Plant Growth Regul (2017) 82:103–110
DOI 10.1007/s10725-016-0242-3

ORIGINAL PAPER

Differential responses to isoprenoid, N^6-substituted aromatic cytokinins and indole-3-butyric acid in direct plant regeneration of *Eriocephalus africanus*

Olwethu Madzikane-Mlungwana[1] · Mack Moyo[1,2] · Adeyemi O. Aremu[1] · Lucie Plíhalová[3] · Karel Doležal[3] · Johannes Van Staden[1] · Jeffrey F. Finnie[1]

Received: 12 May 2016 / Accepted: 15 December 2016 / Published online: 9 February 2017

Abstract *Eriocephalus africanus* is a medicinal and aromatic plant species that is part of South Africa's remarkable diversity. As a result of illegal and over-harvesting, most plant communities have become unsustainable and as such, effective and efficient conservation strategies have to be implemented. In the present study, an isoprenoid cytokinin (CK): isopentenyladenine (iP) and four aromatic CKs namely benzyladenine (BA), *meta*-topolin (*m*T), *meta*-topolin riboside (*m*TR) and 6-(3-hydroxybenzylamino)-9-(tetrahydropyran-2-yl)purine (*m*TTHP) at 1, 5 or 10 µM were evaluated for in vitro plant regeneration in *E. africanus*. Different concentrations of indole-3-butyric acid (IBA) were also evaluated for shoot and root organogenesis. The highest number of shoots was produced by *m*T (1 and 5 µM) treatment, longest shoots were stimulated by iP (1 µM) and the highest fresh mass was obtained in BA (5 and 10 µM), *m*T (5 and 10 µM) and *m*TTHP (5 µM)-treated plants. During acclimatization, all the in vitro plants obtained from the lowest concentration of CKs survived and 1 µM iP regenerants recorded a 100% survival rate. For the rooting experiment, more vigorous adventitious roots were observed in the 1 µM IBA treatment. All IBA treatments had 100% survival rate after 6 weeks of acclimatization. Overall, the concentration and type of plant growth regulators had a remarkable influence on the growth and development of in vitro-derived *E. africanus*.

Keywords Auxins · Cytokinins · Flavonoids · Plant regeneration · Phenolics

Abbreviations

BA	N^6-Benzyladenine
CE	Catechin equivalents
CK	Cytokinin
DW	Dry weight
GAE	Gallic acid equivalents
IBA	Indole-3-butyric acid
iP	N^6-Isopentenyladenine
*m*T	*meta*-Topolin
*m*TR	*meta*-Topolin riboside
*m*TTHP	6-(3-hydroxybenzylamino)-9-(tetrahydropyran-2-yl)purine
MS	Murashige and Skoog (1962) medium
PGR	Plant growth regulator
PPF	Photosynthetic photon flux

Introduction

Eriocephalus africanus L. var. *africanus* (Asteraceae), commonly known as 'wild rosemary' or 'Cape snow

✉ Johannes Van Staden

Jeffrey F. Finnie
rcpgd@ukzn.ac.za

[1] Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg Campus, Private Bag X01, Scottsville 3209, South Africa

[2] Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, P.O. Box 1906, Symphony Way, Bellville, 7535 Cape Town, South Africa

[3] Laboratory of Growth Regulators & Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University and Institute of Experimental Botany AS CR, Šlechtitelů 27, 783 71 Olomouc, Czech Republic

bush' is a bushy, woody evergreen shrub (Njenga et al. 2005; Verdeguer et al. 2009). The genus, which is endemic to South Africa, consists of 32 species that are distributed in the Eastern, Western and Northern Cape (Njenga and Viljoen 2006; Viljoen et al. 2006). *Eriocephalus africanus* has a long tap root and needle-shaped leaves, which helps in water absorption and reduce water loss, respectively. Thus, the plant is adapted to dry, sunny and rocky environments. The species flowers best in winter producing a spectacular view of small snow white flowers (Catarino et al. 2015), which endows the plant species its high ornamental value (Verdeguer et al. 2009). Economically, its leaves are used when preparing food products such as salads and soups as well as for flavouring butter, oil and wine (Catarino et al. 2015).

In traditional medicine, the stem and leaves of the plant are extensively used to treat various diseases. For instance, *E. africanus* infusions are used in the treatment of colds and influenza, stomach disorders and gynaecological conditions, and a reliever in symptoms of colic and gas in babies (Njenga and Viljoen 2006). In addition, it is used to relieve chest pains (Salie et al. 1996) and inflammations (Philander 2011; Lall and Kishore 2014). Tea from *E. africanus* leaves is commonly used for treating cough and colds (Salie et al. 1996). In Western Cape, it is popular for cosmetic purposes such that essential oils derived from it are used in aromatherapy (Makunga et al. 2008; Philander 2011).

Due to the role played by endemic wild rosemary in the medicinal and cosmetic industry as well as the increasing domestic usage, in vitro propagation is important for its conservation even though it is currently classified under least concern by the Red List of South African plants (SANBI 2015). To the best of our knowledge, there are no previous publications on micropropagation of *E. africanus*. The aim of the study was to evaluate the effect of different plant growth regulators (PGRs) for the development of an efficient micropropagation protocol for *E. africanus* using shoot-tip explants. Different types and concentrations of cytokinins (CKs) namely, isopentenyl adenine (iP), benzyladenine (BA), *meta*-topolin (*m*T), *meta*-topolin riboside (*m*TR) and 6-(3-hydroxybenzylamino)-9-(tetrahydropyran-2-yl) purine (*m*TTHP) where evaluated for shoot proliferation and biomass production in *E. africanus*. The auxin, indole-3-butyric acid (IBA) was tested at varying concentrations for its rooting competence. In vitro plantlets, both CK and IBA-derived, were acclimatized to determine survival rate under ex vitro conditions. In addition, the phenolic and flavonoid content in the 8-week-old ex vitro acclimatized IBA-derived in vitro regenerants were quantified on the basis on the essential roles of these phytochemicals during acclimatization.

