



**EPIGENETIC PRIMING AND *in vitro* MUTAGENESIS IN SUGARCANE  
(SACCHARUM SPP. HYBRIDS) FOR RESISTANCE TO *Fusarium* SPECIES AND  
*Eldana saccharina* (LEPIDOPTERA: PYRALIDAE)**

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**Eshani Govender**

February 2022

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*Fusarium* SPECIES AND *Eldana saccharina* (LEPIDOPTERA:  
PYRALIDAE)**

By  
**ESHANI GOVENDER**

Submitted in fulfilment of the academic requirements for the degree of Master of Science  
(MSc) in the Discipline of Biological Sciences, School of Life Sciences, College of  
Agriculture, Engineering and Science, University of KwaZulu Natal, Durban, South Africa.

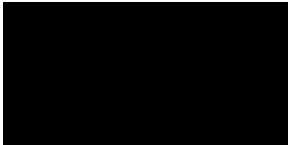
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Supervisor 

Signed: \_\_\_\_\_

Name: M.P Watt

Date: \_\_\_\_28-01-2022

Co-supervisor 

Signed: \_\_\_\_\_

Name: S.J. Snyman

Date: \_\_\_\_28-01-2022

Co-supervisor 

Signed: \_\_\_\_\_

Name: R.S. Rutherford

Date: \_\_\_\_28-01-2022

## Preface

The experimental work described in this dissertation was carried out (part-time) in the Biotechnology Department of the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, Durban, SA from January 2015 to February 2022, under the supervision of Prof. Paula Watt (UKZN), Dr Sandra Jane Snyman (SASRI) and Dr R Stuart Rutherford (SASRI).

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others, it is duly acknowledged in the text.

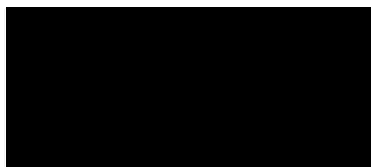
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**FACULTY OF SCIENCE AND AGRICULTURE  
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2. This thesis has not been submitted for any degree or examination at any other university.
  
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## Abstract

In the South African sugar industry, there have been substantial economic losses of R1 billion/annum caused by the indigenous pyralid borer, *Eldana saccharina* (Lepidoptera: Pyralidae). To develop control measures for *E. saccharina* in sugarcane, it is important to understand the interactions between the stalk borer and *Fusarium* spp. In previous studies, *in vitro* assays have shown that *Fusarium* strains may be antagonistic (e.g., *F. sacchari* PNG40) or beneficial (e.g., *F. pseudonyamai* SC17). *F. pseudonyamai* SC17 is a potential endophytic indicator of *E. saccharina* infestation, as the association between borer infestation and infection by the fungus causes *Fusarium* stalk rot in sugarcane. Studies have reported that the presence of endophytic fungi may have several benefits to the host plant, e.g., the production of phytohormones such as indole-3-acetic acid (IAA), which promotes plant development.

The study aimed to: 1) choose a suitable resistance priming agent between hexanoic acid (Hx) and cis-jasmone (CJ); 2) determine an appropriate culture filtrate (CF) concentration for *in vitro* screening of calli and plantlets for tolerance to *F. pseudonygamai*; 3) develop a protocol (epigenetic priming and mutagenesis) to generate mutants: primed only (0.6 mM Hx), a combination of priming and mutagenic agents (100  $\mu$ M 5-AzaC + 16 mM EMS-induced); 4) screen for indole-3-acetic acid production by *F. pseudonygamai*; and 5) characterise *in vitro* selected mutants for *E. saccharina* and *F. pseudonygamai* resistance by comparing the levels of resistance between unprimed, primed, and primed + mutagenic plantlets through *ex vitro* screening. When cis-jasmone (CJ) and hexanoic acid (Hx) were investigated for their effect on priming for pathogen resistance, 0.6 mM Hx was selected as the optimum priming agent concentration for both the callus and plantlet regeneration stages.

At the highest CF concentration (100 ppm) at the embryo germination stage, the number of plantlets was greatly reduced to 58 and 98 plantlets/0.1 g of callus, for cultivars 88H0019 and N41 respectively, compared to more than 600 plantlets/0.1 g of callus in the no CF control. Unexpectedly, in the plantlet regeneration stage all the tested CF concentrations had a significant positive effect on the percentage of plantlets that re-rooted compared with the control. Both cultivars showed a 95 - 100 % rooting ability of the plantlets, which was significantly higher than the percentage of plantlets that rooted in the embryo germination media (EGM1) containing no CF (60 - 70 %) ( $p < 0.001$ ). Likewise, all the concentrations of the CF had a positive effect on the root length of plantlets, with 1500 ppm CF resulting in the highest root length of 31.5 mm  $\pm$  4.3 for 88H0019 and 34.05 mm  $\pm$  3.9 for N41. Hence, *F. pseudonygamai* SC17 could not be used as an *in vitro* selection agent in a root re-growth

assay.

Due to the enhanced effect of *F. pseudonygamai* SC17 CF on root growth, the fungal isolate's potential to produce indole acetic-3-acid (IAA) was assessed. *F. pseudonygamai* produced the highest IAA concentration (743.1 nM) in the presence of L-tryptophan than in the treatment without L-tryptophan (457.2 nM). This suggests that the observed enhanced root growth may be due in part to the production of auxin (IAA) in the *F. pseudonygamai* SC17 CF.

Acclimatised *in vitro* plantlets (8-9 months old) were inoculated only with *F. pseudonygamai* SC17 or dual inoculated: firstly, with *F. pseudonygamai* SC17, then 1-2 2<sup>nd</sup> instar *E. saccharina* larvae that were placed into the leaf whorls 2 weeks later. To confirm tolerance of the putative mutants, fungal isolations were performed on the stem sections above the inoculation lesion from symptomatic and asymptomatic plants. The results revealed that the putative mutant plants that were primed with Hx only and treated with a combination of mutagens (EMS and 5-AzaC) and priming agent exhibited a significant decrease in lesion severity as compared with controls. For both treatments, a mild lesion severity rating was recorded for plants inoculated with only SC17 for cultivars N41 and 88H0019. For the plants that were dual inoculated there was a significant difference in the lesion severity ratings between the two treatments ( $p < 0.001$ ). The lesion severity rating was moderate for cultivar 88H0019 (primed with Hx) and mild for cultivar N41 (primed with Hx). Plants from the combined treatment for both cultivars resulted in a mild lesion severity rating.

This protocol could be valuable in generating commercially important cultivars that are tolerant and resistant to *F. pseudonygamai* SC17 and possibly other sugarcane pathogens. Planting resistant cultivars is recommended as an economical and the best method for controlling diseases and pests. This approach used in this study will have the least impact on the environment and increase yields without the need for expensive chemical applications and labour.

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## **Dedication**

*This dissertation is dedicated to my loving son, Kian Saiyuran Govender – may you never stop learning*

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**Figure 27:** A comparison of lesion severity in the inoculated stems sections of the plants: a) no lesion; b) mild lesion; c) moderate lesion, and d) severe lesion. Plants were stabbed 2-3 cm from the bottom of the stalk with toothpicks colonised with *F. pseudonygamai* SC17. Some plants were dual inoculated after 2 weeks with 2<sup>nd</sup> instar *E. saccharina* larvae, stems were collected after 7 weeks for re-isolation.....86

**Figure 28:** The lesion severity ratings of plantlets that were inoculated with only *F. pseudonygamai* SC17 for cultivars 88H0019 and N41. Dissimilar alphabet characters denote a statistically significant difference amongst treatments for each concentration. Data sets were analysed by One-way ANOVA and Duncan's multiple range test.  $P \geq 0.05$ ,  $n = 6 - 13$ , mean  $\pm$  SE.....87

**Figure 29:** The lesion severity ratings of plantlets that were dual inoculated with *F. pseudonygamai* SC17 and 2 weeks later with the 2<sup>nd</sup> instar *E. saccharina* larvae, after 2 weeks for cultivars 88H0019 and N41. Dissimilar alphabet characters denote a statistically significant difference amongst treatments for each treatment. Data sets were analysed by (One-way ANOVA and Duncan's multiple range test.  $P \geq 0.10$ ,  $n = 11 - 14$ , mean  $\pm$  SE).....87

## List of abbreviations

2,4-D - 2,4-dichloro-phenoxyacetic acid  
ABA - Abscisic acid  
AZA - Azelaic acid  
AFLP - Amplified fragment length polymorphism  
ANOVA - Analysis of variance  
AOC - Allene oxide cyclase  
AOS - Allene oxide synthase  
AUX - Auxins  
AZAC - Azacytidine  
BABA -  $\beta$ -aminobutyric acid  
BABA-IR -  $\beta$ -aminobutyric acid-induced immunity  
BEA - Beauvericins  
CF - Culture filtrate  
CK - Cytokinins  
DNA - Deoxyribonucleic acid  
EGM - Embryo germination media  
EIM - Embryo initiation media  
EMM - Embryo maturation media  
EMS - Ethane methanesulfonate  
ENN - Enniatin  
ET - Ethylene  
ETI - Effector-triggered immunity  
FA - Fusaric acid  
FB1 – Fumonisin B1  
GA - Gibberellins  
GM - Genetic modification  
HF - Hydrogen fluoride  
HR - Hypersensitive responses  
Hx - Hexanoic acid  
IAA - Indole-3-acetic-acid  
IR - Induced resistance  
ISR - Induced systemic resistance  
ISSR - Inter simple sequence repeats  
JAs - Jasmonates  
JA - Jasmonic acid

JA-ACC - Jasmonoyl ACC  
JA-Ile - Jasmonoyl isoleucine  
KZN - Kwa Zulu Natal  
LOX - Lipoxygenase  
LSR - Lesion severity rating  
MAPK - Mitogen-activated protein kinase  
MeJAs - Methyl jasmonates  
MMS - Methyl methanesulfonate  
MNU - N-methyl-N-nitrosourea  
MON - Moniliformin  
MS - Murashige and Skoog  
OPDA - 12- oxo-phytodienoic acid  
PCD - Programmed cell death  
PDB - Potato dextrose broth  
PEMs - Pro-embryogenic masses  
PR - Pathogenesis-related  
PPFD - Photon flux density  
R - Resistance  
RFLP - Restriction fragment length polymorphism  
SA - Salicylic acid  
SA - South Africa  
SAR - Systemic acquired resistance  
SASRI - South African Sugarcane Research Institute  
SE - Somatic embryogenesis  
UV – Ultraviolet

**SI units**

Centimetre – cm

Degree Celsius - °C

Gram per litre – g l<sup>-1</sup>

Grams – g

Hours – hrs

Micromolar - μM

Milligrams per litre – mg l<sup>-1</sup>

Millilitre -ml

Millilitre per litre – ml l<sup>-1</sup>

Millimetre – mm

Millimolar – Mm

Minutes – min

Nanomolar - nM

Parts per million – ppm

Percentage - %

Revolutions per minute – rpm

Volume per volume – v/v

## 1. Introduction and rationale of the study

Sugarcane (*Saccharum* spp. hybrid) is a monocotyledonous crop that belongs to the Poaceae family (Patade and Suprasanna, 2008; Suprasanna et al., 2011; Hailu et al., 2018). It is significant due to its exceptional global and agro-economic value as a well-established multipurpose crop (Naz, 2003; Tolera et al., 2014). Sugarcane is grown in tropical and subtropical regions, and it contributes almost 75 % of the sugar production worldwide, the remaining 25 % being made from sugar beet (*Beta vulgaris*). Hence, a considerable amount of research has focused on sugarcane crop improvement through conventional plant breeding, and in more recent time's modern tools of biotechnology and genetic engineering have been used (Baksha et al., 2002; Tolera et al., 2014; Shabbir et al., 2021).

One of the main challenges faced by the South African sugar industry is the infestation of sugarcane stalks by the indigenous borer *Eldana saccharina* (Lepidoptera: Pyralidae). It is regarded as a major pest and causes direct and indirect losses of up to R900 million per annum in the industry (Conlong, 1994a; Knogge, 1996; Keeping et al., 2014). Several species of lepidopteran stem borers have been found to significantly reduce yields in many crops such as sorghum, maize, sugarcane, and rice (Kleynhans et al., 2017; Péné et al., 2018). They consist of several species belonging to the genera *Sesamia*, *Diatraea*, *Chilo*, and *E. saccharina* (Potgieter et al., 2016; VanWeeldan et al., 2016; Péné et al., 2018)

A close association has been found between *E. saccharina* and *Fusarium* spp. in maize (Schulthess et al., 2002; Péné et al., 2018) and sugarcane (McFarlane et al., 2009; Mahlanza et al., 2013; Rutherford et al., 2021). The *E. saccharina* larvae bore into the sugarcane stalk tissue and create larval galleries around the infected tissue (Goebel and Way, 2003; Mahlanza et al., 2013; Péné et al., 2018). *Fusarium* species are initially present as symptomless endophytes within the sugarcane stalk (Mahlanza et al., 2013; Péné et al., 2018). *E. saccharina* damages the stem tissues which later leads to infection by the *Fusarium* species (McFarlane et al., 2009; Mahlanza et al., 2013; Péné et al., 2018; Rutherford et al., 2021). This is characterized by a dark-red discoloration around the affected areas (McFarlane et al., 2009; Péné et al., 2018). Some *Fusarium* strains have been found to be beneficial to the insect by accelerating development and increasing survival of the *E. saccharina* larvae in artificial diet (McFarlane et al., 2009). However, other isolates negatively affected larval growth and survival (McFarlane et al., 2009; Mahlanza et al., 2014; Rutherford et al., 2021). McFarlane et al. (2009) studied the relationship between various *Fusarium* spp. and *E. saccharina* in sugarcane where it was observed that the *F. pseudonygamai* SC17 isolate was beneficial to

*E. saccharina* growth and survival. *Fusarium* isolates may provide several benefits to *E. saccharina*, such as plant defence suppression by the production of fumonisins, auxins, and cytokinins (De La Torre-Hernandez et al. 2010; Dafoe et al. 2013; Zhang et al. 2015), and antimicrobial inhibition of entomopathogens (Sondergaard et al. 2016; Rutherford et al., 2021). Many studies have demonstrated that endophytic fungi can produce phytohormones, especially indole acetic acid (IAA) and gibberellins, as a direct system of plant growth promotion (Fu et al., 2015; Turbat et al., 2020).

There are many control measures that may be implemented to minimise sugarcane yield losses caused by diseases and pests. *Fusarium* stem rot can be controlled by using well maintained, disinfected and clean equipment that prevents unnecessary damage when cutting seed cane (Croft, 2000; Zhang and Jeyakumar, 2018). Plant diseases caused by *Fusarium* spp. can be controlled by using the correct phosphate, nitrogen, and potassium fertilizers, and using resistant cultivars (Mahlanza et al., 2013; Shabbir et al., 2021). Good farming practices are important but do not always eradicate infections (Zhang and Jeyakumar, 2018). Hence, the extensive damage caused by these pests have resulted in the need for developing *E. saccharina* and *Fusarium* species resistance. To overcome the above-mentioned limitations, it is hypothesised that sugarcane lines tolerant to *F. pseudonygamai* SC17 can be produced, which could still allow endophytic colonisation by the fungus, but resist transition to a necrotrophic phase. Planting resistant cultivars is recommended as an economical and the best method for controlling diseases and pests. This approach will have the least impact on the environment and increase yields without the need for expensive chemical applications and labour (Butterfield et al., 2001; Zhang and Jeyakumar, 2018; Shabbir et al., 2021).

Variety improvement in sugarcane has been achieved mainly by conventional breeding for cultivars with desired characteristics, such as increased yield and disease resistance (Snyman et al., 2011; Zhou, 2013; Dlamini, 2021). However, there are many limitations with regards to sugarcane conventional breeding, as this approach takes up to 10 to 15 years for selection to be completed and it could take much longer to commercially release a cultivar (Snyman et al., 2011; Ming et al., 2006; Tolera et al., 2014; Yadav et al., 2020; Shabbir et al., 2021). The complex genome of sugarcane due to polyploidy (Butterfield et al., 2001; Zhang and Jeyakumar, 2018) and adverse South African climatic conditions to produce pollen limits the progress of conventional breeding (Zhou, 2013; Dlamini, 2021). Over the last decades, various biotechnological approaches have been found to be successful in producing genotypes with improved traits in a variety of crops, including sugarcane. Genetic engineering can also be used to control *Fusarium* spp. by producing plants with improved resistance-conferring genes that target the fungal pathogens (Munkvold et al., 1997; Wang et al., 2021), but the limitations

based on legislative rules affects the use of this approach in sugarcane (Mertens, 2008; Key et al., 2008; Kumar et al., 2020).

*In vitro* tissue culture techniques have been widely used to generate genetic variation in crops (Liang et al., 2019; Diehdhiou et al., 2021). Somaclonal variation is a result of spontaneous mutations that occur during *in vitro* culture systems (Larkin and Scrowcroft, 1981; Lakshmanan, 2006; Snyman et al., 2011) and has been successfully utilized in genetic crop improvement (Heinz, 1973; Diehdhiou et al., 2021). Induced mutagenesis, somatic embryogenesis and organogenesis are *in vitro* techniques that can be used to induce genetic variation for crop improvement.

Genetic variability can be induced by irradiation (Mba et al., 2012; Suhesti et al., 2021), and treatment with physical (Suprasanna et al., 2009; Mir et al., 2020) and chemical mutagens (Koch et al., 2012; Forster and Shu, 2012; Holme et al., 2019; Mullins et al., 2021). This is known as induced mutagenesis. The targeting of mutations conferring desirable traits by imposing a selective pressure *in vitro* is an approach that has already been shown to be successful in sugarcane e.g., to obtain plants tolerant to the herbicide imazapyr (Koch et al., 2012; Munsamy et al., 2013; Rutherford et al., 2017), salt (Luan et al., 2007), drought (Masoabi et al., 2018), *F. sacchari* (Mahlanza et al., 2013) and  $Al^{3+}$  (Purnamanisingh and Hutami, 2016; Snyman et al., 2019). The present study will focus on chemical mutagenesis to produce plants resistant to both *F. pseudonygamai* SC17 and *E. saccharina*.

Recently, the effects of fungal culture filtrates or toxins on resistant and susceptible plants to assess disease resistance, by using *in vitro* techniques have been investigated (Mahlanza et al., 2013; Svabova and Lebeda, 2005; Suthar et al., 2021). The purified toxins or pathogen culture filtrates that contain toxins involved in plant disease development, are suitable for use as *in vitro* selection agents (Svabova and Lebeda, 2005; Suthar et al., 2021). Many studies have confirmed the positive correlation between the tolerance of plants to culture filtrates or toxins and those that are inoculated by the pathogen (Gray et al., 1986; Connell et al., 1990; Suthar et al., 2021).

*Fusarium* spp. are known to produce an extensive array of toxins that vary quantitatively and qualitatively between strains (Zhang et al., 2006; Aly et al., 2011; Wu et al. 2015; Lugtenberg et al., 2016). Infection by some endophytic fungi are regarded as symptomless since they can colonise their host plants in an asymptomatic state (Saikkonen et al. 2004; Yuan et al. 2010; Navarro-Meléndez and Heil, 2014). Endophytic fungi are symptomatic or asymptomatic inside plant tissues, and they share either a symbiotic or antagonistic interaction with the host plant

(Fontana et al., 2021). In a symbiotic interaction the fungal endophytes benefit from nutrition, shelter, and protection in the host plant, whilst the endophyte assists its hosts by stimulating its development, growth, stress tolerance, and adaptation (Saikkonen et al., 2004; Wang et al., 2015; Fontana et al., 2021). Symptomless endophytism is linked with increased jasmonate (JA) and suppressed salicylate (SA) signaling, which suggests that defence against necrotrophic pathogens and insect pests is heightened (many endophytic fungi have the potential to become necrotrophic pathogens) (Navarro-Meléndez and Heil, 2014; Kou et al., 2021). Jasmonates are involved in resistance to necrotrophs and chewing insects such as *E. saccharina* and appear to be involved in maintaining the non-symptomatic state of the endophyte (Glazebrook, 2005; Thaler et al., 2012; Ballare, 2014; Navarro-Meléndez and Heil, 2014; Kou et al., 2021). Necrotrophic colonisation occurs when the fungus produces certain toxins that activate the salicylate pathway (SA) and induces cell death and oxidative stress (De la Torre- Hernandez et al. 2010; Sanchez-Rangel et al. 2012; Arias et al., 2012; Blacutt et al., 2018). The increase in SA levels in a plant intensifies the susceptibility to necrotrophic pathogens (Ghozlan et al., 2020).

In the current study, it was hypothesised that the SA and JA pathways are antagonistic, and SA induction by *Fusarium* spp. likely acts to suppress the JA pathway. The *Fusarium* strains that are beneficial to *E. saccharina* (e.g., *F. pseudonygamai* SC17) (McFarlane et al., 2009) could be suppressing JA-induced defence responses which favour both *E. saccharina* and the fungus (Mahlanza et al., 2013; Ghozlan et al., 2020).

### **Research hypotheses**

A previous study by Mahlanza et al. (2013) focused on the development of sugarcane mutants resistant to the mycotoxins produced by the detrimental strain *Fusarium sacchari* PNG40 (McFarlane et al., 2009) that reduced the suppression of the host plant defence and increased rates of symptomless endophytism. Mahlanza et al. (2013) used ethylmethansulfonate (EMS) and 5-Azacytidine (5-AzaC) to induce mutations, and very few mutant plants were produced. As mentioned above, the presence of valuable endophytic fungi may have some benefits for the host plants. Hence, in the current study, it was hypothesised that the development of plants resistant to the mycotoxins produced by the beneficial *Fusarium* strain, will also have reduced *E. saccharina* infestation. A protocol will be developed to test the above hypotheses. Given the role of JA in maintaining endophytes in a symptomless state, an epigenetic primed and mutagenic protocol had to be established. The established protocol should be able to 'fix' a favourable epigenetic state (e.g., Hx primed) for both *E. saccharina* and *Fusarium* resistance. The selection of putative mutants resistant to mycotoxins (able to induce SA responses and

inhibit JA responses) should result in sugarcane mutant cultivars with enhanced *E. saccharina* and *F. pseudonygamai* resistance. It was hypothesised that the *E. saccharina* beneficial *Fusarium* strain SC17 may be stimulating root growth by the auxin indole-3-acetic acid (IAA) production or increased activity of the enzyme ACC deaminase. An experiment will be conducted to quantify the amount of IAA produced by *F. pseudonygamai* SC17.

#### Aims and Objectives

This research aimed to develop an epigenetic primed and mutagenic protocol to generate and characterise mutant sugarcane cultivars that are resistant to both *E. saccharina* and *F. pseudonygamai* SC17. The cultivars chosen were N41 and 88H0019 which are intermediate-resistant and susceptible to *E. saccharina*, respectively. Once the protocol has been developed, it can be used to generate resistance to *E. saccharina* and *F. pseudonygamai* in sugarcane commercial lines. This was done by using the following *in vitro* techniques: somatic embryogenesis, chemical mutagenesis and priming followed by *ex vitro* screening. Hence, the main objectives of the study were as follows:

- to establish the most suitable priming agent between hexanoic acid and cis-jasmone.
- to determine an appropriate culture filtrate concentration for *in vitro* screening of calli and plantlets for tolerance to *F. pseudonygamai* (SC17).
- to generate additional mutants: primed only (Hx), and primed and mutagenic (5-AzaC + EMS-induced).
- to screen for indole-3-acetic acid production by isolating *F. pseudonygamai* SC17.
- to characterize *in vitro* selected mutants for *E. saccharina* and *Fusarium* resistance by comparing the levels of resistance between un-primed, primed, primed + mutagenic plantlets through *ex vitro* screening.

## 2. Literature Review

### 2.1 Commercial sugarcane cultivation and its importance worldwide

Sugarcane belongs to the genus *Saccharum L.*, which consists of three cultivated species (*Saccharum officinarum*, *S. sinensis* and *S. barberi*) and two wild species (*S. spontaneum* and *S. robustum*) (Kochhar, 1998; Mahmud *et al.*, 2016). It is a large perennial grass cultivated worldwide in subtropical and tropical areas (Ming *et al.*, 2006) for its high content in sucrose (Brumley *et al.*, 2008; Goebel *et al.*, 2018; Péné *et al.*, 2018) and ethanol production (D'Hont *et al.*, 2008; Tolera *et al.*, 2014). Approximately 110 countries generate sugar from either sugarcane or beet, in which sugarcane is used to produce most of the world's sugar, and the rest is produced from sugar beets (*Beta vulgaris L.*) (Grivet and Arruda, 2001; Lakshmanan *et al.*, 2005; Ming *et al.*, 2006; Mirajkar *et al.*, 2019). Nearly 75 % of global sugar production is obtained from sugarcane, and the remaining 25 % is made from sugar beet (Kroger *et al.*, 2006; Shabbir *et al.*, 2021). The top ten producing countries are Brazil, India, China, Thailand, The United States, Pakistan, Mexico, France, Russia, and Australia (Iqbal *et al.*, 2020; Shabbir *et al.*, 2021).

Although sugarcane is primarily required for the food industry for its sucrose content, sugarcane is usually considered to be one of the most efficient and significant sources of biomass for biofuel production (D'Hont *et al.*, 2008; Brumley *et al.*, 2008). The by-products of sugar refining such as molasses, bagasse, furfuryl alcohol, furfural, diacetyl and dextran have many beneficial uses (O'Reilly, 1998; Brumley *et al.*, 2008). The fibrous residue after sugar extraction, which is called bagasse, can be used to produce electricity for the power system, to fuel boilers in the sugar mills, as a livestock feed and to manufacture disposable packaging (Almazan *et al.*, 2001; Martinez-Hernandez *et al.*, 2018). Molasses is used as a substrate for ethanol production as well as in syrups and animal feed (Brumley *et al.*, 2008).

In South Africa in 1852, the first sugar produced was derived from varieties of noble cane (*S. officinarum*) (Zhou, 2013). The modern sugarcane cultivars (*Saccharum* spp.) originated from crosses between *S. spontaneum L.* (wild sugarcane) and *S. officinarum L.* (sweet sugarcane), followed by backcrossing the interspecific hybrids with the noble parent plant, *S. officinarum* (Roach and Daniels, 1987; Brumley *et al.*, 2008; Singh *et al.*, 2010). However, a recent study by Evans and Joshi (2016) described *S. cultum* as a novel species of *Saccharum*. Molecular phylogenetic studies have revealed the need to divide *Saccharum* spp. into four species: *S. spontaneum*, *S. officinarum*, *S. narenga* and the family of recent hybrid cultivars that has been formally named *Saccharum cultum*, originating from a cryptic founder species (Evans and

Joshi, 2016). The basic concept of plant breeding includes the improvement and combination of high sucrose content from *S. officinarum* and disease resistance from *S. spontaneum* (D'Hont et al., 2008).

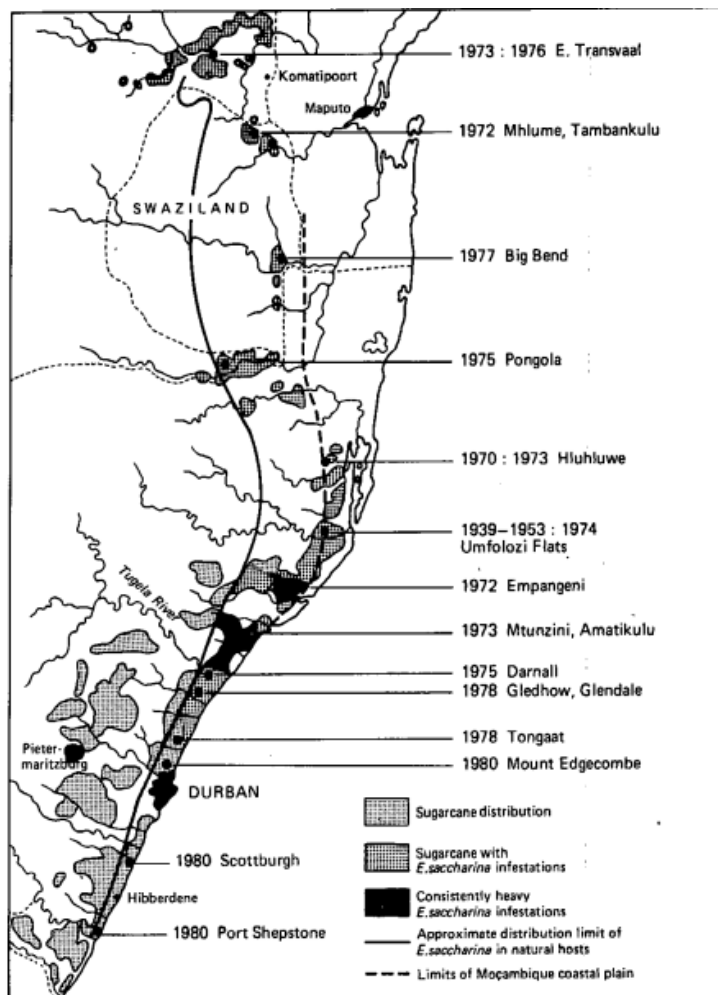
Since ancient eras, sugarcane cultivation has gone through many changes into contemporary noble cane (D'Hont et al., 2008; Mirajkar et al., 2019). At first, the selection of interspecific hybrids and desirable clones introduced many agronomically beneficial traits into the cultivated species. Wild related species has played a significant part as the donor through gene introgression for most of the desirable traits (Brumbley et al., 2008; Singh et al., 2010). The development of intergeneric hybrids and pre-breeding strategies has played a significant role in the production of sugarcane cultivars with high yields (Brumbley et al., 2008; Singh et al., 2010; Mirajkar et al., 2019). Cultivated sugarcane has been improved with useful traits such as high fermentable sugar, high fiber and biotic and abiotic stress tolerance. Sugarcane is a complex crop due to the extreme level of polyploidy, however despite its genomic complexity, long breeding and selection cycles, and hybridization barriers within the genus, notable improvement has been attained to produce a varied range of cultivars, mutants, and hybrids appropriate for dissimilar agroclimatic conditions (Mirajkar et al., 2019).

## 2.2 The main challenges faced by the South African sugar industry

In most of the tropical and sub-tropical regions lepidopteran stem borers have been found to significantly reduce yields of a few crops such as sugarcane, sorghum, rice and corn, thus they are regarded as economically important pests (Kleynhans et al., 2017; Péné et al., 2018). In the South African sugarcane industry, there has been substantial yield losses caused by the indigenous pyralid borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Conlong, 1994a; Knogge, 1996; Keeping et al., 2014). *E. saccharina* has been regarded as a major pest of sugarcane and there was an initial outbreak in 1939 (Dick, 1945; Walton and Conlong, 2016), a second in 1970 (Carnegie, 1974) and the pest has been persistent since then (Fig. 1) (Walton and Conlong, 2016).

Sugarcane has great economic importance and plays major role in contributing to the sugar industry of South Africa. Sugarcane is still very much susceptible to several diseases and pests, causing substantial losses in sugarcane production. Cultivation over large adjacent fields and vegetative propagation of sugarcane make it relatively vulnerable to diseases such as rust and mosaic virus (Dick, 1945; Bailey, 2004). The development of high sugar yielding cultivars in the SA Sugar Industry is limited by the major pest *E. saccharina* (Butterfield and Thomas, 1996; Conlong, 2001). The larvae infest mature cane and their borings provide

access for the fungus *Fusarium* which infects sugarcane growing stalks and seed cuttings following stalk-borer injury, causing *Fusarium* stem rot (Bourne, 1961; McFarlane et al., 2009; Péné et al., 2018).



**Figure 1:** A map illustration of reported *E. saccharina* outbreaks in South Africa and Swaziland (Atkinson et al., 1981).

## 2.3 The history of *E. saccharina* in the sugarcane industry

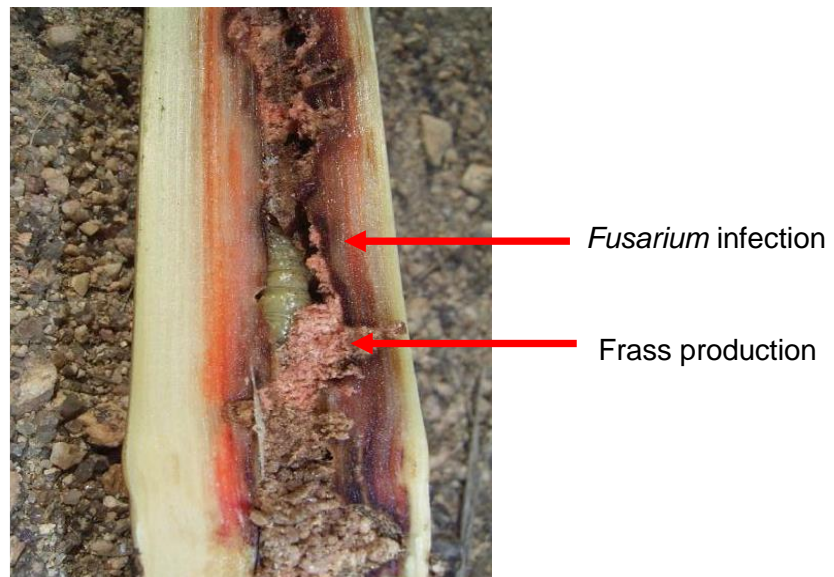
### 2.3.1 History and distribution

The African stalk borer *E. saccharina* Walker (Lepidoptera: Pyralidae) was described over many years ago from specimens obtained from sugarcane in West Africa (Carnegie, 1974). *E. saccharina* is a stalk borer native to Africa which has been found to attack graminaceous crops (e.g., sugarcane, sorghum, and maize) and on various wild sedges and grasses (Waiyaki et al., 1968; Conlong 1994b; Walton and Conlong, 2016). An initial outbreak of *E. saccharina* occurred in 1939 at the Umfolozi Flats, Kwa-Zulu-Natal (Carnegie, 1974; Atkinson et al., 1981). *E. saccharina* was first observed in sugarcane in Sierra Leone, West Africa in

the late 1800's (Betbeder-Matibet, 1981; Walton and Conlong, 2016) and was later reported from sugarcane in East Africa (Girling, 1972; Conlong, 2001; Walton and Conlong, 2016). In 1939, after the first outbreak of *E. saccharina* in South Africa as stated by Dick (1945), the pest disappeared and re-emerged in the 1970s (Carnegie, 1974; Walton and Conlong, 2016). Thereafter *E. saccharina* spread both south and north of Hluluwe, becoming a destructive pest in Pongola, Mpumalanga, Swaziland and in the lower altitudes of KZN (Fig. 1) (Carnegie et al., 1974; Atkinson, 1979). The distribution of stalk borers is reported to be dependent on temperature, rainfall, altitude, and availability of food (Haile and Hofsvang, 2001). However, in recent years, *E. saccharina* has been observed further south, towards the higher altitudes and in colder climates (e.g Midlands North and South), extending to the Limpopo province and observed at the Boskop dam in the North-West province (Assefa et al., 2008). It has been reported that the initial colonisation of sugarcane by *E. saccharina* may have resulted from the removal of *E. saccharina* natural host plants (grasses and sedges) and the subsequent planting of sugarcane in these wetland areas (Conlong, 1994a; Conlong, 2001; Govender et al., 2010).

### 2.3.2 *E. saccharina* and borer-associated *Fusarium* stem rot

*E. saccharina* associated damage and early crop harvesting are major factors limiting sugarcane productivity (Leslie, 2004; Goebel et al., 2005; Mokhele et al., 2009; Keeping et al., 2014; Potgieter et al., 2016; Van Weelden et al., 2016; Péné et al., 2018). As a result of *E. saccharina* infestation due to secondary microbial degradation, sucrose is converted to glucose, thereby lowering the sucrose yields (Geobel, 2005). In sugarcane, the larva enters the stalk by boring through the bud, root primordia or cracked internodes and they then forages on the inner stalk tissue (Mokhele et al., 2009; Potgieter et al., 2016; Van Weelden et al., 2016; Péné et al., 2018). Frass (excrement) expelled from borings (as shown in Fig. 2), is an indication of insect infestation (Girling, 1972; Chinheya et al., 2009). The association between infection by the fungus (*Fusarium* spp.) and borer infestation can result from the wounds created by *E. saccharina* on the stalk, which provide the fungus access to the inner stalk issue, if not already present as a symptomless endophyte (McFarlane et al., 2009; Péné et al., 2018). The infected sugarcane stalks are characterised by a reddish discolouration because of secondary infestation by *Fusarium* spp. an opportunistic fungus which further reduces sugar content, and spreads to the undamaged nodes (Fig. 2) (Bourne, 1961; McFarlane et al., 2009; Chinheya et al., 2009; Péné et al., 2018).



**Figure 2:** *Fusarium* infection in the stem seen by red colour associated with *E. saccharina* infestation (source: SASRI entomology department)

It has been reported that symptomless endophytic colonisation by *Fusarium verticillioides* in maize is related to greater *E. saccharina* damage and infestation in contrast with those plants arising from seeds treated with a fungicide (Schulthess et al., 2002). This suggests a favourable association between the stalk-borer and the fungus (Schulthess et al., 2002). A range of volatiles such as alcohols, aldehydes and esters, which are responsible for insect attraction, were found to be produced by certain *Fusarium* isolates (e.g., *F. verticillioides*) (McFarlane and Rutherford, 2005). *F. verticillioides* has been shown to promote *E. saccharina* development and survival in maize (Ako et al., 2003). In sugarcane, McFarlane et al. (2009) found that although certain endophytic *Fusarium* isolates were beneficial to *E. saccharina* survival and growth rate, other isolates (e.g., *F. sacchari* isolate PNG40) were detrimental. The antagonistic effects of those *Fusarium* strains could possibly be due to the mode of action of metabolites produced by *Fusarium* spp., such as fusaproliferin (Logrieco et al., 1996; Munkvold et al., 2009), beauvericin (Logrieco et al., 2002), and fusaric acid (Ismail and Papenbrock, 2015), which are insecticidal toxins.

It is important to understand the interactions between the stalk borer *E. saccharina* and *Fusarium* spp. since this will help develop control measures for this lepidopteran pest in sugarcane. *Fusarium* isolates that are beneficial to the stalk borer may help in reducing damage so it can be used to control plant infection, whilst harmful strains to the pest may be used as a biological control agent against the pest (Schulthess et al., 2002; Mahlanza et al., 2013).

## 2.4. The interaction between insect pests and *Fusarium* spp. in various economically important crops

### 2.4.1 The pathogen and/or endophyte

*Fusarium* spp. are phytopathogenic fungi that belong to a large complex genus, which are known as worldwide plant pathogens that colonise and infect several crops such as sugarcane, rice, maize, oats and wheat in semi-tropical and temperate areas (Hsuan et al., 2010; Shi et al., 2017). This genus is diverse, and the current classification system has 65 species, 55 varieties, and 22 forms in 16 sections (Leslie and Summerell, 2006, Summerell, 2019). *Fusarium* spp. are widespread in terrestrial ecosystems and are usually found together with higher plants (Ploetz, 2005). Sugarcane (*Saccharum* hybrids), rice (*Oryza Sativa*), bananas (*Musa* spp.) and maize (*Zea mays*) are amongst the most important agricultural crops in several countries (Hsuan et al., 2010; Rebouças et al., 2021). They are commonly infected by plant pathogenic fungi which consist of *Fusarium* species (Leslie and Summerell, 2006; Hsuan et al., 2010). Their infection of sugarcane results in *Fusarium* stem rot and Pokkah boeng (Bourne, 1961; Croft, 2000; Costa et al., 2019). Pokkah boeng in sugarcane is caused by species of the *Fusarium fujikuroi* species complex, characterized by deformation of the top of the plant or stem rot, and is regarded as an important fungal disease (Costa et al., 2019).

Endophytes are microorganisms that, during a variable period of their life cycle colonise plant tissues whilst being symptomless in their host (Carroll, 1988; Stone et al., 2004; Rodriguez et al., 2009; Numponsak et al., 2018). Colonisation of plants by endophytes can occur when host defence mechanisms and pathogen virulence are in balance, so that neither is negatively affected by the association (Bae et al., 2009; Pineda et al., 2010; Numponsak et al., 2018). These plant-endophyte interactions are mutualistic relationships in which the host obtains defence and growth promotion from biotic and abiotic stress, while the fungus acquires its nutrients and habitation from hostile environmental factors and competitors on the plant's exterior surface (Schultz and Boyle, 2005; Rigobelo and Baron, 2021). In previous studies, it has been reported that endophytic fungi have the potential to produce bioactive compounds that builds protection against microbial influencers in the host plant and promotes plant growth in a volatile and non-volatile environment (Morath et al., 2012; Suwannarach et al., 2015; Numponsak et al., 2018). Numerous studies have reported that the secretion of secondary metabolites (phytohormones e.g., auxins, gibberellins, ethylene, siderophore and cytokinins) that are produced by endophytic fungi may be associated with plant growth promotion, as well as their ability of the to move insoluble phosphate and provide their host plants with nitrogen (Dai et al., 2008; Khan et al., 2012; Khan et al., 2015; Numponsak et al., 2018).

A varied range of endophytic fungi has been isolated from various plant species worldwide and found to be the cause of different diseases (Table 1). Most *Fusarium* strains are pathogenic to many crops and cause various diseases such as *Fusarium* wilt (Viswanathan et al., 2017), stalk rot (Mughogho and Rosenberg, 1984; Afolabi et al., 2008; Dela Cueva et al., 2019), and *Fusarium* head blight (Burrows et al., 2008; Shi et al., 2017) while others are endophytic (Bacon and Hinton, 1996; Macia-Vicente et al., 2008; Mahlanza et al., 2013).

#### 2.4.2 *Fusarium*-produced mycotoxins in plant-pathogen interactions

*Fusarium* species produce an extensive range of secondary fungal metabolites known as mycotoxins (Bennett and Bentley, 1989; Marin et al., 2013; Ji et al., 2019), that are either harmful or phytotoxic to plant pathogens and insects, which allows them to act as biological control agents of diseases and pests (McFarlane et al., 2009; Ploetz et al., 2015; Zakaira et al., 2017). *Fusarium* species commonly found as disease causal agents (Table 1) also produce mycotoxins in the field or in storage (Table 2). A wide range of phytotoxic compounds that are chemically different are produced by members of the genus *Fusarium*, such as fumonisins (fumonisin B1, FB1), enniatin (ENN), fusaric acid (FA), beauvericins (BEA), trichothecenes and moniliformin (MON) (Desjardins, 2006; Ismaiel and Papenbrock, 2015; Zakaira, 2017; Dinolfo et al., 2017) (Table 2). Many studies have shown that these secondary metabolites hinder the metabolic processes of animals and plants (Bennet and Klich, 2003; Ismaiel and Papenbrock, 2015), and that they are toxic to several crops (Borras et al., 2001; Leslie et al., 2004; Ismaiel and Papenbrock, 2015). These phytotoxic compounds display various biological activities that cause physiological, metabolic and morphological effects and can be observed through chlorosis, necrosis, wilting, growth inhibition, seed germination inhibition and effects on calli (Desjardins and Hohn, 1997; Ismaiel and Papenbrock, 2015; Suthar et al., 2021).

Several studies have reported on the role of fumonisin B1 (FB1), a mycotoxin produced by *Fusarium verticillioides* that causes serious disease symptoms in maize and other grains (Gilchrist, 1997, 1998; Zhang et al., 2015). Fumonisin B1 has been found to be a strong elicitor of programmed cell death (PCD) in plants (Xing et al., 2013; Zhang et al., 2015). Hypersensitive response (HR) is a form of PCD, which occurs during incompatible plant-birotrophic-pathogen reactions. It also takes place when plant cells around the invasion site(s) rapidly and actively die to limit pathogen growth and stop the nutrient supply, and in doing so prevents the disease from spreading (Coll et al., 2011; Zhang et al., 2015). The sphingolipid biosynthetic pathway is inhibited by FB1, by hindering the ceramide synthase enzyme, which can result in cell death (Stone et al., 2000; Zhang et al., 2015). Additionally, FB1 can induce other HR-like responses including phenolic compounds, callose deposition, reactive oxygen

species (ROS) production and expression of pathogenesis-related (PR) proteins (Wolpert et al., 2002; Zhang et al., 2015). The PR proteins are a group of various proteins that are induced by defense-related signaling molecules and phytopathogens (Ali et al., 2018).

**Table 1:** Examples of *Fusarium* spp. commonly found as causal disease agents in economically important crops

<b>Crop</b>	<b><i>Fusarium</i> spp.</b>	<b>Disease</b>	<b>References</b>
Cotton, <i>Gossypium</i> spp.	<i>F. oxysporum</i>	<i>Fusarium</i> wilt	Davis et al., 2006
Maize, <i>Zea mays</i> L.	<i>F. graminearum</i> <i>F. verticillioides</i> <i>F. proliferatum</i>	<i>Fusarium</i> stalk rot <i>Fusarium</i> cob and root rot	Rahjoo et al., 2008 Hsuan et al., 2010
Pineapple <i>Ananus comosus</i>	<i>F. guttiforme</i>	Fusariosis	Ploetz, 2003
Rice <i>Oryza sativa</i>	<i>F. moniliforme</i>	Bakanal disease	Iqbal et al., 2011
Sugarcane <i>Saccharum</i> hybrids	<i>F. moniliforme</i>	Pokkah boeng	Sanghera et al., 2018
Wheat <i>T. aestivum</i>	<i>F. graminearum</i>	<i>Fusarium</i> head blight	Burrows et al., 2008 Li et al., 2019

Many studies have reported on several factors that may influence the production of mycotoxins. Among the factors are colonisation of the plant host and duration of fungal growth, temperature, host and substrate composition, water activity and moisture content (Ciegler, 1978; Ashiq, 2015; Zakaira, 2017). Stress factors including water shortage and insect/pest infestation might play a role in mycotoxin production (Sanchis and Magan, 2004; Milani, 2013; Zakaira, 2017). According to Atanda et al. (2013) humid and hot conditions are the two most critical factors that promote fungal growth and development including mycotoxin production. Hence, there are many interacting factors involved in promoting mycotoxigenic fungal growth

and mycotoxin production. The latter is dependent on the species of the mycotoxigenic fungi since different species have their own requirements for optimal growth and mycotoxin production (Cast, 2003; Zakaira, 2017).

**Table 2:** Examples of toxic compounds isolated from various crops infected by *Fusarium* spp.

<i>Fusarium</i> spp.	Mycotoxin	Crop	Reference
<i>F. moniliforme</i>	Fusarins	Maize, soybean, rye	Bacon et al., 1996 Gai et al., 2018
<i>F. equiseti</i> , <i>F. sambicinum</i> , <i>F. graminearum</i>	Trichothecenes	Sorgum, wheat, maize	Brian et al., 1961 Wang et al., 2006 Shi et al., 2017
<i>F. graminearum</i> <i>F. culmorum</i> <i>F. equiseti</i>	Zearalenone	Corn, barley, wheat, oats, sorgum	Bennett and Klich, 2003 Ji et al., 2019
<i>F. verticillioides</i> <i>F. subglutinans</i>	Fusaric Acid	Wheat, barley	Brown et al., 2012 Zhang and Jeyakumar, 2018
<i>F. verticillioides</i> <i>F. proliferatum</i> <i>F. nygamai</i>	Fumonisin B1	Soybeans, maize	Thiel et al., 1992 Rheeder et al., 2002, Zhang and Jeyakumar, 2018
<i>F. proliferatum</i>	Fusaproliferin	Rice	Desjardins, 2006
<i>F. sacchari</i>	Moniliformin	Maize	Leslie
<i>F. oxysporum</i>	Beauvericin		and Summerell, 2006 Zakaria et al., 2017

Mycotoxigenic *Fusarium* are responsible for causing mycotoxin contamination of agricultural crops which may occur in direct and indirect ways. Toxigenic fungi were further characterised into two groups namely: “field” fungi and “storage” fungi (Desjardins et al., 1996; Bennett and Klich, 2003; Zakaira, 2017). Mycotoxigenic *Fusarium* spp. that are categorised as field fungi are commonly known to infect crops in the field and contaminate them. These fungi are regarded as the initial phytopathogen that infects the crops and may produce a mycotoxin under suitable conditions as it grows within the crop (Desjardins, 1996; Bennett and Klich,

2003; Zakaira, 2017). There are several mycotoxigenic *Fusarium* spp. that are also phytopathogenic and can infect the plant host (Desjardins, 1996; Bennett and Klich, 2003; Zakaira, 2017). When agricultural products are stored, the mycotoxigenic fungi grows saprophytically on the stored products (Glenn, 2007; Zakaira, 2017).

#### 2.4.3 Plant growth regulators produced by *Fusarium* spp.

The capacity of plants to resist pathogen attack is regulated through various mechanisms which include insect biological control agents (Compant et al., 2005; Petti et al., 2012). A plant's ability to tolerate abiotic and biotic stress is determined by the production of phytohormones (Bari and Jones, 2009; Dinolfo et al., 2017). These are naturally occurring compounds produced by plants that are crucial for reproduction, plant growth regulation, development, and survival (Adie et al., 2007; Bari and Jones, 2009; Dinolfo et al., 2017). There are several phytohormones, which include auxins (AUX), abscisic acid (ABA), cytokinins (CK), gibberellins (GA), jasmonates (JA), ethylene (ET), salicylic acid (SA), brassinosteroids, and peptide hormones which alter their levels during infection as a strategy to prevent colonisation of the pathogen (Adie et al., 2007; Bari and Jones, 2009; Dinolfo et al., 2017). They are responsible for signal transduction/perception, gene expression and cellular homeostasis (Peleg and Blumwald, 2011; Petti et al., 2012; De Vleeschauwer et al., 2013; Pozo et al., 2015; Spence and Bais, 2015; Chanclud and Morel, 2016). Therefore, they play a vital role in plant responses to, and resistance against disease (Peleg and Blumwald, 2011; Petti et al., 2012; De Vleeschauwer et al., 2013; Pozo et al., 2015; Spence and Bais, 2015; Chanclud and Morel, 2016).

The developmental processes in a plant, such as cell division, organ formation and differentiation (Oka et al., 1999; Vanneste, 2005; Benjamins and Scheres, 2008; Chanclud and Morel, 2016), and senescence (Kim et al., 2011; Chanclud and Morel, 2016) involves the use of auxins, which are indole-derived hormones. Auxins control the biotic and abiotic stress responses in plants (Peleg and Blumwald, 2011; Chanclud and Morel, 2017). In bacteria, tryptophan is a pre-cursor for auxin production, which is converted using tryptophan-2-monooxygenase enzymes into indole-3-acetamide (Zhao, 2010; Chanclud and Morel, 2016). Indole-3-acetic acid (IAA) is formed when indole-3-acetamide has been hydrolysed and is also the major active auxin form in plants even though it is produced through dissimilar biosynthesis pathways. Studies have shown that *Fusarium* spp. are involved in the production of fungal toxins (Tsavkelova et al., 2012; Chanclud and Morel, 2016). The phytohormone(s) salicylic acid (SA), jasmonates, abscisic acid and ethylene play an important role protecting plants against fungal pathogens such as *Fusarium oxysporum* (Dempsey and Klessig, 2012; Hinsch et al., 2016; Vrabka et al., 2019).

