in transgenic sugarcane plants acts as an osmoprotectant, stabilizes macromolecule structure, balances integrity of cell membrane and function, and promotes sugarcane acclimatization to drought and salt stress.

The sugarcane was cultivated in the dry land of the experimental station to examine the growth and yield of transgenic plants during a limited water supply. According to the rule for assessing genetically modified organisms, transgenic sugarcane cultivation was done in a constrained and limited field trial system

(GMO). When the stress resistance transgenic sugarcane is compared to wild-type, lateral buds germination, and vegetative growth rate were practically identical, non-transgenic sugarcane showed decrease in stem length as the dry season proceeded. Drought-tolerant sugarcane plants have maximum yield, stalk length, and weight than sensitive plants (de Silva et al. 2008; Machado et al. 2009).

8.5 Stress-Resistance Capacity in Sugarcane Plants

Understanding the agricultural problems of genetic and plant yield aspects is crucial for developing practical and economically beneficial alternatives (Blum 2005). Stress is defined as any barrier to a plant's proper functioning and development during its life cycle. Stress tolerance action mechanisms are major traits in regions with severe water shortages. It improves the variations of absorbing more soil moisture, minimizing water loss, and preserving cellular hydration, allowing crop regeneration after alleviating stress (Tardieu 2012; Cominelli et al. 2013). Resistance mechanisms are beneficial characteristics during mild and severe water deprivation situations because they assist in plant survival under adverse conditions. The excess stomatal conductance, which keeps the photosynthetic rate going, and heat stress resistance, which lowers the leaf canopy temperature, are associated with tolerance characteristics (Blum 2005; Tardieu 2012; Cominelli et al. 2013).

Although the specific mechanism(s) of stress resistance in sugarcane plants is unknown, some traits have been linked to improved crop performance during minimum to medium stress conditions. Silva et al. (2008) found that the maximum number of stalk, height and weight are associated with higher productivity under stress. The diameter of stem varies between cultivars and depends more on genotype than environmental conditions (Soares et al. 2004; Silva et al. 2008; Li et al. 2016; Verma et al. 2020b). Leaf's chlorophyll index, temperature, and photosynthetic responses are the indirect selection factors for drought-resistance sugarcane cultivars (Basnayake et al. 2015; Li et al. 2016; Verma et al. 2020a, b, 2021c). The retention of green leaf area is another essential factor for maintaining the production potential of plant (Blum 2005). Several research studies have found that sugarcane plants under water stress had lower Fv/Fm values (Silva et al. 2014; Da Graça et al. 2010; Verma et al. 2020b). Root characteristics can also indicate plants' ability to resist stressful conditions (Songsri et al. 2008; Wang et al. 2009). Establishing deep and extensive root systems as selection criteria for water stress resistance in sugarcane can be exploited (Smith et al. 2005). When water is scarce, greater root density improves uptake of water, which is a desirable feature for extracting deep soil moisture (Blum 2005; Tardieu 2012).

8.6 Conclusion

Sugarcane growth is divided into different phases, i.e., germination, plant establishment, early tillering, grand growth, maturation, and blooming. Various studies were focused on water stress management during vegetative growth, tillering, and grand growth phases because they are critical stages in crop production. Sugarcane is more sensitive to drought throughout the tillering and stem lengthening periods, with the most impact on stem and leaf growth. Moderate water stress during the maturity period has a beneficial effect on sucrose production because the photosynthetic CO_2 assimilation rate is less resistant to drought than stem development, allowing absorbed CO_2 to be diverted to sucrose accumulation in the stem.

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Impact of Heavy Metal Toxicity on Sugarcane Growth, Development and Productivity

9

Shailly Misra and Brijendra Pratap Singh

Abstract

Sugarcane is one of the world's largest and extremely important crop. It plays a major role in the world economy and is the main source for sugar and ethanol production. The effect on crop growth and development due to soil and water contamination with toxic heavy metals is a serious environmental problem. Heavy metal accumulation in agricultural land is a threat to crop productivity and quality. Heavy metals such as As, Cd, Cu, Cr, Pb, Ni, Zn, Hg, etc., and their various sources like industrial effluents, wastewater irrigation, polluted soil, sewage sludge, and use of pesticides and excessive fertilizers are responsible for the contamination. Increasing levels of heavy metals in soil are absorbed by growing sugarcane, where they reach phytotoxic levels and could lead to severe impacts on plant development. Heavy metals have adverse effects on the ecosystem. The consumption of contaminated crop and juice also causes health issues in humans as the edible parts of crop show a higher accumulation of these toxic metals. This chapter highlights the impact of heavy metal toxicity on sugarcane growth, development and productivity. The focus is laid on sources of heavy metal exposure to sugarcane, their route of exposure, bio uptake, and mechanism of toxicity in the crop. The various toxic effects, symptoms of some heavy metals on sugarcane, and health risks are also discussed.

Keywords

Growth · Productivity · Phytotoxicity · Sugarcane · Heavy metals · Tolerance

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9.1 Introduction

Sugarcane is the highest-ranking crop worldwide and is the primary raw material for producing bioethanol, sucrose, and molasses (Verma et al. 2019, 2020, 2021a, b). Very few studies have been conducted on the effects of heavy metals in sugarcane despite its evident usefulness. Billions of people consume sugar; therefore, lack of information regarding heavy metal accumulation by sugarcane may adversely affect human health. The increasing amount of heavy metals in the urban environment responsible for food contamination is a matter of concern in developing countries. Sugarcane plant is also reported for phytoremediation and is an efficient accumulator of heavy metals. According to World Health Organization (WHO), sugarcane accumulates toxic ions beyond to permitted levels (Abdus-salam et al. 2008).

Heavy metals are those metallic elements with an atomic weight of more than 20 and a density higher than 5 g/cm^3. Typical examples are Arsenic (Ar), Lead (Pb), Cadmium (Cd), Mercury (Hg), Copper (Cu), Chromium (Cr), Nickel (Ni), Manganese (Mn). Heavy metals are classified as essential and non-essential. Some of the essential metals in trace concentrations are important for the growth and metabolic processes in plants, but at specific high concentrations, they can become harmful, e.g., Zinc (Zn), Copper (Cu), Molybdenum (Mo), Iron (Fe), Nickel (Ni), Cobalt (Co), Manganese (Mn). Non-essential metals are hazardous for plants and have damaging effects on the growth and metabolism of plants, e.g., Lead (Pb), Cadmium (Cd), Mercury (Hg), and Arsenic (Ar). The metal itself and its quantity in an organism or in plant determine the difference for an element to be considered essential or toxic. Heavy metals tend to persist in the environment and form soluble compounds, and they are non-biodegradable. Plants absorb and keep these elements over time (Du et al. 2013). The persistence of heavy metals in soils and their high potential risks to ecosystems and human beings have raised significant concerns (Thompson et al. 1988; Elik 2003; Cui et al. 2004). Sugarcane is one of the most important plants grown globally, and information related to heavy metal effects and accumulation in the plant is inadequate (Collin and Doelsch 2010). Furthermore, the adverse impact on human health by consuming sugarcane grown in potentially polluted soil is also a matter of concern. Therefore, this chapter intends to provide information on the effects of heavy metal toxicity on the growth, development, and productivity of sugarcane (Table 9.1).

9.2 Sources of Heavy Metal Exposure to Sugarcane

The leading causes of elevated amounts of heavy metals are urbanization, industrialization, agricultural and mining activities. However, the soil is the sink for most heavy metals. Both natural and anthropogenic factors are responsible for heavy metal origin in soil. The natural source indicates that metal is derived from the parent rock. In contrast, anthropogenic sources indicate that metal originates from wastewater irrigation, sewage sludge, mining activities, pesticides, and excessive use of fertilizers. However, human activities are also responsible for adding elements to

Table 9.1 Impact of heavy metals on Sugarcane (*Saccharum officinarum* L.)

Heavy metals	Concentration range	Effects	Sources
Chromium (Cr)	80 ppm	Significant decrease in bud germination and inhibition in growth parameters. Inhibition was more in root growth than in shoot growth. Decreased activity of CAT and high amount of reducing sugar	Jain et al. (2000)
Cobalt (Co)	>300 μM	Decrease in root weight, cane yield, concentration of sucrose in cane juice, chlorophyll inhibition, decreased activity of CAT in leaves and increased concentration of lipid peroxidation, sugars and high peroxidase enzyme activity	Sinha and Chatterjee (2015)
Copper (Cu)	500 μM	Lethal effects on plant with inhibition in growth parameters. Decrease in biomass. In root and shoot of plant increased MDA activity, POD is activated, and CAT activity is inhibited	Zeng et al. (2019)
Cadmium (Cd)	200 ppm	Inhibitory effects on the growth of roots and shoot of plant. Reduction in plant height, leaf number, and area. Decrease in biomass yield. Inhibitory effect on biosynthesis of chlorophyll. Foliar peroxidase increased activity and catalase decreased activity. Decrease in soluble protein content	Di Toppi and Gabbrielli (1999); Jain and Srivastava (2006)
Lead (Pb)	4 mM	Causes damage to shoot system. Reduced leaf area and bending of leaf margin. Decrease in chlorophyll content. Decreased sugar content. Decreased activity of catalase and enhanced activity of total amylases	Misra et al. (2010)
Manganese (Mn)	150 mg kg^{-1}	Excess Mn leads to structural harm to chloroplast and chlorophyll damage. Mn toxicity induces oxidative stress	Madhumita and Sharma (1991); Huang et al. (2016)
Nickel (Ni)	4 mM	Stimulatory effects in lower amounts but decreasing trend in growth attributes as concentration increases. Decreased biomass. Total protein and sugar content increased and decreased, respectively, at higher concentrations	Misra et al. (2010)
Zinc (Zn)	130 ppm	Exhibited reduction in growth parameters such as plant height, root length and number, leaf length and	Jain et al. (2010)

(continued)

Table 9.1 (continued)

Heavy metals	Concentration range	Effects	Sources
		area, fresh and dry weight of plant. However, increase in pigment concentration such as chlorophyll and carotenoid content was observed. Higher levels of Zn induce oxidative stress and increased activity of enzymes such as SOD, catalase, and peroxidase	

the environment. The soil is the basis on which food crops are grown. Therefore, increased metal levels in soil influence their accumulation in plants, thereby posing long-term environmental hazards with serious health implications on humans and animals.

There is the intensive use of fertilizers, sewage sludge, pesticides, effluents, and irrigation to increase the productivity of sugarcane crops (Verma et al. 2020, 2021a, c). These sources contain heavy metals that increase their level in soil. These heavy metals are absorbed by sugarcane and have toxic impacts on its physiological functions (Gonçalves et al. 2021). The accumulated heavy metals in sugarcane are then transferred to the human body by consumption (Table 9.1).

Sugarcane cultivation adjacent to industries and metal-polluted fills, application of municipal wastes, and use of pesticides and phosphatic fertilizers enhance the toxicity of zinc in sugarcane crop (Jain et al. 2010). Heavy metals such as Ni, Cd, Zn, Cr, and Pb accumulate in surface soil when irrigated with wastewater (Mishra et al. 2009). Continuous use of industrial effluents and sewage, toxic metals such as Cr, Cd, and Ni accumulates in soil and plants. Thus crops become hazardous for consumption (Alghobar and Suresha 2015). Enhanced mining and industrial activities generate Cu, potentially toxic to plants (Asati et al. 2016). Li et al. (2007) reported that sugarcane cultivation in reclaimed Mn mine had Cd and Pb in edible parts beyond the safety limits and therefore unsafe for human consumption. Sugarcane grown along riverbeds dumped with domestic wastes and untreated industrial effluents has elevated amounts of heavy metal accumulation. The problem of heavy metal pollution in agricultural land is expected to intensify due to an increase in human activities, economic development, and activities such as agronomics, mining, and industrial wastewater irrigation (Zhao et al. 2012).

9.3 Mechanism of Heavy Metal Toxicity in Sugarcane

The comprehensible understanding of the route of heavy metal toxicity in sugarcane and the mechanism involved would enable the acquisition of suitable management strategies. Therefore, this section describes the mechanism engaged with the route of exposure of metals, their biouptake, and accumulation in sugarcane. Roots play a

significant role in the plant's uptake and translocation of heavy metals. Heavy metal persistence in the soil allows its entry into plant roots through water intake. These are translocated from roots to aerial parts of the plant via the xylem vascular system and foliar parts via the phloem vascular system. In higher plants, roots act as a barrier for heavy metal translocation to the upper parts of the plant (Wallace and Romney 1975) by retaining toxic metals and preventing their accumulation in shoots.

The absorption and transportation pattern in plants depends on the type of heavy metal. They may be essential or toxic. Some of them are required for normal growth at moderate concentrations, while higher concentrations may hinder growth and metabolism in plants. The metals are absorbed either as chemicals or form complexes with other elements. The extent to which heavy metal is absorbed varies with plant species and varieties. Apart from plant species, the metal uptake also depends upon factors like metal content, organic content, cation exchange capacity, and soil pH (Abdus-Salam et al. 2008; Chandra et al. 2008). The heavy metal translocation from root to shoot also involves a class of multiple transporter proteins such as heavy metal transporting ATPases, cation diffusion facilitator (CDF), multi-drug and toxin efflux (MATE), and zinc-iron permease (ZIP) (Singh et al. 2016).

In sugarcane, the concentration of Zn in its juice, bagasse leaves, and roots decreases with maturity, and its concentration is lowest at the time of harvest (Sampanpanish and Tantitheerasak 2015). Uptake of Zn increases if it is in excess amount in the soil and increases competition over Fe and Mn in the storage site of the root. In the case of Pb, which enters easily into plants because it is in the immobilized form in the soil. The roots do not have any site for Pb, but its uptake through the foliar route by adsorption to the stomatal pores or cuticle has been reported (Schreck et al. 2012, 2013, 2014). The uptake of Hg from soil to roots and then transferred to shoots has been reported (Martíneza et al. 2015), but stomatal pores of leaves may also absorb it during the transpiration stream as gas.

Furthermore, in the case of Cd, foliar uptake has been reported (Santos et al. 2010). However, its uptake is mainly by the roots. The major part of Cr accumulated in the roots, and some parts translocated in shoots of the plant. High concentration of Cu also accumulated in the roots (Fernandes and Henriques 1991), and the plant roots actively take it up. The uptake and translocation of metals also depend on the interaction between two metals, and the interaction could be synergistic, antagonistic, or may not affect each other. Heavy metal content varies in different parts of sugarcane, and the accumulation of metals is lower in juice and bagasse and higher in leaf and roots (Zhang et al. 2014).

In plants, heavy metal toxicity leads to oxidative stress due to the production of ROS (Reactive Oxygen Species) that include free radicals such as hydroxyl radical ($^{\bullet}OH$), superoxide anion ($O_2^{\bullet-}$) as well as non-radical molecules like hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). The oxidative stress results in the destruction of crucial cellular components and leads to various dysfunctions due to damage caused by ROS to proteins, DNA, and lipids. Sugarcane can uptake and retain some heavy metals in significant amounts, but some of its varieties have low ability to uptake and accumulate them in their biomass. Sugarcane is known to be an

efficient accumulator and an excellent biomass producer because of its ability to tolerate some heavy metals (Table 9.1).

9.4 Effects of Heavy Metal Toxicity in Sugarcane

9.4.1 Morphological Symptoms

Heavy metal in high concentration may show conspicuous signs of injury in terms of growth hindrance, chlorosis, and eventually plant death. In sugarcane, the younger leaves show chlorosis, extending to older leaves after prolonged exposure to heavy metals. Zn helps plants produce chlorophyll, but its high soil levels and continued longer exposure may also cause chlorosis (Ebbs and Kochian 1997). It may be due to induced iron deficiency as excess Zn causes Fe and Mn deficiencies in plants (Asati et al. 2016). In sugarcane, severe chlorosis was observed in the case of Mn toxicity, especially in acidic soil. The chlorosis symptoms are similar to Fe deficiency (Alejandro et al. 2020). The common symptoms of manganese toxicity are crinkle leaves showing chlorosis and tissue browning in the youngest leaves of some plants. The leaves show purplish-red color due to phosphorus deficiency (Lee et al. 1996). High levels of Cd inhibit chlorophyll biosynthesis and browning of plant root tips (Di Toppi and Gabbrielli 1999). Excess Co induces interveinal chlorosis and necrotic spots in the middle leaves of sugarcane. Later, the leaves become withered, dry, and necrotic (Sinha and Chatterjee 2015). Younger leaves with Pb toxicity show yellowing leaves and bending of leaf margins (Misra et al. 2010). Leaf chlorosis was observed at 40 ppm Cr concentrations, which turned to necrosis at 80 ppm Cr concentrations in sugarcane (Jain et al. 2000).

9.4.2 Growth, Development and Productivity

Heavy metal accumulation in excess can be toxic to plants. High concentrations of heavy metals exhibit growth depression, dark green leaves and cause immediate stress to the leaves and sugarcane plant roots. Reduction in growth parameters like length and area of leaf, length and the number of roots, fresh and dry weight of sugarcane plant has been reported by several workers. A high level of heavy metals results in retarded growth and causes senescence. Similar to sugarcane, other plants also show reduced plant height and stunted growth. A significant reduction in root length and number was observed at increasing concentrations of Cr in sugarcane (Jain et al. 2000). Toxicity of Cd in sugarcane shows reduction in growth attributes such as plant height, leaf number and area, and dry weight (Jain and Srivastava 2006).

Some heavy metals, such as Ni, also considered an essential micronutrient in lower amount, may cause an increase in plant growth in sugarcane cultivar (Misra et al. 2010). However, its increasing concentrations may be toxic. Heavy metals also have adverse effects on plants' metabolism, such as enzyme activities and mineral

nutrition (Van Assche and Clijsters 1986; Chaoui et al. 1997). These can also cause alterations in various physiological processes such as chlorophyll biosynthesis, photosynthesis, transpiration, and electron transport. The toxic metals present in the antioxidant system could induce biochemical changes in plants (Azevedo et al. 2011). Different toxic metals and their concentrations applied to the plant may show distinct responses. Biochemical parameters such as malondialdehyde (MDA), hydrogen peroxide (H_2O_2); enzymes like catalase (CAT) and peroxidase (POD); and chlorophyll a, b and carotenoids usually show high levels in sugarcane in case of Zn toxicity (Jain et al. 2010).

The increased concentration of Cd in sugarcane causes significant changes in growth and antioxidant responses. The stress induced by Cd affected the antioxidant enzymes of sugarcane seedling, showing an increase in glutathione reductase (GR) and a decrease in CAT activity (Fornazier et al. 2002). Cadmium also induces inhibition of cell growth. The toxicity of Zn and Cd causes oxidative damage in plants. Copper is an essential metal for normal growth and development of the plant, but it is toxic in excess. High concentrations of Cu generate oxidative stress, which disturbs metabolic pathways in plants (Pichhode and Kumar 2015). Chromium has depressive effects on amylase activity; thereby, subsequent transport of sugars to embryo axes is affected and therefore causes a reduction in seed germination and plant biomass. Concentrations of 20 and 80 ppm of Cr exhibit reduction in bud germination by 32–57%, respectively, in sugarcane (Jain et al. 2000). It also induces alteration in the production of pigments, inhibition in photosynthesis, and increase in metabolite production, which causes plant damage (Shanker et al. 2003). The most abundant toxic element in the soil is Pb, which causes morphological abnormalities in plants, induces chlorosis, and increases the production of reactive oxygen species (ROS) in plants. Several cytological studies showed the harmful impact of heavy metals on sugarcane and other plants (Nandi 1985; Lerda 1992; Jain et al. 2000). The chromosomal anomalies, inhibition of cell division, and reduction in mitotic efficiency due to heavy metals indicate the severe cytotoxic effects in sugarcane (Table 9.1).

According to Lakshmanan et al. (2005), the increase in productivity has been attributed to extensive use and development of improved cultivars with high resistance to stress conditions. The availability of relatively cost-effective pesticides and chemical fertilizers better management of nutrients, water, and other resources of increased productivity. Sugarcane has better growth over other crop species and is considered a sturdy tropical and vigorous plant. It has a significant role in the world economy, and area cultivated yields in the last 10 years have increased progressively, explaining 70% of worldwide sugar production (Lakshmanan et al. 2005). The mechanisms of combating damaging effects by excess metal may limit sugarcane plant productivity. Reduction in biomass production due to heavy metal toxicity is a common response by higher plants (Ouariti et al. 1997). The decline in biomass production may be due to inhibition of cell division by heavy metals (Hewitt 1983; Arduini et al. 1994). Cobalt toxicity reduces cane yield and the concentration of sucrose in cane juice (Sinha and Chatterjee 2015). However, some essential metals at lower concentrations alone or in combination with other

metals at specific concentrations may significantly improve plant growth and yield. Application of Zn alone and in combination with Mn showed improved cane and sucrose percentage production in juice (Singh et al. 2002). It could be able to tolerate up to 100 μM of Cu and 500 μM of Cd without showing toxicity symptoms, whereas 250 μM and 500 μM of Cu in solution were lethal (Sereno et al. 2007).

9.5 Risks in Human

Heavy metal accumulation in metal-polluted agricultural soil is a global concern because of potential health risks and food safety issues. The crops absorb these toxic elements, and contaminated crops on ingestion cause harmful effects on human beings. The accumulation of heavy metals in human bones leads to exhaustion of essential nutrients in the body and weakened immunological defenses (Rai et al. 2019). Some heavy metals such as Cd and Pb have carcinogenic effects (Trichopoulos 1997). In contrast, certain ones like Zn, Cu, and Cr can cause non-carcinogenic health hazards in humans, such as liver problems and headaches (US EPA 2000). Sugarcane is an essential plant to human beings as it contains sucrose, fructose, glucose, and other nutrients. However, its prolonged consumption may cause health problems because of its property to accumulate heavy metals in concentrations beyond permitted levels by World Health Organization (Abdus-Salam et al. 2008). Zinc and copper are required for normal body functioning, but excessive exposure may reduce high-density lipoproteins levels and cause gastric problems, respectively. Palladium is highly toxic, and it may lead to disorders related to the immune and nervous systems. Cadmium and chromium have been associated with lung cancer and kidney dysfunction. Nickel is required as a trace element, but higher levels may induce asthma and bronchitis. Iron and manganese are essential elements for human survival, but higher exposure might result in immune malfunction and Parkinson's disease.

9.6 Conclusion

Sugarcane is a major commercial crop cultivated for the production of sugar. Its stem consists of bagasse and juice. Bagasse is used to feed animals, and juice is used to manufacture raw, refined sugar and jaggery. The accumulation of heavy metals in edible parts of sugarcane is related to public health. Therefore, its intoxication by consumption of sugarcane grown in polluted agricultural lands has a high risk to biological systems. The heavy metal transfer from soil to crop and human leads to serious health issues. These are accumulated in the body and damage the body tissues. Heavy metal pollution of soil impacts human health and the environment; therefore, it is a global concern. The heavy metal accumulation in excess is toxic to plants as well. They enter the ecosystem, cause bioaccumulation and biomagnifications along the food chain (Nyatwere 2014), and are hazardous to all food chain components. It is suggested that proper remedial measures should be

taken to minimize soil pollution, and possible sources of contamination should be monitored. Public awareness regarding soil pollution should be raised. Environmental policies should be implemented to protect the future of sugarcane production, and research studies related to sugarcane contamination, soil environment management, and heavy metal control and prevention strategies should be encouraged.

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Defense-Related Proteins in Sugarcane and Their Role in Disease Resistance: Molecular Advancements and Beyond

10

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Abstract

Sugarcane is the major agro-industrial crop, which not only fulfills 80% of the world's sugar needs but is also a valuable source of bioenergy. Crop yield and sugar recovery are continuously under threat owing to consistent infestation by diseases and insect pests. Plants respond to pathogen infection by the activation of constitutive or inducible defense systems, including expression of defense-related proteins, i.e. chitinase, glucanase, chitosanase, metallothionine, peroxidase, thaumatin, and endoproteinase. These pathogen-induced proteins are directly or indirectly involved in plant defense response. Other plant proteins involved in the plant defense system are NBS-LRR, glycoproteins, catalases, and WRKY proteins. Pathogenic diseases are recognized by NBS-LRR, and it induces the production of glycoproteins after infection, which disrupts the physiological activity of the pathogens and make them inactive. Likewise, catalases are involved in the detoxification of reactive oxygen species (ROS). WRKY transcription factors play a crucial role in plant defense systems by regulating PR genes. Molecular interventions provide a swift solution to combat these stresses. Various endogenous genes have been explored in sugarcane to play a pivotal role in biotic stress tolerance. Efforts have also been made to develop GMOs having the potential to survive fungal pathogen infections. Few have reached the commercialization scale, whereas others are at the infancy stage. This chapter highlights defense-related proteins in sugarcane and their potential role to mitigate pathogen infestation through advancements in molecular biology.

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Keywords

Plant defense system · PR proteins · Molecular biology · Sugarcane

10.1 Introduction

Sugarcane belongs to the genus *Saccharum* and the family Poaceae. This crop fulfills more than 80% of world's sugar needs, and it is also a major source of bioethanol. Sugarcane is frequently aneuploid and has a higher ploidy level (Lakshmanan et al. 2005; Verma et al. 2019, 2020a, 2021a). Many genotypes of sugarcane are of much importance in agronomy and industry (Suprasanna et al. 2011). The *Saccharum* spp. hybrids have increased around the globe during the last few decades. Numerous abiotic and biotic factors affect cane production and growth (Verma et al. 2019, 2020b, c). Abiotic stresses include temperature, drought, waterlogging, pH, and nutrients, whereas biotic stresses include pests, diseases, and weeds. These stresses severely affect crop production and may result in complete crop failure. Sugarcane is infected by many diseases caused by viruses, bacteria, phytoplasmas, nematodes, fungi, and miscellaneous syndrome, including stem galls, multiple buds, leaf freckles, etc. (Mehnaz 2013; Song et al. 2021; Verma et al. 2021b).

The defense system is induced in response to certain stresses, including biotic and abiotic stresses. It was observed that many defense-related proteins are induced in plants after viral, bacterial, fungal, oomycetic, or insect attack (Van Loon 1997; Van Loon et al. 2006) (Fig. 10.1). The term "pathogenesis-related" (PR) proteins is used for the proteins induced by the microbial pathogens. In most cases, these proteins are not expressed in the absence of a disease-causing agent (Ryan 1990; Bohlmann 1994; Broekaert et al. 1995; Van Loon et al. 2006).

The first-ever discovered pathogenesis-related (PR) protein was TMV (tobacco mosaic virus) (Van Loon and van Kammen 1970). There are 17 families of PR proteins (PR-1 to PR-17) deployed on their structural configuration and biological activity (Van Loon 1997; Van Loon et al. 2006). In addition, PR-18 and PR-19 have also been worked out in sunflower (Gesell et al. 2011) and Scots pine (Sooriyaarachchi et al. 2011), respectively. Understanding these pathogenesis-related proteins is of pivotal importance in devising strategies to strengthen plants against infectious fungal pathogens.

10.2 PR-1 Family

The most copiously present PR proteins in *Nicotiana tabacum* are members of the PR-1 family which have the ability to be induced up to 10,000 folds in reaction to pathogen infestation (Alexander et al. 1993). During pathogen attack, fungal development is affected by the induction of PR-1 proteins in tobacco, *Arabidopsis*, and tomato (Niderman et al. 1995; Segarra et al. 2013). Transgenic tobacco plants were developed with resistance against two infectious oomycetes: *Peronospora tabacina*,

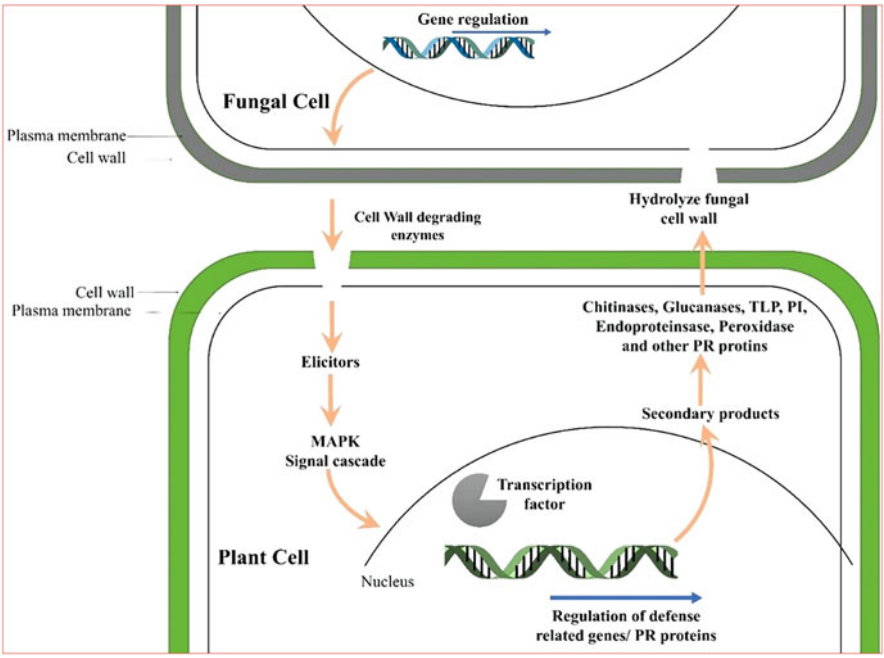

Fig. 10.1 Plant–pathogen interaction showing involvement of various plant proteins in the defense system

Phytophthora parasitica var. *nicotianae*, and over-expressing PR-1a gene (Alexander et al. 1993). Likewise, PR-1 from tomato and tobacco appeared to suppress the germination of *Phytophthora infestans* zoospores and have fungicidal activity (Niderman et al. 1995). Segarra et al. (2013) studied that inoculation of *Botrytis cinerea* fungus enhances the expression of the PR-1 gene in *Arabidopsis*. Sugarcane EST Genome Project (SUCEST) database reports that sugarcane also has PR-1 encoding genes (Kuramae et al. 2002), and respective PR-1 proteins are potential target proteins for future studies against oomycetes as increased activity of these proteins was observed in tomato and tobacco against *Phytophthora* species (Niderman et al. 1995; Alexander et al. 1993).

10.3 PR-2 Family (β-1,3-Glucanase)

β-1,3-glucanases are the enzymes belonging to the PR-2 family. These glucanases cleave β-1,3-glucans by hydrolyzing their 1,3-β-D-glycosidic linkages (Leubner-Metzger 2003). They play a key role in developmental and physiological processes in plants under normal conditions (Balasubramanian et al. 2012; Romero et al. 1998). These enzymes are also activated during biotic (Leubner-Metzger 2003; Kemp et al. 1999) and abiotic (Hincha Jr et al. 1997) stresses. β-glucan is released

from the fungal cell wall by β-1,3-glucanases and induces the production of phytoalexin by acting as elicitors in plant defense (Sharp et al. 1984; Okinaka et al. 1995). In sugarcane, different expression profiles show differential expression of β-1,3-glucanase genes after inoculation of fungus *Sporisorium scitamineum* and *Colletotrichum falcatum* (Prathima et al. 2013). Su et al. (2013) observed down-regulation of ScGluD1 (KC848051) and up-regulation of ScGluA1 (KC848050) gene(s) in response to abiotic stress and *S. scitamineum* infection. Different genotypes have variable levels of susceptibility to *S. scitamineum* as they show variable levels of β-1,3-glucanase activity. In resistant sugarcane varieties, glucanase activity enhanced quickly and stayed longer following the infection with *S. scitamineum*.

10.4 Chitinases (PR-3, PR-4, PR-8, and PR-11 Families)

Chitin, a vital component of the cell wall of numerous fungi and exoskeleton of insects, is cleaved by hydrolyzing β-1,4-linkage among N-acetylglucosamine residues of chitin (Datta et al. 1999). As the defense mechanism activates, chitinases cease the fungal growth by degrading chitin in their cell wall (Schlumbaum et al. 1986). Chitinases are grouped into four PR families based on sequence homology, such as PR-3, 4, 8, and 11. In sugarcane, chitinases are linked with responses to both abiotic and biotic stresses. Su et al. (2015) observed a differential expression pattern of the *ScChiVII1* gene in smut susceptible and resistant sugarcane genotypes. After infection with *Gibberella fujikuroi* (Lin et al. 2010), *C. falcatum* (Rahul et al. 2013), and *S. scitamineum* (Que et al. 2009; Su et al. 2015), differential expression of chitinases was observed by various research groups.

The *ScChiVII1* gene demonstrated a distinct expression pattern in smut-resistant to susceptible *Saccharum* genotypes (Leubner-Metzger 2003). The gene expression was induced at different levels after infection with *C. falcatum*, *S. scitamineum*, and *G. fujikuro*. Moreover, Viswanathan (2012) studied that sugarcane varieties with red rot resistance had higher chitinase activity than susceptible ones. Sugarcane chitinase belongs to the PR-8 family and shows antifungal activity by ceasing the hyphal growth of *Fusarium solani* var. *coeruleum* (Que et al. 2014). Additionally, another study showed that chitinases were linked with *Pseudomonas*-mediated drawn resistance (Viswanathan et al. 2003). Similarly, it was found to be induced by the attack of *C. falcatum* and *Diatraea saccharalis* in sugarcane (Medeiros et al. 2012).

SUGARWIN1 and SUGARWIN2 (present in sugarcane) are class II chitinases associated with the PR-4 family and are homologs of antifungal BARWIN, a barley wound-inducible protein (Medeiros et al. 2012). BARWIN has tridimensional structure containing three disulfide bonds (Zhu et al. 2006) and 125 amino acid residues (Svensson et al. 2002). Several plants, including *Hevea brasiliensis*, *Nicotiana tabacum*, *Solanum lycopersicum*, and *Triticum aestivum*, have proteins with a domain like BARWIN, either with or without chitin-binding domain (Friedrich et al. 1991; Tabei et al. 1998). SUGARWIN and BARWIN proteins have antifungal and antibacterial activity (Kiba et al. 2003; Zhu et al. 2006). In sugarcane, the

expression of SUGARWIN genes was upregulated by treatment of methyl jasmonate and by mechanical wounding of sugarcane borer *Diatraea saccharalis* attack (Medeiros et al. 2012). SUGARWIN2 protein has mycoprotective activity against *C. falcatum* (Franco et al. 2014; Parvaiz et al. 2021) and *Fusarium verticillioides* (Medeiros et al. 2012), but no insecticidal activity although induced by *D. saccharalis* attack. Insect damage regulates the defense mechanism against some fungi by inducing the SUGARWIN2 gene (Franco et al. 2014; Medeiros et al. 2012; Parvaiz et al. 2019). Additionally, SUGARWIN2 shows mycoprotective activity against pathogenic fungus *Ceratocystis paradoxa* and doesn't show any mycoprotective activity against nonpathogenic fungi such as *Saccharomyces cerevisiae* and *Aspergillus nidulans* (Franco et al. 2014). It was observed that SUGARWIN2 affects the sustainability, development, and maturation of fungus by programmed cell death (PCD) followed by vacuolization, excess of intracellular material, and increasing point of fractures (Medeiros et al. 2012; Franco et al. 2014).

10.5 Thaumatin-Like Proteins (PR-5 Family)

Thaumatin-like proteins (TLPs) belong to the PR-5 family, having sequence similarity with thaumatin, a protein isolated from *Thaumatococcus daniellii* (a West African shrub). Thaumatin contains 8 disulfide bonds and comprises 207 amino acid residues (Kim et al. 1988). Both abiotic and biotic stresses induce thaumatin-like proteins (Rajam et al. 2007). Vigers et al. (1992) proved by in vitro studies that the fungal cell plasma membrane was interrupted by the mycoprotective activity of thaumatin-like proteins (Vigers et al. 1992). TLP causes the formation of pores by direct insertion into the plasma membrane of the fungal cell, changes membrane permeability, and disrupts cell wall by hydrolyzing β-1,3-glucans (Roberts and Selitrennikoff 1990; Grenier et al. 1999). In sugarcane, TLP was induced by infection with *D. saccharalis* (Rocha et al. 2007), *C. falcatum*, and *S. scitamineum* (Ramesh Sundar et al. 2008; Heinze et al. 2014). Sathyabhama et al. (2015) observed the differential expression of TLP after *C. falcatum* infection (Sathyabhama et al. 2015).

TLP was first discovered in the extracellular fluid of hyper-sensitively responding tobacco plants but not in the extracellular fluid of uninfected tobacco plants. Despite the fact that some PR proteins are constitutively expressed at low levels in plants, the production of the vast majority of PR proteins is activated in reaction to pathogen attack (Hon et al. 1995). The PR proteins are induced as a result of activation of plant defensive pathways, which prevent the pathogen from entering into the plant or from spreading. Hydrolytic enzymes are expected to act on fungal pathogens immediately after pathogen penetration and weaken them, resulting in no disease development in resistant varieties. In susceptible hosts, the pathogen may penetrate and colonize the tissues before induction of the PR proteins to the required level. It is established that PR proteins are activated early in many host–pathogen interactions. Sugarcane cultivars with varying levels of red rot resistance were tested for the induction of PR proteins. It was shown that some PR proteins are specifically induced in

sugarcane in response to pathogen infection. Furthermore, the study clearly showed that constitutive production of these proteins is low and that their induction requires particular signals such as pathogen infestation. Similarly, in stalk tissues, the resistant variety showed a greater induction of TLP (Farvardin et al. 2020).

10.6 Peptidase Inhibitors (PR-6 Family)

In plants, exogenous and endogenous peptidase activity is controlled by peptidase inhibitors belonging to the PR-6 family. Insects and pathogenic microorganisms secrete peptidases, digested by peptidase inhibitors and activated by plant defense (Habib and Fazili 2007). PhyCys (phytocystatins) is among the most considered protease inhibitors in plants (Benchabane et al. 2010). Peptidase inhibitors are reversible and competitive inhibitors of cysteine proteases. The genes of the cystatin family have the function in response to abiotic stresses (Martinez and Diaz 2008; Hwang et al. 2010), pathogenic attack (Gutierrez-Campos et al. 1999; Bobek and Levine 2016), insect attack (Konrad et al. 2008; Liang et al. 2015), in seed germination (Hwang et al. 2009; Zhao et al. 2013) and PCD (Solomon et al. 1999; Zhao et al. 2013) have been identified and characterized in some plants. Moreover, these genes play an essential role in hypersensitive cell death, plant defense mechanisms and show differential expression in response to biotic and abiotic stresses (Belenghi et al. 2003; Wang et al. 2015). Cane cystatin has 106 amino acid residues and exists in the form of the domain-swapped dimer (Valadares et al. 2013).

Firstly, cane cystatin was characterized by the SUCEST sugarcane genome project (Soares-Costa et al. 2002). Soares-Costa et al. (2002) studied its antifungal activity against *Trichoderma reesei* followed by reduced germination of the filamentous fungus by recombinant expression and purification of this protein. Sugarcane plants may be protected against insects and fungi by the inhibitory effect of thiol peptidases provided by cane cystatin (Vilela et al. 2004). The catalytic activity of cysteine peptidases isolated from coleopteran *S. levis* midgut was affected by Cane CPI-1 purified from transgenic sugarcane (Ribeiro et al. 2008). Moreover, Pechan et al. (2000) studied that the growth of lepidopteran species was inhibited by MIR1 protein (a cysteine peptidase). The role of cystatins in providing resistance to sugarcane against insect pests was also verified, but another type of peptide inhibitor. Sugarcane also has Bowman-Birk type serine peptidase inhibitors besides cysteine peptidase inhibitors (Mello et al. 2003). Bowman-Birk type serine peptidase inhibitors contain many disulfide bonds (BIRK 1985). Almost 14 Bowman-Birk inhibitors with varying amino acid sequences have been identified in sugarcane (Mello et al. 2003). Transgenic sugarcane, expressing Kunitz-type and Bowman-Birk type serine peptidase inhibitors has the better ability to withstand borer (*Diatraea saccharalis*) infection (Mello et al. 2003).

10.7 Endoproteinases (PR-7 Family)

Endoproteinases belong to the PR-7 family and the subtilisin serine protease family and are similar to pathogenesis-related proteins of *Solanum lycopersicum*, i.e., alkaline endoproteinase P-69. This protein is activated in response to CEV (citrus exocortis viroid) infection (Tornero et al. 1997; Vera and Conejero 1988). Endoproteinases are involved in protein degradation by breaking peptide bonds. These proteins are involved in post-translational modifications of defense-related proteins and disrupt the cell wall of microbes that attack plants. However, their importance in abiotic stress tolerance is not well-defined (Tornero et al. 1997; Van Loon et al. 2006). Jordá and Vera (2000) studied P69B and P69C genes of tomato expressed in transgenic Arabidopsis induced *Pseudomonas syringae* infection and salicylic acid application (Jordá and Vera 2000). In sugarcane, involvement of endoproteinases in plant defense has not been explored yet (Ramos and Selistre-de-Araujo 2001; Medeiros et al. 2012).

10.8 Peroxidases (PR-9 Family)

Peroxidases belong to the PR-9 family and are involved in various physiological and plant defense mechanisms. They are actually glycoproteins that use H_2O_2 to catalyze the oxidation of specific inorganic and organic substrates. Peroxidases generate ROS to provide a hostile environment for the growth of pathogens in plants and disrupt the cell wall by affecting the cell wall cross-linking (Passardi et al. 2005). Their role is linked with lignin biosynthesis, a phenolic compound present in the plant cell wall and provides mechanical support to plants, thus helping to defend against pathogen attack. An example of lignin-associated peroxidase in *Arabidopsis thaliana* is ATP A2 peroxidase which is used against pathogens (Østergaard et al. 2000).

After *C. falcatum* infection, peroxidase activity increased in resistant genotypes, while in susceptible genotypes, no change in peroxidase activity was observed (Asthir et al. 2009; Prathima et al. 2013). Moreover, an elicitor extracted from *C. falcatum* enhanced peroxidase activity in sugarcane leaves (Ramesh Sundar et al. 2008). Peroxidase activity was varied in *S. scitamineum* susceptible genotypes to *S. scitamineum* resistant genotypes of sugarcane (Esh 2014). It was found that infection of *Gluconacetobacter diazotrophicus* and *Puccinia melanocephala* induced peroxidase encoding genes in sugarcane (Lambais 2001; Vilela et al. 2004).

10.9 Ribonuclease-Like Proteins (PR-10 Family)

One of the most dominant PR families is PR10-family, and it was first discovered in cultivated parsley cells after being exposed to fungal elicitor therapy. The PR10 family has been identified in a wide range of plant species and is the alone PR protein family that is purely intracellular in nature compared to other PR protein, which are present both as extracellular and intracellular. Ribonuclease-like proteins with

ribonuclease activity belong to the PR-10 family (Van Loon 1997) and are induced by some pathogens in many plants. These genes also show antiviral, antibacterial, antinematode, and antifungal activity (Park et al. 2004; Fernandes et al. 2013). Induction of ribonuclease-like proteins in sugarcane was observed by methyl jasmonate application (Bower et al. 2005) in response to *Puccinia melanocephala* (Oloriz et al. 2012) and *S. scitamineum* infection (Que et al. 2014).

The physicochemical properties of PR-10 proteins showed that they are alkaline in nature. They comprise a highly conserved Betv1 domain and P-loop, a phosphate-binding loop motif found to be intricate in ribonuclease (RNase) activity in vitro. A recent study discovered that the "P-loop" motif seems essential for sustaining the RNase activity of PR10 proteins (Wu et al. 1995).

10.10 Defensins (PR-12 Family)

Defensins belong to the PR-12 family having characteristics β-fold are cysteine-rich, small antimicrobial peptides existing in many organisms (Stotz et al. 2009). They change membrane permeability by electrostatic charge and induce pore formation in pathogens by acting as antimicrobial agents (Thomma et al. 2002). Sd1, Sd3, and Sd5 alleged functional defensins present in sugarcane. These proteins do not show antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Kocuria rhizophila*, and *Bacillus subtilis*; however, they show antimicrobial activity against *Neurospora crassa*, *Fusarium solani*, and *Aspergillus niger* (De-Paula et al. 2008).

10.11 Thionins (PR-13 Family)

Thionins belong to the PR-13 family and are small proteins with antimicrobial properties found in higher plants. Thionins are low molecular weight, basic proteins rich in sulfur-comprising residues (arginine, cysteine, and lysine). Thionins have intracellular location mostly. However, in some instances, thionins may also be found extracellular. Various members of this family are conserved for structure and amino acid sequence. Moreover, they have shown toxicity against fungi, bacteria, yeast, animal, and plant cells. The structural and sequence studies revealed their direct effects on cell membranes of pathogenic organisms. Besides their interaction with cellular membranes, thionins are known to interact with DNA directly as they have a conserved DNA-binding motif. Various transgenic plants like *Oryza sativa* L., *Arabidopsis thaliana* L., and *Nicotiana tabacum* L., when transformed with the thionin gene, showed protection against pathogenic bacteria (Benko-Iseppon et al. 2010).

10.12 Lipid-Transfer Proteins (PR-14 Family)

Just like thionins, the lipid-transfer proteins belonging to the PR-14 family are cysteine-rich, basic, small, and lipid-binding proteins. They are involved in the transformation of lipids among membranes (Rueckert and Schmidt 1990). These proteins are present in plant cell walls, show response against biotic and abiotic stresses, and have a role in cutin biosynthesis (Kader 1997). Some studies show the induction of barley LTP4, a PR-14 type-member after fungal and bacterial infection (Molina and García-Olmedo 1993; Molina et al. 1996). The homologs of TLPs in eyespot-resistant sugarcane differentially induced by inoculation of *Bipolaris sacchari*.

10.13 Oxalate Oxidase and Oxalate Oxidase-Like Proteins (PR-15 and PR-16 Family)

Oxalate oxidases of the PR-15 family and oxalate oxidase-like proteins of the PR-16 family are involved in the creation of hydrogen peroxide (H_2O_2) and subsequently create an environment toxic for pathogens. Moreover, they are known to induce plant defense responses as well (Van Loon et al. 2006).

10.14 PR-17 Family

The PR-17 family of defense-related proteins was reported by Christensen et al. (2002). The study reported two barley proteins as members of the new PR-17 family of PR proteins. Barley was inoculated with *Blumeria graminis*, a causal organism of barley powdery mildew. Six hours post-infection, barley leaves were used for cDNA library construction, and two constructs were found hyper-accumulated. The encoded proteins, namely *Hv*PR-17a and *Hv*PR-17b, were designated as members of a novel PR family called PR-17. Two earlier reported proteins Nt PRp27 from tobacco and WCI-5 from wheat were also included in this family. The members of PR-17 were found to play a key part in plant defense responses either by signal transduction or cell wall metabolism. In this way, these proteins help in the detection of pathogen components and release signal molecules. The possibility to show antibiotic-like properties has also been reported.

10.15 NBS-LRR Proteins

R proteins identify effectors during the activation of effector-triggered immunity/ETI. These R proteins mainly contain a nucleotide-binding leucine-rich repeat receptor named NBS-LRR. Plant NBS-LRR proteins are a large family of plant resistance proteins involved in the recognition of pathogens and insects (Li et al. 2015). These proteins typically have two domains: an LRR, i.e., leucine-rich repeat,

and an NBS, i.e., nucleotide-binding site. Virulence-causing molecules are effector molecules of pathogens, which are sensed by plant NBS-LRR proteins. Based on sequence similarity in the NBS domain, NBS-LRR proteins are classified into TIR (TOLL/interleukin-1 receptor) and non-TIR classes. In TIR class, NBS-LRR proteins are called TNL proteins and are involved in the transportation of the TOLL/interleukin-1 receptor (Joshi and Nayak 2011). This TNL protein class is commonly present in dicotyledonous plants and is absent or rarely present in monocotyledonous plants (Bai et al. 2002). NBS-LRR proteins belonging to the non-TIR class mostly have the RPW8 domain, zinc finger, and coiled-coil (CC) N-terminal domain and are called CNL proteins (DeYoung and Innes 2006). This class is present in dicotyledonous and monocotyledonous plants (Pan et al. 2000). Red-rot-related NBS-LRR genes have a significant role in systemic acquired resistance by upregulating after *C. falcatum* inoculation (Ramesh Sundar et al. 2012). These genes are present in the sugarcane SUCEST database (Gupta et al. 2009). Another fungus, *Puccinia melanocephala*, also cause to induce the NBS-LRR gene in sugarcane variants. Some studies showed the induction of non-TIR-NBS-LRR-type genes in *Saccharum* by inoculation with *S. scitamineum* smut causing agent (Borrás-Hidalgo et al. 2005; Que et al. 2009).

Disease resistance gene analog (RGA) markers were used to identify the resistance-related genes encoding the NBS domain (Sekhwal et al. 2015). Resistance gene analogs (RGA) were disease resistance-related genes having conserved domains amplified by the NBS domain in several plant species (Wang et al. 2001). These genes are linked with resistance against rust caused by *P. melanocephala* and yellow leaf virus (SCYLV) in sugarcane (Glynn et al. 2008). Xa1 and RPS2; non-TIR-NBS-LRR resistance genes, and L6 and N; TIR-NBS-LRR resistance genes were recognized in smut-resistant sugarcane's RGA sequence (QUE et al. 2009). Almost 18 genes having homology with rust-resistant rice and maize were discovered in the sugarcane SUCEST database (Rossi et al. 2003). By analyzing these genes, new markers can be developed by identifying and understanding stress-responsive pathways in sugarcane.

10.16 Glycoproteins

The primary reaction of *S. officinarum* to infection is the creation of glycoproteins, the macromolecules found in the cell wall of plants and classified into two groups; HMMGs (high molecular mass glycoproteins) and MMMGs (mid molecular mass glycoproteins) (Legaz 1998; Fontaniella et al. 2002). As the pathogen attacks, the physiological functions of microbes are modified by both types of glycoproteins produced in response. Sugarcane juice extracted from mechanical injuries was the first source for the isolation of glycoproteins (Legaz 1998). A substantial increase in the concentration of glycoproteins and their component was observed after the infection of sugarcane with *S. scitamineum* (Martinez and Diaz 2008). In sugarcane, both HMMGs and MMMGs perform against smut disease by reducing germination of spores by 50% (Fontaniella et al. 2002), preventing cell polarization (Millanes

et al. 2005), and increasing cyto-agglutination. However, in the sugarcane plant, smut mycelium growth is completely inhibited by both types of glycoproteins (Millanes et al. 2005). Leaf scald is a bacterial disease in *Saccharum* caused by *Xanthomonas albilineans*. During this attack, glycoproteins perform as cell-to-bacterial signal transduction and induce the production of xanthan by *X. albilineans*. Certain glycoproteins are also known to inhibit bacterial proteases, which in response produce xanthan. Glycoprotein protects xanthan biosynthesis responsible enzymes from proteolytic degradation (Legaz et al. 2011).

10.17 Catalases

Catalases, along with SOD and peroxidases, can scavenge ROS produced by HR during pathogen invasion. The first antioxidant enzyme ever characterized and discovered was catalase. ROS (reactive oxygen species) in plants are detoxified by catalases. These enzymes act as heme proteins and catalyze two molecules of H_2O_2 into oxygen and water (Singh et al. 2012). Catalases having similarity with CAT 1, CAT 2, and CAT 3; maize isoforms were found in the sugarcane EST database (SUCEST) (Netto 2001). Gene encoding catalase isoform (CAT3) was upregulated by infection of *S. scitamineum* (Lao et al. 2008), *Gluconacetobacter diazotrophicus* (2.5-fold), and *Herbaspirillum rubrisubalbicans* (fivefold) (Lambais 2001). Kuramae et al. (2002) studied that sugarcane leaves inoculated with rust-causing agent *P. melanocephala* have a significant level of CAT 1 and CAT 3 (Kuramae et al. 2002). Catalases are also induced by elicitors of *C. falcatum* in sugarcane (Ramesh Sundar et al. 2008). High catalases activity was found after red rot inoculation in sugarcane plants susceptible to *C. falcatum* (Asthir et al. 2009). Su et al. (2014) studied the positive correlation between smut-resistant levels in sugarcane and catalase activity (Su et al. 2014). Moreover, plant–fungal interaction induces the catalase gene expression (Que et al. 2014).

10.18 WRKY Proteins

In plant innate immunity, PAMPs perception stimulates the induction of WRKY transcriptional factors and induces expression of defense-related genes, SAR genes, PR genes, and jasmonic acid/ethylene genes. WRKY is a large class of transcription factors because they have a 60 amino acid long conserved domain that has metal chelating zinc finger domain at C-terminal and WRKYGQK, a highly conserved motif at N-terminal (Agarwal et al. 2011). The promoter of several defense-related genes in plants has a W box (TTGACC/T) type DNA sequence recognized by WRKY proteins (Rushton et al. 1996). As a response to biotic stress, WRKY proteins are expressed as transcriptional activators or sometimes as suppressors to pathogen-induced defense mechanisms (Journot-Catalino et al. 2006; Ntui et al. 2013). Effector-triggered immunity/ETI (virulent pathogen effectors) and PAMP-triggered immunity/PTI (pathogen-associated molecular patterns) trigger the

activation of WRKY proteins as plant innate immunity (Jones and Dangl 2006). Gene comparison in this family and other multigene families shows the crucial role of biotic stress for WRKY activation (Ülker and Somssich 2004; Agarwal et al. 2011). WRKY genes were found from data analysis of *Saccharum* defense-related genes with worldwide projects (Wanderley-Nogueira et al. 2012; Que et al. 2014). Some WRKY-like genes in sugarcane regulate the transcription and expression of catalases, peroxidases, chitinases, and β-1,3-glucanases (Dellagi et al. 2000; Hara et al. 2000). Some WRKY-like transcription factors are linked with pathogenesis-related regulons in sugarcane (Lambais 2001). Inoculation of sugarcane with *C. falcatum*, *P. melanocephala*, *S. scitamineum*, and *U. scitaminea* shows strong induction of WRKY genes in sugarcane as depicted by expression analysis (Jinxian 2012; Ramesh Sundar et al. 2012).

10.19 Resistance Gene Analogues (RGAs) Markers

As molecular biology techniques are more reliable, molecular markers associated with disease resistance are always in scope (Seah et al. 1998). The joint venture of molecular and bioinformatics approaches has assisted researchers in putting efforts on molecular markers to screen resistant cultivars. Various PR proteins have been studied in plants to reveal their mycoprotective potential against a broad range of fungal pathogens (Parvaiz et al. 2018; Rasul et al. 2019). Various researchers have focused on the genetic maps established for sugarcane, but the position of resistance gene's loci on these maps is still unidentified. Because of the complexity of the sugarcane genome, very little data of resistance loci are available.

Resistant Gene Analogue Polymorphism (RGAP) is one of the effective molecular markers to discover disease resistance in plants. To date, isolation of such Resistance Gene Analogues (RGAs) employing the conserved motifs and domains of resistance genes has been effective in numerous plants like Arabidopsis (Botella et al. 1997), soybean (Lakshmanan et al. 2005), corn (Collins et al. 1998), rice (Mago et al. 1999), wheat (Seah et al. 1998), tobacco (Gao et al. 2010), and other plants (Wan et al. 2010). RGAs, a hefty class of R-genes, contain conserved domains and motifs, which perform an important part in imparting pathogens resistance. These RGAs may be the actual resistance genes or their homologs involved in imparting resistance. Many of these resistance genes belong to either NBS/LRR or receptor-like proteins as well as kinases, apoplastic peroxidases, and pentatricopeptide repeats. Genetic maps of these RGAs have proved of great worth for developing diagnostic markers and identifying QTLs linked with plant defense response (Sekhwal et al. 2015). Also, plenty of RGAs have been known in sugarcane from cDNA libraries and SUCEST for oxidative stress tolerance, cold tolerance, disease, and insect resistance (Rossi et al. 2001), and the total number of EST sequences in GenBank has reached 366,535. With the help of this, new RGAs can be identified and used for screening disease-resistant cultivars (Sharma and Tamta 2017).

10.20 Potential of Defense-Related Proteins in Sugarcane

ISR (induced systemic resistance) is defined as a possible mechanism involved in the enhancement of resistance in sugarcane. The root-associated rhizobacteria induce systemic resistance against Pseudomonas. They have been reported as potential biocontrol agents against *C. falcatum*, causing red rot in sugarcane (Rahul et al. 2013). These PR proteins uplift chitinase activity, thus enhancing antifungal activity (Sundar et al. 2002; Viswanathan et al. 2003). PR-19, 18, 17, 16, 15, and PR-13 could be the potential RGAs to trigger plant defense systems, hence play a crucial role in protecting plants from harmful pathogens. PR-13 (thionin) is known to break down bacterial and fungal pathogen membranes (Bohlmann 1994) and suppress the growth of phytopathogenic fungi (*Thielaviopsis paradoxa*) in barley (Reimann-Philipp et al. 1989) and *F. oxysporum* in Arabidopsis (Epple et al. 1997). Both of these fungi release toxins that trigger the plant defense system directly or indirectly, resulting in hydrogen peroxide production (Van Loon et al. 2006). Similarly, Nt PRp27 like proteins were detected in barley in response to *Blumeria graminis* infection (Christensen et al. 2002).

In wheat, they were stimulated by the synthetic benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), and in tobacco, they were stimulated upon mosaic virus infection (Görlach et al. 1996). Custers et al. (2004) enhanced the expression of PR-18 (fungus- and SA-inducible carbohydrate oxidases) in transgenic tobacco to combat infection by bacteria. Lately, a novel PR protein having antimicrobial properties was observed in *Pinus sylvestris* and named PR-19. This protein alters the fungal cell wall structure by making bonds with glucans of the cell wall, leading to morphological alteration of hyphae (Sooriyaarachchi et al. 2011). Gene knockdown is also in use for controlling sugarcane diseases. Virus-resistant plants had been developed through RNAi (Kim et al. 2013; Ntui et al. 2013). Gene silencing has been recognized as potential approach to attain multi-strain resistant sugarcane plants for mosaic diseases (Guo et al. 2015).

Studies have stated that microRNA-guided gene expression was vital for resistance to biotic stresses (Gupta et al. 2014). Numerous microRNAs were identified in sugarcane after *Acidovorax avenae* subsp. *Avenae* infestation by Thiebaut et al. (2012). Those unique microRNAs had the potential to be used for genetic engineering/genome editing of stress-resistant plants and can subsidize to an enhanced conception of regulatory pathways for defense-related proteins.

10.21 Conclusion

Sugarcane is a valuable source of sweetener and bioethanol. Despite the complex genome, long breeding cycle, and high delta crop, it has dominated the world sugar market. Fungal pathogens are one of the drastic yield-limiting agents, so need to devise strategies to combat these disease-causing agents. Nature has gifted sugarcane, pathogenesis-related (PR) proteins, which play a crucial role in the plant defense system. Exploring these proteins can help to devise strategies to overcome

these pathogens, thus helping out to increase per hectare yield. This chapter highlights recent interventions to understand PR proteins, their role in the plant defense system, and how they can be manipulated to uplift the immune response of the plants. These updates can be of great value to open up exciting possibilities to manipulate sugarcane as future energy crop.

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Impact of Green and Organic Fertilizers on Soil Fertility and Sugarcane Productivity

11

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Abstract

Sugarcane is grown by small, medium, and large rural farmers in several countries around the globe. In addition, the primary objective is to increase cane yield, sugar recovery and sustainably improve the livelihoods of cane farmers. For producing a large amount of biomass and sugarcane, crop extracts a large amount of nutrients from the soil and accumulate in the plant. The regular harvesting of natural resources consequently from the soil mitigates a high amount of nutrients. Therefore, there is always a need to replace these nutrients with other sources of fertilization. Soil textural properties and fertility status under changing climatic conditions also play an important role. Several alternatives can be utilized to increase the sustainable nutrient use efficiency of both macro and micronutrients to make a balance for the profitability of the crop. Two of these natural alternatives are the use of green and organic manure, i.e., press mud and farmyard manure. This chapter aims to develop an integrated nutrient management approach for the global cane farmers that would improve the quality and productivity of the canes and improve water, nutrients, and pesticide use efficiencies.

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Keywords

Bio-synthetic fertilizer · Plant nutrients · Fertilization strategy · Sugarcane · Sustainable agriculture

11.1 Introduction

Sugarcane is an important crop globally. Brazil, India, and China are the world's major sugarcane producers (Verma et al. 2020a, b, 2021a). Over the last 5 years, the area cultivated with sugarcane in Brazil has increased from 8.5 to 9.0 mha, corresponding to about 30% of the world's production. In Brazil, sugarcane is grown by small, medium, and large farmers. The main products of sugarcane grown in large and medium-sized properties are sugar, alcohol, and energy generated by the burning of sugarcane bagasse (Verma et al. 2020a, 2021b, c). On the other hand, the manufacturing of brown sugar, *rapadura*, *cachaça* and use in animal feed are predominant in small quantities (Oliveira et al. 2021).

Sugarcane fields are managed using a variety of technologies, but producers should seek to maximize the input use efficiency of the applied inputs. This would not only reduce operating costs and increase productivity but also contribute in preserving the natural resources. The industrial production of sugarcane in Brazil is concentrated in the South Central and Northeast regions. The sugar and ethanol production in the South Central region is more than 90% of the Brazilian production. This is due to a greater cultivation area and higher productivity than the Northeast region. Sugarcane crop is harvested from April to May, the following year if planted at the start of the wet season. For this reason, it is called "year sugarcane." When sowing in February or March, the harvest is done after 15–18 months, which is described as "year and a half sugarcane" (Oliveira et al. 2018).

In this chapter, topics related to the green fertilizers, sowing times, soil fertility, growth rate, nutrient accumulation, pest-weed control, and the effects of green manure on sugarcane production are discussed. Regarding organic fertilizers, updates of research conducted under texturally divergent soils and under adverse climatic variables that evaluated the efficiency of agro-industrial residues in crop fertilization and the production and quality of sugarcane juice are also discussed. Research must be focused on the integrated nutrient management of plant and ratoon canes.

11.2 Edaphoclimatic Environments and the Planting of Sugarcane

The edaphoclimatic environments or production environments for sugarcane are defined according to the topography of the land, the microclimate of the region, and the physical, chemical, and mineralogical characteristics of the soils. In the definition of production environments, good cultivation practices of the topsoil, including

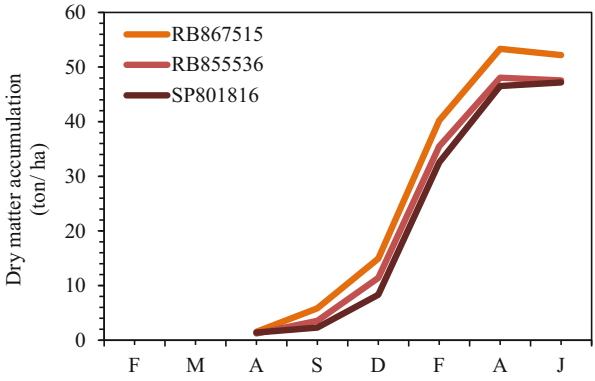

Fig. 11.1 Dry matter buildup in three sugarcane cultivars planted in February and collected in July the following year (Oliveira et al. 2019)

mechanization, liming, and chemical and organic fertilization, are also considered. Thus, the production environment is a set of interactions between climate and the characteristics of topsoil and subsoil layers. The soils of South-Central Brazil are mostly *Latossolos* and *Argissolos*, followed by *Neossolos Quartzarênicos*, *Nitossolos*, and *Cambissolos* (Oliveira et al. 2019).

