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# A review on mechanisms, screening and engineering for pest resistance in sugarcane (*Saccharum* spp)

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**Abstract**
Sugarcane crop remains in field for 12-14 months and is attacked by a large number of insect pests including tissue borers, grasshoppers and termites and some sucking type belonging to fulgorids, coccids, aleurodids or acarina. Many morphological characters of plants have been found obstructing or completely restricting the entry, penetration and feeding of insects on some clones may be called direct resistance. Naturally occurring pest resistant traits in plants can be of either induced or constitutive resistance. Induced resistance requires the plant to recognize that there is an invader, which in turn results in the plant producing proteins or metabolites that are harmful to the invader. With induced resistance the plant detects the pest via at least one molecule produced by the pest. These bio-molecule(s) may be a protein, fatty acid derivative (fatty acid-amino acid conjugates), or other chemical compound secreted by the pest. Further, transgenic research in sugarcane concentrated on the development and evaluation of transgenic lines transformed for resistance to biotic stresses, particularly diseases and insect pests. The genetic complexity of sugarcane coupled with the non-availability of resistance genes in the germplasm has made conventional breeding for insect resistance difficult. In this review, attempt has been made explore different resistance/ defence mechanisms operating in plant system (physical and chemical), role of transgenic technology as handy tool for imparting insect resistance and use of Near-infrared reflectance spectroscopy (NIRS) as a rapid screening tool for pest resistance.

**Keywords:** Sugarcane, induced resistance, morpho-physiological traits, transgenic, pest

## Introduction
Sugarcane (*Saccharum* spp.) is one of the important commercial crops cultivated throughout the world about in 110 countries with a tropical/sub-tropical conditions. It is the primary source for manufacturing crystal sugar, which is a predominant commodity in the global food industry. Besides, the production of crystal sugar as a main product, ethanol, bagasse, press mud and co-generation of power are the other useful by-products. Presently, sugarcane is also looked upon as a feed stock for bio-fuels and would be one of the major sources of energy for the future. Sugarcane is attacked by a range of insects including tissue borers, sucking pests and cane grubs. Losses due to these pests are estimated to be around 10% (Kfir *et al*., 2002; Goebel and Way, 2009) [26, 20]. Sugarcane pests exhibit wide variation in species composition and importance in the diverse agro-climatic conditions of tropics and sub-tropics where the crop is cultivated. Some important tissue borers in sugarcane growing countries of the world include: the sugarcane stem borer *Diatraea saccharalis* (F.) (Rossato *et al*., 2010) [44] and the sugarcane giant borer *Telchin licus* (Drury) (Craveiro *et al.*, 2010) [15] in the Americas including Brazil; the Mexican rice borer *Eoreuma loftini* (Dyar) in south Texas (Tomov and Bernal 2003) [50]; the African stem borer *Eldana saccharina* Walker in South Africa; the Asian spotted stem borer *Chilo sacchariphagus* (Bojer) in Mauritius, Réunion, Madagascar and Mozambique; *Proceras venosatus* Wlk. in China (Weng *et al*., 2006) [54]; and the early shoot borer *Chilo infuscatellus* (Snellen), the internode borer *Chilo sacchariphagus indicus* (Kapur) (Srikanth and Kurup 2011) [49] and the top borer *Scirpophaga excerptalis* Walker in India. Pests are a major constraint affecting the sugarcane productivity world-wide, which includes borer complex, termites, Pyrilla, mites, white grubs, mealy bug and scales. Pest resistance in crop plants is an enigma to be unravelled, in spite of advances made in plant biology. Understanding plant-pest interaction precisely is still a fascinating area, which forms a basis to

