Elicitation of antioxidant metabolites in *Musa* species in vitro shoot culture using sucrose, temperature and jasmonic acid

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Abstract

In vitro culture is not only known for the mass propagation of medicinal plants, it can also be used to boost the accumulation of useful metabolites. This work was aimed at studying the potential of elicitation on the accumulation of antioxidant secondary metabolites in different *Musa* species accessions. Shoot cultures of selected six *Musa* spp. accessions were micropropagated on Murashige and Skoog mineral-based culture medium, supplemented with indole-3-acetic acid and 6-benzylaminopurine. Different temperatures (15, 20 and 26 °C), doses of sucrose (30–50 g/L) and jasmonic acid concentrations (JA, 50–200 µM) were used as elicitors in the culture medium. High-performance thin-layer chromatography (HPTLC) coupled with effect-directed analysis (EDA) was used to compare and characterize all the 71 *Musa* spp. samples from the selected accessions and to reveal compound zones with antioxidant, antidiabetic and anticholinesterase activities. All elicitation and plant tissue culture experiments increased the total phenolic content (TPC) and antioxidant activity of the selected *Musa* spp. accessions. Extracts from plants grown at 20 °C gave the highest TPC and antioxidant activity. Addition of JA (200 µM) and higher sucrose contents (40 g/L or 45 g/L) increased the TPC and antioxidant activity as well. Not only compounds with antioxidant, but also antidiabetic and anticholinesterase activities were detected in the elicited accessions. The system presented in this study could serve as a sustainable, consistent and reliable alternative means of bioactive metabolites supply for the production of biopharmaceuticals.

Key message

Antioxidant and phenolic content in *Musa* spp. can be increased by growing *Musa* shoot cultures at 20 °C, adding sucrose at 40 or 45 g/L and jasmonic acid (200 µM).

Keywords Elicitation · *Musa* species · Bioactive metabolites · HPTLC · Antioxidant · Anticholinesterase · Antidiabetic

Abbreviations

DPPH	2,2-Diphenyl-1-picrylhydrazyl
EDA	Effect-directed analysis
FLD	Fluorescence detection
GAE	Gallic acid equivalent
hR_F	Homologous retention factor ($R_f \times 100$)

HPTLC	High-performance thin layer chromatography
JA	Jasmonic acid
TPC	Total phenolic content
UV/Vis	Ultraviolet/visible

Introduction

In the exploration for new plant-based bioactive secondary metabolites, constant supply of plant material remains a need in the endeavours towards continuous and sustainable preservation and rational usage of biodiversity (Karuppusamy 2009). The importance and expanding commercial use of plant secondary metabolites has led to a great increase in plant bioactive metabolites production, using in vitro plant tissue culture systems. Several secondary metabolites have been produced using in vitro plant tissue culture systems.

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For example, camptothecin, a monoterpene indole alkaloid was produced from cell suspension culture of *Camptotheca acuminata* Decne (Pi et al. 2010). Taraxerol and taraxasterol, anticancer compounds were produced from the root callus suspension culture of *Taraxacum officinale* Weber (Sharma and Zafar 2016). Also, in vitro plant tissue culture system has been reported as one of the notable means for commercial production of artemisinin, the antimalaria and anticancer drug (Kayani et al. 2018). Regeneration of plants via in vitro propagation has a remarkable potential for the production of synchronized, controlled and high-quality plant materials, as a means for large-scale production of medicinal compounds. With the increasing market demand for novel plants products, in vitro culture became a reliable tool for the steady and mass production of plant materials (Hussain et al. 2012).

Research in the pharmaceutical and food industries has focused on developing new techniques that allow secondary metabolites production from in vitro plant tissue culture (Anand 2010). Producing bioactive compounds from this plant tissue culture method is less expensive than extraction from the whole field-grown plant under natural conditions or making them through artificial synthesis. Moreover, this in vitro technique allows the manipulation of the biosynthetic routes to increase the production and accumulation of specific compounds (Dias et al. 2016). One of the means to increase the production of secondary metabolites using in vitro plant cell or tissue cultures is called elicitation.

