Taylor & Francis
Taylor & Francis Group

Botany Letters

ISSN: 2381-8107 (Print) 2381-8115 (Online) Journal homepage: http://www.tandfonline.com/loi/tabg21

Developing a new variety of kentia palms (Howea forsteriana): up-regulation of cytochrome b561 and chalcone synthase is associated with red colouration of the stems

Alba Herraiz, Laura Stokes, Colin Turnbull, Ian Hutton, William Baker & Vincent Savolainen

To cite this article: Alba Herraiz, Laura Stokes, Colin Turnbull, Ian Hutton, William Baker & Vincent Savolainen (2018): Developing a new variety of kentia palms (Howea forsteriana): up-regulation of cytochrome b561 and chalcone synthase is associated with red colouration of the stems, Botany Letters, DOI: 10.1080/23818107.2017.1415818

To link to this article: https://doi.org/10.1080/23818107.2017.1415818

© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group

View supplementary material

Published online: 05 Jan 2018.

Submit your article to this journal

Article views: 31

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=tabg21

Download by: [Imperial College London Library] **Date:** 11 January 2018, At: 06:54

BOTANY LETTERS, 2018
https://doi.org/10.1080/23818107.2017.1415818

Taylor & Francis
Taylor & Francis Group

OPEN ACCESS Check for updates

Developing a new variety of kentia palms (*Howea forsteriana*): up-regulation of cytochrome b561 and chalcone synthase is associated with red colouration of the stems

Alba Herraiz[a], Laura Stokes[a], Colin Turnbull[b], Ian Hutton[c], William Baker[d] and Vincent Savolainen[a,d,e]

[a]Department of Life Sciences, Imperial College London, Silwood Park Campus, Ascot, UK; [b]Department of Life Sciences, Imperial College London, South Kensington Campus, London, UK; [c]Lord Howe Island Museum, Lord Howe Island, Australia; [d]Royal Botanic Gardens, Kew, Richmond, UK; [e]Department of Botany and Plant Biotechnology, University of Johannesburg, Johannesburg, South Africa

ABSTRACT

The kentia palm (*Howea forsteriana*) is endemic to a 12 km^2 volcanic island in the Tasman Sea, Lord Howe Island. It is one of the most traded houseplants in the world. The typical kentia palm presents a dark green-coloured stem. Note that the stem is made of leaf rachis and petioles, which are intermingled towards the base. However, we discovered on Lord Howe Island a new biological variety that has a red stem. Red-stemmed palm species are known and highly demanded as decorative plants. However, these red palm horticultural varieties require tropical or subtropical conditions to grow, hence commercialization is limited. Thus, a red-stemmed variety of *H. forsteriana* may have tremendous market potential. Nonetheless, palm trees grow generally slowly and often reach maturity at 15–20 years old or later, which may make conventional strategies unsuitable for the propagation of a new variety. This difficulty needs to be addressed before commercialization can be achieved. Here, we found that anthocyanin is responsible for the red colouration of the stem in the new variety. Using RNA sequencing and quantitative PCR, we identified two gene isoforms displaying altered expression associated with this red colouration, encoding a cytochrome b561 and a chalcone synthase. The latter protein is known to be part of the anthocyanin biosynthesis pathway, which plays a central role in pigmentation in plants. The levels of cytochrome b561 transcripts accumulated were found to be well correlated with an increased anthocyanin concentration in the red stems.

ARTICLE HISTORY
Received 12 October 2017
Accepted 7 December 2017

KEYWORDS
Howea; horticulture; anthocyanin; RNA-seq; qPCR

Downloaded by [Imperial College London Library] at 06:54 11 January 2018

Introduction

The global horticulture business is growing extremely fast. First, demand from the general public for high quality houseplants, flowers and garden plants have never been so high. Second, the need for new crops is keystone to feeding an ever-growing human population. The horticulture industry is worth several tens of billions of euros worldwide; its value is over two and a half billion pounds in the United Kingdom alone (DEFRA 2017). Palms are some of the most successfully traded plants, with numerous species sold for indoor and outdoor cultivation as ornamentals, and as crops (date palm, oil palm, coconuts, etc.). The kentia palm (*Howea forste-riana* Becc., Figure 1A, B) is one of the most widely exported houseplants in the world and is worth over seven million euros per year in the Dutch horticultural industry alone (Riffle, Craft, and Zona 2012; Savolainen et al. 2006; LHI Nursery, pers. comm.).