develop pest resistance varieties in agriculture. The science of plant pest resistance has undergone a paradigm shift in understanding starting from the gene for gene concept to the present age of guard decoy model to decipher pest resistance genes in crop plants. There are different biological control, cultural practices, chemical control, host plant resistance and genetic transformation tools used to control borers such as *C. saccharіphagus* and *C. partellus*. In Mauritius, cultural and chemical control methods are not considered feasible in controlling *C. saccharіphagus* because it was found that the cultural practice of burning sugarcane before and after harvest actually had a negative impact on the natural enemies of the borer, whereas the larvae and pupae living inside stalks remained unaffected (Rochat *et al*., 2001) [42]. Classical biological control is the favoured method of control whereby a number of natural parasitoids have been introduced from other countries to control the borers (Way and Turner, 1999) [52]. Since *C. saccharіphagus* is not indigenous to Africa, and mainly attacks sugarcane, it should fit the profile for being a target for biological control (Conlong and Goebel, 2002) [14]. Rochat *et al*. (2001) [42] reported that approximately 17 species of natural enemies of *C. saccharіphagus* have been brought into Reunion from different countries, but only a third of them survived and none were able to reduce the levels of *C. saccharіphagus.* The control of *C. partellus* and *C. saccharіphagus* is particularly difficult, because once the larvae enter the plant tissue; it is difficult for natural enemies and insecticides to reach the target. Since *C. saccharіphagus* has a larger impact on cane weight than on sucrose content due to its early attack of sugarcane, control methods should focus on early infestations, with the parasites being released when the crop is still young. In turn this will prevent the insect populations from increasing, which could potentially reduce yield losses (Goebel and Way, 2009) [20]. However, Way *et al*. (2011) [53] made recommendations for the control of *C. saccharіphagus*, including the establishment of a breeding programme for developing resistant varieties. Resistant varieties have a number of advantages over other control methods. Resistant varieties are not influenced by changing weather conditions, do not harm the environment, and in some cases, are the only effective method for controlling certain pests (Kfir *et al*., 2002) [26]. Breeding for resistance to insects in sugarcane lags behind other crops, and this is most likely due to its complex genome and the inheritance of polygenic traits (White *et al*., 2010) [56]. A number of studies have been published reporting on the evaluation of sugarcane varieties for their resistance to sub-species of *C. saccharіphagus*. However, none of the data provides information on the resistance status of Reunion sugarcane varieties, and the gain that could be achieved by improving sugarcane varietie (Nibouche and Tibere, 2009) [35]. The two main sugarcane varieties of sugarcane grown in Reunion are R570 and R579, which have different susceptibilities to *C. saccharіphagus* (Nibouche and Tibere, 2010) [69]. It was shown that R570 is one of the most resistant sugarcane varieties to *C. saccharіphagus* when compared to other sugarcane varieties (Nibouche and Tibere, 2010) [36]. The development of thrips resistant varieties is still in its initial stages. Some of this resistance is based on morphological characters of the plant such as having round or flat leaves, hairy leaves, and an open plant design. In sugarcane it has been shown that those varieties that have a slow initial growth and are slower in unrolling their central leaf rolls are more prone to attack and damage by *F. serrata*.

**Mechanisms of host-plant resistance**

Pest and disease resistance is shown in the inherited ability of an organism to negate the effects, totally or partially, of a pathogen, insect, or other harmful factor. Resistance is shown when symptoms are minimal to none which indicates that the pest cannot spread, or spreads with difficulty on the host (Ahman, 2006) [1]. The resistant characteristic of plants is usually a result of morphological and biochemical attributes of the plant which has an effect on the insect's behaviour and biology and leads to better survival and production of the plant. By comparing resistant plants to more susceptible plants under similar conditions, one can deduce the degree of resistance of that plant. Naturally occurring pest resistant traits in plants can be of either induced or constitutive resistance (Broekgaarden *et al*., 2011) [12]. Induced resistance requires the plant to recognize that there is an invader, which in turn results in the plant producing proteins or metabolites that are harmful to the invader (War *et al*., 2012) [51] (Figure 1). With induced resistance the plant detects the pest via at least one molecule produced by the pest. The molecule(s) may be a protein, fatty acid derivative (fatty acid-amino acid conjugates), or other chemical compound secreted by the pest. Constitutive resistance is the level of resistance already present in the plant, and is not dependent on the attack of a pest. It can be morphological, structural or chemical in nature. Arimura *et al*. (2005) [5] classified and described resistance in terms of direct and indirect defences. Direct defences are those which instantly impact on herbivores attacking the plant, such as physical barriers which include thorns, trichomes, waxes, spines, and chemical means using secondary plant metabolites or special defence proteins. Direct defences can be both constitutive and inducible. Indirect defences are mediated through other species such as natural enemies of the insect pest. There are three components of plant resistance, namely, antixenosis, antibiosis, and tolerance. One or more of these mechanisms may be present in a resistant plant; however it is favourable for all three mechanisms to be present in a resistant variety (Ahman, 2006) [1]. These mechanisms will be discussed in more detail under subsequent sections in this article.