In terms of the edaphic environment, the "year sugarcane" is recommended for more fertile soils with less steep slopes and less erosion due to heavy rains. Due to water and thermal shortage during peak growth periods, nutrient supply should be a limiting factor in achieving biomass yields of more than 120 t of natural matter ha^{-1}. The "year and a half sugarcane" is advised for less fertile soils with more rugged reliefs. The mature crops over time and reaches its maximal growth stage (Fig. 11.1) accords with periods of increased water and light accessibility, which would result in greater soil coverage, as well as a higher rate of photosynthesis and dry matter accumulation. Green manure cultivation before sugarcane planting is another benefit observed on planting "year and a half sugarcane" (Mascarenhas et al. 2008; Oliveira et al. 2018, 2019).

The cutting, loading, and transportation of sugarcane involve the highest cost percentage. For this reason, measures should be implemented to ensure higher land productivity of sugarcane in the plant-cane rotation and smaller diminutions in the succeeding rotation to maximize the use of the main production factors (land, capital, and labor), thus resulting in lower production costs. Sugarcane also takes and stores a vast number of nutrients from the soil since it creates a large amount of biomass. Relative storage of N, P, K, Ca, Mg, and S in plant shoots was observed to be in the tune of 150, 40, 180, 90, 50, and 40 kg, respectively. However, relative shoot storage of iron, manganese, zinc, copper, and boron was reported to be in the tune of 8.0, 3.0, 0.6, 0.4, and 0.3 kg, respectively, for harvesting 120 t of yield (Oliveira et al. 2019).

It is crucial to understand the inherent nutrient supply capacity of the soils, which generally needs to be supported with chemical and organic fertilizers. Green manure in sugarcane reform or implantation areas and organic fertilization in established crops have contributed to the more efficient use of the production factors and

increased sugarcane yields, especially in the first two cuts (Mascarenhas et al. 2008; Silva et al. 2014; Oliveira et al. 2018, 2019).

11.3 Organic Fertilization Using Sugar and Alcohol Industry Waste Residues

The physical, chemical, and biological qualities of the soil are influenced by organic fertilization using residues from the sugar and alcohol industries and green manure. It increases nutrient availability through mineralization and cation exchange capacity, contributing to greater soil aggregate stability by reducing susceptibility to erosion and increasing the capacity for water retention and gas exchange. As a result, the intensive root system has more significance in growth and efficiency for better plant development and crop yield (Oliveira et al. 2018, 2021).

Vinasse, filter cake, and bagasse were recently recognized as alternate sources of fertilizer for improving the soil organic matter, which further helps in enriching the soil properties. Vinasse is the residue of alcohol distillation, and its main constituents are potassium, calcium, and organic matter. Depending on the material used in the fermentation (called must), 10–16 L of vinasse are produced, varying in nutrient concentration for each liter of distilled alcohol. Sugarcane juice, molasses resulting from the industrialization of sugar, or mixture can be used for fermentation. Vinasse from molasses fermentation has a higher nutrient concentration than produced from sugarcane juice's fermentation (Laluce et al. 2016). Table 11.1 shows that the chemical compositions of vinasse from different types of must mean its nutrient stocks varied as per its source. However, K content in the vinasse varied from 1.81 to 2.78 kg m^{-3} from the blended must. Vinasse is more commonly employed in regrowth fertilization and can provide all of the potassium required for sugarcane farming. As a result, the potassium provided by the vinasse application must be eliminated from the mineral fertilizers (Oliveira et al. 2018).

Sugarcane is mostly harvested during the dry season. Therefore, the application of vinasse after the cutting of the sugarcane plants not only provides fertilization but

Table 11.1 Chemical composition of vinasse from different must in South-Central Brazil

	Musts origin		
	Molasses	Mixture	Juice
Chemical composition	kg of the nutrient m^{-3} of vinasse		
N	0.57 – 0.79[a]	0.33 – 0.48[a]	0.25 – 0.35[a]
P	0.05 – 0.15[a]	0.03 – 0.14[a]	0.03 – 0.07[a]
K	3.27 – 6.32[a]	1.81 – 2.78[a]	0.95 – 1.61[a]
Ca	1.32 – 1.70[a]	0.40 – 0.95[a]	0.08 – 0.52[a]
Mg	0.50 – 0.85[a]	0.19 – 0.35[a]	0.13 – 0.25[a]
S	0.30 – 0.40[a]	0.45 – 0.54[a]	0.58 – 0.70[a]
Organic matter	37.0 – 57.0[a]	19.1 – 45.1[a]	15.3 – 34.7[a]

Source: Oliveira M. W. in South-Central Brazil (unpublished data)

Fig. 11.2 Vinasse application with gun sprinkler irrigation in recently harvested sugarcane fields. In addition to fertilizing the soil, when the harvest is carried out under soil water deficiency, the volume of liquid moistens to the field and ensures good regrowth of the sugarcane

also supports in moistening the soil, ensuring good crop regrowth (Fig. 11.2). Depending on the potassium levels, the volume of vinasse applied ranged between 60 and 300 m^3 ha^{-1}. Some states in South-Central Brazil have used the value of 5.0% in the areas of vinasse application as the maximum saturation limit by potassium incapacity for cation exchange at pH 7.0 (CEC_T) so that there is no contamination of the water table. If the potassium content in the soil exceeds this limit, no more than 150 kg ha^{-1} of K should be applied.

The filter cake is the residue of sugarcane juice after chemical treatments to clarify the juice. The chemicals used in the clarification process vary among sugarcane mills although gaseous sulfur (SO_2), calcium hydroxide, and phosphoric acid are the most commonly used ones. Filter cake consists of fragments of sugarcane bagasse, minerals, impurities in the juice, and the chemicals used in the clarification and decantation processes. It can be separated using a rotating vacuum filter, a filter press, and a diffuser. The amount of filter cake produced by crushed cane stalks varies according to the type of filter used. The lowest production is in the diffuser separation (5.0–6.0 kg per t of stalks), followed by the filter press (18–22 kg), and the rotary vacuum filter can reach 28–35 kg t of stalks. The moisture content of the filter cake is high, i.e., 65–75% (Oliveira et al. 2018). In terms of chemical concentrations, viz. carbon, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur vary from 277 to 359, 9.5 to 18.7, 3.3 to 19.1, 1.6 to 117, 8.8 to 17.8, 1.0 to 5.1, and 3.4 to 8.0 g kg^{-1} DM, respectively. The filter cake is commonly used in plant-cane fertilization (Oliveira et al. 2018).

Another way to utilize these organic residues from the sugar and alcohol industries is to enrich the soil and enhance the physical and chemical properties of the land using them as fertigation in sugarcane fields. Approximately 750 L of juice and 250 kg of bagasse are obtained per ton of industrialized stalks. Oliveira et al. (2021) investigated the technical and economic viability of cultivating sugarcane with sugarcane bagasse organic compost where different types of bagasse and poultry deep litter mixes were examined (varied from 1000 to 800 kg bagasse

+200 kg deep litter, all supplemented with 50 kg ammonium sulfate t^{-1} DM). Six tons of dry matter from these composts were applied to planting furrow per hectare following the composting procedure. Fertilizer (06-30-24) was used at a rate of 500 kg per acre to the compost. The highest yield was achieved with a mixture of 1000 kg bagasse DM + 50 kg ammonium sulfate.

The 1000 kg of bagasse dry matter +50 kg of ammonium sulfate nutrient of the mixture was the lowest due to a large amount of lignin and cellulose in the bagasse. Furthermore, the density of this compost was lower, and the volume applied to the bottom of the furrow was higher than in the other treatments. The application of higher volume and the lower nutritional rate must have improved soil capacity and aeration and increased infiltration and water retention capacity for a longer duration. Small changes in the soil water content available to the crop resulted in significant differences in the diffusive flow of the phosphorus. Diffusion is the primary mode of phosphorus transport in the soil. The volumetric water content in the soil, the phosphorus-colloid interaction, the distance to the roots, P content, and soil temperature all have a significant impact. When the soil water content increases, the water film close to the solid soil particles becomes thicker, decreasing the ion-colloid interaction and the tortuosity of the phosphorus. Therefore, there is a direct relationship between soil water content and phosphorus diffusion (van Raij 2011; Oliveira et al. 2018).

Higher phosphorus diffusion is responsible for higher P uptake and endogenous accessibility in the plant, reflecting the nitrogen uptake and metabolism and the assimilation of atmospheric CO_2. Nitrogen is essential for sugarcane nutrition and physiology as it is an important constituent of all proteins, enzymes, and nucleic acids. It is absorbed in larger quantities by crops when combined with potassium. As previously stated, absorbed nitrogen improves shoot meristem activity, resulting in higher sugarcane tillering and leaf area index (LAI), as well as increased leaf length. LAI improves solar radiation usage efficiency, assessed in carbon dioxide fixation rate (μmol CO_2 m^{-2} s^{-1}), resulting in increased dry matter accumulation and sugar production (Oliveira et al. 2018, 2021).

The mixture of 1000 kg of bagasse dry matter +50 kg of ammonium sulfate had extremely low nutrient contents, but the changes it caused in water availability and aeration of the root system have more significant effect on nutrition, metabolism, and crop production than the other composts with higher nutrient contents. It shows that the longevity of organic matter was a key component in increasing the productive capacity of the experimental soil. Another factor to consider is the effect of humic substances originating from the decomposition of the organic compost on plant uptake kinetics and metabolism. Thus, the application of organic compost can improve the nutrition and production efficiency of sugarcane by physical, chemical, and physiological functional mechanisms (Oliveira et al. 2018, 2021). The costs of producing and applying the sugarcane bagasse + ammonium sulfate compost were calculated based on the value of the average price of the sugar in the last decade. Thus, in the soil with high physical variability and phosphorus adsorption capacity, applying organic compost resulted in a net gain of 5200 kg of sugar per hectare.

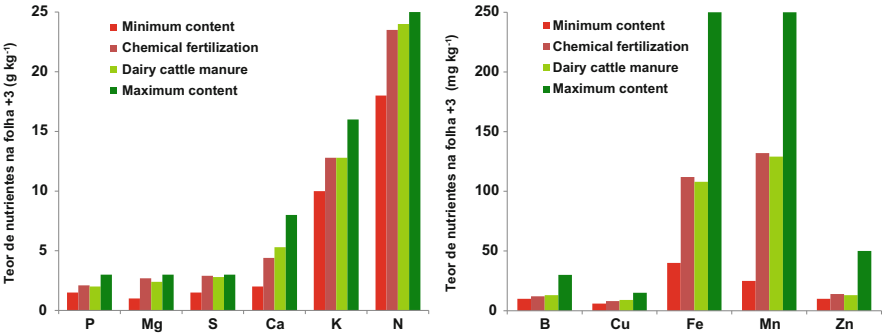

Fig. 11.3 The average concentration of macro and micronutrients in the photosynthetically mature leaf (+3) of the sugarcane variety RB867515 was compared to Brazil's minimum and maximum values reported in the literature

Using bagasse-based compost, residue from the distillery can contribute to this agricultural system's better environmental and economic sustainability. Other interesting findings were much better growth vigor in the area fertilized with the bagasse-based compost than that treated with chemical fertilizer. The sugarcane rhizomes from the treated area with the compost had higher masses of soluble carbohydrates and soluble protein, which were mobilized at the time of regrowth (Oliveira et al. 2018). Sugarcane in small rural properties of South-Central Brazil is used to manufacture brown sugar, *rapadura*, and *cachaca*. The manure of cattle fed with sugarcane can be used to fertilize sugarcane fields, contribute to better nutrient cycling, and reduce crop production costs (van Raij 2011).

In the first and second regrowth cycles, nutritional status, production, forage quality, and production of industrializable stalks of sugarcane variety RB867515 were evaluated. Applied 100 kg of P and 250 kg of K ha^{-1} of sugarcane planted because of the low soil phosphorus and potassium contents. After harvest, the plant cane and two nutrient sources were used as (1) dairy cattle manure and (2) chemical fertilization with urea and potassium chloride. The same amounts of nutrients were applied to fertilize the first and second regrowths (2.2 and 2.5 qt K ha^{-1}), regardless of chemical or organic fertilization. Urea and potassium chloride were used in chemical fertilization, while potassium chloride was needed in organic fertilization. The N: K ratio in the manure was 1.78: 1.0, while the ideal ratio was 1.0: 1.2 (Oliveira et al. 2018). On average, approximately 50% of the K is used in organic fertilization from chemical fertilizers. The amount of dairy cattle manure applied is nearly 12 t of DM ha^{-1} $year^{-1}$. Regarding plant nutritional status, the cycle or the type of fertilization did not affect the macro and micronutrients in the third leaf limbus. For this reason, the average values of the two cycles and two types of fertilization are shown in Fig. 11.3. The plants were well-nourished according to the nutrient concentration ranges stated by van Raij (2011) and Oliveira et al. (2018).

The average accumulation of natural matter in the shoot biomass of sugarcane was 134 t ha^{-1} in the first regrowth cycle and 126 t in the second regrowth rotation.

Table 11.2 The amount of minerals and structural carbs in freshly harvested sugarcane straw samples without burning (1996) and the residual straw a year later (1997) (Oliveira et al. 1999)

Year	DM (t ha^{-1})	Nutrients (kg ha$^{-1)}$ N	P	K	Ca	Mg	S	C
1996	13.9[a]	64[a]	6.6[a]	66[a]	25[a]	13[a]	9[a]	6.255[a]
1997	10.8[b]	53[a]	6.6[a]	10[b]	14[a]	8[b]	8[a]	3.642[b]
Structural carbohydrates (kg ha^{-1})				Nutrient relationship				
	Hemicellulose	Cellulose	Lignin	Cellular content	C/N	C/S	C/P	
1996	3.747[a]	5.376[a]	1.043[a]	3.227[a]	97[a]	695[a]	947[a]	
1997	943[b]	5.619[a]	1.053[a]	2.961[b]	68[b]	455[b]	552[b]	

There was no significant effect on the crop cycle. The percentage of stalks in the natural matter of shoot biomass was about 85%, and the percentage of DM in shoot biomass was nearly 30%. However, these percentages were not influenced by the fertilization and crop cycle. The cycles and types of fertilization also did not affect the bromatological quality of the forage. The average protein concentration in shoot biomass was 29.4 g kg^{-1}DM.

Crude protein and structural carbohydrates values can be considered of high bromatological importance. However, as sugarcane has nutritional limitations, there is a need to complement the dairy cows' diet with protein and some minerals to obtain medium to high animal productivity (Oliveira et al. 2019). Although only two cycles were evaluated, the results show that the fertilization with dairy cattle manure had the same effect as chemical fertilization on plant nutritional status, yield, and forage quality. The sugarcane used for feeding cattle is usually harvested with older leaves, green leaves (tops), and dry leaves. However, when the sugarcane is used to manufacture alcohol, brown sugar, and *rapadura*, the tops, green, and dry leaves remain in the field after they are cut.

After manual harvesting, the cane straw left in the field varies with the productivity of the cultivar and the agricultural practices used, but values generally range between 12 and 18 t ha^{-1} (Oliveira et al. 2018). Oliveira et al. (1999) found that of the nutrients contained in the straw, there was only a significant release of potassium after a year of permanence in the field (Table 11.2). As a result, the minerals in the straw contributed significantly to the K nutrition as compared to other nutrients.

11.4 Organic Fertilization with Poultry Litter

Organic fertilization increases the water retention capacity in the soil, and increased availability can negatively influence the maturation of sugarcane and the quality of juice. When ripe sugarcane is harvested, transportation costs are reduced, and there is an increase in the industrial efficiency for sugar production and alcoholic fermentation. There are several methods to evaluate the ripening of sugarcane, some of which are subjective and require a lot of experience from the evaluator. One of these

is the appearance of the sugarcane fields. For instance, there will be many yellow and dry leaves (consequently, few green leaves). Refractometry and polarimetry are the most popular instrumental methods. The relationship between apparent sucrose concentrations in the juice from the tip and the base of industrially useable stalk determined by polarimetry has also been used to evaluate the ripening of sugarcane. Table 11.3 shows the percentage values of the quotient between the concentrations of apparent sucrose in the juice or soluble solids from the tip and the base of the stalks, as well as the ripening of sugarcane.

Some small farmers also use densitometry to evaluate the ripening of sugarcane, especially to calculate the need to dilute the juice for fermentation. This method is cheaper than refractometry, and Brix density meter or aerometer is used. For small farms, collecting 20 stalks every 0.25–0.30 ha is recommended. These stalks are stripped and cut, after which they are passed through the mill, homogenizing the extracted juice and then determining its density. Thus, it is considered ripe when Brix values are greater than 18° of soluble solids.

Poultry litter is a mixture of poultry manure and material used to cover the coop floor. This waste from poultry production generally has a high concentration of nutrients. As chickens consume 2.5–3.0 kg of feed in the first 35 days of age, approximately 50% N, 70% P, and 80% K consumed are excreted in the feces (Pitta et al. 2012; Souza et al. 2012). Thus, poultry litter can replace chemical fertilization, but there is a need to evaluate further possible changes caused by this waste in the maturation of sugarcane and juice eminence. However, K release is generally faster than other macronutrients, viz. N and P (Oliveira et al. 2018). For this reason, the release of potassium in poultry litter is basically dependent on the volume of rain. In a study conducted in the southwest of Paraná, Pitta et al. (2012) found that 91% K was released just 30 days after applying poultry manure in the field, and the volume of rain in that period was 203 mm. Further, there is significant variation in the literature regarding the percentage of N that is the organic or inorganic form: some researchers have found inorganic nitrogen content in the samples to be small, while others have found percentages exceeding 95%.

The material used to cover the coop floor impacts the nutrient concentration in the poultry litter, and the vast majority of poultry producers utilize rice husks, coffee husks, Napier grass, wood shavings, and corn cobs. Souza et al. (2012) evaluated P mineralization of five poultry beddings. Table 11.4 shows the total nutrient content

Table 11.3 Sugarcane ripening based on the percentage values of the quotient between the concentrations of apparent sucrose in the juice, or of soluble solids, from the tip and the base of the industrializable stalks

Ripening stage of sugarcane	Percentage values of the quotient between the concentrations of apparent sucrose in the juice, or soluble solids, from the tip and the base of the industrializable stalks
Ripe	85–100%
Late ripening	70–84%
Early ripening	60–69%
Unripe or green	Less than 59%

Table 11.4 Dry matter nutrients (g kg^{-1} DM) in five different types of poultry bedding (Souza et al. 2012)

Poultry bed	N	P	K	Ca	Mg	S
Rice husk	34.7^{ab}	15.9^{b}	26.8^{b}	25.7^{a}	6.2^{a}	16^{ab}
Coffee husk	32.8^{a}	14.4^{b}	28.9^{ab}	25.0^{a}	5.5^{b}	15^{b}
Napier grass	34.8^{a}	15.1^{b}	23.3^{c}	25.5^{a}	6.0^{ab}	15^{b}
Wood shavings	30.9^{a}	13.7^{b}	24.4^{c}	25.8^{a}	5.7^{b}	14^{b}
Corn cob	34.2^{a}	18.6^{ab}	29.7^{a}	28.3^{a}	6.7^{a}	18^{a}
Means	33.5^{a}	15.5^{b}	26.6^{a}	26.1^{a}	6.0^{b}	15.0^{b}
C.V. (%)	10.6	12.2	10.0	6.6	8.2	10.1

of these poultry beddings after being used in a flock of broiler chickens with an average age of 48 days, at the density of 15 birds per m^2. About 40% of the P was in the organic form, mainly orthophosphate monoesters. Mineralization of organic phosphorus was relatively fast in the first 15 days, but there was a difference in mineralization rates among bedding types. The coffee husk had the highest mineralization rate (44.7%) in the soil, while the wood shavings had the lowest (4.9%).

Oliveira et al. (2021) investigated the effects of organic fertilization with poultry manure on medium-textured soil to evaluate the cane performance as far as yield, and quality potentials were concerned under subtropical highland climate. Over the last 30 years, the average rainfall has been 1200 mm. From November to April, water surplus while from April to September and September to November, water becomes deficit, and from November to November, well-organized moisture fluctuations are there.

After plant-cane harvest, a study of fertilizing using poultry manure was set up. The sugarcane variety RB867515 was employed in the experiment, with randomized block with four replications. Under treatments, 7.0, 10.0, and 13.0 t of poultry litter DM ha^{-1} $year^{-1}$ applied in both seasons with no chemical or organic fertilization and chemical fertilization. Table 11.5 shows that the poultry litter fertilizer did not affect sugarcane ripening or juice quality. The juice's average soluble solids, sucrose, and purity were reported to be 22, 19, and 88%, respectively. Based on the investigations of Duarte Júnior and Coelho (2008) and Oliveira et al. 2018, the above observations are regarded as a good indicator for evaluating the effect of any treatment on the cane performance.

11.5 Green Manure

Green manure is the cultivation of plants for subsequent incorporation to increase soil organic matter content as well as maintain or even increase soil fertility. As mentioned previously, soil organic matter exerts a protective action against degradation and improves soil physico-chemical and biological properties. There are several ways to improve soil organic matter content, and green manure has been

Table 11.5 As fertilization function, analysis of variance for different quality parameters for sugarcane variety RB867515 of both regrowth cycles

Source of variation	GL	Mean square					
		Brix	POL	Purity	PCC	Fiber	TRS
First regrowth cycle							
Fertilization	4	0.2125^{ns}	0.5161^{ns}	2.753^{ns}	0.237^{ns}	0.618^{ns}	17.5960^{ns}
Waste	15	0.5502	0.3708	4.7551	0.2363	0.1028	25.8885
C.V. (%)	–	3.34	2.70	2.47	2.94	2.23	2.81
Overall mean	–	22.23%	19.62%	88.30%	16.50%	14.37%	162.81 kg t^{-1}
Second regrowth cycle							
Fertilization	4	0.2056^{ns}	0.5012^{ns}	2.543^{ns}	0.223^{ns}	0.607^{ns}	17.4567^{ns}
Waste	15	0.5405	0.3651	4.345	0.2214	0.1124	25.6721
C.V. (%)	–	3.52	2.84	2.63	3.01	2.45	2.98
Overall mean	–	22.04%	19.26%	87.39%	16.12%	14.21%	160.34 kg t^{-1}

ns not significant ($P > 0.05$), *CV* coefficient of variation

used in small, medium, and large farms (Duarte Júnior and Coelho 2008; Mascarenhas et al. 2008; Oliveira et al. 2019, 2021).

The provision of organic and mineral substrates for soil microorganisms is another factor to consider. Microorganisms' ability to fix nitrogen from the atmosphere helps cut down the N fertilizers footprints, which further helps to cultivate sugarcane in a climate-smart way (Oliveira et al. 2021). In addition to these traits, the plant must have a strong and deep root system that helps in the restoration of soil fertility as well as soil decompression. Intensive soil use with conventional practices and excessive mechanization has reduced organic matter and caused soil compaction. As a result, green manure helps to improve inherent soil fertility. A variety of crops are recognized as green crops, but in the South Central region of Brazil, sunn hemp (*Crotalaria juncea* L.) is the most popular (Mascarenhas et al. 2008; Silva et al. 2014; Oliveira et al. 2019).

In evaluation, the productive potential of six green manures (*Crotalaria juncea*, *Cajanus cajan*, *Canavalia ensiformis*, *Mucuna nivea*, *Mucuna terrina*, and native vegetation) for 2 years, Oliveira et al. (2021) found sunn hemp stood out. There was more significant dry matter accumulation and nutrient cycling by sunn hemp than other green manures. During the 2-year experimental period, sunn hemp accumulated on average approximately 15 t DM ha^{-1} in plant shoots, which is statistically higher than the others. Pigeon pea was the second green manure in terms of DM accumulation, averaging 10.5 t ha^{-1}. Dry matter accumulation by *Canavalia ensiformis*, *Mucuna nivea*, and *Mucuna terrina* did not differ from one another (approximately 8.0 t ha^{-1}). An average DM accumulation close to 5.0 t ha^{-1} was found for native vegetation (fallow).

The areas in the aforementioned studies (Oliveira et al. 2021) had predominant vegetation of *Brachiaria decumbens* and *Brachiaria plantaginea* (marmalade grass). The soils in these areas are fertile due to use of acidity correctors, chemical and organic fertilizers, with base saturation oscillating around 60% and average P and K contents. In both years, sowing was carried out in the first week of October, just after the first rainfall events. In choosing the sites, the authors selected soils representative of the farms and developed studies or used the properties as units of validation and diffusion of technologies recommended for sugarcane cultivation, focusing on high yields and use efficiency of production inputs.

11.6 Soil Fertility and Sunn Hemp Growth

Sunn hemp and sugarcane are crops with high productive potential. For this reason, these species are very responsive to restoration and improvement in soil fertility. In terms of soil nutrient availability, sunn hemp is sensitive to low Ca and Mg contents in the soil and high aluminum saturation (Ernani et al. 2001; Meda and Furlani 2005). Thus, improved sugarcane nutrition will positively influence growth, dry mass, and nutrient accumulation by sunn hemp cultivated previously to the cane sowing (Mascarenhas et al. 2008; Oliveira et al. 2018).

Sunn hemp was sown in early October and harvested when the seeds were in the grain filling stage. To assess the effect of soil fertility on DM accumulation by sunn hemp, Oliveira et al. (2021) demonstrated Oxisol red yellow with fertilization of sugarcane in the regrowth. In the experimental field without the use of fertilizers and acidity correctors (control), the authors found average values in the 0–20 cm layer of 18.1% base saturation, 0.96 $cmol_c\ dm^{-3}$ for Al^{3+}, 56.4% aluminum saturation, and 1.3 and 14 mg $dm^{-3}$of P and K, respectively. On the other hand, plots treated with P and K fertilizers and acidity correctives averaged 55.8% base saturation, absence of aluminum, and 8.0 and 52 mg dm^{-3} P and K, respectively.

Mascarenhas and Wutke (2014) experimented on low fertility soil, and they found shoot DM accumulation in sunn hemp of 8.8 t ha^{-1} in the control treatment, increasing 13.9 t ha^{-1} in soil treated with 39 kg Pha^{-1}. Dry matter accumulation on an average in the control plots was 5.6 t ha^{-1}. In contrast, the average 14.2 t ha^{-1} in fields treated with P and K fertilizers and acidity correctives.

Ernani et al. (2001) and Meda and Furlani (2005) found high sensitivity of sunn hemp to aluminum. In a greenhouse experiment, Ernani et al. (2001) used a Brown oxisol with an aluminum saturation of 38.8% and base saturation of 24.5%. Base saturation increased 57% in treatment with liming of 5.0 t ha^{-1}, thus completely neutralizing aluminum. Compared to the control, DM accumulation of sunn hemp increased by 150%. Meda and Furlani (2005) evaluated crop tolerance to aluminum and classified *Lablab purpureus*, *Mucuna nivea*, *Mucuna terrina*, and *Mucuna deeringiana* as highly tolerant and *Cajanus cajan* as tolerant. *Crotalaria mucronata*, *Crotalaria spectabilis*, and *Crotalaria ochroleuca* were classified as moderately tolerant plants. The *Crotalaria juncea* and *Crotalaria breviflora* were the most sensitive to aluminum toxicity. Thus, sunn hemp is sowed before sugarcane planting, lime should be applied to increase base saturation (60%). This will result in complete neutralization of exchangeable aluminum, adequate supply of Ca and Mg, in addition to higher yields of green manure and future sugarcane plantations (van Raij 2011; Oliveira et al. 2018).

11.7 Soil Fertility, Liming and Gypsum

Soil collection of layers 0–20 and 20–40 cm was done in sugarcane implantation areas. The findings of the 0–20 cm layer study were used to determine fertilization and liming, whereas the results of the 20–40 cm layer analysis were used to calculate gypsum requirements. The majority of South-Central Brazil soils have lower soil pH than 7 acidic, which further affected the availability of Ca, Mg, and K, resulting in Al, Fe, and Mn toxicity. Toxic levels ultimately damaged the cane root development and, hence the whole cane. Therefore, timely application of amendments, viz. lime recommended in sugarcane for harvesting potential benefits as far as growth, yield, and quality parameters are concerned (van Raij 2011; Oliveira et al. 2018).

A variety of minerals have been utilized to adjust soil acidity. Further, calcitic and magnesium limestones, as well as calcium and magnesium silicates (commonly known as mill slag), are employed. Magnesium oxide content in these slags was

around 8%, whereas MgO content in calcitic limestones was <5%, magnesium limestone between 6 and 12%, and dolomitic limestone was >12%. The effectiveness of these items in reducing soil acidity is determined by particle size, consistent application, and moisture availability (Oliveira et al. 2018, 2021). In South-Central Brazil, solutions mostly used for determining $H^+ + Al^{+3}$ in the soil are calcium acetate at 1.00 $cmol_c\ L^{-1}$ (pH 7.0) and SMP buffer solution. The determination of soil $H^+ + Al^{+3}$ with the calcium acetate solution considerably undervalues $H^+ + Al^{+3}$ role. This fallout underestimated the exchange capacity of cations (pH 7.0) and, consequently, the liming dose. However, there is no such underestimation with the SMP buffer solution as the amounts of correctives were previously determined for each type of soil, based on incubation studies with calcium carbonate (Kaminski et al. 2002; van Raij 2011; Oliveira et al. 2018). For these reasons, Oliveira et al. (2018) have suggested raising the limestone quantity by 1.5 to 2.0 times to determine soil $H^+ + Al^{+3}$ if the calcium acetate solution is used. The recommendation for sugarcane is to enhance base saturation (60%). The following equation calculates the amount of limestone quantity (LD) to utilize when utilizing the base saturation method.

$$\mathrm{LC}\left(\mathrm{t\ ha^{-1}}\right) = \frac{[(60 - V) \times E]}{\mathrm{RTNP}} \tag{11.1}$$

where,

LC = limestone dose, *V* = current soil base saturation, *E* = exchange capacity of cation (pH 7) and RTNP = relative power of total neutralization of the corrective.

When the Mg content in the 0–20 cm layer of the soil is less than 0.40 $cmol_c\ dm^{-3}$, dolomitic limestone is indicated. However, if the Mg level in the 20 cm layer is larger than 0.40 $cmol_c\ dm^{-3}$ of soil, the most cost-effective soil corrective per ton of RTNP in the field should be used. As a result, the decision-making process for selecting the limestone type includes economic consideration. The usage of gypsum has been recommended based on chemical examination of the 20–40 cm layer, as previously mentioned. When the calcium concentration of the soil is less than 0.40 $cmol_c\ dm^{-3}$, or the aluminum saturation (m%) is greater than 20% (van Raij 2011; Oliveira et al. 2018).

The recommended gypsum dose is typically one-third of the dose of limestone. However, Bernardo van Raij (2011), one of the leading researchers on the use of gypsum in Brazil, has reported seven studies with sugarcane in which the average recommendation values for limestone and gypsum were 2.7 and 2.4 t ha^{-1}, respectively. However, the maximum sugarcane yields were obtained with average limestone and gypsum concentrations of 5.7 and 6.0 t ha^{-1}, respectively. Limestone and gypsum were combined and applied to the soil. The use of gypsum will result in long-term improvement in the root environment of the layers underneath the topsoil. Therefore, gypsum does not need to be applied annually (van Raij 2011; Oliveira et al. 2018).

Plowing and harrowing are commonly preferred field operations used to mix limestone with gypsum in soil (Oliveira et al. 2021).

11.8 Sowing Times of Sunn Hemp

Sunn hemp plant has a distinct growth pattern affected by the duration of night and blooms early as the night lengthens. Plant development is disrupted, and DM buildup and nutrient cycling, particularly N, are reduced. Considering plant physiology alone, the accumulation of DM of sunn hemp depends on the length of the vegetative period before the start of flowering (Oliveira et al. 2021). The effect of sowing times of sunn hemp on the accumulation of DM and nutrients in canes is also influenced by interactions of air temperature, soil water, nutrient availability, and solar radiation. Oliveira et al. (2019) assessed the effect of sowing times on the flowering of sunn hemp for 2 years in Mercês, state of Minas Gerais.

There was practically no difference between the start of plant flowering in the first three sowing duration. However, there was a shortening of the juvenile period for sowing time of mid-November, with adverse effects on the buildup of DM and N. The average plant height was 3 meters and did not differ statistically between sowing times of early mid-October and early November. In addition to greater DM accumulation, taller plants also provided increased shading and improved weed control. When cane sowing times of mid-November, early, and mid-December was compared to early October, DM (%) accumulation reduced by about 20, 35, and 40% (Oliveira et al. 2021). Sowing sunn hemp should occur in early October for full benefits, while March sown is preferred for seed production programs (Oliveira et al. 2019).

Studies on sunn hemp grown in the Zona da Mata Mineira region have revealed that plants can acquire floral induction stimulation about 40 days after emergence. Plants sown in the second half of November will experience an increase in night length (nearly 40 days) following emergence, resulting in early flowering (Oliveira et al. 2019). According to the findings of Brazilian studies on sowing times, sunn hemp should be sown in South-Central Brazil from early October to mid-November to achieve high shoot biomass production (Lima et al. 2010; Oliveira et al. 2019). Lima et al. (2010) reported that the flowering of 50% of the sunn hemp occurred 116 days after sowing in mid-November. However, when sown on January 2, flowering started at 90 days, thus shortening the vegetative period of 15 days.

Santos and Campelo Júnior (2003) also found that sunn hemp growth and DM accumulation were heavily influenced by photoperiod/nictoperiod. As the nights grew longer, there was a reduction in the number of days for flowering. The period between emergence and flowering ranged from 86 to 38 days for plants sown in November and May, respectively. Equations relating day length and DM accumulation were obtained as $Y = 71.45 - 11.223x + 0.4388x^2$, $R^2 = 0.80$ and the length of the day with number of days for sunn hemp to enter flowering as $Y = 3441.2 - 535.18x + 21.035x^2$, $R^2 = 0.93$. Moreover, according to Santos

and Campelo Júnior (2003), the critical photoperiod/nictoperiod for the flower induction of sunn hemp is 10 h and 30 min.

Sunn hemp is typically sown at a depth of 2–3 cm with a spacing of 0.50 m between furrows, at a density of 55–60 seeds per m^2, using 25 kg of seeds ha^{-1}. According to Oliveira et al. (2021), producers should avoid broadcast seeding with subsequent incorporation using a disc plow or dragging branches over the soil. These practices result in uneven germination and plant emergence, leaving some areas without any seedlings and other excess seedlings. Another alternative for small rural properties recommended by Oliveira et al. (2021) is cutting shallow furrows with animal traction, evenly spreading seeds into these furrows, and manually covering the seeds using small hoes or the farmer's own feet, as in most cases, the land will be plowed and harrowed.

11.9 Seed Inoculation of Sunn Hemp

Sunn hemp seed inoculation with bacteria responsible for fixing N from the atmosphere boosts biological nitrogen fixation and N supply in the soil-plant system. Oliveira et al. (2021) observed that inoculating sunn hemp seeds into the soil-plant system in rural properties and sugar mills on the Zona da Mata Mineira do not improve N supply in the soil-plant system (Table 11.6). In a compilation of studies conducted in south-central and northeastern regions of Brazil, the lack of inoculation effect was also found (Oliveira et al. 2021). The inoculants were no more effective than the instinctive strains, with almost similar DM and nitrogen accumulation than uninoculated treatments.

The high native population of these bacteria in soil could be one of the reasons for the lack of response to inoculation. In legumes, strong nodulation with native strains does not suggest the better performance of these bacteria. Further, due to many of these strains' strong competitive capacity, introducing new strains by seed inoculation looked like a challenging task. As a result, Oliveira et al. (2021) believe that seed inoculation of sunn hemp will not result in greater biological nitrogen-fixing unless better strains are produced.

11.10 Accumulation of Dry Matter and Nutrients in the Shoots of Sunn Hemp

The amount of DM and nutrients accumulated by sunn hemp is dependent on several factors. In general, the ones that most interfere are climatic conditions such as photoperiod/nictoperiod, water availability, solar radiation, day and night temperatures, sowing time (winter, spring, or summer), in addition to cultural practices and soil fertility (Oliveira et al. 2021). Oliveira et al. (2019) showed that DM and N storage in the shoot of sunn hemp were statistically equal for planting times from the beginning of the rainy season to early November, studies conducted in the Zona da Mata Mineira (Table 11.7).

Table 11.6 Average values of plant height (H), dry matter accumulation (DM), nitrogen concentration (N), and accumulation (N Ac.) in sunn hemp upper biomass, *Crotalaria spectabilis*, and *Canavalia ensiformis* inoculated with rhizobium. Studies were conducted in the Zona da Mata Mineira (Properties 1, 2, and 3) and the coastal plains of Alagoas (Mills 1 and 2 and Campus of Engineering and Agricultural Sciences—CEAS)

Edaphoclimatic environment and green manure		H (cm)	DM (t/ha)	N (g/kg)	N Ac. (kg/ha)
Property 1	Inoculated (*Crotalaria juncea*)	338	15.2	20.7	315
	Uninoculated (*Crotalaria juncea*)	325	15.9	20.9	332
Property 2	Inoculated (*Crotalaria juncea*)	351	14.9	22.7	338
	Uninoculated (*Crotalaria juncea*)	368	14.1	23.1	326
Property 3	Inoculated (*Crotalaria juncea*)	348	15.4	20.6	318
	Uninoculated (*Crotalaria juncea*)	337	15.7	21.6	337
Mill 1	Inoculated (*Crotalaria spectabilis*)	63	5.6	26.3	147
	Uninoculated (*Crotalaria spectabilis*)	59	6.1	25.2	154
Mill 2	Inoculated (*Crotalaria spectabilis*)	61	5.8	27.2	158
	Uninoculated (*Crotalaria spectabilis*)	67[a]	6.3[a]	26.8[a]	169[a]
CEAS	Inoculated (*Crotalaria juncea*)	111[a]	5.9[a]	25.9[a]	145[a]
	Uninoculated (*Crotalaria juncea*)	103[a]	6.3[a]	25.2[a]	155[a]
	Inoculated (*Crotalaria spectabilis*)	61[a]	5.7[a]	26.2[a]	152[a]
	Uninoculated (*Crotalaria spectabilis*)	69[a]	6.4[a]	27.5[a]	173[a]
	Inoculated (*Canavalia ensiformis*)	81[a]	7.1[a]	28.3[a]	201[a]
	Uninoculated (*Canavalia ensiformis*)	89[a]	7.6[a]	27.2[a]	207[a]

In the phenological stage of grain filling of pods, the assessments were carried out when DM and N in plant shoots were at their peak. If sunn hemp had been incorporated at full flowering, about 4.0 t DM ha^{-1} would not have been incorporated into the soil. Padovan et al. (2014) reported that the incorporation of sunn hemp at full flowering compared to grain filling resulted in 5.0 t less DM to incorporate into the soil. It is important to emphasize that incorporation at the grain filling stage does not risk infesting the area with the legume, as the seeds are not yet viable (Padovan et al. 2014; Oliveira et al. 2021).

There were an average reduction (%) in DM buildup of about 20, 35, and 40% when canes sown in mid-November, early and mid-December to early October, respectively (Oliveira et al. 2021). Oliveira et al. (2021) demonstrated other studies in which sowing from the second half of November onwards resulted in decreased DM buildup and nutrient cycling. Still, after sowing from early to end of October, N

Table 11.7 Deposition of dry mass (Ac. DM) and nitrogen (Ac. N) in sunn hemp stem biomass, as well as plant height (H) in the grain filling stage for different sowing times in Oxisol red, yellow investigation across two agricultural cultivation seasons

	DM (kg ha^{-1})		Ac. N (kg ha^{-1})		H (cm)	
Sowing times	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Early October	14.135	14.789	273	284	293	305
Mid-October	14.768	14.845	297	275	311	298
Early November	14.235	13.785	268	279	287	293
Mid-November	11.985	11.178	220	226	267	256
Early December	9.123	9.545	198	203	247	236
Mid-December	8.523	8.037	174	168	217	208
Native vegetation	6.750	5.348	73	66	–	–

buildups sunn hemp shoots oscillated approximately 300 kg ha^{-1} (Table 11.7). Padovan et al. (2008) stated that sunn hemp accumulated 16.7 t DM ha^{-1} in shoots after 102 days of emergence in Itaquiraí, state of Mato Grosso do Sul. For N, P, K, Ca, Mg, and S, the accumulation values in shoot biomass were 314, 32, 205, 109, 38, and 25 kg ha^{-1}, respectively. In an area of sugarcane reform in Campos dos Goytacazes, state of Rio de Janeiro, Duarte Júnior and Coelho (2008) found DM accumulation of 17.9 t ha^{-1} in the shoots of sunn hemp, in addition to major plant nutrients, i.e., N, P, K, Ca, Mg, and S of 320, 85, 200, 123, 57, and 69 kg ha^{-1}, respectively (Lima et al. 2010).

Most of the studies found lesser DM deposition and nutrients in the shoot biomass of the spontaneous vegetation in the fallow areas (Oliveira et al. 2021). Padovan et al. (2008) found DM accumulation by spontaneous vegetation was only 4.0 t ha^{-1}, and nutrient contents of 64, 8, 92, 26, 15, and 8 kg ha^{-1} were found for N, P, K, Ca, Mg, and S, respectively. These values are close to other studies conducted in South-Central Brazil (Duarte Júnior and Coelho 2008; Mascarenhas et al. 2008; Oliveira et al. 2021). The ^{15}N isotope experiment demonstrated that roughly 60–87% N buildups in the shoots of sunn hemp derived via symbiotic relationships between the roots and N_2 fixing bacteria from the atmosphere air, resulting in vast totals of N being supplied to the soil solution (Silva et al. 2014; Oliveira et al. 2021).

11.11 Sugarcane Production in Areas Previously Cultivated with Sunn Hemp

The incorporation of sunn hemp biomass and the nutrients contained in some of which are rapidly released, i.e., P (Oliveira et al. 2018), has resulted in a significant increase in sugarcane production in areas previously cultivated with this legume compared to fallow areas (Mascarenhas et al. 2008). The cultivation of sunn hemp prior to sugarcane planting resulted in increase in the production of millable canes fluctuating from 26 to 40 t ha^{-1}. Duarte Júnior and Coelho (2008) reported that the cultivation of sunn hemp prior to sugarcane planting increased yields of

industrializable stalks by 33 t $ha^{-1,}$ and sugar production increased by 3.85 t ha^{-1}. Silva et al. (2014) found that the total number of millable canes of first and second sprouts of areas previously cultivated with sunn hemp was 347 t ha^{-1}, which is 77 t more than in fallow areas. As per the studies of Mascarenhas et al. (2008), Duarte Júnior and Coelho (2008), and Oliveira et al. (2021), there was no influence of the cultivation of sunn hemp previous to cane planting on different cane quality parameters. Thus, the increase in sugar production was exclusively due to the increased production of millable canes.

Table 11.8 shows the results of the use of sunn hemp as green manure in an area subsequently used for sugarcane production for cattle feeding. The accumulation of DM shoot matter in sunn hemp was on average 14.5 t ha^{-1}. However, it was less than 5.0 t ha^{-1} in the spontaneous vegetation of the fallow areas. This study used sugarcane variety RB867515, which has high productive potential and is very responsive to improving soil properties and nutrient supply.

Sunn hemp was used as green manure preparatory to planting year and half sugarcane, resulting in an increase in fodder production in both plant and ratoon canes, from 26 to 38 t ha^{-1}. The increase in the production of industrializable stalks ranged from 20 to 30 t ha^{-1}, with stalks accounting for 80–85% of sugarcane shoot biomass (Oliveira et al. 2018). According to a multi-year study, green manure cost ranged from 6 to 12 tonnes of industrializable stalks per hectare in equivalent pricing. As a result, the output increased more than offset the cost of growing sunn hemp.

11.12 Conclusions

Sugarcane is highly productive potential crop and very responsive to the soil's inherent physical, chemical, and biological properties. For this reason, there is more remediation of nutrients at the harvest period, and actions must be taken to ensure the return of these elements to the soil to maintain or increase soil fertility, aiming at smaller decrease in productivity in the regrowth. Using sunn hemp as a green manure in the reform or implantation of sugarcane plantations associated with the use of wastes from the industrialization of sugarcane or from animal production complemented with chemical fertilization has resulted in greater crop productivity. In addition, there is a more efficient use of inputs, land, and human resources,

Table 11.8 Forage production (natural matter) of sugarcane cultivar RB867515 during plant-cane and first regrowth cycles as a function of the previous crop (fallow or cultivated with sunn hemp) in three properties that use sugarcane in the feeding of dairy cattle

	Forage production(t ha^{-1})					
Cycle	Fallow	Sunn hemp	Fallow	Sunn hemp	Fallow	Sunn hemp
Plant cane	156^{b}	177^{a}	138^{a}	153^{a}	146^{b}	165^{a}
First regrowth	139^{a}	150^{a}	126^{a}	137^{a}	123^{b}	142^{a}
Total	295^{a}	327^{b}	264^{a}	290^{b}	269^{a}	307^{b}

reducing production costs. This study highlights the importance of base saturation close to 60% and adequate P and K availability in the soil for high production of DM and biological fixation of atmospheric N_2 by sunn hemp, which further adds to the overall growth, yields, and quality parameters of the sugarcane and finally to the livelihoods of the cane farmers.

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Silicon-Induced Mitigation of Low-Temperature Stress in Sugarcane

12

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Abstract

Sugarcane is a sensitive crop to low temperatures. Although being grown in tropical and subtropical regions, sugarcane is frequently exposed to cold. Cold and frost detrimentally impact sugarcane yield and sugar production in many countries, including China, India, USA, and others. Widespread way to reduce frost-induced damage to cultivate resistant varieties, but they commonly have less productivity and sugar content. Silicon (Si) fertilization for sugarcane is used in Australia, USA, Brazil, and China to increase biomass and the Brix value. For many plant species, supplementation with Si was found to increase the tolerance to low-temperature stress. In short-term greenhouse test, sugarcane plants exposed to cold were treated by two types of Si-Ca slags and diatomite as Si soil amendments, silicon dioxide as Si fertilizer, and organo-silicon compound and concentrated monosilicic acid as Si biostimulators. All Si treatments provided significant increases in the root and shoot weights both under and without cold stress. As a result of 6-h exposure to cold, the contents of photosynthetic pigments were reduced in Si-untreated plants, whereas Si mitigated the cold-induced pigment decrease. These findings suggest that additional plant Si nutrition reinforces the immune system of different cultivated plants. Among tested Si materials, silicon dioxide was the most efficient.

Keywords

Biostimulator · Photosynthetic pigments · Cold stress · Fertilizer · Silicon-rich soil · Sugarcane

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12.1 Introduction

Sugarcane is a perennial crop that requires a large amount of water and high temperatures for stalk formation. Major sugarcane-growing countries are located in the subtropical and tropical zones (Nickell 2018; Verma et al. 2019, 2021a). Sugarcane is widely used for sugar and ethanol production, and the area of its cultivation is growing (Caldarelli and Gilio 2018; Kumar and Singh 2018; Verma et al. 2020b, 2021b). The expansion of the growing area has led to increasing risk of sugarcane exposure to cold, resulting in a decline in the yield and deterioration in the quality of juice (Wang et al. 2014). Frost and chilling are common in many sugarcane-producing regions, such as Louisiana, Florida, India, Australia, Argentina, and the southeast of Brazil. Cold-induced damages to sugarcane have been reported in approximately 25% of the sugarcane-producing countries (Li et al. 2011; Ramburan 2014).

Chilling stress detrimentally impacts plant growth and development in several ways. Firstly, chilling stress influences cell membrane rigidification. Secondly, chilling reduces the stability of proteins and their complexes and negatively impacts enzyme activities, including reactive oxygen species scavenging enzymes. These processes result in photo-inhibition, impaired photosynthesis, and detriment of membranes. Thirdly, it can affect gene expression and hinder the synthesis of proteins and RNA secondary structures. However, such lower-molecular weight solutes as soluble sugars, proline, and others can enhance the plant's protection against chilling (Rasheed et al. 2011).

Frost induces freezing of the cell juice, rupturing the plant cells of sugarcane, and the cane affected by frost stops growing. One of the main reasons for impaired growth is the injury of the growing point that is often observed at temperatures below −2.0 °C (Sakai and Larcher 2012). Low temperatures also induce leaf burning and injury of the eyes down the cane stalk.

Low temperature-exposed sugarcane demonstrates reduced Brix values in stalks (Edme and Glaz 2013). Sugarcane mills have to harvest sugarcane as quickly as possible to prevent sugar loss. The selection of sugarcane cultivar and harvest time is the main strategy to increase the yield under chilling (Youzong et al. 2002; Ramburan 2014). Gravois (2020) from the LSUAg Center has suggested the following gradation of sugarcane variety tolerance to frost (Table 12.1).

Many studies focus on evaluating sugarcane quality parameters (Brix, pol, and sucrose content) as indicators of frost tolerance (Edme and Glaz 2013). Most of the

Table 12.1 Sugarcane variety post-freeze deterioration

Low	Medium	High
HoCP 96-540	HoCP 00-950	L 99-226
L 01-283	L 01-299	L 03-371
HoCP 04-838	Ho 05-961	Ho 07-613
	HoCP 09-804	L 12-201
	L 11-183	Ho 12-615
	Ho 13-739	

investigations do not aim to search biochemical ways to improve sugarcane tolerance to low temperatures. As there seems to be no preventive actions against frost or chilling on a large scale, the development of effective methods for increasing sugarcane productivity in cold conditions remains relevant.

12.2 Influence of Silicon on Growth and Biomass Characteristics

Silicon (Si) is one of the most widely distributed elements in the Earth's crust. Soil is the most Si-enriched layer of the Earth's crust from 20 to 35% of Si in clay soils and 45 to 49% in sandy soils (Kovda 1973). Si is predominantly present in the soil as silica and diverse aluminosilicates (Sokolova 1985). Traditionally, these minerals are considered inert. As a result, many soil scientists, plant physiologists, and agronomists ignore this element as essential for soil fertility and plant nutrition. However, the stability of Si is reflected in the classification of soil elements on their mobility, where Si is shown as an inert element (Perelman 1989). In the same classification, Si is also listed as a mobile element. All-natural waters, including soil solution, contain soluble Si substances. These are the products of mineral weathering or dissolving. They include monosilicic acid (MA), polysilicic acid (PA), and organo-Si compounds that possess chemical and biochemical activities (Matichenkov 1990; Matichenkov et al. 2000; Matichenkov and Bocharnikova 2001). Thus, the soil Si includes two major groups—inert and biogeochemically active compounds.

Orthosilicic acid (H_4SiO_4) and its anions are the most widely distributed variety of MA (Dietzel 2002; Iler 1979). Metasilicic acid (H_2SiO_3) seldom occurs in nature (Babushkin et al. 1972; Mondal et al. 2009). As a weak inorganic acid with a slight buffering capacity at pH ~ 7.0, MA is chemically active (Iler 1979; Lindsay 1979). Monosilicic acid reacts with aluminum, iron, and manganese to form sparingly soluble silicates (Lumsdon and Farmer 1995). Depending on the concentration, MA can interact with heavy metals (Cd, Hg, Pb, Zn, and others), forming soluble complex compounds if its concentration is slight (Schindler et al. 1976) and unsoluble silicates of heavy metals when the MA concentration is elevated (Lindsay 1979). The anion of MA can replace the phosphate-anion in phosphates of calcium, magnesium, aluminum, and iron (Matichenkov and Ammosova 1997).

Natural solutions also contain oligomers of silicic acid that have two and more (up to 100) atoms of Si (Knight and Kinrade 2001). Although their chemical properties are different, these substances are commonly tested together with MA (Matichenkov 2008). The knowledge about this form of soluble Si is inferior. Polysilicic acids with high content of Si atoms (more than 100) are an integral component of natural solutions as well. Unlike MA, PA is chemically inert, acts as an adsorbent, and forms colloidal particles (Yazynin 1994). The chemical inertness of PA results from the molecule's ability "to twist," thus neutralizing a negative charge formed by the dissociation of hydroxyl groups (Iler 1979). Polysilicic acid can create Si bridges between soil particles (Yazynin 1994). Due to permanently

altering moisture content, these bridges are subjected to dehydration with the formation of silica.

On our planet, the biological cycle of Si is the most intensive in terrestrial ecosystems, where plants take up from 0.02 to 7.0 t $ha^{-1}$of Si every year (Bocharnikova and Matichenkov 2012). Silicon is the fourth most abundant element in the plant after oxygen, carbon, and hydrogen (Kovda 1985; Perelman 1989; Bazilevich 1993). Silicon is recognized as a "beneficial" element; however, most cultivated plants absorb Si more than other macronutrients (nitrogen, phosphorus, or potassium).

Starting in 1840, pot and large-scale investigations have shown benefits of Si fertilization for the productivity of *Oryza sativa* L. (15–100%), *Zea mays* L. (15–35%), *Triticum aestivum* L. (10–30%), *Hordeum vulgare* L. (10–30%), *Saccharum officinarum* L. (15–40%), *Cucumis sativus* L. (10–40%), *Fragaria* spp. (10–30%), *Citrus* spp. (5–15%), *Lycopersicon esculentum* L. (10–40%), *Stenotaphrum secundatum*, *Cynodon dactylon*, *Lolium multiflorum*, *Paspalum notatum* (10–25%), *Musa paradisiaca* (20–40%), and other crops (Guntzer et al. 2012; Snyder et al. 2016; Patil et al. 2017; Artyszak 2018).

Today Si-rich agrochemicals are successfully used in USA, Japan, China, India, Australia, Russia, and other countries. During the last 15–20 years, the volume of Si fertilizers and Si-rich soil amendments increased by 15–20% annually. However, despite economic and environmental benefits, Si fertilizers are still rare in the world agricultural practice. The main reason is low information about this element and its role in the soil-plant system. Three main groups of Si-rich materials are currently applied in agriculture: Si-rich soil amendments, Si fertilizers, and Si-based biostimulators.

Soil amendments or soil conditioners do not supply nutrients to the soil but improve the texture (Hamdi et al. 2019; Verma et al. 2020a, 2021b). Si-rich soil amendments primarily impact such soil properties as adsorption capacity, cation exchange capacity, pH, structure and are typically applied at rates more than 500 kg ha^{-1}. Due to the high application rate, these substances improve plant Si nutrition despite the relatively small content of plant-available Si. There are natural Si-rich soil amendments like zeolites, diatomaceous earth, and tuffs. However, the most frequently used Si-rich soil amendments are industrial by-products like calcium silicate slag and ashes (Chaiyaraksa and Tumtong 2019; Matichenkov et al. 2020; Verma et al. 2021c). It should be noted that the use of industrial Si-rich by-products as soil amendments may create a risk of environmental contamination with heavy metals (Ning et al. 2016; Xiaobin et al. 2021).

Fertilizers are natural or artificial substances added to soil to provide nutrients necessary for plant growth and productivity. The main purpose of Si fertilizers is to provide Si nutrition to plants. Silicon fertilizer application rates range between 50 and 500 kg ha^{-1}. Amorphous silicon dioxide (microsilica, fumed silica), silicon gel, and sodium or potassium silicate can be recognized as fertilizer (Ma and Takahashi 2002; Rao et al. 2017).

Plant biostimulators are various non-toxic substances of mainly natural origin that improve and stimulate the vital processes of plants in a differentiated way from

fertilizers or phytohormones. Their effect on plants is not a consequence of their direct ability to regulate metabolism, and their action can be multidirectional. The crucial point is that biostimulants, unlike bio-regulators and hormones, improve the metabolic processes of plants without changing their natural path (Posmyk and Szafrańska 2016).

Four main groups of biostimulants are generally distinguished: organic acids, microorganisms, extracts, and inorganic substances (https://info.agricen.com/growing-for-future-ag-biologicals-booklet). Examples of biostimulants are humic and fulvic acids, amino acids, fatty acids, peptides, chitosan, polyphenols, mycorrhiza, bacteria, polyamides, inorganic salts, and others. The main distinguishing feature of a biostimulant is high efficiency at a low application rate, from a few grams to tens of Kg ha^{-1}, providing yield increases by 5–50%, and sometimes higher.

Many modern studies have reported the ability of some Si-rich substances (organo-silicon compounds, MA, Si-N-compounds, nano-sized Si-rich materials) to induce active defense mechanisms under stressful growth conditions when applied at a low rate (Azad et al. 2021; Hidalgo-Santiago et al. 2021; Shalaby et al. 2021). Due to the low application rate, these substances cannot provide plants Si nutrition but can be classified as biostimulants (Gugała et al. 2019; Constantinescu-Aruxandei et al. 2020; Artyszak et al. 2021; Grankina 2021).

The results of numerous studies have demonstrated that Si-rich soil amendments, fertilizers, and biostimulators positively influence plant growth and protection against biotic and abiotic stresses (Ma and Takahashi 2002; Vivancos et al. 2015; Verma et al. 2020a). Several mechanisms underlying Si-induced plant defense have been suggested as (1) mechanical protection through Si accumulation in epidermal tissue and formation of Si-rich layer that protects leaves against fungi and insect attacks (Alhousari and Greger 2018), (2) physiological protection due to increasing plant viability through optimization of root development and improvement of photosynthesis (Zhang et al. 2018; Frazão et al. 2020), (3) chemical protection via interaction between monosilicic acid and toxic compounds in plant tissue (Ji et al. 2016; Stevic et al. 2016), (4) impact on the transport of elements (Imtiaz et al. 2016), and (5) activation of the stress and reduction of oxidative damage (Balakhnina et al. 2015). These mechanisms are indirectly supported by high concentrations of mono- and polysilicic acids in the plant sap (Matichenkov et al. 2008; Wei et al. 2021). Sugarcane, as a Si-accumulator, favorably responds to Si fertilization (Matichenkov and Calvert 2002; Keeping and Reynolds 2009; Sousa and Korndörfer 2010).

Silicon fertilizers and Si-rich soil amendments promoted tolerance of many plant species to low temperatures (Matichenkov et al. 2001; Zhang et al. 2011). Although no experimental data are available for sugarcane, Si is assumed to benefit from its tolerance to low temperature and frost (Datnoff 2005).

The majority of the EAA soils are organic soils classified as Histosols (suborder: saprist). Histosols were formed under anaerobiotic conditions and are underlain by the Pleistocene-age Fort Thompson formation consisting of alternating beds of limestone, shell, sand, and marl, which are often perforated by solution holes (Snyder and Davidson 1994; Daroub et al. 2011). These organic soils are derived

from hydrophytic vegetative residues and usually contain > 85% of organic matter by weight (Cox et al. 1988; Snyder 1994).

12.3 Si-Rich Soil Amendments

(A) **Phosphorus slag (P-Slag)**—by-product from phosphorus industry, Calcium Silicate Corp., TN; contained Si—18.5–18.6%; Ca—28.0–28.3%; Fe—6.20–6.84 g kg^{-1}; Al—10.5–10.6%; Mg—3.44–3.84 g kg^{-1}; P—4.02–4.15 g kg^{-1}; K—10–14 mg kg^{-1}; Cd, Cr, Ni, Pb, and Hg were not detectable.

(B) **Metallurgical slag (M-Slag)**—by-product from steel production, PRO-CHEM Chemical Company, FL; contained Si—13.5–13.7%; Ca—28.5–28.7%; Fe—2.1–3.0 g kg^{-1}; Al—2.13–2.85 g kg^{-1}; P—0.42–0.5 g kg^{-1}; K—30–-33 mg kg^{-1}; Mg—6.3–6.5%; Cd, Cr, Hg, Ni and Pb were not detectable.

(C) **Diatomite (DE)**—North Queensland, Australia; dense gray-yellow granules containing: SiO_2—88.2–88.6%; CaO—2.0–2.3%; Fe_2O_3—1.4–1.8%; MgO—1.2–1.5%; Na_2O—1.2–1.4%; pH 6.1, particle size <40 μm; average surface area 47 m^2 g^{-1}, porosity 65%.

12.4 Application of Si

Chemically pure SiO_2-Sigma-Aldrich, CAS 14808-60-7; 0.5–10 μm particle sizes, white color, pH 7.0, the average surface area of particles, including pores, was 175 m^2 g^{-1}.

12.4.1 Si-Based Biostimulators

1. Solid Si biostimulator Mival-agro (Mival)—1-(chloromethyl) silatran (LSD Agrosil, Russia).
2. Liquid Si biostimulator Ecosil—stabilized monosilicic acid with 15% Si and 15% Na (Beijing Plum Agrochemical Trading Co, Ltd., China).

The tested Si-rich soil amendments and Si fertilizers were evaluated for their capability to release active forms of Si by the method elaborated (Bocharnikova et al. 2011) (Table 12.2). This method allows the determination of actual Si and potential Si. Actual Si characterizes the amount of Si that passes into the soil solution quickly for several days. Potential Si reflects the ability of Si material to replenish the plant-available soil Si over several months after application.

The actual Si was analyzed as follows: six (6) g of Si material was placed into each flask in 6 replications. Thirty (30) mL of bidistilled water was added. After a 1-h shaking, half of the samples were incubated for 23 h, and the other half was incubated for 4 days. After incubation, samples were centrifuged, followed by the

Table 12.2 Silicon status of the tested Si materials (mg kg^{-1})

Material	Water-extractable Si		Acid-extractable Si	Active Si*
	First day	Fourth day		
P-Slag	22.1	38.2	2105	2708
M-Slag	25.8	40.4	2005	2667
DE	40.2	125.6	895.6	2553.6
SiO_2	215.7	356.3	453.6	6173.6
LSD_{05}	2.5	3.5	15.5	–

solution analysis for Si. Considering that 1 day might not be enough for achieving the equilibrium between solid and soluble forms of Si, a 4-day extraction was also used. 200 mg of material was placed into a flask to analyze potentially plant-available Si. Twenty (20) mL of 0.1 M HCl was added to each flask. After 1-h shaking and subsequent 23-h incubation, the sample was centrifuged, and the cleaned extract was analyzed for Si.

The active Si was calculated by the following equation:

$$^{*}\text{Active Si} = 10^{*}(\text{Actual Si 1 day} + \text{Actual Si 4 days}) + \text{Potential Si}$$

The concentration of Si in all solutions was determined by Mullen and Riley (1955). Soluble P does not interfere with Si determination because the P-molybdenum complex disintegrates by a strong acid (Mullen and Riley 1955).

12.4.2 The Modified Molybdenum Blue Method

Two solutions were prepared prior to the analysis.

Solution A—10 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}$ $4H_2O$) was dissolved in 470 mL of DW, and then 30 mL concentrated HCl (30%) was added and agitated. The solution should be stored in a plastic bottle.

Solution B—20 g of oxalic acid was mixed with 500 mL of DW, and six (6) g of $FeSO_4 \bullet 7H_2O$ was added and then agitated. Concurrently, 250 mL of 18 M H_2SO_4 was carefully blended with 250 mL of DW. After cooling, both solutions were mixed and agitated. The final solution was placed in a plastic bottle.