Gibberellins are diterpenoid acids that are biologically derived from tetracyclic diterpenoid hydrocarbons which are produced by micro-organisms (Srivastava et al., 2003). Gibberellins are essential endogenous hormones found in fungi and plants which control plant development by regulating various physiological mechanisms (Hooley, 1994; Miceli et al., 2019). These phytohormones play a role in stimulating root and stem elongation, flowering, leaf expansion, seed germination, fruit senescence or dormancy (Hedden and Sponsel, 2015). Gibberellic acid ( $GA_3$ ) is the dominant product of gibberellins isolated from bacteria and fungi (Muddapur et al., 2015).

## 2.5 Phytohormone production by fungal endophytes

### 2.5.1 Phytohormones and endophytes

The plant cell wall creates a strong physical barrier that provides protection to the cell against microbial infection (Nafisi et al., 2015). It is made up of four main constituents, cellulose, pectins, hemicelluloses and glycopectins (Carpita and Gibeaut, 1993; Nafisi et al., 2015). There are dramatic chemical and structural changes that occur in the cell wall during pathogen infection (Vorwerk et al., 2004; Voigt, 2014; Nafisi et al., 2015), including deposition of callose (Luna et al., 2012; Nafisi et al., 2015), lignification (Zhao and Dixon, 2014; Nafisi et al., 2015), cell wall protein cross-linking (Bradley et al., 1992; Nafisi et al., 2015), accumulation of antimicrobial compounds (such as phytoalexins) and reactive oxygen species (Franke et al., 2005; O'Brien and Benkova, 2013; Nafisi et al., 2015), which may work to limit the infection and avoid additional pathogen progression. Plants have the ability to sense the cell wall integrity and, when compromised, intracellular events can be activated which involves phytohormone signalling cascades that can trigger defence mechanisms (Hamann, 2012; Nafisi et al., 2015). Plasmamembrane receptor(s) can sense the degradation of cell wall constituents, mainly by necrotrophic pathogens (Hamann, 2012; Nafisi et al., 2015). As a result, defence signalling cascades and subsequent inducible defence responses are activated (Monaghan and Zipfel, 2012; Nafisi et al., 2015).

The ecological adaptability of a plant is enhanced by colonisation of endophytic fungi, or it does so by improving plant tolerance against the abiotic and biotic stresses (Schultz and Boyle, 2005; Mehmood et al., 2018). There is a wide range of endophytic fungi, which does not cause damage when colonising the living plant tissue. Several benefits are provided to the host plants in different ways by endophytic fungi, such as supplementing absorption of minerals, promotion of growth and protection against pests and diseases (Sieber et al., 2002; Mehmood et al., 2018). Fungal endophytes can produce bioactive metabolites which are

responsible for promoting the plant endophyte interaction (Strobel, 2003; Mehmood et al., 2018). The most important effect of fungal symbiosis is plant growth promotion (Hassan et al., 2013), and it occurs via various secondary metabolites, including ammonia and plant growth regulators, particularly indole-3-acetic acid (IAA) (Sieber et al., 2002; Khan et al., 2012; Mehmood et al., 2018) and enzymatic activities such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase which plays a role in plant growth (Nascimento et al., 2014; Hossain and Sultana, 2020). The ethylene precursor, ACC is converted into  $\alpha$ -ketobutyrate and  $\text{NH}_3$  (ammonia), by the ACC deaminase enzyme (Nascimento et al., 2014; Hossain and Sultana, 2020). Plant growth is regulated when the ACC deaminase enzyme cleaves ACC produced by plants and thus reduces the ethylene levels in the plant (Glick et al., 2007; Hossain and Sultana, 2020).

### 2.5.2 IAA in fungal - plant interactions

Indole acetic acid is a renowned plant growth regulator of the auxin class, is produced by plants and microorganisms (Yuan et al., 2008; Fu et al., 2015; Ismail et al., 2021). In plants, it is responsible for the regulation of various aspects of their development and growth (Fu et al., 2015). Roots have been found to be very sensitive to fluctuations in IAA levels, which is essential for both primary and lateral root initiation (Khan et al., 2011; Fu et al., 2015; Turbat et al., 2020). IAA stimulates dose-dependent increase in the formation of lateral roots, adventitious roots and length of epidermal-derived root hairs (Overvoorde et al., 2010; Fu et al., 2015). Additionally, IAA is involved in plant-pathogen interactions such as pathogenesis and defence mechanisms (Kazan and Manners, 2009; Fu et al., 2015; Turbat et al., 2020). It has been suggested by Fu et al. (2015) that in various plant-fungus interaction systems, the fungi may use the fungal produced IAA and related compounds to interrelate with plants for symbiotic strategies or pathogenesis, resulting in promotion of plant growth and basal plant defence mechanism modification.

Fu et al. (2015), reviewed the interaction between plants and rhizosphere-associated microorganisms. Several organic compounds namely sugars, vitamins and organic acids are produced by roots, and these organic compounds have been found to be used as nutrients or signals by fungal populations. Fungi are known to enhance plant growth by increasing the availability of nutrients to their host. They do this by releasing volatile compounds, siderophores and phytohormones. Root formation and root hair development can be induced by fungal-produced IAA. The promotion of root development and growth allows the associated plant to boost its nutrient intake.

## 2.6 Crop improvement strategies for inducing tolerance to biotic and abiotic stress.

### 2.6.1 Conventional plant breeding

Conventional plant breeding is used to develop improved cultivars with specific characteristics suited to the needs of farmers and consumers (Manshardt, 2004; Cheavegatti-Gianotto et al., 2011; Getnet, 2017). Plant breeding is the recombination of desired genes from crop cultivars and closely related species by sexual hybridization (Manshardt, 2004; Cheavegatti-Gianotto et al., 2011; Getnet, 2017). Vegetative or asexual propagation through stem cuttings have been used in modern commercial sugarcane cultivation (Nalawade et al., 2018). Plant breeding programs are based on the development of a segregating population with genetic variability and follows several stages of selection and cloning of the superior genotypes (Gazaffi et al., 2014). There are several factors that determine the success of a breeding program, of which some are the suitable choice of parents to maximize the selection regime, experimental design for good accuracy and the correct choice of desired traits and the assessment period (Cheavegatti-Gianotto et al., 2011; Gazaffi et al., 2014).

Sugarcane has major limitations in terms of conventional breeding due to its polyploidy nature. This means that one cross can result in many progenies that vary in traits, such as yield, agronomic characters, and disease resistance (Berding et al., 2005). Sugarcane breeding programs are time consuming and labour intensive, consequently, it can take 10 - 12 years to develop and release an elite sugarcane cultivar (Gazaffi et al., 2014).

### 2.6.2 Genetic engineering

Genetic modification (GM) is defined as the insertion of specific genes into a genome, to develop plants expressing the desired traits (Birch, 1997; Scott et al., 2016; Kumar et al., 2020). The genes are artificially inserted into the plants rather than using sexual reproduction (Lakshmanan et al., 2005; Kumar et al., 2020). Genetic modification has become an essential biotechnological tool which is used to produce sugarcane with enhanced traits to survive biotic and abiotic stresses as reviewed by Lakshmanan et al. (2005) (Table 3).

While plant breeding results in hybridization between parents with desired characteristics, single traits are usually difficult to target (Scott et al., 2016). However, GM enables gene-by-gene introduction of well-defined characteristics into breeding programmes, and this also allows for the access of genes from a greatly extended range of organisms since hybridization is not reliant on compatibility. As a result, the pool of variation is greatly increased and a much better accuracy is possible when each desirable trait is introduced, without the presence of

undesirable genes (Scott et al., 2016). Furthermore, through genetic engineering the release of unwanted variation that is an inherent feature of hybridization is avoided. In addition, the time required to breed cultivars with improved traits of interest is shortened (Ledford, 2015; Scott et al., 2016).

The first genetically transformed crop was planted in 1996, where 1.7 million hectares of crop were planted (James, 2015; Scott et al., 2016). By 2018, there were over 191.7 million hectares of transgenic crops in 26 countries, mainly of the four crops which include soybean, maize, oilseed rape (canola) and cotton (Scott et al., 2016; ISAAA, 2018, Sieradzki et al., 2021). Some of the genetic traits introduced into transformed crops until 2015 were resistance to insects from *Bacillus thuringiensis* and tolerance to the herbicide glyphosate. In addition to the four main crops others such as beans, eucalyptus, papaya, sugar beet and apple are currently being genetically modified with desirable characteristics such as, disease resistance, salt tolerance, drought tolerance, nitrogen-use efficiency, improved nutritional quality and storage quality (Scott et al., 2016).

Genetic engineering is a great technique for crop improvement, since the resultant genetically modified crop may possess one or more traits of interest, such as disease resistance, herbicide tolerance, insect resistance, abiotic stress tolerance and nutritional improvement (Kumar et al., 2020). The implementation of transgenic technology has been shown to improve crop yields, reduce insecticide and pesticide use, and reduce the cost of crop production (Kumar et al., 2020). However, the possibility of GM for crop improvement has caused much controversy. One of the many concerns raised is about the impact of GM on the environment, also the fact that these transgenic crops carry foreign genes may be a limitation due to concerns of allergenicity and potential toxicity to humans (Mertens, 2008; Key et al., 2008; Lovei and Bohn, 2010; Kumar et al., 2020). Limitations of genetically engineered crops include inadequate knowledge about inheritance of transgene and transgene silencing (Lakshmanan et al., 2005, Kumar et al., 2020). Approaches that can achieve a similar outcome as genetic transformation systems, and do not incorporate foreign genes into the plant, would notably aid in overcoming public and regulatory judgement issues (Arruda, 2011; Kumar et al., 2020).

**Table 3:** Examples of reported applications of genetic modifications for sugarcane.

Trait	Gene of interest	References
Herbicide tolerance: Glufosinate	<i>pat</i>	Leibbrandt and Snyman, 2003
ammonium Glyphosate	<i>cp4 epsps</i>	Snyman et al., 2001 Wang et al., 2017 Nogueira et al., 2019
Virus resistance: Sugarcane Mosaic Virus (SCMV)	SCMV coat protein	Meyer and Snyman, 2011 Yao et al., 2017
Insect resistance: <i>E. saccharina</i>	<i>cry1A (c)</i> , <i>cry1Ab</i>	Meyer and Snyman, 2011 Wang et al., 2017 Gianotto et al., 2019

### 2.6.3 Tissue culture systems as an integral step in crop improvement

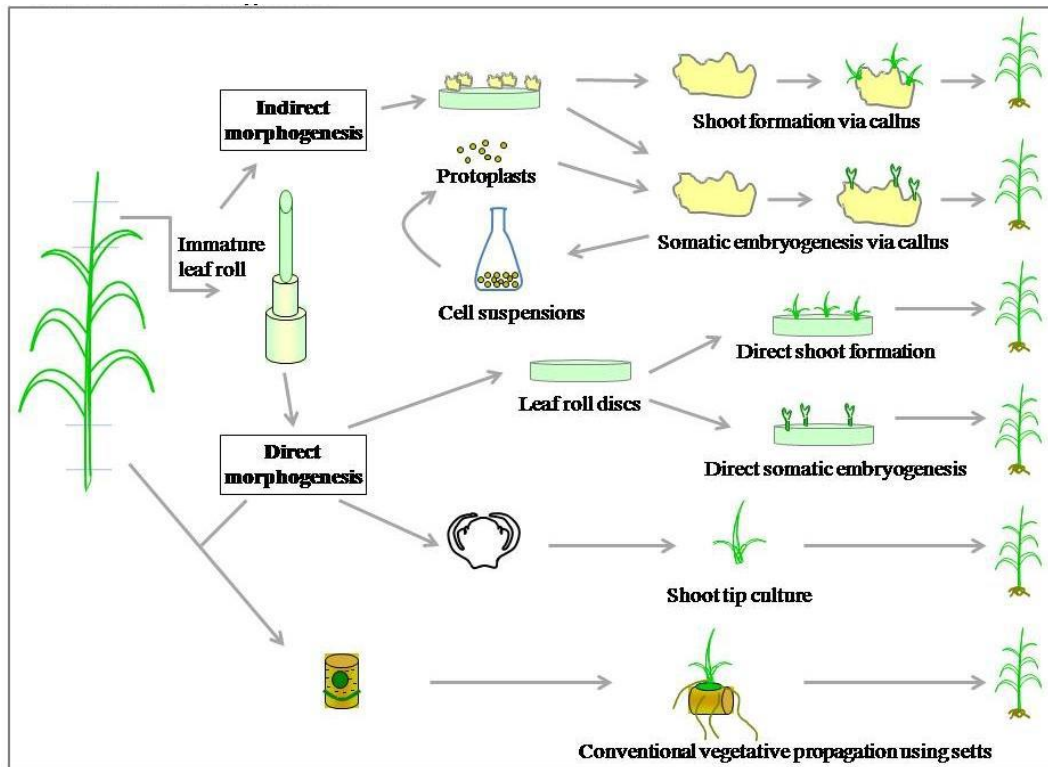
*In vitro* tissue culture is defined as the culture of plant cells, tissues and organs in a nutritive culture media and under a controlled aseptic environment (Thorpe et al., 2007; Trigiano and Gray, 2016; Espinosa-Leal et al., 2018; Diehdhiou et al., 2021). Such techniques are valuable for the rapid multiplication of genotypes of interest, production of disease-free plants, production of plant-derived metabolites of commercial importance and plant genome transformations (Debnath et al., 2006; Altpeter et al., 2016; Espinosa-Leal et al., 2018). Through *in vitro* tissue culture the totipotency of plant cells is manipulated to form a whole plant with a combination of auxins and cytokinins in the culture media (Singh, 2006; Getnet, 2017; Liang et al., 2019; Diehdhiou et al., 2021).

In sugarcane, as for most plant species, there are two individual developmental pathways that can be followed (Fig. 3), somatic embryogenesis and organogenesis, and both have well-proven protocols. The regeneration of plants through organogenesis can be successfully accomplished directly from their apical meristem with no intervening callus phase (Lee, 1987; Grisham and Bourg, 1989; Siddiqui et al., 1994; Lorenzo et al., 1998; Saini et al., 2004; Singh et al., 2006; Ramgareeb et al., 2010; Snyman et al., 2011), or adventitious organs emerging from callus originating from the explant (Grisham and Bourg, 1989; Burner and Grisham, 1995; Gill et al., 2006; Lakshmanan et al., 2006; Ali et al., 2007b; Snyman et al., 2011; Espinosa-Leal et al., 2018). Plant regeneration through direct organogenesis, is the preferred choice for

true-to-type clones as somaclonal variation is minimised (Lakshmanan et al., 2005). Indirect organogenesis is not commonly used because indirect somatic embryogenesis protocols are well established, as discussed below.

Somatic embryogenesis is a developmental process whereby a plant somatic cell dedifferentiates into a totipotent embryogenic stem cell that then, through cell division and differentiation gives rise to an embryo. Somatic embryogenesis can be obtained using a variety of explants and is usually practiced by exposing them to a medium with plant growth regulators, generally the synthetic auxin 2, 4-dichlorophenoxyacetic acid (2,4-D) (Heinz and Mee, 1969; Nadar et al., 1978; Ho and Vasil, 1983; Lee, 1987; Snyman et al., 2001; Franklin et al., 2006; Behera and Sahoo, 2009; Snyman et al., 2011; Feher, 2015; Horstman et al., 2017).

Direct embryogenesis is categorized by the absence of an intermediate callus stage and the embryos are formed directly on the explant (Williams and Maheswaran, 1986; Snyman et al., 2001; Horstman et al., 2017). Indirect somatic embryogenesis is characterised by the presence of an intermediate callus stage, an organized mass of initially vacuolated cells which show vast degrees of compactness and is regarded as the most common pathway (Snyman et al., 2001; Ikeuchi et al., 2013; Horstman et al., 2017; Espinosa-Leal et al., 2018). The production of embryogenic callus follows the formation of pro-embryogenic masses (PEMs) within or on the surface of the callus mass, from which cell clusters or single cells grow into embryos (Halperin, 1966; Toonen et al., 1994; Horstman et al., 2017). The exposure to auxins, especially 2,4-D promotes callus and PEM initiation and proliferation, however, for histogenesis and elongation of the embryo the auxin needs to be removed (Horstman et al., 2017).



**Figure 3:** A depiction of the different routes of somatic embryogenesis and organogenesis by which sugarcane plants can be regenerated *in vitro* (Snyman, 2004).

#### 2.6.4 *In vitro* induced somaclonal variation

Plant tissues that are cultured on medium supplemented with high concentrations of auxins, spend long periods in culture or many repeated subcultures may result in an undifferentiated mass of cells called callus, and later develops into plantlets, as mentioned in section 2.6.3. Plants regenerated via one of the two indirect routes have been reported to show a high frequency of variations (Bairu et al., 2011; Currais et al., 2013; Manchanda et al., 2018). There are several factors that may be responsible for inducing variability *in vitro* such as age of donor plant, explants source, genotype, *in vitro* culture environment (Silvarolla, 1992; Rastogi et al., 2015; Krishna et al., 2016), medium composition and plant growth regulator concentration (Skirvin, 1978; Silvarolla, 1992; Rastogi et al., 2015; Manchanda et al., 2018). Physiological variations are temporary, in response to a stimulus and disappear when it is removed (Rastogi et al., 2015). These induced modifications are best known as somaclonal variation and are defined as epigenetic/genetic changes that are observed among the plantlets regenerated under *in vitro* conditions (Larkin and Scrowcroft, 1981; Ramos et al., 1996; Rani and Raina, 2000; Rastogi et al., 2015; Manchanda et al., 2018). In 1981, Larkin and Scrowcroft were the first to describe somaclonal variation in *in vitro* cultured plants, after observing the resistance of *in vitro* plants that were previously susceptible to the toxin produced by *Helminthosporium sacchari* Butler that causes eyespot disease in sugarcane.

Somaclonal variation may be of three types, namely genetic, epigenetic, or physiological (Rastogi et al., 2015). Genetic variation occurs because of mutations or other changes in the DNA of the cell tissue, these are heritable in the next generation and essential for crop improvement (Larkin and Scrowcroft, 1981; Rastogi et al., 2015; Manchanda et al., 2018). Genetic variations are most useful to plant breeders since these variations are permanent, heritable, and stable during the sexual cycle (Larkin and Scrowcroft, 1981; Manchanda et al., 2018). Epigenetic variations are the physiological changes that are temporarily reversible (plants 'revert' to normal phenotype) and non-heritable (Silvarolla, 1992; Kaeppler et al., 2000; Rastogi et al., 2015; Manchanda et al., 2018). An epigenetic variation is also known as a developmental variation as it is induced during the various stages involved in the tissue culture process (Evans et al., 1984; Manchanda et al., 2018). These changes are non-heritable phenotypic variation.

Somaclonal variation has useful applications in sugarcane genetic improvement. Heinz (1973) was the first to report on *in vitro* screened somaclones of commercial sugarcane resistant to Fiji disease (Gill et al., 2007; Rastogi et al., 2015). Whilst somaclonal variation is undesirable when true-to-type clones are required (Jain, 2001), it may be used for plant improvement (Patade et al., 2006; Rutherford et al., 2014). As previously mentioned, breeding programmes take several years to produce plants with beneficial traits (Lakshmanan et al., 2005) (section 2.4). However, somaclonal variation has many advantages in helping conventional breeding by producing genotypes with desired traits much quicker (Raja et al., 2014; Rastogi et al., 2015). Somaclonal variation occurs by DNA methylation, point mutations, chromosomal rearrangement, and recombination, and altered sequence copy number, these changes usually take place during callus formation (Silvarolla, 1992; Jain, 2001; Khan et al., 2017, Manchanda et al., 2018). Hence, somaclonal variation allows for the production of genetic variants that may be exploited to generate phenotypes with valuable traits (Rutherford et al., 2014; Manchanda et al., 2018).

## 2.7 *In vitro* induced mutagenesis – Principles, types and applications of mutagens

Induced mutagenesis is defined as the exposure of chemicals or radiation to seeds and various plant explants to generate mutants with desirable traits (Mir et al., 2020). Mutations may be induced, or they may arise spontaneously (Jabeen and Mirza, 2004). It is regarded as one of the most useful techniques used in crop improvement to generate abiotic stress tolerant and disease resistant lines (Novak et al., 1992; Oladosu et al., 2016; Yasmeen et al., 2017; Mir et al., 2020) (Table 4). The limitations in case of traditional breeding, such as narrow gene

pool, low vigour, reduced fertility, complex genomes, and the lengthy breeding/selection cycle together hinders plant improvement and can be overcome via induced mutagenesis (Suprasanna et al., 2012; Penna and Jain, 2017). It is a cheap, simple and effective technique used to induce various mutations at the loci which controls economically important traits and/or eliminates undesirable genes present in elite breeding lines (Konzak, 2001; Yasmeeen et al., 2017; Penna, 2017). More than a few desirable traits (e.g., high yield, quality, plant architecture, disease resistance and abiotic stress tolerance) can be found in a single induced mutant (Bugchio et al., 2007; Penna and Jain, 2017; Andrew-Peter-Leon et al., 2021).

Mutagenesis can be carried out using various types of plant material, e.g., whole plantlets, seedlings, *in vitro* cultured cells like stem and leaf explants, tissues, organs, anthers, cell cultures, ovules, microspores, and protoplasts (Oladosu et al., 2016). The induction of mutations can be done on different plant material which are vegetative cuttings, *in vitro* cultured tissues like stem and leaf explants, anthers, cell cultures, calli, ovules, microspores, protoplasts (Oladosu et al., 2016).

Mutation breeding employs three approaches namely physical, chemical or biological agent for the improvement of agronomic traits in crops (Kharkwal and Shu, 2009; Forster and Shu, 2012; Roychowdhury and Tah, 2013; Oladosu et al., 2016; Penna, 2017; Penna and Jain, 2017). As stated by Mba (2013), it is notable that the dosage and rate of exposure or administration of the mutagens is directly related to the types and frequency of mutations (Oladosu et al., 2016).

Ionising radiation can be used to induce mutations, which occurs by the transfer of energy via ionisation or excitation, to sites present within or near the genetic material. This results in increased chemical reactivity of those sites (Sikora et al., 2011; Rutherford et al., 2014). There are various types of radiation used which include high-energy forms such as ultraviolet (UV) light, X-rays, gamma rays and fast-moving neutrons (Waugh et al., 2006, Sikora et al., 2011; Rutherford et al., 2014; Suprasanna et al., 2). There is direct damage since UV light is absorbed by pyrimidines in DNA, and this results in the formation of pyrimidine dimers (TT and CC). This is responsible for causing point mutations (Waugh et al., 2006; Rutherford et al., 2014). X-rays and Gamma rays can penetrate tissue and ionize molecules, radicals are formed, which cause breaks in phosphodiester bonds, resulting in point mutations and deletions (Sikora et al., 2011; Waugh et al., 2006; Rutherford et al., 2014). Nikam et al. (2015) used gamma radiation to generate mutants and salt-tolerant lines in sugarcane and some of which were characterized for various agro-morphological traits and some exhibited improved sugar yield and number of millable canes.

Chemical mutagens are known for inducing mostly point mutations, which are ideal for producing missense and nonsense mutations, thus resulting in a series of change-of-function mutations (Talebi et al., 2012; Purnamaningsih and Hutami, 2016). The most widely used chemical mutagens are sodium azide, EMS, hydrogen fluoride (HF), methyl methanesulfonate (MMS), hydroxylamine and N-methyl-N-nitrosourea (MNU) (Waugh et al., 2006; Sikora et al., 2011; Khalil et al., 2018). EMS (an alkylating mutagen) has been reported to be the most efficient and powerful mutagen in plants, since it creates a high frequency of point mutations and a low frequency of chromosome irregularity (van Harten, 1998; Purnamaningsih and Hutami, 2016; Espina et al., 2018). The alkylation of guanine to form O<sup>2</sup>- ethylguanine which is then able to pair with thymine instead of cytosine is induced by EMS (Waugh et al., 2006). This results in the errors during DNA repair with the A-T pair replacing G-C, particularly during DNA replication (Waugh et al., 2006; Khalil et al., 2018). EMS mutagenesis produces base pair substitutions that result in altered forms of the triplet codon sequence within a protein sequence. This simply changes the physiological characteristics of the protein without stopping its function (Rutherford et al., 2014; Khalil et al., 2018; Dlamini et al., 2021).

In sugarcane, induced mutagenesis has been achieved by exposing embryogenic calli to EMS for a few hours because DNA replication is most rapid at this stage, therefore ensuring the highest probability of incorrect DNA repair (Rutherford et al., 2014). Of relevance to the present study, chemical mutagens were used to treat banana shoot tips to produce variants that were tolerant to *Fusarium* wilt (Jain 2010; Purnamaningsih and Hutami, 2016). Arici et al. (2017) had recently reported on disease resistance of *Fusarium* dry rot by *F. avenaceum*, this study tested the effect of EMS against *F. avenaceum* in potato.

5-Azacytidine (5-AzaC) is a known nucleotide analogue of 5-cytosine, which acts as a demethylating agent. This results in gene expression and activates silenced genes, thus increasing the frequency of somaclonal variation (Christman, 2002; Issa and Kantarjian, 2009; Grzybkowska et al., 2018). Methylation is important in controlling DNA and gene expression (Grant-Downton and Dickinson, 2005; Munsamy et al., 2013; Us-Camas et al., 2014). This takes place by adding a methyl group at the 5' position of the pyrimidine ring of cytosine in DNA (Us-Camas et al., 2014). A gene becomes silenced or is no longer expressed when it has been methylated (Grant-Downton and Dickinson, 2005; Us-Camas et al., 2014). Mutations in DNA may also be created by chromosomal changes because of changes in methylation (Pooggin, 2013; Us-Camas et al., 2014). As sugarcane has a polyploid genome, a change in its methylation activity results in an increase in the frequency of somaclonal variation to obtain traits of interest (Munsamy et al., 2013). Many studies have shown that 5-AzaC behaves strongly as an epimutagen, as a result the frequency of changes induced by 5-AzaC is greater

than sequence-based DNA mutations (Fieldes and Amyot, 1999; Munsamy et al., 2013; Sun et al., 2021).

In mutation breeding the process of classifying individuals with a target mutation is the key point, which involves two main steps: mutant screening and confirmation (Forster and Shu, 2012; Oladosu et al., 2016). When individuals are selected from a large, mutated population that meet a specific criterion, e.g., disease resistance, and early flowering compared with the parent, this process is known as mutant screening (Mba, 2013; Oladosu et al., 2016). However, these are known as false or putative mutants. Mutant confirmation is defined as the process of re-evaluating the putative mutants under a precise and replicated environment using many samples. Many putative mutants are shown to be false mutants through this process. The mutations that are significant for the enhancement of crops usually involve single bases, which may sometimes alter protein synthesis (Mba, 2013; Oladosu et al., 2016). As summarized in Table 4, there has been success using induced mutagenesis to generate sugarcane mutants with desirable traits. Variations can be generated by using different types of mutagens, which induces several forms of mutations (Rutherford et al., 2014; Oladosu et al., 2016).

Sugarcane mutant plants regenerated and multiplied using *in vitro* techniques are acclimatised before being planted out in the field for comparison and assessment of specific beneficial agronomic features (e.g., tiller number, stalk height, fibre content and sucrose content) (Gilbert et al., 2005; Rutherford et al., 2014). However, comparison and assessment can be done on well-developed plants. Molecular and phenotypic assessments of mutant plants are required to confirm if the phenotypic traits of interests are enhanced and positively expressed whilst other traits have not been negatively impacted. To determine if plants obtained *in vitro* for clonal propagation are acceptable for commercial cultivation, the phenotypic traits are further evaluated by *ex vitro* screening trials (Mahlanza et al., 2013; Rutherford et al., 2014; Oladosu et al., 2016).

## 2.8 Epigenetic priming for pest resistance

### 2.8.1 Induced responses in plants to pest attack

Lepidopterans are known to be nocturnal insects that exploit the volatile chemical cues from the plants which allow them to orient themselves towards the host plants (Renwick, 1989; Renwick and Chew, 1994; Zakir, 2011). The preference of the female insects during the selection of appropriate host plants for oviposition is usually important for the survival of offspring in lepidopteran herbivores. The larvae of Lepidopterans are relatively immobile and

do not have a choice to find alternate sources of food, after hatching on plants selected by the female moths (Renwick, 1989; Renwick and Chew, 1994; Zakir, 2011). Immediately after hatching, the larvae begin to feed on the green leaves and stalks of the selected host plant robustly often causing a substantial loss of plant biomass (Pare and Tumlinson, 1996; Kost and Heil, 2008).

Secondary metabolites are produced efficiently by some plant species in response to feeding damage by herbivorous arthropods, e.g., both non-volatile and volatile chemicals. Several studies have shown these secondary metabolites as a requirement for increasing plant resistance against herbivores both at below-ground and above-ground levels (Pare and Tumlinson, 1996; Heil, 2004; Kost and Heil, 2006; Kost and Heil, 2008). When plants are exposed to a range of biotic and abiotic environmental factors it affects their development and growth unfavourably (Rasmann et al., 2012; Bertini et al., 2019). Consequently, plants have evolved sophisticated adaptation and defence mechanisms to protect themselves against a few of the harmful insects and pathogens (Rasmann et al., 2012; Pastor et al., 2014; Lämke and Bäurle, 2017; Bertini et al., 2019). Plants have developed various defence strategies, which are constitutive, but most are specific to a certain type of attack as they are induced in response to a stimulus (Frost et al., 2008; Lämke and Bäurle, 2017; Bertini et al., 2019).

**Table 4:** Examples of the use of induced mutagenesis for desirable traits in sugarcane

Trait	Characteristic	Mutagen used	References
Physiological	Drought tolerance	EMS	Dlamini et al., 2021
	Salt tolerance	EMS	Mallikarjuna et al., 2018
	Salt tolerance	Gamma radiation	Nikam et al., 2015
	Increased aluminium-tolerance	EMS	Purnamaningsih and Hutami, 2016
Disease resistance	Red rot disease	Sodium azide	Ali et al., 2007a
	<i>Fusarium sacchari</i> resistance	EMS, 5-AzaC	Mahlanza et al., 2013, 2014,
	Yellow leaf disease resistance	Sodium azide, sodium nitrite, EMS	Kona et al., 2019
Herbicide tolerance	Imazaypr tolerance	EMS	Koch et al., 2012 Koetle et al., 2018
	Imazypr and smut tolerance	5-AzaC	Munsamy et al., 2013

Most plants respond to herbivory by releasing protective compounds which are lethal to the attacker. The plant's innate immunity controls these inducible defences (Jones and Dangl, 2006; Pastor et al., 2013; Bertini et al., 2019). Recent studies have revealed that plants may be driven to enter a post-challenged state, which allows for an effective and quicker defence response to abiotic or biotic stressors (Conrath et al., 2006; Bertini et al., 2019). Priming is a resistance mechanism, which results in a physiological state that triggers the plants to respond fast and/or robustly upon subsequent pathogen attack and various abiotic stresses, is known as priming (Conrath, 2011; Aranega-Bou et al., 2014, Martinez-Medina et al., 2016; Bertini et al., 2019). This 'primed state' can be stimulated by a pre-exposure of the plants to low doses of biotic and abiotic stress inducers either synthetic or natural compounds (Aranega-Bou et al., 2014; Conrath et al., 2015; Bertini et al., 2019). Hence, in addition to microbe-specific molecules, referred to as pathogen – (PAMPS), microbe – (MAMPS), and host-associated molecular patterns (HAMPS), endogenous plant signalling metabolites can be used to mimic priming. These metabolites are salicylic acid (SA), jasmonic acid (JA), hexanoic acid (Hx) and their useful analogues (Aranega-Bou et al., 2014; Conrath et al., 2015; Bertini et al., 2019). Gene induction is associated with no or minimal priming-increased alertness (Slaughter et al., 2012; Bertini et al., 2019) and may have a long-term effect (Pastor et al., 2013).

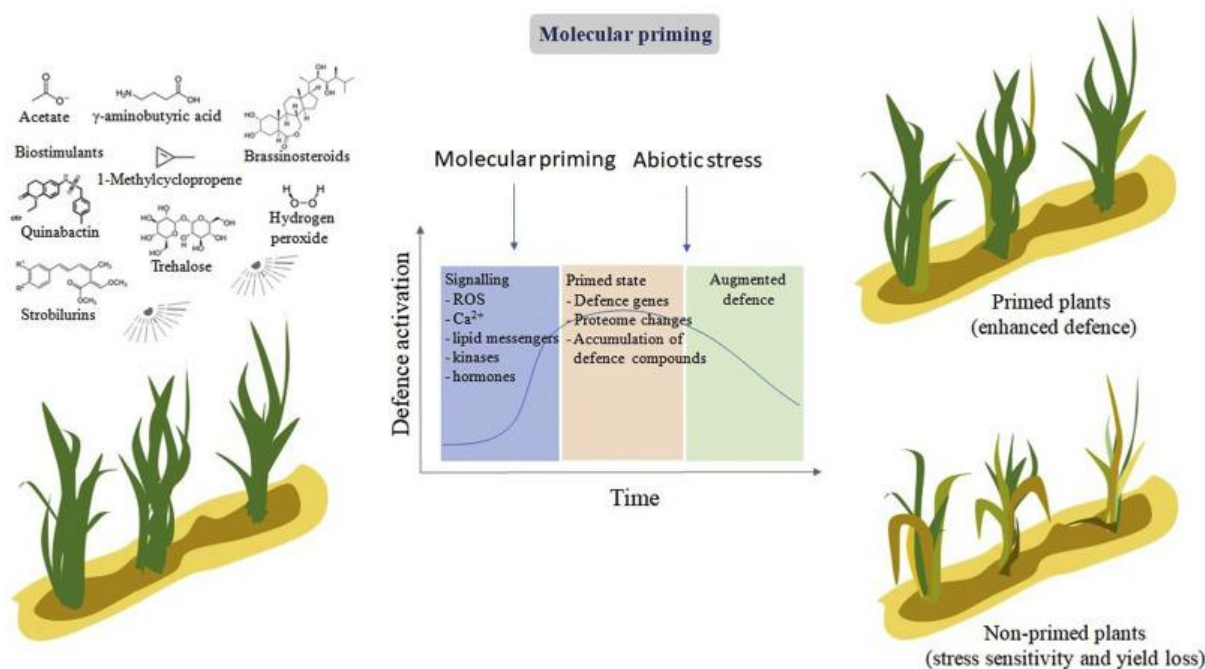
Plants are protected by the pattern-triggered immunity (PTI) against the common potentially destructive micro-organisms. However, a smaller group of infectious plant pathogens, have developed mechanisms to suppress PTI, by using defence-suppressing effector molecules that deregulate the signalling pathways that control plant innate immunity (Jones and Dangl, 2006; Llorens et al., 2017). At early stages of attack, plants have developed the capability to recognise virulent pathogens and counter a suitable defence response, as a co-evolutionary response (Pastor et al., 2013, Pritchard and Birch, 2014; Llorens et al, 2017). Effector-triggered immunity (ETI) is a familiar example of co-evolved plant defence, which allows the plant to detect the presence or activity of pathogen effectors, also known as gene for gene resistance (Boyd, 2006; Qi et al., 2011). Effector-triggered immunity requires resistance (R) genes that are found in the host plant, which can render specific virulent pathogens a-virulent (Boyd, 2006; Llorens et al, 2017). Pest and pathogen attacks are reduced by stimulating downstream responses that may result in a systemic and local induced resistance through PTI and ETI. These inducible defence responses include cell wall reinforcements through lignification and callose deposition, the accumulation of pathogenesis-related (PR) proteins, and the production of secondary antimicrobial compounds (Shah, 2003; Boyd, 2006).

### 2.8.2 Priming for resistance *in vitro*

The most decisive stage for a plant is to first identify that it is being attacked (Nimchuk et al., 2003; Pastor et al., 2014). The time it takes for the plant to sense and accurately recognise a specific stress(or) defines how successful and applicable its reaction will be. In case of pathogen attack, the failure to provide an apt response will result in colonisation of the host tissues and thereafter disease (Ebel and Cosio, 1994; Jones and Takemoto, 2004; Pastor et al., 2014). The colonisation process is slowed down by this basal immunity of the plant, however this may not be able to effectively prevent disease as it is too weak (Nürnbergger and Lipka, 2005; Pastor et al., 2014). The level of basal resistance of a plant can be improved by the treatment of a suitable stimulus. This is known as induced resistance (IR) (Hammerschmidt, 2009; Pastor et al., 2014; Mladenov et al., 2021). Plants have attained the ability to extensively enhance their defence responses against a wide range of pathogens including fungi, viruses, bacteria, and oomycetes toward which they are genetically vulnerable (Hammerschmidt, 2009; Llorens et al., 2017). Plants are treated with several agents including plant extracts, cell wall fragments and synthetic chemicals, which has been found to induce resistance to subsequent pathogen attack both systemically and locally (Hammerschmidt, 2009; Walters and Fountaine, 2009; Llorens et al., 2017). Induced resistance can be divided into two main types, induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Pieterse et al., 2014; Llorens et al., 2017). Some studies have shown that phytohormones such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET), cytokinin, abscisic acid (ABA), auxins and brassinosteroids are the main role players in directing signalling networks that play a role in the adaptive response of plants to its biotic/abiotic environment (Robert-Seilaniantz et al., 2011; Pieterse et al., 2012; Bertini et al., 2019).

Plants have established an alternate approach to cope with various virulent pathogens (Pastor et al., 2013). They have developed the ability to acclimatise to the antagonistic conditions in their environment by alerting their immune system in response to threatening signals. The preliminary phase of resistance induction, in which the plant prepares for a forthcoming attack, but has not yet been challenged by a pathogen, is known as the priming phase (Conrath et al., 2002; Martinez-Medina et al., 2016; Mauch-Mani et al., 2017; Mladenov et al., 2021). Whilst in the primed state, the primed plant may react faster or more robustly to the triggering stress cue when compared with that of a plant in the naïve (unprimed) state (Fig. 4) (Conrath, 2009; Aranega-Bou et al., 2014, Hilke et al., 2016; Kerchev et al., 2020). There are no changes in the DNA sequence, since priming acts at the phenotypic level and is therefore reversible eventually (Conrath, 2009; Aranega-Bou et al., 2014; Hilke et al., 2016; Lämke and Bäumle, 2017). The plant must create and store information that will allow it to utilize a quicker response to stress during this time interval (Pastor et al., 2014, Lämke and Bäumle, 2017). A few stimuli

such as insect pests, avirulent pathogens, synthetic substances, metabolic disturbances of the plant and abiotic stressors, and microbe- and host-derived molecules have been found to induce the priming state (Conrath et al., 2006; Aranega-Bou et al., 2014; Lämke and Bäurle, 2017). This great variety in priming triggers suggests that several strategies may lead to the initiation of the primed state. Studies have shown some of these stimuli to target epigenetic mechanisms (Bruce et al., 2007). Epigenetic mechanisms play a significant role in the regulation of gene expression in response to environmental stress in plants (Boyko and Kovalchuk, 2008; Pontvianne et al., 2010; Sudan et al., 2018). The relation between epigenetic changes and priming is further supported by the occurrence of transgenerational priming, in which the progeny of primed plants show an enhanced defence response (Luna et al., 2012; Martinez-Medina et al., 2016; Mauch-Mani et al., 2017; Mladenov et al., 2021). The demethylation patterns at the coding region of stress-responsive genes which regulates their expression can be modified by abiotic factors like temperature, day length, water, salt, UV and oxidative stress in plants (Beck et al., 2004; Pecinka et al., 2009; Boyko et al., 2010; Zemach et al., 2013; Fang et al., 2014; Xie and Yu, 2015; Sudan et al., 2018). These epigenetic modifications can be very specific to species, tissue, organelle, or age of an organism. To generate plants that have an insignificant effect to a stress stimuli, the mechanisms involved need to be examined carefully (Bhutani et al., 2011; Sudan et al., 2018).



**Figure 4:** Illustration of plant defence activation by priming (Kerchev et al., 2020).

### 2.8.3 Priming-induced signals provide broad-spectrum plant protection

Plants are constantly challenged with a range of various pathogens and pests. These possible attackers apply various tactics to resist the plant defensive system (Balmer et al., 2012, Lämke and Bäumle, 2017). Pathogenic fungi can enter the host cell by breaking the cell walls, bacteria can enter plants through natural openings such as wounds or stomata, and insect herbivores use enzymes to reduce plant toxins (Balmer et al., 2012; Lämke and Bäumle, 2017). In plants, defence priming is activated by signals that indicate forthcoming attack by herbivores or pathogens (Balmer et al., 2012; Lämke and Bäumle, 2017). These defence-priming processes include systemic acquired resistance (SAR). This type of resistance is induced by localized necrotizing pathogen attack and initiates systemic priming of salicylic acid (SA) and pipelicolic acid (PA)-inducible defence mechanisms (Jung et al., 2009; Pastor et al., 2013; Conrath et al., 2015). The defence-priming processes also comprises of induced systemic resistance (ISR), which is activated by fungi in the rhizosphere and growth-promoting bacteria, the changes are associated with modulations of the jasmonate (JA) and ethylene (ET) pathways (Conrath et al., 2015). Hence, ISR is typically effective against pathogens that are controlled by JA-inducible defences (Ton et al., 2002; Pastor et al., 2013).

Most chemicals that induce priming are functional analogues or endogenous plant compounds. Hence, in response to biotic stress signals such as JA (Frost et al., 2008), SA (Kauss and Jeblick, 1995) and azelaic acid (Jung et al., 2009), the chemicals are synthesised by the plant (Pastor et al., 2013). Presently, it is thought that the resistance conferred by arbuscular mycorrhizal fungi is also linked to defence priming (Cameron et al., 2013; Conrath et al., 2015), wound-induced resistance (Chassot et al., 2008) and  $\beta$ -aminobutyric acid-induced immunity (BABA-IR) (Jakab et al., 2001).  $\beta$ -aminobutyric acid (BABA) is a plant xenobiotic compound. It is a non-protein amino acid which can induce resistance in various plant species against a remarkably wide range of abiotic stresses (Pastor et al., 2013). Moreover, BABA acts in an enantiomer-specific manner and is active at low concentrations (Cohen, 2002; Pastor et al., 2013). Based on these characteristics, it has been suggested that BABA either ectopically activates an endogenous signalling compound that regulates several immune responses simultaneously, or that it simulates an endogenous plant signalling compound (Pastor et al., 2013).

## 2.9 Jasmonate signalling and manipulation by insects and pathogens

### 2.9.1 Signals in plant-microbe interactions

Multiple defence responses can be stimulated by a single priming-inducing treatment (Pastor et al., 2013). The response pathways of the central regulatory system of plant defence can be targeted by primed treatments, such as SA and JA (Conrath, 2011; Li et al., 2019). In general,

JA and ethylene (ET) signalling pathways are required for plant defence activation against herbivorous insects and necrotrophic pathogens (Penninckx et al., 1998; Li et al., 2019), whilst SA is a key regulator of defence against biotrophic and hemi-biotrophic pathogens (Pieterse et al., 2012; Campos et al., 2014; Caarls et al., 2015; Zhang et al., 2017). Plants encounter attacks by insects and pathogens with diverse modes of action, and they depend on crosstalk between various hormone signalling pathways to adjust suitable immune responses in contrast to these pathogens (Pieterse et al., 2012; Caarls et al., 2015; Zhang et al., 2017). The SA-JA antagonistic interaction has progressed as a significant strategy for plants to adjust their immune responses depending on the type of attack they experience (Pieterse et al., 2012; Zhang et al., 2017).

Jasmonates are produced from lipid components and sensed by protein receptors that trigger signal transduction pathways (Wasternack and Hause, 2013; Zhang et al., 2017; Wu and Ye, 2020). JA-induced plant defences affect insect herbivores, this includes leaf-eating insects, such as beetles and caterpillars, also piercing-sucking insects, such as leafhoppers, thrips and spider mites (Howe and Jander, 2008; Campos et al., 2014; Lu et al., 2015; Goossens et al., 2016; Zhang et al., 2017). Jasmonate signalling mediates plant defence against necrotrophic pathogens, fungal pathogens such as *Fusarium oxysporum*, *Plectosphaerella cucumerina*, *Botrytis cinerea*, *Alternaria brassicicola*, as well as the bacterial pathogen *Pectobacterium atrosepticum* (Campos et al., 2014; Yan and Xie, 2015; Zhang et al., 2017). In addition to the important role JA plays in plant defence, it also provides a significant role in plant reproduction and other developmental and growth processes, this includes adventitious and lateral root formation, leaf senescence, seed germination, nectaries, and resin ducts, and glandular trichomes formation (Wasternack and Hause, 2013; Campos et al., 2014; Kazan, 2015; Wasternack and Strnad, 2016; Zhang et al., 2017; Wu and Ye, 2020). However, these glandular trichomes, nectaries and resin ducts can generate compounds that are either directly or indirectly involved in plant defence, which connects the dual roles of JA in defence and development (Dicke and Baldwin, 2010; Campos et al., 2014; Zhang et al., 2017).

Jasmonate derivatives have been found to induce the biosynthesis of different defensive chemicals and proteins to counter pathogens and herbivores by functioning as chemical triggers (Wu and Ye, 2020). It is extensively accepted that herbivore and pathogen attacks are linked to a variety of herbivore-associated molecular patterns (HAMPs) such as insect secretions, microbe/pathogen-associated molecular patterns (MAMPs/PAMPs) such as flagellin, and damage-associated molecular patterns (DAMPs) such as systemin or systemin-like peptides and plant cell wall-derived oligogalacturonides (Felton and Tumilson, 2008; Mithöfer and Boland, 2008; Hogenhout and Bos, 2011; Yamaguchi and Huffaker, 2011;

Campos et al., 2014; Heil and Land, 2014; Zhang et al., 2017). The plant pathogen recognition receptors (PRRs) that are positioned at the plant plasma membrane can recognise this attacker associated patterns and thus initiate JA signaling-dependent resistance (Qi et al., 2006; Song et al., 2006; Yamaguchi et al., 2006; Brutus et al., 2010; Yamaguchi et al., 2010; Mousavi et al., 2013; Choi et al., 2014; Zhang et al., 2017; Shi et al., 2019; Wu and Ye, 2020). In numerous genome-wide transcriptome studies, a substantial overlap of gene expression and genes involved in defence hormone signalling was detected across PAMP, HAMP, and DAMP responses (Campos et al., 2014; Zhang et al., 2017). Following the perception of cellular calcium flux and damage signals, the mitogen-activated protein kinase (MAPK) cascades are activated which intensifies the signaling from local to systemic leaves and from the cytosol into the nucleus. The sets of defensive gene expression is transcriptionally reprogrammed at this site JA and its derivatives (Wu and Ye, 2020).

The oxylipin biosynthesis pathway synthesizes JA and the pathway starts with  $\alpha$ -linolenic acid that is released in the chloroplastic membranes following an insect or pathogen attack (Wasternack, 2007; Gfeller et al., 2010). This results in catalysis, the  $\alpha$ -linolenic acid is processed by allene oxide synthase (AOS) and lipoxygenase (LOX), allene oxide cyclase (AOC) and generated in the chloroplast. The 12-oxo-phytodienoic acid (OPDA) is then transported into the peroxisome, where (+)-7-iso-JA is synthesized because of several cycles of  $\beta$ -oxidation (Wasternack and Hause, 2013; Larrieu and Vernoux, 2016; Zhang et al., 2017). This is followed by secretion into the cytosol, thereafter (+)-7-iso-JA is coupled with the amino acid isoleucine (Ile) to produce JA-Ile, known as the most bioactive form of JA (Fonseca *et al.*, 2009; Zhang *et al.*, 2017). The JA cytosolic pool is converted into JA metabolites, through carboxylation and/or hydroxylation, to attenuate JA signalling (Kitaoka et al., 2011; Koo et al., 2011; Koo and Howe, 2012; Zhang et al., 2017).

### 2.9.2 Activation and suppression of JA signalling for fungal pathogens

JA production is a standard feature in many plant-interacting fungal pathogens or symbionts, (Gimenez-Ibanez et al., 2016; Goossens et al., 2016; Zhang et al., 2017). For example, 22 JA and JA-related compounds were found in the *F.oxysporum* (*Fo*) f. sp *mattiolo* culture filtrate (Miersch et al., 1999a, Zhang et al., 2017), and JA biosynthesis has been noted in *Aspergillus niger*, *Laccaria laccata*, *Pisolithus tinctorius* and *Lasiodiplodia theobromae* (Miersch et al., 1999a; Miersch et al., 1999b; Tsukada et al., 2010; Zhang et al., 2017). However, the production of JA has only been described in plant-interacting fungi and this indicates that these fungi may have developed the ability to produce JA over time to colonise its host plants (Goossens et al., 2016; Zhang et al., 2017). A few of the fungal pathogens found in *Arabidopsis*, namely *F. oxysporum* f. sp. *matthioli* (Fomt) and *F. oxysporum* f. sp *conglutinans*

(Focn) produce JA, JA- Leu and JA-II, and exhibit reduced virulence in the *coi1* mutant (Cole et al., 2014; Zhang et al., 2017), this indicates that Fo infection is promoted by JA signaling (Zhang et al., 2017).

In contrast to hemi-biotrophic (a parasitic organism that invades living cells before transitioning to a necrotrophic state) and biotrophic pathogens (fungi that require their nutrients from a living host), necrotrophic pathogens (fungi that secrete toxins to kill the host plant and use the dead plant tissue as a source of nutrients) (Rajarammohan, 2021) and chewing insects suppress JA signaling for their success in host plants. One of the strategies used by the fungal pathogens is to decrease JA accumulation following infection, either by accelerating JA catabolism or by blocking JA biosynthesis. Otherwise, SA-JA antagonism may be utilised for suppression of JA-mediated defence (Zhang et al., 2017). A few fungal species has evolved the ability to break down JA (Patkar et al., 2015; Zhang et al., 2017). It has been reported that, for example the rice blast fungus *Magnaporthe oryzae* produces the antibiotic biosynthesis monooxygenase (Abm) and converts both fungal and plant-derived JA into 12OH-JA to facilitate host colonisation and indicate JA signaling (Patkar et al., 2015; Zhang et al., 2017).

Just as the pathogenic bacteria found in biotrophs and hemi-biotrophs activate JA signalling to inhibit SA signalling, necrotrophic pathogens can manipulate SA-JA antagonism to suppress JA-mediated defence responses. The fungus *Botrytis cinerea* produces an exopolysaccharide ( $\beta$ -(1,3) (1,6)-D-glucan) that stimulates the accumulation of SA and antagonistically suppresses JA-response gene expression, including that of proteinase inhibitors I and II (PI I and PI II) (El Oirdi et al., 2011; Zhang et al., 2017). Proteinaceous effectors are also secreted by pathogens and symbionts to suppress JA signaling. For example, the necrotrophic pathogen *Sclerotinia sclerotiorum* produces an integrin-like (SSITL) protein, which is responsible for suppressing JA-ET signaling mediated resistance at the initial stage of infection (Zhu et al., 2013; Zhang et al., 2017).