Materials and methods

Source of chemicals, plant material, decontamination and culture conditions

Myo-inositol, vitamins (thiamine HCl, nicotinic acid, pyridoxine HCl), glycine, IBA, BA and iP were purchased from Sigma–Aldrich (Steinheim, Germany), while *m*T, *m*TR and *m*TTHP were prepared as previously described (Doležal et al. 2006; Szüčová et al. 2009). Agar bacteriological powder was purchased from Oxoid Ltd., Basingstoke, England. All chemicals used were of analytical grade.

Mature *E. africanus* plants were purchased from the SANBI National Botanical Gardens in Pietermaritzburg, South Africa. The plants were positively identified and a voucher specimen (Olwethu1) was deposited in Bews Herbarium, University of KwaZulu-Natal, South Africa. Healthy plants were transferred to terracotta pots (diameter 200 mm) and maintained in the shade house of the University of KwaZulu-Natal Botanical Gardens at ambient temperature and natural photoperiod. The potted plants were frequently watered and kept weed-free.

Shoot-tips from mature plants were harvested and thoroughly washed with liquid detergent and rinsed with running tap water. The shoot-tips were surface decontaminated in a laminar flow bench using 70% ethanol (v/v) for 60 s followed by sodium hypochlorite (NaOCl; 2% or 3.5%) for either 10 or 20 min. A few drops of Tween 20 were added as a surfactant. The highest decontamination success rate (%) was obtained with 2% NaOCl for 10 min (data not shown) and this treatment was used in all experiments. Decontaminated plant materials were rinsed with sterile distilled water three times. Shoot-tips were excised from the decontaminated plant materials and inoculated onto 10 ml of Murashige and Skoog (MS) medium (Murashige and Skoog 1962) in culture tubes (100 mm × 25 mm, 40 ml volume). The MS media were supplemented with myo-inositol (0.1 g/l) and sugar (30 g/l), pH was adjusted to 5.8 with either 1.0 M potassium hydroxide (KOH) or 1.0 M hydrochloric acid (HCl) before being solidified with 8 g/l agar (No. 1 bacteriological agar). Thereafter, 10 ml of media were dispensed into culture tubes (100 mm × 25 mm, 40 ml volume) and autoclaved at 103 kPa and 121 °C for 20 min. The cultures were incubated in a growth room under 16/8-h light/dark conditions with a photosynthetic photon flux (PPF) of 40–50 µmol m^{-2} s^{-1} at a temperature of 25 ± 2 °C.

Role of CKs on shoot proliferation and growth

Following successful explant decontamination, sterile shoot-tips were used to evaluate the effect of different types and concentrations of CKs on shoot proliferation and growth. An isoprenoid CK, iP, and four aromatic CKs

namely BA, *m*T, *m*TR and *m*TTHP were each tested at three different concentrations (1, 5 and 10 µM). Cultures devoid of CKs served as the control. Sterile shoot-tips excised from in vitro-derived plantlets were cut into 10 mm-long explants. The explants were inoculated randomly onto MS medium supplemented with the different types and concentrations of CKs. The CK treatments and the control each had 25 explants. The cultures were incubated in a growth room as described earlier. After a period of 6 weeks, the number of shoots/explants, shoot length and fresh weight/culture vessel were recorded. The CK experiment was done twice.

To evaluate their acclimatization competence, in vitro-derived plantlets from the respective CK treatments and the control were transferred to ex vitro conditions. In vitro-derived *E. africanus* plantlets were cleaned thoroughly under running tap water. In each case, 15 plantlets were planted in rectangular containers (230 mm × 165 mm × 60 mm) containing a soil:vermiculite (1:1) mixture and kept in the mist house for 2 weeks before being transferred to the greenhouse. The controlled conditions of the mist house had high relative humidity (90–100%), PPF of 450 µmol m^{-2} s^{-1} and temperature of 25 ± 2 °C under natural photoperiod conditions. The greenhouse was maintained at a temperature of 25 ± 2 °C, natural PPF of approximately 1000 µmol m^{-2} $s^{-}1$ under natural photoperiod conditions. After a greenhouse acclimatization period of 8 weeks, survival rate (%) of plants for CK treatments and the control was recorded.

Role of IBA on in vitro rooting and acclimatization of plantlets

Sterile *E. africanus* shoot-tip explants derived from cultures maintained on MS medium devoid of PGRs were used to evaluate the effect of five concentrations (1, 5, 10, 15, 25 µM) of IBA on root proliferation and growth. Cultures without IBA served as the control. In vitro-derived sterile plantlets (2–10 cm in length) were randomly selected, cut into regular 10 mm-long lengths and inoculated on MS medium supplemented with different concentrations of IBA. The IBA treatments and control had 20 explants and the experiment was done twice. Cultures were incubated in a growth room under the same conditions as described earlier. After a period of 6 weeks, the number of roots/explant, root and shoot lengths and plant fresh weight were recorded.

The in vitro-derived plantlets were cleaned thoroughly under running tap water and planted in a soil:vermiculite (1:1) plant growth mixture. In each case, 15 plantlets were potted in rectangular containers (230 mm × 165 mm × 60 mm) and transferred to a high humidity (90–100%) mist house for 2 weeks. Thereafter the plants were transferred to a climate controlled greenhouse where they were watered twice a week. After 8 weeks in the greenhouse, the survival rate (%) and fresh biomass, shoot and root lengths as well as root proliferation were recorded.