**Components of host-plant resistance**

The three components of resistance, namely, antixenosis, antibiosis, and tolerance are they can be either physical or chemical in nature, or a combination of both, they are briefly explained below:

**Antixenosis**

It is a resistant mechanism in plants that can be morphological, physical, or structural in nature and interferes with the behavioural aspect of insects such as mating, laying of eggs and the insects feeding. It therefore results in the insect avoiding the host plant. It could be due to the colour, texture of the leaf surface, certain allele-chemicals, or an interaction between all of these factors in the plant which deters the insect pest and prevents ovipositing from occurring on the plant. Antixenosis has been used in some cases to develop resistant varieties of crops (Kumarasinghe and Jepson, 2003) [29]. Examples are shown in rice that are resistant to *Cnaphalocrocis medinalis* Guenee (Lepidoptera: Crambidae) (rice fodder) as a result of crossing a susceptible cultivar with a wild rice cultivar having antixenotic characteristics; *Chilo infuscatellus* Snellen has a preference for ovipositing on sugarcane plants of 45 days old, whereas older plants will not be used for this purpose (Kumarasinghe

and Jepson, 2003) [29]. In a paper published by Nibouche and Tibére (2010) [36], mechanisms of resistance of two varieties (R570 and R579) to *C. sacchariphagus*, and its location in the plant were identified. In Cultivar R570, plants were artificially infested with *C. sacchariphagus* in the glasshouse. Within 48 hours after infestation there was a reduction in larvae numbers that had established on the plants. Bioassays carried out in the laboratory indicated that the reduction in larvae numbers was due to antixenosis on the lower surface of the leaf sheath. Susceptibility to the antixenosis was seen in the first, second and third instar stages. However, antixenosis was not

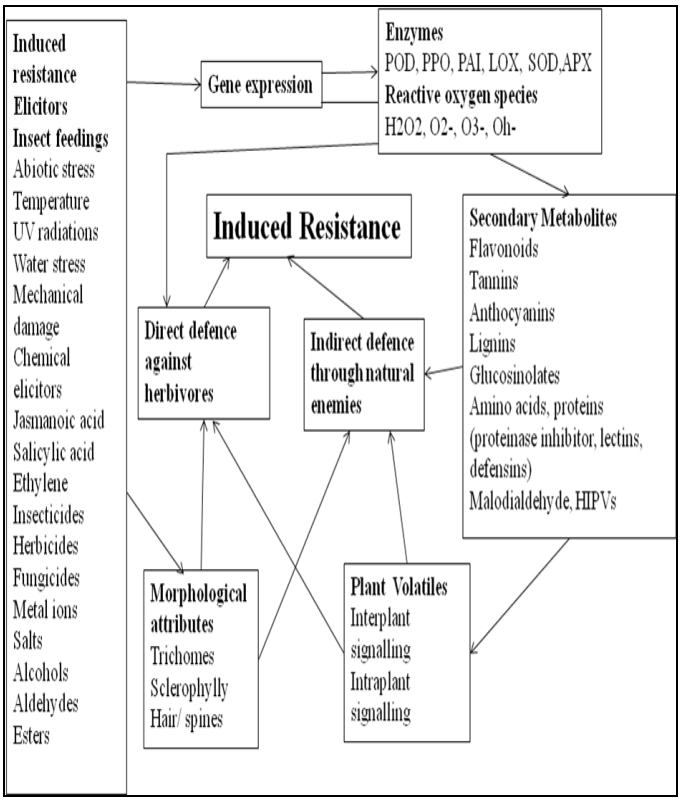


**Fig 1:** Mechanism of induced pest resistance in plants. POD, peroxidase; PPO, polyphenol oxidase; PAL, phenylalanine ammonia lyase; TAL, tyrosine alanine ammonia lyase; LOX, lipoxygenase; SOD, superoxide dismutase; APX, ascorbate peroxidase; HIPVs, herbivore induced plant volatiles. (War *et al*., 2012) [51].

seen on the leaf spindle or on the stalk. It was also concluded that antibiosis could not have been involved in the resistance shown as there was a low number of dead larvae on the plants and thus it was concluded that antixenosis was the main mechanism of resistance to *C. sacchariphagus*.