Wild kentias are only found on a 12 km^2 island in the middle of the Tasman Sea, Lord Howe Island (LHI). The first houses on the island were built from *Howea* trunks and thatched with their leaves (hence their other common name, thatch palm). The seeds of *Howea* have been harvested for export and traded by Lord Howe Island since Victorian times; such was the popularity of kentias that they adorned the room in which Queen Victoria lay in state in the Albert Memorial Chapel in 1901.

Every year, 14 million kentia seedlings are produced worldwide. Historically, the peak production and exportation of kentia palm seedlings from LHI has been reported as 4 million, accounting then for more than 20% of the global market share (LHI Nursery, pers. comm.). Since the global financial crisis of 2008, the LHI has experienced a decline in the exports of kentia palm, predominantly driven by the increasing competition from growers in South Africa, Sri Lanka and California. LHI is now collecting 500,000 seeds a year, with a germination rate of up to 75%, which translates into 375,000 seedlings of green stemmed palms (LHI nursery, pers. comm.). This represents approximately 3% of the global market.

Because LHI is such a unique ecosystem, kentia palms are also a model in speciation research, as *H. forsteriana*

CONTACT Vincent Savolainen v.savolainen@imperial.ac.uk
Supplemental data for this article can be accessed https://doi.org/10.1080/23818107.2017.1415818

© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Figure 1. A young kentia palm has a green stem (A), which later forms a brown trunk as it gets older and trees are mature at around 15–20 years old (B). While they are younger and up to 4–5 metres in height, kentias are used as houseplants. (C) Occasionally, a red-stemmed variety is found in the wild, on *Howea*'s native Lord Howe Island in the Tasman Sea.

Downloaded by [Imperial College London Library] at 06:54 11 January 2018

and its sister species *H. belmoreana* (C.Moore & F.Muell.) Becc. evolved sympatrically on LHI (Savolainen et al. 2006). During field expeditions on LHI, Savolainen and colleagues have also discovered a new mutant variety of the kentia palm: instead of having the typical dark green colouration, it has a reddish stem (note that the stem is made of leaf rachis and petioles, which are intermingled towards the base; Figure 1C). These red-stemmed palms were very rare and found exclusively in *H. forsteriana* (although we heard reports that it could also be found in *H. belmoreana*, Larry Wilson, pers. comm.); it did not seem to be linked to any ecological condition. There are several species of palms that have bright red stems. For example, the red sealing wax palm (*Cyrtostachys renda* Blume) is popular in tropical gardens. A few other red-stemmed species have also been planted in public areas and along streets throughout the tropics (*Areca macrocalyx* Zipp. ex Blume, *Areca vestiaria* Giseke, *Dypsis rivularis* (Jum. & H.Perrier) Beentje & J.Dransf.). However, none of these species would tolerate indoor conditions; instead they require very high humidity levels, warm temperature and often, intensive sunlight. In contrast, kentias are very hardy and grow well under low light and cool temperatures, hence their immense success as decorative items across innumerable indoor public venues and households worldwide. In addition, red is an auspicious colour in Asia. Several red varieties of plants have been seen to quickly inundate the Chinese market, such as red leaved willows; a new red kentia palm has therefore tremendous horticulture potential.

There are, however, difficulties to overcome before the commercialization of a new kentia palm variety could be achieved. Traditional breeding programmes are particularly difficult with these palms, due to them being long-lived and reaching maturity only after 15–20 years. Here, we needed a different approach to develop, and potentially propagate, this variety. To disentangle the underlying genetics of the red-stemmed phenotype in kentia palms, we aimed (i) to identify the chemistry responsible for the red colouration of the stem, (ii) to identify genes potentially responsible for the colouration, and (iii) to analyse the stability of such phenotype in the context of Plant Breeders' Rights (PBR) under the International Convention for the Protection of New Varieties of Plants.

Material and methods

Assessment of varietal stability

For PBR (or Plant Variety Rights, PVR) to grant exclusive control for the propagation and harvest of a new, horticultural plant variety, it needs to be new, distinct, uniform and stable. New, distinct and uniform is relatively easy to demonstrate in the case of red-stemmed kentias; however, we needed to establish whether they are stable. To do so, ca. 1000 seeds of *H. forsteriana* were collected from 12 diverse locations around LHI. Seeds were fumigated before being planted in a perlite/peat soil mixture at the LHI Nursery. The planted seeds were

Downloaded by [Imperial College London Library] at 06:54 11 January 2018

stored in boxes lined with plastic bags in a dark shed for 3–6 months, and then transferred to plastic poly houses until germination. Then, 23 green-stemmed and 180 red-stemmed seedlings were selected and sent to Imperial College, where we planted them in a compost mixture that mimics their native calcareous soil (Hipperson et al. 2016). We visually checked for changes in colouration during four months.