Quantification of total phenolics and flavonoids

After 8 weeks, the greenhouse-acclimatized plants derived from IBA in vitro treatments were harvested, dried and ground into fine powders. The ground samples (0.1 g) were extracted with 50% aqueous methanol (20 ml) in a sonication bath (Branson Model 5210, Branson Ultrasonics B.V., Soest, Netherlands) for 1 h. The aqueous methanolic extracts were filtered under vacuum using Whatman No. 1 filter paper. Thereafter, the filtrates were immediately used for quantification of total phenolics and flavonoids.

Total phenolic content was determined as outlined in the Folin–Ciocalteu assay (Singleton and Rossi 1965) but using gallic acid as a standard. Reaction mixtures consisting of sample, distilled water, Folin & Ciocalteu's phenol reagent (1 N) and 2% sodium carbonate were incubated at room temperature for 40 min. Absorbance at 725 nm was then read using a Cary 50 UV–visible spectrophotometer (Varian, Australia). Total phenolic content was expressed in mg gallic acid equivalents (GAE) per g dry weight (DW).

The aluminium chloride ($AlCl_3$) colorimetric method was used to quantify total flavonoid content (Zhishen et al. 1999). Absorbance at 510 nm was read against a blank that contained 50% aqueous methanol. Suitable aliquots of catechin hydrate were used to derive a standard calibration curve. The samples were assayed in triplicate and total flavonoid content was expressed as mg catechin equivalents (CE) per g DW.

Data analysis

In all cases, data was tested for normality. Data was then subjected to analysis of variance where means were compared and separated using a Post hoc test (Duncan's multiple range test: $P \leq 0.05$) based on SPSS software (Version 22). Graphs were plotted using SigmaPlot 8.0.

Results

Shoot proliferation, in vitro plant growth and acclimatization

The effect of various CKs on shoot proliferation is presented in Fig. 1. The highest number of shoots per explant was obtained at 1.0 and 5.0 µM *m*T (Fig. 1a). A dose-dependent increase in shoot proliferation was observed