Elicitation can be defined as an effective and a notable method for the enhancement and generation of numerous commercially useful medicinal compounds from plants (Perassolo et al. 2011). Elicitors are signals or molecules triggering the formation of secondary metabolites. The elicitation process occurs in response to stress stimuli that activate the protective mechanisms of the plants via secondary metabolite biosynthesis (Rea et al. 2011). The use of elicitors for producing numerous secondary metabolites has been reported in many plants species (Narayani and Srivastava 2017; Thakur et al. 2019). An abiotic elicitor, cobalt nitrate, was used to increase ginsenoside production by two folds compared to the control in suspension culture of *Panax quinquefolius* (Biswas et al. 2016). Yeast extract and silver nitrate (AgNO_3) were used as elicitors in suspension culture of *Ophiorrhiza mungos* Linn. and resulted in 13.3-fold and 8.7-fold increment in camptothecin production when yeast extract (50 mg/L) and AgNO_3 (2.5 μM) were used as elicitors, respectively (Deepthi and Satheeshkumar 2015). Other examples of elicitors used to enhance secondary metabolites production in plant tissue culture include; carbohydrates (sucrose, mannan, chitosan, chitin), light irradiation, nutrient stress, salicylic acid and jasmonic acid among others (Thakur et al. 2019). Temperature, sucrose and jasmonic acid (JA) were used as elicitors in this study. Jasmonic acid is an endogenous plant hormone also

known as a signaling molecule. It is involved in plant stress response (Afrin et al. 2015) that stimulates the biosynthesis and accumulation of secondary metabolites in plants (Pauwels et al. 2009). Apart from the fact that sucrose is one of the nutrients that provides energy and serves as carbon source, it can also affect the physiological activities and metabolism during plant in vitro growth and used as an elicitor. Increasing sucrose content in the in vitro culture medium has been reported to increase the accumulation of phenolic compounds in plants (Fazal et al. 2016).

Musa spp. has many medicinal applications with interesting metabolites. They have been reported for pharmacological activities such as wound healing (Rosida et al. 2014), antidiabetic (Vilhena et al. 2018), antiulcer (Abdullah et al. 2014), antimicrobial (Umamaheswari et al. 2017) and antioxidant (Sonibare et al. 2018), containing many flavonoids and phenolic compounds. Its amenability to in vitro plant tissue culture techniques makes it a good candidate for supply of phytochemicals. Previous study showed that *Musa* spp. leaves had higher phenolic content and antioxidant activity than the fruits (Ayoola et al. 2017). The in vitro-grown leaves were found to contain more phenolic compounds as revealed from high-performance thin layer chromatography analysis, and higher total phenolic content measured as gallic acid equivalent per weight of plant extract as well as higher antioxidant activity than the field-grown ones in all studied accessions (Ayoola-Oresanya et al. 2020). Recently, an anticholinergic alkaloid was detected only from in vitro-grown *Musa* spp. samples, using high-performance thin-layer chromatography (HPTLC) hyphenated with high-resolution mass-spectrometry (HRMS) (Ayoola-Oresanya et al. 2020). High-performance thin-layer chromatography is a reliable, efficient and accurate technique for the bioprofiling of complex samples, allowing the parallel qualitative and quantitative analysis of up to 20 samples. It allows the application of crude extracts and the coupling with bioassays using effect-directed analysis (EDA) as well as spectroscopic and spectrometry techniques (Morlock and Schwack 2010).

This research presents the first elicitation study of antioxidant metabolites obtained from in vitro shoot cultures of six different *Musa* species accessions. Various plant tissue culture parameters, i.e. the effect of temperature, nutrient stress and JA addition were explored to increase the accumulation of secondary metabolites, especially on the accessions previously identified to have relatively low phenolic content and antioxidant activity.

Materials and methods

Plant materials and micropropagation conditions for six *Musa* species accessions

Six *Musa* species accessions were selected from the Taxonomical Reference Collection, which are representatives of the global *Musa* biodiversity (Drapal et al. 2019), at the Genetic Resource Centre, International Institute of Tropical Agriculture, Ibadan, Nigeria (Table 1). Two accessions with high antioxidant activity (Simili radjah and Foconah), two with moderate activity (P. Mas and Red Dacca) and two with low antioxidant activity (Dole and *M. balbisiana* HND), were selected based on the results from a previous study on the total phenolic content (TPC) and antioxidant activity of fifteen taxonomic reference accessions (Ayoola-Oresanya et al. 2020). In vitro-grown plantlets, established from meristem culture, served as explants for the experiments. They were first multiplied on the liquid proliferation media without the solidifying agent gelrite, using the temporary immersion system and RITA® apparatus, as reported by Jekayinoluwa et al. (2019). The temporary immersion system was used for mass multiplication of the plant material, resulting in about 25 shoots per system. The shoots were then transferred to a semi-solid culture medium containing 2.5 g/L of gelrite. The Murashige and Skoog (MS) mineral-based culture medium was used for banana shoot proliferation (Murashige and Skoog 1962; Gueye et al. 2012; Ayoola et al. 2017), supplemented with 30 g/L sucrose, 0.1 g/L myo-inositol, 10 mg/L ascorbic acid, 0.18 mg/L indole-3-acetic acid and 4.5 mg/L 6-benzylaminopurine. Gelrite (2.5 g/L) was used as gelling agent. The pH of the culture media was adjusted to 5.7 prior autoclaving at 121 °C, 100 kPa for 15 min. The cultures were maintained in the culture room at 25 ± 1 °C, with a 12 h light/12 h dark photoperiod given by cool white fluorescent lamps at 38 µmol/m²/s.