12.4.3 Procedure

A sample or Si standard solution containing 2–40 μg Si as MA was placed in a 50 mL volumetric flask. If the pH of tested solution is more than 4.0, several drops of concentrated HCl can be added. Then 10 mL of solution A was added. Ten minutes, 10 mL of solution B was added, and the final volume was brought to 50 mL with DW

and agitated. After standing for 4–5 h, the absorbance of the solution was measured at 660 nm. A blank sample containing all reagents, except the Si solution, was made.

For preparing a standard curve, a serial dilution of the standard Si solution was performed to obtain the concentrations of 0–100 ppm Si. These solutions were used to determine the correlation coefficient between absorbance level and concentration of Si in one-mL aliquot. Silicon concentration was calculated using the formula:

$$\text{g Si kg}^{-1}\text{ dry sample} = \text{Ad} \times \text{Ck} \times \text{Vdx } 1000/(\text{Va} \times \text{Ws})$$

where Ad—absorbance of the sample, Ablk—absorbance of the blank solution, Vd—volume of extractant, Va—volume of tested aliquot, and Ws—weight of dry samples.

Silicon soil amendments and fertilizer were applied to the soil at 0.5 and 1 t ha^{-1} for amendments and 100 and 200 kg ha^{-1} for fertilizer before sugarcane planting. Two and one weeks until chilling and right after, both Si biostimulators were foliar applied at the following rates: 1 and 2 kg ha^{-1}. Twelve hours before application, Mival and Ecosil were diluted with water at 1:100 and 1:500, respectively.

The biomass of roots and shoots was measured 1 week after low-temperature stress. The following method analyzed fresh leaves of sugarcane for pigments (Chl a, b, and carotenoids) (Lichtenthaler and Wellburn 1985). Fresh plant tissue (100 ± 2 mg) was cut with scissors and carefully ground in a mortar with a small amount of $CaCO_3$, quartz sand (on the tip of the spatula), and 80% acetone (20 mL). Then the solution was centrifuged at 15,000 rpm for 5 min. The optical density was measured at λ = 663, 646, and 470 nm. The 80% acetone solution was used as a control.

The pigment concentration was measured according to the formulas (Lichtenthaler and Wellburn 1985):

$$C_{Chl\ a}\ [\text{ppm}] = \mathbf{12.21} \cdot \boldsymbol{D_{663}} - \mathbf{2.81} \cdot \boldsymbol{D_{646}}$$

$$C_{Chl\ b}\ [\text{ppm}] = \mathbf{20.13} \cdot \boldsymbol{D_{646}} - \mathbf{5.03} \cdot \mathbf{D_{663}}$$

$$\boldsymbol{C_{car}}\ [\text{ppm}] = (\mathbf{1000} \cdot \boldsymbol{D_{470}} - \mathbf{3.27} \cdot \boldsymbol{C_{Chl\ a}} - \mathbf{100} \cdot C_{Chl\ b})/\mathbf{229},$$

where

$\boldsymbol{D_{470}}$, $\boldsymbol{D_{646}}$, and $\boldsymbol{D_{663}}$—optical density at 470, 646, and 663 nm, correspondingly;
$\boldsymbol{C}$—concentration of pigment in extract [ppm].

The following formula calculated the final concentration:

$$\boldsymbol{F}\ [\text{mg/g dry mass}] = (\boldsymbol{M}^*(\mathbf{100} - \boldsymbol{W}\%)/\mathbf{100})^*\ (\boldsymbol{V} \cdot \boldsymbol{C})/\boldsymbol{P},$$

where

$\boldsymbol{F}$—the pigment content in plant tissue, mg g^{-1} dry mass
M—mg of fresh weight

Table 12.3 Fresh weight of roots and shoots of sugarcane (g plant^{-1})

Treatment	Control		Treated	
	Roots	Shoots	Roots	Shoots
Control	25.8	35.6	25.5	28.4
P-Slag 1 t ha^{-1}	30.5	43.7	31.3	43.3
P-Slag 500 kg ha^{-1}	29.5	40.3	29.4	38.2
M-Slag 1 t ha^{-1}	31.8	44.3	31.7	42.3
M-Slag 500 kg ha^{-1}	27.8	39.4	28.5	39.2
DE 1 t ha^{-1}	33.6	44.8	34.6	43.2
DE 500 kg ha^{-1}	29.5	40.3	30.2	40.1
SiO_2 200 kg ha^{-1}	39.7	49.5	40.3	48.3
SiO_2 100 kg ha^{-1}	34.7	45.3	35	45.6
Mival 2 kg ha^{-1}	30.7	40.3	30.9	39.4
Mival 1 kg ha^{-1}	28.7	40.1	28.8	38.4
Ecosil 2 kg ha^{-1}	31.5	42.3	31.4	42.6
Ecosil 1 kg ha^{-1}	29.5	40.3	29.6	41.3
LSD_{05}	1.3	0.8	0.5	0.8

W—water content in plant tissue (%)
V—volume of extractant (L)
C—pigment concentration (mg L^{-1})
P—dry weight of plant tissue (g)

Soil samples were analyzed for water- and acid-extractable Si by the following methods. To analyze water-soluble Si: (1) 6.0 ± 0.1 g of fresh soil was placed into a 100-mL plastic vessel and (2) 30-mL of water was added to each vessel; 3) after 1-h shaking, a sample was filtered, and a clean extract was analyzed for Si by described above method. The acid extraction procedure was as follows: (1) two (2.0 ± 0.1) g of an air-dried soil sample was placed in a 100 mL polyethylene cup, (2) 20 mL of HCl (0.1 M) was added, followed by half-hour agitation at 200 rpm, (3) after standing overnight, the mixture was agitated again for a half-hour, then the supernatant was centrifuged at 3000 g during 15 min. Silicon was analyzed in the cleaned extract described above method (Duncan 1957).

The weight of roots and shoots of sugarcane are shown in Table 12.3. The application of all Si materials significantly increased the biomass of roots and shoots, by up to 53 and 39%, respectively, at 200 kg ha^{-1} of SiO_2. Among Si soil amendments, DE was more efficient, increasing the root and shoot weights by 30 and 26%, respectively. Regarding the effect on plant growth, test substances ranged as follows: SiO_2 > DE > M-Slag > Ecosil > Mival > P-Slag for roots and SiO_2 > DE > M-Slag > P-Slag > Ecosil > Mival for shoots. It is important that both Si biostimulators promote root and shoot growth. Short cold stress adversely impacted the shoot weight, reducing by 21%, but had no significant effect on the roots. The Si substances prevented reducing the shoot biomass. The efficiency of both Si biostimulators was more pronounced under stress than non-stress conditions, being similar to that of Si soil amendments or fertilizers.

Table 12.4 Water- and acid-extractable Si in the soil after growing sugarcane (mg kg^{-1})

Treatment	Control	Treated		
	Water-extractable	Acid-extractable	Water-extractable	Acid-extractable
Control	6.7	124	6.8	127
P-Slag 1 t ha^{-1}	21.5	422	21.6	423
P-Slag 500 kg ha^{-1}	12.5	275	12.5	279
M-Slag 1 t ha^{-1}	22.3	456	22.4	465
M-Slag 500 kg ha^{-1}	12.6	286	12.8	284
DE 1 t ha^{-1}	24.6	459	24.7	455
DE 500 kg ha^{-1}	14.7	298	14.8	300
SiO_2 200 kg ha^{-1}	29.5	224	30.1	225
SiO_2 100 kg ha^{-1}	24.7	218	24.6	216
Mival 2 kg ha^{-1}	6.6	129	6.6	127
Mival 1 kg ha^{-1}	6.8	127	6.5	126
Ecosil 2 kg ha^{-1}	6.7	126	6.6	125
Ecosil 1 kg ha^{-1}	6.8	128	6.7	127
LSD_{05}	0.4	10	0.4	11

Silicon soil amendments or fertilizers increased the contents of water- and acid-extractable Si in the soil (Table 12.4). Despite high application rates of all soil amendments (1000 and 500 kg ha^{-1}) compared with SiO_2 (100–200 kg ha^{-1}), SiO_2 provided the more considerable increase in plant-available Si due to its high solubility (Peng et al. 2017). Mival or Ecosil had no significant effect on the soil water- and acid-extractable Si because both biostimulators were applied at a very low rate compared to other Si materials. Cold stress did not influence the soil plant-available Si.

Silicon substances increased the content of all tested pigments (Table 12.5). In general, the increases were by 3–28% for chlorophyll a, by 16–32% for chlorophyll b, and by 3–16% for carotenoids. SiO_2 at a higher rate provided the maximum effects, while P-Slag was the least efficient.

Exposure to cold significantly reduced all pigments by 19, 11, and 18% for chlorophyll a, chlorophyll b, and carotenoids, respectively. All types of Si substances prevented the reduction in pigments, with a higher effect of SiO_2. On average, Si substances increased the content of pigments in stressed plants by 21–49% for chlorophyll a, by 30–46% for chlorophyll b, and by 22–38% for carotenoids. Pigments play a crucial role in the photosynthesis, growth, and development of plants and serve as an essential indicator of plant health (Babenko et al. 2014). Photosynthetic pigments are one of several physiological indicators that correlate with stress tolerance. Silicon agrochemicals contributed to the pigment synthesis and stability, thus improving the plant growth under stress.

Table 12.5 The content of pigments in sugarcane leaves (mg g^{-1})

	Control			Treated		
Treatment	Chl a	Chl b	Carotenoids	Chl a	Chl b	Carotenoids
Control	0.893	0.344	0.384	0.734	0.304	0.312
P-Slag 1 t ha^{-1}	0.943	0.412	0.422	0.903	0.405	0.402
P-Slag 500 kg ha^{-1}	0.922	0.4	0.403	0.887	0.398	0.398
M-Slag 1 t ha^{-1}	0.976	0.432	0.426	0.944	0.412	0.412
M-Slag 500 kg ha^{-1}	0.943	0.412	0.405	0.932	0.402	0.394
DE 1 t ha^{-1}	1.045	0.443	0.439	0.976	0.435	0.422
DE 500 kg ha^{-1}	0.945	0.422	0.403	0.932	0.428	0.403
SiO_2 200 kg ha^{-1}	1.144	0.455	0.445	1.095	0.443	0.432
SiO_2 100 kg ha^{-1}	1.043	0.432	0.423	1.023	0.428	0.421
Mival 2 kg ha^{-1}	0.933	0.402	0.397	0.921	0.398	0.387
Mival 1 kg ha^{-1}	0.932	0.398	0.387	0.921	0.394	0.382
Ecosil 2 kg ha^{-1}	0.987	0.432	0.412	0.932	0.422	0.403
Ecosil 1 kg ha^{-1}	0.976	0.421	0.403	0.922	0.421	0.394
LSD_{05}	0.057	0.032	0.025	0.050	0.031	0.022

12.5 Conclusion

The chapter discussed that all tested types of Si agrochemicals (soil amendments, fertilizer, or biostimulators) benefit the root and shoot biomass of sugarcane and the photosynthetic pigment activity under low-temperature conditions. Silicon-mediated acceleration of pigment activity evidences the participation in the metabolic processes of sugarcane. Silicon fertilizer (amorphous SiO_2) is the best efficient among the other available forms.

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Conflict of Interest The authors declare that they have no competing interests.

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Agro-technologies to Sustain Sugarcane Productivity Under Abiotic Stresses

13

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Abstract

Sugarcane (*Saccharum* spp. hybrid) is a major crop that provides bioenergy, fibre, biofertilizer, and the myriad of by-products/co-products with ecological sustainability. Sugar industries are prominent in India, and they play an essential role in rural socioeconomic development by mobilizing rural resources and producing higher income and employment possibilities. The sugar industry is a seasonal business entirely reliant on the monsoon for optimal sugarcane production. Sugarcane farming has been confronted with multifarious demand, product diversification, and sustainability limitations in the recent past. To meet the escalating demands of sugar, holistic remedial measures in sugarcane farming need to be deployed to address production constraints and, particularly, sustained sugarcane productivity at the farm level. Droughts, shirking soil and water resources, salinity, alkalinity, waterlogging, high temperature, cold, frost, widespread iron and zinc deficiencies, etc., affect cane production significantly in many countries. These issues must be addressed through agronomic interventions and proper management to make sugarcane agriculture sustainable and profitable.

Keywords

Agro-approaches · Environmental variables · Growth · Productivity · Sugarcane · Stress tolerance efficiency

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13.1 Introduction

Sugarcane is a long duration and management responsive crop; therefore, it is highly recommended to balance the congenial soil climate for the proper development of sugarcane crop (Garcia et al. 2020; Misra et al. 2020; Verma et al. 2021a). In drought-prone areas, use of stress-tolerant sugarcane varieties such as Co 0112, Co 09004, Co 10015, Co 10024, Co 10026, and Co 10033 with agronomic interventions. The early planting, soaking setts in lime water, modified trench planting methods, trash mulching, nutrient management, protective irrigation, and anti-transpirants mitigate the negative effects of unfavourable environmental variables and enhance sugarcane productivity (Endres et al. 2018; Misra et al. 2020). High salt contents in the root zone cause loss and delay in the ratoon sprouting, resulting in gaps, lower NMC, and productivity. Sugarcane crop stand is typically low in saline soil with slick or barren patches. Threshold levels are defined as an EC of 4 dS/m and an ESP of 15. For good ratoon, it's important to raise good plant crops. Thus, it has become indispensable to reclaim salt-affected soils. Excess soluble salts are leached from saline soils during the reclamation process. Massive amounts of organic manure, as well as mechanical treatments like deep ploughing, subsoiling, sanding, and profile inversion, can improve leaching and drainage (Yang et al. 2021). One drainage channel must be provided every six to ten rows to remove excess salt and water in the ratoon field. It is necessary to prevent salt accumulation and preferably grow salinity resistant/tolerant varieties such as Co 93005, Co 89010, Co 94008, Co 9401, Co 97008, Co 99004, Co 85019, Co 85019, Co 2001-13, and CoM 0265. Rotation and resistance crops, i.e. cotton, mustard, etc., can enhance soil properties and sustainability. Biological amelioration involving the use of living or dead organisms, organic matter, vegetation, and waste products also helps in improving soil organic matter and soil health. Dead or living organisms, organic manures, green manuring, green cane blanketing, etc., will enhance soil characteristics and internal drainage (Misra et al. 2020; Yang et al. 2021). The modified trench planting system monitored 15% higher cane productivity in contaminated saline soil and water irrigated regions. Irrigation management based on plant requirements, obtained using the temperature of canopy sensors, may also help mitigate the harmful effects of extreme temperature and water-deficit conditions (Misra et al. 2020).

13.2 Effect of Environmental Stresses on Sugarcane Growth, Yield, and Quality

Sugarcane is widely grown as a cash crop in both hemispheres in over 120 countries. Sugarcane is the world's major source of sugar (80%). It plays an important role in the economy, supplying raw materials to the sugar industries as well as over 25 other major industries. Sugarcane is cultivated worldwide between the latitudes of 36.7° N and 31.0° S of the equator, from arid to subarid locations (Srivastava and Rai 2012; Verma et al. 2019b, 2020a, b, 2021b). Sugarcane is often considered a tropical crop

that requires excess ambient air temperature, sufficient solar light, and sufficient water. Due to its versatility, it can be grown in a wide range of agroclimatic conditions. All of the cultivated cultivars/genotypes are grown in hot climates.

The ideal climate for sugarcane is a hot cultivating season, moderately dry, sunny, and frost, but frost-free ripening and crushing season and devoid of storms and high winds. For high yields, a long growing season is required. The growth period should be hot, with average daytime temperatures of about 30 °C with sufficient soil moisture and solar radiation. The ripening and harvest period must be moderate, with average ambient air temperatures around 10–20 °C, but no frost, dry weather, and sufficient sunlight. Low temperatures (12–14 °C) during the ripening stage limit the cane's vegetative growth rate and sucrose enrichment (Fageria et al. 2010).

Sugarcane faces severe demand, product diversification, and sustainability challenges. Sustained improvement in crop productivity needs to be ensured if the growing demand for sugar and sweeteners is met in the coming years. The cost of cultivation has gone up considerably in recent years due to the escalation in the cost of inputs and labours, rendering sugarcane cultivation less profitable. The development of varieties and technologies suited for mechanization has become imperative now because of this. By 2025, worldwide water scarcity is highly like to become a severe issue, particularly in areas with high human density (Cosgrove and Rijsberman 2000). Periodical droughts have resulted in wide fluctuations in cane area and production, adversely affecting the cane industry (Verma et al. 2020e). The natural resources, including water, are dwindling, and soils productivity has also deteriorated. Poor soil physical conditions, especially soil compaction, bulk density and porosity, and other significant physical parameters affect root growth and cane production. Bakker (1999) indicated that the sugarcane root system's development and distribution influenced the genotypes, soil porosity, moisture content, and soil compaction. Soil compaction disrupted the soil properties by breaking continuous open pores.

Environmental stresses, i.e. salinity, alkalinity, drought, flooding, excess ambient air temperature, cold, frost, and widespread iron and zinc deficiencies, affect cane production significantly in many states. Many regions of the world, including the Mediterranean basin and extended areas in low latitudes, may face severe water shortages due to climate change (Palutikof 1993; Verma et al. 2021c). Crops have an intrinsic defence system that allows them to resist certain climatic conditions. The resilience and flexibility to abiotic stressors can vary between species and cultivars. Crops in their early stages have no apparent signs, but their morphology and physiology can change dramatically (Cramer et al. 2011; Verma et al. 2020a, 2021d). The morphological, biochemical, and physiological changes that occur due to high-temperature stress have a significant impact on plant growth and development (Wahid et al. 2007).

Similarly, water stress affects many yield-determining physiological processes in plants, and yield is a complex system that integrates many of these physiological processes (Verma et al. 2020c, d). As a result, it is difficult to understand how plants absorb, integrate, and exhibit the ever-changing and indeterminate physiological action of mechanisms that occur during crops' life cycle (Farooq et al. 2009).

13.2.1 Drought

13.2.1.1 Physiological Response of Sugarcane to Drought

Among various yield-limiting stresses, drought has been a major constraint. Sugarcane is drought resistant, but it produces less sugar when stressed by water (FAO 2004; Verma et al. 2019b, 2020a, 2021a, d). The plants initial response to lack of water is the slowdown in growth, water potential, and photosynthetic efficiency. The density of stomata in crop plants varies dramatically. The number of stomata in the lower epidermis is roughly double that of the upper epidermis (Inman-Bamber et al. 2008; Wilkinson and Davies 2010; Verma et al. 2019a).

Sugarcane has 115 stomata/mm^2 on the adaxial surface and 253 stomata/mm^2 on the abaxial surface. Despite the twofold variation in stomatal density, the upper and lower surfaces have the same transpiration rate (Verma et al. 2019a). External forces, i.e. PAR, ambient air temperature, and relative humidity, significantly impact stomatal activities. Stomata open when exposed to direct sunshine but close to weak or diffuse light. It explains the sugarcane benefits from the early morning sunlight (Verma et al. 2020e). Plant water potential (Ψ) is an acceptable measure of plant water balance (Karamanos 2003). With leaf maturity, growth, stress duration, and severity, the leaf water potential at which stomata close fluctuates. Leaf photosynthetic responses downregulated by 70% when the leaf water potential reduces from −4 to −18 bars. Dehydration is a typical occurrence in various sugarcane-cultivating locations. Thus, it's necessary to consider lowering transpiration and thus lowering consumptive water usage. The leaves account for most transpiration (>90%), while the nodal region, devoid of wax deposition, accounts for modest transpiration rates. The passive curling of leaves, which limits the amount of radiation received by leaves, reduces water loss, and increases WUE, resulted in a significant reduction in water loss (10–20%) (Meyer 1997).

13.2.1.2 Biochemical Crop Response to Drought

Free proline accumulates in water-stressed leaf tissues. Oxidation of proline (to glutamate) in turgid tissues generally prevents accumulation, while in stressed tissue, proline accumulates only to serve as buffer of nitrogenous substances. The progressive accumulation has been accompanied by a fall on leaf water potential. In several studies, proline accumulation was used as a screening test for drought resistance. Proline accumulation promotes membrane integrity by reducing lipid peroxidation, preserving cell redox potential, and lowering ROS levels (Shinde et al. 2016; Verma et al. 2019b, 2020c, 2021d). Betaine, another metabolically inert compound, also accumulates under stress. Abscisic acid (ABA) accumulates in drought-affected leaves. ABA content enhances the leaf water potential by 1–2 bars and thus helps in dehydration postponement. The ABA was also found to possess a direct and stabilizing effect on protoplasm and drought-induced leaves' senescence. Dry matter production by ABA-treated plants was greater than that of control. This was due to a greater shoot development at the expense of roots.

Abscisic acid (ABA) improves plant water-deficit adaptation by activating various signaling pathways (Bücker-Neto et al. 2017). Hyperosmotic stress

exacerbated by water stress, altering overall metabolic activities even plant death (Zhu 2001; Karuppanapandian et al. 2011; Liu et al. 2011). Changes in relative water content (RWC) and membrane stability, osmotic regulators, soluble protein, cell membrane permeability, and other processes are associated with the adaptations that maintain cellular homeostasis (Verma et al. 2021b). Compatible osmolytes are effective osmoprotectants that reduce the consequences of osmotic tension. Recently, interest has been generated on osmotic adjustment, turgor maintenance, and growth. Turgor can be maintained by increasing various osmolytes. An increase in solute concentration or accumulation of solutes causes osmotic adjustment.

During stress, the compounds accumulated are soluble sugars, soluble carbohydrates, proline, potassium, sugar alcohols, and organic acids. The formation of nonhazardous compatible solutes is the prevalent nature of plants during abiotic stresses (Abbasi et al. 2014; dos-Santos and de-Almeida Silva 2015). The osmotic adjustment has a few advantages: maintenance of cell turgor, continued cell elongation, maintenance of stomatal opening and photosynthesis, and survival under dehydration. Enzymes such as nitrate reductase, sucrose phosphate synthase, invertase, etc., are regulated by the tissue water status. Nitrate reductase activity is reversible, and the extent of loss under stress is to the extent of 30%.

13.2.1.3 Drought and Its Impact on Sugarcane Growth, Yield, and Quality

Limited water supply inhibit growth, minerals uptake, photosynthetic capacity, assimilate portioning, growth loss, and high tiller mortality. Sugarcane bud germination does not emerge in airy dried soil (Smit 2011). Soil–water relationships generally affect the rooting depth, distribution, and activity. In sufficient soil moisture, greater root mass occurs with less than 50 cm depth; however, during stress, roots penetrate vertically downwards in the form of a rope. Stress has a significant effect on leaf growth and development (Verma et al. 2020a, c, 2021a, c, d, e). At leaf water potential of −2 bars, leaf expansion begins to slow and eventually stops at potential of −7 to −9 bars. Sugarcane can produce 65 mt of above-ground dry mass per year, about 65% of the cane stalks. When the seasonally available water is used during grand growth, the maximum cane elongation (60–70%) occurs (Venkataramana 2008).

Drought caused a significant reduction in stalk number, length, productivity, and sucrose output (Verma et al. 2020d). The crucial water consumption time was identified as the formative growth stage (60–150 days). In a typical drought year, stress at this early stage of growth directly impacts productivity, juice flavour, and harvest losses of up to 50% have been reported. Limited water irrigated at the formative phase decreased the output and juice parameters, while the stress at the maturity period had a beneficial effect. According to the depth-interval yield technique, Dhanapal et al. (2019) advised irrigation scheduling in plant and ratoon crops at 7- to 15-day intervals throughout the crop's germination, grand developmental, and maturity stages, respectively. Full irrigation at recommended intervals with 100% crop evapotranspiration (ET) replacement produced significantly higher cane yield than deficit irrigation at recommended intervals with 50% crop ET

replacement and skipping alternate irrigations with 50% crop ET replacement, according to the results of experimental trials conducted at the ICAR-Sugarcane Breeding Institute in Coimbatore, India (Tayade et al. 2020).

13.2.2 Salinity

13.2.2.1 Response of Sugarcane to Salinity

It is estimated that one mha of sugarcane land is damaged by salt worldwide (Hunsigi 1993). Salinity stress on sugarcane is caused by salinization and poor irrigation quality of water, as well as water deficit during crucial water demand phases. Chlorides and sulphates of sodium, calcium, magnesium, and potassium largely contribute to salinity (Ham et al. 2000). Salts in soil decrease the osmotic potential of soil water, thereby decreasing its availability to plants. The poor physical characteristics, i.e. low infiltration rate, crusting, and hardening of surface soils upon drying, decreased soil porosity, permeability, soil aeration, water conductance, and water logging for a more extended period, affect the root growth (Rana et al. 2016).

Sugarcane is susceptible to salinity. It is expected to exhibit no growth reduction in soil with salt up to 1.1 dS m^{-1} and 10% reduction in growth at 2.2 dS m^{-1} (Evans 2006). Sugarcane farming is unprofitable in locations where soil salinity is more significant than 4.0 dS m^{-1} (Rozeff 1995). Sugarcane crop's general response to salinity includes poor and delayed germination, reduced tillering, leaf yellowing and burning, stunted growth, poor field stand, extended growth, and reduced yields. The salt interferes with sugar production by affecting growth rate and cane yield and decreasing the sucrose content in the stalk. Due to high salt, vegetative growth is hampered, and the plant can absorb less water, resulting in stunted growth and reduced production. Crops may suffer from the leaf tip and marginal leaf burn, bleaching, and defoliation due to high salt levels (Srivastava and Rai 2012). Also, salinity increases the fibre content of the cane and the juice's electrical conductivity, which affects the jaggery preparation and quality.

13.2.3 Heat Stress and Other Climatic Factors

13.2.3.1 Effect of Heat Stress and Climatic Variables on Sugarcane Productivity and Quality

Ambient air temperature is an important factor in crop productivity, and temperature is also a major environmental attribute influencing crop yields. Sugarcane shoot emergence, leaf morphology, and stalk lengthening are affected by temperature (Inman-Bamber 1994). Germination (0–60 days), developmental (60–150 days), grand development (150–240 days), and maturation (240–360 days) are the four physiological growth phases of sugarcane crop. Each phase required the availability of a precise combination of light, temperature, and water. During germination (300 mm), developmental (600 mm), grand development (1000), and maturation (600 mm), water is required annually. While maximum temperature is necessary for

proper development, metabolism, and final production, high temperature induces significant variations in cellular structural and metabolic functions. The lowest temperature for active sugarcane growth is around 20 °C, but varietal and cultural factors influence it slightly. Crops generally yield the most optimal temperatures, and about 30 °C is the best temperature for proper growth and development.

Temperature plays a crucial role in the germination process. The first sprouting and germination of buds need an ambient air temperature of 26–33 °C and soil temperature of 23–28 °C. The formative phase is characterized by tillering and canopy establishment. The ideal temperature for tillering is between 26 and 33 °C, while higher day temperatures between 32 and 37 °C restrict tillering. Temperatures above 38 °C reduce photosynthetic rate while photorespiration increases with temperature (Hasanuzzaman et al. 2013). When heat stress reaches a certain level of intensity and length, cells are irreparably destroyed, and various living species react differently to higher temperatures. In addition to speeding up phenological events, high temperatures have harmful effects on photosynthesis, respiration, and reproduction, including survival. Thermal adaptation is dependent on genotypes, duration of stress, and growths stage.

The study conducted at ICAR-Sugarcane Breeding Institute demonstrated that excess heat reduced photosynthetic pigments, SPAD values, the chlorophyll fluorescence yield (Fv/Fm), photosynthetic responses, leaf relative water content, and nitrate reductase (NR) and sucrose-metabolizing enzyme activities in a variety of cultivars (Kohila and Gomathi 2018). According to Kaushal et al. (2016), the adverse temperature may have a major impact on leaf gas exchange, respiration, water uptake, and the stability of membranes. The soil temperature is more important than the air temperature, and for optimum growth, the soil temperature should be around 26–27 °C. Cane growth and photosynthetic responses are often limited when soil temperature falls below 21 °C and stops completely below 12 °C (Singels and Inman-Bamber 2011). Sprouting of sugarcane setts is optimum between 20 and 32 °C, and germination is suppressed below 10 °C and above 40 °C.

The cultivating and ripening season are influenced by the duration of the season, with temperatures significantly below (20 °C). During the ripening period, dry and cool weather is required, and mean day ambient air temperature in the range of 10–20 °C is optimal. Climatic conditions are the more efficient approach of cane ripening, as they combat adverse effects such as excess moisture or nitrogen.

13.2.4 Light Stress

Fluctuation in light intensity, quality, and duration interfere with biochemical, physiological, and plant development; however, light intensity and time cannot be changed. High-light stress occurs when a crop is exposed to irradiance levels that are significantly over the photosynthetic light saturation point. Under this situation, the crop may protect chlorophyll molecules by maximizing the biosynthesis and the concentration of carotenoids. These antioxidant compounds guard the plants by avoiding photo-oxidation of chlorophyll from excessive light intensity. High

Table 13.1 The effect of climatic variables on yield and quality of sugarcane

Climatic element		Effect on cane growth
Air	CO_2 concentration	Changes photosynthetic responses
	Ozone and pollutants	Plant damage, growth reduction, possibilities to loss in productivity and juice properties
Light	Day length	Influences in flowering
	Intensity	Controlled leaf gas exchange
Rainfall	–	Causes waterlogging or water deficit; Determines planting and harvesting activities; decides irrigation requirement
Humidity	–	Desired at the vegetative stage; restricts ripening and sugar accumulation; effects evapotranspiration process; encourages fungal diseases
Temperature	Seasonal and daily fluctuations	Changes photosynthetic performance and accumulation of photosynthates
	Low temperature	Cold damage; less germination; decreased tillering process
	High temperature	Heat damage and limited water irrigation
Wind, cyclones, etc.	–	Lodging and uprooting of cane; yield and quality loss

insolation (>1200 h/year) is essential for satisfactory sugarcane growth and yield. Sugarcane can continue to increase the rate of photosynthesis in the field until it reaches full natural light intensity; therefore, the higher radiation, the higher yields. In the development of tillers, light also plays an important role.

The flowering of sugarcane is photo-periodically regulated, and temperature, altitude, water, and nitrogen supply influence flowering. The susceptibility of cane cultivars and clones to light interruption varies greatly (Table 13.1). Flowering was stopped when a 50-ft candle of light was put to H 37-1933 for 1 min during the inductive night. In contrast, 4000 ftca-min at midnight was not inhibitory to *Saccharum spontaneum* var. Mandalay (Julian 1969).

13.2.5 Frost

Sugarcane is considered a cold-sensitive plant that grows in dry and semidry regions where frost is not common. The limits of cane cultivation, by and large, are 30 °N and 30 °S; at higher latitudes, the growing season is unduly restricted by the length of the cold season. For example, the crop is often damaged by frost in several countries, Argentina, Egypt, Iran, Pakistan, Zimbabwe, South Africa, and the continental USA (Florida and Louisiana). It is agreed that the temperature of −1 to −2 °C will kill the leaves and even the meristems, the juice will not freeze, and its quality will remain good for several months, provided ambient temperatures remain low. Low temperature during planting time impedes germination frequency; on the other hand, high temperatures are also undesirable. Sugarcane growth and ripening

processes are inextricably linked to air temperature. When the ambient air temperature was reduced from 23.0 to 13.6 °C, found 84% decline in the rate of sugarcane photosynthesis (Burr et al. 1957). According to Waldron et al. (1967), photosynthetic efficiency reduced linearly when air temperature decreased from 34 to 5 °C. If the temperature falls further (to −7 °C or −8 °C), the juice is freezed and destroys the cells, and even at such low temperatures, sucrose is hydrolysed into glucose and fructose.

13.2.6 Rainfall

An adequate supply of water is required for proper cane development. Ripening (the storing of sucrose in the stems) and harvesting require a dry season or the withholding of water in irrigated areas. Concerning moisture, sugarcane is more adaptive than other varieties of plants, and optimum harvests are achieved when vegetative growth continues without a check under optimum soil moisture conditions. Simultaneously, the crop demonstrates exceptional drought tolerance, particularly in soils that allow for deep-rooted and good moisture retention. Rainfall is essential during the growth stage to ensure larger yields of high-quality cane. The accumulation of sucrose and maturation follows the major growth phase. The sunny day with a temperature of 29–37 °C is beneficial for increasing sucrose storage, lowering nitrogen, and improving juice quality. Rainfall during the maturity phase causes a restoration of growth, making sucrose production and accumulation more difficult. The ripening process is aided by a limited water supply, somewhat low relative humidity, 7–9 h of sunlight per day, and a temperature of 10–14 °C (Table 13.1).

13.2.7 Impact of Climatic Change on Sugarcane Crop Growth

Climate is the compound of weather patterns in a specific region, as measured by long-term statistics for meteorological factors in that area. Climate change, which has resulted in global warming, has become a major source of concern for the survival of life on Earth in recent decades (Abrol et al. 1996; IPCC 2007). According to the Intergovernmental Panel on Climate Change (IPCC) report, the global mean temperature will rise 0.3 °C per decade, reaching approximately 1 and 3 °C over current levels by 2025 and the end of the twenty-first century, respectively, resulting in global warming. CO_2 levels in the atmosphere have risen dramatically from 280 to 370 ppm and are expected to double by 2100 (IPCC 2007). CO_2 levels will double between 2025 and 2070, depending on greenhouse gas emissions (UNFCCC 2012).

Ecosystem services, water availability, agricultural output, food security, and the composition of fauna and flora will be affected by global warming and climate change. Many cane-growing areas are in cyclone or hurricane belts. The mechanical damage caused can be severe. Temperature, rainfall, humidity, and atmospheric gases all interact with plants differently and through different methods. Higher air

humidity and air temperature vastly increase the rate of deterioration of cut cane; efficient logistics can only counter this. Marin et al. (2013) used crop simulation models to show that climate change enhanced sugarcane water usage efficiency and yield in some locations of Brazil. They projected that cane yield in 2050 could be higher (15–59%) than that at the current average level. They increased (CO_2) levels in a controlled situation, increased sugarcane leaf gas exchange, water use efficiency (WUE), biomass, and production (de Souza et al. 2008; Vu and Allen Jr. 2009).

High humidity encourages numerous fungal diseases of the leaf, sheath, and root; the only practical control is the selection of resistant varieties. Rain and flooding assist in spreading fungal, bacterial, and viral disorders. The most striking example of disease infection is the transatlantic movement from Africa to the Caribbean of smut. At maturation, the relative air humidity is required to be below 70%. Damage by lightning has been observed from South Africa, Mauritius, Jamaica, and other areas, but it was not regarded as serious in each case.

13.2.8 The Effect of Climate on Ripening

Sugar synthesis and fast sugar storage occur during the ripening period, while vegetative development is inhibited. Rainfall, humidity, the amount of sunshine, night length, altitude, and temperature influence ripening. High temperatures and rainfall in tropical locations, combined with significant cloud cover and a slight variance in night lengths, promote rapid vegetative growth and prevent ripening. Cool and long nights just before and during harvest enhance the deposition of sucrose in the stems in temperate regions. In arid areas, irrigation is usually discontinued about 62 days before harvest takes place to encourage ripening.

13.2.9 Waterlogging

The requirement of water in sugarcane crop is very high, but more irrigation or persistently heavy rains without proper drainage can lead to waterlogging. Waterlogging or flooding is one of the abiotic stressors that inhibits crop yield. Physical soil deterioration due to waterlogging has been estimated at 11.60 mha in India, with sugarcane agriculture accounting for 10–30% of the land, a key constraint influencing productivity (Gomathi et al. 2015). The primary effect of waterlogging in crop plants is oxygen deprivation or anoxia, and submerged plant parts cannot breathe or photosynthesize.

Furthermore, Rahman et al. (1986) observed that flooding for 1 month decreased stalk elongation rates by 40–88%, and variations were due to genotype. Genotypic variation may be attributed to the presence of root aerenchyma; therefore, root aerenchyma is a key requisite for sustained root activity in waterlogged soil. The roots of 40 sugarcane cultivars assessed contained aerenchyma (Ray et al. 1996; Van Der Heyden et al. 1998). Significant morphological, anatomical, physiological, and biochemical changes are also documented in plants due to waterlogging for

adaptation and survival. Stomatal closure, which can impair carbon uptake, has been observed in other species as a reaction to flooding (Kozlowski 1997). Sugarcane with insufficient water was stomatal closure (Saliendra and Meinzer 1991; Du et al. 1996).

In addition, Du et al. (1998) discovered that stomatal closure in water-stressed sugarcane inhibited photosynthesis. Sugarcane transpiration rate was similar in flood and drainage treatments (Webster and Eavis 1972) until the flood period reached 21 days, after which flooding resulted in a lower transpiration rate. In another study, Chabot et al. (2002) found no variations in sugarcane transpiration rate related to water-table depths of 5, 20, and 45 cm. The crop yield reduction due to floods is believed to be 15–25%, but it can reach 40% depending on the stage of the crop and the length of the flooding (Glaz et al. 2004; Gomathi and Chandran 2009).

13.2.10 Soil Constraint and Its Impact on Sugarcane Growth and Yield

Although sugarcane thrives on well-drained loamy soil with a neutral soil reaction, it is grown in a wide range of soil conditions. Low soil organic carbon level, low available nutrients, unfavourable soil reaction (pH), electrical conductivity (EC), exchangeable sodium percentage (ESP), and poor physical situations, i.e. hard pans, insufficient irrigation, surface crusting and hardening, submergence all have an impact on cane production. To boost their productivity, these soils require reclamation and particular management approaches.

13.2.11 Nutrient Stress

Because nutrient stresses are linked to decreased tiller production and increased tiller mortality, nutrient deficiency directly impacts sugarcane growth, development, and yield. Optimum nutrient supplies have increased the number of millable stalks, a significant contributor to the economic yield. Moreover, the balance of nutrients enhances sugarcane growth through protection from many biotic and abiotic stresses. Iron chlorosis is a common nutrient deficit that occurs in calcareous soils. It intensifies more in ensuing ratoon crops. Chlorosis has been reported in nearly all of India's sugarcane-growing states, primarily in Madhya Pradesh, Maharashtra, Tamil Nadu, and Bihar (Sinha 2016).

13.3 Abiotic Stress Management in Sugarcane

13.3.1 Soil Reclamation and Special Management Practices

In the sodic or saline region, maintaining suitable soil physico-chemical characteristics can be accomplished by using uncontaminated water, the proper selection and/or mix of soil ameliorants, adequate drainage, and appropriate cultural practices (Grattan and Oster 2003). In saline soils, the reclamation process involves the leaching of excess soluble salts. Drainage channels with a depth of 75 cm are constructed all around the land. The physical capabilities of sodic soils should be improved by adding a substantial amount of organic matter, as well as chemical amendments to replace sodium with calcium in the exchange complex and remove carbonate and bicarbonate with sulphate. As additions, gypsum, phosphogypsum, pressmud, sulphur, and pyrites are generally recommended. The most effective and cost-efficient amendment is gypsum. Pressmud, a by-product of the sugar industry, can be used to reclaim sodic soils for benefit. It includes a significant amount of nitrogen (1.20%), phosphorus (3.83%), potassium (1.46%), and calcium (11.10%) and enhances soil fertility. To restore alkali soils, 12.5–20.0 tonnes of pressmud per hectare could be effective. *Pleurotus* and *Trichoderma*, as well as urea (5 kg/t) and cow dung (50 kg/t), can be used to enrich pressmud.

13.3.2 Subsoiling

In sugarcane farming, soil physical properties are deteriorated due to subsoil compaction, which reduces root growth and distribution, thus affecting uptake of water and nutrients. Hence, there is need for soil health management not only for topsoil but also for subsoil to break the stagnant yield barriers of sugarcane. The experiment conducted at Punjab Agricultural University, Regional Station, Faridkot, India, clearly indicated the positive effect of subsoiling over the conventional method of land preparation. Cross subsoiling at 1.0 m × 1.0 m spacing has given significantly higher yield than no subsoiling. This can be attributed to subsoil disturbance in closer spacing, resulting in lower bulk density and higher infiltration rate, ultimately producing more increased root proliferation (Singh et al. 2012). Thus, subsoiling is recommended for higher productivity and soil health improvement in sugarcane. Subsoiling is recommended for improving cane yield and maintaining soil health, particularly cross subsoiling at 1.0 m (Sinha 2016).

13.3.3 Drainage

Nevertheless, sugarcane wants maximum water for irrigation. It is similarly susceptible to flooding, which diminishes overall plant performance and productivity. The yield loss due to waterlogging depends on the duration, i.e. stagnant or moving water, the stage of the crop, drainage facilities, and management practices.

Sugarcane is fairly tolerant to waterlogging for short periods. Therefore, suitable irrigation and drainage facilities are important in sugarcane fields to sustain maximum soil moisture (%) during the course of the growing period and to realize close to higher productivity. The first step is to prevent or eliminate waterlogging by providing adequate drainage facilities wherever possible. The simplest method offers open drains deeper than irrigation channels to draw out the excess water. Subsurface drains at adequate depths below the soil surface, especially in canal irrigated areas, will help to remove the excess water and salt accumulation from the root zone.

13.3.4 Bio-intensive Modulation of Ratoon Rhizosphere

Sugarcane, a long-duration crop, requires repeated tillage, irrigation scheduling, intercultural operations, and mechanical harvesting, which is expected to cause the formation of plough pans to deteriorate soil properties. Sugarcane cultivation enhanced compaction, resulting in pore size distribution, increased water content, and decreased air capacity. Thus, soil pore space for root development and water availability for the plants fell (de Lima et al. 2016; Tormena et al. 2017). Bio-intensive modulation of ratoon rhizosphere technology developed at ICAR–SBI, Coimbatore, could address the soil physical health constraints in sugarcane farming and recorded higher NMC (87.25×10^3), cane yield (100.95 t ha^{-1}), and sugar yield (13.19 t ha^{-1}) over conventional sugarcane cultivation (86.76 and 11.56 t ha^{-1}cane yield and sugar yield, respectively). Bio-intensive modulation of ratoon rhizosphere with off-barring + trash shredding and soil incorporation +100% RDF and microbial consortia amendment helps in the cutting of old and decayed roots during off-barring. The use of shredded trash with microbial consortia has decreased the soil bulk density (1.26 g cc^{-1}) and soil penetration resistance (1.81, 1.69, and 1.75 MPa at the centre and both side of the sugarcane stool, respectively), increased the organic carbon (0.49%), available nutrients, facilitated higher cane growth eventually, and significantly improved 16.35 and 14.10% cane yield.

Higher NMC, taller and thicker cane was attributed to various benefits in terms of N-fixation, P solubilization, plant growth hormones received from microbial consortia amendment. The ISTM (In Situ Trash Management) + Green manuring + 100% RDF application resulted in enhanced OC level of soil from initial soil OC of 0.35–0.52% for 3 years duration, which is attributed to the incorporation of green manure, sunn hemp, and sugarcane trash which might have enhanced the faster decomposition of trash resulting in the build-up of organic carbon. Sunn hemp green manuring and in situ waste management, used in the previous plant crop, had a residual influence on soil EC and pH, with lower values (0.32 ds/m and 8.31) than those used during the last plant crop the other main plot treatments. Thus, trash retention substantially affects the SOM and soil pH and improves soil physical and chemical qualities (Tayade et al. 2020).

13.3.4.1 Early Planting, Using Higher Seed Rate

Early planting would help to decrease the effects of high moisture because, by the time waterlogging occurs, the crop would have put forth sufficient growth to tolerate the excess moisture. To compensate for germination and provide adequate plant stand under soil salinity stress, a higher seed rate of 25% is suggested (Sundara and Vasantha 2004).

13.3.4.2 Crop Rotation, Intercropping, and Green Manuring

Crop rotation using adaptable crops such as cotton, mustard, and other crops promotes soil health and sustainability. The inclusion of green manuring intercrop and in situ incorporation of green manure benefits soil fertility and helps improve productivity in salt-affected soils. Green manuring helps in building up soil health by mineralizing green manuring material, chelation of Ca in alkaline soil and Al in acid soils, and production of organic acids during decomposition of green manuring materials. In sugarcane farming, *Sesbania aculeata* and sugarcane trash mulching enhanced the availability of N and P elements for sustainable soil productivity. The maximum availability of native and amended phosphorous under 'in situ' green mulch was attributed to the reduction in pH value. The in situ green mulch (4 Mg ha^{-1}) and sugarcane trash mulching (6 Mg ha^{-1}) had enhanced the N (11.9%) and P (16.1%) as relative to unmulched for 2 years. Compared to unmulched plots, 'in situ' green mulch and sugarcane trash mulch enhanced natural phosphorus availability by 19.3 and 4.8%, respectively, and added phosphorous by 23.6 and 11.5% (Dahiya and Malik 2002).

13.3.4.3 Earthing Up

High earthing up assists in better root growth and provides proper plant support. By delivering high earthing up, the root zone within the earthed-up soil becomes free of water quickly when floodwater recedes, helping in the recovery of the crop.

13.3.4.4 Planting Methods

Sugarcane responds differentially to different planting methods due to varying soil moisture storage and depletion patterns. In sugarcane, ridges and furrow method, trench method, paired row method, ring or pit method, and wide row planting system are in vogue. Among these in north India, the ridges and furrows method is the most common whereas, in the southern region, a wide row system of planting followed. Planting practices should conserve soil moisture under abiotic challenges to promote sugarcane establishment and crop growth. Sugarcane is one of the most efficient solar energy converters into sugar due to its C4 plant. It can produce nearly half a tonne of dry matter each day during its peak growth period (Yadav 1991).

13.3.4.5 Paired Row Method

An increase in cane yield by 30–40% and saving in 40–45% of irrigation water was reported by Sivanappan (2002) under paired row method with drip irrigation.

13.3.4.6 Pit Method

The data on drip irrigation with fertigation collected from farmers' fields have revealed that the water saving was about 45–50%, and the crop yield varied from 60 to 75 t/acre, indicating that the yield increased is about 15–20 t/acre or 30% more, Sivanappan (2002).

13.3.4.7 Subsurface Drip with Twin Rows Method

As the laterals and emitters are located below the soil surface, this system is called subsurface drip system. Subsurface Drip Irrigation (SDI) system is most recently practiced in sugarcane farming. It recompenses over surface drip irrigation in many ways, i.e. decreased evaporation losses, efficient water use, more water application uniformity, increased growth, productivity, and crop quality. In this method, drip laterals are placed about 15–20 cm below the surface, and the spacing of the lateral line is 150–165 cm. The subsurface drip system with fertigation system is the "triple wonder" technology comprising irrigation, fertigation, and preventing evaporation of water (Sivanappan 2002). Under-settling transplanting technology with drip and irrigation reported the maximum cane yield of 146.56 t/ha in sugarcane with a black gram intercropping system (Vennila et al. 2019). As against the 1500–2500 mm of sugarcane water requirement, only 725 mm of water (excluding the effective rainfall of 494.3 mm) was applied through drip on an alternate day based on pan evaporation.

13.3.4.8 Deep Trench System of Planting

A deep trench planting system can be adopted for early water stress and late flooded conditions. The deep trench system would be useful in deltaic conditions, where early water stress and late flooding are common. In deep trench planting, the roots may easily penetrate in lower soil horizons, and thus under drought conditions, roots absorb more water from the deep soil strata. Under such conditions, the deep trench planting system yielded 19 and 53 t/ha in plants and first ratoon crop, respectively.

13.3.4.9 Modified Trench System of Planting with the Application of Gypsum

Rising sugarcane in 'Modified' trench farming system in saline soils and salty water irrigated regions with the application of gypsum at 2 t/ha and 25% extra N and 'pocket manuring' helps to improve sugarcane and sugar productivity. This technology can increase the productivity of sugarcane by about 15% in areas with saline soil/water problems. In the modified trench, while doing earthing up, furrows are not converted into ridges; instead, a trough is maintained along the row. The irrigation water is let in the cane row itself (Sundara and Vasantha 2004).

13.4 Use of Tolerant/Resistant Varieties and Setts Treatments for the Management of Abiotic Stresses

13.4.1 Sugarcane Varieties Tolerant to Drought

Sugarcane is a durable crop that can tolerate moderate amounts of stress through morphological adaptations and physiological/biochemical modifications. The inward curling of the upper canopy, which is visible in many tolerant cultivars, reflects the irradiance load, allowing for less direct sunlight to be absorbed. The wax layer on the leaf surface helps limit water loss from the leaf and nodal areas of the cane. Growing the varieties of thick cuticle and waxy surfaces can help to reflect solar radiation and prevent heat stress (Bonnett et al. 2006). Furthermore, it was discovered that drought tolerance is linked to less transpiring leaves with a low density of sunken stomata and wide vascular bundles in the roots and stem. Drought-tolerant or drought-resistant cultivars can help to alleviate the water stress caused by high temperatures and low rainfall. Sugarcane cultivar differences in drought tolerance have been documented by Inman-Bamber et al. (2012). According to regression study, the most important parameters for yield build-up under stress are the number of millable canes, cane height, juice extraction (%), and sucrose (%) cane (Gorai et al. 2010).

According to Silva et al. (2011), Cia et al. (2012), and dos-Santos and de-Almeida Silva (2015), susceptible sugarcane cultivars subjected to water stress had a more significant RWC reduction. Water stressors damage the cell membrane in a variety of ways, including damaging its cellular integrity. Membrane stability implies an essential quality of the plant under water stress conditions since it enables plants to adapt to their stress environment (Blum et al. 1981). Rooting depth, distribution, and activity are all affected by soil–water interactions. The sett roots sprout from the root band (located at the nodal region of sugarcane sett) and begin growing within 24 h of planting according to extensive root investigations. As a result, plant breeders have been selecting from enormous populations of different genotypes to obtain or construct desired features in modern varieties. Vasantha et al. (2005) tested 15 sugarcane genotypes for stress resistance and found that drought treatments resulted in significant reductions in leaf area expansion, number of leaves, LAI, and tiller development. The number of millable canes in the drought treatment (67,770/ha) was much lower than in control (82,200/ha). Co 95003, Co 95005, and Co 95006 had higher cane production and sugar yield than the other genotypes tested, demonstrating their drought resistance potential.

13.4.2 Genetic Engineering for Water Stress Resistance

In recent years, numerous genes and gene products activated when plants exposed to diverse abiotic stressors have been revealed. Genes encoding enzymes from several osmolytes' biosynthesis pathways, including proline, glycine betaine, sorbitol, and pinitol, have been cloned and used to improve abiotic stress resistance. Heat shock

proteins (HSPs), late embryogenesis (LEA), responsive to abscisic acid (RAB) protein, and dehydration responsive element (DRE) proteins are examples of potential candidate genes. Currently, osmotin, choline oxidase, and annexin are used in gene transfer and transgenic evolution for water stress resistance capacity.

13.4.3 Setts Soaking in Lime Water

Soaking the setts in a saturated lime solution for 1 h before sowing is very helpful for stress hardening observation. In an experiment, Kathiresan (2000) reported significant increase in the germination percentage, tillers, millable cane number, cane and sugar yields due to setts soaking of 'CoC 671' and 'Co 6304' with lime water. Similarly, Oo et al. (2019) also observed higher germination percentage and higher cane yield in sugarcane with lime water setts treatment (7.5 g/L).

13.4.4 Sugarcane Varieties Tolerant to Salinity

The growth and yield of sugarcane raised in saline soils are very low. However, cane cultivars showed various levels of resistance to salinity. Salinity-resistance sugarcane clones absorbed less Na+ and more K+ than sensitive counterpart clones, resulting in a greater K+: Na + ratio (Wahid and Ghazanfar 2006). Furthermore, when compared to the sensitive counterpart clone, the levels of flavonoids, which appear to be important antioxidants in the environmental stress tolerance process, were higher in tolerant clones, confirming that these substances can also protect sugarcane from ion-induced oxidative stress during salinity stress (Patade et al. 2009). Priming treatments are widely known for improving numerous elements of plant growth under adverse situations (Atreya et al. 2009).

13.4.5 In Situ Trash Mulching in Plant Crops

Trash mulching is an effective way to conserve soil moisture and alleviate moisture stress in sugarcane. Mulching conserves soil moisture by lowering evaporation from the soil surface and helps to adjust soil temperature, improve germination, control weed growth, and improve tiller survival. In an experiment conducted at ICAR-SBI, Coimbatore, India, detrashing was done 5, 7, and 10 months after planting and used for in situ trash mulching in a plant sugarcane crop. The microbial consortium was also applied for the faster decomposition of sugarcane trash, in situ trash mulching combined with the application of microbial consortia resulted in numerically higher single cane weight, height, and girth, as well as a significantly more significant number of millable canes and yield (Tayade et al. 2016).

13.4.6 Green Cane Trash Blanketing in Mechanically Harvested Sugarcane

By and large, good crop of sugarcane produced about 10–15 t/ha of trash. It contains on an average 0.42% N, 0.15% P, and 0.57% K, in addition to other secondary and micronutrients; moreover, it is a potential source of organic matter (46.5%) in sugarcane farming. Thus, to improve the sugarcane production base and harness higher yield per drop of water, the greater thrust needs to be given on conservation measures through using on-farm resources. Green cane trash blanketing (dry leaves, tops, and pieces of stalks retained on soil after mechanized sugarcane harvest) is abundantly available in mechanically harvested fields. It also provides multiple physical, chemical, and biological benefits to the soil and sustains crop yields. However, high C:N ratio (73.1:1), immobilization of soil nutrients up to 100 DAR, high fibre content, lack of proper composting techniques, and prolonged decomposition of sugarcane trash in the field are the main constraint in its recycling. The result of trials revealed that in machine-harvested plant and first ratoon crop 16.29 and 20.11 t/ha of sugarcane trash with an appreciable amount of nutrients, i.e. N (0.5%), P (0.12%), and K (0.73%) was available for recycling for subsequent first and second ratoon crop, respectively. The practice of green cane trash blanketing coupled with the manipulation of upper soil layer by off-barring after machine-harvested first ratoon crop could reduce the soil compaction (2.21 MPa) in surface soil, i.e. 0–15 cm, thereby improving cane weight, cane height, and overall sugarcane growth (Tayade et al. 2017).

13.4.7 Irrigation Management

Many approaches are utilized in agricultural production to conserve water and boost water usage efficiency to combat water constraints. Maximum cane production could be obtained only when the crop is not experiencing prolonged moisture stress. Irrigation schedules must be planned to balance adequate soil moisture in the root zones (Dhanapal et al. 2019). The water requirement of the sugarcane crop increases throughout the summer months due to high evapotranspiration demand and to ensure water-deficit periods. To grow and yield normally, any crop must be provided with optimal soil moisture conditions throughout its growing season. It has been calculated that one tonne of cane requires between 200 and 250 tonnes of water.

The water requirements vary greatly depending on the agricultural yield level and the meteorological circumstances in different parts of the country, ranging from 1200 to 3000 mm. However, depending on the temperature, soil condition, crop length, and application method, the actual water demand differs from location to place. The irrigation effectiveness of surface irrigation is just 30–50%, resulting in significant water waste. In this situation, micro-irrigation techniques become relevant for conserving water and maximizing its use.

13.4.8 Micro-irrigation

Late micro-irrigation, i.e. drip, micro-sprinkler, and subsurface drip, enhanced water productivity considerably. The experiments indicated that water saving from drip varies from 12 to 84% (Narayanmoorthy 2004). In sugarcane, drip irrigation was most economical in effective water usage, and it has a potential role in mitigating the stress caused by high and low temperatures. Kumawat et al. (2016) found that drip irrigation had a 56% higher WUE (5.96 t/ha/cm) than surface irrigation (3.32 t/ha/cm), lower water losses, and higher yields. With the low intensity of weeds and saving in irrigation water, the additional area can be bought under cultivation. There is a tremendous potential to increase the area under micro-irrigation systems in sugarcane crop. Subsurface drip lines have the benefits of decreased soil evaporation, less weeds, and the ability to drive and till throughout the field at any time, regardless of the irrigation pattern. When irrigating salinized soils or irrigating with salty water, drip irrigation permits salts to be continuously drained away from the root system, avoiding salt accumulating in the immediate proximity of the roots. Because the water is delivered directly to the ground using drip irrigation, wastewater can be used, thus reducing health risks.

13.4.9 Fertigation

Because of its long duration and large biomass-producing crop, sugarcane takes a significant amount of plant nutrients. A 100 t/ha cane yield crop utilized 205 kg N, 55 kg P, 275 kg K, and 30 kg S on average. Balanced fertilization at the right time and in the right amount is critical for reducing abiotic stressors and increasing sugarcane productivity. Recent input application technology does not provide the right proportion of nutrients at different growth stages. The major share of fertilizers applied is wasted without fulfilling the plant nutrient requirements. It is generally recognized that only about 50–60% of the complete nutrient enters into the plant systems out of the total fertilizer application. The rest is wasted either by leaching or volatilization. Supply of essential plant nutrients, especially water-soluble fertilizer through micro-irrigation as and when required by the plants directly to the crop's root zone is called fertigation. Fertigation increases the efficiency of fertilizers and therefore can enhance plant growth, escalate the number of effective tillers, encourage cane height and cane girth, and in the long run, increase the millable cane yield. The remaining 70% of N and K was divided equally and fertigated at 90–180 DAT weekly. The fertigation scheduling in sugarcane was found to be far more efficient in fertilizer use than conventional soil application at ICAR-SBI, Coimbatore, India.

13.4.10 Cane Agronomy for Water Scarcity Area

Availability of soil moisture markedly influenced the sugarcane juice quality parameter such as Brix % (total soluble solids in juice) and Pol % (Sucrose content in

juice). Befittingly irrigations are scheduled at 0.75–1.0 IW/CPE ratios to ensure sufficient moisture supply for efficient uptake of nutrients accumulation and conversion to total solids. By and large, under water-limited conditions, the process of uniform ripening of primary cane formed at tillering phase may upset severely and lead to poor juice quality. Water stress at the formative stage deteriorates the quality and reduces the cane yield due to reduced stalk weight and millable cane. According to Bell and Garside (2005), the weight of the stalk and the population of millable cane account for more than 98% of the variation in cane output. Therefore, water stress at critical crop stages should be avoided. When water is sufficient for only one irrigation, it should be scheduled at the third order of tillering similarly; if water is available for two irrigations, it should be given at the second, third order of tillering. Scheduling three irrigation at first, second, and third order of tillering yielded almost the same as in the case of four irrigations (Anonymous 1973).

13.5 Method of Irrigation

Under water scarcity areas, selecting appropriate methods for irrigating sugarcane crop is crucial to achieving the goal of water economy and more crop per drop. Under field conditions, many irrigation experiments have demonstrated the variability in the performance of irrigation systems concerning cane yield, water use efficiency (WUE), and cost of production. Drip irrigation was found beneficial in reducing conveyance losses and deep percolation losses in channels and fields. 19–23% and 30–35% water are lost in surface irrigation methods due to deep percolation and conveyance (Patil 2013). The maximum achievable field application efficiency of water by a furrow irrigated crop is around 60% (Ramos et al. 2011). In sugarcane cultivation in Sri Lanka, it has been estimated at 25–45% (Shanmuganathan 1990). Low irrigation efficiency increases water wastage in farmers' fields and causes water shortage in other irrigable lands.

The higher quantity of irrigation water (2565 mm) in the surface method of irrigation was applied than rain-gun sprinkler irrigation (1744 mm) and drip irrigation (1312 mm); however, it could not realize the higher cane yield. The lowest cane yield was observed in surface irrigation (101.6 t/h) than sprinkler irrigation (117.2 t/ha and drip irrigation (118.5 mm) by Shinde and Deshmukh (2008). Thus, selecting appropriate irrigation methods under drought plays a vital role in sustaining sugarcane yield and water economy. The skip furrow method is highly advocated under the water-scarce or areas prone to drought to economize irrigation water. In the skip furrow method, 45 cm wide and 15 cm deep furrows are made in alternate inter-row spaces. Irrigation scheduling is done in an alternate row by skipping one row. Skip irrigation resulted in 30–40% water saving and increased the WUE (65%). The cane yield was higher in the skip furrow method (Srivastava and Johari 1979).

13.5.1 Use of Trash Mulching

Trash mulching had higher WUE, which was possible due to the effectiveness of trash mulching in economizing irrigation water and promoting cane yield. In sub-surface drip irrigation, the pooled mean cane yield showed 5.6 and 18.2% more output in mulched plots than no-mulch and surface irrigation, respectively (Bhingerdeve et al. 2017).

13.5.2 Adjusting Planting Dates and Population Densities

Adjusting planting dates was one option for preventing high ET during the pre-monsoon season. Delayed planting reduces the length of the pre-monsoon desiccating period, thereby decreasing water requirement. Gulati and Nayak (2002) reported higher cane yield (156.65 t/ha) and water use efficiency with the third week of October planting and irrigation scheduling at 1.2 IW: CPE ratio. Declining trends in cane yield were observed with successive delays in planting. Higher cane yield was also reported by Bhullar et al. (2002) in paired row planting (60:30 cm) of sugarcane by 14.0 and 16.8% over sugarcane planted at 60 and 90 cm row spacing. Both the 90 and 60 cm row planting gave more or less equal cane yield. Therefore, under late sown conditions, paired row planting in subtropical conditions for realizing higher sugarcane productivity is recommended.

13.5.3 Potassium Application Under Stress Condition

The rate at which water is applied and transpired through leaves can be altered by nutrition absorption, especially K. Potassium affects the closing and opening of stomata. Spray application of K either alone or in combination with urea at a deficient concentration produced a considerably higher yield under stress conditions. Experiments have proved that K application is beneficial under early drought conditions because K plays an important role in respiration, transpiration, translocation of sugar and carbohydrate, energy transformation, and enzyme activations. Potassium maintains the turgidity of plant cells, and low availability of K decreases moisture content of the cells and could improve the recoverable quality of juice. Applying 60 kg K_2O at 240 days with trash mulching has improved the yield and juice quality. Sugarcane planting using the pit method in light and medium-textured soils may be used to mitigate drought in light and medium-textured soils (Sinha 2016).

13.5.4 Drip Irrigation

Drip irrigation in sugarcane, compared to furrow irrigation, saves water and nearly doubles water use efficiency (Hapase et al. 1990). Water productivity increased by

9.73 and 10.36 under SSI and 8.05 and 8.38 under sett planting with SSDI in the main and ratoon crops, respectively. Conventional planting had the lowest water productivity, with 5.32 and 5.04 kg/m^3 in the main and ratoon crop, respectively (Anbumani et al. 2020). Subsurface drip may save the most water due to its high application efficiency and low evaporation. Compared to either conventional furrow irrigation or furrow irrigation based on IW/CPE (Irrigation Water Cumulative Pan Evaporation) ratio, subsurface drip (Biwall) at 40/140 cm spacing produced considerably more millable canes, cane length, and single cane weight in the plants. In ratoon crop, biwall irrigation at 60/120 cm resulted in significantly longer canes and single cane weight than other irrigation methods (Ramesh et al. 1994).

13.5.5 Skip Furrow Irrigation

The skip furrow irrigation method is a variation of furrow irrigation in which alternate furrows are skipped by bringing two rows of crop together under a shared furrow for watering and adjusting the gaps between the rows appropriately. Irrigation in the skip furrow saves 30–40% water without reducing cane production (Verma 2004).