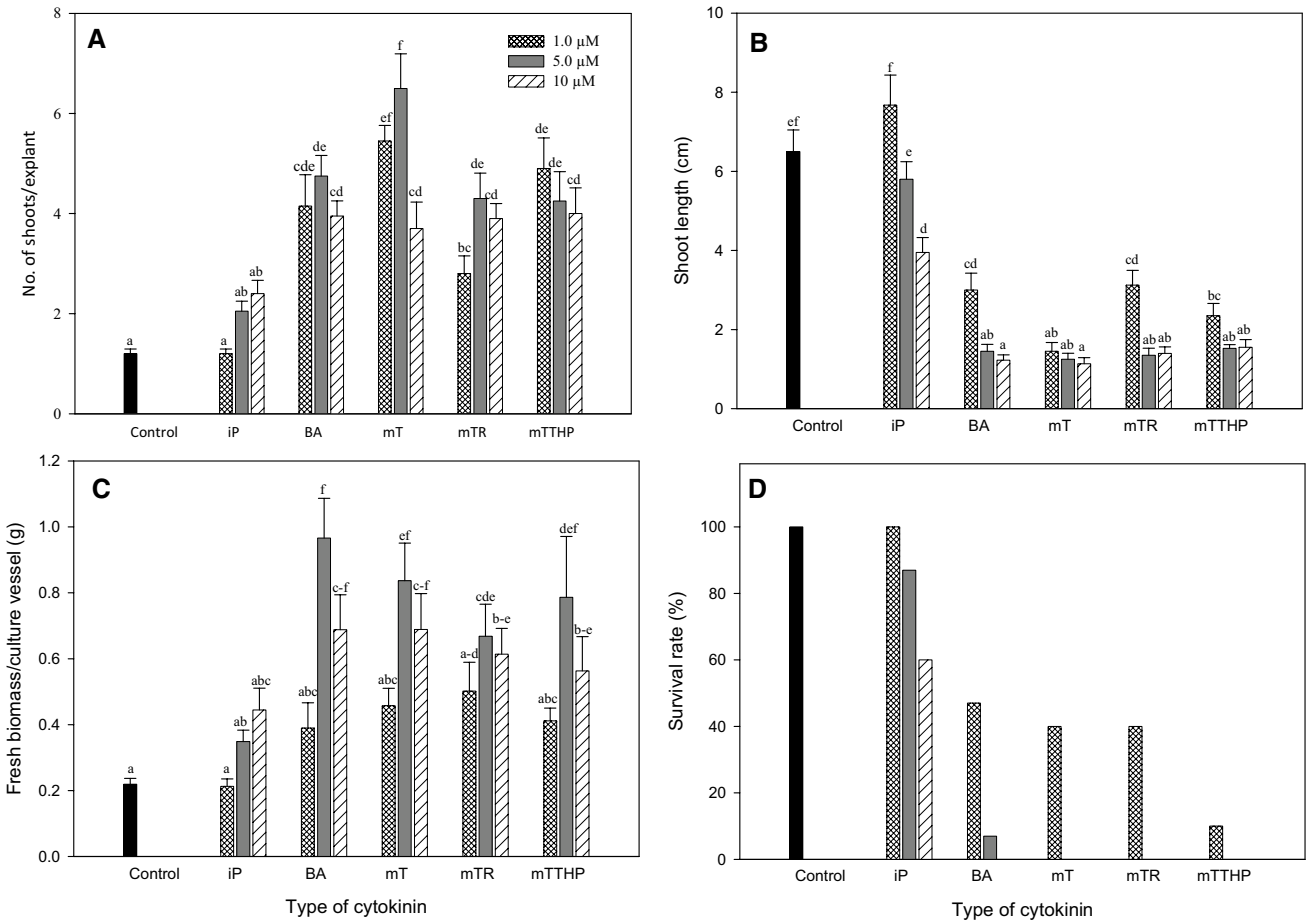

Fig. 1 Effect of type and concentration of cytokinins on shoot growth of *Eriocephalus africanus* after 6 weeks of culture. **a** Number of shoots; **b** shoot length; **c** fresh biomass per culture vessel; **d** percentage survival rate after acclimatization of the in vitro-derived plantlets. iP = isopentenyl adenine, BA = benzyladenine, *m*T = *meta*-topolin, *m*TR = *meta*-topolin riboside and *m*TTHP = 6-(3-hydroxybenzylamino)-9-(tetrahydropyran-2-yl)purine. *Bars* with the same *letter(s)* are not significantly different based on Duncan's multiple range test ($P \leq 0.05$) and n = 50

for iP and *m*TR treatments, whereas an inverse trend was observed for *m*TTHP-treated cultures. In general, the number of shoots per explant was higher for aromatic CKs compared to iP and the control. On the other hand, iP-treated and control plantlets were longer in comparison with the aromatic CKs (Fig. 1b). Compared to the control, there was a significant reduction in shoot length with all the aromatic CKs. For most of the tested CKs, the longest shoot length was observed at the respective lowest concentration (1.0 µM). Correspondingly, the highest ex vitro survival rate (%) was obtained for control and iP-treated plants (Fig. 1d). In addition, survival rate (%) of acclimatized plants was higher for the 1.0 µM treatments for all the CKs. Aromatic CKs exhibited higher biomass production compared to iP treatments and the control (Fig. 1c). The highest level of biomass production was obtained for BA (5.0 and 10 µM), *m*T (5.0 and 10 µM) and *m*TTHP (5.0 µM)-treated plants.

Root proliferation, in vitro and ex vitro plant growth

Figure 2 depicts IBA-induced root proliferation and in vitro growth of *E. africanus* plants following a 6 week incubation period. High concentrations (>5.0 µM) of IBA had a significant inhibitory effect on root proliferation (Fig. 2a) and length (Fig. 2b). The highest number of roots per shoot was obtained on MS supplemented with 1.0 µM IBA and the control. The control produced plants with the longest roots. Shoot length was significantly higher for plants derived from 1.0 µM IBA compared to the control (Fig. 2c). Significantly higher fresh biomass per culture tube was observed for all IBA treatments when compared to the control. Following a dose-dependent response, the highest fresh biomass production was obtained on 5.0 µM IBA (Fig. 2d).

After 8 weeks of acclimatization under greenhouse conditions, all IBA-treated plants and the control

