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ORIGINAL ARTICLE

Methylglyoxal-induced enhancement of somatic embryogenesis and associated metabolic changes in sugarcane (*Saccharum* spp. hybrids)

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Abstract
The production of regenerable embryogenic callus is essential to sugarcane genetic improvement. However, some sugarcane cultivars display poor calli yields using established in vitro protocols. In this study, we tested the impact of methylglyoxal (MG) on embryogenic callus and plantlet development in cultivars NCo376 and N41. Calli were exposed to 0–10 mM MG at embryo maturation and germination stages. For both cultivars, the 2 and 4 mM MG treatments increased callus dry mass by up to 48%, but 70–80% decreases were observed when calli were exposed to 7 and 10 mM. The 2 and 4 mM MG treatments also produced more compact white embryogenic callus than the control. Incorporation of MG at the same levels during embryo germination promoted faster shoot morphogenesis in both cultivars and increase plantlet yield in NCo376 by 130% when treated with 4 mM MG. In both cultivars, MG levels higher than 7 mM had a negative or no effect plantlet production. Metabolic profiling revealed higher levels of sugars in MG-treated than in control calli, which may have contributed to development of more white compact calli. Separate clustering of NCo376 and N41 MG-treated calli in principal component and hierarchical clustering analyses of the metabolic profiles, suggested variations in MG metabolism among the genotypes that may account for variations in the MG-induced effect on somatic embryogenesis between the two cultivars. Although the effect may be genotype-dependant, low MG concentrations can induce improved embryogenic callus and plantlet development in sugarcane.

Keywords Metabolic profiling · Methylglyoxal · Somatic embryogenesis · Sugarcane

Introduction

Sugarcane is cultivated mainly for sugar production and is becoming an increasingly important crop for generation of renewable energy. Sustainable sugarcane production is highly depended on breeding programs that produce pest and disease resistant genotypes. However, conventional breeding of improved sugarcane varieties is complicated by seed sterility, unsynchronised flowering and the polyploid and aneuploid genome of the crop, taking 12–15 years to release a new cultivar (Butterfield et al. 2001; Ming et al. 2006). Biotechnological applications are used to circumvent some of these challenges and in vitro culture techniques, such somatic embryogenesis, play a key role in sugarcane genetic improvement strategies. For instance, embryogenic calli are used in genetic engineering via particle bombardment and *Agrobacterium*-mediated transformation (Snyman 2004; Krishnan and Mohan 2017). In in vitro mutation breeding, embryogenic calli are exposed to mutagens and mutants with traits of interest are regenerated (Patade and Suprasanna 2008; Mahlanza et al. 2013). Production of synthetic seeds is by encapsulation of somatic embryos in alginate (Nieves et al. 2001; Martinez-Montero et al. 2008). Production of regenerable embryogenic calli is therefore essential to biotechnological strategies for sugarcane genetic improvement.

Methods for sugarcane somatic embryogenesis are well developed for some genotypes with embryogenic callus generally forming on medium containing 0.5–3 mg/L

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2,4-dichlorophenoxyacetic acid (Ahloowalia and Maretzki 1983; Ho and Vasil 1983; Snyman 2004; Rodríguez et al. 1996; Mahlanza et al. 2013). To a lesser extent, embryogenic calli has been reported to form on media containing 2,4D in combination with kinetin (Gill et al. 2004) and thidiazuron (Malabadi et al. 2011), and also a combination of kinetin and naphthaleneacetic acid (Desai et al. 2004; Lakshmanan et al. 2006). Plant metabolites associated with somatic embryogenesis in sugarcane under these culture conditions have been studied to a limited extent. However, it has been shown that increased synthesis of sugar compounds in the gluconeogenesis, starch and sucrose metabolic pathways is associated with somatic embryogenesis in sugarcane cultured on media containing 2,4D (Neves et al. 2003; Mahmud et al. 2014). Increased proteolytic activity has also been observed in these sugarcane embryogenic calli (Neves et al. 2003). Nonetheless, somatic embryogenesis is poor in some important sugarcane genotypes, thereby limiting their use in genetic improvement programs. Novel plant growth regulators may alleviate recalcitrance to somatic embryogenesis observed in some genotypes.