Anthocyanin extraction and quantification

Because anthocyanin pigments confer red colouration to many plant tissues, in particular associated to the leaves, flowers and fruits, we measured its concentration in green and red stems of *H. forsteriana*. These plants were collected in the wild on LHI. We used a protocol adapted from Sivitz, Reinders, and Ward (2008). Using scalpel and tweezers, we shaved away the outer layer of the stems of 76 green-stemmed and 13 red-stemmed young palms growing in greenhouses at Imperial College and weighed them out to 100 mg (±5 mg). We added a solution of ethanol containing 1% hydrochloric acid (VWR International Ltd) and grounded the tissue using a pestle and mortar. The homogenate was centrifuged for 6 min at 14,000 rotations per minute, and a solution containing 1 ml of distilled water and 0.5 ml of chloroform (Sigma) was added to the supernatant. Tubes were shaken vigorously and centrifuged for a further 3 min to separate the pigments. The upper phase was used to measure absorbance at 535 nm on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Anthocyanin content was expressed as µg of cyanidin-3-glucoside (C3G) (i.e. the most common type of anthocyanin) per g of tissue, and calculated using the following formula: $C = (A/[\varepsilon \times L]) \times MW \times 1000$; where C is the concentration in µg/g, A is the absorbance at 535 nm, ε is the molar extinction coefficient of C3G (= 26900 M^{-1}), L is the path length of the cuvette in cm, and MW is the molecular weight of C3G (= 445) (Sirén, Sirén, and Sirén 2015). An unpaired *t*-test was used to statistically analyse differences in anthocyanin content among green- versus red-stemmed plants.

Total RNA sequencing

Another set of 12 stem samples were collected on LHI during the first week of September 2015 from palms growing in six different locations on calcareous soil, one of *H. forsteriana*'s typical habitats. Cuttings of the stems of five green and five red stemmed-palms were stored in RNAlater (Sigma) until used. Stem samples in RNAlater were shipped to BGI Tech Solutions (Hong Kong), where RNA was extracted (see below) and one TruSeq Library (Illumina) per sample was constructed. RNA sequencing (RNA-seq) was performed using an Illumina HiSeq 3000 platform, yielding 12.7 million (± 170,000) 50-base pairs single-end reads per library. Data filtering performed at BGI included removing sequencing adaptors, contaminations and low-quality bases from raw reads. After assessing sequence quality with FastQC tool (Andrews 2014), reads were mapped onto the reference transcriptome of *H. forsteriana* (Dunning et al. 2016), and read counts were calculated using the RSEM software package (Li and Dewey 2011). Relative read counts are a proxy for differential gene expression, which were compared between samples using the R package EdgeR (Robinson, McCarthy, and Smyth 2010).

Loci that were significantly differentially expressed (i.e. with $p < 0.01$, False Discovery Rate (FDR) < 0.1) were then aligned against the DNA sequence databases for the oil palm (*Elaeis guineensis* Jacq.) and the date palm (*Phoenix dactylifera* L.) to search for homology using BLASTN (Madden 2002).