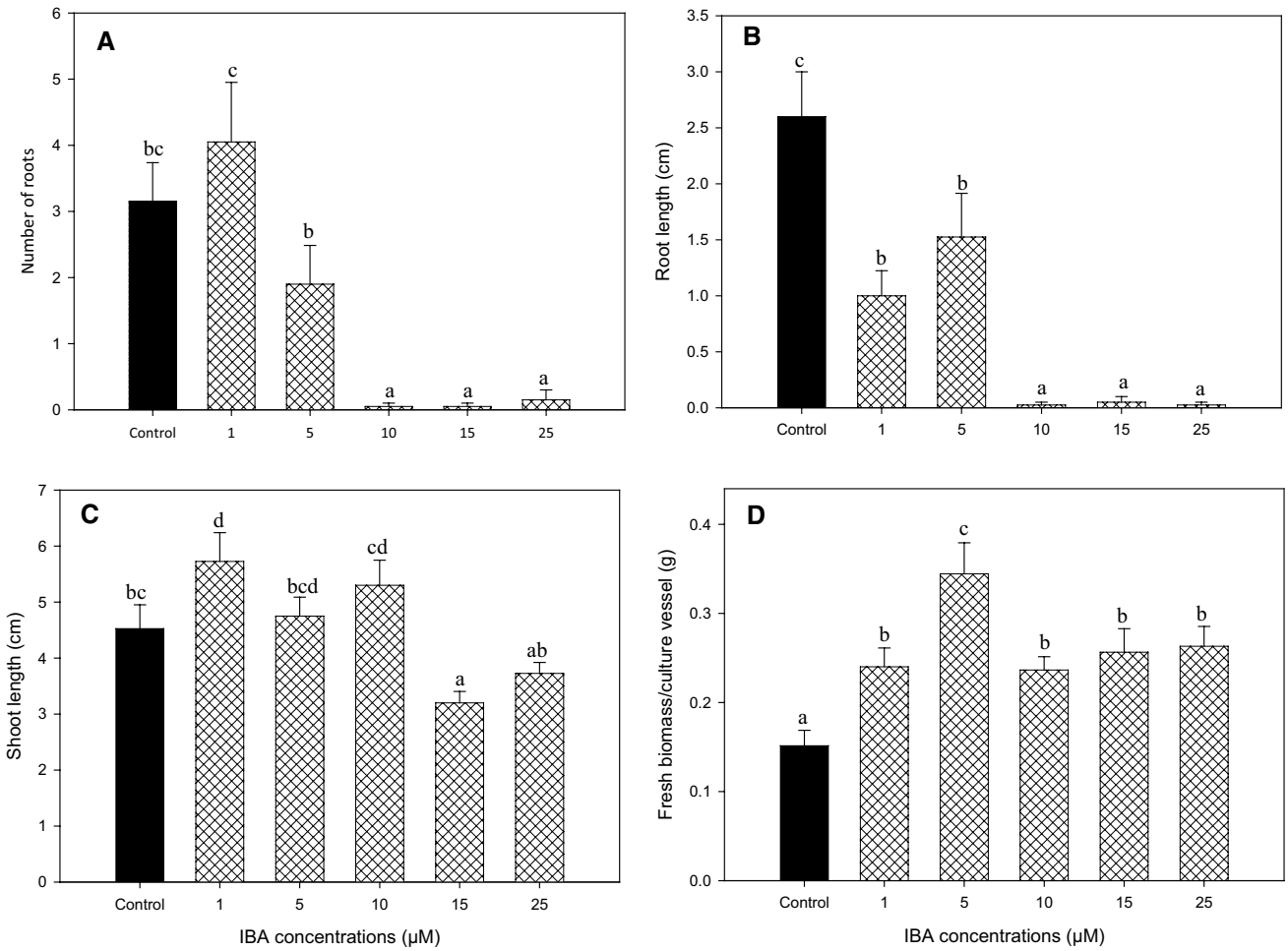

Fig. 2 Effect of different indole-3-butyric acid (IBA) concentrations on in vitro growth of *Eriocephalus africanus*. **a** Number of adventitious shoots. **b** The length of roots for control and different IBA treatments. **c** The length of shoots for control and different IBA treatments. **d** Fresh biomass of regenerants per culture vessel. *Bars* with the same *letter(s)* are not significantly different based on Duncan's multiple range test ($P \leq 0.05$) and n = 40

recorded a 100% survival rate (Table 1). The number of shoots was greater in control compared to 1.0, 5.0 and 10 μM IBA treated plants. The shoot length of 1.0 μM IBA-treated plants was significantly longer than control plants. However, there was no significant difference between the control and IBA-treated plants in root length. Plant fresh weight was significantly higher in the control compared to plants originally treated with IBA in vitro.

Table 1 Effect of indole-3-butyric acid (IBA) applied during in vitro stage on the survival, growth and acclimatization competency of *Eriocephalus africanus* after 8 weeks under greenhouse conditions

IBA concentration (μM)	Survival rate (%)	No. of shoots	Root length (cm)	Shoot length (cm)	Fresh weight (g)
1.0	100	1.4 ± 0.13a	7.9 ± 0.64a	21.0 ± 1.28b	1.54 ± 0.11a
5.0	100	1.5 ± 0.15a	7.3 ± 0.70a	17.5 ± 1.33ab	1.39 ± 0.21a
10	100	1.4 ± 0.14a	7.2 ± 0.43a	17.2 ± 1.63ab	1.35 ± 0.23a
15	100	1.6 ± 0.15ab	8.6 ± 0.72a	17.3 ± 1.00ab	1.59 ± 0.18a
25	100	1.5 ± 0.19ab	7.1 ± 0.57a	17.1 ± 1.07ab	1.37 ± 0.16a
Control	100	2.0 ± 0.13b	8.8 ± 0.77a	15.9 ± 1.85a	2.61 ± 0.51b

In each column, mean value with the same letter(s) are not significantly different based on Duncan's multiple range test ($P \leq 0.05$) and n = 15

Total phenolic and flavonoid contents of IBA-treated plants

Total phenolics and flavonoids for the 8-week-old acclimatized *E. africanus* were significantly higher for IBA-treated plants compared to the control (Fig. 3). Overall, there was higher accumulation of total phenolic compounds in the aerial plant parts compared to underground roots for the control and all IBA treatments (Fig. 3a). On the other hand, an inverse relationship between aerial and underground organs was observed for total flavonoids (Fig. 3b).

Discussion

CK-induced shoot proliferation, biomass accumulation and acclimatization

Cytokinins are known to promote cell division, differentiation, shoot formation and elongation (Gentile et al. 2014). Naturally-occurring CKs are adenine derivatives with either an isoprenoid or aromatic side chain at the N^6 position (Frébort et al. 2011). Compared to the control, all aromatic CK treatments gave a comparatively higher number of shoots. In the current study, *m*T (1 and 5 µM) was the most efficient CK in promoting shoot proliferation of *E. africanus*. Similar observations were reported by Chang et al. (2003), Bairu et al. (2007) and Amoo et al. (2012) for *Zantedeschia albomaculata, Aloe polyphylla* and *A. arborescens*, respectively. In contrast, *m*T did not improve shoot proliferation in *Prunus domestica* and *P. insititia* micropropagation (Gentile et al. 2014). In the present study, within the tested range, an increase in CK concentration reduced ex vitro acclimatization competency of the plants. The effect was more aggravated for aromatic CK-derived plants compared to iP-treated plants. This suggests that *E. africanus* is responsive to low and high exogenous aromatic and isoprenoid CK concentrations, respectively. Differential plant growth responses due to the application of aromatic and isoprenoid CK types have been widely documented (Aremu et al. 2012). Furthermore, CKs belonging to the same structural group may not necessarily have similar effect on plant regeneration (Tubić et al. 2015). In some instances, *m*T was reported to be more effective than BA in shoot induction in *Musa* spp. (Escalona et al. 2003) and *Huernia hystrix* (Amoo and Van Staden 2012) whereas a detrimental response in shoot proliferation was observed in *Sorbus torminalis* (Malá et al. 2009) and in *Citrus* spp. (Niedz and Evens 2010). Different substituents at C6 and/or N9 atoms of the purine moiety of compounds used in the current study were selected with the aim to prove if they cause differences in shoot proliferation response (Plíhalová et al. 2016).