Elicitation experiments

Sucrose content, temperature and JA were used as elicitors in the in vitro shoot cultures of the selected six *Musa* spp. accessions. The effect of the three elicitors on the production

of antioxidant compounds was determined at different doses in the culture medium. All the elicitation experiments were done in replicates. The shoots were harvested at the end of the experiment and kept at – 80 °C for phenolic compound quantification and evaluation of the antioxidant activity.

Increase in sucrose level

The Murashige and Skoog mineral-based culture medium for shoot induction was supplemented with 35, 40, 45 and 50 g/L of sucrose. Culture media with 30 g/L of sucrose was used as control. In vitro-grown explants were sub-cultured on different in vitro culture media and kept in the growth chamber (T 25 ± 1 °C, 12 h light/12 h dark light: 38 µmol/m²/s). The experiment was done in replicates and the growth parameters measured weekly for a period of 6 weeks.

Temperature reduction

The *Musa* spp. plantlets were sub-cultured on the routine proliferation media as described above, but kept in different temperature conditions, i.e. 26 ± 2 °C (control), 20 ± 2 °C and 15 ± 2 °C; using plant growth cabinet (Percival Scientific Perry, USA). These temperature conditions were monitored with a temperature logger. Growth parameters were measured over a period of 8 weeks due to the slow growth of the plants at reduced temperature.

Addition of JA

Different concentrations of this abiotic elicitor JA (50, 100 and 200 µM) were added separately to the *Musa* spp. proliferation culture media. The effect on the secondary metabolite production was investigated. Plantlets of *Musa* spp. exposed to Murashige and Skoog mineral-based culture medium without any elicitor served as control.

Statistical analysis

The growth parameters (number of shoots, number of leaves, shoot length and number of roots) for the different experiments were collected on all the cultured plantlets and analyzed using SAS statistical package. Results are

Table 1 List of the selected six accessions from the Taxonomical Reference *Musa* spp. collection

ID	TRC accession	ITC code	Species	Genome group	Origin
1	Simili Radjah	ITC.0123	<i>Musa acuminata</i>	ABB	Cote d'Ivoire
2	Foconah	ITC.0649	<i>Musa acuminata</i>	AAB	Unknown
3	Pisang Mas	ITC.0653	<i>Musa acuminata</i>	AA	Unknown
4	Red Dacca	ITC.0575	<i>Musa acuminata</i>	AAA	Unknown
5	Dole	ITC.0767	<i>Musa acuminata</i>	ABB	Unknown
6	<i>Musa balbisiana</i> HND	ITC.0080	<i>Musa balbisiana</i>	BBwild	Honduras

expressed as the mean $\pm$ standard error of mean (SEM) of 20 samples. One-way ANOVA at $P < 0.05$ was used to test the significance difference of data followed by Tukey's multiple comparison of means.

HPTLC-UV/Vis/FLD-EDA method

Extraction

The 71 *Musa* spp. samples of the elicitation experiments, collected from the studied accessions, were freeze-dried, grinded and kept in an airtight container. Powdered samples were weighed into 1.5-mL Eppendorf tubes and extracted (1 mg/mL). Four different extraction solvent polarities were investigated (methanol, ethanol–water 7:3, ethyl acetate and *n*-hexane). Ethanol–water 7:3 was selected, as it extracted most compounds (Fig. S-6). The samples were vortexed, sonicated for 10 min, centrifuged at $900\times g$ for 30 min and filtered into 1.8 mL sampler vials.

Chromatographic system

Respective extract solutions were sprayed depending on the number of tracks needed on the HPTLC plates at an 8-mm distance from the bottom edge (ATS 4, CAMAG, Muttenz, Switzerland). For the temperature experiment, 18 extract solutions (1.5 μ L/band, 600 ng/band) were applied as 8-mm bands with a track distance of 10 mm. The JA experiment took 23 extract solutions (1.0 μ L/band, 400 ng/band) applied as 6.5-mm bands with a track distance of 8.2 mm. For the sucrose experiments, 30 extract solutions (1.0 μ L/band, 1000 ng/band) were applied as 8-mm bands on two HPTLC plates with a track distance of 10 mm (15 samples/plate). The HPTLC plate was developed with a polar acidic mobile phase, consisting of ethyl acetate–toluene–formic acid–water, 6.8:1:1.4:1 (V/V/V/V). This took 30 min up to a migration distance of 60 mm in an unsaturated 20 cm $\times$ 10 cm Twin Trough Chamber. The chromatogram was documented at white light illumination (Vis), UV 254 nm (UV) and Fluorescence detection (FLD) 366 nm (FLD; TLC Visualizer, CAMAG). The densitometric absorbance measurement was performed at 546 nm using the mercury lamp and an inverse scan mode (fluorescence mode without optical filter, TLC Scanner 4, CAMAG). For the TPC study, the results were equivalently calculated as gallic acid equivalents (GAE) by linear regression referred to four or five different gallic acid levels (3–30 μ L/band, 10–100 ng/band), as reported. Instruments were operated and data evaluated using the winCATS software version 1.4.7.2018 (CAMAG).