Reverse transcription and quantitative PCR of selected genes

To validate RNA-seq results, we performed quantitative PCR (qPCR) of the 11 gene isoforms showing differential expression in RNA-seq data (Table 1). We used stem samples for which we had measured anthocyanin content (Table 1). Cuttings (<10 mg) of 12 red and 12 green stems were individually homogenised with pestle and mortar, using the following solution: 450 µl Buffer RLT (Qiagen), 4.5 µl β-mercaptoethanol (Sigma), and 50 µl RNA Isolation Aid (Thermo Fisher Scientific). Total RNA was then extracted using the RNeasy Plant Mini Kit (Qiagen) and then DNase-treated using the RNase-Free DNase Set (Qiagen), following the manufacturer's protocol. Concentration and quality of total RNA was assessed with a NanoDrop 2000. A total of 125 ng of RNA per sample was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Quantitative PCR primers specific to isoforms were designed using Geneious version 10.0.5 (Kearse et al. 2012). Primer sequences are 17–24 bp long, have a Tm of 53–65 °C, and whenever possible, primer pairs differed less than two degrees in their Tm (Table S1). The pentatricopeptide repeat-containing protein (PP442) gene of *H. forsteriana* was used as reference for qPCR following Dunning et al. (2016). Quantitative PCR reactions were carried out in a LightCycler® 480 Instrument (Roche), using 1 × Power SYBR Green PCR Master Mix (Applied Biosystems). Cycling parameters were adjusted for each primer pair as follows. For *Inositol oxygenase* and *FAR1-Related Sequence 6* (FRS6) we started with 50 °C for 2 min, 95 °C for 10 min, and then 55 cycles comprising denaturation at 95 °C for 15 s, annealing at 45 °C for 15 s, and extension at 72 °C for 1 min. For *TBC1 domain family member 13* and *Coiled-coil protein WEB1* (WEB1), an initial 50 °C for 2 min and 95 °C for 10 min was followed by 45 cycles comprising denaturation at 95 °C for 15 s, annealing at 54 °C for 15 s, and extension at 72 °C for 40 s. For *Serine/*

Table 1. Differentially expressed isoforms between green- and red-stemmed palms. We show here only the 11 gene isoforms that had FDR < 0.1 in the RNA-seq analysis. LogFC is the Log2 fold change in the red-stemmed samples; a negative sign indicates under expression by comparison with green-stemmed samples. Significance of the differences between green-stemmed and red-stemmed samples is given as P (EdgeR for RNA-seq; REST for qPCR). Note that differential expression for only two isoforms, Cyt b_{561} and CHS (in bold), was significant in both qPCR and RNA-seq analyses. "ID" refers to the unique sequence identifier in the reference transcriptome of Dunning et al. (2016).

		RNA-seq			qPCR	
Gene isoforms identified from BLASTN searches	ID	LogFC	P	FDR	LogFC	P
Chalcone synthase	**comp70005_c0_seq1**	**3.232222**	**<0.0001**	**0.072231**	**3.261531**	**0.006**
FAR1-Related Sequence 6	comp99028_c0_seq3	2.965223	<0.0001	0.004592	0.968644	0.446
CBL-interacting serine/threonine-protein kinase 21	comp95792_c0_seq21	2.896958	<0.0001	0.014846	0.488515	0.134
Protein two-pore potassium channel 3	comp98525_c0_seq3	2.812046	<0.0001	0.029111	0.77905	0.202
Coiled-coil protein WEB1	comp98457_c1_seq2	2.113929	<0.0001	0.079518	0.207893	0.57
Cytochrome b561	**comp77778_c0_seq1**	**1.922622**	**<0.0001**	**0.014846**	**1.128953**	**0.046**
Transmembrane and coiled-coil domain-containing protein 1-like	comp98358_c0_seq8	−2.60818	<0.0001	0.058395	−1.72738	0.112
TBC1 domain family member 13	comp102115_c2_seq3	−2.76956	<0.0001	0.010225	1.799087	0.28
Serine/arginine-rich-splicing factor SR34	comp99765_c0_seq6	−4.52548	<0.0001	0.010225	−0.80339	0.87
Inositol oxygenase	comp99722_c1_seq12	−5.02565	<0.0001	<0.0001	2.176961	0.325
Dicarboxylate transport 2.1	comp55923_c0_seq1	−6.95821	<0.0001	0.029111	−0.34556	0.636

Downloaded by [Imperial College London Library] at 06:54 11 January 2018

arginine-rich-splicing factor SR34 (SR34) and *CBL-interacting serine/threonine-protein kinase 21* (CIPK21), the protocol was: 50 °C for 2 min, 95 °C for 10 min, and 55 cycles of 95 °C for 15 s, 58 °C for 15 s, and 72 °C for 30 s. For *Cytochrome b561* (Cyt b_{561}), *Transmembrane and coiled-coil domain-containing protein 1-like* (TMCO1), and *Chalcone synthase* (CHS), PCR reaction was 50 °C for 2 min, 95 °C for 10 min, and 55 cycles of 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 50 s. Finally, for *Protein two-pore potassium channel 3* (TPK3) and *Dicarboxylate transport 2.1* (DCT1), the PCR reaction included 50 °C for 2 min, 95 °C for 10 min, and 45 cycles of 95 °C for 15 s, 49 °C for 15 s, and 72 °C for 40 s. Relative expression (given as copies of the target gene per hundred copies of the reference gene) was calculated following a simplified Pfaffl method (Pfaffl 2001); fold change and statistical significance of the results was calculated using the REST-2009 software (Pfaffl, Horgan, and Dempfle 2002).