### 2.9.3 Cis-jasmone and hexanoic acid applications for insect resistance

Jasmonates and its by-products, collectively known as jasmonates (JAs), are pervasive plant regulators (Ahmad et al., 2016). Apart from JAs and methyl jasmonates (MeJAs), other JAs particularly jasmonoyl ACC (JA-ACC), cis-jasmone, and jasmonoyl isoleucine (JA-Ile) with numerous biological functions have been mentioned (Ahmad et al., 2016). Additionally, a wide range of JA-induced plant growth, physiological and developmental activities has been reported. This includes biotic and abiotic stress tolerance, fertility, root elongation, storage organ formation, interaction with other hormones, and oxidative defence (Cipollini, 2010, Nafie

et al., 2011). Various other physiological functions related to JA responses include the accumulation of storage proteins, flowering, seed development (Wasternack et al., 2012), herbivory (Erb et al., 2012), wounding (Erb et al., 2012) and systemic resistance (Pieterse et al., 2002, 2012). Various studies have reported on its role in gene expression in different plants such as *Arabidopsis* (Sasaki et al., 2001), sugarcane (Nogueira et al., 2005), rice (Liu et al., 2012), tomato (Boter et al., 2004), which results in defence against environmental stresses (Ahmad et al., 2016).

Necrotrophic pathogen infection leads to the activation of JA dependent defence responses (Avanci et al., 2010). Plants treated with jasmonate have shown improved resistance against pests. Studies have shown that tomato plants treated with JA exhibit reduced numbers of *Frankliniella occidentalis* (thrips), aphids and flea beetles, which were attributed to an increase in the activities of proteinase inhibitors and polyphenol oxidase (Thaler et al., 2001). As reported in *Arabidopsis* and spinach (McConn et al., 1997; Schmelz et al., 2002). Studies on strawberry reported on the reduction of the adverse effects of oxidative and drought stress by MeJA (Wang, 1999). Barley seeds that were pre-treated with MeJA have shown reduced membrane damage than that of the non-treated plants (Bandurska et al., 2003).

According to Kravchuk *et al.* (2011), treatment with Hx was found to protect *Arabidopsis* plants against necrotrophic fungi *B. cinerea*. Similar findings were reported on tomato plants, the roots were treated with Hx, and resulted in protection against *B. cinerea* (Aranega-Bou et al., 2014). The 4-week-old plants root treatment with Hx at concentrations below 1 mM for 48 h before infection considerably reduced the incidence of the disease, as did other non-natural (BABA) and natural (SA) compounds (Vicedo et al., 2009; Aranega-Bou et al., 2014). These concentrations of Hx have shown no antimicrobial effect on the fungi, reduced conditioning times were not suitable for protection of the plant against this pathogen. In addition, it was suggested that plant protection may result from interactions with plant defense systems since there was no accumulation of Hx in the aerial part of the plant (Vicedo et al., 2009; Aranega-Bou et al., 2014). Callose accumulation was induced by Hx treatment upon infection by *B. cinerea* (Kohler et al., 2002; Aranega-Bou et al., 2014). Cell wall fortification by the deposition of callose is an important component of resistance induced by various chemical inducers like benzo (1,2,3) thiadiazole-7-carbothioicacid S-methylester (BTH) and BABA (Aranega-Bou et al., 2014).

Improved resistance against necrotrophs *Alternaria alternata* in Fortune mandarin (Llorens et al., 2013; Aranega-Bou et al., 2014; Llorens et al., 2016) and *A. brassicicola* in *Arabidopsis* (Kravchuk et al., 2011; Aranega-Bou et al., 2014) has been noted with the use of Hx. In this

case, both callose priming and JA-signaling were necessary for Hx-IR. In addition, a rapid accumulation of abscisic acid (ABA) was observed, which could act as a positive regulator of callose deposition, thus supporting the fact that both the JA-signaling pathway and enhanced physical barriers are involved in Hx-IR against necrotrophic pathogens (Aranega-Bou et al., 2014). Hexanoic acid has also been used for inducing defence responses in tomato plants against *Botrytis cinerea*, tomato plants against *Pseudomonas syringae* (Leyva et al., 2008; Vicedo et al., 2009; Scalschi et al., 2013), tomato plants against *Phytophthora citrophthora* and *Alternaria solani* (Flors et al., 2003), and *Cucumis melo* against Melon necrotic ringspot virus (Fernández-Crespo et al., 2017). There is increasing evidence that focuses on seed priming, foliar spraying and hydroponic solutions to induce priming (Levy et al., 2008; Scalschi et al., 2013; Llorens et al., 2016)

## 2.10 Molecular and phenotypic assessment of mutated plants

A wide variety of strategies are available for the characterization and detection of somaclonal variants which are based on the differences in morphological traits (Nhut et al., 2013; Krishna et al., 2016), biochemical (Kar et al., 2014, Krishna et al., 2016), molecular DNA markers (Martínez-Estrada et al., 2017) or their combinations (Dey et al., 2015; Krishna et al., 2016). Somaclonal variation can be achieved by point mutations, changes in chromosome number, somatic crossing over, somatic gene rearrangement, DNA methylation, histone modifications and RNA interference and epigenetic variation (Krishna et al., 2016). Molecular markers (DNA and protein based) are consistent and advantageous as they recognise internal changes that contain a genetic origin (Idrees, 2014). Some examples of DNA markers used for analysis include Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Inter Simple Sequence Repeats (ISSR) and Random Amplified Polymorphic DNA (RAPD) (Jiang, 2013; Nadeem et al., 2018).

Plant species that have been generated through somaclonal variation have been successfully released with improved traits including disease resistance, appearance, yield, abiotic stress tolerance and plant architecture (Anil et al., 2018). Some examples of plant species include maize (*Zea mays* L.) with improved grain quality and, a wheat variant (*Triticum aestivum* L.) with high yield (Anil et al., 2018). A sweet potato cultivar generated through somaclonal variation is comparable to the parent cultivar in disease resistance and yield, however it shows a more desirable darker and more stable skin colour (Moyer and Collins, 1983; Anil et al., 2018).

### 3. Materials and Methods

#### 3.1 Plantlet regeneration using indirect somatic embryogenesis

##### 3.1.1 Plant collection and preparation of material

Sugarcane plants of cultivars N41 and 88H0019 were harvested from the field at the South African Sugarcane Research Institute (SASRI), in Mount Edgecombe, Kwa-Zulu Natal (coordinates: 30 °S, 31 °E). They were selected based on their field ranking for *E. saccharina* tolerance, with 88H0019 and N41 being susceptible and resistant, respectively (Zhou, 2013). The preparation procedures for plant collection followed Snyman et al. (2006). The immature leaf rolls of the sugarcane stalk were surface decontaminated by wiping with 100 % (v/v) ethanol and a 30 cm section was cut transversely on the adaxial end. Thereafter, they were placed in a beaker with 70 % (v/v) ethanol prior to culture initiation in the laminar flow.

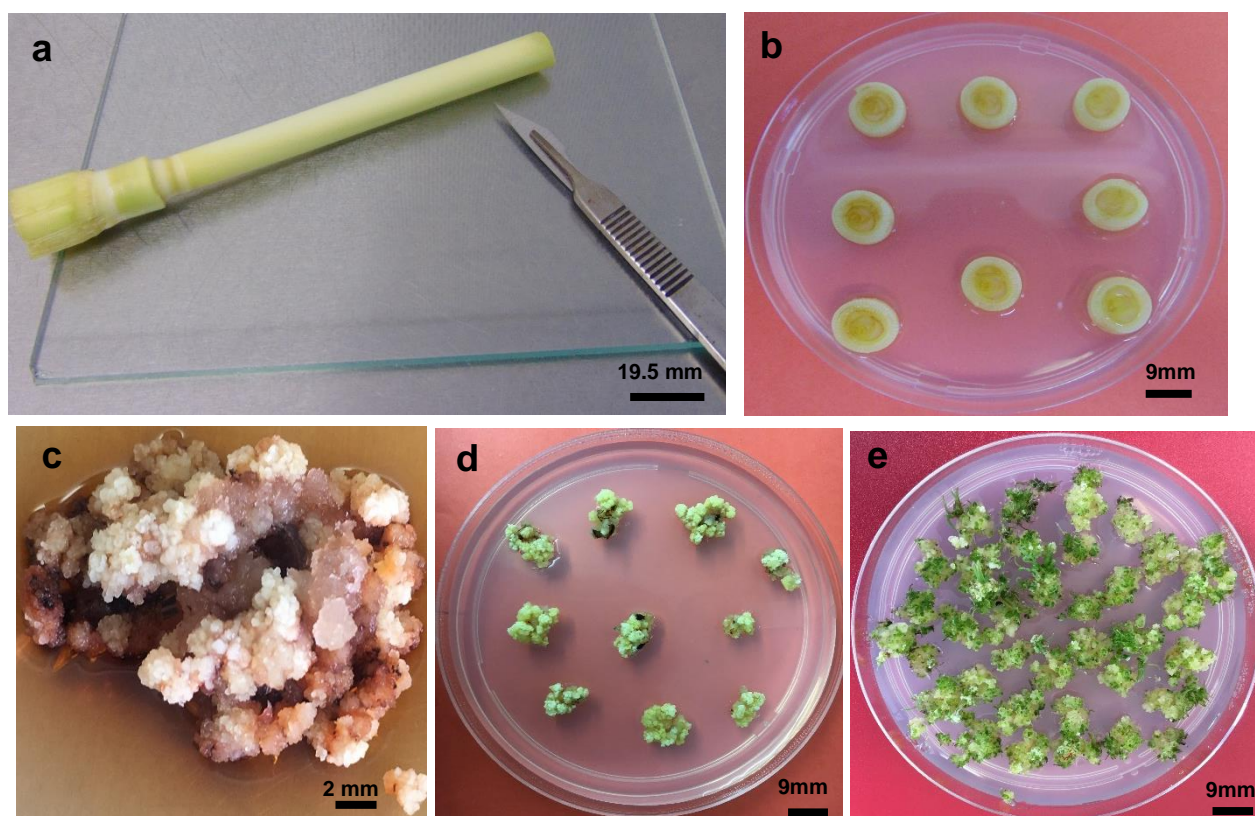
##### 3.1.2 Callus initiation and embryo maturation

Callus initiation followed the protocol described by Snyman et al. (2006). The outer leaf layers of the 30 cm stalk apices were aseptically removed using a sterile scalpel until the inner immature leaf roll was visible (Fig. 5a). After each leaf sheath was removed the leaf rolls were swabbed with 70 % (v/v) ethanol. The inner leaf roll was cut transversely into thirty discs (approximately 2 mm thick) which were placed into Petri plates containing liquid embryo initiation medium (EIM) to remove phenolic exudes from the cut ends. Embryo initiation medium contained MS basal salts and vitamins (Murashige and Skoog 1962, Duchefa, Belgium), 20 g l<sup>-1</sup> sucrose, 3 mg l<sup>-1</sup> 2,4-D, pH 5.6 - 5.8, autoclaved at 121 °C for 20 minutes. Thereafter, 10 leaf discs were cultured per 90 mm Petri dish (Concorde Plastics, Pty Ltd, RSA) with the adaxial surface in contact with semi-solid EIM [25ml semi-solid EIM (as described above) with 8 g l<sup>-1</sup> agar (Neogen, Acumedia, USA)] (Fig. 5b). The plates were sealed with parafilm (Lasec SA) and maintained in the dark at 26 ± 1 °C for 3 weeks, with daily observation for microbial contamination. The cultures were sub-cultured on to fresh medium every 2 weeks.

After 8 weeks on EIM (Fig. 5b), 0.2 g of embryogenic calli were weighed using sterile aluminium weighing boats and placed on to Petri dishes containing 25 ml semi-solid embryo maturation medium (EMM) (Fig. 5d). The media composition for EMM was full strength MS with vitamins, 20 g l<sup>-1</sup> sucrose, 1 mg l<sup>-1</sup> 2,4-D and 8 g l<sup>-1</sup> agar. The plates were sealed with parafilm and incubated in the dark at 26 - 27 °C for 3 weeks.

### 3.1.3 Embryo germination and plantlet regeneration

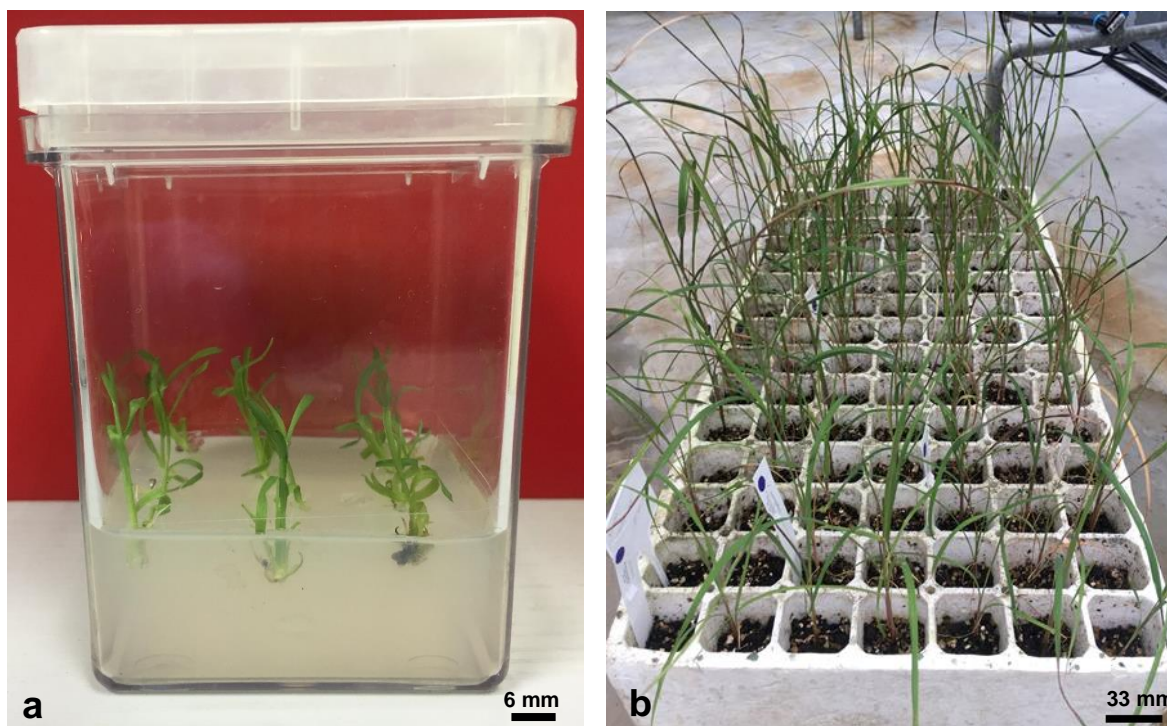
The embryogenic calli were transferred to 90 mm Petri dishes containing 25 ml embryo germination media (EGM1; EIM without 2,4-D) (Fig. 5e). The pieces of calli were evenly spread on the media, sub-cultured twice monthly, and incubated for 4 - 8 weeks at 16 h light (26 °C), 200  $\mu\text{m m}^{-2} \text{s}^{-1}$  photon flux density (PPFD) and 8 h dark (23 °C) photoperiod. The regenerated plantlet clusters were divided and single rooted plantlets of about 20 mm in height were transferred to Magenta® vessels (Sigma-Aldrich, USA) with 100 ml EGM2 (20 plants per vessel). Embryo germination medium (EGM2) contained half strength MS basal salts and vitamins (Murashige and Skoog 1962, Duchefa, Belgium), 10 g l<sup>-1</sup> sucrose, 8 g l<sup>-1</sup> agar and pH 5.6 - 5.8, autoclaved at 121 °C for 20 minutes.



**Figure 5:** Photographs showing plantlet regeneration via indirect somatic embryogenesis from embryo initiation to germination stages: a) sugarcane leaf roll, b) leaf sections on embryo initiation medium (EIM), c) white compact embryogenic callus after 8 weeks, d) embryogenic callus on embryo maturation medium (EMM), and e) plantlet regeneration on embryo germination medium (EGM1).

### 3.1.4 Acclimatisation

Dead leaves were removed from *in vitro* plantlets and the remaining green leaves trimmed to above the growing point and placed into Magenta® vessels with 80 ml semi solid media (Fig. 6a). The plantlets were rinsed with distilled water to remove the media. The control plants and putative *Fusarium* mutants were hardened off *ex vitro* by planting in polystyrene seedling trays [(670 x 330 mm; (Hygrotech, Pretoria, SA)] containing a mix of vermiculite (Coastal Farmer's Co-op, Umhlali, SA) and peat moss (Nirrom, Alberta, Canada) (1:1 v/v) (Fig. 6b). The plants were placed in the glasshouse at 20/34 °C (night/day temperature) and were watered using overhead sprinklers for 1 min twice a day and fertilized every 2 weeks with either N:P:K 5:1:5 pellets (Coastal Farmer's Co-op, Umhlali, SA) or Trelmix, 1 ml l<sup>-1</sup> (Hubers (Pty) Ltd, Howick, SA) alternating bi-monthly for 5 - 6 months.



**Figure 6:** Photographs showing plantlet acclimatisation. a) Plantlet growth in Magenta® vessel, and b) Plantlet acclimatisation in seedling tray.

## 3.2 Establishment of suitable concentrations of priming agents at the callus and plantlet stages for plantlet production

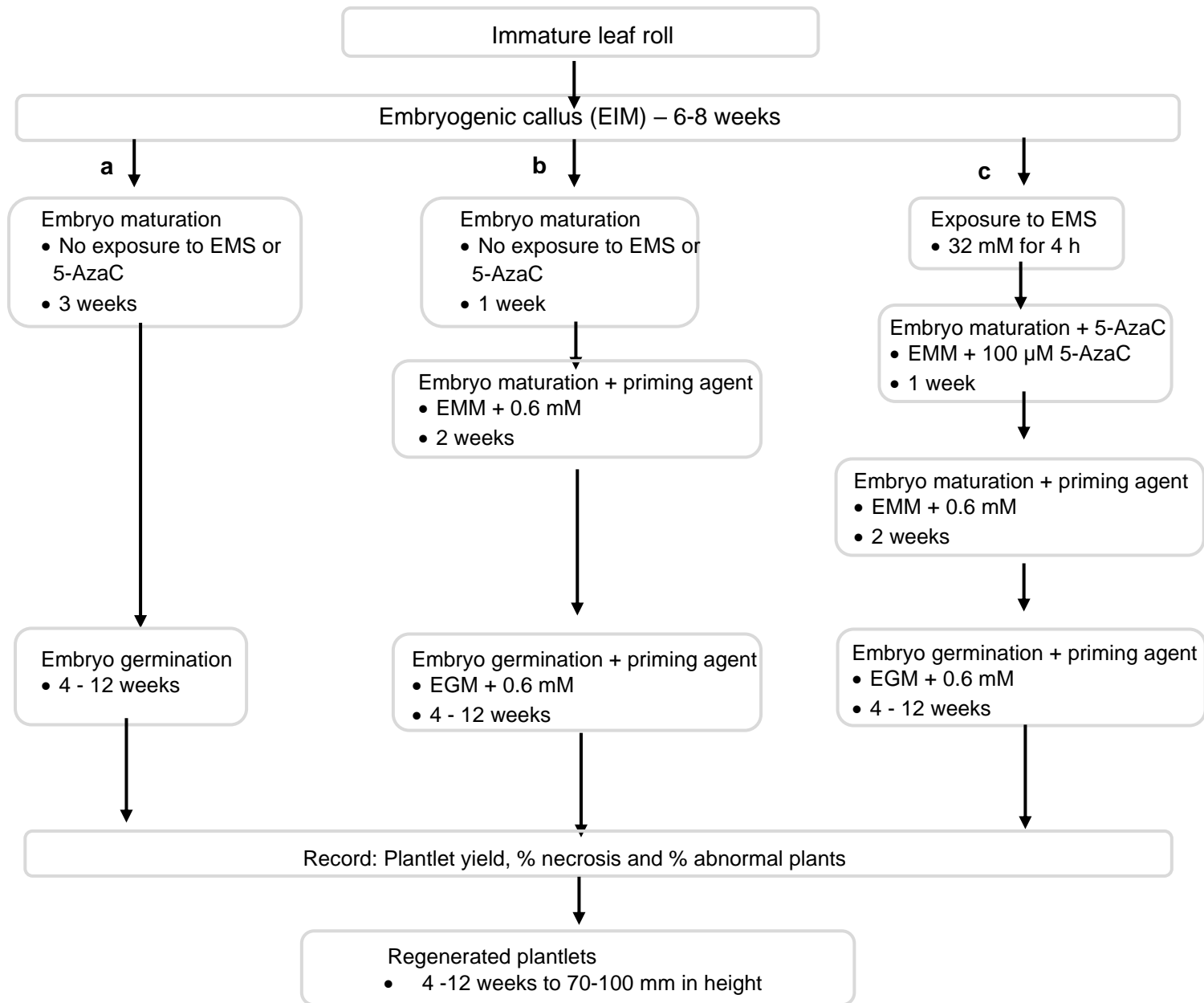
After 8 weeks, embryogenic calli were exposed to two priming agents: hexanoic acid and cis-jasmone at the embryo maturation and germination stages to determine an effective priming agent of the two, and a suitable concentration at each stage. For the control, embryogenic calli were cultured on media without priming agents.

The embryogenic calli were produced as mentioned previously (section 3.1.2). At the embryo maturation stage, EMM (section 3.1.2) was supplemented with various concentrations of *cis*-jasmone (0, 0.2, 1, 5 or 10  $\mu$ M) or hexanoic acid (0, 0.2, 0.6, 2 and 6 mM) in 90 mm Petri dishes for 3 weeks. The concentration range for *cis*-jasmone and hexanoic acid were according to Nieves et al. (2001) and Kravchuk et al. (2011) respectively. The cultures were incubated in the dark at 26 - 27 °C. Embryogenic calli were then transferred on EGM1 (section 3.1.3) containing the above-mentioned concentrations for the priming agents. The cultures were placed in the photoperiod growth room (section 3.1.3) for 4 - 12 weeks, sub-culturing every 2 weeks on EGM1 containing priming agents. The proportion (%) callus necrosis and number of plantlets produced per 0.2 g calli were recorded between 8 - 12 weeks. The most suitable priming agent at its effective concentration was selected for the ensuing *in vitro* mutagenesis and priming manipulations for *E. saccharina* tolerance.

## 3.3 *In vitro* mutagenesis and priming for *E. saccharina* tolerance

### 3.3.1 *In vitro* protocols that involved chemical mutagenesis and epigenetic priming for *E. saccharina* resistance

The experimental design for the exposure of plant tissue from two sugarcane varieties to the two tested mutagens, 5-AzaC (Sigma-Aldrich, St Louis, USA) and EMS (Sigma-Aldrich), and to the priming agents hexanoic acid (Sigma-Aldrich, St. Louis, USA) and cis-jasmonic acid (Sigma-Aldrich, St. Louis, USA) is illustrated in Fig. 7. The mutagenic protocol for the exposure of calli to EMS and 5-AzaC was that reported by Mahlanza et al. (2013). Hexanoic acid was selected as the most effective priming agent at 0.6 mM than cis-jasmone, in terms of callus production and plantlet yield.



**Figure 7:** Experimental design for exposure of calli to priming agents, ethyl methanesulfonate (EMS), and 5- Azacytidine (5-AzaC)

#### a) 5-Azacytidine treatment of calli

A 100  $\mu\text{M}$  stock solution of 5-AzaC was prepared and filter-sterilized using a 0.8/0.2  $\mu\text{m}$  Acrodisc PF syringe filter (Pall Corporation, USA) in a fume-hood cupboard (Munsamy et al., 2013). The pieces of callus (0.2 g) obtained after 3 weeks on EIM as mentioned in section 3.1.2 were transferred to EMM containing 100  $\mu\text{M}$  5-AzaC (4 pieces per Petri dish). The medium was maintained at 45 °C before pouring the media and to avoid 5-AzaC degradation it was used immediately after preparation. After 1 week, the 0.2 g pieces of calli were transferred to standard EMM containing either one of the priming agents as mentioned below (section 3.3) for 2 weeks for callus recovery.

#### b) Treatment of embryogenic calli with ethyl methanesulfonate

A 1M EMS stock solution was prepared in the fume hood in a 50 ml Corning tube (Corning, Massachusetts, USA). Under aseptic conditions, the stock solution was filter sterilised by using a 0.22  $\mu\text{m}$  syringe filter (Pall Corporation, Michigan, USA). The stock solution was used to prepare 10 ml of a 32 mM EMS solution (Koch et al., 2012) in 15 ml Corning tubes by diluting with liquid EMM. White embryogenic calli (0.2 g) were placed into Corning tubes containing the EMS solution for 4 hours (Fig. 7), and the tubes were gently inverted every 30 - 45 min. For the control, calli were transferred to liquid EMM with no EMS for 4 hours. Thereafter, the EMS solution was decanted and the calli were rinsed three times using liquid EMM and then placed on sterile filter paper to soak away the excess media.

#### c) Combination of EMS, 5-AzaC and Hx treatments

In the first protocol (Fig. 7a), embryogenic calli were established for 6 - 8 weeks, as described previously (section 3.1.2). Small pieces of white embryogenic calli were weighed out in the laminar flow and 0.2 g of calli were placed (4 pieces/Petri plate) on 25 ml of EIM for 3 weeks. There was no exposure to EMS or 5-AzaC. The calli were incubated in the dark at  $26 \pm 1$  °C for 2 weeks. After 3 weeks, the calli were then cultured on EGM1 media without priming agents, as described in section 3.1.3 for each treatment. The cultures were placed in the photoperiod growth room (section 3.1.3) for 4 - 12 weeks, sub-culturing every 2 weeks. The number of plantlets per 0.2 g callus was recorded after 4 - 12 weeks because plant regeneration only took place after 4 weeks in culture. Percentage callus necrosis (a piece of callus was considered necrotic if > 50 % appeared brown/black) was recorded for all treatments.

In the second protocol (Fig. 7b) the embryogenic calli were placed on EMM media without mutagens for 1 week (Fig. 7). The calli were then exposed to the priming agent at both the callus maturation and the germination stages. The white embryogenic calli (0.2 g of callus) were then cultured on Petri plates containing 25 ml EMM (section 3.1.2) with 0.6 mM hexanoic acid for 2 weeks. The calli were incubated in the dark at  $26 \pm 1$  °C for 2 weeks. The calli were then transferred to EGM1 containing the same concentration of hexanoic acid. The cultures were placed in the photoperiod growth room, as described in section 3.1.3 for 4 - 12 weeks, sub-culturing every 2 weeks on EGM1 containing the priming agent. The number of plantlets produced and callus necrosis per 0.2 g calli were recorded between 8 - 12 weeks.

In the third protocol (Fig. 7c) the embryogenic calli obtained after 6-8 weeks were exposed to both mutagens. White embryogenic calli (0.2 g of callus) were exposed to 32 mM EMS for 4 hours and then placed on embryo maturation media containing 100  $\mu$ M 5-AZA for 1 week, as described in section 3.2. The calli were then cultured on Petri plates containing 25 ml EMM (section 3.1.2) with 0.6 mM Hx for 2 weeks. The calli were incubated in the dark at  $26 \pm 1$  °C for 2 weeks. The surviving calli were then cultured onto EGM1 containing the same concentration of Hx. The cultures were placed in the photoperiod growth room (section 3.1.3) for 4 - 12 weeks, sub-culturing every 2 weeks. Callus necrosis and number of plants per 0.2 g callus were recorded after 12 weeks.

### 3.4 *Fusarium pseudonygamai* culture and filtrate preparation

#### 3.4.1 Fungal culture and storage conditions

Cultures of *F. pseudonygamai* SC17 were obtained from stock cultures from the Pathology laboratory at SASRI. A colony from each of SC17 and PNG40 were grown on potato dextrose agar (PDA; Biolab, Wadeville, RSA) to produce cultures for storage. After 5 days, mycelial squares (5 x 5 mm) were transferred to Eppendorf microfuge tubes containing 1 ml of 15 % (v/v) glycerol solution (Merck, Wadeville, SA) and stored at - 80 °C. To prepare starter cultures for each isolate, a thawed mycelial square stored in 15 % (v/v) glycerol was placed on PDA for 3 days at 30 °C in an incubator. A 5 x 5 mm mycelial square was excised from the leading edge of the resultant fungal colony and transferred to Erlenmeyer flasks each containing 250 ml of potato dextrose broth (PDB) (Fluka, St Louis, USA). To allow for culture growth, the neck of the flask was plugged with a cotton wool bung, covered with aluminium foil (autoclaved prior to use) and agitated in a shaking incubator at 145 rpm and at 28 - 30 °C for 7 days.

### 3.4.2 Culture filtrate preparation

The culture filtrate preparation protocol was done according to Mahlanza et al. (2013). The 7-day-old liquid fungal culture was transferred into a sterile 250 ml centrifuge tube and centrifuged at 12 000 rpm for 5 min (Heraeus Megafuge 40R, Thermo Scientific). The supernatant was filtered through a sterile muslin cloth to collect the mycelia, the fresh mass of the mycelia was recorded immediately, and its dry mass was determined after incubation at 80 °C for 24 h. The culture filtrate was sequentially filtered through Whatman No. 1 filter paper, 0.45 µM membrane filter (Sartorius Stedim Biotech, Germany), a sintered glass filter unit (Millipore) using a vacuum pump and a 0.2 µM syringe filter (Millipore, Ireland). The culture filtrate (CF) was stored for a maximum of 24 h at 4 °C (Mahlanza et al., 2013). The CF concentration for each batch was expressed as fungal dry mass/volume of PDB used in the *F. pseudonygamai* SC17 liquid culture.

### 3.5 Establishment of culture filtrate selection treatments

#### 3.5.1 Selection media with *F. pseudonygamai* SC17 CF

*F. pseudonygamai* SC17 CF was obtained as described in section 3.3.1. The selection media for calli cultures were prepared by adding the appropriate volume of CF stock solution to the autoclaved EIM medium to obtain the required CF concentrations. The 500 ml Schott bottle containing 350 ml of the medium was swirled gently to allow the media and CF to mix thoroughly before dispensing into Petri dishes. Fresh CF was prepared on a weekly basis, the embryogenic calli were cultured every week onto fresh embryo germination media containing CF.

#### 3.5.2 Exposure of embryogenic callus to medium containing culture filtrate

To determine the effect of CF during the embryo germination stage, embryogenic calli were cultured on selection media (EGM1 containing 0 - 100 ppm CF) (section 3.4.1). For the control, embryogenic calli were exposed to EGM1 without CF.

At the embryo initiation stage (6 - 8 weeks), the calli were cultured on EIM without CF. After 8 weeks, the embryogenic calli (0.1 g per replicate) were then cultured on EMM supplemented with 0, 4, 20, 50 and 100 ppm CF. The cultures were placed in the photoperiod growth room for 4 - 12 weeks, and sub-culturing weekly on EGM1 containing fresh CF. The number of plantlets and percentage callus necrosis were recorded after 6 - 12 weeks. The percentage

callus necrosis (a piece of callus was considered necrotic if <50 % appeared brown/black) was recorded for each treatment.

### 3.5.3 Plantlet rooting in medium with culture filtrate

The root growth with culture filtrate protocol was done according to Mahlanza et al. (2013). *In vitro* plantlets (70 - 100 mm in height) that were maintained on EGM2 in the photoperiod growth room were used for this experiment. All the leaves were trimmed just above the ligule and the roots were trimmed to less than 1 mm, using a pair of forceps and a scalpel (Mahlanza et al., 2013). These trimmed plantlets were transferred to Magenta® vessels (5 plants/vessel), containing EGM2 with 0, 500, 1000 and 1500 ppm CF and incubated in the photoperiod growth room for 3 weeks, as mentioned in section 3.1.3. Root growth was determined after 3 weeks by measuring root length and the percentage of plantlets that rooted.

## 3.6 Screening for indole-acetic acid production using a colorimetric assay

### 3.6.1 Indole-3-acetic acid production

Quantification of indole-3-acetic acid (IAA) produced by *F. pseudonygamai* SC17 was conducted using a colorimetric assay as described by Khan et al. (2016). Due to the enhancing root growth effect by isolate SC17, an experiment was conducted to determine if this enhanced effect was due to the additional levels of IAA in the plants or in the culture filtrate.

The fungus was cultured in 100 ml PDB supplemented with and without 0.1 g l<sup>-1</sup> L-tryptophan (Sigma-Aldrich), and both flasks were placed in a shaking incubator at 30 °C and 200 rpm for 7 days in the dark. This was used for culture filtrate preparation as explained earlier (section 3.4.2). Uninoculated broth was used as a negative control and the experiment was conducted in triplicate for each fungus. A volume of 40 ml of the culture filtrate was taken and adjusted to pH 2.8 by adding 1 N HCl. Ethyl acetate (15 ml) was added three times to the CF and vortexed. The solution was left to separate into two layers after which the top layer of ethyl acetate was transferred to a 100 ml rotary evaporator flask. The ethyl acetate fractions were combined and evaporated under vacuum at 45 °C in a rotary evaporator (Rotavapor-R, Büchi, USA). The residue was re-suspended in 2 ml of 50 % (v/v) methanol and 100 µl of the re-suspended extract was mixed with the Salkowski reagent [200 µl; 0.2 ml 0.5 mol l<sup>-1</sup> FeCl<sub>3</sub>, 4.9 ml water and 4.9 ml 70 % (v/v) perchloric acid] in an ELISA plate (96 well) and kept in the dark for 30 mins (Gordon and Weber, 1951). The resultant solution turned reddish in colour and was measured at an absorbance reading of 530 nm using a plate reader (Synergy-HT, Bio-

Tek, USA). The amount of IAA ( $\text{mg ml}^{-1}$ ) was estimated using a standard curve of pure IAA (Sigma- Aldrich, USA).

### 3.6.2 The effect of indole-3-acetic acid on plantlet regeneration

The leaves and roots of plantlets (70 - 100 mm in height) were trimmed, as described in 3.5.3. They were then placed in Magenta<sup>®</sup> vessels (5 plants/vessel), containing 80 ml of EGM1 with 0, 150, 300, and 460 nm IAA. The plants were incubated in the photoperiod growth room conditions for 3 weeks, as described in section 3.1.3. The root length of the individual plants from all treatments were recorded.

### 3.7 *Ex vitro* selection of primed and EMS-exposed *in vitro* plantlets and *E. saccharina* screening

*In vitro* plantlets were produced using the established mutagenic protocols, as described earlier (Fig. 7). The *F. pseudonygamai* SC17 CF did not inhibit callus growth so it could not be used as a selection agent in this study. The plants were divided into three categories: a) control, b) primed only with Hx, and c) primed with Hx and the use of mutagens EMS and 5-AzaC (Fig 7). Fifty plantlets were randomly selected per 'category' or 'plant family', planted in seedling trays and acclimatised for 8 - 9 months. The plants were placed in the glasshouse at 20/34 °C (night/day temperature) and were watered using overhead sprinklers twice a day for 1 min and fertilized every 2 weeks with either N:P:K 5:1:5 pellets (Coastal Farmer's Co-op, Umhlali, SA) or Trelmix, 1 ml l<sup>-1</sup> [Hubers (Pty) Ltd, Howick, SA]. These plantlets were used for *ex vitro* screening, and to test their response to *E. saccharina* damage and tissue colonisation by *F. pseudonygamai* SC17.

#### 3.7.1 Inoculation of plants using *F. pseudonygamai* culture filtrate

To confirm tolerance of the putative mutants to *F. pseudonygamai* and *E. saccharina*, the plants were acclimatised in the glasshouse and inoculated (Fig. 8). Plants with 1-2 internodes were transferred to pots (100 mm diameter, 90 mm in height) and inoculated by the toothpick inoculation method (McFarlane, pers.comm). Plants were inoculated with only SC17, and dual inoculated with SC17, and after 2 weeks, with 1-2 2<sup>nd</sup> instar *E. saccharina* larvae were placed within the leaf whorl. This was done to demonstrate the effect of the fungus only and both the fungus and eldana larvae on the putative mutant plants. The toothpicks were sterilised in boiling water for 2 h, with the water was being replaced at 30 min intervals and autoclaved them twice with 48 h between autoclaving. PDB was prepared in McCartney bottles (6 ml/bottle) and the toothpicks were immersed in the media (15 toothpicks/bottle) before it was

autoclaved. The media was inoculated with *F. pseudonygamai* by adding a 5 x 5 mm mycelial square from a 3 - day old colony cultured on PDA. The McCartney bottles were placed in a shaking incubator at 145 rpm for 3 weeks at a temperature of 28 - 30 °C. To inoculate the plants, the stems of the plants were stabbed 2-3 cm above the soil surface with *F. pseudonygamai* SC17-colonised toothpicks. The control plants were stabbed with either uncolonised or colonised toothpicks. To avoid secondary pathogen infection, prior to stabbing, the stem was swabbed with 70 % (v/v) ethanol, and the protruding toothpick was cut and parafilm wrapped around the wound after inoculation.

### 3.7.2 Detection and re-isolation of the presence of *F. pseudonygamai*

After 7 - 8 weeks of toothpick inoculation, the stems of the live and dead putative mutant plants (some with chlorotic, crinkled, and necrotic leaves) were removed and placed in Petri dishes. Using aseptic techniques, the leaves and stems were transferred separately to 50 ml Corning tubes (Corning, Greiner Bio-one, SA) and surface sterilised by submerging them sequentially in 95 % (v/v) ethanol for 2 min followed by 10 % (v/v) sodium hypochlorite for 5 min. The stems and leaves were rinsed twice in sterile water to remove the excess sodium hypochlorite, after which they were dried for 5 min by placing them on a sterile paper towel in the laminar air flow cabinet. The surface of the leaves and stems were pressed on PDA to confirm the removal of surface contaminants.

The stems of all the putative mutants were cut into longitudinal segments and the inoculation fungal lesion severity for each individual stem were visually rated on a scale of minimum 0 to a maximum of 3 (0 = no lesions, 1 = mild, 2 = moderate, 3 = severe). Fungal re-isolation from the undamaged area 20 - 30 mm above it or the inoculation lesion, was done by cutting longitudinal stem sections and placing these sections on Nash and Snyder (1962) medium and incubated for 5 days at 28 - 30 °C. The Nash and Snyder semi-selective medium for *Fusarium* spp. consists of 15 g l<sup>-1</sup> peptone, 1 g l<sup>-1</sup> potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>), 0.5 g l<sup>-1</sup> magnesium sulphate heptahydrate (MgSO<sub>4</sub>.7H<sub>2</sub>O), 1 g l<sup>-1</sup> Pentachloronitrobenzene (PNCB), 20 g l<sup>-1</sup> agar, 0.1 % (w/v) streptomycin and 0.012 % (w/v) neomycin, pH 5.5 - 6.5, autoclaved at 121 °C for 20 mins. Once the fungus grew around the stem sections, mycelia was picked off from the colonies with an inoculating loop and cultured on Nash and Snyder medium for 3 days at 28 - 30 °C prior to DNA extraction. *F. pseudonygamai* SC17 mycelial squares from the stock culture were placed on Nash and Snyder media and used as positive controls. Colonies of the isolates were compared visually with SC17 and were subjected to molecular analyse for confirmation of fungal isolate identity.

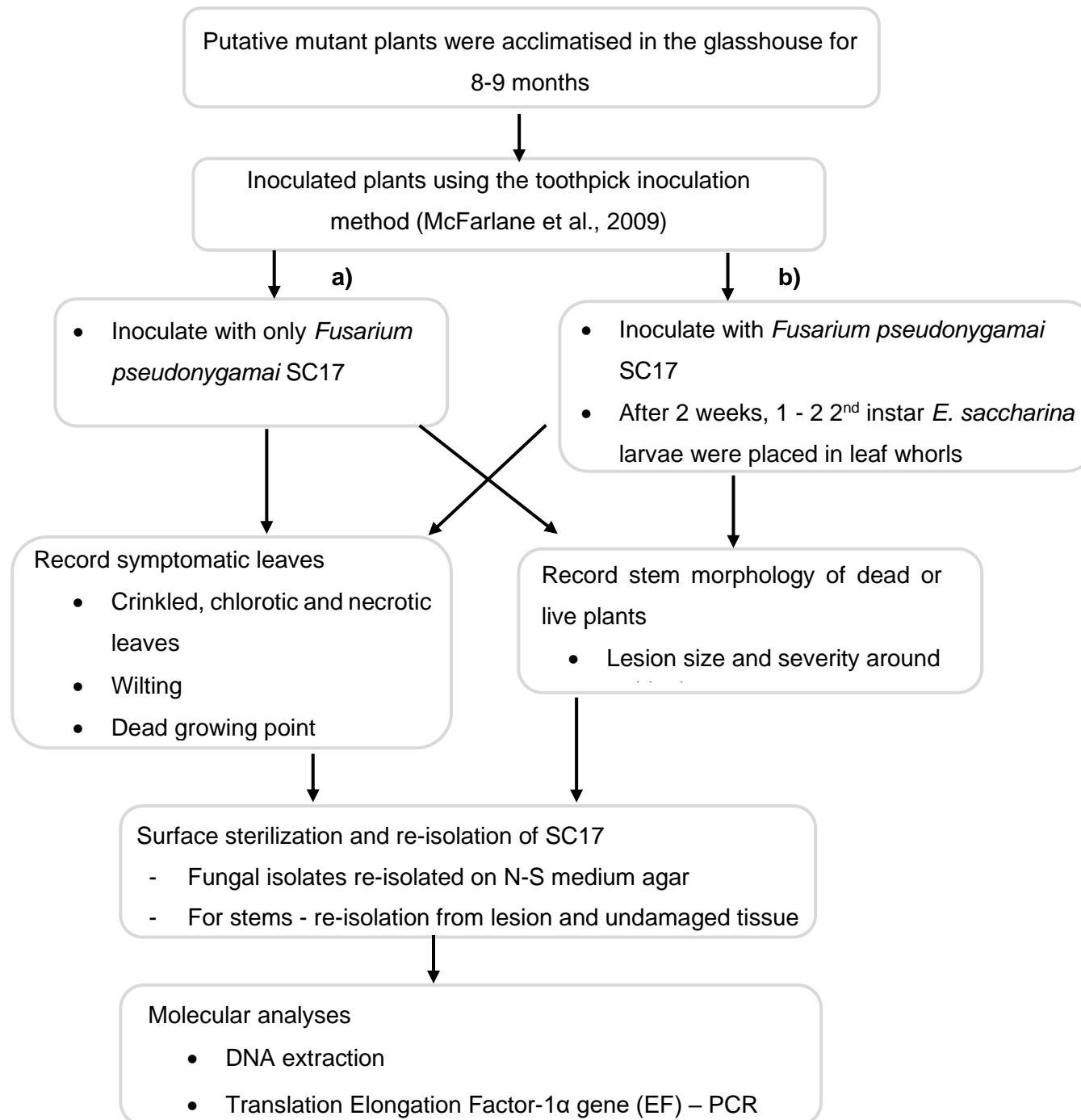


Figure 8: Summary of manipulations undertaken to confirm tolerance of plants to *Fusarium pseudonygamai* (SC17).

## 3.8 Molecular analyses of *Fusarium* isolates that were retrieved from the putative mutants

### 3.8.1 DNA extraction

The PrepMan<sup>®</sup> Ultra Sample (Applied Biosystems, California, USA) protocol was used to conduct fungal DNA extractions. The *F. pseudonygamai* SC17 isolate was grown on corn meal agar and incubated for 3 - 5 days at 28 - 30 °C. The resultant mycelia were aseptically transferred from the fungal colonies to sterile 1.5 ml microfuge tubes, using an inoculating loop. For the DNA extraction process 100 - 200 µl PrepMan<sup>®</sup> Ultra Kit sample preparation reagent (Applied Biosystems, California, USA) was added into the sterile 1.5 ml microfuge tubes (Quality Scientific Plastics, California, USA) containing 0.3 mm glass beads. The tubes were vortexed for 1 minute and the mycelia were ground using sterile plastic pestles. The tubes were then placed in a heating block at 100 °C for 30 min to release the DNA. The tubes were centrifuged (Centrifuge 5415R, Eppendorf, Hamburg, Germany) at 12 000 rpm for 5 min and the supernatant was transferred to clean microfuge tubes. The DNA was purified by adding a 10 % (v/v) of 3 M sodium acetate and 250 % (v/v) of absolute ethanol to the supernatant, which was incubated at - 20 °C and vortexed at 30 min intervals for 2 h. The tubes were centrifuged at 13000 rpm for 20 min for the precipitated DNA to form a pellet. The supernatants were discarded, and the pellets were washed by adding 700 µl of 70 % (v/v) ethanol and centrifuged at 13 000 rpm for 20 mins. The pellets were dried in the laminar flow and then re-suspended by adding 30 µl PrepMan<sup>®</sup> elution buffer and the solutions were vortexed. The concentrations of the DNA samples were determined using a spectrophotometer (NanoDrop Technologies, Delaware, USA).

### 3.8.2 Translation Elongation Factor-1 $\alpha$ gene (EF) – PCR

The re-isolated fungal isolates were inoculated after 3 days on PDA and the extraction of DNA (section 3.8.1) was conducted using the resultant colonies. The elongation factor (EF) primers were used to test the fungal isolates that were retrieved from the putative mutant plants. The PCR was conducted on the resultant colonies. The PCR reactions were done using a PCR kit (Kapa Biosystems, Massachusetts, USA) in 0.2 ml MicroAmp 8-strip PCR tubes (Applied Biosystems, California, USA) containing a final volume of 30 µl composed of 19.85 µl PCR water (Promega, USA), 4 µl Taq buffer with MgCl<sub>2</sub> (1.5 mM), 0.45 µl dNTPs (10 mM), 1 µl primer (EF1) (10 µM), 1 µl primer (EF2) (10 µM), 0.2 µl Taq polymerase and 2 µl DNA template. PCR cycling condition were as follows: initially 3 minutes of denaturation at 95 °C then 35 cycles of denaturation at 94

°C for 30 s, 1 min of primer annealing at 50 °C and extension for 1 min 30 s at 72 °C, and a final extension step at 72 °C for 10 min. The PCR products were visualised using a 1.2 % agarose gel.

### 3.9 Statistical Analyses

To analyse the data, the Genstat statistical package 18th edition (VSN International, Hemel Hempstead, UK) was used. The Shapiro-Wilk test was initially used to test the data for normality. In some instances, before conducting the suitable statistical tests, data had to be transformed ( $\log_{10}$ ), as reported in the results section.

## 4. Results

### 4.1 Establishing a suitable priming agent concentration at the callus initiation and plantlet regeneration stages for plantlet production

This study aimed to establish a suitable concentration of the selected priming agents, cis-jasmone (CJ) and hexanoic acid (Hx) at the callus initiation and the plantlet regeneration stage. The cultivar N41 is known to be intermediate-resistant to *E. saccharina*, whilst cultivar 88H0019 is known to be susceptible to *E. saccharina* in the field (Zhou et al., 2013). The effect of priming agents on callus production, callus necrosis, and plantlet regeneration was investigated.

#### 4.1.1 The effect of priming agents (CJ or Hx) supplied at the callus initiation stage

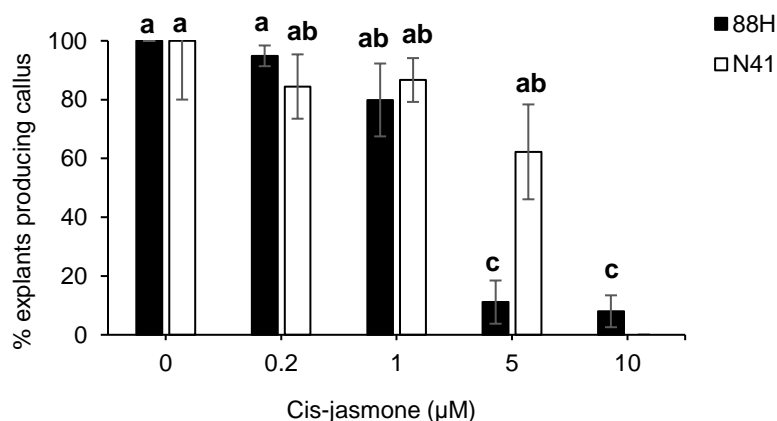
To determine an appropriate priming agent concentration at the callus initiation and plantlet regeneration stage, embryogenic calli (0.2 g per replicate) were transferred to embryo maturation medium (EMM) consisting of CJ (0, 0.2, 1, 5 and 10  $\mu\text{M}$ ) and Hx (0, 0.2, 0.6, 2 and 6 mM) for 2 weeks.

After 8 weeks, a significant difference in the percentage of explants producing callus amongst the CJ treatments was detected for both cultivars ( $p < 0.001$ ) (Fig. 9a). There was a significant decrease in the percentage of explants producing callus for cultivar 88H0019 with increasing concentrations of CJ. For cultivar N41, there were no significant differences in the percentage of explants producing callus between the control, 0.2, 1.0, and 5  $\mu\text{M}$  CJ treatments. For cultivar 88H0019, there was a significant difference between 5  $\mu\text{M}$  CJ ( $11 \% \pm 7.35$ ) and 10  $\mu\text{M}$  CJ ( $8 \% \pm 5.42$ ) compared with the control and other treatments. These two treatments inhibited callus production in cultivar 88H0019 compared with the other treatments that resulted in 60 - 100 % callus production ( $p < 0.001$ , Fig. 9a). For cultivar N41, there was no callus production recorded at 10  $\mu\text{M}$  CJ.

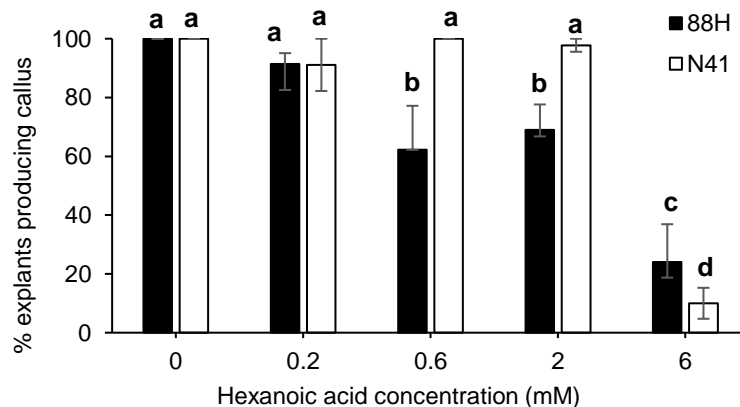
After 8 weeks for each cultivar, there were significant differences in the percentage of explants producing callus when compared with the control and all the tested Hx concentrations at the end of the study ( $p < 0.001$ , Fig. 9b). The percentage of explants producing callus was significantly lower at 6 mM Hx for cultivar 88H0019 ( $24 \% \pm 8.63$ ) and N41 ( $10 \% \pm 2.22$ ) than the control and the other treatments ( $p < 0.001$ ). For cultivar N41, there were no significant differences in the percentage of explants producing callus amongst the control, 0.2, 0.6, and 2 mM Hx treatments. For 88H0019 and N41, there was a significant decrease in the percentage of explants producing

callus with an increase in Hx supplied (Fig. 9b). The 6 mM Hx treatment significantly inhibited the percentage callus production by the explants of both cultivars. Also, callus exposure to the higher priming agents resulted in more severe necrosis than the lower concentrations of both priming agents (Fig. 10a, b). These results indicated that the percentage of explants producing callus by both N41 and 88H0019 explants were positively affected by the tested priming agents concentrations, except for the 5 and 10  $\mu\text{M}$  CJ (Fig. 9a, b).

a)



b)

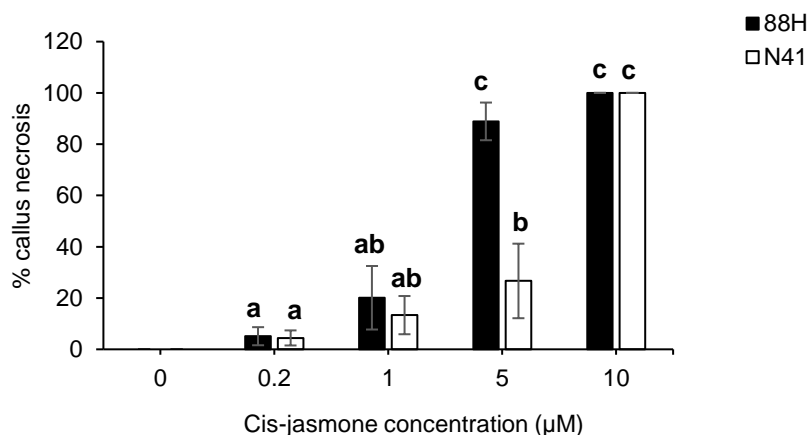


**Figure 9:** The effect of priming agent concentration on % explants producing callus in two sugarcane cultivars after 8 weeks on embryo maturation medium containing either a) cis-jasmone or b) hexanoic acid. Dissimilar alphabet characters denote a statistically significant difference between the cultivars for each concentration. Data sets were analysed by One-way ANOVA, Duncan's multiple range test,  $p < 0.001$ ,  $n = 9$ , mean  $\pm$  SE.