Effect-directed assays

Before the respective assay performance, few microliters of a respective positive control solution (e.g., 0.1 mg/mL gallic acid, 0.1 mg/mL physostigmine, 1 mg/mL acarbose and 0.1 mg/mL nitroquinoline-1-oxide) were applied on each plate. For the radical scavenging (antioxidant) assay, the chromatograms were dipped in a 0.2% methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (immersion speed 3 cm/s, immersion time 2 s, TLC Immersion Device, CAMAG) and documented under white light illumination after 1, 10 and 30 min. The HPTLC- α -glucosidase inhibition assay was performed by piezoelectric spraying (Derivatizer, CAMAG) according to Jamshidi-Aijdi et al. (2019). Concisely, first 2 mL substrate solution (60 mg 2-naphtyl- α -D-glucopyranoside in 50 mL ethanol) were sprayed on the neutralized chromatogram, then after air-drying (2 min), 1 mL sodium acetate buffer (pH 7.2–7.5) for pre-wetting and 2 mL α -glucosidase solution (from *Saccharomyces cerevisiae*, 500 U in 50 mL buffer). After incubation of the plate placed horizontally in a pre-prepared humidity box at 37 °C for 13 min, 0.5 mL Fast Blue Salt B solution was sprayed on the chromatogram and documented under white light illumination after drying (ADC2 humidity control, CAMAG). Analogously, the HPTLC-acetylcholinesterase assay (Azad-niya and Morlock 2018) and HPTLC-SOS-Umu-C bioassay (Meyer et al. 2021) were performed.

Results and discussion

For the six accessions, the different elicitation experiments resulted in 18, 30 and 23 samples were harvested from the temperature decrease, the sucrose increase and JA addition experiments, respectively. The *M. balbisiana* HND accession treated with 50 μ M JA did not grow, and thus one sample is missing. All elicitation experiments resulted in 71 different plant samples from different treatments and accessions. Effects of the different treatments on the vegetative growth of the *Musa* spp. accessions were determined (Fig. 1). After freeze-drying, grinding and extraction, an HPTLC method revealed the antioxidant profile and the accumulation of antioxidant compounds (Figs. 2, 3).

Effect of sucrose increase on growth parameters and antioxidant activity

The increase of sucrose in the culture medium from 30 to 45 g/L led to more leaf initiation in some in vitro materials of *Musa* spp., such as Simili radjah and *M. balbisiana* HND (Fig. 1b). It also increased the number of shoots in most accessions. Particularly, the addition of 40 and 45 g/L of sucrose had a more notable favourable effect on the average

Fig. 1 Growth parameters at the end of 6 weeks of incubation of the six different *Musa* spp. accessions grown under increased sucrose content, reduced temperature and JA additions: **a** average shoot length, **b** average number of leaves and **c** average number of

shoots. Data are presented as mean $\pm$ Standard error (SE) of 20 replicates. Means with the same alphabets are not statistically different at $P < 0.05$

number of leaves (Fig. 1a) and shoots (Fig. 1c) produced by the different accessions, after 6 weeks of sub-culture. At the highest sucrose content of 50 g/L, the average number of leaves was reduced in some accessions. This indicated that the optimum sucrose level that would not affect the plant growth is between 40 and 45 g/L. The favourable effect of intermediary concentration of sucrose on vegetative growth of *in vitro* plantlets was also reported by Fazal et al. (2014) who observed that sucrose level of 20 and 25 g/L among other concentrations (0–50 g/L) increased the biomass accumulation in suspension cultures of *Prunella vulgaris* L. In contrast, Wu et al. (2006) reported that in the adventitious root culture of *Echinacea angustifolia*, the addition of relatively high concentrations (50 and 90 g/L) of sucrose had a stronger positive effect on the biomass accumulation. This implies that the optimum sucrose levels in liquid cultures, for biomass accumulation and vegetative growth, vary according to plant species and type of explants (Cui et al. 2010; Suan See et al. 2011).

