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Gibberellic acid influences growth indices and biochemical parameters in micropropagated *Ocimum gratissimum* L. explants

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Background: *Ocimum gratissimum* L. is a well-known tropical and subtropical plant widely utilised for both medicinal and nutritional purposes. However, its continuous existence is currently threatened because of excessive human exploitation and non-regulated collection.

Aim: This study aimed at mass-producing *O. gratissimum* through its explants cultured on Murashige and Skoog (MS) medium.

Settings: Sterile explants of *O. gratissimum* were used to generate whole plant through the process of somatic embryogenesis and under the influence of different plant growth regulators (PGRs).

Methods: The growth medium was supplemented with various concentrations (0.1 mg/L, 0.5 mg/L and 1.0 mg/L) of PGRs, namely, gibberellic acid (GA_3) and benzyl aminopurine (BAP) in combination with 0.2 mg/L indole acetic acid (IAA), which also served as the control.

Results: The results showed that germination response, shoot and root lengths were significantly enhanced in *O. gratissimum* explants raised in media containing the two PGRs in a concentration-dependent manner after four weeks of culture. *Ocimum gratissimum* explants treated with GA_3 and BAP also experienced reduced lipid peroxidation and ascorbic acid concentration, particularly at the highest tested concentration (1.0 mg/L) as evidenced by the significant drop in malondialdehyde (MDA) concentration. In response to this, the activities of superoxide dismutase (SOD) and catalase (CAT) were significantly increased in *O. gratissimum* explants raised in MS medium supplemented with PGRs.

Conclusion: These results generally suggest that GA_3 in combination with IAA is more favourable than BAP for the micropropagation of *O. gratissimum* explants. Thus, our study revealed that PGRs possess special attributes, which could be exploited in tissue culture for the micropropagation of *O. gratissimum* explants.

Keywords: plant tissue culture; plant growth regulators; explant; micropropagation; antioxidants; somatic embryogenesis.

Introduction

It is a well-known fact that synthetic medications and antibiotics are crucial and fundamental in modern medicines, but it is increasingly being recognised that plants also play a significant role in the pharmaceutical sector (Fowler 2006; Rates 2001). This is because medicinal plants are a valuable source of medicines, and they are very vital in the healthcare system as majority of people all over the world take solace in herbal medicines for their primary healthcare needs (Pei 2001). Apart from the health benefits, medicinal plants also form an important component of the global economy by providing income for individuals and governments, especially in developing countries. As a result of this economic interest coupled with the growing population and lifestyle, the demand for herbal medicines has risen in recent years. In the year 2000, about $60 billion was reported to have been invested in medicinal plants and their products internationally, with an average annual growth rate of 7% and was projected to reach $5 trillion by 2050 (Bhowmik et al. 2009; Nirmal et al. 2013). The nutraceuticals market was also projected to reach over $30bn annually (Nirmal et al. 2013).

Herbal preparations are typically made from field-grown plants, which are usually affected by bacterial, fungal and insect infestations. This can change the therapeutic quality of the resulting

products thereby altering their medicinal potency (Saha et al. 2012). The long-term effectiveness of any healthcare system is dependent on the availability and accessibility of appropriate pharmaceuticals. However, current statistics showed that the supply of plants for medicinal purposes is insufficient to meet the current demand (Saha et al. 2012). This is because several plant species of medicinal interest have become endangered because of excessive human exploitation, non-regulated collection and problems relating to seed viability and seed germination (Saha et al. 2012). One of such threatened plants is *O. gratissimum*.