Methylglyoxal (MG), a α,β-dicarbonyl aldehyde, has been reported to be a natural growth regulator with strong anticancer properties in mammals (Mihich 1963; Szent-Györgyi 1977; Thornalley 1995; Ghosh et al. 2006; Chakraborty et al. 2014). As cancerous cells are in a de-differentiated state, the anticancer activity of MG is possibly derived from its ability to induce differentiation (Roy et al. 2004). Szent-Györgyi (1977) hypothesised that in a primitive earth where a strongly reducing atmosphere existed before oxygen "appeared", early life systems capable of only simple functions used MG or related ketoaldehydes as growth regulators to halt proliferation and induce differentiation and development into increasingly complex life forms. In plants, this cell differentiation-promoting activity of MG has been observed in enhanced somatic embryo maturation and plantlet regeneration in *Pinus* sp. (Niemi et al. 2002), *Solanum nigrum, Daucus carota* (Roy et al. 2004) and *Nicotiana tabacum* (Ray et al. 2013). Hoque et al. (2012) reviewed the role of MG as a signalling molecule in regulation of reactive oxygen species, stomatal conductance and expression of genes involved in hormone signalling, cell-to-cell communications, and chromatin remodelling. Although MG is produced by plants during glycolysis and its accumulation is cytotoxic especially in plants subjected to abiotic stresses, its plant growth regulatory activity may be employed at limited doses to enhance somatic embryogenesis in sugarcane.

In the present study, we therefore tested the effect of MG on sugarcane embryo maturation and germination in vitro towards enhanced production of embryogenic calli and plantlet regeneration in sugarcane genotypes, for use in biotechnological applications in genetic improvement programs. In addition, we studied the metabolic effects related to the MG-associated enhancements to somatic embryogenesis using gas-chromatography-mass spectrometry (GC-MS). This technique is well-established and the most widely used for plant metabolic profiling (reviewed by Jorge et al. 2016).

Materials and methods

Exposure of sugarcane embryogenic calli to methylglyoxal

The cultivars NCo376 and N41 were used in this study. NCo376 was released in 1955 and although its cultivation is on the decline due to susceptibilities to a range of biotic and abiotic stresses, it is often employed in in vitro studies towards genetic improvement of sugarcane in South Africa. N41, released in 2002, is a newer cultivar that is currently extensively grown commercially. For the production of calli via indirect somatic embryogenesis, leaf roll material was obtained from field-grown plants (cultivars NCo376 and N41) from the South African Sugarcane Research Institute, Mount Edgecombe and callus induction was carried out as described by Snyman (2004). The leaf rolls were decontaminated using ethanol, aseptically cut into 1–2 mm transverse sections, placed on callus initiation medium (CIM) [full strength MS salts and vitamins (Murashige and Skoog 1962), casein hydrolysate (0.5 g/L), sucrose (20 g/L), 2,4-dichlorophenoxyacetic acid (3 mg/L), agar–agar (8 mg/L, at pH 5.8)] for 6–8 weeks in the dark, and subcultured every 2 weeks. Thereafter, embryogenic calli (0.2 g) were transferred to embryo maturation medium (EMM) (CIM with 1 mg/L 2,4D) containing 0–10 mM MG (Fluka analytical, St. Louis, USA) for 3 weeks in the dark (Mahlanza et al. 2013). Then they were transferred to embryo germination medium (CIM without 2,4-D) containing 0–10 mM MG (based on preliminary studies) and maintained under 16 h photoperiod (200 lm/m^2/s photon flux density) at 26–30 °C for 4–8 weeks, subcultured every 2 weeks. MG was filter-sterilised and added to autoclaved media and, as it significantly lowered the media pH, sterilised KOH was used to restore the media pH to 5.8. Callus fresh and dry mass were recorded after 3 weeks on EMM and the number of plants regenerated from 0.2 g callus was recorded after 4–8 weeks on EGM.

Metabolic profiling of methylglyoxal-treated calli

The extracts from NCo376 and N41 embryogenic callus cultured on control and 4 mM MG-containing media (three replicates per treatment) were analysed using gas chromatography coupled with mass spectrometry (GC-MS) according a modified method by Glassop et al. (2007). All the chemicals used in the GC-MS-analyses were obtained