In particular, iP (isoprenoid CK) was less active than the aromatic CKs in induction of shoot proliferation and fresh biomass accumulation. In contrast, better plant vigour (Fig. 1b) and a corresponding higher ex vitro survival rate (Fig. 1d) were observed with iP pre-treated in vitro shoots. As one of the features that may dramatically change biological activity of novel generation of aromatic CKs is probably due to the substitution of one or more hydrogen atoms of benzyl ring by -hydroxy, -methoxy, -mercapto or -alkyl group or by their mutual combinations (Plíhalová et al. 2016). Hydroxylated CKs, now commonly known as topolins, have been shown to be more physiologically stable, resistant to CK oxidase and are active at lower concentrations than the isoprenoid CKs (Doležal et al. 2011; Plíhalová et al. 2016). In addition, hydroxylated CKs can form *O*-glucosides, CK storage metabolites, which are amenable to reversible sequestration to active CK bases (Werbrouck et al. 1996). The conversion of *O*-glucosides to active CK bases help maintain CK homeostasis in plant cells (Kamínek et al. 1997).

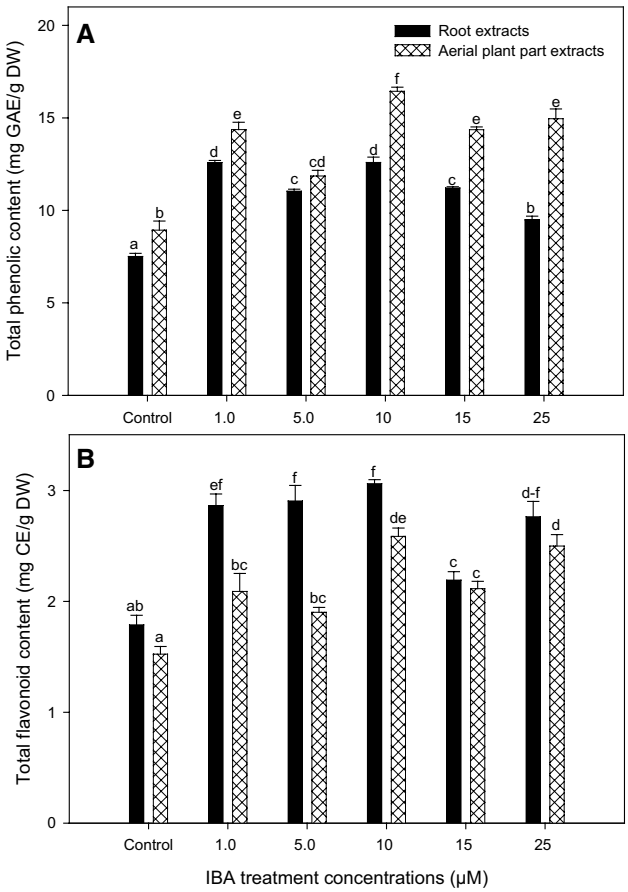

Fig. 3 Effect of indole-3-butyric acid (IBA) applied during in vitro stage on (**a**) phenolic and (**b**) flavonoid content of *Eriocephalus africanus* after 8 weeks under greenhouse conditions. In each graph, *bars* with the same *letter(s)* are not significantly different based on Duncan's multiple range test ($P \leq 0.05$) and n = 3. *GAE* gallic acid equivalents, *CE* catechin equivalents

IBA-induced root proliferation, biomass and phenolics production

The ultimate goal of most micropropagation processes is successful ex vitro acclimatization of in vitro-derived plantlets. The ability to induce rooting, both in vitro and ex vitro, in such plantlets is a key step in vegetative propagation, which enhances success of the acclimatization process and subsequent growth of individuals into mature adult plants (de Klerk et al. 1999). Besides external factors, adventitious rooting is influenced by a number of internal stimuli, in particular the phytohormone, auxin (Štefančič et al. 2005). Although IAA is the main naturally occurring auxin *in planta*, IBA remains the predominant exogenously applied rooting agent due to its high chemical stability and insensitivity to the action of auxin degrading enzymes (Wiesman et al. 1989; de Klerk et al. 1999; Ludwig-Müller 2000; Štefančič et al. 2005; Verstraeten and Geelen 2015). In the present study, although the highest number of roots per shoot and root length were obtained at the lower IBA concentrations (Fig. 2), there was no significant difference in root growth parameters after the 8 weeks acclimatization phase (Table 1). During ex vitro acclimatization, exogenous auxin becomes progressively ineffective, thus root development and growth is largely influenced by endogenous biosynthesis of IAA (Štefančič et al. 2005). Moreover, IBA is known to efficiently release IAA *in planta* via the β-oxidation biochemical pathway (Verstraeten and Geelen 2015). Thus, the in vitro rooting advantage derived from application of exogenous IBA was nullified after 8 weeks ex vitro growth.

Although several studies have demonstrated the effect of PGRs on the production of phenolic compounds under in vitro growing conditions (Amoo et al. 2012; Moyo et al. 2012; Palacio et al. 2012; Szopa and Ekiert 2012, 2014; Khan et al. 2016), there is a paucity of information on their residual effect, particularly that of auxins, after ex vitro acclimatization. The distribution patterns of phenolics and flavonoids observed in the present study may provide insights into their biosynthesis and accumulation sites in *E. africanus*. Although regarded as non-essential for plant growth and development, phenolics and flavonoids are known to possess species-specific roles in defence and UV-B protection (Taylor and Grotewold 2005). Initially, flavonoids were regarded as negative regulators of polar auxin transport (Murphy et al. 2000; Brown et al. 2001; Buer and Muday 2004). However, recent evidence suggests that flavonoids are not essential regulators but only act as modulators in auxin transport (Peer and Murphy 2007). In the present study, the higher concentration of flavonoids in the roots, which are the sites of endogenous auxin biosynthesis (Ljung et al. 2005) may suggest a physiological role between the two molecules.

