



Cytokinin biosynthesis *ISOPENTENYLTRANSFERASE* genes are differentially expressed during phyllomorph development in the acaulescent *Streptocarpus rexii* (Gesneriaceae)

Y.-Y. Chen ^{a,b,c,1}, K. Nishii ^{b,d,*}, A. Spada ^e, C.-N. Wang ^a, H. Sakakibara ^f, M. Kojima ^f, F. Wright ^g, K. MacKenzie ^g, M. Möller ^b

^a Institute of Ecology and Evolutionary Biology, Department of Life Science, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan

^b Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, Scotland, UK

^c University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, UK

^d Tokyo Gakugei University, 4-1-1 Nukuikitamachi, Koganei, Tokyo 184-8501, Japan

^e Dipartimento di Scienze Agrarie e Ambientali, Milan University, Via Celoria 2, 20133 Milan, Italy

^f RIKEN Center for Sustainable Resource Science, 1-7-22, Suehiro, Tsurumi, Yokohama 230-0045, Japan

^g Biomathematics and Statistics Scotland, JCMB, The King's Buildings, Peter Guthrie Tait Road, Edinburgh EH9 3FD, Scotland, UK

ARTICLE INFO

Article history:

Received 17 June 2016

Received in revised form 6 October 2016

Accepted 13 December 2016

Available online xxxx

Edited by K Doležal

Keywords:

Streptocarpus

Cytokinin

Isopentenyltransferase

Leaf

Meristem

ABSTRACT

The enzyme ISOPENTENYLTRANSFERASE (IPT) is responsible for the rate limiting step of cytokinin biosynthesis, an important plant hormone with key roles in meristem maintenance and organ development. In this study, we isolated *IPT* genes from the acaulescent *Streptocarpus rexii*, a plant that shows an unorthodox development starting with post-germination anisocotily, in which cytokinins play an integral role. Three adenosine phosphate-*IPTs* and two tRNA-*IPTs* were isolated from *S. rexii*. Their expression levels and patterns in different tissues were compared by means of realtime-PCR and mRNA *in-situ* hybridization. We found that each *SrIPT* had a distinctive expression pattern. Interestingly, in vegetative tissues as well as in meristems only the adenosine phosphate-*IPT SrIPT5* and the tRNA-*IPT SrIPT9* were found. In addition, they were differentially affected by external hormone application, suggesting their different regulation and expression during meristem formation and maintenance and lamina growth. Our results indicate that *SrIPTs* are involved in shaping the architecture of *S. rexii*, working differentially and redundantly, and show that differentially expressed *IPT* genes regulate plant form.

© 2016 SAAB. Published by Elsevier B.V. All rights reserved.

1. Introduction

Cytokinins are important plant hormones and play key roles in plant development and maintenance (Werner et al., 2001, 2003; Osugi and

Sakakibara, 2015), sink-source balances (Werner et al., 2001; Takei et al., 2001a; Werner et al., 2003), senescence (Gan and Amasino, 1995) and cell division (Riou-Khamlichi et al., 1999). Cytokinins are also important for maintaining the shoot apical meristem (SAM), the source of stem tissue and lateral organs (Werner et al., 2003; Miyawaki et al., 2006).

In *Streptocarpus rexii*, a plant with a unique morphology, cytokinins have important roles in shaping its architecture, since external cytokinin applications severely altered its development and morphology. This acaulescent species lacks a conventional SAM and develops an irregular rosette composed of meristem-bearing leaves termed *phyllomorphs* (Jong, 1970; Hilliard and Burt, 1971; Jong and Burt, 1975; Jong, 1978). Just after germination, both cotyledons are equal in size as in typical dicots (Fig. 1a). However, a SAM is not established between the cotyledons and soon after they unfold, the two cotyledons develop unequally establishing *anisocotily* (Jong, 1970; Hilliard and Burt, 1971; Jong and Burt, 1975; Nishii and Nagata, 2007; Fig. 1b,c). The larger cotyledon, the *macrocotyledon*, continuously expands in size while the smaller cotyledon, the *microcotyledon*, stops any further development and eventually withers away. The macrocotyledon develops into the first phyllomorph (*cotyledonary phyllomorph*)