from Sigma-Aldrich (St Louis, USA). Calli (60 mg) were washed three times in a 2 mL microfuge tube using 300 µL ultrapure water to rinse off excess media, and then macerated in 350 µL methanol using a steel rod. As an internal quantification standard, 15 µL ribitol 2 mg/mL was added to each tube, vortexed and incubated at 70 °C for 15 min. To this, 350 µL water and 300 µL chloroform were added, the mixture vortexed and the polar and non-polar phases separated by centrifugation at 12,000 rpm for 10 min at 4 °C. The supernatant was mixed with 300 µL chloroform, centrifuged and 80 µL of the polar supernatant was dried using a vacuum concentrator. The dried pellet was methoximated by adding 40 µL methoxyamine hydrochloride (20 mg/mL in pyridine) and incubating at 37 °C whilst shaking at 145 rpm for 120 min. Metabolites were trimethylsilylated by adding 60 µL *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) and shaking at 145 rpm/min at 37 °C for 120 min. A 1 µL of the derivitized sample was injected at a split ratio of 4:1 into a QP2010 Ultra GC-MS instrument (Shimadzu Scientific Instruments, Columbia, MD, USA) using an AOC 20i autosampler (Shimadzu Scientific Instruments). The GC column was a SLB 5 ms capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) (Supelco, Bellefonte, PA, USA). The temperatures for the injection, interface and ion source were 230 °C, 250 °C and 250 °C, respectively, with hydrogen as the carrier gas at flow rate of 1 mL/min. The oven temperature program was ramped up from 70 to 76 °C at a rate of 1 °C/min, then to 330 °C at 6 °C/min and finally held at 330 °C for 10 min. Prior to running the samples, the MS was tuned using perfluorotributylamine (PFTBA). The mass spectra were acquired in a scanning range of 50–600 *m*/*z* at a rate of 2 scans/sec. An *n*-alkanes series (C_{10}–C_{40}) was ran as retention time standards. The recorded mass data were analysed using the GCMS solution software (LabSolutions, Shimadzu Scientific Instruments) and AMDIS [National Institute of Standards and Technology (NIST), Gaithersburg, MD, United States]. Metabolite identification was conducted using the NIST library and Golm Metabolic Database (GMD) (Max Planck Institute for Molecular Plant Physiology, Potsdam, Germany). Metabolites were identified by matching the mass spectra by at least 60% and retention index with an allowance of ± 10. Responses from detected peaks were normalised by dividing the peak area by that of the ribitol internal standard and dividing by the dry mass obtained from 0.06 g fresh callus (calculated using the fresh:dry mass ratio for control and 4 mM callus for each cultivar) to obtain the relative quantity of each metabolite per gram of dry mass of callus.

Statistical analyses

Callus mass and plant yield data were tested for normality using the Shapiro–Wilk test and analysis of variance (ANOVA) was conducted to establish statistically significant differences, followed by multiple comparison using a Sidak test. These analyses were conducted using Genstat statistical package (16th edition, VSN International, Hemel Hempstead, UK). Hierarchical cluster and principal component analyses of the GC-MS data and statistical comparisons of the mean metabolite abundances using *t* tests were conducted using XLSTAT statistical software (Addinsoft, USA). Differences were statistically significant at $p \leq 0.05$.

Results

Effect of methlygloxal on callus and plantlet production

After 3 weeks on EMM containing 0–10 mM MG, the fresh mass of NCo376 and N41 calli treated with 2 and 4 mM MG were not significantly different from that of the control calli (Fig. 1a). However, for both cultivars, fresh and dry mass of calli exposed to 7 and 10 mM MG were 80% and 70% lower, respectively, than the untreated controls (Fig. 1a, b). In contrast, in calli treated with 0–4 mM MG, the dry mass increased in both cultivars, with NCo376 and N41 calli treated with 4 mM MG exhibiting dry masses of 0.127 ± 0.005 g and 0.135 ± 0.003 g, respectively, significantly higher than 0.102 ± 0.009 g and 0.091 ± 0.002 g of their controls (Fig. 1b). However, there was no significant difference in fresh and dry mass of NCo376 and N41 calli at each tested MG concentration. Plantlet yield in NCo376 improved as the MG concentration increased from 0 to 4 mM as 406 ± 47, 620 ± 109 and 971 ± 258 plantlets/0.2 g calli were produced from the control, 2 and 4 mM treatments, respectively, with these increases being statistically significant in the 4 mM treatment. There were no differences between the controls and the 7 mM MG treatment, whilst no plantlets were produced in the 10 mM MG treatment (Fig. 1c). In N41 calli, there were no statistically significant differences amongst the control, 2 and 4 mM MG treatments that produced 455 ± 151, 489 ± 41 and 512 ± 26 plantlets/0.2 g calli, respectively. No plantlets were produced in calli treated with 7 and 10 mM MG. Visually, the MG-treated calli produced more white compact embryogenic calli than the untreated controls (Fig. 2a–d). After 4 weeks on embryo germination medium, MG-treated embryogenic calli of both cultivars displayed more rapid shoot growth than non-treated calli (Fig. 2e, f).

