New Biotechnology 33 (2016) 883–890

Contents lists available at ScienceDirect

New Biotechnology

journal homepage: www.elsevier.com/locate/nbt

Full length Article

Auxin-cytokinin interaction and variations in their metabolic products in the regulation of organogenesis in two *Eucomis* species

Adeyemi O. Aremu[a], Lenka Plačková[b], Aleš Pěnčík[b], Ondřej Novák[b], Karel Doležal[b], Johannes Van Staden[a,*]

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

ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form 12 July 2016
Accepted 3 September 2016
Available online 5 September 2016

Keywords:
Asparagaceae
Indole-3-acetic acid
Phytohormones
meta-Topolin
Ornamentals
Rooting

ABSTRACT

In the current study, we evaluated the effect of α-naphthaleneacetic acid (NAA) individually or in combination with different cytokinins (CKs) including benzyladenine (BA), *meta*-topolin (*m*T) and isopentenyladenine (iP) on organogenesis, auxin and CK content in *Eucomis autumnalis* subspecies *autumnalis* (EA) and *Eucomis zambesiaca* (EZ). These species were used as model plants due to their ornamental and medicinal properties. Three leaf explants were inoculated in screw-cap jars containing 30 mL Murashige and Skoog (MS) media supplemented with 5 μM NAA alone or in combination with 5 μM CK (BA, *m*T or iP). After 10 weeks (EA) or 15 weeks (EZ), parameters including shoot and root growth as well as plant fresh weight were recorded. For analysis of auxin and CK content, whole plantlets were harvested, pooled and freeze-dried for the different treatments. In both species, shoot and root proliferation as well as plant biomass were generally higher when NAA was combined with the individual CK than in NAA or CK treatment. The highest concentration of indole-3-acetic acid (IAA, 619 pmol g^{-1} DW) and 2-oxindole-3-acetic acid (OxIAA, 2381 pmol g^{-1} DW) were observed in EA-treated with NAA alone while *m*T treatment (without NAA) had the most abundant indole-3-acetyl-L-aspartic acid (IAAsp, 904 and 582 pmol g^{-1} DW for EA and EZ, respectively) in both species. A significant concentration of total endogenous CK accumulated in both *Eucomis* regenerants from *m*T and *m*T + NAA when compared to the other treatments. The majority of the detected CKs were of the aromatic CK-type, mainly free bases. The potential physiological roles of these quantified phytohormones in relation to the observed morphological responses are discussed.

1. Introduction

Auxins and cytokinins (CKs) are important phytohormones responsible for plant growth and development, playing a vital role in the general life cycle of plants. Researchers have extensively studied and documented the physiological roles of these phytohormones in different plants [1–7]. While auxins and CKs are often known for specific physiological functions, they are also known to mutually coordinate and act as important signalling molecules, thereby regulating growth and development in lower and higher