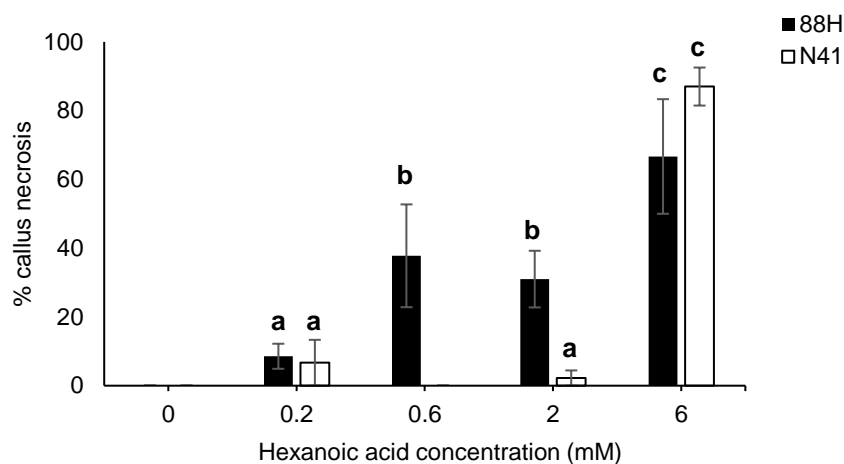
In addition to callus production, callus necrosis was also selected as a parameter to test the effect of the priming agents and select an appropriate concentration for callus production and plantlet regeneration in subsequent studies to establish a protocol for the treatment with selected priming agents. Callus necrosis was recorded if at least 50 % of a callus piece turned brown or black after 3 weeks in culture (Mahlanza et al., 2013). Callus necrosis for each treatment was recorded after 8 weeks in the dark on embryo maturation media containing either CJ or Hx (Fig. 10a, b). No callus necrosis was observed in the control calli of both cultivars (Fig. 10a). For both cultivars, there was a significant difference in callus necrosis between the controls and the CJ treatments ( $p < 0.001$ ), with increasing CJ concentrations significantly increasing necrosis. For both cultivars, the highest negative effect (100 %) on callus necrosis was recorded on media containing 10  $\mu\text{M}$  CJ (Fig. 10a). There were no significant differences in callus necrosis amongst the control and the 0.2 and 1  $\mu\text{M}$  CJ treatments ( $p > 0.001$ ). There were only significant differences in percentage explants producing callus amongst the 5  $\mu\text{M}$  CJ (89 %  $\pm$  7.35 for 88H0019; 27 %  $\pm$  14.53 for N41) and the 10  $\mu\text{M}$  CJ (100 %  $\pm$  0 for 88H0019; 100%  $\pm$  0 for N41) treatments compared with the control ( $p < 0.001$ ) (Fig. 10a).

No callus necrosis was recorded for the control plantlets (Fig. 10b). For cultivar 88H0019, there was a significant increase in callus necrosis with an increase in Hx exposure. The media containing 6 mM Hx had the highest negative effect (67 %) on callus necrosis for both cultivars (Fig. 10b). There were no significant differences in callus necrosis observed amongst the 0, 0.2 (88H0019 and N41), 0.6 (N41), and 2 mM (N41) Hx treatments. For 88H0019, there were significant differences in callus necrosis recorded for cultivar 88H0019 between the 0.6 (38 %  $\pm$  14.95), 2 (31 %  $\pm$  8.24), and 6 mM Hx treatments (67 %  $\pm$  16.67) compared with the control. There was a significant negative effect on callus necrosis by 6 mM Hx (87 %  $\pm$  5.53), compared with the control and the other Hx treatments ( $p < 0.001$ ) (Fig. 12 b). In conclusion, the highest tested concentration of CJ (10  $\mu\text{M}$ ) and Hx (6 mM) resulted in the lowest callus production and highest callus necrosis for both cultivars (Fig. 10a, b).

a)



b)



**Figure 10:** The effect of priming agent concentration on % callus necrosis on embryo maturation medium containing either a) cis- jasmone or b) hexanoic acid. Dissimilar alphabet characters denote a statistically significant difference between the cultivars for each concentration. Data sets were analysed by One-way ANOVA, Duncan's multiple range test,  $p < 0.001$ ,  $n = 9$ , mean  $\pm$  SE.

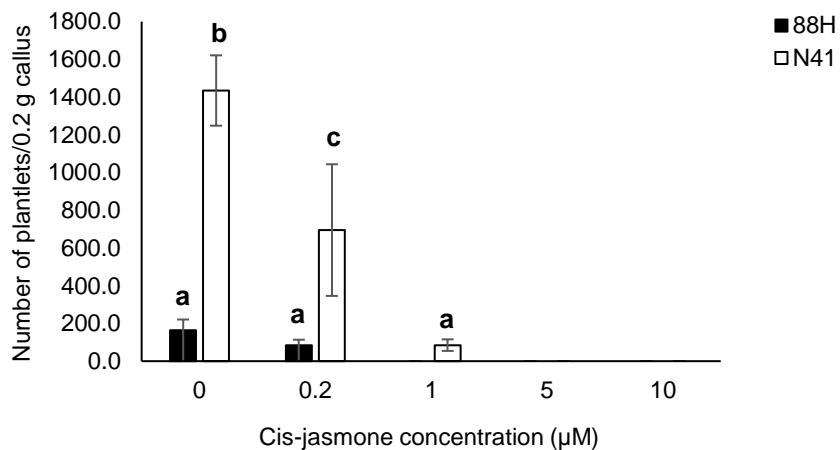
#### 4.1.2 Comparison of plantlet yield for both priming agents

Embryogenic calli were produced on EIM without priming agents for 8 weeks, after which they were transferred to EMM for 1 week. They were then cultured on EGM containing 0, 0.2, 1, 5, and 10  $\mu$ M CJ or 0, 0.2, 0.6, 2, and 6 mM Hx, and number of plants/ 0.2 g callus was recorded after 6 - 12 weeks. Plantlet yield on both the CJ and Hx treatments was negatively affected by high concentrations of the priming agents for the two cultivars (Fig. 11a, b). The plantlet yield of N41

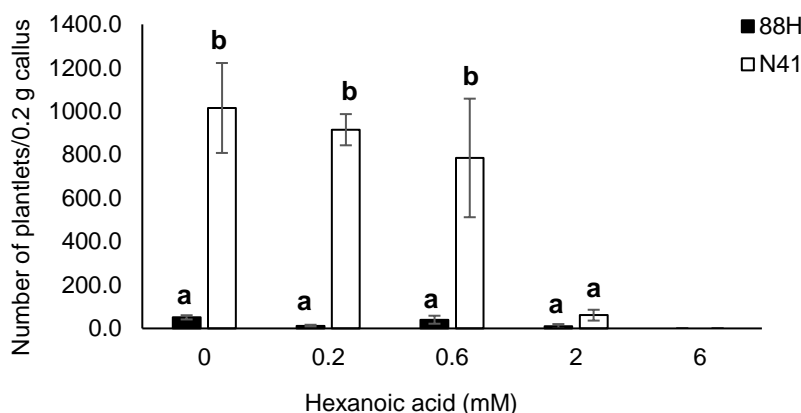
supplied with CJ significantly decreased in the 0.2 (695 plantlets/0.2 g callus  $\pm$  349.0) and 1  $\mu$ M CJ treatment (85 plantlets/0.2 g callus  $\pm$  30.79), when compared with the control (1435 plantlets/0.2 g callus  $\pm$  186.57). For both cultivars, there were no plantlets produced when exposed to the 5 and 10  $\mu$ M CJ treatments and the results indicated a negative effect of the increasing supply of CJ concentrations ( $p < 0.001$ ) (Fig. 11a). There was no significant difference in the number of plants/ 0.2 g of callus produced for cultivar 88H0019 across all the tested concentrations of CJ ( $p > 0.001$ ).

In the Hx treatments, there was no significant difference in plantlet yield between the control and all the treatments for cultivar 88H0019 (Fig. 11b). There was a significant decrease in the number of plantlets/0.2 g of callus amongst the control (1015 plantlets/0.2 g callus  $\pm$  207.1), 0.2 (915 plantlets/0.2 g callus  $\pm$  71.82), 0.6 (785 plantlets/0.2 g callus  $\pm$  272.93) and 2 mM Hx treatments (61 plantlets/0.2 g callus  $\pm$  24.95) for N41 ( $p < 0.001$ ). The callus of cultivar N41 produced significantly more plantlets than that of 88H0019 at the 0.2 and 0.6 mM Hx treatments ( $p < 0.001$ ).

a)



b)



**Figure 11:** The effect of priming agent concentration on the number of plantlets produced/0.2 g of callus on embryo germination medium containing either a) cis- jasmone or b) hexanoic acid. Dissimilar alphabet characters denote a statistically significant difference amongst cultivars for each concentration. Data sets were analysed by One-way ANOVA, Duncan's multiple range test,  $p < 0.001$ , mean  $\pm$  SE  $n = 4$ .

As expected, the number of plantlets/ 0.2 g of callus is inversely proportional to percentage callus necrosis for both cultivars (Fig. 10, 11). The treatments that showed a high percentage of callus necrosis produced low plantlet yields (5 and 10  $\mu$ M CJ treatments; 2 and 6 mM Hx treatments). The research findings from this investigation showed that Hx resulted in better callus production and plantlet yield than CJ. Hence, of the two priming agents tested, 0.6 mM Hx allowed sufficient callus production and plantlet yield, whilst still likely having a priming effect, and was chosen for further studies.

From the two priming agents tested, more plants were produced using Hx as a priming agent (Table 5). The percentage of plantlets for 88H0019 and N41 were expressed as a percentage of the plants produced in the control. For cultivar N41, the highest plantlet yield was 90 % recorded at 0.2 mM Hx, and for cultivar 88H0019 78 % plantlet yield was recorded at 0.6 mM Hx. For both the callus and plantlet production stages, 0.6 mM Hx was selected as the suitable concentration since it was the only concentration that produced greater than 75 % plantlet yield for both cultivars. The research findings from this investigation showed that Hx produced improved callus production and plantlet yield compared with CJ, and therefore was chosen as a priming agent for further studies.

**Table 5:** Table showing the percentage of plantlets produced for each priming agent at different concentrations for cultivars 88H0019 and N41.

Priming agent	Treatment CJ ( $\mu\text{M}$ ) Hx (mM)	Plantlet yield for 88H0019 (% plants)	Plantlet yield for N41 (% plants)
CJ	0 (control)	100	100
	0.2	51	48
	1	0	6
	5	0	0
	10	0	0
HX	0 (control)	100	100
	0.2	24	90
	0.6	78	77
	2	20	6
	6	0	0

#### 4.2 Establishing callus and *in vitro* plantlet screening conditions using *F. pseudonygamai* culture filtrate

This investigation aimed to establish: the concentration of *F. pseudonygamai* SC17 culture filtrate at which somatic embryos could be screened for resistance to the selected *Fusarium* strain at the embryo germination stage; and a suitable culture filtrate concentration for screening putative mutant plantlets at the end of the plantlet production stage.

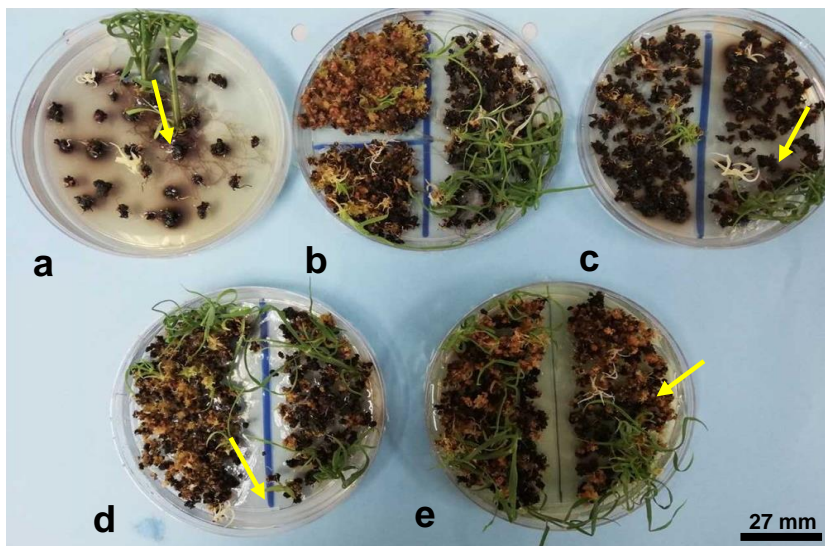
##### 4.2.1 Determining a suitable *F. pseudonygamai* culture filtrate concentration for screening somatic embryos at the embryo germination stage

In this study, different concentrations of *F. pseudonygamai* SC17 CF were incorporated in the embryo germination media to determine the most appropriate one for screening embryos for resistance to the *Fusarium* strain SC17. The embryogenic calli was not treated with priming and mutagenic agents prior to exposure to the CF.

Embryogenic calli were exposed to different concentrations (0, 4, 20, 50, and 100 ppm) of *F. pseudonygamai* CF at the embryo germination stage to determine which CF concentration resulted in callus necrosis, and if it also inhibited embryo germination. The embryogenic callus was produced after 6 - 8 weeks on EIM without CF, which was then transferred to EMM without CF for 3 weeks. Then, embryogenic calli (0.1 g per replicate) was cultured on EGM1 supplemented with 0, 4, 20, 50, and 100 ppm CF. Callus necrosis was measured when at least 50 % of a callus piece had browned, and more calli pieces became necrotic with increasing CF levels in the media (Fig. 12a - e). A significant difference in percentage callus necrosis was recorded between the control (13 %  $\pm$  2) and 100 ppm CF treatment (68 %  $\pm$  10) for cultivar N41 ( $p < 0.001$ ). The only recorded significant difference in percentage callus necrosis between both cultivars was with the 50 ppm CF exposure (88 %  $\pm$  8 for 88H0019; 21 %  $\pm$  9 for N41) ( $p < 0.001$ ; Fig. 13a). After 3 weeks on embryo germination media, some necrotic calli, especially from cultivar 88H0019 that were exposed to 50 - 100 ppm CF, developed root hairs. The percentage callus necrosis for calli transferred to EGM medium containing 4 (73 %  $\pm$  8 for 88H0019; 38 %  $\pm$  7 for N41), 20 (73 %  $\pm$  6 for 88H0019; 35 %  $\pm$  0 for N41) and 50 ppm CF (88 %  $\pm$  8 for 88H0019; 21%  $\pm$  9 for N41) was higher than that of the control (13 %  $\pm$  2) (Fig. 13a). Based on the results obtained, 88H0019 was susceptible to the culture filtrate and produced the highest percentage callus necrosis compared with N41.

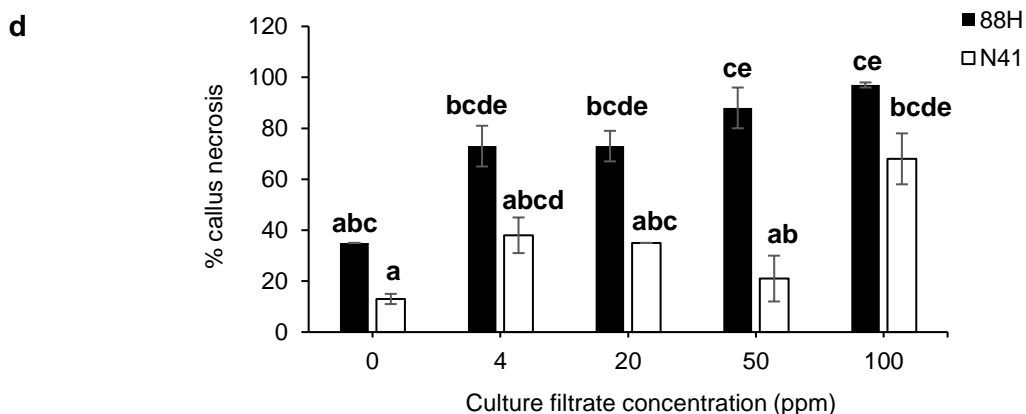
The incorporation of CF in EGM1 resulted in decreased plantlet yield for both cultivars as compared with the EGM1 without CF (Fig. 13b). For cultivar 88H0019, there were no significant differences in the number of plantlets/0.1 g of callus between the control and the 4, 20, 50 and 100 ppm CF. However, the highest CF treatment resulted in significantly fewer plants than the control ( $p < 0.001$ ; Fig. 13b). In cultivar N41, plantlet yield decreased significantly at 100 ppm CF (98 plantlets/0.1 g of callus  $\pm$  29) compared with the control (1020 plantlets/0.1 g of callus  $\pm$  97) and ( $p < 0.001$ ; Fig. 13b). As expected, the results indicate an inverse proportional relationship between the number of plantlets/0.1 g of callus and percentage callus necrosis (Fig. 13a and b). The CF stress at the embryo germination stage resulted in most callus pieces eventually turning black/necrotic, but there was greening of the non-necrotic areas within each callus piece on EGM1 + CF and plantlet yield was recorded by week 12 (Fig. 13b). Plantlets were still produced at the highest CF concentration (100  $\mu$ M) that was tested, however a greater CF concentration should have been analysed. Hence, *F. pseudonygamai* SC17 could not be used as an *in vitro* selection agent at the callus and regeneration stage.

The number of abnormal plantlets/0.1 g of callus from each treatment was also recorded at the end of the embryo germination stage (Fig. 13c). During the germination stage, the CF had a positive effect on the number of abnormal plants/0.1 g of callus produced. There was a significant difference in the number of abnormal plants/0.1 g of callus between both cultivars ( $p < 0.05$ ). In cultivar 88H0019, the number of abnormal plants/0.1 g of callus was significantly higher at 50 ppm CF (21 abnormal plants/0.1 g of callus  $\pm$  10), whereas the 4, 50, and 100 ppm CF treatments did not cause visible abnormalities in N41 plantlets.

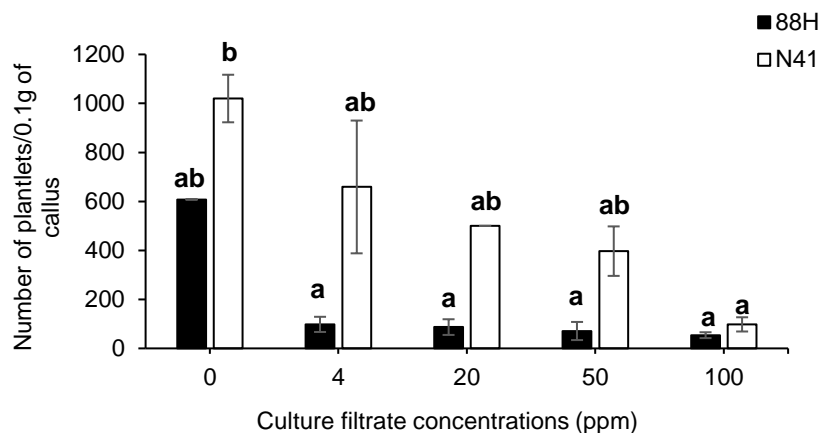


**Figure 12:** The effect of *F. pseudonygamae* CF on callus proliferation and plantlet regeneration in embryo germination media after 7 - 8 weeks with weekly sub-culturing. Embryogenic calli were cultured on EMM-CF for 3 weeks without sub-culturing, before culturing on EGM1 supplemented with: a) 0, b) 4, c) 20, d) 50, and e) 100 ppm. The arrows indicate albino plantlets.

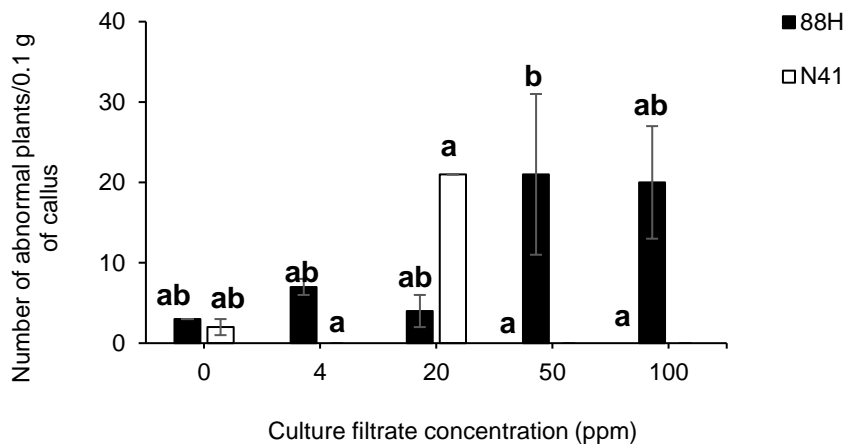
a)



b)



c)

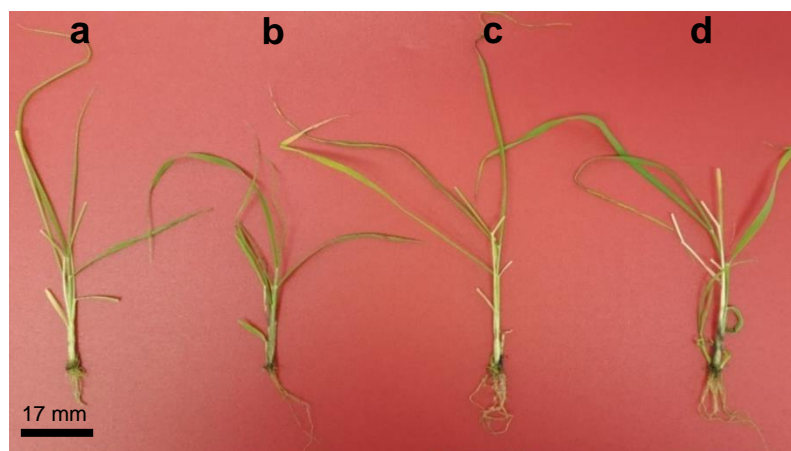


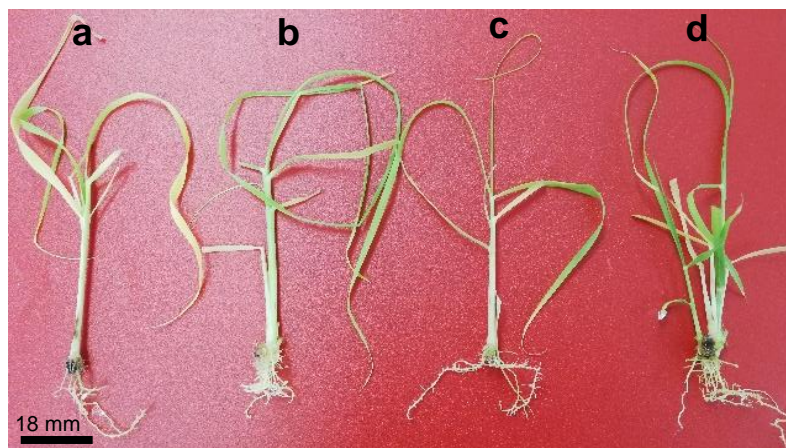
**Figure 13:** The effect of culture filtrate concentration on callus proliferation and plantlet regeneration in embryo germination medium after 4-8 weeks with weekly subculturing. Embryogenic calli were cultured on EMM-CF for 3 weeks without subculturing, before culturing on EGM+CF. a) Percentage callus necrosis; b) Number of plants/ 0.1 g of callus; and c) Number of abnormal plants/0.1 g of callus. Dissimilar alphabet characters denote a statistically significant difference amongst cultivars for each concentration. Data sets were analysed by One-way ANOVA and Sidak post hoc test.  $P \geq 0.05$ ,  $n = 4$ , mean  $\pm$  SE.

#### 4.2.2 Establishing a culture filtrate concentration for screening putative mutant plantlets

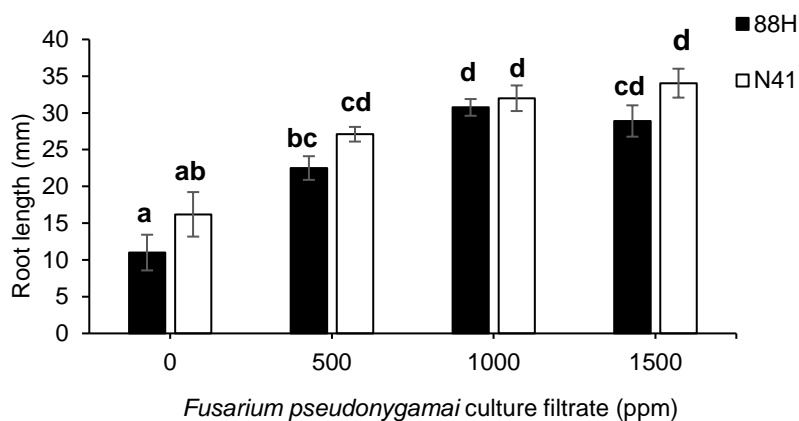
In this investigation, the development of a protocol for selecting plantlets produced from the somatic embryos that survived the CF selection pressure at the embryo germination stage was attempted. This investigation was based on the rooting ability and root length of the plants, under the various treatments, after their roots were trimmed to less than 1 mm in length. Mahlanza et al. (2013) found that CF from the fungal isolate *F. sacchari* PNG40 inhibited root growth of the plants that were trimmed. Those results indicated that improved root growth for mutant plants could be used to select for resistance to toxins present in the CF (Mahlanza et al., 2013).

However, in this study, the addition of different concentrations (0, 500, 1000, 1500 ppm) of CF from the *Fusarium* isolate SC17 to EGM1 enhanced the rooting ability of plantlets (Fig. 14). Plantlets were cultured on EGM1 + CF after their roots were trimmed to less than 1 mm and then transferred to EGM1 containing 0, 500, 1000, and 1500 ppm CF. Root growth was determined after 3 weeks for both cultivars, plants from the CF treatments had an increased root length and slight discoloration at the base of the stem compared with the untreated controls (Fig. 14). Although there was no significant interaction observed between treatment versus cultivar, there was a significant difference in the root length of plantlets amongst the treatments after 3 weeks ( $p < 0.001$ ) (Fig. 15). For both cultivars, root re-growth increased significantly ( $p < 0.001$ ) across all tested CF concentrations. On average, for both cultivars, the controls had significantly shorter root lengths than all other treatments ( $p < 0.001$ ). Plant root length (mm) was significantly higher for cultivar N41 compared with 88H0019 ( $p < 0.001$ ). The results show that all the concentrations of the CF affected the root length of plantlets, with the 1500 ppm CF resulting in the highest root length (31.5 mm  $\pm$  4.3, for 88H0019) and (34.05 mm  $\pm$  3.9, for N41).





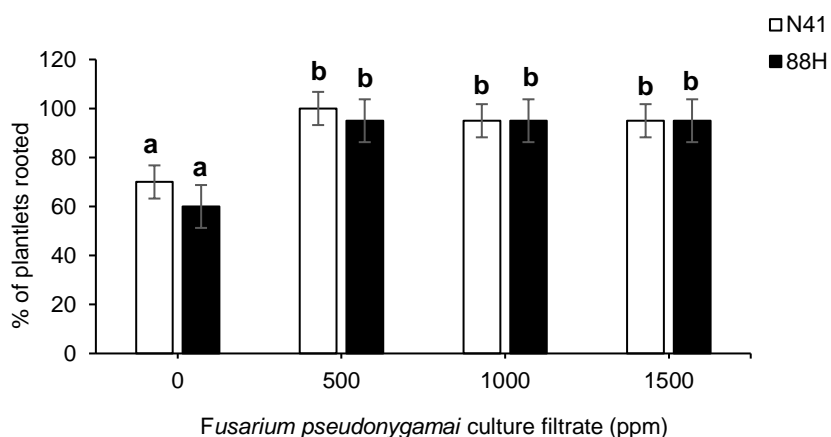
**Figure 14:** The effect of *F. pseudonygamai* culture filtrate (CF) on root growth of plantlets after 3 weeks for cultivars 88H0019 and N41 respectively. a) control, b) 500 ppm, c) 1000 ppm, and d) 1500 ppm CF.



**Figure 15:** The effect of *F. pseudonygamai* culture filtrate (CF) on root growth using plantlets that had their leaves and roots trimmed before being cultured on media with 0 – 1500 ppm CF after 3 weeks. Root length (mm) was measured after 3 weeks. Dissimilar alphabet characters denote a statistically significant difference amongst cultivars for each concentration. Data sets were analysed by One-way ANOVA and Duncan's Multiple Range test,  $P \geq 0.05$ , mean  $\pm$  SE,  $n = 4$ .

The results reported by Mahlanza et al. (2013) indicated that the roots of 50 % of mutant plantlets with trimmed roots cultured on media containing 1500 ppm CF re-regrew to at least 10 mm in length over 3 weeks. In his study he used 10 mm re-growth as the criterion for the selection of CF- tolerant plants. Consequently, this value was set as the criterion for the selection of CF- tolerant plants in the current study.

In this study, there was a significant positive difference in the percentage of plantlets that rooted between the control and all the tested CF concentrations ( $p < 0.001$ ) (Fig. 16). The results recorded for both cultivars indicated a 95 - 100 % rooting ability of the plantlets in EGM1 + CF containing 500, 1000, and 1500 ppm CF, which was significantly higher than the percentage of plantlets that rooted in EGM1 containing no CF (60 - 70 %) ( $p < 0.001$ ). For both cultivars, there was no significant difference between all the tested CF concentrations ( $p > 0.001$ ). In conclusion, the results obtained from this investigation indicated that the *E. saccharina* beneficial strain *F. pseudonygamai* SC17 used in this study cannot be used as an *in vitro* selection agent because of its root promoting property. In many studies the phytotoxicity of *Fusarium* culture filtrates and inhibitory effect on root growth was assessed in crops such as sugarcane (Mahlanza et al., 2013), banana (Rebouças et al., 2021), and maize (Mirsam et al., 2021). Due to the enhanced root growth of the plantlets across all the tested CF concentrations, it was hypothesised that the *E. saccharina* beneficial *Fusarium* strain (SC17) is stimulating root growth by auxin (IAA) production or increased activity of the enzyme ACC deaminase and will be discussed later.



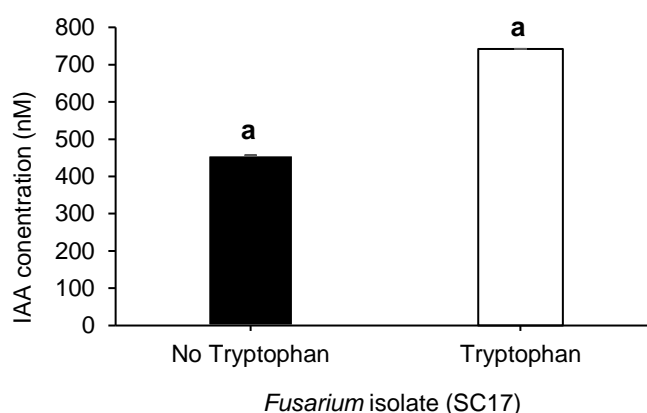
**Figure 16:** The effect of *F. pseudogynamai* culture filtrate (CF) in the re-rooting media on the % of plantlets that re-rooted. Root length (mm) was measured after 3 weeks. Re-rooting defined as roots  $\geq 10$  mm after root triiming to 1mm. Dissimilar alphabet characters denote a statistically significant difference amongst cultivars for each concentration. Data sets were analysed by (One-way ANOVA and Duncan's multiple range test,  $P \geq 0.05$ , mean  $\pm$  SE,  $n = 4$ ).

### 4.3 Screening for indole-3-acetic acid production from isolate *F. pseudonygamai*

Many studies have demonstrated that endophytic fungi can produce phytohormones, especially indole acetic acid (IAA) and gibberellins, as a direct system of plant growth promotion (Fu et al., 2015; Turbat et al., 2020). Therefore, these endophytes are of potential interest since their plant growth-regulating compounds can be used to enhance crop quality and yield. The compounds that are secreted by endophytes can rescue plant growth in a stressful environment and positively influence plant development (Khan et al., 2008; Khan et al., 2011; Khan et al., 2012; Turbat et al., 2020). The results shown in Figs. 15 and 16 (section 4.2.1) indicated a significant positive effect in root growth for cultivars 88H0019 and N41 when exposed to different concentrations of the *F. pseudonygamai* SC17 CF. Hence, an experiment was conducted to determine if this enhanced effect was due to indole-3-acetic acid production by the fungal endophyte by using trimmed plants and culturing them on EGM1 with 0, 150, 300, and 460 nM IAA, and then: a) quantifying the amount of indole-3-acetic acid produced by *F. pseudonygamai* SC17; b) determining the effect of an exogenous supply of indole-3-acetic acid on root growth after 3 weeks; and c) determining the effect of IAA on the fresh and dry mass of roots and shoots after 3 weeks.

#### 4.3.1 To quantify the amount of indole-3-acetic acid produced by *F. pseudonygamai* SC17

The colorimetric method described by Khan et al. (2016) for the quantification of IAA production by microbes was used in this study. To quantify the amount of IAA produced by the *Fusarium* fungal isolate SC17. There was no significant difference in IAA production in the presence or absence of the precursor L-tryptophan by *F. pseudonygamai* ( $p = 0.092$ ) (Fig. 17). However, the results obtained indicated that *F. pseudonygamai* produced the highest IAA concentration (743.1 nM) in the presence of L-tryptophan than in the treatment without L-tryptophan (457.2 nM). The amount of IAA produced from the CF in the rooting experiment was calculated using the concentration of the culture filtrate incorporated in the rooting media (Fig. 15) and the IAA concentration obtained in the treatment without L-tryptophan (Fig. 17) as seen in Table 6. The results indicated that the rooting media containing 1000 and 1500 ppm CF resulted in a significant increase in root length as compared with the control for both cultivars, these were equivalent to 298.2 nM and 447.4 nM IAA, respectively (Table 6).



**Figure 17:** A comparison of the amount of IAA produced by the *F. pseudonygamai* SC17 after incubation for 7 days in potato dextrose broth supplemented with and without 0.1 g L<sup>-1</sup> tryptophan. Dissimilar alphabet characters denote a statistically significant difference. Data sets were analysed by Two-sample T-test; Duncan's multiple range test;  $p > 0.05$ ;  $n = 3$ , mean  $\pm$  SE.

**Table 6:** Indole-3-acetic acid (IAA) production by fungal isolate *F. pseudonygamai* SC17.

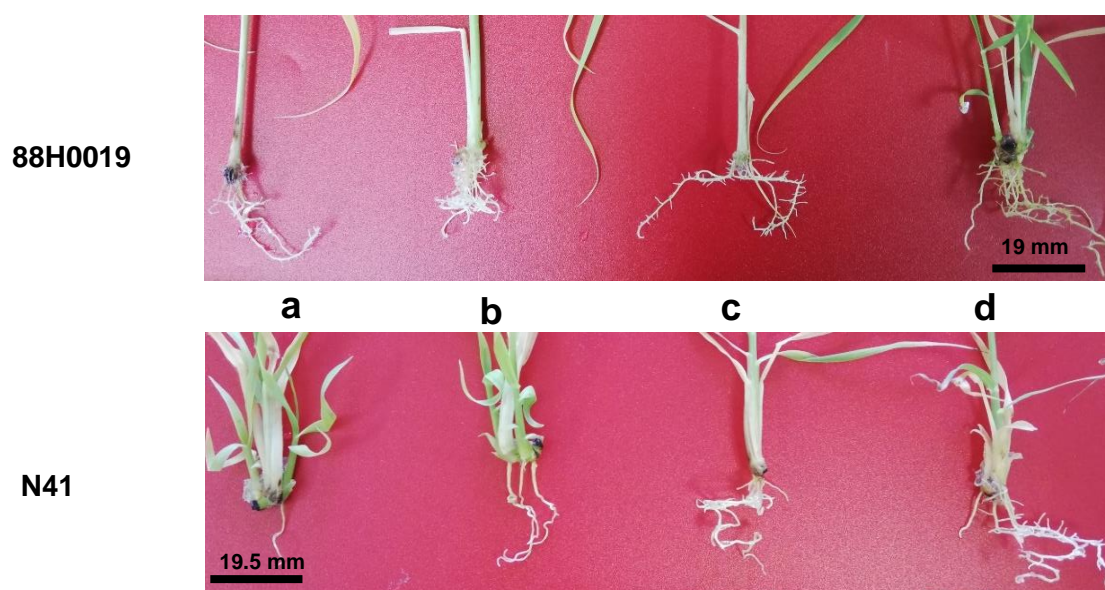
<i>F. pseudonygamai</i> CF concentration in rooting media (ppm)	<i>F. pseudonygamai</i> CF volume (ml) Final volume of 350 ml	Calculated IAA concentration in diluted <i>F. pseudonygamai</i> CF (nM)
0	0	0
500	114.3	149.1
1000	228.6	298.2
1500	342.9	447.4

#### 4.3.2 The effect of exogenous indole-3-acetic acid on root growth after root trimming using *in vitro* plantlets

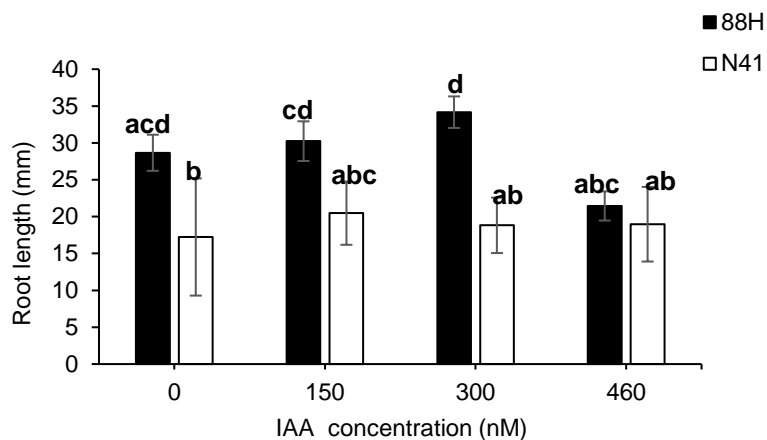
Indole-3-acetic acid is commonly used as a plant hormone to control growth and development in many crops (Cleland, 1987; Rayle and Cleland, 1992; Bunsangiam et al., 2021). Indole acetic acid produced by microbes improves the structure of roots and boosts the availability of nutrients to microbial endophytes (Soliman et al., 2020; Ismail et al., 2021). To determine the effect of IAA on root re-growth, root length, and on the fresh and dry weight of the roots and shoots, different concentrations of IAA (0, 150, 300, and 460 nM) were incorporated into the rooting medium for 3

weeks. The highest concentration of IAA used in this experiment simulates that produced in the 1500 ppm CF. Rooted plantlets were used for this investigation, and were prepared as for previous studies (i.e., roots trimmed to  $\pm 1$  mm, and leaves to just above the ligule as per Mahlanza et al. 2013).

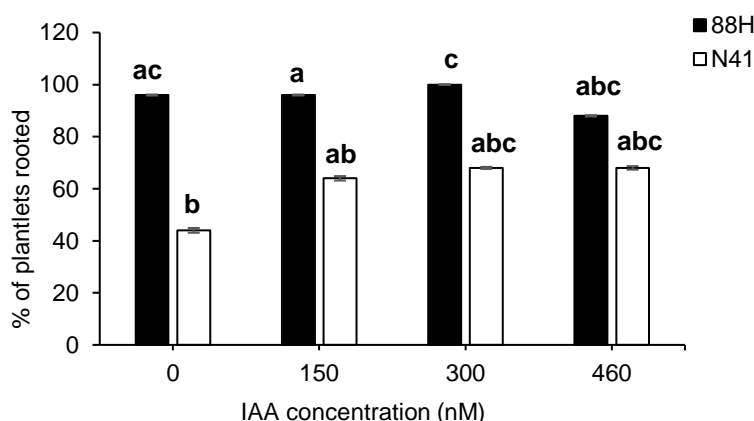
The effect of IAA on the root growth and the percentage of plantlets rooted was recorded after 3 weeks. The results indicated a positive effect on root length of the plantlets that were supplied with exogenous IAA (0, 150, 300, and 460 nM) after 3 weeks in culture (Fig. 18). For cultivar 88H00019, the root length was significantly higher at 300 nM ( $34.17 \text{ mm} \pm 2.14$ ) than 460 nM IAA treatment ( $21.45 \text{ mm} \pm 1.98$ ) (Fig. 19). For cultivar N41, there was no significant difference in root length across all the tested IAA concentrations. However, there were significant differences in root length between both cultivars ( $p < 0.001$ ). In cultivar 88H0019, root length was significantly higher at 300 nM IAA ( $34.17 \text{ mm} \pm 10$ ) compared with the control ( $17.24 \text{ mm} \pm 7.95$ ), 150 ( $20.47 \text{ mm} \pm 4.29$ ), 300 ( $18.82 \text{ mm} \pm 3.76$ ) and 460 nM IAA ( $18.97 \text{ mm} \pm 5.06$ ) treatments for cultivar N41 (Fig. 19). For both cultivars, the results indicated that there were no significant differences in the percentage of plantlets that rooted across all the tested IAA concentrations ( $p > 0.05$ ) (Fig. 20). However, the percentage of plantlets that rooted for cultivar 88H0019 was significantly higher at the 300 nM IAA ( $100 \% \pm 0.00$ ) treatments than control ones ( $44 \% \pm 0.92$ ) and 150 nM IAA ( $64 \% \pm 0.86$ ) for cultivar N41 ( $p = 0.008$ ).



**Figure 18:** The effect of IAA on root growth of plantlets over 3 weeks for cultivars 88H0019 and N41. a) control; b) 150 nM, c) 300 nM, and d) 460 nM IAA.



**Figure 19:** The incorporation of IAA (0 - 460 nM) in the rooting media and its effect on the root length of plantlets for cultivars 88H0019 and N41. The results were obtained after 3 weeks. Root length was only recorded for roots  $\geq 10$  mm. Dissimilar alphabet characters denote a statistically significant difference amongst cultivars for each concentration. Data sets were analysed by One-way ANOVA, Duncan's multiple range test.  $p < 0.05$ ,  $n = 5$ , mean  $\pm$  SE.

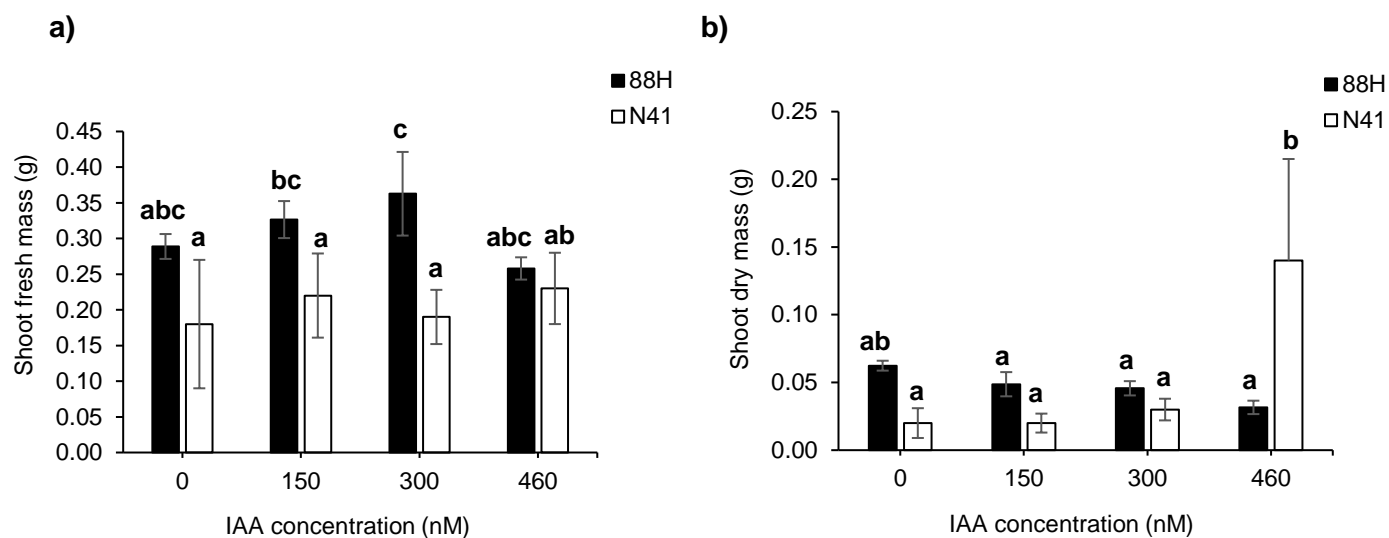


**Figure 20:** The incorporation of IAA (0 - 460 nM) in the rooting media and its effect on the % of plantlets rooted for cultivars 88H0019 and N41. The results were obtained after 3 weeks. Re-rooting is defined as roots  $\geq 10$  mm. Dissimilar alphabet characters denote a statistically significant difference amongst cultivars for each concentration. Data sets were analysed by One-way ANOVA, Duncan's multiple range test.  $p \geq 0.05$ ,  $n = 5$ , mean  $\pm$  SE.

In terms of root and shoot fresh mass for cultivar 88H0019, there were no significant differences in shoot fresh mass (g) amongst all the tested IAA concentrations (Fig. 21a). However, for cultivar 88H0019, the shoot fresh mass (g) at 300 nM IAA ( $0.363 \text{ g} \pm 0.059$ ) was significantly higher than

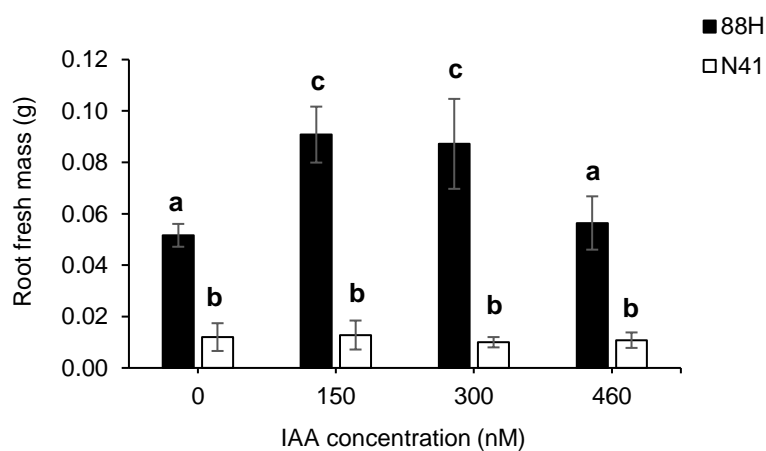
all the tested IAA concentrations for cultivar N41; control ( $0.180 \text{ g} \pm 0.09$ ), 150 ( $0.220 \text{ g} \pm 0.06$ ), 300 ( $0.190 \text{ g} \pm 0.04$ ) and 460 nM IAA ( $0.230 \text{ g} \pm 0.05$ ) ( $p = 0.004$ ). For cultivar N41, there was a significant increase in shoot dry mass (g), recorded at 460 nM IAA ( $0.140 \text{ g} \pm 0.08$ ) when compared with the control and the other IAA treatments ( $p = 0.009$ ) (Fig. 21b).

For cultivar N41, the root fresh mass (g) of plantlets was not significantly affected by the exogenous supply of IAA at any of the tested concentrations (Fig. 22a). However, for cultivar 88H0019 the results recorded indicated a significant increase in root fresh mass (g) for all the tested IAA concentrations ( $p < 0.001$ ). For cultivar 88H0019, the root fresh mass (g) for 150 nM ( $0.091 \text{ g} \pm 0.011$ ) and 300 nM IAA treatments ( $0.087 \text{ g} \pm 0.017$ ) were significantly higher than the control ( $0.052 \text{ g} \pm 0.004$ ) and 460 nM IAA treatments ( $0.056 \text{ g} \pm 0.010$ ) ( $p < 0.001$ ). For cultivar N41, there was no significant difference in root dry mass (g) for all the tested IAA concentrations (Fig. 24b). For cultivar 88H0019, the results indicate a significant decrease in root dry mass (g) across the tested IAA concentrations ( $p < 0.001$ ) (Fig. 22b). The 150 nM IAA treatment ( $0.034 \text{ g} \pm 0.004$ ) had a higher root dry mass (g) than the other IAA treatments (Fig. 22b). In conclusion, for both cultivars, the results obtained indicate that the exogenous application of IAA has no significant positive effect on *in vitro* plantlets in terms of root length and % rooting whereas the CF does, suggesting that the enhanced root growth could be due to auxin (IAA) production or ACC deaminase activity by the CF (Jaroszuk-Ścisiel et al. 2019). The CF was not included as part of the selection protocol because of the confounding effects observed on the root characteristics of plantlets and callus proliferation after exposure to the *E. saccharina* beneficial *Fusarium* strain (SC17).



**Figure 21:** The incorporation of IAA (0 - 460 nM) in the rooting media and its effect on a) shoot fresh mass (g) and b) shoot dry mass (g) of *in vitro* plantlets for cultivars 88H0019 and N41. The results were obtained after 3 weeks in culture. Fresh and dry mass was recorded for plantlets that have re-rooted to  $\geq 10$  mm. Dissimilar alphabet characters denote a statistically significant difference amongst cultivars for each concentration. Data sets were analysed by (One-way ANOVA, Duncan's multiple range test,  $p < 0.05$ ,  $n = 5$ , mean  $\pm$  SE).

a)



b)

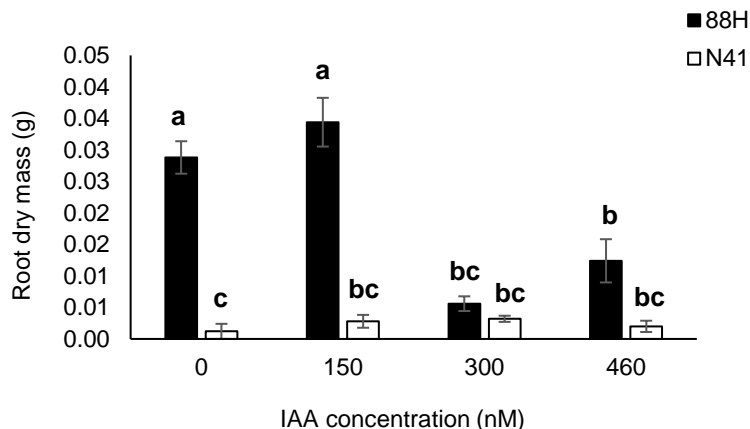


Figure 22: The incorporation of IAA (0 - 460 nM) in the re-rooting media and its effect on a) root fresh mass (g) and b) root dry mass (g) for cultivars 88H0019 and N41. The results were obtained after 3 weeks in culture. Fresh and dry mass was recorded for plantlets that have re-rooted to  $\geq 10$  mm. Dissimilar alphabet characters denote a statistically significant difference amongst cultivars for each concentration. Data sets were analysed by One-way ANOVA, Duncan's multiple range test,  $p < 0.05$ , mean  $\pm$  SE,  $n = 5$ .