Ocimum gratissimum L. is a multipurpose medicinal perennial herb that is also well utilised for its nutritional values. The plant belongs to the family *Lamiaceae* within the order *Lamiales* and the genus *Ocimum* comprising more than 150 species of which *O. gratissimum* is the most abundant. It grows widely and is distributed throughout the tropical and subtropical regions of the world with great abundance in Africa, Asia and South America. The common names of the plant in English are scent leaves, Basil fever plant, tea bush or shrubby basil. In Nigeria, the plant is locally known as *Daidoya tagida* (Hausa), *Nchanwu* (Igbo), *Tanmotswangiwawagi* (Nupe) and *Efinrin* (Yoruba) (Abdullahi et al. 2013; Idris et al. 2011). It is woody at the base with a mean height of 1 m – 3 m. The leaves are broad and narrowly ovate, usually 5 cm – 13 cm long and 3 cm – 9 cm wide. It is a fragrant shrub with lime-green foliage (USDA 2008). The plant has been used to treat a variety of illnesses including rheumatism (Madhu & Harindran 2014), breast tumour (Nangia-Makker et al. 2007), diarrhoea (Orafidiya et al. 2000), sunstroke (Akinmoladun et al. 2007), pneumonia (Akinmoladun et al. 2007), gonorrhoea (Odugbemi & Akinsulire 2006), mental illness and gastrointestinal helminths (Prabhu et al. 2009). In addition, antimicrobial and antimalarial activities have been observed in the plant (Lexa et al. 2008; Matasyoh et al. 2007). It has also been used as mouthwashes, effective gargle (Akinmoladun et al. 2007) for sore throats and tonsillitis and topical ointments (Ugbogu et al. 2021).

Plant growth regulators (PGRs) play a significant role in the development and survival of plants. They are produced naturally by plants to regulate growth and responses to stimuli. Plant growth regulators exist in a variety of forms including auxin, cytokinin, gibberellin, abscisic acid and ethylene with each one having pleiotropic effects (Sidhu 2010). However, auxin and cytokinin are the most widely used PGRs. The former helps plant cells to develop and expand by stimulating cell division and elongation and root differentiation whilst the latter stimulates shoot development. These regulators are also required for a variety of growth phases in tissue culture, such as tropism, elongation and apical dominance (Sidhu 2010).

There is a general fear of the possibility of medicinal plants going into extinction based on their indiscriminate harvesting coupled with the fact that traditional methods of propagation, such as rooting, cuttings and grafting, are insufficient to supply the ever-increasing demands (Gopal et al. 2014). Hence, it is pertinent to devise appropriate ways such as tissue culturing for quick mass production to meet commercial demands, whilst simultaneously preventing genetic erosion of potent plant species. Tissue culture technique is based on the idea that a plant's organ, tissue or cell can be manipulated to develop back into a whole plant and has proven to be a useful tool for mass propagation of plants. Tissue culturing of medicinal plants is widely employed in the herbal and pharmaceutical industries to produce active chemicals. Culturing techniques are also used to conserve the genetic materials of many endangered medicinal plants (Gopal et al. 2014).

To the best of our knowledge as at the time of compiling this report, there was limited information on micropropagation of *O. gratissimum* L., thus requiring that more work needs to be carried out in this important area of research. The main focus of the present study is to produce sterile plants for use in pharmaceutical preparations and establish an efficient genetic transformation protocol *in vitro* for addressing concerns of pest and diseases of *O. gratissimum* explants using the right balance of PGRs. The plant was chosen because of its proven unique health and nutritional benefits. It is hoped that the study would provide a better understanding of the effects of selected PGRs on the micropropagation of *O. gratissimum* from explants.

Materials and methods

Collection and authentication of plant materials

Dried seeds of *O. gratissimum* were collected from the International Technical and Vocational Institute, Department of Science and Technology, Utako, Abuja, FCT, Nigeria in February 2021. A voucher specimen (NIPRD/H/7241) was prepared and deposited at National Institute for Pharmaceutical Research and Development Herbarium after identification by Lateef A. Akeem.

Sterilisation of seeds

The method described by Ehiagbonare (2007) was used to derive explants from dried seeds. Briefly, seeds of *O. gratissimum* were extracted from the fruits and tested for viability by steeping them into a beaker filled with distilled water. The floated seeds were discarded as non-viable whilst the sunken seeds were regarded as viable seeds. The viable seeds were surface sterilised with mild soap followed by a solution of 70% alcohol for 10 min and rinsed three times with sterile distilled water. The seeds were further soaked in a 1.5% solution of commercial bleach for 10 min and finally rinsed three times with sterile distilled water.