Abbreviations: BA, N^6-benzyladenine; BA9G, N^6-benzyladenine-9-glucoside; BAR, N^6-benzyladenine-9-riboside; BAR5′MP, N^6-benzyladenine-9-riboside-5′-monophosphate; CK, cytokinin; *cZ*, *cis*-zeatin; *cZOG*, *cis*-zeatin-*O*-glucoside; *cZR*, *cis*-zeatindata-9-riboside; *cZR5′MP*, *cis*-zeatindata-9-riboside-5′-monophosphate; *cZROG*, *cis*-zeatin-*O*-glucoside riboside; DHZ, dihydrozeatin; DHZOG, dihydrozeatin-*O*-glucoside; DHZR, dihydrozeatin-9-riboside; DHZR5′MP, dihydrozeatin-9-riboside-5′-monophosphate; DHZROG, dihydrozeatin-*O*-glucoside riboside; ESI, electro-spray interface; IAA, indole-3-acetic acid; IAAsp, indole-3-acetyl-L-aspartic acid; IAGlu, indole-3-acetyl-L-glutamic acid; IBA, indole-3-butyric acid; iP, N^6-isopentenyladenine; iP9G, N^6-isopentenyladenine-9-glucoside; iPR, N^6-isopentenyladenine-9-riboside; iPR5′MP, N^6-isopentenyladenine-9-riboside-5′-monophosphate; MS, Murashige and Skoog medium; *m*T, *meta*-topolin; *m*T9G, *meta*-topolin-9-glucoside; *m*TOG, *meta*-topolin-*O*-glucoside; mTR, *meta*-topolin-9-riboside; mTR5′MP, *meta*-topolin-9-riboside-5′-monophosphate; NAA, α-naphthaleneacetic acid; *o*T, *ortho*-topolin; *o*TR, *ortho*-topolin-9-riboside; PPF, Photosynthetic photon flux density; pT, *para*-Topolin; pTOG, *para*-Topolin-*O*-glucoside; pTR, *para*-topolin-9-riboside; pTR5′MP, *para*-topolin-9-riboside-5′-monophosphate; pTROG, *para*-topolin-*O*-glucoside riboside; tZ, *trans*-zeatin; tZ9G, *trans*-zeatindata-9-glucoside; tZOG, *trans*-zeatin-*O*-glucoside; tZR, *trans*-zeatindata-9-riboside; tZR5′MP, *trans*-zeatindata-9-riboside-5′-monophosphate; tZROG, *trans*-zeatin-*O*-glucoside riboside; UHPLC®, Ultra-high performance liquid chromatography.

* Corresponding author.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

http://dx.doi.org/10.1016/j.nbt.2016.09.001
1871-6784/

plants [7–12]. Since the late 1950's, the importance of the appropriate balance between auxin and CK as key regulators of *in vitro* organogenesis was established [13]. Thus, the application of auxins and CKs are often important and desirable in an attempt to propagate different plants efficiently *in vitro* [14,15]. Increasing evidence of the significant influence and importance of these exogenously-added phytohormones on the general plant growth and architecture as well as the endogenous phytohormones cannot be over-emphasised [16–21].

The genus *Eucomis* (family: Asparagaceae, previously Hyacinthaceae) consists of a number of species which are prized for their ornamental values and therapeutic properties [22,23]. In traditional medicine, these bulbous plants are commonly used for a wide range of health conditions by the local population [22]. The increasing benefits derived from these species necessitate a better understanding of their cultivation, general growth and physiology. Furthermore, an increasing awareness of the need to conserve plant genetic resources, particularly those threatened by natural habitat destruction, has made their cultivation an attractive alternative for many valuable plants [24]. Even though a number of micropropagation protocols have been developed for *Eucomis* species [23,25,28], their hormone physiology, particularly during *in vitro* propagation has remained speculative. Improved understanding of the pattern and distribution of these classical phytohormones in *in vitro* propagated plants may provide a viable avenue to easily manipulate and efficiently enhance the propagation protocols [16–18,20].

In the current study, the effects of α-naphthaleneacetic acid (NAA), with either isoprenoid or aromatic CK, on the growth, endogenous auxins and CKs in two *Eucomis* species were investigated. These are known to possess different regeneration cycles and the choice of selected phytohormones were based on *in vitro* responses from previous protocols [23,26,27]. Among the auxins, NAA has the advantage of easy penetration of the plasma membrane without the need for active uptake which allows for a rapid increase in NAA concentration inside the cells [12]. The addition of an isoprenoid CK (N^6-isopentenyladenine, iP) is important due to the observed differences in metabolic fate of aromatic (*meta*-topolin, *m*T and benzyladenine, BA) and isoprenoid CKs [3,5,17]. In addition, these aromatic CKs (*m*T and BA) have been documented as exhibiting different biological responses in several plants [15].