**Antibiosis**

It adversely affects the biology of an insect which tries to use the plant as a host and can be physical or chemical in nature. Damage to the insect can be severe and often affects the larvae and eggs. Insects that survive the effects of antibiosis may be permanently damaged having stunted growth, slower development processes, and a reduction in fecundity. Antibiosis can be attributed to allelochemicals, growth inhibitors, and morphological factors preventing the attack of the insect. Allelochemicals such as glycoalkaloids in potato, γ-tomatine in tomato, gossypol in cotton, rutin and chlorogenic acid in tomato are harmful to insects that attack such plants. In a study conducted by Kumar *et al*. (2006) [28] on antibiosis mechanisms of resistance to *C. partellus* in sorghum, it was found that antibiosis resulted in reduced pupal weight and less pupation. It was suggested that this could be due to secondary plant substances present in the leaves. The levels of amino acids, tannins, phenolics, lignins, acid detergent fiber (ADF), and neutral detergent fiber (NDF) were linked to resistance of sorghum to *C. partellus* (Kumar *et al*., 2006) [28].

**Tolerance**

This mechanism allows for normal growth and an increase in plant biomass, irrespective of the level of insect infestations. Painter (1951) [38] defined tolerance as, "a basis of resistance in which the plant shows an ability to grow and reproduce itself or repair injury to a marked degree in spite of supporting a population approximately equal to that damaging a susceptible host". According to Reese *et al.* (1993) [41], tolerance is the preferred mechanism of resistance because it does not negatively impact on the natural enemy populations, whereas antixenosis and antibiosis increase selection pressure on insect populations, which can lead to the development of more virulent biotypes and can also, have an adverse effect on control methods.

**Role of defence mechanisms in plants against insect pests**

There are different types of physical and chemical structures on the plant surface are important in contributing to pest resistance (Howe and Schaller, 2008) [22] which hinders the establishment of insect pests when they first come into contact with the plant surface.

**Physical defences/ barriers**

The surface of a plant is where organisms first come into contact with the plant in order to establish themselves with the plant, therefore any alteration / barrier on the surface contribute towards pest resistance. Trichomes and/or hairs on the surface of plants have been used to give rise to insect-resistant varieties (Peter *et al*., 1995) [39]. Trichomes can either be non-glandular, tiny hairs which physically deter insects, or they may be specialized glandular trichomes, morphological and chemical in nature, whereby they secrete substances which are stored or volatilized on the surface of the plant and are used to repel pests and prevent them from feeding (Howe and Schaller, 2008) [22]. Recent research also shows that trichomes may be involved in the early detection of pests whereby the trichomes are disturbed by the presence of moths or larvae and this leads to the plant gaining awareness of the pest which allows it to respond to the insect attack more quickly. The nature of response could be to increase the trichome density on new leaves. Epicuticular wax on the surface of leaves may also play a role in protecting plants against insects. In addition to preventing the plant from desiccation, they also result in a more slippery surface which prevents non-specialized insects from inhabiting the plant. The chemical and physical components of the wax layer play an important role in determining resistance (Howe and Schaller, 2008) [22]. In a study conducted on *C. partellus* it has been found that edge spines and leaf surface waxes play an important role in the reorientation of newly hatched *C. partellus* larvae which have drifted out onto leaves, and whose aim is to reach the whorl. The larvae are reorientated to the stalk in order for them to continue their climb. Therefore plant characteristics such as leaf surface waxes can be partially responsible for different levels of resistance between varieties. Silicon in plants can also confer resistance to insects, and it has been shown that constitutive resistance using silicon as a physical defence mechanism is important

against chewing insects. This has been shown for sugarcane that the application of silicon fertilizer resulted in increased resistance to penetration by *E. saccharina* (Kvedaras and Keeping, 2007) [30]. Another important physical attribute of plants involved in pest resistance is leaf toughness. This characteristic has an effect on insect penetration, preventing their piercing and sucking mouthparts from damaging plant tissues. Leaf toughness is generally regarded as a physical factor. However the chemical composition of the cell wall contributes to leaf toughness. Other physical attributes of plants contributing towards insect resistance are shape and colour. In a study was conducted by Kumarasinghe and Jepson (2003) [29] on the antixenotic effect of sugarcane leaves on feeding and ovipositing by *Pyrilla perpusilla* Walker (Lophopidae: Homoptera), leaf colour was found to play an important role in choice of host for feeding.

**Chemical defences**

Chemical factors involved in plant resistance can be used in one of two ways. Firstly, chemicals can decrease the nutritional value of the plant as a food source, and secondly, they can deter insects by producing toxins. Plant primary metabolism gives rise to carbohydrates, amino acids, and lipids that are vital nutrients for insects; and the availability of these nutrients has an effect on the life span, size, productiveness, and mortality of insects (Howe and Schaller, 2008) [22].