#### 4.4 Mutagenesis and priming for *Fusarium pseudonygamai* and *E. saccharina* tolerance *in vitro*

The objective of this study was to establish a protocol for developing putative sugarcane mutants that had been exposed to a suitable priming agent and with/without a mutagenic treatment. The chemical mutagens that was used in this study were 16 mM ethyl methanesulfonate (EMS) and 100  $\mu$ M 5-AzaCytidine (5-AzaC) according to Koch et al. (2012) and Munsamy et al. (2013) respectively. The purpose of using a combination of mutagens was to generate epigenetic (5-AzaC via demethylation; Grzybkowska et al., 2018) and genetic (EMS induces point mutations; Hoffmann, 1980; Lethin *et al.*, 2020) variation in the cells. To supplement this approach, *in vitro* priming of the demethylated epigenetic state during remethylation (Hx primed) could further increase *E. saccharina* resistance both directly, through enhancing JA responses, and indirectly by reducing susceptibility to *Fusarium* mycotoxins (Ravensdale et al., 2014; Llorens et al., 2016).

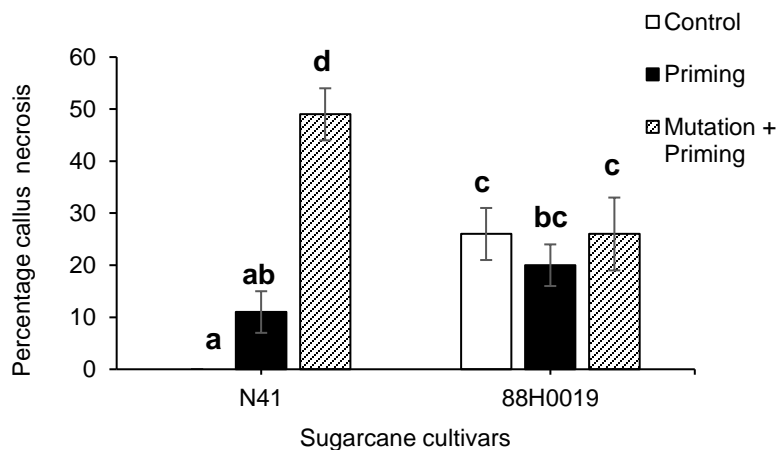
For this study, the priming agent Hx was selected based on the preliminary results obtained for determining the most effective concentration of the priming agent concentration at the embryo maturation and plantlet regeneration stages (section 4.1). To develop a protocol that combined

mutagenesis followed by priming or priming only, the following treatments were investigated: i) no priming and no mutagens (control); ii) primed only with hexanoic acid; and iii) primed with hexanoic acid and exposed to two mutagens, EMS and 5-AzaC. Due to technical difficulties the protocol development for using CF as a selection agent at the embryo germination and regeneration stage was not conducted. Hence, it was decided that mutated/primed plantlets would be produced (as mentioned in section 3.3) as unselected 'families' (not selected using CF *in vitro*) but rather screened with *Fusarium* SC17 and *E. saccharina* larvae *ex vitro* at the plantlet stage.

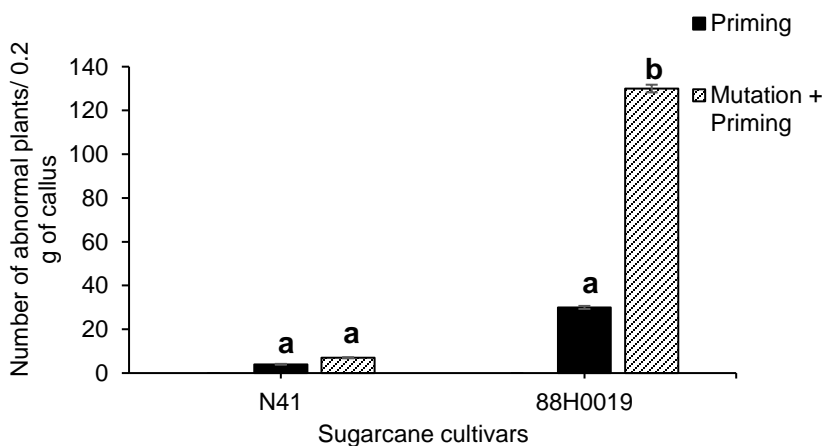
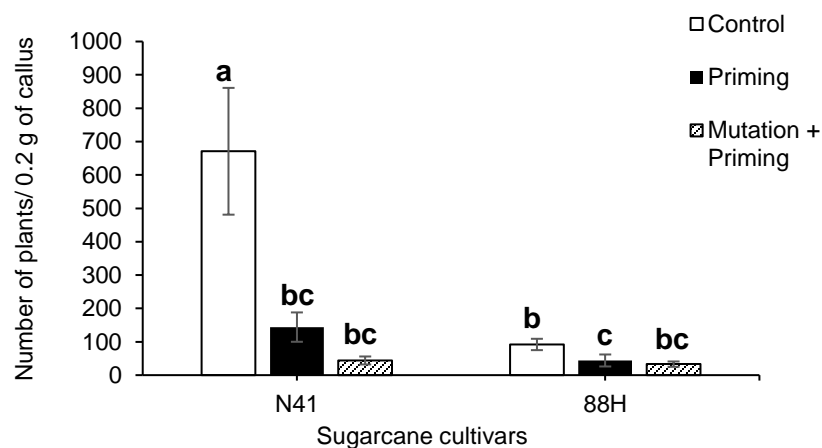
For the protocol that exposed calli to the priming agent only, eight-week-old embryogenic calli were cultured on EMM media with no exposure to mutagens for 1 week (Fig. 7a). The calli were exposed to the priming agent only at the embryo maturation and germination stages. The embryogenic calli (0.2 g) were cultured on EMM with 0.6 mM Hx for 2 weeks, cultured on EGM1, then cultured on EGM1 containing the same concentration of Hx as mentioned above. For the combined treatment (primed + mutated), the embryogenic callus produced after 8 weeks were exposed to both mutagens and the priming agent. Based on the results shown in Munsamy et al. (2013), the callus was exposed to 32 mM EMS for 4 hours and cultured on EMM with 100  $\mu$ M 5-AzaC for 1 week. Thereafter, the calli was cultured on EMM with 0.6 mM Hx for 2 weeks. The surviving calli were cultured on EGM1 with the same concentration of Hx for 4 - 12 weeks and maintained in the light room. The cultures were then assessed in terms of % callus necrosis, the number of plants/0.2 g of callus, and the number of abnormal plants/0.2 g of callus. As before a piece of callus was considered necrotic if greater than 50 % appeared brown to black.

For both treatments, it was observed that each cultivar responded differently (Fig. 23a). For cultivar N41, there was no significant difference in callus necrosis between the control and the treatment that used a priming agent only. As expected, no callus necrosis was recorded for the control for cultivar N41 as they had not been exposed to the priming agent at the maturation and germination stages. However, for cultivar 88H0019 a low percentage of callus necrosis was recorded. The response of different sugarcane cultivars *in vitro* is variable, and this may be the reason for the difference in callus necrosis observed between the controls for both cultivars. For cultivar 88H0019, there were no significant differences in callus necrosis for both treatments when compared with the control ( $p > 0.001$ ) (Fig. 23a). There was a significant negative effect on callus necrosis by the combined treatment (mutagens + priming agent) ( $49 \% \pm 5$ ) than control and the other treatment for both cultivars ( $p > 0.001$ ).

a)



b)



**Figure 23:** The effect of priming calli and *in vitro* plantlets with Hx and a combined treatment (5-AzaC, EMS, and Hx). Results were recorded after 8-12 weeks. a) percentage callus necrosis; b) number of plants/0.2 g of callus; and the number of abnormal plants/0.2 g of callus. Data sets were analysed by One-way ANOVA, Duncan's multiple range test,  $p < 0.001$ ,  $n = 20$ , mean  $\pm$  SE.

The surviving calli were then transferred on EGM1 containing the selected concentration of Hx (0.6 mM) and the number of plants/ 0.2 g callus was recorded (Fig. 23b). Plantlet production was recorded across all treatments and both cultivars. There was a significantly higher number of plants produced/0.2 g of callus for the control for cultivar N41 (671 plantlets/0.2 g of callus) than the other two treatments for both cultivars ( $p < 0.001$ ). There were no significant differences in the number of plants produced/0.2 g callus between the primed and the combined treatment of 5-AzaC, EMS, and Hx for both cultivars ( $p > 0.001$ ). As expected, there was an inverse proportional relationship between percentage callus necrosis and the number of plants/0.2 g callus, with the treatments that recorded the highest callus necrosis produced the lowest number of plants (Fig. 23a, b). In conclusion, the results indicated that the combined treatment (mutagens + priming) resulted in high callus necrosis and a small number of plants/0.2 g of callus.

Plantlets that were derived from the primed, and mutation + primed treatment-derived calli were developed slowly during the germination stage, as it took 10 - 12 weeks to get shoots (approximately 15 mm in height) compared with those from the control treatment which took 8 weeks. For both cultivars, the controls did not produce abnormal plants. A small percentage of abnormal plants, appearing albino and chimeric/dwarf-like were recorded for both treatments (Fig. 23c). There were no significant differences across the treatments for cultivar N41. However, for cultivar 88H0019 the number of abnormal plants produced from the mutation + primed treatment (130 abnormal plantlets  $\pm$  1.76) was significantly higher than the priming treatment ( $p < 0.001$ ). The results obtained indicate that EMS exposure inhibits callus proliferation and development. Kona et al. (2019) reported on the effect of high concentrations of EMS and 2,4-D, in which higher concentrations inhibited callus proliferation.

#### 4.5 Selection of plants tolerant to only *F. pseudonygamai* inoculation, dual inoculation with *E. saccharina* larvae and *F. pseudonygamai* using an *ex vitro* screening method

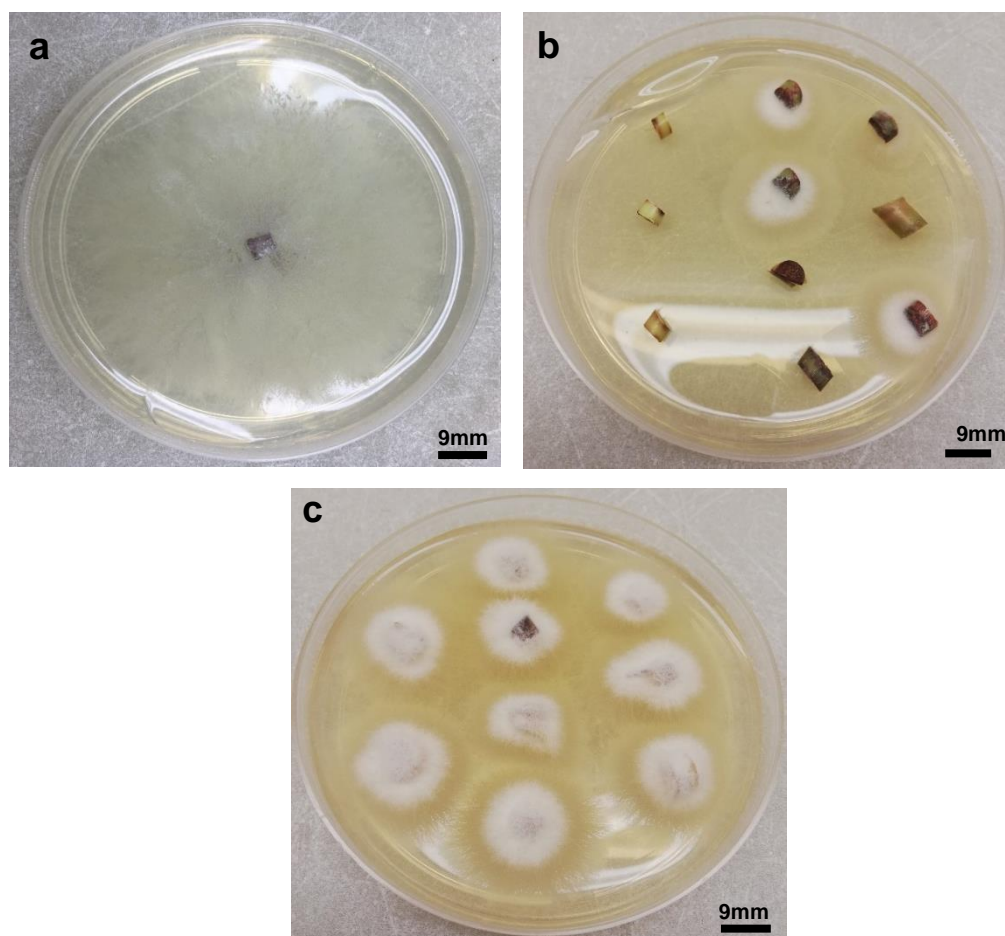
To evaluate the best protocol to achieve *E. saccharina* resistant mutants, chemical mutagenesis followed by priming or priming only was conducted on embryogenic calli and plantlets. Thereafter, the putative mutants were screened *ex vitro* by inoculating the plants with *F. pseudonygamai* SC17 and *E. saccharina* larvae. Plants used for screening were produced by exposing embryogenic calli to 5-AzaC and EMS, and then primed with Hx or exposed to the priming agent only using the established protocol. From each treatment, fifty *in vitro* plantlets were selected

randomly for acclimatisation. Plantlets were acclimatised for 8 - 9 months before they were used for *ex vitro* screening. For each treatment, plants were divided equally depending on the number of plants that survived acclimatisation. These plants were then tested for their response to *E. saccharina* and *F. pseudonygamai* SC17 inoculations in the glasshouse.

The objective of this study was to determine response to tissue colonisation by *Fusarium pseudonygamai* SC17 and combined fungal and *E. saccharina* larval inoculations in sugarcane cultivars N41 and 88H0019. Hence, this investigation focussed on two approaches to test for tolerance *E. saccharina* and *F. pseudonygamai*. Half of the *in vitro* plants were inoculated only with *Fusarium* SC17, using the toothpick inoculation method. The other half of the plants were dual inoculated: once with *Fusarium* SC17, using the toothpick inoculation method and 2 weeks later with 1-2 2<sup>nd</sup> instar *E. saccharina* larvae that were placed into the leaf whorls. To confirm tolerance of the putative mutant plants to *E. saccharina* and *F. pseudonygamai* SC17, fungal isolations were performed on the stem sections from above the inoculation lesion from symptomatic and asymptomatic plants. Lesion severity ratings were recorded for these plants.

#### 4.5.1 Re-isolation of *F. pseudonygamai* SC17 from putative-tolerant plants

The presence of *F. pseudonygamai* SC17 was confirmed in stems of asymptomatic and symptomatic plants two months after inoculation, by surface sterilizing and culturing transverse sections of the stem on selective Nash and Snyder (1962) medium (Fig. 24a - c). To confirm the effectiveness of the surface sterilization, stem sections were pressed on PDA media to test for microbial growth, of which all samples were negative. *Fusarium*-like colonies grew from the lesions of both the asymptomatic and symptomatic stem sections. There was no growth observed from the stem sections of the non-inoculated plants. *F. pseudonygamai* could not be re-isolated from undamaged tissue above the inoculation lesion in two of the fifteen asymptomatic plants from all treatments (Table 7 and 8), for both cultivars that were only inoculated with only SC17. The fungus was retrieved from the other thirteen asymptomatic plants. *F. pseudonygamai* could not be re-isolated from the undamaged tissue in five of the eight asymptomatic plants for N41 and 88H0019 from all treatments for putative mutants that were dual inoculated with the fungus and *E. saccharina* larvae (Table 9 and 10). The fungus was retrieved from the stem sections of both symptomatic and asymptomatic plants.

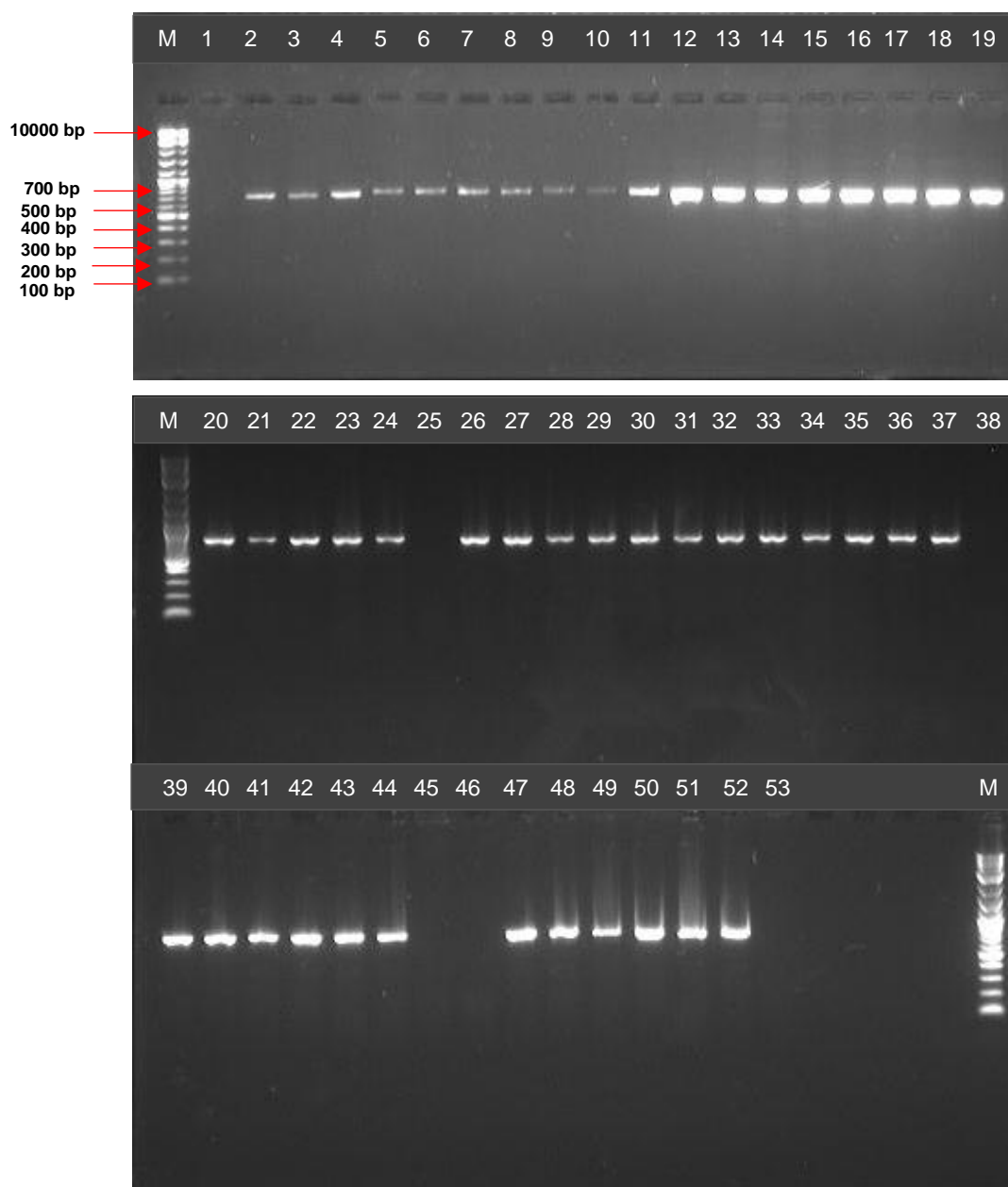


**Figure 24:** A visual comparison of *F. pseudonygamai* SC17 and the fungal isolates that were retrieved from the inoculated plants, after two months. a) SC17; b) fungus re-isolated from damaged stem sections from an inoculated plant treated with only the priming agent Hx (cultivar N41) and c) fungus re-isolated from damaged stem sections from an inoculated control plant (cultivar 88H0019).

#### 4.5.2 Detection and confirmation of the identity of *F. pseudonygamai*

To confirm the identity of the fungal isolates obtained from asymptomatic, symptomatic, and dead plants, fungal genomic DNA was extracted. Molecular analysis was conducted using elongation factor (EF) primers and banding patterns were compared with that of *F. pseudonygamai* SC17 (positive control) to confirm their identity. For both cultivars, the banding patterns generated from all the retrieved fungal isolates were like SC17 (positive control) for all the treatments (Fig. 25). However, some fungi that were re-isolated from a few plants for both cultivars could not be

identified. These isolates that were retrieved and could not be confirmed were from the following treatments: a) Lane 25 - combined mutagenic and primed treatment for cultivar N41; b) Lane 38 - primed with hexanoic acid treatment for cultivar N41; c) Lane 45 - 46 – cultivar 88H0019 control plants; and d) Lane 53 - combined mutagenic and primed treatment for cultivar 88H0019 (Fig. 25).



**Figure 25:** A comparison of banding patterns of the fungal isolates obtained from the inoculated plant tissue and *F. pseudonygamai* SC17 using the elongation factor primers. The isolates were retrieved from symptomatic plants, dead plants and asymptomatic plants by surface sterilizing

leaves and stems and placing cut sections on Nash and Snyder agar. 1kb plus DNA ladder, Lane 1 - negative control (water), 2 - SC17 isolate

#### 4.5.3 *Ex vitro* screening of plantlets using *F. pseudonygamai* SC17 and *E. saccharina* larvae

The infected plants exhibited symptoms after being inoculated with only *F. pseudonygamai* and dual inoculated with *F. pseudonygamai* SC17 and *E. saccharina* (Fig. 26a - d). The reaction to SC17 and *E. saccharina* of the putative mutant plants was confirmed by re-isolating the fungus as well as assessing the lesion severity of the stalks. Lesion severity is a parameter of plant disease intensity that is usually required for the comparison of phenotypes for disease resistance, understanding yield loss, and assessing the effects of different treatments on disease (Kranz, 1988; Bock et al., 2010; Bock et al., 2021). Lesion severity ratings were recorded as 0= no lesions, 1= mild, 2= moderate and 3= severe (Fig. 27a - d).

For cultivar N41, four of the seven control plants and all the 88H0019 control plants, which were inoculated with SC17 colonised toothpicks displayed symptoms. These results are per the current *E. saccharina* rating [N41: intermediate-resistant to *E. saccharina* (Zhou, 2013) and 88H0019: susceptible to *E. saccharina*]. These control plants were regarded as susceptible with a mean lesion severity rating (LSR) of 2. There was only 1 dead plant for cultivar 88H0019 after 2 months. Three plants from the control for cultivar N41 were asymptomatic and showed a mean LSR of 2, these were regarded as resistant. There were no adverse effects seen on the plants that were stabbed with sterile toothpicks (negative control) (Table 7 and 8). For cultivar N41, nine out of the twenty-one putative mutants were asymptomatic and exhibited a mean LSR 1, 8 weeks after toothpick stab inoculation with only the *Fusarium pseudonygamai* SC17 culture filtrate. For cultivar 88H0019, three of the eleven putative mutants were asymptomatic. For both cultivars, re-isolation of the fungal isolates from the lesion area and/or undamaged tissue from all the asymptomatic plants were conducted. Re-isolation of *Fusarium* from the undamaged tissue from seven of these plants indicated that they permitted potential endophytic colonisation by *F. pseudonygamai* SC17 and were regarded as being tolerant to the fungus (Table 7). There was one asymptomatic plant from N41 in which the fungus was re-isolated from the lesion only, with no lesion severity. As a result, this plant was classified as resistant to the fungus as it appeared to limit *F. pseudonygamai* SC17 proliferation and growth (Table 7). All six control plants for 88H were susceptible with a mean LSR of 2 (Table 8).



**Figure 26:** Illustration showing dead plants and plants with symptoms after inoculation with *Fusarium pseudonygamai*. a) dead growing point; b) dead plant 7-8 weeks after inoculation; c) chlorosis and necrosis; d) crinkling and chlorosis 3-7 weeks after stabbing stems with *Fusarium pseudonygamai* colonised toothpick

Some of the putative mutant plants were dual inoculated, i.e., were inoculated with *F. pseudonygamai* SC17 and 2 weeks later with 1-2 2<sup>nd</sup> instar larvae that were placed into the leaf whorls the plants. The tolerance and resistance of these *in vitro* mutated plants were confirmed using the same method for the plants inoculated with only *F. pseudonygamai* SC17. For both cultivars, there were no plants from the control that were asymptomatic (Table 9 and 10). For cultivar N41, two months after the dual inoculation, seven out of the twenty inoculated plants from both treatments were asymptomatic and exhibited a mean LSR of 1. The fungal isolate was isolated from the undamaged tissue of three of these plants and was regarded as being tolerant to the fungus (Table 9). The other five symptomless plants were regarded as resistant since the fungus was only re-isolated from the inoculated lesions, this includes treatments 1-2 for N41 (Table 9). For 88H0019, there was one out of nine plants for both treatments that were asymptomatic with an LSR of 2, this was regarded as resistant. (Table 10). All the 88H0019 control plants exhibited severe symptoms and were regarded as susceptible.

**Table 7:** A summary of the inoculated plants and their responses to tissue colonisation by *F. pseudonygamai* SC17 in sugarcane cultivar N41, 2 months after toothpick stab inoculation method. Plants were inoculated with only *Fusarium* SC17. The plants that were used for *ex vitro* screening were exposed to chemical mutagens followed by priming (treatment 1) or exposed to only priming (treatment 2). The external symptoms were recorded as: 0- no symptoms, 1- crinkled leaves, 2- chlorotic leaves, 3- necrotic leaves, 4- dead growing point, 5- wilting, and 6- all symptoms. Lesion severity ratings were assessed using a scoring system: 0- no lesion, 1- mild lesion, 2- moderate lesion, and 3- severe lesion.

Treatment	Line number	External symptoms	Severity of lesion	Dead (D) or Alive (A)	Re-isolation on NS agar from lesion	Re-isolation on NS agar from undamaged areas above lesion	Tolerant, Susceptible or Resistant
<b>Control</b> (uninoculated)	#1	0	0	A	N	N	-
	#2	0	0	A	N	N	-
	#3	0	0	A	N	N	-
<b>Control</b> (inoculated)	#5	2	2	A	Y	N	S
	#9	0	2	A	Y	N	R
	#9	2	1	A	Y	N	S
	#10	0	2	A	Y	N	R
	#13	2	3	A	Y	N	S
	#15	2	2	A	Y	N	S
	#15	0	3	A	Y	N	R
<b>Treatment 1</b> ( <i>In-vitro</i> mutagenesis with Azac and EMS + <i>in-vitro</i> priming with hexanoic acid)	#6	2	0	A	N	Y	S
	#6	2	0	A	N	Y	S
	#8	2	0	A	N	Y	S
	#11	0	0	A	Y	Y	T
	#11	2	0	A	N	Y	S
	#11	0	1	A	Y	Y	T
	#12	0	1	A	Y	Y	T
	#12	0	0	A	Y	Y	T

	#16	2	1	A	Y	N	S
	#16	0	0	A	Y	N	R
	#17	2	3	A	Y	N	S
	#17	2	1	A	Y	N	S
	#17	2	3	A	Y	N	S
	#17	2	0	A	Y	N	S
<b>Treatment 2</b>	#8	0	0	A	N	Y	T
<i>(In-vitro priming</i>	#9	0	0	A	N	Y	T
<i>with hexanoic</i>	#10	0	0	A	Y	Y	T
<i>acid)</i>	#10	0	0	A	Y	Y	T
	#10	2	3	A	Y	N	S
	#13	1,2,3,5	3	A	Y	N	S
	#17	2	0	A	N	Y	S

**Table 8:** A summary of the inoculated plants and their responses to tissue colonisation by *F. pseudonygamai* SC17 in sugarcane cultivar 88H0019, 2 months after toothpick stab inoculation method. Plants were inoculated with only *F. pseudonygamai* SC17. The plants that were used for *ex vitro* screening were exposed to chemical mutagens followed by priming (treatment 1) or exposed to only priming (treatment 2). The external symptoms were recorded as: 0- no symptoms, 1- crinkled leaves, 2- chlorotic leaves, 3- necrotic leaves, 4- dead growing point, 5- wilting, and 6- all symptoms. Lesion severity ratings were assessed using a scoring system: 0- no lesion, 1- mild lesion, 2- moderate lesion, and 3- severe lesion.

Treatment	Line	External symptoms	Severity of lesion	Dead (D) or Alive (A)	Re-isolation on NS agar from the lesion	Re-isolation on NS agar from undamaged areas above the lesion	Tolerant, Susceptible, Resistant
<b>Control (inoculated)</b>	#16	1,2	0	A	N	N	S
	#18	1,2,3,4	3	A	Y	N	S
	#14	2	3	A	Y	N	S
	#18	1,2,3,5	1	A	N	N	S
	#17	2	3	A	Y	N	S
	#14	3,5	3	D	N	N	S
<b>Treatment 1</b> ( <i>In vitro</i> priming with hexanoic acid + in-vitro mutagenesis with 5-AzaC and EMS)	#21	0	2	A	Y	Y	T
	#1	1,2,3	2	A	N	N	S
	#4	2,3	1	A	N	Y	S
	#1	1,2,3	2	A	N	N	S
<b>Treatment 2</b> ( <i>In vitro</i> priming with hexanoic acid)	#7	1,2	1	A	N	Y	S
	#7	1,2	1	A	N	Y	S
	#17	0	2	A	Y	Y	T
	#11	0	1	A	Y	Y	T
	#19	2	3	A	Y	N	S
	#11	3,4	2	D	Y	N	S
	#7	2	0	A	N	N	S

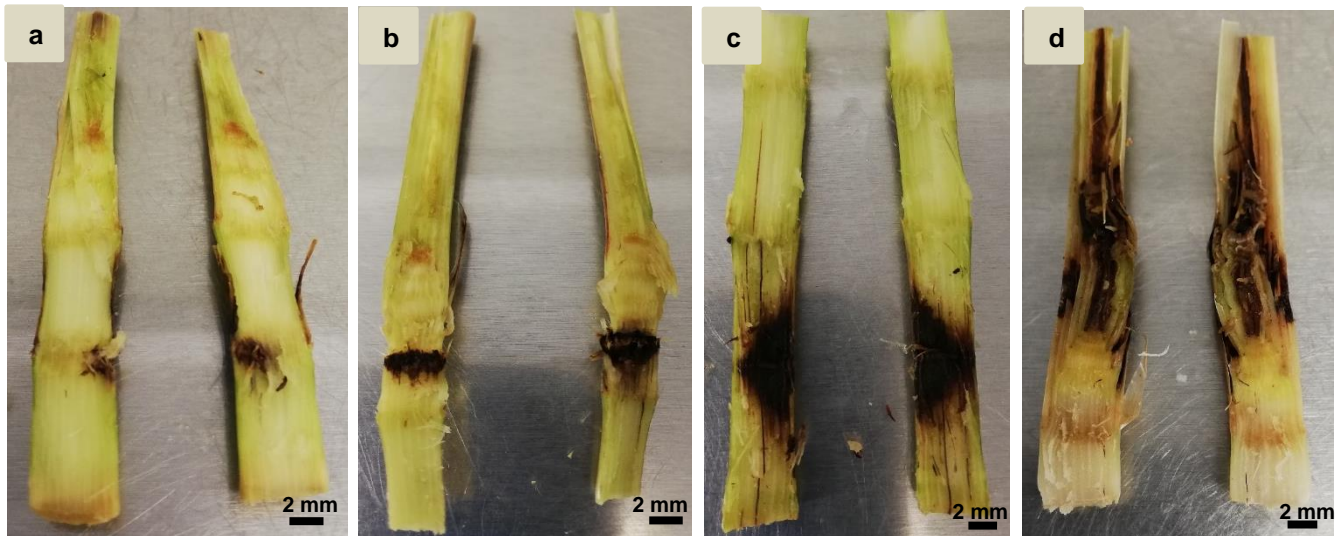
**Table 9:** A summary of the inoculated plants and their response to tissue colonisation by *F. pseudonygamai* SC17 in sugarcane cultivar N41, 2 months after toothpick stab inoculation method. Plants were inoculated with *F. pseudonygamai* SC17, and, 2 weeks later, with 1-2 2<sup>nd</sup> instar *E. saccharina* larvae that were placed into the leaf whorls the plants. The plants that were used for *ex vitro* screening were exposed to chemical mutagens followed by priming (treatment 1) or exposed to only priming (treatment 2). The external symptoms were recorded as: 0- no symptoms, 1- crinkled leaves, 2- chlorotic leaves, 3- necrotic leaves, 4- dead growing point, 5- wilting, and 6- all symptoms. Lesion severity ratings were assessed using a scoring system: 0- no lesion, 1- mild lesion, 2- moderate lesion, and 3- severe lesion.

Treatment	Line	External symptoms	Severity of lesion	Dead (D) or Alive (A)	Re-isolation on NS agar from the lesion	Re-isolation on NS agar from undamaged areas above the lesion	Tolerant, Susceptible, or Resistant
<b>Control</b> (uninoculated)	#1	0	0	A	N	N	-
	#2	0	0	A	N	N	-
	#3	0	0	A	N	N	-
<b>Control</b> (inoculated)	#9	6	2	A	Y	N	S
	#1	6	3	A	Y	N	S
	#6	1,2,3,5	2	A	Y	N	S
	#11	6	3	A	Y	N	S
	#1	1,2,3	3	A	Y	N	S
	#13	1,2,3,5	2	A	Y	N	S
	#10	1,2,3,5	3	D	N	N	S
	#5	6	3	D	N	N	S
	#15	1,2,3	2	D	N	N	S
	#5	1,2,3,4	2	A	Y	N	S
<b>Treatment 1</b> ( <i>In vitro</i> mutagenesis with 5-AzaC and EMS + <i>in vitro</i> priming with hexanoic acid)	#6	6	3	D	N	N	S
	#8	1,3,4	3	A	Y	N	S
	#8	1,2,4,5	2	A	Y	N	S
	#6	2,5	0	A	N	N	S
	#16	0	1	A	Y	N	R
	#17	0	1	A	Y	N	R
	#16	0	1	A	Y	Y	T
#6	1,2	2	A	Y	N	S	
#1	0	1	A	Y	Y	T	

<b>Treatment 2</b> ( <i>In vitro</i> priming with hexanoic acid)	#4	2,4	2	A	Y	N	S
	#13	1,2,3	0	A	N	Y	S
	#17	0	1	A	Y	N	R
	#9	0	1	A	Y	N	R
	#8	1,2,5	2	A	Y	N	S
	#13	2	2	A	Y	N	S
	#9	2	2	A	Y	N	S
	#17	2	1	A	N	N	S
	#2	2,4	0	A	N	Y	S
	#17	1,2,3	0	A	N	Y	S
	#17	0	3	A	Y	N	R
	#9	1,2,3	2	A	Y	N	S

**Table 10:** A summary of the inoculated plants and their response to tissue colonisation by *F. pseudonygamai* SC17 in sugarcane cultivar 88H0019, 2 months after toothpick stab inoculation method. Plants were inoculated with *F. pseudonygamai* SC17, and, 2 weeks later, with 1-2 2<sup>nd</sup> instar *E. saccharina* larvae that were placed into the leaf whorls the plants. The plants that were used for *ex vitro* screening were exposed to chemical mutagens followed by priming (treatment 1) or exposed to only priming (treatment 2). The external symptoms were recorded as: 0- no symptoms, 1- crinkled leaves, 2- chlorotic leaves, 3- necrotic leaves, 4- dead growing point, 5- wilting, and 6- all symptoms. Lesion severity ratings were assessed using a scoring system: 0- no lesion, 1- mild lesion, 2- moderate lesion, and 3- severe lesion.

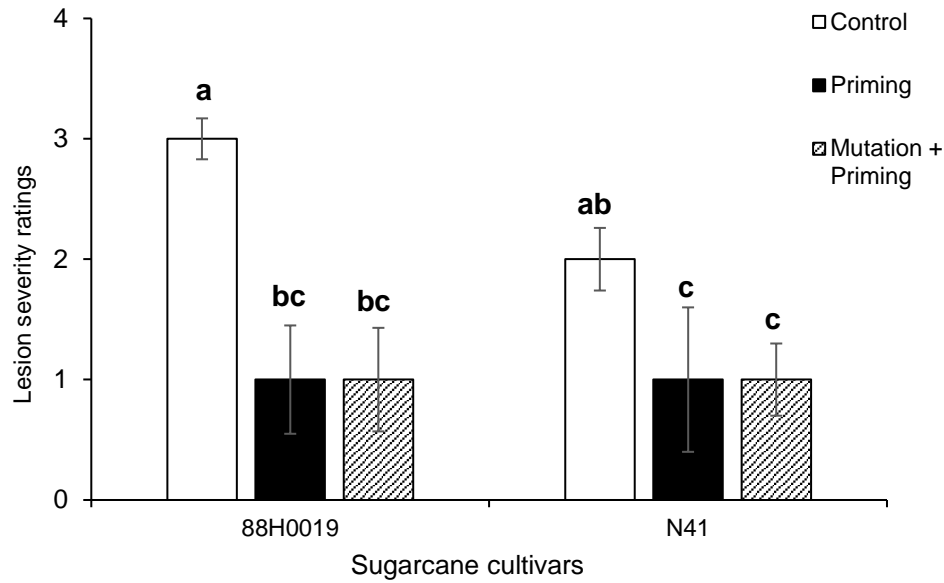
Treatment	Line	External symptoms	Severity of lesion	Dead (D) or Alive (A)	Re-isolation on NS agar from the lesion	Re-isolation on NS agar from undamaged areas above the lesion	Tolerant, Susceptible, Resistant
<b>Control (inoculated)</b>	1	1,2	0	A	Y	N	S
	3	6	3	D	Y	N	S
	1	6	3	D	N	N	S
<b>Treatment 1</b> ( <i>In vitro</i> priming with hexanoic acid + <i>in vitro</i> mutagenesis with 5-AzaC and EMS)	3	1,2	3	A	Y	N	S
	#8	0	2	A	Y	N	R
	#4	1,2,3	2	A	Y	N	S
	#10	1,2,3	0	D	N	N	S
	#3	2	1	A	Y	N	S
<b>Treatment 2</b> ( <i>In vitro</i> priming with hexanoic acid)	#7	1,2,3,4	0	D	N	N	S
	#3	1,2,3,4	3	A	N	N	S
	#15	1,2,3,4	3	A	N	N	S
	#17	1,2,3,5	2	D	N	N	S
	#19	1,2,3,4	3	D	N	N	S



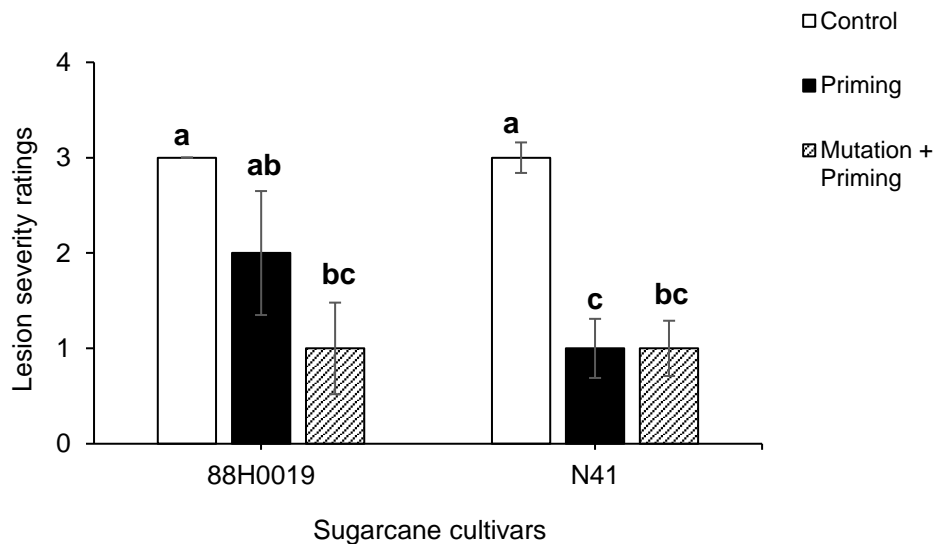
**Figure 27:** A comparison of lesion severity in the inoculated stems sections of the plants: a) no lesion; b) mild lesion; b) moderate lesion, and d) severe lesion. Plants were stabbed 2-3 cm from the bottom of the stalk with toothpicks colonised with *F. pseudonygamai* SC17. Some plants (not shown) were dual inoculated after 2 weeks with 2<sup>nd</sup> instar *E. saccharina* larvae, stems were collected after 7 weeks for re-isolation.

As mentioned above, the tolerance and resistance to *F. pseudonygamai* SC17 and *E. saccharina* of the putative mutant plants was confirmed by re-isolating the fungi and by assessing lesion severity of the stalks. For cultivar 88H0019, there was a significant decrease in the lesion severity ratings of the putative mutant plants that were inoculated with only SC17 for the primed and combined treatment compared with the control ( $p = 0.002$ ) (Fig. 28). For cultivar N41, there was a significant decrease in the lesion severity ratings of the putative mutant plantlets that were inoculated with only SC17 for the primed and combined treatment compared with the control ( $p = 0.002$ ). However, there was no significant difference in the lesion severity ratings between both cultivars across the treatments.

For cultivar 88H0019, there was a significant decrease in the lesion severity ratings of the putative mutant plants that were dual inoculated with SC17 and the 2<sup>nd</sup> instar *E. saccharina* larvae, between the control and mutation + primed treatment ( $p < 0.001$ ) (Fig. 29). For cultivar N41, there was a significant decrease in the lesion severity ratings for the primed and combined treatment when compared with the control ( $p < 0.001$ ). In the primed treatment, cultivar N41 showed a significant decrease in lesion severity ratings when compared with cultivar 88H0019 ( $p < 0.001$ ). In conclusion, these results indicated that the putative mutant plants that were primed with Hx only and treated with a combination of mutagens (EMS and 5-AzaC) and the priming agent Hx exhibited a significant decrease in lesion severity.



**Figure 28:** The lesion severity ratings of plantlets that were inoculated with only *Fusarium* SC17 for cultivars 88H0019 and N41. Dissimilar alphabet characters denote a statistically significant difference amongst treatments for each concentration. Data sets were analysed by One-way ANOVA and Duncan's multiple range test.  $P \geq 0.05$ ,  $n = 6 - 13$ , mean  $\pm$  SE.



**Figure 29:** The lesion severity ratings of plantlets that were dual inoculated with SC17 and 2 weeks later with 2nd instar *E. saccharina* larvae, after 2 weeks for cultivars 88H0019 and N41. Dissimilar alphabet characters denote a statistically significant difference amongst treatments for each treatment. Data sets were analysed by One-way ANOVA and Duncan's multiple range test.  $P \geq 0.10$ ,  $n = 11 - 14$ , mean  $\pm$  SE.

## 5. Discussion

### 5.1 The effect of priming agents on callus production and plantlet yield

Plants have evolved to survive a wide variety of biotic and abiotic stressors by developing inducible defence mechanisms, many of which are triggered by pathogen attack (Scalschi et al., 2013; Lamke and Baurle, 2017; Bertini et al., 2018; Anderson and Kim, 2021). Plant immunity against microbial presence relies on general elicitors, which signal the presence of potential pathogens (Bertini et al., 2018; Anderson and Kim, 2021). Recent studies in plant defence show that plants can be primed, which allows for a rapid and effective defence response to biotic and abiotic stressors (Conrath et al., 2006; Martinez-Medina et al., 2016; Bertini et al., 2018; Anderson and Kim, 2021). Enhanced resistance to biotic and abiotic stressors by using chemical biostimuli or biological organisms to induce molecular priming has been reported (Thakur and Sohal, 2013; Westman et al., 2019; Kerchev et al., 2020). Plants may activate different defence pathways depending on the type of pathogen attack (Garcia-Brugger et al., 2006; Thakur and Sohal, 2013; Anderson and Kim, 2021). The treatment of plants with various chemical elicitors has been found to activate different biosynthetic pathways. Necrotrophs are commonly known to initiate jasmonic acid (JA) and ethylene-dependent responses, while biotrophic pathogens activate salicylic acid (SA) dependent responses (Thakur and Sohal, 2013; Anderson and Kim, 2021).

Priming agents can be applied to whole plants, roots and seeds by means of seed priming (Luna et al., 2012; Thapa et al., 2020), foliage sprays, or addition to a hydroponic culture medium (Levy et al., 2008; Westman et al., 2018). Seed priming with chemicals that induce resistance has emerged as a novel strategy to protect crops against herbivory and disease attacks (Worrall et al., 2012; Luna et al., 2012; Paudel et al., 2020; Thapa et al., 2020). Studies on rice (Bertini et al., 2018), tomato (Luna et al., 2016; Llorens et al., 2020), broccoli and *Arabidopsis* (Venegas-Molina et al., 2020), sugarcane (Patade et al., 2012; Duarte et al., 2018), and citrus (Caccalano et al., 2021) have demonstrated the effective use of several chemical elicitors to induce defence priming.

Hexanoic acid (Hx) has been used for inducing defence responses in *Arabidopsis* and tomato plants against *Botrytis cinerea*, tomato plants against *Pseudomonas syringae* (Leyva et al., 2008; Vicedo et al., 2009; Kravchuk et al., 2011; Scalschi et al., 2013), tomato plants against *Phytophthora citrophthora* and *Alternaria solani* (Flors et al., 2003), *Cucumis melo* against Melon necrotic ringspot virus (Fernández-Crespo et al., 2017), and citrus against *Xanthomonas citri* and *Alternaria alternata* (Llorens et al., 2013, 2015b; Llorens et al., 2016; Caccalano et al., 2021). However, the use of Hx on callus and plantlets is a novel approach

since published studies have mostly investigated seed priming (Borges et al., 2019; Ben Youssef et al., 2021), hydroponic culture systems (Scalschi et al., 2013), foliar spraying (Levy et al., 2008) and soil drench applications (Vicedo et al., 2009; Llorens et al., 2013; Llorens et al., 2015) to induce defence priming (Levy et al., 2008; Scalschi et al., 2013; Llorens et al., 2016). However, Djami-Tchatchou et al. (2017) reported on the comparison of priming-related responses in *Nicotiana tabacum* callus cells triggered by Hx and Azelaic acid (Aza). That study revealed that exposure to Hx and Aza were able to induce many genes of importance to priming, signaling and defence-related responses in plants. There was a significant up-regulation in the *PR-1a* expression in the cells treated with Aza and Hx. *PR-1a* and *Defensin* are important gene transcripts that have been found to be induced after treatment with resistance inducing agents (Shah and Zeier, 2013; Djami-Tchatchou et al., 2017). PR-1 proteins have been used as molecular markers of SA-dependent systemic acquired resistance (SAR), these proteins contribute to increased pathogen resistance by causing harmful effects to microbial attackers directly during SAR (Djami-Tchatchou et al., 2017).

To assess the effectiveness of Hx and CJ as priming agents in the current study, Hx and CJ were applied to calli and regenerated plantlets *in vitro*. Sugarcane leaf discs and embryogenic calli were first exposed to various concentrations of the priming agents to select an appropriate one that induced calli proliferation and plantlet regeneration. The findings revealed that both sugarcane cultivars, 88H0019 and N41 pre-treated with Hx, resulted in better calli production and plantlet yield compared with the CJ treatments. The effect of the priming agents on calli production and plantlet yield was tested using embryogenic calli (0.2 g per replicate) that was transferred to embryo maturation medium (EMM) consisting of CJ (0, 0.2, 1, 5 and 10  $\mu$ M) and Hx (0, 0.2, 0.6, 2 and 6 mM) for 2 weeks. The number of plantlets/ 0.2 g of callus was inversely proportional to percentage callus necrosis for both cultivars, as expected (Fig. 10, 11). The treatments that showed a high percentage of callus necrosis produced low plantlet yields (5 and 10  $\mu$ M CJ treatments; 2 and 6 mM Hx treatments). The percentage of plantlets for 88H0019 and N41 were expressed as a percentage of the plants produced in the control. For cultivar N41, the highest plantlet yield of 90 % was recorded using 0.2 mM Hx, and cultivar 88H0019 recorded a 78 % plantlet yield using 0.6 mM Hx. For both the calli and plantlet regeneration stages, 0.6 mM Hx was selected as an appropriate concentration since it was the only one that produced a greater percentage of plantlets for both cultivars.

Many studies reported on the application of Hx in dicotyledonous plants such as tomato (Scalschi et al., 2013; Scalschi et al., 2014) and citrus (Llorens et al., 2016; Caccalano et al., 2021) to induce resistance against pathogens. Hexanoic acid has also been used to effectively

protect *Arabidopsis* plants (monocotyledonous) against pathogens (Kravchuk et al., 2011; Venegas-Molina et al., 2020). Those studies used 0.6 - 25 mM Hx to induce resistance against different pathogens. Hexanoic acid has been applied using different techniques such as hydroponic culture systems (Scalschi et al., 2013), foliar spray (Levy et al., 2008), and soil drench applications (Vicedo et al., 2009; Llorens et al., 2013; Llorens et al., 2015) to four-week-old tomato, citrus, and *Arabidopsis* plants. The results obtained in the present study revealed Hx at 0.6 mM to be a suitable priming agent in terms of calli production and plantlet regeneration. Hence, this concentration was chosen as a priming agent for further studies.

## 5.2 *Fusarium pseudonygamae* SC17 culture filtrate as a selection agent at the callus and plantlet regeneration stages

The association between fungi and host plants has been discussed in many studies (e.g., Shen et al., 2019; Fadiji and Babalola, 2020; Rigobelo and Baron, 2021). Endophytes have been found to improve plant growth by secreting phytohormones. They also help in improving nutrition using bidirectional nutrient transfer and enhancing plant health by protecting them against phytopathogens (Andreozzi et al., 2019; Shen et al., 2019; Fadiji and Babalola, 2020). The ability of endophytes to enhance host defence responses against diseases and reduce damage caused by pathogen attack is widely acknowledged (Ganley et al., 2008; Mejía et al., 2008; Fadiji and Babalola, 2020). This endophytic interaction is known as balanced antagonism (Schulz et al., 2015), i.e., the endophyte requires the activation of virulence mechanisms for colonisation and the triggering of host defences by these events to recognise the plant as a host. The fungus benefits by surviving off the nutrients from the host plant and, in return, provides benefits to the plants, including tolerance to abiotic and biotic stresses (Bamisile et al., 2018; Rigobelo and Baron, 2021).

Researchers have been investigating the effects of fungal mycotoxins or culture filtrates on resistant and susceptible genotypes of crop plants to assess disease resistance using *in vitro* selection techniques for many years (e.g., Binarova et al., 1990; Mahlanza et al., 2013; Suthar et al., 2021). The evaluation of resistance using *in vitro* techniques is dependent upon a positive relationship between *in vitro* culture filtrate resistance and whole plant disease resistance. The selection for tolerant lines using *Fusarium* culture filtrates and purified toxins has been widely used in callus cultures of sugarcane (*Saccharum* hybrids) (Mahlanza et al., 2013), cumin (*Cuminum cyminum*) (Suthar et al., 2021), turmeric (*Curcuma longa* L) (Gayatri et al., 2005) and sugar beet (*Beta vulgaris* L.) (Yerzhebayeva et al., 2019). *Fusarium* culture filtrates have been used as a selection agent *in vitro* shoot clumps for banana (*Musa* spp.)