Germination experiment

Ten *O. gratissimum* seeds were evenly placed in 12 cm Petri dishes (n = 5) lined with sterile cotton wool moistened with 7.5 mL of distilled water. The Petri dishes were firmly sealed and incubated for 14 days at 27 ± 2 °C in a growth chamber (16 h light and 8 h dark). The resulting explants were subsequently used for the tissue culture experiment.

Tissue culture propagation experiment

The explants (n = 10) were subsequently cultured in Murashige and Skoog (MS) medium containing (per litre) 30 g of sucrose, 0.1 g of inositol and three PGRs, namely, gibberellic acid (GA_3), cytokinin and auxin. The cytokinin and auxin used in the experiment were benzyl aminopurine (BAP) and indole acetic acid (IAA), respectively. Seven growth media were prepared (in triplicates) for the 28-day experiment using the three PGRs in different concentrations and combinations as follows:

Group 1 (Control): 0.2 mg/L IAA

Group 2 (0.1 GA_3): 0.1 mg/L GA_3, 0.2 mg/L IAA

Group 3 (0.5 GA_3): 0.5 mg/L GA_3, 0.2 mg/L IAA

Group 4 (1.0 GA_3): 1 mg/L GA_3, 0.2 mg/L IAA

Group 5 (0.1 BAP): 0.1 mg/L BAP, 0.2 mg/L IAA

Group 6 (0.5 BAP): 0.5 mg/L BAP, 0.2 mg/L IAA

Group 7 (1.0 BAP): 1 mg/L BAP, 0.2 mg/L IAA

Effect of plant growth regulators on growth indices of *Ocimum gratissimum* explants

At the end of the 28-day trial, selected growth indices of *O. gratissimum* explants were evaluated including percentage response, fresh weight, shoot length and root length. Percentage response was determined by comparing the number of plantlets that sprouted over the 28-day period with the number of seeds inoculated. Using a meter rule, the shoot length was measured from the hypocotyl level to the upper point of the terminal bud of the seedling whilst root length was measured from the hypocotyl level to the extreme root end.

Preparation of enzyme extract for biochemical assays

Enzyme extract was prepared following the method described by Sunmonu and Van Staden (2014). Fresh *O. gratissimum* leaves (1 g) were homogenised with 4 mL of 0.1 M Tris-HCl buffer (pH 7.8) under chilled condition using a pestle and mortar. The homogenate was filtered and the resultant filtrate was used as enzyme extract for biochemical assays.

Membrane lipid peroxidation assay

The method described by Heath and Packer (1968) was used to determine lipid peroxidation. An aliquot (0.5 mL) of the enzyme extract was treated with 1 mL of 0.5% thiobarbituric acid prepared with 20% trichloroacetic acid. The mixture was incubated for 30 min at 90 °C in a water bath and then cooled immediately in ice chips before the absorbance was measured at 532 nm. The concentration of malondialdehyde (MDA) was used as the index of lipid peroxidation.

Assay of superoxide dismutase activity

The activity of superoxide dismutase (SOD) was assayed using the method described by Kakkar, Dos and Viswnathan (1984). In a total volume of 2.8 mL, the assay mixture contained 1.2 mL sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 mL of 186 M phenazine methosulfate (PMS), 0.3 mL 300 µM nitroblue tetrazolium (NBT), 0.2 mL enzyme extract and 1.0 mL of water. The reaction was initiated by adding 0.2 mL of NADH (780 M) to the mixture and allowed to stand for 90 s before being stopped with 1.0 mL glacial acetic acid. After that, 4.0 mL of n-butanol was added to the assay mixture and set aside for 10 min. In a spectrophotometer (Model SE 803, India), the chromogen intensity in the butanol layer was measured at 560 nm. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in 1 min.