13.5.6 Alternate Furrow Irrigation

Alternate furrow irrigation is another modification of furrow irrigation wherein irrigations are given in alternate furrows in the first irrigation. The subsequent irrigation is on the other alternate furrows, which do not receive irrigation in the first instance. Irrigations are continually repeating the above cycle. In India, alternate-row furrow irrigation is practiced for sugarcane (Shrivastava et al. 2011). It saves irrigation water by 36% while increasing water use efficiency by 64% compared to every furrow irrigated sugarcane (Visha et al. 2014). According to Shrivastava et al. (2011), the water productivity of alternate furrow irrigated sugarcane was 17 kg/m^3 in India. There was a 31% saving of irrigation water by alternate every furrow irrigation. Pandian et al. (1992) reported that 43–46% reduction in water use was achieved by alternate-row furrow irrigation in irrigated sugarcane in India. Nouri and Nasab (2011) have reported 27% saving of irrigation water by alternate-row furrow irrigation method without significant yield loss in sugarcane in Iran.

13.5.7 Adoption of Water-Saving Techniques

Sugarcane crops require a lot of water when the weather is dry. The ICAR-Sugarcane Breeding Institute in Coimbatore, India, developed a water-saving system to save up to eight irrigations. Applying 10 t/ha composted coir pith or 5 t/ha sugarcane trash in-furrow at the time of planting and scheduling irrigation in

sugarcane at 75% of the recommended level of irrigation saved 387, 344, and 255 mm irrigation water during the plant, first, and second ratoon crops, respectively; in addition, it provided higher irrigation water use efficiency (0.82 t/ha/cm) than scheduling irrigation at 100% level (Dhanapal et al. 2019).

13.5.8 Deficit Irrigation Scheduling with Climate-Smart Sugarcane Genotypes

According to India's growing deficit rainfall scenario, drought is a recurrent issue connected with tropical sugarcane farming, and irrigation water for sugarcane production would be substantially less available in the upcoming years. Irrigation water use efficiency (IWUE), water productivity (WP), and worldwide water security can be improved through more efficient irrigation systems, precise irrigation scheduling, and the proper sugarcane hybrid selection (Tayade et al. 2020; Arun et al. 2020). Full irrigation at recommended intervals with 100% crop evapotranspiration (ET) replacement (I0) produced significantly higher cane yield than deficit irrigation at recommended intervals with 50% crop ET replacement (I1) and skipping alternate irrigations with 50% crop ET replacement (I2). IWUE was similar in I0 and I1, whereas I2 had 23% reduction in IWUE. Sugarcane hybrids with high WP can help maintain sugarcane yield while also reducing the irrigation water used in water-scare tropical India.

13.5.9 Integrated Weed Management with New Generation Herbicide Molecules

Weeds in sugarcane compete for water, and thus sugarcane suffers from water shortage. However, timely weed management practices could control the weeds, thus minimizing water loss. New generation herbicide molecules like topramezone (25.2 g/ha + 656.25 g a.i.ha^{-1}atrazine), tembotrione (120 g/ha + 656.25 g a.i.ha^{-1} atrazine), and halosulfuron methyl (67.5 ga.i/ha + metribuzin 525 g a.i.ha^{-1}) can be used as early post-emergence herbicide (20 days after planting) for weed control in true seed seedling, bud chip settling, and sugarcane setts (Tayade et al. 2020a).

13.6 Fertilizer Management

Sugarcane, C_4 photosynthetic metabolism, required more soil moisture, nutrients, and sunlight for maximum output. To yield about 100 tonnes of sugarcane per hectare, an average sugarcane crop eliminates 208, 53, 280, 30, 3.4, 1.2, 0.6, and 0.2 kg N, P, K, S, Fe, Mn, and Cu from the soil. This will remove many nutrients from the soil, which will need to be replaced to keep the soil productive. Sugarcane yields in Hawaii have decreased due to soil compaction, acidity, nutrient depletion, and changes in soil biological characteristics, according to Humbert (1959).

According to Mathew and Varughese (2007), coupling the use of pressmud @ 5 t ha^{-1} with NPK mineral nutrition at appropriate levels significantly increased the availability of P, K, Ca, Fe, and Zn in sugarcane production. Nutrient management modules based on IPNS–STCR were developed to improve soil health, fertilizer efficiency, productivity, and profitability in the tropical Indian sugarcane plant–ratoon agro-ecosystem (Sinha 2016). For sustaining soil health, sugarcane productivity, and profitability under tropical Indian conditions, application of 10 t ha^{-1} FYM + STCR 150-based fertilizers (390 kg N ha^{-1} and 94 kg P ha^{-1}) + biofertilizers in the plants and application of 20 t ha^{-1} FYM + STCR 150 (390 kg N ha^{-1}, 94 kg P ha^{-1}, and 117 kg K ha^{-1}) in the ratoon crop can be recommended (Tayade et al. 2020a). Small holes of about 10 cm deep and 10 cm away from the clump are made using the crowbar, and fertilizer is covered (Sundara and Vasantha 2004).

Alleviate lime-induced iron chlorosis in sugarcane with nutrient management strategies such as a foliar spray of $FeSO_4$ (2%) along with $MnSO_4$ (0.5%) and urea (0.5%), two to three times (Sinha 2016). Soil amendment of farmyard manure (25 t/ha) + foliar use of $FeSO_4$ (1.5%) with urea (1%) at specific time intervals (weekly) and $ZnSO_4$ (1%) at monthly. In addition, the use of VAM as a biofertilizer helps good crop growth under drought.

13.6.1 Use of Organic Manure

Increased soil pH, electrical conductivity (EC), and exchangeable sodium (%) are affected by excess cations like sodium and anions like carbonate, bicarbonate, and chloride present in irrigation water. Hence, integrated approach of salinity prone areas, like high seed rate, planting in modified trenches, deep ploughing, subsoiling, sanding, profile inversion, etc., improves the soil's physical profile and promotes leaching and drainage (physical amelioration), should be undertaken. The biological amelioration of saline soils consists of living or dead organisms. The application of organic manures (pressmud, farmyard manure, bioearth) has marked influence on amelioration by promoting leaching and reducing soil pH. The salinity of the soil could be considerably reduced by applying waste materials like tamarind seed, safflower hull, and groundnut shell. Coir waste and paddy husk may also be used (Zende 1995). Bakshi et al. (2019) also suggested in situ incorporation of green manure crop (6.25 t/ha) in the soil before planting to improve soil tilth, structure, and water infiltration rate, which provides safeguards against adverse effects of salinity.

Organic manures such as pressmud (10–15 t/ha), farmyard manure (25 t/ha), bioearth, etc., promote essential nutrients like Zn, Fe, Ca, Mg, and Mn. Organic manure reduces the pH of the soil, electrical conductivity, and exchangeable sodium percent in calcareous soil, rendering soil more suitable for sugarcane cultivation (Sundara and Vasantha 2004). Increased availability of P due to the solubilization of insoluble forms of phosphate by organic acids produced during the decomposition of organic matter present in pressmud is a well-demonstrated phenomenon. The availability of N, P, and K in soil was higher and equivalent with the application of 1.25 and 2.50 t ha^{-1} of enriched pressmud compost (Kalaivanan and Hattab 2008). In

addition to structural enhancements, organic or green manures have additional benefits in soil irrigated with saline water in multiple ways, ammonia (NH_3) volatilization losses are exacerbated (Sen and Bandopadhyay 1987). Gypsum application as chemical amelioration was found very good in saline soils. To replace sodium with calcium and to remove carbonate and bicarbonate with sulphate, generally, gypsum, phosphor-gypsum, pressmud, sulphur, pyrite, etc. are recommended.

Various amendment applications, such as pressmud @ 15 t ha^{-1} with 50% (Gypsum) reduced soil pH (1.33%) under-treated paper mill effluent irrigation and 1.25% under saline contaminated irrigation water, registered low EC, soil organic carbon, and nutrients (N, P, and K), enhanced poor irrigation water quality (Paul Sebastian et al. 2009). Also, practices such as irrigation with good quality water, mulching, use of green manures, nutrient management, crop rotation, growing salinity tolerant varieties, etc. were suggested for alleviating salinity stress in sugarcane agriculture.

13.6.2 Foliar Spray of N and K

During the drought, foliar spraying with solution containing 2.5% urea and 2.5% muriate of potash at biweekly intervals improves the crop's drought resistance. Potassium was found to provide abiotic stress resistance in the crop. Crop plants mitigate the adverse effects of drought by exogenous use of salicylic acid, gibberellic acid, putrescine, and cytokinins. In sugarcane, urea and potash spray (2.5 kg/100 L) during the formative phase (90 and 120 days) were also found to alleviate drought stress considerably. Combined use of drought mitigation technologies such as soaking of setts in saturated lime water, application of FYM, and foliar spray of KCl and urea for management of sugarcane during limited water irrigation was also found effective by Mehar et al. (2010) in subtropical Indian states.

Hasanuzzaman et al. (2018) reviewed K involvement in enhancing resistance efficiency during various stress situations. Numerous research suggests that K boosts antioxidant defense in plants, protecting them from oxidative damage in different environmental stresses. When the cane is stressed during late growth and maturity, applying K and mulching the alternate rows is highly cost-effective and beneficial in enhancing yield and quality, especially on small and marginal holdings. Specific management approaches, i.e. soaking of setts in saturated lime water, urea, and potash spray (2.5 kg/100 L) during the formative phase (60, 90, and 120 days), assist in alleviating the drought stress effect considerably.

13.7 Use of Plant Growth Regulators

Of late, chemical variation of plants to increase the tolerance capability to abiotic stress is the possibility currently being investigated. These osmoprotectants include glycine betaine, trehalose, proline, etc. External application of ABA (1×10^{-5} M) exerted a regulatory role on stomatal diffusive resistance and helped maintain

relatively high-water potential (Venkataramana and Naidu 1993). Salicylic acid was also found to play a major role in abiotic stress resistance in plants (Raskin 1992; Pooja and Sharma 2010). In plants, cytokinins and salicylic acid reduce the leaf senescence process and stimulate developing grain to use stem reserves, particularly in drought conditions (Rana et al. 2016). It plays a significant function in regulating proper plant establishment, ripening, flowering, and response to sustain biotic stress (Erdal et al. 2011; Rivas and Plasencia 2011; Hara et al. 2012). In the sugarcane crop, Singh et al. (2018) found that two sprays of 500 ppm Aspirin during drought during the formative stage of sugarcane maintained higher total chlorophyll, leaf water potential, stomatal diffusive resistance with low transpiration rate, resulting in significantly maximum shoot population, number of millable canes and cane yield. Likewise, Miura and Tada (2014) also reported the involvement of salicylic acid (SA), a familiar plant hormone that produces phenolic compound, which is concerned with the regulation of photosynthesis-related protein expression and in plant defense against biographic factors pathogens. Specifically, PGRs like ethephon and gibberellic acid have enormous prospects for better yield and sugar recovery (Li and Solomon 2003; Jain et al. 2011; El-Lattief and Bekheet 2012).

Application of root growth-promoting hormones like IBA and removal of lower leaves to retain only top six to seven leaves, etc., are helpful in mitigating drought. This may be attributed to moderate leaf area index, no or minimum loss in photosynthetic CO_2 assimilation rate, deeper root system, maximum root shoot ratio, and delayed crop senescence that enable it to perform well under drought and will. Under abiotic stresses, tiller production with less mortality plays the main role in sustaining cane productivity. Climatic variables during tillering, genotype, and hormone content significantly impact tiller development senescence (Shrivastava and Misra 1996; Vasantha et al. 2012).

13.8 Use of Antitranspirants

The water requirement of the sugarcane crop increases during the summer months, as well as during water shortage periods, due to increased evapotranspiration demand. The severity of the intermittent drought can reasonably be avoided by using antitranspirants, and the crop can be saved from moisture stress. The role of antitranspirants in checking transpiration rate is well documented. Sugarcane yield responses to silicon (Si) can be linked with induced resistance to biotic and abiotic stresses (Savant et al. 1999).

In a field study in subtropical Indian conditions with different irrigation regimes, trash mulch + silicon @ 0.5% spraying observed maximum sugarcane biomass over other treatments (Singh and Singh 2019). Furthermore, moisture conservation techniques with trash mulch +0.8% kaolin were found to be comparable to Si @ 0.5% + trash mulch. Other experimental findings also have demonstrated that Si improved the water balance of plants by decreasing transpiration rate without affecting photosynthetic responses, which facilitates enlargement of cells due to

turgor pressure and division of cells, thereby enhancing plant performance final output.

13.9 Cultivation of Waterlogged Tolerant Sugarcane

Variety Co 62175 is highly adaptive to more soil water content. Cultivation of resistance genotypes of sugarcane that can withstand flooding such as Co 8231, Co 8232, Co 8145, CoSi 86071, Co Si 776, Co 8371, Co 99006, 93A4, 93A11, 93A145 and 93A21, 93A21, Bo 91, Co 87263, Co 87268, CoTI 8201, and CoTI 88322 should be grown. ICAR-SBI RC, Kannur, India, developed Co 99006 cane variety highly suitable for waterlogging conditions by using waterlogging resistant parent. The clones such as 99WL629, 91WL552, 92WL1029, 98WL1357, 97WL633, and 99WL379 showed better resistance to waterlogging (Gomathi et al. 2010).

13.9.1 Early Planting to Lessen the Surplus Moisture

A high seed rate is required to increase the number of stalks. Early planting is suggested that the crop is entirely established by the time it becomes flooded. The crop that was sown in the first week of February yielded more than the crop planted in March or April. Autumn planting is preferable to spring planting because the autumn crop will have reached adequate vigour and height by the flooding. For early drought and late waterlogging situations, a deep trench planting strategy could be used to boost plant and ratoon productivity.

13.9.2 Planting Approaches

The primary effect of waterlogging in crop plants is oxygen deprivation or anoxia. Plant submerged parts cannot breathe or photosynthesize, and plant roots cannot survive in those conditions. But an artificial aerobic condition created in raised bed planting by cultural practices can improve hydraulic conductivity and decrease soil bulk density, thereby improving root penetration and drainage. To overcome the germination loss under waterlogging situations, a single polybag bud settling raised in the nursery can be planted after the water recedes. Partha method of planting (Planting 3 budded setts in slanting position at 60° angles with one bud into the soil) will be helpful during early flooding conditions.

13.9.3 Earthing Up for Better Root Development

Earthing up may prevent the basal area of cane stool from total flooding in water avoid waterlogging. Earthing up provides some aeration of the stubbles while keeping the crop upright.

13.9.4 Drainage of Excess Water and Providing Field Drains

A drainage trench of 75 cm depth may be built every six to ten rows to remove surplus water during waterlogging.

13.10 Pre-monsoon Field Practices

Before the onset of monsoon season, the field operations, i.e. cleaning the drainage channels and furrows wet earthing up, cane propping to avoid cane lodging, and opening natural drainage outlets, should be carried out.

13.11 Management of Post Waterlogging Crop

The appropriate method for draining water from the field should be developed. To regenerate the root system, enhance crop survival, and prevent pith development, an additional dose of nitrogen and potassium (125 kg urea + 60 kg Muriate of potash) may be given to the crop that is expected to be harvested late. During waterlogging, 2.5% urea foliar spray boosts cane production.

13.12 Conclusions and Future Thrust

Sugarcane agriculture faces severe demand, product diversification, and sustainability challenges. Sustained improvement in crop productivity needs to be ensured if the growing demand for sugar and sweeteners is met in the coming years. The development of varieties and technologies suited for mechanization has become imperative now because of this. Poor soil physical conditions, especially soil compaction, bulk density and porosity, and other significant physical parameters affect root growth and cane production. Environmental stresses, i.e. salinity, alkalinity, drought, flooding, excess temperature, cold, frost, and widespread iron and zinc deficiencies, affect cane production significantly in many states. These issues have to be addressed through agronomic interventions and proper management to make sugarcane agriculture sustainable and profitable. Use of drought, salinity, and waterlogging tolerant varieties with agronomic interventions such as early planting, soaking setts in lime water, modified trench method of planting, trash mulching,

nutrient management, protective irrigation and use of antitranspirants alleviate the negative effects of drought and enhance the sugarcane productivity.

Biological amelioration involves living or dead organisms, organic matter, vegetation, and waste products. The presence of dead or living organisms, organic manures, green manuring, green cane trash blanketing, etc. will maintain soil properties and internal drainage. The modified trench planting system in saline soils and saltwater irrigated areas recorded 15% improvement in cane yield. To mitigate the abiotic stresses and have sustainable sugarcane productivity, the following action points need to be addressed by adopting various breeding, biotechnological, physiological, and agronomic approaches:

- Development of sugarcane genotypes to suit drought, salinity, waterlogging, extreme temperature stress, and other changed global environments.
- Studies to comprehend the regulation at the whole-plant level—A series of experiments need to demonstrate the effect of various climatic factors such as atmospheric CO_2, temperature, water, and crop nutrition both in isolation and in combination.
- Screening of germplasm for excess-temperature resistance through Cell Membrane Thermostability (CMT) test to evaluate and identify temperature tolerant growths. Besides, screening germplasm for temperature tolerant traits such as water retention capacity, leaf wax thickness, chlorophyll stability, synthesis of antioxidants and heat shock proteins, enhanced osmolyte content, and temperature insensitive enzymes.
- Carbon and nutrient dynamics in soil concerning different physical conditions like temperature, moisture, high gases, etc.
- Development of agro-techniques to improve cane productivity under abiotic stresses.

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Biotechnological Approaches to Improve Sugarcane Quality and Quantum Under Environmental Stresses

14

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Abstract

Sugarcane is considered as an important industrial crop to produce sugar, and nearly 80% of sugar production worldwide is produced from this plant. Sugarcane is a C_4 plant that has a higher photosynthetic potential. Abiotic and biotic stresses have a diverse impact on the growth and productivity of sugarcane. Understanding the biochemical and physiological mechanism of these stresses is one of the most important aspects to improve the variety of plants that can meet better quality and quantum. Progress in the development of new sugarcane cultivars by conventional breeding has been hindered by its complex polyploid-aneuploid genome leading to a long breeding period. These types of constraints offer an opportunity to generate new sugarcane cultivars through biotechnological approaches. The new variety of sugarcane with desirable traits, such as drought tolerant and virus resistance, have been attempted to increase the yield of the plant. The inducing accumulation of compatible solutes such as sugar and betaine help sugarcane to adapt and survive in water limited environment. Biotic

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stress causes a significant loss in sugarcane growth and yield. Pathogen-derived resistance (PDR) and RNA interference (RNAi) technologies have been applied to engineered sugarcane cultivars having resistance to the sugarcane mosaic virus. In addition, genetic engineering of sucrose metabolism is also an important means to control carbon flux through the enzyme sucrose-phosphate synthase, which is responsible for the synthesis of sucrose. Here, we summarize recent developments in the biotechnological approaches to improve sugarcane yield by developing stress tolerance efficiency, increased yield, and virus resistance, including potential and challenges of genome editing technological applications.

Keywords

Biotechnological approaches · Biotic-abiotic stress · Carbon partitioning · Stress-tolerant · Virus-resistant · Sugarcane

14.1 Introduction

Sugarcane is a tall perennial tropical grass that produces unbranched stems of 2–4 m or taller and around 5 cm in diameter. It is cultivated to produce sugar (sucrose) which is extracted from the solid stems or stalks. Sucrose is synthesized in the leaves, exported, and accumulated in the stem. The stem is differentiated into joints comprising a node and an internode where sucrose content gradually increases from young immature to mature internode. The length and diameter of the internode are affected by environmental factors such as water supply, nutrition, and temperature (Verma et al. 2020a). The condition favorable to harvest sucrose is dependent on the ripening state that normally takes place during the cooler or drier times of the year. Under the best ripening condition, a tonne of sugar can be produced from 7 to 12 tonnes of cane. After sugarcane harvesting, it is normal to regrow sugarcane once or several times, and this cultivation method is known as ratooning.

Sugarcane, a C_4 plant, is more efficient to use light, water, and nitrogen availability compared to C_3 plants (Kellogg 2013). Under full sunlight, C_4 plants continue to assimilate CO_2 into carbohydrates, which increase as the available light increases. During the daytime, the stomata are slightly closed to minimize transpiration without any effect on carbon assimilation. The C_4 plants also produce more biomass and have a higher photosynthetic rate per unit of water input and nitrogen. The operation of primary carboxylation of phosphoenolpyruvate (PEPC), which is located in mesophyll cell and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) present in bundle sheath cells, makes the CO_2 assimilation more efficient with low rate of photorespiration. Assimilation of CO_2 produces various forms of organic carbon that will produce sucrose as a mobile carbon compound and is distributed to other tissues for carbon and energy source. The key enzyme for the synthesis of sucrose is sucrose-phosphate synthase (SPS), which catalyzes the production of sucrose-6-phosphate, which is converted to sucrose by the action of sucrose-phosphate phosphatase (Huber and Huber 1996). The SPS activity has been reported

to play an important role in sucrose accumulation and biomass production in plants, including sugarcane (Anur et al. 2020). Molecular analysis revealed that SPS activity is structurally regulated by phosphorylation concerning changes in light intensity and water availability. The regulation of PEPC and SPS by environmental conditions such as water supply might express the important role of the enzymes in determining the growth and productivity of sugarcane under water deficit conditions.

Trends in climate change over the past few decades have induced biotic and abiotic stress on plants that have an impact on many agricultural productions, including sugarcane. Plants show a variety of physiological changes under climate change ranging from enhanced abiotic stress to accelerated pathogen infection. Climate change is assumed to cause an increase of temperature, flooding, and drought stress. However, many reports have been focused on the effect of drought stress on plant productivity (Verma et al. 2020a, 2021a, b). The physiological study revealed that plants possess the nature of resilience to survive under a limited water environment. Water stress induces a wide range of changes in gene expression and biochemical alteration to adjust plant growth under the stress condition (Verma et al. 2020b, c, 2021c). Molecular identification classified two groups of drought-inducible genes, the genes for protein abiotic stress tolerance and regulatory proteins such as transcription factors (Shinozaki and Yamaguchi-Shinozaki 2007). In addition, it is well reported that the accumulation of sugar, proline, and betaines helps plants adapt to drought stress (Chen and Murata 2002). These small molecule metabolites perform an essential function to protect cells from damage due to water stress. Glycine betaine (GB) is a non-toxic compatible solute that protects plants under water deficit and osmotic stress (Sakamoto and Murata 2002). Understanding of basic mechanism underlying drought tolerance will be beneficial to anticipate the impact of climate variability and develop genetic engineering for sugarcane (Verma et al. 2019).

Plant pathology has long been considered to study the environmental influence on plant diseases. Temperature change may favor the development of different pathogens such as bacterial diseases and the incidence of vector-borne diseases. In sugarcane, growth and productivity are affected by several diseases such as insects, fungal, bacterial, and viral infections. *Sugarcane mosaic virus* (SCMV) and *Sugarcane streak mosaic virus* (SCSMV) are the most destructive viruses for sugarcane which reduces the yield up to 45% (Putra et al. 2014). The SCMV infection inhibits the development of stem diameter and length of internode from the early growth to the harvest period. This virus has been reported as a dominant pathogen that infects sugarcane in several countries, including Indonesia (Addy et al. 2017). Therefore, several methods have been developed to solve the problems of SCMV infection, such as viral elimination using in vitro meristematic culture, antivirus, and hot water treatments (Dewanti et al. 2016). However, the methods are not found to provide complete protection to sugarcane against viral infection in the field. Molecular study revealed that the SCMV genome contains genes encoding for ten functional proteins, including coat protein (CP) (Zhu et al. 2014). The gene encoding for CP is the most widely used component to induce resistance against viruses using genetic engineering in plants, including sugarcane.

Genetic improvement of sugarcane has been performed through conventional breeding programs, including intercrossing between the hybrids to increase sucrose production, induce stress tolerance, and gain diversity of alternative products. Although the breeding programs were successfully implemented, it is a laborious task and takes around 12 years or more. Modern commercial varieties have also been developed through interspecific hybridization between *Saccharum* species and allied genera of *Miscanthus* and *Erianthus* species. However, the conventional breeding for sugarcane resulted in polyploid and aneuploid with chromosome number of 2n = 80–120 that leads to meiotic instability, production of aneuploid gametes, and production of sterile seeds. Biotechnological tools are required to solve critical problems related to sugarcane improvement for sustainable agriculture.

Progress on molecular techniques and genetic transformation is needed to create new sugarcane cultivars using a biotechnological approach. Biotic and abiotic stresses alter sugarcane metabolism impacting its growth and productivity. To survive, plants exhibit several biochemical and molecular mechanisms which make them withstand or secure stress. Understanding biochemical and physiological mechanisms in response to biotic and abiotic stress is a major challenge for the developing biotechnology for sugarcane. The objective of this chapter is to improve sugarcane quality and quantum under environmental stress using biotechnological approaches.

14.2 Critical Points of *Agrobacterium*-Mediated Transformation in Sugarcane

14.2.1 Sugarcane Micropropagation

An efficient sugarcane tissue culture protocol is a valuable tool for sugarcane research activities, such as large-scale in vitro propagation and cultivar improvement. Conventional vegetative propagation is prone to several diseases, including gumming, Fiji, and other diseases. Therefore, establishing sugarcane tissue culture plays an essential role in producing disease-free plant material and reducing the seed production time. Notably, the sugarcane tissue culture has paved the way in improving sugarcane cultivars via sugarcane genetic transformation. Bower and Birch (1992) developed the first genetic transformation method in sugarcane using tissue culture. This method has been applied for engineering agronomic traits in various sugarcane cultivars (Bower and Birch 1992).

Plant cells have the capacity of totipotency, the ability of cells to regenerate into complete plants containing roots, stems, and leaves. This totipotency capacity can be triggered from meristematic tissue by growth regulators or hormone supplementation in tissue culture media to induce somatic embryogenesis callus and then regenerated to plantlets. In sugarcane, somatic embryogenic callus is derived from meristematic leaf roll tissue grown on Murashige and Skoog (MS) medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4 D) (Lee 1987). Then, the embryogenic sugarcane callus could easily regenerate to plantlets on hormone-free

MS medium (Widuri et al. 2016). This simple micropropagation technique has been considerably applied for providing large-scale sugarcane seed demand. However, somaclonal variation may also arise from somatic embryogenesis in sugarcane that causes the development of variant phenotypes in sugarcane. Interestingly, the phenotypic variation caused by somatic embryogenesis generally reverts to its parental phenotype in sugarcane. The occurrence of somaclonal variations in somatic embryogenesis has been used to obtain new sugarcane varieties that are resistant to biotic or abiotic stress. In addition, somatic embryogenesis has played an essential role in the genetic transformation system to improve sugarcane cultivars.

Sugarcane in vitro propagation is also achieved without callus intervening through direct regeneration and multiplication from apical meristem or axillary buds. Explants from axillary buds can minimize genetic changes and avoid 2,4 D in culture media, which can cause somaclonal variation. In vitro propagation using axillary buds of sugarcane minimizes the somaclonal variation event, so it is used routinely for in vitro propagation of sugarcane (Manickavasagam et al. 2004). However, sterilizing axillary buds from field-grown stalks requires a potent sterilant such as mercury chloride ($HgCl_2$), which is generally avoided because of its toxicity. Alternatively, shoot apical meristem is applied for mass multiplication of sugarcane shoots. Several methods have been developed to improve in vitro sugarcane multiplication from shoot apical meristem in MS media. Temporary immersion of shoot apical meristem into MS media containing high concentration of BAP (benzylaminopurine) resulted in weak, tiny, and non-separable shoots (Biradar et al. 2009). In addition, organic nitrogen sources in MS media may play an essential role in the multiplication of sugarcane shoot apical meristem. Some amino acids such as asparagine, cysteine, casein, glutamine, and glycine are primarily used in culture media as organic nitrogen sources (Saad and Elshahed 2012). The addition of 100 ppm glutamine and 2 ppm glycine into MS media produced robust and healthy sugarcane plantlets (Sugiharto, *unpublished data*). Glutamine and glycine may stimulate the multiplication of shoot apical meristem, which is suitable for micropropagation and genetic transformation of sugarcane.

14.2.2 Agrobacterium-Mediated Transformation

Genetic transformation is a valuable technology based on inserting genes into the genome to improve plant traits such as yield, pathogen resistance, and stress tolerance. Initially, the genes were introduced into plant cells directly using polyethylene glycol (PEG) treatment, electroporation, or particle bombardment (Rathus and Birch 1992). These direct transformation methods were less efficient due to multicopy gene integration, high cost, requiring sophisticated equipment, and skillful labor (Dai et al. 2001). Meanwhile, the transformation method using agrobacterium is a powerful tool to introduce genes of interest into the plant genome. This method has been widely used to introduce genes into numerous dicot crops, including canola, cotton, potatoes, soybeans, and tomatoes. During the initial years, monocot plants were considered recalcitrant to Agrobacterium transformation because of their

narrow host range. However, in recent years, Agrobacterium transformation was successfully carried out even in monocotyledonous plants by improving plant regeneration techniques and manipulation of factors affecting transgene delivery and integration into the plant genome. For example, co-cultivation media supplementing with acetosyringone, a phenolic compound that activates the virulence gene of Agrobacterium, can increase the T-DNA transfer efficiency into rice callus (Xi et al. 2018), maize embryos (Ishida et al. 1996), and banana suckers (May et al. 1995). Agrobacterium-mediated transformation system was also carried out successfully in sugarcane using meristematic explants (Arencibia et al. 1998). This technique provides several advantages, including low copy number of gene integration, low cost, and technical simplicity. However, reproducible transformations using Agrobacterium are required for routine genetic manipulation of sugarcane. So, optimizing critical factors affecting this transformation system is necessary to have a reproducible method, low somaclonal variation, and high efficiency of transformants multiplication.

The embryonic callus was mainly used as an explant in the plant transformation system. However, using in vitro regenerated shoots derived from apical meristem or axillary buds as explant offers several advantages in sugarcane transformation. Agrobacterium-mediated transformation using axillary buds explant resulted in stable transgenic sugarcane with transformation efficiency of about 50% (Manickavasagam et al. 2004). Unfortunately, the preparation of axillary buds from field-grown sugarcane is a tedious process due to the high possibility of bacterial contaminants. Alternatively, apical meristems derived from in vitro shoots are also suitable for obtaining contaminant-free explants (Sugiharto et al. 2005). Micropropagation of shoot from apical meristem has been developed using the MS media supplemented with glutamine and glycine, which results in healthy and rapid shoot growth. The basal segment of the healthy grown shoot was excised traversal around 0.2–0.3 cm and then used as an explant for genetic transformation (Sugiharto 2018). This method produces transgenic sugarcane plants in 4 months with a 4–10% transformation efficiency (Apriasti et al. 2018). Thus, the basal segment of in vitro shoot can act as a suitable and effective explant for routine genetic transformation in sugarcane.

The genetic transformation in sugarcane is not as simple as the preparation of explant but requires fine-tuning of various parameters. Several undetermined factors, such as selection of promoter, a selectable marker, and Agrobacterium strain, should be adjusted to improve transformation efficiency. DNA regulatory elements called promoters control gene expression in particular strengths and patterns. The promoter affects transformation efficiency, and the choice influences transgenic production (Liu et al. 2003). Many plant DNA promoters are well-characterized and classified into constitutive, tissue-specific, cell type-specific, organelle-specific, and inducible promoters. The Cauliflower mosaic virus 35S (CaMV 35S) promoter is a constitutive promoter commonly used in the transformation of dicot and monocot plants, including sugarcane (Apriasti et al. 2018). However, current research shows that ubiquitin, an endogenous plant promoter, effectively directs the constitutive expression in sugarcane. The rice polyubiquitin (RUBQ) 2 promoter increases GUS gene

expression 1.6-fold compared to *Zea mays* polyubiquitin (ZmUbi) 1 promoter in sugarcane callus (Liu et al. 2003). Comparison of the effectiveness of CaMV and ubiquitin promoter showed that the ubiquitin significantly induced a higher expression of the targeted gene in sugarcane (Widyaningrum et al. 2021). Therefore, the ubiquitin promoter is widely used to drive the expression of transgenes in the transformation of sugarcane and other monocotyledonous plants.

The selectable markers and selective agents are critical factors affecting the plant's genetic transformation. The selective agent, such as antibiotic or herbicide, suppressed the growth of the non-transformed cell in the selective media. The selectable marker gene transforms into the plant cell, and the gene of interest facilitates the transformed cells to survive in the selective media. Selectable marker genes that are commonly used in plant genetic transformation are the kanamycin resistance gene (*nptII*), hygromycin resistance gene (*hptII*), and herbicide Basta/phosphinothricin resistance gene (*bar*). Determination of explant sensitivity and appropriate concentration of the selective agent in the media is critical for the success of the genetic transformation. Excessive concentrations of selective agents in the media not only kill non-transformed cells but also suppress the growth of transformed cells (Miki and McHugh 2004). Evaluation of kanamycin and hygromycin as selective agents in Gramineae showed that both antibiotics suppressed cell suspension culture of *Triticum monococcum*, *Panicum maximum*, and *Saccharum officinarum* (Hauptmann et al. 1988). The hygromycin showed more effectiveness than kanamycin as a selective agent in the genetic transformation of rice (Lin and Zhang 2005) and maize (Que et al. 2014). In addition, herbicide Basta has also been used as the selective agent in the genetic transformation of monocots, such as rice (Rathore et al. 1993) and oil palm (Parveez et al. 2007). The comparative studies of *nptII*, *hptII*, and *bar* effectivity in sugarcane genetic transformation are limited. However, the agrobacterium-mediated transformation of sugarcane generally uses *the hptII* gene as a selectable marker because a low concentration of hygromycin, 25 mg/L, in a selective media is sufficient to discriminate between transformant and non-transformant plants (Arencibia et al. 1998).

The Agrobacterium strain and its density during explant infection contribute to the efficiency of the plant's genetic transformation. The LBA4404 strain is commonly used for the genetic transformation of monocot plants. While the GV3101 strain has the highest transformation efficiency than AGL1, EHA105, and MP90 strains in the dicot plant (Chetty et al. 2013). The agrobacterium density at $OD_{600} = 0.5$ during infection processes increases cotton's transformation efficiency (Jin et al. 2005), and higher density also leads to bacterial overgrowth that is difficult to eliminate from the explant after co-cultivation. The use of GV3101 strain for the infection of in vitro shoot explant at $OD_{600} = 0.5$ is the best example for routine transformation of sugarcane.

14.3 DNA Recombinant Technology

14.3.1 Cloning Gene

Recombinant DNA technology is defined as combining DNA fragments from different sources or inserting foreign DNA into the genome to obtain valuable characters or products from a living organism. These technologies include gene isolation, cloning, genetic transformation, and gene insertion into the genome of living organisms. Recombinant DNA technology has developed since the discovery of DNA polymerase, reverse transcriptase, DNA ligase, and restriction endonucleases enzymes that can copy, cut, and ligate DNA fragments. DNA polymerase and reverse transcriptase provide the possibility to copy DNA from the genome or messenger RNA, respectively. DNA ligase acts as glue for joining two adjacent DNA fragments via a phosphodiester bond. More than 600 commercially available restriction endonucleases serve as scissors that are able to cut DNA at a particular site (Roberts et al. 2007). Cutting and re-ligation using restriction endonucleases and DNA ligase facilitate the transfer of one DNA fragment to another. The isolated DNA fragment can then be inserted into a plasmid, a circular DNA molecule distinct from the bacterial chromosome. The plasmid can replicate independently during cell division, thereby enabling the amplification of the inserted DNA fragment.

Using these techniques, several genes from sugarcane have been cloned and characterized. The gene encoding *sucrose-phosphate synthases (SPS)*, *SoSPSl*, and *SoSPS2* were isolated from the cDNA of sugarcane leaves. *SoSPSl* is expressed predominantly in leaves, whereas SoSPS2 is expressed in both leaves and roots (Sugiharto et al. 1997). The drought-inducible gene *SoDip22*, which is expressed in bundle sheath cells, was also isolated from the cDNA of sugarcane leaves (Sugiharto et al. 2002). In addition, the genes encoding for sucrose transporter protein (Novita et al. 2007) and coat protein of SCMV (Apriasti et al. 2018) have also been successfully isolated from sugarcane.

14.3.2 Gene Overexpression

Gene overexpression is defined as an attempt to increase the transcript level of a coding gene using a promoter or other regulatory element. This technique intends to achieve higher levels of RNA transcription and protein expression. Several promoters have been known as constitutive promoters, such as *CaMV35S*, *ZmUbi1*, *OsAct1*, *OsTubA1*, and *OsUbq1*. Some promoters have been used to generate transgenic sugarcane with high sucrose content, virus tolerance, cold tolerance, and drought tolerance. For example, the *CaMV35S* promoter drives *SoSPS1* expression to increase sucrose content in sugarcane (Anur et al. 2020), while *ZmUbi1* controlled the expression of RNAi constructs to generate virus-resistant sugarcane (Widyaningrum et al. 2021). In addition, the *ZmUbi1* promoter

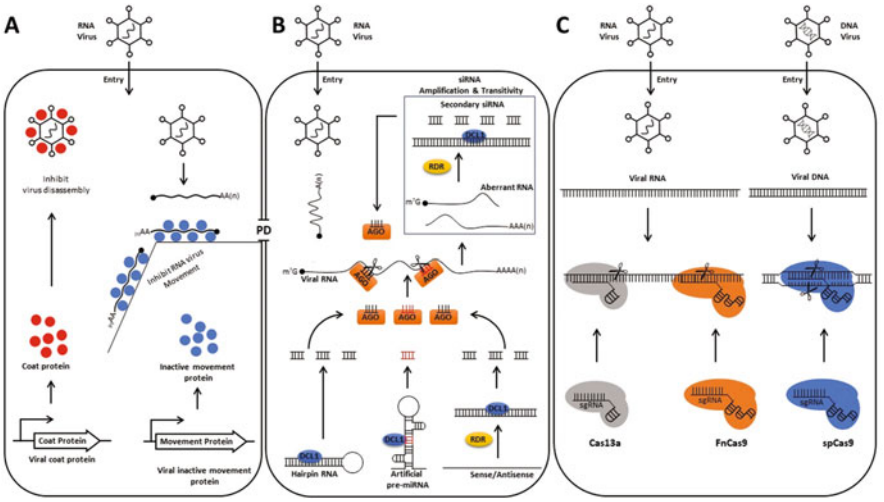

Fig. 14.1 Model of PDR, RNA silencing, and CRISPR/Cas strategy for inducing virus resistance in the plant. (**a**) PDR strategy achieved by expressing viral coat protein or inactive movement protein in the transgenic plant. Coat proteins inhibit virion disassembly in the initial infection, while inactive movement protein (MP) inhibits cell to cell RNA virus movement through plasmodesmata (PD). (**b**) RNA silencing is triggered by hairpin RNA, artificial pre-miRNA, or the activity of RDR on sense/antisense RNA. The dsRNA is processed to small interference RNA (siRNA). The siRNA incorporates into AGO protein effectors, which provide sequence specificity to cleave homolog RNA target. The activity of RDR enables amplification and production of secondary siRNA corresponding to regions outside of the primary siRNA target (transitivity). (**c**) Three variants of Cas protein were used in the strategy to target plant viruses. The spCas9 was utilized for targeting DNA virus while FnCas9 and Cas13a were employed for targeting RNA virus. All Cas variants required specific sgRNA, which provide sequence specificity to the virus genome

was also utilized to drive *Alpha (α)–tubulin* (*TUA*) and *ATP citrate lyase* (*ACL*) to develop cold and drought tolerance sugarcane (Chen et al. 2021; Zhu et al. 2021).

Sanford and Johnson (1985) described a concept that inserting a gene from a virus into the host genome would confer resistance to the host against the virus, which was then known as pathogen-derived resistance (PDR). For example, the viral coat protein expressed in plants provides resistance to the virus by inhibiting the virion disassembly in the early infection event (Baulcombe 1996). This mechanism is supported by experiments that plants expressing the coat protein show resistance to virion inoculation but are sensitive to virus inoculation in the form of RNA, indicating that coat protein inhibits early infection events (Powell-Abel et al. 1986; Hemenway et al. 1988). Virion disassembly is required to allow viral genome replication and RNA expression in the host cell to produce organelles of new viruses. Reassembly of virus organelle into virion is essential for the long-distance movement to allow virus entry into vascular tissue (Saito et al. 1990). So, the inhibition of virion disassembly by coat protein in the initial infection event prevents virus replication and long-distance movement (Fig. 14.1a).

Plasmodesmata, channels connecting cytoplasm between adjacent cells, mediate the spreading of viruses from one cell to another. The movement of viruses between adjacent cells is facilitated by movement proteins (MPs) encoded by the viral genome. The MPs complex polymer binds to virus RNA to facilitate movement along microtubules toward plasmodesmata (Carrington et al. 1996). Then, MPs modify the plasmodesmata component to increase its size exclusion limits (SEL), facilitating the movement of either naked RNA or virion to cross plasmodesmata channels (Lazarowitz and Beachy 1999). Unlike CP (coat proteins), plants expressing a functional MP are more susceptible to *tobacco mosaic virus* (TMV) infection, whereas overexpression of inactive MPs (lacking movement function) confers resistance to the TMV virus (Lapidot et al. 1993; Cooper et al. 1995). The inactive MPs and wild-type MPs possibly compete for the binding site at the plasmodesmata component, resulting in inhibition of virus dispersal (Baulcombe 1996). Interestingly, inactive MPs confer resistance to various virus groups (Cooper et al. 1995). It seems that inactive MPs complex can recognize genome RNA from several viruses and prevent cell to cell movement (Fig. 14.1a).

14.3.3 RNA Interference

Gene silencing is a conserved mechanism in the eukaryotic organism that employs small interference RNA (siRNA) and protein effectors to suppress homolog gene expression at the transcriptional or post-transcriptional levels. Gene silencing was initiated by forming double-strand RNA (dsRNA) and subsequently processed to small interference RNA (siRNA). One of the two strands of siRNA incorporates into protein effectors to form RNA-induced silencing complex (RISC) and provide sequence specificity to cleave homolog RNA target in post-transcriptional gene silencing (PTGS) or mediate chromatin methylation in transcriptional gene silencing (TGS). PTGS is later known as RNA interference (RNAi). Although TGS and PTGS are mechanistically related and share molecular machinery, in this chapter, the discussion is focused on PTGS/RNAi in relation to virus resistance traits in sugarcane.

The dsRNA is naturally found in replication intermediates or highly structured genomic RNA of the virus. RNA virus replication was mediated by viral RNA-dependent RNA polymerase (RdRP), resulting in perfectly paired dsRNAs known as replication intermediates. On the other hand, the RNA genome of the virus arranges in highly base-paired structure with several imperfect dsRNA and hairpin loop structures. DICER processes replication intermediates and imperfect dsRNA to 21 and 22 primary siRNAs (Molnár et al. 2005). The formation of primary siRNA by DICER is the initiation phase of the RNA silencing mechanism to deal with viruses.

The DICER protein comprises three functional domains lying from the N- to C-terminus: RNA helicase, PAZ (Piwi/Argonaut/Zwille), RNAse III a b, and dsRNA-binding domain. The plant genome generally encodes four different DICER-LIKE (DCL) proteins that produce a distinct length of siRNAs (Song and Rossi 2017). DCL1 produces variable size microRNA (miRNA), a small RNA

encoded in the genome (Bartel 2004). DCL2, DCL3, and DCL4 process dsRNA to 22, 24, and 21-nucleotide (nt) siRNA, respectively (Nagano et al. 2014; Benoit 2020; Wu et al. 2020). The 21-nt and 22-nt siRNA guide RNA degradation in PTGS, while 24 nt siRNA mediate chromatin methylation in TGS (Tan et al. 2020). The DCL2 and DCL4 have a redundant function in processing viral-derived RNA and play an essential role in systemic antiviral silencing in the plant (Qin et al. 2017; Chen et al. 2018).

The siRNA assembles into Argonaute (AGO) protein and provides specificity to Argonaute (AGO) protein effectors to cleave homolog RNA. The AGO protein comprises two domains, the PAZ domain for binding single-stranded nucleic acid and PIWI-domain containing RNAse-H-like fold (Hutvagner and Simard 2008). Seven AGO are critical players of gene silencing and viral defense, i.e., AGO1, AGO2, AGO5, AGO7, and AGO10 play a role in targeting RNA degradation in PTGS; AGO4 and AGO6 mediate chromatin methylation in TGS (Carbonell and Carrington 2015). The AGO1 is a significant player in plant defense mechanisms against invading viruses, indicated by its upregulation in response to the viral attack (Várallyay et al. 2010). However, the activity of AGO1 has interfered with the protein suppressors encoded by the virus by inhibiting its transcription level or cleavage activity (Xiuren Zhang et al. 2006; Csorba et al. 2010; Várallyay et al. 2010). When the AGO1 is inactivated, the plant activates the second layer of defense mechanism against invading virus by expressing AGO2 (Harvey et al. 2011).

The amplification of virus siRNA is required to ensure the efficiency of RNA silencing against virus attacks. RNA-dependent RNA polymerase (RDR) mediates the formation of secondary siRNA from cleaved RNA of the virus. RDR synthesizes dsRNA from RNA lacking a 5′ triphosphate cap or poly-A tail and then processed into 21 or 22 secondary siRNA by DICER (Luo and Chen 2007; Willmann et al. 2011). The mRNA lacking poly-A tail or 5′ triphosphate cap is converted to dsRNA by RDR through a primer-independent or dependent approach, respectively (Curaba and Chen 2008). The activity of RDR enables the production of secondary siRNA corresponding to regions outside the primary siRNA target (Fig. 14.1b) (Moissiard et al. 2007). Amplification and transitivity of siRNA determine the strength and persistence of antiviral defense against the virus (Baulcombe 2007). Three homologous plant *RDR* genes, *RDR1*, *RDR2*, and *RDR6*, are required in the biogenesis of secondary siRNA from RNA viruses. For example, the biogenesis of secondary siRNA from the *Tobacco rattle virus* (TRV) required the combined activity of the three RDR genes (Livia et al. 2008).

Several techniques had been reported to generate dsRNA, such as co-suppression, sense-antisense construct, and hpRNA (Fig. 14.1b) (Waterhouse et al. 1998). The dsRNA using co-suppression or sense-antisense construct usually results in low silencing efficiency (Stoutjesdijk et al. 2002). A more effective approach to produce dsRNA is to clone both sense and antisense sequences, separated by an intron, under the control of a promoter to generate self-complementary RNA (Wesley et al. 2001). The approach was initially known as hpRNA, but later it was known as RNAi construct. This technology was applied to confer resistance against several families of RNA viruses in soybean, tomato, and tobacco (Andika et al. 2005; Hu et al. 2011;

Zhang et al. 2011; Ammara et al. 2015). The RNAi was also reported to mediate effective resistance against SCMV and *sorghum mosaic virus* (SrMV) in sugarcane. The trait introduced by RNAi was inherited in plant progeny, indicating that the RNAi construct is stable in the offspring of the transgenic plant (Chuang and Meyerowitz 2000).

14.3.4 Genome Editing

Genome editing provides flexibility and effectivity to manipulate plant genomes for diverse purposes such as gene study, increased productivity, conferring resistance to biotic or abiotic stress, and improving plant quality. Genome editing is a genetic engineering technique that employs engineered nuclease to generate double-strand breaks (DSB) at the specific location, which are then repaired by the cell's internal mechanisms through non-homologous end joining (NHEJ) or homologous recombination (HR), resulting in a mutation or insertion. Four genome editing systems have recently been developed to manipulate plant genomes, such as ZFN, TALEN, and the CRISPR/Cas9 system. The CRISPR/Cas9 system has been widely used because of its simplicity, robustness, and cost-effectiveness.

The CRISPR/Cas9 is an adaptive immune system against invading viruses or foreign genetic elements in prokaryotes. In this immune system, the bacteria acquire a short sequence from viruses known as a spacer and integrate it between two sequences repeat of the CRISPR array in the genome, allowing them to remember and develop immunity against viruses. Bacteria transcribe the spacer-repeat array into a long precursor CRISPR RNA (pre-crRNA) and subsequently process it to a small mature crRNA guide. Repeat sequence in the crRNA forms base pairs with an additional small non-coding RNA known as trans-activating crRNA (tracrRNA) to form dual-RNA structure. The tracrRNA is encoded by trans-activating the CRISPR RNA gene located upstream of the CAS operon in the CRISPR locus. The Cas9 nuclease protein recruits a dual crRNA-tracrRNA structure to identify the complementary target sequences in viral DNA. The Cas9-crRNA-tracrRNA complex recognizes a short DNA motif termed Protospacer Adjacent Motif (PAM), where Cas9 binds and unwinds the dsRNA to facilitate duplex formation between spacer of crRNA and DNA target sequence (Jiang and Doudna 2017; Hille et al. 2018). PAM is a short-conserved sequence adjacent to the crRNA target site, recognized specifically by the Cas9 protein. The commonly used *Streptococcus pyogenes* Cas9 (spCas9) protein recognizes PAM motif 5′-NGG-3′, where N can be any nucleotide of DNA (Sternberg et al. 2014). The PAM motif appears only in adjacent target sequences but not in crRNA, facilitating discrimination of self and non-self DNA, thereby preventing the immune system from attacking the host (autoimmunity) (Sashital et al. 2012; Rath et al. 2015). This immune system confers resistance to the bacterial population and is inherited vertically to their progeny (Marraffini 2015).

The CRISPR/Cas9 immune system is adopted as a genetic engineering tool to manipulate the sequence of a eukaryotic genome. The CRISPR/Cas9 system comprises two components, i.e., single guide RNA (sgRNA) and

CRISPR-associated 9 (Cas9) protein. The sgRNA is a single RNA transcript formed by a combination of crRNA and tracrRNA separated by a linker loop, mimicking the dual-RNA structure required by Cas9 to direct site-specific DNA cleavage. By modifying the 20 bp spacer sequence at 5′ end of gRNA, it is possible to target any sequence in the genome (Martin et al. 2012). Cas9 gene is constitutively expressed in plant cells driven byCaMV35S or ZmUbi promoter for modifying the plant genome. Nuclear localization signals (NLS) are fused to Cas9 protein to direct its expression to the nucleus. The sgRNA is expressed under U6 or U3 promoters to facilitate transcription, which starts with nucleotides G for U6 or A for U3 promoters. For targeting plant genomes, the sgRNA spacer follows a consensus sequence $G(N)_{19–22}$ or $A(N)_{19–22}$, where the first G or A may or may not pair with the target sequence (Belhaj et al. 2013). Recently, CRISPR/Cas9 was utilized to generate sugarcane herbicide resistance by generating DSB in the *Acetolactate synthase* (*ALS*) gene and introduced *ALS* sequence containing amino acid substitutions W574L and S653I via HDR mechanism (Oz et al. 2021).

The causal agent of disease that causes a significant loss in sugarcane production is mostly mediated by RNA viruses. Since spCas9 is targeting DNA, a variant that targets RNA is required to engineer resistance against viral RNA. Two Cas9 variants, *Francisella novicida* Cas9 (FnCas9) and *Leptotrichia shahii* Cas13a are components of prokaryotic adaptive immunity against RNA viruses. The FnCas9 recognizes PAM 5′-NGG-3′ at its target RNA locus, whereas Cas13a does not require PAM, making it more flexible than FnCas9 (Sampson et al. 2013; Abudayyeh et al. 2017). Both FnCas9 and Cas13a provide the possibility to develop plants resistance to RNA viruses (Fig. 14.1c). An example from the monocot plant, the Cas13a and sgRNA targeting *southern rice black-streaked dwarf virus* (SRBSDV) or r*ice stripe mosaic virus* (RSMV) expressed in rice showed mild symptoms and less virus accumulation (Zhang et al. 2019). A similar approach using FnCas9 or Cas13a might be applied to combat RNA virus attacks in sugarcane.

14.4 Biotechnology to Increase Sucrose Production

Sucrose is a dominant mobile sugar that has a crucial role in plant growth and development, various types of gene expressions, and sugar signaling pathway (Gifford et al. 1984). However, the function of sucrose in microorganisms still remains unclear. Recent biochemical and molecular studies reported that sucrose synthesis in prokaryotic cells provides new insight into sugar metabolism in terms of its origin (Salerno and Curatti 2003). Sucrose is the common form of sugar that is generated from photosynthesis products. Further, sucrose is exported from source leaves to carbon-importing sink tissues for allocation of carbon resources. There is evidence that sucrose not only provides the fuel for plant growth but also has an important influence on the expression of genes that are involved in signaling function and cell differentiation (Lunn and MacRae 2003). Sucrose accumulates in the stem as the primary storage reserve in sugarcane. Thus, it is suggested that the

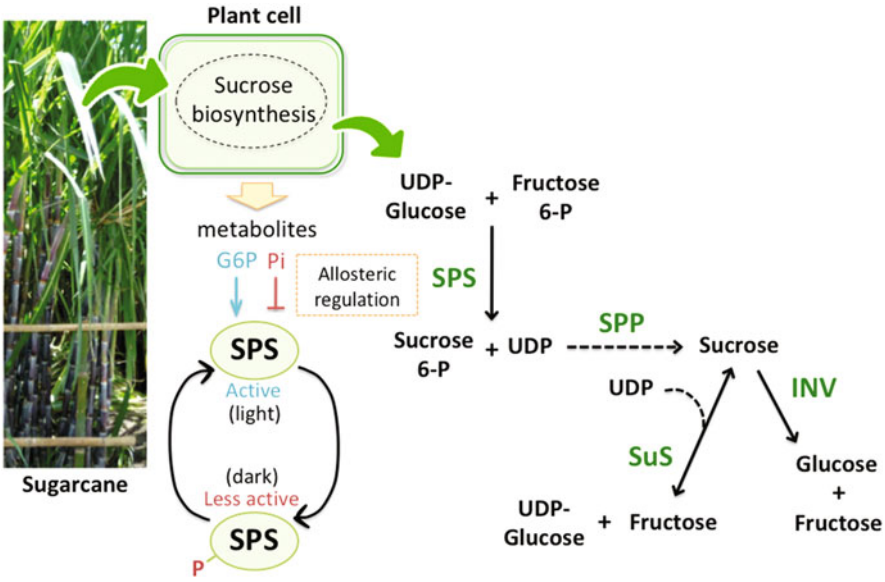

Fig. 14.2 Model of sucrose metabolism pathway in plant cell

activity of sucrose biosynthesis enzymes influences sucrose loading into the phloem and sink (Zhu et al. 1997; Castleden et al. 2004).

Based on the phylogenetic origin, the enzymes involved in sucrose metabolism have been characterized as sucrose biosynthesis-related proteins (SBRPs). The group of enzymes that are classified under SBRP comprised of sucrose-phosphate synthase (SPS; EC 2.4.1.14), sucrose synthase (SuS; EC 2.4.1.13), and sucrose-phosphate phosphatase (SPP; EC 3.1.3.24). SPS is responsible for yielding sucrose with inorganic phosphate (Pi), whereas SPP catalyzes an irreversible pathway for producing free sucrose. Subsequently, SuS catalyzes a reversible reaction in which sucrose is hydrolyzed into fructose and uridine diphosphate glucose (UDP-G). In addition, invertase (INV; EC 3.2.1.26) is involved in their reversible cleavage of sucrose (Cumino et al. 2002; Salerno and Curatti 2003). Both SuS and INV are assigned a role in breaking down sucrose under most physiological conditions in plant cells (Fig. 14.2).

It is well known that SPS is a key enzyme in the sucrose synthesis pathway. SPS catalyzes the reaction of S6P (sucrose-6-phosphate) formation from the substrate UDP-G and fructose-6-phosphate (F6P) (Leloir and Cardini 1955; Amir and Preiss 1982). SPS plays a physiological role by modulating photosynthetic carbon flux into sucrose. The activity of plant SPS is under complex regulation involving allosteric effectors, glucose-6-phosphate (G6P), and Pi. Plant SPS is activated by G6P in a concentration-dependent manner up to 5 mM. An increase of G6P concentration is correlated with increased sucrose formation and decreasing concentration of the

cytosolic Pi (Doehlert and Huber 1983; Huber and Huber 1996; Sawitri et al. 2016) (Fig. 14.2).

The active form of SPS occurred as a result of the dephosphorylated enzyme. It has been previously postulated that phosphorylation at certain serine residue modulates SPS activity in response to dark-light transition. Thus, the accumulation of G6P might be associated with light conditions. In order to determine the residues responsible for phosphorylation, alteration of a serine residue at position 162, which corresponds to residue S158 in spinach, has been attempted in sugarcane SPS. Substitution of S158 to alanine in spinach SPS showed consistency with dephosphorylated form and is not regulated by light modulation (Lunn et al. 1999; Toroser et al. 1999). However, there was no insight into the phosphorylation state of sugarcane SPS except that loss of S162 in the N-terminal domain deletion mutant has no significant effect on SPS activity (Sawitri et al. 2016).

The gene encoding SPSs have been successfully cloned from various C_3 and C_4 plants, such as Arabidopsis (Park et al. 2008) and maize (Worrell et al. 1991), respectively. In addition, the response of photosynthetic SPS is more sensitive to G6P rather than non-photosynthetic SPS. In some plants, including sugarcane, SPSs have different isomeric forms with different deduced amino acid sequences. The comparison between sugarcane SPS and SPSs from other species showed that *So*SPS1 has the highest homology of about 95% identical to maize SPS and less but significant homology to spinach SPS (56%), sugar beet SPS (56%), and potato SPS (55%). Sugarcane *So*SPS2 has significant homology to maize (50%), spinach (58%), sugar beet (57%), and potato (56%). The corresponding sequences revealed 49% identity between *So*SPS1 and *So*SPS2 (Sugiharto et al. 1997). Consequently, *So*SPS1 provides a potential application to be engineered since it has been considered as a representative enzyme for photosynthetic carbon allocation with the regulatory function (Sawitri et al. 2016).

The protein stability and abundance of *So*SPS1 in plant cells are relatively poor (Huber and Huber 1996). Therefore, constructing a recombinant protein expression system offers new prospects to enhance its protein production level for enzyme characterization and biotechnology application. Several studies reported the expression of plant and cyanobacteria SPS genes (Worrell et al. 1991; Sonnewald et al. 1993; Lunn et al. 1999; Chen et al. 2007) in *Escherichia coli* but resulting recombinant enzymes did not show a clear property of enzyme regulation. Previous reports revealed that deletion of the N-terminal domain tends to increase the specific activity by tenfold as compared to full-length plant SPS. Although N-terminal deletion in SPS is not allosterically regulated by G6P, the application of these mutants will be one of the strategies to increase the sucrose accumulation in sugarcane.

Many studies demonstrate to elucidate the role of SPS in controlling carbon partitioning in plants. Overexpression of SPS showed an increase of photosynthetic rate and sucrose: starch ratio in leaves of transgenic tomato (Worrell et al. 1991; Galtier et al. 1993) and Arabidopsis (Signora et al. 1998). Whereas, overexpression of SPS also contributes to enhancing sucrose accumulation in tomato fruit (Nguyen-Quoc et al. 1999), while overexpression of SPS in cotton resulted in improved fiber quality (Haigler et al. 2007). The effect of overexpressed SPS in plant growth and

biomass has also been investigated in transgenic Arabidopsis, poplar, and tobacco (Park et al. 2008; Maloney et al. 2015). These reports revealed that overexpression of SPSs affected not only increased sucrose accumulation in leaves but also played a pivotal role in starch metabolism and carbon partitioning in sink tissue.

It will also be interesting to determine the regulatory mechanism of *So*SPS1 involved in carbon partitioning. Carbon partitioning is a critical process in distributing chemical energy converted by the plant through photosynthesis. In sugarcane, overexpression of the *So*SPS1 gene revealed that SPS accumulation and its activity increased, followed by increased sucrose accumulation and improved growth traits, such as increased plant biomass in transgenic sugarcane. The elevated sucrose levels showed that SPS is not only modulating sucrose synthesis but also concomitant with degrading INV activity in the leaves (Anur et al. 2020). It suggested that INV controls the sucrose levels so as not to exceed the level of photosynthesis gene suppression; therefore, INV plays an important role in maintaining the balance between the sucrose signaling pathway and metabolism.

Although high sucrose content is accumulated in the sugarcane stalk, the sucrose translocation and accumulation mechanism remains unclear. Synthesis of sucrose is predominantly reported in leaves and translocated to the sink tissues through several types of sugar transporters, such as sucrose transporter (SUT) and SWEET proteins (Wang et al. 2013). Several studies showed that the overexpression of a sucrose transporter gene increased sucrose unloading and sink strength (Rosche et al. 2002; Cheng et al. 2018). Manipulation of the SUT and SWEET genes was reported to increase the SPS activity and sucrose unloading in the sink tissue (Lin et al. 2014). Multiple target genes are considered for genetic engineering to increase sucrose accumulation in sugarcane. Therefore, the engineering of sugar transporters in cooperation with increased SPS activity generates a new alternative for enhancing sucrose accumulation and improving crop yield, including sugarcane.

14.5 Biotechnology of Water Stress Tolerance

14.5.1 Biochemical and Molecular Aspects of Water Stress Responses

Water deficit or drought stress is one of the most important environmental factors limiting sugarcane growth and productivity. The drought stress significantly affects sugar production, which is determined by Brix, Pol, and reducing sugar (Begum et al. 2012). On the other hand, gradual water deficit during sugarcane maturation reduces growth but increases sucrose accumulation in the stem (Inman-Bamber and Smith 2005). A new perspective to the sugarcane production system has been reported that sugarcane previously exposed to drought stress will perform better under water stress on the next cultivation (Marcos et al. 2018). However, these controversial issues lead to studies on the effect of drought stress in sugarcane at biochemical and molecular levels.

Sugarcane is a C_4 plant and is considered to have a higher water use efficiency. The operation of the C_4 cycle with PEPC in mesophyll cell and Rubisco in bundle sheath cell generate suppression of photorespiration. PEPC is believed to have high affinity for CO_2 assimilation from the atmosphere and allows high-rate carbon assimilation when stomata are slightly closed (Lopes et al. 2011). During midday, with a high temperature and light intensity, the C_4 plants leaves are slightly rolling to reduce transpiration. The PEPC is a primary enzyme for carbon assimilation, and that activity is affected by water stress (Ghannoum 2009).

Measurement of the carbon assimilating enzyme activity showed that sucrose content and shoot dry weight fluctuated according to the SPS activity in *Saccharum* species (Sugiharto 2005). Furthermore, observation of sugarcane grown in the field revealed that the SPS activity, as well as sucrose contents, was higher in dry land than in wet land. The biochemical analysis found that halting the process of watering resulted in increased SPS activity and sucrose content in sugarcane leaves (Sugiharto 2018). The activity of SPS is enhanced by water stress due to covalent modification of the enzyme that is caused by protein phosphorylation of serine residue at position 424 (Huber and Huber 1996). In addition, identification of drought-response genes showed that water deficit is associated with changed gene expression associated with sucrose accumulation in sugarcane (Iskandar et al. 2011). These results suggested that sucrose may act as an osmoregulator and helped the sugarcane getting adapt to water deficit conditions.