Conclusions

The effect of different types and concentrations of PGRs was evaluated on plant regeneration and acclimatization competency of *E. africanus*. Overall, for optimum in vitro shoot and root proliferation, low concentrations (≤5 μM) of PGRs were generally more effective and efficient in the micropropagation of *E. africanus*. Application of different concentrations of IBA during the in vitro rooting stage had a significant influence on some growth parameters and the level of total phenolics and flavonoids on the greenhouse acclimatized plants after 8 weeks of ex vitro growth. For future research, combining lower concentrations of CKs and IBA should be investigated for potential enhancement as well as more effective and efficient micropropagation of *E. africanus*. In addition, quantification of the endogenous phytohormone content may also offer an avenue to further enhance the proliferation of *E. africanus*.

Acknowledgements The University of KwaZulu-Natal and National Research Foundation, South Africa provided financial support. LP and KD thank the Ministry of Education, Youth and Sports, Czech Republic (Grant LO1204 from the National Program of Sustainability I.) for financial support. We thank Mrs Alison Young (UKZN Botanical Garden, Pietermaritzburg, South Africa) and her Staff for their assistance in maintaining the greenhouse facilities.

Author contributions OMM conducted the experiments, collected and analysed data and wrote the draft paper with the help of MM and AOA. LP and KD synthesized *meta*-topolin, *meta*-topolin riboside and 6-(3-hydroxybenzylamino)-9-(tetrahydropyran-2-yl)purine. JFF and JVS provided funding and research facilities, respectively. All authors edited the paper.

References

Amoo SO, Van Staden J (2012) Influence of plant growth regulators on shoot proliferation and secondary metabolite production in micropropagated *Huernia hystrix*. Plant Cell Tiss Organ Cult 112:249–256

Amoo SO, Aremu AO, Van Staden J (2012) In vitro plant regeneration, secondary metabolite production and antioxidant activity of micropropagated *Aloe arborescens* Mill. Plant Cell Tiss Organ Cult 111:345–358

Aremu AO, Bairu MW, Doležal K, Finnie JF, Van Staden J (2012) Topolins: a panacea to plant tissue culture challenges? Plant Cell Tiss Organ Cult 108:1–16

Bairu MW, Stirk WA, Dolezal K, Van Staden J (2007) Optimizing the micropropagation protocol for the endangered *Aloe polyphylla*: can *meta*-topolin and its derivatives serve as replacement for benzyladenine and zeatin? Plant Cell Tiss Organ Cult 90:15–23

Brown DE, Rashotte AM, Murphy AS, Normanly J, Tague BW, Peer WA, Taiz L, Muday GK (2001) Flavonoids act as negative regulators of auxin transport in vivo in *Arabidopsis*. Plant Physiol 126:524–535

Buer CS, Muday GK (2004) The transparent testa4 mutation prevents flavonoid synthesis and alters auxin transport and the response of *Arabidopsis* roots to gravity and light. Plant Cell 16:1191–1205

Catarino MD, Silva AMS, Saraiva SC, Sobral AJFN, Cardoso SM (2015) Characterization of phenolic constituents and evaluation of antioxidant properties of leaves and stems of *Eriocephalus africanus*. Arab J Chem. 10.1016/j.arabjc.2015.04.018

Chang HS, Charkabarty D, Hahn EJ, Paek KY (2003) Micropropagation of calla lily (*Zantedeschia albomaculata*) via in vitro shoot tip proliferation. In Vitro Cell Dev Biol-Plant 39:129–134

de Klerk G-J, van der Krieken W, de Jong JC (1999) Review the formation of adventitious roots: new concepts, new possibilities. In Vitro Cell Dev Biol-Plant 35:189–199

Doležal K, Popa I, Kryštof V, Spíchal L, Fojtíková M, Holub J, Lenobel R, Schmülling T, Strnad M (2006) Preparation and biological activity of 6-benzylaminopurine derivatives in plants and human cancer cells. Bioorg Med Chem 14:875–884

Doležal K, Malá J, Máchová P, Cvrcková H, Karady M, Novák O, Szucova L, Mikulik J, Spichal L, Strnad M. 2011. New cytokinin derivatives—their discovery, development and use for micropropagation of endangered tree species. BMC Proceedings. BioMed Central Ltd, p O46

Escalona M, Cejas I, González-Olmedo J, Capote I, Roels S, Cañal M, Rodríguez R, Sandoval J, Debergh P (2003) The effect of *meta*-topolin on plantain propagation using a temporary immersion bioreactor. InfoMusa 12:28–30

Frébort I, Kowalska M, Hluska T, Frébortová J, Galuszka P (2011) Evolution of cytokinin biosynthesis and degradation. J Exp Bot 62:2431–2452

Gentile A, Gutiérrez MJ, Martinez J, Frattarelli A, Nota P, Caboni E (2014) Effect of *meta*-topolin on micropropagation and adventitious shoot regeneration in *Prunus* rootstocks. Plant Cell Tiss Organ Cult 118:373–381

Kamínek M, Motyka V, Vaňková R (1997) Regulation of cytokinin content in plant cells. Physiol Plant 101:689–700

Khan T, Abbasi BH, Khan MA, Shinwari ZK (2016) Differential effects of thidiazuron on production of anticancer phenolic compounds in callus cultures of *Fagonia indica*. Appl Biochem Biotechnol 179:46–58