Assay of catalase activity

The activity of catalase (CAT) was assayed using the method described by Luck (1965). An aliquot of 40 µL of enzyme extract was added to a 3 mL hydrogen peroxide–phosphate buffer (0.067 M, pH 7.0) and thoroughly mixed. Using a spectrophotometer (Model SE 803, India), the time taken for a 0.05 unit drop in absorbance was measured at 240 nm. As a control, an enzyme solution containing hydrogen peroxide-free phosphate buffer was used. The amount of enzyme necessary to reduce the absorbance at 240 nm by 0.05 units was taken as one enzyme unit.

Determination of ascorbic acid concentration

Ascorbic acid concentration was determined according to the method described by Roe and Keuther (1943). Firstly, ascorbic acid was extracted from 1 g of the plant sample using 10 mL of 4% trichloroacetic acid. The supernatant obtained after centrifugation at 2000 rpm for 10 min was treated with a pinch of activated charcoal, shaken vigorously using a cyclomixer and kept for 5 min. The charcoal particles were removed by centrifugation and the supernatant was used for the estimation of ascorbic acid concentration. Briefly, standard ascorbate (0.5 mL) ranging between 0.2 mM – 1.0 mM and 1.0 mL of the extract was taken. The volume was made up to 2.0 mL with 4% trichloroacetic acid. Dinitrophenylhydrazine (DNPH) reagent (0.5 mL) was added to all the tubes, followed by two drops of 10% thiourea solution. The contents were mixed and incubated at 37 °C for 3 h in a water bath resulting in the formation of osazone crystals, which were subsequently dissolved in 2.5 mL of 85% sulphuric acid. Similarly, to the blank, DNPH reagent and thiourea were added after the addition of sulphuric acid. The tubes were cooled in ice and absorbance was read at 540 nm in a spectrophotometer (Model SE 803, India). A standard calibration graph was constructed from where the concentration of ascorbic acid in the samples was determined and expressed as milligram per gram of sample.

Statistical analysis

The experiment was carried out in a completely randomised design with three replicates. Data were analysed using SPSS Statistics 20 Statistical software (IBM United Kingdom,

Portsmouth, UK) with results expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was employed to assess the significance of treatment means. In all cases, the confidence coefficient was set at 0.05.

Ethical considerations

This article followed all ethical standards of research without direct contact with human or animal subjects.

Results

Effect of plant growth regulators on germination and growth indices of *Ocimum gratissimum* explants

The response of *O. gratissimum* explants to treatment with PGRs is presented in Table 1. Gibberellic acid in combination with IAA in MS medium had a higher percentage response after 4 weeks of culture compared with medium containing BAP. Gibberellic acid (1.0 mg/L) had the highest percentage of explants with 98% response followed by 0.5 mg/L GA_3 and 1.0 mg/L BAP with percentage responses of 88.66% and 66%, respectively. There was no significant difference in the response of explants raised in MS media supplemented with 0.1 mg/L and 0.5 mg/L BAP. Compared with the control, all the culture media positively impacted the growth indices tested (Table 2). Evidently, the fresh weight, shoot length and root length were significantly increased in *O. gratissimum* explants raised PGR-supplemented MS media in a concentration-dependent manner. However, GA_3 produced a better effect compared with BAP-treated explants.

TABLE 1: Effect of different concentrations of gibberellic acid and benzyl aminopurine in combination with indole acetic acid in Murashige and Skoog medium on percentage response of *O. gratissimum* explants after 28 days of culture.

Growth regulators (mg/L)	Percentage response (%)
Control (0.2 IAA)	42.00 ± 3.25[a]
0.1 GA_3	46.66 ± 2.58[a]
0.5 GA_3	88.66 ± 2.18[b]
1.0 GA_3	98.42 ± 1.89[c]
0.1 BAP	53.33 ± 2.62[d]
0.5 BAP	58.33 ± 2.05[d]
1.0 BAP	66.00 ± 1.05[e]

Note: Data are mean ± s.d. Values with different superscripts indicate significant difference at $p < 0.05$.

GA_3, gibberellic acid; BAP, benzyl aminopurine; IAA, indole acetic acid.