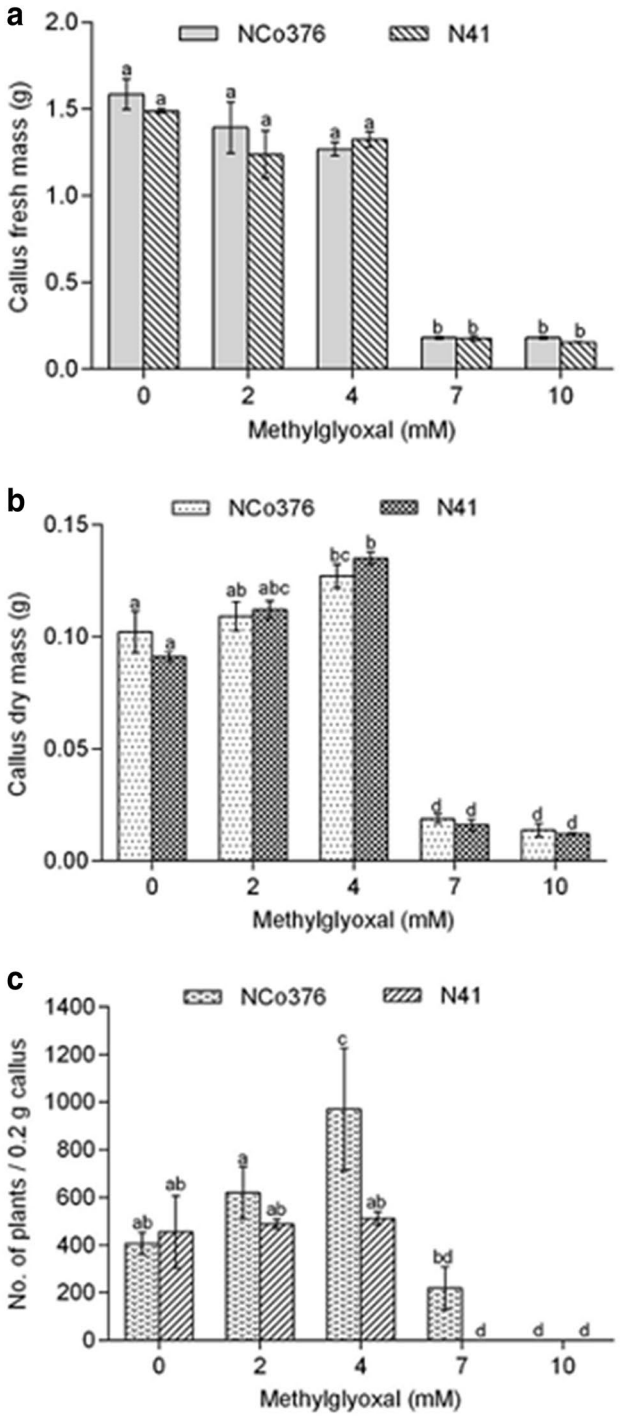

Fig. 1 The effect of 0–10 mM methylglyoxal on callus **a** fresh, **b** dry masses, and **c** plantlet yield of cultivars NCo376 and N41. Different letters denote statistical significance. ANOVA and Sidak tests; fresh mass: $p < 0.001$, dry mass: $p < 0.001$, number of plants: $p < 0.001$. Mean ± SE, n = 3–4

Metabolic profiling in MG-treated and non-treated calli

To study the metabolic responses associated with the improved somatic embryogenesis in calli exposed to MG, metabolic profiling using GC-MS was conducted in MG-treated and non-treated embryogenic calli of NCo376 and N41. The analysis yielded 56 and 64 metabolites in the controls and 4 mM MG treated calli of both cultivars, respectively with a total of 66 metabolites being detected amongst the treatments. To gain an overview of the data, hierarchical cluster analysis (HCA) was performed and showed N41 and NCo376 clustering into main clusters. In addition, two subordinate clusters separating the metabolite profiles of the control and MG-treated calli were observed within the N41 cluster (Fig. 3). Within the NCo376 cluster, the profiles of the control and MG-treated calli did not separate into distinct sub-clusters. The metabolites detected in control and MG-treated calli of both varieties were also clustered into two main clusters, each consisting of sugars, amino acids and organic acids (Fig. 3). The principal component analysis (PCA) confirmed results from the HCA, that N41 and NCo376 separated in distinct clusters and the N41 control and MG samples clustered separately, but not the NCo376 samples (Fig. 4). The first component represented 34.29% of the total variance whilst the second differentiated samples with 22.57% of the variation. The levels of glucose, fructose, trehalose, sucrose and galactinol in the N41 calli were significantly greater in the MG-treated calli than the controls, whilst increases in galactose and raffinose were not statistically significant (Table 1). In NCo376, the concentrations of glucose, fructose, galactose and galactinol were significantly higher in the MG-treated calli than in the controls, while those of sucrose, threose, raffinose and trehalose were similar in both. The glycine, alanine and proline levels in the N41 calli exposed to MG were lower than in the controls and a similar trend was observed for glycine in NCo376. The alanine levels in the NCo376 controls and MG-treated calli were the same, whilst proline was not detected in calli treated with MG (Table 1). The amount of aminobutanoic acid, phosphoric acid and malic acid in MG-treated calli of genotypes were lower than the controls (Table 1).