Water deficit induces gene expression for the protein responsible for the drought stress tolerance in plants. The molecular study revealed that a drought-inducible protein named SoDip22 was identified in the water stress-tolerant sugarcane phenotype (Sugiharto et al. 2002). The amino acid sequence of SoDip22 exhibited similarity to ABA, stress, and ripening-inducible protein from various plant species. However, further study on the function of the protein on water stress response has never been conducted.

It is well established that drought stress regulates several genes expression, including the transcription factors (TFs) in plants. The TFs are the proteins that play the vital molecular switches of gene expression and regulate plant development in responses to various types of stress. The key TFs regulating drought-responsive gene transcription have been identified in plants such as MYB, MYC, DREB/CBF, ABF/AREB, NAC, and WRKY (Osakabe et al. 2014). For example, CBF/DREB1 and DREB2 from rice have been identified, and their overexpression improved drought tolerance in rice (Shinozaki and Yamaguchi-Shinozaki 2007). The overexpression of GmDREB1 from soybean consistently improved the yield performance of transgenic wheat when grown under limited water conditions in the field (Zhou et al. 2020). Recently, the TFs of R2R3-MYB have been identified and play a positive role in responding to drought-induced senescence in sugarcane (Guo et al. 2017). Therefore, the potential use of TFs families such as WRKY, NAC, MYB is an important clue for the engineering of stress-tolerant sugarcane (Javed et al. 2020). However, the research on TFs as a target to genetically engineer drought tolerance sugarcane is still meager. Most recently, it was reported that overexpression of AtBBX29, a member of B-box proteins, increased drought tolerance and delayed

senescence under the water deficit condition in transgenic sugarcane (Mbambalala et al. 2021).

Measurement of enzymes activity responsible for scavenger and detoxification of reactive oxygen species (ROS) during drought stress showed that superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) are higher in drought stress-tolerant plants as compared to sensitive sugarcane cultivars (Cia et al. 2012; dos Santos and Silva 2015). The activities of ROS scavenging enzymes were suggested as a marker of drought stress tolerance in sugarcane. Genetic engineering of ROS enzymes has been conducted to increase drought tolerance in plants but has not been developed in sugarcane.

14.5.2 Genetic Engineering to Enhance Glycine Betaine Biosynthesis

Plants have various strategies to survive under water deficit by inducing the accumulation of small molecules referred to as compatible solutes or osmoprotectants (Rhodes and Hanson 1993). Glycine betaine (GB) (*N,N,N*-trimethyl glycine) is one of the most studied osmoprotectants which helps plants to acclimate to drought conditions (Chen and Murata 2002). The GB stabilizes the structure of macro molecules and helps in the proper functioning of the cell membrane (Sakamoto and Murata 2002). The accumulation of GB has been reported in some species such as *Amaranthus*, sorghum, sugar beet under drought stress conditions, and that accumulation contributes to the acclimation of the plant cell to water stress (Bohnert et al. 1995). The addition of exogenous GB at 10 mM has been reported to increase the growth and yield of maize under salt-stress conditions (Yang and Lu 2005). However, the economic analysis and other disadvantages of the exogenous application should be well considered. Although the detailed function of GB has not been established well, the genetic engineering to enhance GB synthesis in sugarcane that naturally does not accumulate GB is discussed in this section.

GB is synthesized from two-step reactions, conversion of choline to betaine aldehyde and the betaine aldehyde to GB that is catalyzed by choline dehydrogenase (CDH) and betaine aldehyde dehydrogenase (BADH) in the microorganism and mammalian cells. In plants, the conversion of choline to betaine aldehyde is catalyzed by choline monooxygenase (CMO) and then converted into GB by BADH. In addition, a single-step reaction for the conversion of choline into GB by choline oxygenase (COD) was found in *Arthrobacter globiformis* and *Arthrobacter pascens*. Interestingly, the microbial CDH was found capable to catalyze two-step reactions, the oxidation of choline into BADH, and further conversion to GB (Cánovas et al. 2000). This result has been confirmed using purified CDH from *Halomonas elongata* that showed a similar substrate specificity to both choline and BADH (Gadda and McAllister-Wilkins 2003).

The genes involved in the pathway of GB biosynthesis have been cloned from various organisms. The genes for CDH and BADH referred to as *betA* and *betB* were isolated from *Escherichia coli* (Andresen et al. 1988) and bacteria *Halomonas elongata* (Gadda and McAllister-Wilkins 2003). The COD gene that catalyzes a

single-step reaction for GB synthesis was also successfully cloned from bacteria *Arthrobacter pascens* and *Arthrobacter globiformis* (Rozwadowski et al. 1991). In higher plants, genes for CMO and BADH have been isolated from GB accumulator species such as spinach, sugar beet, and amaranth (Rathinasabapathi 2000). The genes encoding for the pathway of GB synthesis from microorganisms have become a major target to develop environmental stress-tolerant plants. Genetic engineering of GB accumulation using the genes from microorganisms has been reported in tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), rice (*Oryza sativa*), and maize (*Zea mays*) (Quan et al. 2004).

The genetic engineering of GB biosynthesis has been conducted mainly with the gene for a single-step reaction of GB synthesis in plants. Overexpression of gene for COD targeted in chloroplast accumulated low level of GB in *Arabidopsis* (Hayashi et al. 1998) and rice (Sakamoto and Murata 1998). In addition, constitutive expression of bacterial COD in naturally lacking GB plant species also reported lower levels of GB. The low GB level in transgenic plants was caused by a low level of choline substrate in the site targeted GB synthesis. A substantial increase in GB content was obtained when the transgenic plants were supplied with choline or phosphocholine (Huang et al. 2000).

Introducing *betA* encoding for CDH from *E.coli* showed increased activity of CDH and resulted in salt tolerance in tobacco (Lilius et al. 1996) and maize (Saneoka et al. 1995) plants. The increased CDH activity elevated GB content which is correlated with the degree of salt tolerance. These results indicated that the GB plays a key role in osmotic adjustment. Furthermore, the overexpression of *betA* resulted in drought-tolerant maize, and the grain yield was significantly higher than the wild-type control after water-deficit conditions (Quan et al. 2004). A similar result reported that overexpression of the *betA* gene elevated the GB content and created drought tolerance in transgenic cotton (Lv et al. 2007). It was established that CDH from microorganisms has the capacity to catalyze the oxidation of choline to betaine aldehyde and further converted into GB (Cánovas et al. 2000). These results indicated that overexpression of the *betA* gene from microorganisms enhanced GB level and salt-drought tolerances and improved plant growth and productivity.

Genetic engineering to enhance GB synthesis and drought tolerance has been developed using the *betA* gene in sugarcane. The *betA* isolated from *Rhizobium meliloti* was constructed into the binary vector by Ajinomoto Co. Inc. Japan (Australian Patent Office No. 737600) and introduced into *Agrobacterium tumefaciens* LBA4404 for sugarcane transformation. The *Agrobacterium*-mediated transformation was performed using embryogenic explant callus in the Laboratory of Biotechnology PT. Perkebunan Nusantara XI in collaboration with Ajinomoto company and University of Jember. After selection using appropriate antibiotics, the selected sugarcane transformant was acclimatized in the greenhouse for further analysis.

PCR and Southern hybridization analysis showed stable betA gene integration in the transgenic sugarcane leaves genome. The GB content was elevated in the leaves of transgenic lines ranging from 182 to 880 ppm after drought treatment but not detected in the wild-type or non-transgenic sugarcane parental. The transgenic lines

showed prolonged wilting symptoms compared to the wild-type sugarcane after drought stress. Interestingly, the root morphology was longer and deeper distributed in the soil media, showing the character of drought-tolerant sugarcane (Smith et al. 2005). Moreover, the elevation of GB content also enhanced salt tolerance in the transgenic sugarcane lines. These results indicated that overexpressing the *betA* gene enhances GB content and helps the sugarcane to get adjusted to drought and salt stress.

Evaluation of the growth and productivity of the transgenic sugarcane lines were conducted in the confine limited field trial under the supervision of the Indonesian Genetically Modified Product Biosafety Commission. The stem internode of transgenic lines was grown normally and not affected by drought stress, but the internode of non-transgenic shortened due to growth retardation. Total cane yield significantly increased in the transgenic lines compared to the non-transgenic counterpart. The sugar production in the transgenic lines is 10–30% higher than wild-type parental sugarcane in non-irrigated dry land (Waltz 2014). The transgenic sugarcane has been completed with biosafety certifications by the Indonesian Biosafety Commission, released by the Indonesian Ministry of Agriculture (No 4571/Kpts/SR.120/8/2013), and cultivated by the sugarcane company.

14.6 Biotechnology of Virus Resistance

14.6.1 Viruses and Sugarcane Mosaic Disease

Mosaic diseases are a major constrain causing significant losses in sugarcane yield and have become a severe problem for sugarcane plantations. The disease reduces total leaf chlorophyll content and photosynthetic capacity, affecting sucrose accumulation and ultimately causing yield losses in sugarcane (Irvine 1971). The causal agents of mosaic disease in sugarcane are three viruses: SCMV, SrMV, and SCSMV.

SCMV and SrMV are classified into genus *Potyvirus* in the *Potyviridae* family based on serological tests and the host range of viruses (Hall et al. 1998; Gibbs and Ohshima 2010). SCMV and SrMV are naturally transmitted by aphids from plant to plant in a non-persistent manner (Gadhave et al. 2020). *Potyvirus* genome is positive single-strand RNA (+ ssRNA) attributed with genome-linked protein at 5′ terminal and poly-A tail at 3′ terminal (Gell et al. 2014). This RNA genome encoded a single large polyprotein, which is cleaved by self-encoded protease into ten individual mature proteins, i.e., protein 1 (P1), helper component proteinase (HC-pro), protein 3 (P3), 6 K protein 1 (6 K1), cylindrical inclusion protein (CI), 6 K protein 2 (6 K2), viral protein genome-linked (VPg), nuclear inclusion a (NIa) protein, nuclear inclusion b (NIb) protein, and coat protein (CP) (Revers et al. 2007). To distinguish between SCMV and SrMV, Yang and Mirkov (2007) developed RT-PCR coupled RFLP method. Sequence alignment confirmed the gaps and nucleotide differences between SCMV and SrMV at the 3′-terminal of the genome that spanned the Nib, CP, and 3′-untranslated regions. Two sets of specific primers were designed using

the gaps and nucleotide differences and then used in the RT-PCR coupled RFLP method to distinguish between SCMV and SrMV (Yang and Mirkov 2007).

SCMV and SrMV are major pathogens causing a severe threat to sugarcane plantations globally. SCMV could reduce sugarcane yield up to 45% in India for susceptible varieties. Mosaic diseases caused by SCMV are reported with an incidence of up to 78% in East Java and Indonesia (Addy et al. 2017). SCMV infection cases, including new strains or genome variation, are still reported from many countries, indicating that the virus is a severe problem in the sugarcane-based industry (Wu et al. 2012). While SrMV is the most common pathogen associated with sugarcane mosaic disease compared to SCMV in China. It is also reported that SrMV is a causal agent for mosaic disease in Louisiana, with incidences ranging from 0 to 10% (Rice et al. 2019). High incidence of coinfection of SCMV and SrMV was reported from China, in which coinfection resulted in heavy mosaic symptoms. In contrast, a single virus infection showed symptomatic or asymptomatic conditions indicating that coinfection is more virulent than a single infection (Xu et al. 2008). The incidence of coinfection of SCMV and SrMV is also common in Tucumán, Argentina (Perera et al. 2008). SCMV and SrMV are common pathogens for sugarcane and can also infect sorghum, maize, and Columbus grass (*Sorghum almum*) (Fan et al. 2003; Xu et al. 2010; Mollov et al. 2016; Klein and Smith 2020).

SCSMV was previously known as sugarcane mosaic virus-strain F (SCMV-F) and classified into genus Potyvirus in the Potyviridae family. The virus was identified from quarantined sugarcane exhibiting mosaic symptoms imported from Pakistan. The SCMV-F is transmitted from plant to plant in a mechanical mode rather than a vector-transmitted fashion (Damayanti and Putra 2010; Putra et al. 2015). Phylogenetic study shows that protein encoded by 3′ terminal sequence of the SCMV-F is highly similar to *Wheat streak mosaic virus* (WSMV) and *Brome streak mosaic virus* (BSMV). To reflect this similarity, the SCMV-F was renamed to *Sugarcane streak mosaic virus* (Hall et al. 1998). However, the serological test revealed no cross-reaction between SCSMV and members of *Potyvirus* (SCMV, SrMV) and *Rymovirus* (WSMV, BSMV). The genomic structure of SCSMV is identical to the member of the *Potyviridae* family, including *Ipomovirus*, *Potyvirus*, *Rymovirus*, and *Tritimovirus* (Xu et al. 2010). However, the sequence similarity of SCSMV and potyviral-related genera was comparatively low, indicating that SCSMV does not belong to *Potyvirus* and should be classified into a new genus in the family *Potyviridae* (Rabenstein et al. 2002; Viswanathan et al. 2008a). International Committee on Taxonomy of Viruses (ICVT) has designated *Poacevirus* as the new genus name for SCSMV, *Triticum mosaic virus*, and *Caladenia virus A* (Wylie et al. 2017). The identification of SCSMV from an unknown field sample or germplasm is carried out using an RT-PCR-based method using a specific primer amplified CP region at the 3′ end of the virus genome (Hema et al. 2003).

Mosaic diseases caused by SCSMV infection are mostly reported from Asian countries such as India (Chatenet et al. 2003), China (Li et al. 2011; He et al. 2013), and Indonesia (Damayanti and Putra 2010), but recently, it was also identified in Côte d'Ivoire, Africa (Sorho et al. 2020). SCSMV was observed in 30% sugarcane fields across Java, Indonesia, and found to reduce sugar yield by about 20% in highly

susceptible varieties (Putra et al. 2014, 2015). Sugarcane mosaic diseases caused by a single infection of SCMV rarely occur (Xu et al. 2008). SCSMV predominantly infects sugarcane in a coinfection manner with SCMV (Rao et al. 2006) and SrMV (Luo et al. 2016). Coinfection is a common incident that causes mosaic disease, so that method is required to identify several viruses simultaneously. An RT-PCR-based method was developed by designing two primer sets in the single tube RT-PCR reaction (Duplex RT-PCR) to identify 860 bp and 690 bp coat proteins corresponding to SCMV and SCSMV, respectively (Viswanathan et al. 2008b). Similarly, Feng et al. (2020) also developed multiplex RT-PCR methods to identify multiple viruses in a single tube reaction from sugarcane samples (Feng et al. 2020).

14.6.2 Strategy to Develop Virus-Resistant Plants

The mosaic disease is reported globally and has become a severe threat to sugarcane plantations. SCMV, SrMV, and ScSMV are the primary causative agent of mosaic disease, in which a single dominant virus infection or mixed infection occurs depending on time and place. SCMV infection is dominant in India, China, and Indonesia in the 1980s. In recent years, the mixed infection has been frequently observed in China (SCMV-SrMV and SrMV-SCSMV) (Xu et al. 2008; Luo et al. 2016), India (SCMV-SCSMV) (Rao et al. 2006), and Indonesia (SCMV-SCSMV) (Putra et al. 2015; Addy et al. 2017). SCMV is still the most severe and prevalent virus observed in sugarcane plantations worldwide. Aphids naturally spread SCMV and SRMV, so they are more easily transmitted than SCSMV, which are mechanically transmitted.

SCMV and SrMV are transmitted by aphids in a non-persistent manner, while SCSMV spreads from plant to plant in a mechanical manner. Controlling the dispersal of the virus using chemicals is impossible, and regulating aphids as vectors is impractical (Wu et al. 2012). Therefore, the cultivation of resistant varieties is the most effective way to control the mosaic disease (Gonçalves et al. 2012). Natural resistance traits to SCMV, SrMV, and SCSMV were exploited from sugarcane germplasm and may serve as basis of sugarcane breeding programs (Li et al. 2018a, b). However, introducing resistance traits to the elite sugarcane cultivar by conventional breeding is complicated due to its poor fertility and complex polyploid-aneuploid genome, resulting in an extended breeding period (Lakshmanan et al. 2005). Therefore, genetic engineering has become an essential tool to introduce virus resistance traits into elite sugarcane cultivars by utilizing molecular approaches, such as PDR, RNAi, and CRISPR/Cas9.

The first genetic engineering approach in sugarcane to introduce virus resistance traits is PDR. This approach uses a sequence from the pathogen's genome and is introduced into the plant's genome under the control of a specific promoter. The most widely used viral sequence in PDR is coat protein. Joyce et al. (1998) used CP sequence under the control of either Emu (synthetic promoter) or Ubi (ubiquitin promoter) and introduced it into sugarcane using particle bombardment. Only one line from the Emu transgenic line showed resistance to SCMV. In comparison, Ubi

Table 14.1 Biotechnology approach used for generating virus-resistant sugarcane

Virus target	Molecular approach	Viral genetic component	References
SCMV	PDR	Coat protein	Joyce et al. (1998)
SrMV	RNAi	Coat protein	Ingelbrecht et al. (1999)
SrMV	RNAi	Coat protein	Guo et al. (2015)
SCMV	RNAi	Coat protein	Aslam et al. (2018)
SCMV	PDR	Coat protein	Apriasti et al. (2018)
SCMV	RNAi	Coat protein	Widyaningrum et al. (2021)

transgenic plants indicated ten lines of resistance, four lines with a mild symptom, and ten lines with the ability to recover from the SCMV infection in the challenge test. The data indicated that the CP sequence in the sugarcane genome could confer resistance to SCMV infection (Joyce et al. 1998). The promoter also plays an essential role in controlling CP expression to acquire resistance.

Apriasti et al. (2018) compared the efficiency of complete (927 bp) and N-terminal truncated (702 bp) sequence of CP gene to induce PDR against SCMV infection in sugarcane. Both sequences were introduced into sugarcane via Agrobacterium-mediated transformation. The complete and truncated CP genes were expressed at protein levels in the transgenic sugarcane. As a result, the complete sequence of CP generated a higher resistance to SCMV infection than its truncated version, indicating that complete coat protein is possibly required for effective blockage of viral disassembly and replication (Apriasti et al. 2018).

RNAi was more widely used than PDR to introduce virus resistance traits in sugarcane. The RNAi mechanism is initiated by the formation of siRNA, which plays a role in degrading viral RNA through a complex process known as PTGS. The siRNAs are processed from dsRNA, generated from hairpin repeat, sense, and antisense RNA. Ingelbrecht et al. (1999) have constructed an untranslatable sense CP gene from SrMV cassette driven by ubiquitin promoter. The cassette was transformed into sugarcane using particle gun bombardment to generate transgenic plants. In the SrMV infection test, plants with susceptible phenotype, recovery phenotype, and completely resistant phenotype were observed among transgenic plants (Table 14.1). The resistant plants show a high transcription rate of CP transgene, but its mRNA levels are low or undetectable (Ingelbrecht et al. 1999). Probably, the gene silencing machinery processed mRNA of CP transgene immediately into siRNA so that the mRNA was undetectable. The untranslatable form of sense CP can induce virus resistance in sugarcane through the PTGS mechanism.

Guo et al. (2015) constructed 423 bp CP gene from SrMV in hairpin structures driven by CaMV35S promoter in RNAi vector. The RNAi construct was delivered to sugarcane callus via agrobacterium-mediated transformation to generate a stable transgenic plant. The transgenic plant with the interference sequence shows a resistance rate of 87.5% in the artificial SrMV inoculation challenge (Guo et al. 2015). Aslam et al. (2018) engineered a stable short hairpin (shRNA) carrying siRNA driven by polyubiquitin promoter for targeting the CP gene of SCMV. The shRNA constructs were introduced into sugarcane callus via particle bombardment

to generate a transgenic plant. Upon SCMV virus inoculation challenge, the transgenic sugarcane shows virus RNA reduction ranging from 10 to 90%, indicating that most transgenic sugarcane lines expressing shRNA were resistant to SCMV infection (Aslam et al. 2018).

Widyaningrum et al. (2021) compared the effectivity of CaMV35S and ZmUbi promoter in controlling the hairpin structure of CP (997 bp) to induce resistance against SCMV infection. Both the CaMV35S and ZmUbi promoters driving the CP hairpin structure were introduced to sugarcane by Agrobacterium-mediated transformation. In the SCMV infection test, hairpin CP driven by the CaMV promoter generated 57.69% resistant lines, whereas the ZmUbi promoter generated 82.35% resistant lines. The result indicated that the ZmUbi promoter is more effective than the CaMV35S promoter in driving CP RNAi expression to induce SCMV resistance in sugarcane (Widyaningrum et al. 2021).

Recently, Hidayati et al. (2021) performed a comparative study examining the efficacy of PDR and RNAi in generating sugarcane resistance against SCMV infection and found that RNAi is more effective than PDR (Hidayati et al. 2021). This finding implies that gene silencing-induced virus RNA degradation is more effective in combating virus attack than inhibiting virion disassembly by coat protein. Possibly, sugarcane carrying RNAi of coat protein accumulated a high level of siRNA inducing virus RNA degradation that operates by a mechanism similar to PTGS. In agreement with this hypothesis, the use of RNAi in downregulating endogenous genes is more efficacious than co-suppression because of its effectiveness in producing dsRNA and siRNA to trigger gene silencing (Stoutjesdijk et al. 2002).

14.7 Conclusion

A new sugarcane cultivar with essential traits such as drought tolerance, disease resistance, and high biomass yield has been developed employing novel strategies using biotechnological approach. To develop drought and stress-tolerant sugarcane, overexpression of the gene encoding bacterial *bet*A for increasing betaine content helps sugarcane to acclimate to water deficit environment. In addition, pathogen-derived resistance (PDR) and RNA interference (RNAi) technologies have been applied to engineer sugarcane cultivars having resistance to mosaic virus. Along with improving sugarcane traits through abiotic stress tolerance and biotic stress resistance, improving the efficiency of carbon partitioning by genetic engineering of SPS can be utilized as an essential strategy for increasing yield and biomass allocation.

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Biotic Stresses in Sugarcane Plants and Its Management

15

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Abstract

Sugarcane is a strategic cash crop having a deep impact on social and governmental issues on many people around the globe. Rapid climatic change and intensification as mono-culture cropping of sugarcane, world trade, and extensive use of chemical products have amplified the risk of regular recurrence of disease/pest outbreaks and incursions. Any sugarcane variety development program must consider adaptation to biotic stressors. Understanding the causes of biotic stress resistance implies knowledge of sugarcane taxonomy. Various wild species are still being studied for their ability to withstand biotic stresses. The major issues involving the most widely spread diseases, such as ratoon stunting, rust, and smut, as well as its history and explanation, have been thoroughly examined.

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Plants respond to pathogen infection by upregulating the expression of glucanases, chitinases, thaumatins, peptidase inhibitors, defensins, catalases, and glycoproteins, among other proteins. Pathogen-induced proteins are engaged in plant defense either directly or indirectly, resulting in pathogen death or generating additional plant defense responses. Effective management of pests/diseases in sugarcane agroecosystems is based on integrated crop managing scenarios. This chapter focuses on agricultural practices and their influence on pests/disease, biological, chemical control, transgenic varieties, and the use of GIS in sugarcane integrated pest management.

Keywords

Biotic stress · Biocontrol · Biotechnological approaches · Diseases · Sugarcane

15.1 Introduction

Sugarcane is a key commodity and bioenergy sourced crop in tropical and subtropical countries and is afflicted by a variety of diseases and pests (Viswanathan 2020). Sugarcane is currently susceptible to about various type of diseases caused by pathogens such as fungi, viruses, bacteria, phytoplasma, and nematodes and cause severe losses globally (Bhuiyan et al. 2021). Various conditions such as red rot, wilt, pokkah boeng, and smut caused by fungi; ratoon stunting disease (RSD) and leaf scald disease (LSD) caused by bacteria; grassy shoot disease (GSD) and white leaf disease (WLD) caused by phytoplasmas; and different types of mosaics and leaf yellows caused by viruses have plagued the world since last 100 years (Viswanathan and Rao 2011; Srivastava 2014; Sharma et al. 2019; Holkar et al. 2020). As a result, the world suffers tremendous economic losses due to the emergence and re-emergence of different diseases (Manavalan 2021; Verma et al. 2021; Song et al. 2021).

15.2 Fungal Diseases of Sugarcane

Red rot of sugarcane was first recorded in Java more than 100 years back (Went 1893). It is one of the most devastating sugarcane diseases in Asia, Argentina, the USA, and other countries (Viswanathan 2021). The key diagnostic sign to signify the development of the disease in the stalk at later stages is reddening the interior tissues with alternating red and white patches (with an alcoholic odor) (Hossain et al. 2020). Red rot-affected canes cause about 29–83% weight and 24–90% juice loss (Viswanathan 2010). Sugarcane yield is reduced by 5–50% due to this disease, and only 31% sugar recovered (Ghazanfar and Kamran 2016). The red rot decreases the quality of sugarcane juice (such as sucrose concentration, purity, and Brix) and commercial cane sugar (Thangamanil et al. 2013) (Fig. 15.1). This disease is the most severe disease, with its destructive effects being the primary reason for the

Fig. 15.1 Effects of red rot disease on the stalk and infected field in India. (Photo credit: A. K. Tiwari)

elimination of numerous sugarcane varieties from cultivation around the world (Malathi et al. 2002; Tiwari et al. 2010; Singh et al. 2014; Hossain et al. 2020). During the 2020–2021 season, roughly 0.5 mha out of total 2.6 mha in Uttar Pradesh had severe red rot (Viswanathan 2021).

Sugarcane smut caused by *Sporisorium scitamineum* (Syn. *Ustilago scitaminea*) is recognized by the emergence of a long, elongated whip-like structure from the growing point of shoots or new tillers covered with black spores, and later, the affected plant tillers develop profusely with bearing spindly and erect shoots (Ramesh Sundar et al. 2012; Amrate et al. 2019). This disease led to severe losses to yield and juice quality (Viswanathan et al. 2009; Srivastava et al. 2016). Significant quantitative yield losses and cane quality reduction can occur due to whip smut disease in sugarcane (Ferreira and Comstock 1989; Indi et al. 2012; Amrate et al. 2019).

The fungus that causes the wilt disease in sugarcane stalks is *Fusarium sacchari*, which significantly impacts cane yield and productivity (Viswanathan 2020). First described by Butler and Khan (1913) explained that wilt is also major cane productivity affecting sugarcane disease. Many commercial cultivars were withdrawn from cultivation due to disease epidemics in the previous century (Subba Raja and Natarajan 1972; Singh and Singh 1974; Viswanathan and Rao 2011). Another disease of sugarcane caused by different *Fusarium* species is pokkah boeng. Pokkah boeng (a Javanese term) was first described in Java by Walker and Went in 1896 (Wang et al. 2017). It is a re-emerging sugarcane disease that has recently been discovered to inflict significant crop losses in most sugarcane-producing countries, including India, South Africa, Malaysia, and China (Lin et al. 2014; Tiwari et al.

2021). The disease is caused by *Fusarium* spp. with various workers reporting *F. moniliformae*, *F. sacchari*, *F. verticillioides*, *F. proliferatum*, *F. fujikuroi*, and *F. andiyazi* being the most common species found in different parts of the world (Vishwanathan et al. 2011; Kumar et al. 2018). Pokkah boeng is now a severe fungal infection that affects sugarcane worldwide (Siddique 2007; Srivastava et al. 2019, 2020). Disease severity in different sugarcane cultivars ranged from 5 to 90% (Vishwakarma et al. 2013). It can result in a significant quality decline in high sugar-yielding cultivars, decreasing the sugar content by 40.8–64.5% (Siti Nordahliawate et al. 2008; Tiwari et al. 2021).

Pineapple disease is one of the most damaging diseases that affect sugarcane (Talukder et al. 2007), specifically known to damage the plant's root system (Vuyyuru et al. 2019). The pathogen of the disease is *Thielaviopsis paradoxa* (Borges et al. 2019). The fragrance of a matured pineapple from the infected cane setts gave rise to the name pineapple disease (Chhama et al. 2014). This odor is caused due to the synthesis of ethyl acetate because of the metabolic activities of the causative agent, *Ceratocystis paradoxa* (Chhama et al. 2014). Pathogen invades cane pieces through the cut ends, producing seed deterioration and irregular germination widespread in sequentially planted sugarcane soils (Raid and Rott 2018). South Africa, China, the Philippines, Colombia, Mexico, Cuba, India, the Dominican Republic, and Haiti have recorded the incidence of the disease (Tiwari et al. 2012; Farr and Rosmman 2018). In Florida, an outbreak was reported to destroy sugarcane stands (Raid 1998). The disease is seen in most areas of Brazil, where sugarcane is cultivated for industrial purposes. The research found that this disease could reduce sprouting by 50%, lowering sugarcane yields by 31–42% in a sugarcane field (Chapola et al. 2014).

15.3 Bacterial Diseases of Sugarcane

Leaf scald disease, caused by *Xanthomonas albilineans* (Ashby) Dowson, is one of the most important bacterial diseases of sugarcane, with significant economic implications for sugarcane industries around the world (Rott and Davis 2000a). The production of interspecific hybrids decreased the disease's significant impact (Govindaraju et al. 2019). Leaf scald produces large losses in tonnes of cane per hectare and lowers juice quality, particularly in the ratoon crop (Ricaud and Autrey 1989; Rott and Davis 2000a; Gutierrez et al. 2018; Tiwari et al. personal communication). *X. albilineans* colonizes the vascular system of sugarcane leaves and stalks, but it may also infect sugarcane parenchyma cells, which sets it apart from other bacterial diseases with a closed similar genome (Mensi et al. 2014). According to a recent study, antibiotic therapies can help manage the sugarcane plant's condition at an early stage of the disease (Tiwari et al. unpublished).

Acidovorax avenae causes red stripe (RS) and top rot (TR) symptoms in sugarcane (*Saccharum* spp. hybrids) (Hernández-Juárez et al. 2021). Stripes emerge along with the leaves of diseased sugarcane plants, which later turn into a crimson stripe with top rot. RS and TR can occur separately or concurrently in a single plant under

specific environmental factors, viz. humidity and temperature (Rakesh and Bipen 2015). Increased prevalence and severity of red stripe disease have contributed to global economic losses in the recent decade (Rott and Davis 2000b). Climate change promotes infection and the dissemination of the pathogen to new sugarcane plantation regions, which aids disease development (Yonzone and Devi 2018). Furthermore, innovative production techniques (Fontana et al. 2016), the use of susceptible cultivars, and the emergence of virulent and aggressive pathogen strains have all had a role in the disease prevalence (Fontana et al. 2013; Grisham and Johnson 2014). In vulnerable cultivars, the disease causes loss of sugarcane stems for grinding or milling, limiting output, and affecting sugarcane juice quality (Fontana et al. 2013, 2016).

Ratoon Stunting Disease (RSD), caused by the bacterium *Leifsonia xyli* subsp. *xyli* (*Lxx*) lowers sugarcane yield by inhibiting culm growth and tillering and is particularly severe in plants with high *Lxx* titers (Garcia et al. 2021). Ratoon stunting is one of the most serious diseases affecting sugarcane production worldwide, with yield losses ranging from near zero to 30% or more depending on variety and growth conditions as per the observations recorded by several sugarcane pathologists (Davis and Bailey 2000; Comstock 2002; Rott et al. 2018). Ratoon stunting (RS) is a severe threat to all sugarcane-cultivating countries worldwide. Although this disease was initially depicted in Australia in 1944, its actual cause was not discovered until 1980 (Teakle et al. 1973; Davis et al. 1980). Annual losses due to RSD have been reported to vary from 1 to 11 million US dollars (Fegan et al. 1998; Croft 2002; Urashima et al. 2017). Its prevalence in commercial sectors has now been higher than 60% in Brazil and China (Urashima and Marchetti 2013; Fu et al. 2016). Ratoon stunting disease causes reduced yields by lowering stalk weight and number (Steindl 1950), albeit not all stalks within a stool, nor stools within a crop, are diseased, resulting in a patchy appearance (Young 2016).

15.4 Phytoplasma Disease of Sugarcane

Because of the overall reduction of millable cane yield, Sugarcane Grassy Shoot Disease (SCGS) is considered the most damaging (Kadirvel et al. 2020). The disease is predominantly documented in South and South-East Asian countries (Gautam et al. 2019). SCGS infection is associated with *Candidatus* Phytoplasma sacchari, a member of 16SrXI group phytoplasmas spread by different species of leafhoppers (Tiwari et al. 2016, 2017a). Based on its symptomatology, it is known as grassy shoot in India, Pakistan, and white leaf in Thailand, Vietnam, Myanmar, China and known by both names in Sri Lanka. The grassy shoot disease has been reported to contribute losses of 5–20% in the main crop, and these losses are up to 100% in ratoon crop (Rao et al. 2008; Viswanathan and Rao 2011; Tiwari et al. 2012; Iqbal et al. 2015; Anuradha et al. 2019). Primarily SCGS infected plants are limited in number, but incidence increases by up to 60–80% in ratoon crops through secondary

spread by insect vectors (Srivastava et al. 2006; Rao et al. 2014; Anuradha et al. 2019; Sharma et al. 2020). Because sugarcane is a vegetatively propagated crop, the disease spreads through seed and phloem-feeding leafhoppers (Kavakita et al. 2000). *Saccharosydne saccharivora, Matsumuratettix hiroglyphicus* (Hanboonsong et al. 2002), *Deltocephalus vulgaris* (Srivastava et al. 2006), and *Yamatotettix flavovittatus* (Hanboonsong et al. 2006) have been identified as vectors for this phytoplasma disease of sugarcane. The use of hot water-treated propagating materials, substituting resistant cultivars, and implementing enhanced and specific agronomic approaches to manage this phytoplasma disease is suggested.

15.5 Viral Diseases of Sugarcane

A variety of virus species afflicts sugarcane, including Sugarcane yellow leaf virus (SCYLV), which causes yellow leaf disease (YLD); sugarcane streak virus (SSV), which causes streak disease; sugarcane Fiji disease virus (SFDV), which induces the famous Fiji disease; sugarcane bacilliform virus (SCBV), which causes fleck leaf disease (Braithwaite et al. 1995); sugarcane streak mosaic virus (SCSMV) and sugarcane mosaic virus (SCMV) (Rott and Davis 2000a; Singh et al. 2009; Viswanathan and Rao 2011) are associated with mosaic disease (Holkar et al. 2020). The detailed information on critical viral diseases of sugarcane is mentioned in Table 15.1.

Sugarcane mosaic virus (SCMV) is found all over the globe as one of the most common viral diseases of sugarcane. After invading sugarcane, the virus causes systemic damage (Wu et al. 2012; Lu et al. 2021). Mosaic symptoms appear on infected plants, most noticeable on the lowest section of the younger leaves. Extremely vulnerable cultivars have pronounced chlorosis, accompanied by a red striped pattern (Signoret 2008). SCMV is spread via aphids and mechanical means. Young leaf spots and brilliant green or yellow-green leaf spots are important diagnostic symptoms of this disease (Sivanesan and Waller 1986; Mishra et al. 2010).

Sugarcane yellow leaf virus (SCYLV) is a member of the Luteoviridae family's Polerovirus genus. SCYLV is a serious constraint on sugarcane yield worldwide, and it is currently present in most sugarcane-growing countries (Holkar et al. 2020). Yellow leaf disease (YLD) is a newly discovered sugarcane disease that substantially impacts sugarcane productivity in all sugarcane-growing regions globally. Yellow leaf disease (YLD) of sugarcane was initially documented in 1989 on variety H65-0782 in Hamakua (Hawaii) as yellow leaf syndrome (Schenck 1990) and has since spread to the United States mainland (Comstock et al. 1994) and many other sugarcane-growing countries. The disease has been documented in more than 30 countries around the world (Lockhart and Cronjé 2000; Schenck 2001). The severe prevalence of YLD on numerous sugarcane cultivars resulted in crop losses of up to 50% in Brazil, 37% in Reunion Island, 30% in Thailand, and 15% in the United States (Holkar et al. 2020). Due to its extensive incidence of YLS in India, the

Table 15.1 Major viral diseases of sugarcane globally

Name of the disease	Virus involved	Family of virus	Report	Genetic material	Transmission	Mode	Yield losses	References
Sugarcane mosaic disease	Sugarcane mosaic virus (SCMV) and sorghum mosaic virus (SrMV)	Potyviridae	The disease was first described by Musschenbroek (1893) in Java as "yellow stripe disease"	Positive-sense ssRNA	Several species of aphids or by planting infected stalks	Non-persistent manner	10–50% or even 60–80% yield loss has been reported worldwide	Musschenbroek (1893); Viswanathan and Balamuralikrishnan (2005); Lu et al. (2021)
Sugarcane yellow leaf disease (YLD)	Sugarcane yellow leaf virus (SCYLV)	Luteoviridae	Reported from Hamakua (Hawaii) in 1989 on variety H65-0782	Positive-sense ssRNA	Several species of aphids, viz. *Melanaphis sacchari*, *Ceratovacuna lanigera*, *Rhopalosiphum maidis*, and *R. rufiabdominalis* or by infected to healthy sugarcane	Persistent, circulative, and non-propagative manner	SCYLV has been linked to yield losses of 11–50% in commercial fields, as well as a negative impact on sugarcane growth	Mishra et al. (2010); Boukari et al. (2019); Holkar et al. (2020)
Sugarcane streak mosaic disease (SCSMD)	Sugarcane streak mosaic virus (SCSMV)	Potyviridae	Firstly reported by Hall et al. (1998)	Positive-sense ssRNA	Infected cane cuttings and mechanically by sap inoculation are effective ways to spread the virus, or it can easily be transmitted by using the cutting knife and other agricultural tools	–	Cane tonnage and sugar yield are both reduced by 16–17% and 19–21%, respectively, due to this disease	Putra et al. (2014); Daugrois et al. (2020)

(continued)

Table 15.1 (continued)

Name of the disease	Virus involved	Family of virus	Report	Genetic material	Transmission	Mode	Yield losses	References
Sugarcane Fiji disease (SFD)	Sugarcane Fiji disease virus (SFDV)	Reoviridae	Firstly, described by Egan and Ryan (1986)	Positive-sense dsRNA	Transmitted by the planthopper *Perkinsiella saccharicida* and by planting infected seed cane	Persistent and propagative manner	This viral disease can result in 100% yield loss and ratoon crop failure in vulnerable types. The yield loss is proportional to the percentage of infected plants	Zhang et al. (2021)
Sugarcane bacilliform virus disease (SCBVD)	Sugarcane bacilliform Guadeloupe A virus (SCBGAV), sugarcane bacilliform Guadeloupe D virus (SCBGDV), sugarcane bacilliform MO virus (SCBMOV), and sugarcane bacilliform IM virus (SCBIMV)	Caulimoviridae	It was first reported in Cuba (Rodriguez-Lema et al. 1985) and was later purified in 1988	dsDNA	Insect vectors include the mealy-bug species *Saccharicoccus sacchari* and *Dysmicoccus boninsis*, as well as crude virus-containing sap and *Agrobacterium*-mediated inoculation in the laboratory	Semi-persistent manner	Fresh yield reduction of up to 93–98%	Rodriguez-Lema et al. (1985); Lockhart and Autrey (1988); Lockhart et al. (1996); Ahmad et al. (2019)

severity of this disease in sugarcane fields intensify the reduction of cane quality (Bertasello et al. 2021). During the last two decades, notable research has been conducted on diagnostics employing cutting-edge molecular techniques, genome characterization, genetic diversity, and management through meristem tip culture and a three-tier seed production program (Holkar et al. 2020).

Sugarcane streak mosaic virus (SCSMV) is a member of the family Potyviridae that induces pale green symptoms on sugarcane leaves. It was initially discovered by Hall et al. (1998) in quarantined germplasm material transported from Pakistan exhibiting mosaic symptoms. Since then, SCSMV has been recorded in several Asian nations, including Bangladesh, India, Indonesia, Iran, Sri Lanka, Thailand, Vietnam, and China, but no cases have been documented beyond Asia (Chatenet et al. 2005; Xu et al. 2010; Putra et al. 2014; Sorho et al. 2020). Streak mosaic is a severe threat to the sugar industry as a whole, and it has to be investigated more since it could disrupt sugarcane crops and local economies (Sorho et al. 2020). The rate of SCSMV infection increased as well, with disease incidence varying from 0.44 to 86.75%. SCSMV spreads quickly due to its transmissibility through cane cuttings and the movement of planting materials from one location to another, regardless of the health of the cane cuttings (Putra et al. 2015).

Sugarcane Fiji disease virus (SCFDV) (previously known as Fiji disease virus) causes Fiji leaf gall (FLG) (erstwhile known as Fiji disease). It is one of the most important sugarcane diseases in Australia and several other sugar-producing areas of Asia and the Pacific region (Smith and Candy 2004). SCFDV is a dsRNA virus belonging to the genus Fiji virus of the Reoviridae family (Matthews 1982). Sugarcane infected with SCFDV develops leaf galls and deformation, which leads to the death of meristematic tissue and stunting, resulting in significant productivity losses (Egan and Ryan 1986). SCFDV was found in gall and non-gall tissues. However, gall tissue had more viruses than non-gall tissue (Dhileepan et al. 2006). Fiji disease is treated by identifying and exploiting plant resistance (Egan and Fraser 1977; Egan and Ryan 1986; Ryan 1988).

Sugarcane bacilliform viruses (SCBV) are a genetically diverse badnavirus species complex that infects sugarcane. The International Committee on Taxonomy of Viruses (ICTV) has classified four badnaviruses as separate species in the badnavirus genus: sugarcane bacilliform Guadeloupe A virus (SCBGAV), sugarcane bacilliform Guadeloupe D virus (SCBGDV), sugarcane bacilliform MO virus (SCBMOV), and sugarcane bacilliform IM virus (SCBIMV) (Adams and Carstens 2012; Geering and Hull 2012; Adams et al. 2016). SCBV (Sugarcane Bacilliform Virus) was initially discovered in sugarcane in Cuba in 1985 and many other sugarcane-growing nations (Autrey et al. 1995). It causes symptoms such as mottling, chlorosis, and leaf freckles. However, many diseased plants are asymptomatic (Fig. 15.2). SCBV-infected sugarcane had very low juice content, sucrose content, gravity, purity, and stalk weight (Li et al. 2010).

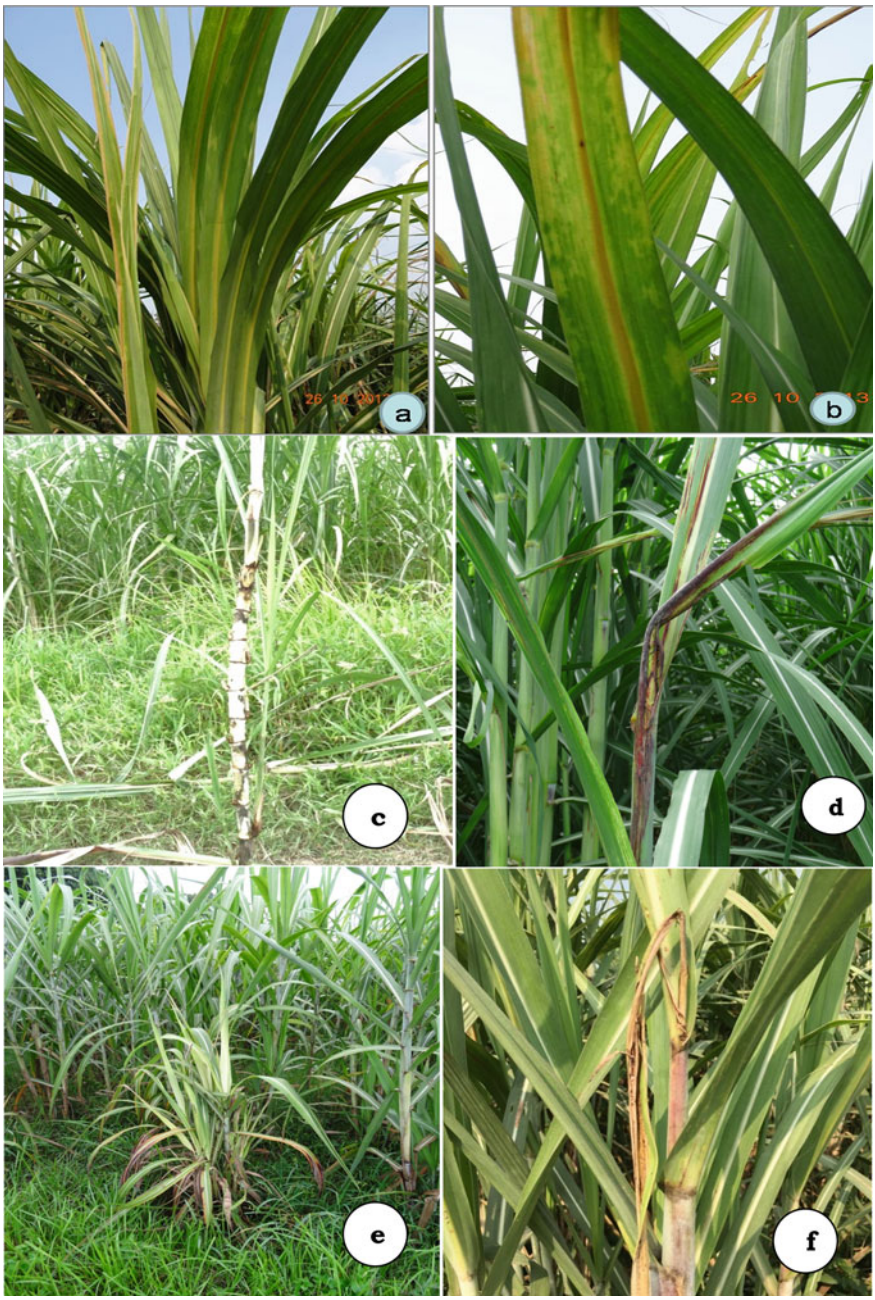


Fig. 15.2 Symptoms of some sugarcane plant diseases such as (**a**, **b**) sugarcane yellow leaf virus, (**c**, **d**) sugarcane leaf scald, (**e**) sugarcane red stripe, and (**f**) sugarcane grassy shoot disease (GSD)

15.6 Management of the Sugarcane Diseases Through Biotechnological Approaches

Because biotic stress restricts plants' normal physiological and metabolic processes, it thereby acts as a serious barrier in sugarcane production. Disease-related crop losses appear as lower yields, worse quality produce, and less post-harvest storage. Research has shown infections' ongoing ability to evolve new pathotypes and strains, some of which are break-resistant kinds or less vulnerable to chemical management. Farmers are presently recommended to combine multiple plant disease management measures into an integrated plant disease management approach (He et al. 2021). Cultural control, the use of disease-free material, resistant types, physical control, biological control, and fungicidal control are examples of such measures. However, some of these technologies are costly and significantly raise production costs. Plant disease management has benefited from advances in molecular biology and biotechnology. It includes everything from detection through control, including gene transfer, mutation breeding, and RNA interference, among other things. The present breakthroughs in the applications of molecular techniques and biotechnology to treat plant diseases are discussed in this study and their potential for future applications and improved plant disease management (Dayou et al. 2018). New, more accurate molecular techniques emerged over time. Proteomics, metabolomics, transcriptomics, plant tissue culture, and genetic engineering are only a few examples. Gene transfer, gene silencing, mutation breeding, and transcription factor modulation are all examples of genetic engineering (Sankaran et al. 2010; Ocsoy et al. 2013; Mahlein 2016).

15.6.1 Physical Management

To prevent seed-borne diseases, sugarcane seed should be treated with hot water (Table 15.2). This treatment aids in reducing seed-borne diseases caused by some fungi like *Colletotrichum* spp. and by bacterial pathogens (*Pseudomonas* spp., and *Xanthomonas* spp.). However, to keep seed viability, the temperature and time intervals must be rigorously adhered to. It is a good idea to test the germination of hundreds of heat-treated and hundred untreated seeds to ensure that the seed is not damaged. For a long time, disease-free plant propagation materials have been obtained by hot air and hot water treatment (Damayanti and Putra 2010). Practically, it was proved by many scientists that the efficiency in eradicating all infections improves by combining plant tissue culture and chemotherapy with hot water treatment (Mink et al. 1998).

15.6.2 Biocontrol of Sugarcane Diseases

The fact that red rot and wilt diseases are soil (debris) and sett-borne favors the accumulation of pathogenic inocula during epidemics of these diseases

Table 15.2 Hot air/hot water treatment of some important diseases of sugarcane

Disease name	Causal organism	Physical management		Reference
		Hot air treatment	Hot water treatment	
Red rot	*Colletotrichum falcatum*	Sett transmitted diseases can be entirely eradicated by using moist hot air therapy (54 °C for 3 h and RH of 95%). The use of moist hot air at 54 °C for 2 h was more successful in preventing red rot	The pathogen can be removed from contaminated setts using an aerated stream at 52 °C or a sett soaked in cold running water for 48 h followed by 150–180 min of hot water treatment at 50 °C	Stoll et al. (2008); Talukder et al. (2010); Hossain et al. (2020)
Whip smut	*Sporisorium scitamineum*	Hot air treatment at 54 °C for 2 h 30 min	Hot water treatment at 50 °C for 45 min (2 h)	Varma et al. (2020a, b); Bhuiyan et al. (2021)
Pineapple disease	*Ceratocystis paradoxa*	–	During late planting, soak setts in hot water for 30 min at 50–51 °C	Wijeratnam et al. (2005)
Sugarcane leaf scald	*Xanthomonas albilineans*	–	Planting materials are disinfected by using hot water treatments (seed cane). To manage leaf scald bacteria before planting, soak setts for 40 h in ambient-temperature flowing water followed by 3–4 h at 50 °C can give 95% management	Govindaraju et al. (2019)
Ratoon stunting	*Leifsonia xyli* subsp. *Xyli*	The pathogen is inactivated by treating seed canes with hot air for 4 h at 54 °C	A traditional aerated steam therapy treatment at 50 °C for 1–3 h provides 100% control. A temperature that is higher than this will kill the cane, while a temperature that is lower than this will allow the disease to survive	Reddy and Rama (2021)

(continued)

Table 15.2 (continued)

Disease name	Causal organism	Physical management		Reference
		Hot air treatment	Hot water treatment	
Grassy shoot disease (GSD)	*Phytoplasma*	For 8 h, setts were treated with hot air at 54 °C	One hour before planting, pretreat the healthy setts with hot water at 50–52 °C	Anuradha et al. (2019)
Sugarcane mosaic disease	Sugarcane mosaic virus (SCMV) and sorghum mosaic virus (SrMV)	–	Because the virus is spread by setts, Aerated Steam Therapy (AST) at 56 °C for 3 h is recommended for setts before planting	Lu et al. (2021)
Sugarcane streak mosaic disease	Sugarcane streak mosaic virus (SCSMV)	–	Setts were treated for 10 min with hot water at 53 °C, which significantly reduced disease severity while maintaining 100% plant viability. The effect of SCSMV infection during the tillering period could be reduced if the virus was suppressed earlier before planting by hot water treatment	Damayanti and Putra (2010)

(Viswanathan and Malathi 2019). The prevalent fungal diseases for which biological treatment could be a viable strategy for integrated disease management are red rot, wilt, sett rot, and seedling rot (Table 15.3). Effective fungal and bacterial antagonists have been discovered, and their efficacy has been demonstrated in vitro and in vivo. Fungal bioagents such as *Chaetomium*, *Trichoderma*, and bacterial antagonists were found to be effective exclusively and in combination with bacterial antagonists and fungicide in protecting the crop from red rot (Poveda et al. 2020). The ability of bacterial antagonists to develop resistance against red rot through induced systemic resistance has been demonstrated, and the antagonists' delivery through sett therapy was standardized for field use. In addition, under field conditions, a *Trichoderma* press-mud formulation was efficient against wilt.

Similarly, seedling rot induced by *Pythium* spp. was well treated with *Trichoderma* formulation, which is now used in seedling trays to manage the disease. Antifungal genes/proteins proficient in lowering red rot pathogen's pathogenic ability, i.e., *Colletotrichum falcatum*, have been isolated and characterized,

Table 15.3 Transitory information of some important diseases of sugarcane in which biocontrol can be possible

S. No.	Disease name	Causal organism	Mode of survival and spread	Biocontrol used	Reference
Fungal diseases					
1.	Red rot	*Colletotrichum falcatum*	Fungal spores dispersed from infected seed canes through rain splashes, wind, or soil	The enzymatic actions of the metabolites generated by biocontrol agents like *Trichoderma viride* and *T. harzianum* not only manage red rot but may also induce systemic resistance in sugarcane. *Pseudomonas*, *Enterobacter*, *Burkholderia*, *Ochrobactrum*, *Gluconacetobacter*, and *Bacillus* have recently been shown to be linked with sugarcane rhizospheres and have the potential to manage *C. falcatum*	Singh et al. (2011); Tiwari et al. (2017b); Patel et al. (2019)
2.	Whip smut	*Sporisorium scitamineum* (formerly known as *Ustilago scitaminea*)	Through infected seed cane or wind-borne teliospore of the fungus	Sett treatment with *Trichoderma viride* culture filtrate proved effective in managing whip smut disease. Two endophytic bacteria, i.e., *Bacillus axarquiensis* ESR 7 and *B. pumilus* ESR 21 are consistently used in suppressing smut disease on a highly smut susceptible variety under artificially pathogen-inoculated condition	Lal et al. (2009); Jayakumar et al. (2019)

3.	Wilt	*Fusarium sacchari*	Soil, infected seed canes, rain splashes, wind and irrigation water spread the fungal spores	*Trichoderma* species, viz. *T. viride*, *T. harzianum*, and *T. pseudockei* have been recognized as a potential biocontrol agent against *Fusarium moniliforme*	Deshmukh et al. (2016); Jena and Panigrahi (2017)
4.	Pineapple disease	*Ceratocystis paradoxa*	Fungal spores survive in the soil and spread the disease. Rain-splashed or wind-blown spores from infected canes are also a matter of concern	The mycelium of *Gliocladium virens*, *Trichoderma koningii*, *T. viride*, and *T. hirsuta* were found growing over the pathogen of sugarcane pineapple disease	Mahalingam et al. (2011)
5.	Pokkah boeng disease	*Fusarium moniliforme*	Through airborne spores or infected seed pieces	As compared to bioagents alone, soil application with *Trichoderma*-enriched FYM had an additive effect in decreasing the incidence of sugarcane pokkah boeng disease	Srivastava et al. (2019)
6.	Seedling root rot	*Pythium* spp. (*Pythiumarrhenomanes*)	Soil borne	The use of *Trichoderma* formulation against *Pythium* seedling rot proved highly effective, and to treat the biotic infection, it is now used in seedling trays	Viswanathan and Malathi (2019)
7.	Sugarcane ringspot disease	*Leptosphaeria sacchari*	Wind- or rain-born spores of the fungus	Different *Trichoderma* species, viz. *Trichoderma viride*, *T. harzianum*, and *T. virens* were most effective against *L. sacchari*	Nanjundaswamy et al. (2020)

(continued)

Table 15.3 (continued)

S. No.	Disease name	Causal organism	Mode of survival and spread	Biocontrol used	Reference
Bacterial diseases					
8.	Sugarcane leaf scald	*Xanthomonas albilineans*	Through infected seed cane and agricultural implements, rain- or irrigation water suspensions having bacterial pathogen	*Xanthomonas albilineans*, a pathogen that causes leaf scald, was suppressed in vitro by *Gluconacetobacter diazotrophicus*. Sugarcane stems infected with *G. diazotrophicus* were also resistant to infection by *X. albilineans. Pantoea dispersa* strain SB1403, which detoxifies albicidin, provided nearly perfect biocontrol against leaf scald disease when co-inoculated with a ten-fold excess of *X. albilineans* cells into a very sensitive sugar cane variety	Arencibia et al. (2006); Zhang and Birch (2008)
9.	Sugarcane red stripe disease	*Acidovorax avenae* subsp. *avenae*	Bacterial pathogen suspensions from the surface of leaf wounds in the rain or in the water	The bacteria *Curtobacterium flaccumfaciens*, *Microbacterium oxydans*, *Pseudomonas oryzihabitans*, and *Pseudomonas fluorescens* are found extremely efficient in managing this disease of sugarcane	Horuz and Aysan (2018)

Viral diseases					
10.	Sugarcane mosaic disease	Sugarcane mosaic virus (SCMV) and sorghum mosaic virus (SrMV)	Several species of aphids or by planting infected stalks	Several parasitoids (*Aphelinus maidis*, *Enrischia comperei*, *Bracon* sp., *Lioadalia flavomaculata*, *Lysiphlebus delhiensis*) and predators (*Allograpta exotica*, *Brumus suturalis*, *Chrysoperla* sp., *Coelophora inaequalis*, etc.) in addition to the entomogenous fungus, viz. *Verticillium lecanii* have showed a very efficient biocontrol agent for *M. sacchari* infesting sugarcane mosaic disease	Hall (1987); Singh et al. (2004)
	Sugarcane yellow leaf disease (YLD)	Sugarcane yellow leaf virus (SCYLV)	Several species of aphids, viz. *Melanaphis sacchari*, *Ceratovacuna lanigera*, *Rhopalosiphum maidis*, and *R. rufiabdominalis* or by infected to healthy sugarcane	The spraying of the gray fungus (*Verticillium lecanii*) spores resulted in 45% drop of aphids' population. Furthermore, predators such as *Olla v-nigrum* (Mulsant), *Allograpta exotica* (Wiedemann), *Coleomegilla maculata fuscilabris* (Mulsant), *Hippodamia convergens* (Guerin), *Diomus terminates* (Say), *Lysiphlebus testaceipes* (Cresson), *Micromus subanticus* (Walker), *Chrysoperla externa* (Hagan) (L.) have been shown to be effective biocontrol agents against *M. sacchari*	Hall (1987); Hall (1988); White et al. (2001); Holkar et al. (2020)

with promising results. More research is needed to uncover specific markers for plant growth promotion, antagonistic potential, rhizosphere competency, endophytic colonization, and other features that might be used to select effective biocontrol strains (Viswanathan and Malathi 2019).

15.6.3 Chemical Control

Few diseases, particularly those caused by fungal infections, may be controlled chemically. The pathogen that causes sett rot can persist in the soil, and the setts should be dipped in fungicide solution as a preventative precaution to protect the cut ends from the disease. According to recent research, a sett application of thiophanate methyl fungicide in combination with the biocontrol bacterium *Pseudomonas* reduces soil-transmitted infection of the red rot pathogen surviving in debris (Peng et al. 2021). Between November and March, five to six sprayings of Mancozeb (0.2%) are recommended to manage severe rust under certain conditions. Similarly, ocular spots can be controlled by spraying copper oxychloride or mancozeb (0.2%) once every 30 days throughout the starting period. When the sickness is severe, fungicidal treatments should be applied every 18–20 days (Table 15.4).

15.7 Genetic Resources of Resistance/Tolerance Genes

Biological diversity is not equitably spread either geographically or biologically. Crops were domesticated in the centers of respective species variety, which Nikolai I. Vavilov improved and expanded (1926). Genetic diversity is physiologically dispersed throughout primary, secondary, and tertiary gene pools, distinguished by their hybridization compatibility and thereby non-uniformly available to cultivated crops (Harlan and de Wet 1971). Finally, genetic diversity is unequally distributed among chromosomes within a genome and is related to recombination rates (Gaut et al. 2007). Domestication at the genesis of agriculture acts as a centric/punctuated process (Abbo and Gopher 2017) vs. many origins across long periods (Fuller et al. 2012; Civáň et al. 2013) have recently been the subject of heated dispute. There may have been single or several origins, a linear or reticulate descent from an ancestral population(s), and gene flow between wild and domesticated populations throughout the history of domesticated crops. All of these hypotheses appear to be supported by evidence from domesticated crops such as sugarcane (Smýkal et al. 2018).

The sugarcane genome's complexity and bulk are important impediments to genetic innovation. While persistent selective breeding for increased sucrose accretion has achieved more than half of the yield growth in the last 50 years, the gene pool studied in traditional breeding programs has been said to have reached a plateau (Mariotti 2002). On the other hand, individual research initiatives have been shown to achieve significant genetic progress every year by maintaining a diversified gene pool (Edme et al. 2005). By assisting in the association of phenotypes with genetic

Table 15.4 List of chemical treatments and their recommended dose against some sugarcane diseases

S. No.	Disease name	Causal organism	Chemical management	Reference
Fungal diseases				
1.	Red rot	*Colletotrichum falcatum*	In vitro studies reveal that the use of chemicals completely inhibits the growth of *C. falcatum*. Benomyl® 50 WP, Folicar®, and Radomil® 75WP (100%) @ 5–50 μg mL^{-1} totally inhibited the fungal growth. Using Bavistin® and Topsin® M against *C. falcatum* also proved effective. Treatment of infected setts with carbendazim and benomyl for 30–60 min is probably also suggested to prevent red rot incidence. Dip treatment of sugarcane setts with 0.25% suspension of thiophanate methyl and carbendazim metabolite (managing debris borne infection 24 h before planting) effectively handled red rot disease	Subhani et al. (2008); Rahman et al. (2009); Malathi and Viswanathan (2013); Bharadwaj and Sahu (2014); Hossain et al. (2020)
2.	Whip smut	*Sporisorium scitamineum* (Formerly known as *Ustilago scitaminea*)	For efficient management of sett transmitted sugarcane smut disease, sett dip with Tilt, Bavistan, and Bayletan (0.15%) can be recommended. Dipping of seed cane in azoxystrobin+tebuconazole@0.1% for 15 min has reduced the smut incidence in plant crop and spraying twice with tebuconazole@0.1% immediately on ratoon initiation and second spray at 30 days after ratooning had effectively managed whip smut of sugarcane under field conditions and resulted in enhanced cane yield compared to untreated control	Rajput et al. (2019); Varma et al. (2020a, b); Rajput et al. (2021)

(continued)

Table 15.4 (continued)

S. No.	Disease name	Causal organism	Chemical management	Reference
3.	Wilt	*Fusarium sacchari*	The disease intensity is reduced by dipping the setts in 40 ppm boron or manganese or spraying the plants with either of these elements. Sett treatment with fungicide like Bavistin, 0.1% before planting. Apply carbendazim @ 2 gm/L of water at the root zone area and same as follow at 15 days interval. Sett treatment with 0.5% Agallol also reduces disease incidence	Deshmukh et al. (2016); Md. Minnatullah et al. (2021)
4.	Pineapple disease	*Ceratocystis paradoxa*	It is unlikely that fungicides would be an economic option against this disease; but if it is extremely needed then use carbendazim. Dip applications with benomyl, thiophanate-methyl, propiconazole, flusilazole, and ethyltrianol also provided significant levels of control in greenhouse tests	Raid (1990)
5.	Pokkah boeng disease	*Fusarium moniliforme*	Spraying fungicides such as Bavistin (1 gm/L of water), Blitox (0.2%), or copper oxychloride or 0.3% dithane M-45 (3 gm/L of water) is successful in suppressing the pokkah boeng disease	Kumar et al. (2018); Srivastava et al. (2019); Srivastava et al. (2020)
6.	Seedling root rot	*Pythium* spp. (*Pythiumarrhenomanes*)	@ 0.2%, Blitox and Bavistin inhibited mycelial growth of the test fungus and reduced disease incidence	Deshmukh et al. (2016)
7.	Rust	*Puccinia melanocephala*	Propineb @ 0.25% and mancozeb @ 0.20% are found to be effective against rust, should be sprayed on the foliage just after the appearance of rust pustules, thrice at 15 days interval	Selvakumar and Viswanathan (2019)

Bacterial diseases				
8.	Sugarcane leaf scald	*Xanthomonas albilineans*	At 2 months after planting, spraying antibiotics such as streptomycin + tetracycline (60 g/ha/500 L water) at 2-week intervals was shown to effectively manage the pathogen in the field. Spraying these antibiotics minimizes the severity of leaf scald in the early stages	Govindaraju et al. (2019)
9.	Sugarcane red stripe disease	*Acidovorax avenae* subsp. *avenae*	The most effective treatments against red stripe bacterium is ampicillin and vancomycin @ 75 and 25 g/mL, respectively	Hussnain et al. (2011); Yonzone and Devi (2018)
10.	Ratoon stunting	*Leifsonia xyli* subsp. *Xyli*	Chemical disinfectants that may be used on cane cutting knives include, lysol, dettol, ethanol, Mirrol, and Roccal. At least 5 min of contact with the cutting surface is needed to assure disinfection	Yulianti et al. (2020)
Phytoplasma disease				
11.	Grassy shot disease (GSD)	*Phytoplasma*	To control insect vectors, spray 1 mL dimethoate in 1 L of water. To manage aphids, use methyl-demeton at a rate of 2 mL/L of water	Anuradha et al. (2019)
Viral diseases				
12.	Sugarcane mosaic disease	Sugarcane mosaic virus (SCMV) and sorghum mosaic virus (SrMV)	When population reaches 70 and 155 aphids per plant at 50 and 80 days after planting in spring and 60 and 90 days after planting in autumn, foliar sprays of demeton-S-methyl, dimethoate, endosulfan, parathion, diazinon, malathion, metasystox, phosdrin, quinalphos, carbofenthion, and carbofuran were effective	Singh et al. (2004)

(continued)

Table 15.4 (continued)

S. No.	Disease name	Causal organism	Chemical management	Reference
13.	Sugarcane yellow leaf disease (YLD)	Sugarcane yellow leaf virus (SCYLV)	The use of a 0.05% dimethoate 30 EC solution was shown to be beneficial in controlling the aphid population. Endosulfan 35 EC at 0.07%, monocrotophos 36 WSC at 0.04%, and chlorpyriphos 20 EC at 0.05%, on the other hand, can effectively manage aphid populations. When the crop in the field is more than 5–6 months old, however, applying insecticide treatments to control aphids is not possible, for which automatic aerial sprays are helpful	Balikai (2004); Viswanathan et al. (2017)

markers and genetic maps, modern technologies can assist breeding programs in achieving even greater yield advances (Dillon et al. 2007).