Results and discussion

As expected, red stems accumulated, on average, 2.8 times more C3G equivalent than the green stems ($p < 0.001$) (Table S2). It is known that anthocyanin pigments confer red coloration to many plant tissues such as fruits (Wei et al. 2011; Yang et al. 2015), flowers (Sheehan, Hermann, and Kuhlemeier 2012), and leaves (Feild, Lee, and Holbrook 2001). Cyanidin-3-glucoside being the most widespread anthocyanin in nature (Mulder-Krieger and Verpoorte 1994), it is not surprising that red-coloured stems indeed accumulated C3G.

For the RNA-seq data, between 68.9% and 77.5% (mean = 73.2%, SD = 2.5%; Table S3) of the Illumina reads mapped onto the reference transcriptome of *H. forste-riana*, corresponding to 13,868 genes and 31,693 gene isoforms. A total of 322 isoforms were assessed as being differentially expressed ($p < 0.01$; Table S4), although only 11 matched our significant threshold of FDR < 0.1. Six of the latter isoforms were up regulated and five were down regulated in red-stemmed samples (Table 1).

Using qPCR to study transcript accumulation for these 11 isoforms, we found that nine isoforms mirrored the results of the RNA-seq above (Table 1), but only two significant so: *Cytochrome b561* (Cyt b_{561}) and *Chalcone synthase* (CHS), which were both up regulated in the red stems. (Table 1, Figure 2).

Cyt b_{561} is a cytochrome found in the plasma membrane and transfers electrons from cytoplasmic ascorbate (ASC) to an extracellular acceptor. It is known to be involved in ASC recycling and iron homeostasis, and to have Fe^{3+}-reductase activity (Asard et al. 2013). In wild watermelon Cyt b_{561} is over-expressed under drought and high light conditions, helping to dissipate the excess of light energy (Nanasato, Akashi, and Yokota 2005). However, the potential links with an increase in anthocyanin content remain unclear.

CHS is a key enzyme in the anthocyanin biosynthesis pathway, catalysing the first step of the pathway that leads to the synthesis of flavonoids (Sun et al. 2015). Therefore, it is not surprising that we found higher levels of its transcript in red tissues both in the RNAseq and in the qPCR analysis. CHS expression has shown to be increased by biotic and abiotic stimuli, such as wounds, pathogens, and UV light (Dao, Linthorst, and Verpoorte 2011).

We also found that the expression of two isoforms, *Protein two-pore potassium channel 3* (TPK3) and Cyt b_{561}, correlated well with anthocyanin content ($R^2 = 0.242$, $p < 0.01$ for TPK3 and $R^2 = 0.225$, $p < 0.05$ for Cyt b_{561}) (Figure 3). However, CHS expression was not significantly correlated with anthocyanin content, which may be explained because the expression of the first enzyme of the pathway and the accumulation of anthocyanins would happen sequentially.

Finally, after four months, the stems of 149 of the 178 red-stemmed plants (85.6%) had lost their red colouration and turned green, while 100% of the green-stemmed plantlets did not show changes in colouration. This means that further work is needed to select for plants that are stable with regard to the red colouration