Antioxidant compounds of the accession extracts were detected as bright bands on a purple background in the HPTLC-DPPH[•] autogram measured at 546 nm (Fig. 2).

Antioxidant signal, along with TPC, amplified with increasing sucrose doses across all accessions, with 40 and 50 g/L giving the highest activity (Table 2), although a similar compound zone pattern was observed. For the accession Foconah, TPC increased most, by 4.9-fold from 39.4 ng/g GAE (30 g/L) to 138.6 ng/g GAE (50 g/L), whereas the increase was 1.2- to 2.8-fold for other accession extracts. Ali et al. (2016) reported that total phenolic content increased sequentially with increase in sucrose concentration from 1 to 5% in the cell suspension cultures of *Artemisia absinthium* L. Other studies have also reported an increase in the TPC when higher doses of sucrose were added to culture media (Wu et al. 2006; Cui et al. 2010), as sucrose may induce osmotic stress, which can in turn increase the accumulation of phenolics in plant cells (Suan See et al. 2011).

Effect of temperature reduction on formation of antioxidative metabolites

The three different temperature conditions studied (15, 20 and 26 °C) had an impact on the growth rate and accumulation of antioxidative compounds. The lower the temperature

Fig. 2 HPTLC-DPPH⁺ autograms (antioxidants as bright bands on purple background) and respective densitograms at 546 nm for evaluation of the antioxidant activity of the six different *Musa* spp. accessions elicited by increased sucrose contents (1000 ng/band sample,

gallic acid (GA) calibration levels 10–100 ng/band, detailed information of the track assignment is found in Table 2), on HPTLC plates silica gel 60 F₂₅₄ with ethyl acetate–toluene–formic acid–water, 6.8:1:1.4:1

Fig. 3 HPTLC-DPPH⁺ autograms (antioxidants as bright bands on purple background) and respective densitograms at 546 nm for evaluation of the antioxidant activity of the six different *Musa* spp. accessions elicited by **a** reduced temperatures (600 ng/band sample, GA calibration levels 3–30 ng/band, detailed information of the track

assignment is found in Table 3) and **b** JA additions (400 ng/band sample, GA calibration levels 5–30 ng/band, detailed information of the track assignment is found in Table 4) on HPTLC plates silica gel 60 F₂₅₄ with ethyl acetate–toluene–formic acid–water, 6.8:1:1.4:1

Table 2 Sucrose increase experiment: peak area and TPC obtained by the densitometric measurement and calculated equivalently to gallic acid (GAE) showing the increase in antioxidants compared to the control (set as 1.0; respective highest increase in bold)

ID track	TRC accession	Sucrose (g/L)	Peak area	TPC (ng/g GAE)	Increase factor
1	Simili radjah	30	3167.85 ± 0.9	36.83 ± 0.0	1.0
2	Simili radjah	35	8271.29 ± 0.6	70.20 ± 0.0	1.9
3	Simili radjah	40	9942.00 ± 0.3	81.12 ± 0.1	2.2
4	Simili radjah	45	1989.98 ± 0.3	29.13 ± 0.3	0.8
5	Simili radjah	50	6341.98 ± 0.6	57.58 ± 0.3	1.6
6	Foconah	30	3565.71 ± 0.3	39.43 ± 0.6	1.0
7	Foconah	35	2562.33 ± 0.3	32.87 ± 0.6	0.8
8	Foconah	40	1874.88 ± 0.3	28.38 ± 0.6	0.7
9	Foconah	45	5397.44 ± 0.3	51.41 ± 0.6	1.3
10	Foconah	50	18,737.61 ± 0.3	138.64 ± 0.6	3.5
11	P. Mas	30	9599.75 ± 0.6	78.89 ± 0.6	1.0
12	P. Mas	35	12,360.41 ± 0.3	96.94 ± 0.6	1.2
13	P. Mas	40	14,258.19 ± 0.3	109.35 ± 0.6	1.4
14	P. Mas	45	16,399.14 ± 0.3	123.35 ± 0.3	1.6
15	P. Mas	50	17,939.55 ± 0.3	133.42 ± 0.3	1.7
16	Red Dacca	30	11,523.83 ± 0.3	68.72 ± 0.3	1.0
17	Red Dacca	35	18,072.33 ± 0.3	103.42 ± 0.3	1.5
18	Red Dacca	40	25,930.94 ± 0.3	145.05 ± 0.3	2.1
19	Red Dacca	45	23,575.89 ± 0.3	132.57 ± 0.3	1.9
20	Red Dacca	50	25,717.73 ± 0.3	143.93 ± 0.3	2.1
21	Dole	30	13,243.66 ± 0.7	77.83 ± 0.3	1.0
22	Dole	35	22,095.84 ± 0.6	124.73 ± 0.3	1.6
23	Dole	40	21,438.37 ± 0.3	121.25 ± 0.3	1.6
24	Dole	45	23,337.77 ± 0.3	131.31 ± 0.3	1.7
25	Dole	50	17,093.48 ± 0.3	98.23 ± 0.6	1.3
26	<i>M. balbisiana</i> HND	30	12,786.31 ± 0.3	75.41 ± 0.6	1.0
27	<i>M. balbisiana</i> HND	35	22,985.30 ± 0.3	129.45 ± 0.6	1.7
28	<i>M. balbisiana</i> HND	40	24,350.91 ± 0.0	136.68 ± 0.6	1.8
29	<i>M. balbisiana</i> HND	45	23,131.05 ± 0.0	130.22 ± 0.3	1.7
30	<i>M. balbisiana</i> HND	50	23,523.12 ± 0.3	132.29 ± 0.3	1.8