TABLE 2: Effect of different concentrations of gibberellic acid and benzyl aminopurine in combination with indole acetic acid in Murashige and Skoog medium on fresh weight.

Growth regulators (mg/L)	Fresh weight (g)	Root length (cm)	Shoot length (cm)
Control (0.2 IAA)	2.00 ± 0.14[a]	3.50 ± 0.05[a]	4.00 ± 0.20[a]
0.1 GA_3	2.50 ± 0.20[a]	4.20 ± 0.26[b]	5.60 ± 0.06[b]
0.5 GA_3	3.00 ± 0.05[b]	4.60 ± 0.12[b]	6.50 ± 0.15[c]
1.0 GA_3	5.60 ± 0.15[c]	6.60 ± 0.25[c]	8.60 ± 0.15[d]
0.1 BAP	1.20 ± 0.04[d]	3.60 ± 0.15[a]	4.60 ± 0.10[e]
0.5 BAP	1.30 ± 0.11[d]	5.00 ± 0.07[d]	5.60 ± 0.15[b]
1.0 BAP	3.60 ± 0.01[e]	5.60 ± 0.05[e]	6.30 ± 0.20[c]

Note: Data are mean ± s.d. values with different superscripts along with the same column for each parameter indicate significant difference at $p < 0.05$. Root length and shoot length of *O. gratissimum* explants after 28 days of culture.

GA_3, gibberellic acid; BAP, benzyl aminopurine; IAA, indole acetic acid.

Effect of plant growth regulators on malondialdehyde and ascorbic acid concentrations in *Ocimum gratissimum* explants

Tables 3 and 4 show the effect of PGRs on lipid peroxidation and ascorbic acid concentration, respectively, in *O. gratissimum* explants. The data revealed that explants treated with lower concentrations of PGRs (0.1 mg/L and 0.5 mg/L) experienced higher oxidative stress based on the accumulation of MDA concentration when compared with those raised in 1.0 mg/L (Table 3). Accordingly, there was a significant reduction in ascorbic acid concentration in *O. gratissimum* explants cultured in MS media containing 1.0 mg/L of GA_3 and BAP compared with 0.1 mg/L and 0.5 mg/L concentrations. Generally, the accumulation of ascorbic acid is lower in GA3-treated explants compared with BAP treatments (Table 4).

Effect of plant growth regulators on superoxide dismutase and catalase activities in *Ocimum gratissimum* explants

The effect of the application of PGRs on antioxidant enzyme activities during *in vitro* proliferation of *O. gratissimum* explants in MS culture media is presented in Tables 5 and 6. Considering that these enzymes are stress indicators in plants, the activities of SOD and CAT were assayed in *O. gratissimum* explants (Tables 5 and 6, respectively). In response, the activities of the two enzymes were significantly increased in explants raised in culture media supplemented with 1.0 mg/L of GA_3 and BAP when compared with control and lower concentrations of the two PGRs. However, GA_3 produced a better result than BAP.

TABLE 3: Effect of different concentrations of gibberellic acid and benzyl aminopurine in combination with indole acetic acid in Murashige and Skoog medium on malondialdehyde concentration in *O. gratissimum* explants after 28 days of culture.

Growth regulators (mg/L)	MDA concentration (mmol/g fresh weight)
Control (0.2 IAA)	15.48 ± 1.24[a]
0.1 GA_3	5.16 ± 0.01[b]
0.5 GA_3	4.73 ± 0.12[c]
1.0 GA_3	3.01 ± 0.02[d]
0.1 BAP	10.96 ± 0.75[e]
0.5 BAP	9.03 ± 0.98[e]
1.0 BAP	6.45 ± 0.34[f]

Note: Data are mean ± s.d. Values with different superscripts indicate significant difference at $p < 0.05$.

GA_3, gibberellic acid; BAP, benzyl aminopurine; IAA, indole acetic acid, MDA, malondialdehyde.