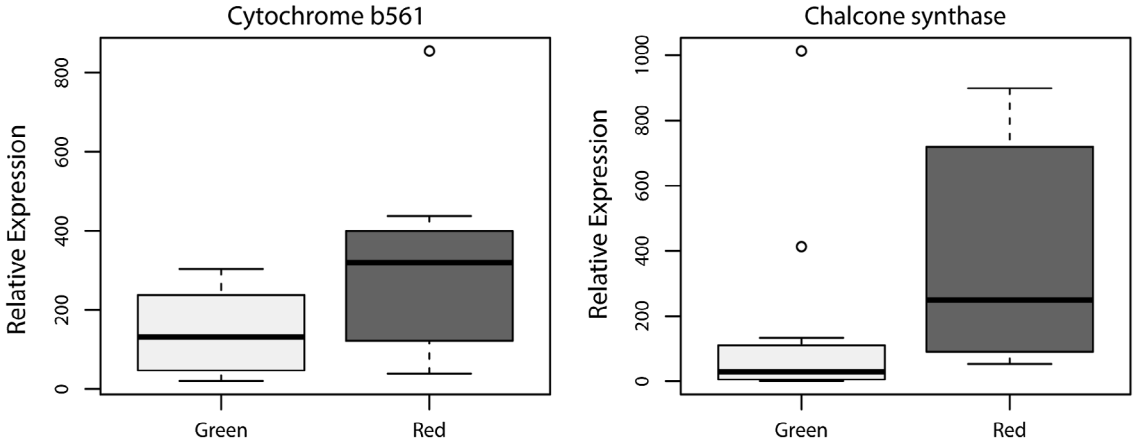


Figure 2. Relative isoform expression in green and red stems, given as mRNA copies of the gene of interest per 100 copies of PP442 mRNA (reference gene). Fold change was statistically significant in both cases ($p < 0.05$).

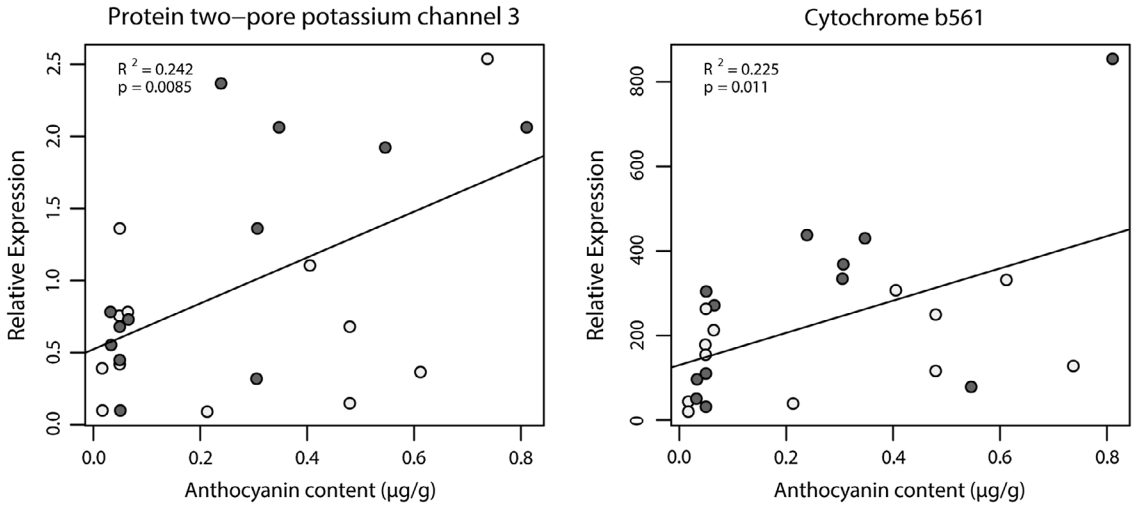


Figure 3. Correlation between anthocyanin content and relative gene (isoform) expression. Relative expression is given as mRNA copies of the gene of interest per 100 copies of PP442 mRNA (reference gene). Anthocyanin content is expressed in µg of C3G per g of tissue. White dots correspond to green stems and grey dots to red stems.

Downloaded by [Imperial College London Library] at 06:54 11 January 2018

of the stem; stability being a requirement for granting PBR or PVR.

We have characterized the genetic underpinning of red stems in kentias and, assuming stable plants can be bred, what is the commercial potential of such a variety? It is quite challenging to have specific figures for the consumption of kentia palms. However, knowing that the worldwide demand for flowers and plants is concentrated in the European countries, China, Japan and the USA (Altmann 2017), we could assume that share for the kentia palm market could equal to roughly 11 million seedlings. LHI's current capacity is only 3% of the global market. However, given that LHI has already registered maximum production capacity of 4 million, we believe that overtime and with the right infrastructures in place we could go back to those high levels of production and establish LHI as the primary supplier of red-stemmed kentia plants. The annual export of 4 million seedlings of the red-stemmed variety could generate £1.2 million to LHI directly, considering the current £0.31 price per seedling applied for the green-stemmed kentia palm (LHI nursery, pers. comm.). Assuming a conservative estimate of £20 for the retail of the new kentia variety (based on the current price of the green variety), we anticipate a global market of £80 million for the new variety. However, given there are, to our knowledge, no other providers of red-stemmed kentia palm, it is possible that the variety could attract premium pricing. This would be an important income for the island, which could benefit its conservation programmes. Indeed, LHI is a UNESCO-designated World Heritage Site, with over 70% of the island protected, and comprising high levels of endemic plants and numerous animals. Today, invasive rats and mice, as well as climate change, threaten the native flora and fauna. A new launch of kentia's business on LHI would be very welcome.