*Values are expressed as mean ± SEM (*n* = 3)

was, the lower was the growth rate. Plants grown at 26 °C had the highest shoot length, number of leaves and shoots, followed by plants grown at 20 °C. Plants grown at 15 °C had relatively slower growth rate across all accessions (Fig. 1), except for the average shoot length and shoot number for the accessions P. Mas and Red Dacca, respectively. However, the temperature increase had a reverse effect on the phytochemical accumulation. Reduction in temperature led to an increase in the total phenolic content and antioxidant activity. Extracts of the *Musa* spp. accessions grown at 26 °C gave the lowest TPC (Table 3), while more antioxidant bands were observed on extracts of *Musa* spp. material grown at 20 °C, followed by those grown at 15 °C (Fig. 3a). The TPC of Simili radjah increased 2.9 times, from 6.3 (26 °C) to 18.5 ng/g GAE (20 °C), whereas it increased by 4.8-fold from 8.6 (26 °C) to 41.6 ng/g GAE (20 °C) for Dole (Table 3). These results showed that 20 °C was the best

concession for both, vegetative growth as well as secondary metabolite production of the studied *Musa* spp. in vitro plantlets, it allowed the highest antioxidant activity at a moderate growth rate. The highest and lowest temperature are not ideal for natural product production in *Musa* spp., an intermediate temperature was more optimal for production while plantlets grew better in the highest temperature. This correlation between temperature growth conditions and production of natural production is contrasting with the report made by Ochoa-Villarreal et al. (2016) who stated that the growth of plant cells is inversely related to the production of natural products. Environmental conditions such as light, temperature, humidity are known to affect the phytochemical and secondary metabolite profiles and thus antioxidant activity (Yang et al. 2018). Plants generally adapt to changes in abiotic stresses such as temperature by adjusting their metabolism. Secondary metabolites are also usually produced as a

Table 3 Temperature of culture conditions reduction experiment: peak area and TPC obtained by the densitometric measurement and calculated equivalently to gallic acid (GAE) showing the increase in antioxidants with regard to control (set as 1.0; respective highest increase in bold)

ID track	TRC accession	Temperature (°C)	Peak area	TPC (ng/g GAE)	Increase factor
1	Simili radjah	26	458.91 ± 0.6	6.30 ± 0.6	1.0
2	Simili radjah	20	2271.95 ± 0.6	18.40 ± 0.6	2.9
3	Simili radjah	15	590.12 ± 0.6	7.19 ± 0.6	1.1
4	Foconah	26	342.58 ± 0.0	5.52 ± 0.6	1.0
5	Foconah	20	1851.96 ± 0.3	15.66 ± 0.6	2.8
6	Foconah	15	1480.50 ± 0.6	13.17 ± 0.6	2.4
7	P. Mas	26	561.61 ± 0.3	6.99 ± 0.3	1.0
8	P. Mas	20	1948.34 ± 0.6	16.31 ± 0.3	2.3
9	P. Mas	15	1783.95 ± 0.6	15.21 ± 0.3	2.2
10	Red Dacca	26	2357.17 ± 0.6	19.06 ± 0.3	1.0
11	Red Dacca	20	4835.15 ± 0.3	35.70 ± 0.3	1.9
12	Red Dacca	15	2116.89 ± 0.3	17.44 ± 0.3	0.9
13	Dole	26	798.52 ± 0.3	8.59 ± 0.3	1.0
14	Dole	20	5708.75 ± 0.3	41.57 ± 0.3	4.8
15	Dole	15	1217.34 ± 0.6	11.40 ± 0.3	1.3
16	<i>M. balbisiana</i> HND	26	2277.03 ± 0.3	18.52 ± 0.3	1.0
17	<i>M. balbisiana</i> HND	20	7256.71 ± 0.3	51.97 ± 0.3	2.8
18	<i>M. balbisiana</i> HND	15	3194.43 ± 0.3	24.68 ± 0.3	1.3