Discussion

In this study, the inclusion of MG in the culture media during embryo maturation at 4 mM or lower enhanced growth and development of embryogenic calli in NCo376 and N41 as evidenced by higher dry mass and development of more compact white calli in MG-treated calli. Incorporation of MG at the same levels during embryo germination also promoted rapid shoot growth in both cultivars with significantly

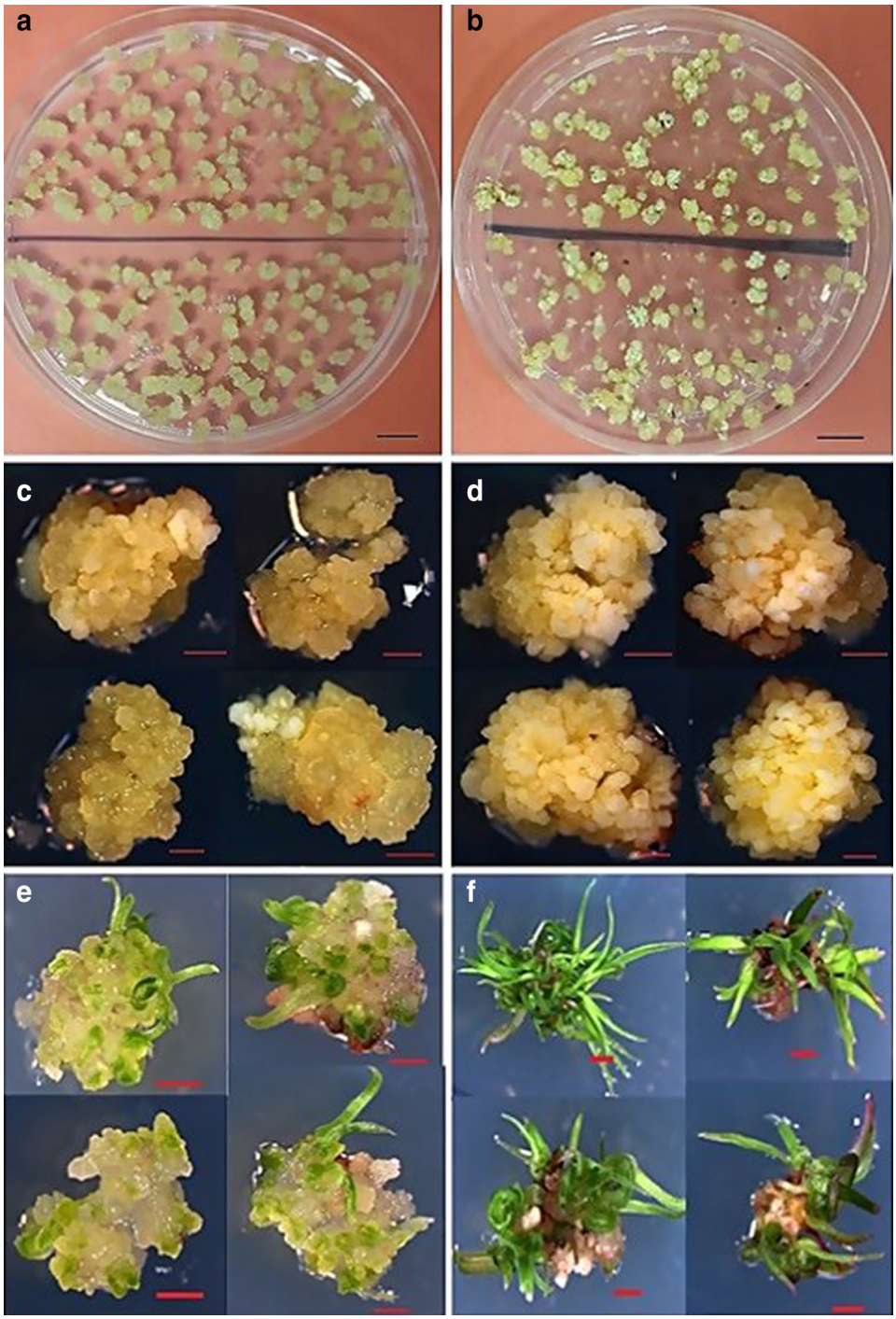

Fig. 2 Visual illustration of the impact of 4 mM methylglyoxal on embryo maturation and germination in calli of NCo376. Calli after 3 weeks of culture on embryo maturation media containing **a** 0 mM and **b** 4 mM MG; somatic embryo development in calli exposed to **c** 0 mM and **d** 4 mM MG; and germination of embryos after 4 weeks on media containing **e** 0 mM and **f** 4 mM MG. Size bar = 10 mm (**a, b**), 1 mm (**d–f**)

higher plantlet yields in NCo376 in the 4 mM MG treatment (Figs. 1c, 2e, f). Levels at 7 mM and above retarded callus growth and development of embryos, and either had no effect or negatively affected plantlet production (Fig. 1b, c).