TABLE 4: Effect of different concentrations of gibberellic acid and benzyl aminopurine in combination with indole acetic acid in Murashige and Skoog medium on ascorbic acid concentration in *O. gratissimum* explants after 28 days of culture.

Growth regulators (mg/L)	Ascorbic acid concentration (mg/g fresh weight)
Control (0.2 IAA)	102.00 ± 3.16[a]
0.1 GA_3	89.00 ± 2.88[b]
0.5 GA_3	75.33 ± 2.01[c]
1.0 GA_3	67.33 ± 1.05[d]
0.1 BAP	60.33 ± 1.10[e]
0.5 BAP	54.33 ± 1.05[f]
1.0 BAP	51.33 ± 1.35[f]

Note: Data are mean ± s.d. Values with different superscripts indicating significant difference at $p < 0.05$.

GA_3, gibberellic acid; BAP, benzyl aminopurine; IAA, indole acetic acid.

TABLE 5: Effect of different concentrations of gibberellic acid and benzyl aminopurine in combination with indole acetic acid in Murashige and Skoog medium on superoxide dismutase activity in *O. gratissimum* explants after 28 days of culture.

Growth regulators (mg/L)	SOD activity (U/g fresh weight)
Control (0.2 IAA)	2.16 ± 0.2.8[a]
0.1 GA_3	3.45 ± 0.18[b]
0.5 GA_3	4.86 ± 0.15[c]
1.0 GA_3	7.01 ± 0.81[d]
0.1 BAP	3.00 ± 0.11[e]
0.5 BAP	3.04 ± 0.03[e]
1.0 BAP	3.10 ± 0.24[e]

Note: Data are mean ± s.d. Values with different superscripts indicate significant difference at $p < 0.05$.

GA_3, gibberellic acid; BAP, benzyl aminopurine; IAA, indole acetic acid; SOD, superoxide dismutase.

TABLE 6: Effect of different concentrations of gibberellic acid and benzyl aminopurine in combination with indole acetic acid in Murashige and Skoog medium on catalase activity in *O. gratissimum* explants after 28 days of culture.

Growth regulators (mg/L)	CAT activity (U/g fresh weight)
Control (0.2 IAA)	102.66 ± 2.36[a]
0.1 GA_3	113.66 ± 1.69[b]
0.5 GA_3	117.33 ± 1.65[b]
1.0 GA_3	134.33 ± 3.01[c]
0.1 BAP	110.00 ± 1.92[b]
0.5 BAP	115.66 ± 2.16[b]
1.0 BAP	124.00 ± 1.06[d]

Note: Data are mean ± s.d. Values with different superscripts indicate significant difference at $p < 0.05$.

GA_3, gibberellic acid; BAP, benzyl aminopurine; IAA, indole acetic acid; CAT, catalase.

Discussion

The growing global population and attendant dependence on medicinal plants have tremendously endangered some plant species because of excessive human exploitation. Consequently, there is an urgent need for massive production of medicinal plants for pharmaceutical and nutritional purposes. The present study clearly provides an insight into the development of a protocol for micropropagation of *O. gratissimum* using selected PGRs via tissue culture to meet the ever-increasing demand for this important medicinal plant.

Seed germination and growth are important stages in the development of plants and studies have implicated PGRs in these activities (Khan, Bano & Babar 2019; Rademacher 2015). Consequently, the significant increase in growth indices of *O. gratissimum* explants observed in this study may be attributed to the presence of GA_3 and BAP in the culture medium. Specifically, GA_3 induces the production of α-amylase (an enzyme responsible for the degradation of reserved carbohydrate to soluble sugars) during germination (Damaris et al. 2019; Lee, Chen & Yu 2014). The hormone also enhances the mobility of stored compounds, thus leading to an increase in growth rate (Hadi, Bano & Fuller 2010). Therefore, the observed increase in percentage response, shoot length and root length in *O. gratissimum* explants may be attributed to supplementation of the growth media with GA_3. The reduction in plant growth indices of *O. gratissimum* explants with a reduction in PGR concentration may be an indication of stressful conditions, which in turn affected carbohydrate storage, translocation and metabolism (Mishra & Dubey 2008) leading to a reduced biomass accumulation (Gong et al. 2013). Thus, the reduction in fresh weight recorded with PGR-treated *O. gratissimum* explants at 0.1 mg/L concentration may be associated with low photochemical efficiency coupled with a relatively high oxidative stress in the leaves of these plants when compared with higher PGR treatments.