(Rebouças et al., 2021) and vanilla plants (*Vanilla planifolia* Jacks.) (Ramírez-Mosqueda et al., 2019).

Programmed cell death is an important biological process which occurs under stress conditions during normal growth and development (Lam, 2004; Dickman and Fluhr, 2013; Zhang et al., 2015). Hypersensitive response (HR) is an extensively studied form of PCD in plants, which takes place during incompatible plant-pathogen interactions. Reaction oxygen species (ROS) that are associated with PCD of host cells are generated by the plant during the HR response when an oxidative burst occurs (Howlett, 2006). This form of PCD also follows when plant cells around the invasion site(s) rapidly and actively die to limit pathogen growth and prevent nutrient supply and, consequently, preventing the disease from spreading through the whole plant (Coll et al., 2011; Zhang et al., 2015).

There are two major pathways required for plant innate immunity, the jasmonic acid (JA) and salicylic acid (SA) pathway, induced by necrotrophs and biotrophs, respectively (Thomma et al., 1998; Glazebrook, 2005; Klemme et al., 2019). In the case of biotrophs, the plant responds to these pathogens via the SA pathway, inducing SA-specific pathogen response genes that result in HR which inhibits pathogen growth and development (Shah, 2003; Klemme et al., 2019). In a resistant plant genotype, necrotrophic fungi have been found to activate the JA signaling pathways, which triggers the JA-specific pathogen response genes that block the hypersensitive response (HR) (Van Loon and Van Strien, 1999; Clarke et al., 2000; Mur et al., 2006; Klemme et al., 2019). However, the pathogens find a way to avoid activation of the JA signaling pathway (responsible for promoting plant resistance) by increasing SA which inhibits the JA pathways (Klemme et al., 2019). The SA pathway has been induced to stimulate HR, this allows the necrotrophic fungal pathogens to secrete toxins in the host plant, promoting cell death, to access nutrition from the dead host tissue (Howlett, 2006; Zhang et al., 2015). In a susceptible plant, *Fusarium* spp. can use this mechanism by producing fumonisins since this mycotoxin causes programmed cell death (PCD) (Zhang et al., 2015; Daou et al., 2021).

There are numerous species of known fungi, although only a few of them, namely *Penicillium* spp., *Fusarium* spp., and *Aspergillus* spp. have been found to produce most of the mycotoxins that significantly affect agricultural crops (Kabak, 2009; Freire and de Rocha, 2017; Daou et al., 2021). The mycotoxin fumonisin B1 (FB1) produced by *Fusarium verticillioides* (*moniliforme*) is a strong sphingolipid-like programmed cell death (PCD) elicitor that causes severe disease symptoms in maize and other grain crops (Gilchrist 1997, 1998; Zhang et al., 2015; Daou et al., 2021), all monocotyledonous species such as sugarcane. The sphingolipid biosynthetic pathway is inhibited by FB1 which hinders the production of ceramide synthase,

which results in cell death (Stone et al., 2000; Desai et al., 2002, Daou et al., 2021). Chivasa et al. (2005) stated that in *Arabidopsis* fumonisin B1 mediates cell death by altering the abundance of specific intracellular proteins in *Arabidopsis* by depleting extracellular adenosine triphosphate (ATP). The exogenous application of FB1 to germinated maize seeds resulted in a dose-dependent increase of SA, which was associated with an increase in sphingoid bases and inhibition of ceramide synthase (De La Torre-Hernandez et al., 2010; Blacutt et al., 2018). As a result, FB1 increased maize PCD and necrotrophic fungal colonisation of the plant by activating the SA pathway (Arias et al., 2012; Blacutt et al., 2018). Williams et al. (2007) also stated that in maize plants there was a dose-dependent reduction in root mass that was inversely correlated with fumonisin B1.

Since no work on sugarcane has been conducted to determine the effect *F. pseudonygamai* SC17 on calli proliferation and plantlet production, it was necessary to determine a suitable CF concentration for screening somatic embryos at the embryo germination stage and plantlets at the plantlet regeneration stage. The effect of different concentrations (0, 4, 20, 50 and 100 ppm) of *F. pseudonygamai* SC17 CF on calli production, plantlet yield and production of abnormal plantlets was recorded after 3 weeks. The incorporation of *F. pseudonygamai* SC17 CF in the EGM1 culture media resulted in necrosis of the embryogenic calli but plantlets were still obtained at all the tested concentrations of the CF (Figs 13a, b and c). There was a dose-dependent effect on callus necrosis and decrease in plantlet yield. For cultivar N41, there was a significant decrease in plantlet yield between the control (1020 plantlets/0.1 g of callus) and 100 ppm CF (98 plantlets/0.1 g of callus). There were no significant differences in plantlet yield observed for cultivar 88H0019 between the control and all the tested CF concentrations. Similarly, Mahlanza et al. (2013) reported an increase in callus necrosis and a decrease in plantlet yield when sugarcane callus cultures were placed in medium containing increasing concentrations of *F. sacchari* PNG40 CF. Similar results were also found in callus cultures of cumin (Suthar et al., 2021) and shoot clumps of banana (Rebouças et al., 2021). According to Daub (1986), selection agents used for *in vitro* screening for disease tolerance played a role in pathogenesis and showed a negative effect on plant tissues, cells, organs or whole plants. The selection agent used in the current study, *F. pseudonygamai* SC17, did not result in complete necrosis at the embryo germination stage as compared with the fungal strain *F. sacchari* PNG40 used in the study of Mahlanza et al. (2013).

Mahlanza et al. (2013) also stated that the production of sugarcane plants from the calli exposed to CF during embryo germination may have been due to light-dependent activity of mycotoxins (Asai et al., 2000), or different effects of the CF on the various physiological and biochemical processes that occur at the embryo germination stage. It is assumed that the

toxicity of the CF during the embryo germination stage under light conditions could likely be due to secondary metabolites (e.g., fumonisins) produced by *Fusarium spp.* which cause cell death. In a study established by Asai et al. (2000) an *Arabidopsis* protoplasts model was used to understand plant cell death signalling. Programmed cell death (PCD) was induced in wild-type protoplast by the toxin. When the protocol was established to understand plant cell death, the results indicated that FB1 induced PCD is light-dependent and it required the jasmonate, salicylate, and ethylene dependent signalling pathways (Williams et al., 2007; Asai et al., 2000). This explains the plants need for light during the oxidative burst to produce reactive oxygen (Govin and Levin, 2002; Zhang et al., 2015).

In the current study, greatly reduced numbers of plantlets were obtained at the highest CF concentration (100 µM) for cultivars 88H0019 and N41, 58 and 98 plantlets/0.1 g of callus, respectively compared to more than 600 plantlets/0.1 g of callus in the no CF control. Unexpectedly, all the tested CF concentrations had a significant positive effect on the percentage of plantlets that re-rooted compared with the control. Plantlets were still produced at the highest CF concentration (100 µM) that was tested, however a greater CF concentration should have been analysed. Hence, *F. pseudonygamai* SC17 could not be used as an *in vitro* selection agent in a root re-growth assay.

When the protocol for determining a suitable CF concentration for screening somatic embryos was established, it was then necessary to determine a suitable CF concentration for screening putative mutant plants at the end of the regeneration stage under *in vitro* conditions. Mahlanza et al. (2013) reported on the inhibition of root growth by *F. sacchari* PNG40 CF and this indicated that root re-growth can be used for selection of putative mutant lines *in vitro*. Many researchers doing studies on maize (Yoder, 1973), *Amaranthus hybridus* (Chen and Swart, 2002), and winter cherry (*Withania somnifera*) (Chakraborty et al., 2020) found that culture filtrates have an inhibitory effect on callus or root growth and proved to be a reliable and quick technique to produce resistant lines against biotic stresses.

A root re-growth test was established to screen putative mutant lines at the whole plant level. This test has been used by Mahlanza et al. (2013) and demonstrated that the roots of 50 % of the mutant plantlets with trimmed roots cultured on media containing 1500 ppm CF re-grew to at least 10 mm in length over 3 weeks. In his study he used 10 mm re-growth as the criterion for the selection of CF- tolerant plants. Subsequently, this value was set as the criterion for the selection of CF- tolerant plants in the current study. The results obtained from this investigation indicated that the eldana beneficial strain *F. pseudonygamai* SC17 could not be used as an *in vitro* selection agent because the presence of CF in the culture media

promoted root growth for cultivars N41 and 88H0019 instead of inhibiting growth. The addition of different concentrations (500, 1000, 1500 ppm) of *F. pseudonygamai* SC17 CF to the EGM2 medium significantly enhanced the rooting ability of plantlets compared with the controls for both cultivars. The results revealed that all the tested CF concentrations had a significant positive effect on the percentage of plantlets that re-rooted between the control and all the tested CF concentrations (Fig. 16). The results recorded for both cultivars indicated a 95 - 100 % rooting ability of the plantlets in EGM1 + CF containing 500, 1000, and 1500 ppm CF, which was significantly higher than the percentage of plantlets that rooted in EGM1 containing no CF (60 - 70 %).

Although there was no significant interaction observed between treatment versus cultivar, there was a significant difference in the root length of plantlets amongst the treatments after 3 weeks (Fig. 15). The results show that all the tested concentrations of the CF affected the root length of plantlets, with the 1500 ppm CF resulting in the highest root length (31.5 mm  $\pm$  4.3, for 88H0019) and (34.05 mm  $\pm$  3.9, for N41). The root growth of *in vitro* plantlets were inhibited in the presence of 750 ppm and 1500 ppm *F. sacchari* CF in sugarcane (Mahlanza et al., 2013). Liao et al. (2017) reported that the use of the insect-pathogenic fungus *Metarhizium robertsii* culture filtrate promoted lateral root growth and root hair development of *Arabidopsis* seedlings.

In the present study, due to the percentage of plants that re-rooted and root length of the plantlets across all the tested CF concentrations, *F. pseudonygamai* SC17 could not be used as an *in vitro* selection agent because of its root promoting property. As previously mentioned Williams et al. (2007) reported on a dose-dependent (increasing fumonisin = decreasing root growth) reduction in root mass of maize plants that was inversely correlated with fumonisin B<sub>1</sub>, sphingoid base 1-phosphate in roots, and sphingoid bases. Therefore, *F. pseudonygamai* SC17 CF may not contain fumonisins since it promoted root growth.

It was then hypothesised that the *E. saccharina* beneficial strain *F. pseudonygamai* SC17 CF stimulated root growth by auxin (IAA) production and/or through the presence of active ACC deaminase in the CF. Assays were conducted to screen the *F. pseudonygamai* SC17 fungal isolate for IAA production. For future work a toxicological profile for *F. pseudonygamai* SC17 will be required to determine the toxins present that inhibits plantlet regeneration at the callus and regeneration stages.

### 5.3 Fungal endophytes and plant growth promotion *in vitro*

The association between the host plant and some endophytic fungi is mutualistic (Khan et al., 2015; Rigobelo and Baron, 2021). The inner part of the host plant is a protected niche for endophytes since this contains the essential nutrients for fungal growth and survival, consequently the fungi improve the plant's fitness by several mechanisms (Khan et al., 2015; Lugtenberg et al., 2016; Chitnis et al., 2020; Rigobelo and Baron, 2021). The positive aspects of endophytic colonisation in host plants are well documented (Berg, 2009; Rigobelo and Baron, 2021). The direct benefits of endophytic colonisation include an increase in the production of phytohormones and acquisition of nutrients, which are associated with the improved root development, plant height and increase in biomass production (Bamisile et al., 2018). On the other hand, the indirect ones is the activation of systemic resistance, production of secondary metabolites and antibiotics, production of siderophores and protection against biotic and abiotic stresses (Rigobelo and Baron, 2021).

Indole-3-acetic acid (IAA) is the main auxin produced by endophytic fungi that plays a significant role in plant growth and development (e.g., Jaroszuk-Ścisiel et al. 2014; Fadiji and Babalola, 2020; Fontana et al., 2021; Rigobelo and Baron, 2021). Low levels of IAA have been found to stimulate root elongation, whilst high concentrations result in the formation of adventitious and lateral roots (Jaroszuk-Ścisiel et al., 2019). Some plant growth-promoting fungi (PGPF) also play an important role in plant growth by producing the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Nascimento et al., 2014; Hossain and Sultana, 2020). The ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) is converted into  $\alpha$ -ketobutyrate and  $\text{NH}_3$  (ammonia), by the ACC deaminase enzyme (Nascimento et al., 2014; Hossain and Sultana, 2020). Plant growth is regulated when the ACC deaminase enzyme cleaves ACC produced by plants and thus reduces the ethylene levels in the plant (Glick et al., 2007; Hossain and Sultana, 2020). The production of ACC deaminase has been reported in some fungi, such as *Phytophthora sojae* (Jia et al., 1999; Singh and Kashyap, 2012), *Fusarium graminearum* (Svoboda et al., 2019), and *Issatchenkia occidentalis* (Palmer et al., 2007). Jaroszuk-Ścisiel et al. (2019) reported on the ability of the mycoparasitic *Trichoderma* (DEMTkZ3A0) strain to synthesize phytohormones GA, IAA and the enzyme-ACC deaminase.

Due to the observed enhanced root growth of the plantlets across all the CF concentrations tested in the present study, it was hypothesised that the beneficial *Fusarium* strain (SC17) stimulated root growth by auxin (IAA) production and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. To test the former, a quantitative colourimetric was conducted to screen for IAA production by the *F. pseudonygamai* fungal isolate SC17 (Khan et al., 2016).

The *F. pseudonygamai* isolate was able to synthesise IAA in both the presence and absence of L-tryptophan. The isolated *Fusarium* endophyte SC17 produced 743.1 nM and 457.2 nM IAA in the presence and absence of L-tryptophan, respectively (Fig. 19). The addition of 0.1 g L<sup>-1</sup> L-tryptophan enhanced the IAA production by the isolate. Similar findings on the production of IAA by fungi isolated from sugarcane (Asrul et al., 2020), coffee (Numponsak et al., 2018), *Arabidopsis* (Liao et al. 2017), *Sophora flavescens* (Turbat et al., 2020), maize (Vrabka et al., 2019), and rice (Kuswinanti et al., 2015; Restu and Payangan, 2019) have been reported. Meents et al. (2019) reported on the production and accumulation of IAA in the mycelia of two beneficial endophytic fungi, *Mortierella hyaline* and *Piriformospora indica* found in *Arabidopsis* roots. Contreras-Cornejo et al. (2009) reported on the direct correlation between lateral root development in *Arabidopsis* seedlings inoculated with *T. virens* and increased levels of fungal IAA. Depending on growth conditions, microbial endophytes can produce IAA in varying quantities. The results recorded in those studies support the hypothesis that the observed enhanced root growth in the plantlets could have been due to the production of auxin (IAA) by the SC17 CF. However, as only screening for IAA production by the *F. pseudonygamai* isolate SC17 was conducted, the activity of ACC deaminase will need to be determined in future studies. Plant growth-promoting fungi (PGPF) also play an important role in plant growth by producing the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Nascimento et al., 2014; Hossain and Sultana, 2020).

#### 5.4 *Ex vitro* screening of putative mutant sugarcane plants for tolerance to *F. pseudonygamai* and *Eldana saccharina*

Plants have the ability to develop an enhanced state of resistance, known as induced resistance (IR), in addition to basal resistance (Llorens et al., 2015; Llorens et al., 2020). Basal resistance also known as innate immunity, is the first line of inducible defences that protect plants against attack by pathogens (Freeman and Beattie, 2008; Llorens et al., 2015; Llorens et al., 2020). Many attempts have been made to enhance stress tolerance in crops by inducing stress memory progressively. The most promising example of improving stress tolerance is by activating priming responses and targeting epigenome modifications (Martinez-Medina et al., 2016; Mauch-Mani et al., 2017; Mladenov et al., 2021). Defence priming is triggered by a priming stimulus, which is also responsible for inducing a persistent primed state of enhanced plant defence readiness (Martinez-Medina et al., 2016; Mauch-Mani et al., 2017; Mladenov et al., 2021). Priming is a state in which, during a second attack, the plant displays a quicker and more vigorous response in contrast to the initial one. Consequently, this robust response increases the plants chances of survival (Kinoshita and Seki, 2015; Mladenov et al., 2021). Priming occurs at the phenotypic level and does not involve changes to the DNA sequence and is therefore reversible (Conrath et al., 2015; Mladenov et al., 2021). Defence priming can

be induced using a diverse-range of mechanisms such as, a mild abiotic stress, treatment with synthetic or natural chemicals, infection by pathogens and colonisation of roots by beneficial microbes (Mauch-Mani et al., 2017; Balestrini et al., 2018; Ashapkin et al., 2020; Ioannou et al., 2020; Mladenov et al., 2021).

When a plant encounters a priming stimulus, a period of stress memory follows, which induces altered transcriptional regulation (Balmer et al., 2015; Mladenov et al., 2021). This modification results in sustained changes in gene expression or an improved transcriptional reaction to a secondary stimulus and is one possible indicator of stress memory (Oberkofler et al., 2021; Mladenov et al., 2021). Depending on the type of initial stimulus and the pathosystem involved there are a diverse set of defence mechanisms used by primed plants against pathogen attack (Balmer et al., 2015; Mladenov et al., 2021). It has been observed that defence mechanisms depend strongly on the priming state, which has been divided into a 'priming phase, a 'post-challenge primed state', and a 'transgenerational primed state' (Gamir et al., 2014; Pastor et al., 2014; Balmer et al., 2015). A priming stimulus triggers the priming phase which lasts until the plant is exposed to a challenging stress. During the primed phase, the levels of several primary and secondary metabolites, hormones, enzymes and other molecules are slightly changed, which puts the plant in a standby state (Gamir et al., 2014; Pastor et al., 2014; Balmer et al., 2015). When exposed to a challenging stress, the plant moves into the post-challenged primed state in which suitable reactions to combat the given stressor are induced quickly. The transgenerational primed state is the heritability of transcriptional changes from a parent to its progeny (Gamir et al., 2014; Pastor et al., 2014; Balmer et al., 2015; Martinez-Medina et al., 2016).

The use of Hx to enhance resistance to pathogens has been effectively demonstrated in *Arabidopsis* against *B. cinerea* (Kravchuk et al., 2011) and tomato plants against *P. syringe* and *B. cinera* (Leyva et al., 2008; Vicedo et al., 2009; Scalschi et al., 2013). Some studies have reported on the effect of Hx against the necrotrophic fungus *A. alternata* in *Arabidopsis* (Venegas-Molina et al., 2020) and 'Fortune' mandarin (Llorens et al., 2015).

In this study, Hx was used in combination with the chemical mutagen 5-AzaC. 5-Azacytidine has been widely used in many studies which generates epigenetic changes by inhibiting DNA methylation (Munsamy et al., 2013; Kakoulidou et al., 2021). Epigenetic mechanisms such as histone modifications and DNA methylation may be carriers of stress memory and trigger immune responses, therefore has been confirmed as key factors responsible for inducing resistance to biotic/abiotic stresses (Espinass et al., 2016; Lämke and Baurle, 2017; Ashapkin et al., 2020; Turgat-Kara et al., 2020). There is increasing experimental evidence that suggests the involvement of epigenetic modification with defence priming against stressors (He and Li,

2018; Mladenov et al., 2021). Epigenetic modifications have been found to cause prolonged stress memory, specifically in seed priming (Sani et al., 2013; Mladenov et al., 2021). A few studies have reported that defence priming can be inherited across sexual generations, a phenomenon referred to as 'transgenerational priming' (Martinez-Medina et al., 2016). It has been suggested that DNA methylation regulates priming by contributing to transgenerational priming (Luna et al., 2012; Martinez-Medina et al., 2016). Kim et al. (2017) reported on the involvement of DNA methylation in improving drought tolerance in primed seedlings exposed to a cycle of mild drought and re-watering treatments. Martinez-Medina et al. (2016) has shown the significance and dynamic involvement of epigenetic mechanisms, this indicated the possibility that epigenetic changes could be the main factor in establishing a priming effect. Several molecular markers were found to be useful for detecting the primed state, in *Arabidopsis*. These molecular markers include high levels of pathogen-recognition receptors, increased expression of transcription factor genes, enhanced accumulation of the mitogen-activated protein kinases MPK3 and MPK6, specific modifications to histones and DNA hypomethylation (Conrath et al., 2015; Martinez-Medina et al., 2016).

A protocol was established to develop putative mutant sugarcane lines that were primed with only Hx, or treated with a combination of Hx, mutagens EMS, and 5-AzaC. Following the establishment of the protocol for chemical mutagenesis and priming, embryogenic calli were exposed to, (32 mM EMS for 4 hrs, 100 µM 5-AzaC in embryo maturation media (EMM) for 1 week, and EMM containing 0.6 mM Hx for 2 weeks, followed by a sub-culture on embryo germination media (EGM1) containing 0.6 mM Hx for 4-12 weeks), and (no exposure to EMS and 5-AzaC, sub-cultured in EMM containing 0.6 mM Hx for 2 weeks, followed by a sub-culture onto EGM1 containing 0.6 mM Hx for 4-12 weeks). From each treatment, 50 *in vitro* plantlets were randomly selected for acclimatisation for 8 - 9 months, before they were used for *ex vitro* screening. To confirm tolerance of putative mutant lines, plants were inoculated with only *F. pseudonygamai* SC17 and dual inoculated with *F. pseudonygamai* SC17 and, after 2 weeks, 2<sup>nd</sup> instar eldana larvae were placed inside the leaf whorls. The main reason for using a combination of mutagens was to generate epigenetic (5-AzaC; Grzybkowska et al., 2018) and genetic (EMS; Lethin et al., 2020) variations in calli. It was hypothesised that *in vitro* priming with Hx could increase resistance to eldana and *F. pseudonygamai* SC17 whilst mutation (epimutagenesis in particular) could 'fix' this resistance during subsequent cycles of clonal propagation and chimera dissolution.

In this study, for N41 and 88H0019, embryogenic calli that were primed with Hx, and exposed to the mutagens + Hx, resulted in increased calli necrosis and a lesser number of plants/0.2 g of callus than the control. For cultivars N41 and 88H0019, there were no significant differences

in the number of plants produced/0.2 g callus between the primed and the combined treatment of 5-AzaC, EMS, and Hx. For the primed only treatment, there were 144 and 44 plants produced/0.2 g of callus for N41 and 88H0019, respectively. For the combined treatment, there were 44 and 33 plants produced/0.2 g of callus for N41 and 88H0019, respectively.

Lesion severity is an effective parameter of plant disease intensity for disease resistance (Bock et al., 2010; Bock et al., 2021), hence it was used in the present study to assess tolerance to *E. saccharina* and *F. pseudonygamai*. Lesion severity was recorded 2 months after inoculation, and the results obtained indicated that the putative mutant plants from both treatments exhibited a significant decrease in fungal lesion severity when compared with the controls for both N41 and 88H0019 (Figs 28 and 29). The lesion severity rating was as follows: 0- no lesion; 1- mild lesion; 2- moderate lesion; and 3- severe lesion. For both treatments, a mild lesion severity rating was recorded for plants inoculated with only SC17 for cultivars N41 and 88H0019. For the plants that were dual inoculated there was a significant difference in the lesion severity ratings between the two treatments. The lesion severity rating was moderate for cultivar 88H0019 (primed with Hx) and mild for cultivar N41 (primed with Hx). Plants from the combined treatment for both cultivars resulted in a mild lesion severity rating.

Similar findings have been recorded in previous studies, whereby exposure to Hx via soil drench, foliar spray or hydroponic conditions have resulted in reduced lesion severity caused by various pathogens in *Arabidopsis* (Kravchuk et al., 2011; Venegas-Molina et al., 2020), tomato (Levy et al., 2008; Scalschi et al., 2013), and citrus (Llorens et al., 2013, 2015). Novel studies in citrus ('Fortune' mandarin) demonstrated that Hx applied as a soil drench reduced the incidence of the necrotrophic fungus *Alternaria alternata* by 50 % (Llorens et al., 2013; Llorens et al., 2017). Scalschi et al. (2013) reported that Hx treatments protected tomato plants against *Pseudomonas syringae* in a concentration-dependent manner in all tomato cultivars analysed when Hx was applied by soil drench or in hydroponic culture systems. Four-week-old tomato plants that were treated with 0.6 mM Hx in hydroponic conditions showed reduced symptoms by 50 % (Scalschi et al. 2013). Several studies have reported on the enhancement of resistance mechanisms using Hx, and it is known that this priming agent induces resistance against necrotrophic pathogens by activating the JA pathway (Vicedo et al., 2009; Llorens et al., 2013; Venegas-Molina et al., 2020). These results are supported by previous studies on the association between JA-signaling pathways and attack by necrotrophic pathogens in different plant species. For example, Llorens et al. (2016) reported on the alteration in metabolic pathways after treatment with Hx, inoculation with *A. Alternata*, and inoculation with *A. Alternata* and treatment with Hx. Published literature suggests that treatment with Hx can induce the JA pathway against necrotrophic pathogens and increase compounds such as 12-

oxo-phytodienoic acid (OPDA), jasmonic acid (JA) or jasmonic-isoleucine (JA-Ile) (Vicedo et al., 2009; Camañes et al., 2015; Llorens et al., 2016). The results obtained in the current study have proven that *in vitro* priming with Hx only or in combination with the mutagens 5-AzaC and EMS produced plants that were resistant to *E. saccharina* and *F. pseudonygamai* SC17.

## 6. Conclusion

A protocol for producing sugarcane cultivars resistant to *F. pseudonygamai* SC17 and *E. saccharina* using two approaches was achieved. These two approaches were epigenetic priming (Hx) and chemical mutagenesis (EMS, 5-AzaC) or priming only (Hx). The established protocol for selecting a suitable priming agent concentration was accomplished. The relevant established concentration was 0.6 mM Hx that was used at both, the calli production and plantlet regeneration stages. To generate sugarcane lines tolerant to *F. pseudonygamai* and *E. saccharina*, *in vitro* plantlets produced from the established mutagenic/priming protocol needed to be selected on media containing the selection agent (i.e., *F. pseudonygamai* CF). The use of *in vitro* screening techniques using selective agents is an important strategy to select putative mutants before *ex vitro* screening and molecular analysis. The *F. pseudonygamai* SC17 CF was incorporated in the embryo germination media to determine its effect as a selection agent at the calli and plantlet regeneration stages. Root growth was enhanced by the increasing levels of the CF for cultivars 88H0019 and N41. As mentioned previously, fungal endophytes produce several secondary metabolites and toxins that are beneficial or antagonistic to pests or host plants. Hence, it was important to determine the plant growth-promoting activities of fungal pathogens.

In addition, this study revealed that there was an increase in root growth after 3 weeks, when plants were trimmed and transferred to semi solid embryo germination media (EGM2) containing increasing CF concentrations. It was suggested that the increase in root growth (1500 ppm CF) was likely due to the production of IAA or the regulation of the ACC deaminase enzyme by the endophyte. However, only screening for IAA production by the *F. pseudonygamai* fungal isolate SC17 was conducted and detecting the activity of ACC deaminase still needs to be done. To determine if the fungal culture filtrate produced IAA, assays were conducted to screen *F. pseudonygamai* SC17 for plant growth-promoting traits. The *F. pseudonygamai* isolate was able to synthesise IAA in both the presence and absence of L-tryptophan. *F. pseudonygamai* CF produced higher levels of IAA in the presence than in the absence of L- tryptophan. The established protocol produced putative mutants that were randomly selected and acclimatised *ex vitro* for 8 - 9 months. The putative mutants that were

only primed with Hx and the combined treatment (mutagens + Hx) resulted in decreased lesion severity ratings upon infection with only SC17, and dual inoculated with SC17 and 2<sup>nd</sup> instar eldana larvae.

In summary, the sugarcane putative mutants were randomly selected from each treatment without *in vitro* selection, this was due to the enhanced root growth caused by *F. pseudonygamai* in the study. The plant-growth promoting traits of the fungal isolate was assessed and it was found to produce IAA. The use of Hx to induce resistance against the necrotrophic pathogen *F. pseudonygamai* was effective in sugarcane. It would be valuable to investigate the different changes in transcript levels of JA/ET and SA-responsive marker genes in response to Hx. The established protocols can be used to assess resistance to *F. pseudonygamai* and *E. saccharina* in other commercially important sugarcane cultivars.

Further studies on the following should be conducted:

- Develop an *in vitro* selection protocol using *F. pseudonygamai* CF at higher concentrations than those used here during plantlet regeneration.
- A toxicological profile for *F. pseudonygamai* is required.
- Determining the activity of ACC deaminase of the fungal isolate *F. pseudonygamai*.

## References

- Adie, B.A.T., Pérez-Pérez, J., Pérez-Pérez, M.M., Godoy, M., Sánchez-Serrano, J.J., Schmelz, E.A., Solano, R., 2007. ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defences in *Arabidopsis*. *The Plant Cell* 19, 1665-1681.
- Afolabi, C.G., Ojiambo, P.S., Ekpo, E.J.A., Menkir, A. and Bandyopadhyay, R., 2008. Novel sources of resistance to *Fusarium* stalk rot of maize in tropical Africa. *Plant Disease* 92(5), 772-780.
- Ahmad, P., Rasool, S., Gul, A., Sheikh, S.A., Akram, N.A., Ashraf, M., Kazi, A.M., Guzel, S., 2016. Jasmonates: multifunctional roles in stress tolerance. *Frontiers in Plant Science* 7, 1-15.
- Ako, M., Schulthess, F., Gumedzoe, M.Y., Cardwell, K.F., 2003. The effect of *Fusarium verticillioides* on oviposition behaviour and bionomics of lepidopteran and coleopteran pests attacking the stem and cobs of maize in West Africa. *Entomologia Experimentalis et Applicata* 106(3), 201-210.
- Ali, A., Naz, S., Alam, S.S., Iqbal, J., 2007a. *In vitro* induced mutation for screening of red rot (*Colletotrichum falcatum*) resistance in sugarcane (*Saccharum officinarum*). *Pakistan Journal of Botany* 39(6), 1979-1994.
- Ali, A., Naz, S., Iqbal, J., 2007b. Effect of different explants and media compositions for efficient somatic embryogenesis in sugarcane (*Saccharum officinarum*). *Pakistan Journal of Botany* 39(6), 1961-1977.
- Ali, S., Ganai, B.A., Kamili, A.N., Bhat, A.A., Mir, Z.A., Bhat, J.A., Tyagi, A., Islam, S.T., Mushtaq, M., Yadav, P. and Rawat, S., 2018. Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbiological Research* 212, 29-37.
- Almazan, O., Gonzalez, L., Galvez, L. 2001. The sugarcane, its by-products and co-products. Annual Meeting of Agricultural Scientists. Sugar Cane International, 3-8.
- Altpeter, F., Springer, N.M., Bartley, L.E., Blechl, A.E., Brutnell, TP., Citovsky, V., Conrad, L., Gelvin, SB., Jackson, D., Kausch, A.P., Lemaux, P.G., Medford, JI., Orozco-Cardenas M, Tricoli, D., VanEck, J., Voytas, DF., Walbot, V., Wang, K., Zhang Z.J., Stewart, C.N., 2016. Advancing crop transformation in the era of genome editing. *Plant Cell* 28, 1510-1520.
- Andrew-Peter-Leon, M.T., Ramchander, S., KK, K., Muthamilarasan, M. and Pillai, M.A., 2021. Assessment of efficacy of mutagenesis of gamma-irradiation in plant height and days to maturity through expression analysis in rice. *Plos One* 16(1), 1-20.

- Anderson, A.J. and Kim, Y.C., 2021. The Plant-Stress Metabolites, Hexanoic Acid and Melatonin, Are Potential. *The Plant Pathology Journal* 37(5), 415-427.
- Andreozzi, A., Prieto, P., Mercado-Blanco, J., Monaco, S., Zampieri, E., Romano, Asai, T., Stone, J.M., Heard, J.E., Kovtun, Y., Yorgey, P., Sheen, J. and Ausubel, F.M., 2000. Fumonisin B1–induced cell death in *Arabidopsis* protoplasts requires jasmonate-, ethylene-, and salicylate-dependent signaling pathways. *The Plant Cell* 12(10), 1823-1835.
- Anil, V.S., Lobo, S., Bennur, S., 2018. Somaclonal variations for crop improvement: Selection for disease resistant variants *in vitro*. *Plant Science Today* 5(2), 44-54.
- Aranega-Bou, P., de la O Leyva, M., Finiti, I., García-Agustín, P., González-Bosch, C., 2014. Priming of plant resistance by natural compounds. Hexanoic acid as a model. *Frontiers in Plant Science* 5, 1-12.
- Arici, S., Tuncel, Z., Kara, A., Caltili, O., 2017. *In vitro* mutagenesis and selection of potato mutants resistance to *Fusarium* dry root. *Communications in Applied Biological Sciences* 82(3), 378-385.
- Arias, S.L., Theumer, M.G., Mary, V.S. and Rubinstein, H.R., 2012. Fumonisin: probable role as effectors in the complex interaction of susceptible and resistant maize hybrids and *Fusarium verticillioides*. *Journal of agricultural and food chemistry* 60(22), 5667-5675.
- Arruda, P., 2011. Perspective of the sugarcane industry in Brazil. *Tropical Plant Biology* 4, 3-8.
- Asai, T., Stone, J.M., Heard, J.E., Kovtun, Y., Yorgey, P., Sheen, J. and Ausubel, F.M., 2000. Fumonisin B1–induced cell death in *Arabidopsis* protoplasts requires jasmonate-, ethylene-, and salicylate-dependent signaling pathways. *The Plant Cell* 12(10), 1823-1835.
- Ashapkin, V.V., Kutueva, L.I., Aleksandrushkina, N.I. and Vanyushin, B.F., 2020. Epigenetic mechanisms of plant adaptation to biotic and abiotic stresses. *International Journal of Molecular Sciences* 21, 1-33.
- Ashiq,S., 2015. Natural occurrence of mycotoxins in food and feed: Pakistan perspective. *Comprehensive Reviews in Food Science and Food Safety* 14(2), 159-175.
- Asrul, L., Kuswinanti, T. and Musa, Y., 2020, April. Isolation of fungi producing hormone Indole Acetic Acid (IAA) on sugarcane bagasse and filter cake. In *IOP Conference Series: Earth and Environmental Science*, IOP Publishing 486(1), 1-7.
- Assefa, Y., Conlong, D.E., Van den Berg, J., Le Rü, B.P., 2008. The wider distribution of *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa and its potential risk to maize production. *Proceedings of the South African of Sugarcane Technologists' Association* 81, 290-297.

- Atanda, S.A., Pessu, P.O., Aina, J.A., Agoda, S., Adekalu, O.A., Ihionu, G.C., 2013. Mycotoxin management in agriculture. *Greener Journal of Agricultural Science* 3(2), 176-184.
- Atkinson, P.R., 1979. Distribution and natural hosts of *Eldana saccharina* Walker in Natal, its oviposition sites and feeding patterns. South African Sugar Technologists' Association Proceedings of the fifty-ninth annual congress held at Durban and Mount Edgecombe, 17 to 21 June 1985. South African Sugar Technologists' Association 59 Annual congress, South Africa
- Atkinson, P.R., 1981. Mating behaviour and activity patterns of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Journal of the Entomological Society of South Africa* 44, 265-280.
- Avanci, N.C., Luche, D.D., Goldman, G.H., Goldman, M.H.S., 2010. Jasmonates are phytohormones with multiple functions, including plant defense and reproduction. *Genetic Molecular Research* 9(1), 484-505.
- Bacon, C.W., Hinton, D.M., 1996. Symptomless endophytic colonization of maize by *Fusarium moniliforme*. *Canadian Journal of Botany* 74, 1195-1202.
- Bae, H., Sicher, R.C., Kim, M.S., Kim, S.H., Strem, M.D., Melnick, R.L., Bailey, B.A., 2009. The beneficial endophyte *Trichoderma hamatum* isolate DIS219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. *Journal of Experimental Botany* 60, 3279-3295.
- Bailey, R.A. 2004. Diseases. In: Sugarcane, 2nd Edition. James, G. (ed). Blackwell Science, Oxford, 55-77.
- Bairu, M.W., Aremu, A.O., Staden, J.V., 2011. Somaclonal variation in plants: causes and detection methods. *Plant Growth Regulation* 63, 147-173.
- Balestrini, R., Chitarra, W., Antoniou, C., Ruocco, M. and Fotopoulos, V., 2018. Improvement of plant performance under water deficit with the employment of biological and chemical priming agents. *The Journal of Agricultural Science* 156(5), 680-688.
- Ballaré, C.L. 2014. Light regulation of plant defense. *Annual Review Plant Biology* 65, 335-363
- Balmer, D., Planchamp, C., Mauch-Mani, B., 2012. On the move: induced resistance in monocots. *Journal of Experimental Botany* 64(5), 1249-1261.
- Baksha, R., Alam, R., Karim, M.Z., Paul, S.K., Hossain, M.A., Miah, M.A.S. and Rahman, A.B.M.M., 2002. *In vitro* shoot tip culture of sugarcane (*Saccharum officinarum*) variety LSD28. *Biotechnology* 1(2-4), 67-72.
- Bamisile BS, Dash CK, Akutse KS, Keppanan R, Wang LD. 2018. Fungal endophytes: beyond herbivore management. *Front Microbiology* 9, 544.

- Bandurska, H., Stroiński, A. and Kubiś, J., 2003. The effect of jasmonic acid on the accumulation of ABA, proline and spermidine and its influence on membrane injury under water deficit in two barley genotypes. *Acta Physiologiae Plantarum* 3, 279-285.
- Bari, R., Jones, J.D., 2009. Role of plant hormones in plant defence responses. *Plant Molecular Biology* 69, 473-88.
- Beck, E.H, Heim, R., Hansen, J., 2004. Plant resistance to cold stress: mechanisms and environmental signals triggering frost hardening and dehardening. *Journal of Biosciences* 29(4), 449-459
- Behera, K.K., Sahoo, S., 2009. Rapid in vitro micropropagation of sugarcane (*Saccharum officinarum* L. cv-Nayana) through callus culture. *Nature and Science* 7(4), 1-10.
- Benjamins, R., Scheres, B., 2008. Auxin: the looping star in plant development. *Annual Review of Plant Biology* 59, 443–465.
- Bennett, J.W., Bentley, R., 1989. What's in a name? Microbial secondary metabolism. *Advances in Applied Microbiology* 34, 1-28.
- Bennett, J.W., Klich, M., 2003. Mycotoxins. *Journal of Clinical Microbiology* 16(3), 487-516.
- Ben Youssef, R., Jelali, N., Boukari, N., Albacete, A., Martinez, C., Alfocea, F.P. and Abdelly, C., 2021. The Efficiency of Different Priming Agents for Improving Germination and Early Seedling Growth of Local Tunisian Barley under Salinity Stress. *Plants*, 10(11), 1-20.
- Berding, N., Hurney, A.P., 2005. Flowering and lodging, physiological-based traits affecting cane and sugar yield: What do we know of their control mechanisms and how do we manage them? *Field Crops Research* 92(2-3), 261-275.
- Berg, G., 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology* 84(1), 11-18.
- Bertini, L., Palazzi, L., Proietti, S., Pollastri, S., Arrigoni, G., Polverino de Laureto, P., Caruso, C., 2019. Proteomic analysis of MeJa-induced defense responses in rice against wounding. *International Journal of Molecular Sciences* 20(10), 1-24.
- Betbeder-Matibet, M., 1981. *Eldana saccharina* Walker, African sugarcane stalk borer. *Tropical Agronomy* 36, 279-293.
- Bhutani, N., Burns, D.M. and Blau, H.M., 2011. DNA demethylation dynamics. *Cell* 146(6), 866-872.
- Binarová, P., Nedělník, J., Fellner, M., Nedbálková, B., 1990. Selection for resistance to filtrates of *Fusarium spp.* in embryogenic cell suspension culture of *Medicago sativa* L. *Plant Cell, Tissue and Organ Culture* 22(3), 191-196.

- Birch, R.G., 1997. Transgenic sugarcane: Opportunities and limitations. In: Intensive sugarcane production: Meeting the challenges beyond 2000. Keating, B.A. and Wilson, J. R. (Eds), CAB International, Wallingford, 125-140.
- Blacutt, A.A., Gold, S.E., Voss, K.A., Gao, M., Glenn, A.E., 2018. *Fusarium verticillioides*: Advancements in understanding the toxicity, virulence, and niche adaptations of a model mycotoxigenic pathogen of maize. *Phytopathology*, 108(3), 312-326.
- Bock, C.H., Poole, G.H., Parker, P.E., Gottwald, T.R., 2010. Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. *Critical Reviews in Plant Sciences* 29(2), 59-107.
- Bock, C.H., Pethybridge, S.J., Barbedo, J.G., Esker, P.D., Mahlein, A.K., Del Ponte, E.M., 2021. A phytopathometry glossary for the twenty-first century: towards consistency and precision in intra-and inter-disciplinary dialogues. *Tropical Plant Pathology*, 1-11.
- Borges, A.A., Jiménez-Arias, D., Expósito-Rodríguez, M., Sandalio, L.M., Pérez, J.A., 2014. Priming crops against biotic and abiotic stresses: MSB as a tool for studying mechanisms. *Frontiers in Plant Science* 5 (642), 1-4.
- Borras, O., Santos R., Matos, A.P., Cabral, R.S., Arzola, M., 2001. A first attempt to use a *Fusarium subglutinans* culture for the selection of pineapple cultivar resistant to fusarirose disease. *Plant Breeding* 120, 435-438.
- Boter, M., Ruíz-Rivero, O., Abdeen, A., Prat, S., 2004. Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and *Arabidopsis*. *Genes & Development* 18(13), 1577-1591.
- Bourne, B.A., 1961. *Fusarium* sett or stem rot. In: Sugarcane disease of the world. Martin, J.P. Abbott, E.V., Hughes, C. G. (Eds.) Elsevier, New York, 186-202.
- Boyd, L.A., 2006. Can the durability of resistance be predicted? *Journal of the Science of Food and Agriculture* 86(15), 2523-2526.
- Boyko, A. and Kovalchuk, I., 2008. Epigenetic control of plant stress response. *Environmental and Molecular Mutagenesis* 49(1), 61-72.
- Boyko, A., Blevins, T., Yao, Y., Golubov, A., Bilichak, A., Ilnytskyy, Y., Hollander, J., Meins Jr, F., Kovalchuk, I., 2010. Transgenerational adaptation of *Arabidopsis* to stress requires DNA methylation and the function of Dicer-like proteins. *PLoS One* 5(3), 1-12.
- Boyko, A., Blevins, T., Yao, Y., Golubov, A., Bilichak, A., Ilnytskyy, Y., Hollander, J., Meins, F.J., Kovalchuk, I., 2010. Transgenerational adaptation of *Arabidopsis* to stress requires DNA methylation and the function of Dicer-like proteins. *PLoS One* 5(3), 1-12.
- Bradley, D.J., Kjellbom, P., Lamb, C.J., 1992. Elicitor-and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. *Cell* 70(1), 21-30.

- Brian, P.W., Dawkins, A.W., Grove, J.F., Hemming, H.G., Lowe, D., Norris, G.L.F., 1961. Phytotoxic compounds produced by *Fusarium equiseti*. *Journal of Experimental Botany* 12, 1-12.
- Brown, D.W., Butchko, R.A.E., Busman, M., Proctor, R.H., 2012. Identification of gene clusters associated with fusaric acid, fusarin, and perithecial pigment production in *Fusarium verticillioides*. *Fungal Genetics Biology* 49, 521–532.
- Bruce, T.J., Matthes, M.C., Napier, J.A., Pickett, J.A., 2007. Stressful “memories” of plants: evidence and possible mechanisms. *Plant Science* 173(6), 603-608.
- Brumbley, S.M., Snyman, S.J., Gnanasambandam, A., Joyce, P., Hermann, S.R., da Silva, J.A.G., McQualter, R.B., Wang, M., Egan, B.T., Patterson, A.H., Albert, H.H and Moore, P.H. 2008. Sugarcane. *A Compendium of Transgenic Crop Plants: Sugar, Tuber and Fiber Crops*, 7-55.
- Brutus, A., Sicilia, F., Macone, A., Cervone, F., De Lorenzo, G., 2010. A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. *Proceedings of the National Academy of Sciences United States of America* 107, 9452-9457.
- Bughio, H.R., Asad, M.A., Odhano, I.A., Bughio, M.S., Khan, M.A., Mastoi, N.N., 2007. Sustainable rice production through the use of mutation breeding. *Pakistan Journal of Botany* 39(7), 2457-2461.
- Bunsangiam, S., Thongpae, N., Limtong, S., Srisuk, N., 2021. Large scale production of indole-3-acetic acid and evaluation of the inhibitory effect of indole-3-acetic acid on weed growth. *Scientific Reports*, 11(1), 1-13.
- Burner, D.M. and Grisham, M.P., 1995. Induction and stability of phenotypic variation in sugarcane as affected by propagation procedure. *Crop science* 35(3), 875-880.
- Burrows, M., Grey, W., Dyer, A., 2008. *Fusarium* head blight (scab) of wheat and barley. *Montana State University Extension, Bozeman, Montana*, 1-4.
- Butterfield, M.K. and Thomas, D.W., 1996. Sucrose, yield and disease resistance characteristics of sugarcane varieties under test in the SASEX selection programme. *Proceedings of the South African of Sugarcane Technologists' Association* 70, 103-105.
- Butterfield, M.K., D'Hont, A., Berding, N., 2001. The sugarcane genome: a synthesis of current understanding, and lessons for breeding and biotechnology. In *Proceedings of the South African Sugarcane Technologist' Association* 75, 1-5.
- Caarls, L., Pieterse, C.M., Van Wees, S., 2015. How salicylic acid takes transcriptional control over jasmonic acid signaling. *Frontiers in Plant Science* 6, 170.

- Caccalano, M.N., Dilarri, G., Zamuner, C.F.C., Domingues, D.S., Ferreira, H., 2021. Hexanoic acid: a new potential substitute for copper-based agrochemicals against citrus canker. *Journal of Applied Microbiology* ISSN 1364-5072, 1-12.
- Camañes, G., Scalschi, L., Vicedo, B., González-Bosch, C., García-Agustín, P., 2015. An untargeted global metabolomic analysis reveals the biochemical changes underlying basal resistance and priming in *Solanum lycopersicum* and identifies 1-methyltryptophan as a metabolite involved in plant responses to *Botrytis cinerea* and *Pseudomonas syringae*. *The Plant Journal* 84(1), 125-139.
- Cameron, D.D., Neal, A.L., van Wees, S.C., Ton, J., 2013. Mycorrhiza-induced resistance: more than the sum of its parts? *Trends in Plant Science* 18(10), 539-545.
- Campos, M.L., Kang, J.H., Howe, G.A., 2014. Jasmonate-triggered plant immunity. *Journal of Chemical Ecology* 40(7), 657-675.
- Carnegie, A.J.M., 1974. A recrudescence of the borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Proceedings of the South African of Sugarcane Technologists' Association* 48, 107-110.
- Carpita, N.C. and Gibeaut, D.M., 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *The Plant Journal* 3(1), 1-30
- Carroll G. 1988 Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology* 69, 2-9.
- Cast, 2003. Mycotoxins: risks in plant, animal and human systems. Task Force Report No. 139, Ames, Iowa: Council for Agricultural Science and Technology, 53.
- Chakraborty, N., Banerjee, M., Acharya, K., 2020. *In vitro* selection of elite clone of *Withania somnifera* against leaf blight disease caused by *Alternaria alternata*. *Physiological and Molecular Plant Pathology* 112, 1-9.
- Chanclud, E. and Morel, J.B., 2016. Plant hormones: a fungal point of view. *Molecular plant pathology* 17(8), 1289-1297.
- Chassot, C., Buchala, A., Schoonbeek, H.J., Métraux, J.P., Lamotte, O., 2008. Wounding of *Arabidopsis* leaves causes a powerful but transient protection against *Botrytis* infection. *The Plant Journal* 55(4), 555-567.
- Cheavegatti-Gianotto, A., de Abreu, H.M.C., Arruda, P., Bespalhok Filho, J.C., Burnquist, W.L., Creste, S., di Ciero, L., Ferro, J.A., de Oliveira Figueira, A.V., de Sousa Filgueiras, T., de Fátima Grossi-de-Sá, M., 2011. Sugarcane (*Saccharum X officinarum*): a reference study for the regulation of genetically modified cultivars in Brazil. *Tropical Plant Biology* 4(1), 62-89.
- Chen, W.Q. and Swart, W.J., 2002. The *in vitro* phytotoxicity of culture filtrates of *Fusarium oxysporum* to five genotypes of *Amaranthus hybridus*. *Euphytica*, 127(1), 61-67.

- Chinheya, C.C., Mutambara-Mabveni, A.R.S., Chinwada, P., 2009. Assessment of damage due to *Eldana saccharina walker* (Lepidoptera: Pyralidae) in sugarcane. Proceedings of the South African of Sugarcane Technologists' Association 82, 446-456
- Chitnis, V.R., Suryanarayanan, T.S., Nataraja, K.N., Prasad, S.R., Oelmüller, R., Shaanker, R.U., 2020. Fungal endophyte-mediated crop improvement: the way ahead. Frontiers in Plant Science 11, 1588.
- Chivasa, S., Ndimba, B.K., Simon, W.J., Lindsey, K., Slabas, A.R., 2005. Extracellular ATP functions as an endogenous external metabolite regulating plant cell viability. The Plant Cell 17(11), 3019-3034.
- Choi, J., Tanaka, K., Cao, Y., Qi, Y., Qiu, J., Liang, Y., Lee, S.Y., Stacey, G., 2014. Identification of a plant receptor for extracellular ATP. Science 343, 290-294
- Christman, J.K., 2002. 5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. Oncogene 21, 5483-5495
- Ciegler, A., 1978. Fungi that produce mycotoxins: conditions and occurrence. Mycopathologia 65(1-3), 5-11.
- Cipollini, D., 2010. Constitutive expression of methyl jasmonate-inducible responses delays reproduction and constrains fitness responses to nutrients in *Arabidopsis thaliana*. Evolutionary Ecology 24(1), 59-68.
- Clarke, J.D., Volko, S.M., Ledford, H., Ausubel, F.M., Dong, X., 2000. Roles of salicylic acid, jasmonic acid, and ethylene in cpr-induced resistance in *Arabidopsis*. The Plant Cell, 12(11), 2175-2190.
- Cleland, R.E., 1987. Auxin and cell elongation. In Plant hormones and their role in plant growth and development. Springer, Dordrecht, 132-148.
- Cohen, Y.R., 2002.  $\beta$ -aminobutyric acid-induced resistance against plant pathogens. Plant Disease 86(5), 448-457.
- Cole, S.J., Yoon, A.J., Faull, K.F., Diener, A.C., 2014. Host perception of jasmonates promotes infection by *Fusarium oxysporum* formae speciales that produce isoleucine-and leucine-conjugated jasmonates. Molecular Plant Pathology 15(6), 589-600.
- Coll, J.E., Weiss, E.L., Yarvis, J.S., Oh, H., 2011. No one leaves unchanged: Insights for civilian mental health care professionals into the military experience and culture. Social Work in Health Care 50(7), 487-500.
- Compant, S., Duffy, B., Nowak, J., Clement, C., Ait, Barka., 2005. Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action and future prospects. Applied Environmental Microbiology 71(9), 4951-4959.