Studies have reported that MG may serve as a signalling molecule, especially in response to stress (Yadav et al. 2005; Singla-Pareek et al. 2006; Hossain et al. 2009; Kaur et al. 2015). Stress is known to induce morphogenic responses in plant cells, including embryogenesis, (Zavattieri et al. 2010). In vitro culture conditions themselves are known to induce stress in plant cells (Desjardins et al. 2009). The reported stress response signalling activity of MG in in vitro cultured plant cells may explain its role in somatic embryogenesis.

The role of MG in cell differentiation has been observed in different plant species. Ray et al. (2013) reported improved growth and differentiation of organogenic callus of *Nicotiana tabacum* L. when cultured on media containing up to 0.1 mM MG, but growth decreased at higher

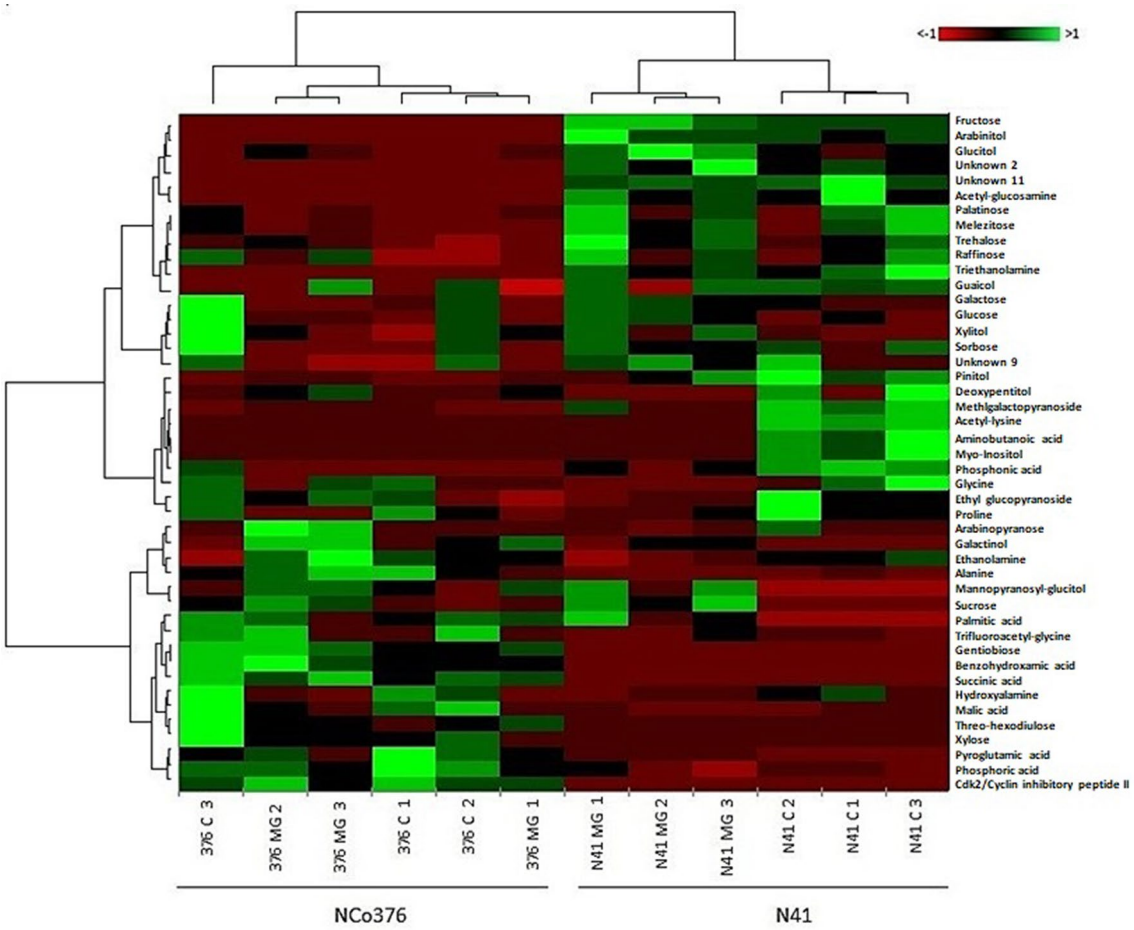

Fig. 3 Hierarchical cluster analysis of metabolites detected using GC-MS in embryogenic (EM) calli of NCo376 and N41 cultured on embryo maturation medium containing 4 mM methylglyoxal (MG) and without MG (C). The analysis clustered replicates of each treatment of N41 and NCo376 based on the similarity of their metabolite abundance profiles. Data were $\log_{10}$ transformed and normalised. For each metabolite, the colours represent values of the relative metabolite abundance normalised in a range between 1 (red = low abundance) to 1 (green = high abundance). (Color figure online)