Key questions that need to be answered are now: What type of phenomenon are we looking at (a genetic mutant, a gene-environment interaction or maybe even an epigenetic phenomenon)? How could the phenotype

be "fixed" for commercial use? Do the plants that stayed red provide a clue? Would a search for genetic polymorphisms linked to the red phenotype be of any help? How feasible would be tissue culture to propagate the variety? Date palms are supposedly a lot easier to propagate than oil or coconut palms, what is the situation in *Howea*? These are applied research avenues for the future.

Author contribution statement

VS designed the research, AH conducted the RNA-seq, qPCR and stability analysis, LS performed the anthocyanin measurements, CT and WB provided advice on methods, IH contributed to field collections, VS and AH wrote the manuscript with comments from co-authors.

Acknowledgments

We thank the Lord Howe Island Board and the New South Wales National Park and Wildlife Services for granting permission for our research on LHI, and Imperial Innovation for their help with the market research. We thank Hank and Sue Bower, Christo Haselden, Larry Wilson for their help in the field, Mike Maxwell, Ellen Briand and Rob van Staalduinen for the help with the nursery, two anonymous reviewers for their comments, and the European Research Council, NERC and the Leverhulme Trust for funding.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the FP7 Ideas: European Research Council; Leverhulme Trust; Natural Environment Research Council.

Notes on contributors

Alba Herraiz is the genomic analyst and laboratory manager at the Wolfson Ecological Genomics Laboratories at Silwood Park, Imperial College London.

Laura Stokes was an undergraduate student at Imperial College London.

Colin Turnbull is a plant molecular physiologist at Imperial College London.

Ian Hutton is the curator of the Natural History Museum on Lord Howe Island.

William Baker is the head of Comparative Plant Biology at Kew Gardens.

Vincent Savolainen is in charge of the Ecological Genomics Laboratories at Silwood Park, Imperial College London.

References

Altmann, M. 2017. "Market Description of the Flower Markets: Horticultural Trade Follows the Overall Political Situation." *Floriculture*. Accessed 2 September 2017.http://www.florinews.com/index.php/past-featured-articles/40-past-featured-articles/258-market-description-of-the-flower-markets

Andrews, S. 2014. *FastQC A Quality Control tool for High Throughput Sequence Data*. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Asard, H., R. Barbaro, P. Trost, and A. Berczi. 2013. "Cytochromes b561: Ascorbate-Mediated Trans-Membrane Electron Transport." *Antioxidants & Redox Signaling* 19 (9): 1026–1035. doi:10.1089/ars.2012.5065.

Dao, T. T., H. J. Linthorst, and R. Verpoorte. 2011. "Chalcone Synthase and Its Functions in Plant Resistance." *Phytochemistry Reviews* 10 (3): 397–412. doi:10.1007/s11101-011-9211-7.

DEFRA. 2017. *Agriculture in the United Kingdom 2016*. London: Department for Environment, Food & Rural Affairs.

Dunning, L. T., H. Hipperson, W. J. Baker, R. K. Butlin, C. Devaux, I. Hutton, J. Igea, et al. 2016. "Ecological Speciation in Sympatric Palms: 1. Gene Expression, Selection and Pleiotropy." *Journal of Evolutionary Biology* 29 (8): 1472–1487. doi:10.1111/jeb.12895.

Feild, T. S., D. W. Lee, and N. M. Holbrook. 2001. "Why Leaves Turn Red in Autumn. The Role of Anthocyanins in Senescing Leaves of Red-Osier Dogwood." *Plant Physiology* 127 (2): 566–574. doi:10.1104/pp.010063.

Hipperson, H., L. T. Dunning, W. J. Baker, R. K. Butlin, I. Hutton, A. S. Papadopulos, C. M. Smadja, T. C. Wilson, C. Devaux, and V. Savolainen. 2016. "Ecological Speciation in Sympatric Palms: 2. Pre- and Post-Zygotic Isolation." *Journal of Evolutionary Biology* 29 (11): 2143–2156. doi:10.1111/jeb.12933.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, et al. 2012. "Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data." *Bioinformatics* 28 (12): 1647–1649.