- Conlong, D.E., 1994a. A review and perspectives for the biological control of the African sugarcane stalkborer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Agriculture, Ecosystems & Environment* 48(1), 9-17.
- Conlong D.E., 1994b. Biological control of *Eldana saccharina* Walker in South African sugarcane: Constraints identified from 15 years of research. *Insect Science and its Application* 17, 69-78.
- Conlong, D.E., 2001. Biological control of indigenous African stem borers: What do we know? *Insect Science and its Application* 21, 267-274.
- Connell, S.A., Legg, T., Heale, J.B., 1990. Sensitivity of cells and protoplasts of Hop cultivars to cytotoxic components of culture filtrates of *Verticillium albo-atrum* isolates from Hop. *Plant Pathology* 39, 92-101.
- Conrath, U., Pieterse, C.M., Mauch-Mani, B., 2002. Priming in plant-pathogen interactions. *Trends in Plant Science* 7(5), 210-216.
- Conrath, U., Beckers, G.J., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., Newman, M.A., Pieterse, C.M., Poinssot, B., Pozo, M.J., Pugin, A., 2006. Priming: getting ready for battle. *Molecular Plant-Microbe Interactions* 19(10), 1062-1071.
- Conrath, U., 2009. Priming of induced plant defense responses. *Advances in Botanical Research* 51, 361-395.
- Conrath, U., 2011. Molecular aspects of defence priming. *Trends in Plant Science* 16(10), 524-531.
- Conrath, U., Beckers, G.J., Langenbach, C.J., Jaskiewicz, M.R., 2015. Priming for enhanced defense. *Annual Review of Phytopathology*, 53, 97-119.
- Contreras-Cornejo, H.A., Macías-Rodríguez, L., Cortés-Penagos, C. and López-Bucio, J., 2009. *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. *Plant physiology* 149(3), 1579-1592.
- Costa, M.M., Melo, M.P., Guimarães, E.A., Veiga, C.M.O., Carmo Sandin, F., Moreira, G.M., Costa, S.S., Pfenning, L.H., 2019. Identification and pathogenicity of *Fusarium* species associated with pokkah boeng of sugarcane in Brazil. *Plant Pathology* 68(7), 1350-1360.
- Croft, B.J., 2000. *Fusarium* sett or stem rot. In: *A guide to sugarcane diseases*. Rott, P., Jack, R.A., Comstock, C., Croft, B.J., Saumtally, A.S. (Eds.). CIRAD and ISSCT, 107-110.
- Currais, L., Loureiro, J., Santos, C., Canhoto, J.M., 2013. Ploidy stability in embryogenic cultures and regenerated plantlets of tamarillo. *Plant Cell, Tissue and Organ Culture* 114,149-159.
- D'Hont, A., Sousa, G.M., Menossi, M., Vincentz, M., Van Sluys, M., Glaszmann, J.C., Ulian, E., 2008. Sugarcane: A major source of sweetness, alcohol, and bio-energy. In:

- Genomics of tropical crop plants. Moore, P.H., Ming, R. (Eds.). Springer, New York, pp 483-518.
- Dai, C.C., Yu, B.Y., Li, X., 2008. Screening of endophytic fungi that promote the growth of *Euphorbia pekinensis*. *African Journal of Biotechnology* 7, 3505-3509.
- Dafoe, N.J., Thomas, J.D., Shirk, P.D., Legaspi, M.E., Vaughan, M.M., Huffaker, A., Teal, P.E., Schmelz, E.A., 2013. European corn borer (*Ostrinia nubilalis*) induced responses enhance susceptibility in maize. *PLoS One*, 8(9), 1-18.
- Daou, R., Joubrane, K., Maroun, R.G., Khabbaz, L.R., Ismail, A., El Khoury, A., 2021. Mycotoxins: Factors influencing production and control strategies. *AIMS Agriculture and Food* 6(1), 416-447.
- Davis, R.M., Colyer, P.D., Rothrock, C.S., Kochman, J.K., 2006. *Fusarium* wilt of cotton: population diversity and implications for management. *Plant Disease* 90(6), 692-703.
- Debnath, M., Malik, C.P. and Bisen, P.S., 2006. Micropropagation: a tool for the production of high-quality plant-based medicines. *Current Pharmaceutical Biotechnology* 7(1), 33-49.
- Dela Cueva, F., De Torres, R., de Castro, A., Mendoza, J., Balendres, M.A., 2019. Susceptibility of sugarcane to red rot caused by two *Fusarium* species and its impact on stalk sugar level. *Journal of Plant Pathology*, 101(3).
- De La Torre-Hernandez, M.E., Rivas-San Vicente, M., Greaves-Fernandez, N., Cruz-Ortega, R., Plasencia, J., 2010. Fumonisin B1 induces nuclease activation and salicylic acid accumulation through long-chain sphingoid base build-up in germinating maize. *Physiological and Molecular Plant Pathology*, 74(5-6), 337-345.
- De Vleeschauwer, D., Gheysen, G., Hofte, M. 2013. Hormone defense net-working in rice: tales from a different world. *Trends in Plant Science* 18, 555-565.
- Dempsey, D.M.A. and Klessig, D.F., 2012. SOS—too many signals for systemic acquired resistance? *Trends in Plant Science* 17(9), 538-545.
- Desai, K., Sullards, M.C., Allegood, J., Wang, E., Schmelz, E.M., Hartl, M., Humpf, H.U., Liotta, D.C., Peng, Q., Merrill Jr, A.H., 2002. Fumonisin analogs as inhibitors of ceramide synthase and inducers of apoptosis. *Molecular and Cell Biology of Lipids* 1585(2-3), 188-192.
- Desjardins, A.E., Plattner, R.D., Proctor, R.H. 1996. Linkage among genes responsible for fumonisin biosynthesis in *Gibberella fujikuroi* mating population A. *Applied and Environmental Microbiology* 62, 2571-2576.
- Desjardins, A.E. and Hohn, T.M., 1997. Mycotoxins in plant pathogenesis. *Molecular Plant-Microbe Interactions* 10, 147-153.
- Desjardins, A.E., 2006. *Fusarium* Mycotoxin: Chemistry, genetics, and biology. American Phytopathology Society, Minnesota, USA, 260.

- Dey, T., Saha, S., Ghosh, P.D., 2015. Somaclonal variation among somatic embryo derived plants—evaluation of agronomically important somaclones and detection of genetic changes by RAPD in *Cymbopogon winterianus*. South African Journal of Botany 96, 112-121.
- Dick, J., 1945. Some data on the biology of the sugarcane borer (*Eldana saccharina* Wlk.). Proceedings of the South African of Sugarcane Technologists' Association 19, 75-79.
- Dicke, M. and Baldwin, I.T., 2010. The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. Trends in Plant Science 15(3), 167-175.
- Dickman, M.B. and Fluhr, R., 2013. Centrality of host cell death in plant-microbe interactions. Annual Review of Phytopathology 51, 543-570.
- Diehdiou, A.G., Arora, N.K., Tawfeeq Al-Ani, L.K., Ngom, M., Fall, S., Hafidi, M., Ouhdouch, Y., Kouisni, L., SY, M.O., 2021. Potential role and utilization of plant growth promoting microbes in plant tissue culture. Frontiers in Microbiology 12, 1-13.
- Dinolfo, M.I., Castañares, E. and Stenglein, S.A., 2017. *Fusarium*-Plant Interaction: State of the Art- a Review. Plant Protection Science 53(2), 61-70.
- Djami-Tchatchou, A.T., Ncube, E.N., Steenkamp, P.A., Dubery, I.A., 2017. Similar, but different: Structurally related azelaic acid and hexanoic acid trigger differential metabolomic and transcriptomic responses in tobacco cells. BMC plant biology 17(1), 1-15.
- Dlamini, P.J., 2021. Drought stress tolerance mechanisms and breeding effort in sugarcane: A review of progress and constraints in South Africa. Plant Stress, 1-18.
- Duarte, R.P., Rezende, R.K.S., Pinto, F., Jesus, M.V., da Silva Junior, I.R., Scoton, A.M., 2018. *In Vitro* Priming of Sugarcane Varieties (RB966928 and RB867515). American Journal of Plant Sciences 9(04), 763-774.
- Ebel, J., and Cosio, E. G., 1994. Elicitors of plant defense responses. International Review of Cytology 148, 1-36.
- El Oirdi, M., El Rahman, T.A., Rigano, L., El Hadrami, A., Rodriguez, M.C., Daayf, F., Vojnov, A., Bouarab, K., 2011. *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in tomato. The Plant Cell 23, 2405-2421.
- Erb, M., Meldau, S., Howe, G.A., 2012. Role of phytohormones in insect-specific plant reactions. Trends in Plant Science 17(5), 250-259.
- Espinas, N.A., Saze, H., Saijo, Y., 2016. Epigenetic control of defense signaling and priming in plants. Frontiers in Plant Science 7 (1201), 1-7.
- Espina, M.J., Ahmed, C.M., Bernardini, A., Adeleke, E., Yadegari, Z., Arelli, P., Pantalone, V., Taheri, A., 2018. Development and phenotypic screening of an ethyl methane sulfonate mutant population in soybean. Frontiers in Plant Science 9 (394), 1-12.

- Espinosa-Leal, C.A., Puente-Garza, C.A., García-Lara, S., 2018. *In vitro* plant tissue culture: means for production of biological active compounds. *Planta* 248(1), 1-18.
- Evans, D.A., Sharp, W.R., Medina-Filho, H.P., 1984. Somaclonal and gametoclonal variation. *American Journal of Botany* 71, 759-774.
- Evans, D.L. and Joshi, S.V., 2016. Complete chloroplast genomes of *Saccharum spontaneum*, *Saccharum officinarum* and *Miscanthus floridulus* (Panicoideae: Andropogoneae) reveal the plastid view on sugarcane origins. *Systematics and Biodiversity* 14(6), 548-571.
- Fadiji, A.E. and Babalola, O.O., 2020. Elucidating mechanisms of endophytes used in plant protection and other bioactivities with multifunctional prospects. *Frontiers in Bioengineering and Biotechnology* 8(467), 1-20.
- Fang, H., Liu, X., Thorn, G., Duan, J. and Tian, L., 2014. Expression analysis of histone acetyltransferases in rice under drought stress. *Biochemical and Biophysical Research Communications* 443(2), 400-405.
- Feher, A., 2015. Somatic embryogenesis-stress-induced remodelling of plant cell fate. *BBA Gene Regulation Mechanisms* 1849, 385-402.
- Felton, G.W., Tumlinson, J.H., 2008. Plant-insect dialogs: complex interactions at the plant-insect interface. *Current Opinion in Plant Biology* 11, 457-463.
- Fernández-Crespo, E., Navarro, J.A., Serra-Soriano, M., Finiti, I., García-Agustín, P., Pallás, V. and González-Bosch, C., 2017. Hexanoic acid treatment prevents systemic MNSV movement in Cucumis melo plants by priming callose deposition correlating SA and OPDA accumulation. *Frontiers in Plant Science* 8 (1793), 1-15
- Fieldes, M.A., Amyot, L.M., 1999. Evaluating the potential of using 5-azacytidine as an epimutagen. *Canadian Journal of Botany* 77, 1617-1622.
- Flors, V., Miralles, C.M., González-Bosch, C., Carda, M., García-Agustín, P., 2003. Induction of protection against the necrotrophic pathogens *Phytophthora citrophthora* and *Alternaria solani* in *Lycopersicon esculentum* Mill. by a novel synthetic glycoside combined with amines. *Planta* 216(6), 929-938.
- Fonseca, S., Chini, A., Hamberg, M., Adie, B., Porzel, A., Kramell, R., Miersch, O., Wasternack, C., Solano, R., 2009. (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nature Chemical biology* 5, 344-350.
- Forster, B.P., and Shu, Q.Y., 2012. Plant mutagenesis in crop improvement: Basic terms and applications, in: Shu, Q.Y, Foster, B.P and Nakagawa, H (Eds.), *Plant mutation breeding and biotechnology*. Centre for Agriculture and Bioscience International, 9-21.
- Fontana, D.C., de Paula, S., Torres, A.G., de Souza, V.H.M., Pascholati, S.F., Schmidt, D., Dourado Neto, D., 2021. Endophytic Fungi: Biological Control and Induced Resistance to Phytopathogens and Abiotic Stresses. *Pathogens* 10(5), 570.

- Franke, R., Briesen, I., Wojciechowski, T., Faust, A., Yephremov, A., Nawrath, C., Schreiber, L., 2005. Apoplastic polyesters in *Arabidopsis* surface tissues-a typical suberin and a particular cutin. *Phytochemistry* 66(22), 2643-2658.
- Franklin, G., Arvinth, S., Sheeba, C.J., Kanchana, M., Subramonian, N., 2006. Auxin pretreatment promotes regeneration of sugarcane (*Saccharum spp.* hybrids) midrib segment explants. *Plant Growth Regulation* 50(2-3), 111-119.
- Freire, F.D.C.O. and da Rocha, M.E.B., 2017. Impact of mycotoxins on human health. *Fungal Metabolites*, 239-261.
- Freeman, B.C. and Beattie, G.A., 2008. An overview of plant defences against pathogens and herbivores. *The Plant Health Instructor*.
- Frost, C.J., Mescher, M.C., Carlson, J.E. and De Moraes, C.M., 2008. Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiology* 146(3), 818-824.
- Fu, S.F., Wei, J.Y., Chen, H.W., Liu, Y.Y., Lu, H.Y., Chou, J.Y., 2015. Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. *Plant Signaling & Behavior* 10(8), 1-9.
- Gai, X., Dong, H., Wang, S., Liu, B., Zhang, Z., Li, X., Gao, Z., 2018. Infection cycle of maize stalk rot and ear rot caused by *Fusarium verticillioides*. *PLOS one* 13(7), 1-11.
- Garcia-Brugger, A., Lamotte, O., Vandelle, E., Bourque, S., Lecourieux, D., Poinssot, B., Wendehenne, D. and Pugin, A., 2006. Early signaling events induced by elicitors of plant defenses. *Molecular Plant-Microbe Interactions* 19(7), 711-724.
- Gamir, J., Sánchez-Bel, P. and Flors, V., 2014. Molecular and physiological stages of priming: how plants prepare for environmental challenges. *Plant Cell Reports* 33(12), 1935-1949.
- Ganley, R.J., Sniezko, R.A., Newcombe, G., 2008. Endophyte-mediated resistance against white pine blister rust in *Pinus monticola*. *Forest Ecology and Management* 255(7), 2751-2760.
- Gayatri, M.C. and Kavyashree, R., 2005. Selection of turmeric callus tolerant to culture filtrate of *Pythium Graminicolum* and regeneration of plants. *Plant Cell, Tissue and Organ Culture* 83(1), 33-40.
- Gazaffi, R., Oliveira, K.M., de Souza, A.P., Garcia, A.A.F., 2014. Sugarcane: Breeding methods and genetic mapping. In Luis Augusto Barbosa Cortez (Coord.). *Sugarcane bioethanol - R&D for Productivity and Sustainability*, São Paulo: Editora Edgard Blücher, 333-344.
- Getnet, B., 2017. Review on *in vitro* propagation of sugarcane to advance the value of tissue culture. *Agricultural Research & Technology Open Access Journal* 5 (4), 99-105.

- Gfeller, A., Liechti, R., Farmer, E.E., 2010. *Arabidopsis* jasmonate signaling pathway. *Science Signaling* 3(109), 1-2.
- Ghozlan, M.H., Eman, E.A., Tokgöz, S., Lakshman, D.K., Mitra, A., 2020. Plant Defense against Necrotrophic Pathogens. *American Journal of Plant Sciences*, 11(12), 2122-2138.
- Gianotto, A.C., Rocha, M.S., Cutri, L., Lopes, F.C., Dal'Acqua, W., Hjelle, J.J., Lirette, R.P., Oliveira, W.S., Sereno, M.L., 2019. The insect-protected CTC91087-6 sugarcane event expresses Cry1Ac protein preferentially in leaves and presents compositional equivalence to conventional sugarcane. *GM Crops & Food*, 10(4), 208-219.
- Gilbert, R. A., Gallo-Meagher, M., Comstock, J. C., Miller, J.D., Jain, M., Abouzid, A., 2005. Agronomic evaluation of sugarcane lines transformed for resistance to Sugarcane mosaic virus Strain E. *Crop Science* 45, 2060-2067.
- Gilchrist, L., Vivar, H., Franco, J., Crossa, J., 1997. Comparing *Fusarium graminearum* infection period in wheat and barley. *Cereal Research Communications* 25(3), 739-740.
- Gilchrist, D.G., 1998. Programmed cell death in plant disease: the purpose and promise of cellular suicide. *Annual Review of Phytopathology* 36(1), 393-414.
- Gill, R., Malhotra, P.K., Gosal, S.S., 2006. Direct plant regeneration from cultured young leaf segments of sugarcane. *Plant cell, Tissue and Organ Culture* 84(2), 227-231.
- Gill, R.S., Meeta, M., Thind, K.S., Kumar, B., 2007. Identification of red rot resistant promising crosses. *Sugar Tech*, 9(4), 321-324.
- Gimenez-Ibanez, S., Chini, A. and Solano, R., 2016. How microbes twist jasmonate signaling around their little fingers. *Plants* 5(9), 1-12.
- Girling., DJ. 1972. *Eldana saccharina* Walker (Lepidoptera: Pyralidae), a pest of sugarcane in East Africa. *Proceedings of the International Society of Sugar Cane Technologists* 14, 429-434.
- Glazebrook, J., 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* 43, 205-227.
- Glick, B.R., Cheng, Z., Czarny, J., Duan, J., 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. *New perspectives and approaches in plant growth-promoting Rhizobacteria Research*, 329-339.
- Glenn, A.E., 2007. Mycotoxigenic *Fusarium* species in animal feed. *Animal Feed Science and Technology* 4, 213-240.
- Goebel, F.R. and Way, M.J., 2003. Investigation of the impact of *Eldana saccharina* (Lepidoptera: Pyralidae) on sugarcane yield in field trials in Zululand. *Proceedings of the South African of Sugarcane Technologists' Association* 77, 256-265.

- Goebel, F.R., Way, M.J., Gossard, C., 2005. The status of *Eldana saccharina* (Lepidoptera: Pyralidae) in the South African sugar industry based on regular survey data. Proceedings of the South African Sugarcane Technologists Association 79, 337-346.
- Goebel, F.R., Beuzelin, J.M., Way, M.J., 2018. Progress in understanding and managing insect pests affecting sugarcane. In: Achieving sustainable cultivation of sugarcane. Vol 2: Breeding, pest and diseases. P Rott (Eds.), University of Florida, Burleigh Dodds series in Agricultural Science, 363-94.
- Goossens, J., Fernández-Calvo, P., Schweizer, F., Goossens, A., 2016. Jasmonates: signal transduction components and their roles in environmental stress responses. Plant Molecular Biology 91(6), 673-689.
- Govender, P., McFarlane S.A., Rutherford, R.S., 2010. *Fusarium* species causing Pokkah boeng and their effect on *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Proceedings of the South African of Sugarcane Technologists Association 83, 267-270.
- Grant-Downton, R.T., Dickinson, H.G., 2005. Epigenetics and its implications for plant biology. Annals of Botany 96, 1143-1164.
- Gray, L.E., Guan, Y.Q., Widholm, J.M., 1986. Reaction of soybean callus to culture filtrates of *Phialophora gregata*. Plant Science 47(1), 45-55.
- Grisham, M.P. and Bourg, D., 1989. Efficiency of in vitro propagation of sugarcane plants by direct regeneration from leaf tissue and by shoot-tip culture. Journal of American Society Sugar Technology 9, 97-102.
- Grivet, L. and Arruda, P. 2001. Sugarcane genomics: depicting the complex genome of an important tropical crop. Current Opinion in Plant Biology 5, 122-127.
- Grzybkowska, D., Morończyk, J., Wójcikowska, B., Gaj, M.D., 2018. Azacytidine (5-AzaC)-treatment and mutations in DNA methylase genes affect embryogenic response and expression of the genes that are involved in somatic embryogenesis in *Arabidopsis*. Plant Growth Regulation 85(2), 243-256.
- Haile, A. and Hofsvang, T., 2001. Survey of lepidopterous stem borer pests of sorghum, maize and pearl millet in Eritrea. Crop Protection 20(2), 151-157.
- Halperin, W., 1966. Alternative morphogenetic events in cell suspensions. American Journal of Botany 53, 443-453.
- Hailu, M., Chimdessa, M. and Muthswamy, M., 2018. *In vitro* propagation of selected sugarcane (*Saccharum officinarum* L.) Varieties (C 86-165 and C 86-12) through shoot apical meristem. International Journal of Horticulture and Agriculture 3(1), 1-7.
- Hamann, T., 2012. Plant cell wall integrity maintenance as an essential component of biotic stress response mechanisms. Frontiers Plant Sciences 3 (77), 1-5.

- Hammerschmidt, R., 2009. Systemic acquired resistance. *Advances in Botanical Research* 51, 173-222.
- Hassan, S.E.D., 2017. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium L.* *Journal of Advanced Research* 8(6), 687-695.
- He, Y. and Li, Z., 2018. Epigenetic environmental memories in plants: establishment, maintenance, and reprogramming. *Trends in Genetics* 34(11), 856-866.
- Hedden, P., Sponsel, V. A., 2015. Century of Gibberellin Research. *Journal of Plant Growth Regulation* 34, 740-760.
- Heil, M., 2004. Direct defense or ecological costs: responses of herbivorous beetles to volatiles released by wild lima bean. *Journal of Chemical Ecology* 30, 1289-1295.
- Heil, M., Land, W.G., 2014. Danger signals - damaged-self recognition across the tree of life. *Frontiers in Plant Science* 5, 578.
- Heinz, D.J. and Mee, G.W.P., 1969. Plant differentiation from callus tissue of *Saccharum species*. *Crop Science* 9, 346-348.
- Heinz, D.J., 1973. Sugarcane improvement through induced mutations using vegetative propagules and cell culture techniques. In: *Induced mutations in vegetatively propagated plants. Proc. of a Panel, Intern. Atomic Energy Agency, Vienna*, 53-59.
- Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., 2016. Priming and memory of stress responses in organisms lacking a nervous system. *Biological Reviews of the Cambridge Philosophical Society* 91, 1118-33.
- Hirsch, J., Galuszka, P., Tudzynski, P. 2016. Functional characterization of the first filamentous fungal tRNA-isopentenyltransferase and its role in the virulence of *Claviceps purpurea*. *New Phytologist* 211, 980-992.
- Ho, W.J. and Vasil, I.K., 1983. Somatic embryogenesis in sugarcane (*Saccharum officinarum L.*) I. The morphology and physiology of callus formation and the ontogeny of somatic embryos. *Protoplasma* 118(3), 169-180.
- Hoffmann, G.R., 1980. Genetic effects of dimethyl sulfate, diethyl sulfate, and related compounds. *Mutation Research/Reviews in Genetic Toxicology* 75(1), 63-129.
- Hogenhout, S.A., Bos, J.I., 2011. Effector proteins that modulate plant– insect interactions. *Current Opinion in Plant Biology* 14, 422-428.
- Holme, I.B., Gregersen, P.L. and Brinch-Pedersen, H., 2019. Induced genetic variation in crop plants by random or targeted mutagenesis: convergence and differences. *Frontiers in Plant Science* 10 (1468), 1-9.
- Hooley, R., 1994. Gibberellins: Perception, transduction and responses. *Plant Molecular Biology* 26, 1529-1555.

- Horstman, A., Bemer, M., Boutilier, K., 2017. A transcriptional view on somatic embryogenesis. *Regeneration* 4(4), 201-216.
- Hossain, M.M. and Sultana, F., 2020. Application and mechanisms of plant growth promoting fungi (PGPF) for phytostimulation. *Organic Agriculture*, 1-31.
- Howe, G.A. and Jander, G., 2008. Plant immunity to insect herbivores. *Annual Review of Plant Biology* 59, 41-66.
- Howlett, B.J., 2006. Secondary metabolite toxins and nutrition of plant pathogenic fungi. *Current Opinion in Plant Biology* 9(4), 371-375.
- Hsuan, H.M., Zakaria, L., Salleh, B., 2010. Characterization of *Fusarium* isolates from rice, sugarcane and maize using RFLP-IGS. *Journal of Plant Protection Research* 50 (4), 409-415.
- Idrees, M and Irshad, M., 2014. Molecular markers in plants for analysis of genetic diversity: a review. *European Academic Research* 2(1), 1513-1540.
- Ikeuchi, M., Sugimoto, K., & Iwase, A., 2013. Plant callus: mechanisms of induction and repression. *Plant Cell* 25, 3159-3173
- Ioannou, A., Gohari, G., Papaphilippou, P., Panahirad, S., Akbari, A., Dadpour, M.R., Krasia-Christoforou, T. and Fotopoulos, V., 2020. Advanced nanomaterials in agriculture under a changing climate: the way to the future? *Environmental and Experimental Botany* 176, 1-43.
- Iqbal, M., Javed, N., Sahi, S.T. and Cheema, N.M., 2011. Genetic management of bakanae disease of rice and evaluation of various fungicides against *Fusarium moniliforme* in vitro. *Journal of Phytopathology* 23, 103-107.
- Iqbal, M.S., Tabassum, B., Awan, M.F., Tariq, M., Ali, Q., Sharif, M.N., Nasir, I.A., 2020. Genetic variability of sugarcane genotypes for red rot. *Genetics and Molecular Research* 19 (1), 1-12.
- Ismail, A.A., Papenbrock, J., 2015. Mycotoxins: Producing fungi and mechanisms of phytotoxicity, *Journal of Agriculture* 5, 492-537.
- Ismail, M.A., Amin, M.A., Eid, A.M., Hassan, S.E.D., Mahgoub, H.A., Lashin, I., Abdelwahab, A.T., Azab, E., Gobouri, A.A., Elkelish, A., Fouda, A., 2021. Comparative Study between Exogenously Applied Plant Growth Hormones versus Metabolites of Microbial Endophytes as Plant Growth-Promoting for *Phaseolus vulgaris* L. *Cells* 10(5), 1059.
- Issa, J.P.J., Kantarjian, H.M., 2009. Targeting DNA methylation. *Clinical Cancer Research* 15, 3938-3946.
- ISAAA. 2018. Global Status of Commercialized Biotech/GM Crops in 2018: Biotech Crops Continue to Help Meet the Challenges of Increased Population and Climate Change. ISAAA Brief No. 54. ISAAA: Ithaca, NY.

- Jabeen, N. and Mirza, B., 2004. Ethyl methane sulfonate induces morphological mutations in *Capsicum annuum*. *International Journal Agricultural Biology* 6, 340-345.
- Jain, S.M. 2001. Tissue culture-derived variation in crop improvement. *Euphytica* 118, 153-166.
- Jain, S.M., 2010. Mutagenesis in crop improvement under the climate change. *Romanian biotechnological letters* 15(2), 88-106.
- Jakab, G., Cottier, V., Toquin, V., Rigoli, G., Zimmerli, L., Métraux, J.P., Mauch-Mani, B., 2001.  $\beta$ -Aminobutyric acid-induced resistance in plants. *European Journal of Plant Pathology* 107(1), 29-37.
- James, C., 2015. Global Status of Commercialized Biotech/GM Crops: 2015. ISAAA Brief no. 51. International Service for the Acquisition of Agri-biotech applications, Ithaca, New York.
- Jaroszuk-Ścisel, J., Kurek, E., Trytek, M., 2014. Efficiency of indoleacetic acid, gibberellic acid and ethylene synthesized *in vitro* by *Fusarium culmorum* strains with different effects on cereal growth. *Biologia* 69(3), 281-292.
- Jaroszuk-Ścisel, J., Tyśkiewicz, R., Nowak, A., Ozimek, E., Majewska, M., Hanaka, A., Tyśkiewicz, K., Pawlik, A., Janusz, G., 2019. Phytohormones (auxin, gibberellin) and ACC deaminase *in vitro* synthesized by the mycoparasitic *Trichoderma* DEMTkZ3A0 strain and changes in the level of auxin and plant resistance markers in wheat seedlings inoculated with this strain conidia. *International Journal of Molecular Sciences* 20(19), 1-35.
- Ji, F., He, D., Olaniran, A.O., Mokoena, M.P., Xu, J., Shi, J., 2019. Occurrence, toxicity, production and detection of *Fusarium* mycotoxin: A review. *Food Production, Processing and Nutrition* 1(1), 6.
- Jiang, GL., 2013. Molecular markers and marker-assisted breeding in plants, In: Andersen SB, editor. *Plant breeding from laboratories to fields*. Rijeka: InTech, 45-83.
- Jia, Y.J., Kakuta, Y., Sugawara, M., Igarashi, T., Oki, N., KisAKi, M., Shoji, T., Kanetuna, Y., Horita, T., Matsui, H. and Honma, M., 1999. Synthesis and degradation of 1-aminocyclopropane-1-carboxylic acid by *Penicillium citrinum*. *Bioscience, Biotechnology, and Biochemistry* 63(3), 542-549.
- Jones, J.D. and Dangl, J.L., 2006. The plant immune system. *Nature* 444 (7117), 323.
- Jones, D. A., and Takemoto, D., 2004. Plant innate immunity - direct and indirect recognition of general and specific pathogen-associated molecules. *Current Opinion in Immunology* 16, 48-62.
- Jung, H.W., Tschaplinski, T.J., Wang, L., Glazebrook, J., Greenberg, J.T., 2009. Priming in systemic plant immunity. *Science* 324 (5923), 89-91.

- Kabak, B., 2009. The fate of mycotoxins during thermal food processing. *Journal of the Science of Food and Agriculture* 89, 549-554.
- Kaeppeler, S.M., Kaeppeler, H.F., Rhee, Y., 2000. Epigenetic aspects of somaclonal variation in plants. *Plant Molecular Biology* 43, 179-188.
- Kakoulidou, I., Avramidou, E.V., Baránek, M., Brunel-Muguet, S., Farrona, S., Johannes, F., Kaiserli, E., Lieberman-Lazarovich, M., Martinelli, F., Mladenov, V., Testillano, P.S., 2021. Epigenetics for Crop Improvement in Times of Global Change. *Biology* 10(766), 1-46.
- Kar, B., Kuanar, A., Singh, S., Mohanty, S., Joshi, R.K., Subudhi, E., Nayak, S., 2014. In vitro induction, screening, and detection of high essential oil yielding somaclones in turmeric (*Curcuma longa* L.). *Plant Growth Regulators* 72(1), 59-66.
- Kauss, H. and Jeblick, W., 1995. Pretreatment of parsley suspension cultures with salicylic acid enhances spontaneous and elicited production of H<sub>2</sub>O<sub>2</sub>. *Plant Physiology* 108(3), 1171-1178.
- Kazan, K. and Manners, J.M., 2009. Linking development to defense: auxin in plant–pathogen interactions. *Trends in Plant Science*, 14(7), 373-382.
- Kazan, K., 2015. Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends in Plant Science* 20(4), 219-229.
- Keeping, M.G., Miles, N., Sewpersad, C., 2014. Silicon reduces impact of plant nitrogen in promoting stalk borer (*Eldana saccharina*) but not sugarcane thrips (*Fulmekiola serrata*) infestations in sugarcane. *Frontiers in Plant Science* 5, 289.
- Kerchev, P., van der Meer, T., Sujeeth, N., Verlee, A., Stevens, C.V., Van Breusegem, F., Gechev, T., 2020. Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. *Biotechnology Advances* 40, 1-9.
- Key, S., Ma, J.K., Drake, P.M., 2008. Genetically modified plants and human health. *Journal of the Royal Society of Medicine* 101(6), 290-298.
- Kinoshita, T. and Seki, M., 2014. Epigenetic memory for stress response and adaptation in plants. *Plant and Cell Physiology* 55(11), 1859-1863.
- Khalil, F., Naiyan, X., Tayyab, M., Pinghua, C., 2018. Screening of EMS-Induced Drought-Tolerant Sugarcane Mutants Employing Physiological, Molecular and Enzymatic Approaches. *Agronomy* 8(10), 1-13
- Khan, S.A., Hamayun, M., Yoon, H., Kim, H.Y., Suh, S.J., Hwang, S.K., Kim, J.M., Lee, I.J., Choo, Y.S., Yoon, U.H., Kong, W.S., 2008. Plant growth promotion and *Penicillium citrinum*. *Biomed Central Microbiology* 8(1), 1-10
- Khan, A.L., Hamayun, M., Kim, Y.H., Kang, S.M., Lee, J.H., Lee, I.J., 2011. Gibberellins producing endophytic *Aspergillus fumigatus* sp. LH02 influenced endogenous

- phytohormonal levels, isoflavonoids production and plant growth in salinity stress. *Process Biochemistry* 46(2), 440-447.
- Khan, A.L., Hamayun, M., Kang, S.M., Kim, Y.H., Jung, H.Y., Lee, J.H., Lee, I.J., 2012. Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomyces formosus* LHL10. *Biomed Central Microbiology* 12(1), 1-14.
- Khan AR, Ullah I, Waqas M, Shahzad R, Hong SJ, Park GS, Jung BK, Lee IJ, Shin JH. 2015. Plant growth promoting potential of endophytic fungi isolated from *Solanum nigrum* leaves. *World Journal of Microbiology and Biotechnology* 31, 1461-1466.
- Khan, A.L., Halo, B.A., Elyassi, A., Ali, S., Al-Hosni, K., Hussain, J., Al-Harrasi, A., Lee, I.J., 2016. Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. *Electronic Journal of Biotechnology* 21, 58-64.
- Khan, M.T., Seema, N., Khan, I.A., Yasmine, S., 2017. Characterization of somaclonal variants of sugarcane on the basis of quantitative, qualitative, and genetic attributes. *Pakistan Journal of Botany*, 49(6), 2429-2443.
- Kharkwal, M.C. and Shu, Q.Y., 2009. The role of induced mutations in world food security. *Induced plant mutations in the genomics era*. Food and Agriculture Organization of the United Nations, Rome, 33-38.
- Kim, J.I., Murphy, A.S., Baek, D., Lee, S.W., Yun, D.J., Bressan, R.A., Narasimhan, M.L. 2011. YUCCA6 over-expression demonstrates auxin function in delaying leaf senescence in *Arabidopsis thaliana*. *Journal of Experimental Botany* 62, 3981–3992.
- Kim, M. and Costello, J., 2017. DNA methylation: an epigenetic mark of cellular memory. *Experimental & molecular medicine* 49(4), 322-322.
- Kitaoka N, Matsubara T, Sato M, Takahashi K, Wakuta S, Kawaide H, Matsui H, Nabeta K, Matsuura H. 2011. Arabidopsis CYP94B3 encodes jasmonyl-L-isoleucine 12-hydroxylase, a key enzyme in the oxidative catabolism of jasmonate. *Plant & Cell Physiology* 52, 1757-1765.
- Klemme, S., De Smet, Y., Cammue, B., De Block, M., 2019. Selection of salicylic acid tolerant epilines in brassica napus. *Agronomy* 9(2), 92.
- Kleynhans, E., Barton, M.G, Conlong, D.E., Terblanche, J.S., 2017. Population dynamics of *Eldana saccharina* Walker (Lepidoptera: Pyralidae): application of a biophysical model to understand phenological variation in an agricultural pest. *Bulletin of Entomological Research* 108, 283-294.
- Knogge, W., 1996. Fungal infection of plants. *The Plant Cell* 8(10), 1711.
- Koch, A.C., Ramgareeb, S., Rutherford, R.S., Snyman, S.J., Watt, M.P., 2012. An in vitro mutagenesis protocol for the production of sugarcane tolerant to the herbicide imazapyr. *In Vitro Cellular & Developmental Biology-Plant* 48(4), 417-427.

- Kochhar, S.L., 1998. Economic Botany in the tropics. Second Edition, Macmillan. India Ltd, 1-476
- Koetle, M.J., Evans, D.L., Singh, V., Snyman, S.J., Rutherford, R.S., Watt, M.P., 2018. Agronomic evaluation and molecular characterisation of the acetolactate synthase gene in imazapyr tolerant sugarcane (*Saccharum* hybrid) genotypes. Plant Cell Reports, 1-13.
- Kohler, A., Schwindling, S., Conrath, U., 2002. Benzothiadiazole-induced priming for potentiated responses to pathogen infection, wounding, and infiltration of water into leaves requires the NPR1/NIM1 gene in *Arabidopsis*. Plant Physiology 128, 1046-1056.
- Kona, P., Kumar, M.H., Reddy, K.H.P., Hemalatha, T.M., Reddy, D.M., Reddy, N.E., Latha, P., 2019. Regeneration and evaluation of somaclones of sugarcane variety Co86032 for yellow leaf disease resistance and yield traits. Journal of Biosciences 44(2), 29.
- Konzak, C. F. 2001. Breeding in Crop Plants–Mutations and *In Vitro* Mutation Breeding. Crop Science 41, 253-253.
- Koo, A.J, Cooke, T.F, Howe, G.A. 2011. Cytochrome P450 CYP94B3 mediates catabolism and inactivation of the plant hormone jasmonoyl-L-isoleucine. Proceedings of the National Academy of Sciences United States of America 108, 9298-9303.
- Koo, A.J., Howe, G.A., 2012. Catabolism and deactivation of the lipid derived hormone jasmonoyl-isoleucine. Frontiers in Plant Science 3 (19), 1-7.
- Kost, C. and Heil, M., 2008. The defensive role of volatile emission and extrafloral nectar secretion for lima bean in nature. Journal of Chemical Ecology 34(1), 2-13.
- Kost, C., and Heil, M. 2006. Herbivore-induced plant volatiles induce an indirect defense in neighbouring plants. Journal of Ecology 94 (3), 619-628.
- Kou, M.Z., Bastías, D.A., Christensen, M.J., Zhong, R., Nan, Z.B., Zhang, X.X., 2021. The plant salicylic acid signaling pathway regulates the infection of a biotrophic pathogen in grasses associated with an *Epichloë* endophyte. Journal of Fungi, 7(8), 633.
- Kravchuk, Z., Vicedo, B., Flors, V., Camañes, G., González-Bosch, C., García-Agustín, P., 2011. Priming for JA-dependent defenses using hexanoic acid is an effective mechanism to protect *Arabidopsis* against *B. cinerea*. Journal of Plant Physiology 168(4), 359-366.
- Krishna, H., Alizadeh, M., Singh, D., Singh, U., Chauhan, N., Eftekhari, M., Sath, R.K., 2016. Somaclonal variations and their applications in horticultural crops improvement. 3 Biotech 6(1), 54.
- Kroger, M., Meister, K. and Kava, R., 2006. Low-calorie sweeteners and other sugar substitutes: a review of the safety issues. Comprehensive Reviews in Food Science and Food Safety 5(2), 35-47.

- Kumar, K., Gambhir, G., Dass, A., Tripathi, A.K., Singh, A., Jha, A.K., Yadava, P., Choudhary, M. and Rakshit, S., 2020. Genetically modified crops: current status and future prospects. *Planta* 251, 1-27.
- Kuswinanti, T., Syam'un, E. and Masniawati, A., 2015. The potency of endophytic fungal isolates collected from local aromatic rice as indole acetic acid (IAA) producer. *Procedia Food Science* 3, 96-103.
- Lakshmanan, P., Geijskes, R.J., Aitken, K.S., Grof, C.L.P., Bonnett, G.D., Smith, G.R., 2005. Sugarcane biotechnology: The challenges and opportunities. *In Vitro Cellular and Developmental Biology* 41, 345-363.
- Lakshmanan, P., Geijskes, R.J., Wang, L., Elliott, A., Grof, C.P., Berding, N., Smith, G.R., 2006. Developmental and hormonal regulation of direct shoot organogenesis and somatic embryogenesis in sugarcane (*Saccharum* spp. interspecific hybrids) leaf culture. *Plant cell reports* 25(10), 1007-1015.
- Lam, E., 2004. Controlled cell death, plant survival and development. *Nature reviews Molecular Cell Biology* 5(4), 305-315.
- Lämke, J. and Bäurle, I., 2017. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. *Genome Biology* 18(1), 124.
- Larkin, P.J. and Scowcroft, W.R. 1981. Somaclonal variation - a novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics* 60(4), 197-214.
- Larrieu, A., Vernoux, T., 2016. Q&A: How does jasmonate signaling enable plants to adapt and survive? *BMC Biology* 14, 79.
- Ledford, H., 2015. CRISPR, the disruptor. *Nature* 522, 20-24.
- Lee, T.S.G., 1987. Micropropagation of sugarcane (*Saccharum* spp.). *Plant Cell, Tissue and Organ Culture* 10(1), 47-55.
- Leibbrandt, N.B. and Snyman, S.J., 2003. Stability of gene expression and agronomic performance of a transgenic herbicide-resistant sugarcane line in South Africa. *Crop Science* 43(2), 671-677.
- Leslie, J.F and Summerell, B.A., 2006. *The Fusarium laboratory manual*. Blackwell Publishing, USA, 1-388.
- Leslie, J.F., Zeller, K.A., Lamprecht, S.C., Rheeder, J.P., Marasas, W.F.O., 2004. Toxicity, pathogenicity and genetic differentiation of five species of *Fusarium* from sorghum and millet. *Phytopathology* 95, 275-283.
- Lethin, J., Shakil, S.S., Hassan, S., Sirijovski, N., Töpel, M., Olsson, O., Aronsson, H., 2020. Development and characterization of an EMS-mutagenized wheat population and identification of salt-tolerant wheat lines. *BMC Plant Biology* 20(1), 1-15.

- Leyva, M., Vicedo, B., Finiti, I., Flors, V., Del Amo, G., Real, M. 2008. Preventive and post-infection control of *Botrytis cinerea* in tomato plants by hexanoic acid. *Plant Pathology*. 57, 1038–1046.
- Li, G., Zhou, J., Jia, H., Gao, Z., Fan, M., Luo, Y., Zhao, P., Xue, S., Li, N., Yuan, Y., Ma, S., 2019. Mutation of a histidine-rich calcium-binding-protein gene in wheat confers resistance to *Fusarium* head blight. *Nature Genetics* 51(7), 1106-1112.
- Liang, C., Wu, R., Han, Y., Wan, T., Cai, Y., 2019. Optimizing suitable antibiotics for bacterium control in micropropagation of cherry rootstock using a modified leaf disk diffusion method and E test. *Plants* 8(66), 1-3.
- Liao, X., Lovett, B., Fang, W., St Leger, R.J., 2017. *Metarhizium robertsii* produces indole-3-acetic acid, which promotes root growth in *Arabidopsis* and enhances virulence to insects. *Microbiology* 163(7), 980-991.
- Liu, H., Li, X., Xiao, J., Wang, S., 2012. A convenient method for simultaneous quantification of multiple phytohormones and metabolites: application in study of rice-bacterium interaction. *Plant Methods* 8(2), 1-12.
- Llorens, E., Fernández-Crespo, E., Vicedo, B., Lapeña, L., García-Agustín, P., 2013. Enhancement of the citrus immune system provides effective resistance against *Alternaria* brown spot disease. *Journal of Plant Physiology* 170(2), 146-154.
- Llorens, E., Camañes, G., Lapeña, L., García-Agustín, P., 2016. Priming by hexanoic acid induce activation of mevalonic and linolenic pathways and promotes the emission of plant volatiles. *Frontiers in Plant Science* 7 (495), 1-12.
- Llorens, E., García-Agustín, P., Lapeña, L., 2017. Advances in induced resistance by natural compounds: towards new options for woody crop protection. *Scientia Agricola* 74(1), 90-100.
- Llorens, E., González-Hernández, A.I., Scalschi, L., Fernández-Crespo, E., Camañes, G., Vicedo, B., García-Agustín, P., 2020. Priming mediated stress and cross-stress tolerance in plants Concepts and opportunities, In: *Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants*. Academic Press, 1-20.
- Logrieco, A., Moretti, A., Fornelli, F., Fogliano, V., Ritieni, A., Caiaffa, M.F., Randazzo, G., Bottalico, A., Macchia, L., 1996. Fusaproliferin production by *Fusarium subglutinans* and its toxicity to *Artemia salina*, SF-9 insect cells, and IARC/LCL 171 human B lymphocytes. *Applied and Environmental Microbiology* 62(9), 3378-3384.
- Logrieco, A., Rizzo, A., Ferracane, R., Ritieni, A., 2002. Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight. *Applied Environmental Microbiology* 68(1), 82-85.

- Lorenzo, J.C., González, B.L., Escalona, M., Teisson, C., Borroto, C., 1998. Sugarcane shoot formation in an improved temporary immersion system. *Plant Cell, Tissue and Organ Culture* 54(3), 197-200.
- Lovei, G.L., Bohn, T., Hilbeck, A., Lim, L.C., Traavik, T., 2010. Biodiversity, ecosystem services and genetically modified organisms 10. Third World Network.
- Lugtenberg, B.J., Caradus, J.R., Johnson, L.J., 2016. Fungal endophytes for sustainable crop production. *FEMS Microbiology Ecology* 92, 1-17.
- Lu, J., Robert, C.A.M., Riemann, M., Cosme, M., Mène-Saffrané, L., Massana, J., Stout, M.J., Lou, Y., Gershenzon, J., Erb, M., 2015. Induced jasmonate signaling leads to contrasting effects on root damage and herbivore performance. *Plant Physiology* 167(3), 1100-1116.
- Luan, Y.S., Zhang, J., Gao, X.R., An, L.J., 2007. Mutation induced by ethyl methane sulphonate (EMS), *in vitro* screening for salt tolerance and plant regeneration of sweet potato (*Ipomoea batatas* L.). *Plant Cell, Tissue and Organ Culture*, 88(1), 77-81.
- Luna, E., Bruce, T.J., Roberts, M.R., Flors, V., Ton, J., 2012. Next-generation systemic acquired resistance. *Plant Physiology* 158(2), 844-853.
- Maciá-Vicente, J.G., Jansson, H.B., Abdullah, S.K., Descals, E., Salinas, J. and Lopez-Llorca, L.V., 2008. Fungal root endophytes from natural vegetation in Mediterranean environments with special reference to *Fusarium* spp. *FEMS Microbiology Ecology*, 64(1), 90-105.
- Mahlanza, T., Rutherford, R.S., Snyman, S.J., Watt, M.P., 2013. *In vitro* generation of somaclonal variant plants of sugarcane for tolerance to *Fusarium sacchari*. *Plant Cell Reports* 32(2), 249-262.
- Mahlanza, T., Rutherford, R.S., Snyman, S.J., Watt, M.P., 2014. *Eldana saccharina* (Lepidoptera: Pyralidae) resistance in sugarcane (*Saccharum spp.*): effects of *Fusarium* spp., stalk rind, fibre and nitrogen content. *African Entomology* 22(4), 810-822.
- Mahmud, K., Nasiruddin, K.M., Hossain, M.A., Hassan, L., 2016. Development of mutants in sugarcane through callus culture. *Plant Tissue Culture and Biotechnology* 26(1), 123-130.
- Mallikarjuna, S.J., Kumar, M.H., Reddy, D.M., Sudhakar, P., 2018. Effects of different mutagenic chemicals on callogenesis in sugarcane (*Saccharum officinarum*) clones 20088T42 and 2009T5. *International Journal of Current Microbiology and Applied Sciences* 7(6), 1404-1411.
- Manchanda, P., Kaur, A., Gosal, S.S., 2018. Somaclonal variation for sugarcane improvement, in: *Biotechnologies of Crop Improvement, Volume 1*. Springer, Cham, 299-326.

- Manshardt, R., 2004. Crop Improvement by Conventional Breeding or Genetic Engineering: How Different Are They? *Biotechnology*, 1-3.
- Marin, S., Ramos, A. J., Cano-Sancho, G., & Sanchis, V., 2013. Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology: an international journal published for the British Industrial Biological Research Association* 60, 218-237.
- Martínez-Estrada, E., Caamal-Velázquez, J.H., Salinas-Ruíz, J., Bello-Bello, J.J., 2017. Assessment of somaclonal variation during sugarcane micropropagation in temporary immersion bioreactors by intersimple sequence repeat (ISSR) markers. *In Vitro Cellular & Developmental Biology-Plant* 53(6), 553-560.
- Martinez-Hernandez, E., Amezcua-Allieri, M.A., Sadhukhan, J., Anell, J.A., 2018. Sugarcane bagasse valorisation strategies for bioethanol and energy production. *Sugarcane-Technology and Research* Chapter 4, 70-79.
- Martinez-Medina, A., Flors, V., Heil, M., Mauch-Mani, B., Pieterse, C.M., Pozo, M.J., Ton, J., van Dam, N.M., Conrath, U., 2016. Recognizing plant defense priming. *Trends in Plant Science* 21(10), 818-822.
- Masoabi, M., Lloyd, J., Kossmann, J., van der Vyver, C., 2018. Ethyl methane sulfonate mutagenesis and in vitro polyethylene glycol selection for drought tolerance in sugarcane (*Saccharum* spp.). *Sugar Tech*, 20(1), 50-59.
- Mauch-Mani, B., Baccelli, I., Luna, E., Flors, V., 2017. Defense priming: an adaptive part of induced resistance. *Annual Review of Plant Biology*, 68, 485-512.
- Mba, C., Afza, R., Shu, Q.Y., 2012. Mutagenic radiations: X-rays, ionizing particles, and ultraviolet. *Plant Mutation Breeding and Biotechnology*, 83-90.
- Mba, C., 2013. Induced mutations unleash the potentials of plant genetic resources for food and agriculture. *Agronomy* 3(1), 200-231.
- McConn, M., Creelman, R.A., Bell, E., Mullet, J.E., 1997. Jasmonate is essential for insect defense in *Arabidopsis*. *Proceedings of the National Academy of Sciences* 94(10), 5473-5477.
- McFarlane, S.A. and Rutherford, R.S., 2005. *Fusarium* species isolated from sugarcane in KwaZulu-Natal and their effect on *Eldana saccharina* (Lepidoptera: Pyralidae) development *in vitro*. *Proceedings of the South African of Sugarcane Technologists' Association* 79, 120-124.
- McFarlane, S.A., Govender P., Rutherford, R.S., 2009. Interactions between *Fusarium* species from sugarcane and the stalk borer, *Eldana saccharina* (Lepidoptera: Pyralidae). *Annals of Applied Biology* 155, 349-359.
- Meents, A.K., Furch, A.C., Almeida-Trapp, M., Özyürek, S., Scholz, S.S., Kirbis, A., Lenser, T., Theißen, G., Grabe, V., Hansson, B. and Mithöfer, A., 2019. Beneficial and

- pathogenic *Arabidopsis* root-interacting fungi differently affect auxin levels and responsive genes during early infection. *Frontiers in Microbiology* 10 (380), 1-14.
- Mehmood, A., Irshad, M., Khan, N., Hamayun, M., Javed, I., Javed, H., Javed, A., Hussain, A., 2018. *In Vitro* Maize Growth Promotion by Endophytic *Fusarium Oxysporum* WLW. *Journal of Agricultural Biological and Environmental Statistics* 8(4), 1-7.
- Mejía, L.C., Rojas, E.I., Maynard, Z., Van Bael, S., Arnold, A.E., Hebbbar, P., Samuels, G.J., Robbins, N., Herre, E.A., 2008. Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens. *Biological Control* 46(1), 4-14.
- Mertens, M., 2008. Assessment of environmental impacts of genetically modified plants. *Implementation of the Biosafety Protocol Development of Assessment Bases*, 234.
- Meyer, G.M. and Snyman, S.J., 2011. Progress in research on genetically modified sugarcane in South Africa and associated regulatory requirements. In *II Genetically Modified Organisms in Horticulture Symposium* 974, 43-50.
- Miceli, A., Moncada, A., Sabatino, L., Vetrano, F., 2019. Effect of Gibberellic Acid on Growth, Yield, and Quality of Leaf Lettuce and Rocket Grown in a Floating System. *Agronomy* 9(7), 382.
- Miersch, O., Porzel, A., Wasternack, C., 1999a. Microbial conversion of jasmonates-hydroxylations by *Aspergillus niger*. *Phytochemistry* 50(7), 1147-1152.
- Miersch, O., Regvar, M., Wasternack, C., 1999b. Metabolism of jasmonic acid in *Pisolithus tinctorius* cultures. *Phyton (Horn)* 39(3), 243-248.
- Milani, J.M., 2013. Ecological conditions affecting mycotoxin production in cereals: a review. 2013. *Veterinari Medicina* 58(8), 405-411.
- Ming, R., Moore, P.H., Wu, K.K., D'Hont, A., Glaszmann, J.C., Tew, T.L., 2006. Sugarcane improvement through breeding and biotechnology. *Plant Breeding Reviews* 71, 15-118.
- Mirsam, H., Kalqutny, S.H., Aqil, M., Azrai, M., Pakki, S., Muis, A., Djaenuddin, N., Rauf, A.W., 2021. Indigenous fungi from corn as a potential plant growth promoter and its role in *Fusarium verticillioides* suppression on corn. *Heliyon* 7(9), 1-12.
- Mir, A.S., Maria, M., Muhammad, S., Ali, S.M., 2020. Potential of Mutation Breeding to Sustain Food Security. In *Genetic Variation*. IntechOpen, London.
- Mirajkar, S.J., Devarumath, R.M., Nikam, A.A., Sushir, K.V., Babu, H., Suprasanna, P., 2019. Sugarcane (*Saccharum spp.*): breeding and genomics. In *Advances in Plant Breeding Strategies: industrial and food crops*. Springer, 363-406.
- Mithöfer, A., Boland, W., 2008. Recognition of herbivory-associated molecular patterns. *Plant Physiology* 146, 825-831.