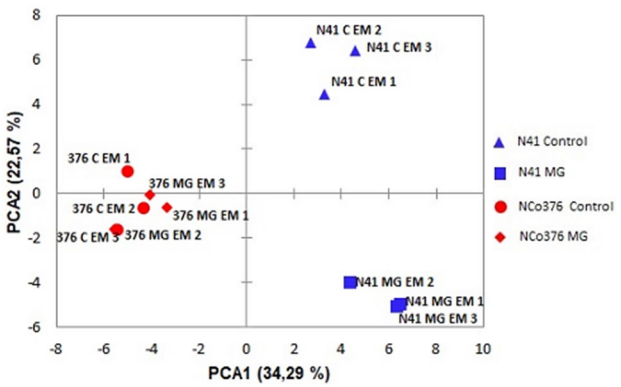

Fig. 4 Principal component analysis of relative abundance of metabolites detected in extracts of embryogenic calli of NCo376 and N41 cultured on maturation media containing 4 mM methylglyoxal

concentrations. Those authors reported that MG used in combination with glycine and succinate, precursors of chlorophyll biosynthesis, improved shoot length and proliferation. Cell histology in MG-treated calli showed vigorous development of corm-like structures and shoots. Similarly, Roy et al. (2004) observed that organogenic calli of *Solanum nigrum* and *Daucus carota* cultured on media containing 0.5 mM MG produced more shoots which were greener than those from the controls and kinetin-containing media. They suggested that MG was functionally similar to kinetin, albeit superior in inducing cell differentiation. Inclusion of MG in 2,4D-containing media may produce a 'auxin-cytokinin' balance that enhances somatic embrogenesis. It is possible that MG may serve in signalling a switch from cell division to a cell differentiation. Maturation of somatic embryos involves accumulation of storage products (Santa-Catarina et al. 2003; Cangahuala-Inocente et al. 2009), which in the present study, may have resulted in the recorded higher

Table 1 Relative abundance of metabolites detected by GC-MS in embryogenic calli of N41 and NCo376 cultured on embryo maturation medium only (control) and with methylglyoxal (4 mM MG)

Metabolite	Relative abundance			
	N41		NCo376	
	Control	4 mM MG	Control	4 mM MG
Glycine	130.0 ± 49.7^{a}	7.9 ± 1.9^{b}	61.2 ± 17.2^{A}	16.4 ± 7.2^{B}
Alanine	19.1 ± 8.4^{a}	4.5 ± 2.5^{b}	61.0 ± 22.0^{A}	31.6 ± 12.8^{B}
Proline	68.0 ± 32.7^{a}	18.1 ± 4.4^{b}	44.8 ± 12.4	ND
Phosphoric acid	139.05 ± 14.0^{a}	74.3 ± 24^{b}	204 ± 22.6^{A}	69.9 ± 7.4^{B}
Malic acid	566.3 ± 102.8^{a}	254.8 ± 49.3^{b}	2113.6 ± 361.8^{A}	311.1 ± 98.7^{B}
Aminobutanoic acid	8011.7 ± 1904.3^{a}	13.8 ± 1.9^{b}	469.0 ± 200.0^{A}	22.2 ± 7.3^{B}
Glucose	56.3 ± 12.2^{a}	244.7 ± 42.9^{b}	80.2 ± 19.6^{A}	149.8 ± 67.5^{B}
Galactose	25.5 ± 3.9^{a}	34.8 ± 8.1^{a}	7.2 ± 2.2^{A}	22.1 ± 10.6^{B}
Fructose	17.0 ± 0.9^{a}	20.7 ± 2.2^{b}	5.2 ± 1.8^{A}	15.5 ± 8.6^{B}
Myo-inositol	18329.3 ± 3419.4^{a}	1885.5 ± 313.2^{b}	2383.2 ± 412.6^{A}	951.2 ± 129.0^{B}
Threose	12.3 ± 4.2^{a}	6.5 ± 0.5^{b}	6.8 ± 2.4^{A}	4.0 ± 1.1^{A}
Trehalose	85.4 ± 9.5^{a}	131.7 ± 15.6^{b}	35.8 ± 10.0^{A}	26.1 ± 3.7^{A}
Melezitose	1459.2 ± 552.8^{a}	1164.7 ± 314.7^{a}	473.6 ± 128.6^{A}	216.1 ± 14.9^{B}
Sucrose	17735.6 ± 1338.2^{a}	39978.9 ± 6420.5^{b}	18857.8 ± 4128.4^{A}	14461.5 ± 3857.2^{A}
Raffinose	75.0 ± 22.8^{a,b}	59.0 ± 17.5^{a}	30.2 ± 17.4^{A}	19.2 ± 5.8^{A}
Galactinol	36.5 ± 11.1^{a}	84.2 ± 30.1^{b}	87.4 ± 30.8^{A}	141.3 ± 15.1^{B}