Li, B., and C. N. Dewey. 2011. "RSEM: Accurate Transcript Quantification from RNA-Seq Data with or without a Reference Genome." *BMC Bioinformatics* 12: 323. doi:10.1186/1471-2105-12-323.

Madden, T. 2002. "The BLAST Sequence Analysis Tool." In *The NCBI Handbook [Internet]*, edited by J. McEntyre and J. Ostell. National Center for Biotechnology Information (US).

Mulder-Krieger, T., and R. Verpoorte. 1994. *Anthocyanins as Flower Pigments: Feasibilities for Flower Colour Modification*. Dordrecht: Springer.

Nanasato, Y., K. Akashi, and A. Yokota. 2005. "Co-Expression of Cytochrome b561 and Ascorbate Oxidase in Leaves of Wild Watermelon under Drought and High Light Conditions." *Plant and Cell Physiology* 46 (9): 1515–1524. doi:10.1093/pcp/pci164.

Pfaffl, M. W. 2001. "A New Mathematical Model for Relative Quantification in Real-Time RT-PCR." *Nucleic Acids Research* 29 (9): 45e.

Pfaffl, M. W., G. W. Horgan, and L. Dempfle. 2002. "Relative Expression Software Tool (REST) for Group-Wise

Downloaded by [Imperial College London Library] at 06:54 11 January 2018

Comparison and Statistical Analysis of Relative Expression Results in Real-Time PCR." *Nucleic Acids Research* 30 (9): 36e.

Riffle, R., P. Craft, and S. Zona. 2012. *The Encyclopedia of Cultivated Palms*. 2nd ed. Portland: Timber Press.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. "EdgeR: A Bioconductor Package for Differential Expression Analysis of Digital Gene Expression Data." *Bioinformatics* 26 (1): 139–140. doi:10.1093/bioinformatics/btp616.

Savolainen, V., M. C. Anstett, C. Lexer, I. Hutton, J. J. Clarkson, M. V. Norup, M. P. Powell, D. Springate, N. Salamin, and W. J. Baker. 2006. "Sympatric Speciation in Palms on an Oceanic Island." *Nature* 441 (7090): 210–213. doi:10.1038/nature04566.

Sheehan, H., K. Hermann, and C. Kuhlemeier. 2012. "Color and Scent: How Single Genes Influence Pollinator Attraction." *Cold Spring Harbor Symposia on Quantitative Biology* 77: 117–133. doi:10.1101/sqb.2013.77.014712.

Sirén, H., K. Sirén, and J. Sirén. 2015. "Evaluation of Organic and Inorganic Compounds Levels of Red Wines Processed from Pinot Noir Grapes." *Analytical Chemistry Research* 3: 26–36.

Sivitz, A. B., A. Reinders, and J. M. Ward. 2008. "Arabidopsis Sucrose Transporter AtSUC1 is Important for Pollen Germination and Sucrose-Induced Anthocyanin Accumulation." *Plant Physiology* 147 (1): 92–100. doi:10.1104/pp.108.118992.

Sun, W., X. Meng, L. Liang, W. Jiang, Y. Huang, J. He, H. Hu, J. Almqvist, X. Gao, and L. Wang. 2015. "Molecular and Biochemical Analysis of Chalcone Synthase from Freesia Hybrid in Flavonoid Biosynthetic Pathway." *PLoS One* 10 (3): e0119054. doi:10.1371/journal.pone.0119054.

Wei, Y. Z., F. C. Hu, G. B. Hu, X. J. Li, X. M. Huang, and H. C. Wang. 2011. "Differential Expression of Anthocyanin Biosynthetic Genes in Relation to Anthocyanin Accumulation in the Pericarp of Litchi Chinensis Sonn." *PLoS One* 6 (4): e19455. doi:ARTN e19455/10.1371/journal.pone.0019455.

Yang, Y. N., G. F. Yao, D. M. Zheng, S. L. Zhang, C. Wang, M. Y. Zhang, and J. Wu. 2015. "Expression Differences of Anthocyanin Biosynthesis Genes Reveal Regulation Patterns for Red Pear Coloration." *Plant Cell Reports* 34 (2): 189–198. doi:10.1007/s00299-014-1698-0.

Downloaded by [Imperial College London Library] at 06:54 11 January 2018