- Mladenov, V., Fotopoulos, V., Kaiserli, E., Karalija, E., Maury, S., Baranek, M., Segal, N., Testillano, P.S., Vassileva, V., Pinto, G., Nagel, M., 2021. Deciphering the epigenetic alphabet involved in transgenerational stress memory in crops. *International Journal of Molecular Sciences* 22(13), 1-20.
- Mokhele, T.A., Ahmed, F., Conlong, D.E., 2009. Detection of sugarcane African stalk borer *Eldana Saccharina Walker* (Lepidoptera: Pyralidae) using hyperspectral remote sensing (Spectroradiometry) *Proceedings of the South African of Sugarcane Technologists' Association* 82, 457-470.
- Monaghan, J. and Zipfel, C., 2012. Plant pattern recognition receptor complexes at the plasma membrane. *Current opinion in plant biology* 15(4), 349-357.
- Morath, S.U., Hung, R., Bennett, J.W., 2012. Fungal volatile organic compounds: A review with emphasis on their biotechnological potential. *Fungal Biology Reviews* 26, 73-83.
- Mousavi, S.A., Chauvin, A., Pascaud, F., Kellenberger, S., Farmer, E.E., 2013. Glutamate receptor-like genes mediate leaf-to-leaf wound signalling. *Nature* 500, 422-426.
- Moyer, J.W., Collins, W.W., 1983. Scarlet sweet potato. *Horticulture Science* 18, 111-112.
- Muddapur, U.M., Gadkari, M.V., Kulkarni, S.M., Sabannavar, P.G., Niyonzima, F.N., More, S.S., 2015. Isolation and characterization of Gibberellic acid 3 producing *Fusarium* sp. from Belgaum agriculture land audits impact on green pea and rice growth promotion. *Aperito Journal of Advanced Plant Biology* 1(2), 1-9.
- Mughogho, L.K., 1984. Sorghum Root and Stalk Rots A Critical Review. *Proceedings of the consultative group discussion on research needs and strategies for control of sorghum root and stalk rot diseases*, 73-78.
- Mullins, E., Bresson, J.L., Dalmay, T., Dewhurst, I.C., Epstein, M.M., Firbank, L.G., Guerche, P., Hejatko, J., Moreno, F.J., Naegeli, H., 2021. *In vivo* and *in vitro* random mutagenesis techniques in plants. *EFSA Journal* 19(11), p.e06611.
- Munkvold, G.P. and Desjardins, A.E., 1997. Fumonisin in maize: can we reduce their occurrence?. *Plant Disease* 81(6), 556-565.
- Munkvold, G.P., Logrieco A., Moretti A., Ferracane R., Ritieni A., 2009. Dominance of Group 2 and fusaproliferin produced by *Fusarium subglutinans* from Iowa maize. *Food Additives and Contaminants* 26(3), 388-394
- Munsamy, A., Rutherford, R.S., Snyman, S.J., Watt, M.P., 2013. 5-Azacytidine as a tool to induce somaclonal variants with useful traits in sugarcane (*Saccharum* spp.). *Plant Biotechnology Reports* 7(4), 489-502.
- Mur, L.A., Kenton, P., Atzorn, R., Miersch, O., Wasternack, C., 2006. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiology* 140(1), 249-262.

- Nadar, H.M., Soeprapto, S., Heinz, D.J., Ladd, S.L., 1978. Fine Structure of Sugarcane (*Saccharum* spp.) Callus and the Role of Auxin in Embryogenesis, *Crop Science* 18(2), 210-216.
- Nadeem, M.A., Nawaz, M.A., Shahid, M.Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., 2018. DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment* 32(2), 261-285.
- Nafie, E., Hathout, T., Mokadem, A., Shyma, A., 2011. Jasmonic acid elicits oxidative defense and detoxification systems in *Cucumis melo* L. cells. *Brazilian Journal of Plant Physiology* 23(2), 161-174.
- Nafisi, M., Fimognari, L., Sakuragi, Y., 2015. Interplays between the cell wall and phytohormones in interaction between plants and necrotrophic pathogens. *Phytochemistry* 112, 63-71.
- Nalawade, S.M., Mehta, A.K., Sharma, A.K., 2018, January. Sugarcane planting techniques: a review. In Special Issue: National Seminar "Recent Trends in Plant Sciences and Agricultural, 98-104.
- Nascimento, F.X., Rossi, M.J., Soares, C.R., McConkey, B.J., Glick, B.R., 2014. New insights into 1-aminocyclopropane-1-carboxylate (ACC) deaminase phylogeny, evolution and ecological significance. *PLOS one* 9(6), 1-17.
- Navarro-Meléndez, A.L., Heil, M., 2014. Symptomless endophytic fungi suppress endogenous levels of salicylic acid and interact with the jasmonate-dependent indirect defense traits of their host, lima bean (*Phaseolus lunatus*). *Journal of Chemical Ecology* 40(7), 816-825.
- Naz, S., 2003. Micropropagation of promising varieties of sugarcane and their acclimatization response. *Activities on Sugar Crops in Pakistan*. In Proceeding. Fourth Workshop Research. & Development, 1-9.
- Nhut, D.T., Hai, N.T., Thu, P.T.M., Thi, N.N., Hien, T.T.D., Tuan, T.T., Nam, N.B., Huy, N.P., Chien, H.X., Jain, S.M., 2012. Protocol for inducing flower color somaclonal variation in torenia (*Torenia fournieri* Lind.). In *Protocols for Micropropagation of Selected Economically-Important Horticultural Plants*, Humana Press, Totowa, NJ, 455-462.
- Nikam, A.A., Devarumath, R.M., Ahuja, A., Babu, H., Shitole, M.G., Suprasanna, P., 2015. Radiation-induced *in vitro* mutagenesis system for salt tolerance and other agronomic characters in sugarcane (*Saccharum officinarum* L.). *The Crop Journal* 3(1), 46-56.
- Nogueira, G.F., Luis, Z.G., Pasqual, M and Scherwinski-Pereira, J.E., 2019. High-efficiency somatic embryogenesis of a broad range of Brazilian *Saccharum* spp. hybrids (sugarcane) varieties using explants from previously established *in vitro* plants. *In Vitro Cellular and Developmental Biology* 55, 26-35.

- Nogueira, F.T., Menossi, M., Ulian, E.C., Arruda, P., 2005. Identification of methyl jasmonate-responsive genes in sugarcane using cDNA arrays. *Brazilian Journal of Plant Physiology* 17(1), 173-180.
- Nimchuk, Z., Eulgem, T., Holt III, B. F., Dangl, J. F., 2003. Recognition and response in the plant immune system. *Annual Review of Genetics* 37, 579-609.
- Novak, F.J. and Brunner, H., 1992. Plant breeding: Induced mutation technology for crop improvement. *International Atomic Energy Agency Bulletin* 4, 25-33.
- Nüernberger, T. and Lipka, V., 2005. Non-host resistance in plants: new insights into an old phenomenon. *Molecular Plant Pathology* 6(3), 335-345.
- Numponsak, T., Kumla, J., Suwannarach, N., Matsui, K., Lumyong, S., 2018. Biosynthetic pathway and optimal conditions for the production of indole-3-acetic acid by an endophytic fungus, *Colletotrichum fructicola* CMU-A109. *PloS one* 13(10), 1-17.
- O'Reilly, G., 1998. The South African sugar industry. *International Sugar Journal* 100, 266-268
- O'Brien, J.A. and Benková, E., 2013. Cytokinin cross-talking during biotic and abiotic stress responses. *Frontiers in Plant Science* 4, 451.
- Oberkofler, V., Pratx, L., Bäurle, I. 2021 Epigenetic regulation of abiotic stress memory: Maintaining the good things while they last. *Current Opinion Plant Biology* 61, 1-7.
- Oka, M., Miyamoto, K., Okada, K., Ueda, J., 1999. Auxin polar transport and flower formation in *Arabidopsis thaliana* transformed with indole acetamide hydrolase (*iaaH*) gene. *Plant Cell Physiology* 40, 231-237.
- Oladosu, Y., Rafii, M.Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H.A., Miah, G., Usman, M., 2016. Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnology & Biotechnological Equipment* 30(1), 1-16.
- Overvoorde, P., Fukaki, H., Beeckman, T., 2010. Auxin control of root development. *Cold Spring Harbor Perspectives in Biology* 2(6), 1-9.
- Palmer, C., Golden, K., Danniels, L., Ahmad, H., 2007. ACC deaminase from *Issatchenkia occidentalis*. *Journal of Biological Sciences*, 7, 188-193.
- Paré, P.W. and Tumlinson, J.H., 1996. Plant volatile signals in response to herbivore feeding. *Florida Entomologist*, 93-103.
- Pastor, V., Luna, E., Mauch-Mani, B., Ton, J., Flors, V., 2013. Primed plants do not forget. *Environmental and Experimental Botany* 94, 46-56.
- Pastor, V., Balmer, A., Gamir, J., Flors, V., Mauch-Mani, B., 2014. Preparing to fight back: generation and storage of priming compounds. *Frontiers in Plant Science* 5 (295), 1-12.

- Patade, V.Y., Suprasanna, P., Bapat, V. A. 2006. Selection for abiotic (salinity and drought) stress tolerance and molecular characterization of tolerant lines in sugarcane. *Bhaha Atomic Research Centre Newsletter* 273, 244-257.
- Patade, V.Y., Suprasanna, P., 2008. Radiation induced *in vitro* mutagenesis for sugarcane improvement. *Sugar Tech* 10, 14-19.
- Patade, V.Y., Bhargava, S., Suprasanna, P., 2012. Halopriming mediated salt and iso-osmotic PEG stress tolerance and, gene expression profiling in sugarcane (*Saccharum officinarum* L.). *Molecular Biology Reports*, 39(10), 9563-9572.
- Patkar, R.N., Benke, P.I., Qu, Z., Chen, Y.Y.C., Yang, F., Swarup, S., Naqvi, N.I., 2015. A fungal monooxygenase-derived jasmonate attenuates host innate immunity. *Nature Chemical Biology* 11(9), 733-740.
- Paudel Timilsena, B., Seidl-Adams, I., Tumlinson, J.H., 2020. Herbivore-specific plant volatiles prime neighboring plants for nonspecific defense responses. *Plant, Cell & Environment* 43(3), 787-800.
- Pecinka, A., Rosa, M., Schikora, A., Berlinger, M., Hirt, H., Luschnig, C., Scheid, O.M., 2009. Transgenerational stress memory is not a general response in *Arabidopsis*. *PLoS one* 4(4), 1-10.
- Peleg, Z. and Blumwald, E., 2011. Hormone balance and abiotic stress tolerance in crop plants. *Current Opinion Plant Biology* 14, 290-295.
- Péné, C.B., Boua, B.M., Coulibaly-Ouattara, Y., Goebel, F.R., 2018. Stem borer (*Eldana saccharina* W) infestation outbreak in Sugarcane plantations of northern Ivory Coast: Management strategies under implementation. *American Journal of Bioscience Bioengineering* 6(4), 27-35.
- Penna, S. and Jain, S.M., 2017. Mutant Resources and Mutagenomics in crop plants. *Emirates Journal of Food and Agriculture*, 651-657.
- Penna, S., 2017. Induced mutations and food security-prospective progress. *Insights on Global Challenges and Opportunities for the Century Ahead*, 261.
- Penninckx, I.A., Thomma, B.P., Buchala, A., Métraux, J.P., Broekaert, W.F., 1998. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. *The Plant Cell* 10(12), 2103-2113.
- Petti, C., Reiber, K., Ali, S.S., Berney, M., Doohan, F.M., 2012. Auxin as a player in the biocontrol of *Fusarium* head blight disease in barley and its potential as a disease control agent. *BioMed Central Plant Biology* 12(224), 1-9.
- Pieterse, C.M.J., Van Wees, S.C.M., Ton, J., Van Pelt, J.A., Van Loon, L.C., 2002. Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *Plant Biology* 4(05), 535-544.

- Pieterse, C.M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., Van Wees, S.C., 2012. Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* 28, 489-521.
- Pieterse, C.M., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C., Bakker, P.A., 2014. Induced systemic resistance by beneficial microbes. *Annual review of phytopathology* 52, 347-375.
- Pineda, A., Zheng, S.J., van Loon, J.J., Pieterse, C.M., Dicke, M., 2010. Helping plants to deal with insects: the role of beneficial soil-borne microbes. *Trends in Plant Science* 15(9), 507-514.
- Ploetz, R.C., 2005. *Fusarium*-induced diseases of tropical, perennial crops. *Phytopathology* 96, 648-652.
- Ploetz, R.C., 2015. *Fusarium* wilt of banana. *Phytopathology* 105, 1512–1521.
- Ploetz, R.C., Lim, T.K., Menge, J.A., Kenneth, G., Rohrbach, K.G., Michaelides, T.J., 2003. Common pathogens of tropical fruit crops. *Diseases of Tropical Fruit Crops*. Wallingford, UK: CABI Publishing, 1-19.
- Pontvianne, F., Blevins, T., Pikaard, P.S., 2010. *Arabidopsis* histone lysine methyltransferases. *Advances in Botanical Research* 53,1-22.
- Pooggin, M., 2013. How can plant DNA viruses evade siRNA-directed DNA methylation and silencing? *International Journal of Molecular Sciences* 14(8), 15233–15259.
- Potgieter L, Van Vuuren JH, Conlong DE, 2016. Simulation modelling as a decision support in developing a sterile insect inherited release strategy for *Eldana saccharina* (Lepidoptera: Pyralidae). *Florida Entomologist* 99(1), 13-22.
- Pozo, M.J., Lopez-Raez, J.A., Azcon-Aguilar, C., Garcia-Garrido, J.M. 2015. Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. *New Phytology* 205, 1431-1436.
- Pritchard, L. and Birch, P.R., 2014. The zigzag model of plant–microbe interactions: is it time to move on? *Molecular Plant Pathology* 15(9), 865-870.
- Purnamaningsih, R. and Hutami, S., 2016. Increasing Al-Tolerance of Sugarcane Using Ethyl Methane Sulphonate and In Vitro Selection in the Low pH Media. *Hayati. Journal of Biosciences* 1, 1-6.
- Qi, Y., Tsuda, K., Glazebrook, J., Katagiri, F., 2011. Physical association of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) immune receptors in *Arabidopsis*. *Molecular Plant Pathology* 12(7), 702-708.
- Qi, Z., Stephens, N.R., Spalding, E.P., 2006. Calcium entry mediated by GLR3.3, an *Arabidopsis* glutamate receptor with a broad agonist profile. *Plant Physiology* 142, 963-971.

- Rahjoo, V., Zad, J., Javan-Nikkhah, M., Gohari, A.M., Okhovvat, S.M., Bihamta, M.R., Razzaghian, J., Klemsdal, S.S., 2008. Morphological and molecular identification of *Fusarium* isolated from maize ears in Iran. *Journal of Plant Pathology*, 463-468.
- Raja, S., Qamarunnisa, S., Jamil, I., Naqvi, Q., Azhar, A., Qureshi, A. 2014. Screening of sugarcane somaclones of variety BL4 for Agronomic characteristics. *Pakistan Journal of Botany* 46(4), 1531-1535.
- Rajarammohan, S., 2021. Redefining Plant-Necrotroph Interactions: The thin line between hemibiotrophs and necrotrophs. *Frontiers in Microbiology* 12, 1-4.
- Ramgareeb, S., Snyman, S.J., Van Antwerpen, T., Rutherford, R.S., 2010. Elimination of virus and rapid propagation of disease-free sugarcane (*Saccharum spp. cultivar NCo376*) using apical meristem culture. *Plant Cell, Tissue and Organ Culture* 100(2), 175-181.
- Ramos Leal, M.A., Maribona, R.H., Ruiz, A., Korneva, S., Canales, E., Dinkova, T.D., Izquierdo, F., Coto, O., Rizo, D., 1996. Somaclonal variation as a source of resistance to eyespot disease of sugarcane. *Plant Breeding* 115, 37-42
- Rani, V and Raina, S., 2000. Genetic fidelity of organized meristem derived micropropagated plants: a critical reappraisal. *In Vitro Cellular Developmental Biology Plant* 36, 319-330.
- Rasmann, S., De Vos, M., Casteel, C.L., Tian, D., Halitschke, R., Sun, J.Y., Agrawal, A.A., Felton, G.W., Jander, G., 2012. Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant physiology* 158(2), 854-863.
- Ramírez-Mosqueda, M.A., Iglesias-Andreu, L.G., da Silva, J.A.T., Luna-Rodríguez, M., Noa-Carrazana, J.C., Bautista-Aguilar, J.R., Leyva-Ovalle, O.R., Murguía-González, J., 2019. *In vitro* selection of vanilla plants resistant to *Fusarium oxysporum f. sp. vanillae*. *Acta Physiologiae Plantarum*, 41(3), 1-8.
- Rastogi, J., Siddhant, P.B., Sharma, B.L., 2015. Somaclonal variation: a new dimension for sugarcane improvement. *GERF Bulletin of Biosciences* 6(1), 5-10.
- Ravensdale, M., Rocheleau, H., Wang, L., Nasmith, C., Ouellet, T., Subramaniam, R., 2014. Components of priming-induced resistance to *Fusarium* head blight in wheat revealed by two distinct mutants of *Fusarium graminearum*. *Molecular Plant Pathology* 15(9), 948-956.
- Rayle, D.L. and Cleland, R.E., 1992. The Acid Growth Theory of auxin-induced cell elongation is alive and well. *Plant physiology* 99(4), 1271-1274.
- Rebouças, T.A., de Jesus Rocha, A., Cerqueira, T.S., Adorno, P.R., Barreto, R.Q., dos Santos Ferreira, M., Lino, L.S.M., de Oliveira Amorim, V.B., dos Santos-Serejo, J.A., Haddad, F., Ferreira, C.F., 2021. Pre-selection of banana somaclones resistant to *Fusarium oxysporum f. sp. cubense*, subtropical race 4. *Crop Protection* 147, 1-10.

- Renwick, J.A.A., Chew, F.S., 1994. Oviposition Behaviour in Lepidoptera. Annual Review of Entomology 39, 377- 400.
- Renwick, A. and Poole, N., 1989. The environmental challenge to biological control of plant pathogens. Biotechnology of Fungi for Improving Plant Growth (16), 277.
- Restu, A.M. and Payangan, R.Y., 2019. Production of IAA (Indole Acetic Acid) of the rhizosphere fungus in the Suren community forest stand. In IOP Conference Series: Earth and Environmental Science, 343(1), 1-6.
- Rheeder, J.P., Marasas, W.E.O., Vismer, H.E., 2002. Production of fumonisin analogs by *Fusarium* species. Applied Environmental Microbiology 68, 2101-2105.
- Rigobelo, E.C. and Baron, N.C., 2021. Endophytic fungi: a tool for plant growth promotion and sustainable agriculture. Mycology, 1-17.
- Roach, B.T and Daniels, J. 1987. A review of the origin and improvement of sugarcane. In: Copersucar International Sugarcane Breeding Workshop, 1-32.
- Robert-Seilaniantz, A., Grant, M., Jones, J.D., 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annual Review of Phytopathology 49, 317-343.
- Rodriguez, R.J, White, J.F Jr., Arnold, A.E., Redman, R.S. 2009. Fungal endophytes: diversity and functional roles. New Phytologist 182, 314-330.
- Roychowdhury R, Tah J. 2013. Mutagenesis potential approach for crop improvement. In: Hakeem KR, Ahmad P, Ozturk M. (Eds.), Crop improvement: new approaches and modern techniques. New York (NY), Springer, 149-187.
- Rutherford, R.S., Snyman, S.J., Watt, M.P., 2014. In vitro studies on somaclonal variation and induced mutagenesis: progress and prospects in sugarcane (*Saccharum spp.*)- a review. The Journal of Horticultural Science and Biotechnology 89(1), 1-16.
- Rutherford, R.S., Maphalala, K.Z., Koch, A.C., Snyman, S.J., Watt, M.P., 2017. Field and laboratory assessments of sugarcane mutants selected *in vitro* for resistance to imazapyr herbicide. Crop Breeding and Applied Biotechnology 17, 107-114.
- Rutherford, R.S., McFarlane, S., Memela, N., Snyman, S., 2021. Harnessing the sugarcane microbiome for improved resistance to the stalk borer *Eldana saccharina Walker* (Lepidoptera: Pyralidae), 1-5.
- Saikkonen, K., Wäli, P., Helander, M., Faeth, S.H., 2004. Evolution of endophyte–plant symbioses. Trends in plant science 9(6), 275-280.
- Saini, N., Saini, M.L., Jain, R.K., 2004. Large scale production, field performance and RAPD analysis of micropropagated sugarcane plants. Indian Journal of Genetics 64(2), 102-107.

- Sánchez-Rangel, D., Sánchez-Nieto, S., Plasencia, J., 2012. Fumonisin B1, a toxin produced by *Fusarium verticillioides*, modulates maize  $\beta$ -1, 3-glucanase activities involved in defense response. *Planta* 235(5), 965-978.
- Sanchis, V. and Magan, N., 2004. Environmental conditions affecting mycotoxins. *Mycotoxins in food: Detection and Control*, 174-189.
- Sanghera, G.S., Lenika, K., Rajinder, K., Rajinder, P., Paramjit, S., 2018. Incidence of Pokkah Boeng disease on sugarcane clones and varieties in Punjab. *Agricultural Research Journal* 55(4), 754-756.
- Sani, E., Herzyk, P., Perrella, G., Colot, V., Amtmann, A., 2013. Hyperosmotic priming of *Arabidopsis* seedlings establishes a long-term somatic memory accompanied by specific changes of the epigenome. *Genome Biology* 14(6), 1-24.
- Sasaki, Y., Asamizu, E., Shibata, D., Nakamura, Y., Kaneko, T., Awai, K., Amagai, M., Kuwata, C., Tsugane, T., Masuda, T., Shimada, H., 2001. Monitoring of methyl jasmonate-responsive genes in *Arabidopsis* by cDNA macroarray: self-activation of jasmonic acid biosynthesis and crosstalk with other phytohormone signaling pathways. *Dna Research* 8(4), 153-161.
- Scalschi, L., Vicedo, B., Camañes, G., Fernandez-Crespo, E., Lapeña, L., González-Bosch, C., García-Agustín, P., 2013. Hexanoic acid is a resistance inducer that protects tomato plants against *Pseudomonas syringae* by priming the jasmonic acid and salicylic acid pathways. *Molecular Plant Pathology* 14(4), 342-355.
- Scalschi, L., Camañes, G., Llorens, E., Fernández-Crespo, E., López, M.M., García-Agustín, P., Vicedo, B., 2014. Resistance inducers modulate *Pseudomonas syringae* pv. tomato strain DC3000 response in tomato plants. *PLoS One* 9(9), 1-12.
- Schmelz, E.A., Grebenok, R.J., Ohnmeiss, T.E. and Bowers, W.S., 2002. Interactions between *Spinacia oleracea* and *Bradysia impatiens*: a role for phytoecdysteroids. *Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America* 51(4), 204-221.
- Schulthess, F., Cardwell, K.F., Gounou, S., 2002. The effect of endophytic *Fusarium verticillioides* on infestation of two maize varieties by lepidopterous stemborers and coleopteran grain feeders. *Phytopathology* 92(2), 120-128.
- Schultz, B., Boyle, C., 2005. The endophytic continuum. *Mycological Research* 109, 661-686.
- Schulz, B., Haas, S., Junker, C., Andrée, N., Schobert, M., 2015. Fungal endophytes are involved in multiple balanced antagonisms. *Current Science*, 39-45.
- Scott, P., Thomson, J., Grzywacz, D., Savary, S., Strange, R., Ristaino, J.B., Korsten, L., 2016. Genetic modification for disease resistance: a position paper. *Food Security* 8 (4), 865-870.

- Shabbir, R., Javed, T., Afzal, I., Sabagh, A.E., Ali, A., Vicente, O., Chen, P., 2021. Modern Biotechnologies: Innovative and Sustainable Approaches for the Improvement of Sugarcane Tolerance to Environmental Stresses. *Agronomy* 11(1042), 1-20.
- Shah, J., 2003. The salicylic acid loop in plant defense. *Current Opinion in Plant Biology*, 6(4), 365-371.
- Shah, J. and Zeier, J., 2013. Long-distance communication and signal amplification in systemic acquired resistance. *Frontiers in Plant Science*, 4 (30), 1-16.
- Shen, F.T., Yen, J.H., Liao, C.-S., Chen, W.C., Chao, Y.T. 2019. Screening of rice endophytic biofertilizers with fungicide tolerance and plant growth promoting characteristics. *Sustainability* 11(11113), 1-13.
- Shi, W., Tan, Y., Wang, S., Gardiner, D.M., De Saeger, S., Liao, Y., Wang, C., Fan, Y., Wang, Z. and Wu, A., 2017. Mycotoxigenic potentials of *Fusarium* species in various culture matrices revealed by mycotoxin profiling *Toxins* 9(1), 6.
- Siddiqui, S.H., Khan, I.A., Abdullah, K., Nizamani, G.S., 1994. Rapid multiplication of sugarcane through micropropagation. *Pakistan Journal of Agricultural Research* 15(1), 134-136.
- Sieber, T.N., 2002. Fungal root endophytes. In *Plant roots*, CRC Press, 1369-1418.
- Sieber, T.N., Waisel, Y., Eshel, A., Kafkafi, U., 2002. Fungal root endophytes. In *Plant roots: Sikora, P., Chawade, A., Larsson, M., Olsson, J., Olsson, O.* 2011. Mutagenesis as a tool in plant genetics, functional genomics, and breeding. *International Journal of Plant Genomics* 2011, 13.
- Silvarolla, M.B., 1992. Plant genomic alternations due to tissue culture. *Journal of Brazil Association. Advance Science.* 44, 329-335.
- Singh, N., Kumar, A., Garg, G., 2006. Genotype dependent influence of phytohormones combination and sub culturing on micro propagation of sugarcane varieties. *Indian Journal of Biotechnology* 5(1), 99-106.
- Singh, N. and Kashyap, S., 2012. In silico identification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from *Phytophthora sojae*. *Journal of Molecular Modeling*, 18(9), 4101-4111.
- Singh, R.K., Mishra, S.K., Singh, S.P. Mishra, N., Sharma, M.L., 2010. Evaluation of microsatellite markers for genetic diversity analysis among sugarcane species and commercial hybrids. *Australian Journal of Crop Science* 4, 116-125.
- Sieradzki, Z., Mazur, M., Król, B., Kwiatek, K., 2021. Prevalence of genetically modified soybean in animal feedingstuffs in Poland. *Journal of Veterinary Research* 65(1), 93-99.
- Skirvin, R.M., 1978. Natural and induced variation in tissue culture. *Euphytica* 27,241-266.

- Slaughter, A., Daniel, X., Flors, V., Luna, E., Hohn, B., Mauch-Mani, B., 2012. Descendants of primed *Arabidopsis* plants exhibit resistance to biotic stress. *Plant Physiology* 158(2), 835-843.
- Snyman, S.J., Monosi, B.B., Hockett, B.I., 2001. New developments in the production of herbicide-resistant sugarcane. *Proceedings of the South African of Sugarcane Technologists' Association* 75, 112-114.
- Snyman, S.J. 2004 Sugarcane transformation. In: *Transgenic crops of world: Essential protocols*. Curtis, I.S. (ed). Kulwer Academic Publishers, Dordrecht. 103-114.
- Snyman, S.J., Meyer, G.M., Richards, J.M., Haricharan, N., Ramgareeb, S., Hockett, B.I., 2006. Refining the application of direct embryogenesis in sugarcane: effect of the developmental phase of leaf disc explants and the timing of DNA transfer on transformation efficiency. *Plant Cell Reports* 25(10), 1016-1023.
- Snyman, S.J., Meyer, M.G., Koch, A.C., Banasiak, M., Watt, M.P., 2011. Applications of *in vitro* culture systems for commercial sugarcane production and improvement. *In Vitro Cellular and Developmental Biology Plant* 47, 234-249.
- Snyman, S.J., Naidoo, M., Watt, M.P., Rutherford, R.S., 2019. An *in vitro* screening system to assess aluminum toxicity in sugarcane (*Saccharum spp.*) cultivars. *In Vitro Cellular & Developmental Biology Plant* 55(4), 403-408.
- Song, C.J., Steinebrunner, I., Wang, X., Stout, S.C., Roux, S.J., 2006. Extracellular ATP induces the accumulation of superoxide via NADPH oxidases in *Arabidopsis*. *Plant Physiology* 140, 1222-1232.
- Sondergaard, T.E., Fredborg, M., Oppenhagen Christensen, A.M., Damsgaard, S.K., Kramer, N.F., Giese, H., Sørensen, J.L., 2016. Fast screening of antibacterial compounds from fusaria. *Toxins* 8(355), 1-9.
- Soliman, M., Elkesh, A., Souad, T., Alhaithloul, H., Farooq, M., 2020. Brassinosteroid seed priming with nitrogen supplementation improves salt tolerance in soybean. *Physiology and Molecular Biology of Plants* 26(3), 501-511.
- Spence, C. and Bais, H., 2015. Role of plant growth regulators as chemical signals in plant–microbe interactions: a double-edged sword. *Current Opinion in Plant Biology* 27, 52-58.
- Srivastava, A.C., Ahamad, S., Agarwal, D.K., Sarbhoy, A.K., 2003. Screening of potential gibberellin producing *Fusarium* strains for the hybrid rice production. *Food, Agriculture and Environment* 1(2), 250-253.
- Stone, J.M., Heard, J.E., Asai, T., Ausubel, F.M., 2000. Simulation of fungal-mediated cell death by fumonisin B1 and selection of fumonisin B1–resistant (fbr) *Arabidopsis* mutants. *The Plant Cell*, 12(10), 1811-1822.

- Stone, J.K., Polishook, J.D., White, J.R.J., 2004. Endophytic fungi. In: Mueller G, Bills GF, Foster MS, editors. Biodiversity of fungi: Inventory and monitoring methods. Elsevier: Burlington, 241–270.
- Strobel, G.A., 2003. Endophytes as sources of bioactive products. *Microbes and Infection*, 5(6), 535-544.
- Sudan, J., Raina, M., Singh, R., 2018. Plant epigenetic mechanisms: role in abiotic stress and their generational heritability. *3 Biotech* 8(3), 1-12.
- Suhesti, S., Syukur, M., Husni, A., Hartati, R.S., 2021. Increased genetic variability of sugarcane through gamma ray irradiation. In IOP Conference Series: Earth and Environmental Science. IOP Publishing 653 (1), 1-8.
- Summerell, B.A., 2019. Resolving *Fusarium*: current status of the genus. *Annual Review of Phytopathology* 57, 323-339.
- Sun, C., Ali, K., Yan, K., Fiaz, S., Dormatey, R., Bi, Z., Bai, J., 2021. Exploration of Epigenetics for Improvement of Drought and Other Stress Resistance in Crops: A Review. *Plants* 10(6), 1-16.
- Suprasanna, P., Sidha, M., Bapat, V.A., 2009. Integrated approaches of mutagenesis and *in vitro* selection for crop improvement. *Plant tissue culture and molecular markers: their role in improving crop productivity*. IK International Publishing House, New Delhi, 73-92.
- Suprasanna, P., Patade, V.Y., Desai, N.S., Devarumath, R.M., Kavar, P.G., Pagariya, M.C., Ganapathi, A., Manickavasagam, M., Babu, K.H., 2011. Biotechnological developments in sugarcane improvement: an overview. *Sugar Tech* 13, 322-335.
- Suprasanna, P., Jain, S.M., Ochat, S.J., Kulkarni, V.M., Predieri, S., 2012. Applications of *in vitro* techniques in mutation breeding of vegetatively propagated crops. In: Shu, Q.Y., B. P. Forster and H. Nakagawa (Eds.), *Plant Mutation Breeding and Biotechnology*, CABI Publishing, Wallingford, 371-385.
- Suthar, R., Bhatt, P.N., Bhatt, D.P., 2021. Selection of vascular wilt resistance cumin callus to culture filtrate of *Fusarium equiseti* and regeneration of plants. *Vegetos* 34(2), 318-324.
- Suwanarach, N., Kumla, J., Matsui, K., Lumyong, S., 2015 Characterization and efficacy of *Muscodora cinnamomi* in promoting plant growth and controlling *Rhizoctonia* root rot in tomatoes. *Biological Control* 90, 25-33.
- Švábová, L. and Lebeda, A., 2005. *In vitro* selection for improved plant resistance to toxin-producing pathogens. *Journal of Phytopathology* 153(1), 52-64.
- Svoboda, T., Parich, A., Güldener, U., Schöfbeck, D., Twaruschek, K., Václavíková, M., Hellinger, R., Wiesenberger, G., Schuhmacher, R. and Adam, G., 2019. Biochemical characterization of the *Fusarium graminearum* candidate ACC-deaminases and

- virulence testing of knockout mutant strains. *Frontiers in Plant Science* 10 (1072), 1-17.
- Thakur, M. and Sohal, B.S., 2013. Role of elicitors in inducing resistance in plants against pathogen infection: A Review. *International Scholarly Research Notices. ISRN Biochemistry*, 1-10.
- Talebi, A.B., Talebi, A.B., Shahrokhifar, B., 2012. Ethyl methane sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. *American Journal of Plant Sciences* 3 (12), 1661-1665.
- Thaler, J.S., Stout, M.J., Karban, R., Duffey, S.S., 2001. Jasmonate-mediated induced plant resistance affects a community of herbivores. *Ecological Entomology* 26(3), 312-324.
- Thaler, J.S., Humphrey, P.T., Whiteman, N.K., 2012. Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science* 17(5), 260-270.
- Thapa, S., Adhikari, J., Kumari Limbu, A., Joshi, A., Nainabasti, A., 2020. Significance of seed priming in agriculture and for sustainable farming. *Tropical Agroecosystems (TAEC)* 1(1), 1-6.
- Thiel, P.G., Marasas, W.F., Sydenham, E.W., Shephard, G.S., Gelderblom, W.C., 1992. The implications of naturally occurring levels of fumonisins in corn for human and animal health. *Mycopathologia*, 117(1), 3-9.
- Thomma, B.P., Eggermont, K., Penninckx, I.A., Mauch-Mani, B., Vogelsang, R., Cammue, B.P., Broekaert, W.F., 1998. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proceedings of the National Academy of Sciences* 95(25), 15107-15111.
- Thorpe, T.A., 2007. History of plant tissue culture. *Molecular biotechnology* 37(2), 169-180.
- Tolera, B., Diro, M. and Belew, D., 2014. *In vitro* aseptic culture establishment of sugarcane (*Saccharum officinarum* L.) varieties using shoot tip explants. *Advances in Crop Science and Technology* 2(3), 1-6.
- Ton, J., Van Pelt, J.A., Van Loon, L.C., Pieterse, C.M., 2002. Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in *Arabidopsis*. *Molecular Plant-Microbe Interactions* 15(1), 27-34.
- Toonen, M. A. J., Hendriks, T., Schmidt, E. D. L., Verhoeven, H. A., Vankammen, A., & Devries, S. C. 1994. Description of somatic-embryo-forming single cells in carrot suspension-cultures employing video cell tracking. *Planta* 194, 565-572.
- Trigiano, R.N and Gray, D.J. 2016. *Plant tissue culture, development, and biotechnology*. United States: CRC Press, 608
- Tsavkelova, E., Oeser, B., Oren-Young, L., Israeli, M., Sasson, Y., Tudzynski, B. and Sharon, A., 2012. Identification and functional characterization of indole-3-acetamide-mediated

- IAA biosynthesis in plant-associated *Fusarium* species. *Fungal Genetics and Biology* 49(1), 48-57.
- Tsukada, K., Takahashi, K., Nabeta, K., 2010. Biosynthesis of jasmonic acid in a plant pathogenic fungus, *Lasiodiplodia theobromae*. *Phytochemistry* 71(17-18), 2019-2023.
- Turbat, A., Rakk, D., Vigneshwari, A., Kocsubé, S., Thu, H., Szepesi, Á., Bakacsy, L., D Škrbić, B., Jigjiddorj, E.A., Vágvölgyi, C., Szekeres, A., 2020. Characterization of the plant growth-promoting activities of endophytic fungi isolated from *Sophora flavescens*. *Microorganisms* 8(5), 683-697.
- Turgut-Kara, N., Arikan, B., Celik, H., 2020. Epigenetic memory and priming in plants. *Genetica*, 148(2), 47-54.
- Us-Camas, R., Rivera-Solís, G., Duarte-Aké, F., De-la-Pena, C., 2014. *In vitro* culture: an epigenetic challenge for plants. *Plant Cell, Tissue and Organ Culture* 118(2), 187-201.
- Van Harten, A.M., 1998. *Mutation Breeding: Theory and Practical Applications*. New York: Cambridge University Press, 353.
- Vanneste, S., 2005. Auxin coordinates cell division and cell fate specification during lateral root initiation. *Physiology Plant* 123, 139-146.
- Van Loon, L.C. and Van Strien, E.A., 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiological and Molecular Plant Pathology* 55(2), 85-97.
- Van Weelden, M.T., Wilson, B.E., Beuzelin, J.M., Reagan, T.E., Way, M.O., 2016. Impact of nitrogen fertilization on Mexican rice borer (Lepidoptera: Crambidae) injury and yield in bioenergy sorghum. *Crop Protection* 84, 37-43.
- Venegas-Molina, J., Proietti, S., Pollier, J., Orozco-Freire, W., Ramirez-Villacis, D., Leon-Reyes, A., 2020. Induced tolerance to abiotic and biotic stresses of broccoli and *Arabidopsis* after treatment with elicitor molecules. *Scientific Reports* 10(1), 1-17.
- Vicedo, B., Flors, V., de la O Leyva, M., Finiti, I., Kravchuk, Z., Real, M.D., García-Agustín, P., González-Bosch, C., 2009. Hexanoic acid-induced resistance against *Botrytis cinerea* in tomato plants. *Molecular Plant-Microbe Interactions* 22(11), 1455-1465.
- Viswanathan, R., Balaji, C.G., Selvakumar, R., Malathi, P., Sundar, A.R., Prasanth, C.N., Chhabra, M.L., Parameswari, B., 2017. Epidemiology of *Fusarium* diseases in sugarcane: a new discovery of same *Fusarium sacchari* causing two distinct diseases, wilt and Pokkah Boeng. *Sugar Tech* 19(6), 638-646.
- Voigt, C.A., 2014. Callose-mediated resistance to pathogenic intruders in plant defense-related papillae. *Frontiers in Plant Science* 5, 168.
- Vorwerk, S., Somerville, S., Somerville, C., 2004. The role of plant cell wall polysaccharide composition in disease resistance. *Trends in Plant Science* 9, 203-209.

- Vrabka, J., Niehaus, E.M., Münsterkötter, M., Proctor, R.H., Brown, D.W., Novák, O., Pěňčík, A., Tarkowská, D., Hromadová, K., Hradilová, M., Oklešť'ková, J., 2019. Production and role of hormones during interaction of *Fusarium* species with maize (*Zea mays* L.) seedlings. *Frontiers in Plant Science* 9 (1936), 1-16.
- Waiyaki, J.N., 1968. Studies on Chemical Control and General Biology of Sugar-Cane Moth Borers at Tanganyika Planting Company, Arusha-Chini, Tanzania. 1966-1967. *International Journal of Pest Management A* 14(1), 153-154.
- Walton, A.J. and Conlong, D.E., 2016. General biology of *Eldana saccharina* (Lepidoptera: Pyralidae): A target for the sterile insect technique. *Florida Entomologist* 99(1), 30-35.
- Walters, D.R. and Fountaine, J.M., 2009. Practical application of induced resistance to plant diseases: an appraisal of effectiveness under field conditions. *The Journal of Agricultural Science* 147(5), 523-535.
- Wang, S.Y., 1999. Methyl jasmonate reduces water stress in strawberry. *Journal of plant growth regulation* 18(3), 127-134.
- Wang, H., Hwang, S.F., Eudes, F., Chang, K.F., Howard, R.J., Turnbull, G.D., 2006. Trichothecenes and aggressiveness of *Fusarium graminearum* causing seeding blight and root rot in cereals. *Plant Pathology* 55, 224-230.
- Wang, X., Zhang, X., Liu, L., Xiang, M., Wang, W., Sun, X., Che, Y., Guo, L., Liu, G., Guo, L., Wang, C., 2015. Genomic and transcriptomic analysis of the endophytic fungus *Pestalotiopsis fici* reveals its lifestyle and high potential for synthesis of natural products. *BMC genomics* 16(1), 1-13.
- Wang, W.Z., Yang, B.P., Feng, X.Y., Cao, Z.Y., Feng, C.L., Wang, J.G., Xiong, G.R., Shen, L.B., Zeng, J., Zhao, T.T., Zhang, S.Z., 2017. Development and Characterization of Transgenic Sugarcane with Insect Resistance and Herbicide Tolerance. *Frontiers in Plant Science* 8, 1535.
- Wang, X., Yu, R., Li, J., 2021. Using genetic engineering techniques to develop banana cultivars with *Fusarium* Wilt resistance and ideal plant architecture. *Frontiers in Plant Science* 11,
- Wasternack, C., 2007. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of botany* 100(4), 681-697.
- Wasternack, C. and Strnad, M., 2016. Jasmonate signaling in plant stress responses and development—active and inactive compounds. *New biotechnology* 33(5), 604-613.
- Wasternack, C., and Hause, B., 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Annals of Botany* 111(6), 1021-1058.

- Wasternack, C., Goetz, S., Hellwege, A., Forner, S., Strnad, M., Hause, B., 2012. Another JA/COI1-independent role of OPDA detected in tomato embryo development. *Plant Signaling & Behavior* 10, 1349-1353.
- Waugh, R., Leader, D., McCallum, N., Caldwell, D., 2006 Harvesting the potential of induced biological diversity. *Trends in Plant Science* 11, 71-79.
- Westman, S.M., Kloth, K.J., Hanson, J., Ohlsson, A.B., Albrechtsen, B.R., 2019. Defence priming in *Arabidopsis*—a meta-analysis. *Scientific Reports* 9(1), 1-13.
- Williams, E. G., and Maheswaran, G., 1986. Somatic embryogenesis – factors influencing coordinated behavior of cells as an embryogenic group. *Annals of Botany* 57, 443-462.
- Williams, L.D., Glenn, A.E., Zimeri, A.M., Bacon, C.W., Smith, M.A., Riley, R.T., 2007. Fumonisin disruption of ceramide biosynthesis in maize roots and the effects on plant development and *Fusarium verticillioides*-induced seedling disease. *Journal of Agricultural and Food Chemistry* 55(8), 2937-2946.
- Wolpert, T.J., Dunkle, L.D., Ciuffetti, L.M., 2002. Host-selective toxins and avirulence determinants: what's in a name? *Annual review of phytopathology* 40(1), 251-285.
- Worrall, D., Holroyd, G.H., Moore, J.P., Glowacz, M., Croft, P., Taylor, J.E., Paul, N.D. and Roberts, M.R., 2012. Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens. *New Phytologist* 193(3), 770-778.
- Wu, C., Kim, H.K., Van Wezel, G., 2015. Metabolomics in the natural products field - a gateway to novel antibiotics. *Drug Discov Today Technology* 13, 11-7.
- Wu, X. and Ye, J., 2020. Manipulation of jasmonate signaling by plant viruses and their insect vectors. *Viruses* 12(2), 148.
- Xie, M., Yu, B., 2015. SiRNA-directed DNA methylation in plants. *Current Genomics* 16(1), 23-31.
- Xing, F., Li, Z., Sun, A., Xing, D., 2013. Reactive oxygen species promote chloroplast dysfunction and salicylic acid accumulation in fumonisin B1-induced cell death. *Federation of European Biochemical Societies* 587(14), 2164-2172.
- Yadav, S., Jackson, P., Wei, X., Ross, E.M., Aitken, K., Deomano, E., Atkin, F., Hayes, B.J., Voss-Fels, K.P., 2020. Accelerating genetic gain in sugarcane breeding using genomic selection. *Agronomy* 10(585), 1-21.
- Yamaguchi Y, Huffaker A. 2011. Endogenous peptide elicitors in higher plants. *Current Opinion in Plant Biology* 14, 351-357.
- Yamaguchi, Y., Huffaker, A., Bryan, A.C., Tax, F.E., Ryan, C.A., 2010. PEPR2 is a second receptor for the Pep1 and Pep2 peptides and contributes to defense responses in *Arabidopsis*. *The Plant Cell* 22, 508-522.

- Yamaguchi, Y., Pearce, G., Ryan, C.A., 2006. The cell surface leucine rich repeat receptor for AtPep1, an endogenous peptide elicitor in *Arabidopsis*, is functional in transgenic tobacco cells. *Proceedings of the National Academy of Sciences United States of America* 103, 10104-10109.
- Yan, C. and Xie, D., 2015. Jasmonate in plant defence: sentinel or double agent? *Plant biotechnology journal* 13(9), 1233-1240.
- Yao, W., Ruan, M., Qin, L., Yang, C., Chen, R., Chen, B., Zhang, M., 2017. Field performance of transgenic sugarcane lines resistant to sugarcane mosaic virus. *Frontiers in Plant Science* 8(104), 1-9.
- Yasmeen, S., Rajput, M.T., Khan, I.A., Hasseney, S.S., 2017. Induced mutations and Somaclonal variations in three sugarcane (*Saccharum Officinarum L.*) varieties. *Pakistan Journal of Botany* 49 (3), 955-964.
- Yerzhebayeva, R.S., Abekova, A.M., Bersimbaeva, G.H., Konysbekov, K.T., Bastaubaeva, S.O., Roik, N.V., Urazaliev, K.R., 2019. *In vitro* cell selection of sugar beet for resistance to culture filtrate of the fungus *Fusarium oxysporum*. *Cytology and Genetics* 53(4), 307-314.
- Yoder, O.C., 1973. Selective toxin produced by *phyllosticta-maydis*. *Phytopathology* 63(11), 1361-1366.
- Yuan, Z.C., Haudecoeur, E., Faure, D., Kerr, K.F., Nester, E.W., 2008. Comparative transcriptome analysis of *Agrobacterium tumefaciens* in response to plant signal salicylic acid, indole-3-acetic acid and  $\gamma$ -amino butyric acid reveals signalling cross-talk and *Agrobacterium*–plant co-evolution. *Cellular microbiology* 10(11), 2339-2354.
- Yuan, Z.L., Zhang, C.L., Lin, F.C., Kubicek, C.P., 2010. Identity, diversity, and molecular phylogeny of the endophytic mycobiota in the roots of rare wild rice (*Oryza granulata*) from a nature reserve in Yunnan, China. *Applied and environmental microbiology* 76(5), 1642-1652.
- Zakaria, L., 2017. Mycotoxigenic *Fusarium* species from agricultural crops in Malaysia. *JSM Mycotoxins* 67(2), 67-75.
- Zakir, A.Z., 2011. Inducible defenses in herbivore-plant interactions: functions mechanisms and manipulations. *Swedish University of Agricultural Sciences*, 1-25.
- Zemach, A., Kim, M.Y., Hsieh, P.H., Coleman-Derr, D., Eshed-Williams, L., Thao, K., Harmer, S.L., Zilberman, D., 2013. The *Arabidopsis* nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. *Cell* 153(1), 193-205.
- Zhang, H.W, Song, Y.C and Tan, R.X., 2006. Biology and chemistry of endophytes. *Natural Product Report* 23, 753- 771.
- Zhang, X., Wu, Q., Cui, S., Ren, J., Qian, W., Yang, Y., He, S., Chu, J., Sun, X., Yan, C., Yu, X., 2015. Hijacking of the jasmonate pathway by the mycotoxin fumonisin B1 (FB1) to

- initiate programmed cell death in *Arabidopsis* is modulated by RGLG3 and RGLG4. *Journal of experimental botany* 66(9), 2709-2721.
- Zhang, H., Zhang, Q., Zhai, H., Li, Y., Wang, X., Liu, Q., He, S., 2017. Transcript profile analysis reveals important roles of jasmonic acid signalling pathway in the response of sweet potato to salt stress. *Scientific reports* 7(1), 1-12.
- Zhang, M. and Jeyakumar, J.M.J., 2018. *Fusarium* Species Complex Causing Pokkah Boeng in China. In *Fusarium-Plant Diseases, Pathogen Diversity, Genetic Diversity, Resistance and Molecular Markers*. Intech Open, 139-154.
- Zhao, Y., 2010. Auxin biosynthesis and its role in plant development. *Annual Review of Plant Biology* 61, 49-64.
- Zhao, Q. and Dixon, R.A., 2014. Altering the cell wall and its impact on plant disease: from forage to bioenergy. *Annual Review of Phytopathology* 52, 69-91.
- Zhou, M., 2013. Conventional sugarcane breeding in South Africa: Progress and future prospects. *American Journal of Plant Sciences* 4(2), 189.
- Zhu, W., Wei, W., Fu, Y., Cheng, J., Xie, J., Li, G., Yi, X., Kang, Z., Dickman, M.B., Jiang, D., 2013. A secretory protein of necrotrophic fungus *Sclerotinia sclerotiorum* that suppresses host resistance. *PLoS One* 8(1), 1-18.