15.8 Sugarcane Pests Introduction

Worldwide, more than 1500 species of pests are cited in sugarcane (Box 1953), both in tropical and subtropical regions, where international trade, changes in climatic conditions, simplification, and intensification of agricultural systems have increased the risk of outbreaks of new pest species (Goebel and Nikpay 2017). Pest groups in sugarcane include stem borers, sap feeders, leaf feeders, and subterranean pests (Leslie 2004; Kumar et al. 2019). However, not all insect pests are of economic importance and depend on favorable conditions for their growth and development in each region (Santies-Herrera et al. 2017). For example, in Mexico, more than 150 species of pests and diseases (insects, rodents, nematodes, fungi, bacteria, and viruses) are classified that causing severe stress to sugarcane crops. However, the most important pests that cause serious damage and economic losses to sugarcane are stem borers and spittlebugs, locusts, leaf feeders, weevils, sap feeders, and white grubs (Mendoza 1996; Flores 2007; Rodríguez-del-Bosque et al. 2014; Santies-Herrera et al. 2017).

15.8.1 Biological Control of Insect Pests

Within the integrated pest management (IPM) context, natural enemies (parasitoids, predators, and entomopathogens) play a key role in reducing damage to their ecological regulation of pest populations during crop development (Stehr 1992). The absence or reduction of natural enemies due to stressful environmental conditions (temperature, drought, wind, etc.) or human activities (agricultural practices, harmful insecticides, etc.) favor the increase of pests and, therefore, cause damage to sugarcane crops. The biological control of sugarcane pests has been studied in different sugarcane regions worldwide with different results of successes, and it is considered as the basis of pest management in this crop (Mendoza 1996; Flores 2007; Terán 2009; Meagher and Gallo 2008; Rodríguez-del-Bosque et al. 2014; Nikpay and Goebel 2016).

Stem borers are the main insect pests in sugar-producing countries in the world, except Australia, and their management implementation requires multi-tactics. Several strategies should be used to significantly reduce the population to obtain sustainable production of canes (Nikpay et al. 2020). Most borers around the world are Lepidopterans (Leslie 2004; Goebel and Nikpay 2017) belonging to the families Crambidae, Pyralidae, Noctuidae, and Castniidae, and the most important genera in sugarcane include *Bissetia*, *Chilo*, *Diatraea*, *Eoreuma*, and *Scirpophaga* within Crambidae, while *Elasmopalpus* and *Eldana* are found in Pyralidae. *Busseola* and *Sesamia* are important genera of Noctuidae, while Telchin is the only important genus in Castniidae (Smith et al. 1993). Important genera in America include

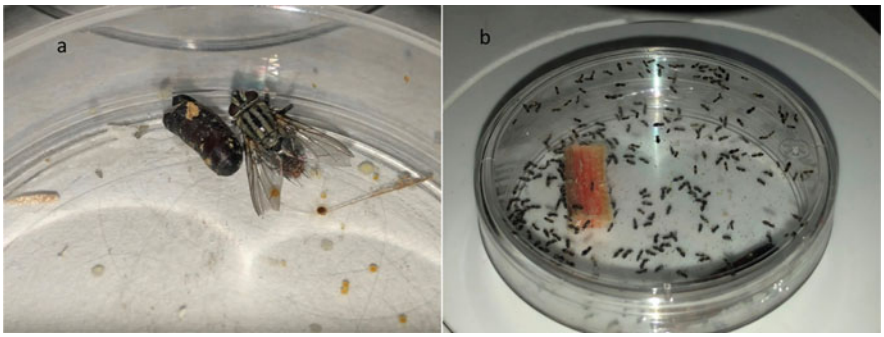


Fig. 15.3 *Billaea claripalpis* (Diptera: Tachinidae) (**a**) and *Braconid wasps* (**b**). (Photos credit: G. Vejar-Cota)

Diatraea, *Eoreuma*, *Telchin*, and *Elasmopalpus*. However, the last genus has different lifestyles and should be treated separately.

The main species of stem borer widely distributed in America is *Diatraea saccharalis* (F.) (Mendoza 1996). However, some other species have importance in specific sugarcane areas and during some phenological stages of the plant, such as *D. indigenella* Dyar & Heinrich, *D. tabernella* Dyar, *D. busckella* Dyar & Heinrich, *D. grandiosella* Dyar, *D. considerata* Heinrich, *D. crambidoides* Grote, *D. magnifactella* Dyar, and *Eoreuma loftini* (Dyar) (Flores 2007; Rodríguez-del-Bosque et al. 2014; Vargas et al. 2015). For more details on the biology and damage, see Smith et al. (1993), Leslie (2004), Meagher and Gallo (2008), Goebel and Nikpay (2017).

The biological control of stem borers in America started in the early twentieth century, and intensity increased in the last four decades. Since then, actions have been implemented in different countries and agroclimatology conditions, in most cases through international cooperation (Williams et al. 2013; Rodríguez-del-Bosque et al. 2014; Leslie 2004; Vargas et al. 2015). Natural enemies of stem borers, parasitoids, have received the most attention, possibly for their ecological diversity, host specificity, and the ability to attack cryptic feeding hosts inside the stalk (Smith et al. 1993) (Fig. 15.3). Mendoza (1996) provided a list of stem borer larvae and egg parasitoids reported in Latin America and the Caribbean. Recently, Rodríguez-del-Bosque et al. (2014) listed 39 species of parasitoids from México, and new species and their distributions data for each year are provided (Vejar-Cota 2016; Robles-Pérez et al. 2021).

The introduction of parasitoids and their conservation has been a tremendous success achieved so far to control insect pests in sugarcane (Meagher and Gallo 2008). However, some borers have become pests due to the implementation of some agricultural practices that negatively impact ecological processes. One of them is reducing or eliminating natural enemies by the inappropriate use of insecticides (Smith et al. 1993). Frequently, when the use of conventional insecticides is reduced

Fig. 15.4 Established plots of cane to serve as reservoirs for natural enemies in Jamaica. (Photo credit: T. Falloon, Jamaica)

or avoided, it can help to restore the beneficial effect of natural enemy populations; for example, Vejar-Cota et al. (2005) reported the recovery of *Conura acuta* (F.) (Hymenoptera: Chalcididae) populations when the use of insecticides was suspended. They mention that parasitism was practically zero in 1993, and the population increased progressively after discontinuing applications of chemical insecticides on the overall sugarcane area, reaching an annual average of 3.2% in 1997 and subsequently stabilizing at 2–3%. They also observed a parasitism rate of up to 43.3% during the middle of September 1996. The preservation of natural enemies is a key factor for a successful biological program. It can be achieved by creating suitable reservoirs as green-patch areas in or out of sugarcane fields to provide food for adult parasitoids and predators (Fig. 15.4). When fields are harvested, these undisturbed cane plots can act as reservoirs for natural enemies, especially parasitoids.

Another stressful cause why stem borer can become a pest is due to changes in agronomic practices and their effect on natural or introduced enemies. An example is cited by Macedo and Araujo (2000), who evaluated the impact of cane burning on parasitoids of *D. saccharalis* larvae and eggs in two consecutive crop cycles. They concluded that cane burning negatively affects natural enemies of the larval parasitoids *Metagonistylum minense* Townsend, *Billaea* (*Paratheresia*) *claripalpis* (Wulp), and *Cotesia flavipes* (Cameron), as well as the egg parasitoid *Trichogramma* spp. On the other hand, Vejar-Cota et al. (2008) found that the braconid larval parasitoid *Macrocentrus prolificus* Wharton survives the burning inside the stem borer larvae located underground, showing parasitism of 0.5% above ground versus 18.4% underground, which may explain the appearance of this parasitoid in the following crop cycle. Apparently, not burning the cane (e.g., in the mechanical harvesting of green cane) favors the conservation and increase of natural enemies and decreases the damage caused by stem borers (Araújo and Macedo 1998; Goebel and Nikpay 2017). The entomopathogenic fungi are the most promising biocontrol agents in agroecosystems. Under the sugarcane scenario, they can infect borers'

Fig. 15.5 Infected larvae of *D. saccharalis* by entomopathogenic fungus *Beauveria bassiana*. (Photo credit: G. Vejar-Cota)

larvae and cause death and reduction in population dynamics of stem borers (Fig. 15.5).

The classical biological control, which introduces and establishes an exotic natural enemy against an introduced pest species, is a well-known technology and an essential component in pest management in sugarcane. However, the classical biological control can also reduce the impact of existing native pests, which is called "new association" (Smith et al. 1993; Goebel and Nikpay 2017; Alleyne and Wiedenmann 2001). When the native natural enemies do not control a pest, introducing a newly associated parasitoid species may be the most appropriate biological control strategy. The most successful and documented case of the new association, biological control against stem borers, involves the old world braconid *C. flavipes* for use against the new world stem borer *D. saccharalis* (Smith et al. 1993; Mendoza 1996; Rodriguez-del-bosque and Vejar-Cota 2008; Williams et al. 2013; Rodríguez-del-Bosque et al. 2014; Vargas et al. 2015). Due to the success of *C. flavipes* against *D. saccharalis* in different countries of America, its use quickly became popular in Mexico to control other species of existing stem borers, mainly *Diatraea*. However, after some years of attempts and without previous studies of specificity on native stem borer species, the releases were discontinued, mainly caused by poor or no parasitism (Flores 2007). Later studies on *D. considerata* demonstrated that the poor parasitism was due to the host's immune response, mainly that did not yield parasitoids or pupate within the appropriate time interval, suggesting encapsulation of the parasitoid progeny. It also resulted in essential implications for the narrow host range of *C. flavipes* (Wiedenmann et al. 2003). Currently, America's sugarcane areas with *D. saccharalis* as the unique or main pest species still conserve releases of *C. flavipes* as part of their management strategy for stem borers (Macedo et al. 1993; Vargas et al. 2015; Aya et al. 2017) (Fig. 15.6).

Fig. 15.6 *Cotesia flavipes* in sugarcane fields in Brazil (**a**) and affected stem borer larvae by Macrocentrus spp. (**b**). (Photo credit: A. M. Vacari and G. Vejar-Cota)

15.8.2 Chemical Control of Sugarcane Pests

Chemical control has deserved special attention within the management tactics of stemborers in sugarcane because it is the only means of suppressing rapidly and economically (Metcalf and Luckmann 1992; Chelliah and Bharathi 1994). However, this control tactic requires precise knowledge of economic thresholds, application methods, application timing, dose, pest evaluation, and damage reduction variables.

Since insecticide applications began in the United States in the 1940s, their use has shown different levels of control in stem borers (Hensley et al. 1961). They have been more effective when new active ingredients were developed (Metcalf and Luckmann 1992). Products such as cryolite (inorganic) and ryania (botanical) were replaced by organochlorines and later organophosphate insecticides; however, repeated and excessive use of them soon generated contamination problems and high selection pressure in primary and secondary pests (Metcalf and Luckmann 1992; Peshin and Pimentel 2014). Indiscriminate use of insecticides and their unwanted effects triggered a return to a combination of tactics such as cultural control and greater emphasis on sustainable pest management strategies, such as varietal resistance, biological control, and "green chemicals" (Reagan and Mulcahy 2019). Thus, the greatest sustainability is achieved within Integrated Pest Management (IPM) through the balanced use of different control tactics.

Pest management, including insecticides, is the selective activity mainly to beneficial arthropods that reduce the population with minimal effects on other environmental components (Metcalf and Luckmann 1992). Within this selectivity, several authors agree on the use of "green molecules" due to their characteristics, less impact on the environment, and pest control (Metcalf and Luckmann 1992; Terán 2009; Gavkare et al. 2013; Reagan and Mulcahy 2019; Vejar-Cota 2019). In this sense, Gavkare et al. (2013) mentioned a list of safer molecules that could undergo photodegradation, microbial and chemical degradation, leaving fewer amount of residues in the environment. The molecules which can replace conventional insecticides more selectively obtain crop protection, ensure production, and

maintain security for natural enemies of different pests for a long time. The use of insecticides to control stem borers offers several challenges to researchers because the harmful stage is the larva, which feeds inside the stem, where contact with foliar insecticides is reduced and thereby reduces their effectiveness (Litsinger et al. 2005; Goebel and Nikpay 2017).

Applications directed to young larvae (external or exposed larvae) and adults can be effective, but once the larva makes galleries in the stalk (internal larvae), control is difficult (Meagher and Gallo 2008); therefore, applications must be precisely scheduled to coincide with these developmental stages (Goebel and Nikpay 2017). For the above reasons and the lack of further research, insecticides for stem borer control have provided limited damage or have failed in many sugarcane countries, thus their use was discontinued and largely replaced by biological control and varietal resistance programs (Mendoza 1996; Flores 2007; Terán 2009; Vargas et al. 2015).

In some countries, where population densities of stem borers in sugarcane cause very significant damage and biological control agents do not reduce population, the use of insecticides is justified as long as the molecules are selective and do not affect the natural enemies (Vejar-Cota 2019). Recent studies with novel molecules and better application programs have improved the reduction of stem borer populations and associated damages, offering the farmers healthier fields to harvest and better qualities for environmental protection. One of the first compounds developed for stem borer control and widely used in the United States was tebufenozide (Insect Growth Regulator or IGR), which acts only during molt when insects change the outer cuticle (Reagan and Mulcahy 2019). In the United States, tebufenozide was commercially used to control stem borer *Diatraea saccharalis* (F.) spread in the states with sugarcane areas, after its first evaluation in 1993 (Rodriguez et al. 1994). It replaced products such as lambda-cyhalothrin due to its adverse impacts on beneficial arthropods in the agroecosystem, pest resurgence, and resistance problems (Beuzelin et al. 2010).

After a decade of extensive use of tebufenozide (ca. 90% in Louisiana), early resistance studies showed 27 folds increase in LC50 after 12 generations of selection in the laboratory (Akbar et al. 2008). The results of Akbar et al. (2008) showed the need to manage resistance in stem borers in the sugar industry and preserve biorational molecules for much longer within multiple control tactics (Beuzelin et al. 2010). Tebufenozide continues to be used for commercial applications in several Latin American countries, while its widespread use has declined in the United States due to resistance problems. Novaluron, a new IGR (chitin synthesis inhibitor), was developed. It is currently commercially available throughout America with the advantage of being a molecule with minimal environmental impact and no effect on non-target arthropods (Wilson et al. 2017). A group of insecticides that have shown selectivity and are relatively harmless to non-target arthropods are namely diamides (Chlorantraniliprole, flubendiamide), which act at the level of ryanodine receptors in insects and have been evaluated for the control of stem borers, as well as other lepidopteran pests (Lahm et al. 2009; Gavkare et al. 2013; Sidhu et al. 2014; Padmasri et al. 2014; Wilson et al. 2017; Vejar-Cota 2019).

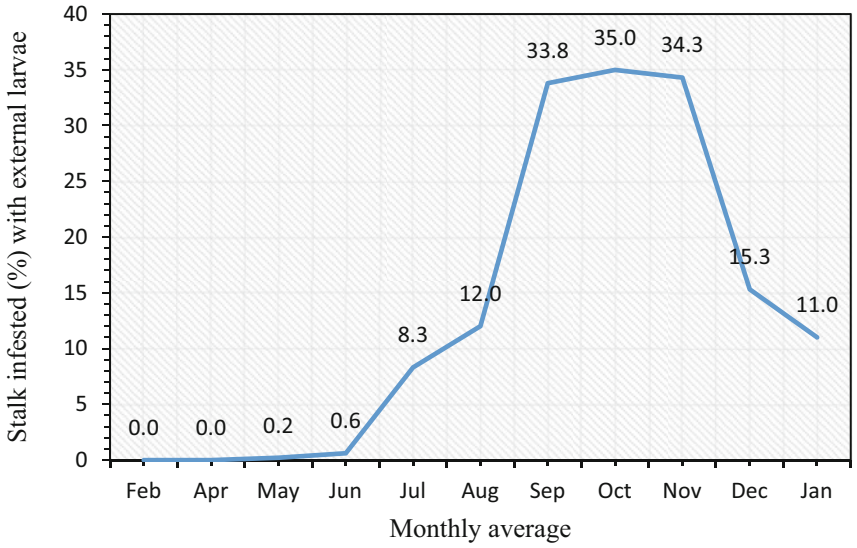


Fig. 15.7 External larvae of stem borer complex in northern Sinaloa, Mexico. Scouting method: percentage of stalks infested by searching for external larvae on 100 randomly selected stalks at five sampling sites each week (monthly average) during one year of sugarcane growth

Among some desirable characteristics of insecticides to control stem borers are the selectivity to the target pest, mobility within the plant (systemic), high persistence in the plant, minimal impact on the environment, and easy use by farmers within a sustainable management program. Gavkare et al. (2013) mention new groups of insecticides, however, not all are systemic or selective for non-target arthropods. The use to control stem borers combining different molecules can provide farmers with multiple management options to better control infestations and reduce the probability of insecticide resistance through reduced selective pressure (Reagan and Mulcahy 2019). The developmental stages of stem borers exposed to insecticide activity are the eggs and neonate larvae that live on the leaves, while most of the larval and pupae stages inside the stem escape application, except for insecticide runoff that make contact with the larvae when they are near the holes. In this sense, it is important to have field data and population dynamics of eggs and external larvae of stem borers to be more assertive during the precise schedule of the application (Fig. 15.7).

At first, within the bioecology of stem borers, it is possible to control different generations considering the behavior during the developmental stages in their life history and the densities in their age structure, combining it with the various management tactics together with insecticides. In this sense, it is difficult to control the first generation of stem borers with insecticides that appear during the tillering phase because the larvae are hidden deep in the gallery. Many of them are located in underground stalks (Vejar-Cota et al. 2008; Nikpay et al. 2020). Considering the above, the first application of insecticides should be carried out from the

differentiation of the first cane internodes to protect the stalks from new infestations in the next generation of stem borers, taking into consideration the moment in which the density of the external larvae begins to increase. Overlapping generations are present, as seen in Fig. 15.7. Sugarcane growers in Louisiana typically make one to three insecticide applications annually against *D. saccharalis.* A study carried out in Mexico by Vejar-Cota (2019) for the control of the stem borers *D. considerata* Heinrich, *D. grandiosella* Dyar, and *Eoreuma loftini* (Dyar) indicates that the use of selective insecticides is the way to reduce population and damage in sugarcane regions where the existing and induced natural biological control is not yet sufficient to maintain densities below the economic threshold.

In the same way, it indicates that the eight active ingredients evaluated in this study (chlorantraniliprole, indoxacarb, methoxyfenozide, spinetoram, azadirachtin, neem oil, thiamethoxam, and monocrotophos) affected the external larval densities of the stem borers in different degrees of effectiveness, Chlorantraniliprole stands out both in aerial application and in the drip irrigation system, with damage reduction from 53.9 to 85.2% with two or three applications in the grand growth period. In addition, a 75.9% decrease in dead hearts in the treated areas compared to untreated areas was found 2 months after harvest, which has repercussions on the size of the stem borer population that began in the next crop cycle. It was also found that the agro-industrial variables Brix, sucrose (%), purity, cane height, and weight showed positive results.

In contrast, sugar reducers and fiber content variables were negative when borer damage decreased. A study conducted in India by Padmasri et al. (2014) evaluated seven molecules for the control of *Chilo infuscatellus* Snellen and *Chilo sacchariphagus* indicus (Bojer) (rynaxypyr, spinosad, acephate, chloropyriphos, chlorantraniliprole, indoxacarb, and flubendiamide), finding that chlorantraniliprole significantly reduced the incidence and intensity of stem borers (93.23%), and in the same treatment, the highest sugarcane yield per hectare was obtained.

On the other hand, Wilson et al. (2017) evaluated four selective insecticides for biological control agents (tebufenozide, novaluron, chlorantraniliprole, and flubendiamide) for the control of the stem borers *D. saccharalis* and *E. loftini* in Texas and Louisiana, in the United States, finding that all of them reduced the damaged stalks. Chlorantraniliprole's case reduced injury to the top portion of sugarcane stalks. These authors suggest that the molecules tested (IGRs and diamides) can improve control of *E. loftini*, but more research into application strategies is needed to achieve consistent efficacy. In recent years, drones have been evaluated increasingly as part of precision agriculture in sugarcane fields (Zhang et al. 2019). This practice has been performed successfully in China and Mexico, with satisfactory stem borers management, time efficiency, and significant reduction in water use through pesticide application (Zhang et al. 2019) (Fig. 15.8).

Scouting stem borers for insecticide application involves walk through the sugarcane field to detect eggs, external larvae, and visible damage on leaves sheaths, and stalks; however, the scouting for stem borers is time-consuming, laborious, and when farmers can see the effects of stem borer damage, it is too late to treat fields (Schexnayder et al. 2001). Although dead hearts are a symptom of stem borer

Fig. 15.8 Application of insecticides by drone for stem borers in Mexico. (Photo credit: G. Vejar-Cota)

Fig. 15.9 Using pheromone traps as scouting procedure to evaluate population dynamics of stem borers. (Photos credit: Y. Hu and G. Vejar-Cota)

damage in the tillering stage, it is not used to determine the appropriate time for insecticide applications (Vejar-Cota 2019). Whereas the appropriate time for insecticide applications is during the population growth of the external larvae in the grand growth period (key to achieving a more significant impact on reducing damage). Other scouting techniques that can help detect increases in stem borer populations include the black light and pheromone traps (Hammond and Hensley 1971; Nikpay et al. 2020) (Fig. 15.9).

Sexual attraction pheromones are of particular interest since adult captures can be associated with the presence of eggs and external larvae, as well as damage caused by stem borers. Pheromones can also be used for mass trapping and mating disruption, as well as detecting the invasion of a new species in sugarcane regions (Campion and Nesbitt 1983; Wilson et al. 2012; Reagan and Mulcahy 2019; Nikpay et al. 2020).

In addition to the safety of insecticides for natural enemies, economic and action thresholds, systematicity and high persistence in the plant, scouting methods and application techniques, new technologies such as the use of drones and digital applications (smartphones and tablets) for pest scouting could be tools that will be put into practice to find better ways to make efficient applications for the control of sugarcane stem borers in the future.

15.9 Agroecological Options for the Management of Sugarcane Stem Borers: The Case of *Chilo sacchariphagus* (Lepidoptera: Crambidae) and *Sesamia spp.* (Lepidoptera: Noctuidae)

In many regions, sugarcane is attacked by insect pests, and some of them are very damaging such as Lepidoptera stem borers causing economic losses. In Reunion Island, the two major pests are the white grub, *Hoplochelus marginalis* (Coleoptera: Scarabaeidae) introduced from Madagascar in the 70s; and the spotted stemborer, *Chilo sacchariphagus*, originally from Java. While the control of the white grub by an entomopathogenic fungus, *Beauveria hoplocheli*, has been successful, *C. sacchariphagus* remains problematic in some sugarcane areas in Reunion, where susceptible varieties are grown, such as R 579. Stalk and internodes bored by larval stages result in significant crop losses up to 30% (tons cane per ha) in case of severe infestations (Goebel et al. 2010). Recently introduced In Mozambique, the spotted stem borer has become a major pest in the sugarcane estates of Mafambisse, Xinavane, and Marromeu. It is a potential threat to the sugarcane industry in South Africa. Originally from South-East Asia, *C. sacchariphagus* is widely distributed in all sugarcane region areas and is a key pest in Indonesia (Java and Sumatra), China, and Thailand. *Sesamia calamistis*, *S. nonagriodes*, and *S. cretica* (Lepidoptera, Noctuidae) are other key moth borer species of cereal and sugarcane crops.

15.9.1 Trap Crops, Companion Plants, and Intercropping

The push-pull approach is a strategy that uses plant diversity for control of pests by attracting them and sometimes kill them (push) or attracting parasitoids and predators (pull) to kill the pest (Fig. 15.10).

By integrating new plant species (service plants) into the agroecosystem, it is possible to mitigate the impact of insect pests through several methods which can also be combined. These service plants can thus develop a push-pull system, which can become a valuable part of agroecological crop protection (Goebel et al. 2018). In Reunion Island, the choice of the control strategy against this pest is directed toward mixing biocontrol and the use of companion plants. Today, the use of the trap crop

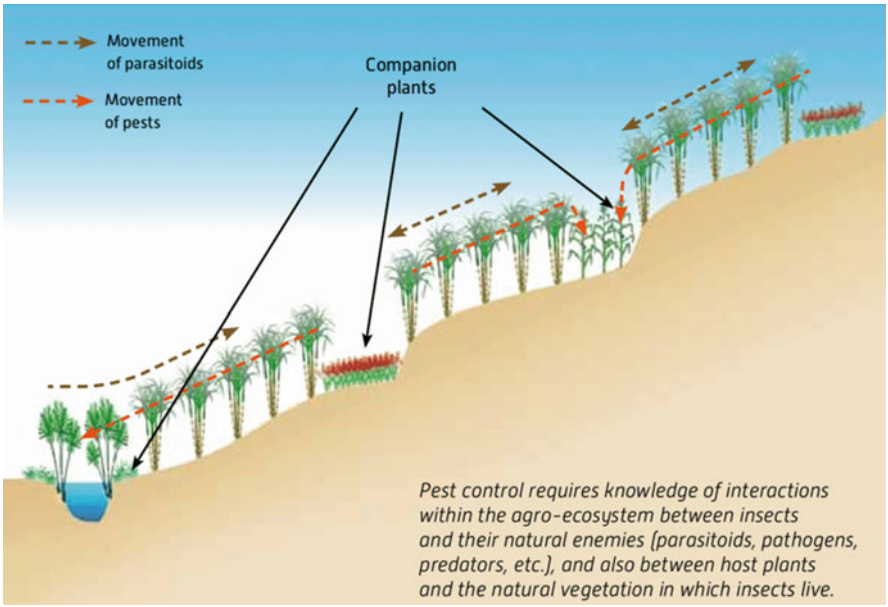


Fig. 15.10 Schematic photo on the interaction among companion plants, pests, and natural enemies (Conlong and Rutherford 2009)

Fig. 15.11 *Erianthus arundinaceum* was used as push-pull strategy in Reunion Island. (Photo credit: S. Nibouche)

Erianthus arundinaceum, a close relative of sugarcane, is at the heart of the research activities in Reunion (Fig. 15.11).

The females of the sugarcane borer *Chilo sacchariphagus* prefer to lay their eggs on *E. arundinaceum* rather than on sugarcane (Nibouche et al. 2012). At the same time, the survival of the larvae on this grass is meager. Our work in Reunion Island

Fig. 15.12 Use of companion plant *Canavalia ensiformis* between furrows and in the border of sugarcane fields in Reunion Island. (Photos credit: F. R. Goebel)

has shown that planting a row of *Erianthus* around sugarcane plots reduces damage to the cane. Chemical mechanisms underlying this insect–plant interaction are also explored. The use of companion plants in many countries has received more attention. These plants can serve and maintain crop biodiversity and be a natural host for adult parasitoids and predators (Fig. 15.12). Cultivation of companion plants as ecological service plants is an environmentally sound strategy in sugarcane fields, which not only provide external foods and nectars for adult natural enemies but also can maintain soil quality, weeds suppression, attract beneficial arthropods, and act as a repellent for notorious pests such as stem borers (Nikpay et al. 2020).

Recently, volatile compounds released by intact plants were collected at dusk and analyzed with a thermodesorber, a gas chromatograph, and a mass spectrometer. This protocol was repeated on seven accessions of *Erianthus* and one sugarcane cultivar susceptible to *C. sacchariphagus*. Eighty compounds were identified and tested in a Y tube olfactometer to test the attractivity of *C. sacchariphagus* females (Nikpay et al. 2020).

Another type of combination is intercropping, which means the cultivation of sugarcane with other plants such as pepper, beans, canola, especially on small-holder farmers. This practice is common in India, Pakistan, Vietnam, and Bangladesh (Nikpay et al. 2020) (Figs. 15.13 and 15.14). Increasing soil fertility, improving the efficacy of soil-borne microorganisms, and raising farmers' income are the main reasons for intercropping.

Fig. 15.13 Cultivation of flowering plants around sugarcane fields in Vietnam for maintaining biodiversity. (Photo credit: Cao A. Duong)

Fig. 15.14 Sugarcane intercropping with beans in Vietnam. (Photo credit: Cao A. Duong)

15.10 Nitrogen and Silicon Are Key Elements to Influence Borer Infestation

Recent studies on three borer species, *Eldana saccharina* (Ivory Coast, Senegal, South Africa) *Diatraea* spp. (Argentina and Panama), and *Chilo sacchariphagus* (Indonesia) have shown that these pests are susceptible to silicon and nitrogen content in the plant. For example, the use of silicon-based products has shown a significant reduction of borer damage levels by up to 50%, which confirms the positive effect of silicon as a physical barrier to borer penetration. However, excessive nitrogen applied in the soil led to a reverse situation, attracting borer populations and increasing damage. Over-application of nitrogen is common in sugarcane, and it is necessary to conduct soil analysis for checking nitrogen levels in the soil before applying this fertilizer (Nikpay et al. 2020).

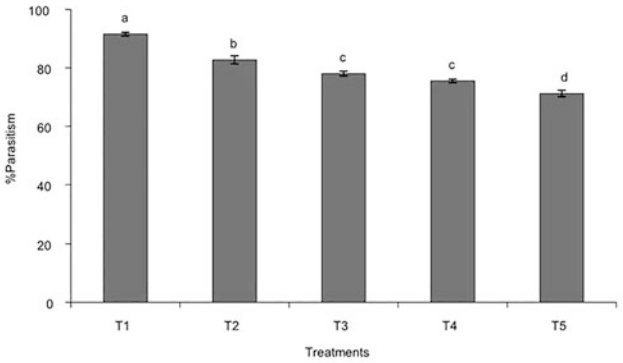
15.10.1 Silicon Reinforce the Resistance of Sugarcane Varieties

One recently novel approach to manage stem borers in sugarcane agroecosystems is the application of silicon fertilizers as a nutritional soil amendment. This scenario is classified as nutritional integrated pest management as it encompasses improving crop resistance by increasing crop vigor (Reynolds et al. 2016). Silicon is the second element in the earth's crust and is considered a major nutritional element that may positively affect the growth and development of crops. Silicon is absorbed by higher plants in the form of mono-silicic acid ($Si(OH)_4$). After transportation via roots to vegetative shoots, silicon becomes concentrated in cell walls as silica gel (Ma and Yamaji 2006). Silicon may act mechanically and biochemically in plant defense against arthropod pests. Silicon depositions under leaf cuticles provide a mechanical barrier that increases rigidity and abrasiveness of plant tissues and may decrease palatability and digestibility to arthropod pests. Eventually, food intake becomes reduced (Reynolds et al. 2009, 2016).

Observations indicated that silicon fertilization boosts levels of defense-related genes; moreover, increasing the activities of plant defense enzymes leading to enhanced accumulation of defensive compounds such as phenolics and phytoalexins (Reynolds et al. 2016). Silicon fertilization in accumulating plants such as sugarcane proved to provide satisfactory results against arthropods pests (stem borers, spittle bugs, and mites) in several countries (Korndörfer et al. 2011; Keeping et al. 2013; Nikpay and Soleyman Nejadian 2014; Nikpay et al. 2015, 2017; Nikpay 2016a; Nikpay and Laane 2017; Atencio et al. 2019). The primary target pest in the sugarcane agroecosystem is stem borers, and they are managed efficiently by the application of silicon fertilizers. The common type of silicon prevalent in sugarcane is solid silicon formulations in the form of calcium silicate (Nikpay and Goebel 2015; Reynolds et al. 2016).

In 2015, Nikpay et al. applied calcium silicate to protect three sugarcane varieties, CP69-1062, SP70-1143, and IRC99-01, under field conditions. Silicon fertilizer was sprinkled in the furrow and mixed thoroughly in the soil to a depth of 35 cm. The results showed that by applying calcium silicate fertilizer, the percentage of stalk damage, percentage of internode bored, length of borer tunnel, percentage of borer exit holes, and the number of lived borer per stalks were significantly reduced in comparison with control. Silicon can be incorporated successfully with other environmentally sound practices such as beneficial parasitoids. Nikpay (2016a) evaluated the potential efficacy of silicon for improving the biological control of Scelionid parasitoid *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae) on susceptible variety CP69-1062. The results of this study indicated that the application of silicon as a soil amendment plus half release of parasitoids provided a significant reduction of percentage stalk damage and percentage of bored internodes caused by *Sesamia* spp. stem borers. Moreover, the cane quality characteristics, including Brix (%), pol (%), and purity, increased as compared to control. Interestingly, the parasitism rate was higher in silicon with parasitoid treatment than in check plots (Fig. 15.15).

Another aspect of silicon fertilization is its effects on the tri-trophic level. Silicon properties may affect the influence of beneficial arthropods (parasitoids and



Mean percent parasitism of *T. busseolae* on stalk borers ±SE for all treatments: T1 – calcium silicate (1,200 kg · ha^{-1}) and 2,500 *T. busseolae*; T2 – 5,000 *T. busseolae*; T3 – 2,500 *T. busseolae*; T4 – 1,250 *T. busseolae*; T5 – untreated control. Means followed by the same letter in each column are not significantly different using Tukey's HSD test at $p < 0.05$ (Nikpay 2016, Journal of Plant Protection Research).

Fig. 15.15 Improving parasitism by combining silicon and releasing parasitoids (Nikpay 2016a)

predators) on insect pests. Silicon may alter the emissions of herbivore-induced plant volatiles (HIPVs), concerning the attraction of natural enemies to treated plants (Reynolds et al. 2016). There is only one published paper on silicon fertilization and its effect on natural enemies in sugarcane. In 2017, Nikpay et al. investigated the efficacy of three silicon formulations on the rate of parasitism on five sugarcane commercial varieties. The parasitism rate on treated and untreated sugarcane varieties was recorded for two consecutive years. The results showed significant differences between silicon treatments and control in all sugarcane tested varieties. The results confirm that silicon fertilization may positively enhance biological control effectiveness, which is shown as parasitism level.

15.11 Cane Burning Is Not Compatible with an Agroecological Approach

In several sugar-producing countries in Africa, South America, and South-East Asia, including the USA (Florida), cane burning is still employed mainly before harvest. This practice is known to have a substantial negative impact on biodiversity, thus disturbing the entire biological equilibrium in the fields and at the vicinity (sometimes including the natural environment at the edge) (Fig. 15.16).

In Reunion, the ban of cane burning in infested areas following high pollution effects by flying ashes and the implementation of green harvesting has reduced *C. sacchariphagus* damage by 50%. Numerous surveys in Reunion, Indonesia, South Africa, and West Africa have proved that borer larvae can survive in the internodes as the fire passes too quickly to kill them inside the tunnels (Goebel et al. 2010). Due to key environmental considerations, more and more countries tend to stop this practice which also harms the health of workers involved in the harvesting process. This practice is incompatible with agroecology principles, aiming to

Fig. 15.16 Cane burning in a large sugar estate in Sudan, North-East Africa. (Photo credit: F. R. Goebel)

preserve and promote functional biodiversity and ecosystem services. This has also been encouraged by the growing demand for cane trashes for field blanketing and/or energy purposes and bioplastics.

15.12 Biocontrol of *Chilo sacchariphagus* Using Natural Enemies and How to Preserve Them

Leading research institutions and sugarcane mills have been using biocontrol for many years with augmentative or inundative releases of parasitoids in the sugarcane fields, such as *Trichogramma* spp., *Cotesia* spp., *Lyxophaga* spp., *Telenomus* spp., *Tetrastichus* spp., and others, with success stories but also several failures. For example, the inability of borer control using *Trichogramma* spp. in the 1960s and 1970s was partly due to lack of research on parasitoids themselves (species, bionomics, and efficacy), but also lack of quality control of mass production (Goebel et al. 2010). During this period, exotic parasites were introduced from different countries (mainly India) and released without evaluating their impact on pests (Goebel et al. 2010). These facts have led to a negative image of biocontrol with *Trichogramma* spp. and other parasitoids and loss of interest in this strategy (Goebel et al. 2010).

In Reunion and Indonesia, biocontrol programs implemented there showed the need for proper research, strict evaluation protocols, and a better understanding of parasitoids' ecology. For example, biocontrol of *C. sacchariphagus*, using *Trichogramma chilonis*, has been constantly improved by spending more time on research and development. One of the key elements is choosing the right strain with the best performances. After more than 10 years of laboratory studies and field experiments (biology, natural parasitism, ecology, time and rates of field releases, mass production, etc.), the strategy adopted in Reunion was to release 100,000 *T. chilonis* per hectare and per week at the beginning of the crop growth (between 1 and 4 months). This strategy allowed the reduction of 50% of damage with an economic gain estimated up to 1400 €/ha (Goebel et al. 2010).

In many other countries, such as Indonesia (Java), China, and India, this parasitoid is used as the main component of their biocontrol strategy. Indonesia is still producing millions of *Trichogramma* associated with the sugar factories, while India has seen small farmers taking over the production and release of *Trichogramma* wasps (cards) in their fields. However, the number of egg cards (*Corcyra cephalonica* eggs) released in the field is often under 50,000 trichogramma/ha, and the efficacy on borer damage reduction is lower than expected. In China, *T. chilonis* has been widely produced and released on sugarcane fields throughout southern regions, especially in Guangxi, and promising results have been achieved during recent years (Pan et al. 2020, 2021). Proper monitoring of sugarcane borers as well as the time of releasing parasitoids are key factors for gaining satisfactory control (Nikpay et al. 2020; Pan et al. 2021).

Another good example in biocontrol using parasitoids is Brazil, which has succeeded in controlling *Diatraea saccharalis* using the combination of two parasitoids: *Cotesia flavipes*, *a larval parasitoid*, and *Trichogramma galloi* parasitizing eggs. This example is noteworthy because using key parasitoids allows optimal control of stem borer population. Some countries also use pupal parasitoids such as *Tetrastichus howardi* (Hymenoptera: Eulophidae) or *Xanthopimpla stemmator* (Hymenoptera, Ichneumonidae). Biocontrol using parasitoids will continue in most sugarcane-producing countries. However, in the meantime, research and development activities should continue to improve biocontrol in all its components: quality control, cost reduction, conditioning, packaging, efficacy, economic feasibility, and adoption by growers (Goebel et al. 2010).

15.13 Predation by Ants and Other Beneficial Arthropods: Better Understanding of Their Impact

In Reunion and Indonesia, the importance of predation of *C. sacchariphagus* eggs by ants *Pheidole megacephala* has been reported as an essential component of the natural control of this pest (Goebel et al. 2010), as it is the case for other stem borer species (Atencio et al. 2019). The presence of generalist predators such as ants or even spiders must be reconsidered for the biocontrol with field releases of egg parasitoids. Knowing that predatory ants feeding on parasitized eggs will increase cane growth, it may be important to plan Trichogramma releases. At the beginning of moth borer oviposition, timely release on younger canes should be privileged (when ant predation is still low). Ant colonies tend to build up rapidly, particularly when the cane fields become dense, generally between 6 and 10 months, and natural predation of *C. sacchariphagus* is significant, making field releases of *T. chilonis* redundant or wasteful. In la Reunion, to decrease this negative impact, new dispensers with tiny holes to prevent ants from penetrating and feeding on parasitized eggs were tested with the help of a private company in France (Goebel et al. 2010). In Indonesia, to prevent ant predation on *Trichogramma* cards, truck grease is applied on leaves where these cards are placed (Goebel et al. 2010).

Finally, for farmers and practitioners keen to implement agroecological practices, it could be interesting to enhance biological pest control by natural enemies by planting flower strips around the sugarcane fields as a part of agroecological innovations. Therefore, farmers can create so-called flower strips for pollinators and other beneficial biodiversity-promotion areas (BPAs) for ecological compensation in sugarcane agro systems.

15.14 Field Releases of *Telenomus Busseolae* Against *Sesamia* spp.

Another interesting example of biocontrol was implemented in Iran to reduce *Sesamia* infestation. Under field conditions in Iran, mass rearing and releasing of *Telenomus busseolae* are the primary management strategy against moth stem borers (Nikpay and Goebel 2016). *Telenomus busseolae* was first collected by Daniali in 1970 at Haft-Tappeh sugarcane agro-industry, and this parasitoid is now active on maize, sugarcane, rice, sorghum, and weeds and can successfully parasitize *Sesamia* spp. eggs. *T. busseolae* is a solitary and pro-ovigenic parasitoid and can oviposit 78% of its eggs in the first 3 days. This parasitoid has been released in sugarcane fields for more than 15 years. The results of natural parasitism of *T. busseolae* indicated that this parasitoid establishes in sugarcane fields and can parasitize egg batches of *Sesamia cretica* Lederer and *Sesamia nonagrioides* Lefebvre up to 90% (Nikpay et al. 2014). However, climatic conditions may affect *T. busseolae* life parameters (Jamshidnia and Sadeghi 2014; Cheraghi et al. 2018). In a recent study, Cheraghi et al. (2018) conducted laboratory experiments on the effects of temperature on life cycle of *T. busseolae*. This research illustrated that temperature is one of the major crucial factors on the life of *T. busseolae*. The authors showed that the optimum temperature for population growth and suitable mass rearing of this parasitoid wasp was 28 °C.

15.15 Cultivar Resistance in Sugarcane Stem Borers Integrated Pest Management

Sugarcane cultivars with resistance to stem borers have been a part of sugarcane IPM programs for nearly a century (Holloway 1935). Resistant cultivars are currently used in IPM programs for *Diatraea saccharalis* (Reagan and Mulcahy 2019), *Eoreuma loftini, Eldana saccharina* (Keeping 2006), *Chilo sacchariphagus,* and *Sesamia* spp. (Nikpay 2016b). Although no sugarcane cultivars are immune to stem borers, resistant cultivars often have 60–80% lower injury levels than susceptible cultivars (Keeping 2006). Cultivar resistance is unique among IPM tactics, and it is compatible with all other management approaches, including chemical, cultural, and biological controls. In cases where resistant cultivars have prolonged exposure of stem borer larvae by impeding stalk entry, resistance can be synergistic through enhancing mortality from insecticides or natural enemies (Wilson et al. 2012).

Mechanisms of cultivar resistance have traditionally been placed into three categories: antibiosis, antixenosis (non-preference), and tolerance. However, resistance mechanisms to sugarcane stem borers cannot always be placed definitively into a single category. The following sections discuss separate cultivar resistance into categories as physical or mechanical resistance and chemical resistance.

15.16 Physical and Mechanical Resistance

Traits that impede the processes of oviposition and larval feeding, typically related to physical attributes of sugarcane cultivars, are the most common mechanisms of resistance to sugarcane stem borers. Of these, physical characteristics which impede the establishment of neonates are the most important for imparting resistance to *D. saccharalis*, *E. loftini* (Wilson et al. 2012), and *C. sacchariphagus* (Nibouche et al. 2012). Unfortunately, these physical characteristics are often undesirable from agronomic production and milling perspectives. High fiber in stalks has been demonstrated to be strongly associated with resistance in sugarcane cultivars to *D. saccharalis*. However, because of the reduction in milling efficiency associated with increase in fiber content, high-fiber cultivars have little potential for widespread commercial sugarcane production regardless of the level of stem borer resistance (Wilson et al. 2012).

Similarly, the presence of increased levels of pith in stalks is correlated with resistance to *D. saccharalis*, but sucrose content declines with increasing pith. Thus, selecting for resistance based on high levels of fiber and pith would result in reduced sugar yield in resistance cultivars. Indeed, recurrent selection for cultivars with low stem borer injury produced cultivars with a high level of *D. saccharalis* resistance but low suitability for commercial production (White et al. 2001). However, highly productive commercial varieties have been developed which possess resistance levels sufficient to reduce insecticide usage by approximately 50% (Wilson et al. 2012). These cultivars possess other physical characteristics which can impart resistance with little detrimental to yield potential and milling.

Stalk rind hardness has been most consistently associated with resistance to *D. saccharalis*, *E. saccharina* (Keeping 2006), and *C. sacchariphagus.* Increased hardness results from increased silicate or lignin in plant tissues (Keeping et al. 2009). Unlike fiber and pith, rind hardness does not reduce sucrose recovery. Leaf characteristics may also hinder larval feeding without negative impacts on milling quality. Leaf-sheath tightness (or oppression) has also been suggested as a resistance mechanism (Coburn and Hensley 1972). However, this characteristic has not been measured quantitatively. Physical traits that influence larval feeding are often independent, and multiple mechanisms may be functioning in a single cultivar. Indeed, independent leaf and stalk resistance mechanisms were identified for cultivars with resistance to *C. sacchariphagus* (Nibouche et al. 2012). Differences in

C. sacchariphagus leaf-feeding lesions among cultivars allow for assessment of resistance using non-destructive observational methods (Conlong et al. 2004).

Physical characteristics of sugarcane cultivars can also influence oviposition preference among stem borer species. Ovipositional preferences for adult females vary considerably among stem boring species. *E. loftini* and *E. saccharina* prefer cryptic oviposition sites, often within the folds of dry leaves low in the sugarcane canopy (Mabulu and Keeping 1999). This is in contrast to *D. saccharalis* that oviposits on newly formed vegetative leaves high in the canopy of rapidly growing sugarcane (Fuchs and Harding 1978). These differences result in substantial variation in the role of oviposition preference in cultivar resistance among species.

The strong preference for *E. loftini* to lay eggs in folds of dry leaves has increased the importance of oviposition preference in cultivar resistance related to other borer species. Cultivars with an increased prevalence of senescent leaf tissue are more susceptible relative to cultivars with more green leaves or cultivars that shed senescent leaves. Accordingly, susceptibility to *E. loftini* increased in sugarcane under drought or salt stress conditions. Cultivars that naturally shed leaves as they senesce may have increased levels of *E. loftini* resistance. Physical mechanisms of resistance are often variably expressed under differing environmental conditions. Genotypes by environment interactions have been reported for cultivars with resistance to *E. loftini* and *C. sacchariphagus* (Conlong et al. 2004). Resistance was expressed differently between wet and irrigated environments relative to dryer conditions. No differences in *E. saccharina* oviposition were reported among cultivars when young plants with similar amounts of dry leaf tissue were assessed (Mabulu and Keeping 1999). However, drought stress later in the growing season is thought to influence susceptibility to *E. saccharina* (Keeping 2006).

15.17 Chemical Resistance

Though much less common than physical resistance mechanisms, some studies have suggested that the chemical composition of sugarcane cultivars influences susceptibility. Reduced larval weights and developmental rates have been reported for resistance related to susceptible cultivars for *E. loftini* and *D. saccharalis.* Both studies suggested the antibiotic effects attributed to antinutritional components or allelochemicals, but the chemical composition of cultivar tissues was not examined in either study. Conversely, reduced ovipositional preference by *E. loftini* was associated with lower concentrations of the specific free amino acid (FAAs) between irrigated and non-irrigated sugarcane, but effects were confounded with differences in dry leaf tissue availability. Further, the influence of FAA concentrations on larval development was not assessed by though studies. A subsequent study that examined oviposition preference among various host grass species found no association between FAA concentration and oviposition preference while reporting dry leaf tissue availability as the chief characteristic associated with increased oviposition (Beuzelin et al. 2010). Fructose has also been suggested as a limiting nutrient

affecting *E. loftini* development, though fructose concentrations have not been compared between borer-resistant and susceptible sugarcane cultivars.

Perhaps the best examples of chemical resistance in sugarcane occur in transgenic cultivars expressing insecticidal compounds. Transgenic sugarcane was developed to express the snowdrop lectin protein of the snowdrop lily (*Galanthus nivalis*) by incorporating the gene into commercial cultivar CP 65-357 (Irvine and Mirkov 1997). This cultivar's leaf and stalk tissue had inconsistent and non-lethal effects on *D. saccharalis* and *E. loftini* and thus was not used in pest management programs. Transgenic sugarcane cultivars have been more successful by expressing the insecticidal *cry* proteins from *Bacillus thuringiensis* (Bt). Varieties of corn (*Zea mays*) expressing Bt proteins have been successfully managed *D. saccharalis* and other stem borers for decades (Huang et al. 2006). The potential to utilize the same strategy in sugarcane has long been identified (Arencibia et al. 1997). However, the unique challenges faced by sugarcane breeding, production, and concerns about the development of Bt resistance delayed the production of transgenic sugarcane. Bt sugarcane expressing Cry1Ab and Cry2Ab has recently been developed and successfully deployed for *D. saccharalis* management in Brazil (Cristofoletti et al. 2018). Resistance management strategies include implementing a 20% refuge that hoped to delay the development of resistance for more than 15 years the average duration of production of a commercial sugarcane cultivar in Brazil (Cristofoletti et al. 2018). The production of Bt sugarcane in Brazil may provide a model for utilizing the technology in other production regions. The use of transgenic sugarcane may affect the marketability of the sugar produced so that adoption may be limited globally.

Cultivar resistance will continue to be a critical component of sugarcane stem borer IPM. It provides a practical and economic management tactic that has been immensely important in sustainable sugar production worldwide. Research into resistance mechanisms will improve understanding of plant–insect interactions and enhance the deployment of this valuable management strategy.

15.18 New Tools and Emerging Technologies to Optimize IPM in Sugarcane: Remote Sensing and GIS for Early Detection of Pest Damage

Precision agriculture can be applied to pest management. With the growing use of remote sensing, electronic- and computer-based technologies, there is a real opportunity to understand the temporal and spatial movements of the insect populations at levels never before possible. Relating insect distributions to physiographical landscape elements is essential to predicting future pest population dynamics and management (Hunter 2002). Geographic Information System (GIS) is becoming increasingly more important in pest management programs because they can be used to create maps and conduct geo-statistical analysis of spatial interactions that occur at larger scales (Becker et al. 2005). Field observations on environmental conditions, including vegetation, water, and topography, can be combined in a GIS to enhance interpretation of remote sensing data and facilitate characterization of the

Fig. 15.17 Cane grub *Dermolepida albohirtum* damage on sugarcane and aggregation on *F. oppisita* in Australia. (Photos credit: F. R. Goebel)

landscape in terms of pest movements and resulting infestation (Leibhold and Rossi 1993). It makes remote sensing/GIS a robust set of tools for pest surveillance, predicting potential pest outbreaks, targeting intervention programs, and improving scouting practices (Lefko et al. 1998). Remote sensing using satellite images to detect pest and disease problems is in progress and is generally implemented at a landscape scale.

Satellite imagery was investigated as a method for detecting infestations over large areas of white grubs (Coleoptera: Scarabaeidae) in Australia. High spatial resolution multispectral and panchromatic satellite images were acquired in May–June 2013–2015, corresponding with the months when symptoms of feeding by the greyback canegrubs *Dermolepida albohirtum* were most visible (Fig. 15.17).

Images taken over 3 years were processed using geographic object-based image analysis (GEOBIA). Results indicated that very high-resolution imagery could detect grub damage within cane fields (Fig. 15.18). However, cane grub damage was difficult to specify in some situations because other problems such as water-logging, pig damage, or weed infestation appeared similar.

Nevertheless, these studies in Australia using satellite images have revealed interesting results and allowed to establish risk maps of infestation in the Mulgrave area (Zellner et al. 2014). A landscape map of the study site's land cover has first produced a map with seven classes: buildings, bare soil, sugarcane, herbals, isolated woody vegetation, riparian woods, and rainforests. Good results were obtained on the discrimination between vegetation and non-vegetation areas due to the high spatial resolution of the panchromatic channel and fine-scale segmentation (mean object size = 1.5 m^2), which includes single trees in the vegetation domain.

The separation of sugarcane and other vegetation in the second level (mean object size = 490 m^2) is a crucial processing step since all types of non-sugarcane vegetation border the target classes. The overall accuracy of 97% is reached at this level, with a kappa coefficient of 0.95 representing a stable basis for more detailed classification of non-sugarcane vegetation. The remaining problems are the loss of single small trees into neighboring objects, the classification of herbaceous surfaces

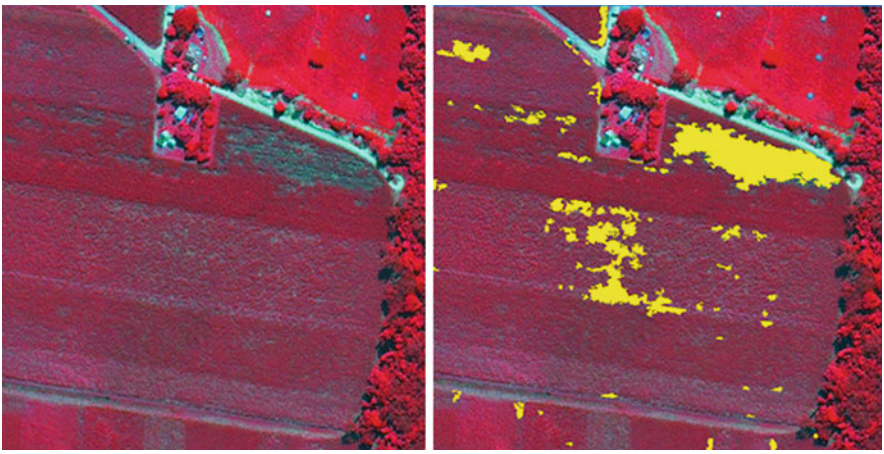


Fig. 15.18 Checking for grub damage by image processing. Left: texture analysis by infrared and Right: resegmentation of potential grub damage object (yellow)

to forest and sugarcane classes, and the confusion along with transitions from sugarcane to woody vegetation.

The map derived from the model allowed us to develop a risk map of grub damage—Figure 15.19 shows the spatial distribution of sugarcane fields with three risk levels of damage: low, medium, and high class. Risk is essentially higher, close to the forest and near the palm plantations.

All the results showed that the landscape elements played a significant role in the ecology of this pest. The adults (beetles) can fly from and to the sugarcane fields after a period of mating and feeding on specific trees called "feeding and roosting" trees. A list of these trees is already available and can be updated with the progression of the knowledge (Goebel et al. 2010). If the vegetation mapping can help, ground-truthing to identify the botanic groups of trees in a given area is a key point. Knowing that feeding trees (food source) and roosting trees (aggregating source) are key elements of vegetation surrounding sugarcane paddocks, surveys should be done on a regular basis to inspect these "hotspot" trees and estimate the beetle population. For example, through this work, an almond tree (*Calophyllum inophyllum*) was discovered near sugarcane fields in the Mulgrave area (north Queensland) that attracts hundreds of beetles each year. However, this tree is unusual as there is no leaf damage! The beetles are just swarming there, calling for each other and then mating. Therefore, this type of tree can serve as a population indicator in the main flight period. Based on numerous data and observations, it is established that preferred trees near sugarcane fields heavily contribute to increased damage in the local area.

The distance that a beetle can fly is still poorly documented, but the distance from feeding trees to highly infested patches in paddocks is relatively short. This is probably why the most damaged areas (Mulgrave but also Burdekin) are the ones

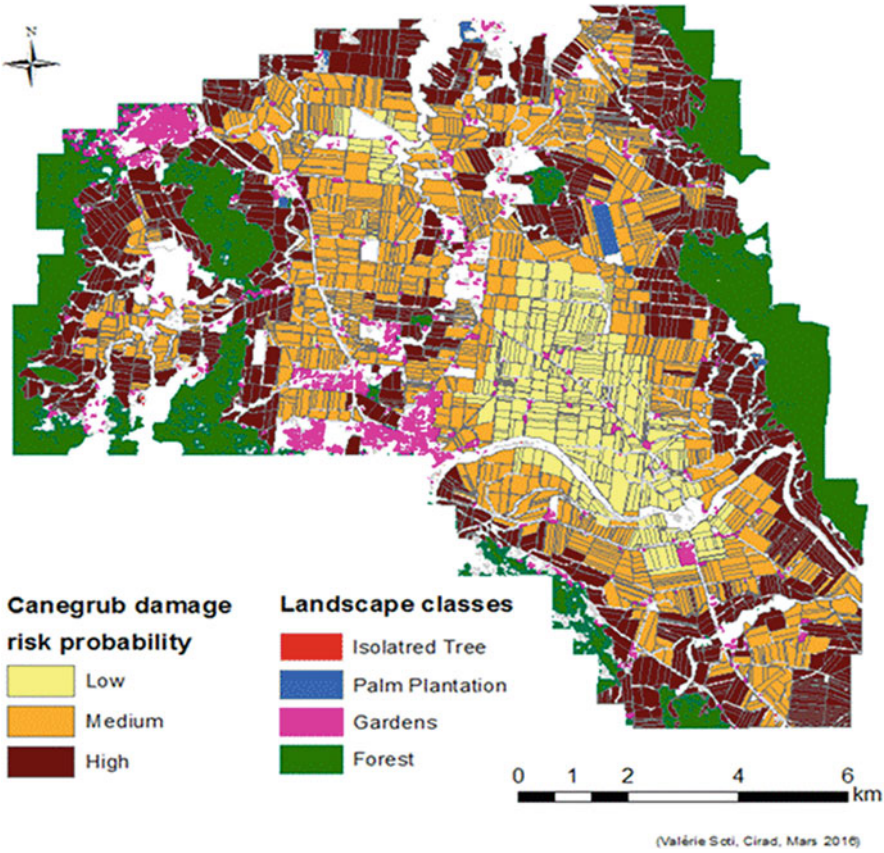


Fig. 15.19 Risk of cane grub damage in the Mulgrave study area

located along the river creeks. Therefore, if strips could treat these areas from the vegetation edge to 200 meters inside the paddocks, this would significantly reduce the damage on a wide scale.

15.19 Conclusion

Finally, remote sensing is a very useful tool to help growers concentrate their control strategy on specific areas based on risk maps. These maps can include an additional component using the presence or absence of vegetation natural vegetation in the damage occurrence.

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Weeds Management in Sugarcane: Recent Developments and Future Perspectives

16

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Abstract

Among the sugarcane production constraints, weed interference is dominant. Weeds compete with sugarcane crops for water, light, and nutrients, demanding better and more accurate control measures. Chemical weed control with pre- and/or post-emergence herbicides is mainly used, as sugarcane fields are usually large, requiring fast, efficient, and economically feasible weed control approaches. Furthermore, various weed species evolved resistance to different herbicidal mechanisms of action, and some herbicides effective earlier are now

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ineffective. Sugarcane is planted in numerous geographic systems. Depending on the application approaches, there will be changes in the composition of weed species, thus demanding specific herbicides and duration of application. To achieve high sugarcane yields, suitable genotypes with high productivity should be planted and as result, these varieties could be adapted to stress environmental conditions and interaction with selective herbicides. Furthermore, alternative weed management strategies such as integrated weed management, crop rotation, and alternative herbicide mechanisms of action will reduce problems with weed resistance in sugarcane fields and herbicide damages to crop plants.

Keywords

Chemical weed control · Herbicides · Integrated weed management · Sugarcane genotype

16.1 Introduction

Weeds are one of the most harmful challenges in sugarcane production, and their severity varies depending on weed density and sugarcane plant age (Mehra et al. 1990; Aekrathok et al. 2021). Weeds compete with sugarcane for resources, i.e., water, minerals, sunshine, and area, reducing sugarcane harvest (Zafar et al. 2010; Aekrathok et al. 2021). Such losses have been reported as highest as 70–84% in Ethiopia. Weed-crop competition reduced sugarcane yields by 78, 51, and 42% in the United States of America 3–9 weeks after planting (Zimdhal 1980; Yirefu et al. 2013; Farooq et al. 2014; Aekrathok et al. 2021). In the first 4 months after sugarcane planting in the rainy season, weeds resulted in production damages of over 70% in Thailand (Suwanarak 1990). The weed species varied at different locations due to various factors, including soil composition, pH, seasonal variations, and the choice and use of synthetic fertilizers and herbicides (Marshall et al. 2003; Pinke et al. 2010; Nagy et al. 2018; Aekrathok et al. 2021).

About 1000 weed species infest sugarcane agroecosystems worldwide (Araldi et al. 2015). The expansion of the sugarcane area will undoubtedly lead to a greater demand for pesticides, especially herbicides for weed control. In 2020, around 11.99 billion dollars were spent, considering all pesticides used in Brazil, and herbicides represented approximately 45% of the total. Sugarcane is the second most demanding crop for herbicides in Brazil (Sindag 2021). This extensive herbicide use in sugarcane occurs due to the slow initial development, whose Period Prior to Weed Interference (PPWI) is long. Even though sugarcane is very efficient in the use of environmental resources (water, light, and nutrients) available for its growth and development as it presents a C_4-type photosynthetic metabolism, its initial growth rate demands protection from weed competition (Procópio et al. 2003, 2016; Galon et al. 2012; Cabrera et al. 2020; Conab 2021).