*Values are expressed as mean ± SEM ($n=3$)

defense mechanism against biotic and abiotic stresses (Khare et al. 2020). Several researchers have reported that for plants grown in vitro or on the field, lowering the temperature is able to enhance the accumulation of plant phenolics (Leyva et al. 1995; Janas et al. 2002; Akula and Ravishankar 2011). For example, *Melastoma malabathricum* cell cultures incubated at a lower temperature range (20 ± 2 °C) grew better and had higher anthocyanin production than those grown at 26 ± 2 °C and 29 ± 2 °C (Chan et al. 2010).

Effect of elicitation by JA on antioxidant secondary metabolites

JA has been used in many plant species for the accumulation of important secondary metabolites such as ginsenoside, vinblastine, nicotine and taxol, among others (Afrin et al. 2015). Hence, the effect of different doses of JA (50–200 µM) on plant growth and accumulation of antioxidant compounds in the six *Musa* spp. accessions was studied. Generally, the addition of different doses of JA affected the plant growth of the accessions differently (Fig. 1). For some accessions (Simili radjah, Foconah and Red Dacca), it was observed that the highest JA dose (200 µM) led to longest or equivalent shoot lengths compared to the control (without JA) but the lowest dose (50 µM) led to longest shoot length for accessions P. Mas and Dole. But, antioxidant activity and TPC of the *Musa* spp. accessions increased as JA is added or increased in the culture medium. The highest JA dose (200 µM) gave the highest TPC across all accessions (Table 4). Although, there was no

qualitative difference in the produced antioxidant compound profile between the control plants and the elicited accessions, a quantitative increase in the TPC and the antioxidant activity was observed for all accessions (Fig. 3b). The TPC of Dole and *Musa balbisiana* HND was 2.2 and 1.7 times higher than the controls (Table 4). This increase is supported by the study of Jalalpour et al. (2014), which showed that methyl jasmonate (100 µM) was able to enhance the accumulation of phenolics and flavonoids in *Taxus baccata* cell culture. In the same line, the study of Krzyzanowska et al. (2012) detected the highest concentration of rosmarinic acid (1.5 times higher than control) in cell suspension cultures of *Mentha piperita* after 48 h of addition of JA at 200 µM, among the various tested concentrations (50, 100 and 200 µM). However, Ali et al. (2015) observed that higher doses (10 µM) of methyl jasmonate and JA inhibited the accumulation of phenolic compounds in suspension cultures of *Artemisia absinthium* L. This suggests that the effective dose of an elicitor varies with plant species and can only be determined empirically (Vasconsuelo and Boland 2007). As signaling molecule, JA is known to trigger catalytic reactions by specific enzymes involved in the biosynthesis of phenolic and other antioxidant compounds when a plant is under biotic or abiotic stress (Saeed et al. 2017; Manivannan et al. 2016; Mendoza et al. 2018).

Table 4 Addition of JA in the *Musa* spp. culture medium experiment: peak area and TPC obtained by the densitometric measurement and calculated equivalently to gallic acid (GAE) showing the increase in antioxidants with regard to control (set as 1.0; respective highest increase in bold)

ID track	TRC accession	JA added (μ M)	Peak area	TPC (ng/g GAE)	Increase factor
1	Simili radjah	0	508.32 $\pm$ 0.3	7.00 $\pm$ 0.6	1.0
2	Simili radjah	50	1138.88 $\pm$ 0.3	9.38 $\pm$ 0.6	1.3
3	Simili radjah	100	846.17 $\pm$ 0.3	8.28 $\pm$ 0.6	1.2
4	Simili radjah	200	1359.95 $\pm$ 0.3	10.21 $\pm$ 0.6	1.5
5	Foconah	0	1244.78 $\pm$ 0.3	9.78 $\pm$ 0.6	1.0
6	Foconah	50	1410.15 $\pm$ 0.3	10.40 $\pm$ 0.6	1.1
7	Foconah	100	2360.80 $\pm$ 0.6	13.98 $\pm$ 0.6	1.4
8	Foconah	200	2301.61 $\pm$ 0.0	13.76 $\pm$ 0.6	1.4
9	P. Mas	0	1079.36 $\pm$ 0.3	9.16 $\pm$ 0.6	1.0
10	P. Mas	50	1601.44 $\pm$ 0.0	11.12 $\pm$ 0.3	1.2
11	P. Mas	100	2878.81 $\pm$ 0.3	15.94 $\pm$ 0.3	1.7
12	P. Mas	200	3822.35 $\pm$ 0.3	19.49 $\pm$ 0.3	2.1
13	Red Dacca	0	2196.06 $\pm$ 0.3	13.36 $\pm$ 0.3	1.0
14	Red Dacca	50	4357.05 $\pm$ 0.3	21.50 $\pm$ 0.0	1.6
15	Red Dacca	100	4803.77 $\pm$ 0.3	23.19 $\pm$ 0.0	1.7
16	Red Dacca	200	7250.76 $\pm$ 0.3	32.41 $\pm$ 0.6	2.4
17	Dole	0	994.66 $\pm$ 0.3	8.84 $\pm$ 0.0	1.0
18	Dole	50	1722.59 $\pm$ 0.3	11.58 $\pm$ 0.6	1.3
19	Dole	100	2884.76 $\pm$ 0.3	15.96 $\pm$ 0.0	1.8
20	Dole	200	3911.28 $\pm$ 0.3	19.82 $\pm$ 0.0	2.2
21	<i>M. balbisiana</i> HND	0	1803.31 $\pm$ 0.3	11.88 $\pm$ 0.6	1.0
22	<i>M. balbisiana</i> HND	100	3730.04 $\pm$ 0.3	19.14 $\pm$ 0.6	1.6
23	<i>M. balbisiana</i> HND	200	3962.59 $\pm$ 0.3	20.02 $\pm$ 0.3	1.7