Statistical comparisons are within each cultivar; different superscript letters indicate statistical differences for each metabolite, Student's *t* test, $p < 0.05$, mean ± SE, n = 3

callus dry masses in the 2 and 4 mM MG treatments than the controls. Kaur et al. (2015) studied global gene expression in rice plants exposed to MG and observed over-expression of several protein kinases and transcription factors which are involved in signal transduction in various biological processes. As a transition from vegetative to embryogenic developmental processes in plant cells requires reprogramming of gene expression patterns (Karami et al. 2009), it is possible that MG-mediated cell signalling triggers such mechanisms. The limited effect of MG on plantlet yield in N41 in comparison with NCo376, may be due to more active MG-detoxifying glyoxalase 1 and 2 enzymes that converted the MG into lactic acid and glutathione more effectively in the stress tolerant N41. The differences in metabolic profile data clustering of N41 and NCo376 MG-treated and non-treated calli in the PCA and HCA suggest that the cultivars have different efficacies of metabolising MG.

In this study, metabolic profiling of embryogenic calli revealed mainly accumulation of sugars in MG-treated embryogenic calli. Neves et al. (2003) reported that high levels of soluble sugars were associated with somatic embryogenesis in sugarcane. Sugars are essential as signalling molecules, osmoticum and sources of carbon and energy (Lipavská and Konrádová 2004). Sucrose is one of the most important component of maturing seeds and somatic embryos as a carbon source. Whilst sucrose was supplied in the growth media, embryogenic calli exposed to MG accumulated higher levels of sucrose than the controls. The MG-treated calli also accumulated higher levels of glucose and fructose than the controls. Studies in different plant species, including sugarcane, have shown that somatic embryogenesis is associated with higher levels of sugars in embryogenic callus than non-embryogenic callus (Neves et al. 2003; Pescador et al. 2008; Mahmud et al. 2014). In the present study, this accumulation of sugars was higher in MG-treated calli than untreated ones. These higher levels may be due accumulation of storage products that occurs in embryo maturation. Roy et al. (2004) observed that the differentiation-enhancing activity of MG in *S. nigrum* and *D. carota* was associated with its influence on the activities of NADP-dependent glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase and NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, enzymes involved in the pentose phosphate pathway and glycolysis. In the calli of both cultivars, methylgloxal induced the accumulation of galactinol, a precursor of raffinose family oligosaccharides (RFOs). Blochl et al. (2004) demonstrated that improved somatic embryogenesis in *Medicago sativum* calli exposed to abscisic acid (ABA) was associated with accumulation of galactinol and its RFO derivatives. In addition, Hoque et al. (2012) showed that some ABA-responsive genes in *Arabidopsis* were dependant on treatment of cells with MG, suggesting co-ordination of endogenous ABA and MG in regulation these genes. Accumulation of sugars is a mechanism of desiccation tolerance in seeds and is essential for protecting macromolecules and membranes as water is gradually lost during seed maturation (Hoekstra et al. 2001; Zhang et al. 2016). However,

somatic embryos do not spontaneously acquire such desiccation tolerance, but require plant growth regulators and stress treatments to induce desiccation tolerance (Hoekstra et al. 2001; Maruyama and Hosoi 2012). It is possible that treatment with MG triggers the accumulation of sugars in embryogenic calli in order to achieve desiccation tolerance in sugarcane somatic embryos. The lower levels of malic acid in MG-treated calli may indicate the negative impact of MG on the tricarboxylic acid cycle, potentially explaining the lower amounts of amino acids, products of the TCA cycle, observed in the calli exposed to MG. This was also associated with lower levels of aminobutanoic acid, a signalling molecule involved in cell growth (Kinnersley and Turano 2000) and phosphoric acid, an important component of nucleic acids and phospholipids (Hayakawa 1991).

The enhanced somatic embryogenesis and subsequent plantlet regeneration by inclusion of MG in the culture medium as observed in NCo376 in this study, may aid production of regenerable embryogenic callus for use in various biotechnological applications in sugarcane genetic improvement programs. The observations indicated that MG has an impact on sugar metabolism to the benefit of somatic embryogenesis. This MG-induced improvement in embryogenic callus production and subsequent plantlet regeneration will assist utilisation of genotypes that exhibit poor somatic embryogenesis in sugarcane biotechnology.

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Author contributions TM, SJS, RSR and MPW designed the experiments. TM conducted the experimental work, data analyses and writing the manuscript. SJS, RSR and MPW contributed to data interpretation, writing and reviewing the article. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

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