Lall N, Kishore N (2014) Are plants used for skin care in South Africa fully explored? J Ethnopharmacol 153:61–84

Ljung K, Hull AK, Celenza J, Yamada M, Estelle M, Normanly J, Sandberg G (2005) Sites and regulation of auxin biosynthesis in *Arabidopsis* roots. Plant Cell 17:1090–1104

Ludwig-Müller J (2000) Indole-3-butyric acid in plant growth and development. Plant Growth Regul 32:219–230

Makunga N, Philander L, Smith M (2008) Current perspectives on an emerging formal natural products sector in South Africa. J Ethnopharmacol 119:365–375

Malá J, Máchová P, Cvrčková H, Karady M, Novák O, Mikulík J, Hauserová E, Greplová J, Strnad M, Doležal K (2009) Micropropagation of wild service tree (*Sorbus torminalis* [L.] Crantz): the regulative role of different aromatic cytokinins during organogenesis. J Plant Growth Regul 28:341–348

Moyo M, Finnie JF, Van Staden J (2012) Topolins in *Pelargonium sidoides* micropropagation: do the new brooms really sweep cleaner? Plant Cell Tiss Organ Cult 110:319–327

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497

Murphy A, Peer AW, Taiz L (2000) Regulation of auxin transport by aminopeptidases and endogenous flavonoids. Planta 211:315–324

Niedz RP, Evens TJ (2010) The effects of benzyladenine and *meta*-topolin on in vitro shoot regeneration of a *Citrus citrandarin* rootstock. Res J Agric Biol Sci 6:45–53

Njenga E, Viljoen A (2006) In vitro 5-lipoxygenase inhibition and anti-oxidant activity of *Eriocephalus* L.(Asteraceae) species. S Afr J Bot 72:637–641

Njenga E, Van Vuuren S, Viljoen A, Eloff J (2005) Antimicrobial activity of *Eriocephalus* L. species. S Afr J Bot 71:81–87

Palacio L, Cantero JJ, Cusidó RM, Goleniowski ME (2012) Phenolic compound production in relation to differentiation in cell and tissue cultures of *Larrea divaricata* (Cav.). Plant Sci 193–194:1–7

Peer WA, Murphy AS (2007) Flavonoids and auxin transport: modulators or regulators? Trends Plant Sci 12:556–563

Philander LA (2011) An ethnobotany of Western Cape Rasta bush medicine. J Ethnopharmacol 138:578–594

Plíhalová L, Vylíčilová H, Doležal K, Zahajská L, Zatloukal M, Strnad M (2016) Synthesis of aromatic cytokinins for plant biotechnology. New Biotechnol 33:614–624

Salie F, Eagles P, Leng H (1996) Preliminary antimicrobial screening of four South African Asteraceae species. J Ethnopharmacol 52:27–33

SANBI (2015). Red list of South African plants. http://redlist.sanbi.org/

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am J Enol Vitic 16:144–158

Štefančič M, Štampar F, Osterc G (2005) Influence of IAA and IBA on root development and quality of *Prunus* 'GiSelA 5' leafy cuttings. HortScience 40:2052–2055

Szopa A, Ekiert H (2012) In vitro cultures of *Schisandra chinensis* (Turcz.) Baill.(Chinese Magnolia Vine)—a potential biotechnological rich source of therapeutically important phenolic acids. Appl Biochem Biotechnol 166:1941–1948

Szopa A, Ekiert H (2014) Production of biologically active phenolic acids in *Aronia melanocarpa* (Michx.) Elliott in vitro cultures cultivated on different variants of the Murashige and Skoog medium. Plant Growth Regul 72:51–58

Szüčová L, Spíchal L, Doležal K, Zatloukal M, Greplová J, Galuszka P, Kryštof V, Voller J, Popa I, Massino FJ, Jørgensen J-E, Strnad M (2009) Synthesis, characterization and biological activity of ring-substituted 6-benzylamino-9-tetrahydropyran-2-yl and 9-tetrahydrofuran-2-ylpurine derivatives. Bioorg Med Chem 17:1938–1947

Taylor LP, Grotewold E (2005) Flavonoids as developmental regulators. Curr Opin Plant Biol 8:317–323

Tubić L, Savić J, Mitić N, Milojević J, Janošević D, Budimir S, Zdravković-Korać S (2015) Cytokinins differentially affect regeneration, plant growth and antioxidative enzymes activity in chive (*Allium schoenoprasum* L.). Plant Cell Tiss Organ Cult 124:1–14

Verdeguer M, Blázquez MA, Boira H (2009) Phytotoxic effects of *Lantana camara, Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils in weeds of Mediterranean summer crops. Biochem Syst Ecol 37:362–369

Verstraeten I, Geelen D (2015) Adventitious rooting and browning are differentially controlled by auxin in rooting-recalcitrant *Elegia capensis* (Burm. f.) Schelpe. J Plant Growth Regul 34:475–484

Viljoen AM, Njenga EW, Van Vuuren SF, Bicchi C, Rubiolo P, Sgorbini B (2006) Essential oil composition and in vitro biological activities of seven Namibian species of *Eriocephalus* L.(Asteraceae). J Essent Oil Res 18:124–128

Werbrouck SPO, Strnad M, Van Onckelen HA, Debergh PC (1996) *Meta*-topolin, an alternative to benzyladenine in tissue culture? Physiol Plant 98:291–297

Wiesman Z, Riov J, Epstein E (1989) Characterization and rooting ability of indole-3-butyric acid conjugates formed during rooting of mung bean cuttings. Plant Physiol 91:1080–1084

Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64:555–559