Abbreviations: BAP, 6-Benzylaminopurine; cZ, *cis*-Zeatin; cZR, cZ riboside; cZRPs, cZR 5'-phosphates; DAU, days after cotyledon unfolding; DIG, digoxigenin; DMAPP, dimethylallyl diphosphate; DMSO, dimethylsulfoxide; DZ, dihydrozeatin; DZR, DZ riboside; DZRPs, DZR 5'-phosphates; GA, gibberellic acid; GA2ox, GA2-oxidase; GA20ox, GA20-oxidase; iP, N⁶-(Δ²-isopentenyl)adenine; iPR, iP riboside; iPRPs, iPR 5'-phosphates; IPT, isopentenyltransferase; KNOX1, class 1 KNOX genes; MS medium, Murashige and Skoog medium; NAA, 1-Naphthaleneacetic acid; RAM, root apical meristem; SAM, shoot apical meristem; STM, SHOOTMERISTEMLESS; tZ, *trans*-Zeatin; tZR, tZ riboside; tZRPs, tZR 5'-phosphates.

* Corresponding author at: Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, Scotland, UK.

E-mail addresses: y.chen@rbge.ac.uk (Y.-Y. Chen), kanaenishii@gmail.com, k.nishii@rbge.ac.uk (K. Nishii), alberto.spada@unimi.it (A. Spada), leafy@ntu.edu.tw (C.-N. Wang), sakaki@riken.jp (H. Sakakibara), mikiko@riken.jp (M. Kojima), Frank.Wright@hutton.ac.uk (F. Wright), Katrin.MacKenzie@hutton.ac.uk (K. MacKenzie), m.moeller@rbge.ac.uk (M. Möller).

¹ Both authors contributed equally: KN, YY.

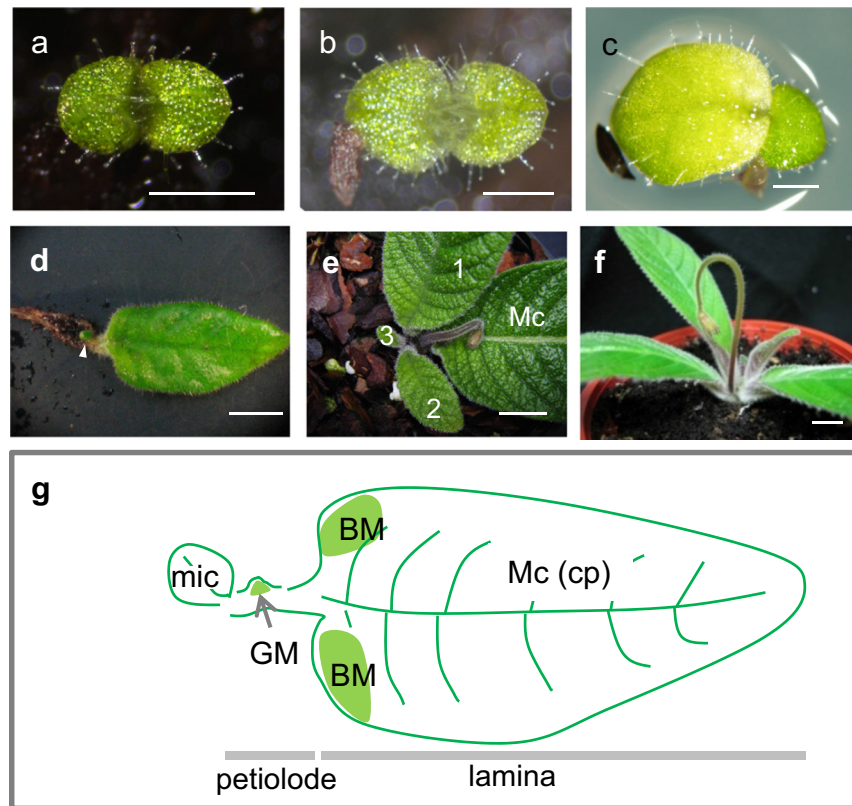


Fig. 1. The unique development of the rosulate *Streptocarpus rexii*. a Seedling with fully unfolded cotyledons representing ‘day 1 after cotyledon unfolding’ (1 DAU). b 10 DAU, beginning of the anisocotylous phase. c 30 DAU, strongly anisocotylous seedling. Bars: 0.5 mm. d Anisocotylous seedling with large cotyledonary phyllomorph (macrocotyledon) and microcotyledon (arrowhead). e Plant with several additional phyllomorphs, formed in numbered succession. f Inflorescence initiated at the base of the phyllomorph in the reproductive stage. Bars: 1 cm. g Schematic illustration of a phyllomorph (modified from Jong and Burtt, 1975). The macrocotyledon retains the basal meristem and the groove meristem, and grows to become the cotyledonary phyllomorph. BM: basal meristem, GM: groove meristem, Mc: macrocotyledon, mic: microcotyledon, cp: cotyledonary phyllomorph.