The significant increase in MDA level in the leaves of IAA-regenerated *O. gratissimum* explants (control) indicates a high production of reactive oxygen species (ROS) beyond the scavenging efficiency of the antioxidant enzymes, leading to oxidative stress. Although reactive oxygen species can serve as signalling molecules in plants, overproduction can be highly destructive to lipids, proteins and nucleic acids (Gaspar et al. 2002). Lipid peroxidation because of oxygen radicals can lead to increased membrane permeability and subsequent low nonphotochemical chlorophyll fluorescence quenching (NPQ) (Kalaji et al. 2014). Nonphotochemical chlorophyll fluorescence quenching is a process that occurs in the photosynthetic membranes of plants during which excess absorbed light energy is dissipated as heat (Demmig-Adams, Stewart & Adams 2014). The reduction in MDA concentration in PGR-treated explants is a clear indication that GA_3 and BAP are able to attenuate lipid peroxidation by reducing membrane permeability. This possibly enhanced NPQ with GA_3 demonstrating more potency than BAP.

A significantly high antioxidant enzyme activity was recorded in GA_3- and BAP-derived plants when compared with the control (plants regenerated with only IAA). In most cases, the activities of the antioxidant enzymes increased with a rise in GA_3 and BAP concentrations. A high antioxidant enzyme activity with increased cytokinin content was also reported in transgenic Pssu-ipt tobacco plants (Synková et al. 2006). Dı´az-Vivancos et al. (2011) also observed a stimulatory effect of cytokinins on SOD activity in *Crocus sativus*, a perennial bulbous plant. The enhancement of SOD activity, which is the first line of defence against reactive oxygen species in the detoxification process (Alscher, Erturk & Heath 2002), particularly indicates a high production of superoxide anion radicals possibly because of oxygen photoreduction by electron leakage at Photosystem I (PSI) in the leaves. Similarly, in the present study, there was a significant increase in the activities of SOD and CAT enzymes in GA_3- and BAP-regenerated plants when compared with the control. The reduction in SOD and CAT activities at low PGR concentration (0.1 mg/L) suggests oxidative stress, which is most likely because of the production and accumulation of reactive oxygen species in an attempt by the plants to control excess excitation energy (Neill et al. 2002). This also accounted for the high MDA concentration recorded for explants in the medium.

Ascorbic acid possesses powerful antioxidant activity by enhancing redox reactions in cells thereby contributing to non-enzymatic transport of hydrogen to the plant tissues (Georgeseu, Gavat & Voinescu 2019). It is also a potent Reactive Oxygen Species (ROS) scavenger that neutralises peroxide radicals whilst promoting enzymatic activity in plant tissues (Al-Majidi & Al-Qubury 2016; Logan 2005). The reduction in ascorbic concentration in *O. gratissimum* explants as the concentration of PGR increases

is indicative of reduced activity ROS and neutralisation of hydrogen peroxide brought about by GA_3 and BAP.

Conclusion

In conclusion, the results obtained from the study showed that a medium containing GA_3 is more favourable for the micropropagation of *O. gratissimum* explants compared with BAP. The protocol could be exploited to produce disease-free and healthy *O. gratissimum* plants on a large scale for the nutraceutical and pharmaceutical industries.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

All authors participated in the design of the study and approved the final manuscript. M.M.O. prepared the extracts and carried out the germination experiment and biochemical assays. G.M. conceived and supervised the study. C.C.O. coordinated the manuscript preparation and revised the manuscript. T.O.U. coordinated the research, performed the statistical analysis and drafted the manuscript.

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Data availability

Data created or analysed in this study were included in this manuscript.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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