Secondary metabolites play a vital role in defense mechanisms of plants (i.e., antixenosis, antibiosis and tolerance) (Wink, 1988) [57]. Phenolic compounds are secondary metabolites that are major compounds in plants (Mazid *et al*., 2011) [33]. Phenolic compounds include coumarin, lignin, flavonoids, and tannins (Mazid *et al*., 2011) [33]. Coumarins occur extensively in plants and are known to act as natural defense compounds against insects, fungi, and bacteria. There are a number of cases where flavonoids are used in resistance against insects in plants. The C-glycosyl flavone, maysin, in maize silk tissues has insecticidal activity against *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae). Tannins can affect the growth and development of insects, and can also behave as feeding repellents to a number of animals. Terpenes are the largest group of secondary products and have a number of functions in plants, which include the formation of oils and resins involved in the defense against other organisms (Mazid *et al*., 2011) [33]. It has been shown that individual terpenes behave as insect antifeedants. They have also been found to play a role in antibiosis in sugarcane against the woolly aphid *Ceratavacuna lanigera* Zehntner (Hemiptera: Aphididae) (Hunsigi *et al*., 2006) [23].

Non-protein, nitrogen containing secondary metabolites includes alkaloids, cyanogenic glycosides, polyamines, polyamine phenylpropanoid conjugates (PPCs) and benzoxazinoids (Mazid *et al*., 2011) [33]. Dhurrin, a cyanogenic glycoside has been found in sugarcane leaves using HPLC, and it plays a role in constitutive and inducible resistance whereby the activity of IDP-glucose:p-hydroxymandelonitrile-O-glucosyltransferase and dhurrinase enzymes increases when sugarcane is attacked by the stalk borer *Diatraea saccharalis* Fabricius (De Rosa-Junior *et al*., 2007) [16]. Arginine is an important amino acid in plants and is involved in defense mechanisms against insects and pathogens. Arginine can be broken down by Jasmonic acid (JA)-induced arginase which in turn has an effect on the insect's nutrition. Benzoxazinoids occur predominantly in the family Poaceae which includes maize, wheat and sugarcane (Singh *et al*., 2003) [48]. Benzoxazinoids concentrations are higher in younger plants and young, exposed tissues of older plants. Aphids and stalk borers are deterred by benzoxazinoid compounds, which could be due to an anti-feeding effect of the compound and may also result in the inability of insects to detoxify other defense compounds in plants. Benzoxazinoids are known to be constitutive compounds but can also be synthesized due to an induced response from insect attack.

**Protein based defence**

There are four classes of proteinases in insects, namely, serine, cysteine, aspartic acid proteinases and the metallo proteinases (Falco *et al*., 2001) [18]. Serine proteinase is found in Lepidoptera and cysteine proteinase activity in Coleopteran insects (Mohan *et al*., 2008) [34]. Plant proteinase inhibitors (PIs) are found in a number of plants and form part of their natural defence mechanisms against herbivores. The inhibitors are more commonly found in plant parts more prone to attack, such as bulbs, leaves and seeds and may be of constitutive or wound-induced in nature (Falco *et al*., 2001) [18]. A number of papers have shown that these proteinase inhibitors have an effect on larval development but do not cause their death (Wolfson and Murdock, 1995) [58]. It is thought that the inhibitors have an effect on the digestive system of insects by inhibiting the activity of midgut enzymes therefore resulting in the reduced availability of amino acids required for protein synthesis, which in turn negatively impacts on growth, development, and reproduction of the insect (Falco *et al*., 2001) [18]. Alternatively, an indirect effect on insects can be caused whereby there is an increase in production of digestive proteinases to make up for the low levels of available amino acids, and this results in a reduction in amino acids needed for essential proteins. It has been shown in artificial diets incorporating soybean proteinases fed to sugarcane borers that a reduction in growth and development occurs in the borers (Falco *et al*., 2001; Christy *et al*., 2009) [18,13]. Chitinase may also play a protective role against insects in plants by interrupting chitin-containing glycoproteins of the peritrophic matrix, which is a system that guards the gut cells from being damaged by digestive enzymes and microorganisms. Lectins are sugar-binding proteins that occur in plants and other organisms, predominantly in legumes, that play a role in the defense mechanisms of the plant. The expression of lectin-like genes in sugarcane has been shown to be specific to certain tissues where expression is lower in the stalk and higher in the leaf roll, apical meristem and lateral buds (Falco *et al*., 2001) [18].