*Values are expressed as mean $\pm$ SEM ($n=3$)

Acetylcholinesterase and α -glucosidase inhibitor zones and absence of genotoxic effects

Acetylcholinesterase and α -glucosidase inhibitors are used in the Alzheimer's disease and diabetes mellitus management (Lane et al. 2006; Hanefeld and Schaper 2007), and information on their presence in the studied accessions was of interest. In the HPTLC autograms, enzyme inhibitors were revealed as bright zones on a purple background. In all five selected accessions (that showed high total phenolic content in the different elicitation experiments), two acetylcholinesterase and two α -glucosidase inhibiting compound zones were found, when 10 μ g/band extract was applied. Acetylcholinesterase was inhibited most intense at hR_F 42 but also at hR_F 28 (Fig. 4a) while α -glucosidase most intense inhibition was at hR_F 34 but also at hR_F 42 (Fig. B). A recently developed HPTLC-SOS-Umu-C assay (Meyer et al. 2021) was applied to study the presence of potential genotoxic compounds. The same chromatographic HPTLC system was used on RP 18 W plates, which ensures band sharpness even after 3 h of incubation in the aqueous bioassay (Klingelhöfer and Morlock 2014). The separated bands were similarly distributed along the

migration distance and thus the transfer to the apolar plate was successful (Fig. 4c). The HPTLC-genotoxicity assay showed that there is no genotoxic compound in the elicited *Musa* spp. accessions for the applied extract amount of 10 μ g/band.

Conclusions

The amenability of *Musa* spp. to plant tissue culturing and elicitation of antioxidants was demonstrated by the results of this study. The antioxidant and phenolic content in *Musa* spp. can be increased by growing *Musa* shoot cultures at 20 °C and adding sucrose at 40 or 45 g/L and JA at 200 μ M. This parameter design was a good compromise between optimum growth and increased secondary metabolite production. The HPTLC-effect directed analysis was exploited to reveal and quantify compounds with antioxidant, anticholinesterase and antidiabetic activities. Such elicitation methods can be further utilized to target the production of important pharmaceuticals. Thus,

Fig. 4 HPTLC-autograms of the **a** acetylcholinesterase and **b** α -glucosidase inhibition of the *Musa* spp. accessions P. Mas grown at 50 g/L sucrose (1) and Red Dacca grown at 20 °C (2; 10 μ g/band each; developed as in Fig. 3; documented at FLD 366 nm, and after the assay under white light illumination) as well as **c** HPTLC-SOS-

Umu-C assay on RP 18 W plate at FLD 366 nm before and after the assay; respective positive control C, applied as track pattern before the assay: physostigmine (0.1–1.5 ng/band for **a**), acarbose (1–10 ng/band for **b**) and 4-nitroquinoline-1-oxide (0.1–0.3 ng/band for **c**)

in vitro *Musa* spp. material constitute an alternative system for sustainable, consistent and mass production of plant materials for extraction of antioxidant biochemicals. This system also ensures the rational use of natural resources and reduces the dependence of the pharmaceutical industry on field collection of plant materials thereby conserving natural resources.

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Author contributions IOO performed most experiments, analyzed the results and drafted the manuscript; MAS was involved in the experimental design; BG designed and supervised the in vitro plant tissue culture elicitation experiments; MTA revised the manuscript; GEM supervised the HPTLC research performed at JLU Giessen and revised the manuscript.

Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

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