2. Materials and methods

2.1. Source of chemicals

meta-Topolin was synthesised as previously described by Doležal et al. [29] while iP, BA and NAA were purchased from Sigma-Aldrich (Steinheim, Germany). Formic acid and methanol used for preparing mobile phases for phytohormone analytical procedure were from Merck (Darmstadt, Germany). The CK standards, authentic and deuterium-labelled were from Olchemim Ltd. (http://www.olchemim.cz/), Czech Republic. [indole-$^{13}C_6$]-IAA was obtained from Cambridge Isotope Laboratories (http://www.isotope.com) while other auxin standards were purchased from Olchemim Ltd (http://www.olchemim.cz/) Czech Republic. All chemicals used in the current study were of analytical grade.

2.2. Explant source and in vitro organogenesis experimental design

Regularly maintained laboratory stock of aseptically-obtained leaves of *Eucomis autumnalis* subspecies *autumnalis* (EA) and *Eucomis zambesiaca* (EZ) were used for the current experiments. Three leaf explants (approximately $1 \times 1\,cm^2$) were inoculated in screw-cap jars (110×60 mm, 300 mL volume) containing 30 mL Murashige and Skoog (MS) medium [30]. For treatment regimes, the media were supplemented with 5 μM NAA alone or in combination with 5 μM CK (BA, *m*T or iP). The controls consisted of MS alone (hormone-free) or NAA alone. All media were adjusted to pH 5.8 and solidified with agar ($8\,g\,L^{-1}$) prior to autoclaving at 121 °C for 20 min. Each treatment had 24 explants and the experiments were conducted twice. The cultures were incubated in 16/8 h light/dark conditions with a photosynthetic photon flux density (PPF) of $45\,\mu mol\,m^{-2}\,s^{-1}$ at 25 ± 2 °C for 10 (EA) or 15 weeks (EZ). Thereafter, parameters including shoot (number and length) and root (number and length) growth as well as plant fresh weight were recorded.

2.3. Analysis of endogenous auxin and CK content in plant materials

Whole plantlets were harvested, pooled and freeze-dried on the basis of treatment types. Lyophilized plant materials were used for the quantification of auxin and CK content. Previously established protocols by Pěnčík et al. [31] and Novák et al. [32] as described for auxins and CKs, respectively were used. Additional modifications applied in the experiment have been fully outlined recently [18,33]. The samples were analyzed in triplicate and the quantification was conducted using an ultra-high performance liquid chromatograph (UHPLC®; Waters, Milford, MA, USA) coupled to a Xevo® TQ-MS™ (Waters, Milford, MA, USA) triple quadrupole mass spectrometer equipped with an electro-spray interface (ESI).

2.4. Data analysis

Auxin and CK content were analyzed using Masslynx 4.1 software (Waters, Milford, MA, USA) as determined by the standard isotope-dilution method in the samples [34,35]. The statistical differences between the mean values of untreated and NAA-treated plants with or without CK were determined by subjecting the data to the Student's *t* test. The analysis was performed with SigmaPlot software (version 8.0) and the significance level was determined at $P \leq 0.05$ (*), $P \leq 0.01$ (**) and $P \leq 0.001$ (***).

3. Results

3.1. Growth responses

The addition of NAA to the media generally increased shoot number in both species investigated (Fig. 1A). In EA and EZ plantlets, the number of shoots was doubled when NAA was combined with *m*T treatment compared with the application of *m*T alone. In terms of the effectiveness of the applied CKs individually, BA treatment produced more shoots in both *Eucomis* species.

Both *Eucomis* plantlets from NAA-containing media (with or without CK) had longer shoots than those lacking NAA (Fig. 1B). However, no significant difference was observed in shoot length of EZ regenerated with or without NAA in the absence of CK treatment. When compared to CK-free media, the use of CK, especially the aromatic (BA and *m*T) ones, reduced the shoot length in the regenerants.

The number of roots observed in the *in vitro*-derived *Eucomis* species was remarkably higher in the presence of NAA with or without CKs (Fig. 1C). Among the tested CKs, BA had the most noticeable inhibitory effect on number of roots in both EA and EZ. In contrast, iP had the least inhibitory rooting (number) effect among the tested CKs.

When compared to NAA-absent treatments, NAA-supplemented media generally produced longer roots which was more evident in EZ (Fig. 1D). In contrast, the addition of NAA caused a