Polyphenol oxidase (PPO) enzymes result in the browning of plant extracts and tissues that have been damaged by herbivory, wounding and JA (Falco *et al*., 2001) [18]. It has been suggested that there is a role for PPOs in defending plants against insects. During feeding of the insect on the plant, o-quinones are produced due to mixing of PPO and phenolics and this leads to the modification of free amino acids and sulfhydryl groups in dietary proteins in the insect's mouth and gut. The combination of PPOs with a phenolic substrate in glandular trichomes results in a glue-like substance which traps tiny insects (Falco *et al*., 2001)[18]. When PPOs are present in mesophyll tissue they result in proteins being modified and this leads to reduced digestibility of the protein in the insects gut during feeding. The gene has been found to be expressed in the sugarcane callus, seeds, the root transition zone, and in the stalk of the plant.

**Indirect defences**

Within the last few years another type of defence has been identified, which was first found in maize, and is now referred to as indirect defence. It has now been identified in a number of plant species in which the attack of insect results in the plant giving off complex amounts of volatiles into the atmosphere from their vegetative plant parts and these volatiles are known as herbivore induced plant volatiles (HIPVs). Enemies of these herbivores can be attracted to these HIPVs, and this is known as constitutive indirect defence (Baldwin *et al*., 2002) [7].

There is a range of HIPVs known to exist in plants, including alkenes, alkanes, and two jasmonates (cis-jasmone and methyl jasmonate), but the main compounds seems to be C6 green leaf volatiles (GLVs), terpenes and products derived from the shikimic acid pathway (Arimura *et al*., 2004; Ferry *et al*., 2004) [5,19]. The volatiles can work in a number of different ways against herbivores; either attracting predators and parasites of the target herbivores, directly deterring the herbivore, or by priming the healthy plant parts of the plant under attack, or of the neighbouring vulnerable plants so that more efficient defense can take place in future attacks. Although indirect defence mechanisms are seen in natural hosts of *E. saccharina*, there have been no known records of sugarcane displaying indirect defense to this stalk borer. This could be due to loss of this trait during sugarcane plant breeding where only direct defence is actively selected.

**Screening Techniques**

Initial host-plant resistance screening studies should take place under controlled conditions in a glasshouse, or in the laboratory to increase precision (Ahman, 2006) [1]. Characteristics in the field such as soil, moisture, climate, and variable pest numbers reduce precision of field trials. Field assessments are generally performed at the last two stages of sugarcane selection programmes (Keeping, 2006) [25]. Designing experiments where the conditions are optimized for determining differences between sugarcane varieties in terms of their resistances takes into account the background of resistance mechanisms. For assessing both induced and constitutive resistance, bioassays using insects on plants can be used to compare insect numbers, plant symptoms, antibiosis, and antixenosis resistance components (Ahman, 2006) [1]. Numerous methods have been used and explored to distinguish sugarcane varieties for resistance against stalk borers (Mathes and Charpeinter, 1969) [32]. These include measurement of internode rind hardness, forced penetration of larvae into stalks, ovipositing tests using moths, trials conducted under a controlled environment for artificial infestation of plants, and the incorporation of leaf powders into an artificial diet (Nibouche and Tibere, 2010; Goebel and Way, 2009) [36, 20].

Black head stage egg masses and neonate larvae of *C. partellus* have been used to artificially infest maize varieties to determine their different resistances or susceptibilities (Kumar, 1997) [27]. A camel hair paint brush can also be used to manually deposit larvae onto the plant (Nibouche and Tibere, 2010) [36]. The use of larvae for infestation of plants has been reported to be more effective than egg masses in host-plant resistance screening studies. The stage of the plant to be used in host-plant resistance screening studies must also be considered. However, it has also been found that plants of 2 weeks of age can distinguish between resistant and susceptible sugarcane varieties (Kumar, 1997) [27]. *C. partellus* has been known to attack sorghum plants from two weeks after germination (Kumar *et al*., 2006) [28].

**Use of artificial diet bioassays**

Artificial diets are vital in arthropod research. Incorporating leaf material into an artificial diet can be useful in determining any constitutive resistance mechanisms in different plant varieties. The use of artificial diets in resistance screening studies is also useful in comparing sugarcane varieties under uniform conditions where variations from the environment are excluded. In order for successful resistance screening studies to take place, a large supply of insects in sufficient numbers is required, as well as a suitable artificial diet for rearing and maintain [ing insects to be used in resistance screening studies. There are numerous commercial diets that have been developed to maximize insect growth and development by meeting all the nutritional requirements of the target insect.