Weed species infesting sugarcane usually present high competitive ability, with efficient use of water, light, and nutrients, grow fast, and occur in high densities

(Procópio et al. 2016; Aekrathok et al. 2021). Furthermore, they may serve as hosts for diseases and insects, in addition to releasing allelopathic substances that may harm sugarcane (Cabrera et al. 2020). In addition to the expected reduction in sugarcane tillering, stalk, and sucrose productivity, other evident negative aspects such as the decrease in field longevity, drop in raw material quality, and difficulty in harvesting and transport operations are often reported for highly infested fields (Procópio et al. 2003, 2016; Cabrera et al. 2020).

Weed control expenses in sugarcane can represent about 30% of production cost (ratoon cane) and 15–25% for plant cane (Lorenzi 1996). For several reasons, such as speed of operation, the best cost-benefit, higher safety for the crop, and increased control efficiency even in rainy seasons, weed control with herbicides is the most used (Procópio et al. 2016; Aekrathok et al. 2021). There is a need to know herbicide's chemical and physical properties, their effects on the crop and the environment, the appropriate application technology, herbicide mixtures, and handling, among others.

16.2 Main Weed Species Infesting Sugarcane Fields

Major weeds of sugarcane consist of sedges, grasses, and broad-leaved weeds. Sedges are perennial, grass-like weeds grown in bunches or clusters, and *Cyperus rotundus* comes under this category and can be found in sugarcane crops. Grasses are weeds having short stems with long narrow leaves. Example of grasses are *Cynodon dactylon*, *Sorghum halepense*, *Panicum* sp., and *Dactyloctenium aegyptium*. As the name indicates, broad-leaved weeds have broader leaves compared to sedges and grasses. For instance, *Chenopodium*, *Convolvulus*, *Amaranthus*, *Portulaca*, *Commelina*, *and Trianthema* are common botanical genera with important broad-leaved weeds of sugarcane. Fahim and Zafarulla (2015) reported *Scandix* spp. to be a big problem in sugarcane fields, cited by 96% of the surveyed farmers in Pakistan. It was followed by *Sorghum halepense* and *Cirsium arvense*.

Usually, the diversity of weed species is high in sugarcane fields. However, most of these weeds are considered highly invasive and hard-to-control as they are adapted to the sugarcane cropping system. They are either tolerant or resistant to the most used herbicides in most cases. Some weed species are location-specific, while others are widespread in most sugarcane-producing regions.

Several damages are reported in sugarcane plantations as consequences of weed interference, mainly the following ones:

- **Reduction in stalk and sugar yield:** Weed competition can cause losses in crop performance ranging from 10 to 80% (Procópio et al. 2016). This wide range can be attributed to differential varietal/clonal competitive ability, as well as the sanitary status; the harvesting cycle (1-year harvest, 1½-year harvest, sett sugarcane, ratooning sugarcane); weed species established; plant density and timing of weed emergence; in addition to the availability of light, nutrients, and moisture in the soil.

- **Decrease in field longevity:** High weed infestation levels associated with poor control can accelerate the need for sugarcane renewal. The natural yield loss in sugarcane fields is accentuated in areas with poor weeding techniques, forcing many farmers to start stump destruction after only three cuttings when the original schedule was to carry out plantation renewal at least after five cuttings. This occurs as a result of the field's premature depletion process, associated with the lack of proper fertilization soil compaction occurrence of insect pests, nematodes, among others (Pinke et al. 2010; Nagy et al. 2018).
- **Difficulty and harvesting costs increase:** Weed presence in harvesting, whether manual or mechanical, causes operational inconvenience and increases in costs. When fields are infested with weeds, labor cost increases. In mechanized harvesting, weeds cause constant interruptions for cleaning and unclogging the harvester's cutting and supply mechanisms. There is also premature machinery wear and difficulty adjusting the proper cutting height and damage the sprouting.
- **Decrease in the industrial quality of the raw material:** When a cane field infested with weeds is harvested, it is inevitable that seeds and plant parts of weeds are transported along with stalks to the industrial unit.
- **Shelter for insect pests and sugarcane diseases:** Many weed species commonly found in sugarcane fields can host insect pests or serve as hosts for certain species of fungi, bacteria, and nematodes that cause significant damage to sugarcane plantations.
- **Land value:** Certain weed species such as purple nutsedge (*Cyperus rotundus*) and crabgrass (*Rottboellia exaltata*), especially in high densities, can depreciate the land's market value or even harm the agreement of lease contracts. Special care must be taken to avoid the weed spread of these species in areas with no occurrence history.

16.3 Planting Timings and Critical Period of Interference

In Midwestern Brazil, sugarcane is planted at two different times. September–November: vegetative cycle with ~12 months, called "1-year sugarcane." January–April: vegetative cycle ~14–18 months, being called "1½-year sugarcane." Variations in cycle duration depend on climate, planting date, etc. (Procópio et al. 2016). After the first harvest, all subsequent cuttings/harvests in the same field, regardless of whether they originate from 1-year or 1½-year sugarcane, will have an average duration of 12 months, being called "sugarcane ratoon."

Weed interference on the crop will depend on variety/clone, seedling quality, weed species, soil fertility, planting depth and spacing, and cultural management, factors that accelerate or delay sugarcane development. Table 16.1 shows averages for the period prior to weed interference (PPWI), total interference prevention period (TIPP), and critical interference prevention period (CIPP) in Midwestern Brazil, as reference.

The PPWI is approximately 20–30 days after the emergence of the primary stalk in sett-cane. Plant maintenance depends almost exclusively on its reserves in the first

Table 16.1 Period prior to weed interference (PPWI), total interference prevention period (TIPP), and critical interference prevention period (CIPP) for sugarcane plantations in the Midwestern region of Brazil

Planting time	TIPP (days)	PPWI (days)	CIPP (days)
1½-year sett sugarcane	90–150	20–50	20–150[a]
1-year sett sugarcane	90–120	20–40	20–120
Ratooning (sprout. May/Sep.)	90–100	30–40	30–100
Ratooning (sprout. Oct./Dec.)	70–90	20–30	20–90
Pre-sprouted seedlings	0–195	0–19	19–195[b]

[a] Sugarcane planted in April, infested by *Brachiaria decumbens* and *Panicum maximum*
[b] Sugarcane planted with pre-sprouted seedlings in December, infested by *Merremia aegyptia* and *Brachiaria decumbens*. Source: adapted from Procópio et al. (2016) and Amaral et al. (2019)

cycle days. With ratooning sugarcane, reserves at the base of old stumps sustain the new shoots for the initial period.

16.4 Chemical Weed Control in Unburned Sugarcane

The current trend of increasing areas of sugarcane harvested without traditional fire burning (unburned sugarcane), whether due to environmental aspects or even by market demand, and the current weed management strategies in these areas present significant changes. The majority of weed management expertise in this new technology has yet to be developed. Sugarcane production for mechanized harvesting of unburned sugarcane has been grown in the recent decades (Velini and Negrisoli 2000; Ferreira et al. 2010). Harvesting without burning causes some beneficial agronomical factors such as reduction of soil erosion; better soil moisture conservation; more remarkable nutrient recycling; increase in soil organic matter and soil microbial activity; improvement of soil physicochemical properties; less stalk lodging caused by burning; decreased weed infestation due to the presence of crop straw, and the loss of sugars via exudation from the stalks during and/or immediately after burning is avoided. The straw from the preserved sugarcane provides thick ground cover that hinders weed emergence, reducing light incidence to the soil. There may also be the release of exudates from the straw, which may have allelopathy on the germination of some weed species (Velini and Negrisoli 2000; Ferreira et al. 2010).

Several major issues including retardation in tillering stages in some varieties, severity of pest incidence, increasing of using nitrogen fertilizers and low temperature may reduce sugarcane growth and development. In lowlands, due to excessive humidity, incidence of weeds are inevitable, but by application of precision agriculture, everity of weeds has been managed (Ferreira et al. 2010).

This mulching is very significant for weed control as it influences the dormancy, germination, and seed mortality of weeds (Trezzi and Vidal 2004; Ferreira et al. 2010). Such mulching also reduces erosion and evaporation and increases water infiltration and moisture retention (Reddy 2003). The physical impediment caused

by the mulching also causes etiolation and makes weed seedlings susceptible to mechanical damage (Victória Filho 1985; Correia and Durigan 2004). There is also a reduction in the emergence of positively photoelastic weeds when they grow from seeds and require specific wavelengths (Correia and Durigan 2004).

Toledo et al. (2005) compared unburned sugarcane with traditional, burned sugarcane in Mexico; they reported that the former resulted in lower weed aggressiveness, greater biomass production (larger and thicker stalks, in addition to greater quantity), juice purity, and sugar production, as well as positive differences in organic matter, nitrogen, phosphorus, potassium, and soil pH levels. The economic analysis also showed higher income for unburned sugarcane. Núñez and Spaans (2008), in a similar study comparing the two systems, achieved 35% lower cots with weed control when harvesting unburned sugarcane.

In Brazil, sugarcane straw mulching drastically reduced soil temperature between day and night at 1 and 5 cm depth (Velini and Negrisoli 2000; Ferreira et al. 2010). According to them, this effect decisively contributes to reducing weed germination in unburned sugarcane fields, as thermal amplitude is determinant in the seed germination of many species.

The greater effectiveness of straw mulching in reducing weed emergence depends fundamentally on its uniform distribution on the soil surface, as small stand failures are sufficient to provide favorable conditions for the emergence of positively photoelastic weeds (Ferreira et al. 2010). Among the species whose population has been increasing in surveys carried out in areas of unburned sugarcane, mainly in the Southeastern region of Brazil, *Euphorbia heterophylla*, *Ipomoea* ssp., *Merremia* ssp., *Senna obtusifolia*, *Cissampelos glaberrima*, *Pyrostegia venusta*, *Momordica charantia*, *Neonotonia wightii*, and *Cyperus rotundus* are highlighted. Sedges are reduced by mulching but at unsatisfactory levels (Ferreira et al. 2010; Procópio et al. 2016). These reports show the apparent trend of flora change in sugarcane production areas, previously dominated by grasses in burned sugarcane fields and now with the preponderance of dicotyledons, especially those with large seeds and some sedges (Ferreira et al. 2010; Procópio et al. 2016).

Besides modifying weed species composition, straw from the unburned harvest can alter the efficiency of active soil herbicides—those with residual effect. This change is mainly the result of the interception of spray droplets during herbicide application, preventing them from reaching the soil where they are supposed to prevent weed emergence. Some alternatives to improve herbicide management and efficiency where unburned sugarcane was harvested are in progress.

16.4.1 Post-emergence Herbicide Application

It has the advantage of identification of the species that emerged and then choosing the best treatment. It helps in reducing control costs and the impact on non-target organisms. A disadvantage may be the need for a second herbicide application. This can happen because many weed species present delayed emergence that may cause a new emergence flow after the first application, and this new flow may occur before the critical interference prevention period (CIPP) end.

16.4.2 Herbicide Application Prior to Straw Deposition

It is possible to carry out this technique by adapting the sprayer to the mechanical harvester, where the product is applied before releasing the harvest residues to the soil. This technique has been studied by cooperation between agricultural machinery companies, pesticide manufacturers, and research institutions.

16.4.3 Application of Specific Herbicides Over the Straw Mulching

Studies have shown satisfactory efficiency of some pre-emergence herbicides, even when applied to sugarcane straw. The main property of suitable herbicides for this technique is their water solubility. All herbicides that performed well experimentally when used over the straw mulching are highly soluble in water. The occurrence of rain after the application has also been identified as an important factor for washing the herbicide from the straw to the soil. Furthermore, the influence of straw on herbicide dynamics is dependent on the mulching volume. Often, for low-solubility herbicides, less than 5 t ha^{-1} of straw is enough to affect their efficiency.

16.4.4 Pre-emergence Herbicide Application

This type of herbicide application is evenly sprayed on the soil layer of a certain thickness when the bud, seedling, and root of weed seeds absorb it by contact to play a weed-killing role. The advantage of soil treatment is that the weed is killed before emergence so that the weeds cannot be excavated to harm at certain period. After the application of soil treatment, the sugarcane field has been in a grassless state for a long time, which is conducive in promoting the growth of sugarcane. The time of application is also less limited by weather, even on rainy days, but the disadvantage is that it is easily affected by soil type, organic matter content, and weed composition. After the herbicide is sprayed on the soil surface, a layer of herbicide film can be formed on the field surface, called the drug film layer. When the weeds begin to germinate and encounter the drug film layer, the germ or radicle of weeds will absorb the herbicide and die of poisoning, and the weeds will be sealed in the soil (for example, Atrazine, S-metolachlor, etc.).

Under Iranian sugarcane fields, Alion®, a new promising herbicide, was registered in 2016 and successfully applied on ratoon fields for control of annual grass and broadleaf weed species and this herbicide was used in Asian-African sugarcane-producing countries (Nikpay et al. 2015; Abin et al. 2017; Sharafizadeh and Nikpay 2018). Alion® provides long-lasting, unique management for pre-emergence control of a wide range of grass and broadleaf weeds, including those resistant to other herbicides, all without phytotoxic symptoms. The active ingredient in this herbicide is a cellulose-biosynthesis inhibitor (CBI), which affects cell wall formation, cell elongation, and division. The main drawback of this herbicide is that the limited application for ratoon fields and spraying at planting

Table 16.2 Main herbicides used in sugarcane plantations as a function of location, mode of action, and application timing

Herbicide	Mode of action	Application Timing	Country			
			BR	CN	PK	IR
2,4-D	Auxin	Post	√			√
2,4-D + Picloram	Auxin	Pre/post	√			
Ametryn	FSII	Pre/post	√	√	√	√
Atrazine	FSII	Pre/post		√	√	√
Ametryn + Atrazine	FSII	Pre/post			√	
Diuron	FSII	Pre/post	√	√	√	√
Ethoxysulfuron	ALS	Early post	√			
Metribuzin	FSII	Pre/post	√	√	√	√
Tebuthiuron	FSII	Pre	√		√	√
Hexazinone + Diuron	FSII	Pre/post	√	√	√	
Amicarbazone	FSII	Pre/post	√			√
S-metolachlor	Cell division	Pre	√	√	√	
Imazapic	ALS	Pre	√	√	√	
Imazapyr	ALS	Pre	√		√	
Halosulfuron	ALS	Post	√	√	√	
Trifloxysulfuron-sodium	ALS	Post	√	√	√	
Clomazone	Carotenoids	Pre	√		√	
Isoxaflutole	Carotenoids	Pre	√			
Mesotrione	Carotenoids	Post	√	√	√	
Sulfentrazone	Protox	Pre	√	√	√	
Oxyfluorfen	Protox	Pre	√	√	√	
Saflufenacil	Protox	Post	√		√	
MSMA	Respiration	Post	√		√	
Trifluralin	Mitosis	Pre/PPI	√		√	
Pendimethalin	Mitosis	Pre/PPI	√	√	√	
Indaziflam	Cellulitis	Pre			√	√
Paraquat	FSI	Post/DIR			√	√
Glyphosate	EPSPs	Post/DIR	√	√	√	√
EPTC	Lipids	Pre			√	√

ALS acetolactate synthase; *Pre* pre-emergence; *post* post-emergence; *PPI* pre-planting application, incorporated to the soil; *DIR* non-selective herbicides, application directed to inter-rows; *BR* Brazil; *CN* China; *PK* Pakistan; *IR* Iran

must be avoided due to highly adverse effects on cane germination and severe dwarf. Table 16.2 lists the main herbicides applied to sugarcane plantations as a function of location, mode of action, and application timing. Commercially available herbicide mixtures were not included.

16.5 Climatic Factors Affecting Herbicide Activity in Sugarcane Fields

There is practically herbicide in use for every sugarcane weed considering the available herbicides. However, the results have sometimes been not conclusive in the field due to the lack of knowledge about the application, equipment, and the disregard for the environmental conditions (solar radiation, temperature, air and soil humidity, wind, dew). These factors on herbicide effectiveness are complex because they interact with each other (Procópio et al. 2016). Some remarks are made regarding the impact of these factors on the action and efficiency of herbicides applied to sugarcane fields.

16.5.1 Sun Radiation

According to Monquero et al. (2004) and Galon et al. (2013), light can increase herbicide translocation as it promotes photosynthesis and, consequently, its movement together with these into the plant. However, the high light intensity increases the cuticle and leaf mesophyll thickness in certain situations. A more significant number of trichomes or even leaf curling in grasses can hinder the absorption of herbicides.

16.5.2 Rains

Rains interfere with herbicide action depending on intensity, duration, and frequency. Ferreira et al. (2005) report that raining a few days before herbicide application post-emergence increases weed susceptibility, thus improving control efficiency. This is mainly due to the increase in soil water content and the washing of part of the waxes and alkanes from the leaf surface. This, on the other hand, may also reduce herbicide selectivity to specific sugarcane clones, which needs to be taken into account when applying herbicides to sugarcane fields (Ferreira et al. 2005; Galon et al. 2009, 2013). Herbicides applied in pre-emergence need to be effective that the soil has good water content, as the presence of water facilitates the absorption of these products by plants (Nunes et al. 2018; Takeshita et al. 2019).

16.5.3 Air Relative Humidity

The relative air humidity is probably the factor affecting the life length of spray droplets and herbicide activity, especially those that target emerged weeds, applied post-emergence (Procópio et al. 2016). The relative humidity of the air affects herbicide absorption and translocation when applied to the leaf. It involves the permanence time of the droplet on the leaf and influences cuticle hydration (Meyer et al. 2016; Almeida et al. 2017). The low relative humidity causes the droplet to evaporate quickly, hinders penetration via cuticle, and can cause water stress on the plant.

16.5.4 Temperature

Air temperature influences herbicide action in several ways, as it can modify its properties and alter physiological processes (Almeida et al. 2017). Gupta and Lamba (1978) found that low (below 10 °C) or very high temperatures can reduce plant metabolism, herbicide action, and weed control efficacy. The crop's lower herbicide selectivity can also occur when applied at extreme temperatures. This is mainly because the herbicide's selectivity to the crop is very often attributed to the differential metabolism promoted by the crop plant.

16.5.5 Wind Speed

Wind indirectly affects the herbicide uptake by plants, as it increases spray droplet evaporation from the leaf surface (Galon et al. 2021). Plants growing in high wind speed and high temperatures usually have a thicker and more pubescent cuticle, hindering herbicide absorption.

In the application of pesticides, wind can cause a droplet drift. Drift can cause chemical deposition in unwanted areas, negatively affecting crops sensitive to these molecules, especially herbicides (Ferreira et al. 2006; Galon et al. 2021).

16.5.6 Managing to Reduce Adverse Climatic Effects on Herbicide Efficiency

Some techniques can be adopted to reduce the negative impact of unfavorable environmental conditions on the effectiveness of herbicides applied to sugarcane, such as:

- Do not apply pesticides under unsuitable ecological conditions (relative humidity, temperature, wind speed greater than 10 km h^{-1}, or less than 3 km h^{-1});
- Do not apply herbicides to weeds under stress conditions (difficult herbicide absorption and translocation);
- It is recommended to apply early in the morning, or at late afternoon, or, if the herbicide and technological conditions allow, apply at night;
- Mechanically incorporate to soil sensitive herbicides to photo-decomposition when soil is dry or with low humidity;
- Use, if possible, large droplets in spraying;
- Do not exceed pumping pressure for the spray nozzle to avoid drift in particular;
- Use the adjuvants or surfactants recommended by the herbicide manufacturer for each situation.
- The best time to apply stem and leaf treatment herbicides is when most weeds are 3–5 leaves or 10–15 cm tall.

16.6 Tolerance of Sugarcane Genotypes to Herbicides

Sugarcane cultivars can present distinct responses to herbicides for weed control, which as a consequence leads to phytotoxicity problems, even causing losses in production (Ferreira et al. 2005; Galon et al. 2009, 2010). A sugarcane variety or clone can show a different behavior, depending on the herbicide used and according to the climatic, soil, and management conditions. In the field, some herbicide symptoms in sugarcane are commonly reported (Figs. 16.1, 16.2, 16.3, and 16.4), such as:

- Leaf bleaching (pigment inhibitors);
- Leaf chlorosis and necrosis on leaf edges and tips (photosynthesis inhibitors that are absorbed applied to leaves, and respiration inhibitors);
- Reduced crop growth (amino acid inhibitors and photosynthesis inhibitors);

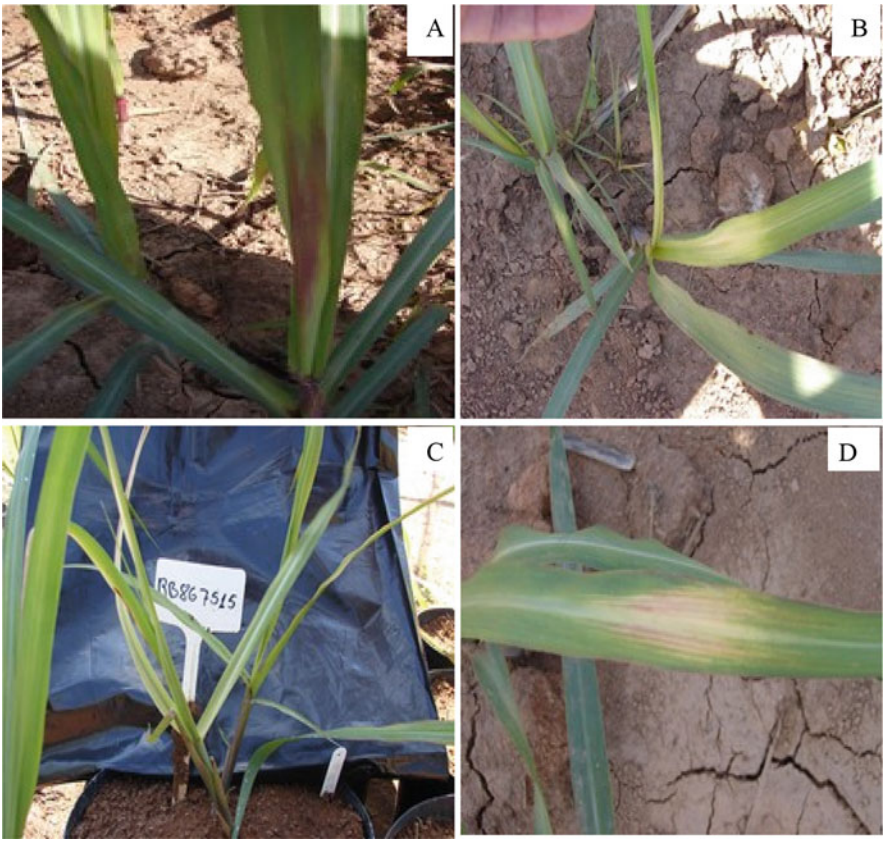

Fig. 16.1 Phytotoxicity of trifloxysulfuron-sodium to sugarcane cv. RB867515 plants. (Photo source: Leandro Galon)

Fig. 16.2 Phytotoxicity of Ametryn to sugarcane cv. RB855113 plants. (Photo source: Leandro Galon)

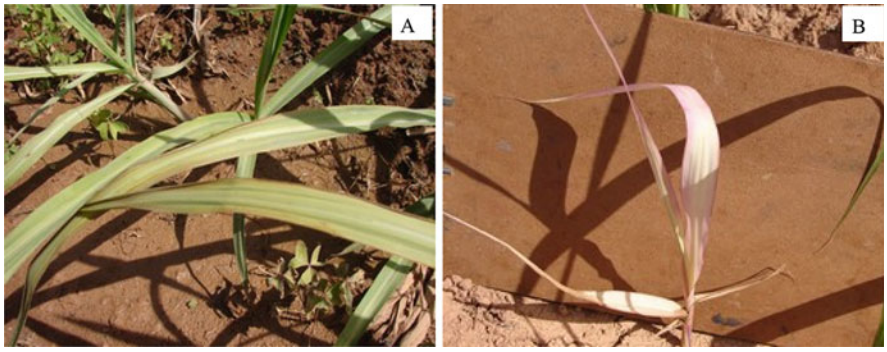

Fig. 16.3 Phytotoxicity of the commercially available mixture of [Trifloxysulfuron-sodium + Ametryn] to sugarcane plants. (Photo source: Leandro Galon)

- Teratogenesis in stalks and roots, thinner or curved internodes, thickened and tumored nodes, curved elbow-shaped stalks, roots with less development, and meristematic necrosis near nodes (growth regulators).

Typically, these phytotoxicity symptoms are expected to disappear within 15–90 days of their onset. However, the period necessary for the recovery of

Fig. 16.4 Phytotoxicity of 2,4-D on sugarcane variety CP69-1062 stems causing galls. (Photo source: Amin Nikpay)

sugarcane plants depends mainly on the type of phytotoxicity symptom, the intensity of the symptoms, and climatic, soil, and management conditions (Ferreira et al. 2005; Galon et al. 2009, 2010).

16.6.1 Visible Impacts of Herbicides on Sugarcane Genotypes

Ferreira et al. (2005) reported differential genotype susceptibility to (the commercial mixture of) [trifloxysulfuron-sodium + ametryn]. RB855113 was the genotype most susceptible, while SP80-1816, SP80-1842, SP79-1011, and RB957689 showed medium susceptibility to the herbicide mixture. The susceptibility was considered low for the other cultivars (Table 16.3).

Azania et al. (2006) reported that herbicides were more phytotoxic to sugarcane when applied at late post-emergence. In early post-emergence, plants fully recovered from herbicide intoxication with a smaller impact on stalk yield. This could be explained, at least in part, by greater leaf number at a more advanced stage, maximizing herbicide interception. Concenço et al. (2007) claim that as plant ages, morpho-anatomical traits cause the herbicide to be less absorbed, among them highlights the reduction in plasmodesmata pore diameter as one of those responsible for the lower absorption or deficient translocation of herbicides in plants. In sugarcane, however, the lower ability in herbicide translocation by plants is apparently compensated by the higher number of leaves able to intercept the herbicide.

Barroso et al. (2008), working with the sugarcane cultivar SP80-1816, reported that some herbicides promoted accentuated phytotoxicity to crop (Table 16.4). The authors noted that treatments that caused the highest toxicity early after the application were [ametryn + clomazone] (1800 + 1200 g ha^{-1}) and clomazone (1250 g ha^{-1}). It was also reported that sulfentrazone (900 g ha^{-1}) resulted in the lowest damage level to the crop, a behavior maintained in later evaluations.

Table 16.3 Effect of the commercially available mixture of [ametryn + trifloxysufuron-sodium] on sugarcane genotypes

Cultivar/clone	Phytotoxicity (%)		SDM (%)[a]	Herbicide susceptibility
	13 DAT	34 DAT		
RB855113	13.75	44.40	33. 32	High
SP80-1842	7.50	21.16	50.29	Mean
SP80-1816	5.75	13.17	58.73	Mean
RB855002	6.25	8.33	94.79	Low
RB928064	3.75	5.83	90.51	Low
SP79-1011	8.50	16.60	40.35	Mean
SP81-3250	2.50	2.83	95.88	Low
RB867515	4.25	5.83	94.45	Low
RB957712	2.50	6.33	88.53	Low
RB72454	7.50	7.50	91.76	Low
RB845210	7.50	10.83	85.30	Low
RB947643	5.00	4.17	89.24	Low
RB855536	2.75	4.20	93.76	Low
RB835486	1.25	6.67	86.05	Low
RB957689	15.00	24.17	49.52	Mean

[a] SDM = relative shoot dry mass, comparatively to the respective control without herbicide application. Assessments were conducted 45 days after herbicide application (Ferreira et al. 2005)

Table 16.4 Herbicide toxicity to sugarcane genotype SP80-1816. Santa Helena de Goiás, Brazil, 2006/07

Treatment	Dose g ha^{-1}	Phytotoxicity (%)			
		7 DAA	14 DAA	21 DAA	35 DAA
[Clomazone + hexazinone]	1000 + 250	19.0	9.3	3.5	0.0
[Clomazone + hexazinone]	1200 + 300	20.0	11.3	5.8	0.0
Sulfentrazone	900	12.5	9.8	2.8	0.0
[Ametrina + clomazone]	1800 + 1200	24.5	14.5	4.3	0.0
Clomazone	1250	23.3	15.5	5.3	0.0
[Sulfentrazone + clomazone]	1000 + 500	17.3	14.3	4.5	0.0
Control—infested	–	0.0	0.0	0.0	0.0
Control—hoeing (clean)	–	0.0	0.0	0.0	0.0

Source: Barroso et al. (2008)

Phytotoxic effects should not be determined just by checking visual symptoms, as herbicides can reduce crop yields without causing visually detectable effects. On the other hand, some herbicides can cause severe injuries, which disappear with sugarcane development (Velini and Negrisoli 2000; Negrisoli et al. 2004; Galon et al. 2010). Therefore, the selectivity of herbicides applied both pre-emergence and post-emergence will depend on climate, soil, herbicide physicochemical properties and dose, genotype and development stage, application technology, and crop management.

16.6.2 Invisible Impacts of Herbicides on Sugarcane Genotypes

There is a piece of minimal knowledge about the impact of herbicide application on crop physiology. Although not causing visually detectable phytotoxic symptoms to sugarcane, some herbicides may impair physiological processes and cause damages only noticed as reduced stalk or juice yields. Galon et al. (2010) reported impacts of ametryn, trifloxysulfuron-sodium, and its commercial mixture, on sugarcane physiological performance (Table 16.5).

The concentration of CO_2 within the leaf (Ci), available for photosynthesis, was affected by the herbicide treatments and reported differences among genotypes when assessed early in crop development (Fig. 16.5). As expected, the application of ametryn (PSII) resulted in higher CO_2 concentrations within the leaf once photosynthesis was most severely affected. CO_2 concentration within the leaf was approximately 50% higher in treatments with ametryn compared to hoeing. Trifloxysulfuron-sodium also impacted Ci, but lower magnitudes (Table 16.5).

Photosynthesis rate (A) reported for trifloxysulfuron was similar to the control, while treatments involving ametryn presented photosynthesis rate inferior to those noted for the control. When considering the treatment containing ametryn + trifloxysulfuron, it was possible to highlight the genotype RB947520 due to its ability to deal with the treatment and keep the photosynthesis rate.

Chemical herbicides can effectively control major weeds in sugarcane fields and affect the chlorophyll content, photosynthetic rate, and active enzymes of some sugarcane varieties (Wang et al. 2012). Liu (2016) reported that spraying different concentrations of dimethyltetrachloride at 3~4 leaf stage had different effects on photosynthesis, chlorophyll content, and stomatal conductance of rice. Spraying a specific concentration of herbicides can effectively control the spread and growth of weeds in sugarcane fields and improve the yield and quality of sugarcane. But at the same time, it also has a certain influence on the development of cane seedlings. This effect mainly manifests in agronomic characters, physiological characteristics, and chemical residues.

Huang et al. (2013) found that cultivars Yuetang 00-236, Yuetang 55, Yuetang 93-159, and Xintai Sugar 22 showed no symptoms of herbicide injury in terms of plant height, tillering rate, leaf shape, survival rate, total fresh weight, shoot fresh weight, and effective stem at low doses, and their growth was the same as that of the control sprayed with clear water. When the dosage reached a certain degree, MCPA-sodium 56% showed an inhibitory effect on the agronomic traits of the four varieties.

Even the damages caused by ametryn being visually identified more accessible, the photosynthesis rate under trifloxysulfuron-sodium was also impacted. In other words, herbicide damage on crops maybe not be visually detectable but still harm crop development.

Ametryn (2000 g ha^{-1}), trifloxysulfuron-sodium (22.5 g ha^{-1}), and the commercially formulated mixture [ametryn + trifloxysulfuron-sodium] (1463 + 37 g ha^{-1}) applied to 10 sugarcane genotypes (RB72454, RB835486, RB855113, RB855156,

Table 16.5 Physiological variables assessed in sugarcane genotypes as a function of the application of the herbicides ametryn, trifloxysulfuron-sodium, and its commercial mixture

	Sugarcane genotype					
Treatment	RB72454	RB835486	RB855113	RB867515	RB947520	SP80-1816
Internal CO_2 concentration (Ci, µmol mol^{-1})						
Hoeing	AB 102	A 177	AB 104	AB 123	B 68	AB 120
Ametryn 2000 g ha^{-1}	A 165	A 136	A 169	A 178	A 134	A 157
Trifloxysulfuron 22.5 g ha^{-1}	AB 126	AB 127	AB 128	AB 114	B 88	A 137
Ame. + Trifl. 1673 + 37 g ha^{-1}	AB 146	B 877	AB 125	A 179	AB 145	A 158
Photosynthesis (µmol m^{-2} s^{-1})						
Hoeing	AB 45.1	AB 51.2	B 41.3	AB 47.9	A 60.7	AB 48.0
Ametryn 2000 g ha^{-1}	A 37.5	B 25.8	A 36.1	B 28.9	A 37.0	A 37.3
Trifloxysulfuron 22.5 g ha^{-1}	B 41.1	B 36.6	B 38.9	B 40.3	A 49.5	B 38.8
Ame. + Trifl. 1673 + 37 g ha^{-1}	A 42.1	A 36.4	A 38.4	B 32.8	A 40.5	A 40.1

Source: Galon et al. (2010)

Fig. 16.5 (**a**) Infrared Gas Analyzer (IRGA) and (**b**) its application under field conditions to assess the physiological performance of sugarcane genotypes following pre- or post-emergence herbicide application. (Photo source: Evander A. Ferreira)

Table 16.6 Sugarcane stem productivity (% of control) as a function of genotype treated with trifloxysulfuron-sodium, ametryn, or its commercial mixture. Source: Galon et al. (2009)

	Herbicide			
Genotype	Hoeing	Ametryn (2000 g ha^{-1})	Trifloxysulfuron (22.5 g ha^{-1})	Ametryn + Trifloxysulfuron (1673 + 37 g ha^{-1})
RB72454	100.00	95.92	90.86	100.00
RB835486	100.00	100.00	83.10	99.82
RB855113	100.00	98.84	95.34	86.53
RB855156	100.00	92.24	81.69	90.69
RB867515	100.00	96.30	100.00	99.11
RB925211	100.00	100.00	99.32	90.36
RB925345	100.00	98.51	95.93	100.00
RB937570	100.00	96.38	99.31	100.00
RB947520	100.00	99.46	100.00	100.00
SP80-1816	100.00	100.00	100.00	100.00

RB867515, RB925211, RB925345, RB937570, RB947520, and SP80-1816) demonstrated that the genotype RB855156 was the most susceptible one while RB925345, RB947520, and SP80-1816 were the most tolerant ones (Galon et al. 2009). The same study reported complete crop recovery within 60 days after application; however, there were still differences in stalk productivity (Table 16.6). Thus, the authors concluded that the selectivity of ametryn, trifloxysulfuron-sodium, and [ametryn + trifloxysulfuron-sodium] to sugarcane is dependent on genotype (Table 16.7).

Table 16.7 Weed species with resistance to herbicides in Brazil, common to sugarcane and other dryland crops

Weed species	Resistance to	Weed species	Resistance to
Euphorbia heterophylla	ALS, PROTOX, EPSPs	*Bidens pilosa*	ALS
Digitaria insularis	ACCase, EPSPs	*Bidens subalternans*	ALS, FSII
Digitaria ciliaris	ACCase	*Urochloa plantaginea*	ACCase
Amaranthus palmeri	ALS, EPSPs	*Eleusine indica*	ACCase, EPSPs
Amaranthus retroflexus	ALS, FSII, PROTOX	*Conyza bonariensis*	EPSPs
Amaranthus viridis	ALS, FSII	*C. canadensis*	EPSPs
Amaranthus hybridus	ALS, EPSPs	*Conyza sumatrensis*	ALS, Auxin, EPSPs, FSI, FSII, PROTOX

Source: Heap (2021)

16.7 Weed Resistance and Tolerance to Herbicides in Sugarcane

16.7.1 Weed Resistance to Herbicides in Sugarcane

Farmers prefer to use herbicides over other weed management methods due to the high efficiency, practicality, and relatively lower cost than other control methods. However, the indiscriminate and inappropriate use of herbicides led to the development of resistance to these compounds by various weeds (Vrbničanin et al. 2017).

The plant is considered susceptible to a herbicide when it grows and develops differently by its action, causing plant death when subjected to a specific dose. On the other hand, tolerance is the innate ability of species to reproduce and grow after herbicide treatment. This is based on the species' natural ability to avoid the herbicide's effect in some way. The species is considered resistant to a given herbicide when it acquires to survive certain herbicide treatments that control other individuals of the same species (Christoffoleti et al. 2016; Vrbničanin et al. 2017).

The repeated use of the same compound can select preexisting resistant biotypes into the community, increasing the proportion of resistant individuals. It can increase to a point where it compromises the control efficiency in just a few years (Christoffoleti et al. 2016).

The first cases of herbicide resistance were reported in the year of 1957 (USA and Canada). Many other cases have been reported since then, and currently, there are more than 500 resistant weed biotypes distributed in more than 55 countries (Heap 2021). There are weed species resistant to more than one herbicide mode of action. The largest number refers to herbicides that inhibit ALS, ACCase, and FSII enzymes (Heap 2021). The largest number of resistant biotypes to these modes of action is due

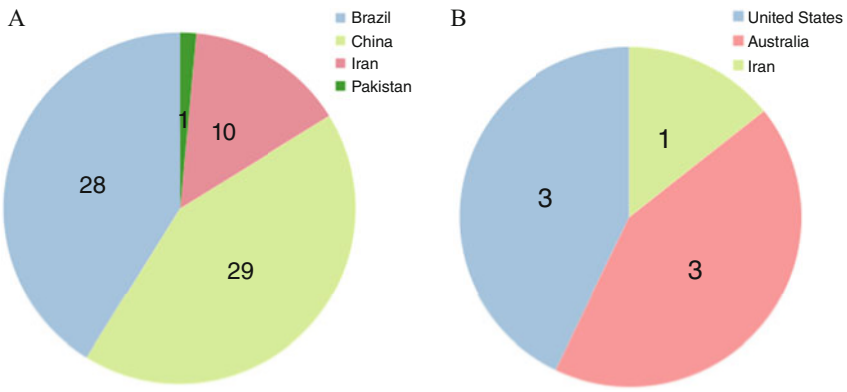

Fig. 16.6 Unique weed species with resistance to herbicides in some sugarcane-producing countries, common to various dryland crops (**a**); weed species with resistance to herbicides occurring specifically in sugarcane plantations worldwide (**b**) (Heap 2021)

to their higher specificity in their mechanism of action and efficiency. They are also applied to large areas in consecutive years in different crops.

Several weed species have a straightforward cross and multiple resistance to different herbicides infesting sugarcane, as shown in Fig. 16.6a (Heap 2021). In many cases, weeds show various resistances, and they are resistant to more than one mechanism of action, which makes chemical control more difficult in crops where these species appear. Among the resistant weeds occurring in sugarcane plantations, only a few species are specific to sugarcane (Fig. 16.6b); most of them are weed species adapted to other dryland crops (soybean, maize, pastures, orchards, etc.) whose occurrence is also highly reported in sugarcane fields. This makes sense when considering that weeds are usually competitive-type plant species, with the ability to easily adapt to other crops and cropping systems in similar edaphic and climatic conditions (Concenço et al. 2014). Considering the Brazilian sugarcane weed scenario (Table 16.7), there are 14 weed species common to several crops, severely infesting sugarcane plantations.

Resistance prevention and management aim to reduce selection pressure, control hard-to-kill individuals before they seed, and expand the possible control alternatives. This can be achieved by adopting some management practices:

- Using herbicides with different mechanisms of action
- Carrying out sequential applications of the same herbicide with the interval of 9–14 days interval between applications
- Applying herbicide mixtures with different mechanisms of action and detoxification
- Adopting crop rotation and alternating herbicides with distinct mechanisms of action
- Limiting the number of applications of the same herbicide into an agricultural year

- Choosing herbicides with innate lower selection pressure (lower residual and lower efficiency)
- Promote rotation and integration of weed control methods
- Monitor changes in flora by means of periodic phyto-sociological surveys
- Preventing suspicious plants from producing seeds by identifying, locating, and destroying them
- Diversify land use and soil tillage systems

16.7.2 Weed Tolerance to Herbicides in Sugarcane

The factors responsible for selecting herbicide-tolerant species are more similar to those observed in selecting resistant biotypes from normally susceptible populations (Owen 2006; Concenço et al. 2014). Environmental factors also influence changes in weed flora composition. Thus, when there is a predominance of tolerant plant species in a population, it can become more challenging to control tolerant biotypes than to reduce the frequency of individuals of a given resistant biotype. Repeated application of the same herbicide, or herbicides with the same mechanism of action, creates selection pressure. The two main ways of weed response are specific changes in flora, through the selection of more tolerant weed species, or intraspecific selection of herbicide-resistant biotypes (Christoffoleti and Caetano 1998; Concenço et al. 2014).

According to Christoffoleti et al. (2016), any plant population that shows a variable genetic basis regarding tolerance to a particular control measure will, over time, change its population composition towards tolerance as an escape mechanism for survival. According to the same authors, the use of the plow eliminated at first most weeds, but new and more adapted species began to infest plowed crop fields over time. Another example was the no-till system, which caused a drastic reduction in weed incidence; however, it led to the selection of species adapted to the new condition after some time.

Tolerance of weeds to herbicides occur by mechanisms also attributed to resistance and herbicide selectivity to crops. It may occur due to the developmental stage, differences in leaf morphology and anatomy, differential absorption and translocation rates and compartmentalization; and to improve the metabolism of the herbicide (Westwood and Weller 1997; Vargas et al. 1999; Concenço et al. 2014). In sugarcane, herbicides belonging to triazine and substituted urea groups have been used to control crabgrass (*Digitaria* spp.). The genus *Digitaria* has 13 morphologically similar species, and these include some of the main weeds in Brazilian sugarcane crops in central-southern Brazil (*D. nuda*, *D. ciliaris*, *D. horizontalis*, and *D. bicornis*) (Dias et al. 2007). According to the same authors, crabgrass variants are being selected by herbicide misuse in sugarcane.

Dias et al. (2007) also reported *D. nuda* as most tolerant to imidazolinones and substituted ureas, compared to *D. ciliaris*. The former was reported to be most tolerant to diuron, imazapyr, and tebuthiuron, than the latter. Both species' comparative absorption and translocation of diuron (leaf-applied), imazapyr, and metribuzin

(root-applied) demonstrated that absorption and translocation mechanisms differed between species. The same authors reported that hexazinone + diuron, tebuthiuron, and imazapic had the lowest controls for *D. nuda* pre-emergence, and diuron and hexazinone + diuron, the lowest controls for *D. nuda* post-emergence. Therefore, it is clear that this species is one of the most tolerant to these herbicides.

16.8 Technology of Herbicide Application in Sugarcane

There are many compounds for weed control in sugarcane, both pre- and post-emergence (early, intermediary, or late post-). In addition, there are systemic or contact herbicides available, some very selective and some demanding special care for not harming the crop. Furthermore, the difficulty in getting machinery for herbicide application into the area after a certain crop height, the presence of straw mulching (unburned sugarcane) make weed management in the sugarcane field a complex task. In the following, some herbicide application methods to sugarcane fields and the special care needed will be described.

16.8.1 Aircraft Applications

Application of weedicides through aircraft and drone are widely used in developed countries in large cultivated areas and is recommended pre-emergence and initial post-emergence herbicide applications. This method is not recommended for weed control in intermediary or late post-emergence, as it is impossible to achieve good weed coverage. The success of this type of application depends upon favorable wind conditions such as preponderant, convective currents, air temperature, and also humidity.

16.8.2 Tractor-Towed and Self-Propelled Sprayers

When carried out in the broad area, it is made with tractor-towed equipment or self-propelled sprayer, with spraying bars ranging from 7 to 20 m in width, moving on average at 4–10 km h^{-1}, depending on equipment and terrain topography. Applications can be accomplished pre-emergence or early to late post-emergence.

16.8.3 Backpack Sprayers

This type of application is widely used in areas with irregular topography, in small regions of sugarcane production, in localized infestations, and in fixing small problematic plots after an overall application. Equipment for this type of application can be manually pumped or electrically pressurized backpack sprayers, allowing greater application yield and less effort for workers. Some small-sized sprayers with

Table 16.8 Control efficiency of Guinea grass (*Panicum maximum*) in four assessment timings after glyphosate application, alone or added with adjuvants

Treatment	Dose kg ha^{-1}	Assessment (days after application)			
		7	14	31	45
Glyphosate	1.80	86.0	96.0	98.5	100.0
Glyphosate + vegetable oil	1.44 + 1 L	85.1	95.2	97.2	98.7
Glyphosate + vegetable oil	1.08 + 2 L	68.3	70.6	95.3	96.0
Glyphosate + vegetable oil	0.72 + 3 L	54.0	60.2	72.1	70.0
Glyphosate + urea	1.44 + 0.2%	90.0	92.0	94.0	97.5
Glyphosate + urea	1.08 + 0.3%	61.2	77.0	86.7	89.0
Glyphosate + urea	0.72 + 0.4%	51.0	53.0	68.1	60.0

Source: Duringan (1992)

a combustion engine are also available. Sprayer accessories for drift prevention in non-selective herbicide applications, such as diquat, ammonium-glufosinate, glyphosate, and MSMA, are practical and help reduce the intensity of phytotoxicity symptoms in sugarcane plants (Procópio et al. 2016).

16.8.4 Application Over Straw Mulching (Unburned Sugarcane)

The great advantage of applying herbicides under the straw mulching would be the control of weeds that emerge even when undercover. Foloni (2008), working with herbicide application over and under the straw mulching at the time of sugarcane harvesting, did not report differences. However, the efficiency of over straw applications depends on the characteristics of the weed species present. The choice for specific spray nozzles is essential for successful herbicide application. In the field, it has been observed that the use of inappropriate nozzles, added to incorrect water volume and errors in sprayer calibration, are the most common factors responsible for failures in herbicide application to sugarcane, mainly in intermediary and late post-emergence. The weed species and its developmental stage should be considered for determining the herbicide, rate, and moment of application.

Another point to be observed when spraying is the weather conditions, since wind speed, ambient air relative humidity, and temperature directly influence the application quality. Furthermore, aspects related to water quality and the proper adjuvant, in some instances, improve the herbicide effect on weeds usually challenging to control. Table 16.8 shows the impact of adjuvants added to glyphosate in the control of Guinea grass (*Panicum maximum*).

16.9 Future Perspectives for Weed Control in Sugarcane

The challenge of agricultural sustainability requires a balance between the satisfactory quali-quantitative production of agricultural products, the reduction of environmental impacts, and the demand for non-renewable resources. Weed management is

a fundamental issue as herbicides are the most used pesticides globally. Therefore, it is necessary to adopt correct strategies for weed management; for this, it is necessary to know the competitive ability of weeds against the crop to compete for water, light, and nutrients and opt for the most competitive sugarcane varieties against weeds. Simple measures such as choosing the most competitive sugarcane genotype against weeds and adopting management practices technically based on sustainable principles such as cover crops and crop rotation can help reduce the use of herbicides in sugarcane and, consequently, lower costs environmental impact.

New technologies emerged and developed for other crops such as maize, soybeans, cotton, etc. should be applied in weed management in sugarcane. For example, soybean resistance to the herbicide glyphosate was made available in the last years of the previous century; more recently, there has been resistant soybean to glyphosate, 2,4-D, ammonium-glufosinate, and dicamba. For now, these technologies are still not available for sugarcane, which may be of significant and should be attempted.

It is clear that the way of cropping sugarcane has changed, remarkably the harvesting of unburned sugarcane, the use of quality seedlings, and state-of-the-art agricultural machinery. Combined with the harvest of unburned sugarcane, there is a greater amount of straw on the ground which interferes with the action of many herbicides, sometimes requiring an increase in doses or the necessity for positioning them under the straw mulching. This is an aspect that needs further attention, aiming to reduce expenses with weed control, and at the same time, the environmental impact of herbicides uses. There are still many problems in worldwide sugarcane plantations related to the occurrence of resistant weeds or even greater dissemination of tolerant species; both begin to infest large sugarcane areas.

Many herbicide problems are still reported, with injuries to sugarcane, thus reducing productivity or even the longevity of sugarcane fields. This could be alleviated by developing the most tolerant cultivars to herbicides or even introducing resistance genes into sugarcane, as occurred with soybean, maize, cotton, and other crops. On a global scale, many fundamental problems are still to be solved related to weed management in sugarcane plantations. Herbicide application technology needs to be improved to help reduce costs, increase weed control efficiency, and mitigate the environmental impact of pesticide applications. By using deep plowing (powder ridge technique) in the field preparation operation of machine tillage before sugarcane planting, the germination of some weed seeds is effectively controlled by burying them. Deep soil turning is accomplished by deep plowing with the traction of large and medium-sized tractors.

Furthermore, the biological control uses natural biological enemies that are not conducive to the growth of weeds, such as some insects, pathogenic fungi, bacteria, viruses, nematodes, herbivores, or other higher plants, to control the occurrence, growth, spread, and harm of weeds. The aim is not to eradicate weeds but to control them so that their damage is below economically acceptable levels. Biological grass control has the advantages of no pollution, no harm, and high economic benefit. These techniques, however, are still at the early stage of development, and their success is also limited to specific edaphoclimatic conditions, as the biological control

agents should be locally adapted to survive and to be effective in their predation ability.

16.10 Final Remarks

Weed control is one of the primary management that can be improved and optimized in sugarcane fields. The correct use of herbicides, combined with other control methods such as preventive, cultural, mechanical, and physical, has become important and should be focused. An integrated weed control approach should always be the priority. Soil tillage quality should be fine so that the field surface is flat, the soil is fine, and there is no exposed seed in the sowing and covering the soil. The herbicide mixture should be uniform; spray and distribute the herbicide solution evenly in the field. Field area must be measured accurately, and herbicide dosage prepared and applied accordingly; weed seedling emergence should be continuous, and herbicides should be applied at the right timing.

New technologies such as transgenic crops conferring tolerance to herbicides, already available for other crops, are still in early stage of development in sugarcane. The so-called conventional herbicides are starting to skid for weed control in fields with no crop rotation or at least an integrated approach for crop management. Given the concern to produce more and better, aiming to smaller environmental impact, there is a need to rethink how we could manage weeds in this crop—from a herbicide-based system to an integrated approach. In this context, the most competitive sugarcane varieties, coupled with the adoption of varieties most tolerant to the herbicides, will play a significant role.

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17 Synergistic Integration of Sugarcane Proteomics with Genomics: Proteogenomics to Decipher the Mechanism of Disease Resistance in Sugarcane

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Abstract

Deciphering disease resistance in sugarcane is a challenge by virtue of its genomic complexities like autopolyploidy, heterozygosity, etc. In the past few decades, most of the researchers employed a gamut of genomic tools to elucidate the mechanism of disease resistance in sugarcane. However, because of various hurdles in decoding the whole genome information, the progress thus far made to delineate the mechanism of disease resistance is not encouraging even with the deployment of robust genomic tools like high throughput next-generation sequencing (NGS) technologies. In the meantime, the application of proteomics to understand sugarcane–pathogen interaction is progressing steadfast with robust gel-free platforms and advanced mass spectrometric approaches, but at a relatively slow pace compared to other monocots. With the evolving de novo protein sequencing approaches, precise identification and establishment of comparative quantitative proteome maps are becoming more expedient. Hence, there is a pertinent need for employing proteomics to add momentum, gain leverage, and bridge the gaps in sugarcane genomics wherever possible. Nevertheless, synergistic integration of proteomics with the ameliorating support from sugarcane

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genomics will provide an additional impetus to understand the mechanism of disease resistance in sugarcane.

Keywords

Sugarcane · Proteomics · Disease resistance · Genome sequencing · Proteogenomics

17.1 Introduction

Sugarcane is one of the important commercial crops cultivated in more than 100 countries to produce sugar, energy (biomass, bioethanol, and electricity), and other value-added products. It is the major source for around 80% and 25% of global sugar and ethanol production, respectively (OECD/FAO 2019; Verma et al. 2020a, b). In recent years, increment in the area of sugarcane cultivation is not very encouraging, which would hardly meet the growing global demand for sugar and bioethanol in the near future. Among the limiting factors of crop productivity, biotic stress alone accounts for over 20% yield loss. More than 200 diseases have been recorded in sugarcane. The impact of diseases varies considerably according to the cultivars in practice, the prevailing pathogenic races, growing conditions, and geographic location (Verma et al. 2021). Ratoon stunting disease, smut, red rot, leaf scald, brown rust, and wilt are the few major diseases limiting worldwide sugarcane production.

Ensuring food security, clean energy, and economic stability is possible only by cultivating high-yield sugarcane varieties with durable disease resistance. To develop such disease-resistant cultivars, the molecular factors that govern these disease resistance mechanisms need to be comprehended. Despite the advent of various cutting-edge technologies in this "omics" era and the focus of research thrust on this crop, the progress made thus far in understanding the various aspects of sugarcane disease resistance is not appreciable. Particularly, when considering the recent developments in the proteomic approaches, revolutionizing progress in genomic technologies, and the substantial outcomes being achieved by integrating genomic/transcriptomic information with proteomics (even in non-model pathosystems), it is appropriate to state that the potential of proteomics is yet to be harnessed in sugarcane pathosystems. With this backdrop, this chapter is focused on the status of sugarcane genomics, challenges, and the extent of proteomics that could leverage toward deciphering disease resistance.

17.2 Complexities, Challenges, and Status of Sugarcane Genome Sequencing

Modern sugarcane cultivars are the hybrids derived from successive crosses involving *Saccharum officinarum* and *S. spontaneum* to introgress desirable traits such as high sugar, yield, vigor, adaptability, and disease resistance. As a consequence of this process of nobilization, the genome of these modern inter-specific hybrid cultivars features approximately 100–130 chromosomes with a staggering size of 10 Gb, which together contributed to its highly complex polyploidy nature (Piperidis et al. 2010; Piperidis and D'Hont 2020). Notwithstanding their estimated monoploid genome size as just 1 Gb, the degree of polymorphism among the 10 uneven homoeologous loci in the autopolyploidy genome could not be ascertained (Souza et al. 2011; Thirugnanasambandam et al. 2018). For instance, it is estimated that single gene may have around 8–15 homo(eo)logous copies in the genome of modern hybrid sugarcane cultivars, and it may vary from genotype to genotype because of random sorting of chromosomes during the crossing (Souza et al. 2011, 2019). These complexities put together hampered the progress of deciphering the whole genome information of sugarcane. Hence, genome mapping and identification of trait-specific loci for disease resistance have become a herculean task, unlike the case in other crops. To address these challenges, efforts are currently underway to draft a reference genome of sugarcane by the sugarcane genome sequencing initiative (SUGESI) consortium by integrating BAC cloning and next-generation sequencing (NGS) approaches (SUGESI 2020).

Meanwhile, researchers worldwide began to develop new approaches/strategies beyond the basic de novo approach to assemble and retrieve the genomic information of sugarcane either directly or indirectly and achieved considerable success. For instance, the genome of sugarcane shares an extensive synteny and genome-wide colinearity with sorghum, and so, it is considered as a reference genome for annotations, mappings, and identification of genetic loci of any sugarcane genetic element (Wang et al. 2010; Figueira et al. 2012; de Setta et al. 2014). However, assembling a monoploid sugarcane genome based on the available genetic information of closely related monocots like sorghum necessitated the development of novel strategies and algorithms capable of assembling and scaffolding high allelic variations and repetitive sequences as stated above (Dal-Bianco et al. 2012). Accordingly, Garsmeur et al. (2018) utilized the microcollinearity property of sorghum to construct the gene-rich part of the monoploid reference sequence of around 382 Mb (single tiling path) of sugarcane cultivar-R570 based on the strategy of WGP™. Intriguingly, this microcollinearity strategy has rendered only a mosaic monoploid genome that represents around 30–40% of the actual monoploid genome.

On the other hand, Zhang et al. (2018) employed the approach of sequencing the haploid genome of AP85-441 ($1n = 4\times = 32$) culture generated from the wild-type octoploid *S. spontaneum* accession SES208 using the high throughput chromatin conformation technology (Hi-C) with newly developed Hi-C-based scaffolding algorithm (ALLHIC). Mapping the available sequence information of the hybrid cultivar SP80-3280 that contributes approximately 12.25% of *S. spontaneum*

genome against the 32 pseudo-chromosomal assemblies of haploid genome indicated random distribution of genes throughout the genome. Similarly, Nascimento et al. (2019) developed a polyploid gene assembler that integrates reference-assisted loci and de novo assembly strategies to sequence *S. spontaneum.* Souza et al. (2019) have reported an improved representative gene space assembly of SP80-3280 with >4 GB of sugarcane genome information, which predicted 373,869 putative genes. All these studies have created a fundamental niche towards deciphering the high-resolution chromosome assembly of modern hybrid cultivars.

17.3 Disease Resistance in Sugarcane: A Comprehensive Lookout Involving Genomics and Proteomics

Considering the status and strenuous efforts to unveil the sugarcane genome, it is imperative that many genomics-related crop improvement strategies, especially breeding for disease resistance traits, cannot be effectively employed. This is because of the two major hurdles—lack of whole genome information and the complexity in genome mapping as stated above (Dal-Bianco et al. 2012; Cardoso-silva et al. 2014). Hence, many of the well-demonstrated genomic strategies and techniques were adversely implicated or counteracted by the aforesaid hurdles, which otherwise could significantly contribute to delineate disease resistance. For instance, identification of various trait-specific markers and regulatory sequences for marker-assisted selection, genetic interaction mapping, RNAi, epigenetics, prediction of isoforms by alternative splicing, and genome editing with CRISPR are not easy task for accomplishment in sugarcane with the present status on genomic information.

Regardless of the hurdles of sugarcane genomics, transcriptomics of sugarcane has substantially progressed with the help of NGS technologies and contributed to a basic understanding of sugarcane disease resistance as evinced by the number of publications (Table 17.1). Incidentally, the largest collection of sugarcane expressed sequence tags (ESTs) by the SUCEST consortium served as the primary genetic reference resource for further exploitation of many "omic" tools for more than a decade. Despite the initiation of molecular studies on sugarcane disease resistance a few decades back, it has gained momentum only in the recent past with the application of NGS approaches. Besides that, very few but significant milestones have been attained using marker approaches. Identification of three QTL markers led to fetch1574 putative *R* genes that were found to be significantly associated with orange rust resistance in sugarcane (Yang et al. 2018). Another study on marker-trait associations using linkage disequilibrium and association mapping identified many defense-related proteins that are putatively associated for red rot disease resistance (Singh et al. 2016). Expression profiling of candidate defense genes and differential expression analysis through cDNA-AFLP, DDRT-PCR, and SSH have led to the identification of many defense-related genes in sugarcane. However, only few of them were functionally characterized (Muthiah et al. 2013; Prathima et al.

Table 17.1 List of publications on NGS-based transcriptome analysis on sugarcane disease resistance

Brief description of work	No. of transcripts/ unigenes assembled	Reference
Transcriptome analysis of sugarcane responses to smut infection	72,812	Wu et al. (2013)
Transcriptome analysis of sugarcane responses to smut infection	65,852	Que et al. (2014)
Transcriptome analysis in response to sugarcane red stripe disease	168,767	Santa et al. (2016)
Transcriptome analysis of whip development in sugarcane smut disease	88,487	Schaker et al. (2016)
Transcriptome analysis of sugarcane response to the infection by sugarcane steak mosaic virus (SCSMV)	63,025	Dong et al. (2017)
Sequencing of miRNAs during smut infection	–	Su et al. (2017)
Transcriptional profiling during sugarcane-sorghum mosaic virus interaction	89,338	Ling et al. (2018)
Transcriptome analysis of smut infected buds	138,062	McNeil et al. (2018)
Transcriptome sequencing for six contrasting sugarcane genotypes involved in leaf abscission, tolerance to pokkah boeng disease, and drought stress	471,654	Xu et al. (2018)
Comparative transcriptome profiling of pokkah boeng resistant and susceptible sugarcane genotypes	76,175	Wang et al. (2019)
Differential expression analysis of smut-resistant and susceptible genotypes	72,078	Rody et al. (2019)
Degradome sequencing of miRNAs during smut infection	–	Su et al. (2019)
Differential expression analysis of leaf scald resistant and susceptible genotypes	614,270	Ntambo et al. (2019)
Transcriptional profiling of sugarcane leaves infected with *Puccinia kuehnii* (Orange rust)	451,462	Correr et al. (2020)

2013; Selvaraj et al. 2014; Sathyabhama et al. 2015; Ashwin et al. 2018, 2020a; Huang et al. 2018).

Though the application of transcriptomics has improved the conceptual understanding of sugarcane–pathogen interaction, proteomics, which represents the actual functional role of the transcriptome at the molecular level, was relatively underutilized. The study of proteomics gains importance over genomics in many aspects such as an abundance of transcripts may not reflect their actual role as they may be degraded rapidly or translated inefficiently due to post-transcriptional controlling processes. Even after translation also, the activities of many proteins depend on alternative splicing events, interaction complex formation events (interactome), and post-translational modifications like phosphorylation, acetylation, methylations, and sumoylation (Bludau and Aebersold 2020). Witnessing the successful application of proteomics in several model/non-model plants, including crops, and realizing its

potential in providing insights on disease resistance mechanisms, proteomics-based approaches have started to blossom only recently to understand the defense mechanisms operative against fungal and bacterial diseases of sugarcane. Despite the limited availability of information on proteomics-based studies on sugarcane defense responses, we consider these milestones as landmark achievements as "All big things have small beginnings." Ab initio, some researchers have used isoenzyme pattern analysis on SDS-PAGE gels and immune-biochemical estimations of defense-related enzymes like peroxidase, chitinase, glucanase, etc. (Viswanathan et al. 2003; Ramesh et al. 2008). However, the establishment of a standard protein extraction methodology compatible with two-dimensional gel electrophoresis (2DGE) and mass spectrometry (MS) by Amalraj et al. (2010) has laid the primary platform for subsequent proteomics-based studies on biotic and abiotic stress of sugarcane, including disease resistance.

Presently, there are very few research groups worldwide actively utilizing proteomics as a tool to understand sugarcane disease resistance. Thus, there is a considerable paucity of literature available on the biotic stress of sugarcane (Table 17.2). Most of them are focused on identifying the differentially expressed proteins during sugarcane × *Sporisorium scitamineum* (causative agent of sugarcane smut) interaction since sugarcane smut disease is a severe production constraint worldwide (Nalayeni et al. 2021). Further, superimposing the accumulated information of comparative proteomics onto the well-established concepts of plant disease resistance is gaining traction as a way forward in elucidating disease resistance in sugarcane. For instance, through comparative proteomics, CfEPL1, a cerato-platanin pathogen-associated molecular pattern (PAMP), and CfPDIP1, a putative effector of *Colletotrichum falcatum* have been identified, and their possible role in PAMP-triggered immunity and effector-triggered immunity against sugarcane was successfully demonstrated (Ashwin et al. 2017a, 2018).