(Fig. 1). Phyllomorphs consist of a lamina and *petiolode*, a stem-like petiole (Jong, 1970; Hilliard and Burtt, 1971; Jong and Burtt, 1975; Fig. 1g) and their development is governed by three meristems, the *basal meristem* at the proximal end of the lamina for lamina growth, the *petiolode meristem* for petiolode and midrib extension and thickening, and the *groove meristem* located at the juxtaposition between the lamina and petiolode that forms new phyllomorph primordia (Fig. 1g), the reiteration of this process results in a false rosette (Fig. 1e, f).

Previous studies (Rosenblum and Basile, 1984; Nishii et al., 2004; Mantegazza et al., 2009) demonstrated that the synthetic cytokinin 6-benzylaminopurine (BAP) causes both cotyledons to develop into cotyledonary phyllomorphs creating *macrocotyledonary isocotily* in several *Streptocarpus* species (i.e. *S. prolixus*, *S. wendlandii* and *S. rexii*), suggesting a pivotal role for cytokinin in the establishment of anisocotily.

The enzyme ISOPENTENYLTRANSFERASE (IPT), is responsible for the rate limiting step of cytokinin biosynthesis (Kakimoto, 2001; Takei et al., 2001b). IPTs transfer the isoprenoid group from isoprenyl donor dimethylallyl diphosphate (DMAPP) to the N^6 -position of adenosine, forming isopentenyladenosine, which is a precursor for cytokinin biosynthesis (Sakakibara, 2006). There are two different forms of IPTs in plants, adenosine phosphate-IPTs and tRNA-IPTs. Adenosine phosphate-IPTs in plants use primarily ATP, ADP as substrates and are responsible for the synthesis of N^6 -(Δ^2 -isopentenyl)adenine (iP) or *trans*-Zeatin (tZ) type cytokinins. tRNA-IPTs, on the other hand, catalyse the addition of prenyl-moiety to a tRNA-bound adenine nucleotide and contributes to the formation of certain cytokinins, mainly *cis*-Zeatin (cZ) in *Arabidopsis* (Miyawaki et al., 2006; Sakakibara, 2006).

Seven adenosine phosphate-IPTs and two tRNA-IPTs (Kakimoto, 2001; Takei et al., 2001b; Golovko et al., 2002; Miyawaki et al., 2004) are known to belong to the multigene ISOPENTENYLTRANSFERASE (IPT)

family in *Arabidopsis thaliana*. Each *AtIPT* gene showed a specific expression patterns and different hormonal feedback regulations (Miyawaki et al., 2004). At the same time, *AtIPTs* work highly redundantly, since single or double *IPT* gene mutants did not show phenotypes, only multiple (triple or quadruple) mutants did (Miyawaki et al., 2006). Although there is a high degree of redundancy, some *IPT* genes have specific roles in meristems, with some under the control of class 1 *KNOX* genes (*KNOX1*). *KNOX1* genes are responsible for the formation and maintenance of the SAM (Hake et al., 2004) and these induced up-regulation of *AtIPT7* but not of other *AtIPTs* (Yanai et al., 2005).

The present study investigates the role of the cytokinin biosynthesis genes ISOPENTENYLTRANSFERASE in the morphogenesis of *Streptocarpus*, with particular focus on the meristems. We isolated *IPT* genes from the rosulate *S. rexii* and characterized their expression patterns during plant growth with special emphasis on macrocotyledon and phyllomorph development, in an attempt to find specific *IPT* genes linked to meristem formation and maintenance. The results are compared with those obtained for model plants, particularly *A. thaliana*, and the role of cytokinin and *IPTs* in the phyllomorph morphogenesis in *S. rexii* discussed.

2. Materials and methods

2.1. Plant material

Seeds of *Streptocarpus rexii* (originally collected from Tsitsikamma, Cape Province, South Africa, and subsequently grown at the Royal Botanic Garden Edinburgh, RBGE, accession number 20030814) were cultivated at 22 °C–24 °C under 16 h of light and 8 h of darkness, and a relative humidity of 80%, on sterilized soil (Potgrond H, Klasmann-Deilmann GmbH, Germany). The plants were fertilized once a week

Download English Version:

<https://daneshyari.com/en/article/5763038>

Download Persian Version:

<https://daneshyari.com/article/5763038>

[Daneshyari.com](https://daneshyari.com)