**Engineering sugarcane against pests**

Sugarcane is the most suitable candidate for genetic engineering because of its complex polyploidy nature, variable fertility and genotype versus environment interactions. The availability of high frequency *in vitro* regeneration system from various explants makes this crop as a suitable candidate for genetic manipulation (Arencibia *et al*., 1997; Sanghera *et al*., 2016) [4, 45]. Several genes (for disease/pest resistance, salt and drought tolerance, and sugar accumulation) targeted towards sugarcane improvement have been introduced into sugarcane (Altpeter and Oraby 2010; Arvinth *et al*., 2010; Hotta *et al*., 2011) [2, 6, 21]. The success of transgenic sugarcane plant production depends on the method used for transformation, the target tissue/explants and tissue culture regeneration system used (Weng *et al*., 2006) [54]. Various explant types (axillary buds, apical meristems, immature inflorescences, leaf segments)) have been used successfully to regenerate full plants in sugarcane indicating that a wide range of totipotent target tissues are available for genetic transformation (Sanghera *et al*., 2018) [46]. The sugar industry throughout the world suffers considerable economic loss due to various insects and diseases although precise quantification has not been reported for many of the pests. Although chemical control and integrated pest management are regularly practiced for the control of insect pests, success is often limited due to practical difficulties (Braga *et al*., 2001; 2003) [10, 11]. The sugarcane canopy and internal habitat pose serious limitations to deployment of chemical control measures. Since larvae of borers enter the plants soon after eclosion, typically remain on the inner side of the leaf sheath or inside the stem, burrow tunnels and feed on the stem tissues, they are inaccessible to insecticides. Systemic insecticides are also largely ineffective due to poor translocation within the plant. Alternative control measures, including mechanical methods, cultural practices and biological agents, followed under specific situations, are limited by costs and only moderate efficacies. The genetic complexity of sugarcane coupled with the non-availability of resistance genes in the germplasm has made conventional breeding for insect resistance difficult. Advances in genetic transformation technology and knowledge of gene expression have led to rapid progress ingenetic engineering of crop plants for protection against insect pests (Weng *et al*., 2010; Sanghera and Kumar, 2018) [55, 46]. Advantageous use of this technology to produce plants for pest control using different molecules, such as proteinase inhibitors (PI), plant lectins, ribosome inactivating proteins, secondary plant metabolites,

delta endotoxins and vegetative insecticidal protein from *Bacillus thuringiensis* (Bt) and related species, either alone or in combination with the Bt genes (Bates *et al.*, 2005) [9] has now been widely recognized. Transgenic sugarcane plants expressing high levels of modified cry1Ac provide effective control against stem borers under field trials have been documented by Weng *et al.* (2010) [55]. Similarly, Deng *et al*., (2008) [17] reported fusion of insect resistant gene (s) mediated by matrix attachment region (MAR) sequence in transgenic sugarcane. Therefore, engineering insect resistance in the sugarcane plant through expression of such molecules appears to be a realistic approach to mitigate potential damage caused by pests. Engineering crop plants for enhanced resistance to insect pests has been one of the successes of transgenic technology; stem borer (Weng *et al.*, 2010) [55] and cane grubs (Nutts *et al*., 1999) [37]. As a trait, insect resistance, either alone or stacked with herbicide resistance, currently ranks second in terms of the global area occupied by biotech crops (James, 2010) [24].

**Use Near-infrared reflectance spectroscopy (NIRS) for pest detection**

The analysis of plant constituents (e.g. proteins, carbohydrates, and lipids) is an integral part of numerous agricultural studies. However, chemical analyses are time consuming and expensive (Purcell *et al*., 2009) [40]. In plant breeding trials, large numbers may be required for analysis. This can lead to the analyst being forced to bulk samples in order for a sufficient amount to be obtained, and this in turn results in the accuracy of the experimental design being compromised. The use of NIRS has proven to be useful to overcome such issues. NIRS makes use of optical data, and is based on the reflectance from a sample in relation to the amount of radiation hitting it. NIRS is associated with the absorption of electromagnetic radiation in the wavelength region from 750 to 2500 nm, next to the mid-infrared region and up to the visible region (Workman and Shenk, 2004) [59]. The types of bonds that occur between atoms in plant tissues reflect the composition of the tissue, and spectroscopy can be used to determine information about the bonds between the atoms or groups of atoms (functional groups) (André and Lawler, 2003) [3] The exposure of a sample to irradiation results in vibrations between the bonds which in turns results in stretching and bending. This results in a wave motion occurring in the bond at a frequency specific to the functional group. Absorption occurs of the incident light whose frequency matches that of the vibrations of the waves, and reflection or transmittance occurs of those frequencies that do not match. Vibrations of C-H, -O-H, S-H, and N-H bonds predominantly absorb NIR. These bonds are major constituents of organic compounds in plant tissues (André and Lawler, 2003) [3]. The type and number of bonds in the tissues are determined by the chemical make-up of the tissue and hence it is the chemical constituents in the tissues that determine the wavelengths and the amount of light that is absorbed. It is therefore the light that is reflected from a sample that gives information on the chemical composition of that specific sample.