For further information on sugarcane proteomics and the various proteomic strategies for understanding plant–pathogen interactions, the readers are advised to refer to the comprehensive reviews by Barnabas et al. (2015) and Ashwin et al. (2017b, 2020b). Similarly, for the recent comprehensive updates on sugarcane "omics," including metabolomics, the readers may refer to Ali et al. (2019).

17.4 Unveiling the Avenues of Proteomics and the Significance of Integrating It with Genomics

With the hurdles of genomics and the vantage of transcriptomics deliberated, we would like to redirect the focus on how proteomics could supplement in light of the exemplary accomplishments made in other related crops, viz. maize, sorghum, and rice, and also discuss the feasibility of similar approaches in sugarcane with the synergistic integration of proteomics with genomics, termed "proteogenomics." Adequate information is available on maize and rice defense proteomics with the establishment of specific proteome maps for individual tissues, sub-cellular proteomics, and secretome profiles with appropriate corroboration from its intracellular and

Table 17.2 List of publications related to sugarcane biotic stress or disease resistance using proteomic approaches

Brief description of work	Proteomic approach used	Reference database for protein identification	Number of proteins identified	Reference
Differential protein expression during sugarcane—*S. scitamineum* interaction	2DGE and MALDI-TOF-TOF/MS	NCBInr database of related species	23 differentially expressed proteins	Que et al. (2011)
Proteomic analysis of sugarcane seedling in response to *S. scitamineum* infection	2DGE and MALDI-TOF-TOF/MS	NCBInr database of related species	18 differentially expressed proteins	Song et al. (2013)
Differential protein expression during compatible interaction of sugarcane—*S. scitamineum* interaction	2DGE and MALDI-TOF-TOF/MS	In-house developed *Saccharum*-specific amino acid database containing 150,247 EST-based coding sequences	53 differentially expressed proteins	Barnabas et al. (2016)
Differential protein expression analysis of smut resistant and susceptible genotypes during interaction with *S. scitamineum*	LC-ESI-MS/MS with iTRAQ labeling	65,852 sugarcane unigenes identified by Que et al. (2014)	4251 proteins	Su et al. (2016)
Secretomic analysis of *S. scitamineum* in response to host signals	2DGE and MALDI-TOF-TOF/MS	*S. scitamineum* specific databases	16 differentially expressed proteins	Barnabas et al. (2017)
Proteomic analysis of two sugarcane varieties with contrasting susceptibility to smut during infection	2DGE and MALDI-TOF-TOF/MS	BLASTp and tBLASTn against NCBInr databases and sugarcane EST databases	30 differentially expressed proteins	Singh et al. (2019)
Differential protein expression of sugarcane proteins during red rot infection	2DGE and Nano Frontier eLD-IT-TOF-MS/MS	NCBInr database and the Swiss-Prot database	136 differentially expressed proteins	Kumar et al. (2020)
Comparative proteomic analysis of sugarcane × *Xanthomonas albilineans* interaction	LC-MS/MS with iTRAQ labeling	*Saccharum* spp. unigene database (P101SC18020747-01)	6891 proteins	Meng et al. (2020)

secretome map with and without interaction with respective pathogens (Agrawal and Rakwal 2011; Pechanova et al. 2013; Pechanova and Pechan 2015; Jiang et al. 2019; Meng et al. 2019). These comprehensive studies encompassing developmental, comparative, quantitative, and functional (PTMs) proteomics have provided insights on pathogenicity and host disease resistance mechanisms that helped develop a comprehensive snapshot of dynamic alterations during host–pathogen interaction.

Similar accomplishments are possible in sugarcane proteomics with the advancements in de novo sequencing and high-throughput quantitative proteomic technologies. Notably, the algorithms of de novo sequencing of peptides/proteins with tandem mass spectrometry data are evolving rapidly with a higher degree of precision in deep learning techniques, thus becoming a key technology in protein identification without reference databases in high throughput gel-free global and targeted proteomic approaches (Yang et al. 2019). Even specific PTMs can be identified in targeted proteomic approaches by certain enrichment strategies like ion exchange, immobilized affinity chromatography, and co-immuno precipitation methods. There are no hitches in establishing proteomic profiles of major sugarcane pathogens, given the availability of most of their genome information in public databases. However, these de novo-based sequencing technologies still have some pitfalls like inaccurate mass determination, confusion with similar residue substitution, and differentiation of isoforms (Timp and Timp 2020).

To alleviate these glitches and proceed forward, the proteogenomics approach gains leeway significance. Integrating genomic datasets with proteomics is gaining momentum in many crops, including sugarcane. This synergistic integration will increase the investigation power and provide more insights as the transcriptional data may not always go in tandem with the proteomics data, thus inciting further probing into the regulatory and functional aspects of specific proteins (Kumar et al. 2016). The proteogenomic approach can be systemically utilized for different applications, viz. sequence-centric proteogenomics, analysis of proteogenomic relationships, and integrative modeling of proteogenomic data (Ruggles et al. 2017). This proteogenomics approach relies on streamlined data integration and appropriate bioinformatics software, including protein quantification for reproducible and reliable identification. Through this proteogenomics approach, few groups have employed the sugarcane transcriptomic data for comparative proteomics studies, which drastically improved protein identification (Su et al. 2016; Meng et al. 2020). By integrating the accumulated transcriptomic information on sugarcane–pathogen interaction(s) with the de novo-based high throughput proteomic approaches, sugarcane proteomics has the potential and scope for growing leaps and bounds from this budding stage.

This collective proteomic information based on the proteogenomic approach would unlock the secrets of functional molecular players that determine the outcome of sugarcane–pathogen interactions. Further, this would supplement and alleviate the bottlenecks of genomic-based expression profiling tools like microarray, identifying disease resistance markers, functional interactome mapping, etc., in delivering quantifiable information to elucidate the signaling pathways involved in disease

resistance and establishing the dynamic interactome complex of PAMPs, effectors, and R proteins.

17.5 Conclusion

Despite phenomenal efforts exerted in sugarcane genome sequencing and establishing genome maps, this challenging and laborious task is expected to take a few more years for near completion. Because of this, the path of genomic-centered approaches alone would not be sufficient in proceeding towards unraveling disease resistance in sugarcane. At this juncture, a natural question arises: can proteomics overhaul the recited hurdles of genomics to decipher sugarcane disease resistance? No, tapping the potential of proteomics alone could not be a promising alternative to address the hurdles of genomics, as proteomics strategy itself has some pitfalls in protein identification. As stated earlier, this issue can be addressed by the proteogenomics approach. With this approach, establishing quantitative proteome maps, especially tissue-specific and comparative proteome profiling in response to various biotic and abiotic stress factors, is becoming more expedient. Moreover, new proteomic techniques that are currently evolving, like fluoro-sequencing and Nanopore 5D fingerprinting, are also promising and may revolutionize proteomics/protein identification in the near future (Timp and Timp 2020). Conclusively, besides treading on sugarcane proteomics and genomics in isolation, synergistic integration of both can override the above said hurdles and may pave a new path to possibly decode the mechanism of disease resistance in sugarcane.

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The Metabolic Interaction of Potassium Salt of Active Phosphorus (PSAP) and Its Stimulatory Effects on the Growth and Productivity of Sugarcane Under Stressful Environment

18

Prashant Nandargikar, Narendra Jani, Govind P. Rao, and S. Solomon

Abstract

The world's population has been increasing rapidly day by day and would demand more food from the limited natural resources such as land and water. Agricultural productivity will have to be increased substantially by using available resources, which are being depleted rapidly. Therefore, it is a challenging and herculean task for farming communities and agricultural technologists to fulfill the basic needs of the ever-increasing population. Agricultural scientists are engaged in developing improved varieties of crops along with their matching agro-technologies. Enhancing productivity and improving the quality of agricultural produce are the prime objectives of all the agricultural development organizations and funding agencies, and they are striving hard to achieve the same. Plant nutrients play a very important role in crop growth, development, and production. The role of phosphorus (P) in metabolic processes and potash (K) for inducing ability in plants is very significant to tolerate major abiotic and biotic stresses. These major crop nutrients are supplied traditionally through chemical fertilizers through soil irrigation, resulting in only 10–20% absorption by crop plants. The share of 80–90% of phosphate gets fixed in soil which is not available for the plants. To overcome these challenges on phosphorus and potash, the potassium salt of active phosphorus (PSAP) was invented using catalytic technology. The technical molecule of PSAP is 180% water-soluble and easily

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absorbed by the plant roots and leaves and plays a vital role in plant metabolism by inducing tolerance to the major biotic and abiotic stresses. Application of PSAP increases plant productivity from 30 to 50% with remarkable improvement in product quality along with the reduction in the cost of cultivation. The inclusion of PSAP in farming will certainly enhance the farmers' income due to earning substantial additional profits. In conclusion, PSAP has emerged as a molecule of choice for enhancing the farmers' income by improving the yield and quality and reducing the cost of crucial inputs.

Keywords

Abiotic stress · Growth · Physiological traits · Biochemical-molecular aspects · PSAP · Sugarcane

18.1 Introduction

One of the major challenges before the globe is the ever-increasing population of the world. It is estimated that there will be about ten billion people by 2050, resulting in additional demand for food. Apart from it, adverse effects of the activities performed such a large population on the climate will also hamper global food security (FAO 2015; Kagan 2016; Adisa et al. 2019). The increasing world population is putting pressure by way of extra demand for food grains. The only source of providing food and feed for such a large human and livestock population is agriculture (Verma et al. 2020, 2021).

The natural resources are shrinking faster than before. Soil health has also been deteriorating fast due to over-emphasis on synthetic fertilizers as well as very low or zero application of organic matter to the soil. Farm productivity is declining due to the deleterious effects of various forms of stress (Boyer 1982; Zhao and Li 2015; Adisa et al. 2019). Minimizing these losses has emerged as a great concern for all countries to increase food availability. Low or high temperature is the prime factor adversely influencing the growth and development of the crop by inducing morphological, physiological, and biochemical changes. The number of abiotic stresses like waterlogging, drought, salinity, and acidity of the soil, extremities in temperature, imbalanced use of essential plant nutrients, metal toxicity, and UV radiation adversely affect the soil and the crops' productivity across the globe (Lawlor and Cornic 2002; Flexas et al. 2004; Jewell et al. 2010; Vilela et al. 2017; Etesami and Jeong 2018). The financial loss and inefficiency in the yield of food grains further aggravate the sincere efforts targeted for food security and safety (Oerke and Dehne 2004; Adisa et al. 2019).

18.2 Forms and Application of Phosphorus

18.2.1 Nutrient-Based Phosphorus: Phosphate (PO_4^-)

Phosphates are applied to crop plants as a source of phosphorus in the form of chemical fertilizers such as superphosphate, 00-52-34, 10-26-26, DAP (diammonium phosphate) (12-61), 19-19-19, and organic matter. The application of phosphorus· and potash-based fertilizers is associated with several solubility problems, fixation/leaching, availability, and uptake. Even after spraying, these fertilizers are very poorly absorbed by the foliage of most crop plants, and if they remain on the plant, the residue supports fungal growth. Therefore, it is challenging to manage PO_4 under field conditions (Table 18.1).

Table 18.1 Various forms of phosphorus

Parameter	Nutrient-based phosphorus (PO_4-)	Fungicide-based phosphorus (PO_3-)	Stress alleviator-based active phosphorus
Base	(a) Phosphate: PO; synthetic fertilizer base (b) Organic phosphorus	(a) Alkali metal salts fungicide base (b) Carbon compound growth regulator base	Molecular combination of active phosphorus and potash catalytic base
Function	Phosphorus is a major plant nutrient that induces virtually all the biochemical processes and development phases of crop plants.	These products have fungicide mode of action and/or regulate some metabolisms. However, PO_4 and PO_3 phosphorus share antagonistic relationships and do not replace each other.	Phosphorus inactive form has an important role in stress alleviation. Role of active phosphorus is complementary and supplementary to nutrient base phosphorus PO_4^{2-}. Phosphorus and potash from PSAP rapidly absorb and quickly translocate in crop plants.
Limitation	Synthetic fertilizers • Solubility • Fixation/leaching • Uptake • Absorption • Availability • Soil and water pollution Organic phosphorus • Very slowly available • Inadequately available • Soil bacteria are required • Poor source of phosphorus	Alkali metal salts • Crop-wise specific application • Phytotoxic • No direct role in the growth • PO_3^- unsuited in ATP generation Carbon compounds • Some compounds have MRL • May hinder growth metabolism • Debate is going on towards its environment-friendly utilization	It can be applied at any given stage and condition of crop plants

18.2.2 Fungicide-Based Phosphorus: Phosphite (PO_3^-)

Mono and dipotassium salt of phosphorous acid and/or potassium salt of phosphoric acid or potassium phosphite are some of the major molecules generally applied by the growers in combination with some fungicides such as captan and mancozeb. The PO_3-molecule has been reported to have some specified role in the management of diseases, apart from balancing pH in the above-mentioned fungicides. However, in case of excess application, it has been found highly phytotoxic. Carbon-phosphite molecules are phosphonates, also refer as PGR or fungicides like fosetyl-Al and N-(phosphonomethyl) glycine herbicide (Table 18.1).

18.2.3 Stress Alleviator-Based Phosphorus: Inactive Phosphate

Potassium salt of active phosphorus (PSAP) is an autonomous form of phosphorus, playing a crucial role in the biosynthesis of primary and secondary metabolites, including the shikimic acid pathway (SAP), which overcomes the limitation of phosphate and phosphite molecules when applied to crop plants (Cramer et al. 2009; Tariq et al. 2017; Verma et al. 2021). A synergistic effect is created with the application of PSAP along with fertilizers. PSAP supports bio-energy generation, storage, and translocation in ATP/NADP bonds. The availability of ATP and a reductant in the form of NADPH helps the plants to scavenge ROS and adapt to stress. Hence, recovery of various metabolic processes from stress in PSAP-treated plants is very fast and effective. Active phosphorus in crop plants alleviates biotic and abiotic stresses (Table 18.1).

18.3 Potassium Salt of Active Phosphorus (PSAP): Autonomous Combination of Phosphorus and Potash

Plants frequently cope with rapidly fluctuating and adverse environmental factors due to their intrinsic metabolic capabilities. The plant metabolism could be put out of homeostasis by any minor or major variations in the outside environment. The plants essentially harbor advanced metabolic and genetic techniques within their cellular system. The plants possess an array of protective mechanisms to combat unfavorable situations of the environment during the course of evolution, which results in metabolic re-programming in cells facilitating routine physico-biochemical processes without taking the cognizance of the external situations. It is essential to have phosphorus in the environment for the existence of any living organism. The tissues of all the plants, as well as animals, contain phosphorus. Being the basic necessity of life, phosphorus is essentially required for all the important physiological activities like photosynthesis, the synthesis and carbohydrates breakdown, and internal transfer of energy. Plants absorb phosphorus from the soil (Cortina et al. 2013; Tariq et al. 2017). Plants will not be able to grow satisfactorily if the soil does not contain sufficient level of phosphorus or phosphorus is not provided to the soil

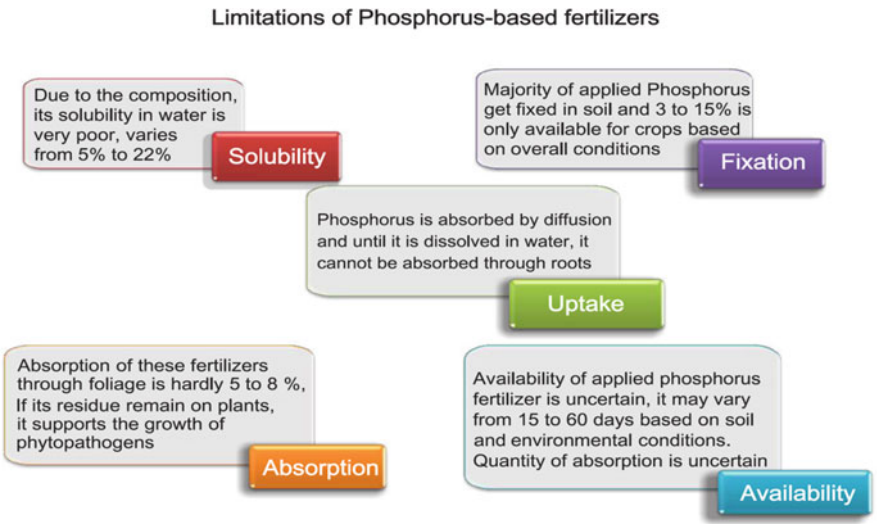

Fig. 18.1 Limitations of phosphorus-based fertilizers

from external sources. Unlike abundance availability of major nutrients like nitrogen and potassium, phosphorus does not remain available in abundance. Soil contains phosphorus in organic as well as organic forms. A very small fraction of the total phosphorus remains available for the plants (Verma et al. 2021; Fig. 18.1).

18.4 Impact of PSAP on Crop Plants During Abiotic Stress

For the optimal development, growth, and reproduction of plants, they require water, light, carbon, and mineral nutrients. Plant growth and development are hampered by extreme conditions (below or above the optimal levels). The stress conditions are posed for the plants by unfavorable environments comprising extreme low or high temperatures as well as drought or salinity (Cakmak 2005; Verma et al. 2021a). By sensing, the plants react to stresses in several ways to survive. Plant do not forget past exposure to abiotic stresses and recall the coping mechanisms. Therefore, plants can respond to repeated stresses differently.

The cellular level followed by physiological symptoms on the plants initially witnessed the adverse effects of unconducive conditions. The physiology of the plants, including photosynthesis, is adversely affected by water stress (Verma et al. 2019, 2020a, 2021a, b). The sharp decline in leaf water potential, stomatal opening, reduced leaves, suppressed growth of roots, reduced number, size, and viability of seeds, delayed flowering and fruiting, restricted plant growth, and low productivity are recorded under prolonged water stress. (Chaves et al. 2009; Tariq et al. 2017; Verma et al. 2020b).

Therefore, the plants have developed different mechanisms optimizing water consumption for growth until they are faced with unfavorable conditions. The physiological activities of plants are reduced by exposure to high or light intensities, which results in poor growth and development (Sangakkara et al. 2000; Zhao et al. 2001; Bahrami-Rad and Hajiboland 2017). Excess light induces photooxidation, which enhances the production of highly reactive oxygen intermediates for manipulating biomolecules and enzymes. Crops suffer heavily due to freezing (cold) injury and high temperature. Several edaphic factors like alkalinity, salinity and acidity contamination with pollutants and anthropogenic perturbation adversely affect the plant growth, resulting in poor performance and productivity (Barbosa et al. 2015; Dinh et al. 2017; Zhang and Govindaraju 2018).

The soil nutrients are adversely affected by varying levels of acidic conditions by restricting the ease with which they are available, resulting in nutrient deficiency in plants, and their normal physiological growth pattern is lost (Taylor et al. 2011; Tripathi et al. 2019; Li et al. 2019). The early exposure to salinity notices the ion toxicity within the cells, leading to disruption of osmotic balance if the stress persists for a more extended period. The growth and development of the plants are restricted due to the dual effect of these ionic and osmotic shocks. There is a need to maintain the tolerance to salinity or ionic as well as osmotic homeostasis within the cells by quick adjustments. Plants combat the high salinity level by keeping the plant tissues far from the place, or the roots exude ions or compartmentation away from the cytoplasm of physiologically active cells (Cornic et al. 2000; Lawlor and Cornic 2002; Flexas et al. 2004; Tariq et al. 2017; Verma et al. 2021).

Under stress conditions, the plants reduce the adverse effect of stress by understanding the machinery as well as the physiology of the molecules, including elucidation of the abundance of metabolic pathways and their regulatory genes in different varieties (Ibrahim et al. 2008; Ripley et al. 2007, 2010; Zhao and Li 2015; Verma et al. 2020). Strategies for reducing the adverse effect of stress involve identifying multigenic traits engaged in response to stress, exploring the linked markers for the above-mentioned genes, and investigating the possibilities of important pooling genes through breeding programs; supposed to be the focus of stress mitigation strategies. Another supporting strategy to alleviate stresses from abiotic sources in plants includes the split application of PSAP in foliage. Although various techniques of tolerating stress in plants are well known, there is a need to explore the knowledge of the "on-field response" of PSAP-treated crops exposed to multiple forms of stress.

18.4.1 Stress Mitigation Process of the Crops

The variation in external environmental conditions compels the plants to sense, manage, maintain, or escape changing. Within pathways and diverse biosynthetic networks, interactive metabolic crosswalk is involved in response to abiotic forms of stress (Meena et al. 2017; Adisa et al. 2019; Verma et al. 2021). The architecture of the roots, which is considered to be more sensitive to abiotic stimuli, reacts in the

Table 18.2 The strategic defense mechanisms adopted by plants during abiotic stress

Stress	Effects	Defense response
Salinity	Disturbed osmotic and ion homeostasis, membrane damage, nutrient imbalance	Synthesis of osmolytes, enzymes-detoxification responsive to stress, transporters of ion
Heat	Higher transpiration, water deficiency, elevated evaporation	Induction of acclimation, synthesis of heat-shock proteins, induction of protein repair mechanisms
Drought	Decreased photosynthesis, water transport inhibition	Closing of stomata, leaf rolling, enzymes responsive to stress, induction of osmolytes synthesis, responsible for lowering water potential
Chilling and cold	Slow rate of biochemical reactions, decreased CO_2 fixation, ice-crystal mediated damage, formation of free radicals	Increased synthesis and accumulation of osmolytes, hydrophilic proteins, termination of growth
Intense light	Inhibited photosynthesis, increased photooxidation, elevated generation of ROS	Increased production of scavengers of ROS, inactivation of photosynthesis, oxidation of proteins and lipids, etc.
Heavy metals	Bio-accumulation and protein damage	Reactive oxygen radicals production, excess metal deposition in vacuoles
Submergence of flood	Anaerobiosis, respiration in mitochondria inhibited	Aerenchyma development

soil. The involvement of real times, as well as dynamic changes at genetic, cellular, metabolic, transcriptional and physiological stages, makes it a very complex process (Table 18.2). Water-deficient stages within the cells are generally created by the direct impact of salinity, drought, frost, and heat, followed by the parallel development of molecular, biochemical, and phenotypic responses to stress (Etesami and Jeong 2018; Adisa et al. 2019; Verma et al. 2020, 2021). Plants witness various sources of stress in the environment and express it differently. Plants express in the environment to various stresses in many forms (Vilela et al. 2017; Zhang and Govindaraju 2018). The sole source of stress is much simpler than the multiple forms of stress. The response given to a particular form of stress is a complex phenomenon, where the expression of specific genes is activated, followed by intracellular metabolic programming (Sardans and Peñuelas 2012; Tariq et al. 2017).

Several growth stages in the development of the plants involve dynamic phenomena of susceptibility or tolerance to stress. Most of the crops cultivated in the sub-optimal conditions of the environment also restrict the growth and development of the plants by limiting their genetic potential. Plants resist to stress by defending, repairing, acclimatizing, and adapting (Etesami and Jeong 2018; Adisa et al. 2019).

18.4.2 Plant Secondary Metabolism and Improved Metabolite Biosynthesis

In their habitats, plants being sessile organisms continuously interact with a number of variable factors ranging from biotic to abiotic stresses. Within an ecosystem, the survival of floral diversity necessitates a number of appropriate defense mechanisms (Taylor et al. 2011; Adisa et al. 2019). Out of these, the chemical defense mechanism involves the major trait of an immune system to combat the unfavorable environment. An ambit of inherent techniques is developed and exploited by their metabolic plasticity to create an enriched repertoire of complex metabolites of adaptive importance that help the plants survive in different ecological niches (Ibrahim et al. 2008; Zhao and Li 2015). A large number of adaptive and evolutionary benefits are bestowed to the producing plants by the phytochemical derivatives of secondary metabolism. As an elaborate and systematic plan of action for endurance and production of diversity at the organismic level, the power to synthesize specific classes of secondary metabolites is generally limited to a few taxonomic groups. Secondary metabolites help regulate the interaction between plants and their abiotic and biotic environment (Cakmak 2005; Glick 2014; Meena et al. 2017). In addition to it being an integral component of the wall or lignin, they also mediate specific aspects of their physiology of growth and development, symbiosis, and reproduction (Table 18.2).

Secondary metabolism is the functional level of plant metabolism, which is not essentially required for growth and development but essentially required for the survival of the species. Against the nature of primary metabolism, high degree of plasticity of secondary metabolism permits chemical as well as structural modifications with the emphasis of utmost restrictions as the mechanical basis for generating chemical diversity (Zhao et al. 2001; Damon and Rengel 2007; Suriyagoda et al. 2011; Verma et al. 2020).

The various changes at the molecular level associated with metabolism are preserved structurally, functionally, and genetically with bestowed adaptive as well as selective benefits their hosts in diverse ecosystems (Adisa et al. 2019). Despite the vast diversity of structure, the synthesis of secondary metabolites is derived from a very small range of products derived from primary metabolism. Recent research studies have clearly defined the molecular biology and biochemistry of few biosynthetic pathways of secondary metabolism (Etesami and Jeong 2018). Most of the results revealed that elaboration of some central intermediates is the original source of the diversification of secondary metabolism (Hawkesford et al. 2012; Tariq et al. 2017).

Although drought, heat, chilling, and salinity result in different impacts, there are more or less similar biochemical responses. Almost identical effects are also generated by high light intensity and heavy metal toxicity. Still, degenerative responses are witnessed in the plants under flooding or submergence conditions with aerenchyma development to combat anaerobiosis (Boyer 1982; Vilela et al. 2017; Verma et al. 2020, 2021). Thus, it is crystal clear that plants adopt adaptive correspondent strategies to combat the impact of stress caused by the number of

abiotic sources. This observation may offer a solution in PSAP-treated plants for developing a strategic tolerance to combined sources of abiotic stress.

18.4.3 Plant to PSAP empirical interactions and metabolic modifications

After making secondary metabolic pathways crystal clear, regulating genes involved enzymes, and the number of factors influencing different crucial metabolites, accrued certificates have made the modeling of these systems and engineering of metabolic pathways of plants possible to enable increased metabolite production (Adisa et al. 2019; Verma et al. 2021a). The number of factors, the complicated unified regulative techniques, and metabolic route networks result in specific metabolites synthesis along with general plasticity and the ability to change for different biosynthetic pathways, shaping of profiles, and fluxing of secondary metabolites of plants (Zhang and Govindaraju 2018).

The exploitation of the ability of plants to synthesize metabolites provides several good prospects along with equally complex challenges (Zhao and Li 2015; Meena et al. 2017; Adisa et al. 2019). Rich chemical diversity is originated from a circumscribed pool of chemical scaffolds. These chemical scaffolds are later transformed through specific chemical substitutions as catalyzed by substrate and/or regio-specific enzymes. The remunerative key points of exploitation include the reactivity and regio- and stereo-chemistry controlled by the enzyme in converting substrates into specific products through the number of steps in the bio-catalytic landscape of secondary metabolism (Ripley et al. 2007; Zhang and Govindaraju 2018). It is interesting to note the process of exploitation of biomimetic enzymes in production, explicitly exhibiting particular stereospecificity. Synthesis of new metabolites through protein engineering to modify the substrate specificity of biosynthetic enzymes is also equally challenging (Etesami and Jeong 2018; Adisa et al. 2019).

Metabolites and proteins production can be improved by modifying pathway distributions and rates through using recombinant deoxyribonucleic acid method store structure metabolic networks (Meena et al. 2017). To obtain new chemical products, modify post translational protein processing, and recalcitrant takedown wastes, present pathways extension can be enabled by recruitment of heterologous proteins. To study the architecture of secondary metabolites, transgenic plants with modified enzymes activities have emerged as a potent tool. Under the impact of the environment, the synthesis and accumulation of secondary metabolites lead to the multitude of dimensions of the metabolic manipulation level points for increasing production, which seems to be very effective in PSAP-treated crop plants (Tariq et al. 2017; Verma et al. 2020, 2021). Due to this impact, metabolic perturbation of different stratum through management of a single environmental factor or its combinations triggers precipitous positive activation of quantitative as well as qualitative changes in the accumulation of secondary metabolites.

Understanding the physiology of pathways is mandatory, and it is almost identical to understanding transport, pH, and cellular and subcellular compartmentation (Wang et al. 2013; Glick 2014; Dinh et al. 2017). For obtaining more profound insights into the mechanisms which help in reducing stress in PSAP-treated plants, it is important to go for using proteomics and metabolomics as potent tools for associating the genes with the secondary metabolite pathways for genome sequencing of the target plant species, which will emerge as a valuable and promising approach for increasing productivity. Studying the alleviation of abiotic stress in PSAP-treated field-grown plants will open new avenues for researchers to unearth innovative strategies for mitigating such stresses. Studies on omics may also help provide deeper insights for understanding such complex PSAP–plant interactions and metabolic alterations.

For synthesis and aggregation of desirable bioactive compounds through modifying the complex secondary metabolic pathways, there are bright prospects for biochemical and genomic techniques along with admiration of molecular evolution and environmental stresses (Munns and Tester 2008; Liu et al. 2015; Adisa et al. 2019). These are required to be established in PSAP-treated crop plants. But, the regulatory architectures of different pathways along the paths of integrating the same for broadening the metabolic networks are still not fully known. Therefore, it becomes difficult to foretell the conclusion about the expression of single or multiple genes in a specific pathway.

Although a number of sincere efforts have been made for dissecting secondary metabolism to improve bioactive metabolites with the help of classical genetics, they have given positive results in some species (Verma et al. 2021). It must also be tried in PSAP-treated crop plants. It is important to have deeper insights into the elementary network of metabolic intermediates and enzymes to unravel these attributes. Similarly, for explaining the modalities of action of PSAP at the molecular level, it is pertinent to have cognition of the temporal as well as spatial regulatory architectures of secondary metabolic pathways along with the possible paths of their integration for broadening the metabolic networks. A diverse grouping of proteins having the capacity to acknowledge particular DNA sequences in the genes promotions, regulating the gene expression by interacting at the level of transcription is known as transcription factors (TFs) (Boyer 1982; Zhao and Li 2015; Tripathi et al. 2019). Mediating the gathering of the basal transcription machinery leads to activating the RNA polymerase II and mRNA synthesis. The particular groups of genes controlled within the metabolic network are performed by the interaction among different TFs, between non-DNA proteins and transcription factors, and cis-regulatory elements and TFs in a well-structured hierarchical network of gene regulation (Lawlor and Cornic 2002; Verma et al. 2020, 2021).

Empirical observations on data of PSAP-treated crop plants revealed the enormous potential to an extensive range of applications, starting from enhancing the specific secondary metabolites production to exploring new pathways. Therefore, it is essential to enrich our deep insight information regarding the secondary metabolism of plants at the level of the intermediates, enzymes, and genes in PSAP-treated plants further to realize the recent potential of metabolic engineering.

18.4.4 Impact of Abiotic Stress Factors on Sugarcane Yield and Productivity

Sugar and ethanol are produced from the plant source of sugarcane produced in over 80 nations across the globe. But the sugarcane yield can decline due to an unfavorable environment which can jeopardize the bright future prospects to meet the additional demand for sugarcane-derived by-products and bio-ethanol (Zhao and Li 2015; Vilela et al. 2017; Verma et al. 2020). To enhance the productivity of sugarcane, it is fundamental to develop stress-tolerant plants. To increase cane yield and stress tolerance, biotechnological interventions in sugarcane production may offer a comprehensive account of practical and theoretical aspects, providing exhaustive coverage of genome mapping and molecular breeding in sugarcane and showing the status of the elucidation and improvement of plant genomes with economic consideration (Zhang and Govindaraju 2018). The average sugarcane yield in India is 30–32 tonnes per acre annually (75–80 tonnes/ha). Losses in sugarcane yield are estimated at 70–80% because of marginal conditions (Moore 2009; Verma et al. 2020, 2021). Metabolic toxicity, generation of ROS, membrane disorganization, inhibition of photosynthesis, reduced nutrient acquisition, and altered hormonal levels are caused by limited water supply, salinity, extremely high and low temperatures, heavy metals, and other abiotic stresses (Kandel et al. 2018).

18.5 High-Temperature Stress-Induced Effects on Sugarcane

Anatomical as well as morphological, physiological, and biochemical variations are witnessed in plants induced by high-temperature stress, ultimately resulting in variations in water relations, accumulation of compatible osmolytes, slow photosynthesis, hormonal changes, and cell membrane thermostability (Zhao and Li 2015; Zhang and Govindaraju 2018). Scorched twigs and leaves, sunburning of stems, branches and leaves, inhibited root and shoot growth, leaf senescence, and abscission, low yields are caused by high-temperature stress, i.e., over 40 °C. All cellular compounds are harmed by high levels of ROS and negatively influence cellular metabolic processes (Fig. 18.2) (Meena et al. 2017; Etesami and Jeong 2018). It is pertinent to detoxify these ROS, and the plants also have evolved appropriate strategies to cope up with them (Verma et al. 2020, 2021). By enhancing the expression and activity of ROS-scavenging enzymes and enhancing antioxidants production for maintaining redox homeostasis, cells of the plants reveal their response. Production of activated forms of oxygen, including singlet oxygen, hydrogen peroxide (H_2O_2), superoxide, and hydroxyl radical, is associated with environmental stress in plants (Glick 2014; Meena et al. 2017; Etesami and Jeong 2018; Verma et al. 2021).

ROS are continuously generated and located in various cellular compartments like mitochondria, chloroplasts, peroxisomes as by-products of different metabolic pathways. At the global level, the accumulation of ROS caused by high-temperature

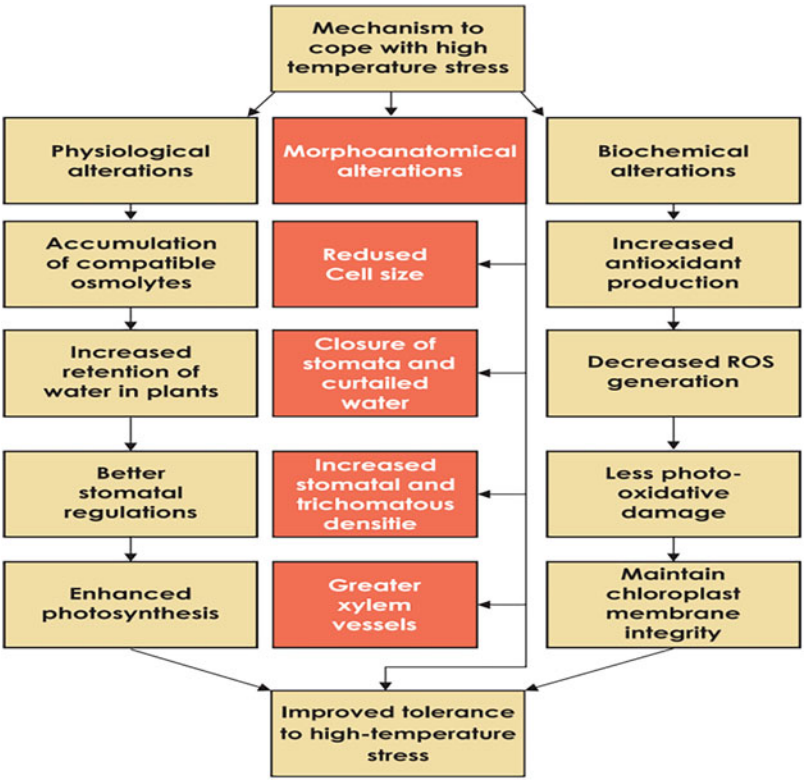

Fig. 18.2 Physiological, morphological, and biochemical alterations during excess ambient air temperature in the sugarcane plants

stress is the prime reason responsible for low crop yield (Cakmak 2005; Zhao and Li 2015).

18.5.1 Techniques for Inducing Tolerance to High-Temperature Stress

The preconditioning of plants and foliar application and pre-sowing or pre-planting seed treatment with low-concentration of inorganic salts, signaling molecules like growth hormones, osmoprotectants, and oxidants like hydrogen peroxide are the most common techniques to develop tolerance to high-temperature stress in plants (Cornic et al. 2000; Cakmak 2005; Adisa et al. 2019). Similarly, sugarcane leaves manifest enhanced thermostability and decreased lipid peroxidation. The malondialdehyde (MDA) reduced damage to chloroplast upon exposure to high-temperature stress in heat-acclimated in comparison to plants that are non-acclimated (Etesami and Jeong 2018). It is observed that the exogenous application of PSAP can

promote the tolerance of plants to heat. Before any stress treatment, the application of PSAP may increase MDA content by stimulating the activity of SOD, catalase, and guaiacol peroxidase, the probable cause of inducting heat tolerance. PSAP is being used in different plant species to induce heat tolerance successfully.

It has been observed that PSAP-treated plants have recorded lower membrane damage, higher rate of photosynthesis, enhanced leaf water potential, and higher shoot dry mass than untreated plants (Verma et al. 2020, 2021). In the number of plants of other crops, it has been observed that exogenous use of PSAP provides better resistance against heat by improving chlorophyll fluorescence parameters, hardening, and better resistance to thermal loss of the pigment–protein complexes structure and greater activity of PSII during the smooth temperature rise. PSAP is needed for the general maintenance of antioxidant activity under heat stress. Therefore, to minimize the adverse effects of stress on growth, a higher quantity of PSAP is required.

18.6 Effect of Cold (freezing) Temperature Stress on Sugarcane Plants

The lower temperature may restrict or reverse sucrose aggregation in subtropical India during autumn or early cold season. During the spring season, the productivity is further reduced due to freezing by delaying and suppressing crop growth, further reducing growth span and the plant population. Therefore, various tissues need to develop resistance to stress from freezing at different crop growth stages (Boyer 1982; Glick 2014; Meena et al. 2017). However, the level of productivity loss due to moderate pre-harvest freezes is insignificant, whereas total loss of yield can be observed in the case of severe freezes (Fig. 18.3). Due to poor tillering caused by extreme cold, shoot population and yield decrease by 78 and 87%, respectively, in the case of underground buds unprotected from freezing. Although cold and heat both bring down the level of number of metabolites changes, its intensity is higher in cold in comparison to heat, clearly highlighting the great impact of low temperature on plant metabolism (Acquaah 2007; Etesami and Beattie 2017).

Metabolic and biochemical processes rate decreases slowly with the reduction in temperature, which can cease during severe cold (Etesami and Jeong 2018; Adisa et al. 2019). Cellular parts and the metabolic process of sugarcane plants suffer due to extremely cold temperature stress (0–10 °C). The stress of variable severity is caused by extremely low temperature, which depends on the intensity and duration of stress (Verma et al. 2020). It has been revealed by large number of studies that the primary spot of freezing injury in plants is the membrane systems of the cells, and the damage is caused by severe dehydration associated with freezing. The formation of ice starts in intercellular spaces in the extracellular fluid when the temperature goes down below 0 °C as the extracellular fluid has higher freezing point than intracellular fluid (Fig. 18.3) (Cakmak 2005; Zhao and Li 2015).

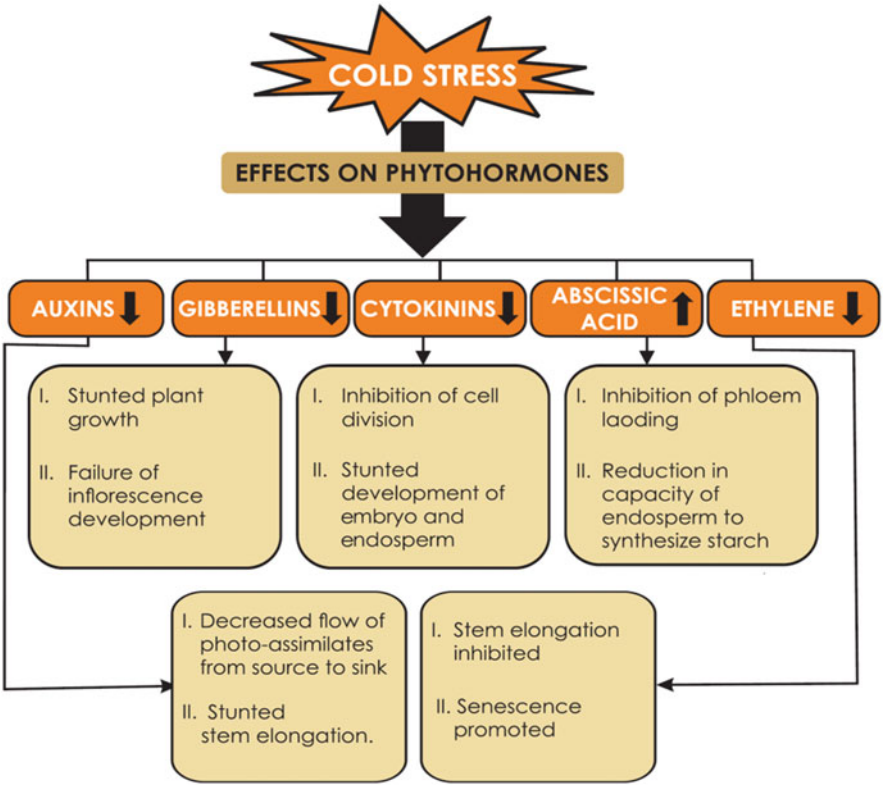

Fig. 18.3 Influence of plant hormones on sugarcane during cold stress

Several forms of membrane damage can happen due to freeze-induced cellular dehydration comprising expansion-induced lysis, lamellar to hexagonal-II phase transitions, and fracture jump lessons. During cold stress in plants, cold temperature-induced bring changes in membrane fluid and provide a potential site of perceptions and/or injury. Freeze-induced ROS production damage the membrane, and intercellular ice can cause adherence formation with membrane and cell walls, ultimately resulting in the rupture of cells. Several evidence suggests that protein denaturation in plants due to cold temperatures can cause cellular damage (Boyer 1982; Pantin et al. 2012).

18.6.1 Approaches for Inducing Tolerance to Cold Stress

Proper membrane fluidity is important for cold stress, delineated by transgenic, mutation analysis, and physiological studies. Higher unsaturation of membranes lipids is found important for optimizing membrane function during low temperature. To combat various cold stresses, the plants develop the number of approaches

(Campbell and Sage 2006; Sardans and Peñuelas 2012; Meena et al. 2017). Cold acclimatization is the primary technique for stabilizing membranes against freezing injury. Tolerance of the plants to cold restricts expansion-induced lyses along with the formation of hexagonal-II phase lipids. This indicates the involvement of the number of mechanisms in this stabilization. Changes in the composition of lipids are considered the best-documented changes (Etesami and Jeong 2018).

Similarly, the aggregation of sucrose and other simple sugars happens with cold acclimation contributing to the stabilization of membranes such as molecules can protect membranes against freeze-induced loss under the in vitro situation. Another technique of plants for combating cold temperature stress may be the extensive binding capacity of water of hydrophilic proteins for offering a safe environment in the proximity of stabilization. Although membrane lesions cause freezing injury due to cellular dehydration, certain other factors are also responsible for freezing-induced cellular damage (Meena et al. 2017; Adisa et al. 2019). The enhancement of growth, development and improve water use efficiency (WUE), the reduction of freeze-induced cellular damage, the increase of antioxidative mechanisms, enhanced sugar in the apoplastic space, and the induction of genes coding molecular chaperones may provide sufficient protection.

18.7 Salinity Stress

The number of irrigated areas is being affected by salinization due to the use of salty brackish water (Lawlor 1995; Munns and Tester 2008; Adisa et al. 2019). More than 45 mha area of irrigated land across the globe has already been damaged by salt. High salinity is the major cause responsible for annually taking about 1.5 mha area. The survival, growth, and development of the plants are influenced by the above-mentioned effects altogether (Boyer 1982; Cakmak 2005; Zhao and Li 2015). The major physiological processes like photosynthesis, protein synthesis and energy, and lipid metabolism are severely affected by all the major functions during the onset and development of salt stress within a plant. Excess Na^+ and the important chloride can adversely affect plant enzymes with the swelling of cells, leading to reduced production of energy and other forms of physiological changes (Fig. 18.4) (Rasool et al. 2013; Meena et al. 2017; Etesami and Jeong 2018).

18.8 Water Deficit Stress

In severe to moderate drought stress, cane yield decreases by 20–30%, respectively, compared to well-irrigated plants. The morphological and biochemical changes caused by drought for acclimatizing the plants are reduced leaf expansion, inspissation of leaves the earliest and most prevalent appearing anatomical acclimation, decreased activity of stomata, enhanced roots shoot ratio, reduction in cell size, acclimation includes changes in enzymatic and non-enzymatic actions, nitrogen and carbohydrates pools, aggregation of stress indicators like glycine betaine (GB),

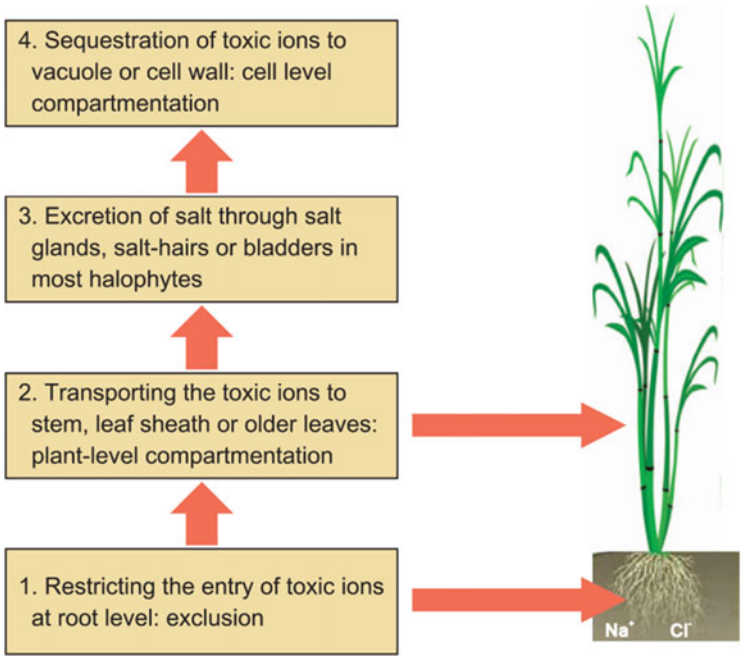

Fig. 18.4 Predominant salt-tolerance mechanisms in plants

abscisic acid, proline and the metabolites of the compounds mentioned above (Bodner et al. 2015; Etesami 2017; Verma et al. 2020, 2021). Size of leaf, exposure, structural modifications in the stomata, cuticle, and bulliform cells regulated the potential rate of water loss by transpiration (Verma et al. 2020). The low density of stomata, narrow band bulliform cells, and thick cuticle check transpiration. Several stomatal characteristics like low frequency and small size restrict water loss restrict carbon assimilation and ultimately restrict growth (Fig. 18.5) (Verma et al. 2020, 2021a, b).

18.8.1 Metabolic Adaptation Strategies

If severe and prolonged, water deficit will affect most of the functions of the plant.

- Proline accumulates significantly in stressed sugarcane plant leaves.
- Stress hormone-like abscisic acid may increase 75 times in stressed plants of sugarcane.
- ABA production is triggered by drought, and therefore, for combating drought, both ABA-independent and ASA-dependent pathways are involved in the plant.
- In all, there are 64 dehydration-enhanced metabolites. Out of them, 16 are regulated by ABA-dependent pathways comprising few amino acids,

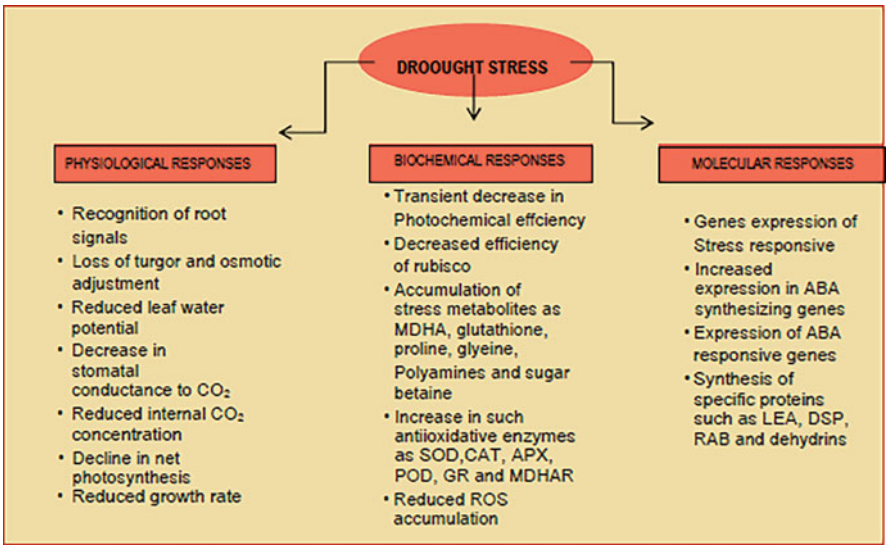

Fig. 18.5 Impact of limited water irrigation and its consequences on molecular, physiological, and biochemical responses on sugarcane plants

ethanolamine, fructose, and glucose. ABA-independent pathways like galactinol and raffinose metabolites belonging to the TCA cycle and GABA shunt regulate 35 dehydration-increased metabolites. In contrast, the rest 13 are regulated by agmatine, proline, lysine, methionine, phenylalanine, and saccharopine, comprising ABA-dependent and ABA-independent pathways.

18.9 Abiotic Stress vs. PSAP

Apart from it, transcriptome, proteome along with metabolome studies using sensitive as well as tolerant sugarcane lines to salt stress may clarify the major steps involved in gene expression for understanding the mechanism of salt tolerance in PSAP-treated sugarcane. The protective mechanisms against chilling injury in PSAP-treated sugarcane plants still require exploration although they may depend on a complex antioxidant system. Further genomic and molecular studies involved with biochemical unveiling are needed to explain the mechanism of sugarcane responding to high temperatures when treated with PSAP (Fig. 18.6). The expression profiles of cold-inducible genes have disclosed proteins directly involved in chilling and freezing tolerance. For example, one EST of sugarcane encoding a putative NAO-dependent xanthine dehydrogenase (XDH) gene has been identified as inducing after cold exposure for protecting against oxidative stress (Zhao and Li 2015; Zhang and Govindaraju 2018; Tripathi et al. 2019).

Thus, to improve crop management through water use efficiency (WUE) and ensure the economic viability of sugarcane farming, it is necessary to understand the

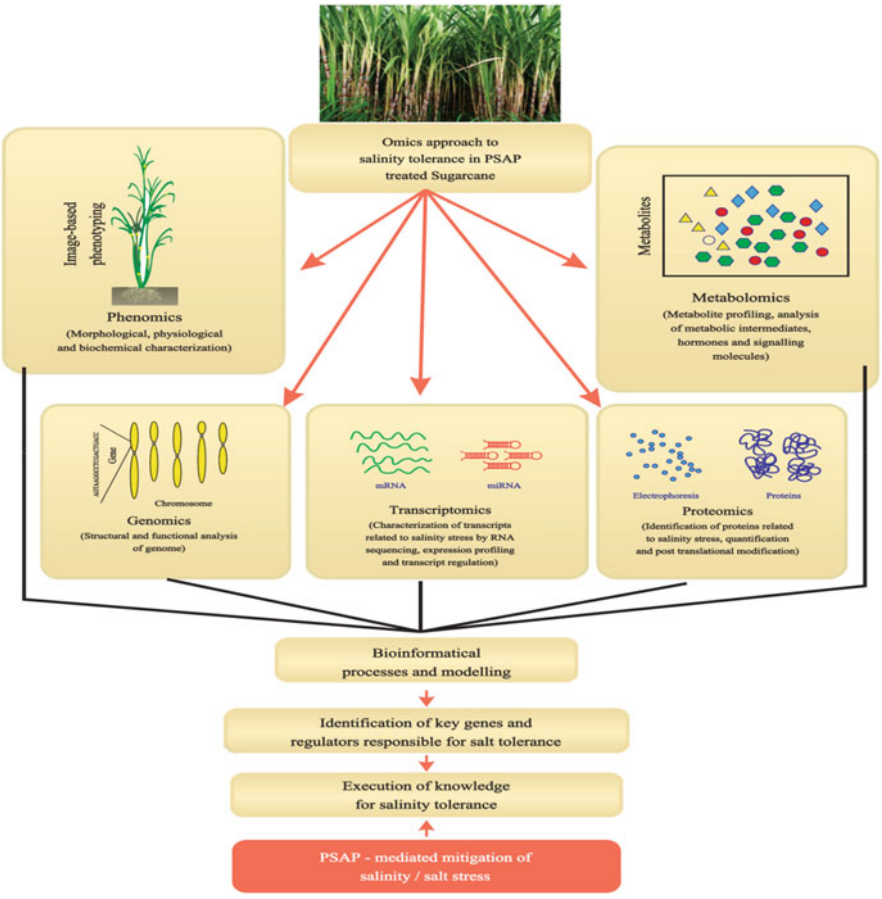

Fig. 18.6 Impact of PSAP on sugarcane plants during unfavorable environmental conditions

mechanism of PSAP-treated sugarcane plants response to drought. Plants respond to drought initially by retarded growth with the decreased photosynthetic rate as the reduction in plant water potential (Damon and Rengel 2007; Tariq et al. 2017; Verma et al. 2021). Drought responses in sugarcane were found to be analogous to those induced by exogenous ABA application (Verma et al. 2020). Expression of genes encoding a PP2C such as S-adenosylmethionine decarboxylase, protein phosphatase, and two delta-12 oleate desaturases was influenced by drought and ABA (Cramer et al. 2009; Zhao and Li 2015; Meena et al. 2017). An ethylene-responsive factor (ERF) SodERF3 of sugarcane is induced by ABA under drought stress which may also be involved in drought and salt tolerance (Zhang and Govindaraju 2018). However, acclimatization of the plant under drought conditions is a complex phenomenon, particularly with a polyploidy genome such as sugarcane, in addition to the involvement of biochemical networks under drought stress that is still being elucidated. For example, phosphorus and potash supply through PSAP improved the

drought tolerance of sugarcane by influencing water status and photosynthetic rate leading to network modulation under drought conditions.

As in the past, environmental problems like contamination of water, soil, and sediments with toxic metals will continue in the near future, which needs to be dealt with. Nowadays, it is being realized that besides implementation of intensive programs and continuous and sincere plant breeding efforts for enhancing cane yield, the pollution originated from contaminated water, chemical fertilizers, herbicides, pesticides, industrial residues, and sewage sludge, containing various concentrations of toxic metals may be firmly dealt with, as these metals severely affect plant growth (Meena et al. 2017; Etesami and Jeong 2018; Adisa et al. 2019). The large number of reports have been published in the recent past, focusing on the adverse effects of toxic metals on the number of plant species (Jewell et al. 2010; Paul and Lade 2014; Etesami 2017).

Although the aggregation of these compatible solutes in the sugarcane plant leaf tissues is not efficient enough to prevent reduction in dry matter production (Zhao and Li 2015). Molecular analyses along with biochemical data are the need of the hour for understanding the mechanisms of Al toxicity in the case of PSAP-treated sugarcane. For studying biochemical pathways related to A1 toxicity, sugarcane expressed sequence tag (SUCEST) data bank can be used (http://www.sucest-fun.org). A more comprehensive view has to be taken and must necessarily include. Studies on gene expression, enzyme activity, and protein translation must be retained for a more comprehensive view. These are the most important tools for getting a wide range of information in case of responses of PSAP-treated sugarcane to heavy metals stresses. For identification of the genes expression involved with metal tolerance and nutrient uptake, molecular genetics approaches may be helpful (Fig. 18.6).

Excess iron (Fe) and aluminum (Al) responsible for ion stress in sugarcane can be removed with additions of phosphorus (P) and potassium (K) instantly made available with the application of PSAP. Hence, it is essential in sugarcane to have sufficient potassium to utilize unassimilated nitrogen (N) and bring the maturity where the sucrose is converted from reducing sugars. Nutrient deficiency is detrimental to sugarcane growth and development and can reduce yields (Paul and Lade 2014; Etesami 2017; Etesami and Jeong 2018), a phenomenon that continues to be the subject of extensive research. The quantum yield for carbon dioxide uptake declined linearly with decreasing leaf nitrogen (N) content and the rate of photosynthesis reduced with increased severity of K deficiency (Kaya et al. 2006; Chen et al. 2016).

Therefore, enhancing sucrose recovery through the reduction in fiber content, the application of K fertilizers along with PSAP can be helpful in K deficient soil. It has been confirmed now that balanced use of all the essential nutrients can enhance cane yield and increase sugar recovery by making the plant resistant to biotic and abiotic both forms of stress and through better synthesis and storage of sugar. For instance, the adverse effect of water stress in sugarcane can be mitigated or removed by P supply, possibly due to increased proline content (Verma et al. 2020, 2021a, b). Although higher free-proline content in drought-tolerant sugarcane genotypes was

recorded compared to drought-sensitive plants (Verma et al. 2020), more investigations required to studies are needed to affirm that PSAP more efficiently modulates the above response in sugarcane.

It is also interesting to note that stomatal diffusive resistance is enhanced significantly when sugarcane setts are treated with PSAP before planting under water stress, thereby reducing the rate of transpiration and enhancing the leaf water potential and cane length sucrose content of the juice along with cane yield.

18.10 Excess Nutrients Can Trigger Extreme Stress Responses in Sugarcane

Plants respond to stress in both the case of excess and deficiency of nutrients by involving complex mechanisms for modulating the uptake and accumulation of ions (Compant et al. 2005; Paul and Lade 2014; Etesami 2017; Etesami and Jeong 2018). Therefore, there is a need to identify and understand the expression of genes responsible for or associated with nutrient uptake and distribution resulting in efficient nutrient management in sugarcane. However, in comparison of sugarcane to other crops, we find some of the crops less economically important with limited contribution, and still, a lot has to be done. Research on the biochemical and molecular modifications associated with adaptation responses to extreme temperature, drought, salinity, and excess nutrients and metals in PSAP-treated sugarcane plants is required. As sugarcane is one of the most important cash crops produced across the globe for sugar and ethanol, in-depth studies on the independence of the source and nature of the abiotic agent, anthropogenic or natural, are required (Fig. 18.7).

Only limited studies on the impact of high temperature on sugarcane have been conducted so far, possibly due to the cultivation of sugarcane species in subtropical and tropical areas (Zhao and Li 2015; Zhang and Govindaraju 2018). But taking the cognizance of possible risks involved in sugarcane cultivation due to global warming and its potential impact on the production of sugar as well as ethanol, there is an urgent need to undertake research work on metabolic pathway regulation, development of the plant, and productivity of sugarcane under stressed conditions (Fig. 18.7).

To develop improved varieties with increased tolerance of biotic and abiotic stresses, integration of genomics, breeding techniques, and physiology is required to study such traits in PSAP-treated plants.

18.11 Conclusion

It was formulated after 6 years of untiring and in-depth rigorous research efforts. PSAP technology has been proved to increase cane yield and improve sugarcane quality. It induces diseases, pests, and various types of stress tolerance in sugarcane. Besides, this product is nontoxic environmentally friendly, having a wide range of

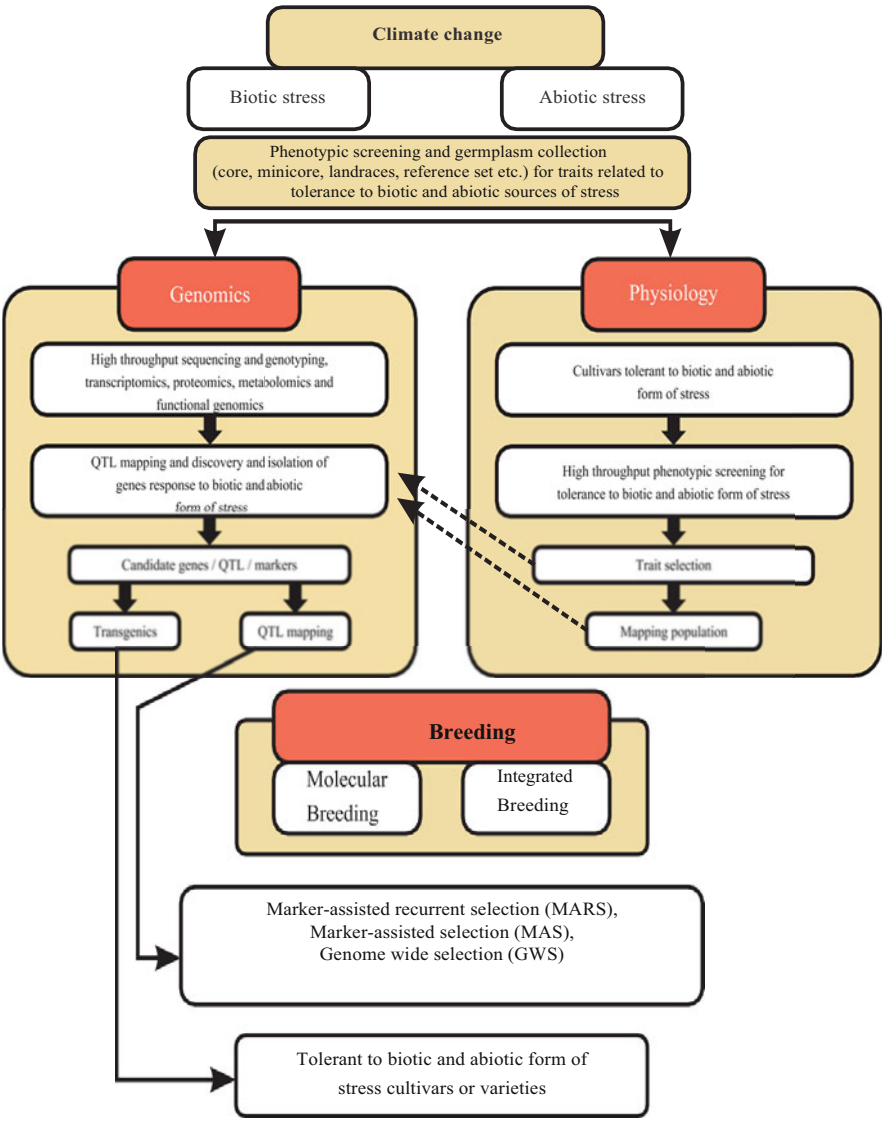

Fig. 18.7 PSAP technology, genetic science, soil health, and omics combined can endorse sustainable agriculture

crops applicability. The application of PSAP is easy to manage and can be used without much changes in the agricultural practices in vogue. The application of PSAP is complementary to the existing agricultural production technology as well as emerging technologies such as precision agriculture. Sustainable agriculture can be endorsed with PSAP. It is very effective in various crops in improving plant health, inducing stress tolerance, higher yield (30–100%), quality of produce (sweetness,

keeping quality, luster). Cane yield improvement to the tune of 100–200 qt./acre (around 30% higher than unsprayed). Per acre, sugarcane yield improvement, as well as sugar recovery enhancement, helps to reduce cane area requirement to fulfill the crushing needs of sugar mill and also helps to increase the production of side products like ethanol, co-generation (due to additional bagasse availability), bio-manures, etc. It is eco-friendly, nontoxic, and has no residual effect, and the agricultural produce is very safe for humans and livestock.

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