NIRS has a number of advantages in that it provides a rapid and accurate analysis of materials, is non-destructive to samples being analysed, does not require expensive and hazardous chemicals, is able to analyse a large number of non-homogeneous samples, and a number of components in each sample can be seen in its spectrum from a single measurement, which in turn also reduces costs (Mark and Campbell, 2008) [31]. However, there are also accompanying disadvantages with using NIRS. The initial cost of the instrumentation is large, and it is also a necessity to calibrate the instrument for each sample component to be analysed (Workman and Shenk, 2004; Roggo *et al*., 2007) [59, 43]. However, a number of handheld NIRS instruments are also being used, mainly for measuring nitrogen contents of leaves to assist in optimizing fertilizer applications to crops. Portable NIRS instruments are also available that can scan the entire NIR spectrum. These specific instruments make use of fibre optic probes.

Overtones and amalgamations occur in the NIR spectrum due to scattering of light and the occurrence of absorptions in the mid-infrared region (Barton, 2002) [8]. This makes the direct interpretation of the NIR spectrum difficult because there are only a few areas of absorbance that are due to only one functional group. Thus statistical models are needed to confirm the intensity of the relationship between a specific absorbance and a laboratory assay of a specific constituent in a number of different tissues for the sample of interest. Near infrared spectroscopy (NIRS) is therefore a secondary method used, whereby chemical composition is determined by comparisons of the spectra with samples whose composition has been determined using different laboratory methods.

**Conclusions**

Sugarcane gains economic importance by virtue of its industrial potential in terms of products like crystal white sugar, bagasse, press mud, power etc. Among the various production constraints of the crop, diseases and pests are seen as a major threat for sustaining the productivity of sugarcane. About 1,500 species of insects attack sugarcane worldwide resulting in yield losses in all sugar industries. Borer complexes are significant pests of sugarcane because they feed directly on the vegetative tissue in which sucrose is stored, effecting both yield and quality of the crop. The rise in trade and the change in global climate have resulted in the easier spread and establishment of insects and diseases in previously unaffected areas. The use of insecticides for controlling pests raises environmental concerns, and can have a negative impact on beneficial insects and natural enemies of pests. The use of host plant resistance has a number of advantages over other control methods, and is one of the most effective means of controlling insects. Resistant varieties do not harm the environment, and in some cases, plant resistance is the only effective method for controlling certain pests. Breeding for resistance to insects in sugarcane lags behind other crops, and this is most likely due to its complex genome and the inheritance of polygenic traits. Screening for resistance to pests and diseases is presently limited to later selection stages within the sugarcane breeding programme. The development of new varieties can take up to 14 years, and is a resource intensive process. The breakdown of pest resistance is attributed to the possible emergence of new virulent biotypes. This situation has warranted a pertinent need to have a thorough understanding on inheritance pattern and different mechanism of pest resistance (physical, chemical and biochemical) in sugarcane, which would aid for quick screening of pest resistant clones and their successful management, respectively. Overall, there is a paradigm shift in the understanding of plant pest resistance, thanks to the advent of robust molecular and transformation tools. An integration of the tools of conventional and non-conventional has further strengthened in deciphering plant-pathogen/pest interactions at the molecular level. Applications of new

screening tools at earlier selection stages will reduce costs, increase productivity, and increase the number of resistant clones progressing to later selection stages. With an increasing number of potential pests of sugarcane, the need for rapid and less costly methods to screen sugarcane varieties increases in importance. Near infrared reflectance spectroscopy (NIRS) is a rapid, non-invasive, and reliable technique which has the potential to examine the interaction between sugarcane and its related pests. With the accomplishments in elucidating NIRS in sugarcane ably supported by employing the next generation screening platforms to unlock the secrets of pest management in sugarcane, it is now made possible to further improve our understanding on pest resistance in sugarcane.

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